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# Integrated Second Peristaltic Pump for Improved Sample Throughput and Reduced Matrix Effects

Technical Overview

## Introduction

As a simpler, less expensive alternative to Agilent's Integrated Sample Introduction System (ISIS), the Second Peripump Option permits both rapid sample uptake and constant flow nebulization for improved throughput and matrix tolerance and is fully compatible with all the most recent hardware and software improvements on the 7500 Series ICP-MS.

### Uncoupling Sample Uptake from Sample Nebulization

With the introduction of its ISIS in 1999, Agilent pioneered the use of constant flow nebulization in ICP-MS. By uncoupling the sample uptake rate (using a separate, high-capacity peristaltic pump) from the nebulizer flow rate (using a small, close-coupled peristaltic pump), very high sample throughput could be achieved without the concurrent overloading of the sample introduction system and plasma. Total matrix load on the plasma could be reduced by a factor of 5x or more over a conventional single pump system with the added benefit of significantly higher sample throughput. While ISIS supported many other functions, including autodilution and matrix elimination, for many users, constant flow nebulization was the primary advantage.

### Second Peristaltic Pump Option

Based on the input of dozens of ISIS users, Agilent decided to introduce a simplified, more economical solution for rapid sample uptake and constant flow nebulization: a computer-controlled second peristaltic pump that is fully compatible with recent software and hardware enhancements to improve matrix tolerance and productivity.

The second peristaltic pump option works seamlessly with both Pre-emptive and Intelligent Rinse functions, as well as with the new High Matrix Sample Introduction (HMI) accessory. The result is simple, fully integrated, high sample throughput for even the most difficult samples, with no danger of plasma overloading or sample carryover.

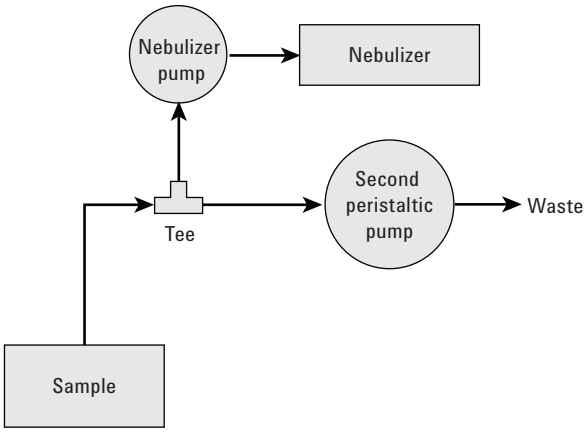


**Figure 1. Schematic of the second peristaltic pump arrangement.**

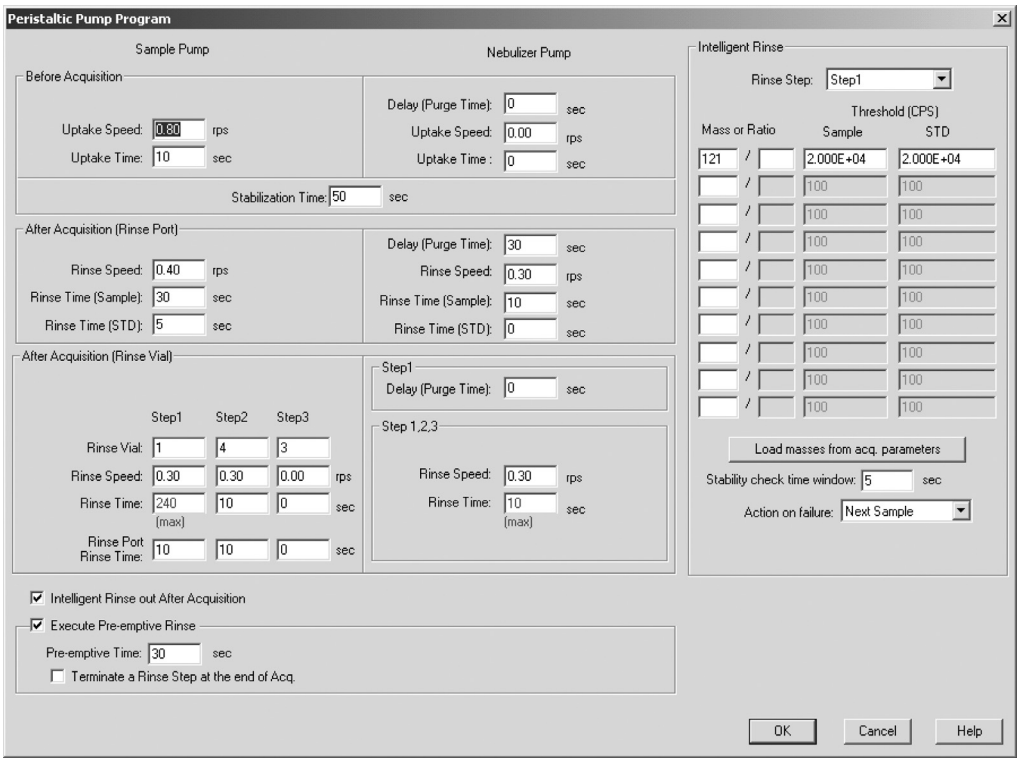


**Figure 2. The second peristaltic pump option allows completely independent control of both the sample uptake pump (left) and the nebulizer pump (right) and fully supports all intelligent and pre-emptive rinse functions.**

Operation of the second peristaltic pump is simple and fully controlled by the ChemStation software. The second peristaltic pump is used to rapidly pull the sample to the Tee shown on Figure 1. After the sample reaches the Tee, the nebulizer pump transports it to the nebulizer at a constant flow, without the need to alter the pump speed. This constant flow eliminates extended stabilization time and the introduction of excessive sample into the plasma, interface, and mass spectrometer.

The ChemStation pre-emptive rinse feature will move the autosampler probe to the rinse station to begin cleaning the probe and sample line while the nebulizer pump delivers the remaining sample to complete the analysis. In addition, Intelligent Rinse will ensure that rinse time is sufficient by monitoring up to 10 predefined element signals, but avoid excessive rinse times when they are not needed (for example, after a blank). A significant advantage to this system is that high throughput is accomplished without the need to use a six-port valve or additional vacuum pumps.

## Ordering Information

Order Agilent product number G3146B to add a second three-channel peristaltic pump integrated into the mainframe. ISIS tubing kit G3138-65006 is recommended with G3146B. It is preconfigured for easy set up. Please note that G3146B is not compatible with G3148B (ISIS).

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Agilent Technologies

# Eliminate the Dilution Step from ICP-MS Sample Prep with the Agilent High Matrix Introduction System

## Introduction

Agilent has developed the High Matrix Introduction (HMI) accessory for ICP-MS as an alternative to conventional dilution. The HMI modifies the sample introduction system of the Agilent 7500 Octopole Reaction System (ORS) ICP-MS, making it possible to directly measure sample solutions with total dissolved solids (TDS) exceeding 1%.

## Analytical Challenges

Analysts at TestAmerica Savannah labs in the USA tested the HMI for the analysis of soil and Toxicity Characteristic Leaching Procedure (TCLP) samples. For ICP-MS analysis in the Savannah Labs, these types of samples are typically diluted by a factor of 1:5 for liquids and 1:10 for soils. The dilution steps reduce the matrix effects contributed by both the acids used in the preparation and the samples themselves. There are several disadvantages associated with this practice:

- Possibility of introducing contamination from the pipette tips or the diluent
- Chance of “human” error of simply preparing an inaccurate dilution
- Sample prep time and cost of reagents
- Increased waste disposal volume

## HMI Methodology

With the HMI unit installed on the 7500ce, TestAmerica analysts are now able to introduce most typical environmental samples to the ICP-MS directly, without a dilution step, and thus eliminate the disadvantages associated with conventional dilution.

## High Acid Matrices

A significant hurdle to running undiluted environmental samples is related to the final acid concentration specified in USEPA method 3050B for soils and wastes using the "hot-plate" procedure, which includes 10% HCl. Method 3010A for liquids specifies a final concentration of 5% HCl. Prior to using the HMI, matrix matching during the dilution step was used to reduce and normalize the acid concentration in standards and both types of samples. Not only does the HMI eliminate the matrix effects from varying acid concentrations, it also removes the need for expensive platinum cones when analyzing high acid concentrations. TestAmerica now uses 5% $HNO_3$ /5% HCl for standards and blanks with all sample types run using HMI.

The HMI was set-up for typical “ultra-robust” analysis. Table 1 shows comparison Method Detection Limit (MDL) data (mg/kg) for soils with and without the HMI. With the HMI, the digestates were analyzed directly (undiluted); without the HMI, the digestates were diluted 1/10. The results highlight an improvement in MDLs for the majority of elements when the HMI is used.

| Element (Mass) | HMI MDL mg/kg | w/o HMI MDL (1/10 dil.) mg/kg |
|---|---|---|
| Al (27) | 4.1 | 5.8 |
| Sb (121) | 0.026 | 0.031 |
| As (75) | 0.03 | 0.15 |
| Ba (137) | 0.053 | 0.17 |
| Be (9) | 0.021 | 0.018 |
| B (11) | 0.33 | 1.3 |
| Cd (111) | 0.029 | 0.031 |
| Ca (40) | 5.7 | 8.4 |
| Cr (52) | 0.031 | 0.13 |
| Co (59) | 0.0064 | 0.0094 |
| Cu (63) | 0.078 | 0.14 |
| Fe (56) | 1.7 | 4.6 |
| Pb (208) | 0.055 | 0.03 |
| Mg (24) | 1.3 | 1.9 |
| Mn (55) | 0.037 | 0.11 |
| Mo (95) | 0.036 | 0.058 |
| Ni (60) | 0.024 | 0.036 |
| K (39) | 3.8 | 6.5 |
| Se (78) | 0.13 | 0.044 |
| Ag (107) | 0.005 | 0.0071 |
| Na (23) | 1.8 | 15 |
| Sr (88) | 0.051 | 0.066 |
| Tl (205) | 0.026 | 0.015 |
| Sn (118) | 0.105 | 0.24 |
| Ti (47) | 0.17 | 0.12 |
| V (51) | 0.041 | 0.096 |
| Zn (66) | 0.84 | 0.64 |

**Table 1. Comparison of MDL for a suite of elements using HMI and normal dilution.**

## Conclusions

The addition of the High Matrix Introduction system to the Agilent 7500ce ICP-MS allows the analysis of soils without the complications of a dilution step and with slightly improved Method Detection Limits (MDL). This ensures that soils analyzed according to USEPA methodologies can now be done so with significantly improved productivity and reduced costs.


## Acknowledgements

Cliff Eaton & Ernie Walton, TestAmerica Inc. Savannah, Ga., USA

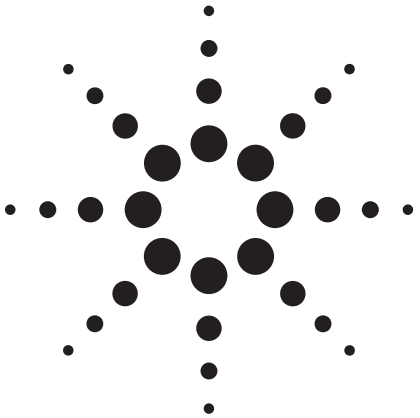

## More Information www.agilent.com/chem/hmi

**www.agilent.com/chem/icpms**



Agilent Technologies

# Retention Time Locking with the MSD Productivity ChemStation



Technical Overview

## Introduction

A retention time is the fundamental qualitative measurement of chromatography. Most peak identification is performed by comparing the retention time of the unknown peak to that of a standard. It is much easier to identify peaks and validate methods if there is no variation in the retention time of each analyte.

However, shifts in retention time occur frequently. Routine maintenance procedures such as column trimming alter retention times. In a multi-instrument laboratory running duplicate methods, the retention times for each instrument will differ from each other, even when run under nominally identical conditions. These differences in retention times mean that each instrument must have a separate calibration and integration event table, making it time-consuming to transfer methods from one instrument to another. Differences in retention time also complicate comparison of data between instruments over time.

## What Is Retention Time Locking?

Retention time locking (RTL) allows a close match of retention times on one Agilent Technologies GC/MSD or GC system to those on another like system with the same nominal column. By making an adjustment to the inlet pressure, the retention times on one system can be closely matched to those on another system using the same nominal column. The ability to very closely match retention times from one system to another can greatly reduce the time it takes to develop and transfer methods. “RTLocked” methods can also compensate for degradations in chromatographic performance. The ability to correct for degrading chromatographic performance, optimize lab resources, and still provide the correct answer saves time, money, and results in significant productivity gains.

Using GC/MS, it is also possible to screen samples for the presence of target compounds using a mass spectral database of RTL spectra. The RTL mass spectral database provides additional confirmatory information in spite of changes to the chromatographic system. One such database is the G1672AA Pesticide RTL Library. This database allows for quick and easy screening of pesticide samples.

## When Should I Lock My Methods?

Locking or relocking your methods should be done whenever you make any changes to the chromatographic system or move methods from one system to another (GC or GC/MS). To establish and maintain a locked method, RTL should be performed whenever:

- The column is changed or trimmed
- The method is installed on a new instrument
- A detector of different outlet pressure is used (GC vs. MS)
- System performance is validated
- Troubleshooting chromatographic problems

## How Does It Work?

The process of RTL is to determine what adjustment in inlet pressure is necessary to achieve the

desired match in retention times. To lock a given method for the first time or for the reasons above, you must first develop a Retention Time vs. Pressure (RT vs. P) calibration curve. Using an established method, multiple (five) injections of the standard are used to calculate the retention times at predefined inlet pressures. The use of an automatic sampler simplifies this process. The RT vs. P calibration data for each of the five injections are saved and used to correct locked methods.

The five defined pressures are:

- Target pressure –20%
- Target pressure –10%
- Target pressure (nominal method pressure)
- Target pressure +10%
- Target pressure +20%

Even when using columns with the same part number (same id, stationary phase type, phase ratio, and same nominal length), separate and different locking calibration curves may be needed. Other examples of when separate locking calibration curves are required include:

- Systems with different column outlet pressures (MSD/vacuum, FID/atmospheric)
- Columns differing from the "nominal" length by more than 15% (for example, due to trimming)
- Systems where the predicted locking pressure falls outside the range of the current calibration

### Selecting the Standard Compound for RTLocking

A specific compound (usually one found in the normal method calibration standard) must be chosen and then used for both developing the locking calibration and locking all future systems. The compound, or target peak, should be easily identifiable, symmetrical, and should elute in the most critical part of the chromatogram. Compounds that are very polar or subject to degradation should be avoided.

Once the target compound has been chosen and all other chromatographic parameters of the method have been determined, the five calibration runs are performed. The resulting RT vs. P calibration curve data are saved. The software is then used to select and integrate the peak used for locking.

## Creating an RTL Method

The following is an overview of the actual steps taken to acquire the RT vs. P calibration curve data, selecting the compound/peak to use and locking the method.

### Acquiring the RTLock Data

From **Instrument Control**, select the **Instrument** menu and **Acquire RTLock Calibration Data....** Agilent Technologies GCs are the only ones supported. See Figure 1.

If you are using an automatic sampler, the five sample injections will be made automatically. This example illustrates how RTL works on a GC/MS system. Upon completion of the five-sample analysis, Data Analysis will begin and display the nominal MS total ion chromatogram (TIC). If the system was configured as a stand-alone GC, all five chromatograms will be displayed. From these displays, you will select the compound that will be used for RTLocking of the method.

### Selecting the RTLock Compound

Use the mouse to select the compound or peak that you would like used for locking. For GC-only mode, you must select one peak from each of the five chromatograms for RTLocking. Once you have made your selection, you will be asked to allow the software to automatically find the remaining peaks. You may choose to zoom the display for better visibility. See Figure 2.

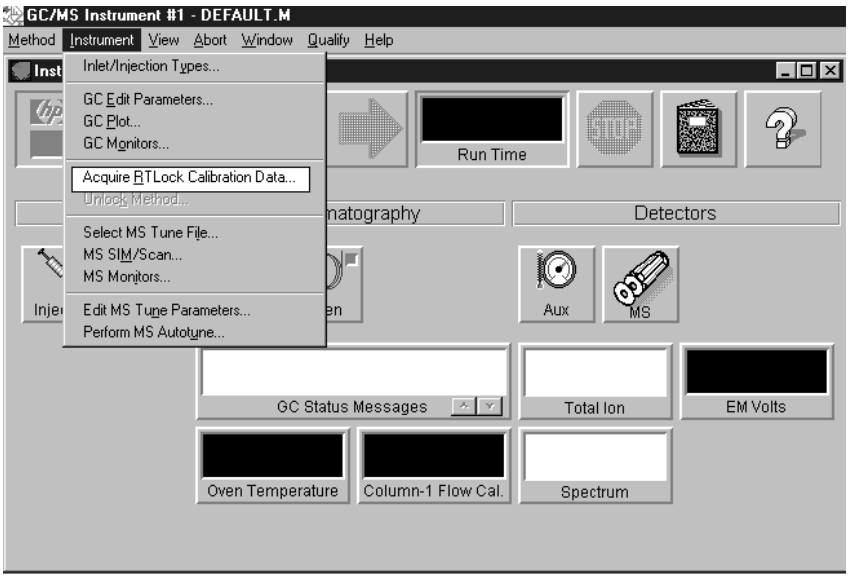



**Figure 1. From instrument control, you select the mode of data acquisition for RTL.**

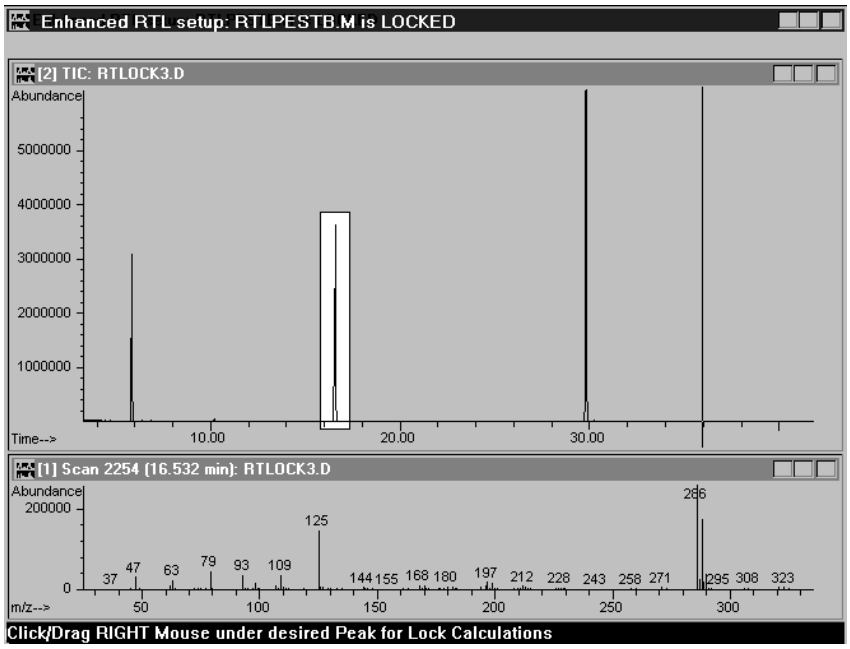



**Figure 2. From this panel, the user selects the peak used for RTL. In this example the second peak has been selected. The spectrum of the selected compound is also displayed and is used to confirm the RTL compound.**

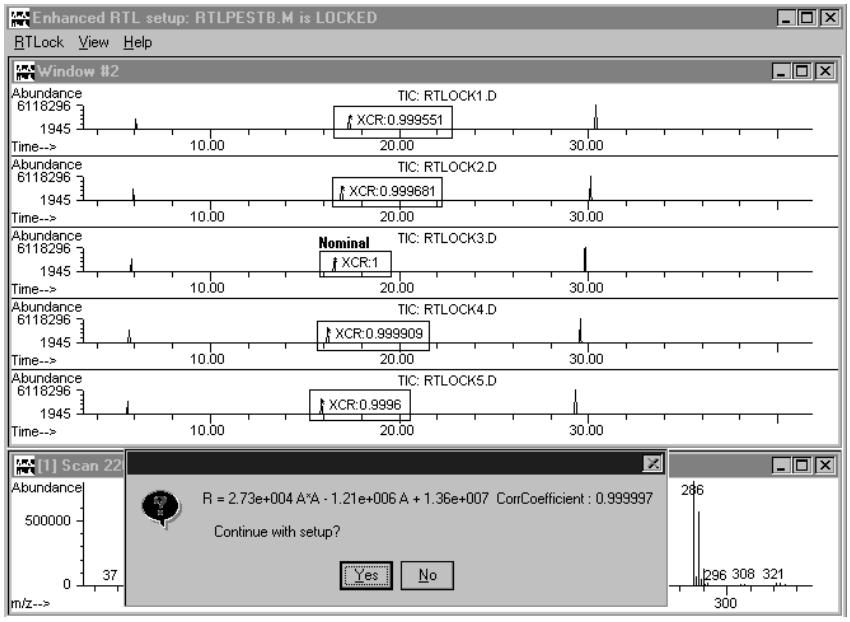



**Figure 3. When the RT vs. P calibration curve equation is calculated, the correlation coefficient is determined for the RTLocked compound in each of the five calibration samples. The resulting coefficient is displayed at each peak. The "nominal," or no change to pressure calibration sample, has a correlation of 1.**

### Calculating the RTLock Curve and Saving a Method

Once the RTLock compound has been selected, the new RT vs. P curve for each compound will be displayed. To select a new compound and calculate new RT vs. P curves, reset the nominal MSTIC from the **RTLock** menu item. Select a new compound as the RTL compound and from the **RTLock** menu item, select **Calculate New Curve from Selected Peaks**. This will generate new RT vs. P calibration curves. The new RT vs. P calibration curve equation will be displayed on the screen along with the correlation coefficient. Select **Yes** to either create a new, or update an existing, RT vs. P calibration file. See Figure 3.

Next, you can enter the name and retention time of the RTLock compound. See Figure 4 and Figure 5.

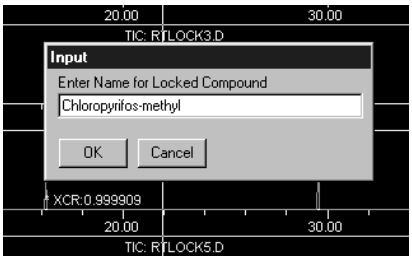



**Figure 4. Enter or confirm the name of the RTLock compound.**

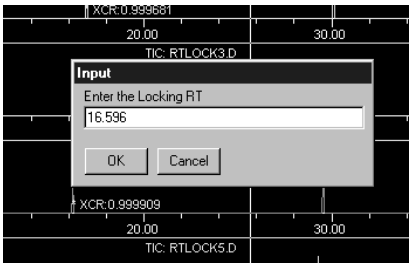



**Figure 5. Enter or confirm the retention time of the RTLock compound.**

You will then be asked to confirm the RTLock pressure that has been calculated and will be used for that method. Select **Yes** to confirm the new RTLock pressure and save the method. See Figure 6.

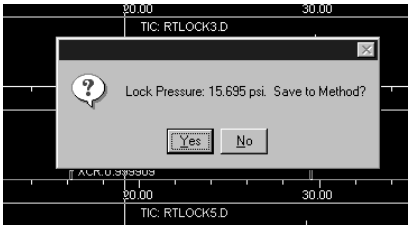



**Figure 6. By selecting Yes, you RTLock and save the method.**

### View Current RTLock Method Setpoints and Report

Once the system is calibrated and locked, you can view and confirm the RTLock setpoints by selecting **View Current Method Setpoints.** See Figure 7.

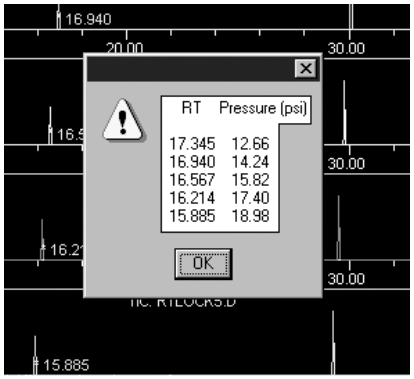



**Figure 7. Viewing the RTLock retention times and corresponding pressures for the RTLock method.**

A report is also available that provides detailed information regarding the RTLock method. See Figure 8. The report includes:

- Method name
- Calibration date
- Instrument name
- Operator name
- Status of method (on or off)
- RTLock compound name
- Tabular retention time/ pressure calibration table
- Maximum deviation
- RTLock curve equation and correlation coefficient data
- Locked retention time information (file name, acquisition date, instrument name, and operator name)
- Report date

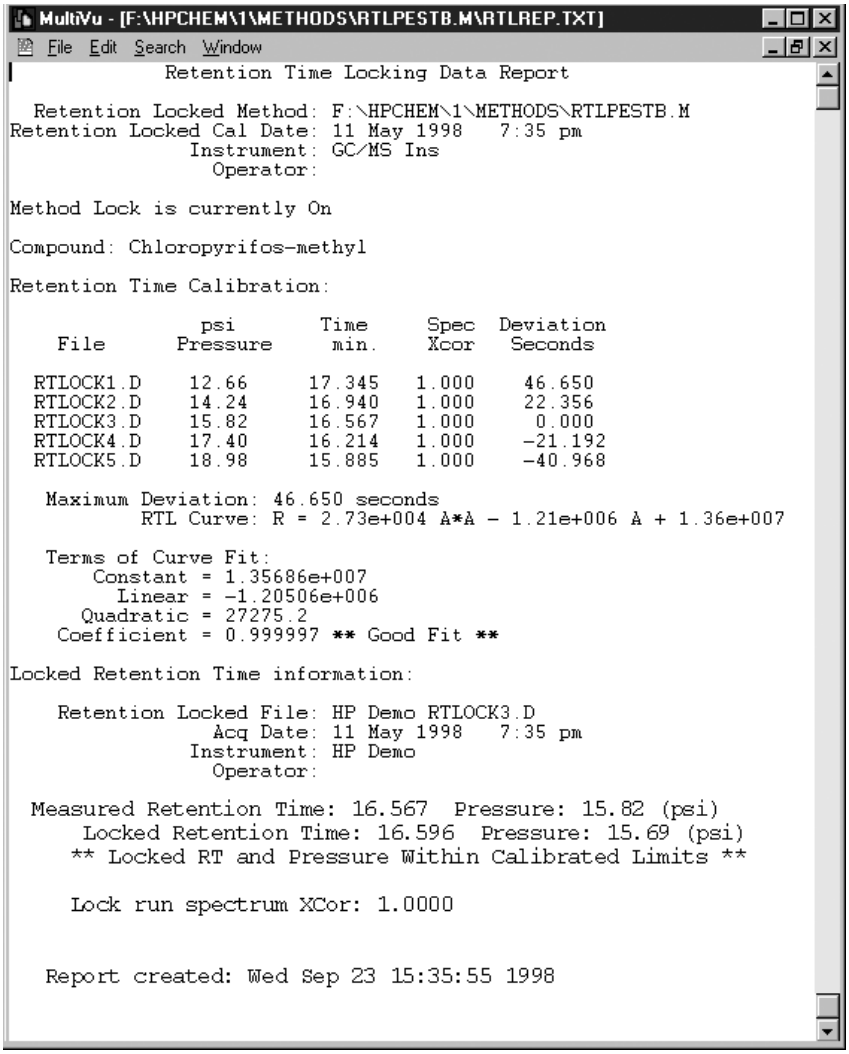



**Figure 8. Example RTLock Report**

### Running an RTLock Method

Once the RTL method is created, you can analyze and process new samples. This requires that you unlock and relock the methods without editing the quantitation calibration data. The following example demonstrates how retention times might change when column maintenance is performed or a method is moved to a new GC/MS system. See Figure 9. Once the method is RTLocked, new samples are analyzed and retention times corrected. See Figure 10.

### Unlocking and Relocking a Method

Once a method is locked you may unlock or relock it using the same or different compounds or after additional maintenance. To define a new compound for RTLocking, select **RTLock Setup** from the **View** menu in **Data Analysis**. See Figure 11. The nominal RTLock sample that represents the method you are working on will be displayed. Once again, use the mouse to identify a new RTLock compound.

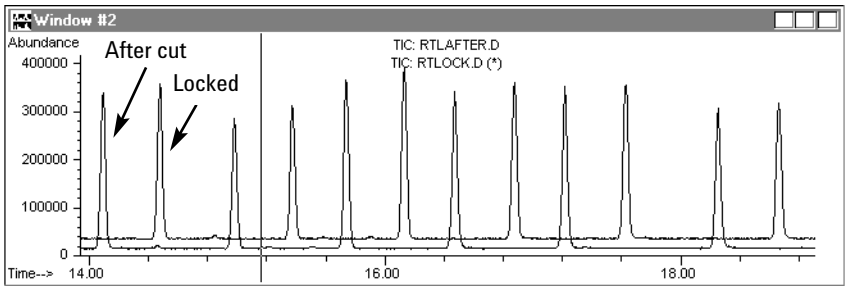



**Figure 9. Overlay of an RTLocked data file (labeled Locked) and the resulting data file after clipping one meter off the column (labeled After Cut).**

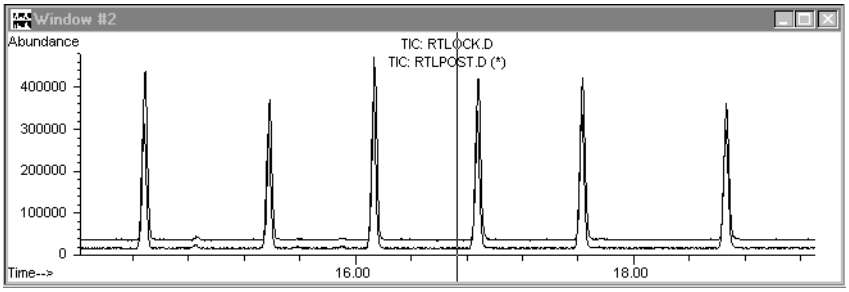



**Figure 10. Offset overlay of RTLocked data file and the RTCorrected datafile show peaks overlapped and corrected for column maintainence.**

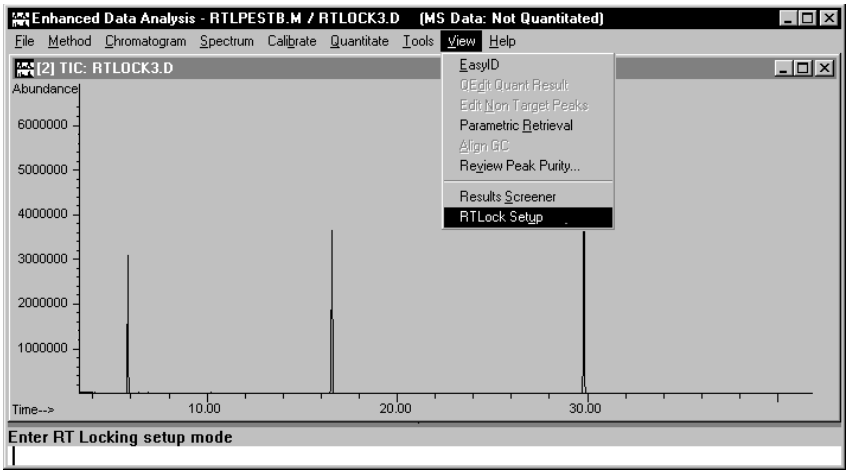



**Figure 11. To define a new compound for RTLocking, select** RTLock Setup **from the** View **menu in data analysis.**

After selecting the compound to RTLock, from the **RTLock** menu item, select **Calculate New Curve** from **Selected Peaks**. This will generate a new RT vs. P calibration curve. The new RT vs. P calibration curve equation will be displayed on the screen along with the correlation coefficient. Select **Yes** to create a new or update an existing RTLock calibration. See Figure 12. From the RTLock menu you can also:

- View current setpoints
- Calculate the RT vs. P curve
- Restore the original chromatograms
- Report the RTLock calibration
- Unlock the method
- Relock the method

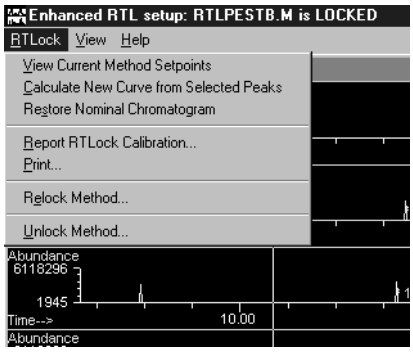



**Figure 12. RTLock view and menu choices.**

## Summary

RTLocking provides an easy and flexible tool that can be used to reduce the time and complexity often associated with routine chromatographic maintenance. It allows methods to be transferred between like GC/MS systems without time-intensive edits to the quant database and reacquisition of standards. It also simplifies the process for executing routine chromatographic maintenance. RTLocking can minimize mistakes and provide a productivity improvement for most applications by reducing the time and setpoint changes required to update a method.

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# Case Study for Agilent Lab Monitor and Diagnostic Software

Application

**HPI, Environmental, Food Safety**

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## Abstract

**Agilent Lab Monitor & Diagnostic (LMD) software can monitor in real time all the Agilent GCs and LCs in your lab. It automatically tracks supply usage, monitors chromatographic quality, and notifies you of maintenance needs before a problem occurs by keeping track of injections, hours of operation, and other parameters that you specify. Two case studies are given demonstrating some of the features and benefits of this software.**

## Introduction

Agilent Lab Monitor & Diagnostic (LMD) software is an innovative tool to help you manage your lab to ensure performance, productivity, and reliability. LMD software can monitor in real time a single Agilent GC or LC or all the Agilent GCs and LCs in your lab. It automatically tracks supply usage, monitors chromatographic quality, and notifies you of maintenance needs before a problem occurs by keeping track of injections, hours of operation, and other parameters that you specify. LMD software "knows" when it's time to replace consumables or perform basic upkeep. It provides full diagnostic capabilities with an extended list of tests and calibration procedures and automates basic diagnostic routines that help verify proper instrument performance. The software is coupled with an extensive suite of user information Help functions that provide quick, easy access to maintenance information, such as manuals and videos, so that you can get the information you need right when you need it.

LMD provides the following features and benefits:

- Increases your lab's uptime by alerting you to problems before they happen
- Provides intuitive help with diagnostic capabilities and easy-to-follow repair procedures in case of a problem
- Enhances diagnostics and troubleshooting functionality with searchable, complete user information capability
- Keeps your systems in top condition and helps with routine troubleshooting
- Maximizes column and consumables utilization by optimizing replacement schedules
- Helps you meet regulatory requirements by keeping all maintenance, event, and run logs in a single, easily accessible location
- Provides a link to the optional, Web-enabled Remote Advisor to back up your internal service and support resources

The following two case studies demonstrate some of the features and benefits of the Agilent LMD software.

### Configuration for Case Studies

- One PC with LMD monitors two Agilent 7890A GCs
- Agilent LMD software (A.01.03, Advanced)
- Firmware (A.01.06)
- ChemStation (B.03.02)

The first case study demonstrates the feature/benefit of right advice/alarm by tracking resource counters before results go bad.

### Problem

Oxygen is an enemy to most capillary GC columns, especially polar columns. In this study, a polar column is used for analyzing trace oxygenates. The baseline is getting worse after 400 runs because of septum leakage, which may impact quantitative analysis for trace-level analysis (see Figure 1, upper chromatogram).

### Tracking Resource Counters and Giving Alarm Before Results Go Bad

Because LMD is configured to track the GC's inlet septum, it reminds the user to change the septum when the limit has been reached, before the GC baseline goes bad. After the septum is changed, the expected baseline is obtained. Figure 1 shows the baseline of the polar column after 400 runs (top) and after the septum has been changed (bottom).

LMD can track not only the inlet septum, but also other GC resources, like the inlet o-ring, oxygen traps, inlet gold seal, and so on. As Figure 2 shows, LMD gives real-time indicators and alerts of preventive maintenance needs.

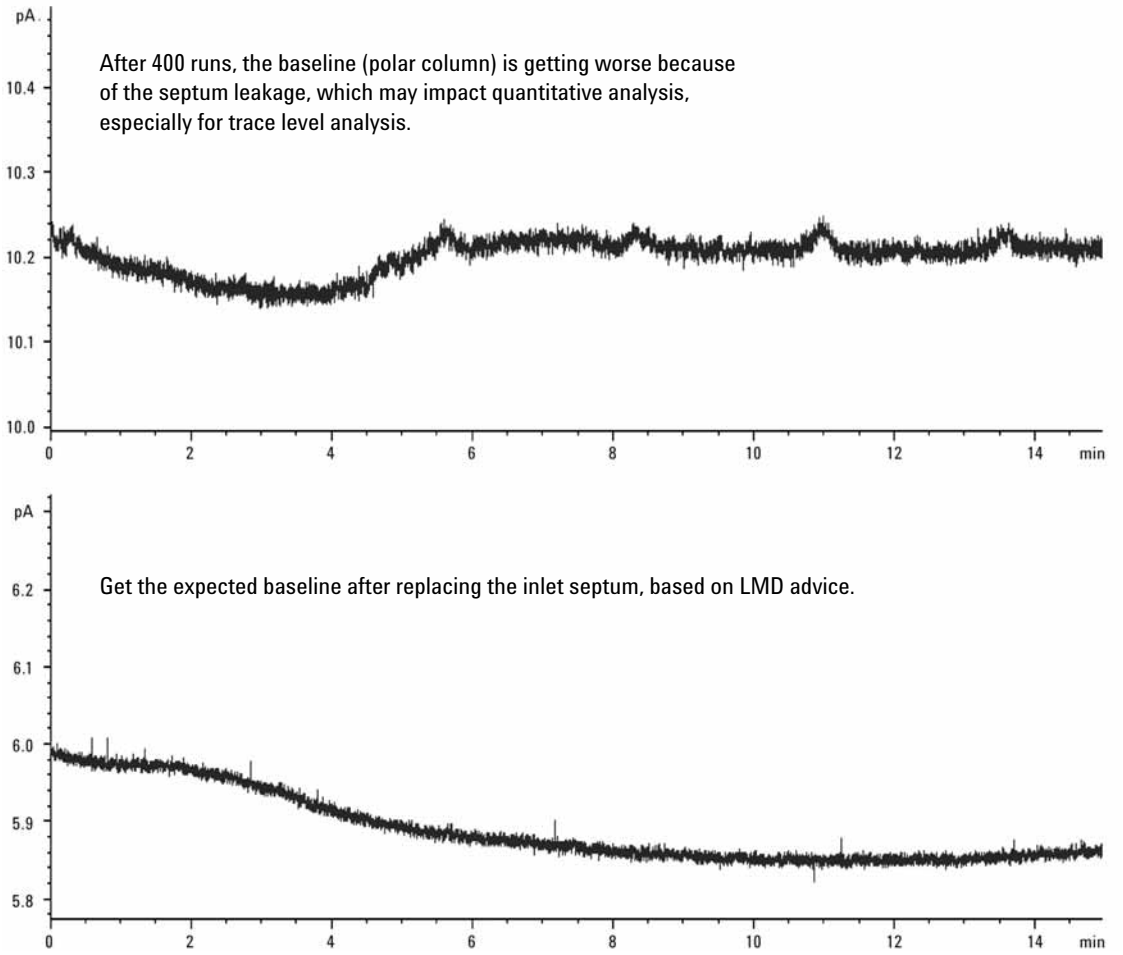



**Figure 1. Baseline chromatograms on polar column after 400 runs and after the inlet septum has been replaced.**

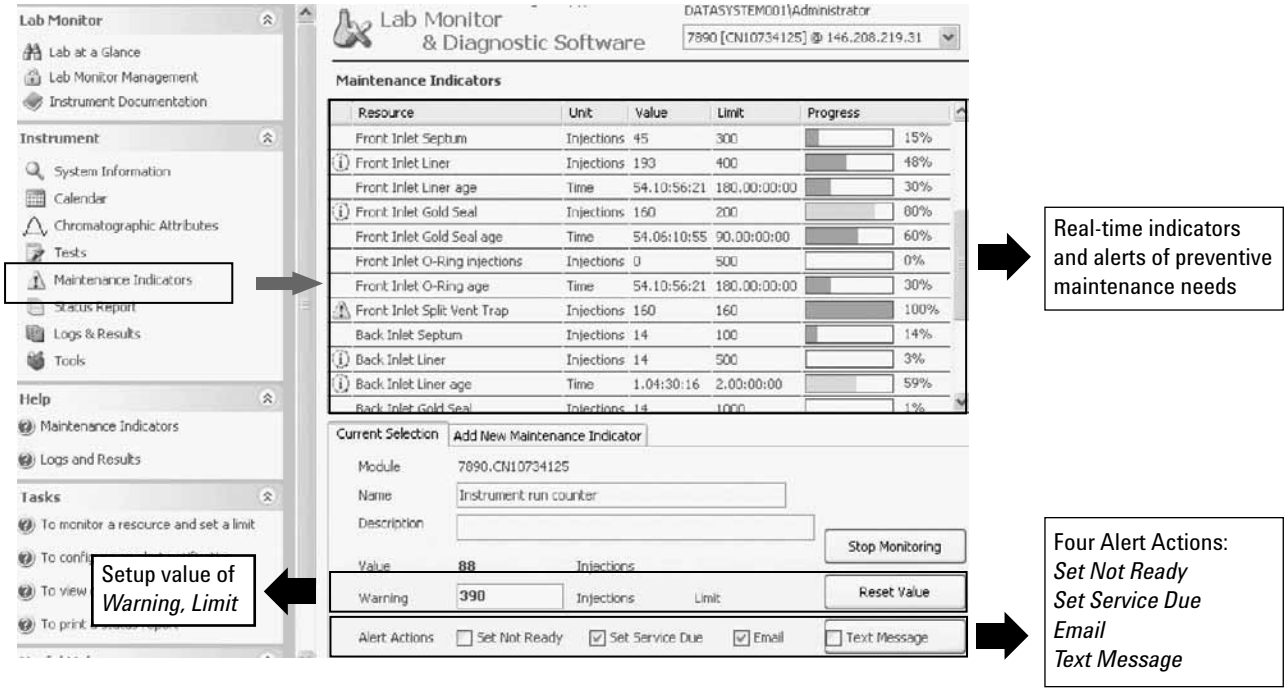



**Figure 2. LMD real-time indicators and alerts of preventive maintenance needs.**

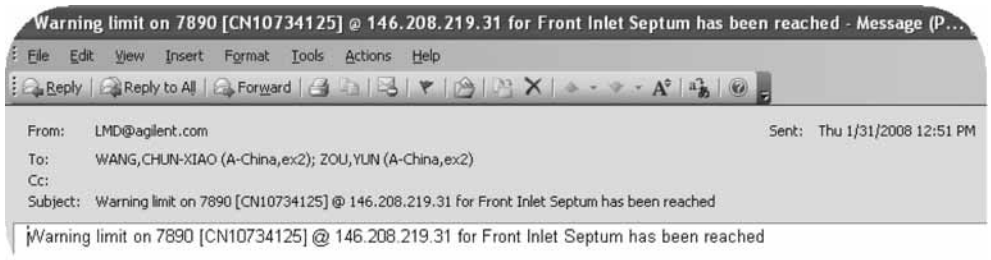



**Figure 3. Alert email for when the front inlet septum limit has been reached.**

Four alert actions are available: Set Not Ready, Set Service Due, Email, and Text Message. For example, when the email alert action is selected, LMD will send an alert email to the users you specify. Figure 3 shows an example email alert informing a user that the front inlet septum's limit has been reached.

An email alert is configured in only three steps:

1. Manage the users (see Figure 4)
2. Manage the alerts (see Figure 5)
3. Configure the maintenance indicators

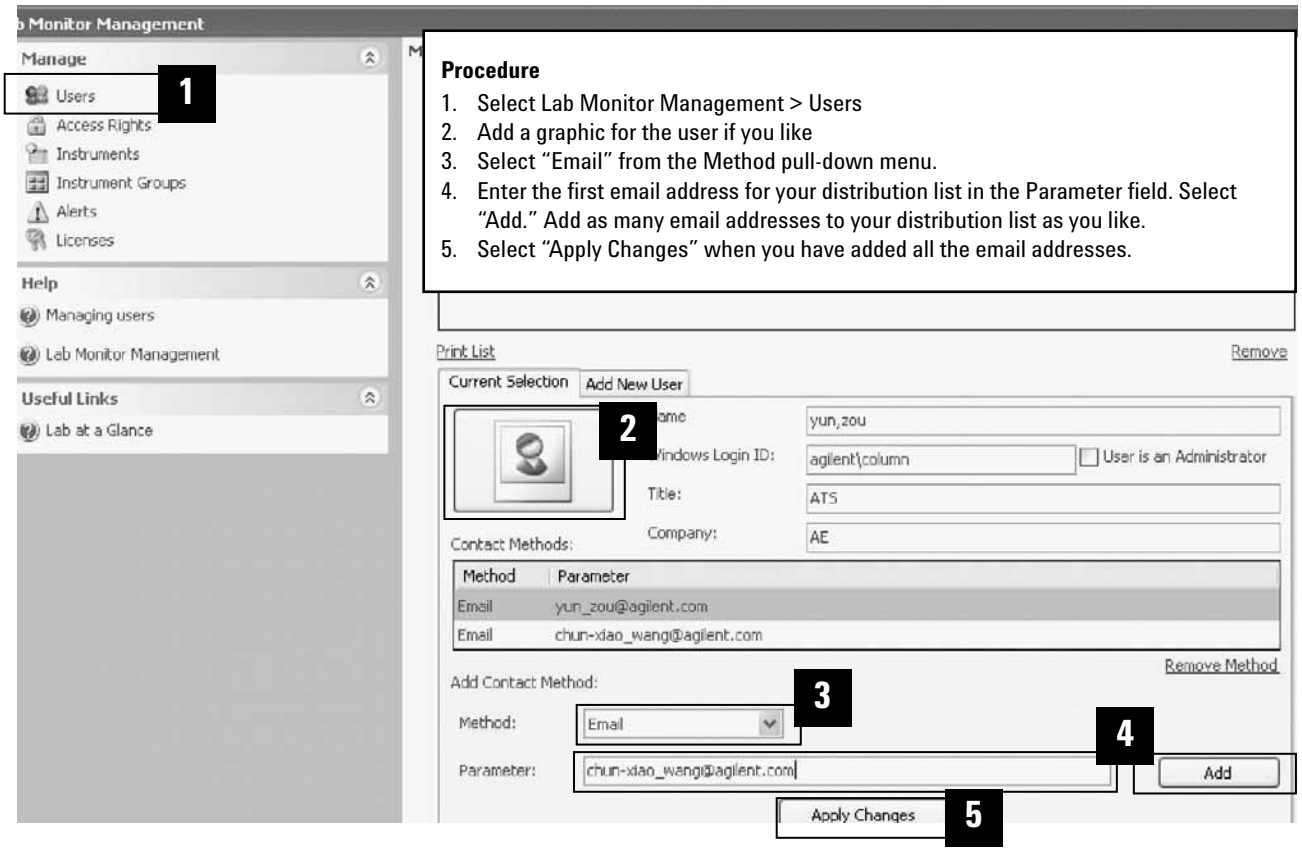



**Figure 4. Procedure for managing users.**

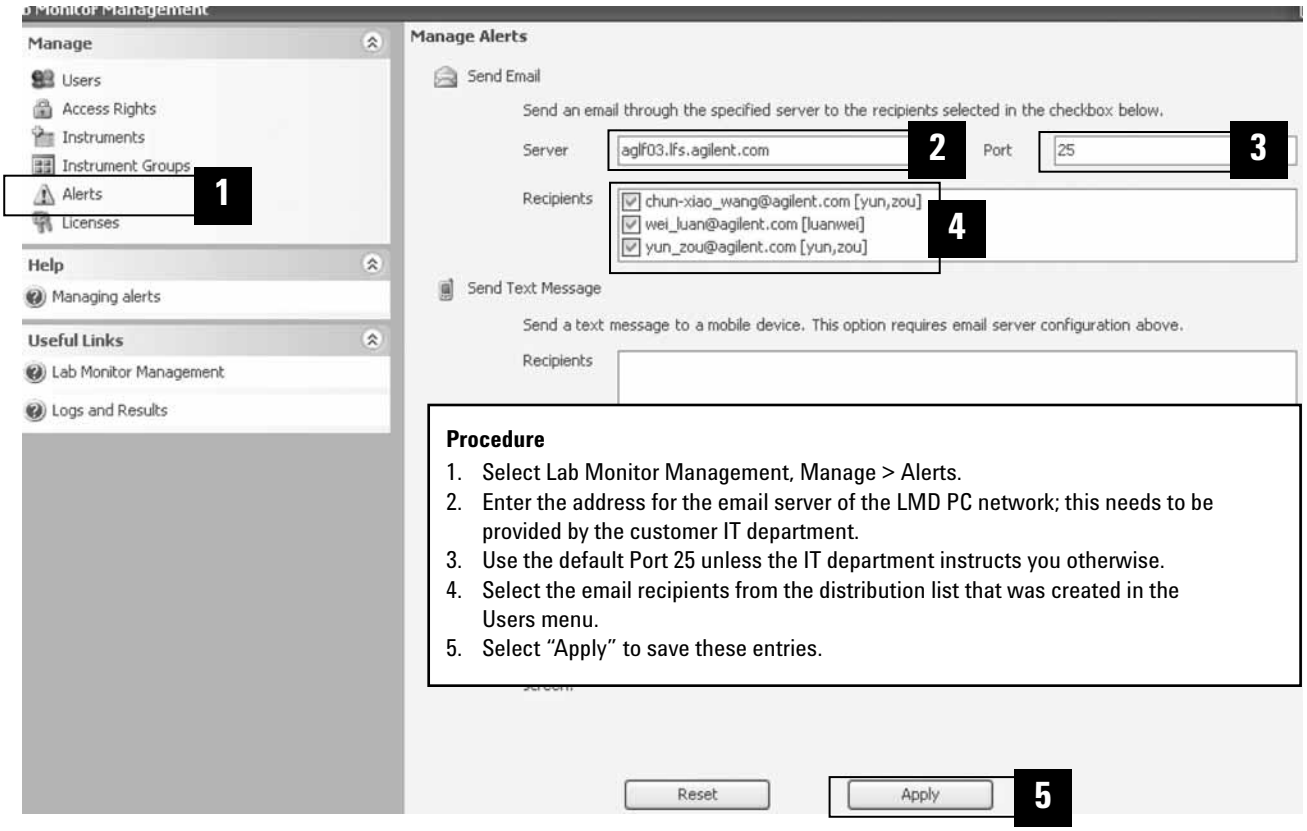



**Figure 5. Procedure for managing alerts.**


**Procedure**

1. Edit parameters and click the icon that allows for selection of diagnostic counters.
2. Select the counters that you want to use within LMD.

**Figure 6A. Procedure for configuring maintenance indicators. Set up the resource counter within ChemStation/Workstation.**

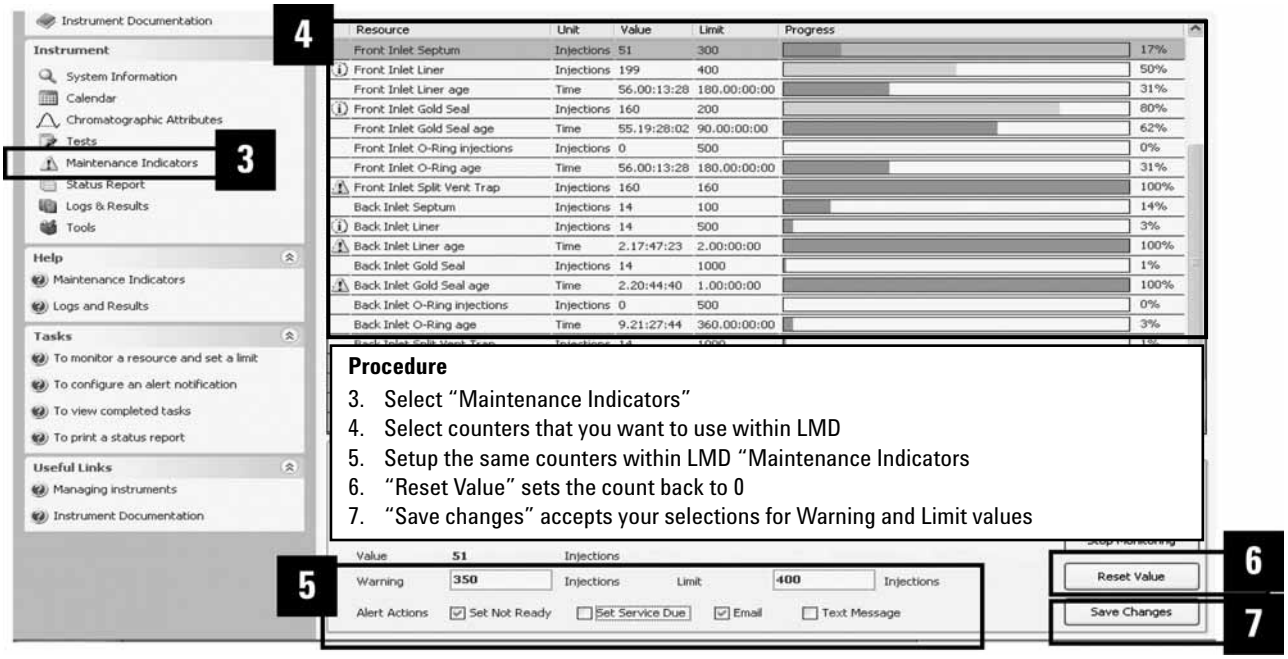



**Figure 6B. Procedure for configuring maintenance indicators. Set up the same counter within LMD.**
In this example, a warning email will be sent at 350 injections on the front inlet liner. At 400 injections the GC will be set to "Not Ready," thus stopping the sequence, and an email will be sent to the distribution list that was set up in the previous procedure (see Figure 4).

For the 7890A GC, the resource counters must first be enabled from ChemStation before they will count within LMD (see Figure 6A). Next, the same counters are set up within LMD maintenance indicators (see Figure 6B).

The next case study demonstrates how LMD can provide intuitive help and easy-to-follow procedures in case there is a problem with diagnostic capabilities. It also illustrates how the complete, searchable user information enhances the diagnostic and troubleshooting functionality of LMD.

### Problem

The trace level peak was lost or its response reduced on the 7890A GC with FID and capillary column. In this scenario, the response of ethylbenzene is reduced and m-oxylene is lost, as shown in Figure 8.

### Designing Diagnostics and Tests to Solve Specific Problems

This problem may be caused by inlet leakage. The diagnostic tests of the LMD software simplify complex troubleshooting tests for the user by automatically performing specific troubleshooting-related tasks. For example, the inlet leak and inlet decay tests helps the user with quick diagnostics, and the FID checkout test helps verify proper performance with convenient guidance. The problem can be fixed efficiently with the intuitive help of LMD.

When an LMD test is running the GC Remote light comes on, indicating control of the GC. At this time, the ChemStation must be closed to run the LMD tests.

1. Run SS inlet 7890A leak check. Result: Failed. The procedures and result are shown in Figure 8.
2. Run SS inlet 7890A pressure decay test to confirm a leakage. Result: Failed. The above tests indicate that there is leakage in the inlet system. The procedures and result are shown in Figure 9.
3. Solve the inlet leak problem by tightening the column fittings, base gold seal fitting, split vent trap housing, septum nut, and the latch to the SSL inlet. Result: These actions don't work.
4. Replace the inlet septum and the liner O-ring. User document search capability makes it easy to find instructions on how to change the consumables, including the inlet septum and liner O-ring. Also, detailed consumable information, including part number, helps you select the right part (see Figure 10).
5. Run the SS inlet pressure decay and leak check tests. Result: Passed. See Figure 11.
6. Run the FID checkout test to confirm that the GC system is operating properly. Before the detector checkout test can run, you have to install the appropriate consumable parts; that is, an evaluation column, HP-5 30 m x 0.32 mm x 0.25 µm (p/n 19091J-413); an FID performance evaluation (checkout) sample (p/n 5188-5372) is also needed. The software will prompt you when you are required to perform a task or answer a question. When the FID detector checkout test is finished, restore the instrument to normal operating conditions. Result: Passed. See Figure 12.
7. Run trace-level aromatic sample. The expected result is obtained as shown in Figure 13 after diagnostics and troubleshooting.
8. The GC system is fixed.

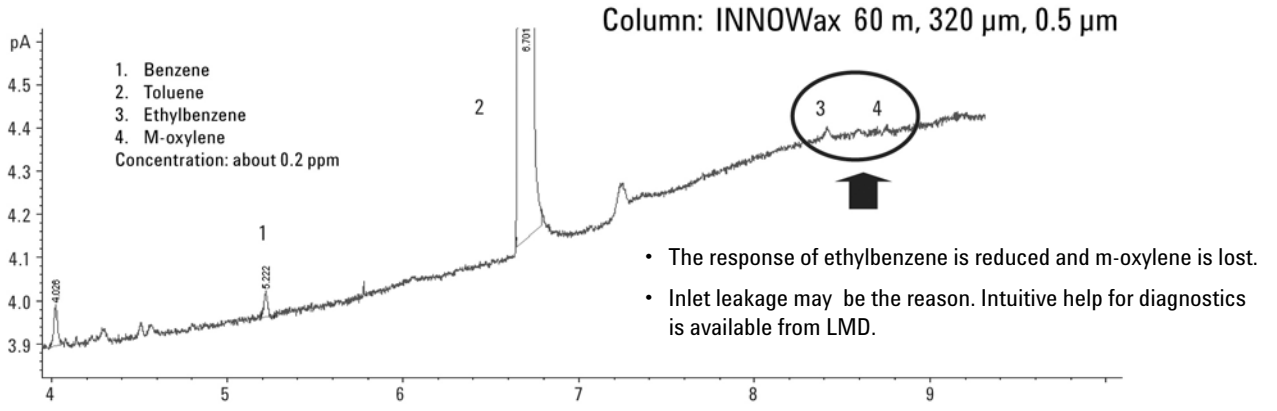



**Figure 7. Trace level peak lost or response reduced on 7890GC with FID and capillary column.**

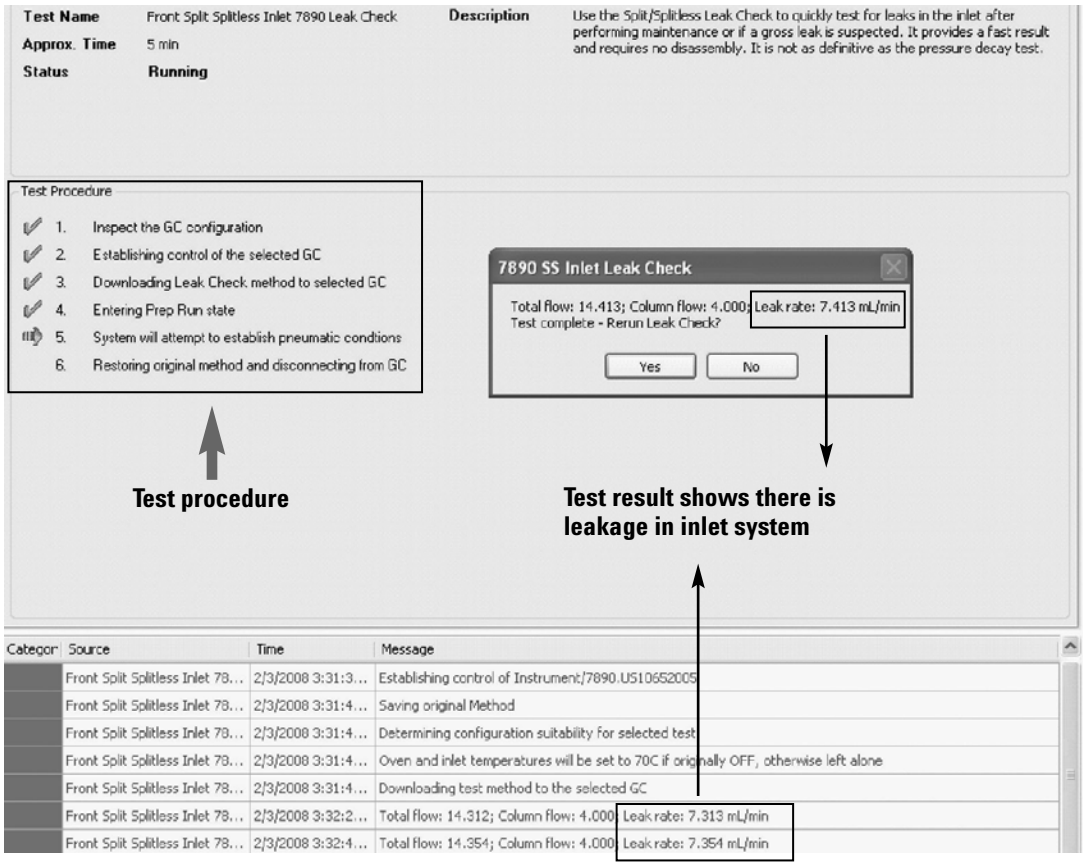



**Figure 8. Procedure and result for SS inlet leak check test.**

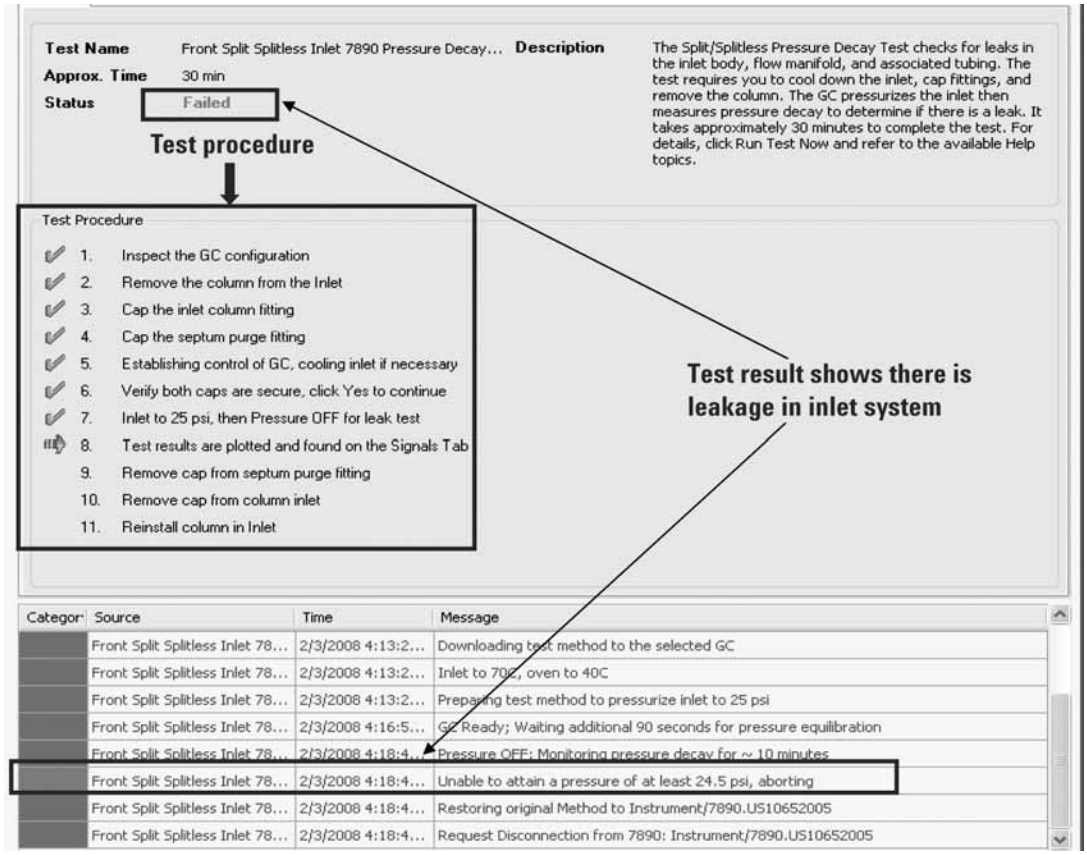
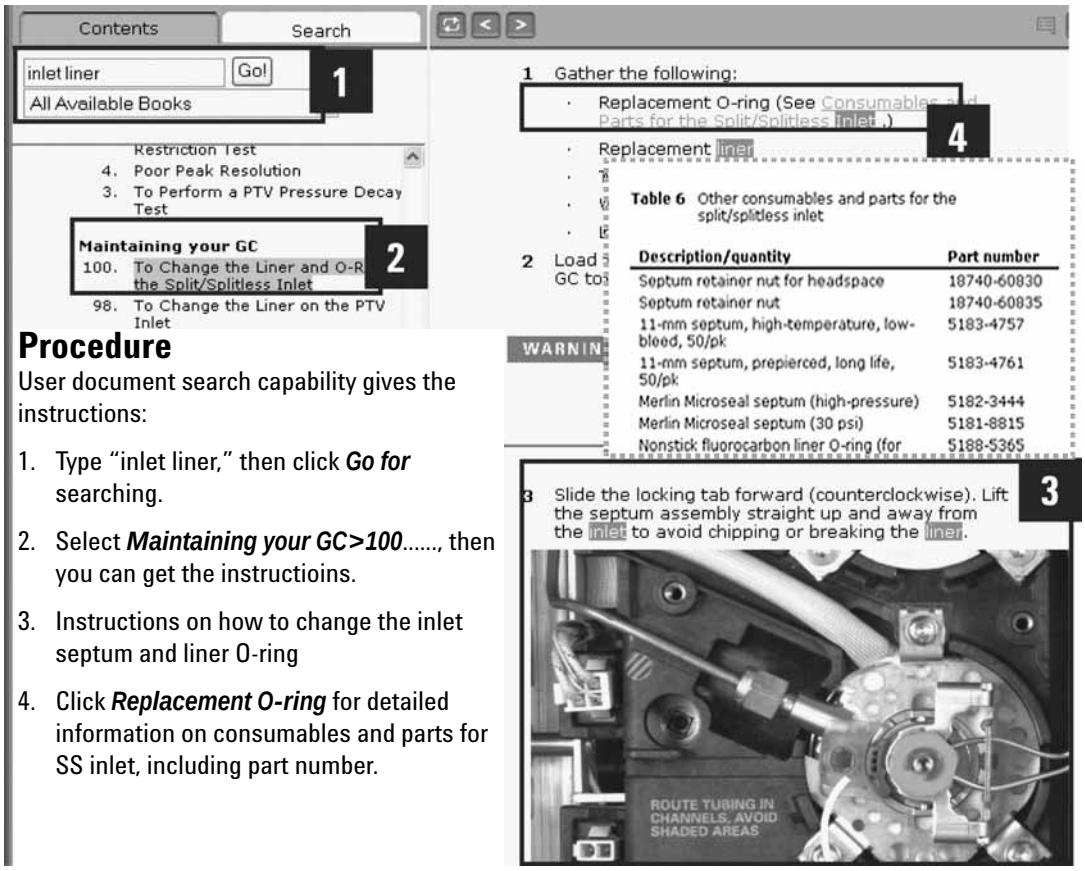



**Figure 9. Procedure and result for SS inlet pressure decay test.**




## Procedure

User document search capability gives the instructions:

1. Type "inlet liner," then click ***Go for*** searching.
2. Select ***Maintaining your GC>100***....., then you can get the instructioins.
3. Instructions on how to change the inlet septum and liner O-ring
4. Click ***Replacement O-ring*** for detailed information on consumables and parts for SS inlet, including part number.

**Figure 10. Searchable, complete user information capability allows for enhanced diagnostics and troubleshooting functionality.**

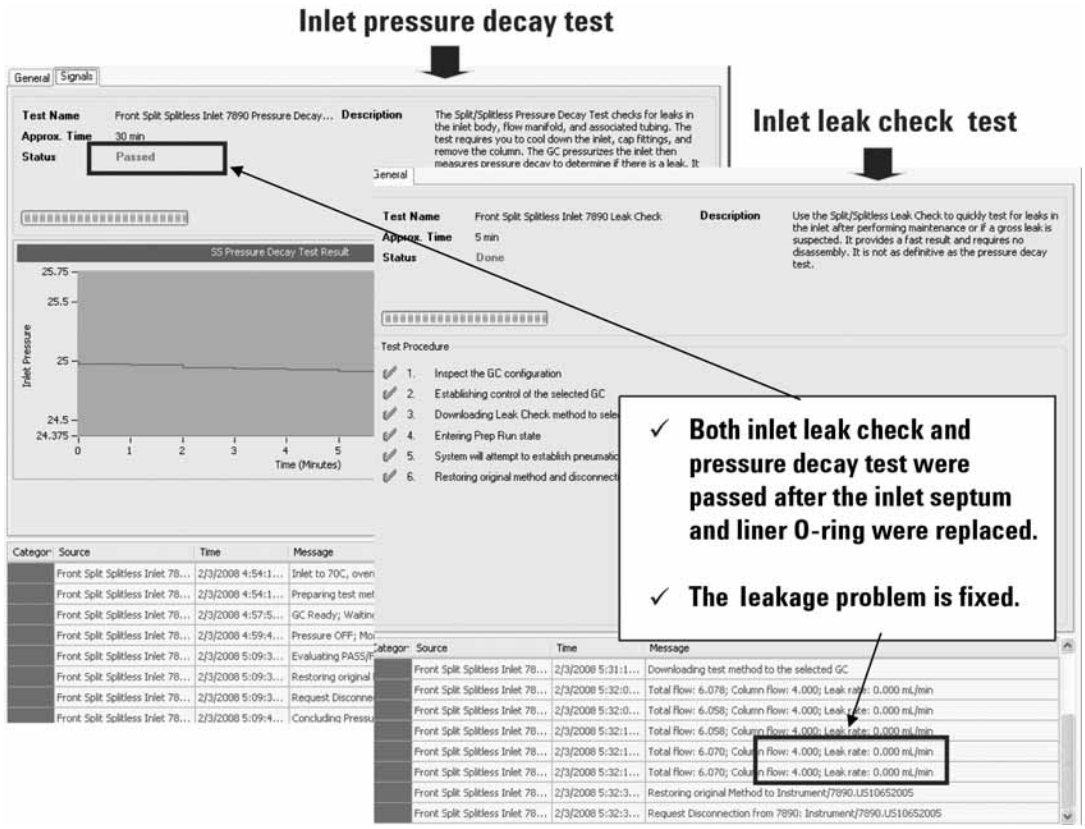



**Figure 11. The result passed both inlet leak check and pressure decay test after troubleshooting.**

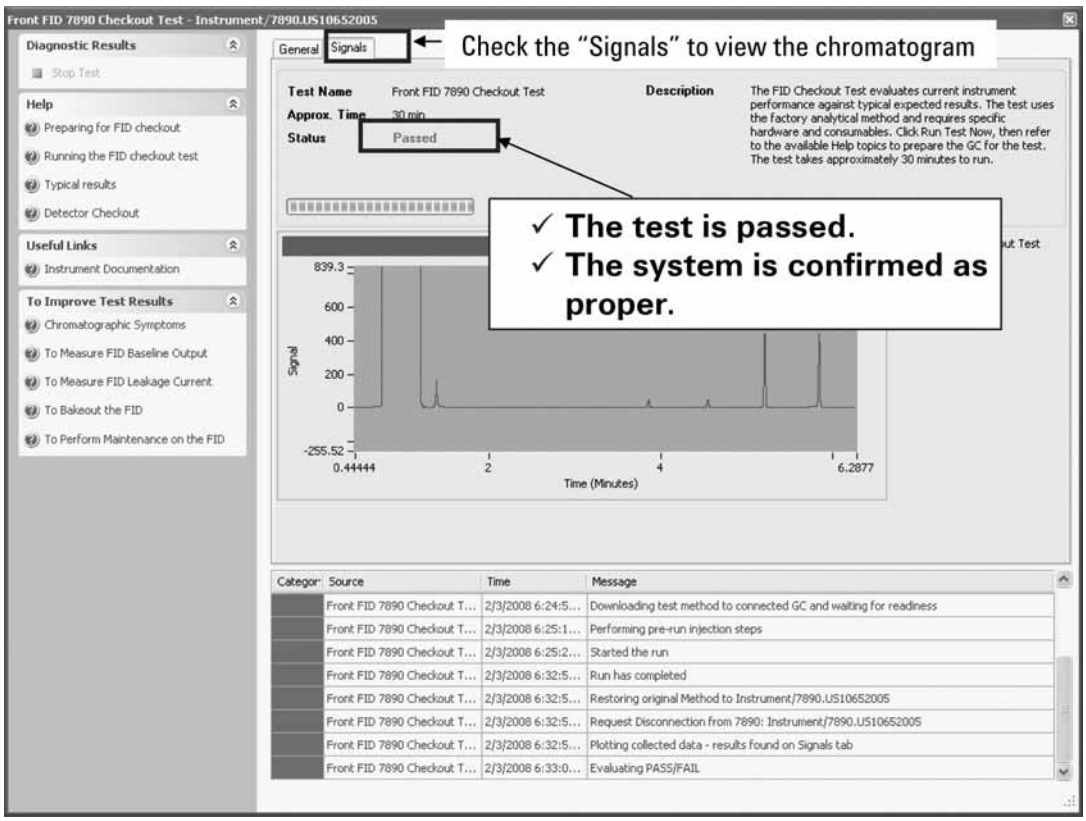



**Figure 12. The result has passed FID checkout test.**

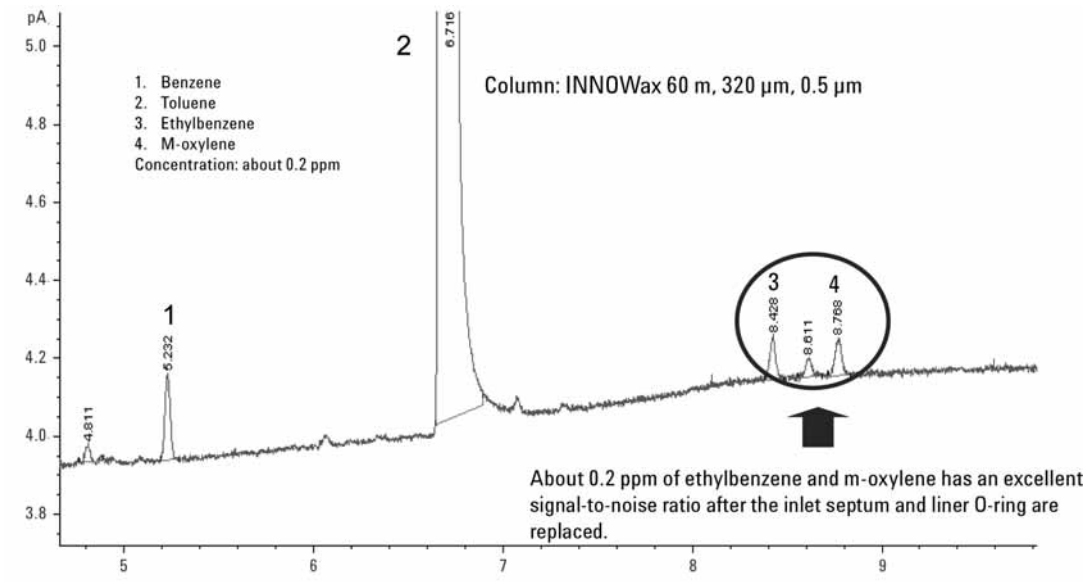



**Figure 13. Expected result for trace-level aromatic sample is obtained after troubleshooting with intuitive help from LMD.**

## Conclusions

Agilent Lab Monitor & Diagnostic (LMD) software is a new tool to help ensure the productivity, performance, and reliability of instruments in the lab. LMD monitors multiple instruments in the lab continuously in real time and detects maintenance needs and instrument problems before a problem occurs. These case studies demonstrated the benefits of Agilent LMD software for customers.

- **Right advice before results go bad**

  Real-time instrument monitoring tracks the number of runs or the life of GC resources, such as inlet septa, liners, and o-rings. Then, when the user-specified limit has been reached, LMD generates an Alert Action, for example, it sends an alert email to specified user(s).

- **Intuitive help in case of a problem**

  - Diagnostics and tests will help you solve problems and verify proper performance.

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  - Easy-to-use system tests, including an inlet leak test and inlet decay test, will help you do quick diagnostics.
  - A detector checkout test will provide con venient guidance to help you verify proper performance.

- **Extensive user document search capability**

  LMD offers easy-to-find and easy-to-use instructions on how to change the consumables, including the inlet septum and liner O-ring, and detailed consumable information, including part numbers, to help you select the right parts.

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Agilent Technologies

# Capillary Flow Technology for GC/MS: A Simple Tee Configuration for Analysis at Trace Concentrations with Rapid Backflushing for Matrix Elimination

Application

**Environmental, Drug Testing, and Forensics**


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## Abstract

**Capillary Flow Technology devices offer the potential to enhance GC/MSD operation and robustness. In operation, they can allow rapid service of the GC column and inlet, including liner and septum, without venting or subjecting the MSD to air. In terms of robustness, late eluting compoundscan be removed from the column by "backflushing," which forces components to retreat through the column into the injection port before they damage the MSD source or compromise the next analysis. This leads to higher analytical integrity as both the column phase and the MSD can be protected. This application describes a simple arrangement for Capillary Flow Technology devices that provides ventless maintenance features with highly accelerated backflushing and minimal losses in the MSD signal. This solution supports GC analysis in constant flow mode with pressure pulsed injections and is recommended for all MSD users (in both electron impact or chemical ionization modes), including those with diffusion pump systems.**


## Introduction

The introduction of Electronic Pressure Control (EPC) was a major advance for GC and especially GC/MS analysis. EPC allowed development of the constant flow mode of analysis, which generates chromatographic peaks of consistent width (time) and allows optimization of MS cycle times to meet either qualitative or quantitative requirements. Also, splitless injections gained pressure pulsing or ramped flow modes, which lowered the analytes' residence time in the hot injection port and confined the expansion of the injection solvent (avoiding overfilling of the liner). The power of this approach lead to continued evolution of EPC technology with the present state of the art represented in the new 7890A GC.

The recent addition of Capillary Flow Technology (CFT) devices has reinvigorated and recast Deans switching and other pressure control approaches to GC analysis. One such CFT device, the QuickSwap [1–3], provides two important capabilities to GC/MS:

1) The ability to service and/or replace the entire analytical column or the injection port liner and septum without venting the MSD (yet still retaining high vacuum integrity)

2) The ability to remove from the column late-eluting, highly retained components that elute after the target compounds of analytical interest by reversing the carrier flow direction through the column in what is called “backflushing.” With the oven temperature elevated and the flow reversed, these very high boiling interferences can be pushed off the column into the split vent and thereby prevent degradation of the column phase or the detector.

A schematic representation of the arrangement that makes this possible is shown in Figure 1.

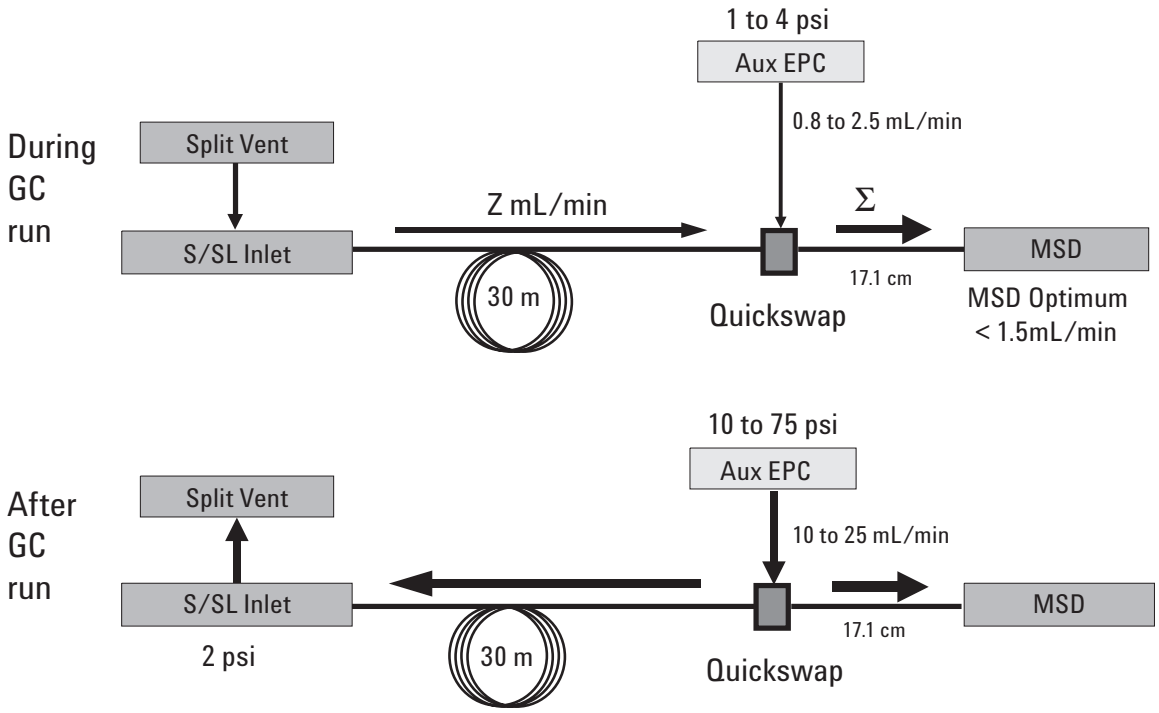


**Figure 1. Schematic of QuickSwap arrangement.**

Every new approach has a downside and for QuickSwap it is the additional makeup flow required to purge the QuickSwap device during analysis which dilutes the signal in the GC/MSD. This is not an issue for many users since the sensitivity of the MSD is usually more than adequate. However, analysis at trace concentrations has more stringent requirements and maintaining a signal closely comparable to that of a single continuous column is essential.

Another CFT configuration for GC/MSD applications designed specifically for trace GC/MS analysis where customers do not wish to surrender signal is possible using the QuickSwap or any of several other CFT devices. In this alternate configuration, the CFT device is located in the middle of the analytical column, essentially splitting the column in half. For example, a 15-m column preceeds and follows a CFT tee. Schematically this arrangement is illustrated in Figure 2. The auxiliary EPC device adds just enough pressure (flow) to match the flow (pressure) from the first column, so there is little flow addition and therefore less “dilution” and loss in the GC/MSD signal. Backflushing is similarly simple; the pressure or flow is dropped in the first column section while the second section column flow is increased.

Advantages of this pressure controlled tee (PCT) approach are similar to those of QuickSwap, such as:

- Service of injection port liner and septum without venting the MSD
- Column cutback or replacement of the “front” or first column without venting the MSD

But additional advantages of the PCT arrangement over QuickSwap are:

- Minimal or no signal loss (in EI- or CI-MS) is obtained because of the very small additional “makeup” gas flow.
- Constant flow mode and pressure pulsed injections are straightforward.
- This configuration is suitable for diffusion pumped systems and allows backflushing in diffusion pumped systems.
- Backflushing is more rapid and can be initiated earlier.

This application details some configurations and provides an example of backflushing.

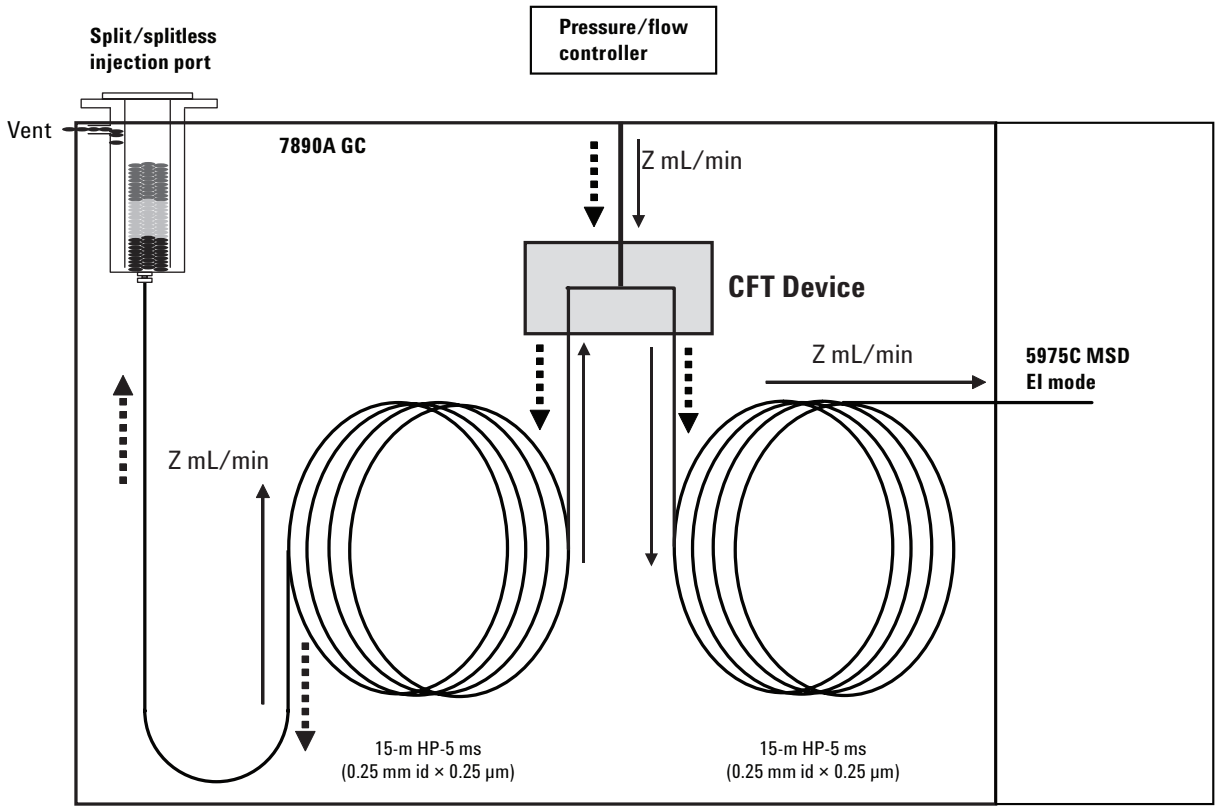


**Figure 2. Schematic of pressure controlled tee arrangement for the GC-MSD: solid lines indicate the forward flow during GC/MSD analysis and the dashed lines indicate backflushing flows.**

## Experimental

A number of devices can be used in this approach and those arrangements will be cited later, but for these experiments the instrument configuration was as follows:

- 7890A GC with split/splitless ports in front and back and a 7683B ALS
- 5975C MSD with performance turbomolecular pump
- 2 HP-5ms 15 m × 0.25 mm id × 0.25 µm film columns (19091S-431)
- CFT device: 2-way unpurged splitter (G3181-60500) with SilTite ferrules and nuts
- CFT GC mounting hardware: dual-wide mounting bracket (G2855-00140) or single-wide mounting bracket kit (G2855-00120)
- Deactivated 0.25 mm id column approximately 1 m long
- 2 CFT blanking plugs (G2855-60570 or as G2855-20550 with G2855-20593)

As an overview of the configuration, the 1-m column was connected to the back injection port and to the first position on the CFT splitter using the appropriate SilTite fittings. (This CFT device has three connection points and is really best thought of as a simple tee reminiscent of glass Y- or T-connectors and will be referred to as a "CFT device" or "CFT tee" from here forward).

One of the 15-m HP-5ms columns was connected at the uppermost position on the CFT tee and the other end through the transfer line into the MSD as usual. The other 15-m HP-5ms column was connected to the midpoint of the CFT device and the front injection port.

In detail, the arrangements were as follows. The CFT tee was attached to the forward position on the mounting hardware on the right side in the GC oven. The 1-m long section of guard column was wound on a spare column cage and hung on the column hanger in the back of the oven. (This could simply be added to one of the 15-m HP-5ms column

cages to avoid the extra cage.) Using a Vespel/graphite ferrule, one end was connected to the back injection port and the other end to the lowest connection of the CFT device with a SilTite ferrule and nut. The other two CFT tee connections were sealed with CFT blanking plugs and the back injection port was pressure tested as described in the 7890A *Advanced User Guide* (part number G3430-90015).

One of the 15-m columns was then hung on the cage carrying the 1-m column and installed with one end through the MSD transfer line. Since this column (column #2) can be expected to have a rather long life as it will be protected by the upstream column, a SilTite ferrule is recommended for the transfer-line seal. These ferrules do not develop leaks as the transfer-line temperature is cycled; however, the Vespel/graphite ferrules can shrink and develop leaks. (Note that if the surface of the transfer line is very worn it may fail to seal well, in which case the Restek *Agilent interface cleaner* [P/N 113450] can be used to resurface the sealing surface if very carefully employed). The other end of this GC column was connected to the uppermost connection on the CFT tee with the SilTite ferrule.

The "upstream" 15-m GC column (column #1) was hung on the other 15-m column cage and installed in the front split/splitless injection port with a Vespel/graphite ferrule, liner, and BTO septum, as usual. The other end was connected to the CFT tee middle post and, after temporarily removing the other connected columns, blanked off and pressure tested as above.

All connections were then re-established to the CFT tee with the 1-m column in the lower position; the front, first column (#1) connected in the middle position; and the rear, second or MSD column (#2) in the uppermost connection. Helium was supplied to both the front and back ports, and a helium leak detector was used to check for any leaks.

A picture of the arrangement is shown in Figure 3.

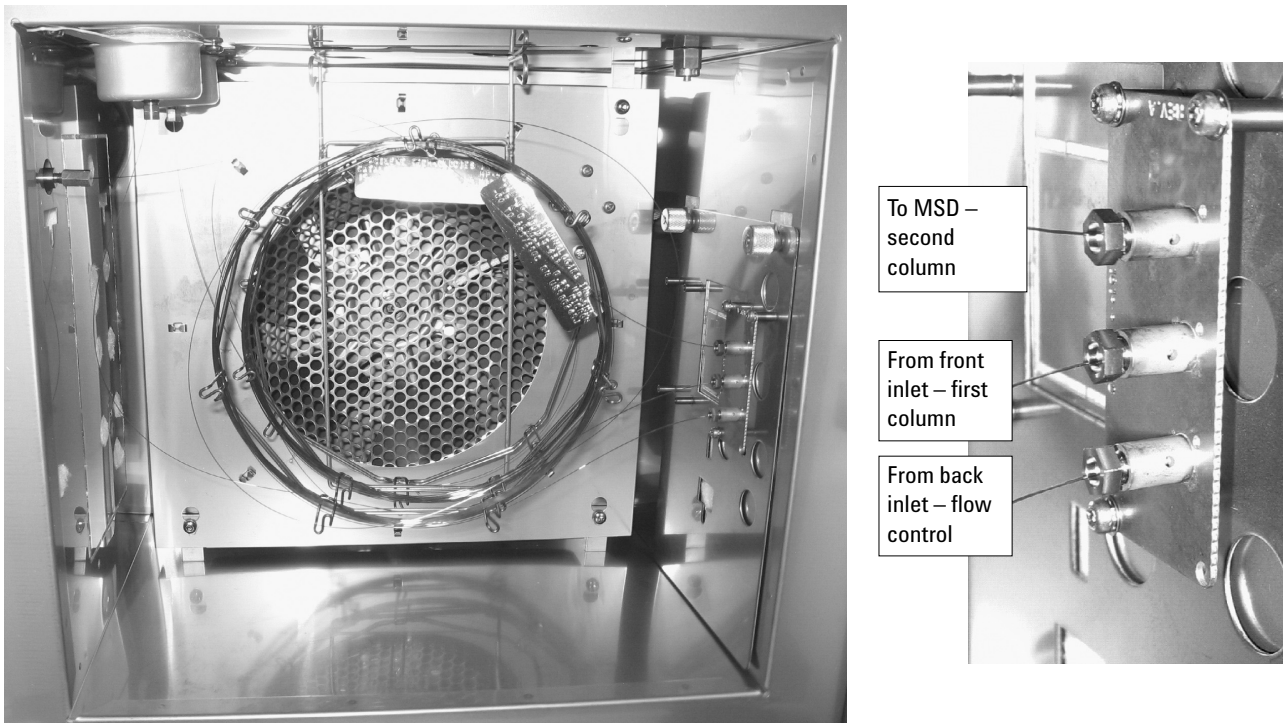


**Figure 3. Picture of the installed pressure controlled tee arrangement for the GC/MSD.**

### GC Configuration

The GC can be configured in several ways. However, for instructional purposes and those of these experiments, the GC was configured as follows:

| | |
|---|---|
| Column #1: | 30 m × 0.25 mm id × 0.25 µm column |
| Inlet: | Front injection port: pulsed splitless mode, split flow 15 mL/min |
| Outlet: | MSD (vacuum) |
| Mode: | Constant flow |
| Column #2: | 15 m × 0.25 mm id × 0.25 µm column |
| Inlet: | Back injection port: split mode, split flow 15-mL/min |
| Outlet: | MSD (vacuum) |
| Mode: | Constant flow |

The flows were set to 1.2-mL/min, all zones were left cold, and the MSD power was turned on. With the MSD and GC zones still "cold," the MSD background was checked to be sure *m/z* 28 was decreasing, indicating that the system was tight. Only after there was confidence that there was no leak were other zones brought up to temperature.

### Operating with Pressure Pulsed-Splitless Injection

Figures 4A and 4B show screen captures of the 7890A GC configuration for a standard pressure-pulsed splitless injection with constant flow mode operation; they show the front and back injection port parameters. Remember, the arrangement is set up such that the front port, into which the sample will be injected, is configured as if a 30-m column were installed into the MSD. Typical pressure-pulse conditions are set for these parameters: a 25 psi pulse for 0.5 minutes; split flow on at 0.75 minutes at 50-mL/min; with gas saver on at 2 minutes at 15-mL/min. The general rules apply for pressure-pulsed splitless injections: given a particular liner, inlet temperature, injection volume, and solvent, the expansion of the solvent is confined to a fraction of the interior volume (< 0.75) of the liner by the pressure applied.

Figure 4B shows that the back injection port is in split mode, at 120 °C (to remove water background), with split flow and gas saver set at 15-mL/min flow.

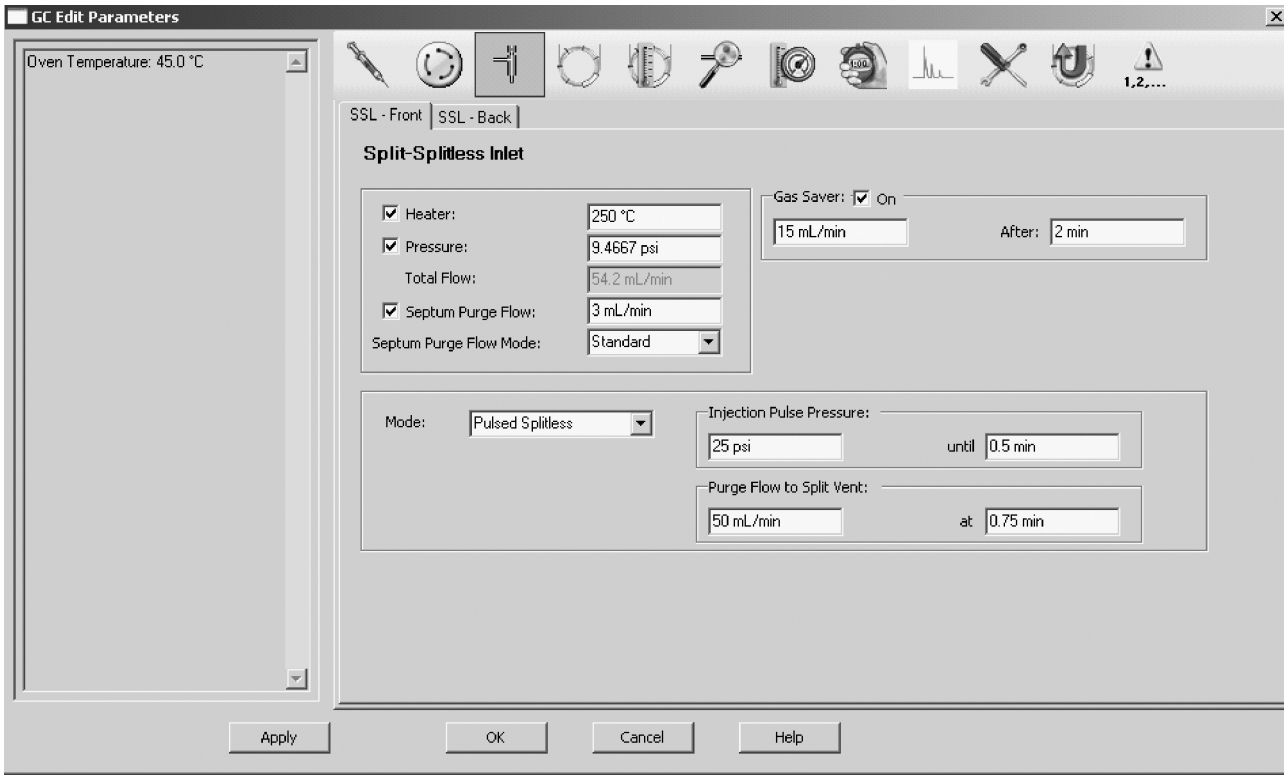


**Figure 4A (upper panel). Typical pressure-pulsed splitless injection parameters for constant flow: front injection port.**

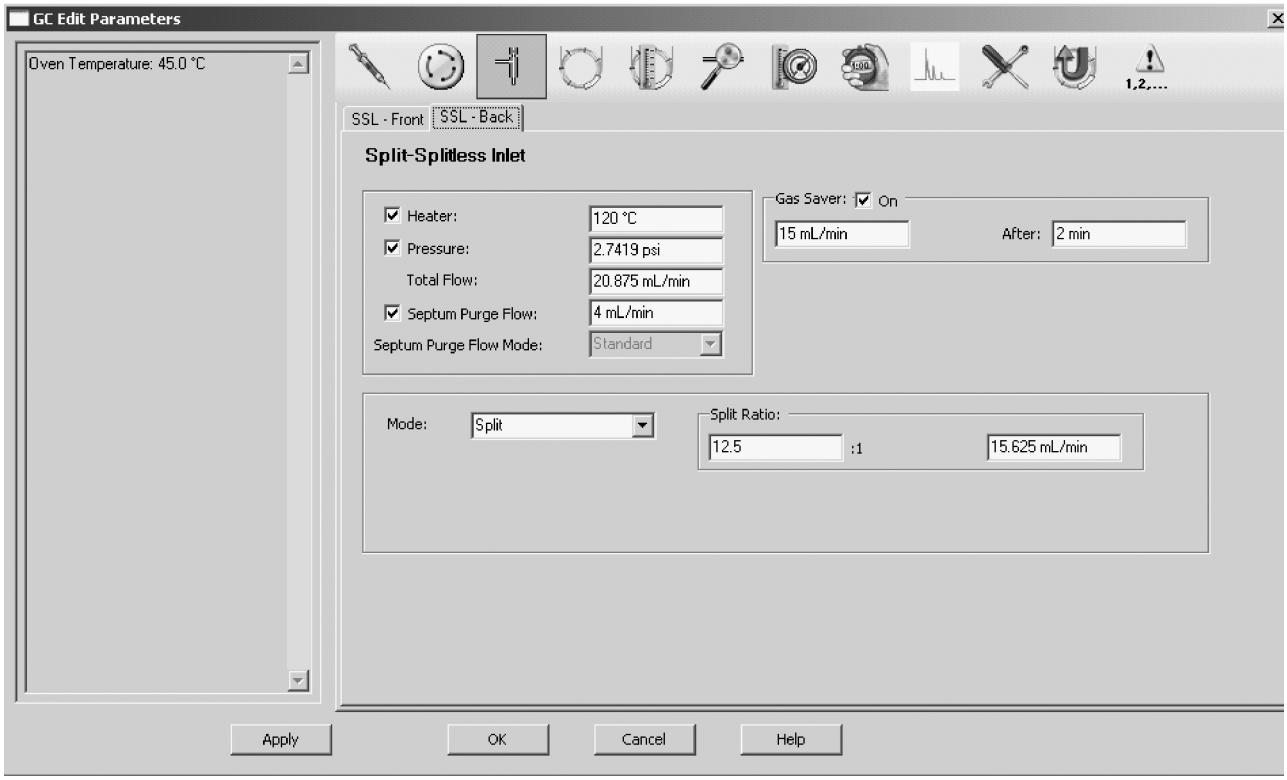


**Figure 4B (lower panel).** **Typical pressure-pulsed splitless injection parameters for constant flow: back injection port (not used for injection but for column control).**

Figures 5A and 5B show the constant flow mode settings for the two columns. The front column flow is the typical 1.20 mL/min, but the back column flow is slightly higher at 1.25 mL/min to prevent any backflow. Essentially the additional flow is equivalent to an extra meter of column length.

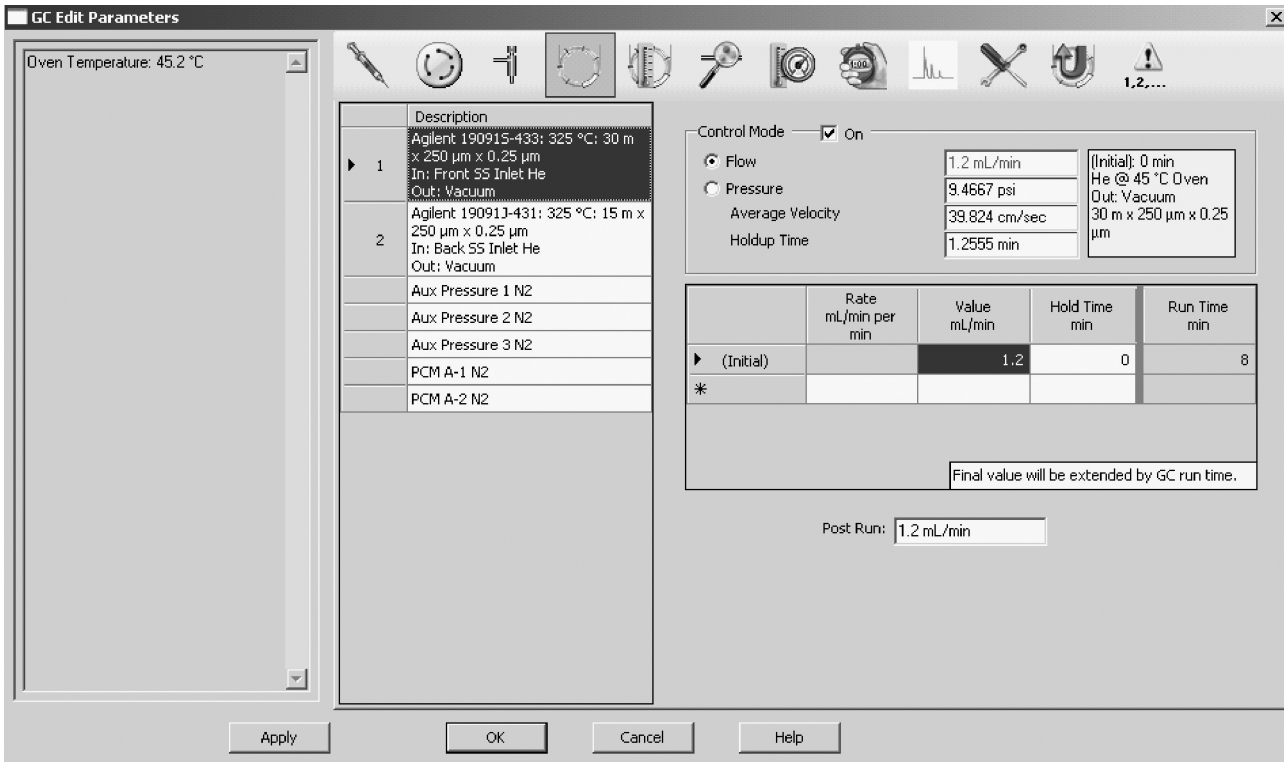


**Figure 5A (upper panel).** **Typical pressure-pulsed splitless injection parameters for constant flow: First column section (configured as a 30-m column).**

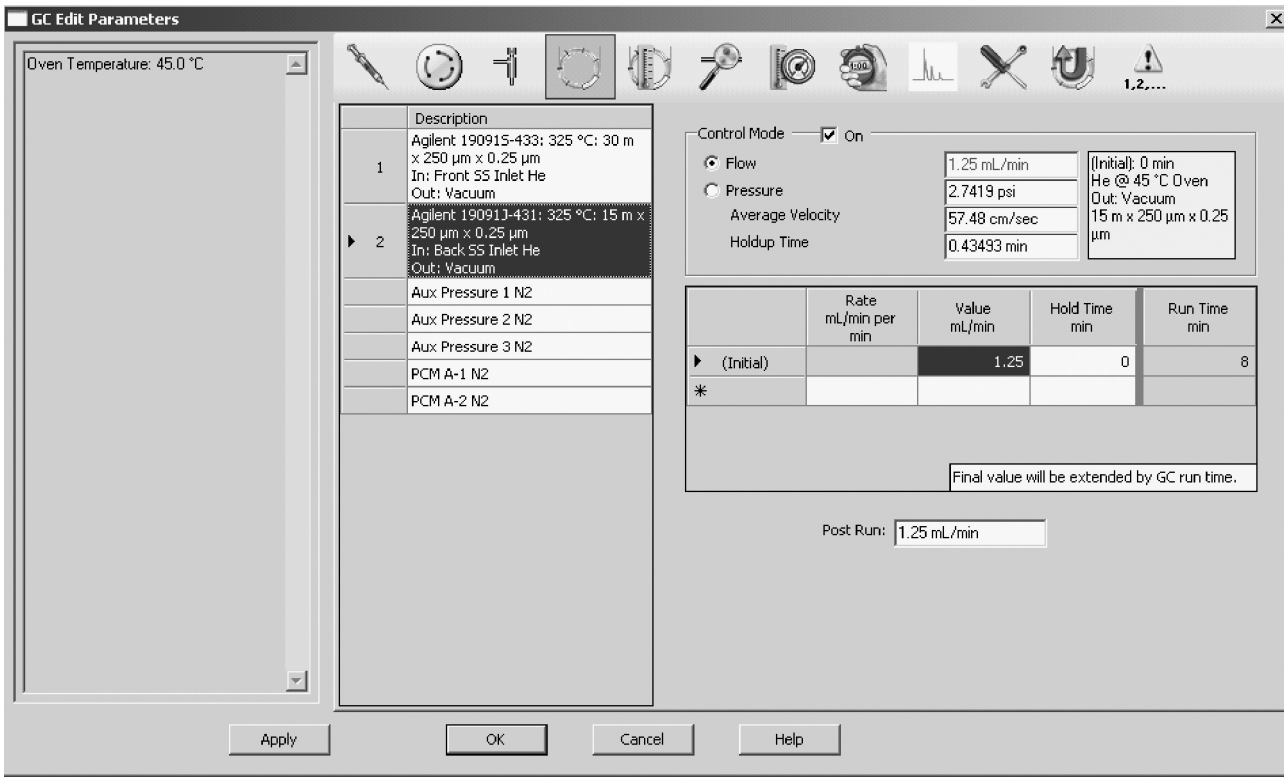


**Figure 5B (lower panel). Typical pressure-pulsed splitless injection parameters for constant flow: Second column section (configured as a 15-m column).**

## Results and Discussion

Figure 6 shows the results for pressure-pulsed splitless injections of octafluoronaphthalene (OFN) at 1-pg/µL acquired in selected ion monitoring (SIM) with the two 15-m column and CFT tee configuration and the standard 30-m continuous column configuration. Both peak height and area remain the same, indicating that there is no loss in signal. This is as expected since no signal dilution is taking place. There is a slight degradation in S/N for the CFT tee results as the background noise is raised by about 35% due to the additional flow controller. The important point is that the signal is preserved at trace levels.

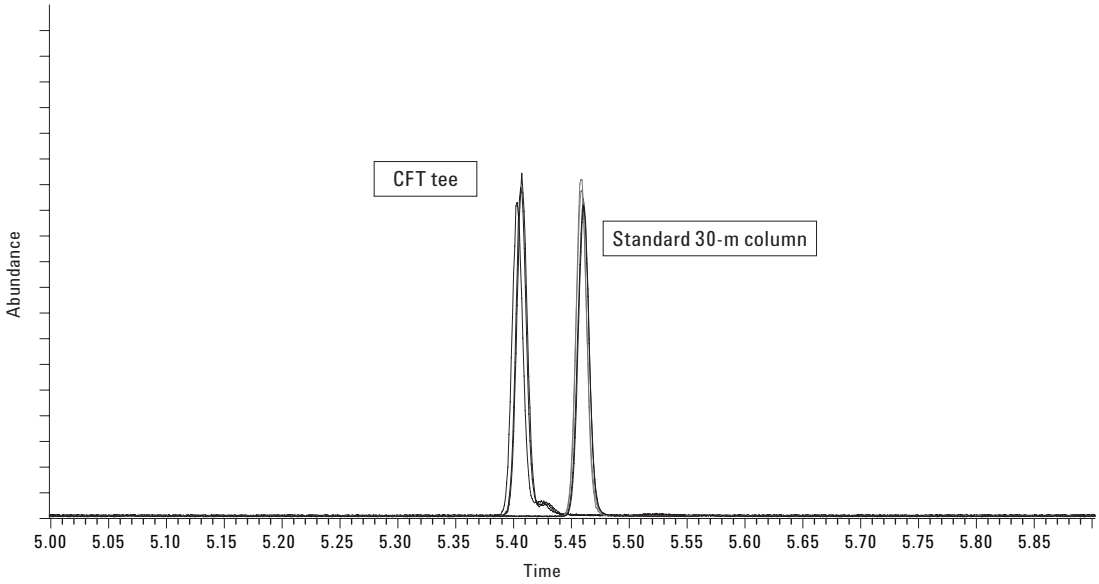


**Figure 6. Reconstructed total ion chromatogram (RTIC) of three replicate SIM acquisitions of octafluoronaphthalene using pulsed splitless injection with CFT tee (left profiles) and with a standard 30-m continuous column configuration (right profiles).**

### Chromatographic Character

Beyond preserving signal, the CFT device should exhibit reasonable chromatographic performance. One indication of chromatographic integrity is the peak shape profiles of the fatty acid methyl esters (FAMEs). The result for GC/MS analysis of a FAMEs standard acquired using a metabolomics method is shown in Figure 7 and suggests very little degradation of chromatography using this PCT. This can be expected as the path is deactivated and the path length in the channels in the PCT relative to the linear velocity suggests a relatively rapid transit through the device.

Another common chromatographic test used in organochlorine pesticide analysis (as in USEPA method 8081) examines degradation of 4,4'-DDT and Endrin. This degradation test was developed to indicate the degree of activity of the injection port by examining the amounts of DDD and DDE products of DDT and the ketone and aldehyde products of Endrin. The situation is complicated here as the degradation products can be generated in both the injection port and the CFT tee. How-

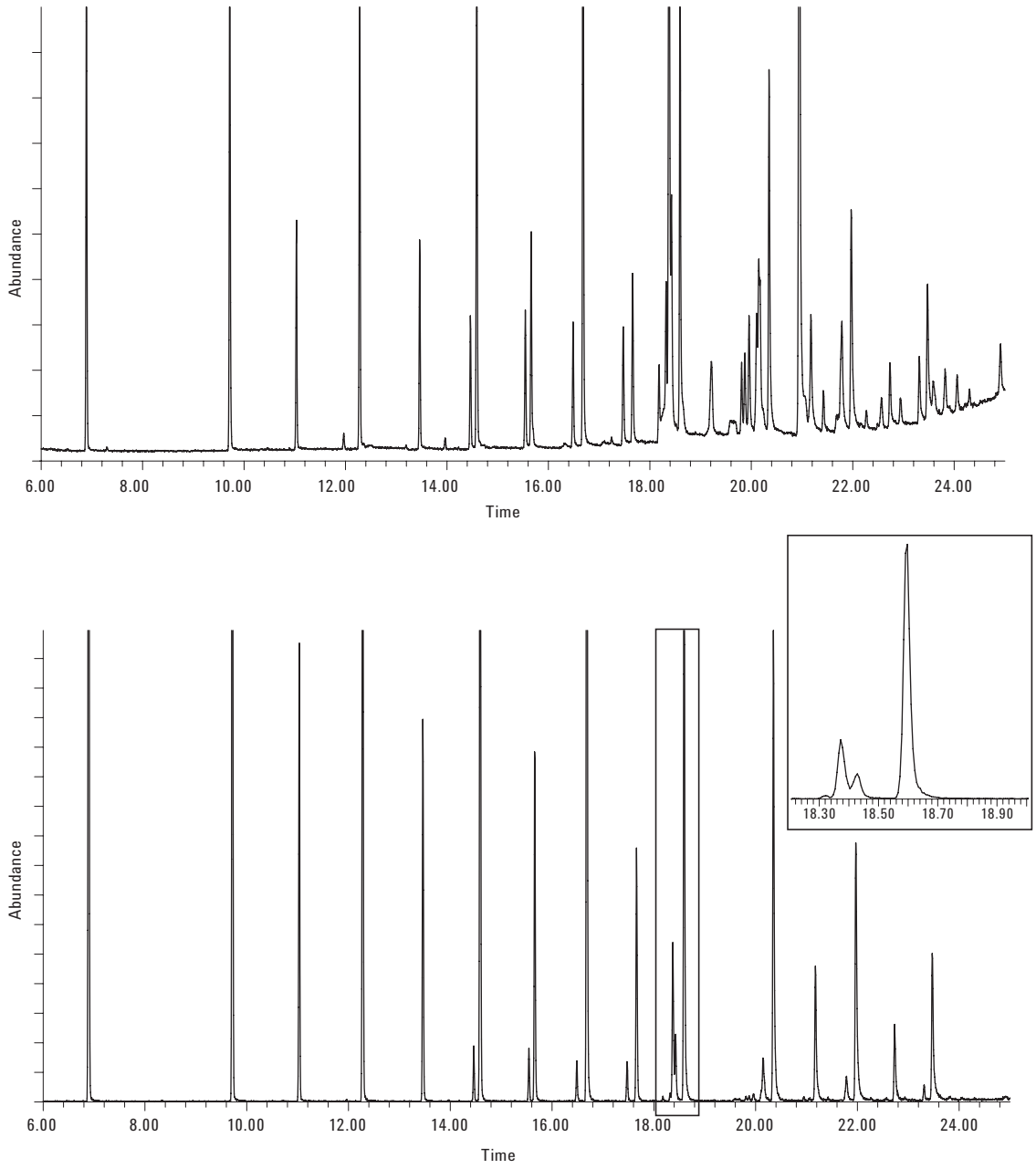


**Figure 7. Reconstructed total ion chromatogram (RTIC) of a multicomponent FAMEs standard using pulsed splitless injection with CFT tee (upper) and the reconstructed extracted ion chromatogram (REIC) for *m/z* 74. The enlarged panel is for octadecanoic methyl ester.**

ever, because those products formed in the injection port and those formed at the CFT device will have different retention times due to differing lengths of column, the degradation contributions from the two origins should be discernable. By analyzing these known breakdown products in the PCT and then injecting the DDT and Endrin agents themselves, an estimate of the activity contributed by the CFT device can be calculated. The upper panel of Figure 8 presents the reconstructed total ion current (RTIC) for the selected ion monitoring (SIM) signals of the four breakdown products. These were acquired in SIM-scan mode with a single SIM group composed of one or two major ions for each compound so there was no time selection for the compounds' appearance. On the basis of summed areas, the total breakdown for Endrin is less than 13% with the CFT device contributing less than 10% of the total breakdown area or less than 1.2% to the area total. The DDT breakdown is less than 4% for the system; however, the CFT device contributes about 46% of the total observed breakdown and is about double the breakdown generated by the port. It is possible some DDD breakdown is "hidden" under the DDT peak. On the basis of the DDT to DDE contribution from the CFT tee, however, it is likely to increase the breakdown perhaps less than about another 2%. A better study would use on-column injection

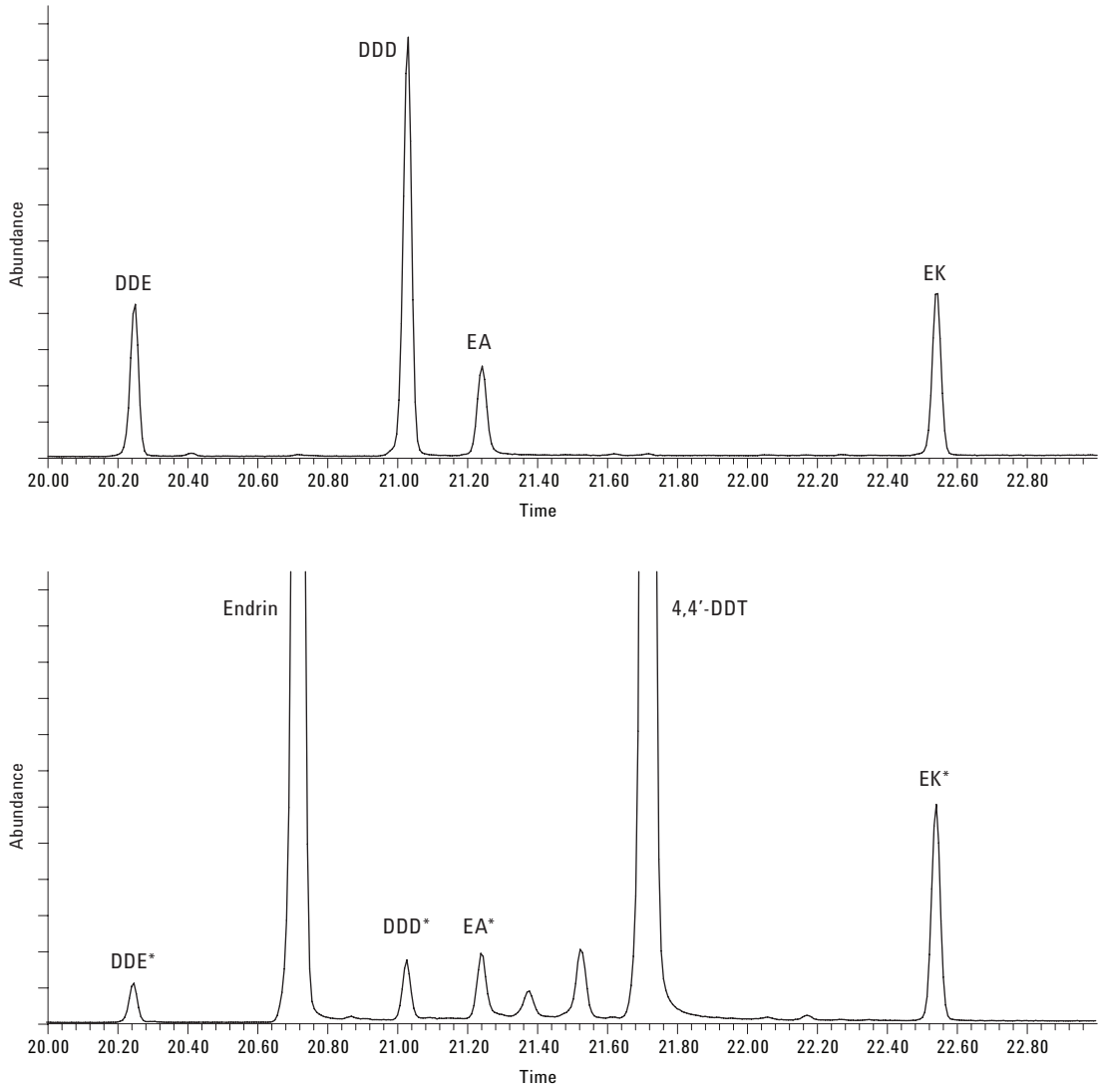


**Figure 8. CFT tee activity. A: the REIC of a GC-MS SIM acquisition using pulsed splitless injection with the PCT configuration of the expected degradation products of DDT and Endrin at 0.2 ng on column : 4,4′-DDE (DDE), 4,4′-DDD (DDD), Endrin aldehyde (EA), and ketone (EK). B: REIC for an injection of 2.0 ng of 4,4′-DDT and Endrin identifying degradation products. Those with an asterix (*) are attributed to the injection port and due to the CFT device activity such as; from Endrin (5 as ketone) and from 4,4′-DDT (6 as DDE). Note 7 is tenatively identified as DDMU, source unknown.**

of all components, but the verdict is likely the same: the CFT device has some activity but is comparable to that of other elements (for example, in the inlet and liner). It is worth noting that this CFT device has a very long path compared to others (see the *Alternative Configurations* section) and that air intrusion in any part of the system will be a major issue in considering activity problems.

### Adding Backflush

Figures 9A, 9B, and 9C show the GC parameters for adding backflush. They are quite simple. The oven temperature can remain the same as the temperature at the end of the oven program or can be raised to the isothermal or programmed temperature limits in Post Run for backflushing. Raising the column temperature during Post Run helps condition the column and removes some column bleed but is not necessary. The front column (column #1) flow is dropped to 0.3 mL/min and the back column (column #2) flow is raised to 4 mL/min.

To quickly estimate the duration of the Post-Run time parameter, notice that the back column (column #2) in Figure 9C cites the column Holdup Time at a given flow. At the 1.25-mL/min shown, the Holdup Time is roughly 0.4 minutes. When the column #2 flow is raised to 4 mL/min, the Holdup Time for back flow through column #1 will be less than this (actually around 0.26 min). But estimating that every 0.4 minute the front 15-m column section would be flushed at least once is very conservative and an adequate approximation. Five to 10 column volumes will flush this front 15-m section in less than 2 to 4 minutes, which is relatively rapid. Choose a time in this range (for example, 3 minutes) and test the effectiveness of the backflush method by injecting a sample and follow this with a solvent blank injected under the non-backflush GC/MSD method. There should be no sign of carryover. Extend this Post-Run time if there is carryover or further raise the Post-Run temperature or both. This is a very conservative approach.

### Column or Inlet Servicing and Maintenance

To change the liner, septum, cutback the column, or replace the front 15-m column, simply cool the inlet(s) and increase the flow on the back column (column #2) to 4 mL/min and set the front injection port pressure to OFF. It is worth saving this method (such as SERVICE-Front.M). When the head of the column is removed from the injection port, one can confirm that the carrier is flowing back up the column by immersing the tip in liquid.

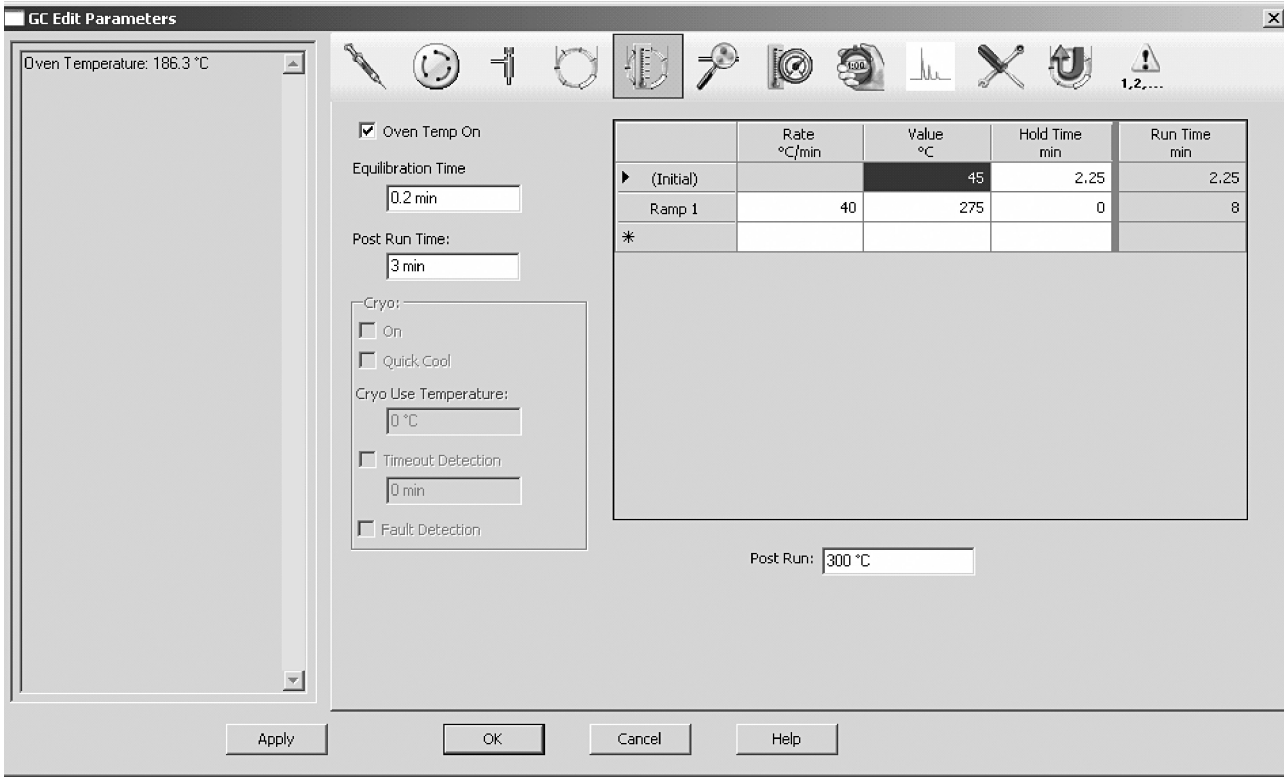


**Figure 9A (upper panel). Adding backflushing in Post Run: oven parameters.**

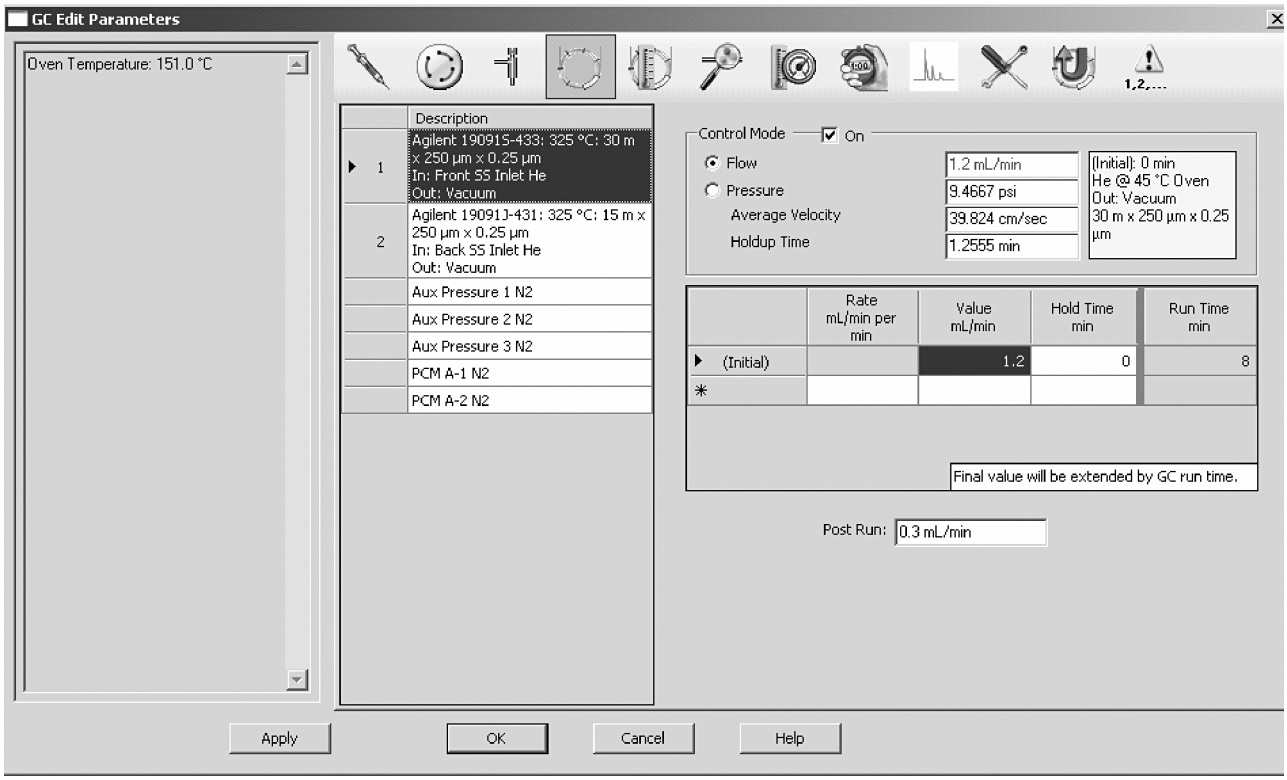


**Figure 9B (middle panel). Adding backflushing in Post Run: front column (column #1) parameters.**

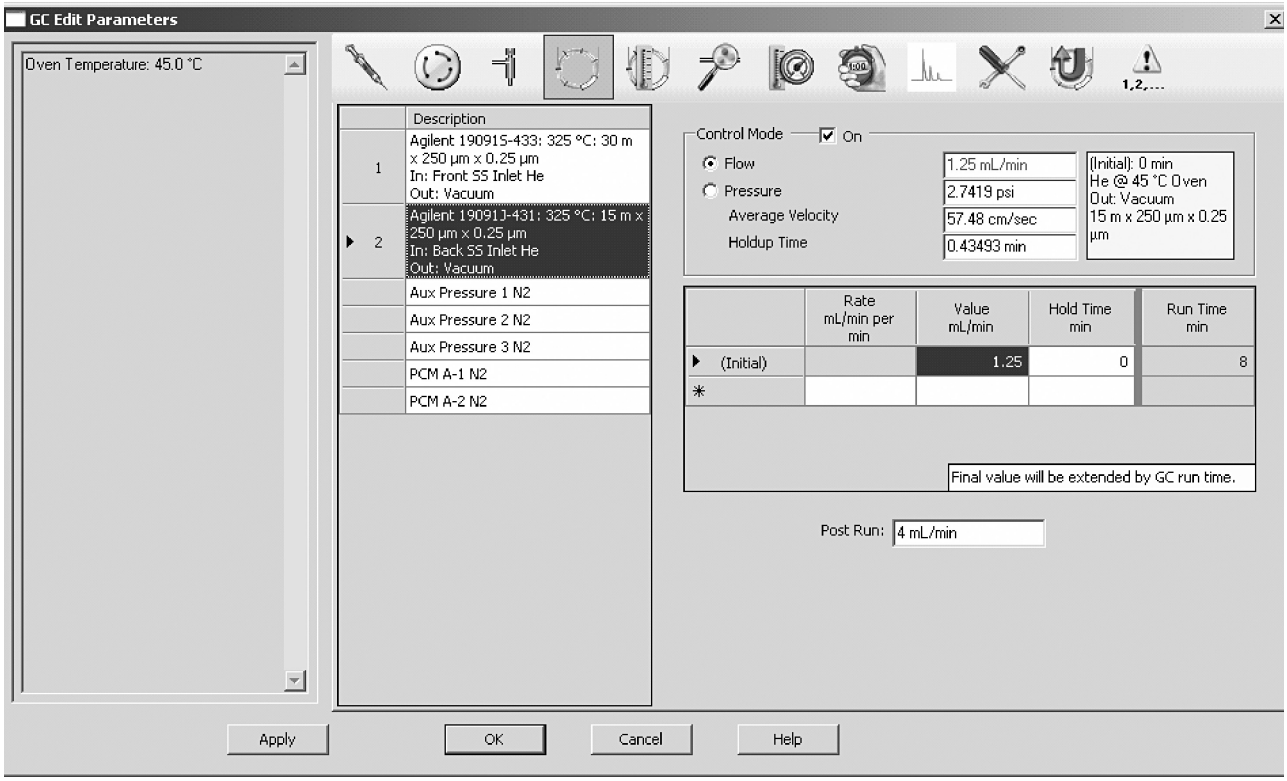


**Figure 9C (lower panel). Adding backflushing in Post Run: back column (column #2) parameters.**

This backflow also prevents fines from the column cutting from entering the column. Make the necessary service and reattach and reload the analytical method.

If a completely new 15-m column (#1) is installed, it can be conditioned *in situ* by setting up the backflow condition with the oven at the conditioning column temperature.

#### Advanced Techniques: Concurrent Backflushing

If the fastest possible total analytical time is the highest priority, one will realize that backflush can begin earlier than the elution of the last component. In other words, backflushing can occur during the analytical acquisition, thereby increasing productivity. After the last compound of interest has passed the CFT tee and entered the back 15-m column, the pressure or flow through the earlier 15-m column can be dropped and compounds will cease moving forward and actually begin to retreat. When the last compound elutes, then the flow in the back column can be raised to complete backflushing. This is demonstrated in Figure 10.

The calculations are also very simple. To calculate when the flow (pressure) in the front column (column #1) is to be reduced, simply subtract the Holdup Time (Figure 9C) from the last compound's

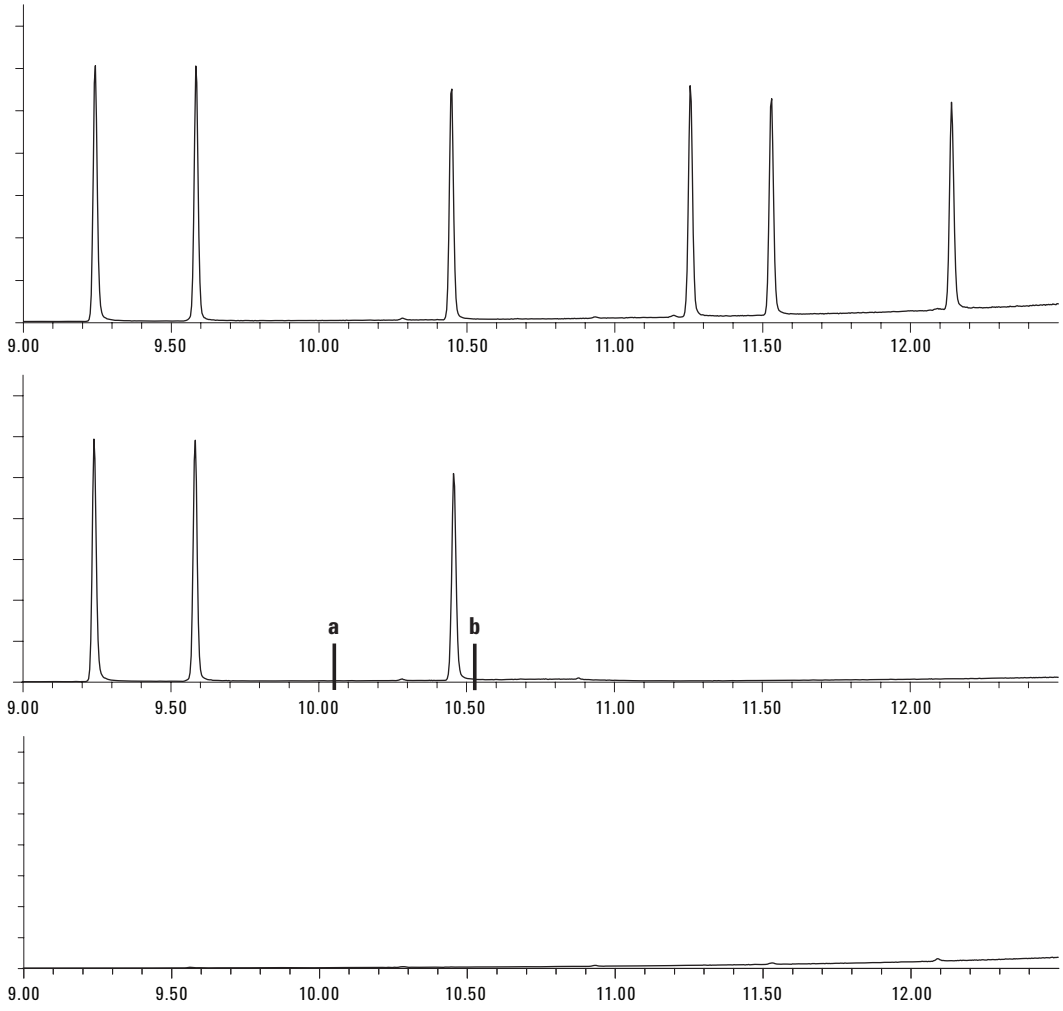


**Figure 10. Example of backflushing with flow or pressure control. Upper panel: RTIC of original six-component standard. The third peak is considered the last analyte and the fourth peak the beginning of the late-eluting interferences. Middle panel: RTIC of the same standard with backflushing beginning at 10.1 min (a), where the first 15-m column (column #1) flow is dropped and at (b) where column #2 flow is increased to 4 mL/min. Note that the last analyte is retained but the late eluters never enter the MSD. Lower panel: solvent blank run without backflush after the backflush method which shows no carryover.**

elution time. After this last compound has eluted, go into Post Run and set the second 15-m column (#2) flow to 4-mL/min (or the pumping system maximum) with the front column (#1) pressure remaining low and the oven at the final programmed temperature. This can best be accomplished in ramped flow mode or in pressure programming. Do this for two to three column volumes and test with a sample followed by a solvent blank to see if this is sufficient. Experimentation with particular samples will enable setting these requirements more efficiently.

## Conclusions

### Alternative Configurations

The CFT is very rich and allows many possible arrangements; these are only a few suggestions or alternatives. The CFT tee used here can be replaced by a purged two-way splitter with one channel plugged (G3180-61500) or even the QuickSwap itself can be moved back from the MSD interface and suspended in the oven.

However, the best CFT tee device appears to be the new Purged Ultimate Union (G3186-60580), Figure 11. As the name describes, this is essentially a union with a gas purging line, making it a very low dead volume tee. It occupies very little space and can be suspended from the column cage, the oven wall, or through the upper GC wall. Preliminary tests of this Purged Ultimate Union using DDT and Endrin have shown very little breakdown. Chromatographic behavior is also very good.

Similarly, the carrier control need not be the back injection port split/splitless module; a Pressure Control Module (PCM) or EPC module can be used. Of the two, the Pressure Control Module may be more convenient.

Most importantly, the CFT tee position itself does not need to be exactly in the middle. The best arrangements can be considered on the basis of selection against components and the rapidity of backflushing. In other words, rapid backflushing suggests a shorter upstream column #1. So another arrangement is at the two-thirds mark or a 10-m column, then the CFT tee, and then a 20-m column to create a 30-m analytical column. Here

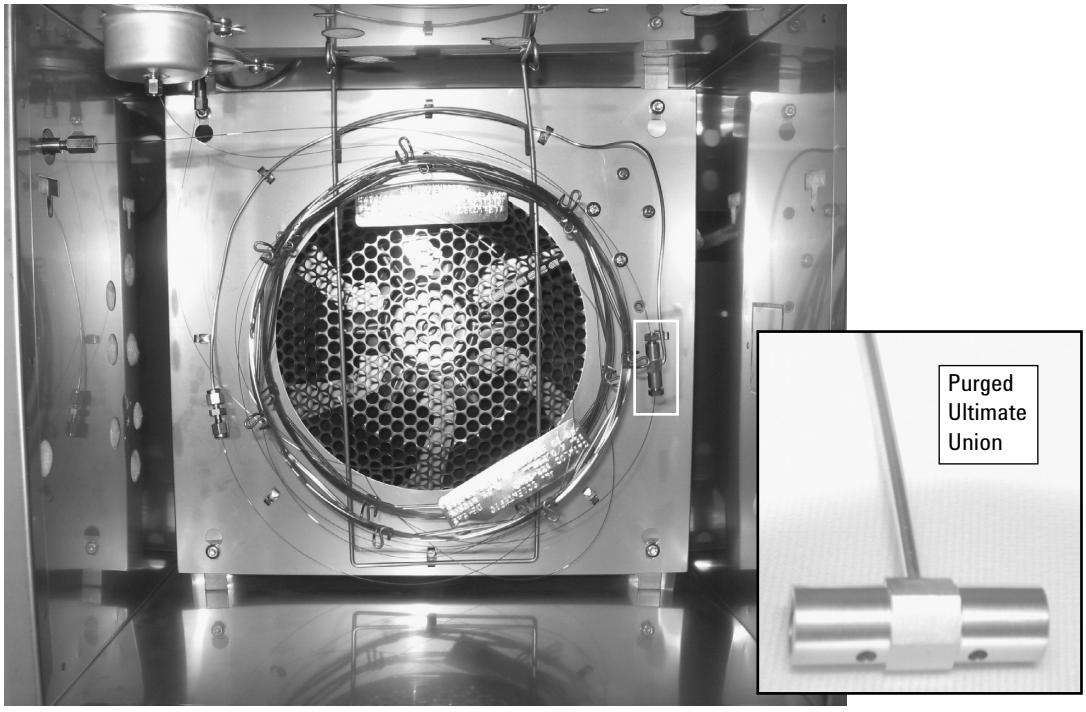


**Figure 11. Purged Ultimate Union.**

backflushing would be nearly 10 times faster than the arrangement with QuickSwap and more than twice as fast as the 15-m column for the same pressure. This would be the best arrangement for the MSD with a diffusion pump. Also, in terms of analytical time, this approach would provide even higher efficiency since 10 column volumes could be flushed in about 2 minutes. If backflushing begins before the analytical run ends (as shown in Advance Techniques and in Figure 10), then in many cases the Post-Run time would be very short or entirely unnecessary, yet still provide sufficient backflushing. This would further reduce total cycle times.

The joined columns need not match in many aspects. For example, a 0.32-mm id may be the first column and a 0.25-mm id the second column. In this situation it will be better to have the columns configured and described as they actually exist in the 7890A. For example, column #1 inlet is the splitless port and the outlet is the PCM module A; column #2 inlet is the PCM module A and the outlet is the MSD. Considerations of capacity, resolution, robustness, etc., can be entertained in several innovative ways to enhance productivity and data quality.

This solution can also be implemented on the Agilent 6890 GC. Of course, the PCT tee configuration is not confined to the Agilent GC/MS detector, but is suitable for other detection schemes as well.

Future software releases will contain a key command that will allow more functionality and greater ease of use: it will allow the user to apply the IGNORE READY = TRUE condition to the EPC device controlling the CFT tee. This will prevent the pressure pulse or other flow conditions from producing a “not ready” condition for the instrument.



## References

1. The 5975C Series GC/MSD, Agilent Technologies publication 5989-7827EN
2. Frank David and Matthew S. Klee, “Analysis of Suspected Flavor and Fragrance Allergens in Cosmetics Using the 7890A GC with Column Backflush,” Agilent Technologies publication 5989-6460EN
3. Frank David and Matthew S. Klee, “GC/MS Analysis of PCBs in Waste Oil Using the Backflush Capability of Agilent QuickSwap Accessory,” Agilent Technologies publication 5989-7601EN

(These references are available in the Literature Library at www.chem.agilent.com.)

## Acknowledgements

The author is very grateful to Bruce Quimby, Wes Norman, and Matthew Klee for several informative and encouraging discussions. Also, a special thanks to Wes Norman for providing superb CFT devices tailored to the needs of the GC/MSD and an advance example of the Purged Ultimate Union.

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Agilent Technologies

# Reliable Transfer of Existing Agilent 6890/5973 GC/MSD Methods to the New 7890/5975 GC/MSD

Application

**Gas Chromatography/Mass Spectroscopy**


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## Abstract

**The performance of the Agilent Technologies' new series of instruments, the 7890 Series GC and 5975 Series MSD, is compared to that of the previous versions, the 6890 Series GC and the 5973 Series MSD. Performance is shown to be fully equivalent in test comparisons using a 16-component polynuclear aromatic hydrocarbon (PAH) sample. Excellent reproducibility and calibration characteristics were obtained. These results indicate that one can migrate a method currently running on a 6890/5973 system to a 7890/5975 system with high confidence that the performance will be equivalent or better.**


## Introduction

Agilent Technologies recently introduced new gas chromatograph and mass spectrometer platforms, the 7890 Series GC and the 5975 Series MSD, containing improved software, firmware, and hardware over the prior series, the 6890 GC and the 5973 MSD.

It was necessary to validate instrument performance of the 7890 Series GC for methods previously created and run on 6890 Series instrument to ensure equivalence. It was also desirable to demonstrate the ease of method transfer from existing 6890 GC methods using the prior ChemStation software to new 7890A GC methods using the new ChemStation software.

**Table 1. System Configurations Compared**

| 6890 System configuration | 7890A System configuration |
|---|---|
| Agilent 6890A GC | Production prototype 7890 Series GC |
| S/SI inlet | S/SI inlet |
| ALS + tray | 7683 ALS + tray |
| Agilent 5973N MSD | Agilent 5975B MSD |
| Diffusion pump | Standard turbo |
| Inert E.I. source | Inert E.I. source |
| ChemStation 1701 DA version D.00.01 | ChemStation 1701DA version D.03.00 |

The method conditions used for this comparison were similar and are summarized in Table 2.

**Table 2. Semivolatiles Method Conditions**

| | |
|---|---|
| Column | HP-5 MS, 25 m x 250 µm id (p/n 19091S-433) |
| Carrier gas | He, constant pressure mode, nominal 13 psig |
| RTL | Anthracene @ 8.300 min |
| Split/splitless inlet | 300 °C, pulsed splitless: 25 psig for 0.3 min, 30 mL/min purge @ 0.75 min |
| Oven | 55 °C (1 min) → 320 °C (3 min) @ 25 °C/min; total time 14.60 min |
| Sample | 1-µL injection of PAHs in 0.32 to 10 ppm concentration range |
| MSD | Scan 45 to 400 u<br>Samples = $2^2$<br>Autotune EM offset +200 V<br>Source = 230 °C<br>Quad = 150 °C<br>Transfer line = 280 °C |

## Experimental

Our goals were to determine the performance metrics on a current 6890 GC for a typical retention time locked method, to transfer method conditions to a new 7890A GC and relock, and to determine the 7890A performance metrics and compare them to those of the 6890. The system configurations used are shown in Table 1.

## Results and Discussion

The test sample contained 16 different polynuclear aromatic hydrocarbons (PAHs), covering a wide range of physical properties. Total ion chromatograms (TICs), derived from each of the Agilent systems are compared in Figure 1. The comparison reveals a very high level of reproduciblity.

More detailed comparisons for selected PAHs are shown in Figures 2 and 3.

We note from the TICs shown in Figures 1 to 3 that the performance of both systems is nearly identical. The reproducibility data for all 16 PAHs are shown in Table 3, along with the delta RT, illustrating the ability to move methods between systems without the need for method redevelopment.

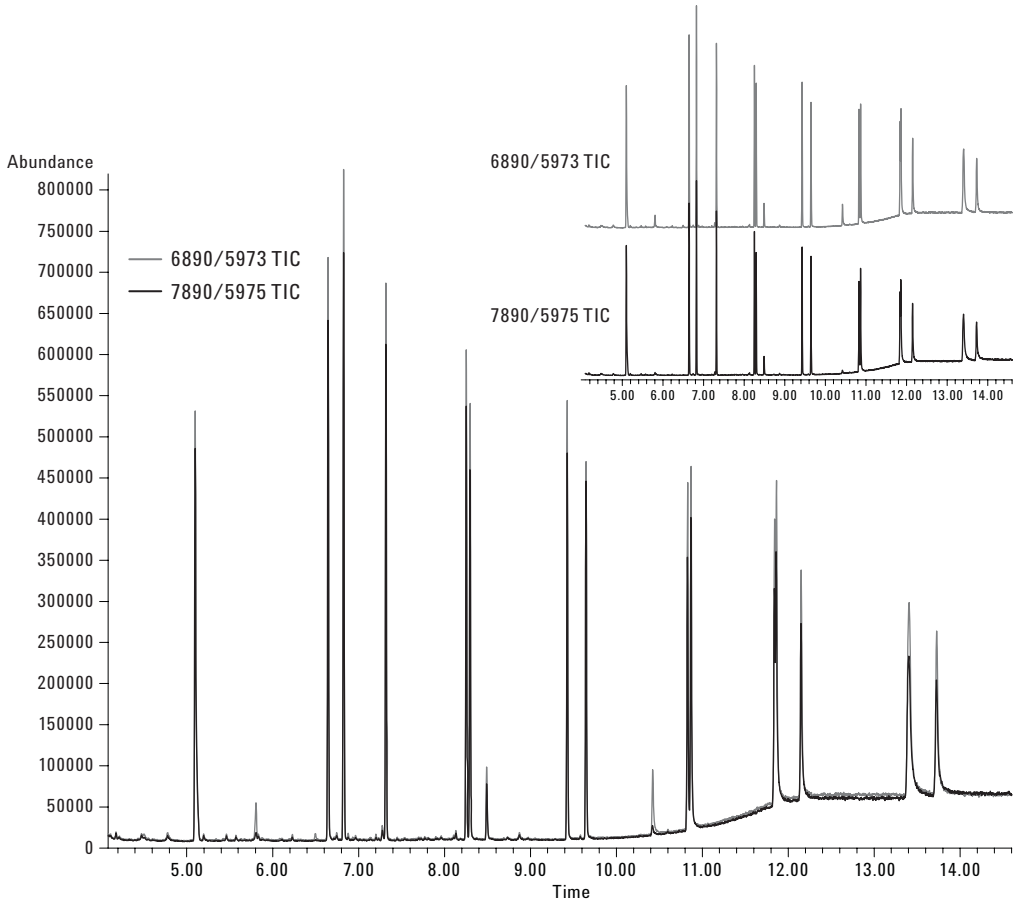


**Figure 1. Overlays of TICs of the same sample of PAHs are virtually indistinguishable. Inset shows the TICs in separated format.**

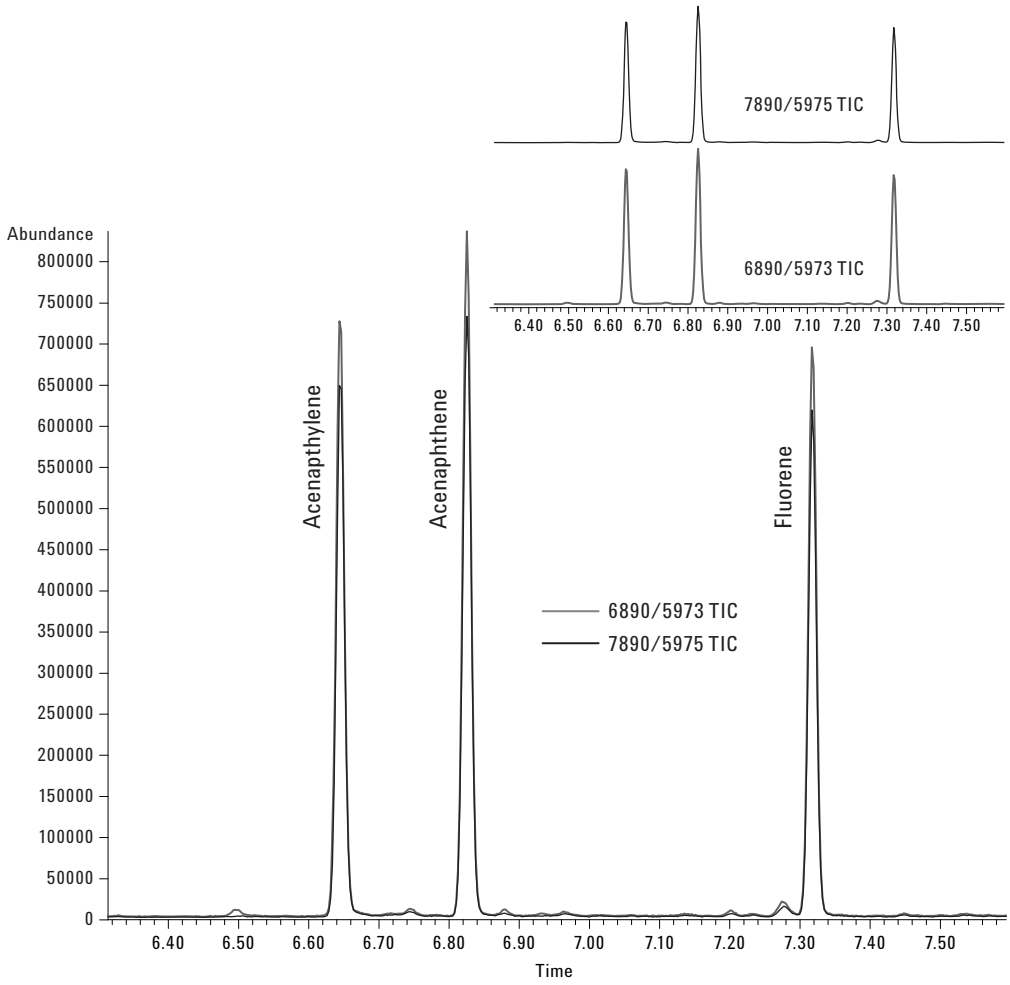


**Figure 2. System TICs for acenapthylene, acenaphthene, and fluorene are compared.**

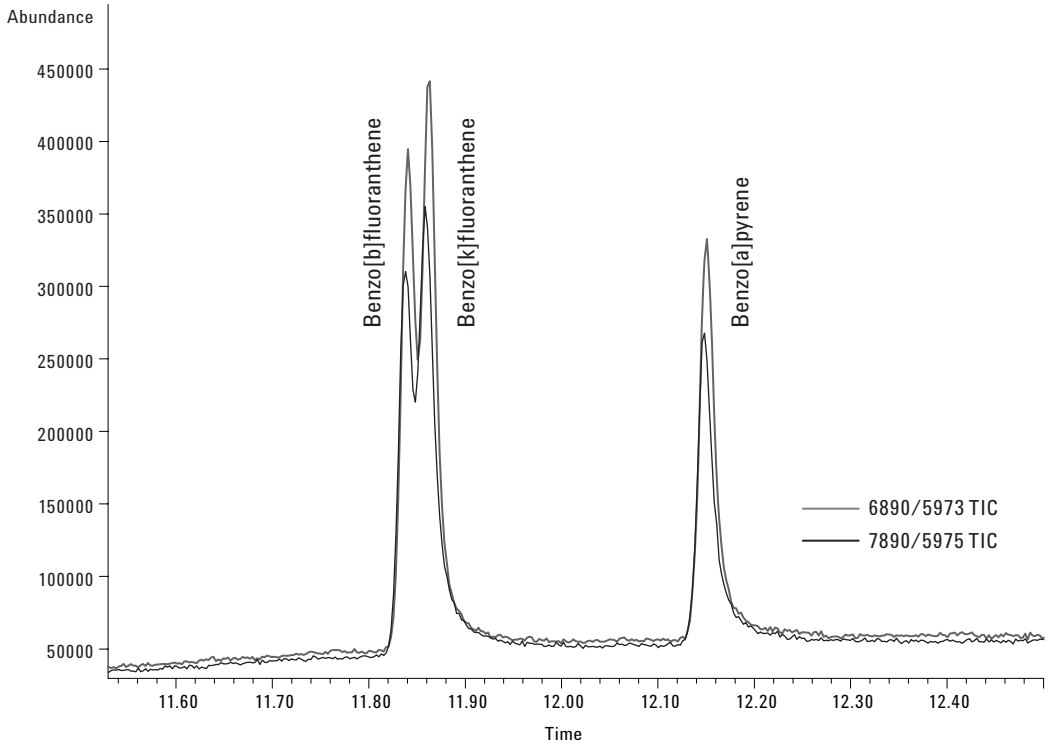


**Figure 3. System TICs for benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[a]pyrene are compared.**

**Table 3. Comparison of Performance Metrics**

| Solute | 6890/5973 | | | | 7890/5975 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Avg RT (min) | SD | RSD | Calibration linearity, $r^2$ | Avg RT (min) | SD | RSD | Calibration linearity, $r^2$ | ΔRT |
| Naphthalene | 5.103 | 0.005 | 0.098 | 0.992 | 5.103 | 0.005 | 0.098 | 0.995 | 0.000 |
| Acenapthylene | 6.650 | 0.000 | 0.000 | 0.998 | 6.649 | 0.003 | 0.050 | 0.998 | 0.001 |
| Acenaphthene | 6.830 | 0.000 | 0.000 | 0.993 | 6.830 | 0.000 | 0.000 | 0.995 | 0.000 |
| Fluorene | 7.320 | 0.000 | 0.000 | 0.997 | 7.320 | 0.000 | 0.000 | 0.998 | 0.000 |
| Phenanthrene | 8.256 | 0.005 | 0.064 | 0.999 | 8.251 | 0.003 | 0.040 | 0.999 | 0.004 |
| Anthracene | 8.300 | 0.000 | 0.000 | 0.999 | 8.300 | 0.000 | 0.000 | 0.998 | 0.000 |
| Fluoranthene | 9.430 | 0.000 | 0.000 | 0.997 | 9.430 | 0.000 | 0.000 | 0.995 | 0.000 |
| Pyrene | 9.650 | 0.000 | 0.000 | 0.997 | 9.650 | 0.000 | 0.000 | 0.996 | 0.000 |
| Chrysene | 10.830 | 0.000 | 0.000 | 0.991 | 10.830 | 0.000 | 0.000 | 0.992 | 0.000 |
| Benz[a]anthracene | 10.870 | 0.000 | 0.000 | 0.995 | 10.870 | 0.000 | 0.000 | 0.994 | 0.000 |
| Benz[b]fluoranthene | 11.848 | 0.010 | 0.082 | 0.999 | 11.849 | 0.011 | 0.089 | 0.997 | -0.001 |
| Benz[k]fluoranthene | 11.862 | 0.004 | 0.037 | 0.997 | 11.862 | 0.004 | 0.037 | 0.999 | 0.000 |
| Benzo[a]pyrene | 12.151 | 0.003 | 0.027 | 0.999 | 12.150 | 0.000 | 0.000 | 0.999 | 0.001 |
| Indeno[1,2,3-cd]pyrene | 13.412 | 0.008 | 0.062 | 0.998 | 13.412 | 0.004 | 0.033 | 0.995 | 0.000 |
| Dibenz[a,h]anthracene | 13.404 | 0.005 | 0.039 | 0.994 | 13.396 | 0.005 | 0.039 | 0.996 | 0.009 |
| Benzo[ghi]perylene | 13.732 | 0.004 | 0.032 | 0.993 | 13.729 | 0.008 | 0.057 | 0.995 | 0.003 |

Figure 4 shows that the response characteristics for all PAH components are similar on both the 6890/5873 and 7890/5975C systems.

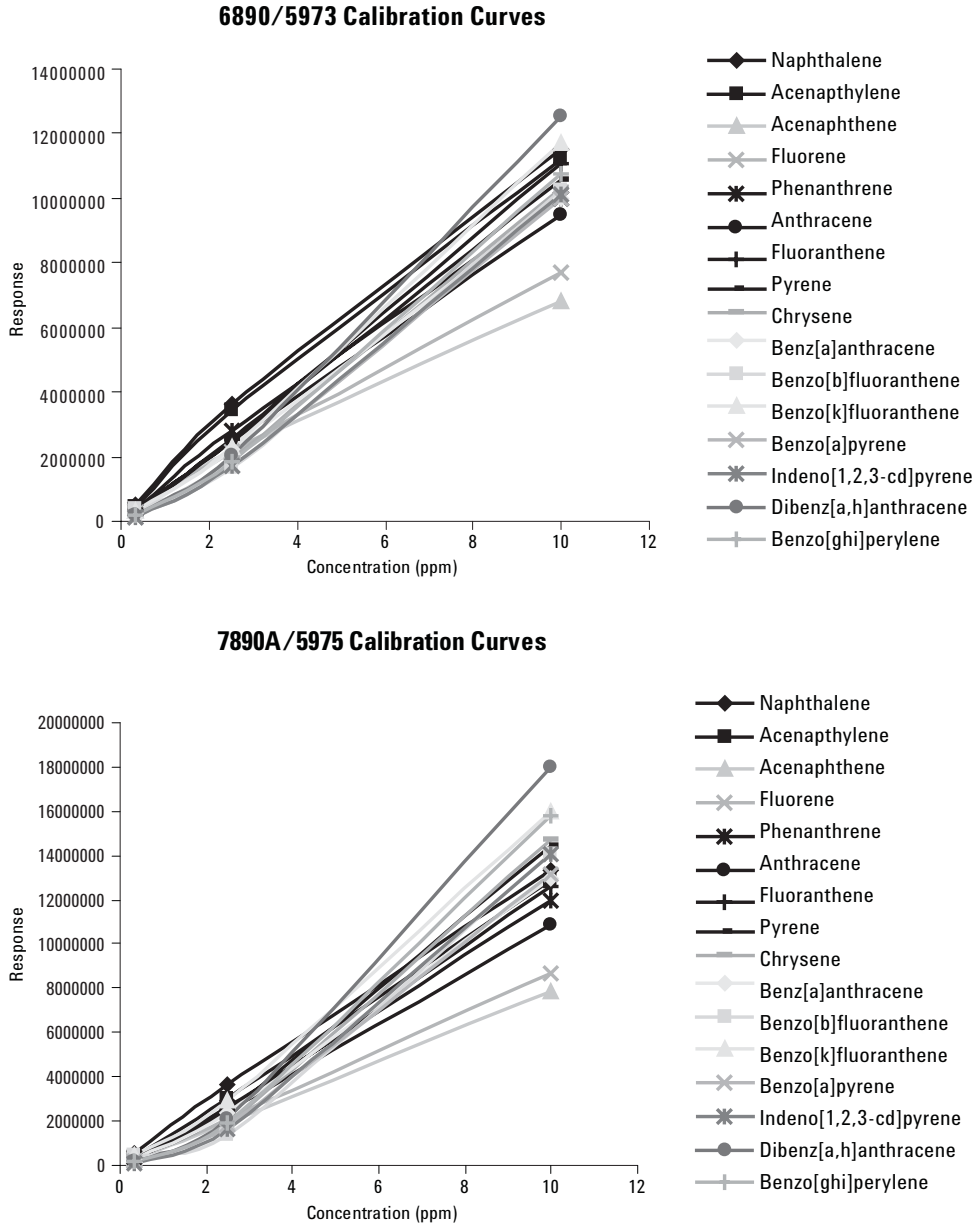


**Figure 4. PAH calibration plots over the 0.32-10 ppm concentration range using both the 6890/5973 (upper) and 7890A/5975C (lower) GC/MSD systems.**

## Conclusions

System equivalence is demonstrated. The new Agilent 7890 Series GC and 5975 Series MSD system easily reproduced method characteristics of the prior Agilent 6890 Series GC and 5973 Series MSD systems, using a 16-component PAH sample and retention time locking. This demonstrates that methods can be confidently migrated to the new systems without loss in performance, allowing rapid and trouble free implementation of the new platforms.

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**Agilent Technologies**

# Direct Injection of Fish Oil for the GC-ECD Analysis of PCBs: Results Using a Deans Switch With Backflushing

Application

**Environmental and Pharmaceutical**


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## Abstract

**A Deans switch, employing Agilent's Capillary Flow Technology, was configured on an Agilent 7890A gas chromatograph (GC) equipped with dual electron capture detectors (ECDs). A method was developed for the analysis of fish oil for polychlorinated biphenyl (PCB) contamination. The Deans switch was used to heart cut 7 indicator PCBs (IUPAC congeners 28, 52, 101, 118, 138, 153, and 180) from the primary DB-XLB column onto a DB-200 column for further separation. Fish oil from a supplement capsule was simply diluted 1:10 in isooctane and injected directly. In a separate experiment, the fish oil was analyzed by GC with a flame ionization detector (GC/FID) without backflushing. From these analyses, it was estimated that about two-thirds of the fish oil components would remain on the column after the 17.4-minute GC/ECD run. To prevent carryover, contamination, and retention time shifts, the Deans switch was used to backflush the primary column at the end of each run. Evidence shows that backflushing removed the fish oil residue, which otherwise would quickly degrade the chromatography.**


## Introduction

Fish oils contain high levels of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), omega-3 fatty acids that are thought to have beneficial health affects. In addition to eating fish, many people take fish oil as a supplement to their daily diet. However, fish, especially those high on the aquatic food chain, can bioaccumulate fat-soluble pollutants. Among these are polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). Therefore, fish oil used in supplements undergoes a variety of analyses, including tests for halogenated pollutants.

One of the quality assurance tests is to analyze fish oil for PCB contamination. This is complicated by the fact that fish oil is a very complex mixture containing high-boiling fatty acids and triglycerides of fatty acids; chain lengths are mostly between 14 and 22 carbons. They also contain varying amounts of phospholipids, glycerol ethers, wax esters, and fatty alcohols. PCB analysis is complex by itself, with 209 possible congeners. Of these, 140 to 150 have been observed in commercial PCB mixtures called Aroclors. PCB analysis usually focuses on the 12 planar, dioxin-like PCBs and/or on seven indicator PCBs (IUPAC Numbers 28, 52, 101, 118, 138, 153, and 180).

To obtain sufficient sensitivity and selectivity for these compounds, analysts have traditionally employed very expensive techniques such as high-resolution mass spectrometry (HR/MS) or HR/MS/MS. Analysis of the fish oil generally follows a series of extraction and cleanup steps. This paper focuses on the analysis of the seven indicator PCBs in fish oil using an Agilent 7890A GC configured with a Deans switch, two columns of differing selectivity, and dual electron capture

detectors (ECDs). Fish oil from a commercially available supplement was simply diluted 10:1 in isooctane and injected into the GC. No cleanup steps were employed.

## Experimental

The fish oil supplement was obtained from a local grocery store. According to the bottle's label, each gelatin capsule contains 1.0 g of fish oil of which 180 mg is EPA and 120 mg is DHA. Oil was removed from a capsule and diluted with isooctane (pesticide grade from Sigma-Aldrich, Milwaukee, WI, USA) to make a 10% solution. This solution was spiked with various Aroclors (Supelco, Bellefonte, PA, USA) or with individual PCB congeners (AccuStandard, New Haven, CT, USA).

Table 1 lists the instrumentation and experimental conditions for the analysis.

**Table 1. Instrumentation and Experimental Conditions**

| | |
|---|---|
| **Instrumentation and Software** | |
| Gas chromatograph | Agilent 7890A |
| Automatic sampler | Agilent 7683B Series injector and tray |
| Primary column | J&W 30-m × 0.18-mm × 0.18-µm DB-XLB (P/N 121-1232) |
| Primary column connections | Split/splitless inlet to Deans switch |
| Secondary column | J&W 30-m × 0.25-mm × 0.50-µm DB-200 (P/N 122-2033) |
| Secondary column connections | Deans switch to back ECD |
| Restrictor | 76.8-cm × 0.100-mm deactivated fused silica tubing |
| Restrictor connections | Deans switch to front ECD |
| Inlet liner | Agilent deactivated single taper with glass wool (P/N 5062-3587) |
| Auxiliary pressure control device | Agilent 7890A Pneumatic Control Module (PCM) Option # 309 |
| Deans switch calculator software | Agilent Technologies Deans Switch Calculator (Rev. A.01.01) |
| Software for data acquisition and analysis | Agilent GC ChemStation (Rev. B.03.01) |
| **Instrumental Conditions for Analysis** | |
| Inlet | Split/splitless at 330 °C |
| Oven temperature program | 80 °C (1 min), 50 °C/min to 200 °C (0 min), 10 °C/min to 290 °C (5 min) |
| Detectors | Dual ECD at 340 °C |
| ECD make-up gas | $N_2$ at 60 mL/min |
| Inlet pressure | $H_2$ at 41.040 psig (constant pressure mode) |
| PCM pressure to Deans switch | $H_2$ at 20.610 psig (constant pressure mode) |
| **Post-Run Backflush Conditions** | |
| Post-run duration | 2.4 min |
| Inlet pressure | $H_2$ at 0 psig |
| PCM pressure | $H_2$ at 80 psig |
| Oven temperature during backflush | 290 °C for 2.4 min |

## Results and Discussion

Without backflushing, the high-boiling components of fish oil can be retained by the GC column, causing severe carryover problems from one run to the next. After a few injections, so much of the fish oil residue builds up on the column that it causes PCB retention times to shift by a minute or more. Such dramatic retention time shifts would prevent the use of the Deans switch, where heart cuts are just a few seconds wide.

### Deans Switch–Heart Cutting

The Deans switch is one of Agilent's new devices that employ Capillary Flow Technology. These devices have extremely low dead volumes, are inert, and do not leak, even with large cycles in oven temperature. Columns are easy to install into the Deans switch, which is mounted on the side of the oven wall (Figure 1).

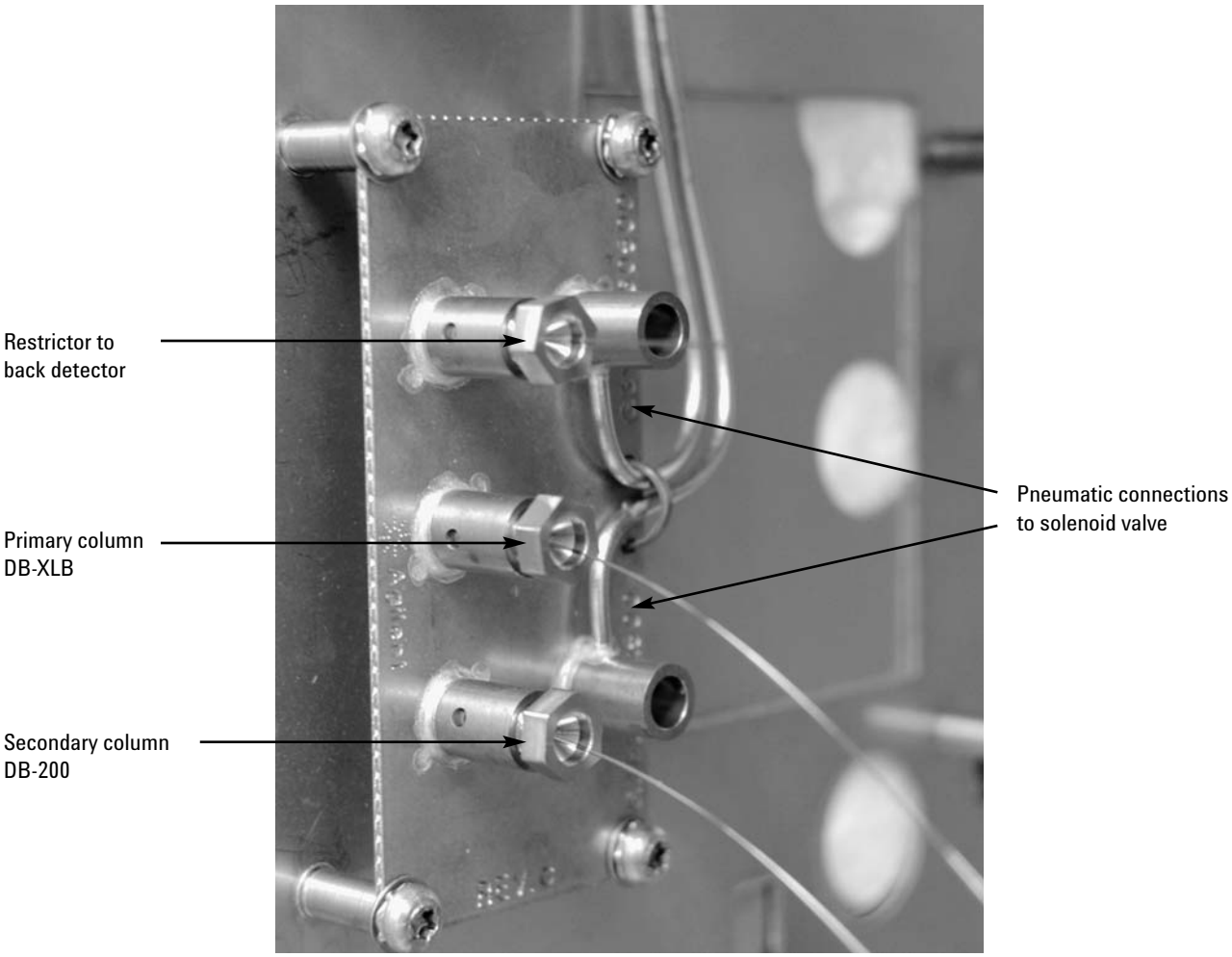


**Figure 1. Photograph of the Deans switch installed on the side of the 7890A GC oven. The column and restrictor connections are indicated by an * in Figure 2a.**

As shown in Figure 2a, the 30-m × 0.18-mm × 0.18-µm DB-XLB column is connected between the split/splitless inlet and the Deans switch. A short length of deactivated fused silica tubing (76.8 cm × 0.100 mm) connects the Deans switch to the front ECD. The secondary column (30-m × 0.25-mm × 0.5-µm DB-200) was chosen because it is more polar than the DB-XLB column and has a different selectivity for PCBs. It has an upper temperature limit of 300 °C, which is high enough to elute the PCBs of interest.

Figure 2a shows the Deans switch in the "normal" mode with the solenoid valve in the off position. In this mode, the effluent from the primary DB-XLB column is directed through the restrictor to the front ECD. When the solenoid valve is switched, the effluent is directed through the secondary DB-200 column to the back ECD (Figure 2b). The retention times for the seven indicator PCBs were initially determined with the valve in the *off* position. Using the timed events table in the ChemStation, the valve was switched to *on* just before each PCB peak and *off* immediately after. This produced seven heart cuts that were directed through the DB-200 column to the back ECD.

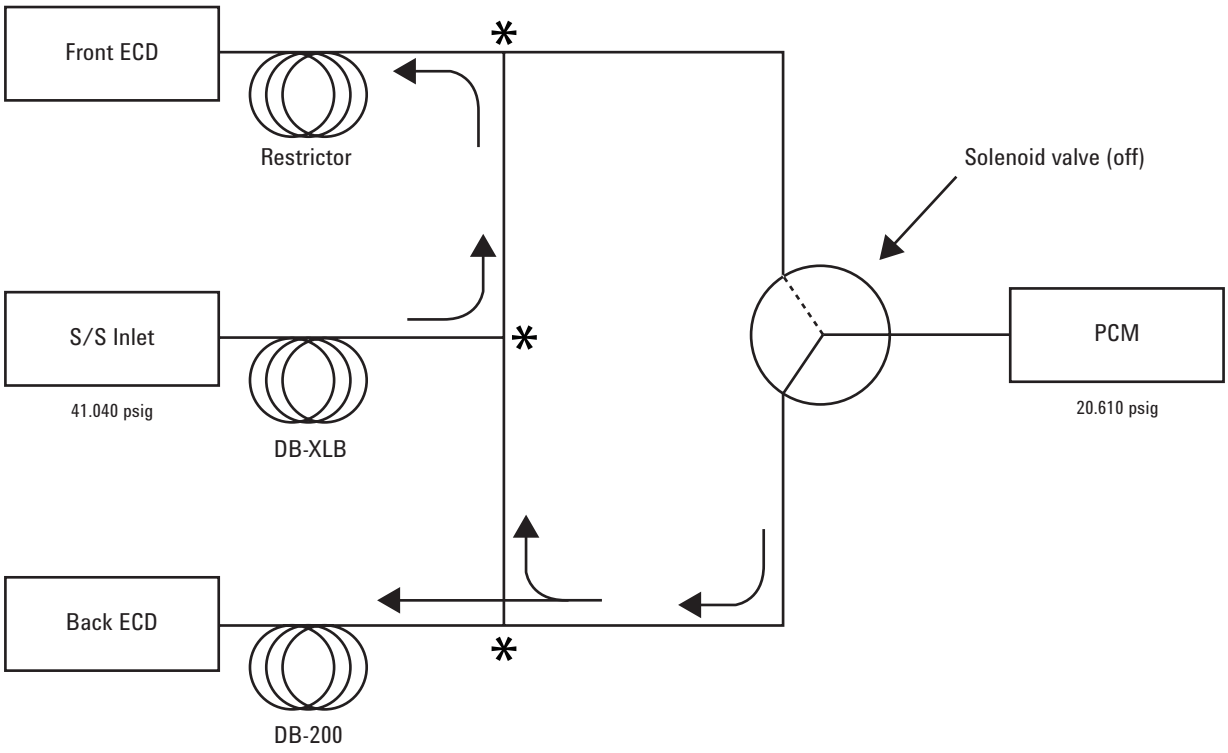


**Figure 2a. Deans switch in the "no cut" position. The effluent from the DB-XLB column goes directly to the front ECD through the short restrictor. The intersections marked with an * are column and restrictor connections to the Deans switch plate (Figure 1).**

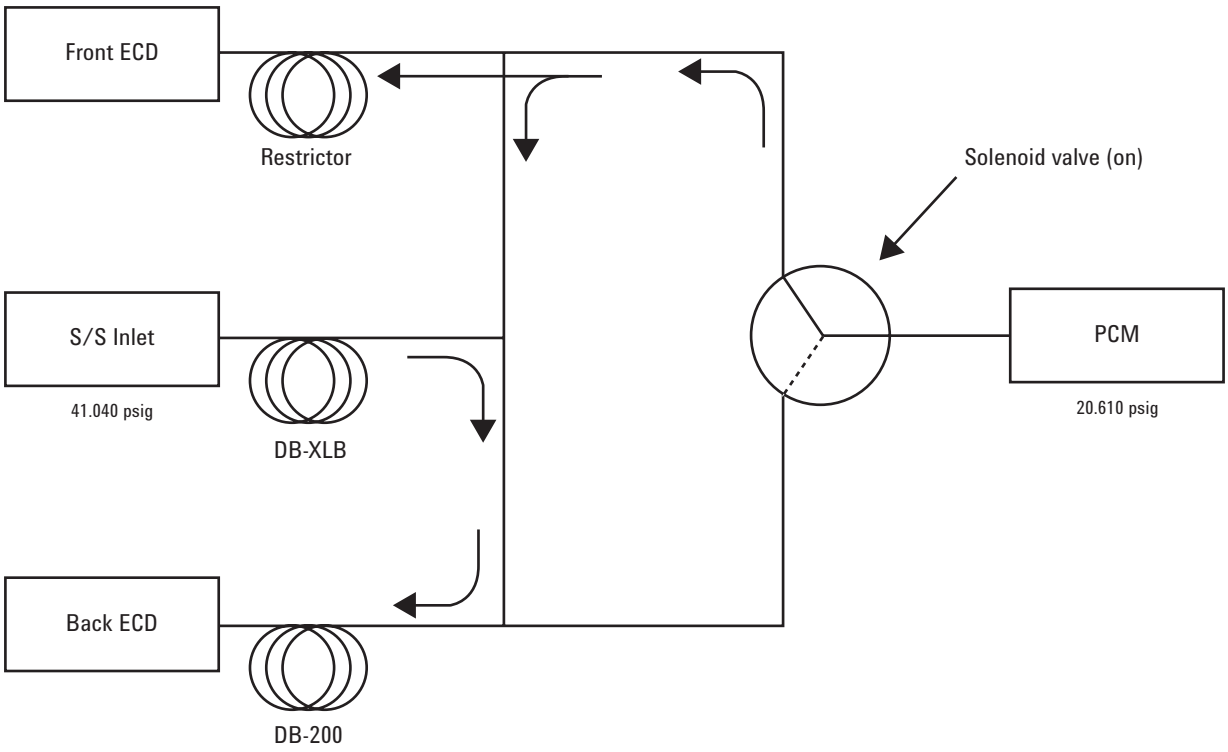


**Figure 2b. Deans switch in the "cut" position. The effluent from the DB-XLB column goes to the DB-200 column and then to the back ECD.**

In some Deans switch systems, the second column is placed in a separate GC oven or cryogenic cooling is used to trap the heart cut components at the head of the second column. In this case, both columns were mounted inside of the 7890A oven and cooling was not used to focus compounds at the head of the DB-200 column.

Figure 3a shows the chromatogram for a fish oil sample spiked with Aroclor 1260. PCBs 28, 52, 101, 118, 138, 153, and 180 were cut out of the primary chromatogram (Figure 3b) and sent to the second column (Figure 3c). The purpose of the DB-200 column is to resolve the target PCBs from other PCBs and matrix components that co-elute with them on the DB-XLB column. Six of the 7 PCBs appear to be well resolved on the DB-200 column. PCB 118 is only partially resolved by this method.

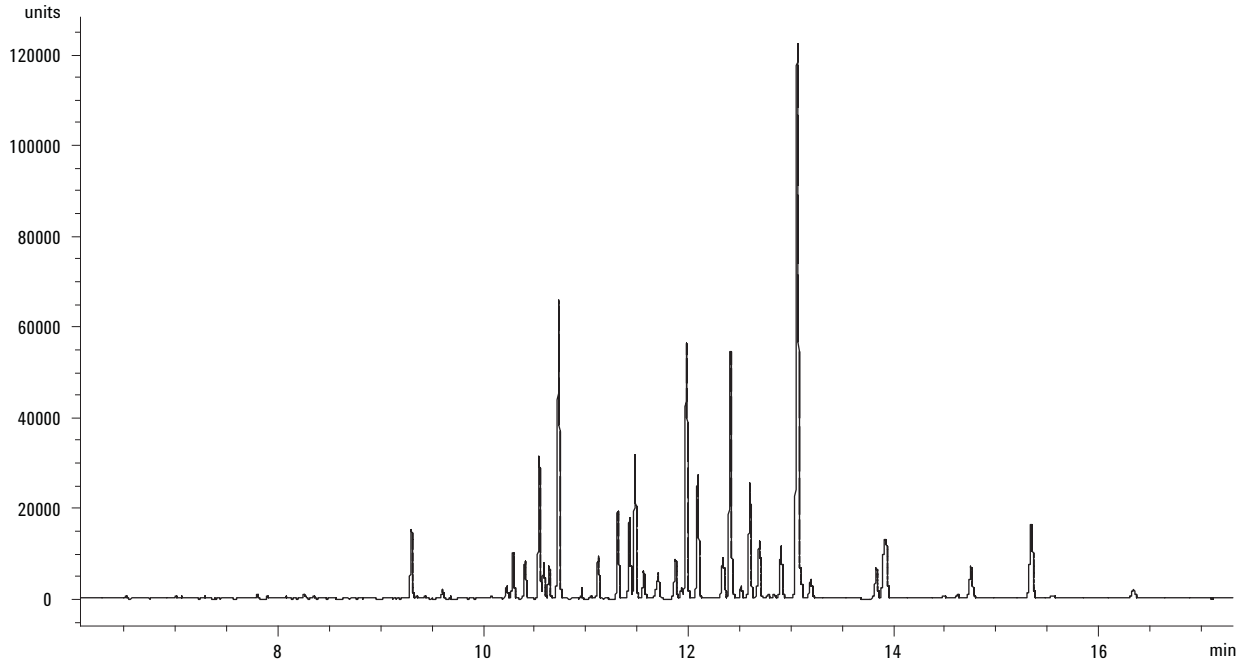


**Figure 3a. GC/ECD chromatogram of Aroclor 1260 spiked into fish oil. This is the effluent from the primary DB-XLB column with seven heart cuts.**

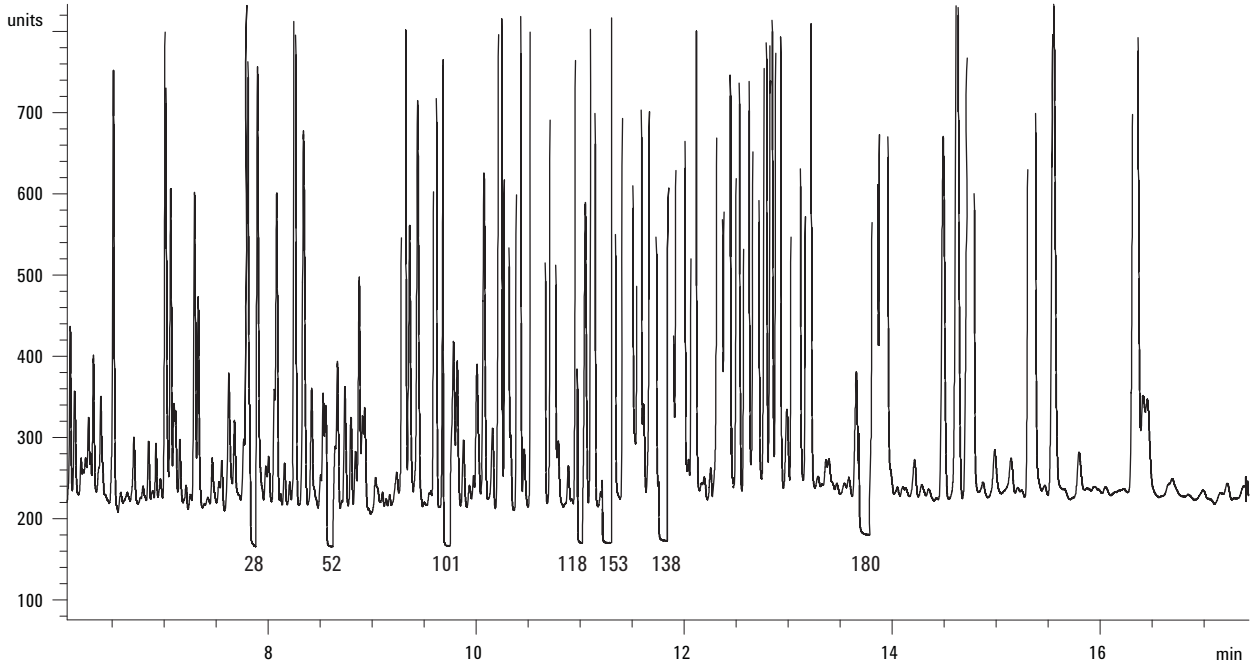


**Figure 3b. Enlargement of the chromatogram in Figure 3a showing where heart cuts were made for the seven target PCBs.**

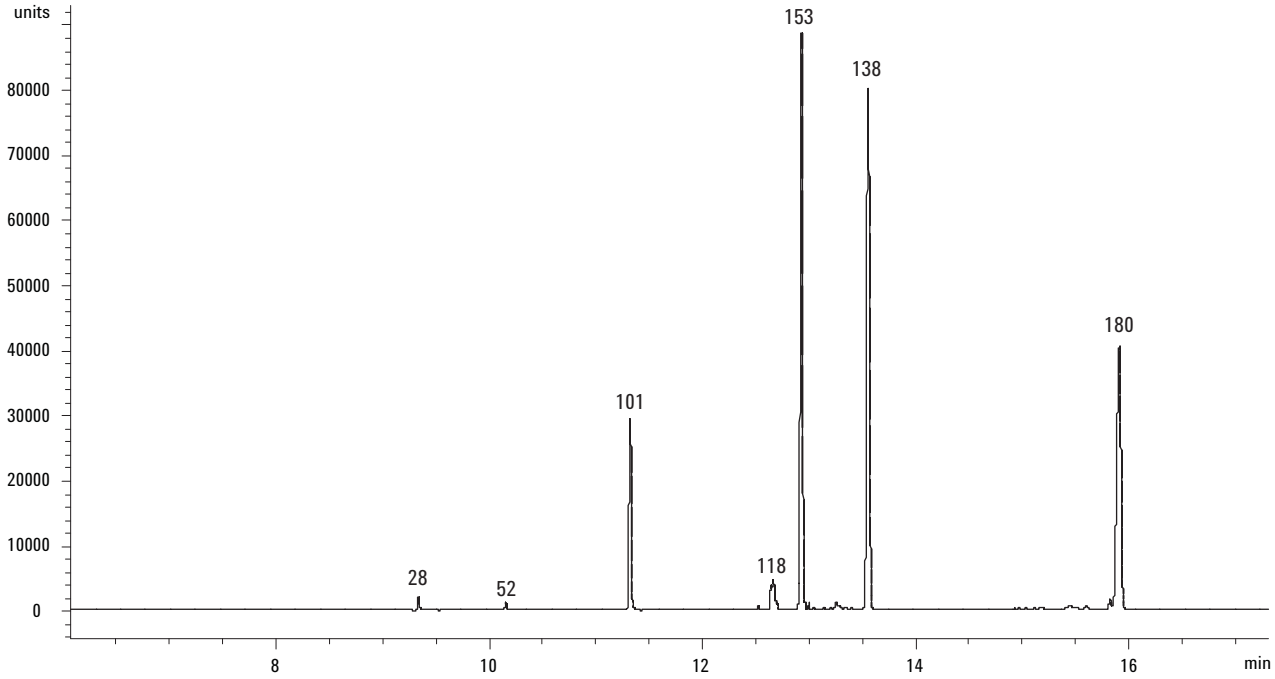


**Figure 3c. GC/ECD chromatogram from the DB-200 column. The peaks in this chromatogram were heart cut from the DB-XLB column. Except for congener 118, the target PCBs were separated from co-eluting interferences by the DB-200 column.**

### Deans Switch–Backflushing

Data collection with the Deans switch system ended at 17.4 min with the oven at 290 °C. While it was assumed that a lot of the fish oil components remained on the column at this point, it was impossible to tell because the ECD responds poorly to these compounds. The fish oil does contribute some small peaks (both positive and negative) to the chromatogram, but it is impossible to see the full contribution of the fish oil. So a sample of the fish oil was analyzed on an identical DB-XLB column using a flame ionization detector (FID) with no Deans switch installed. The temperature was held at 290 °C for an extra 25 minutes to determine if high boiling compounds were still eluting.

Figure 4 shows that a great deal of the fish oil continued to elute after 17.4 minutes (arrow in figure). When a blank run was made with a final oven temperature of 310 °C, much more of the fish oil eluted from the column (Figure 4, middle chromatogram). A second blank run (Figure 4, top chromatogram) showed that fish oil components were still eluting from the column. In actuality, only about a third of the fish oil comes off the column under the Deans switch conditions. This is why other fish oil methods begin with a solvent extraction followed by solid phase extraction for sample cleanup.

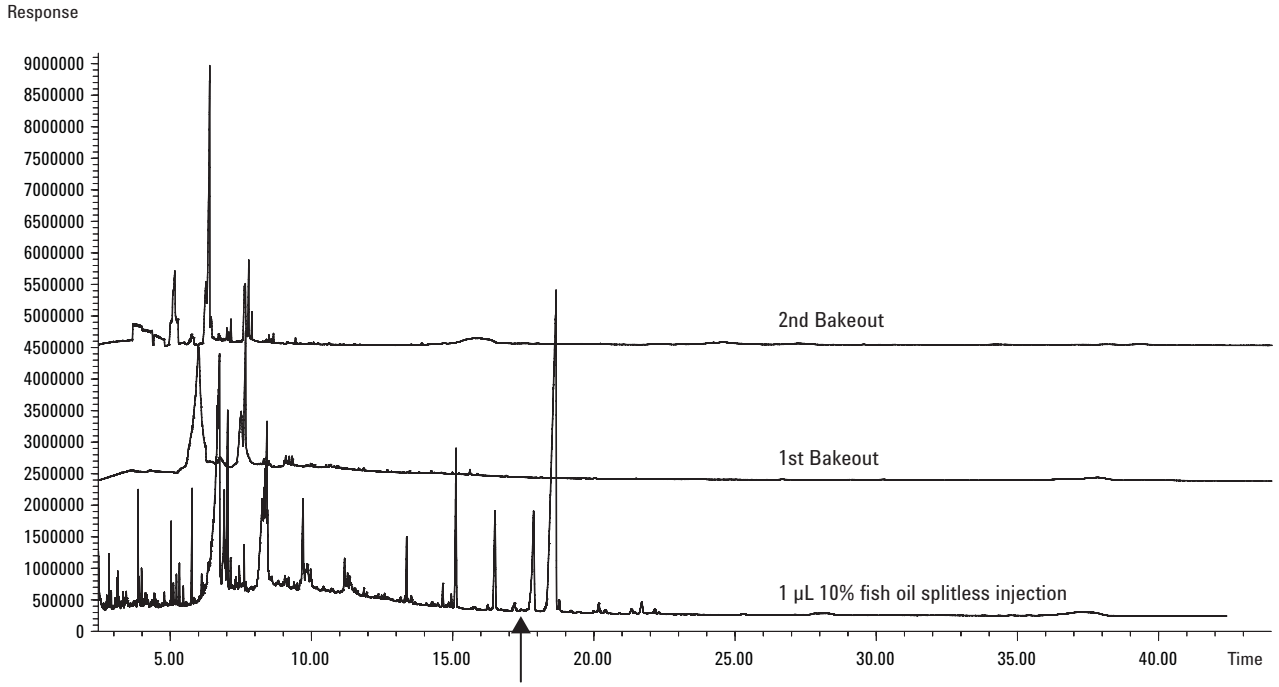


**Figure 4. GC/FID chromatogram from a 1 µL splitless injection of 10% fish oil using a 30-m × 0.18-mm × 0.18 µm DB-XLB column. The arrow indicates where the GC/ECD method ends and the post-run backflush begins. In this case, there was no backflushing so the oven was held at 290 °C for an extra 25 min. The run was repeated two more times without injection but with the oven held at 310 °C for 30 minutes at the end of the run. Residue from the fish oil injection continued to elute, even during a second bakeout step.**

The 7890A has been designed to make column backflushing a routine process. It has been shown empirically that backflushing should continue for about five times the holdup time. In this case the column was held at 290 °C during the post run backflush. At the same time, the inlet pressure was dropped to 0 psig while the PCM pressure was increased to 80 psig. Using Agilent's GC Pressure/Flow Calculator software, the $H_2$ flow rate backwards through the column was 3.81 mL/min and the holdup time was 0.466 min. Backflushing was, therefore, continued for 2.4 minutes, which is slightly more than five times the calculated holdup value. Figure 5 shows the Deans switch in the backflush mode.

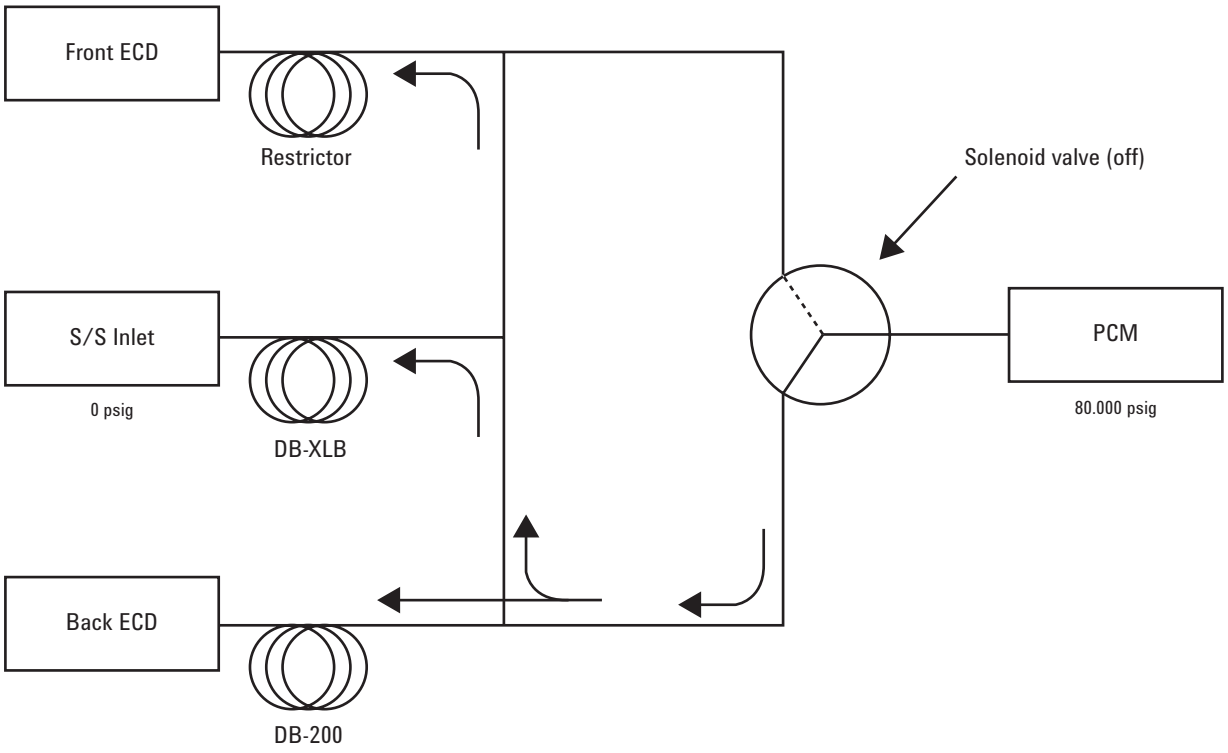


**Figure 5. Deans switch in the "backflush" mode. The inlet pressure is dropped to 0 (or 1) psig while the PCM pressure is raised to 80 psig. This causes the carrier gas to flow backwards through the DB-XLB column. The reverse flow sweeps high-boiling fish oil components off the head of the column and out the split vent.**

As mentioned earlier, just a few injections of fish oil can cause dramatic shifts in PCB retention times. Backflushing forces the remaining fish oil components backwards through the primary column and out through the split vent. This prevents fish oil buildup on the column, thus eliminating carryover and retention time shifts. Figure 6a compares the first and last chromatograms in a six-run sequence. One-µL splitless injections were made of 10% fish oil spiked with Aroclor 1260. This sequence was run after many previous injections of fish oil using this method, and it is clear that the retention times did not shift.

Figure 6b shows the seven PCBs that were heart cut from the two analyses shown in Figure 6a. Figure 6b shows no differences in the first and last heart cut chromatograms, providing further proof that there were not even subtle shifts in the PCB retention times. Each heart cut was just 4 to 5 seconds wide, so very small RT shifts in the first column would dramatically alter the results in the second.

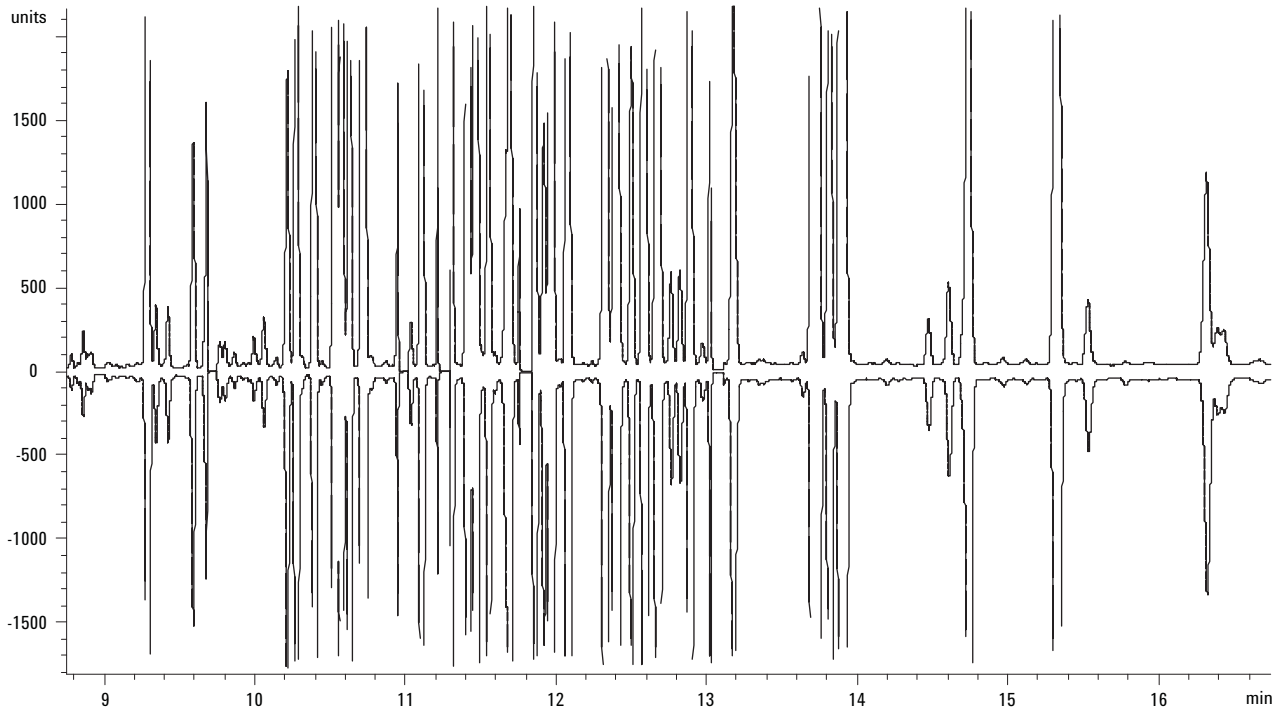


**Figure 6a. First (top) and sixth (inverted) injections of 10% fish oil spiked with Aroclor 1260. Seven Deans switch cuts were made from this DB-XLB column in order to isolate PCBs 28, 52, 101, 118, 138, 153, and 180. The DB-XLB column was back-flushed after each run, preventing build-up of fish oil residue. The comparison shows that there was no shift in retention times caused by fish oil accumulation.**

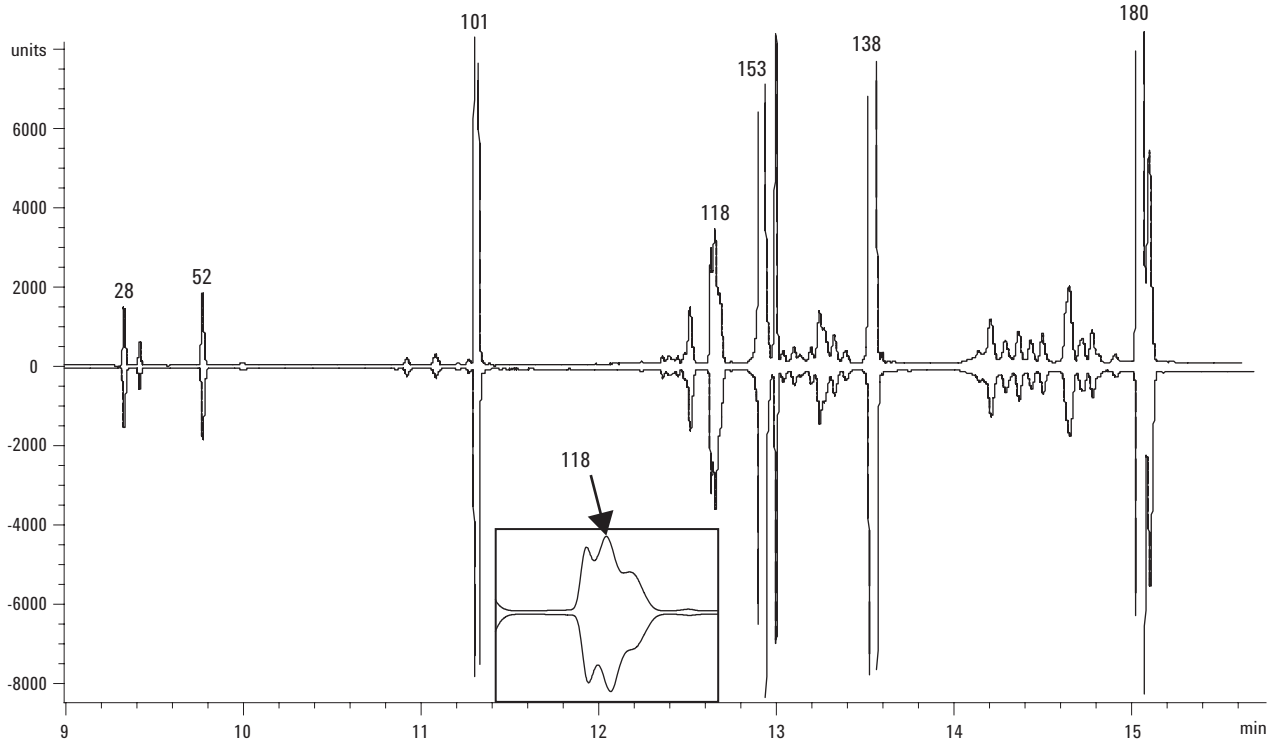


**Figure 6b. Chromatogram of the seven PCB congeners and interferences that were cut from the DB-XLB column to the DB-200. The first chromatogram (top) and sixth (inverted) are identical, providing further proof of retention time stability. Any retention time shift on the primary column would severely alter the appearance of the secondary chromatogram.**

## Conclusions

This paper demonstrates that it is possible to analyze PCBs in fish oil without performing laborious sample cleanup prior to GC injection. A Deans switch was used to cut seven target PCBs (28, 52, 101, 118, 138, 153, and 180) from a DB-XLB column for further separation on a DB-200 column. This produced nearly baseline separation of the target PCBs. Only congener 118 was not well separated from co-eluting PCBs. Further refinement of the oven temperature program would be needed to isolate this congener.

It has been estimated that about two-thirds of the fish oil remained on the primary GC column at the end of the run. By setting the Deans switch to the backflush mode for just 2.4 minutes at the end of each run, this material was swept backwards through the column and out the split vent. There was no evidence for retention time shifts or carryover from run to run.

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Agilent Technologies

# Improving Productivity and Extending Column Life with Backflush

## Application Brief

Chin-Kai Meng

**All Industries**

A previous application note [1] has shown that multiple GC signals and MS signals can be acquired from a single sample injection. When a 3-way splitter is connected to the end of a column, column effluent can be directed proportionally to two GC detectors as well as the MSD. This multi-signal configuration provides full-scan data for library searching, SIM data for quantitation, and element selective detector data for excellent selectivity and sensitivity from complex matrices.

The system used in this study consists of a 7683ALS, a 7890A GC with split/splitless inlet, 3-way splitter, μECD, dual flame photometric detector (DFPD), and a 5975C MSD. Figure 1 shows four chromatograms from a single injection of a milk extract. The synchronous SIM/scan feature of the 5975C MSD provides data useful for both screening (full scan data) and quantitation (SIM data). DFPD provides both P and S signals without the need to switch light filters.

Noticeably in the full scan TIC in Figure 1, a significant number of matrix peaks were observed after 32 minutes. It is not uncommon to add a "bake-out" oven ramp to clean the column after analyzing complex samples. The bake-out period is used to quickly push the late eluters out of the column to be ready for the next injection. Therefore, it is common to use a higher oven temperature than required for the analysis and an extended bake-out period at the end of a normal

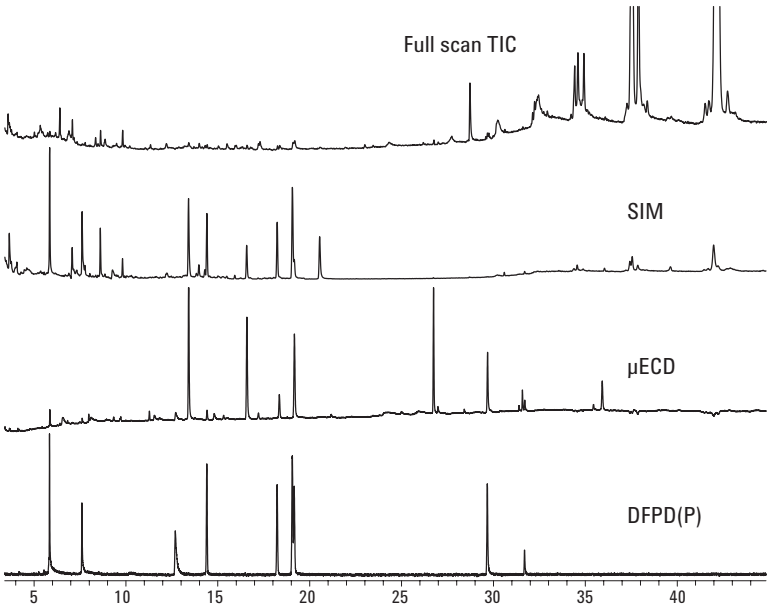


**Figure 1. Four chromatograms collected simultaneously from a single injection of a milk extract.**

## Highlights

- Backflush – a simple technique to remove high boilers from the column faster and at a lower column temperature to cut down analysis time and increase column lifetime.
- The milk extract example shows that a 7-minute 280 °C backflush cleaned the column as well as a 33-minute 320 °C bake-out. The cycle time was reduced by more than 30%.
- Using backflush, excess column bleed and heavy residues will not be introduced into the MSD, thus reducing ion source contamination.

over program to clean out the column, which adds to the cycle time and shortens the column lifetime. Adding the bake-out period to the milk extract analysis, additional matrix peaks were observed even up to 72 minutes, while target compounds already eluted before 42 minutes. This means that 30 minutes were lost in productivity for each injection.

Backflush [2] is a simple technique to drastically decrease the cycle time by reversing the column flow to push the late eluters out of the inlet end of the column. Late eluters stay near the front of the column until the oven temperature is high enough to move them through the column. When the column flow is reversed before the late eluters start to move down the column, these late eluters will take less time and at a lower oven temperature to exit the inlet end of the column.

There are many benefits in using backflush:

- Cycle time is reduced (no bake-out period, cooling down from a lower oven temperature)
- Column bleed is reduced (no high-temperature bake-out needed), resulting longer column life
- Ghost peaks are eliminated (no high boilers carryover into subsequent runs)
- Contamination that goes into the detector is minimized, which is especially valuable for the MSD (less ion source cleaning)

Figure 2 shows three total ion chromatograms from the Agilent 7890A GC/5975C MSD. The top chromatogram is a milk extract analysis with all the target compounds eluted before 42 minutes (over program goes to 280 °C). However, an additional 33-minute bake-out period at 320 °C was needed to move the high boilers out of the column. This bake-out period was almost as long as the required time to elute all target compounds. The middle chromatogram is the same milk extract analysis stopped at 42 minutes with a 7-minute backflush post-run at 280 °C added to the analysis. The bottom chromatogram is a blank run after the backflushing was completed. The blank run shows that the column was very clean after backflushing. The example shows that a 7-minute backflush cleaned the column as well as a 33-minute bake-out.

The milk extract example in Figure 2 illustrates the backflush technique in reducing cycle time and column bleed. The cycle time was reduced by more than 30% and the column was kept at 280 °C, without going to the bake-out temperature of 320 °C. A column effluent splitter or QuickSwap is required to do the backflush.

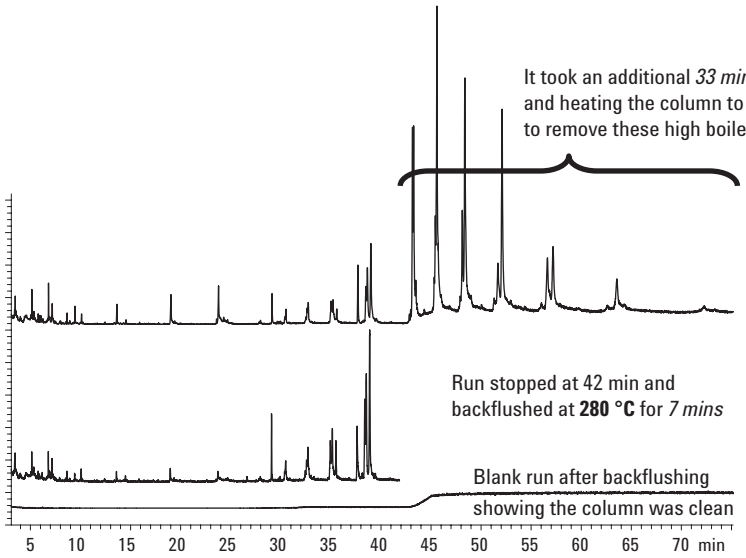


**Figure 2. Three total ion chromatograms comparing the results with and without backflush.**

## References

1. Chin-Kai Meng and Bruce Quimby, "Identifying Pesticides with Full Scan, SIM, μECD, and FPD from a Single Injection," Agilent Application Note, 5989-3299EN, July 2005.
2. Matthew Klee, "Simplified Backflush Using Agilent 6890 GC Post Run Command," Agilent Application Note, 5989-5111EN, June 2006.

## Acknowledgement

Milk extract is courtesy of Dr. Steven Lehotay from USDA Agricultural Research Service in Wyndmoor, Pennsylvania, USA.

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# Using RTL and 3-Way Splitter to Identify Unknown in Strawberry Extract

## Application Brief

Chin-Kai Meng

**Food Safety and Environmental**

Fruit and vegetable extracts are usually very complex to analyze. It is common to use the very selective GC detectors, for example NPD, µECD, and FPD, to look for trace pesticide residues in the extracts. Mass spectrometry is most often used to confirm the hits from GC detectors. A previous application note [1] describes a GC/MS system with a three-way splitter added to the end of the column. The column effluent could be split three ways to two GC detectors and the MSD. The splitter system is therefore capable of providing up to four signals (two GC signals, SIM, and full-scan chromatograms) from a single injection. The combination of element selective detectors, SIM/scan, and Deconvolution Reporting Software (DRS) makes a very powerful pesticide analysis system [2]. The trade-off is the decrease of analyte concentration in any detector due to the flow splitting at the end of the column.

The system used for this study consists of an Agilent 7890A GC with split/splitless inlet, a three-way splitter, µECD, dual flame photometric detector (DFPD), and 5975C MSD. Figure 1 shows chromatograms from 2 separate injections (each injection provides two GC signals) of the same strawberry extract without any hardware or filter changes. All of the target compounds were found and confirmed by DRS, GC, and MS signals except the unknown peak at about 41 minutes. The peak shows responses from µECD, DFPD(S) and DFPD(P). However, no peak was observed in the MS full-scan signal. This makes it difficult to confirm the unknown peak using the full-scan TIC.

Since the analysis was retention time locked, it is therefore possible to find potential matches by examining the RTL pesticide database (part number G1672AA). The unknown compound, containing electron-capturing atoms (for example, Cl or O), P, and S atoms, would have a target retention time inside the

## Highlights

Splitter—an inert, easy-to-use capillary flow technology that splits column effluent to multiple detectors (for example, MSD, DFPD, and µECD). The splitter configuration provides a comprehensive screening and quantitative system.

By combing RTL, element-selective detector chromatograms, and the RTL pesticide database, a trace level pesticide residue was identified without the full-scan mass spectrum.

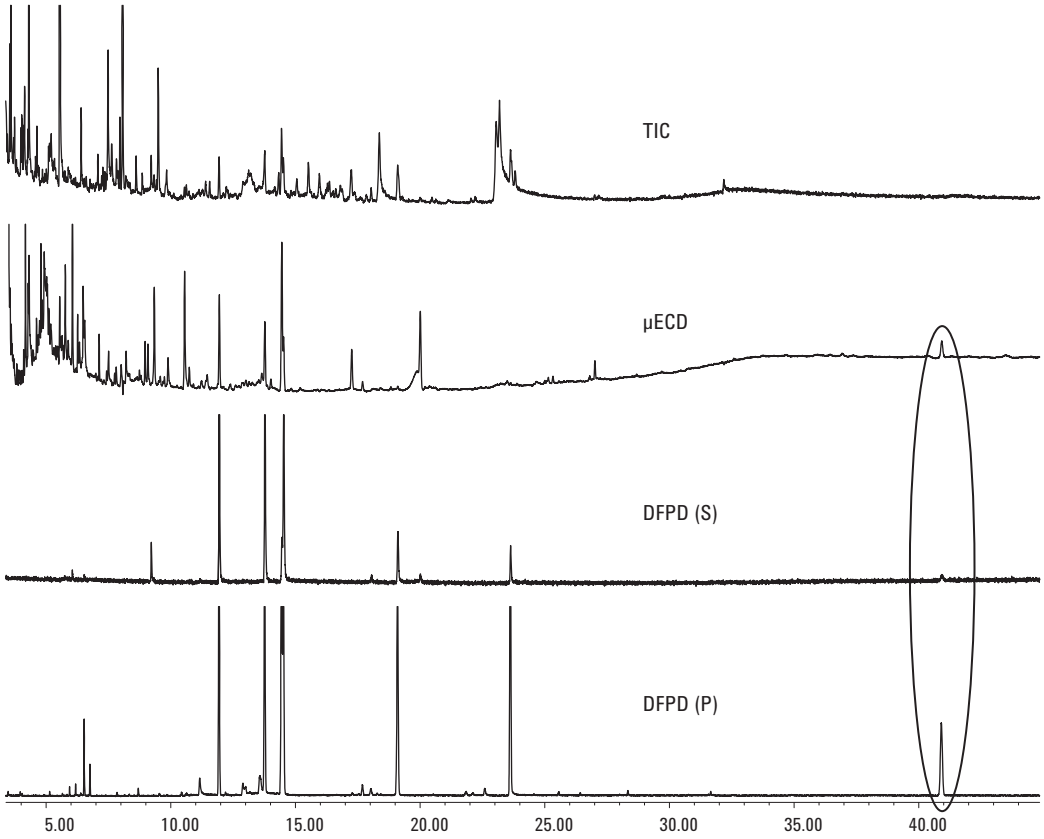


**Figure 1. Unknown compound detected by GC signals not found in strawberry extract TIC.**

**Table 1. Compound List Extracted from the RTLPest3.tab File**

| Name | CAS | Mol form | Mol wt | R.T. | Target Ion | Q1 | Q2 | Q3 |
|---|---|---|---|---|---|---|---|---|
| Fluthiacet-methyl | 117337196 | $C_{15}H_{15}ClFN_3O_3S_2$ | 403.9 | 39.10 | 403 | 56 | 405 | 232 |
| Benzo[g,h,i]perylene | 191242 | $C_{22}H_{12}$ | 276.3 | 39.13 | 276 | 277 | 138 | 275 |
| Temephos | 3383968 | $C_{16}H_{20}O_6P_2S_3$ | 466.5 | 40.74 | 466 | 125 | 93 | 109 |
| PBB 169 hexabrombiphenyl | 60044260 | $C_{12}H_4Br_6$ | 627.6 | 40.93 | 308 | 468 | 148 | 154 |
| Rotenone | 83794 | $C_{23}H_{22}O_6$ | 394.4 | 41.70 | 192 | 191 | 394 | 177 |

41 ± 0.5-minute window (that is, 40.5 to 41.5 min) in the database, if it is included in the database. Table 1 is a portion of the RTLPest3.tab file[1] opened in Microsoft Excel. The compound temephos at locked retention time 40.74 min meets all the criteria for the unknown peak. To further confirm peak identity, extracted ion chromatograms (EICs) of the four major ions of temephos were plotted. Figure 2 shows EICs of target ion and three qualifiers (ions 466, 125, 93, and 109 from Table 1) of temephos. Although the ion intensities were weak, the noticeable presence of all four ions at 40.9 min helped to confirm that the unknown peak was temephos.

1. The RTLPest3.tab file is created in the C:\Database directory while executing the Tools\List Screen Database... command (in MSD Enhanced Data Analysis software) and selecting the RTLPest3.scd from the C:\Database directory.

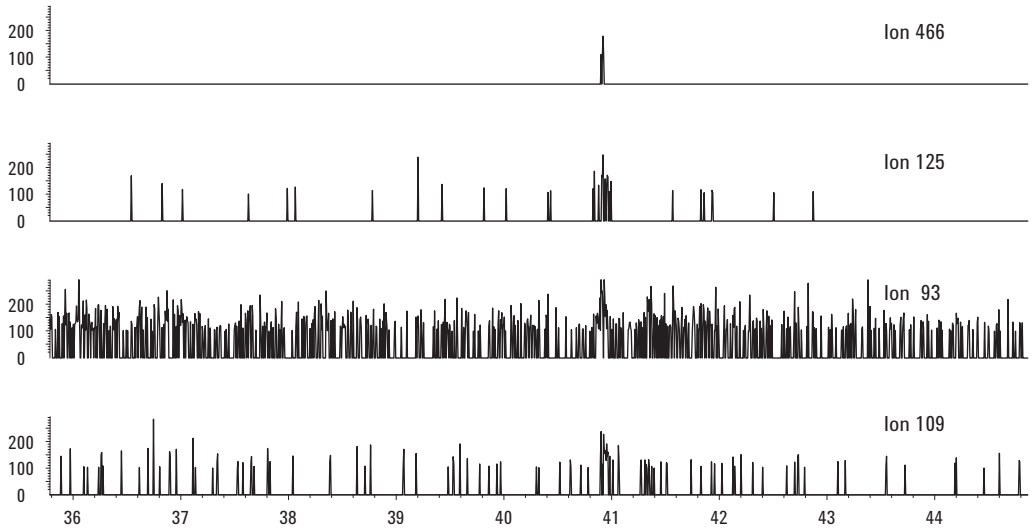


**Figure 2. EICs of target ion 466 (temephos) and three qualifier ions.**

## References

1. Chin-Kai Meng and Bruce Quimby, "Identifying Pesticides with Full Scan, SIM, μECD, and FPD from a Single Injection," Application Note, 5989-3299, July 2005.
2. Mike Szelewski and Bruce Quimby, "New Tools for Rapid Pesticide Analysis in High Matrix Samples," Application Note, 5989-1716, October 2004.

## Acknowledgement

Strawberry extract is courtesy of Dr. Steven Lehotay from USDA Agricultural Research Service in Wyndmoor, Pennsylvania, USA.

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Agilent Technologies

# Fast and Ultra-fast Analysis with the Agilent 1200 Series Rapid Resolution LC System Compared to a Conventional Agilent 1100 Series LC System Using Sub 2-µm Particle Columns

Application Note

A. G. Huesgen

**Abstract**

Due to an increasing workload in many analytical laboratories, a need to develop analytical methods faster has arisen. Furthermore, developing faster methods for standard columns is critical. Faster method development for faster LC methods is a requirement that can be met with state-of-the-art LC equipment. Even though conventional LC equipment can also provide fast methods, better performance and time savings can be obtained on specially designed LC systems with wider pressure and temperature ranges and lower delay volume - predominantly with 2.1-mm ID columns, where typically lower flow rates are used than on 4.6-mm ID columns. This Application Note shows that shorter run times, shorter equilibration times, and consequently shorter cycle times and more sample throughput are obtained using the Agilent 1200 Series Rapid Resolution LC (RRLC) system.

Agilent Technologies

## Introduction

Due to an increasing workload in many analytical laboratories, a need to develop analytical methods faster has arisen. Furthermore, developing faster methods for standard columns is critical. Increasingly more applications are carried out using LC/MS systems, therefore there is also a demand to use narrow-bore columns for full compatibility with most MS engines. Narrow-bore columns with an internal diameter of 2.1 mm and lower have high demands in respect to low delay volumes and dispersion volumes before and after the column. In the following experiment an example is given, showing how fast methods can be developed on an LC system taking advantage of higher pressure and temperature limits of state-of-the-art equipment. In addition, speed and performance comparisons are made between a conventional Agilent 1100 Series LC system and an Agilent 1200 Series Rapid Resolution LC system, using 4.6-mm ID columns and 2.-mm ID columns packed with 1.8-µm particles.

## Experimental

An Agilent 1200 Series RRLC system was used with the following modules:

- Agilent 1200 Series binary pump SL with vacuum degasser for applications using 1.8-µm particle columns up to 150-mm length and with internal diameters from 2.1 to 4.6 mm
- Agilent 1200 Series high-performance autosampler SL for highest area precision
- Agilent 1200 Series thermostatted column compartment SL with wide temperature range from 10 degrees below ambient up to 100 °C
- Agilent 1200 Series diode-array detector SL for 80-Hz operation, including new data protection tool
- ZORBAX SB C-18 columns with different internal diameters and 50-mm length, packed with 1.8-µm particles
- Low dispersion kit for optimized conditions for 2.1-mm ID columns (Agilent part number G1316-68744)

An Agilent 1100 Series LC system was used with the following modules:

- Agilent 1100 Series binary pump with vacuum degasser
- Agilent 1100 Series well-plate autosampler
- Agilent 1100 Series thermostatted column compartment
- Agilent 1100 Series diode-array detector B
- Low dispersion kit for optimized conditions for 2.1-mm ID columns (Agilent part number 5065-9947)

## Results and discussion

In the past the Agilent 1100 Series LC system was frequently used for fast and ultra-fast analysis[1]. The instrument is very well suited specifically for the analysis of compounds using short 4.6-mm ID column packed with 1.8-µm particles, and run times below one minute. Cycle times below two minutes were achieved. The Agilent 1200 Series RRLC system is a newly developed LC system with a wider pressure and temperature range, lower system delay volumes and improved noise for the DAD system. Due to these advancements, speed and performance have improved compared to an Agilent 1100 Series LC system, especially for columns with an internal diameter of 2.1 mm.

**Experiments using a 4.6-mm ID column**

Both instruments were set up in a standard configuration with mixers and 0.17-mm ID flow capillaries installed. Typically the same parameters can be used to optimize an LC method for speed and resolution. These parameters are flow rate, column temperature, gradient profile and other instrument-specific parameters such as switching the autosampler delay volume out of the flow path after the sample has reached the top of the column (ADVR=automatic delay volume reduction). Gradient changes can therefore reach the column much faster. A typical example of how a fast method can be developed is given in figure 1. The objective is to achieve fast cycle times and a minimum resolution of 2 for all peaks.

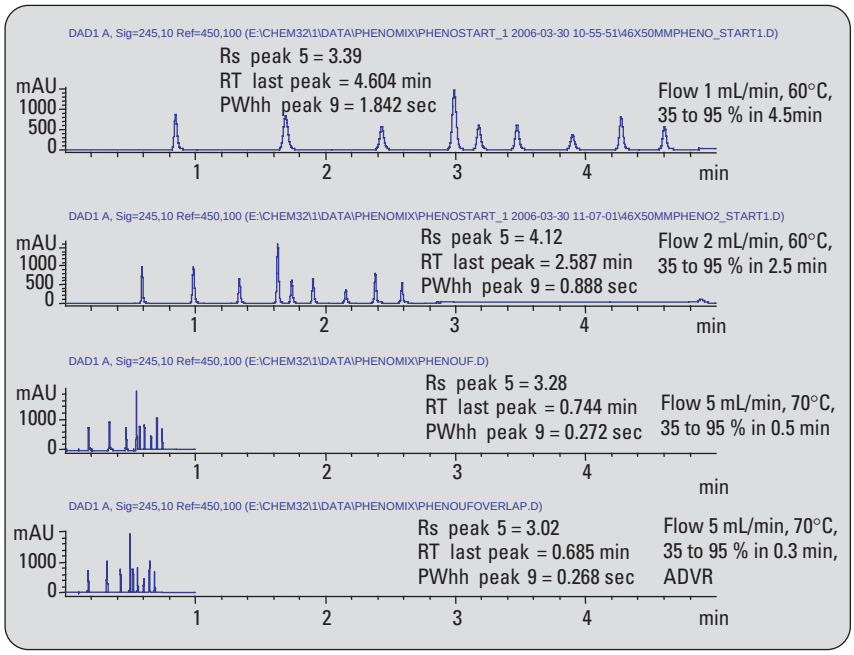


**Figure 1**
**Method development of an ultra fast LC method.**

**Chromatographic conditions:**

| | |
|---|---|
| Test sample: | Set of 9 compounds; 100 ng/µL each; dissolved in water/ACN (65/35)<br>1. Acetanilide, 2. Acetophenone, 3: Propiophenone, 4. Butyrophenone,<br>5. Benzophenone, 6.Valerophenone, 7. Hexanophenone, 8. Heptanophenone,<br>9. Octanophenone |
| Column: | 50 x 4.6 mm ZORBAX SB C-18, 1.8 µm for 600 bar operation |
| Pump: | Solvent A: $H_2O$ + Solvent B: ACN<br>Gradient: 35 to 95 % B using different profiles |
| Autosampler: | Injection volume: 1 µL<br>Wash 5 sec for needle exterior<br>flush out factor 20 |
| Thermostatted column compartment: | Temperature: different temperatures |
| Diode array detector | B and diode-array detector SL:<br>Signal: 245/10 nm Ref 450/100 nm |

Optimization of all of the above-mentioned parameters on both systems resulted in the chromatograms shown in figure 2. The pressure limit of 400 bar on the Agilent 1100 Series LC system restricts the maximum possible flow. 5 mL/min flow was not possible, even though the column temperature was set to 80 °C, which is the upper limit for the 1100 Series column compartment. The Agilent 1200 Series RRLC system can be operated with up to 600 bar and up to 100 °C. Applying a flow rate of 5 mL/min can be done without reaching the 600 bar pressure limit at elevated temperatures. In addition, due to design changes, the noise level of the Agilent 1200 Series DAD SL has significantly improved compared to the Agilent 1100 Series DAD B.
The performance for both systems is shown in table 1.

Resolution and noise have improved with the Agilent 1200 Series RRLC system, whereas run and cycle times are comparable. The noise level of the 1200 Series RRLC system can be further reduced using the post column cooling device[2]. The device adapts the temperature of the column effluent to the temperature of the optical unit. This further reduces the noise level, especially if high flow rates and high temperatures are used. Another possibility to reduce cycle time is to enable the overlapped injection features, which is possible with both systems.

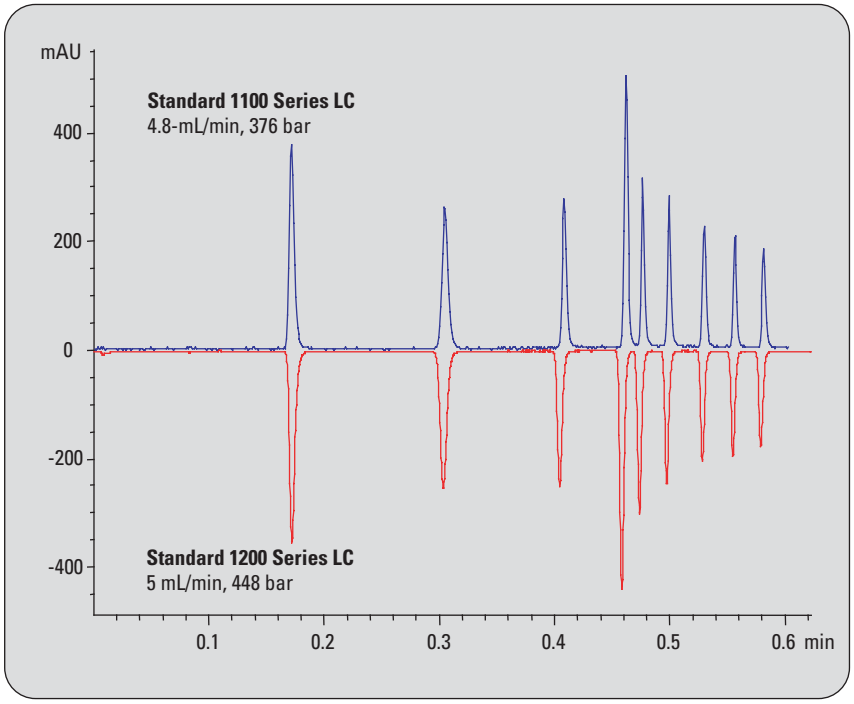


**Figure 2**
**Standard Agilent 1200 Series RRLC system vs. Agilent 1100 Series LC system: analysis of phenone mix on 4.6-mm ID column packed with 1.8-µm particles.**

**Chromatographic conditions:**

| | |
|---|---|
| Test sample: | Set of 9 compounds, 100 ng/µL each, dissolved in water/ACN (65/35)<br>1. Acetanilide, 2. Acetophenone, 3: Propiophenone, 4. Butyrophenone, 5. Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone |
| Column: | 50 x 4.6 mm ZORBAX SB C-18, 1.8 µm for 600 bar operation |
| Pump: | Solvent A: $H_2O$, Solvent B: ACN<br>Gradient: 35 to 95 % B in 0.3 min |
| Autosampler: | Injection volume: 1 µL<br>Wash 5 sec for needle exterior, flush-out factor 20 |
| Thermostatted column Compartment: | Temperature: 80 °C |
| Detector DAD B and DAD SL: | Signal: 245/10 nm Ref 450/100 nm |

| _Parameter | Standard 1100 Series 80 °C 4.8 mL/min | Standard 1200 Series 80 °C 5 mL/min |
|---|---|---|
| Flow rate | 4.8 mL/min | 5 mL/min |
| Run time | 0.60 min | 0.60 min |
| Cycle time | 1 min 37 sec | 1 min 37 sec |
| Rs Peak 5 | 2.22 | 2.30 |
| PW1/2 peak 9 | 0.00378 min | 0.00375 min |
| PW1/2 peak 1 | 0.00458 min | 0.00486 min |
| Noise PtoP | 6.2021mAU | 0.7930 mAU |
| Backpressure | 376 bar | 448 bar |
| Injection volume | 1 µL | 1 µL |
| DAD data rate | 20 Hz, path 10 mm | 80 Hz, path 10 mm |

**Table 1**
**Performance comparison for 4.6-mm ID column.**

Furthermore, column switching valves can be installed in the ovens, which provides even higher sample throughput using 2 columns for analysis. A sample is analyzed on the first column, while the second column is regenerated using a second pump. If the analysis on the first column is completed, the next injection can be immediately performed on the previously equilibrated second column.

**Experiments using 2.1-mm ID column**

Columns with an internal diameter of 2.1 mm and lower have high demands regarding low delay volumes and dispersion volumes before and after the column. Using columns with an internal diameter of 2.1 mm, the Agilent 1100 Series binary LC system must be optimized without using a mixer or only a mixer with a significantly smaller volume and capillaries with smaller IDs for all flow connections. Nevertheless, cycle times below 2 minutes could barely be achieved using columns packed with 1.8 µm particles and 50 mm length. This was mainly due to the pressure limitation of 400 bar for the Agilent 1100 Series LC system. In addition, the delay volume of the 1100 Series LC system is a drawback for fast run and equilibration times. With the introduction of the Agilent 1200 Series RRLC system this gap was closed. Now using narrow bore columns packed with 1.8-µm particles, run times below 0.5 min are possible, with higher flow rates and elevated temperatures. Both systems are compared using the same column and optimized instrument configurations. To allow for optimized conditions for both systems, the following set-ups were used:

Configuration of the Agilent 1100 Series LC system:

- The mixer was replaced by a short capillary with an internal diameter of 0.12 mm (Agilent part number G1312-67301)
- Seat and seat capillary were replaced by 0.12-mm ID parts (well-plate seat, Agilent part number G1367-87104, and seat capillary, Agilent part number G1313-87103)
- The capillary from the injector to the column compartment was replaced with a 0.12-mm ID capillary (Agilent part number 01090-87610)
- The 0.17-mm ID capillary from the column compartment to the column was exchanged with a capillary with an internal diameter of 0.12 mm (Agilent part number G1316-87303)
- The column was connected to the detector using the detector inlet capillary.
- A 1.7-µL cell with a path length of 6 mm was used as the detector cell.

Configuration of the Agilent 1200 Series RRLC system:

- The low delay volume configuration for the pump was set up with a 120-µL delay volume (mixer and damper were moved out of the flow path).
- Two flow capillaries were replaced with 0.12-mm ID capillaries, all included in the Agilent 1200 Series low dispersion kit (Agilent part number G1316-68744).
- The seat capillary was also replaced with a 0.12-mm ID capillary (included in kit Agilent part number G1316-68744)
- The DAD SL 2 µL flow cell with a 3-mm path length was used. The inlet capillary was directly connected to the column outlet.

The same 2.1 x 50 mm column was used for both systems. The flow rate was set so that the backpressure was close to the limit of each system. Automated delay volume reduction (ADVR) was selected in the injector setup screen for both systems. The injection volume was set to 1 µL for the Agilent 1100 Series LC system, and to 2 µL for the Agilent 1200 Series RRLC system to compensate for the lower path length of the 1200 Series 2-µL flow cell.

In figure 3 an overlay of the chromatograms obtained from both systems is shown. In table 2 the performance for both system is recorded.

The chromatograms in figure 3 clearly demonstrate the advantages of the Agilent 1200 Series RRLC system, using 2.1-mm ID columns, packed with 1.8-µm particles. Faster run times and cycle times are possible, due to the fact that higher flow rates can be obtained with the Agilent 1200 Series RRLC system. Table 2 indicates that the cycle time for the Agilent 1200 Series RRLC system is only half that of the Agilent 1100 Series LC system. In addition, the resolution of the 5th peak and also peak width at half height is significantly improved at higher flow rates.

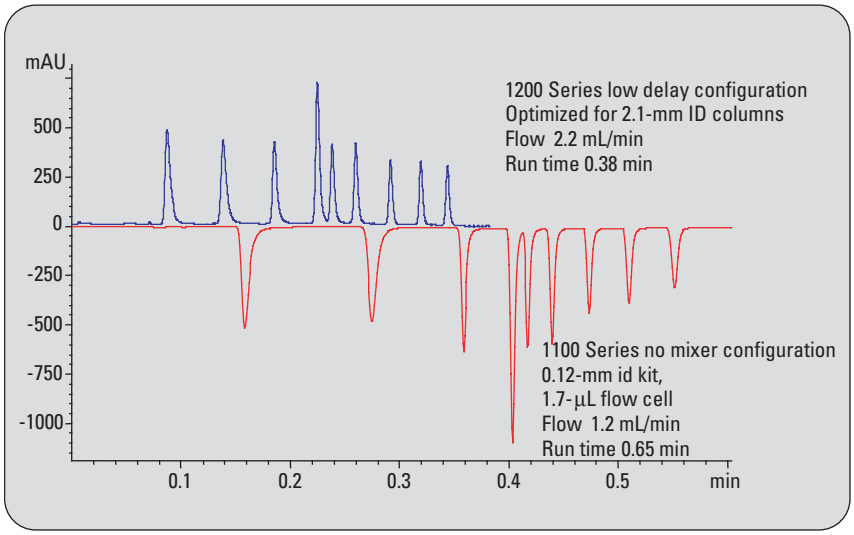


**Figure 3**
**Analysis performed with a 2.1-mm ID column with the optimized Agilent 1200 Series RRLC system and the optimized Agilent 1100 Series LC system using automated delay volume reduction for both systems.**

**Chromatographic conditions:**

| | |
|---|---|
| Test sample: | Set of 9 compounds, 100 ng/µL each, dissolved in water/ACN (65/35)<br>1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone, 5. Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone |
| Column: | 50 x 2.1 mm ZORBAX SB C-18, 1.8 µm for 600 bar operation |
| Pump: | Solvent A: $H_2O$ , Solvent B: ACN<br>Gradient: 35 to 95 % B in 0.3 min |
| Autosampler: | Injection volume: 1 and 2 µL<br>Wash 5 sec for needle exterior, flush out factor 20 |
| Thermostatted column compartment: | Temperature: 80 and 95 °C |
| Detector DAD B and DAD SL: | Signal: 245/10 nm Ref 450/100 nm |

| Parameter | 1100 Series, optimized, no mixer, ADVR, 80 °C | 1200 Series, optimized, low delay volume configuration, ADVR, 95 °C |
|---|---|---|
| Flow rate | 1.2 mL/min | 2.2 mL/min |
| Run time | 0.65 min | 0.38 min |
| Cycle time | 2 min 33 sec | 1 min 16 sec |
| Rs Peak 5 | 1.86 | 2.15 |
| PW1/2 peak 9 | 0.00556 min | 0.00328 min |
| PW1/2 peak 1 | 0.00729 min | 0.0049 min |
| Noise PtoP | 0.1 mAU | 0.2 mAU |
| Backpressure | 370 bar | 570 bar |
| Injection volume | 1 µL | 2 µL |
| DAD data rate | 20 Hz, path 6 mm | 80 Hz, path 3 mm |

**Table 2**
**Performance comparison using a 2.-mm ID column.**

## Conclusions

Faster method development for faster LC methods is a requirement that can be met with state-of-the-art LC equipment. Even though conventional LC equipment can also provide fast methods, better performance and time savings can be obtained on specially designed LC systems with wider pressure and temperature ranges. Predominantly with 2.1-mm ID columns, where typically lower flow rates are used than on 4.6-mm ID columns, an LC system like the Agilent 1200 Series RRLC system provides significantly lower delay volumes. Shorter run times and shorter equilibration times, and consequently shorter cycle times and more sample throughput are obtained.

## References


1.
Anabel Fandino, "Ultra-fast liquid chromatography using the Agilent 1100 Series HPLC system and 1.8-um ZORBAX SB C18 Rapid Resolution HT columns", *Agilent Application Note, publication number 5989-1603EN*, **2004.**

2.
A.G.Hüsgen, "Agilent 1200 Series column compartment SL with temperature control up to 100 °C and post-column cooling for lowest baseline noise", *Agilent Application Note, publication number 5989-5034EN*, **2006.**

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**www.agilent.com/chem/1200rr**



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**Agilent Technologies**

# Improving the Effectiveness of Method Translation for Fast and High Resolution Separations

Application


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## Abstract

**The increased availability of sub-2-micron (STM) columns and increased demand for methods friendly to mass spectrometers has led to strong trend toward conversion of existing HPLC methods to smaller diameter and smaller particle size columns. While the conversion is a simple mathematical exercise requiring the scaling flow rates, gradient times and injection volumes, many users observe less than perfect results. Here we look closely at the problem and propose calculations that improve the speed and/or resolution in a more predictable and beneficial way.**


## Introduction

Methods developed on older columns packed with large 5- or 10-µm particles are often good candidates for modernization by replacing these columns with smaller dimension columns packed with smaller particle sizes. The potential benefits include reduced analysis time and solvent consumption, improved sensitivity and greater compatibility with mass spectrometer ionization sources.

Simplistically, a column of 250-mm length and containing 5-µm particles can be replaced by a 150-mm length column packed with 3-µm particles. If the ratio of length to particle size is equal, the two columns are considered to have equal resolving power. Solvent consumption is reduced by L1/L2, here about 1.6-fold reduction in solvent usage per analysis. If an equal mass of analyte can then be successfully injected, the sensitivity should also increase by 1.6-fold due to reduced dilution of the peak as it travels through a smaller column of equal efficiency.

LC/MS (Liquid Chromatography/Mass Spectrometry) ionization sources, especially the electrospray ionization mode, have demonstrated greater sensitivity at lower flow rates than typically used in normal LC/UV (UltraViolet UV/VIS optical detection) methods, so it may also be advantageous to reduce the internal diameter of a column to allow timely analysis at lower flow rates. The relationship of flow rate between different column diameters is shown in Equation 1.

$$\text{Flow}_{\text{col. 1}} \times \left[\frac{\text{Diam.}_{\text{column2}}}{\text{Diam.}_{\text{column1}}}\right]^2 = \text{Flow}_{\text{col. 2}} \quad \text{(eq. 1)}$$

The combined effect of reduced length and diameter contributes to a reduction in solvent consumption and, again assuming the same analyte mass can be injected on the smaller column, a proportional increase in peak response. We normally scale the injection mass to the size of the column,

though, and a proportional injection volume would be calculated from the ratio of the void volumes of the two columns, multiplied by the injection volume on the original column.

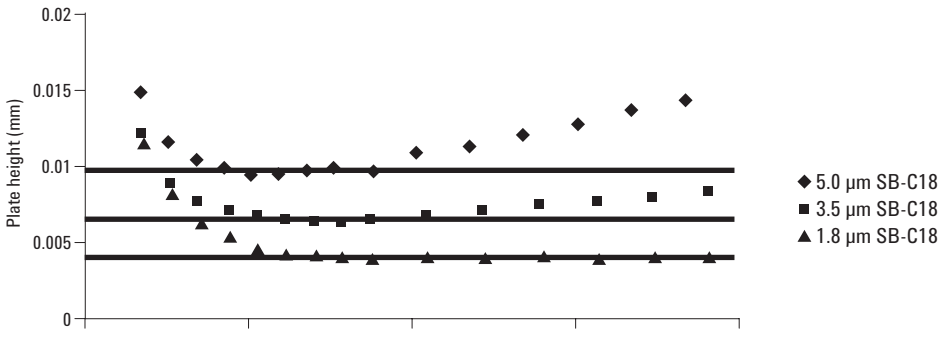
$$\text{Inj. vol.}_{\text{col. 1}} \times \left[\frac{\text{Volume}_{\text{column2}}}{\text{Volume}_{\text{column1}}}\right] = \text{Inj. vol.}_{\text{col. 2}} \quad \text{(eq. 2)}$$

For isocratic separations, the above conditions will normally result in a successful conversion of the method with little or no change in overall resolution. If one wishes to improve the outcome of the method conversion, though, there are several other parameters that should be considered. The first of these parameters is the column efficiency relative to flow rate, or more correctly efficiency to linear velocity, as commonly defined by van Deemter [1] and others, and the second is the often overlooked effect of extracolumn dispersion on the observed or empirical efficiency of the column.

Van Deemter observed and mathematically expressed the relationship of column efficiency to a variety of parameters, but we are most interested here in his observations that there is an optimum linear velocity for any given particle size, in a well-packed HPLC column, and that the optimum linear velocity increases as the particle size decreases. Graphically, this is often represented in van Deemter plots as shown in Figure 1, a modified version of the original plot [2].

In Figure 1 we observe that the linear velocity at which 5-µm materials are most efficient, under the conditions used by the authors, is about 1 mm/sec. For 3.5-µm materials the optimum linear velocity is about 1.7 mm/sec and has a less distinct optimum value, suggesting that 3.5-µm materials would give a more consistent column efficiency over a wider flow range. For the 1.8-µm materials, the minimum plate height, or maximum efficiency, is a broad range beginning at about 2 mm/sec and continuing past the range of the presented data. The practical application of this information is that a reduction in particle size, as discussed earlier, can often be further optimized by increasing the linear velocity which results in a further reduction in analysis time. This increase in elution speed will decrease absolute peak width and may require the user to increase data acquisition rates and reduce signal filtering parameters to ensure that the chromatographic separation is accurately recorded in the acquisition data file.

The second important consideration is the often overlooked effect of extracolumn dispersion on the observed or empirical efficiency of the column. As column volume is reduced, peak elution volumes are proportionately reduced. If smaller particle sizes are also employed there is a further reduction in the expected peak volume. The liquid chromatograph, and particularly the areas where the analytes will traverse, is a collection of various connecting capillaries and fittings which will cause a measurable amount of bandspreading. From the injector to the detector flow cell, the cumulative dispersion that occurs degrades the column performance and results in observed efficiencies that can be far below the values that would be estimated by purely theoretical means. It is fairly typical to see a measured dispersion of 20 to 100 µL in an HPLC system. This has a disproportionate effect on the smallest columns and smallest particle sizes, both of which are expected to yield the smallest




| Lin. vel. | mm/sec | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| 4.6 mm | mL/min | 0.7 | 1.4 | 2.1 | 2.8 | 3.5 |
| 3 mm | mL/min | 0.3 | 0.6 | 0.9 | 1.2 | 1.5 |
| 2.1 mm | mL/min | 0.14 | 0.29 | 0.44 | 0.58 | 0.73 |
| 1 mm | mL/min | 0.033 | 0.066 | 0.1 | 0.133 | 0.166 |

**Figure 1. van Deemter plot with various flow rates and particle sizes.**

possible peak volumes. Care must be taken by the user to minimize the extracolumn volume and to reduce, where practical, the number of connecting fittings and the volume of injection valves and detector flow cells.

For gradient elution separations, where the mobile phase composition increases through the initial part of the analysis until the analytes of interest have been eluted from the column, successful method conversion to smaller columns requires that the gradient slope be preserved. While many publications have referred to gradient slope in terms of % change per minute, it is more useful to express it as % change per column volume. In this way, the change in column volume during method conversion can be used to accurately render the new gradient condition. If we think of each line of a gradient table as a segment, we can express the gradient by the following equation:

$$\%\ \text{Gradient slope} = \left[ \frac{(\text{End}\% - \text{Start}\%)}{\#\text{Column volumes}} \right] \quad \text{(eq. 3)}$$

Note that the use of % change per column volume rather than % change per minute frees the user to control gradient slope by altering gradient time and/or gradient flow rate. A large value for gradient slope yields very fast gradients with minimal resolution, while lower gradient slopes produce higher resolution at the expense of increased solvent consumption and somewhat reduced sensitivity. Longer analysis time may also result unless the gradient slope is reduced by increasing the flow rate, within acceptable operating pressure ranges, rather than by increasing the gradient time.

Resolution increases with shallow gradients because the effective capacity factor, k*, is increased. Much like in isocratic separations, where the capacity term is called k', a higher value directly increases resolution. The effect is quite dramatic up to a k value of about 5 to 10, after which little improvement is observed. In the subsequent examples, we will see the results associated with the calculations discussed above.

### Experimental Conditions

#### System

Agilent 1200 Series Rapid Resolution LC consisting of:
G1379B micro degasser
G1312B binary pump SL
G1367C autosampler SL, with thermostatic temperature control
G1316B Thermostatted column compartment SL
G1315C UV/VIS diode array detector SL, flow cell as indicated in individual chromatograms
ChemStation 32-bit version B.02.01

#### Columns

Agilent ZORBAX SB-C18, 4.6 mm × 250 mm, 5 µm
Agilent ZORBAX SB-C18, 3.0 mm × 150 mm, 3.5 µm

#### Mobile phase conditions

| | |
|---|---|
| Organic solvent: | Acetonitrile |
| Aqueous solvent: | 25 mm phosphoric acid in Milli-Q water |

#### Gradient Conditions

| | |
|---|---|
| Gradient slope: | 7.8% or 2.3% per column volume, as indicated. See individual chromatograms for flow rate and time |

#### Sample

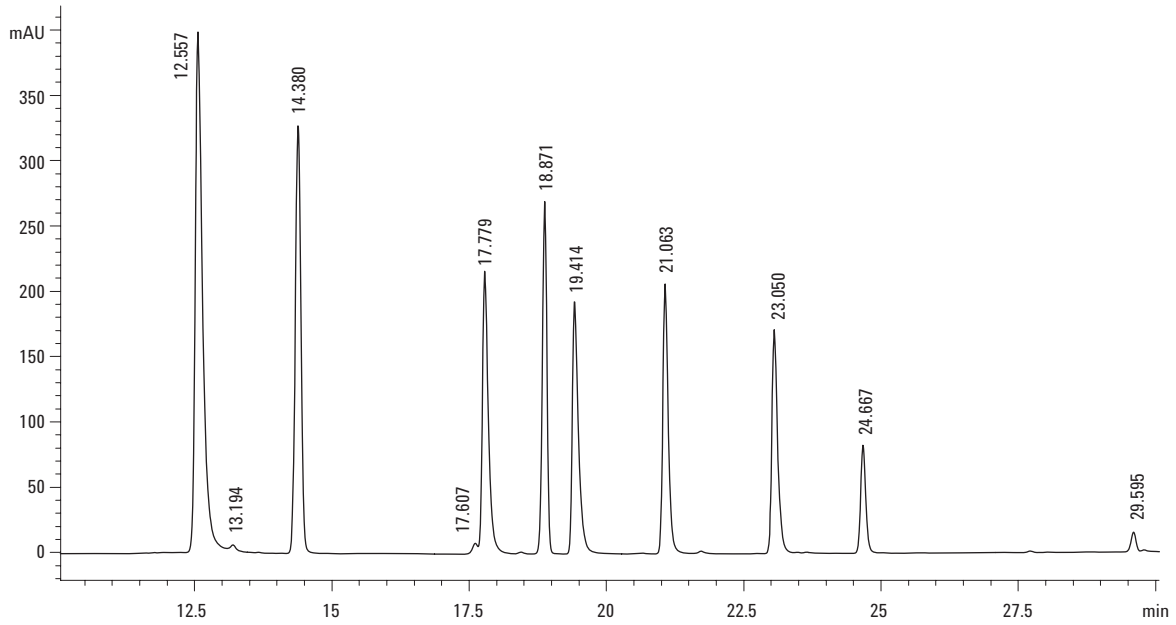
Standard mixture of chlorinated phenoxy acid herbicides, 100 µg/mL in methanol

## Results

The separation was initially performed on a standard 4.6 × 250 mm, 5-µm ZORBAX SB-C18 column thermostatted to 25 °C (Figure 2) using conditions referenced in US EPA Method 555. The method was then scaled in flow and time for exact translation to a 3.0 × 150 mm, 3.5-µm column (Figure 3). Solvent consumption is reduced from 60 mL to 15.5 mL per analysis.

The separation was then re-optimized for faster separation with the identical slope, 7.8%, by increasing the flow rate from 0.43 to 1.42 mL/min, and proportionately reducing the gradient time (Figure 4). Finally, increased resolution is demonstrated by keeping the original times used in Figure 3 with the increased flow rate (Figure 5). This yields a gradient with identical time but a reduced slope of 2.3%. The increased resolution of peaks 4 and 5 is readily apparent.

The conditions in Figure 4, 7.8% slope at increased linear velocity on 3.0 × 150 mm, 3.5-µm material, yield a separation with comparable resolution to the original 4.6 × 250 mm method, but with only a 12-minute total analysis time. This is excellent for




**Conditions**

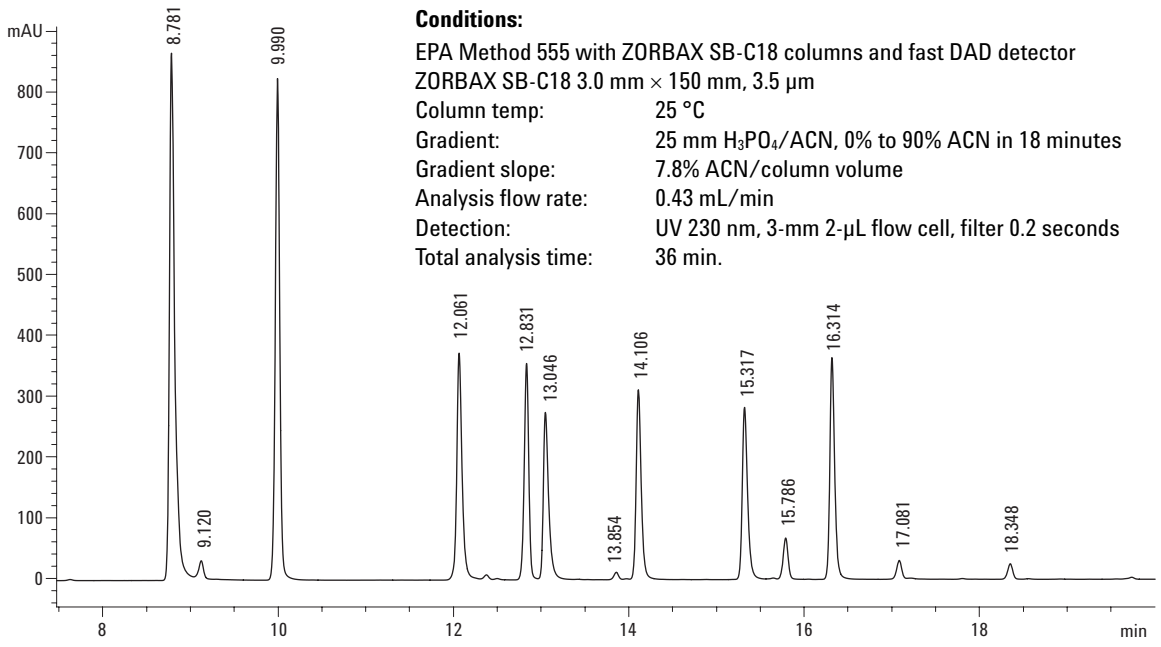
EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector
ZORBAX SB-C18 4.6 mm × 250 mm, 5 µm

| | |
|---|---|
| Column temp: | 25 °C |
| Gradient: | 10% to 90% ACN vs. 25 mM $H_3PO_4$ |
| Gradient slope: | 7.8% ACN/column volume |
| Analysis flow rate: | 1 mL/min |

Group A Compounds

| | |
|---|---|
| Total analysis time: | 60 min |
| Detection: | UV 230 nm, 10-mm 13-µL flow cell, filter 2 seconds (default) |

**Figure 2. Gradient separation of herbicides on 4.6 250 mm 5-µm ZORBAX SB-C18.**




**Conditions:**

EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector
ZORBAX SB-C18 3.0 mm × 150 mm, 3.5 µm

| | |
|---|---|
| Column temp: | 25 °C |
| Gradient: | 25 mm $H_3PO_4$/ACN, 0% to 90% ACN in 18 minutes |
| Gradient slope: | 7.8% ACN/column volume |
| Analysis flow rate: | 0.43 mL/min |
| Detection: | UV 230 nm, 3-mm 2-µL flow cell, filter 0.2 seconds |
| Total analysis time: | 36 min. |

**Figure 3. Gradient separation of herbicides on 3.0 150 mm, 3.5-µm ZORBAX SB-C18.**

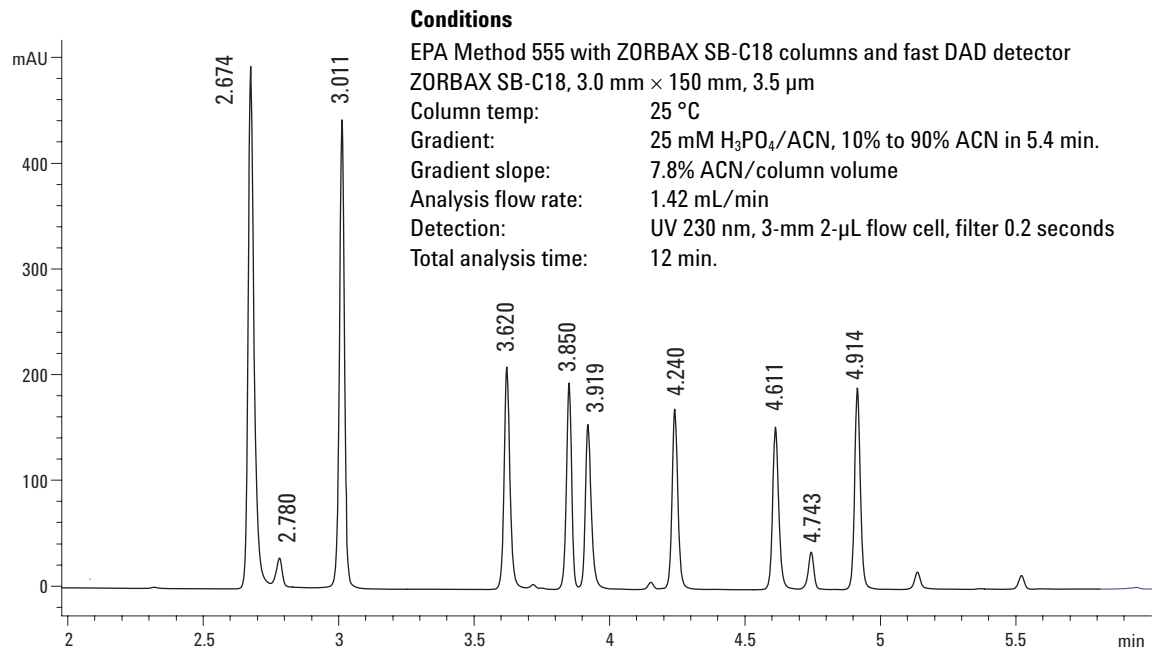



**Figure 4. High speed gradient separation of herbicides on 3.0 150 mm, 3.5-µm ZORBAX SB-C18.**

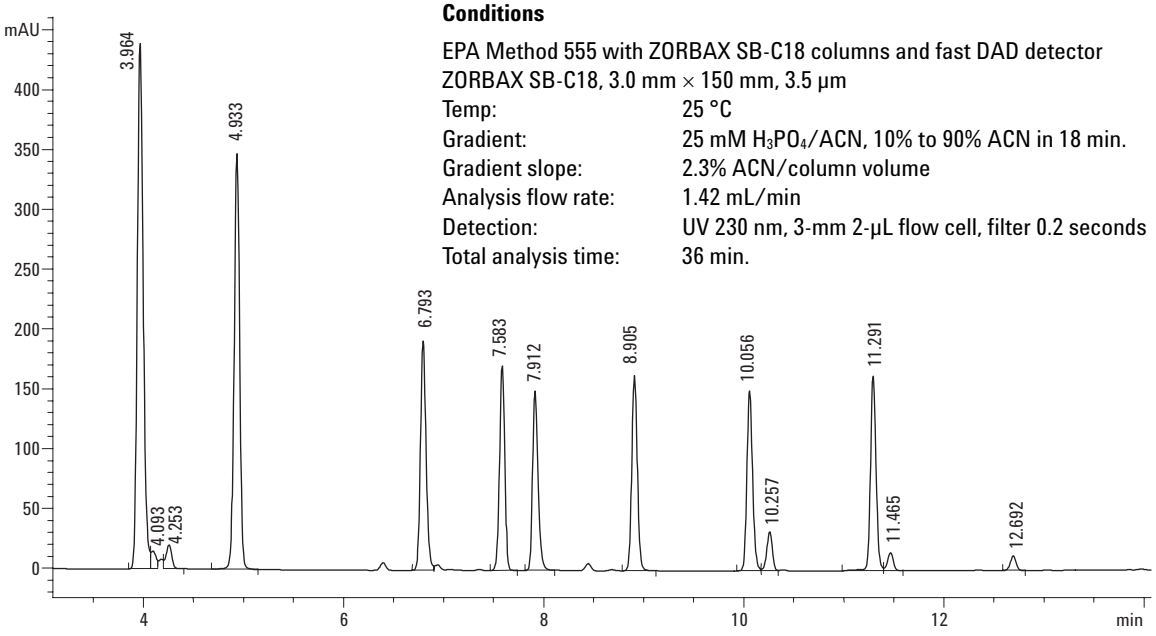



**Figure 5. Reduced slope gradient separation of herbicides on 3.0 150 mm, 3.5-µm ZORBAX SB-C18.**

high throughput screening and quantitation of a large number of samples. Figure 5, with the gradient slope reduced to 2.3%, results in a high-resolution separation with a calculated R value of 3.3 vs. the standard 3.0 × 150 mm separation value of 1.9, for the critical pair seen in Figure 5 at 7.5 to 8 minutes.

In Table 1 the column has been replaced with a low dead volume connecting union in a system fitted with 0.12-mm id capillary tubing at all points of sample contact. A 1-µL injection of dilute actone is made to determine the bandspreading contribution of the system, with various flow cells. Multiple flow cells were tested, and the average result reported, where possible. The elution volume summarizes the total volume of all tubing in the system. While the absolute volume from the 2-µL to the 13-µL flow cells is 11 µL, we observe an increase of 15 to 16 µL because of the larger diameter inlet tubing integral to the larger volume flow cells.

**Table 1. Volumetric Measurements of Various Flow Cells**

| Flow cell | Elution volume (µL) | Half height width (µL) | 5 Sigma width (µL) |
|---|---|---|---|
| New SL 2 µL 3 mm | 11 | 5 | 12 |
| Micro 6 mm 1.7 µL (n = 2) | 14 | 6 | 18 |
| Semi-micro 6 mm 5 µL (n = 2) | 13 | 6.5 | 18.5 |
| Standard 10 mm 13 µL | 26 | 11 | 26 |
| New SL 10 mm 13 µL | 27 | 11 | 25 |

## Conclusion

Careful analysis of the existing gradient conditions, coupled with an awareness of the need to accurately calculate new flow and gradient conditions can lead to an easy and reliable conversion of existing methods to new faster or higher resolution conditions. In addition, awareness of extracolumn dispersion, especially with small and high resolution columns, will ensure good column efficiency which is critical to a successful translation of the method.

## References

1. J. J. van Deemter, F. J. Zuiderweg, A. Klinkenberg, Chemical Engineering Science 1956, 5, 271–289
2. The Influence of Sub-Two Micron Particles on HPLC Performance, Agilent Technologies, application note 5989-9251EN, May 2003

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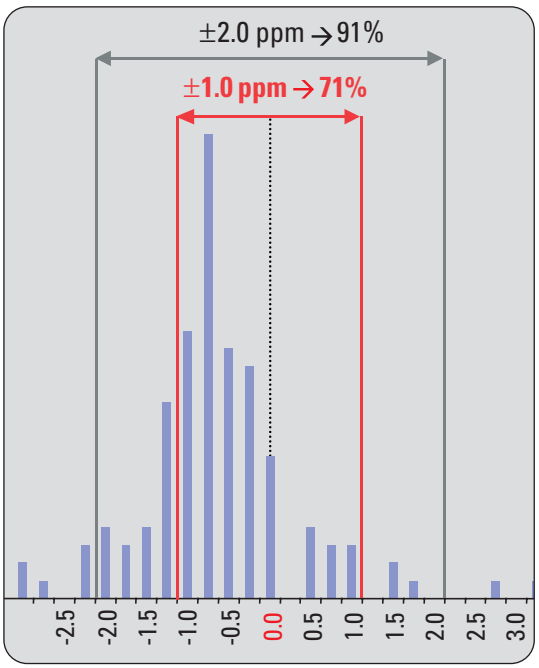
5989-5177EN

# Agilent 1200 Series Rapid Resolution LC system and the Agilent 6210 TOF MS – Highest data content with highest throughput

## Application Note




Michael Frank

**Abstract**

Fast and unambiguous determination of purity and identity of compounds derived from screening libraries is a common task for many analytical labs in the pharmaceutical industriy. The method of choice to determine the identity of compounds is mass spectrometry, preferably with accurate mass. As yet, data quality was usually compromised by gaining higher throughput. This Application Note demonstrates how a daily throughput of far more than 1000 samples can be achieved together with full spectral data acquisition and accurate mass information with close to FT-MS mass accuracy.

Agilent Technologies

## Introduction

In the quest to achieve highest throughput in LC/MS analyses, the quality of the data is often compromised. There are certain approaches to increase the throughput of LC/MS systems. One approach is to do flow injection analysis. This probably delivers the highest possible throughput, however since no chromatographic separation occurs, the probability to loose compounds by the ion suppression effect during the ionization process is high. Orthogonal detection methods like UV detection do not succeed at all in flow injection analysis as all compound signals are overlaid. Approaches to achieve at least minimal chromatographic separation by using very short columns with 5 µm particles and ballistic gradients are an improvement in view of data quality, however, not state-of-the-art. Some manufactures have established parallel working instrumentation with a shared mass spectrometer and shared UV detector. Obviously, this also compromises data quality as the full acquisition rate of each instrument has to be shared on each LC channel[1].

With the introduction of an LC/MS system which facilitates the use of columns with sub two micron particles it is now possible to achieve short analyses times as well as high chromatographic resolution. Furthermore the system is able to acquire full UV spectral data and mass spectral data with accurate masses.

## Experimental

The Agilent 1200 Series Rapid Resolution LC system is set up for alternating column regeneration (ACR)[2] using 2.1-mm id columns. The pumps are in the low delay volume configuration with an internal volume of only ca. 120 µL. All other modules are optimized for lowest delay volumes by using the low delay volume capillary kit (G1316-68744) and the alternating column regeneration kit (G1316-68721). Consequently, from the injection valve on only capillaries of 0.12 mm id are used. In the thermostatted column compartment the newly introduced low dispersion heat exchangers consisting of 1.6 µL internal volume have been used as well as the high pressure rated 2-position/10-port valve.

The instrument set-up is shown in figure 1:

- Two Agilent 1200 Series binary pumps SL with the new Agilent 1200 Series micro vacuum degasser placed between the two pumps eliminates the need for long tubing to the pumps.
- Agilent 1200 Series high performance autosampler SL.
- An Agilent 1200 Series thermostatted column compartment SL, equipped with a high pressure, 2-position/10-port valve, facilitating alternating column regeneration.
- An Agilent 1200 Series diode-array detector SL allowing a data acquisition rate of 80 Hz and equipped with a 500 nano liter flow cell with 0.12-mm id connecting capillaries.
- Agilent 6210 Time-of-Flight mass spectrometer allowing a maximum data acquisition rate of 40 Hz and equipped with a dual ESI source for parallel ionization of the analyte and a reference mixture.
- Two ZORBAX SB C18, 2.1 mm id x 50 mm, 1.8 µm columns
- As mobile phase gradient grade water with 0.1 % trifluoro acetic acid and acetonitrile with 0.08 % trifluoro acetic acid was used. No additional filtering of the solvents was made.

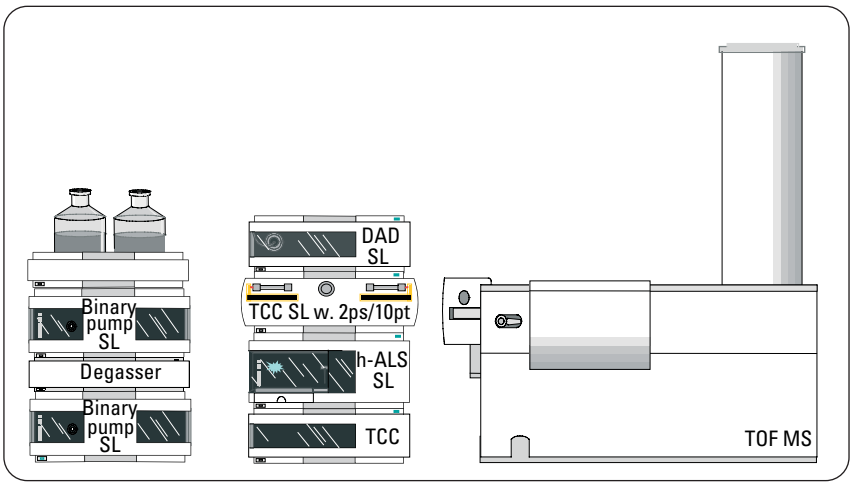



**Figure 1**
**Agilent 1200 Series Rapid Resolution LC system with Agilent 6210 TOF-MS with low delay volume for high speed applications using 2.1-mm id columns with lengths ranging from 20 to 50 mm.**

Instrument control and data acquisition was done by the Agilent TOF-software A02.01 running on a Hewlett-Packard xw 4300 workstation with an Intel dual core Pentium™ D840 CPU at 3.2 GHz.

## Results and discussion

By applying elevated temperatures the viscosity of the solvent can be reduced which allows higher flow rates and therefore shorter gradient times. A maximum temperature of 80 °C was applied, which allowed a flow rate of 1.8 mL/min without hitting the pressure limit of the pump. This results in a linear velocity of approximately 11 mm/s for the 2.1 mm x 50 mm column (1.8 µm). With the help of the regeneration pump and the 2-position/10-port valve in the column compartment cycle times could be reduced significantly because one column is flushed with high organic content solvent and then re-equilibrated again with the starting composition of the gradient while on the second column the separation of a sample occurs. After this sequence the 10-port valve is switched and both columns are exchanged in the flow path. Details of alternating column regeneration and the correct setting of time points are described in another Application Note[2]. Despite the high flow rate (1.8 mL/min), the column effluent was not split prior to reaching the mass spectrometer. The standard ESI source specifies a maximum flow rate of up to 1 mL/min, however even these higher flows are tolerated if the drying gas temperature and flow rate are set to maximum and little condensation occurs. Condensation of water is practically eliminated when using ACR because equilibration is done on the column which is not connected to the detector. Generally the use of an Agilent multi mode source with a specified flow rate up to 2 mL/min even with pure water is recommended. The chromatographic conditions in table 1 were used to achieve gradient times of 0.5 min. Under these conditions, the peak capacity for the MS detection is in the range of >40 in 39 s. With the use of a 5-µm particle size column of the same dimension the peak capacity would only be half!

| Data File | Sample Type | Inj Vol (µl) | Capillary | Fragmentor | Skimmer |
|---|---|---|---|---|---|
| opt_4000_215_60.wiff | Unknown | 1 | 4000 | 210 | 60 |
| opt_4000_215_60.wiff | Unknown | 1 | 3000 | 210 | 60 |
| opt_4000_215_60.wiff | Unknown | 1 | 2000 | 210 | 60 |
| opt_4000_215_60.wiff | Unknown | 1 | 4000 | 180 | 60 |
| opt_4000_215_60.wiff | Unknown | 1 | 3000 | 180 | 60 |
| opt_4000_215_60.wiff | Unknown | 1 | 2000 | 180 | 60 |
| opt_4000_215_60.wiff | Unknown | 1 | 4000 | 150 | 60 |
| opt_4000_215_60.wiff | Unknown | 1 | 3000 | 150 | 60 |
| opt_4000_215_60.wiff | Unknown | 1 | 2000 | 150 | 60 |
| opt_4000_215_60.wiff | Unknown | 1 | 4000 | 210 | 40 |
| opt_4000_215_60.wiff | Unknown | 1 | 3000 | 210 | 40 |

**Figure 2**
**Feature of the TOF software to modify the MS parameter from run to run.**

| | | | | |
|---|---|---|---|---|
| **Method:** | | | | |
| **Solvent:** | A = water (0.1% TFA), B = ACN (0.08% TFA) | | | |
| **Temperature:** | 80 °C | | | |
| **Flow:** | 1.8 mL/min | | | |
| **Gradient:** | 0.00 min 5%B<br>0.50 min 90%B<br>0.51 min 5%B<br>0.65 min 5%B | | **Regeneration:** | 0.00 min 5%B<br>0.01 min 95%B<br>0.20 min 95%B<br>0.21 min 5%B<br>0.65 min 5%B |
| **Stoptime:** | 0.65 min | | | no limit |
| **Posttime:** | off | | | off |
| **DAD:** | Wavelength: | 210 nm (8), ref. off | | |
| | Peak width: | >0.0025 min (0.05s responsetime), 80 Hz | | |
| | Spectra: | no | | |
| | Slit: | 8 nm | | |
| | Balance: | pre-run | | |
| **MS:** | Scan range: | 100-1000 m/z | | |
| | Acquisition rate: | 5, 20, 30 and 40 cycles/s | | |
| | Data type: | profile data | | |
| | Capillary voltage: | 3000 V | | |
| | Fragmentor: | 180 V | | |
| | Skimmer: | 40V | | |
| | Gas temperature: | 350 °C | | |
| | Gas flow: | 13 L/min | | |
| **Injection volume:** | 1µL | | | |
| **Injector:** | Overlapped injection, Automatic delay volume reduction,<br>Sample flush out factor = 10 | | | |
| **Valve position:** | Next position | | | |

**Table 1**
**LC/MS method used for the data shown in figures 3-5. The method was also used to achieve the values in table 2.**

The detector of the Agilent 6210 TOF MS would be saturated if the compound concentrations used here to give also significant UV signals would be injected into the MS without special settings. Saturation of the MS detector would produce incorrect results in mass determination. The solution is to intentionally desensitize the TOF MS. This can be done quite easily by applying the functionality of the TOF software to alter the MS parameters from one run to the other,

simply by adding one or more "MS-parameter" columns to the worklist (figure 2). Select "add columns" from the worklist and then chose "MS-parameter" and the desired parameter. As the reference mixture is also affected by these settings, the concentration of the reference mixture was increased. Only the capillary voltage, the fragmentor voltage and the skimmer voltage were varied. The optimal conditions determined by this approach can be found in the method parameters in table 1.

In figure 3 the total ion chromatogram and the UV chromatogram achieved with conditions above (80 Hz DAD, 30 Hz TOF data acquisition rate) is shown for a five-component sample (58 ng/µL atenolol, 85 ng/µL primidon, 62 ng/µL metoprolol, 125 ng/µL verapamil and 75 ng/µL beclomethasone-dipropionat). The peaks of the total ion chromatogram are inherently broader than the peaks of the UV chromatogram because of additional extra column volume from the flow cell and also from connecting the capillary between the UV detector and ESI interface. But as can be seen in figure 3, the additional peak broadening of the MS peaks is only minor. The peak widths at half height of the MS peaks obtained under the highest data acquisition rate (40 Hz) are shown in figure 4 with values from as little as 0.34 to 0.42 s. The chromatograms shown in figure 5 were produced under the same chromatographic conditions, but with different data acquisition rates of the time-of-flight MS. The peak form and resolution are improved by having high data acquisition

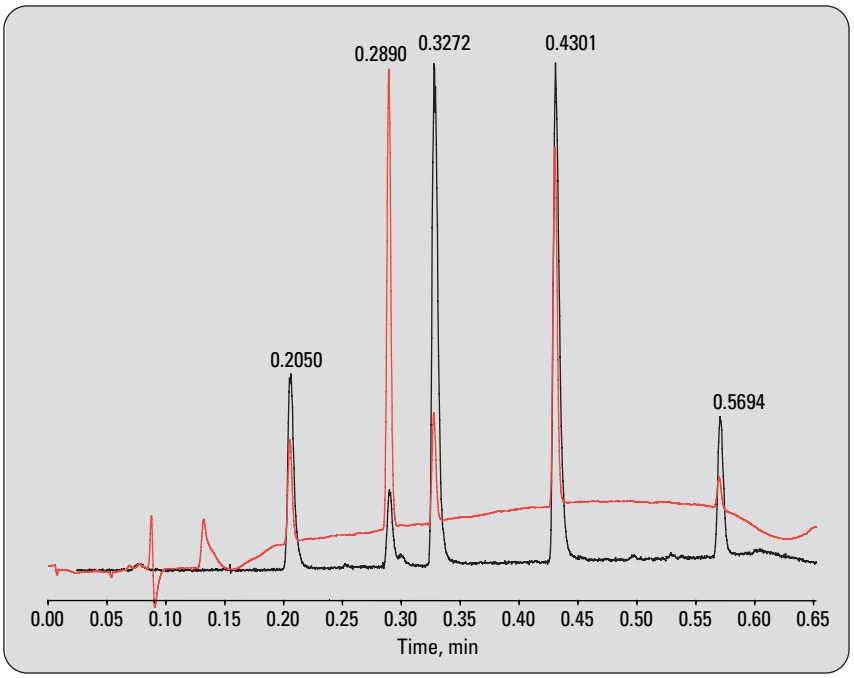



**Figure 3**
**Comparison of corresponding peaks in the UV (red trace) and the MS detection (black trace).**

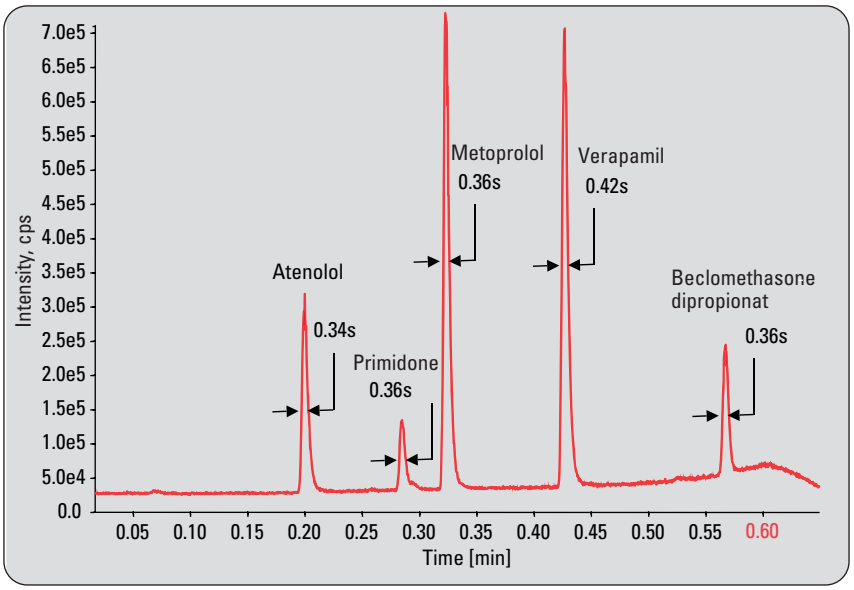



**Figure 4**
**MS total ion chromatogram of highest speed LC-TOF-MS analysis (40 Hz TOF data acquisition rate).**

rates in the MS which shows clearly in figure 5. The effect is nicely demonstrated on the little side peak next to the primidon peak – with 40-Hz data acquisition rate it is obvious that an additional compound shows up but with 5 Hz data acquisition rate this could not be differentiated from tailing of the primidon! The advantage, especially if MS quantization is necessary, is clear.

By applying the chromatographic conditions of table 1 and 80 Hz signal data acquisition of one wavelength and 30 Hz TOF centroid data, a cycle time of 49 s was achieved. The achievable cycle time is not only dependent on the used run time (that is the gradient time plus additional flush and re-equilibration times, or in Agilent terminology the stop time plus post time) but also very much dependent on the instrument overhead time. This is usually caused by communication between the data system and the individual LC/MS modules as well as the data system writing data to the hard disc and initiating certain processes. The overhead time caused by the data system can be significant if the computer's performance is not sufficient to handle the data amount or if other software programs or processes are consuming the power available. To decrease the cycle time it might be worth decreasing the amount of data acquired.

Table 2 shows the cycle times and the possible daily throughput depending on the DAD and MS settings. Since the MS data are constantly written to the hard disc during data acquisition, whereas the UV data are buffered and added to the data file after the stop time of

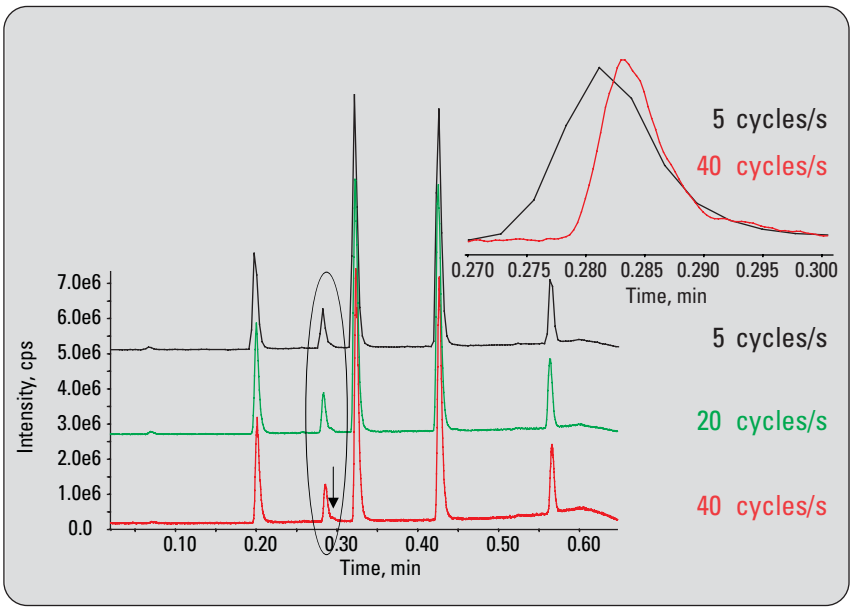



**Figure 5**
**Total ion chromatograms recorded with varying data acquisition rates – dependence of the MS peak shape and resolution on the data acquisition rate.**

| DAD (80 Hz) | | TOF (100 – 1000 Da) | | | Cycletime | Throughput |
|---|---|---|---|---|---|---|
| Type | Wavelength | Centroide | Profile | Data rate [Hz] | [s] | [Samples/day] |
| spectral | 190-900 (1) | | x | 20 | 62 | 1394 |
| spectral | 190-900 (1) | x | | 20 | 62 | 1394 |
| spectral | 190-400 (2) | | x | 20 | 59 | 1464 |
| spectral | 190-400 (2) | | x | 40 | 59 | 1464 |
| spectral | 190-400 (2) | | x | 30 | 58 | 1490 |
| signal | 210/254 | | x | 20 | 50 | 1728 |
| signal | 210 | x | | 30 | 49 | 1763 |

**Table 2**
**Dependence of the cycle time on the DAD and MS data acquisition settings, method stop-time was 0.65 min (39 s), pre-run balance was applied (ca. 2 s). The number in brackets for the DAD wavelength range stands for the scan width in nm.**

the method, the cycle time depends more on the UV data amount than on the MS data amount. The cycle time was calculated from the time stamp each file gets assigned from the WindowsXP™ operating system after closing the file following data acquisition.

If using a TOF MS the attention is certainly focused on the accurate mass. The question may arise if the possibility to obtain low mass accuracy errors might suffer from these high speed conditions. Figure 6 shows the achieved mass accuracy errors of the analysis of 140 members of a chemical library used in a screening campaign by a pharmaceutical company. The shown error-values have been extracted from an automated empirical formula confirmation report and involved no manual interference. Sixteen of the compounds could not be ionized under positive ESI conditions and two compounds showed large mass errors of 11 and 15 ppm, probably caused by co-eluting isobaric impu-

rities. The cycle time was 90 s and was determined by a required injector program which allowed an on-line dilution of the samples directly prior to the analysis. Chromatographic conditions applied a 5-100 % water-acetonitrile (0.1 % TFA) gradient in 0.7 min at a flow rate of 1.5 mL/min and 60 °C column temperature. UV data acquisition to determine purity was done in the wavelength range of 210 to 500 nm with an acquisition rate of 80 Hz. The MS data acquisition rate was at 8 Hz to reduce the file size. The scan range was 120 – 1200 Da, capillary voltage 4000 V and the fragmentor voltage at 215 V. No ACR was applied and the flow to the MS was splitted in a 1:7.5 ratio.

More compelling is the histogram of the mass errors of these samples as shown in figure 7. More than 91 % of the ionizable compounds (outliers included) have a mass accuracy error in the range of ±2.0 ppm. Excluding the outliers even 93 % of the analyzed samples lie in-between the ±2.0 ppm range. In the ±1.0 ppm range which is FT-MS-like mass accuracy 71 % of the samples can be found (72 % excluding the outliers).

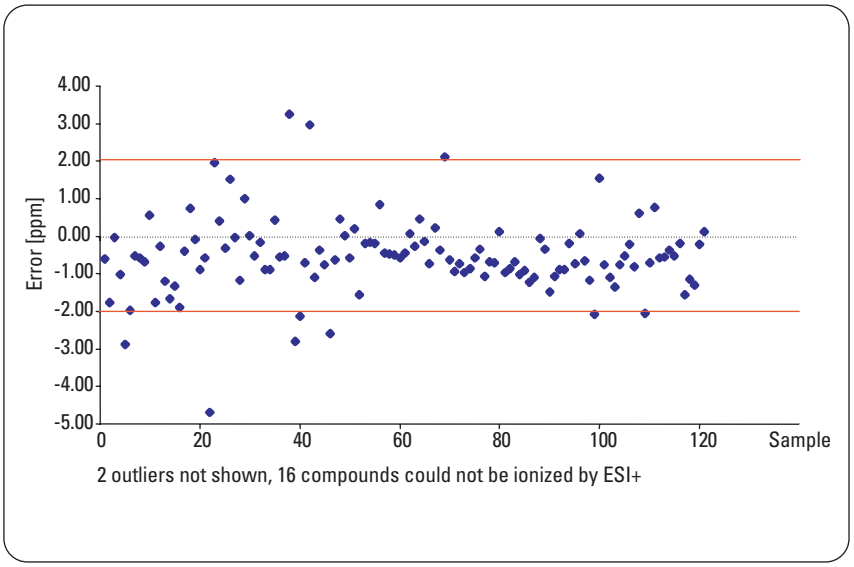



**Figure 6**
**Mass accuracy errors of the analyses of a set of chemical library members under fast-LC conditions.**

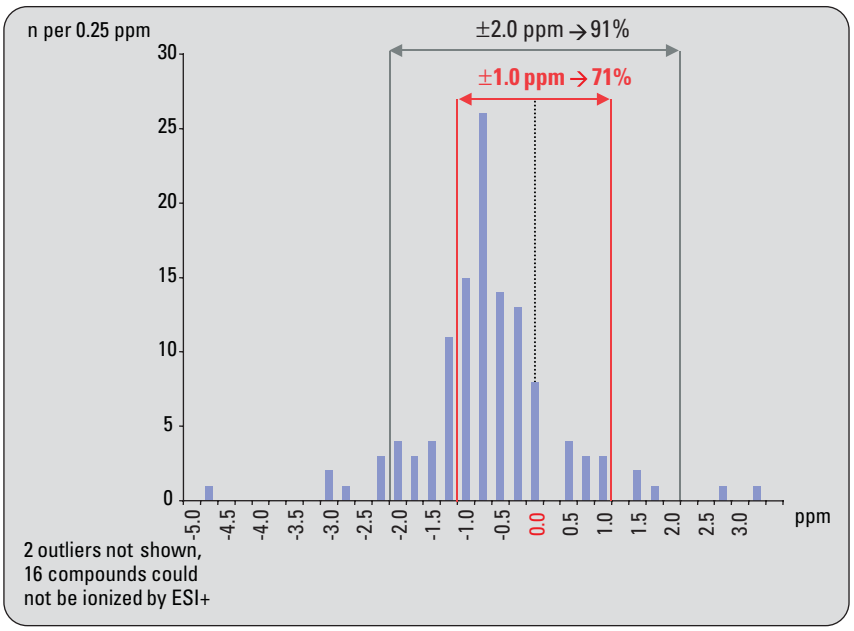



**Figure 7**
**Histogram of the mass accuracy errors of the analyses of a set of chemical library members under fast LC conditions. The given populations of the ±1.0 ppm and ±2.0 ppm range include the outliers.**

## Conclusion

The Agilent 1200 Series Rapid Resolution LC system together with the Agilent 6210 Time-of-Flight mass spectrometer allows acquisition of a wealth of data to unambiguously determine the purity and identity of compounds in samples as they are typical for the high throughput analytical departments of pharmaceutical companies. In the time range of one minute high chromatographic resolution, full spectral diode-array data from 190-900 nm wavelength in a band width of 1 nm at an 80 Hz acquisition rate plus full MS spectral data from 100-1000 m/z with high acquisition rate and with an accurate mass with a mass error below ±2.0 ppm for more than 91 % of the samples could be acquired.

Using features like alternating column regeneration, overlapped injection, high temperatures, high flow rates together with highest data acquisition rates and most importantly stable and easy-to-use accurate mass, this system outperforms other high throughput LC/MS techniques used as yet in throughput and/or data quality. The linear velocities achieved were in the range of 11 mm/s and cycle times were as fast as 49 s for a run time of 41 s. Due to the columns with particle sizes of 1.8 µm, the UV peak capacities were still in the range of fifty and even the MS peak capacities were in the range of forty for a gradient time of 39 s.

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2.

Udo Huber, „High throughput HPLC – Alternating column regeneration with the Agilent 1100 Series valve solutions" *Agilent Application Note, Publication number 5988-7831EN;* **2002.**



*Most of the data herein was presented as a poster, titled "Non-multiplexed DAD-ToF Analysis of 1400 Samples/day" by Michael G. Frank, Edgar Naegele (Agilent Technologies, Waldbronn, Germany), Doug McIntyre (Agilent Technologies, Santa Clara, USA), Thilo A. Fligge, Stefan Buehler, Markus Christ (Boehringer-Ingelheim, Biberach, Germany), CO-1152, at the Pittcon conference 2006 in Orlando, Florida, USA.*



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**Agilent Technologies**

# Achieving fastest analyses with the Agilent 1200 Series Rapid Resolution LC system and 2.1-mm id columns

Application Note

Michael Frank


## Abstract

The need to increase the daily throughputs of LC systems is a constant desire. Now, with the Agilent 1200 Series Rapid Resolution LC system highest throughputs are possible, and in combination with the Agilent ZORBAX RRHT columns and the increased pressure and temperature range of the LC system, excellent chromatographic resolution can be achieved even at run times below one minute.

This Application Note describes the correct set-up of the instrument which is the key for optimal results with narrow bore columns, such as a 2.1 mm x 50 mm column packed with sub two micron particles. Peak capacities in the range of fifty in analysis times as short as 24 seconds and peak widths as narrow as 200 milliseconds are shown. The well-balanced use of all possible module options to achieve shortest cycle times with throughputs far beyond 1500 samples per day is described.


Agilent Technologies

## Introduction

Particularly analytical service laboratories in the pharmaceutical industry, responsible for analyzing chemical libraries[1] or performing MS based quantifications of certain ADME-properties and drug metabolism studies of drug candidates[2] are faced with the challenge to increase their throughput, but also to maintain a high chromatographic resolution. In 2003 Agilent Technologies introduced sub two micron particles in their RRHT column series. Because of the small particle size, the chromatographic resolution obtainable with these columns is superior to standard particle sizes such as 3.5 µm or even 5 µm. Due to a unique silica manufacturing process, Agilent ZORBAX RRHT columns show a significantly reduced backpressure, if compared to similar column dimensions of other manufacturers. Excellent chromatographic results are achieved in a very short analysis time with the Agilent 1200 Series Rapid Resolution LC system, which facilitates an increased pressure range and flow rates from 0.05 up to 5 mL/min using column diameters ranging from 2.1-mm id up to 4.6-mm id. This Application Note will focus on 2.1-mm id columns only.
Not only are the run times of the analyses important for high throughput, but also the overhead time. The Agilent 1200 Series Rapid Resolution LC system can be optimized to achieve highest throughputs with exceptionally good overall system performance.

## Experimental

An important issue when dealing with narrow bore columns, especially in gradient mode where smallest peak widths can be achieved, is to have small extra column volumes. This also includes any volumes in front of the sampling device, because any volume after the solvent mixing point will increase the time for the gradient composition to reach the column. This results in an increased run time. The Agilent 1200 Series Rapid Resolution LC system can be reconfigured within a few minutes to provide appropriate system volumes for different column ids. Here, the pumps are set-up in the low delay volume configuration with an internal volume of approximately 120 µL. All other modules are optimized for lowest delay volumes by using the low delay volume capillary kit (G1316-68744). Consequently, only capillaries of 0.12 mm id are used beyond the injection valve. In the Agilent 1200 Series thermostatted column compartment SL the newly introduced low dispersion heat exchangers with 1.6 µL internal volume were used. In some experiments, the Agilent 1200 Series Rapid Resolution LC is set up for alternating column regeneration to achieve highest throughput using the ACR-capillary kit (G1316-68721) and 2.1-mm id columns[3]. The high pressure rated 2-position/10-port valve in the thermostatted column compartment was only placed into the flow path if alternating column regeneration was used indeed.

The instrument set-up is as follows (figure 1):

- Agilent 1200 Series binary pump SL with the new Agilent 1200 Series micro vacuum degasser
- Agilent 1200 Series high performance autosampler SL
- Agilent 1200 Series thermostatted column compartment SL, equipped with a high pressure, 2-position/10-port valve, facilitating alternating column regeneration
- Agilent 1200 Series diode-array detector SL with a 2-µL/3-mm cell
- ZORBAX SB C18, 2.1 mm id x 50 mm, 1.8 µm

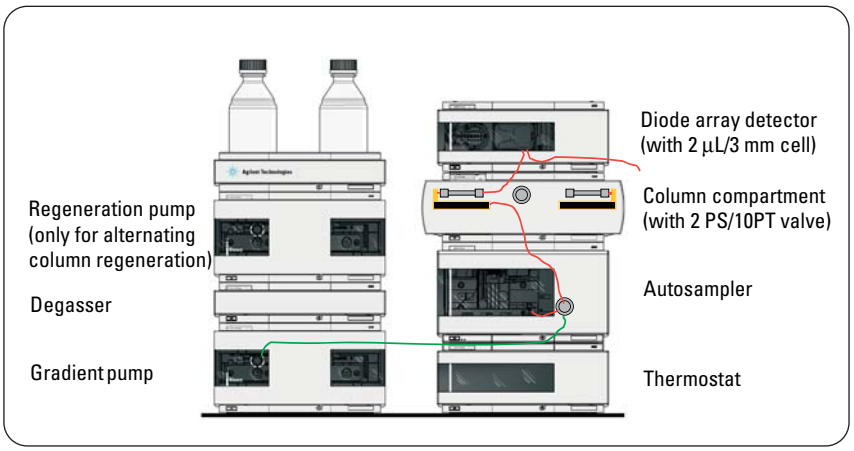


**Figure 1**
**System setup with low delay volume for high speed applications using 2.1-mm id columns with lengths from 20 to 50 mm.**

The Agilent 1200 Series binary pump SL is designed to fulfill the demands for high throughput, highest performance, optimum resolution and lowest pump ripple. The pump hardware is significantly different from the standard binary pump. In the Agilent 1200 Series binary pump SL the pressure transducer is separate from the damper which has been modified to have a lower delay volume (pressure dependent ranging from 80-280 µL). In this study the pumps were used in the low delay volume configuration without the mixer and damper in the flow path. In contrast to the standard binary pump the pump heads of the binary pump SL have an additional damping coil (500 µL volume each) to allow damping in the low delay volume configuration. This does not add to the gradient delay volume because it is before the mixing point. Anyhow, pressure ripples are also strongly suppressed by the Electronic Damping Control (EDC). The pressure range of the pump and all other modules is increased to 600 bar.

Only one sample, the so-called "phenone-mix", was used in the course of this study to keep variations low. The sample consists of nine compounds: acetanilid, acetophenone, propiophenone, butyrophenone, benzophenone, valerophenone, hexanophenone, heptanophenone and octanophenone. Unless otherwise stated, the concentration was 0.1 µg/µL for each compound except butyrophenone which was 0.2 µg/µL. The solvent was water-acetonitril 2:1.

## Results and discussion

The most frequently sold particle size in chromatographic columns today is 5 µm. Of course, fast and ultra fast LC is also possible with columns packed with particles of these larger diameters – the reduced back pressure is even beneficial to allow higher flow rates. However, resolution will be sacrificed because conditions are usually far on the right side of the van-Deemter-optimum. Here, the big advantage of the RRHT columns with particles of less than 2 µm diameter is proven. The van Deemter optimum is shifted further to the right and the curve is much flatter at the onset because the "resistance of mass transfer" term is diminished (figure 2). In figure 3 the analysis on a 2.1-mm id column with 1.8-µm particles is compared to the linear scaled analysis on the same stationary phase but on 5 µm particles packed in a 4.6-mm id-column. The gain in resolution is obvious – from Rs = 2.1 up to Rs = 3.5 for the critical pair which matches the theoretically expected value of a 1.66 fold increase in resolution. Also note that there is a saving in solvent consumption of 8.6 mL in the "standard" HPLC analysis and only 1.8 mL in the ultra fast HPLC analysis.

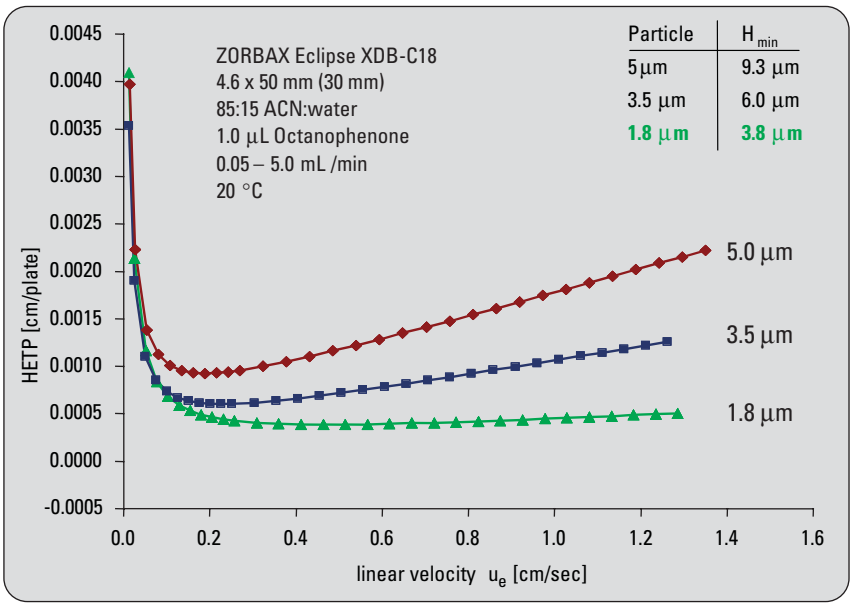


**Figure 2**
**Van Deemter curves of columns packed with 1.8 µm, 3.5 µm and 5.0 µm particles.**

For gradient separation the dependencies of the capacity factor can be expressed as:

$$k^* = 0.87 \cdot tg \cdot \frac{F}{Vm \cdot \Delta\%B \cdot S}$$

*(tg = gradient time, F = flow rate, Vm = column void volume, △%B = gradient steepness, S = solvent and solute dependent factor)*

If the product of the gradient time and flow rate, the so-called gradient volume, is kept constant together with all other parameters, the gradient time might be decreased while the flow rate is increased. Thus, the capacity factors of two compounds will stay constant and if no large alteration of the plate height occurs, the resolution will not change significantly, either. The final point is the big advantage of the sub two micron particles – the van-Deemter curve is nearly flat on the right side of the minimum (figure 2) and flow rates can be increased with only little increase in plate heights. However, the equation is an empirical one and deviations may occur especially under extreme conditions.

With a two-step approach, highest gradient speeds with virtually no loss or only little loss in resolution can be achieved. In the first step, start from a medium temperature and begin to increase the flow rate up to the pressure maximum. Subsequently the temperature should be increased to lower the viscosity of the solvent and then the flow rate is increased again. It may be worthwhile to check the resolution with two identical gradients but with different temperatures to see the influence of the temperature change on the resolution which may be very compound dependent. In figure 4 the result of this approach is shown. A nearly 7-fold increase in separation speed could be achieved with still baseline separation of the critical pair before meeting the pressure and temperature limit (the maximum temperature is a function of flow, temperature, number of controlled Peltier elements and of the heat capacity of the solvent used).

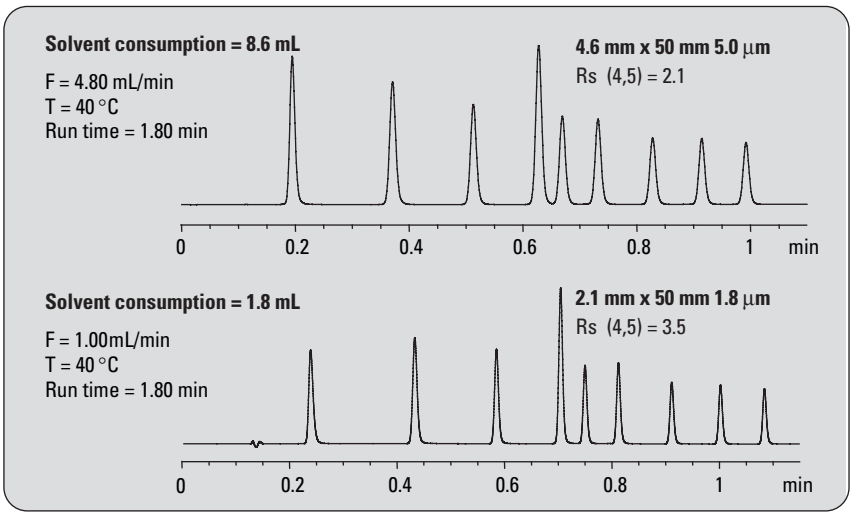


**Figure 3**
**Analysis with 1.8-µm particle column vs. 5.0 µm particle column.**

| | | |
|---|---|---|
| **Conditions:** | 4.6-mm id column used on standard Agilent 1200 system | |
| Solvent: | A = Water, B = ACN | |
| Temperature: | 40 °C | |
| **Column:** | **2.1 mm x 50 mm, 1.8 µm** | **4.6 mm x 50 mm, 5.0 µm** |
| Flow: | 1.0 mL/min | 4.8 mL/min (scaled from 2.1 mm col.) |
| Gradient: | 0.00 min 35 %B | 0.00 min 35 %B |
| | 0.90 min 95 %B | 0.90 min 95 %B |
| | 1.10 min 95 %B | 1.10 min 95 %B |
| | 1.11 min 35 % B | 1.11 min 35 % B |
| Stoptime: | 1.15 min | 1.15 min |
| Posttime: | 0.70 min | 0.70 min |
| Wavelength: | 245 nm (8), ref. 450 nm (100) | 245 nm (8), ref. 450 nm (80) |
| Peakwidth: | >0.0025 min (0.05 s res.time), 80 Hz | >0.01 min (>0.2 s), 20 Hz |
| Injection volume: | 1 µL | 5 µL (not scaled) |

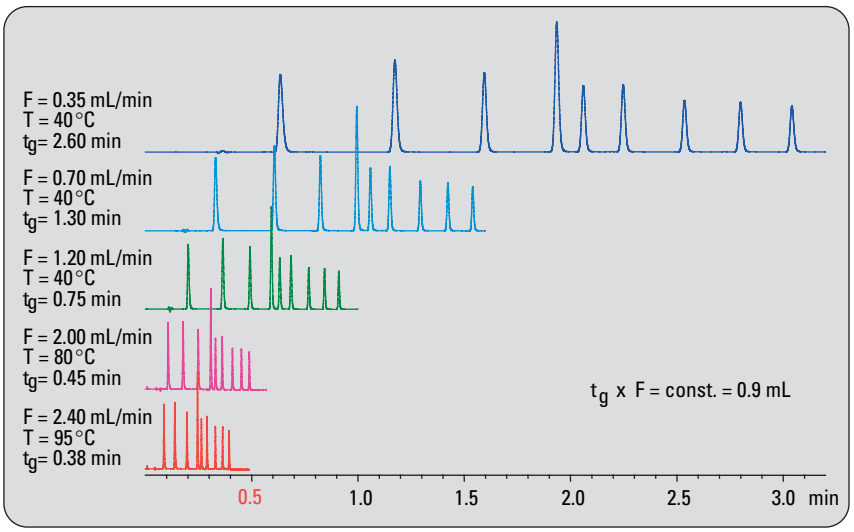


**Figure 4**
**Increasing separation speed by increasing temperature and flow rate while decreasing gradient time.**

**Conditions:**
Solvent: A = water, B = ACN
Temp.: 40 °C, 80 °C, 95 °C
Flow: 0.35, 0.70, 1.20, 2.00, 2.40 mL/min
Gradient: 0.00 min 35 %B
2.60 min 95 %B
3.20 min 95 %B
3.21 min 35 %B
*Time values for F = 0.35 mL/min. For all other flow rates times are scaled so that (tg x F) = 0.90 mL*
Stop time: 3.20 min
Post time: 2.00 min
Wavelength: 245 nm (8), Ref. 450 nm (100)
Peak width: >0.0025 min (0.05 s response time), 80 Hz

The last chromatogram is enlarged in figure 5 and reveals the details of this separation. The first peak is eluted after only five seconds and peaks with a width at half height of less than 200 ms are achievable. Within twenty-four seconds nine compounds are separated with a peak capacity in the range of fifty.

### Retention time precision at highest analysis speed

High analysis speed is meaningless without precision. One basic performance criteria for HPLC pumps is the precision of gradient formation measured by the precision of retention times of repeated gradients. However, the stability of the column temperature must also be taken into consideration, because temperature fluctuations will also influence the retention times of a given sample. In table 1 and figure 6 the results from the 10-fold repeated analysis of a standard sample are listed and since the deviation between individual runs is so small, the octanophenone peak is enlarged in a separate window. This sample contains compounds that are both not retained and refer to isocratically eluted compounds found at the starting conditions of the gradient, as well as highly unpolar and strongly retained compounds. The analyses

**Conditions:**

| | |
|---|---|
| Solvent: | A = Water, B = ACN |
| Temp.: | 40 °C, 80 °C |
| Flow: | 0.35 mL/min, 1.20 mL/min, 2.0 mL/min |
| Gradient: | 0.00 min 35%B |
| | 2.60 min 95%B |
| | 3.20 min 95%B |
| | 3.21 min 35%B |
| | *Time values for F = 0.35 mL/min.* |
| | *For all other flow rates times are* |
| | *scaled so that (time x flow) = 0.90 mL* |
| Stop time: | 3.20 min |
| Post time: | 2.00 min |
| Injection vol.: | 1.0 µL |

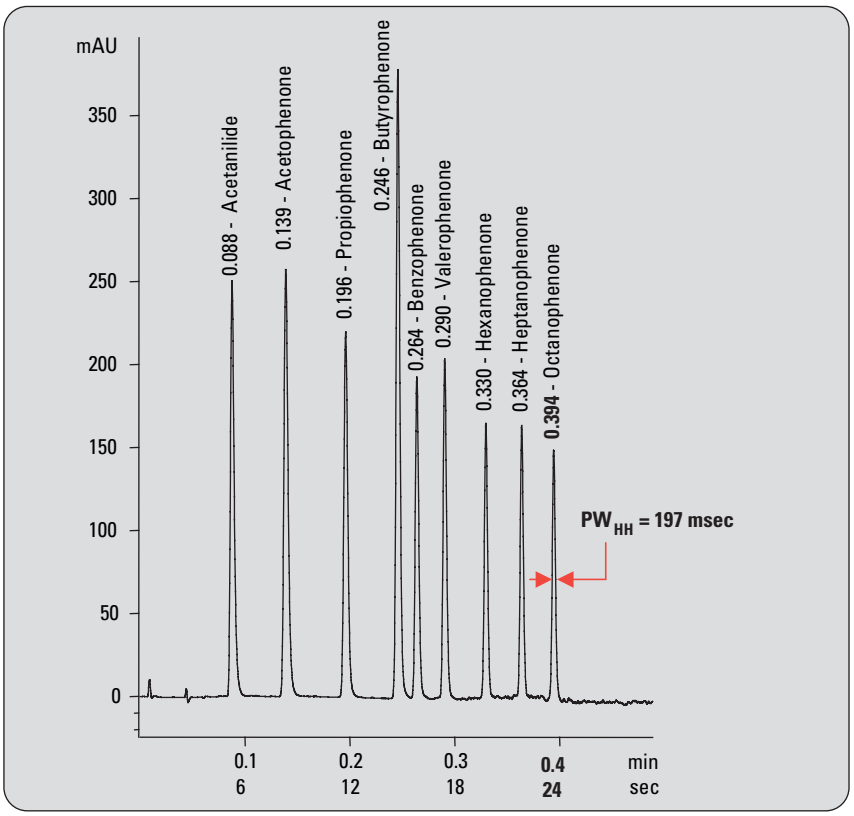


**Figure 5**
**Separation of a nine compound mixture under ultra fast conditions.**

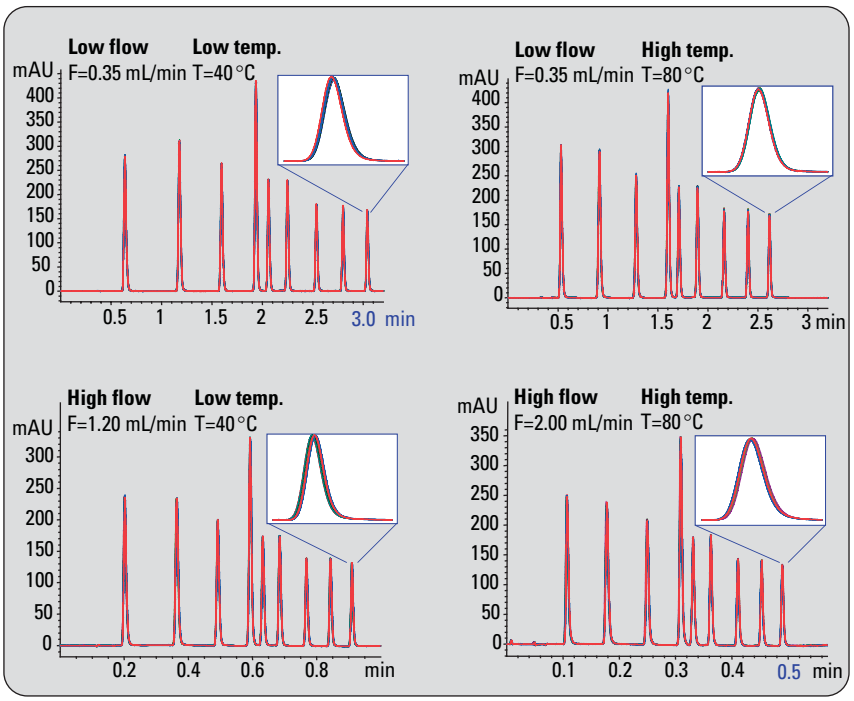


**Figure 6**
**Overlaid chromatograms of the repeated analysis of a 9 compound mixture under various conditions.**

were done at high and low flow rates as well as with high and low temperatures as in the examples shown earlier. In all cases the mean retention time precision is below 0.3 % RSD, which was the specification of the Agilent 1100 Series LC system. Of course, the results are also in line with the specifications for the new Agilent 1200 Series Rapid Resolution LC system which is < 0.07 % RSD or < 0.02 min SD, whichever is met first. At these high gradient speeds, the SD criteria are always met. The RSD criteria are also met for both fast-LC gradients of 2.6 min duration (0.35 mL/min flow rate). Even at ultra-fast gradient speeds, the retention time precisions are still below or only slightly higher than 0.1% RSD (table 1).

### Improving the cycle-time

Not only is the gradient speed important when dealing with high-throughput analysis but furthermore the over all cycle time of the entire system, which is the time between two consecutive analyses. A good method to measure the cycle time is by using the time stamp the data file is assigned by the operating system of the computer. Clearly, optimizing the cycle time has some drawbacks. For example, extensive needle cleaning procedures are in contradiction with a high sampling speed. Table 2 gives an overview of important parameters influencing the cycle time. Using 1.8-µm particle size columns together with an optimized HPLC system very short run times can be achieved without sacrificing chromatographic resolution. Combining short run times together with low overhead times will result in a high daily throughput. In figure 7 the cycle time and daily throughput is shown for two

| | 0.35 mL/min, 40°C | | 0.35 mL/min, 80°C | | 1.20 mL/min, 40°C | | 2.00 mL/min, 80°C | |
|---|---|---|---|---|---|---|---|---|
| | SD | % RSD | SD | % RSD | SD | % RSD | SD | % RSD |
| Average | 0.00107 | 0.067 | 0.00084 | 0.070 | 0.00048 | 0.098 | 0.00031 | 0.134 |

**Table 1**
**Standard deviations (mAU) and %RSD (n=10) of the retention times under different chromatographic conditions in temperature and flow.**

| Module | Parameter | Effect on cycle time | Other effects |
|---|---|---|---|
| **Pump** | Low delay volume setting | Reduced retention times, run time can be shortened, reduced cycle time | Increased pressure ripple, slightly increased mixing noise if modifiers such as TFA are used. |
| **Autosampler** | Automatic Delay Volume Reduction (ADVR) – activated | Reduced delay volume, reduced retention times, run time can be shortened, reduced cycle time | Increased carry-over |
| | ADVR activated and Overlapped Injection (OI) | Enables parallel sampling, thus reduces the cycle time independently of the below listed settings (as long as the overall sampling speed does not exceed the gradient and post time) | Increased carry-over |
| | no OI – Needle Wash | Increased sampling time with increasing wash time | Reduced carry-over with longer needle wash time |
| | no OI – Equilibration time | Increased sampling time with increased equilibration time | Better injection precision with longer equilibration time |
| | no OI – Draw/Eject speed | Low speed causes increased sampling time | Low speed results in better injection precision |
| **Column compartment** | Alternating column regeneration | Saves column wash-out and equilibration time, reduces cycle time enormously | Additional hardware required, slightly increased extra column volume, slightly different retention times between columns possible |
| **Detector** | Pre-run and/or post-run balance | Increased cycle time | Baseline drifts possible if not applied |
| | Spectral data acquisition with high data rate, small band width and broad wavelength range large data files | Depending on computer power and additional processes running might increase cycle time because of writing speed | Reduced information content if no spectral data acquired or with lower resolution |
| **Software** | Data analysis with acquisition | Increased cycle time, depending on computer power and number of peaks | Data analysis has to be done offline is no set |
| | Save method with data | Slightly increased cycle time | Information is missing if method is not saved |
| | Execution of pre-run or post-run macros | Increased cycle time, depending on macro | Depending on macro |
| **System** | LC controlled over local network between computer and LC (and MS) only | Faster data and method transfer between computer and LC because of reduced net work traffic reduced cycle time | Additional hardware might be necessary (use independent acquisition computer) |
| | Number of detectors | More detectors produce a higher data amount and lower the data transfer speed, resulting in higher cycle times | More detectors higher information content |

**Table 2**
**Influence of various parameters on the overall cycle time.**

different methods – both giving virtually the same resolution. The first method (0.45 min gradient) utilizes alternating column regeneration and high temperatures to allow high flow rates and speed optimized settings. A cycle time of 49 s could be achieved, resulting in a theoretical daily throughput of more than 1700 samples per day. The second method (0.90 min gradient) does not use high temperatures or alternating column regeneration and the time saving of some simple and often forgotten method options are shown. By optimizing these parameters the real cycle time gets as close to 8 s to the run time (stop time plus post time) and allows a daily throughput of more than 700 samples per day. By sub-optimal method set up this can easily drop to below 500 samples per day if options like automatic delay volume reduction, overlapped injection or offline data-analysis are not used.

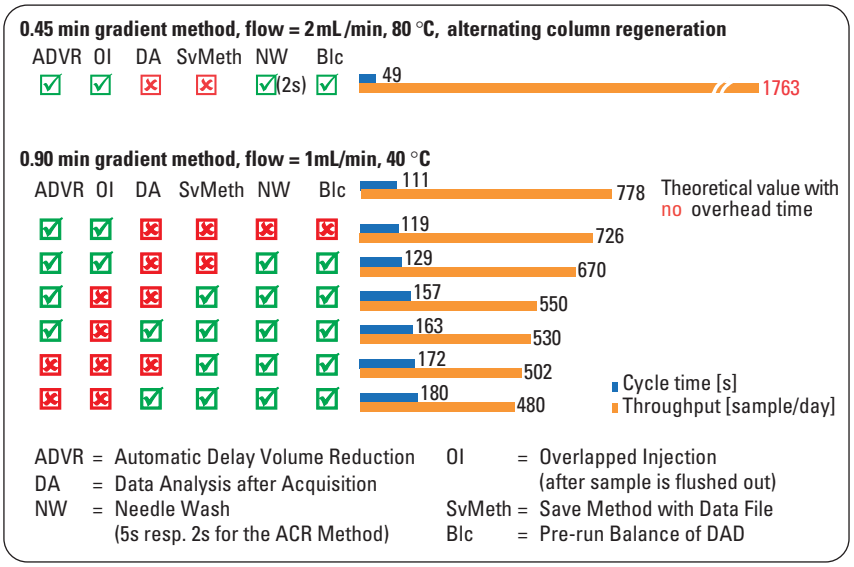


**Figure 7**
**Cycle time and daily throughput optimization.**

**Chromatographic conditions:**

**Alternating Column Regeneration Method**

| | | |
|---|---|---|
| Solvent: | A = Water, B = ACN | |
| Temp.: | 80 °C | |
| Flow: | 2.0 mL/min | |
| ADVR: | Yes | |
| Gradient: | **Gradient-Pump** | **Regeneration-Pump** |
| | 0.00 min 35 %B | 0.00 min 35 %B |
| | 0.45 min 95 %B | 0.01 min 95 %B |
| | 0.46 min 35 %B | 0.11 min 95 %B |
| | 0.57 min 35 %B | 0.12 min 35 %B |
| Stoptime: | 0.57 min | no limit |
| Posttime: | off | off |
| Wavelength: | 245 nm (8), ref. 450 nm (100) | |
| Peak width: | > 0.0025 min (0.05 s response time), 80 Hz | |
| Spectra: | none | |
| Injection volume: | 1.0 µL | |
| Injector: | Overlapped injection, 2 s needle wash, sample flush-out factor = 10, draw/eject speed = 100 µL/min | |
| Valve: | next position | |

**No Alternating Column Regeneration Method**

| | | |
|---|---|---|
| Solvent: | A = Water, B = ACN | |
| Temp.: | 40 °C | |
| Flow: | 1.0 mL/min | |
| ADVR: | Yes | No |
| Gradient: | 0.00 min 35 %B | 0.00 min 35 %B |
| | 0.90 min 95 %B | 0.90 min 95 %B |
| | 1.10 min 95 %B | 1.10 min 95 %B |
| | 1.11 min 35 %B | 1.11 min 35 %B |
| Stoptime: | 1.15 min | *1.40 min (add. 300 µL extra column volume, increased retention times)* |
| Posttime: | 0.70 min | 0.70 min |
| Wavelength: | 245 nm (8), ref. 450 nm (100) | |
| Peak width: | > 0.0025 min (0.05 s response time), 80 Hz | |
| Spectra: | all, 190-500 nm, BW = 1 nm | |
| Injection volume: | 1.0 µL | |
| Injector: | See figure 7, 2 s equilibration time | |

## Conclusion

The Agilent 1200 Series Rapid Resolution LC system is a powerful tool to achieve highest chromatographic resolutions and also highest throughputs. The extended pressure range allows the usage of columns packed with stationary phases with particles sizes below 2 µm, for example, Agilent RRHT columns with particle sizes of 1.8 µm. These columns not only allow an increase in linear flow rates with virtually no loss in resolution but also have an inherently higher resolution compared to 3.5 µm or even 5.0 µm particle sizes. The possibility to switch the pump into its low delay volume configuration allows the use of the entire bandwidth of today's widely used column ids – from 4.6 mm down to 2.1 mm and even 1.0 mm. As illustrated above, the system has uncompromised performance characteristics even at highest gradient speeds.

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**Agilent Technologies**

# Screening for Hazardous Chemicals in Homeland Security and Environmental Samples Using a GC/MS/ECD/FPD with a 731 Compound DRS Database

Application Note

**Homeland Security, Environmental**


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## Abstract

**Response to homeland security or environmental incidents involving hazardous chemicals requires first, the rapid and accurate identification of the chemical agent(s) involved and second, the quantitative measurement of that agent in large numbers of samples to aid in managing the response. Given the unknown nature of the analytes and the complexity of matrices that could be encountered, developing analytical methods for this analysis is challenging. The approach described in this work uses a gas chromatography/mass spectrometry (GC/MS) system with a micro-fluidic splitter added to the end of the column. The splitter divides the column effluent between the MS and either a dual-wavelength flame photometric detector (DFPD) or a micro electron-capture detector (μECD) and a single-wavelength FPD. This approach allows the simultaneous collection of MS and two channels of selective GC detector data from a single injection. This multisignal configuration provides: full-scan MS data for library searching, selective ion monitoring (SIM) data for trace analysis, μECD and FPD data for excellent selectivity and sensitivity in complex matrices. The systems use retention time locking (RTL) to produce retention times (RTs) that precisely match those in a 731 compound database of hazardous chemicals. Deconvolution Reporting Software (DRS) is used to provide fast and accurate interpretation of the MS data, especially in samples with high matrix contamination. The combination of selective GC detectors, SIM/Scan, and deconvolution makes a very powerful hazardous chemical analysis system that shows significant progress toward the above goals.**


## Introduction

In recent years, there has been increasing concern over the release of hazardous chemicals through either accidental or intentional acts. Both the homeland security and environmental communities recognize the need for preparing analytical laboratories that can respond quickly to such incidents. The terms toxic industrial chemicals/toxic industrial materials (TIC/TIM) are used in homeland security to refer to hazardous chemicals, while the environmental community uses different terminology like hazardous materials. In either case, the challenge is to develop laboratory methods with the capability of identifying any hazardous chemical(s) involved in an incident and to be able to measure its concentration in collected samples.

There are several significant challenges to face when developing methods for this analysis. The methods must able to:

- Rapidly and accurately identify the specific toxic agents involved
- Measure concentration correctly at high levels of agent at the epicenter (high dynamic range)
- Measure concentration correctly at low levels of agent at perimeters and during decontamination (low detection limits)

- Be highly selective over matrix interferences (wood smoke, fuels, burning tires, etc.) to minimize both false positives and false negatives
- Indentify as many toxic agents as possible
- Handle large numbers of samples

It is clear that there is no single analytical technique that can be used for detecting all possible hazardous chemicals. However, one technique that is widely applicable for the identification and measurement of broad classes of hazardous chemicals is GC/MS. GC/MS is widely used in laboratories worldwide for the analysis of thousands of different chemicals.

GC/MS methods are typically developed to analyze between 10 and 100 individual compounds. A target compound is deemed to be present if the target ion and two or three qualifier ions, with specific abundance ratios, fall within a defined RT window. The identity of the target may be further confirmed by comparison of the scan at the apex of the peak with a library reference spectrum.

Matrix interferences are usually minimized by optimizing a combination of the sample preparation, GC, and MS parameters. Since most methods only deal with at most a few matrix types, the ions chosen for identification purposes can be selected such that they are minimized in the matrix. With the limited number of targets addressed by the method, recalibration of response factors, RTs, and qualifier ion abundance ratios can be accomplished with the injection of a few calibration mixtures.

General screening methods for very large numbers of targets in widely varying and complex matrices offer a new set of challenges for the method developer. When screening for hundreds of targets, several factors must be addressed:

- Use of sample preparation to reduce matrix interferences is now significantly limited because rigorous cleanup steps may unintentionally remove targets. This reduced level of cleanup can result in significantly higher levels of matrix interferences to contend with.
- Recalibration of response factors, RTs, and qualifier abundance ratios is difficult or impossible because of the large number of targets.
- The methods may be deployed in laboratories without access to standards for all of the targets.
- The time required for data review of hundreds of targets in complex matrices can become unmanageably large.
- Even with a very large database of targets, it is possible that hazardous chemicals not in the target list could be present in a sample.

Recently, several techniques have become available to help address the above set of challenges. RTL produces RTs that precisely match from instrument-to-instrument and to those in a database [1]. This eliminates the need for recalibration of the individual RTs and timed events. The introduction of reliable and inert microfluidic splitters allows for the simultaneous collection of mass spectral data and, for example, phosphorus, sulfur, and/or electron capture data [2]. The selective detector chromatograms can highlight suspect compounds even if they are not in the MS target list. They can also offer an alternative means for quantitation of target analytes.

The introduction of the synchronous SIM/Scan feature allows for the simultaneous acquisition of both full scan and SIM data from the same injection [2, 3]. The scan data can be used for screening the full list of targets in the database while the SIM data looks for a high priority subset of compounds down to very low levels.

One of the most significant tools developed for dealing with complex matrices is Agilent's Deconvolution Reporting Software (DRS) [4]. It uses advanced computational techniques to extract the spectra of targets from those of overlapped interference peaks. It then compares the extracted spectrum with a library to determine if the target is present. Any hits are confirmed by searching against the main NIST MS reference library. This process is automated and provides significant time savings in data interpretation. Since it deals with the entire spectrum instead of just four ions, DRS can often correctly identify a target in the presence of interferences where the typical approach would fail. The use of DRS substantially reduces the number of both false positives and false negatives.

This application note describes the combination of the above techniques with a database of 731 hazardous chemicals, the Agilent Hazardous Chemical DBL (HCD), to be used for screening purposes. The compounds were chosen because of their significance in environmental or food safety analysis. The reasoning is that if the materials are manufactured in significant quantities and are toxic, they would be likely to appear in an

environmental method. The pesticides are included because many exhibit toxicity.

The list is comprised of:

- Chlorinated Dioxins and Furans: EPA 8280A, 10 compounds
- Polychlorinated biphenyls: EPA 8082, 19 compounds
- Volatiles: EPA 502/524, 60 compounds
- Semivolatiles: EPA 8270C Appendix IX, 140 compounds
- Pesticides: Agilent RTL Pesticide Database (adapted), 567 compounds
- Total: 796 compounds, with 65 compounds in two groups, or 731 individual compounds

The names of all the compounds in the database are listed in Appendix A at the end of this note.

The above list by no means contains all of the hazardous chemicals that could be encountered. However, it does screen for a large number of known hazards and with the addition of selective detection can highlight other nontarget compounds that may be of interest.

The chromatographic conditions chosen for development of the database are general in nature and are compatible with the analysis of other types of compounds beyond those in the table. For example, laboratories with access to calibration standards for chemical warfare agents (CWA) can add CWA data to the tables and screen for them as well.

The RTs for compounds in the database were collected with the column outlet pressure at 3.8 psig using a microfluidic splitter. This was done to assure that the RTs observed during sample analysis would closely match those in the database when a microfluidic splitter or QuickSwap is used.

The chromatographic conditions for the database were chosen to be compatible with the method translation technique. Constant pressure mode was used in the GC inlet so that method translation can be used to precisely time scale the methods for faster operation [5]. Provided with the Agilent Hazardous Chemicals DBL are the files to run the analysis precisely threefold (3X) and sevenfold (7X) faster than the primary database (1X). Also, each of the three-speed variations of the database are provided in two forms: one with the entire set of 731 compounds and one with the 36 aromatic hydrocarbons removed. The latter is provided for use with samples known to contain fuels and where the fuel components are not of interest. In the examples shown below the database with hydrocarbons removed was used, since fuels were used as prototype matrices.

## System Configuration

The system configurations used are shown in Figure 1A and 1B.

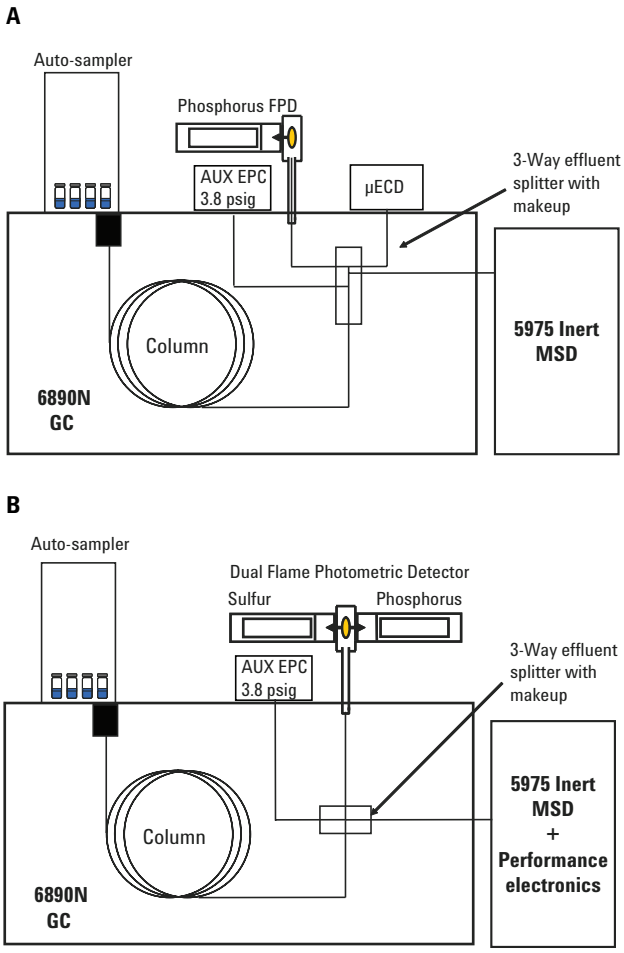



**Figure 1. System configurations. A). GC/MS/ECD/FPD system used for 1X and 3X screening analyses. B). GC/MS/DFPD system used for 7X screening analyses.**

Key components are:

### Fast Oven

The primary 1X method only requires the 120V oven. With the 6890N 240V oven (option 002), the screening analysis method can be run precisely three times faster (14.33 min) using a 15-m HP-5MS column. If the 240V GC is further equipped with SP1 2310-0236 (puts MSD interface in back of oven under rear injection port) and

using the G2646-60500 oven-insert accessory, the speed can be increased to seven times faster (6.14 min) with a 5-m HP-5MS column. Note that use of the oven insert prevents use of the front inlet and detector positions. Only one detector is available for splitting. The DFPD is a good choice for this configuration, as it uses only one detector position but generates two signals.

#### µECD

The 6890N Option 231 is a µECD. The signal from the electron capture detector (ECD) is collected, stored, and processed by the MS ChemStation simultaneously with the MS data. ECDs are selective in nature and exhibit very sensitive response to halogenated compounds, with detection limits below 1 pg for polyhalogenates. They also respond to several other functional groups like nitro compounds. They do, however, also respond to some fairly low-priority compounds, like phthalate esters. The ECD data can be used in several ways. Nontarget halogenated or nitro compounds are highlighted. The presence of an electrophore at the RT of an identified compound can be used to support confirmation of identity. The response on the ECD can be used for quantitative analysis, but only after calibration with a standard, as the response factors are compound dependent and can vary significantly with compound class.

#### Single FPD

The 6890N Option 240 is a single FPD. It is used to selectively detect either sulfur or phosphorus. The detector is usually run in the phosphorus mode to highlight such compounds as organophosphorus pesticides and nerve agents. In the phosphorus mode, the detector is highly selective ($>10^6$) with a very low (~0.050 pg) detection limits for phosphorus. The ability of the FPD to uncover nontarget organophosphorus compounds like new pesticides or designer nerve agents is especially helpful. The presence of phosphorus at the RT of an identified compound can be used to support confirmation of identity. Because the response per unit weight of phosphorus is relatively consistent from compound to compound, the FPD can be used for semi-quantitative analysis in situations where no calibration standard is available for an identified analyte.

#### Dual FPD

The 6890N Option 241 is a DFPD with two optical detection channels that measures sulfur and phosphorus simultaneously. The DFPD sulfur response is also selective ($>10^4$) and sensitive (detection limits <10 pg) , although not as much as phosphorus. The sulfur signal is also quadratic with respect to the amount of sulfur injected. It is often used to detect sulfur-mustard agents and for confirmation of sulfur-containing pesticides. The response per unit weight of sulfur is relatively consistent from compound to compound, but varies more than that of the phosphorus signal.

#### Microfluidic Splitter

The 6890N Option 890 (3-way splitter) or Option 889 (2-way splitter) uses diffusion-bonded plate technology combined with metal column ferrules to make an inert, easy-to-use, leak free, high-temperature column-effluent splitter. The splitter uses Auxiliary EPC for constant pressure makeup (6890N Option 301). The Auxiliary EPC makeup can be pressure programmed at the end of the run to higher pressure, while at the same time the inlet pressure is lowered to near ambient. This causes the flow in the column to reverse direction, backflushing heavy materials out the split vent of the inlet. Backflushing can greatly reduce analysis times for samples that contain high-boiling matrix components [6]. The Aux EPC also allows column changing and maintainance without venting the MSD. When the column fitting is removed from the splitter, helium from the makeup supply purges the fitting, preventing air from entering the MSD. If the column is attached to the splitter but removed from the inlet, helium flows backwards through the column and out the inlet end. Inlet maintainance or column headtrimming can be done without cooling and venting the MSD to prevent sucking air into a hot source.

#### MSD System

The 5975 inert MSD with performance turbo (G3243A) or 5973N inert MSD with performance electronics and performance turbo (G2579A), EI (electron impact ionization mode) MSD is used. These configurations provide faster full scan rates while maintaining sensitivity. The scan rates are compatible with the narrower peaks generated by fast chromatography. The performance turbo pump is required to handle the higher flows associated with the screening method.

#### Synchronous SIM/Scan

The D.02.00 (or higher) revision of the Agilent MSD ChemStation is used because it supplies the synchronous SIM/Scan feature. SIM/Scan operates by collecting SIM data every other cycle and scan data on alternate cycles throughout the entire chromatogram. The signal-to-noise performance of the collected SIM and scan data is virtually identical to that obtained with SIM-only and scan-only

methods. As with conventional SIM methods, not all 731 targets can be monitored in a single run due to the required time separation between SIM groups. In general, the acquisition of SIM data is set up to collect high-priority targets at very low levels. Examples would be the chlorinated dioxins and CWAs.

**DRS Software** (G1716AA)

Spectral deconvolution of the MS data enables identification of analytes in the presence of overlapped matrix peaks [4]. This significantly reduces chromatographic resolution requirements, which allows detection of targets in higher levels of matrix or can be used with fast chromatography to shorten analysis times. DRS uses the AMDIS deconvolution program from NIST, originally developed for trace chemical-weapons detection in complex samples. DRS presents the analyst with three distinct levels of compound identification:

- ChemStation, based on RT and four-ion agreement
- AMDIS, based on "cleaned spectra" full-ion matching and locked RT
- NIST05 search using a 163000 compound library

**Hazardous Chemical DBL** (G1671AA)

This supplies the mass spectral library, method, and DRS files for the 731 compound-screening method.

## Instrument Operating Parameters

The instrument operating parameters used (unless noted otherwise) are listed in Table 1. These are starting conditions and may have to be optimized.

The split/splitless injection port was used for all work described here. It was chosen for its flexibility, allowing splitless injections for clean samples and split injections for dirty or high-concentration samples. It is also compatible with column backflushing. For all cases (except ambient headspace), the inlet liner used was the 4-mm id Siltek Cyclosplitter (Restek, part number 20706-214.1). This inlet liner was found to be of low activity, as it does not contain glass wool. Proper mixing for split injections is done by the internal liner geometry. Except as noted, split injections with a split ratio of 10:1 were used. For high matrix samples, this roughly matches the amount of matrix injected with the column capacity. If excess amounts of matrix are injected, the RTs of targets can shift. Split injection is also the easiest and most reliable way of screening samples for analytes ranging in volatility from gases to large polynuclear aromatic hydrocarbons (PAHs). Splitless injections are usually incompatible with the lowest boiling volatiles due to problems with the solvent. For low matrix samples where semivolatiles are of interest, splitless injections can be used.

For ambient headspace analysis [7], the conditions are listed separately at the bottom of Table 1. The liner used for ambient headspace was 1-mm id straight through (no glass wool) and Siltek coated (Restek, part number 20973-214.5). The auto injector parameters are critical in ambient headspace and are listed in Table 1. The volatiles samples run by ambient headspace were prepared as described in Reference 7.

While the targets in the table cover a very broad range of boiling points, it is usually not practical to screen for all of them in one run. This is because an analysis for semivolatile compounds would be done with a solvent that would occlude the lowest boiling volatiles in the table. Conversely, a method for injecting the lowest boiling compounds would usually not be suitable for the highest boiling. The MSD solvent delays listed in Table 1 are based on isooctane as the solvent in a semivolatiles analysis. If a lower boiling solvent is used, it may be possible to reduce these delays accordingly.

Some of the target compounds were found to have sufficiently high boiling points to require higher inlet and detector temperatures. These were the higher molecular weight PAHs, the polychlorinated dioxins, and the polychlorinated furans. For these compounds the inlet temperature, MS source, and transfer line were also raised to 300 °C. Without this increase in temperature, the compounds would exhibit tailing and in some cases reduction in signal. The trade-off with temperature is that the performance of some thermally labile compounds is degraded at the higher temperatures.

The MSD data acquisition sampling rates listed in Table 1 are for scan mode only. For volatiles analysis, the scan rate is increased one step. It is also increased one step when SIM/Scan is used. In SIM/Scan mode the SIM dwell time was set to 40 milliseconds for each ion monitored.

The microfluidic splitter parameters are chosen to provide the desired flow ratio between detectors while meeting the flow requirements of the detectors used. A primary consideration is to make sure that the flow to the MSD does not exceed ~4 mL/min while collecting analyte data. It was also desired to split the effluent equally between the DFPD and MSD in the 2-way split configuration. In the 3-way configuration, the split to the μECD was reduced

**Table 1. Gas Chromatograph and Mass Spectrometer Conditions**

| | Original 1X Method | 3X Method | 7X Method |
|---|---|---|---|
| **GC** | | | |
| Agilent Technologies 6890N | | | |
| 7683 Autoinjector and Tray | | | |
| **Inlet** | EPC Split/Splitless | EPC Split/Splitless | EPC Split/Splitless |
| Mode | Constant pressure | Constant pressure | Constant pressure |
| Injection type | Split | Split | Split |
| Injection volume (uL) | 1.0 | 1.0 | 1.0 |
| Inlet temp ( °C) | 250 | 250 | 250 |
| Pressure, nominal (psig) | 31.17 | 23.96 | 8.84 |
| RT Locking compound | Tripropyl phosphate | Tripropyl phosphate | Tripropyl phosphate |
| RT Locking time (min) | 12.874 | 4.291 | 1.839 |
| Split ratio | 10:1 | 10:1 | 10:1 |
| Gas saver | Off | Off | Off |
| Gas type | Helium | Helium | Helium |
| **Oven** | | | |
| Voltage (VAC) | 120 or 240 | 240 | 240 (and pillow) |
| Initial oven temp (°C) | 40 | 40 | 40 |
| Initial oven hold (min) | 2 | 0.667 | 0.286 |
| Ramp rate (°C/min) | 10 | 30 | 70 |
| Final temp (°C) | 300 | 300 | 300 |
| Final hold (min) | 15 | 5 | 2.143 |
| Total run time (min) | 43.00 | 14.33 | 6.14 |
| Equilibration time (min) | 0.5 | 0.5 | 0.5 |
| **Column** | | | |
| Type | HP 5-MS inert | HP 5-MS | HP 5-MS |
| Agilent part number | 19091S-433i | 19091S-431 | Custom |
| Length (m) | 30 | 15 | 5 |
| Diameter (mm) | 0.25 | 0.25 | 0.25 |
| Film thickness (um) | 0.25 | 0.25 | 0.25 |
| Outlet pressure (AUX EPC, psig) | 3.8 | 3.8 | 3.8 |
| **FPD or DFPD** | | | |
| Type | Single, Phosphorus | Single, Phosphorus | Dual, S and P |
| Temperature (°C) | 250 | 250 | 250 |
| Hydrogen flow (mL/min) | 75 | 75 | 75 |
| Air flow (mL/min) | 100 | 100 | 100 |
| Mode: Constant makeup flow | | | |
| Nitrogen makeup flow (mL/min) | 60 | 60 | 60 |
| Data rate (Hz) | 5 | 10 | 10 |
| **µECD** | | | |
| Temperature (°C) | 30 0 | 300 | N/A |
| Nitrogen makeup flow (mL/min) | 60 | 60 | N/A |
| Mode: Constant makeup flow | | | |
| Data rate (Hz) | 5 | 10 | N/A |
| AUX EPC Pressure | | | |
| Pressure (psig) | 3.8 | 3.8 | 3.8 |
| Gas type | Helium | Helium | Helium |

**Table 1. Gas Chromatograph and Mass Spectrometer Conditions (Continued)**

| | | | |
|---|---|---|---|
| **MSD** | | | |
| Agilent Technologies | 5975 inert MSD | 5975 inert MSD | 5973 inert with Performance Electronics |
| Tune file | Atune.U | Atune.U | Atune.U |
| Mode | Scan | Scan | Scan |
| Solvent delay (min) | 2.20 | 0.82 | 0.40 |
| EM voltage | Atune voltage | Atune voltage | Atune voltage |
| Low mass (amu) | 35 | 35 | 35 |
| High mass (amu) | 565 | 565 | 565 |
| Threshold | 0 | 0 | 0 |
| Sampling | 1 | 1 | 0 |
| Scans/s | 5.23 | 5.23 | 9.46 |
| Quad temp (°C) | 150 | 150 | 150 |
| Source temp (°C) | 230 | 230 | 230 |
| Transfer line temp (°C) | 280 | 280 | 280 |
| **Splitter** | | | |
| Type | 3 way | 3 way | 2 way |
| 6890N option number | 890 | 890 | 889 |
| Flow ratio | 1:1:0.1 MSD:FPD:ECD | 1:1:0.1 MSD:FPD:ECD | 1:1 MSD:DFPD |
| [Deactivated fused silica tubing] | | | |
| MSD restrictor length (m) | 1.44 | 1.44 | 1.44 |
| MSD restrictor id (mm) | 0.18 | 0.18 | 0.18 |
| FPD/DFPD restrictor length (m) | 0.53 | 0.53 | 0.53 |
| FPD/DFPD restrictor id (mm) | 0.18 | 0.18 | 0.18 |
| ECD restrictor length (m) | 0.51 | 0.51 | N/A |
| ECD restrictor id (mm) | 0.10 | 0.10 | N/A |
| **Ambient Headspace** | | | |
| Inlet | EPC Split/Splitless | | |
| Mode | Constant pressure | | |
| Injection type | Split | | |
| Inlet temp ( °C) | 200 | | |
| Pressure, nominal (psig) | 31.17 | | |
| RT locking compound | Tripropyl phosphate | | |
| RT locking time (min) | 12.874 | | |
| Split ratio | 1:1 | | |
| Gas saver | Off | | |
| Gas type | Helium | | |
| **Autoinjector** | | | |
| Sample washes | 0 | | |
| Sample pumps | 3 | | |
| Injection volume (µL) | 50 | | |
| Syringe size (µL) | 100 | | |
| PreInj Solvent A washes | 0 | | |
| PreInj Solvent B washes | 0 | | |
| PostInj Solvent A washes | 1 | | |
| PostInj Solvent B washes | 3 | | |
| Viscosity delay (s) | 5 | | |
| Plunger speed | Fast | | |
| Pre-injection dwell (min) | 0 | | |
| Post-injection dwell (min) | 0 | | |
| Sampling depth (mm) [critical!] | 20 | | |

to 1/10th that going to the MSD and FPD because of the extreme sensitivity of the detector. The lengths and diameters of the detector restrictors were calculated using the spreadsheet calculator included with the splitter.

The peak recognition windows used in the Agilent ChemStation were set to ±0.2 min and in AMDIS to 12 s. these values were found to be sufficiently wide enough to compensate for some RT drift yet narrow enough to minimize the number of false positives. The minimum match factors setting in AMDIS was set to 45. This value seemed to give the least number of false positives and false negatives.

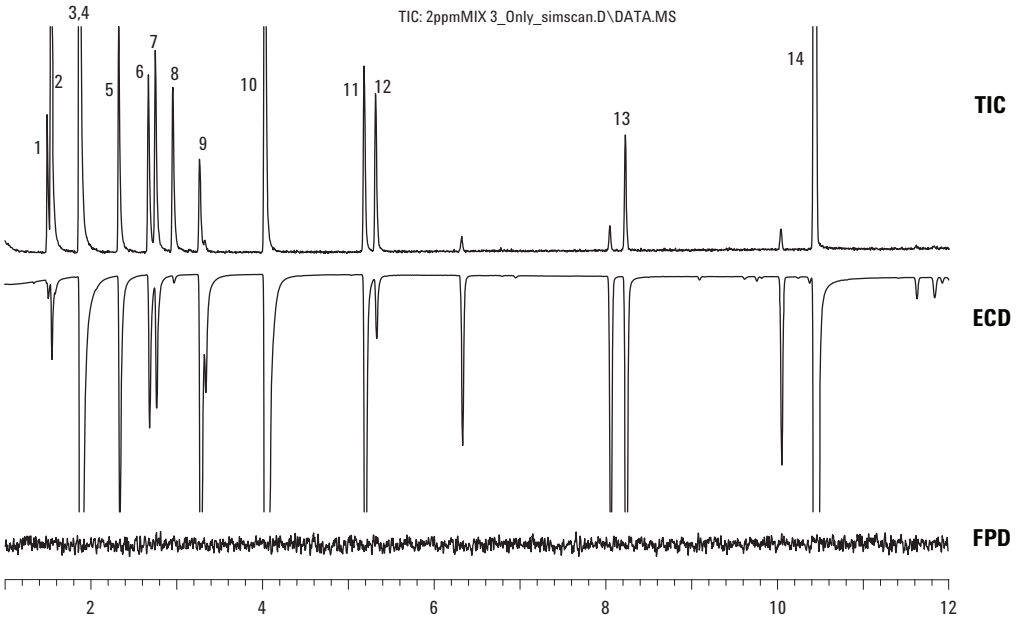
## Results

### Volatiles

To evaluate the HCD method for volatiles analysis, headspace injection was chosen. Headspace injections are usually done with an automated heated sampler specifically designed for the purpose. Ambient headspace [7] is a variant of the technique that uses a gastight syringe in the liquid autosampler and injects the headspace from a 2-mL vial. It is unheated, and is thus limited to compounds that are volatile at room temperature. Ambient headspace works well for the analysis of relatively non-polar volatiles in water. It is convenient for labs that need to screen samples for volatiles but do not have a dedicated headspace sampler. The conversion from liquid sampling to ambient headspace simply requires changing the inlet liner and the autosampler syringe.

Figure 2 shows the chromatograms from a run using the system in Figure 1A. A mixture of 14 halogenated volatiles was spiked into water at 2 ppm. Fifty microliters of the approximately 1 mL of headspace in the vial was injected. With the exception of peaks 3 and 4, which coelute, the compounds are well separated. The ECD chromatogram is inverted for comparison with the MS total ion chromatogram from the full-scan data. All of the volatiles respond on the ECD, although the response to compounds 1, 2, and 8 is significantly lower than for the rest of the compounds. In general with an ECD the response to a compound increases dramatically with the number of halogens in the molecule. Since none of the compounds contain phosphorus, there is no response on the FPD.

Figure 3 shows the DRS report for the sample. For each compound identified, the RT, Chemical Abstracts number (CAS#), and compound name are listed. A line is generated in the report if a compound is found by either the Agilent ChemStation, AMDIS, or both.




**Peak identities**

1) 1,2-Dichloroethane
2) 1,1-Dichloropropylene
3) 1,2-Dichloropropane
4) Trichloroethylene
5) *cis*-1,3-Dichloropropylene
6) *trans*-1,3-Dichloropropylene
7) 1,1,2-Trichloroethane
8) 1,3-Dichloropropane
9) 1,2-Dibromoethane
10) 1,1,1,2-Tetrachloroethane
11) 1,1,2,2-Tetrachloroethane
12) 1,2,3-Trichloropropane
13) 3-Chloro-1,2-dibromopropane
14) Hexachlorobutadiene

**Figure 2. Ambient headspace analysis of volatile organics in water, spiked at 2 ppm per component.**

MSD Deconvolution Report
Sample Name: 2 ppmVOA MIX 3 Only
Data File: C:\msdchem\1\DATA\042205AMHS_SimScan\2ppmMIX 3_Only_simscan.D
Date/Time: 05:13:03 PM Friday, Dec 9 2005

The NIST library was searched for the components that were found in the AMDIS target library.

| | | | Agilent | AMDIS | | NIST | |
|---|---|---|---|---|---|---|---|
| R.T. | Cas # | Compound Name | ChemStation Amount (ng) | Match | R.T. Diff sec. | Reverse Match | Hit Num. |
| 1.497 | 107062 | 1,2-Dichloroethane | 2.27 | 97 | 0.6 | 94 | 1 |
| 1.540 | 563586 | 1,1-Dichloropropylene | 7.6 | 100 | 0.5 | 96 | 1 |
| 1.867 | 78875 | 1,2-Dichloropropane | 4.92 | 95 | 0.7 | 90 | 1 |
| 1.871 | 79016 | Trichloroethylene | 7.58 | 99 | 0.6 | 91 | 1 |
| 2.330 | 10061015 | cis-1,3-Dichloropropylene | 4.39 | 98 | 1.0 | 92 | 2 |
| 2.677 | 10061026 | trans-1,3-Dichloropropylene | 3.3 | 97 | 1.5 | 94 | 1 |
| 2.758 | 79005 | 1,1,2-Trichloroethane | 2.82 | 99 | 1.7 | 92 | 1 |
| 2.961 | 142289 | 1,3-Dichloropropane | 3.39 | 98 | 1.5 | 92 | 1 |
| 3.273 | 106934 | 1,2-Dibromoethane | 2.6 | 91 | 1.5 | 76 | 4 |
| 4.032 | 630206 | 1,1,1,2-Tetrachloroethane | 5.15 | 100 | 1.9 | 94 | 1 |
| 5.187 | 79345 | 1,1,2,2-Tetrachloroethane | 2.38 | 99 | 2.3 | 89 | 1 |
| 5.322 | 96184 | 1,2,3-Trichloropropane | 1.89 | 98 | 2.5 | 94 | 1 |
| 6.323 | 76017 | pentachloroethane | 0.08 | 63 | 2.0 | 76 | 1 |
| 8.232 | 96128 | 3-Chloro-1,2-dibromopropane | 1.62 | 93 | 1.6 | 87 | 1 |
| 10.439 | 87683 | hexachlorobutadiene | 16.46 | 94 | 0.9 | 95 | 1 |
| | | | | | | | |
| | | | | | | | |

**Figure 3. DRS report for the analysis in Figure 2.**

The report shows that a compound has been determined as present by the Agilent ChemStation if a value appears in the Agilent ChemStation Amount column. This means the identification criteria set in the DATA ANALYSIS section of the method have been met. Typically the criteria are that the target ion is present and all three qualifier ions are present in ratios that fall within the percent uncertainty values for that compound.

The Agilent ChemStation Amount listed is a very rough approximation of the amount of the compound, in nanograms, reaching the MS. This is based on the response factor originally observed when the HCD table data was collected. Since valid quantitation requires recent recalibration of response factors on the specific instrument used for analysis, the numbers in this column should never be used to report concentrations of identified analytes. The error in these values can easiliy be a factor of 10 or higher. The purpose of the listed values is to give an approximate amount that can be used to guide standard preparation for quantitative calibration of the compound, if needed.

The match value listed under the AMDIS column is the degree to which the extracted (deconvolved) spectrum of the peak at that RT matched the spectrum in the HCD AMDIS target library. The higher this number, the better the spectra agree. The column “R.T. Diff sec.” lists the difference in seconds between the observed RT and that in the AMDIS target library. The lower this number, the better the RTs agree.

The NIST column lists the reverse-match quality of the extracted spectrum compared with the NIST05 main library spectrum with the same CAS#. The entry “Hit Num.” is the number of the hit in the NIST search results that has the same CAS# as the identified compound. The higher the reverse-match value and the lower the hit number, the better the extracted spectrum matches with NIST05. The NIST column serves as a second opinion on the identity of the extracted spectrum.

The analysis in Figure 2 is of course an easy one, but serves to demonstrate how the system works. All 14 spiked compounds were found by both the Agilent ChemStation and AMDIS. The certainty of identification is very high because:

- The target ion and three qualifier ions are present in appropriate ratios and at the appropriate time as determined by the Agilent ChemStation
- The deconvolved spectrum and the RT at which it appears closely matches the data in the AMDIS target library.

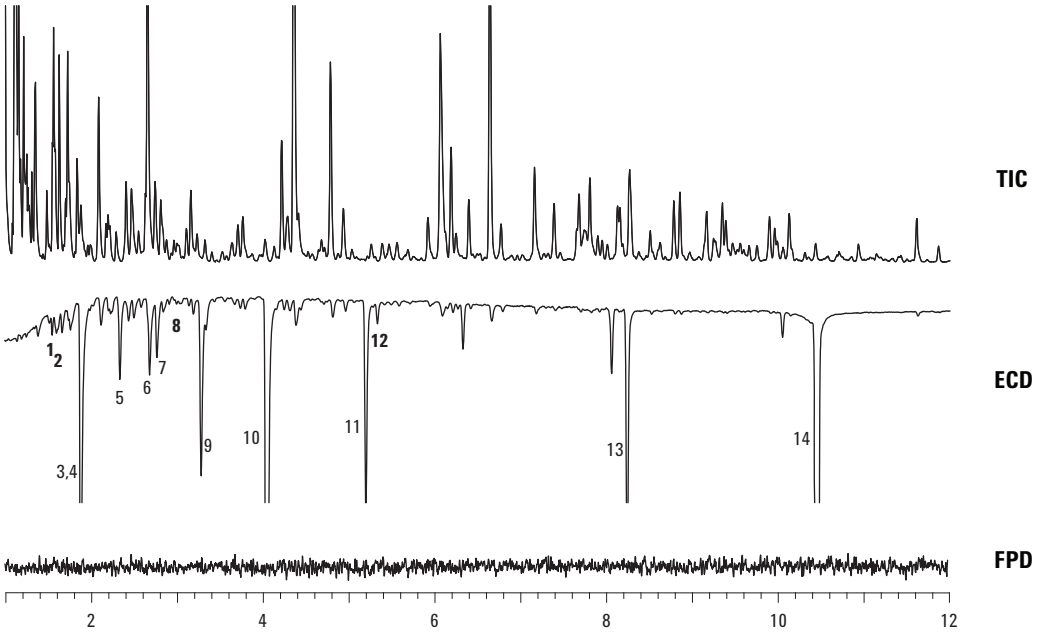
- The extracted spectrum of the identified compound also matches the spectrum with the same CAS # in the NIST05 library.
- The compounds all have a significant response on the ECD, as expected from their halogen content.

To challenge the system in a more realistic way, the effect of matrix and dilution of the analytes was studied. Additional samples were prepared that contained: the same 2-ppm mixture of analytes plus 100 ppm of pump gasoline; 100 ppb of analytes only; and 100 ppb of analytes plus 100 ppm of pump gasoline.

Figure 4 shows the chromatograms from the 100 ppb of analytes with 100 ppm of gasoline. The complexity of the TIC chromatogram illustrates the severe matrix challenge presented by the thousand-fold excess of gasoline. In the ECD chromatogram, interference peaks are now apparent. However, with the exception of peaks 1, 2, 8, and 12, all of the analytes peaks are still visible above the matrix interferences.

Table 2 summarizes the results from the matrix and dilution experiments. In the sample that was 2 ppm of analytes with 100 ppm of gasoline, the Agilent ChemStation (column labeled Quant) found all but two of the compounds. Those two compounds had qualifier ions out of range due to interferences from the matrix. AMDIS successfully found all 14 compounds. Also, with the exception of compound 8, all of the analytes were clearly visible above the matrix responses on the ECD chromatogram.

In the sample that contained 100 ppb of analytes but without gasoline, quant found 7 of the 14 analytes. Using full-scan data, the signal to noise ratio for most of the analytes at the 100-ppb level is very low. This results in difficulties with finding the qualifier ions in ratios that fall within the specified uncertainty range in the quant calibration table. AMDIS found 11 of the 14 compounds. Peak 3 was not found due to a severe overlap with the coeluting peak number 4. Peaks 9 and 13 were missed by AMDIS because the signal to noise ratio was too low.




**Peak identities**

1) 1,2-Dichloroethane
2) 1,1-Dichloropropylene
3) 1,2-Dichloropropane
4) Trichloroethylene
5) *cis*-1,3-Dichloropropylene
6) *trans*-1,3-Dichloropropylene
7) 1,1,2-Trichloroethane
8) 1,3-Dichloropropane
9) 1,2-Dibromoethane
10) 1,1,1,2-Tetrachloroethane
11) 1,1,2,2-Tetrachloroethane
12) 1,2,3-Trichloropropane
13) 3-Chloro-1,2-dibromopropane
14) Hexachlorobutadiene

**Figure 4. Ambient headspace analysis of volatile organics in water. Analytes at 100 ppb plus pump gasoline at 100 ppm.**

**Table 2. Effect of Matrix and Concentration on DRS Results**

| | | | 2 ppm STD only | | 2 ppm STD with 100 ppm gasoline | | 100 ppb STD only | | 100 ppb STD with 100 ppm gasoline | |
|---|---|---|---|---|---|---|---|---|---|---|
| RT (min) | Compound | Peak Number | Quant (ng) | AMDIS (match) | Quant (ng) | AMDIS (match) | Quant (ng) | AMDIS (match) | Quant (ng) | AMDIS (match) |
| 1.491 | 1,2-Dichloroethane | 1 | 2.27 | 97 | 2.47 | 93 | | 73 | | 65 |
| 1.536 | 1,1-Dichloropropylene | 2 | 7.60 | 100 | 7.34 | 98 | 0.37 | 89 | | 85 |
| 1.793 | 1,2-Dichloropropane | 3 | 4.92 | 95 | 5.59 | 64 | 0.21 | **Overlap** | | **Overlap** |
| 1.863 | Trichloroethylene | 4 | 7.58 | 99 | 7.71 | 97 | 0.40 | 90 | 0.30 | 82 |
| 2.317 | *cis*-1,3-Dichloropropylene | 5 | 4.39 | 98 | 4.81 | 98 | 0.21 | 88 | 0.23 | 74 |
| 2.658 | *trans*-1,3-Dichloropropylene | 6 | 3.30 | 97 | | 84 | | 53 | | **Overlap** |
| 2.735 | 1,1,2-Trichloroethane | 7 | 2.82 | 99 | 3.05 | 96 | 0.12 | 72 | | **Overlap** |
| 2.938 | 1,3-Dichloropropane | 8 | 3.39 | 98 | 3.50 | 97 | | 66 | 0.22 | 46 |
| 3.250 | 1,2-Dibromoethane | 9 | 2.60 | 91 | | 95 | | **S/N** | | 66 |
| 4.003 | 1,1,1,2-Tetrachloroethane | 10 | 5.15 | 100 | 5.32 | 99 | | 89 | 0.31 | 88 |
| 5.151 | 1,1,2,2-Tetrachloroethane | 11 | 2.38 | 99 | 2.41 | 98 | | 48 | 0.19 | 53 |
| 5.283 | 1,2,3-Trichloropropane | 12 | 1.89 | 98 | 1.85 | 98 | 0.07 | 79 | 0.14 | 75 |
| 8.208 | 3-Chloro-1,2-dibromopropane | 13 | 1.62 | 93 | 2.40 | 90 | | **S/N** | | 59 |
| 10.435 | Hexachlorobutadiene | 14 | 16.46 | 94 | 3.54 | 89 | 0.65 | 75 | 0.36 | 52 |
| | Total Found | | 14 | 14 | 12 | 14 | 7 | 10 | 7 | 11 |

With 100 ppm of gasoline added to the 100-ppb sample, quant again found 7 of the 14 compounds and AMDIS again found 11 of the 14. Curiously, in both cases some of the compounds missed in the absence of matrix were now found. It is possible that the presence of matrix enhances the concentration of some of the analytes in the headspace. The compounds missed in quant were again the result of low signal to noise and/or interference. In AMDIS the three missed peaks were due to severe interferences from the gasoline. As indicated above, the ECD response from 10 of the 14 compounds was still visible above the peaks due to interferences.

### SIM/Scan

The quant data in Table 2 was generated using full scan mode. Peak 13 was missed in quant due to low signal to noise ratio. SIM/Scan mode can be used to collect SIM data simultaneously with the scan data. The 100 ppb plus 100-ppm gasoline sample was run in SIM/Scan mode with SIM groups for each of the 14 analytes. Figure 5 compares the target and qualifier extracted ion chromatograms in both modes with the ECD response for peak 13.

The signal-to-noise (peak to peak) for the target ion increases from 34 in full scan mode to 433 in SIM mode. The peaks lost in quant due to low signal-to-noise were all recovered in SIM mode. This example demonstrates the power of SIM/Scan when looking for high-priority targets at low levels. If necessary, the ECD could also be used for quantitation, as it has a high signal to noise ratio and is free from interference.

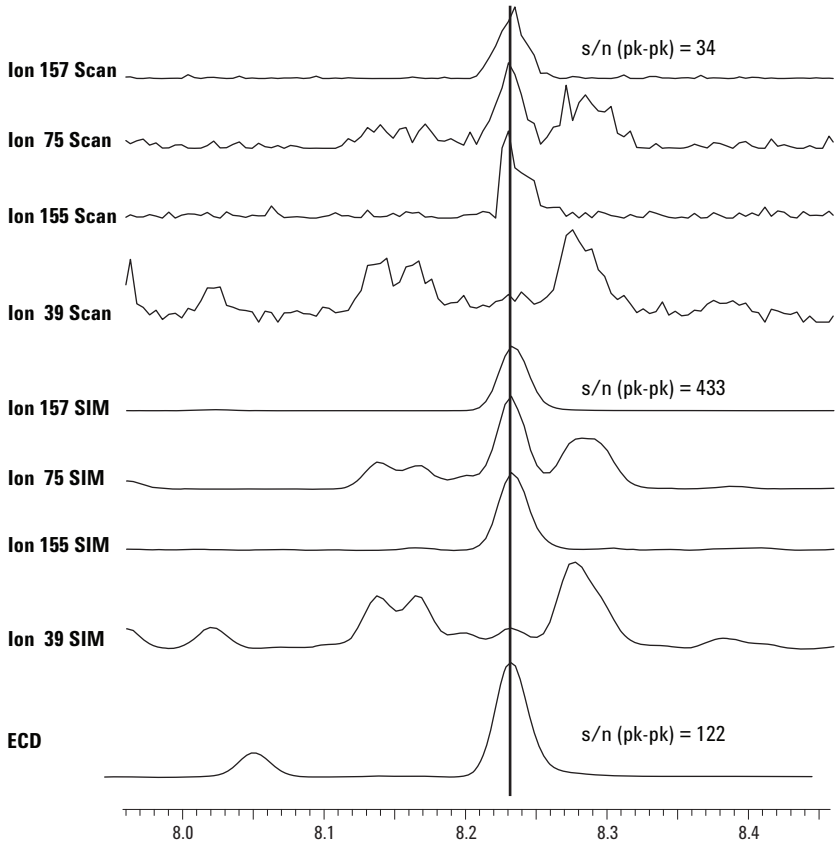



**Figure 5. Target and qualifier extracted ion chromatograms for peak 13 (3-Chloro-1,2-dibromopropane) in Figure 4. SIM, scan, and ECD data collected simultaneously.**

## AMDIS

Figure 6 illustrates the ability of AMDIS to clean the interference ions from the spectrum of an analyte. The raw spectrum at the top of Figure 6 was taken at the apex of peak 13 in the 100 ppb plus 100-ppm gasoline sample. When searched against the NIST05 library using the NIST search program, the actual compound (3-Chloro-1,2-dibromopropane) was the 70th hit in the search results. Using manual subtraction of nearby spectra in the Agilent ChemStation data analysis program improved the quality of the spectrum so that it was now the second hit when searched in NIST. This is a tedious process, however, when dealing with a large number of analytes. The spectrum as deconvolved by AMDIS is shown in Figure 6 above the NIST05 library spectrum. When this spectrum is searched, it is the first hit in the results. The automated deconvolution provided by AMDIS saves an enormous amount of time in the data review process.

### Fast Methods

When a retention time locked database is constructed, the RTs are (or at least should be) collected under the highest resolution conditions expected for the application. If the database is collected under constant pressure mode, method translation can then be used to adjust the speed of the method to meet the needs of different situations.

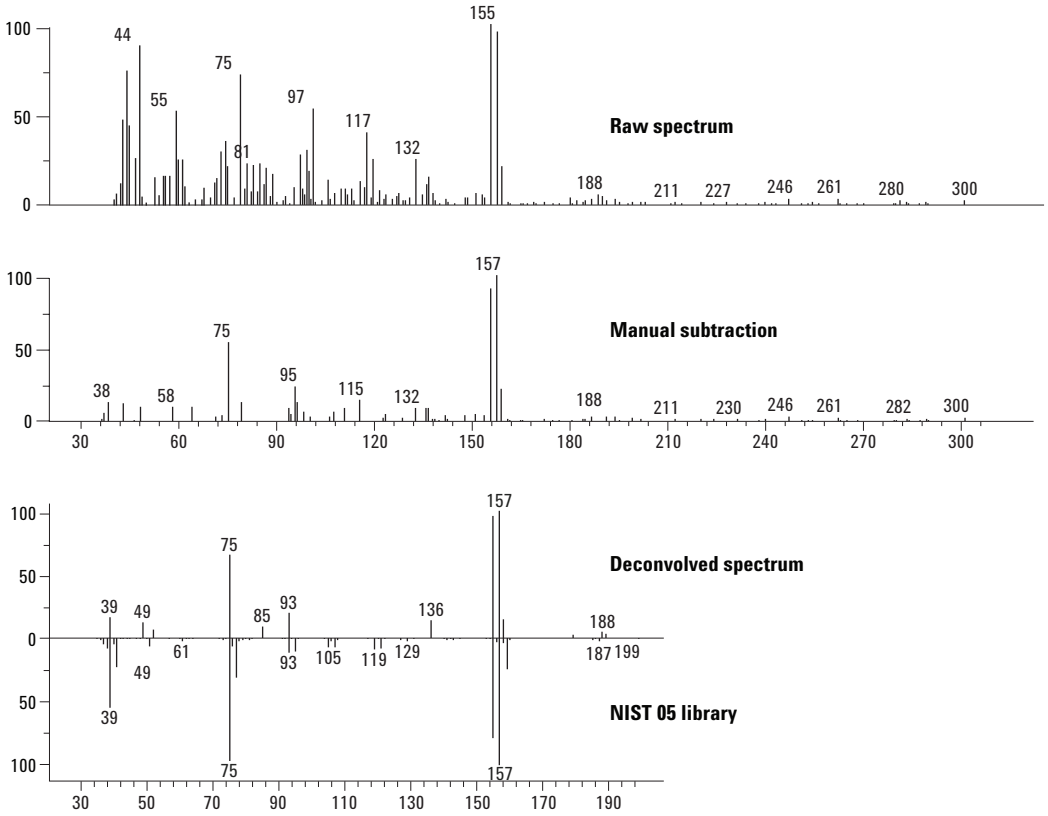



**Figure 6. Comparison of raw, manually subtracted, AMDIS deconvoluted, and NIST05 reference spectra for peak 13 (3-Chloro-1,2-dibromopropane) in Figure 4.**

The 3X method uses RTs in its database that are simply the RTs from the 1X method divided by exactly 3. The 7X method likewise uses RTs that are 1/7 of those in the original database. The quality of RTs matching between the two new faster methods and the new divided databases is demonstrated in Figure 7. Three different mixtures containing 13 chlorinated hydrocarbons and 36 pesticides were run with the two methods. The RTs were compared to those in the two new databases. The graph at the top of Figure 7 plots the database RT on the x-axis versus the difference of the measured RT from the database on the y axis.

If the RT matching were perfect, the plot would be a straight horizontal line at zero height on the y axis. The maximum deviation from the table values for the 3X method was –0.047 min. The plot indicates that a peak recognition window of ±0.1 min should be sufficient. The maximum deviation in the 7X plot at the bottom of Figure 8 is +0.032 min indicating that the same peak recognition window could be used here as well. In general the RTs in scaled methods agree very well with the predicted RTs.

The conditions for the two higher-speed methods were chosen to increase speed while maintaining the same column capacity. The capacity is important for both the dynamic range of quantitative measurements and for minimizing analyte RT shifts in samples with high levels of matrix. In gas chromatography, the well-known triangle of speed, resolution, and capacity dictates that if the capacity is to be maintained and the speed is to be increased, then the resolution will decrease.

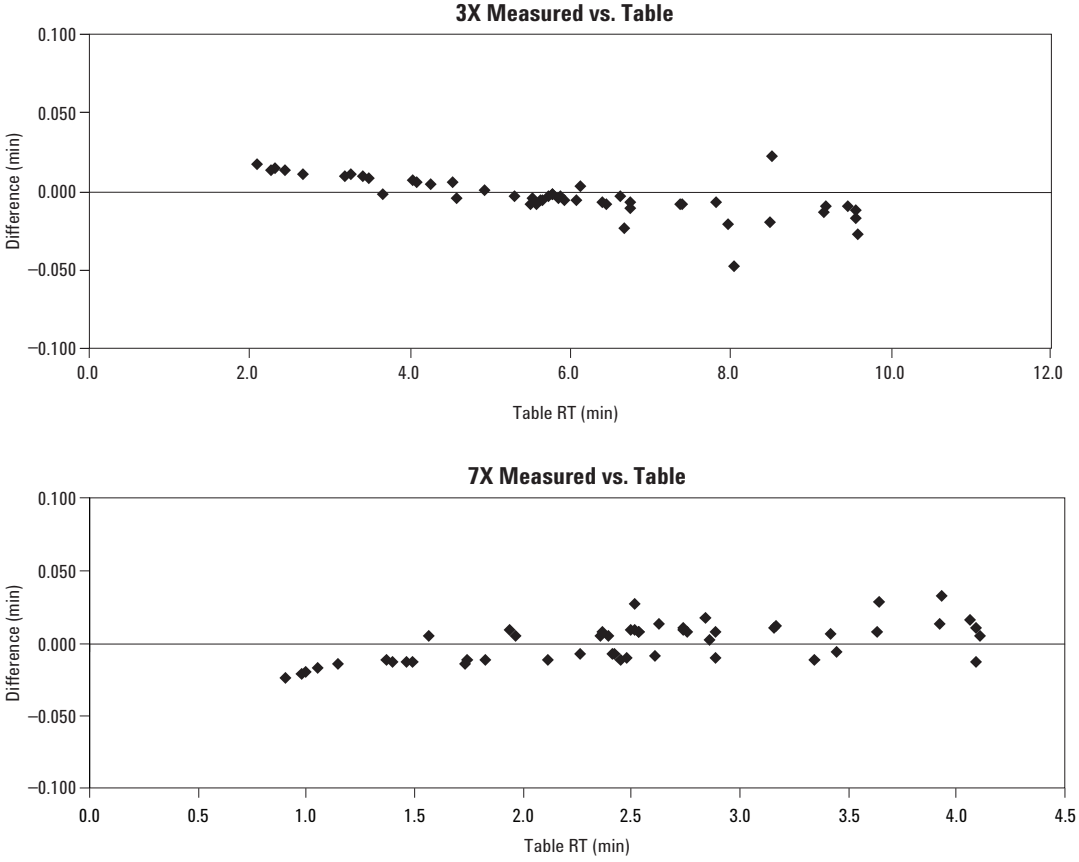
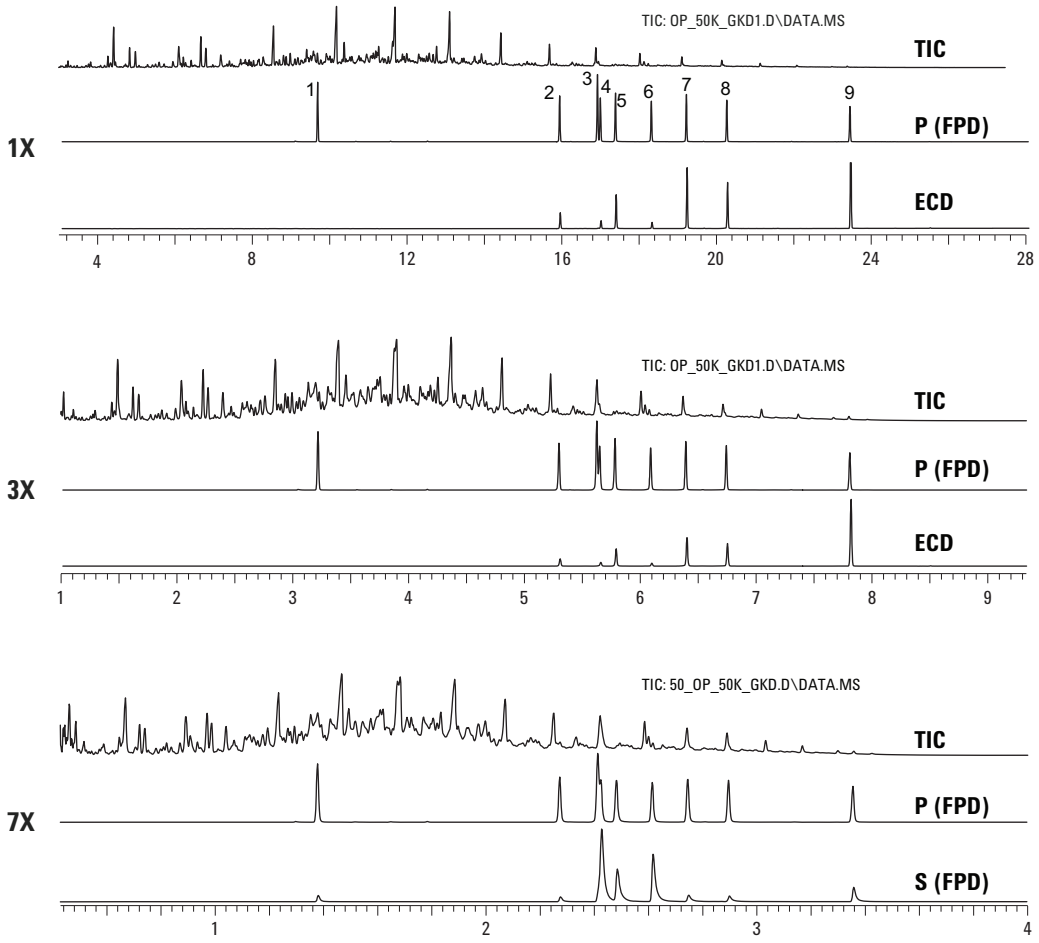



**Figure 7. Difference between scaled HCD table and experimental retention times for 50 compound test set. Y axis is table value minus experimental, X axis is table RT. Top plot is 3X, bottom is 7X.**

Figure 8 shows three sets of chromatograms using the HCD database at three different speeds. The sample consists of nine organophosphorus pesticides (identified in the caption to Figure 8) at 50 ppm and a matrix consisting of an equal volume mixture of gasoline, kerosene, and diesel fuel spiked at 50,000 ppm total mixture. The 1X and 3X data were collected on the three-way splitter instrument and the 7X was collected on the DFPD instrument. All nine compounds also contained sulfur as can be seen in the DFPD sulfur chromatogram at the bottom of Figure 8. Note that the sulfur tails somewhat compared to the phosphorus.




**Peak identities**

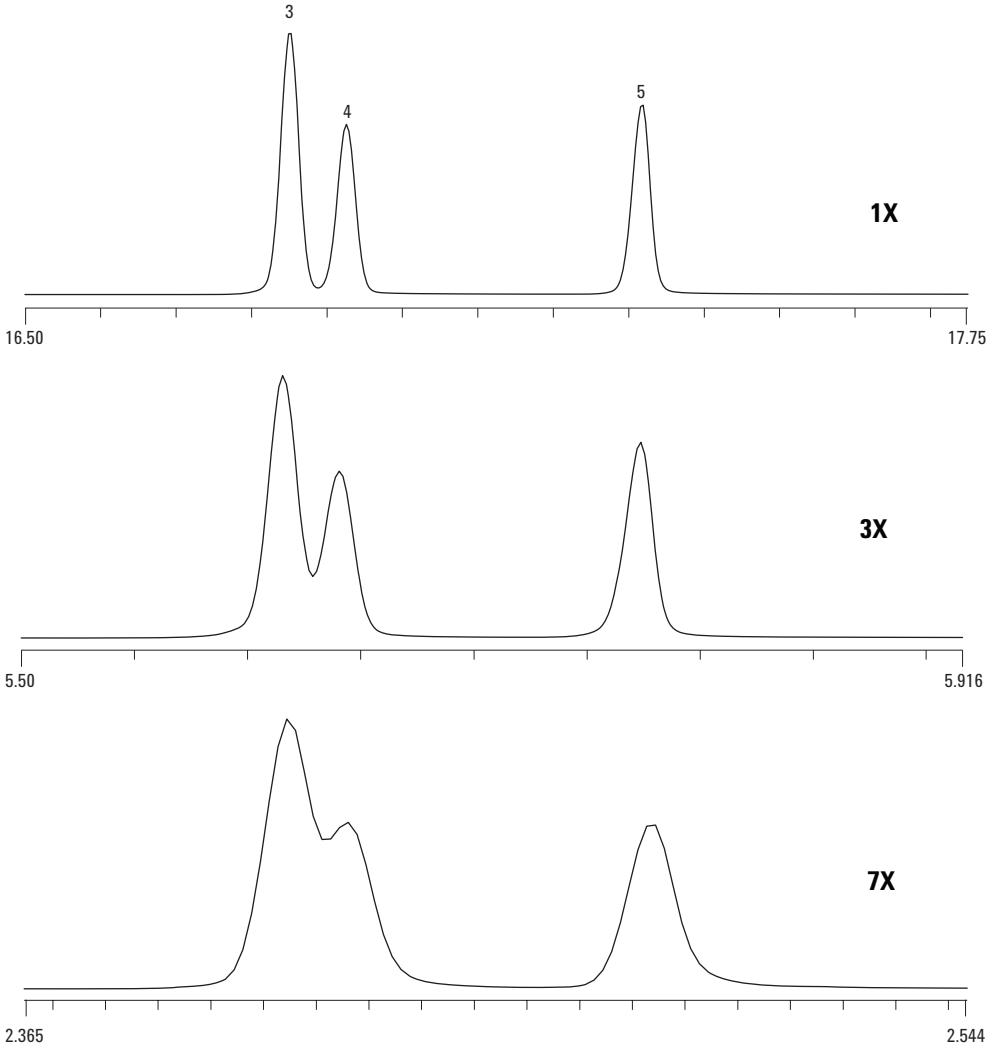
1) O,O,O-triethyl phosphorothioate
2) Thionazin
3) Sulfotepp
4) Phorate
5) Dimethoate
6) Disulfoton
7) Methyl parathion
8) Parathion
9) Famphur

**Figure 8. Comparison of 1X, 3X, and 7X chromatograms. 1X and 3X were run on GC/MS/ECD/FPD system, 7X on GC/MS/DFPD.**

Figure 9 expands the RT region of the phosphorous chromatogram containing peaks 3, 4, and 5 from Figure 8. The decrease in resolution with increasing speed is clearly evident.

If only the standard target and three qualifier ion approach is used, the loss in resolution causes a significant problems. With the 1X method, all nine of the analytes are identified and eight false positives are reported. With the 3X method, all analytes are again found but now with 25 false positives. With the significantly decreased resolution of the 7X method, only seven of the nine analytes are identified and 48 false positives are reported.

The situation is much different when using the approach described here. Even in the worst situation, the 7X method, AMDIS finds all nine analytes with high-quality matches and only three false positives. The DRS report for the 7X analysis is shown in Table 3. To simplify the table, the 48 false positives that only appear in the quant column are not shown. The analyte compounds are shown in bold. All show close RT and high-quality spectral matches to both the AMDIS target library and to the NIST05 library.




**Peak identities**
3) Sulfotepp
4) Phorate
5) Dimethoate

**Figure 9. Comparison of FPD phosphorus chromatograms from 1X, 3X, and 7X runs in Figure 8.**

**Table 3. DRS Report for 7x Analysis of 50 ppm Pesticides In 50,000 ppm Gasoline/Kerosine/Diesel Matrix**

| RT | Cas # | Compound name | Agilent ChemStation amount (ng) | AMDIS match | RT Diff (sec.) | NIST reverse match | Hit number |
|---|---|---|---|---|---|---|---|
| 0.973 | 98862 | Acetophenone | | 71 | –9.5 | 74 | 50 |
| **1.380** | **126681** | **O,O,O-triethyl phosphorothioate** | **13.92** | **69** | **0.7** | **71** | **1** |
| 1.520 | 94597 | Safrole | | 46 | –7.6 | | |
| 1.520 | 52417502 | Benzeneacetaldehyde, à,2,5-trimethyl- | | | | 74 | 1 |
| 2.113 | 132649 | Dibenzofuran | 0.35 | 64 | 0.6 | 80 | 3 |
| 2.138 | 90437 | o-Phenylphenol | | 55 | 2.3 | | |
| 2.138 | 2131411 | Naphthalene, 1,4,5-trimethyl- | | | | 85 | 1 |
| **2.275** | **297972** | **Thionazin** | **89.2** | **91** | **0.5** | **85** | **1** |
| **2.417** | **3689245** | **Sulfotepp** | | **88** | **0.5** | **83** | **1** |
| **2.427** | **298022** | **Phorate** | **23.31** | **90** | **0.6** | **85** | **1** |
| **2.485** | **60515** | **Dimethoate** | **27.34** | **84** | **0.7** | **85** | **1** |
| **2.619** | **298044** | **Disulfoton** | **22.7** | **92** | **0.6** | **88** | **1** |
| **2.748** | **298000** | **Methyl parathion** | **25.12** | **92** | **0.6** | **82** | **1** |
| **2.901** | **56382** | **Parathion (ethyl)** | | **91** | **0.7** | **85** | **1** |
| **3.360** | **52857** | **Famphur** | | **93** | **0.8** | **85** | **1** |
| | | (48 quant-only hits not shown) | | | | | |

The peak at 0.973 minutes is a reasonable spectral match to acetophenone, but the large time difference and being the 50th hit in the NIST search results suggests that this is not the compound. The peak at 1.520 min is a poor spectral match with a large time difference. The absence of a NIST reverse search and hit entry means that the listed compound was not in the top 100 hits in the NIST search. The next compound listed at 1.520 min is the top entry from the NIST search. It is quite clear that safrole is not present.

The peak at 2.113 min, dibenzofuran, was not one of the analytes added to the sample. However, it probably is present in the diesel fuel matrix. Its presence is supported by both reasonably good spectral matches and close time matching with a database.

The last extraneous peak at 2.138 min is also questionable. The time match is somewhat poor and the NIST reverse search suggests the identification is not correct.

All nine analytes are detected with the FPD on both the phosphorus and sulfur chromatograms. All analytes except peak 1 are detected selectively on the ECD as well.

These results suggest that while the loss of resolution in going to 7X is unacceptable when using only conventional screening approaches, with the method discussed here, it is a viable option. By using the DRS report combined with the selective detector data, the number of false positives and false negatives are significantly reduced. For those situations where speed is a critical factor, for example in response to homeland security incidents, the fastest method may be the one of choice.

For many laboratories, the 3X method would be an attractive choice. It has higher resolution than the 7X and higher speed than the 1X and still allows the use of two GC detectors in parallel with the MSD. It also only requires a 240V oven, not the repositioning of the MSD to the back position.

## Conclusions

The systems described here offer several advantages when screening samples for the presence of hazardous chemicals. The advantages derive from a combination of techniques that result in both faster and more accurate screening results.

- Retention time locked target database of 731 hazardous chemicals for screening with MS
- Microfluidic splitter - using selective detection simultaneous with MS data for added confirmation, finding non-target suspect compounds, and alternate quantitation
- SIM/Scan - Acquire SIM data on high priority targets simultaneously with scan data. Saves time by eliminating need to run samples in both modes.
- DRS - automated deconvolution dramatically increases accuracy of target identification, even in the most challenging matrices. The reduction of data interpretation from hours to minutes is especially useful for response to hazardous chemical incidents.
- Fast chromatography using shorter columns, faster ovens, and backflushing to greatly reduce run times.

This combination of techniques offers a viable solution to the hazardous chemicals challenge.

## References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# Lists of Compounds in Databases

**Volatiles:**
EPA 502/524, 60 compounds

1,1,1,2-Tetrachloroethane
1,1,1-Trichloroethane
1,1,2,2-Tetrachloroethane
1,1,2-Trichloroethane
1,1-Dichloroethane
1,1-Dichloroethylene
1,1-Dichloropropylene
1,2,3-trichlorobenzene
1,2,3-Trichloropropane
1,2,4-trichlorobenzene
1,2,4-trimethylbenzene
1,2-Dibromoethane
1,2-dichlorobenzene
1,2-Dichloroethane
1,2-Dichloropropane
1,3,5-trimethylbenzene
1,3-dichlorobenzene
1,3-Dichloropropane
1,4-dichlorobenzene
2,2-Dichloropropane
2-chlorotoluene
3-Chloro-1,2-dibromopropane
4-chlorotoluene
Benzene
Bromobenzene
Bromochloromethane
Bromodichloromethane
Bromoform
Bromomethane
Carbon Tetrachloride
Chlorobenzene
Chlorodibromomethane
Chloroethane
Chloroform
Chloromethane
*cis*-1,2-Dichloroethylene
*cis*-1,3-Dichloropropylene
Dibromomethane
Dichlorodifluoromethane
Ethylbenzene
Hexachlorobutadiene
Isopropylbenzene
Methylene Chloride
m-xylene
Naphthalene
n-butylbenzene
n-propylbenzene
o-Xylene
p-isopropyltoluene
p-xylene
Styrene
*tert*-butylbenzene
Tetrachloroethylene
Toluene
*trans*-1,2-Dichloroethylene
*trans*-1,3-Dichloropropylene
Trichloroethylene
Trichlorofluoromethane
Vinyl chloride

**Semivolatiles:**
EPA 8270C Appendix IX,
140 compounds
1,2,4,5-tetrachlorobenzene
1,2,4-trichlorobenzene
1,2-dichlorobenzene
1,3,5-trinitrobenzene
1,3-dichlorobenzene
1,4-dichlorobenzene
1,4-naphthoquinone
1-naphthylamine
2,3,4,6-tetrachlorophenol
2,4,5-trichlorophenol
2,4,6-trichlorophenol
2,4-dichlorophenol
2,4-dimethylphenol
2,4-dinitrophenol
2,4-dinitrotoluene
2,6-dichlorophenol
2,6-dinitrotoluene
2-acetylaminofluorene
2-chloronaphthalene
2-chlorophenol
2-methyl-4,6-dinitrophenol
2-methylnaphthalene
2-naphthylamine
2-nitroaniline
2-nitrophenol
2-picoline
3,3'-dichlorobenzidine
3,3'-dimethylbenzidine
3-methylcholanthrene
3-nitroaniline
4,4'-DDD
4,4'-DDE
4,4'-DDT
4-aminobiphenyl
4-bromophenyl phenyl ether
4-chloro-3-methylphenol
4-chloroaniline
4-chlorophenyl phenyl ether
4-nitroaniline
4-nitrophenol
4-nitroquinoline-1-oxide
5-nitro-o-toluidine
7,12-dimethylbenz[a]anthracene
a,a-dimethylphenethylamine
Acenaphthene
Acenaphthylene
Acetone
Acetophenone
Aldrin
Alpha-BHC (alpha-HCH)
Aniline
Anthracene
Aramite (total)
Benz[a]anthracene
Benzene
Benzo[a]pyrene
Benzo[b]fluoranthene
Benzo[ghi]perylene
Benzo[k]fluoranthene
Benzyl alcohol
Beta-BHC (beta-HCH)
Bis(2-chloroethoxy)methane
Bis(2-chloroethyl) ether
Bis(2-chloroisopropyl) ether
Bis(2-ethylhexyl)phthalate
Butyl benzyl phthalate
Chlorobenzilate
Chrysene
Delta-BHC (delta-HCH)
Diallate (total)
Dibenz[a,h]anthracene
Dibenzofuran
Dieldrin
Diethyl phthalate
Dimethoate
Dimethyl phthalate
Di-n-butyl phthalate

Di-n-octyl phthalate
Dinoseb
Diphenylamine
Disulfoton
Endosulfan I
Endosulfan II
Endosulfan sulfate
Endrin
Endrin aldehyde
Ethyl methanesulfonate
Famphur
Fluoranthene
Fluorene
Gamma-BHC (lindane)
Heptachlor
Heptachlor epoxide -isomer B
Hexachlorobenzene
Hexachlorobutadiene
Hexachlorocyclopentadiene
Hexachloroethane
Hexachlorophene
Hexachloropropene
Indeno[1,2,3-cd]pyrene
Isodrin
Isophorone
Isosafrole
Kepone
m-cresol (3-methylphenol)
m-dinitrobenzene
Methapyrilene
Methoxychlor
Methyl methanesulfonate
Methyl parathion
Naphthalene
Nitrobenzene
N-nitrosodiethylamine
N-nitrosodimethylamine
N-nitrosodi-n-butylamine
N-nitrosodi-n-propylamine
N-nitrosodiphenylamine
N-nitrosomethylethylamine
N-nitrosomorpholine
(4-nitrosomorpholine)
N-nitrosopiperidine
(1-nitrosopiperidine)
N-nitrosopyrrolidine (1-nitrosopyrrolidine)
O,O,O-triethyl phosphorothioate
o-cresol (2-methylphenol)
o-toluidine
p-(dimethylamino)azobenzene
Parathion (ethyl)
p-cresol (4-methylphenol)
Pentachlorobenzene
Pentachloroethane
Pentachloronitrobenzene
Pentachlorophenol
Phenacetin
Phenanthrene
Phenol
Phorate
p-phenylenediamine
Pronamide
Pyrene
Pyridine
Safrole
Sulfotepp
Thionazin

**Chlorinated Dioxins and Furans:**
EPA 8282, 19 compounds

2,3,7,8-Tetrachlorodibenzo-p-dioxin
1,2,3,7,8-Pentachlorodibenzo-p-dioxin
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin
Octachlorodibenzo-p-dioxin
2,3,7,8-Tetrachlorodibenzofuran
1,2,3,7,8-Pentachlorodibenzofuran
1,2,3,4,7,8-Hexachlorodibenzofuran
1,2,3,4,6,7,8-Heptachlorodibenzofuran
Octachlorodibenzofuran

**Polychlorinatedbiphenyls:**
EPA 8082, 19 compounds

2-chlorobiphenyl
2,3-dichlorobiphenyl
2,2',5-trichlorobiphenyl
2,4',5-trichlorobiphenyl
2,2',5,5'-tetrachlorobiphenyl
2,2',3,5'-tetrachlorobiphenyl
2,3',4,4'-tetrachlorobiphenyl
2,2',4,5,5'-pentachlorobiphenyl
2,2',3,4,5'-pentachlorobiphenyl
2,3,3',4',6-pentachlorobiphenyl
2,2',3,5,5',6-hexachlorobiphenyl
2,2',4,4',5,5'-hexachlorobiphenyl
2,2',3,4,5,5'-hexachlorobiphenyl
2,2',3,4,4',5'-hexachlorobiphenyl
2,2',3,4',5,5',6-heptachlorobiphenyl
2,2',3,4,4',5',6-heptachlorobiphenyl
2,2',3,4,4',5,5'-heptachlorobiphenyl
2,2',3,3',4,4',5-heptachlorobiphenyl
2,2',3,3',4,4',5,5',6-nonachlorobiphenyl

**Pesticides:**
Agilent RTL pesticide database
(adapted), 567 compounds
1,2,4-Trichlorobenzene
1,2-Dibromo-3-chloropropane
17a-Ethynylestradiol
2-(1-naphthyl)acetamide
2-(2-Butoxyethoxy)ethyl thiocyanate
2-(Octylthio)ethanol
2,3,4,5-Tetrachlorophenol
2,3,4,6-Tetrachlorophenol
2,3,5,6-Tetrachlorophenol
2,3,5-Trichlorophenol
2,3,5-Trimethacarb
2,3,5-Trimethylphenyl methyl carbamate
(Trimethacarb)
2,3,7,8-Tetrachlorodibenzofuran
2,3,7,8-Tetrachlorodibenzo-p-dioxin
2,4,5-T methyl ester
2,4,5-Trichlorophenol
2,4,6-Trichlorophenol
2,4-D methyl ester
2,4-D sec-butyl ester
2,4-DB methyl ester
2,4-Dichlorophenol
2,4-Dichlorophenyl benzenesulfonate
2,4-Dimethylaniline
2,6-Dichlorobenzonitrile
2,6-Dimethylaniline
2-[3-Chlorophenoxy]propionamide
2-Ethyl-1,3-hexanediol
2-Hydroxyestradiol
2-Methylphenol
2-Phenoxypropionic acid
3,4,5-Trimethacarb
3,4-Dichloroaniline
3,5-Dichloroaniline
3-Chloroaniline
3-Hydroxycarbofuran
4,4'-Dichlorobenzophenone
4,6-Dinitro-o-cresol (DNOC)
4-Chloroaniline
4-Methylphenol
5,7-Dihydroxy-4'-methoxyisoflavone
9,10-Anthraquinone
Acephate
Acetochlor
Acifluorfen methyl ester
Alachlor
Aldrin
Allidochlor
Ametryn
Amidithion
Aminocarb
Amitraz
Ancymidol
Anilazine
Aniline
Atraton
Atrazine
Azaconazole
Azamethiphos
Azinphos-ethyl
Azinphos-methyl Aziprotryne
Azobenzene
Barban

Benalaxyl
Benazolin-ethyl
Bendiocarb
Benfluralin
Benfuresate
Benodanil
Bentazone
Bentazone methyl derivative
Benthiocarb
Benzo(a)pyrene
Benzophenone
Benzoylprop ethyl
b-Estradiol
BHC alpha isomer
BHC beta isomer
BHC delta isomer
Bifenox
Bifenthrin
Binapacryl
Bioallethrin
Bioallethrin S-cyclopentenyl isomer
Bioresmethrin
Biphenyl
Bis(2-ethylhexyl)phthalate
Bisphenol A
Bitertanol I
Bitertanol II
Bromacil
Bromobutide
Bromocyclen
Bromophos
Bromophos-ethyl
Bromopropylate
Bromoxynil
Bromoxynil octanoic acid ester
Buprofezin
Butachlor
Butamifos
Butoxycarboxim
Butralin
Butyl benzyl phthalate
Butylate
Butylated hydroxyanisole
Captafol
Captan
Carbaryl
Carbetamide
Carbofuran
Carbofuran-3-keto
Carbophenothion
Carbosulfan
Carboxin
Chinomethionat
Chloramben methyl ester
Chloranocryl
Chlorbenside
Chlorbromuron
Chlorbufam
Chlordecone
Chlordimeform
Chlorfenethol
Chlorfenprop-methyl
Chlorfenson
Chlorfenvinphos
Chlorflurecol-methyl ester
Chlormefos
Chlornitrofen
Chlorobenzilate
Chloroneb
Chloropropylate
Chlorothalonil
Chlorotoluron
Chlorpropham
Chlorpyrifos
Chlorpyrifos Methyl
Chlorthal-dimethyl
Chlorthiamid
Chlorthion
Chlorthiophos
Chlorthiophos sulfone
Chlorthiophos sulfoxide
Chlozolinate
*cis*-Chlordane
Clomazone
Coumaphos
Crimidine
Crotoxyphos
Crufomate
Cyanazine
Cyanofenphos
Cyanophos
Cycloate
Cycluron
Cyfluthrin I
Cyfluthrin II
Cyfluthrin III
Cyfluthrin IV
Cyhalothrin I (lambda)
Cymoxanil
Cypermethrin I
Cypermethrin II
Cypermethrin III
Cypermethrin IV
Cyprazine
Cyprofuram
Cyromazine
d-(*cis-trans*)-Phenothrin-I
d-(*cis-trans*)-Phenothrin-II
Dazomet
Decachlorobiphenyl
Deltamethrin
Demephion
Demeton-S
Demeton-S-methylsulfon
Desbromo-bromobutide
Desmedipham
Desmetryn
Dialifos
Di-allate I
Di-allate II
Diamyl phthalate
Diazinon
Dibrom (naled)
Dicamba
Dicamba methyl ester
Dicapthon
Dichlofenthion
Dichlofluanid
Dichlone
Dichlormid
Dichlorophen
Dichlorprop
Dichlorprop methyl ester
Dichlorvos
Diclobutrazol
Diclofop methyl
Dicloran
Dicrotophos
Dicyclohexyl phthalate
Dicyclopentadiene
Dieldrin
Diethatyl ethyl
Diethofencarb
Diethyl dithiobis(thionoformate) (EXD)
Diethyl phthalate
Diethylene glycol
Diethylstilbestrol
Difenoconazol I
Difenoconazol II
Diflufenican
Dimefox
Dimethachlor
Dimethametryn
Dimethipin
Dimethoate
Dimethylphthalate
Dimethylvinphos(z)
Dimetilan
Di-n-butylphthalate
Diniconazole
Dinitramine
Dinobuton
Dinocap I
Dinocap II
Dinocap III
Dinocap IV

Dinoseb
Dinoseb acetate
Dinoseb methyl ether
Dinoterb
Dinoterb acetate
Di-n-propyl phthalate
Dioxacarb
Dioxathion
Dioxydemeton-S-methyl
Diphacinone
Diphenamid
Diphenylamine
Dipropetryn
Disulfoton
Ditalimfos
Dithiopyr
Diuron
Dodemorph I
Dodemorph II
Drazoxolon
Edifenphos
Endosulfan (alpha isomer)
Endosulfan (beta isomer)
Endosulfan ether
Endosulfan lactone
Endosulfan sulfate
Endrin
Endrin aldehyde
Endrin ketone
EPN
Epoxiconazole
EPTC
Erbon
Esfenvalerate
Esprocarb
Etaconazole
Ethalfluralin
Ethiofencarb
Ethiolate
Ethion
Ethofumesate
Ethoprophos
Ethoxyquin
Ethylenethiourea
Etridiazole
Etrimfos
Famphur
Fenarimol
Fenazaflor
Fenbuconazole
Fenchlorphos
Fenfuram
Fenitrothion
Fenobucarb
Fenoprop
Fenoprop methyl ester
Fenoxycarb
Fenpropathrin
Fenson
Fensulfothion
Fenthion
Fenthion sulfoxide
Fenuron
Fenvalerate I
Fenvalerate II
Fepropimorph
Flamprop-isopropyl
Flamprop-methyl
Fluazifop-p-butyl
Flubenzimine
Fluchloralin
Flucythrinate I
Flucythrinate II
Flumetralin
Fluometuron
Fluorodifen
Fluotrimazole
Flurenol-butyl ester
Flurenol-methylester
Fluridone
Flurochloridone I
Flurochloridone II
Fluroxypyr-1-methylheptyl ester
Flusilazole
Flutolanil
Flutriafol
Fluvalinate-tau-I
Fluvalinate-tau-II
Folpet
Fonofos
Formothion
Fuberidazole
Furalaxyl
Furathiocarb
Furmecyclox
Heptachlor
Heptachlor epoxide
Heptachlor exo-epoxide isomer B
Heptenophos
Hexabromobenzene
Hexachlorobenzene
Hexachlorophene
Hexaconazole
Hexazinone
Hexestrol
Imazalil
Ioxynil
Iprobenfos
Iprodione
Isazophos
Isobenzan
Isobornyl thiocyanoacetate
Isocarbamide
Isodrin
Isofenphos
Isomethiozin
Isoprocarb
Isopropalin
Isoprothiolane
Isoproturon
Isoxaben
Isoxathion
Jodfenphos
Kinoprene
Lenacil
Leptophos
Leptophos oxon
Lindane
Linuron
Malathion
Malathion-o-analog
MCPA methyl ester
MCPB methyl ester
m-Cresol
Mecarbam
Mecoprop methyl ester
Mefenacet
Mefluidide
Menazon
Mephosfolan
Mepronil
Metalaxyl
Metamitron
Metasystox thiol
Metazachlor
Methacrifos
Methamidophos
Methfuroxam
Methidathion
Methiocarb
Methiocarb sulfone
Methiocarb sulfoxide
Methomyl
Methoprene I
Methoprene II
Methoprotryne
Methoxychlor
Methyl paraoxon
Methyl parathion
Methyl-1-naphthalene acetate
Methyldymron
Metobromuron
Metolachlor
Metolcarb
Metribuzin
Mevinphos
Mirex
Molinate
Monalide
Monocrotophos
Monolinuron

Myclobutanil
N,N-Diethyl-m-toluamide
N-1-Naphthylacetamide
Naphthalic anhydride
Napropamide
Nicotine
Nitralin
Nitrapyrin
Nitrofen
Nitrothal-isopropyl
N-Methyl-N-1-naphthyl acetamide
Norflurazon
Nuarimol
o,p'-DDD
o,p'-DDE
o,p'-DDT
Octachlorostyrene
o-Dichlorobenzene
Omethoate
o-Phenylphenol
Oryzalin
Oxabetrinil
Oxadiazon
Oxadixyl
Oxamyl
Oxycarboxin
Oxychlordane
Oxydemeton-methyl
Oxyfluorfen
p,p'-DDD
p,p'-DDE
p,p'-DDT
Paclobutrazol
Paraoxon
Parathion
p-Dichlorobenzene
Pebulate
Penconazole
Pendimethalin
Pentachloroaniline
Pentachloroanisole
Pentachlorobenzene
Pentachloronitrobenzene
Pentachlorophenol
Pentanochlor
Permethrin I
Permethrin II
Perthane
Phenamiphos
Phenkapton
Phenoxyacetic acid
Phenthoate
Phorate
Phosalone
Phosfolan
Phosmet
Phosphamidon I
Phosphamidon II
Phthalide
Picloram methyl ester
Pindone
Piperalin
Piperonyl butoxide
Piperophos
Pirimicarb
Pirimiphos-ethyl
Pirimiphos-methyl
Plifenat
p-Nitrotoluene
Pretilachlor
Probenazole
Prochloraz
Procymidone
Profenofos
Profluralin
Promecarb
Prometon
Prometryn
Propachlor
Propamocarb
Propanil
Propargite
Propazine
Propetamphos
Propham
Propiconazole-I
Propiconazole-II
Propoxur
Propyzamide
Prothiofos
Prothoate
Pyracarbolid
Pyrazon
Pyrazophos
Pyrazoxyfen
Pyributicarb
Pyridaben
Pyridaphenthion
Pyridate
Pyridinitril
Pyrifenox I
Pyrifenox II
Pyrimethanil
Pyroquilon
Quinalphos
Quinoclamine
Quizalofop-ethyl
Resmethrin
S,S,S-Tributylphosphorotrithioate
Sebuthylazine
Secbumeton
Simazine
Simetryn
Sulfotep
Sulfur (S8)
Sulprofos
Swep
Tamoxifen
TCMTB
Tebuconazole
Tebutam
Tecnazene
Temephos
Terbacil
Terbucarb
Terbufos
Terbumeton
Terbuthylazine
Terbutryne
Tetrachlorvinphos
Tetradifon
Tetraethylpyrophosphate (TEPP)
Tetramethrin I
Tetramethrin II
Tetrapropyl thiodiphosphate
Tetrasul
Thenylchlor
Thiabendazole
Thiofanox
Thiometon
Thionazin
Tiocarbazil I
Tiocarbazil II
Tolclofos-methyl
Tolylfluanid
trans-Chlordane
Triadimefon
Triadimenol
Tri-allate
Triamiphos
Triazophos
Tributyl phosphate
Tributyl phosphorotrithioite
Trichlorfon
Trichloronate
Triclopyr methyl ester
Tricyclazole
Tridiphane
Trietazine
Triflumizole
Trifluralin
Tryclopyrbutoxyethyl
Tycor (SMY 1500)
Uniconizole-P
Vamidothion
Vernolate
Vinclozolin

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# RoHS/ELV Directives - Measurement of Heavy Metals Using ICP-MS

Application

**Environmental**


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## Abstract

**This application note describes a robust analytical method for the determination of heavy metals and substances regulated by the EU Restriction of Hazardous Substances/End of Life Vehicles (RoHS/ELV) directives. Based on the experience of Nihon Environmental Services Co., Ltd in Japan using an Agilent Technologies 7500c ORS (Octopole Reaction System) collision/reaction cell ICP-MS, this note summarizes:**

- **An overview and validation of ICP-MS methodology for RoHS applications**
- **Benefits of ICP-MS methodology to the analysis of trace elements in high matrix samples and limitations of EDXRF (Energy Dispersive X-Ray Fluorescence) for the measurement of lead in flame resistant plastics**
- **Advantages of ICP-MS for RoHS applications.**


## Introduction

The European Union (EU) RoHS directive (Restriction of the use of certain Hazardous Substances, Directive 2002/95/EC effective July 2006) aims at effectively eliminating the use of hazardous chemical materials used in electric/electronic products. The EU End of Life Vehicles (ELV) Directive 2000/53/EC covers the use of certain substances in the manufacture of new vehicles and automotive components. In China and Japan, the respective equivalents of the RoHS directive will be enforced at the same time as in the EU. The Japanese version is currently being prepared by the Ministry of Economy, Trade and Industry.

Table 1 shows the substances/metals that are regulated by the RoHS/ELV directives along with their thresholds. The RoHS directive restricts the use of three heavy metals: cadmium (Cd), lead (Pb), mercury (Hg), plus hexavalent chromium ($Cr^{6+}$) and two brominated flame retardants. The ELV directive only targets the metals stated above. In accordance with these directives, careful control is required during the manufacture of sub components to prevent the above substances entering into the process. Polymeric resin materials are particularly susceptible to contamination. Nevertheless, other than British Standard/European Standard BS EN1122:2001: Plastics – Determination of Cadmium - Wet Decomposition Method [1], a standard method for analyzing metal impurities in resins does not exist. A National Standardization Technical Committee (TC111) working group 3 organized in the International Electrotechnical Commission (IEC) was set the task of developing standard RoHS analysis methods.

**Table 1. Substances Restricted by the RoHS/ELV Directives and Their Thresholds**

| Restricted substance | RoHS directive/ mg/kg | ELV directive/ mg/kg |
|---|---|---|
| Cd | 100 | 100 |
| Pb | 1000 | 1000 |
| Hg | 1000 | 1000 |
| $Cr^{6+}$ | 1000 | 1000 |
| PBBs* | 1000 | – |
| PBDEs** | 1000 | – |

* Polybrominated biphenyls

** Polybrominated diphenyl ethers

## Methodology

Table 2 summarizes the methods of analysis that are performed at Nihon Environmental Services, along with the minimum quantitative detection limits. Official analysis methods for hexavalent chromium include EPA3060A/7196A, (alkaline digestion of hexavalent chromium, colorimetric method), plus JIS H 8625 (hexavalent chromium by a diphenylcarbazide colorimetric method), ISO3856/5 (determination of hexavalent chromium content of the pigment portion of liquid paint or paint in powder form, diphenylcarbazide spectrophotometric method) and DIN53314 (determination of content of chromium (VI) in leather by colorimetric analyses using diphenyl carbazide). None of these, however, have been sufficiently validated for a wide variety of materials. For this reason, it has become general practice at Nihon Environmental Services to measure the total concentration first, and then measure the concentration of the hexavalent form only if the total concentration exceeds the $Cr^{6+}$ minimum quantitation limit. Nihon Environmental Services uses US EPA SW-846 Method 3052 (microwave assisted acid digestion of siliceous and organically based matrices, hereafter "EPA3052") [2]. This screening method specifies the simultaneous pretreatment procedures for cadmium, lead, mercury, and total chromium.

Nihon Environmental Services Co., Ltd uses an Agilent 7500c equipped with an ORS collision/ reaction cell to analyze cadmium, lead and chromium, see Table 3 for operating conditions. Quantitative determination of mercury is also possible provided that the memory effect (the element's adsorption to the container) and the volatility of the material are taken into account. The instrument was calibrated using a mixed standard solution (SPEX, Metuchen NJ, USA). Table 4 shows the analytical isotopes and internal standards monitored.

**Table 2. Minimum Quantitative Detection Limits and Methods of Analysis**

| Restricted substance | Minimum Level of Quantitation mg/kg | Analysis method | |
|---|---|---|---|
| | | Sample preparation | Quantitative Analysis (Technique) |
| Cd<br>Pb<br>Hg<br>Cr | 1<br>10<br>1<br>1 | In accordance with the US EPA SW-846 Method 3052<br>Acid decomposition method with microwave oven | ICP-MS method, see Table 3 for operating parameters |
| $Cr^{6+}$ | 10 | In accordance with the US EPA SW-846 Method 3060A<br>Hot alkali extraction method | In accordance with the US EPA SW-846 Method 7196A, using Diphenylcarbazide absorption spectroscopy |
| PBBs | 10 | Solvent extraction method | GC/MS |
| PBDE | 10 | Solvent extraction method | GC/MS |

**Table 3. ICP-MS Operating Parameters, Agilent 7500c ICP-MS**

| | |
|---|---|
| Nebulizer type | Babington |
| RF power | 1600 W |
| Sampling depth | 8.5 mm |
| Carrier gas flow rate | 1.2 L/min |
| Makeup gas flow rate | 0 L/min |
| Spray chamber temp | 2 °C |
| Peristaltic pump speed | 0.1 rps |
| Reaction gas | $H_2$ (3.5 mL/min) |

**Table 4. Isotopes Used for Measurements by ICP-MS**

| Measured element | *m/z* | Internal Standard (*m/z*) |
|---|---|---|
| Cr | 52<br>53* | Ga (71,69) or Y (89) |
| Cd | 106<br>111*<br>112 | In (115*) or Y (89)<br>or Te (125) |
| Hg | 200<br>202* | Tl (205) |
| Pb | 207<br>208* | Tl (205) |

* Isotope that is used primarily for quantitative determination.

## Validation of Methodology

### Analysis of Standard Reference Materials

A standard polyethylene substance CBR-680 supplied by the Community Bureau of Reference (CBR) was prepared for analysis by ICP-MS according to the procedures detailed in methods EN1122 and EPA3052. The certified value for cadmium in the standard is 140.8 mg/kg. The measured values were subjected to Grubbs' test for outliers to confirm that no samples were to be rejected. The results were then tested at the 5% significance level to check if there was a difference between the averages calculated by the two methods. The average resulting from the EN1122 method was 143.2 mg/kg (CV: 3.2%, recovery: 101%) and the average from the EPA3052 method was 138.0 mg/kg (CV: 2.4%, recovery 98%). No significant difference was observed between the results.

### Results of a Proficiency Test

To test the validity of their ICP-MS methodology the lab took part in the proficiency test in 2004 (IIS04P02): "Cadmium and Lead in Plastics" that was administered by the Institute for Interlaboratory Studies (IIS), in the Netherlands. At present, this is the only international proficiency test that is based on the ISO/IEC Guide 43. According to the report, this test attracted the participation of 56 analytical institutions from 20 countries around the world, with a large number of participants from Japan, Hong Kong, China and other areas in Asia [3]. Three participating labs used ICP-MS, 17 used atomic absorption spectroscopy and 33 used ICP-OES.

Three samples were used in the test. Cadmium was the target metal for #0454 and #0455, while lead was the target for #0456. All samples were polyvinyl chloride (PVC) moldings. Samples #0454 and #0456 contained over 10 times the regulated value of Cd and Pb respectively, while Sample #455 had only a moderate concentration of Cd. The results that Nihon Environmental Services obtained were close to the average for both the EN1122 and EPA Method 3052, as shown in the example histograms for Cd and Pb in Figures 1 and 2. The calculated z-scores were within a range of ±1.

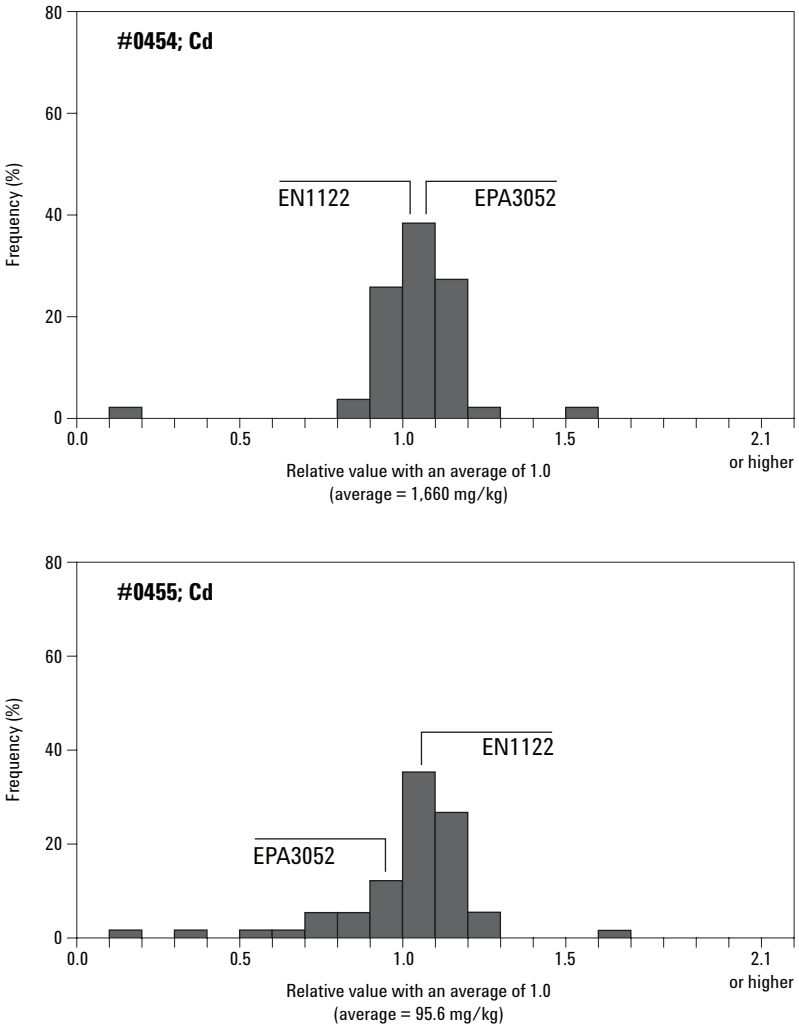


**Figure 1. Histograms of results for Cd in sample #0454 and #0455.**

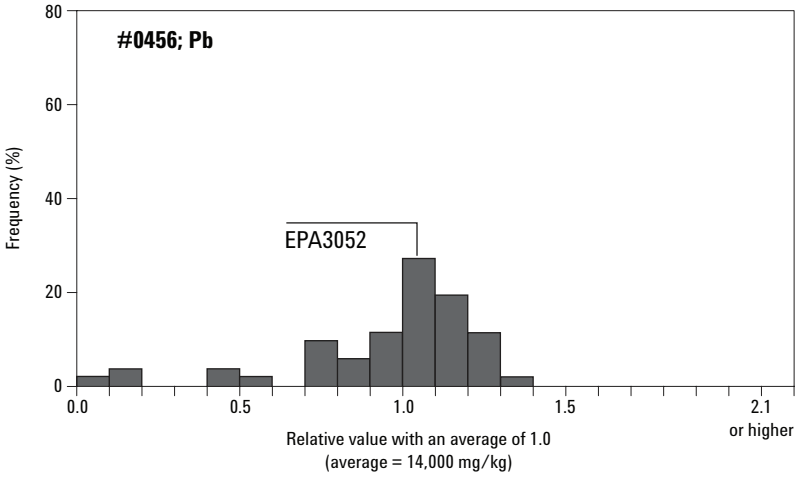


**Figure 2. Histogram of results for Pb in sample #0456.**

### High-Concentration Matrix Samples

The materials covered by the RoHS/ELV directives include various metals and ceramics, as well as polymers. Some resin products contain a large amount of inorganic components as a result of additives such as metal fillers. While resins and other organic components can be decomposed using microwave digestion, their inorganic constituents often remain in high concentrations. It is difficult for some types of analytical instrumentation to measure trace elements in the presence of such high concentration matrices. To overcome this problem, pretreatment methods such as solvent extraction and ion exchange are proposed. Nihon Environmental Services, however, prefers direct quantitative determination and uses ICP-MS with minimum sample pretreatment. By making use of the relative freedom from spectral interferences provided by the 7500c ICP-MS, complicated sample pretreatment procedures are simplified, and the risk of introducing contaminants is minimized. Turnaround time is also improved.

### Managing Interferences in ICP-MS

Spectral interferences caused by overlaps of polyatomic species on elements of interest (cadmium, lead, chromium, and mercury) were overcome by operating the 7500c ORS collision/reaction cell in helium mode (4.5 mL/min flow rate). Physical interferences were corrected through the use of appropriately selected internal standards. Internal standards were selected with similar ionization potential and a similar mass-to-charge ratio to the analytes being measured. Note that the internal standards recommended by the USEPA are not always the most suitable for all matrices.

### Limitations of EDXRF for Measurement of Lead in Flame Resistant Plastics

EDXRF spectroscopy can provide quick, low cost analyses. For this reason, this instrument is widely used for screening tests in the electronic, electric appliance and automobile industries, where the number of parts and components used extends from several hundreds to several tens of thousands. Modern EDXRF instruments have improved significantly in sensitivity and are currently capable of measuring at ppm levels under optimized conditions. However, their application is limited because of interferences on key analytes.

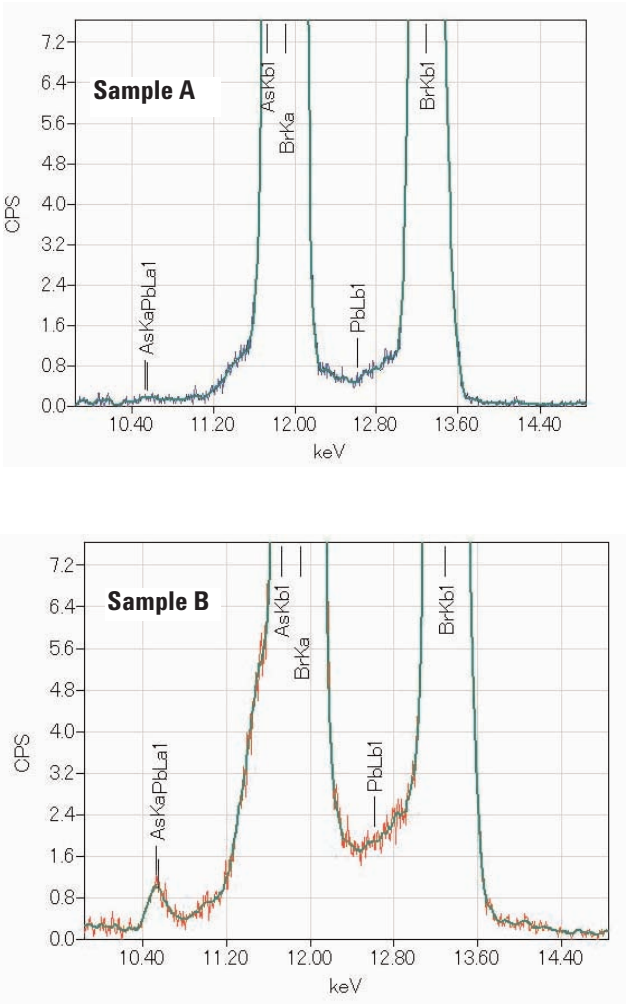


**Figure 3. EDXRF spectra of brominated flame retardant containing plastics A (top) and B (bottom).**

Figure 3 shows an example of the measurement of lead contained in two flame resistant plastic samples (A and B) using an EDXRF spectrometer. The presence of the prominent peaks associated with bromine suggests that both samples A and B contain brominated flame retardants. EDXRF results also showed less than 10 mg/kg of lead for sample A and 70 mg/kg of lead for sample B. However, analysis by ICP-MS showed less than 10 mg/kg for both samples. The difference in the results for lead in Sample B can be accounted for as follows:
Table 5 shows how the wavelength of the lead peak $L_{\alpha 1}$ is close to $K_{\alpha}$ of arsenic, and the $L_{\beta 1}$ and $L_{\beta 2}$ of lead are close to $K_{\alpha}$ and $K_{\beta 1}$ of bromine. This means that it is difficult to determine lead in a sample

which contains arsenic and bromine. Brominated compounds are generally added as a flame retardant and antimony trioxide ($SbO_3$) is sometimes used to enhance performance. Arsenic compounds are not intentionally added to plastics, however it can occur as a contaminant in the antimony compound. The ICP-MS results for Sample B showed arsenic was present at the 50-ppm level. The comparison demonstrates that ICP-MS provides more reliable results for the determination of heavy metals and other elements such as arsenic, in flame-resistant materials.

**Table 5. EDXRF Wavelengths for Quantitative Analysis and Interference Identification**

| Elements | Spectrum | keV |
|---|---|---|
| Pb | $L_{\alpha 1}$ | 10.552 |
| | $L_{\beta 1}$ | 12.614 |
| | $L_{\beta 2}$ | 12.623 |
| As | $K_{\alpha}$ | 10.532 |
| | $K_{\beta 1}$ | 11.727 |
| Br | $K_{\alpha}$ | 11.909 |
| | $K_{\beta 1}$ | 13.292 |

### Advantages of ICP-MS

A major advantage of ICP-MS for RoHS applications is its multi-element capability which enables it to determine a full suite of elements including mercury. This eliminates the need for a peripheral technique such as cold vapor atomic absorption (CVAA) for ICP-optical emission spectroscopy (OES). Antimony (Sb) and arsenic (As) are not currently listed in RoHS/ELV, but these elements are listed along with beryllium (Be) and selenium (Se) in the Green Procurement Guidelines issued by JIG (Joint Industry Guideline) [4] prepared jointly by the industry groups in Japan, the United States and the EU. ICP-MS allows the determination and quantification of all elements specified in current directives and guidelines as well as impurity elements which provide valuable information on the content of the sample.

Another advantage of the technique is its sensitivity. The RoHS/ELV maximum allowable concentration is 100 ppm (Cd) and 1000 ppm (Pb, Cr, Hg). Some manufacturers set specific requirements at, for example, 1/100 or 1/1000 of the RoHS/WEE threshold which requires determination at lower than the 1–10 ppm level. Because ICP-MS is significantly more sensitive than ICP-OES or AAS, only 1/10–1/100 of the sample amount is required for ICP-MS. This reduces the time required for sample preparation and allows small sample volumes to be analyzed. This is an advantage if the sample itself is very small or the sample is a high value part, such as a noble metal. Further benefits of the simplified sample preparation procedure arise from the reduced volume of acid that is required to digest the sample and that needs to be disposed of at the end of the analysis.

ICP-MS also provides the possibility of employing isotope dilution mass spectrometry (IDMS) as a high caliber reference calibration technique for more accurate quantitative analysis. IDMS involves spiking samples before the dissolution process, which allows accurate calculation of sample preparation recovery.

## Conclusions

The ICP-MS method developed at Nihon Environmental Services Co., Ltd. using the 7500c ORS is capable of measuring the four metals regulated by the RoHS/ELV directives efficiently, as well as the 10 heavy metals specified in the guidelines currently prepared jointly by the industries concerned in Japan, the United States and the EU.

To assure the quality of the data acquired according to the RoHS/ELV directives, it is desirable to have reliable third parties perform the same measurements to ensure that the results will be commercially acceptable worldwide. Nihon Environmental Services is ISO9001 accredited and conducts method development according to ISO management. The company also obtained laboratory accreditation (ISO/IEC17025) from the Japan Accreditation Board for Conformity Assessment (JAB) for the measurement of cadmium according to the EN1122 method, which employs ICP-MS as the determination method. IEC TC111 will also include ICP-MS. This experience provides further confidence in the ICP-MS method as applied to the RoHS/ELV directives.

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2. US EPA SW-846 Method 3052, http://www.epa.gov/SW-846/pdfs/3052.pdf
3. Results Proficiency Test Cadmium and Lead in Plastics October 2004. For more information contact: masahiko_endo@agilent.com
4. Guideline for Standardization of Material Declaration by Japan Electronics and Information Technology Association (JEITA JGPSSI), http://home.jeita.or.jp/eps/green2.htm


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# Precise Time-Scaling of Gas Chromatographic Methods Using Method Translation and Retention Time Locking

Application

Gas Chromatography

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## Abstract

**Complete development of a gas chromatographic method often involves a significant amount of effort. Once a method is completed, retention time locking (RTL) can be used to implement the method and to obtain the same retention times on multiple systems. This application note describes how to use method translation combined with RTL to implement precise time-scaled versions of a method on multiple instrument types. This allows the original method to be re-used with minimal effort, while optimizing the method for a given sample type or instrument setup. In this way, the utility of the original method is extended greatly, increasing the payback on the investment in its development and optimizing its use for specific analyses. In this note, the Agilent RTL Pesticide Library method is used as an example. The steps involved in precise time-scaling of the method to different speeds, detectors, and columns are presented.**



## Key Words

Pesticides, GC, GC-AED, retention time locking, RTL, method translation, scalable RT libraries


## Introduction

Interest in the analysis of pesticide residues has been increasing recently, in part due to the discovery that some of these compounds act as endocrine disrupters. Agilent Technologies has responded to the need for rapid, accurate, and comprehensive screening analysis for pesticides by developing a method to screen for 567 pesticides and suspected endocrine disrupters. The method uses element- selective detection and a retention time locked library of retention times to find and identify pesticides in a sample.[1]

In the method, sample extracts are run with element-selective detection using a prescribed set of chromatographic conditions and with the column retention time locked to the retention times in a table. If any peaks containing heteroatoms are observed, the section of the table corresponding to a small time window around the observed peak is searched. The time search results are further sorted using the observed element content of the peak. The combination of time and element content narrows rapidly the possible compounds that could have produced the heteroatom response to a few pesticides.

The element-selective detection is done with either gas chromatography-atomic emission detection (GC-AED), which can screen for all the individual elements found in pesticides, or with a combination of other selective detectors like the electron capture detector (ECD), the nitrogen-phosphorus detector (NPD), the flame photometric detector (FPD), or the electrolytic conductivity detector (ELCD).

The GC-AED technique can also be used to calculate element ratios and to quantitate unknown peaks that are detected because of its equimolar element response factors. The measured element ratios can be used to further distinguish between possible identities of detected heteroatomic compounds, often resulting in a single entry as the likely identity of a given peak. With compound-independent calibration, the amount of the unknown can be calculated using element response factors generated with a different standard compound.

Once the element-selective screen is completed, samples that contain any suspect compounds are run on a GC with mass spectral detection (GC-MS) system that is retention time locked to the pesticide method, thus having the same retention times as the element-selective detectors. Using the possible identities generated from the element screen, the GC-MS data is evaluated to decide which (if any) of the possible identities for suspect peaks is correct. The confirmation process is simplified greatly because the element screen usually yields only a few possibilities and because the retention time in the GC-MS run is accurately known. In practice, extracted ion chromatograms for characteristic ions of each possible compound are used to determine the identity of suspect compounds.

This screening method minimizes false negatives, even in dirty samples, by using element-selectivity and time in the initial screen. With element-selective detection, all compounds containing chlorine, phosphorus, nitrogen, etc. are detected. Even if a detected heteroatomic compound is not in the table, its presence is known, and it can be marked for further GC-MS evaluation. By using GC-MS for confirmation, false positives are also minimized.

The RTL Pesticide Library method is a good example of a method in which a substantial investment of time and material has been made. As with many methods intended for use in multiple laboratories, it would be desirable to be able to scale the method for use in different situations of sample type and instrument setup. Because the method relies on the measured retention times of 567 compounds, it would be impractical to re-measure all the retention times whenever the method is modified, for example, to increase its speed.

Method translation[2–4] is a calculation technique developed at Agilent Technologies that allows a capillary column GC method to be translated to different chromatographic conditions. The technique calculates the required changes in inlet pressure and oven temperature ramp rates and hold times required to maintain peak elution order identical to that of a reference method. In this way, the speed of an analysis can be scaled predictably to accommodate the needs of a specific sample or instrument type.

The inlet pressure calculated for the new version of a method by the method translation software is based on the assumed or nominal dimensions of the column. As such, the calculated inlet pressure will provide a close, but not exact, match to the desired scaled retention times. To match precisely the retention times of the scaled method to the desired scale factor, the new method must be retention time locked. Retention time locking[3] (RTL) is a technique developed by Agilent Technologies whereby the inlet pressure required to match retention times precisely is calculated from a calibration curve of inlet pressure versus retention time.

Using method translation followed by RTL allows a method to be scaled by a precisely known factor. Once the chromatography has been scaled, a retention time table, such as the RTL Pesticide Library, can then be scaled by the same factor, resulting in a new library whose retention times match those of the scaled method precisely.

The steps required to scale the method are:

1. Determine the desired scale factor for the new method.
2. Use the method translation software[4] to calculate the inlet pressure and oven temperature adjustments to obtain the desired scaling of the method. The scale factor is the “speed gain” value reported in the method translation software. Make sure that the new method parameters are consistent with the hardware capabilities of where the new method will be used.
3. Perform the RTL calibration runs for the new method. Alternatively, the method translation software can be used to calculate the RTL calibration points for the new method using those from the original method.
4. Retention time lock the new method using the locking reference standard from the original method. The new method should be locked to the original reference standard retention time divided by the scale factor.
5. Export the retention time table as a text file using the EXPORT function in the RTL SEARCH menu of the RTL ChemStation software.
6. Divide the retention times in the table by the scale factor in a spreadsheet program like Microsoft® Excel™.
7. Re-import the new, scaled table.
8. Run a representative test mixture to validate the scaled method.

Several examples of scaling the HP RTL Pesticide Library are presented below.

## Experimental

All data were collected on Agilent 6890 Series GC systems. All systems were equipped with:

- Electronic pneumatics control (EPC)

- Split/splitless inlet
- Automatic liquid sampler

The GC-AED system also included an Agilent G2350A atomic emission detector with GC-AED ChemStation software (rev B.00.00) for Microsoft® Windows NT®.

The GC-micro-ECD system was controlled by Agilent GC ChemStation software (rev A.05.04). Both the GC-AED and the GC-micro-ECD ChemStations contained RTL software for GC ChemStation (G2080AA) and the Retention Time Locking Pesticide Library for GC ChemStation (G2081AA).

The GC-MS system (G1723A) used consisted of an 6890 Series GC equipped with an Agilent 5973 mass selective detector (MSD). The process for retention time locking the GC-MS system is described in reference 2.

All systems except the micro-ECD instrument used 30 m × 0.25 mm id × 0.25 µm HP-5MS columns (part no. 19091S-433). The Agilent micro-ECD instrument used 10 m × 0.1 mm id × 0.1 µm HP-5 column (part no. 19091J-141).

RTL measurements were made with a solution of dichlorvos, methyl chlorpyrifos, and mirex, each at 10-ppm concentration in acetone. All injections were 1-µL splitless, except for the micro-ECD experiments, which were 1-µL split 100:1. In all methods, inlets were operated at 250 °C and detectors at 300 °C.

Method translation requires inlets to be run in constant pressure mode to obtain precise scaling of retention times. Thus, all methods discussed in the note were run in this mode.

## Results and Discussion

### Locking GC-MS with Other GC Detectors

When using selective GC detectors in conjunction with GC-MS, one problem that is encountered is knowing the relationship between retention times on the selective detector and that of the GC-MS. In GC-MS, the outlet pressure of the column is vacuum, while with most other GC detectors, the outlet pressure of the column is at or near atmospheric pressure. This difference in outlet pressures results in large differences in retention time between GC with MS detection and GC with other detectors. Comparison of GC-FID, a general detector, with GC-MS is reasonably straightforward, because the total ion chromatogram (TIC) of the GC-MS system has similar response to the FID. Retention times on the GC-MS system corresponding to those on the GC-FID can be determined by looking for similar patterns of response. With selective detectors, this is much more difficult because the response patterns from selective detectors usually do not resemble the TIC. For this reason, matching the retention times of selective detectors precisely with the GC-MS system simplifies data analysis greatly.

In this first example of scaling the RTL Pesticide Library, the method will be scaled from the GC-AED method to the GC-MS method. In this case, the desired scale factor is exactly 1, that is, the GC-MS retention times are desired to be exactly the same as those of the GC-AED. The first step is to use the method translation software to determine the GC conditions to use for GC-MS.

Figure 1 shows the method translation software. The original method conditions for the GC-AED pesticide method are entered in the column labeled “Original Method.” The column dimensions, carrier gas type, inlet pressure, outlet pressure, ambient pressure, and oven temperature program are entered here. Note that the inlet pressure is in psi (gauge), while the outlet pressure and ambient pressure are psi (absolute). The original method here is being used on a GC-AED system, so the outlet pressure is entered as atmospheric pressure plus 1.5 psi, the operating pressure of the GC-AED.

The “Criterion” parameter is set to “None,” which allows the user to select a specific value of “speed gain” by adjusting the value of hold-up time for the translated method (see figure 1). In the column labeled “Translated Method,” the parameters of column dimensions, carrier gas type, outlet pressure, and ambient pressure for the GC-MS method are entered. Note that the inlet pressure and oven program are not entered; they are calculated by the program. To set the speed gain to a desired value, take the calculated value of hold-up time in the first column (0.996060 minute) and divide it by the scale factor. Because in this case the desired scale factor (“speed gain”) is 1, the same hold-up time for both the GC-AED and the GC-MS methods is required. Clicking the radio button next to the hold-up time in the “Translated Method” column will do this automatically.

The method translation indicates that to obtain the same retention times on the GC-MS system as on the GC-AED, use all the same method parameters

except inlet pressure. Instead of using 27.6 psi as is used on the GC-AED, method translation calculates that 17.93 psi on the GC-MS system will result in matching retention times. As mentioned above, this inlet pressure is calculated on the assumed dimensions of the column in the GC-MS system. To get the retention times to match precisely, RTL[3] is used.

To retention time lock the GC-MS method to the GC-AED method in this example, it is necessary to construct an RTL calibration file for the GC-MS system. Construction of this file only needs to be done once. All subsequent users of the GC-MS method will then be able to use this calibration file for a similarly configured GC-MS instrument.

The RTL calibration file is constructed by running five calibration runs of the target compound, in this case methyl chlorpyrifos, at five different inlet pressures. The runs are made at conditions identical to the nominal method except that four of the runs are made at different pressures. The pressures used are typically:

- Target pressure – 20%
- Target pressure – 10%
- Target pressure (nominal method pressure)
- Target pressure + 10%
- Target pressure + 20%

The retention time of the target compound is determined for each run. The resulting set of five pressures and corresponding retention times is then entered in the RTL calibration dialog box for the method and saved with the method.

To lock the method on the GC-MS setup, the target compound is run at

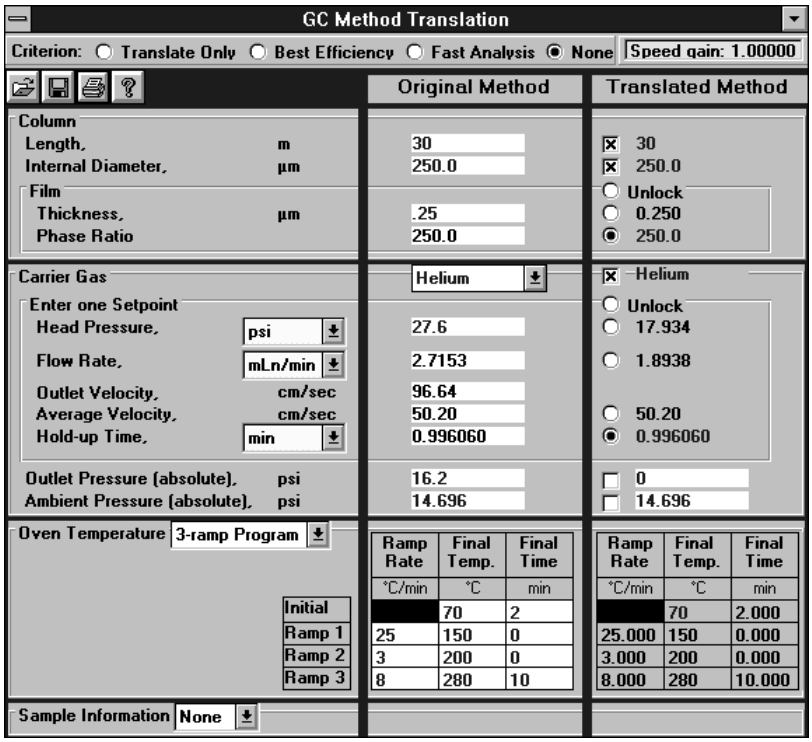


**Figure 1. Method translation software showing scaling HP RTL Pesticide Method from GC-AED conditions to GC-MS with a scale factor of 1.**

the nominal method pressure, and the retention time is observed. The pressure and resulting retention time are then entered into the "(Re)Lock New Column" menu item of the RTL software to calculate the correct pressure for obtaining locked retention times.

Normally, the RTL calibration for a new method is determined by actually making the five calibration runs. In the current example, methyl chlorpyrifos would be run at:

- 17.93 psi – 20% = 14.34 psi
- 17.93 psi – 10% = 16.14 psi
- 17.93 psi (nominal method pressure)
- 17.93 psi + 10% = 19.72 psi
- 17.93 psi + 20% = 21.56 psi

However, because the new GC-MS method is scaled from an existing GC-AED method that already has RTL calibration data, method translation can be used to calculate the new RTL calibration points. This is useful when you want to try a scaled method rapidly and save the time required in making the five runs. (Note: For methods that will be used extensively, the five-runs approach may provide a somewhat better calibration. It is recommended that for these methods, the standard calibration be performed.)

To calculate the five RTL calibration pairs of pressure and retention time for the GC-MS method from those of the GC-AED method:

- Take the inlet pressure used for each original GC-AED RTL calibration run, and enter it into the method translation software for the inlet pressure of the original method. Make sure the hold-up times are locked, giving a "speed gain" of 1.

- The inlet pressure calculated in the "Translated Method" column will now change to a new value, corresponding to the pressure that would be obtained if the calibration run were made on a GC-MS system. This pressure is used with the retention time obtained for the corresponding GC-AED calibration run as a calibration point for the GC-MS method.

When all five points have been calculated in this way, they are entered into the RTL calibration dialog box for the GC-MS method and saved with the method. Table 1 lists the original RTL calibration pressures and times with the calculated pressures and times for the GC-MS method.

To test the accuracy of using a predicted RTL calibration file for GC-MS, a real calibration set was measured on the GC-MS system. The data is shown in the first two columns of table 2. (Note: The calibration points are spaced ~ 5% apart in pressure instead of the typical 10%.) A GC-MS RTL calibration file was constructed with these measured points. For each point, the locking pressure required to lock the method was calculated and is shown in column 3 of table 2.

The locking pressure is the pressure determined by the RTL software that would make methyl chlorpyifos have a retention time of 16.596 minutes. This is determined by entering the pressure and retention time for each point into the "(Re)Lock New Column" menu item of the RTL software. If the calibration is done correctly, the locking pressures determined from each point should be very similar, as they are in column 3 of table 2.

Column 4 of table 2 shows the locking pressures for the same set of runs but determined using the GC-MS RTL calibration points calculated using method translation. The calculated data provide locking pressures that agree well with those based on measured data. The range in locking pressures pressure is only from 17.72 to 17.75 psi. This range of 0.03 psi corresponds to only about a 0.006-minute range in the retention time of methyl chlorpyrifos.

Figure 2 shows the locked chromatograms from a three-component mixture run on GC-AED and GC-MS systems. As can be seen, the retention times are well matched between the two methods.

The RTL Pesticide Library contains the retention times of the 567 pesticides measured with GC-FID. The values measured with the FID would be the same observed with any detector that is operated at or near atmospheric pressure. Because retention time matching is critical in this application, the retention times for all the compounds in the table were also measured on the GC-MS system after scaling as described here. Figure 3 is a plot of the difference between the retention times measured on the GC-FID and the GC-MS systems. The plot shows the retention times match well within ± 0.1 minute out to 30 minutes. A few compounds at the end deviate outside this window, with one compound 0.2-minute different. The

**Table 1. RTL Calibration Points from Original GC-AED Method and Calculated Points for GC-MS**

| GC-AED RTL Calibration | | GC-MS RTL Calibration | |
|---|---|---|---|
| Pressure (psi) | Ret Time (min) | Calculated Pressure (psi) | Calculated Ret Time (min) |
| 33.1 | 15.346 | 24.27 | 15.346 |
| 30.4 | 15.919 | 21.18 | 15.919 |
| 27.6 | 16.578 | 17.934 | 16.578 |
| 24.8 | 17.338 | 14.654 | 17.338 |
| 22.1 | 18.242 | 11.449 | 18.242 |

**Table 2. Comparison of Locking Pressures Calculated Using Measured and Predicted GC-MS RTL Calibration Data**

| GC-MS Locking Runs | | Locking Pressures | |
|---|---|---|---|
| Measured GC-MS RTL Cal Points | | Using Measured RTL Cal Points | Using Calculated RTL Cal Points |
| Pressure ( psi) | Ret Time (min) | Pressure (psi) | Pressure (psi) |
| 20 | 16.127 | 17.73 | 17.75 |
| 19 | 16.326 | 17.72 | 17.73 |
| 18 | 16.536 | 17.72 | 17.72 |
| 17 | 16.760 | 17.74 | 17.74 |
| 16 | 16.988 | 17.72 | 17.74 |

deviation is clearly largest in the isothermal hold region, which starts at 31.87 minutes. This effect is seen with GC-MS, but not with scaling to other atmospheric pressure detectors. While the cause is not yet clearly understood, it appears related to the vacuum outlet pressure of the GC-MS column. Although this level of matching is very good, the table includes both the GC-FID and GC-MS retention times so that smaller time windows can be used in searching unknowns.

### Locking GC-AED with Other GC Detectors

When the method translation step is done to scale the GC-AED method to other atmospheric pressure detectors, the only different parameter to enter is the outlet pressure. The outlet pressure for the GC-AED method is 16.2 psi and that for the others is 14.696 psi. The method translation calculates that the nominal GC-AED inlet pressure of 27.6 psi would be changed to 26.29 psi for the other atmospheric detectors. This difference (<5%) is so small that it can be neglected, because corrections in this range are compensated easily by the retention time locking step. Thus, the method conditions and RTL calibration points used with GC-AED are interchangeable with FID, NPD, ECD, FPD, and other atmospheric detector methods.

Note that this would not always be the case. If for example, a method is being scaled that uses a very low inlet pressure, the 1.5-psi difference in outlet pressure could become significant. It is best to check the method with method translation and see if the inlet pressure will change by >10%. If it does, it would be advisable to collect (or translate) a new RTL calibration centered around the translated nominal inlet pressure.

### Gaining Speed in the Same Instrument Setup

In the analysis of pesticide residues in food, there are usually only a few compounds encountered in any one sample. Because the screening method uses selective detectors, it makes sense to consider trading speed for chromatographic resolution. Selective detectors respond to only those compounds containing a specific heteroatom(s), and the chromatography only needs to resolve those compounds from each other, not from every other compound in the matrix. This approach can save a significant amount of analysis time.

In this example of scaling the RTL Pesticide Library, the method will be increased in speed at the expense of chromatographic resolution. The first consideration is by what factor to increase the speed. The method translation software is useful for determining this. A candidate speed gain, in this example threefold, is entered into the method translation software. The resulting inlet pressure and oven temperature ramp rates are then inspected to see if the instrument on

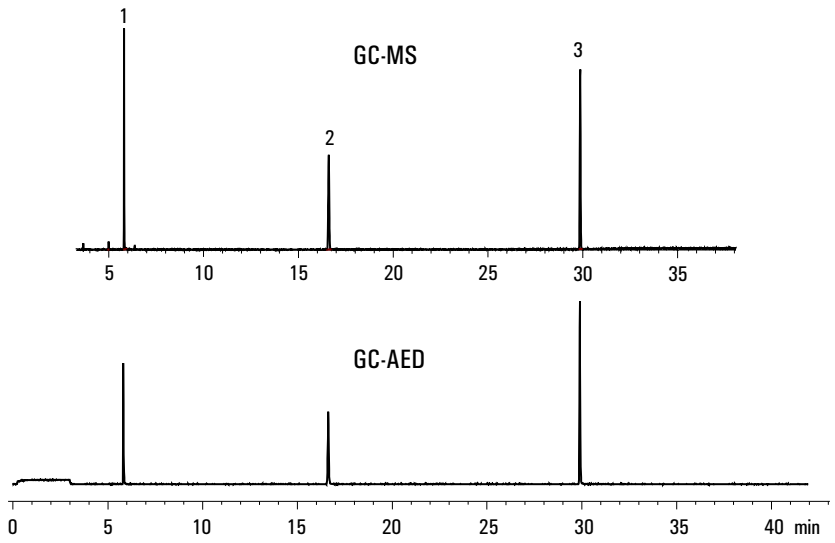


**Figure 2. GC-AED chlorine and GC-MS TIC chromatograms of three-component locking mixture. Peak identifications: 1. dichlorvos, 2. methyl chlorpyrifos, 3. mirex.**

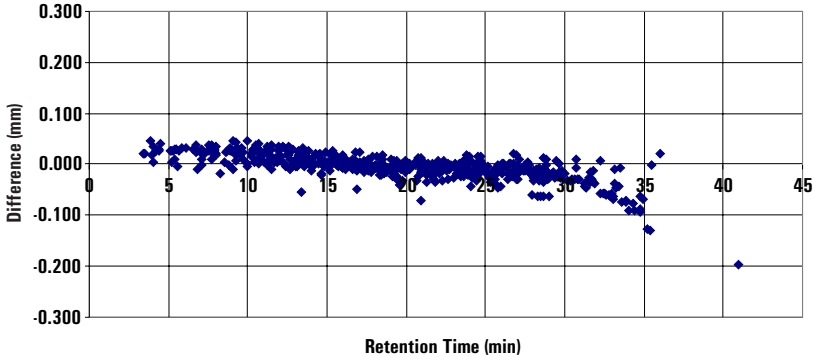


**Figure 3. Difference plot of GC-MS and GC-FID retention times in RTL Pesticide Library.**

which the new method will be run is compatible with those parameters.

Figure 4 shows the method translation software with the data entered for a speed gain of 3. Note that columns for "Original Method" and "Translated Method" are set up as in the previous example with two exceptions. Because the scaling is from GC-AED to GC-AED, the outlet pressure in both columns is entered as 16.2 psi. The second and most significant difference is the holdup time. The desired "speed gain" is 3.

To set the speed gain, the calculated value of hold-up time in the first column (0.996060 minute) is divided by exactly 3. This value (0.33202 minute) is entered for the hold-up time in the second column. This will force the speed gain to exactly 3.

The inlet pressure and oven temperature ramp for the new threefold speed method are now calculated. The calculated inlet pressure is 87.862 psi, which is compatible with the EPC module on the current system (maximum 100 psi). Note that the helium source supplying the GC must be capable of reaching 100 psi of helium. An optional 150-psi EPC module is available for the HP 6890 GC to provide additional inlet pressure, if necessary.

The oven temperature program calculated for the new method has the first ramp listed as 75 °C/min. This ramp rate is compatible with the 240-V oven option on the current instrument but would not work with a 120-V oven, which is limited to about 50 °C/min in this temperature range. With a 120-V oven, the speed gain would be limited to about 2.

The next step is to calculate the RTL calibration points from the original GC-AED method. This is done by the same process as shown in the GC-MS scaling above. In this case, when one of the original method RTL calibration pressures is entered, the resulting holdup time must be divided by 3 and entered for the holdup time in the "Translated Method" column. This will force the "speed gain" back to 3. The resulting inlet pressure is then paired with the retention time of the corresponding original GC-AED calibration run, but divided by 3 as a calibration point for the new method.

Table 3 shows the RTL calibration points from the original GC-AED method and calculated points for the threefold speed gain (3×) method.

When the calibration data is entered into the RTL calibration dialog box, the target time for methyl chlorpyrifos is entered as 5.532 minutes, which is 16.596 minutes divided by 3.

Table 4 compares the locking pressures determined with measured and with calculated RTL calibration points. As in the above GC-MS example, the range of the locking pressures from the calculated data is only 0.11 psi (87.88 to 87.99), which corresponds to ~ 0.003 minute.

Figure 5 compares the chromatograms of the RTL locking mixture from both the original and the 3× scaled methods. Note that while the chromatographic resolution is reduced, the speed is increased by a factor of 3.

Figure 6 shows a plot of the difference between the RTL Pesticide Library retention times, divided by 3, and those of the 3× method. The data were taken with a 36-component subset of the library. The plot shows the retention times match well within ± 0.05 minute for all compounds, even

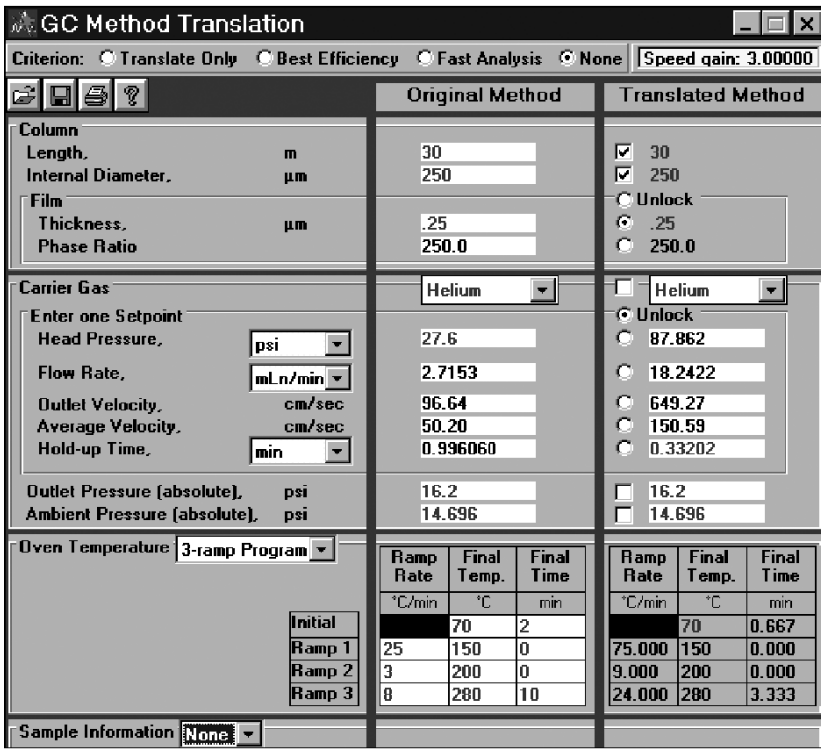


**Figure 4. Method translation software showing scaling RTL Pesticide method scaled to threefold faster method.**

those in the 3.3-minute hold time at the end of the run.

## Gaining Speed with a Small-Bore Column

In the previous example, speed was gained at the expense of resolution. In this example, speed will be gained while maintaining most of the resolution but sacrificing capacity. This is done by scaling the original method to a 0.1-mm id column.

In scaling to columns of a different diameter, there are two important considerations that must be obeyed to obtain precise matching to a library or reference method. The first is that the stationary phase composition must be the same as that used in the original method. The second is that the phase ratio of the column being scaled to must be the same as that of the reference method. Columns of the same phase ratio have the same ratio of inner diameter to film thickness. Because the reference method was developed on a column with 0.25 mm id × 0.25 µm film thickness, scaling to a 0.1-mm id column will require a 0.1-µm film thickness. A 10-m column of these dimensions was chosen for this example.

The micro-ECD for the 6890 GC is extremely sensitive, with detection limits in the low femtogram range for polyhalogenated pesticides. These detection limits are so low that it is reasonable to consider using split mode for a rapid screening method. Using split mode with a split ratio of 100 still gives a detection limits in the range of a few picograms. The split is also more compatible with the relatively low capacity of the column.

**Table 3. RTL Calibration Points from Original GC-AED Method and Calculated Points for Threefold Speed Gain (3×) Method**

| GC-AED RTL Calibration | | 3x GC-AED RTL Calibration | |
|---|---|---|---|
| Pressure (psi) | Ret Time (min) | Calculated Pressure (psi) | Calculated Ret Time (min) |
| 33.1 | 15.346 | 106.21 | 5.115 |
| 30.4 | 15.919 | 97.23 | 5.306 |
| 27.6 | 16.578 | 87.86 | 5.526 |
| 24.8 | 17.338 | 78.44 | 5.779 |
| 22.1 | 18.242 | 69.31 | 6.081 |

**Table 4. Comparison of Locking Pressures Calculated Using Measured and Predicted 3× GC-AED RTL Calibration Data**

| 3x GC-AED Locking Runs | | Locking Pressures | |
|---|---|---|---|
| Measured 3x GC-AED RTL Cal Points | | Using Measured RTL Cal Points | Using Calculated RTL Cal Points |
| Pressure ( psi) | Ret Time (min) | Pressure (psi) | Pressure (psi) |
| 97 | 5.319 | 87.99 | 87.99 |
| 92 | 5.433 | 87.94 | 87.95 |
| 87 | 5.557 | 87.99 | 87.99 |
| 82 | 5.689 | 87.99 | 87.96 |
| 77 | 5.832 | 87.97 | 87.88 |

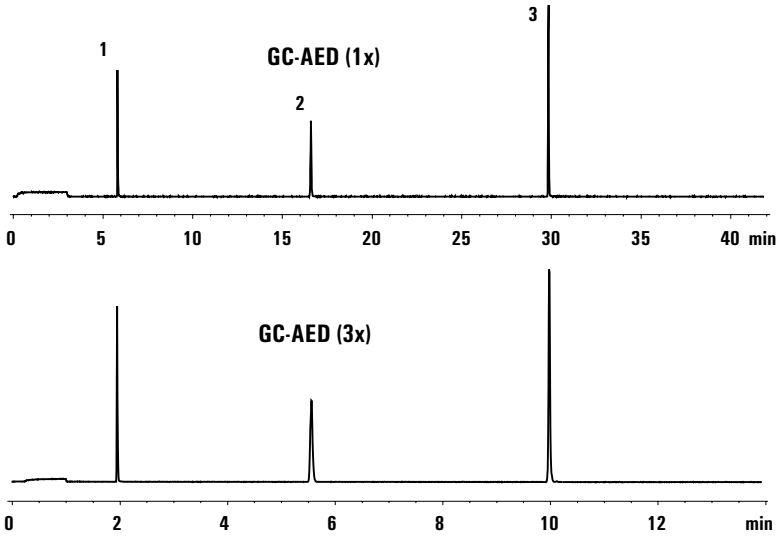


**Figure 5. Chlorine chromatograms from original and 3x GC-AED methods of three-component locking mixture. Peak identifications: 1. dichlorvos, 2. methyl chlorpyrifos, 3. mirex.**

Figure 7 shows the method translation from the GC-AED method to the 0.1-mm id column with a scale factor of 3. A speed gain of 3 was again chosen based on oven and inlet limitations as described above. The same scaling process as used above is followed.

The RTL calibration points for the new 3× 0.1-mm micro-ECD method were both calculated with method translation and measured. Table 5 shows the calculated values.

When the locking pressures from the measured and calculated values were examined, the calculated values provided much poorer predictions of locking pressure than expected. The pressure required to actually lock the column was confirmed to be 65.95 psi, as predicted by the measured RTL calibration data. Method translation had predicted the inlet pressure would be 58.514 psi for an assumed 10-m column length. Because the actual locking pressure was noticeably higher, this suggests that the actual column length was longer and/or the column diameter was smaller and/or the film thickness larger than the assumed values.

As an experiment, it was assumed that the problem was in the assumed length of the column used in calculating the RTL calibration points. The column length entry for the 0.1-mm column was iteratively adjusted until the calculated inlet pressure matched the actual locking pressure, 65.95 psi. This resulted in a calculated column length of 10.5622 m. A new set of calculated RTL calibration points were calculated using 10.5622 m as the length of the 0.1-mm column. The results are shown in table 6.

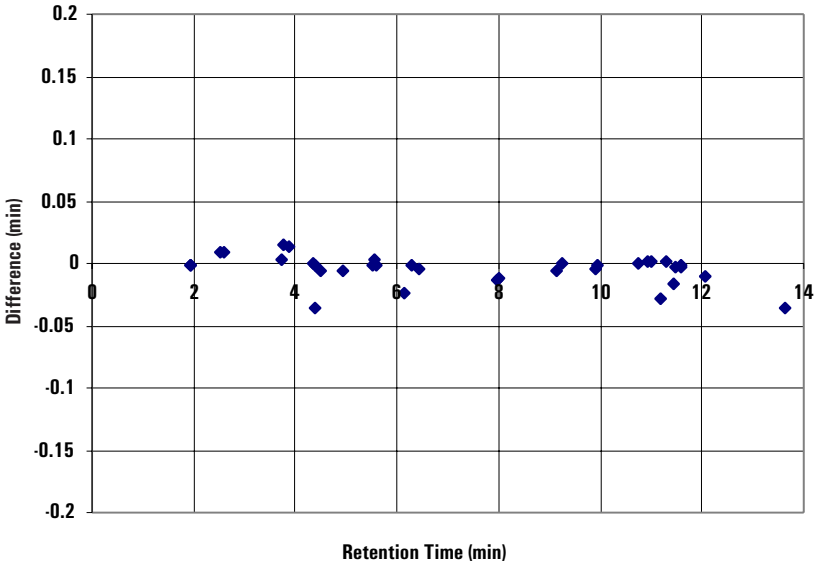


**Figure 6. Difference plot of RTL Pesticide Library (GC-FID) retention times divided by 3 minus 3× GC-AED retention times for 36-compound subset of the library.**

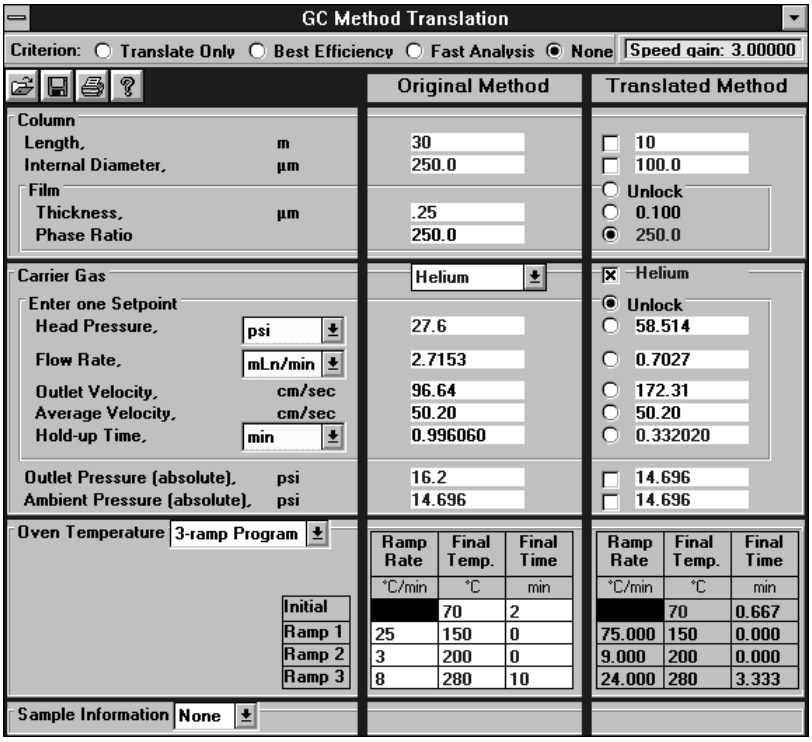


**Figure 7. Method translation software showing scaling RTL pesticide method scaled to a threefold faster method on a 10-m × 0.1-mm id column.**

Table 7 shows a comparison of locking pressures calculated using measured and predicted 3× 0.1-mm id micro-ECD calibration data. The range of locking pressures from the measured data (66.03 to 65.93) only corresponds to a spread in retention times of about 0.004 minute. However, with the data calculated based on a 10-m assumed length, the spread (66.38 to 63.18) is much larger and would correspond to a time range of 0.14 minute. The locking pressures calculated using the 10.5622 value are much more consistent with the measured values. The range in retention times would be ~ 0.03 minute if all the calculated points are used, and if the first value in column 5 is ignored, the range drops to ~ 0.005 minute.

The fact that the agreement in locking pressures is much improved by using 10.56 m instead of 10 m suggests that length is probably the largest contributor to the discrepancy. These results should reinforce the recommendation that if a method is to be used extensively, it is prudent to obtain measured RTL calibration data. It should be noted, however, that even with the RTL calibration from the 10-m assumed length, the worst consequence would be that the RT locking step would need to be repeated an extra time to get a more precise match.

Figure 8 compares the chromatograms of the RTL locking mixture from both the original and the 3 × 0.1-mm id micro-ECD methods.

**Table 5. RTL Calibration Points from Original GC-AED Method and Calculated Points for 3× 0.1-mm id Micro-ECD Method Assuming 10-m Column Length**

| GC-AED RTL Calibration | | 3x Micro-ECD RTL Calibration | |
|---|---|---|---|
| Pressure (psi) | Ret Time (min) | Calculated Pressure (psi) | Calculated Ret Time (min) |
| 33.1 | 15.346 | 71.03 | 5.115 |
| 30.4 | 15.919 | 64.90 | 5.306 |
| 27.6 | 16.578 | 58.51 | 5.526 |
| 24.8 | 17.338 | 52.11 | 5.779 |
| 22.1 | 18.242 | 45.91 | 6.081 |

**Table 6. RTL Calibration Points from Original GC-AED Method and Calculated Points for 3× 0.1-mm id Micro-ECD Method Assuming 10.5622-m Column Length**

| GC-AED RTL Calibration | | 3x Micro-ECD RTL Calibration | |
|---|---|---|---|
| Pressure (psi) | Ret Time (min) | Calculated Pressure (psi) | Calculated Ret Time (min) |
| 33.1 | 15.346 | 80.03 | 5.115 |
| 30.4 | 15.919 | 73.13 | 5.306 |
| 27.6 | 16.578 | 65.95 | 5.526 |
| 24.8 | 17.338 | 58.74 | 5.779 |
| 22.1 | 18.242 | 51.75 | 6.081 |

**Table 7. Comparison of Locking Pressures Calculated Using Measured and Predicted 3× 0.1-mm id Micro-ECD Calibration Data**

| 3x Micro-ECD Locking Runs | | Locking Pressures | | |
|---|---|---|---|---|
| Measured 3x Micro-ECD RTL Cal Points | | Using Measured RTL Cal Points | Using 10-m Calculated RTL Cal Points | Using 10.56-m Calculated RTL Cal Points |
| Pressure (psi) | Ret Time (min) | Pressure (psi) | Pressure (psi) | Pressure (psi) |
| 48.81 | 6.323 | 65.95 | 66.38 | 65.30 |
| 52.66 | 6.041 | 66.03 | 65.77 | 65.85 |
| 58.51 | 5.797 | 65.95 | 65.12 | 65.96 |
| 64.36 | 5.585 | 65.93 | 64.36 | 65.95 |
| 70.22 | 5.396 | 66.00 | 63.18 | 65.90 |

Note that while the most of the chromatographic resolution is preserved, the speed is increased by a factor of 3.

After being locked, the three peaks in the 3× micro-ECD method had retention times of 1.924, 5.533, and 9.963 minutes, respectively. These values are very close to the RTL Pesticide Library retention times for the three compounds divided by 3: 1.932, 5.532, and 9.949. The fact that the largest difference between the scaled table and the 3× micro-ECD method is only 0.014 minute again demonstrates the precision of retention time matching achievable with the scaling technique described here.

## Conclusions

Using method translation combined with retention time locking provides a means of extending the usefulness of existing capillary GC methods. The ability to precisely scale a method to meet the needs of different samples and instrument types greatly reduces the effort required to re-use methods, thus saving time and money.

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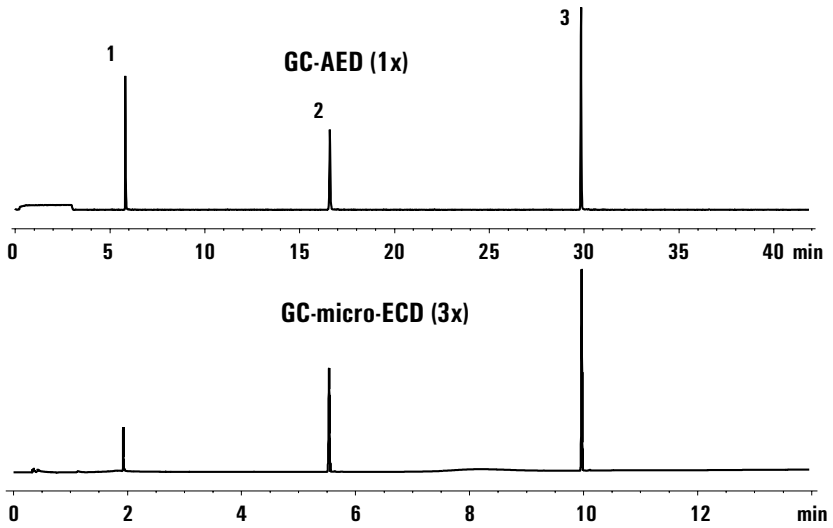


**Figure 8. Chlorine chromatogram from 1× GC-AED method (top) and 3× micro-ECD method (bottom) of three-component locking mixture. Peak identifications: 1. dichlorvos, 2. methyl chlorpyrifos, 3. mirex.**

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**Agilent Technologies**
Innovating the HP Way

# Performance Characteristics of the Agilent 7500ce - The ORS Advantage for High Matrix Analysis

**Part 1 of a 3 part series on Environmental Analysis**

Application

**Environmental Analysis**


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## Abstract

**The Agilent 7500ce ICP-MS was specifically designed and optimized for the analysis of trace metals in high matrix samples including environmental, clinical, geological, and others. The 7500ce uses enhanced Octopole Reaction System (ORS) technology for improved sensitivity and robustness over previous generation inductivity coupled plasma mass spectrometry (ICP-MS) instruments. This application note outlines the theory of interference removal using the ORS, the design enhancements employed, and the typical performance of the Agilent 7500ce.**


## Introduction

This application note is Part One of a three part series on environmental analysis using the Agilent 7500ce ICP-MS system. Part Two is an application note demonstrating the ability of the Agilent 7500ce ICP-MS system to measure trace elements in drinking water, at substantially below regulated levels, under challenging real-world conditions [1].

Part Three is an application note covering the analysis of various high matrix environmental samples using the Agilent 7500ce ICP-MS [2].

This application note details the advances in ion optics and octopole reaction system (ORS) design that were incorporated into the 7500ce. These advances came about as a result of extensive testing and development of its predecessor (Agilent 7500c) with difficult, high-matrix samples. The design goals of the 7500ce were:

- Develop an ICP-MS system specifically to meet the needs of analytical laboratories to analyze unknown, variable, high-matrix samples, which are currently depending on inductively coupled plasma-optical emission spectroscopy (ICP-OES), graphite furnace atomic absorption (GFAA), and hydride and cold vapor techniques, in addition to ICP-MS.
- Maintain the simple, effective interference removal characteristics of the ORS - successfully introduced in the 7500c.
- Improve the overall sensitivity to allow ultra-trace analysis of mercury (Hg) and other low level elements, which were previously difficult in some very high matrix sample types.

These goals were achieved through enhancements in the sample introduction system, interface, ion optic, and ORS regions of the instrument. In common with all the other models in the 7500 Series, the 7500ce uses highly efficient 27 MHz plasma coupled to a low-flow nebulizer and cooled-spray chamber to minimize plasma and interface matrix effects. This approach has been

successfully used in all Agilent ICP-MS instruments since the 4500 Series in 1994, but recent enhancements with the development of a new digitally driven, all solid-state RF generator have further increased plasma robustness. This serves to reduce metal oxide interferences, as evidenced by a very low $CeO^+/Ce^+$ ratio of <1.5% (<0.8% in He cell gas mode). Following the successful strategy of the 7500c, all ion lenses with the exception of the octopole are outside the high vacuum region and can be serviced without venting the mass analyzer. This design greatly reduces downtime for routine system maintenance. The 7500ce maintains a linear, axial flow of ions from the sampler and skimmer cones through a pair of on-axis extraction lenses, enhancing ion transmission and reducing the effects of matrix accumulation on the extraction lenses. Borrowed from the successful 4500 and 7500a systems, the 7500ce uses a simplified Omega lens to eliminate photons and neutrals from the ion beam before entering the octopole. Unlike older photon stop designs, the Omega lens eliminates photons and neutrals while maintaining high ion transmission, particularly at low masses. After the Omega lens, ions enter the octopole reaction cell, which is now located on-axis to the quadrupole and detector, further enhancing ion transmission. The redesigned ion lens and ORS provide improved ion transmission without compromising the tight control of ion energy, which is essential for efficient interference removal by energy discrimination (ED).

Figure 1 compares the 7500c and 7500ce configurations, highlighting the simplification in the ion trajectory that has led to the improved performance specifications of the 7500ce. Enhancements in software designed specifically for routine high matrix analyses add additional capability and ease of use. These include the introduction of "Virtual Internal Standardization" (VIS) which allows the user to interpolate between internal standard (ISTD) response factors to create a VIS at a mass where no appropriate ISTD exists. Intelligent calibration resloping can automatically fine-tune a calibration curve, if needed, during a long sequence of high matrix samples, without the time consuming recalibration. This can be accomplished in the same process as monitoring a required continuing calibration verification (CCV).

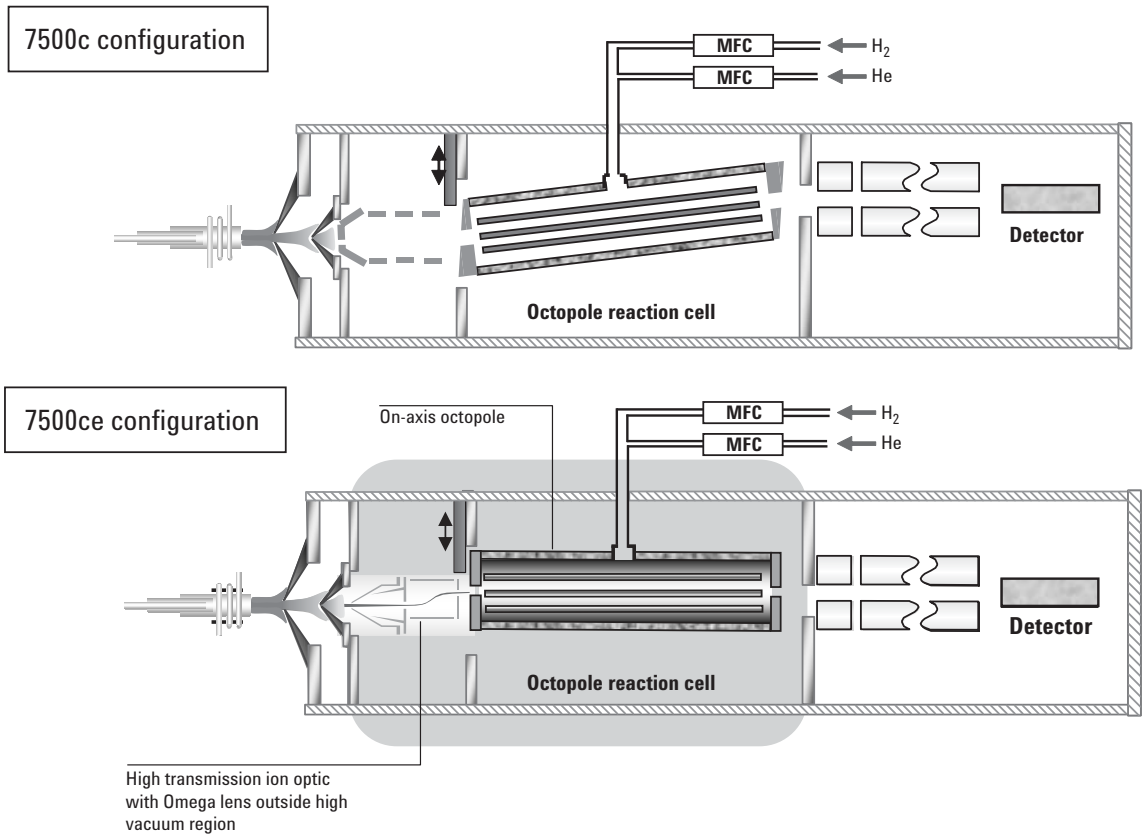


**Figure 1. A comparison of the ion optic and octopole configurations between the Agilent 7500c and 7500ce ICP-MS systems.**

### Enhanced ORS

Like its predecessor the 7500c, the 7500ce uses collision/reaction cell (CRC) technology in the form of the ORS to remove polyatomic interferences. The use of CRC technology to reduce interferences in ICP-MS is well-documented [3]. However, until now, there have been compromises associated with the use of some designs of CRC ICP-MS for trace level, multi-element analysis in unknown or variable matrices. These compromises included poor sensitivity for low-mass analytes, poor stability, and the necessity for matrix matching of samples and standards to avoid unexpected new interferences caused by complex, sequential reaction chemistry in the cell. As a result, some CRC systems allow only the analysis of a small number of analytes under a specific set of conditions for a single sample matrix.

Numerous publications [3, 4, 5] have discussed the mechanisms for polyatomic interference removal using CRC technology including:

- Collisionally induced dissociation (CID)
- Chemical reaction
  - Charge transfer
  - Atom transfer
- Kinetic energy discrimination (KED)

Mechanism 1, CID, does not occur to any great degree with the relatively light gases typically used in the collision cell because the combined kinetic energy of the collision does not generally exceed the bond energy of the polyatomic species. In most CRC ICP-MS systems, chemical reaction mechanisms including charge transfer and atom transfer are the predominant mechanisms [4, 5]. However, in order to provide sufficient reduction of interferences, the reaction must be highly favored, which can require the use of very reactive gases for many interferences. Such gases can also react with analyte ions, so reducing sensitivity and compromising multi-element analysis, or form reaction by-products that can interfere with other analytes [4]. In this case, reaction cell conditions must be matched to a specific analyte/matrix combination and cannot be used simultaneously for multiple analytes in variable matrices. Mechanism 3, KED, relies on the fact that at the exit of the collision cell polyatomic species will possess lower kinetic energy than atomic ions at the same mass-to-charge ratio [3, 4]. This is due to the fact that collision cross sections of polyatomic ions are larger than for atomic ions, so that polyatomic species suffer more collisions with the cell gas, thus losing more of their initial energy. A bias voltage at the cell exit is then used to reject the low-energy polyatomic species, while allowing the high-energy atomic ions to enter the quadrupole for analysis and detection

### Three Modes of Operation - One Set of Conditions

Table 1 lists the typical instrument conditions used for high-matrix analysis for the 7500ce. Instrument parameters are essentially the same for all three modes of operation[1]. This is because no complex procedures are required to remove newly created interferences or to avoid the reactive loss of analyte in any ORS mode.

[1]Slightly higher bias voltages are required in the octopole and quadrupole to maintain ion velocity in a pressurized collision cell compared with a nonpressurized cell. Other parameters, with the exception of the cell gas flow, are identical in all modes of operation.

**Table 1. Instrument Parameters for Robust Plasma Conditions Used with the 7500ce**

| Instrument parameter | Normal mode | Hydrogen mode | Helium mode |
|---|---|---|---|
| RF Power | 1500 W | <Same | <Same |
| Sample depth | 8 mm | <Same | <Same |
| Carrier gas | 0.85 L/min | <Same | <Same |
| Makeup gas | 0.2 L/min | <Same | <Same |
| Spray chamber temp | 2 °C | <Same | <Same |
| Extract 1 | 0 V | <Same | <Same |
| Extract 2 | –160 V | <Same | <Same |
| Omega bias | –24 V | <Same | <Same |
| Omega lens | –0.6 V | <Same | <Same |
| Cell entrance | –30 V | <Same | <Same |
| QP focus | 3 V | –11 V | <Same as $H_2$ |
| Cell exit | –30 V | –44 V | <Same as $H_2$ |
| Octopole bias | –7 V | –18 V | <Same as $H_2$ |
| QP bias | –3.5 V | –14.5 V | <Same as $H_2$ |
| Cell gas flow | 0 | 3.0 mL/min $H_2$ | 4.5 mL/min He |

### The Hydrogen Reaction Mode

In hydrogen reaction mode, the ORS is pressurized using a small flow of pure hydrogen at 1–5 mL $min^{-1}$. Simple reactions with hydrogen remove argon-based polyatomics according to the following examples.

Charge ($e^-$) transfer:

$Ar^+ + H_2 \rightarrow H_2^+ + Ar$ (interference on $^{40}Ca^+$)

Proton transfer:

$Ar_2^+ + H_2 \rightarrow Ar_2H^+ + H$ (interferences on $^{78}Se^+$ and $^{80}Se^+$)

$ArO^+ + H_2 \rightarrow ArOH^+ + H$ (interference on $^{56}Fe$)

Atom transfer:

$ArO^+ + H_2 \rightarrow H_2O^+ + Ar$ (interference on $^{56}Fe$)

In all cases the Ar interference is removed from mass 40, 56, 78, and 80. Since Ca, Fe, and Se do not react with $H_2$, there is no loss of analyte signal.

$Ca^+ + H_2 \rightarrow$ No reaction

$Se^+ + H_2 \rightarrow$ No reaction

$Fe^+ + H_2 \rightarrow$ No reaction

Note that some of these reaction processes lead to new polyatomic ion species, principally hydrides of the original interference. However, these new, cell-formed species all have low energy and are removed from the ion beam using the same bias voltage, as was discussed above, under interference removal by KED.

Reaction mechanisms can be highly efficient, as evidenced by the calibration curves in Figure 2 for $^{78}Se$, $^{40}Ca$ and $^{56}Fe$, which show that all the "normal" background species are reduced significantly under a single set of cell conditions.

Figure 3 illustrates the reduction in background from $Ar^+$ at $m/z$ = 40 as hydrogen flow in the cell is increased, yielding a $10^9$ reduction in background. Since the reaction chemistry is specific to argon polyatomics, no signal is lost due to reaction of the analyte with hydrogen, as could occur with other more reactive gases. However, due to the specificity of reaction mode, there are numerous examples where it is not useful. For example, in samples where the matrix composition is unknown, or there are multiple polyatomic interferences at a single $m/z$, it is not possible to use reaction mode effectively. In this case, a more generic method of interference removal is needed.

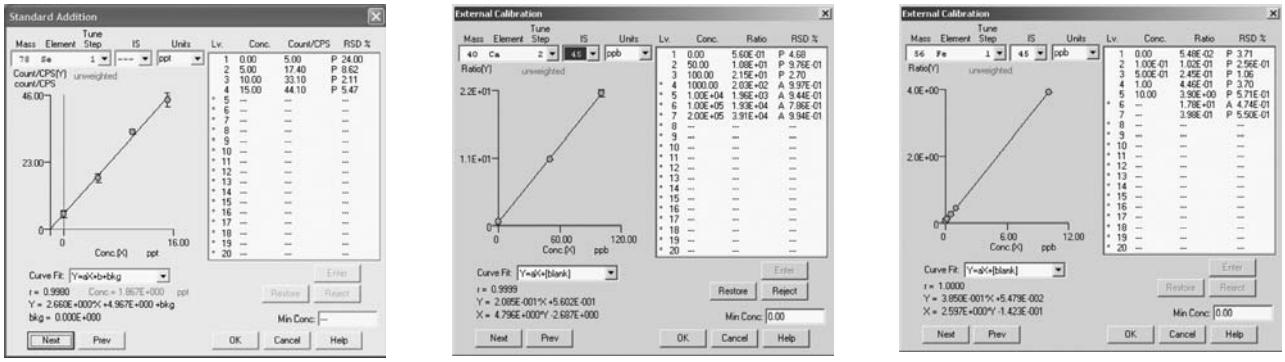


**Figure 2. Calibration plots for $^{78}Se$, $^{40}Ca$, and $^{56}Fe$ under hydrogen reaction conditions.**

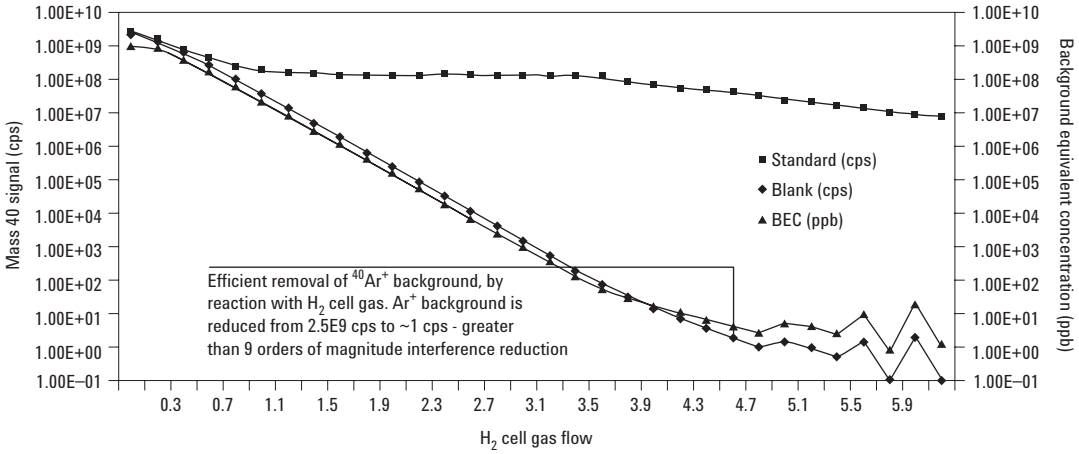


**Figure 3. Reduction in background on mass 40 for calcium using hydrogen reaction mode. In this case, as the hydrogen flow is increased to about 5 mL/min, the background at mass 40 decreased from approximately 2.5 billion cps to about 1 cps, a >$10^9$ reduction.**

### The Helium Collision Mode

Helium collision mode can reduce or eliminate polyatomic interferences by one of two mechanisms; either CID or KED. Both are nonreactive mechanisms and so they do not form any new polyatomic ionic species that must be managed. CID can occur when the collision energy between the polyatomic ion and the collision gas (typically He) is sufficient to break the polyatomic bond. The result is two (usually atomic) fragments at lower mass, one of which will retain the charge of the original ion. A few common polyatomic interferences are bound weakly enough for this to occur. They include $NaAr^+$, which can interfere with the measurement of $^{63}Cu$ in high sodium samples and $ArO^+$ which interferes with iron. However, when ion energies are properly controlled, KED is the more useful of the two techniques. Kinetic energy discrimination depends on the fact that polyatomic ions are always larger in collisional cross section than monatomic ions (Figure 4), and as a result undergo more collisions and so lose more energy when traversing a pressurized collision cell. Figures 5 and 6 depict the KED process.

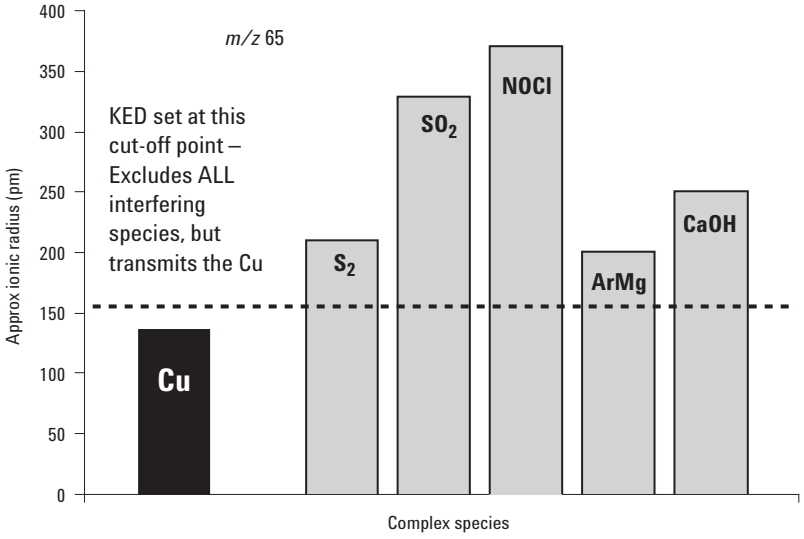


**Figure 4. Comparison of approximate ionic radii (in picometers) for copper and several polyatomic species.**

Figure 5 shows the greater loss of energy of the polyatomic ion relative to the atomic ion, in this case $ArCl^+$ relative to $As^+$. However, for KED to be effective, it must remove the polyatomic ion effectively while not significantly reducing the response of the atomic ion. This means there must be minimal overlap in ion kinetic energies between the polyatomic and atomic ions at the exit of the octopole. For this to be the case, the energy spread of incoming ions must be less than the energy difference between analyte and polyatomic interference at the octopole exit.

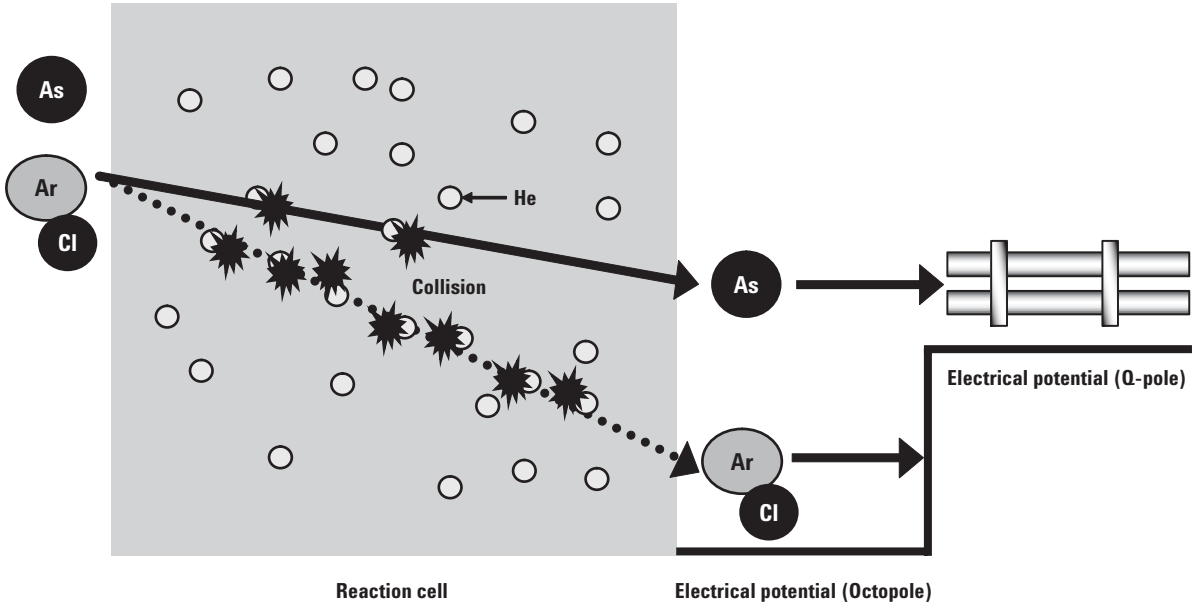


**Figure 5. KED. Polyatomic species have a larger collision cross section, and so experience more energy dissipating collisions and exit the cell with lower kinetic energy. A small stopping potential between the exit of the octopole and the entrance of the quadrupole keeps the polyatomic ions from entering the quadrupole and being detected.**

Only the Agilent ORS can accomplish this, as a result of the use of the ShieldTorch system, which minimizes plasma potential and eliminates secondary ionization in the interface, which would otherwise cause broadening of the ion energy spread. It is also essential to avoid band-broadening collisions induced by high extraction voltages in the high-pressure region immediately behind the skimmer cone. On the 7500ce, this is accomplished by using soft-extraction, (extract 1 operates at 0 to +5 V), as a result of which, the mean ion energy is maintained at less than 2 eV with an ion energy spread of about 0.5 eV, ideal for the KED of plasma-source polyatomic interferences.

A simplified schematic representation of ion kinetic energy and energy distribution for a typical ICP-MS system and from an Agilent 7500 ORS ICP-MS is shown in Figure 6.

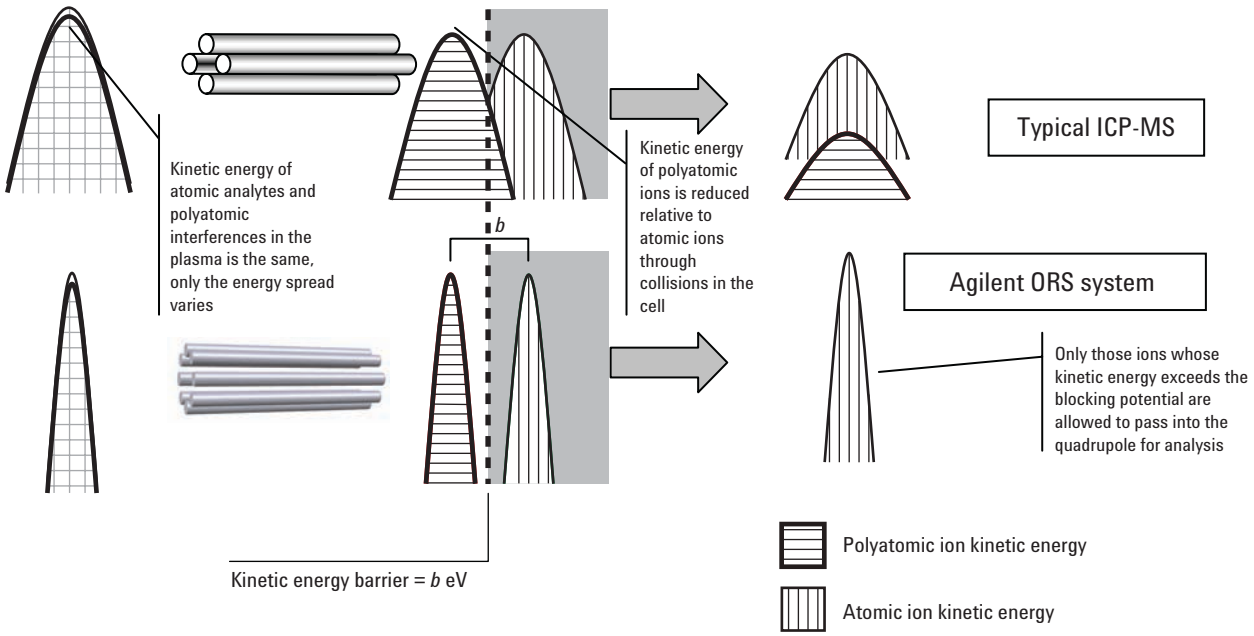


**Figure 6. Simplified schematic representation of ion kinetic energy and energy distribution of a typical ICP-MS system (upper), and from an Agilent 7500 ORS ICP-MS (lower).**

After multiple collisions in the CRC, in both cases the average kinetic energy of the larger polyatomic ions is decreased relative to the smaller atomic ions by *b* eV. If a kinetic energy barrier is applied to the ion beam at the exit to the collision cell that is equivalent to the kinetic energy difference, *b*, (indicated by dashed line), then any ions whose kinetic energy is lower than the barrier will be blocked. If the energy distribution of ions is larger than the difference in average energies of the ions, only partial rejection of polyatomic ions occurs accompanied by a loss of atomic ions.

While ED was described on other designs of ICP-MS systems, these systems do not have the tight control of ion energy provided by the ShieldTorch System and so the ED is only effective for reducing the very low energy polyatomics formed within the cell, typically as a result of sequential reaction chemistry, which is characteristic of the use of a highly reactive cell gas, such as $NH_3$. Table 2 shows the reactants and products for a number of polyatomic interferences using both hydrogen and ammonia reaction mode. As can be seen, in the presence of common matrix components such as carbon and sulfur, the use of $NH_3$ can create multiple new interferences, which must be removed. Avoiding the use of $NH_3$ eliminates the possibility of creating new, cell-formed, polyatomic cluster ions in the first place.

Reaction of hydrogen with plasma-based polyatomics such as $Ar^+$ is highly favored and results in elimination of the interferent. Neither hydrogen nor $NH_3$ are effective at removing the interference from $ClO^+$ on vanadium. Additionally, the use of ammonia can lead to reaction with other common matrix elements such as carbon and sulfur, creating new interferences such as $HCN^+$ on aluminum and $NHSH^+$ on titanium. Using the Agilent system with an inert cell gas and KED would eliminate the interference from $ClO^+$ on vanadium AND, for example, $ArC^+$ on Cr, without producing any new interferences.

An excellent test of the efficiency of interference removal can be seen in low-level calibration plots. When interferences are present, the response curve will be offset in the y direction by the magnitude of the interference, increasing the background equivalent concentration (BEC) and the detection limit (DL). When the interference is removed, the calibration curve intersects the y-axis at a point much nearer to zero with a correspondingly lower BEC and DL. Figure 7 depicts sub-ppb calibration curves for chromium and vanadium in 1% each methanol, HCl, and $HNO_3$, with and without the use of helium collision mode. Since KED does not depend on chemical reaction, it is independent of matrix concentration as well as composition.

**Table 2. Comparison of Reaction Products for Several Possible Reactants Involving Hydrogen and Ammonia**

| Reactants | | Interfered analytes | Products | |
|---|---|---|---|---|
| $Ar^+$ | $H_2$ | $Ca^+$ | $H_2^+$ | Ar |
| $Ar_2^+$ | $H_2$ | $Se^+$ | $ArH^+$ | Ar, H |
| $ArO^+$ | $H_2$ | $Fe^+$ | $H_2O^+$, $ArOH^+$ | |
| $ClO^+$ | $H_2$ | $V^+$ | None | None |
| $ClO^+$ | $NH_3$ | $V^+$ | None | None |
| $HCN^+$ | $H_2$ | $Al^+$ | $HCNH^+$ (28) | H |
| $C^+$ | $NH_3$ | | $NH_3^+$ (17) | C |
| | | | $HCN^+$ (27) | $H_2$ |
| | | | $HCNH^+$ (28) | H |
| $S^+$ | $NH_3$ | | $NHSH^+$ (48) | |

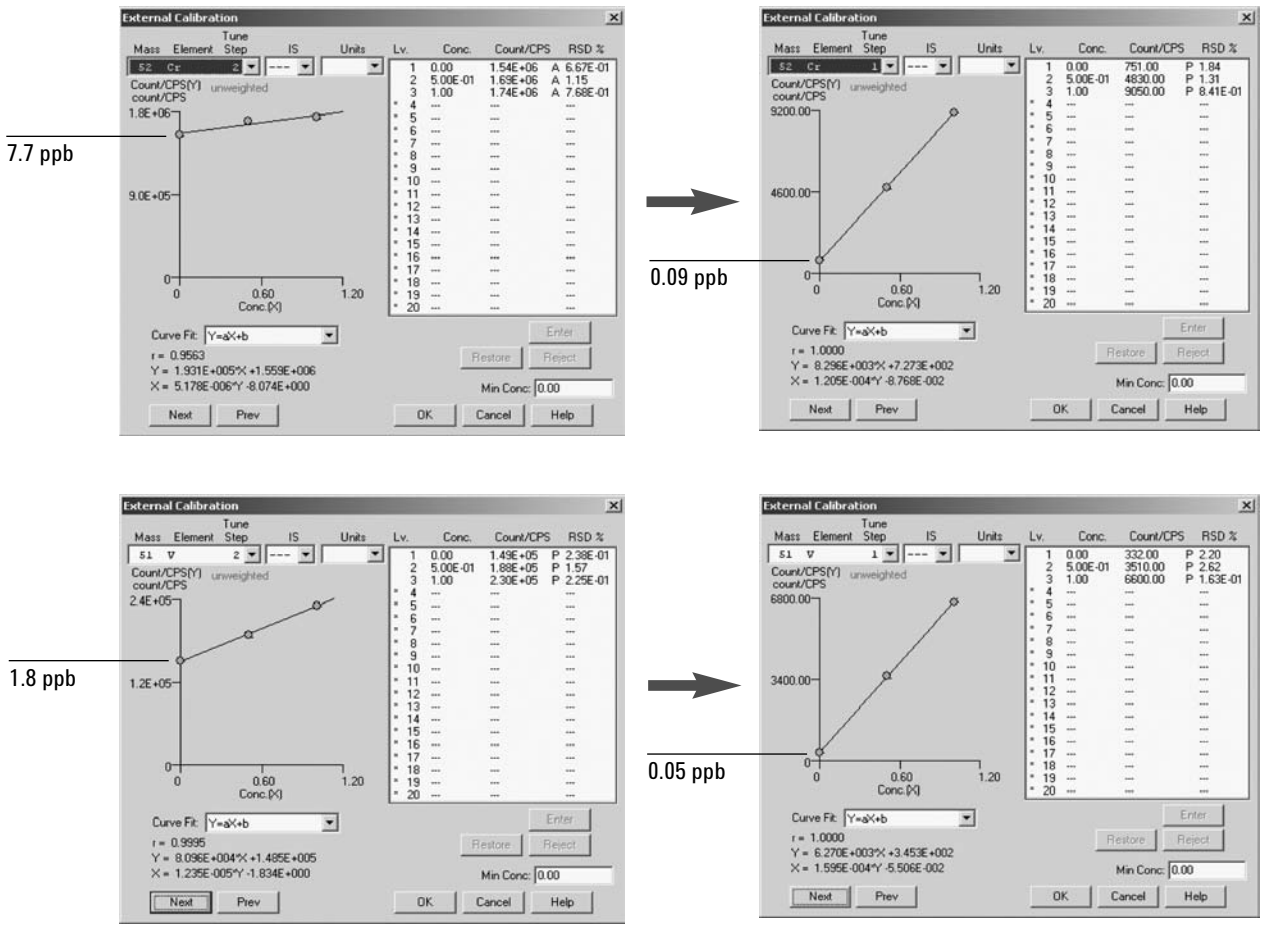


**Figure 7. Calibration plots of $^{52}$chromium and $^{51}$vanadium in 1% nitric, 1% hydrochloric, 1% methanol showing the contribution of interferences from $ArC^+$ and $ClO^+$ in normal mode on the left and after removal of interferences by the ORS using He on the right.**

Figure 8 depicts the effect of increasing HCl on the measured concentration of a 5-ppb solution of vanadium in both helium and normal (no gas) mode. Increasing the HCl from 0% to 1% causes an 80% increase in measured vanadium concentration in the no-gas mode. There is no increase in the V concentration reported for the variable sample matrix, when measured in He mode.

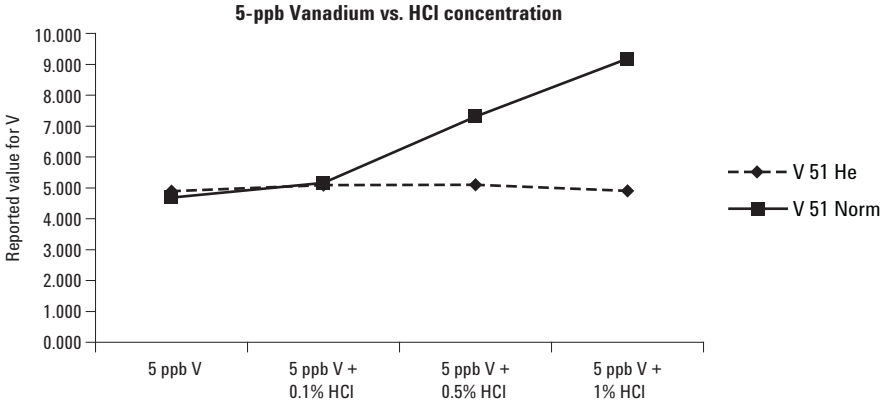


**Figure 8. Effects of increasing HCl concentration on vanadium response in both normal and helium modes.**

### The Normal Mode

Normal mode uses the octopole as an ion guide only with no additional gas added. In this mode, the Agilent 7500ce ICP-MS operates as a conventional (non-collision cell) instrument. Because an octopole is a highly efficient ion guide as compared with a lower-order multipole, such as a hexapole or quadrupole, there are no compromises in ion transmission efficiency and the addition of a collision gas to promote collisional focusing in normal mode is not required. Because of this, the Agilent 7500ce exhibits exceptional sensitivity for uninterfered low mass elements, such as lithium, beryllium, and boron. Typically, normal mode is used only for these elements, though it is also acceptable for other elements that do not require interference removal such as lead, mercury, thallium, and uranium. Examination of Table 3 will show that the DLs for the interference-free heavy metals are essentially the same in all three modes, giving the user the flexibility to select as appropriate.

**Table 3. Three Sigma Detection Limits (ppt). All Isotopes 1 s Total Integration Time Except Ca and Hg Which Were 3 s Total Integration Time**

| | | STD Mode (No gas) | | $H_2$ Mode (5 mL/min) | | He Mode (4 mL/min) | |
|---|---|---|---|---|---|---|---|
| **Mass** | **Element** | **DL (3-Sigma)** | **BEC** | **DL (3-Sigma)** | **BEC** | **DL (3-Sigma)** | **BEC** |
| 6 | Li | 4.99 | 17.09 | 161.491 | 95.83 | 331.32 | 142.58 |
| 7 | Li | 1.67 | 14.36 | 23.755 | 26.31 | 9.38 | 20.55 |
| 9 | Be | 0.19 | 0.11 | 6.932 | 2.62 | 7.812 | 2.61 |
| 11 | B | 5.88 | 47.26 | 83.182 | 128.03 | 48.21 | 107.28 |
| 23 | Na | 3.36 | 148.40 | 62.682 | 313.64 | 37.65 | 299.38 |
| 24 | Mg | 0.27 | 0.72 | 2.570 | 1.75 | 3.37 | 1.41 |
| 27 | Al | 3.05 | 50.70 | 8.079 | 5.51 | 37.56 | 53.52 |
| 31 | P | 418.27 | 12521.95 | – | – | 1903.62 | 3800.52 |
| 39 | K | 1347.50 | 47564.25 | 29.532 | 118.74 | 2838.73 | 27943.17 |
| 40 | Ca | – | – | 2.936 | 7.13 | – | – |
| 43 | Ca | 460.04 | 8520.74 | 129.640 | 121.68 | 191.85 | 742.92 |
| 44 | Ca | 2932.48 | 50407.443 | 102.104 | 121.94 | 48.01 | 352.38 |
| 45 | Sc | 7.95 | 183.06 | 6.446 | 19.41 | 1.34 | 6.46 |
| 47 | Ti | 4.07 | 40.86 | 32.197 | 19.88 | 4.98 | 3.52 |
| 49 | Ti | 11.49 | 57.33 | 17.535 | 10.46 | 5.69 | 1.70 |
| 51 | V | 0.40 | 2.52 | 1.309 | 0.73 | 0.42 | 0.19 |
| 52 | Cr | 5.53 | 212.670 | 19.919 | 68.63 | 3.10 | 22.70 |
| 53 | Cr | 7.98 | 52.87 | 28.504 | 82.65 | 8.70 | 21.60 |
| 55 | Mn | 1.69 | 25.24 | 1.362 | 4.10 | 4.25 | 12.23 |
| 56 | Fe | 1443.70 | 55093.34 | 5.034 | 20.21 | 53.99 | 451.26 |
| 57 | Fe | 444.36 | 23132.21 | 66.614 | 261.56 | 71.06 | 215.90 |
| 59 | Co | 0.21 | 2.15 | 0.816 | 0.26 | 0.38 | 0.30 |
| 60 | Ni | 26.523 | 672.20 | 71.224 | 742.27 | 41.70 | 491.67 |
| 63 | Cu | 1.32 | 37.48 | 20.271 | 20.14 | 6.37 | 68.35 |
| 65 | Cu | 2.70 | 47.46 | 27.845 | 27.76 | 7.52 | 59.76 |
| 66 | Zn | 2.85 | 9.01 | 1.612 | 1.83 | 1.84 | 2.18 |
| 69 | Ga | 0.30 | 3.03 | 0.273 | 0.19 | 0.82 | 1.04 |
| 71 | Ga | 1.22 | 7.40 | 0.125 | 0.15 | 1.80 | 2.22 |
| 72 | Ge | 2.60 | 53.69 | 1.448 | 1.14 | 3.32 | 7.55 |
| 73 | Ge | 3.72 | 32.74 | 5.556 | 2.74 | 7.30 | 6.04 |
| 75 | As | 23.24 | 660.78 | 14.130 | 14.00 | 10.72 | 65.72 |
| 78 | Se | 48.10 | 6351.29 | 2.396 | 2.556 | 48.93 | 195.45 |
| 82 | Se | 26.92 | 251.29 | 15.225 | 56.51 | 20.63 | 116.01 |
| 85 | Rb | 0.27 | 1.06 | 0.349 | 0.57 | 0.72 | 0.34 |
| 88 | Sr | 0.19 | 0.84 | 0.072 | 0.04 | 0.38 | 0.13 |
| 89 | Y | 0.10 | 0.26 | 0.054 | 0.04 | 0.16 | 0.04 |
| 90 | Zr | 0.07 | 0.09 | 0.709 | 0.18 | 0.15 | 0.06 |

**Table 3. Three Sigma Detection Limits (ppt). All Isotopes 1 s Total Integration Time, Except Ca and Hg Which Were 3 s Total Integration Time (Continued)**

| | | STD Mode (No gas) | | $H_2$ Mode (5 mL/min) | | He Mode (4 mL/min) | |
|---|---|---|---|---|---|---|---|
| **Mass** | **Element** | **DL (3-Sigma)** | **BEC** | **DL (3-Sigma)** | **BEC** | **DL (3-Sigma)** | **BEC** |
| 93 | Nb | 0.12 | 0.20 | 0.68 | 0.53 | 0.15 | 0.05 |
| 95 | Mo | 0.32 | 0.67 | 14.53 | 4.33 | 0.31 | 0.1 |
| 101 | Ru | 0.60 | 1.40 | 19.27 | 5.74 | 0.28 | 0.09 |
| 103 | Rh | 0.08 | 0.11 | 4.24 | 1.75 | 0.05 | 0.0 |
| 105 | Pd | 0.30 | 0.27 | 10.11 | 6.39 | 0.33 | 0.15 |
| 107 | Ag | 0.23 | 0.33 | 1.42 | 1.61 | 0.28 | 0.4 |
| 111 | Cd | 0.56 | 0.83 | 0.32 | 0.20 | 0.86 | 0.54 |
| 115 | In | 0.07 | 0.11 | 0.05 | 0.03 | 0.08 | 0.05 |
| 116 | Cd | 0.33 | 0.41 | 0.40 | 0.47 | 0.34 | 0.23 |
| 118 | Sn | 0.24 | 0.43 | 0.55 | 0.54 | 0.91 | 0.73 |
| 121 | Sb | 0.11 | 0.08 | 0.21 | 0.010 | 0.46 | 0.25 |
| 125 | Te | 1.96 | 0.94 | 2.05 | 1.29 | 9.57 | 4.12 |
| 126 | Te | 1.12 | 1.64 | 2.08 | 1.67 | 7.33 | 4.27 |
| 127 | I | 2.02 | 21.73 | 3.57 | 22.30 | 7.71 | 20.41 |
| 133 | Cs | 0.09 | 0.04 | 0.06 | 0.04 | 0.15 | 0.06 |
| 137 | Ba | 0.22 | 0.20 | 0.38 | 0.16 | 0.9 | 0.38 |
| 139 | La | 0.17 | 1.94 | 2.49 | 2.46 | 0.66 | 2.14 |
| 140 | Ce | 0.223 | 2.65 | 2.18 | 3.21 | 0.47 | 2.88 |
| 141 | Pr | 0.11 | 0.25 | 0.12 | 0.28 | 0.12 | 0.31 |
| 146 | Nd | 0.39 | 0.44 | 0.43 | 0.50 | 0.70 | 0.73 |
| 147 | Sm | 0.22 | 0.17 | 0.11 | 0.04 | 0.58 | 0.24 |
| 153 | Eu | 0.02 | 0.03 | 0.04 | 0.02 | 0.11 | 0.03 |
| 157 | Gd | 0.17 | 0.14 | 0.15 | 0.05 | 0.35 | 0.22 |
| 159 | Tb | 0.03 | 0.02 | 0.01 | 0.01 | 0.055 | 0.03 |
| 161 | Dy | 0.18 | 0.15 | 0.05 | 0.05 | 0.23 | 0.17 |
| 163 | Dy | 0.15 | 0.08 | 0.08 | 0.04 | 0.23 | 0.16 |
| 165 | Ho | 0.04 | 0.00 | 0.02 | 0.01 | 0.05 | 0.02 |
| 166 | Er | 0.15 | 0.06 | 0.05 | 0.02 | 0.17 | 0.09 |
| 169 | Tm | 0.02 | 0.02 | 0.02 | 0.01 | 0.03 | 0.03 |
| 172 | Yb | 0.11 | 0.09 | 0.07 | 0.02 | 0.30 | 0.18 |
| 175 | Lu | 0.04 | 0.020 | 0.02 | 0.01 | 0.06 | 0.03 |
| 178 | Hf | 0.13 | 0.08 | 0.06 | 0.06 | 0.32 | 0.15 |
| 181 | Ta | 0.04 | 0.06 | 0.06 | 0.049 | 0.11 | 0.08 |
| 182 | W | 0.32 | 0.35 | 1.39 | 0.5 | 0.56 | 0.33 |
| 183 | W | 5.07 | 1.07 | 0.87 | 0.42 | 0.43 | 0.47 |
| 185 | Re | 0.12 | 0.07 | 0.07 | 0.05 | 0.12 | 0.08 |
| 193 | Ir | 0.09 | 0.10 | 0.25 | 0.08 | 0.33 | 0.15 |
| 195 | Pt | 0.14 | 0.17 | 1.94 | 0.52 | 0.22 | 0.18 |
| 197 | Au | 0.22 | 0.11 | 1.76 | 0.43 | 0.18 | 0.07 |
| 200 | Hg | 0.82 | 2.00 | 1.04 | 1.78 | 1.15 | 2.18 |
| 201 | Hg | 1.11 | 2.54 | 2.07 | 2.29 | 1.56 | 2.58 |
| 202 | Hg | 0.86 | 1.84 | 0.75 | 1.77 | 0.59 | 1.91 |
| 205 | Tl | 0.20 | 0.24 | 0.13 | 0.22 | 0.35 | 0.30 |
| 206 | Pb | 0.33 | 0.84 | 0.28 | 0.64 | 0.34 | 0.73 |
| 207 | Pb | 0.51 | 0.94 | 0.25 | 0.69 | 0.95 | 1.05 |
| 208 | Pb | 0.47 | 0.712 | 0.40 | 0.55 | 0.53 | 0.755 |
| 209 | Bi | 0.05 | 0.04 | 0.03 | 0.02 | 0.06 | 0.05 |
| 232 | Th | 0.04 | 0.04 | 0.03 | 0.01 | 0.06 | 0.05 |
| 238 | U | 0.05 | 0.04 | 0.04 | 0.01 | 0.05 | 0.044 |

## Conclusions

The Agilent 7500ce ICP-MS has achieved its design goals of providing sensitive, robust, interference-free analysis of difficult, high-matrix samples. With five times the sensitivity of its predecessor, nine operating orders of dynamic range and increased matrix tolerance, it is capable of replacing both GFAA and ICP-OES instruments in addition to older generation ICP-MS systems. The 7500ce is unique in offering a single solution for multi-elemental analysis of complex and variable, high matrix samples, while allowing the operator the freedom to use simple and consistent sets of instrument conditions for almost all elements in almost any matrix.

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# Interference-Free Semiquantitative Analysis Using the Agilent 7500ce ICP-MS

Application

**Elemental Analysis**


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## Abstract

**Rapid and accurate semiquantitative elemental screening of a wide range of sample types is shown to be possible using the Agilent 7500 ORS Series ICP-MS, in the helium-only collision mode. Polyatomic interferences are eliminated in the helium pressurized Octopole Reaction System cell, enabling greater accuracy and data integrity in complex sample matrices.**


## Introduction

One of the benefits of inductively coupled plasma mass spectrometry (ICP-MS) is the simplicity of the spectra. These are easy to interpret and are frequently used to “fingerprint” unknown samples, using the relative intensities of the various observed isotopes. This data can also provide a means of generating approximate concentrations for uncalibrated elements in a sample - referred to as semiquantitative (or semiquant) analysis. Quantification is done by comparing the target elements to an internal standard element (or elements) of known concentration.

Semiquant analysis is useful in situations where semiquant calibration standards are unavailable, as is often the case, for example, with solids analysis by laser ablation, and also as a very powerful screening technique. While recent advances in collision/reaction cell (CRC) technology have all but eliminated interferences from quantitative ICP-MS, little is published on their applicability for semiquantitative analysis.

Semiquant elemental analysis by ICP-MS operates on the principle that the relative response (counts per unit concentration) of each element follows a consistent pattern, so the concentration for each element can be calculated from the measured response of any other element. This is because when acquired under consistent operating conditions, the relative elemental response is independent of the species of the element and relatively free from the effects of matrix. Furthermore, the relative response for a given isotope can be predicted with a reasonable degree of accuracy, based on the relative isotopic abundance of the measured isotope and the ionization potential (degree of ionization) of the element.

The Agilent ICP-MS ChemStation contains a database of relative response factors (RFs) for all of the naturally occurring elements that can be

measured by ICP-MS. This database can be updated and resloped by analyzing a semiquant calibration standard or certified reference material and/or including one or more reference elements at known concentration (effectively internal standards - ISTDs) in the sample to be measured.

In common with fully quantitative analysis by conventional ICP-MS, there are several elements or isotopes that do not give accurate results using semiquant analysis because of either isobaric or polyatomic interferences. These of course include the isotopes that have overlaps from ionized argon species, including $^{40}$Ca, $^{56}$Fe, $^{78}$Se, and $^{80}$Se. Depending on the sample matrix being analyzed, other common polyatomic interferences from N, C, Cl, S, etc. can also give increased backgrounds and poorer accuracy in the determination of $^{54}$Fe, $^{52}$Cr, $^{51}$V, $^{75}$As, $^{77}$Se, and many others.

### CRC ICP-MS and Semiquant

Recent advances in CRC technology have all but eliminated interferences from quantitative ICP-MS for most matrix types. However, while highly reactive gases may be used for the removal of many interfering species, they have two important limitations: namely, they may cause the loss of some analytes by reaction, and they will cause some new interferences as a result of secondary reaction chemistry. As a result, these gases can be used effectively only when the analyst has some detailed knowledge of the interference that is being targeted, so that the thermodynamics of the reaction pathways can be predicted and a suitable cell gas and set of conditions can be selected for each target interference.

Even reaction gases that are effective at removing several different interferences (the so-called universal reaction gases) must rely on reaction chemistry to remove the interferences, and so they will not be effective against interfering ions that are unreactive with that particular gas. As a result, errors will be introduced if any highly reactive cell gas is used for the analysis of unknown or variable high matrix samples where, typically, the interferences are not known in advance.

For the most accurate semiquantification results to be achieved, it is essential that all elements are acquired under the same conditions, so the consistent relationship between the response of adjacent elements is maintained. As a result, it is not possible to use CRC technology in reaction mode to reduce interferences in semiquant in the same way as in full quantification.

However, the unique ability of the Agilent 7500 ORS (Octopole Reaction System) to eliminate polyatomic interferences using carefully controlled kinetic energy discrimination (KED) in helium collision mode allows accurate semiquantification in complex, unknown sample matrices. Since helium is a nonreactive gas, no new interferences are formed in the cell, no analytes are lost by reaction, and the plasma-formed and matrix-formed polyatomic interferences are eliminated through a physical "molecular filtering" process, as a function of their larger ionic radii (Figure 1).

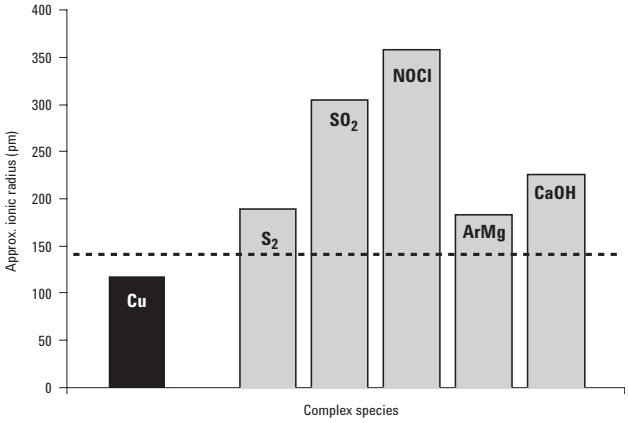


**Figure 1. Graphic representation of the relative diameter of an atomic ion (Cu) compared with the polyatomic (molecular) ions that can interfere with the Cu at mass 65 [1, 2]. Most elemental ions are smaller than 150-picometer radius, while most polyatomic ions are larger.***

*Actual ion radii may vary according to conditions, but the relative relationships are constant.

Since only a single set of ORS cell parameters is used, consistent RFs are maintained, regardless of sample matrix. Since helium collision mode eliminates only polyatomic interferences, interference-free isotopes of elements suffering from isobaric interferences must still be used. For the most part,

the only significantly affected isotope is $^{40}Ca$. In this case, $^{44}Ca$ can be used. KED eliminates the transmission of the larger polyatomic ions to the quadrupole by placing an energy barrier between the octopole and quadrupole (Figure 2).

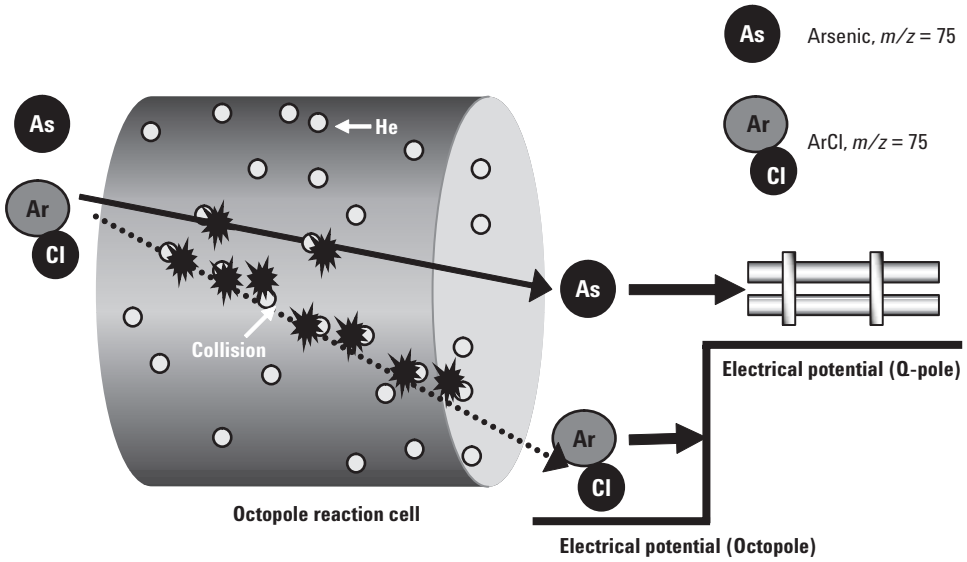


**Figure 2. Diagrammatic representation of KED after energy-depleting collisions within the ORS cell.**

Since the larger polyatomic ions undergo more energy-depleting collisions with He in the cell than do the smaller atomic ions, they lack sufficient energy to cross the barrier. Figure 3 depicts the effects of KED on ion energy. Only the high-energy atomic ions exceed the stopping potential and are transmitted.

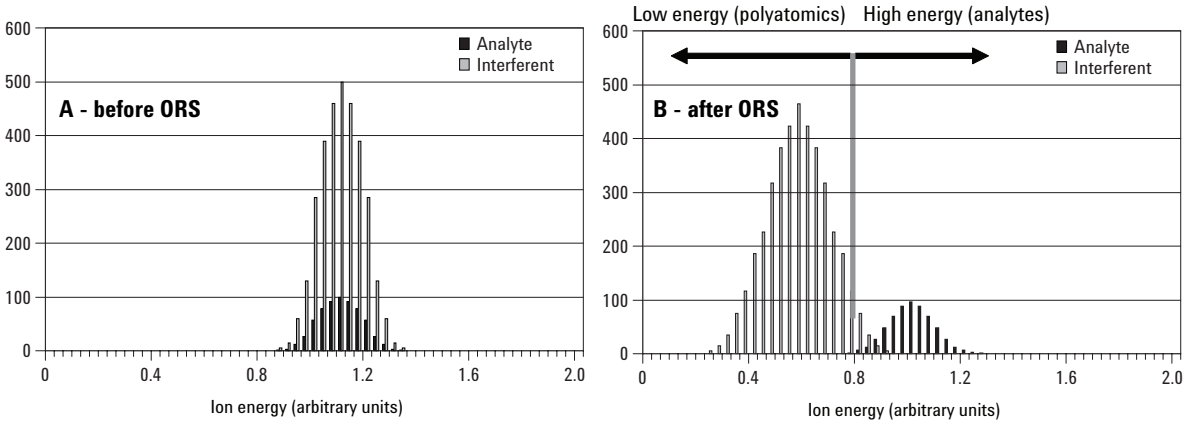


**Figure 3. Effects of KED on the larger diameter polyatomic ions relative to the smaller atomic ions.**

Figures 4–7 illustrate the effective removal of multiple polyatomic interferences on several interference-prone elements. In this case, a standard containing 5 ppb of the elements of interest was measured in a matrix containing increasing concentrations of sulfuric acid, hydrochloric acid, and butanol. This matrix was designed to create sulfur, carbon, chlorine, and oxygen based interferences. Measurements were taken in the "normal" mode (no gas in the collision cell), and in helium collision mode. Instrument conditions are listed in Table 1.

Since polyatomic interferences are removed simply due to their size and not their reactivity, He collision mode works effectively with all matrices. And since only a single set of ORS cell parameters is used, consistent RFs are maintained, regardless of sample matrix.

**Table 1. Experimental Conditions Used for Acquisitions Depicted in Figures 4–7**

| | |
|---|---|
| Instrument | Agilent 7500ce |
| Mode of operation | He cell gas (5 mL/min) |
| Cell gas flow variation | None |
| Cell voltage variation | None |
| Calibration matrix | 1% $HNO_3$ |
| Sample matrix components | $H_2SO_4$ + HCl + BuOH |
| Sample matrix levels (each) | 0%, 0.1%, 0.5%, 1.0% |
| Analyte spike level | 5 ppb |
| Internal standard | $^{72}Ge$ |
| Interference correction equations used | None |

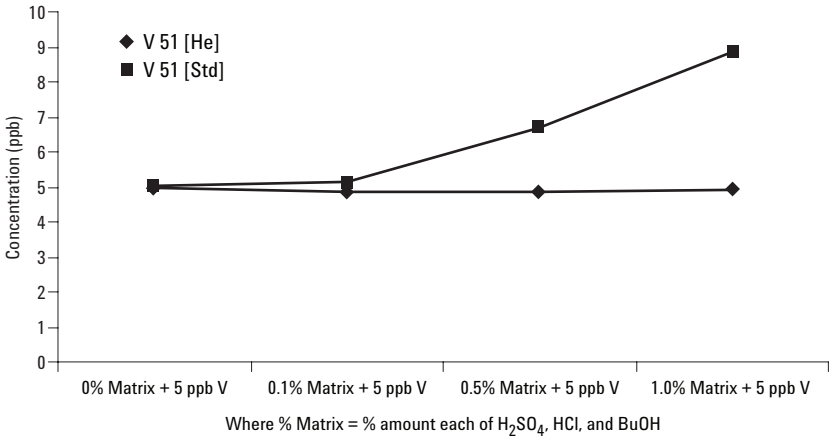


**Figure 4. Measurement of vanadium ($m/z = 51$) and $ClO^+$ ($m/z = 51$) in a matrix of increasing concentrations of sulfuric acid, hydrochloric acid, and butanol with and without the use of interference removal by KED in He collision mode.**

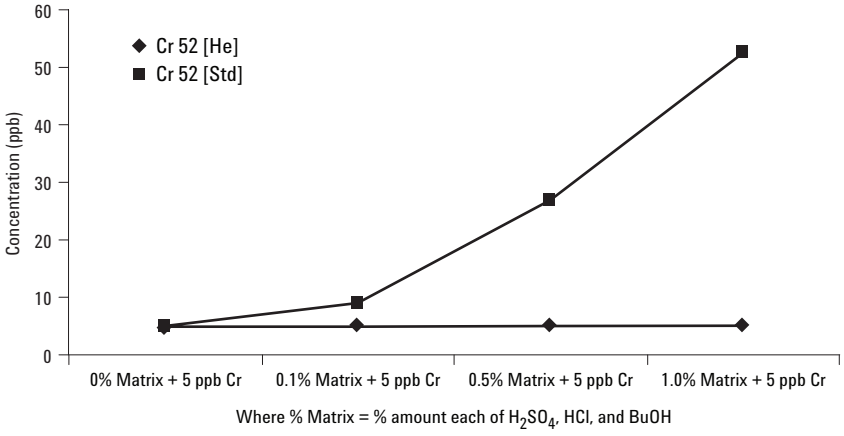


**Figure 5. Potential interferences at mass 52 in this matrix include $^{34}S^{18}O$, $^{35}Cl^{16}O^{1}H$, and $^{40}Ar^{12}C$. All are removed, regardless of reactivity, under a single set of conditions, allowing accurate quantification of Cr at the major isotope.**

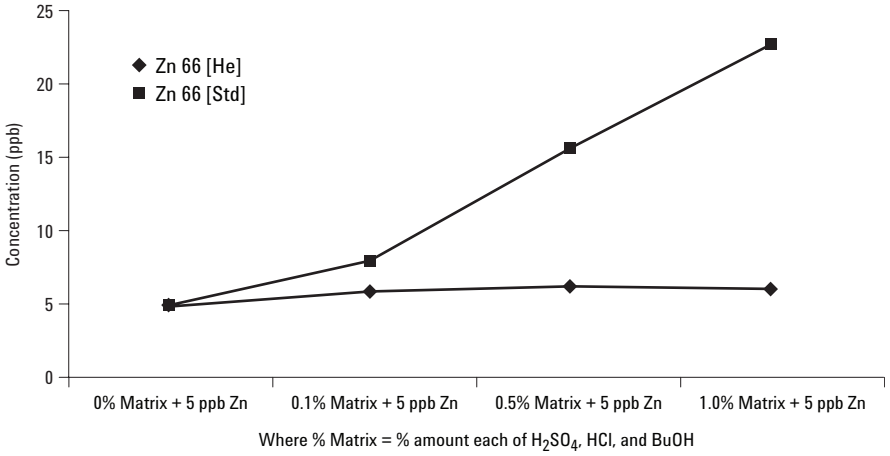


**Figure 6. Potential interferences at mass 66 in this matrix include $^{34}S^{16}O_2$, $^{32}S^{34}S$, and $^{40}Ar^{12}C^{14}N$. All are removed, regardless of reactivity, under a single set of conditions, allowing accurate quantification of Zn at the preferred isotope.**

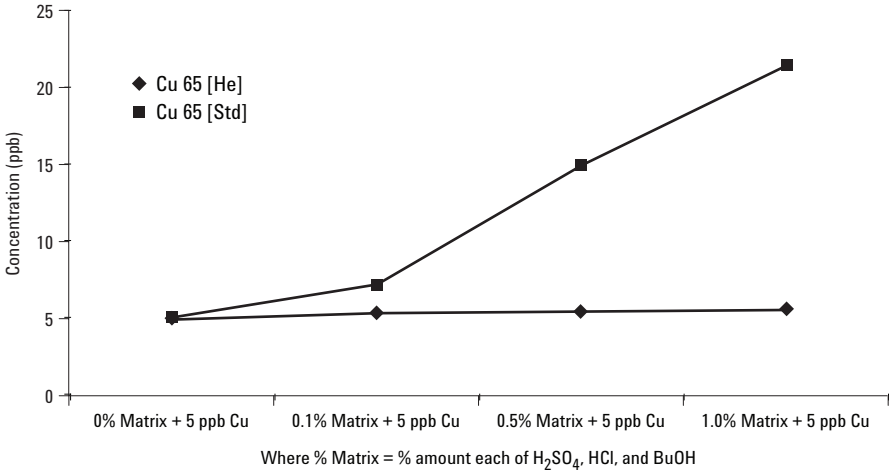


**Figure 7. Potential interferences at mass 65 in this matrix include $^{33}S^{16}O_2$, $^{32}S^{33}S$, and $^{40}Ar^{12}C_2{}^1H$ [3]. All are removed, regardless of reactivity, under a single set of conditions, allowing accurate quantification of Cu at this isotope.**

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### Semiquant Analysis - Method

The instrument was tuned for typical robust plasma conditions, as indicated in Table 2.

**Table 2. Typical Instrumental Conditions Used for Semiquant Analysis in He Collision Mode**

| | |
|---|---|
| RF power | 1550 W |
| Carrier gas flow rate | 0.85 L/min |
| Makeup gas flow rate | 0.2 L/min |
| Sampling depth | 8 mm |
| Peristaltic pump speed | 0.05 rps |
| Spray chamber temperature | 2 °C |
| Helium flow | 3–5 mL/min |

The following acquisition parameters were used (Figure 8).

| | |
|---|---|
| Integration time: | 0.1 s |
| Mass range: | 5–240* |
| Replicates | 1 |
| Peak pattern | Peak hopping, single point per mass |

* The "empty masses," for example between 210–230 and masses for elements including C, O, N, F, and the noble gases, were skipped to reduce total acquisition time. Total acquisition time is less than 60 s for one replicate.

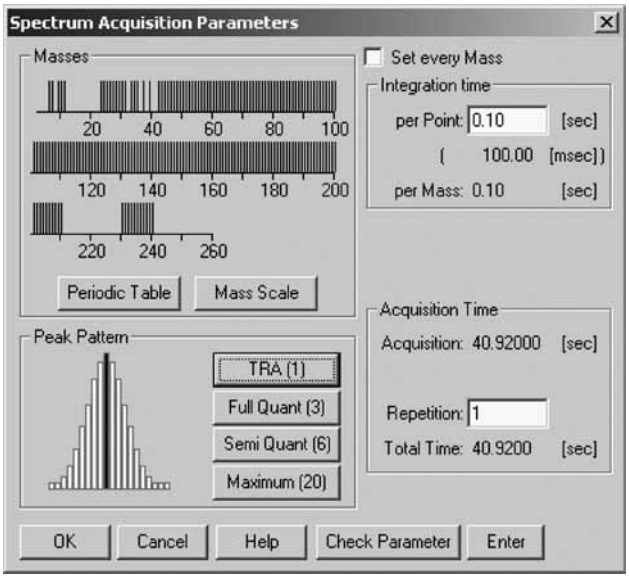


**Figure 8. Semiquant acquisition parameter dialog box.**

A single calibration standard at 100 ppb, made up in 1% $HNO_3$/0.5% HCl, was used to update the semiquant RF database for a range of elements across the mass range. Noncalibrated elements are updated by interpolating between calibrated isotopes, which the ICP-MS ChemStation software does automatically. Any number of calibration elements may be used, but increasing the number of calibration elements will reduce the mass range over which the factors are interpolated between measured reference elements and, therefore, improve semiquantitative accuracy. Internal standardization was applied using a typical suite of ISTD elements distributed across the mass range.

## Results

Prior to the analysis of some reference materials to test the accuracy of semiquant analysis using the ORS, a further study was made to confirm matrix interference removal.

A very challenging test matrix consisting of 1% $HNO_3$, 1% HCl, 1% $H_2SO_4$, 1% Butanol, and 100 ppm each of Ca and Na was prepared, and spectra obtained in both non-gas and He modes. Figure 9 is the spectrum obtained when the matrix is analyzed with the ORS operating without any gas. In this mode, the instrument behaves like a standard non-CRC ICP-MS and peaks from polyatomic species such as $^{16}O^{35}Cl$ (at 51), $^{40}Ar^{12}C$ (at 52), $^{40}Ar^{16}O$/$^{40}Ca^{16}O$ (at 56), $^{32}S^{16}O_2$ (at mass 64), $^{40}Ar^{32}S$ (at mass 72), $^{40}Ar^{35}Cl$ (at 75), and $^{40}Ar^{40}Ar$ (at 80), etc. can be seen. In this matrix, analysis of many transition elements would be severely compromised.

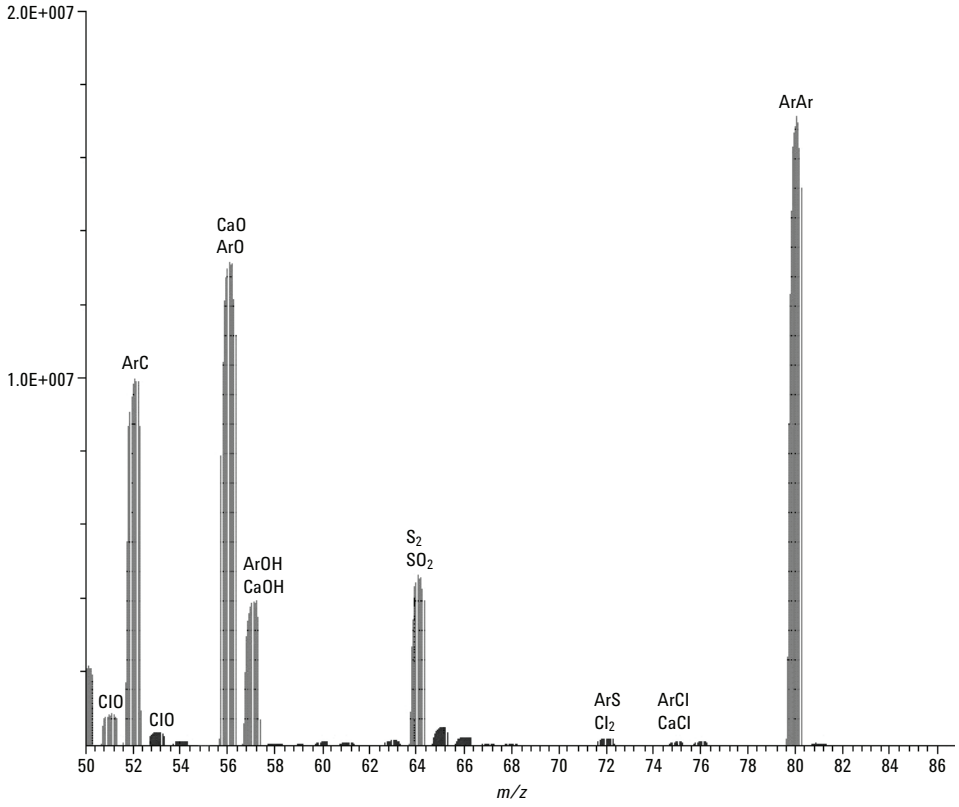


**Figure 9. Blank containing 1% each of $HNO_3$, HCl, $H_2SO_4$ and BuOH, plus 100-ppm Ca and Na acquired on a 7500ce in non-gas mode. Note polyatomic peaks.**

Figure 10 shows the spectra obtained from the same matrix, under the same operating conditions, except with He in the cell. Note the elimination of most of the polyatomic species to sub-ppb levels (the He mode spectrum is on an intensity scale 400 times lower than the non-gas mode spectrum). The analyte peaks are due to trace contamination in the matrix. The lower spectrum demonstrates the interference removal power of the ORS in He mode. In this very challenging matrix, the ORS simultaneously removes all polyatomic interferences. At the same time, no new, cell-formed interferences are generated. The ORS offers greater data integrity in high matrix samples, in both semiquantitative and quantitative modes.

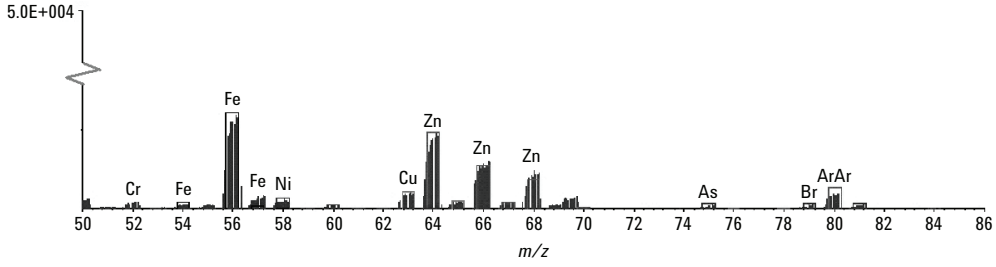


**Figure 10. Blank containing 1% each of $HNO_3$, HCl, $H_2SO_4$, and BuOH, plus 100-ppm Ca and Na acquired on a 7500ce in He mode. Note polyatomic peaks were eliminated and the residual metal peaks arising from impurities in the matrix, Fe approx. 1.5 ppb, Zn approx. 5 ppb, Cu approx. 0.2 ppb. The isotopic fingerprint is overlaid to illustrate this point. Peak at *m/z* 75 is due to As and not ArCl, since there is no ArCl peak at *m/z* 77.**

Tables 3 and 4 show the results of a semiquant measurement of two standard reference materials, NIST 1640 water and LGC 6177 landfill leachate. No attempt was made to matrix-match and the plasma conditions shown in Table 2 were used with the same He collision mode for all elements in all matrices. In all cases, for every certified element, the measured response was very close to the certified concentration from as low as 2 ppb for Rb in NIST 1640 to over 1700 ppm for Na in the LGC 6177 landfill leachate.

**Table 3. Results for NIST 1640 Standard Reference Water Using He Semiquant Mode, in ppb**

| Element mass | SQ conc. | Ref | Element mass | SQ conc. | Ref | Element mass | SQ conc. | Ref |
|---|---|---|---|---|---|---|---|---|
| Li 7 | IS Element | 50.7 | Se 78 | 21 | 21.96 | Sm 147 | 0.072 | N/A |
| Be 9 | 35 | 34.94 | Br 79 | <0.1600 | N/A | Eu 153 | 0.0098 | N/A |
| B 10 | 280 | 301.1 | Rb 85 | 1.8 | 2 | Gd 157 | 0.065 | N/A |
| Na 23 | 30,000 | 29,350 | Sr 88 | 120 | 124.2 | Tb 159 | 0.0017 | N/A |
| Mg 24 | 5,700 | 5,819 | Y 89 | 0.051 | N/A | Dy 163 | 0.021 | N/A |
| Al 27 | 54 | 52 | Zr 90 | 0.091 | N/A | Ho 165 | 0.006 | N/A |
| Si 28 | 4,800 | 4,730 | Nb 93 | 0.0035 | N/A | Er 166 | 0.016 | N/A |
| P 31 | 35 | N/A | Mo 95 | 46 | 46.75 | Tm 169 | <3.600E-4 | N/A |
| S 34 | 110 | N/A | Ru 101 | <3.400E-3 | N/A | Yb 172 | 0.0082 | N/A |
| K 39 | 1,000 | 994 | Rh 103 | IS Element | N/A | Lu 175 | 6.50E-04 | N/A |
| Ca 44 | 9,300 | 7,045 | Pd 105 | <2.900E-3 | N/A | Hf 178 | <1.400E-3 | N/A |
| Sc 45 | <5.800E-3 | N/A | Ag 107 | 7.7 | 7.62 | Ta 181 | 3.10E-04 | N/A |
| Ti 49 | 0.089 | N/A | Cd 114 | 22 | 22.79 | W 182 | 0.017 | N/A |
| V 51 | 13 | 12.99 | In 115 | 0.0046 | N/A | Re 185 | 0.0067 | N/A |
| Cr 52 | 37 | 38.6 | Sn 118 | 2.1 | N/A | Os 189 | <1.600E-3 | N/A |
| Mn 55 | 120 | 121.5 | Sb 121 | 15 | 13.79 | Ir 193 | IS Element | N/A |
| Fe 56 | 29 | 34.3 | Te 125 | <0.1200 | N/A | Pt 195 | <1.300E-3 | N/A |
| Co 59 | 19 | 20.28 | I 127 | 0.17 | N/A | Au 197 | 0.0065 | N/A |
| Ni 60 | 26 | 27.4 | Cs 133 | 0.078 | N/A | Hg 202 | 0.012 | N/A |
| Cu 63 | 87 | 85.2 | Ba 137 | 140 | 148 | Tl 205 | 0.035 | N/A |
| Zn 66 | 55 | 53.2 | La 139 | 0.42 | N/A | Pb 208 | 27 | 27.89 |
| Ga 69 | 32 | N/A | Ce 140 | 0.52 | N/A | Bi 209 | 0.0015 | N/A |
| Ge 72 | IS Element | N/A | Pr 141 | 0.076 | N/A | Th 232 | 0.16 | N/A |
| As 75 | 24 | 26.67 | Nd 146 | 0.35 | N/A | U 238 | 0.85 | N/A |

**Table 4. Results for LGC 6177 Standard Reference Landfill Leachate Using He Semiquant Mode, in ppb**

| Element mass | SQ conc. | Ref | Element mass | SQ conc. | Ref | Element mass | SQ conc. | Ref |
|---|---|---|---|---|---|---|---|---|
| Li 7 | IS Element | N/A | Se 78 | <16.00 | N/A | Sm 147 | <0.05000 | N/A |
| Be 9 | <2.400 | N/A | Br 79 | 6,900 | N/A | Eu 153 | 0.036 | N/A |
| B 10 | 6,700 | 9,800 | Rb 85 | 400 | N/A | Gd 157 | 0.036 | N/A |
| Na 23 | 1,500,000 | 1,750,000 | Sr 88 | 980 | N/A | Tb 159 | 0.0098 | N/A |
| Mg 24 | 62,000 | 73,500 | Y 89 | 0.28 | N/A | Dy 163 | 0.14 | N/A |
| Al 27 | 110 | N/A | Zr 90 | 49 | N/A | Ho 165 | 0.061 | N/A |
| Si 28 | 22,000 | N/A | Nb 93 | 1.4 | N/A | Er 166 | 0.052 | N/A |
| P 31 | 12,000 | 11,500 | Mo 95 | 6.6 | N/A | Tm 169 | 0.011 | N/A |
| S 34 | 1,600 | N/A | Ru 101 | <0.04200 | N/A | Yb 172 | 0.044 | N/A |
| K 39 | 810,000 | 780,000 | Rh 103 | IS Element | N/A | Lu 175 | 0.01 | N/A |
| Ca 44 | 77,000 | 74,800 | Pd 105 | <0.03500 | N/A | Hf 178 | 0.44 | N/A |
| Sc 45 | 0.21 | N/A | Ag 107 | 1.8 | N/A | Ta 181 | 0.0066 | N/A |
| Ti 49 | 18 | N/A | Cd 114 | 0.94 | N/A | W 182 | 58 | N/A |
| V 51 | 63 | N/A | In 115 | 0.18 | N/A | Re 185 | 0.061 | N/A |
| Cr 52 | 160 | 180 | Sn 118 | 48 | N/A | Os 189 | <0.01700 | N/A |
| Mn 55 | 130 | 140 | Sb 121 | 5 | N/A | Ir 193 | IS Element | N/A |
| Fe 56 | 3,300 | 3,800 | Te 125 | 3 | N/A | Pt 195 | <0.01300 | N/A |
| Co 59 | 40 | N/A | I 127 | 1,200 | N/A | Au 197 | 5.4 | N/A |
| Ni 60 | 170 | 210 | Cs 133 | 3.5 | N/A | Hg 202 | 0.28 | N/A |
| Cu 63 | 41 | N/A | Ba 137 | 770 | N/A | Tl 205 | <7.600E-3 | N/A |
| Zn 66 | 250 | 260 | La 139 | 0.24 | N/A | Pb 208 | 17 | N/A |
| Ga 69 | 130 | N/A | Ce 140 | 0.7 | N/A | Bi 209 | 2.4 | N/A |
| Ge 72 | IS Element | N/A | Pr 141 | <1.000E-2 | N/A | Th 232 | 0.1 | N/A |
| As 75 | 86 | N/A | Nd 146 | 0.2 | N/A | U 238 | 0.35 | N/A |

## Conclusions

Semiquant has always been a powerful tool available to the ICP-MS analyst for quickly reporting the concentration of unknown, uncalibrated elements in a variety of simple matrices. However, in complex matrices, polyatomic interferences could introduce inaccuracies in the reported results for several elements. CRC technology which requires specific conditions for different elements or different sample matrices (for example, specific reaction processes to remove pre-identified interferences), cannot be used since this would result in deviation from the standard relative response tables upon which semiquant is based. Helium collision mode coupled with kinetic energy discrimination in the Agilent 7500 ORS Series instruments can overcome these limitations. By effectively removing all polyatomic interferences regardless of source and reactivity, rapid, accurate semiquantitative screening of a wide range of sample types for most possible analyte elements is possible.

CRC-ICP-MS systems that rely on the use of reaction gases such as $CH_4$ and $NH_3$ have limited applicability in unknown complex matrix samples due to the risk of interference from new polyatomic species generated by reaction with the cell gas. Typically in environmental analysis, reaction mode can only be used for 4–5 analytes. Since He collision mode is nonreactive and produces no new interferences, it can be used for all analytes and in any matrix. He collision mode applies equally well in quantitative analysis, enabling the 7500ce to quantitate previously difficult-to-measure analytes at trace levels in complex, unknown sample matrices.

## References


1. Inorganic Chemistry: Principles of Structure and Reactivity (4th Edition) James E. Huheey, Ellen A. Keiter, Richard L. Keiter, 1997 p. 292.
2. "Structural Inorganic Chemistry," (5th Edition) A.F. Wells, Clarendon Press, Oxford, 1984, p. 1288.


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# Extending the Dynamic Range of Environmental ICP-MS Using the Integrated Sample Introduction System and Octopole Reaction System

## Replacing Graphite Furnace AA and Optical ICP

Application

**Environmental**


## Author

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## Abstract

**The Octopole Reaction System in the Agilent 7500c inductively coupled plasma mass spectrometer (ICP-MS) can be used to reduce the interferences on critical trace elements, such as arsenic and selenium in environmental samples. It can also be used to reduce the response of certain high concentration matrix elements, such as Na, K, Ca and Mg. As such, it can extend the dynamic range of ICP-MS over a range of elements to exceed that of the traditional combination of graphite furnace atomic absorbtion plus ICP optical emission spectroscopy (ICP-OES). When used in conjunction with the Integrated Sample Introduction System, dynamic range and productivity are further enhanced.**


## Introduction

While ICP-MS has become the predominant technique for environmental metals analysis, until recently, limitations in practical dynamic range have still necessitated the use of ancillary techniques such as graphite furnace atomic absorbtion (GFAA) and ICP-OES for measurements at extremely high or low concentrations. This has been especially true for very high-concentration analytes or very high-matrix concentrations, which typically required dilution before analysis. To meet the dynamic range needs of environmental laboratories using a combination of GFAA and ICP-OES for each sample, the ICP-MS must be able to tolerate typical matrix concentrations with little or no dilution, measure high-concentration elements at ICP-OES levels (~100 ppm), and measure trace elements at GFAA limits of detection.

### Dynamic Range in ICP-MS

Dynamic range as it pertains to ICP-MS can have many meanings depending on the context or application. It can mean the range of concentrations for a given analyte in a given matrix for which the instrument response is linear. It is also commonly defined by the linear range of the detector because this is well defined and independent of sample, plasma and other instrument conditions. However, in the analysis of environmental samples, neither of these definitions actually defines the actual useful dynamic range of the ICP-MS system. Environmental samples vary significantly in their composition and concentration, both of matrix and analyte. It is generally desirable to be able to measure certain elements at trace (ppb) or ultratrace (ppt) levels while measuring other elements in the same sample at much higher concentrations. As a result, the practical linear dynamic range of ICP-MS in environmental applications is limited by the interaction of many system processes and components.

### Limitations to Linear Dynamic Range

The detector ultimately limits the range of analysis for a given element in a given matrix. Employing both pulse and analog counting techniques, the linear range of modern ICP-MS detectors is ~$10^9$. Depending on the response of the element, this may be a concentration range from less than a ppt to hundreds of ppm. Element response is, in turn, dependent on many factors including relative isotopic abundance, ionization potential, the composition of the matrix, and various instrument design and tune parameters that affect ion transmission efficiency.

In practice, however, the dynamic range is limited to much narrower ranges of concentration by other factors. These factors include the effects of matrix, both on the plasma and on the transmission of ions from the plasma to the detector. A matrix high in easily ionized elements, such as sodium or potassium, can suppress the ionization of more difficult to ionize elements, such as Hg or Se. Also, high concentrations of heavy ions can suppress the response of lower mass ions through space charge effects in the ion optic region of the ICP-MS. Memory effects or carryover can also significantly limit the practical dynamic range. Typical washout after introducing a high-concentration sample is 2–4 orders of magnitude reduction in measured concentration per minute of rinse depending on the element and instrument configuration. Where analysis time is important, these memory effects limit the dynamic range per element significantly. Other limitations include the effects of deposition of matrix on the interface and lens components. Due to the extremely high sensitivity of ICP-MS, linear range is also limited by the availability of suitably clean blanks. It is very difficult in the production environmental laboratory to maintain blanks at the actual instrument background. For example, sensitivities of 100 million counts per second per ppm (100 MCPS/ppm) with instrument backgrounds of 1 to a 5 CPS are now quite possible. Such signal to noise yields an instrument background equivalent concentration (BEC) of 10–50 ppq. In this case, a typical environmental laboratory, whose blanks may contain a single-to double-digit ppt levels or higher of some elements, is sacrificing three or more orders of magnitude of its instruments maximum dynamic range.

From a practical standpoint, the single element dynamic range required by most environmental labs is only about three to at most four orders of magnitude. This is based on current required detection limits and concentration ranges typically found in environmental samples as well as limitations due to memory effects. However, the element-to-element dynamic range is much higher. For example, it is desirable to have very low limits of detection for highly toxic elements, such as mercury, and at the same time in the same sample, very high linear range for common matrix elements, such as calcium or sodium. In this case, the interelement dynamic range could be from 1–5 ppt for Hg to upwards of 1000 ppm for Na or Ca, or about $10^9$, while the single element range is still only 3–4 orders of magnitude. If we factor in the loss of range at the low end due to interferences or blank contamination, we have exceeded the range of the detector. To reduce this limitation, it is necessary to reduce both the background for certain elements and the count rates for others. It is also necessary to minimize the effects of sample matrix on the plasma and interface components.

### Maximizing Dynamic Range

The combined application of several techniques is necessary to achieve the desired dynamic range. These techniques work together to:

1. Minimize memory effects
2. Minimize blank background
3. Minimize matrix-based spectroscopic interferences
4. Minimize matrix-based nonspectroscopic interferences
5. Minimize count rates for high-response, high-concentration analytes while maintaining maximum response for trace analytes
6. Minimize matrix accumulation on sample introduction and interface components

Most of these effects can be achieved through the implementation of two features of the Agilent 7500 series ICP-MS. One is the Integrated Sample Introduction System (ISIS). The other is the Octopole Reaction System (ORS), which defines the Agilent 7500c ORS system. Together, they comprise the Agilent 7500ci Environmental Analysis System.

## ISIS

Two features of ISIS (see Figure 1) serve to minimize the effects of matrix on dynamic range and instrument performance. They are constant flow nebulization and rapid sample uptake. Constant flow nebulization takes advantage of very close coupling of the nebulizer peripump to the nebulizer. By minimizing the associated swept volume, it is not necessary to use high nebulizer flow during sample uptake and rinseout. At the same time, sample is delivered to the nebulizer peripump at very high flows via an open-split interface at the nebulizer peripump.

By uncoupling the sample uptake and rinse flow from the nebulizer flow, it is possible to have very rapid sample uptake and rinseout without overloading the sample introduction system (nebulizer, spray chamber, and torch), and interface (interface cones and ion lenses). As a result, the total matrix loading on the ICP-MS is significantly reduced while uptake speeds and rinseout are improved. Furthermore, because all Agilent ICP-MS systems are designed for optimum performance using low-flow nebulizers, performance is never compromised to reduce matrix.

ISIS also has the ability to autodilute high-concentration samples, as needed, to further reduce matrix effects. Thus, the use of ISIS with an efficient low-flow nebulizer can address items 1, 4 and 6 above. Additional design features of all Agilent ICP-MS systems, which permit the analysis of high-matrix samples with minimum detrimental effects, are discussed in an Agilent ICP-MS Technical Note, publication 5968-1897E .

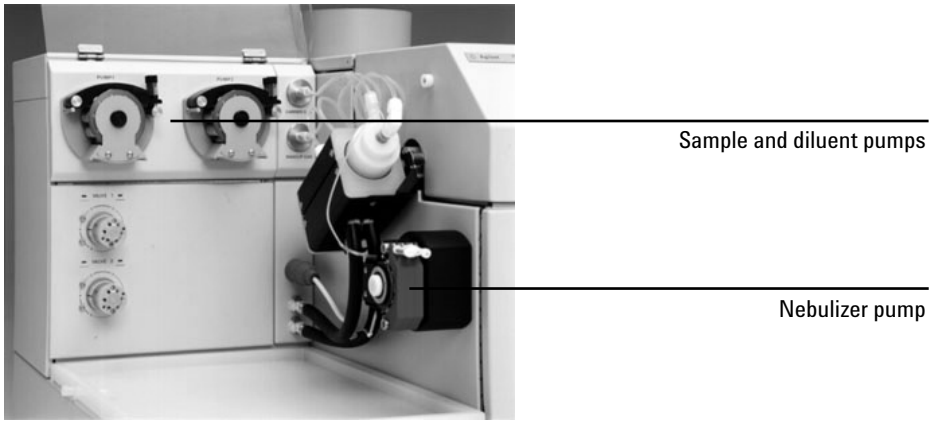



**Figure 1: ISIS depicting large sample uptake and diluent pumps is at the upper left, and the small three-channel nebulizer pump is at the lower right.**

## ORS

Spectroscopic interferences from the plasma or sample matrix and excessive count rates for high-concentration analytes can be addressed through the use of the ORS. See Figure 2.

The ORS is a static, radio frequency (RF) only multipole composed of eight parallel stainless steel rods arranged in a linear octagon. The octopole is contained in a vessel that can be pressurized relative to the surrounding vacuum with a reaction gas, normally either helium or hydrogen. The octopole functions both as an ion guide, replacing many of the ion-focusing lenses on the non-octopole system, and as a collision/reaction cell.

When used in the pressurized collision/reaction mode, the ORS has the ability to significantly reduce or remove spectroscopic interferences from ionized argon and plasma or matrix-based polyatomic ions. By reducing the background, the signal to noise of the analyte is improved. Eliminating certain interferences also allows the measurement of alternate, more abundant analyte isotopes. For example, reduction of the argon-based interferences at $m/z$ 40 and 80 allows the measurement of the major isotopes of Ca and Se, which is not possible without the use of the ORS in most environmental matrices. Use of the ORS to remove NaAr+ allows the measurement of the most abundant copper isotope at $m/z$ = 63.

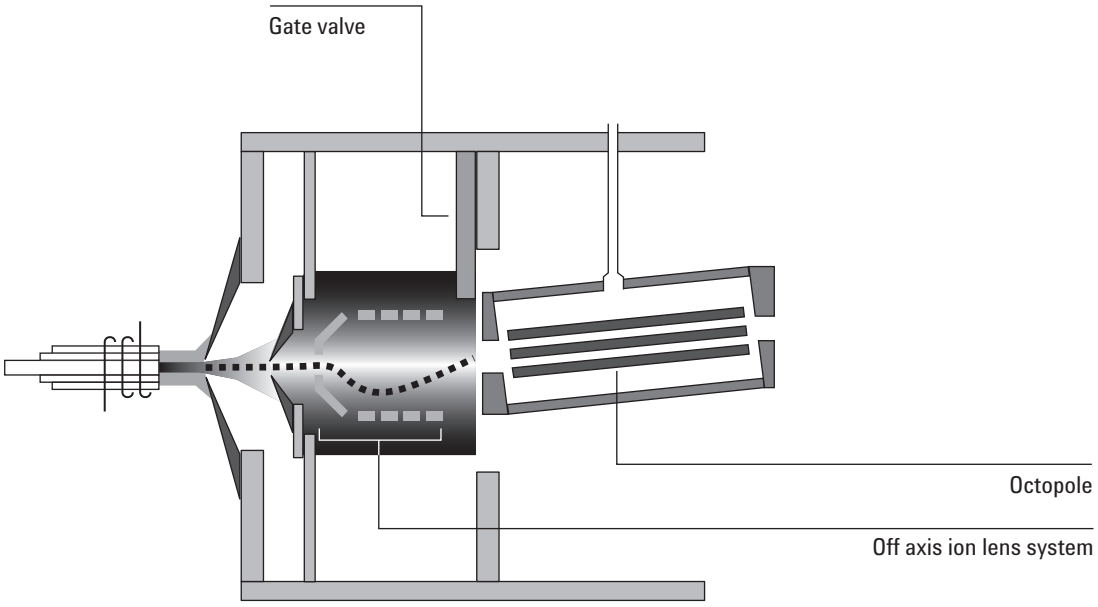



**Figure 2. Agilent 7500c ion optic region with octopole collision/reaction cell. Ion path is depicted by dark squares.**

## Experimental

Six-level external calibration plots were generated for Na-23, Mg-25, K-39, Ca-40, Fe-56, and Se-78. They are shown in Figures 3 through 8, with linear and logarithmic curve fits.

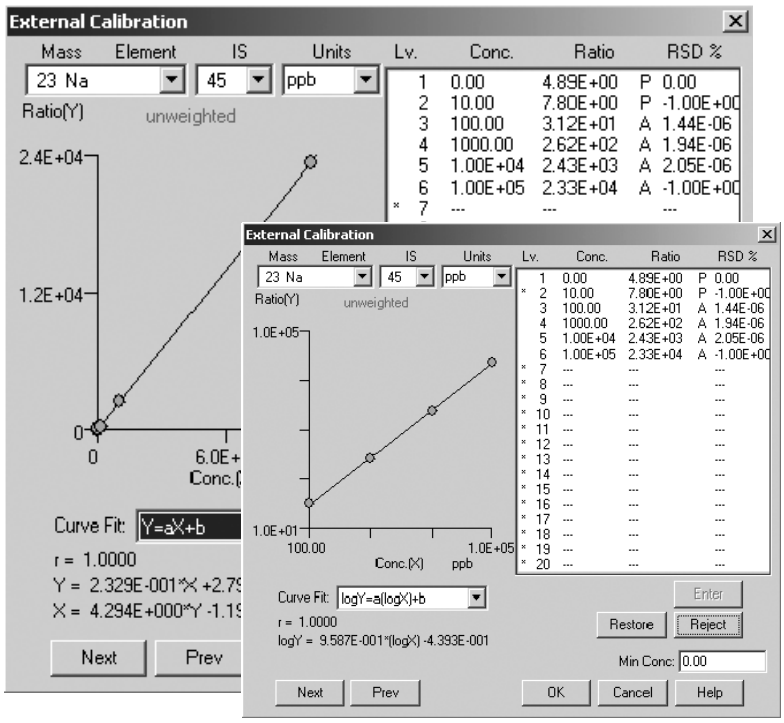



**Figure 3. Calibration plot for sodium from 10 ppb to 100 ppm, hydrogen mode.**

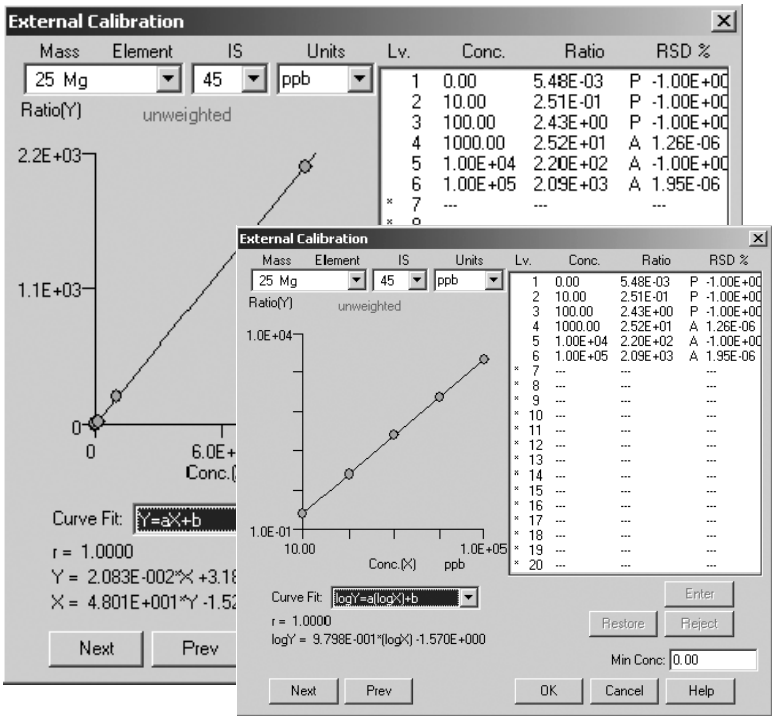



**Figure 4. Calibration plot for magnesium from 10 ppb to 100 ppm, hydrogen mode.**

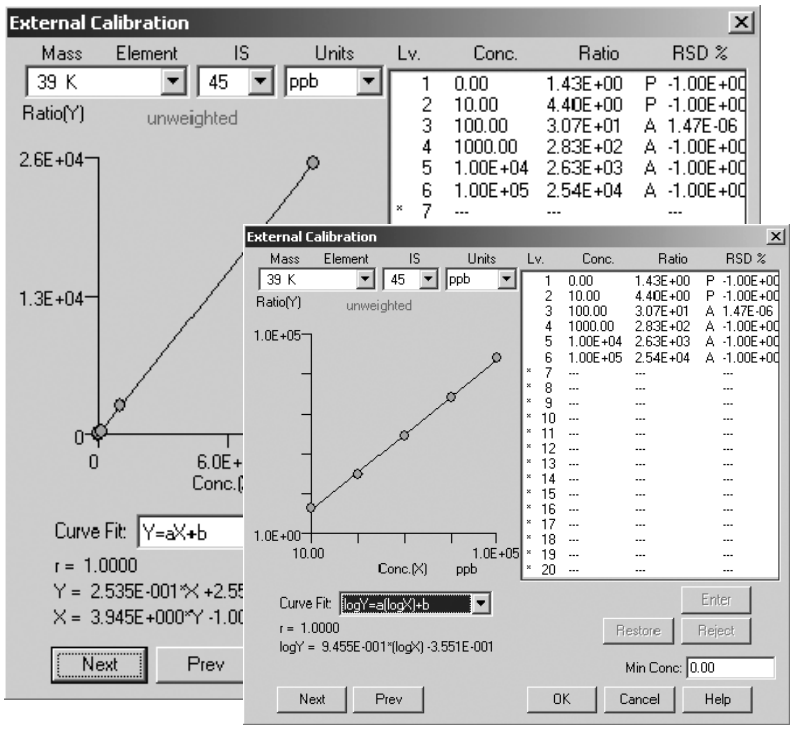



**Figure 5. Calibration plot for potassium from 10 ppb to 100 ppm, hydrogen mode.**

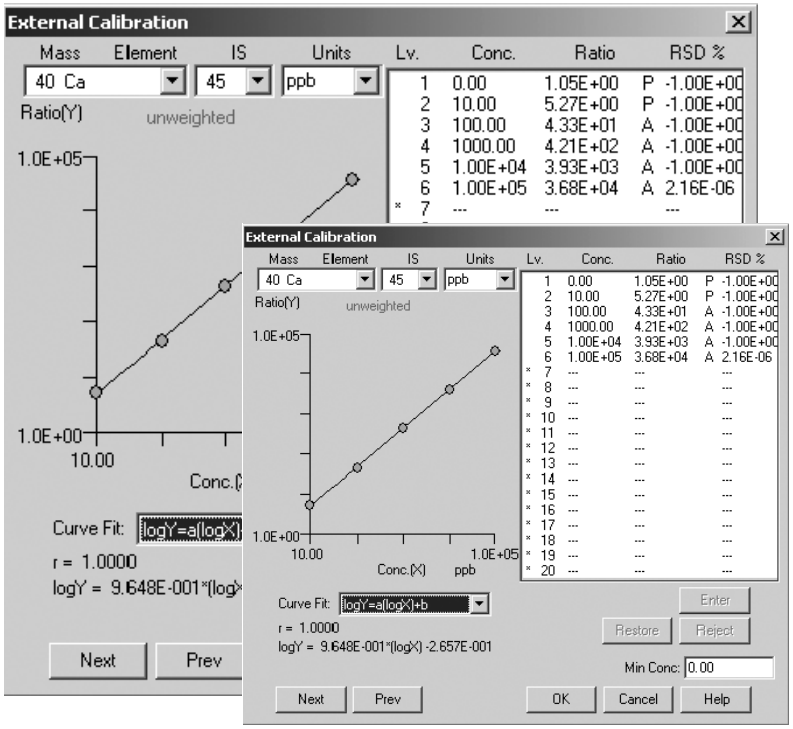



**Figure 6. Calibration plot for calcium from 10 ppb to 100 ppm, hydrogen mode.**

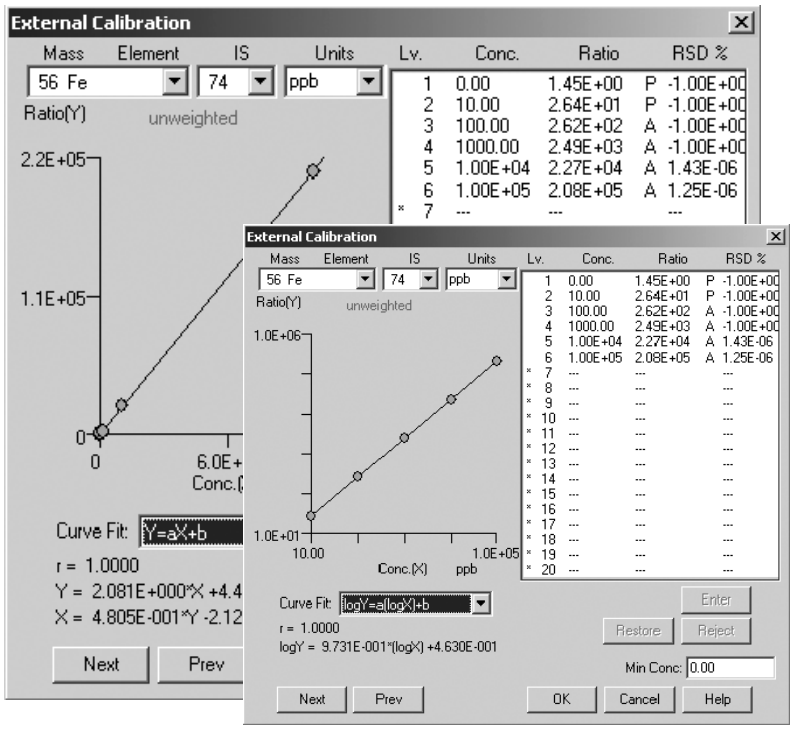



**Figure 7. Calibration plot for iron from 10 ppb to 100 ppm, hydrogen mode.**

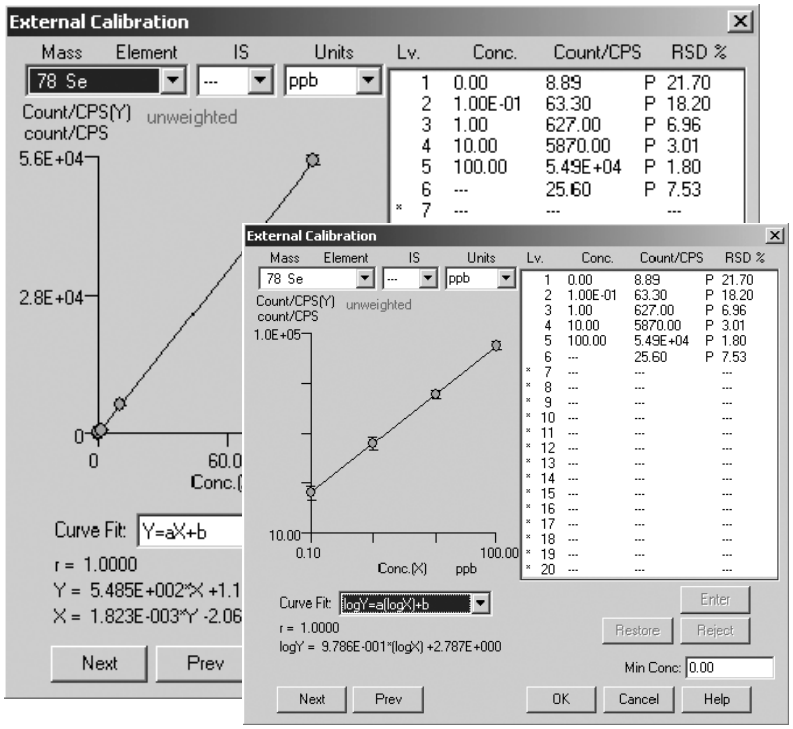



**Figure 8. Calibration plot for selenium from 0.1 ppb to 100 ppm, hydrogen mode.**

A response plot for the scandium internal standard at six different calibration levels is shown in Figure 9.

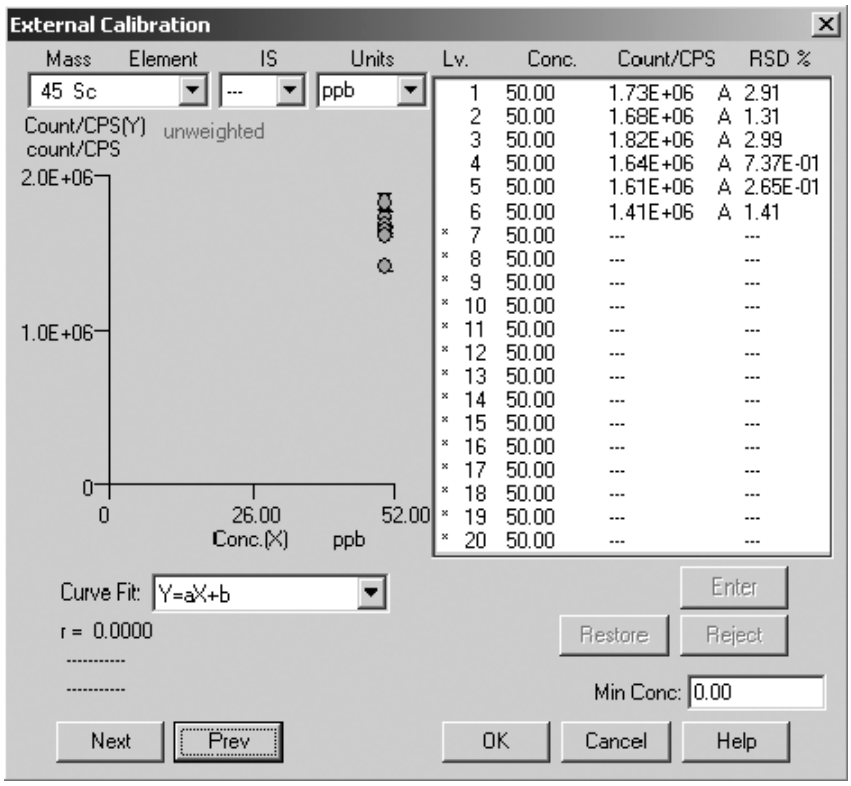



**Figure 9. Response plot for scandium internal standard at six calibration levels.**

### Extending the Dynamic Range

We take advantage of two properties of the ORS to extend the practical dynamic range of the ICP-MS instrument in both directions. By using the ORS in hydrogen mode:

- We eliminate the argon based interference on Se 78 (or 80). This permits the measurement of a more abundant isotope, which is normally free of other matrix-based interferences. The other commonly measured isotopes of Se, *m/z* 77 and 82, in addition to being less abundant than 78 and 80, suffer from interferences from ArCl at 77 and Kr or HBr at 82, respectively. As a result, measurement of selenium in typical environmental samples at ppt levels is easily accomplished.
- We take advantage of the low-mass signal attenuation due to collisions with hydrogen gas within the cell. In this way, elements, which normally have very high response and can have high concentrations in environmental samples such as sodium and calcium, can be attenuated before the signal reaches the quadrupole and detector. This can be used to increase the linear range upward by a factor of 10. By reducing the ion current for these common elements, detector wear and tear is reduced as well.

In summary, use of the ORS in hydrogen mode for the ‘mineral elements’, Na, K, Ca, Mg, and Fe, and trace elements such as selenium, can, under a single set of conditions, extend the practical dynamic range of the ICP-MS by a factor of 10 or more downward for selenium and a factor of 10 upward for the minerals. The majority of the other analyte elements can be run in ‘normal’ mode, for example, no gas in the cell.

In special cases where matrix-based interferences prohibit the measurement of other analytes at sufficiently low levels, helium collision mode can be used as well. Examples include the measurement of $^{63}Cu$ in the presence of high concentrations of Na or As when Germanium is used as an internal standard. $^{74}GeH$ as well as ArCl can interfere with the measurement of As in some samples.

Figure 10 shows the acquisition parameter setup and Table 1 shows the results of analysis of a standard reference water sample, NIST 1640. Normally, this sample would require two analyses to bracket both the trace elements, such as silver, and the matrix elements, such as sodium, within the calibration range. Also, minimizing the interference from CaO and ArO on iron is difficult at this level. In this example, the sample was analyzed only once, undiluted, and without the use of interference equations. The dynamic range was sufficient to measure sodium directly at ~30 ppm and the trace elements at ppb levels. Interferences on As, Fe, Se, Cu, and V were eliminated.

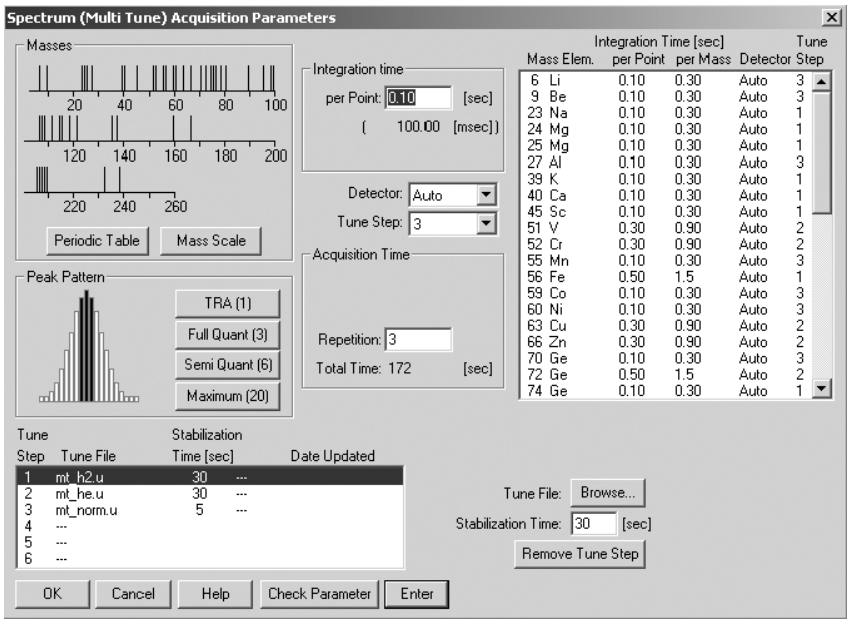


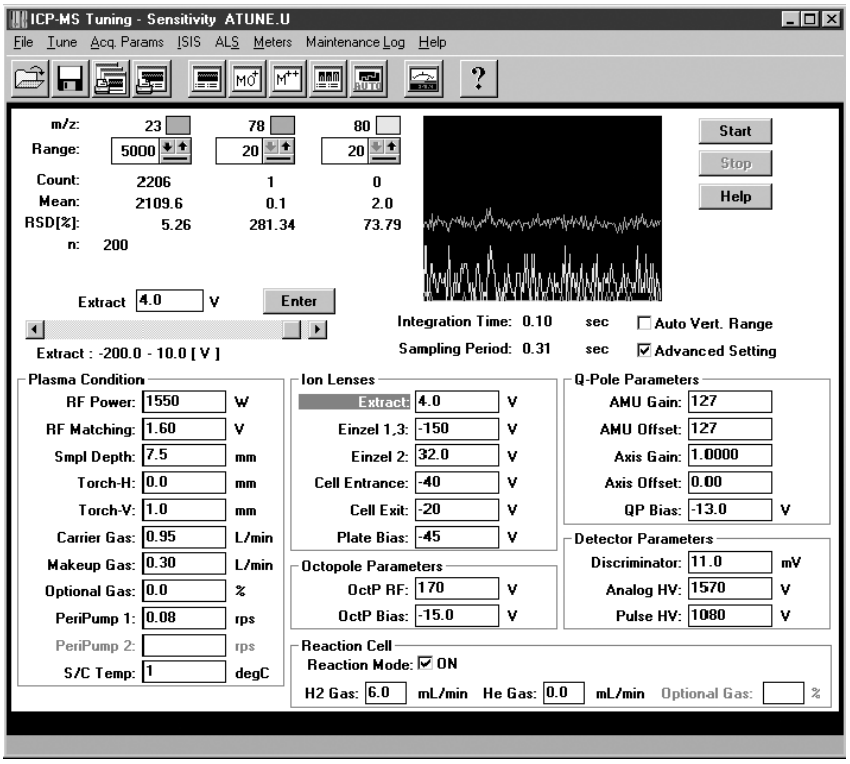

**Figure 10. Acquisition parameters setup panel showing multi tune setup. Tune step 1 is hydrogen reaction mode, step 2 is helium collision mode, and step 3 is "normal mode", for example, the ORS is unpressurized.**

**Table 1. Analysis of NIST 1640: Trace Metals in Water without Dilution Using Multi Tune Acquisition**

| | |
|---|---|
| Sample: | NIST 1640 |
| Date/Time: | 2/12/2002 17:05 |
| Method: | ENV7500C.M |

| Element | Measured value | Certified value | % Recovery |
|---|---|---|---|
| Be 9 | 35.49 | 34.94 | 101.6 |
| Na 23 | 29250 | 29350 | 99.7 |
| Mg 24 | 5679 | 5819 | 97.6 |
| Mg 25 | 5686 | 5819 | 97.7 |
| Al 27 | 52.49 | 52 | 100.9 |
| K 39 | 966.6 | 994 | 97.2 |
| Ca 40 | 6938 | 7045 | 98.5 |
| V 51 | 12.81 | 12.99 | 98.6 |
| Cr 52 | 36.07 | 38.6 | 93.4 |
| Mn 55 | 113.5 | 121.5 | 93.4 |
| Fe 56 | 31.18 | 34.3 | 90.9 |
| Co 59 | 21.41 | 20.28 | 105.6 |
| Ni 60 | 26.9 | 27.4 | 98.2 |
| Cu 63 | 84.64 | 85.2 | 99.3 |
| Zn 66 | 59.69 | 53.2 | 112.2 |
| As 75 | 28.98 | 26.67 | 108.7 |
| Se 78 | 22.13 | 21.96 | 100.8 |
| Mo 95 | 44.89 | 46.75 | 96.0 |
| Mo 98 | 47.07 | 46.75 | 100.7 |
| Ag 107 | 7.954 | 7.62 | 104.4 |
| Cd 111 | 22.41 | 22.79 | 98.3 |
| Cd 114 | 22.7 | 22.79 | 99.6 |
| Sb 121 | 13.8 | 13.79 | 100.1 |
| Ba 135 | 147.2 | 148 | 99.5 |
| Ba 137 | 145.7 | 148 | 98.4 |
| Pb 208 | 29.14 | 27.89 | 104.5 |

Figure 11 shows the Tuning Panels for both a water blank (A) and a mixed sodium (1000 ppm)/ selenium (10 ppb) sample (B), under hydrogen collision conditions.



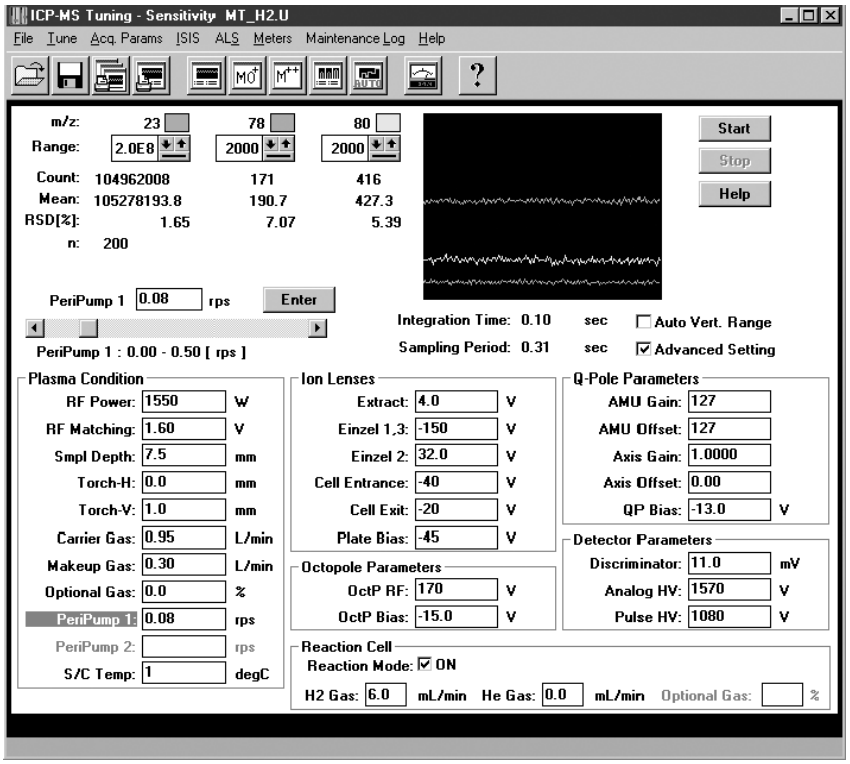




**Figure 11. Tune Panel showing (A) blank water and (B) 1000 ppm sodium plus 10 ppb selenium simultaneously under hydrogen collision conditions.**

Figure 12 displays the reaction gas autotuning panel for three elements, FE-56, Se-78, and Pb-208. The hydrogen gas flow rate, which yields the lowest BEC, is calculated automatically.

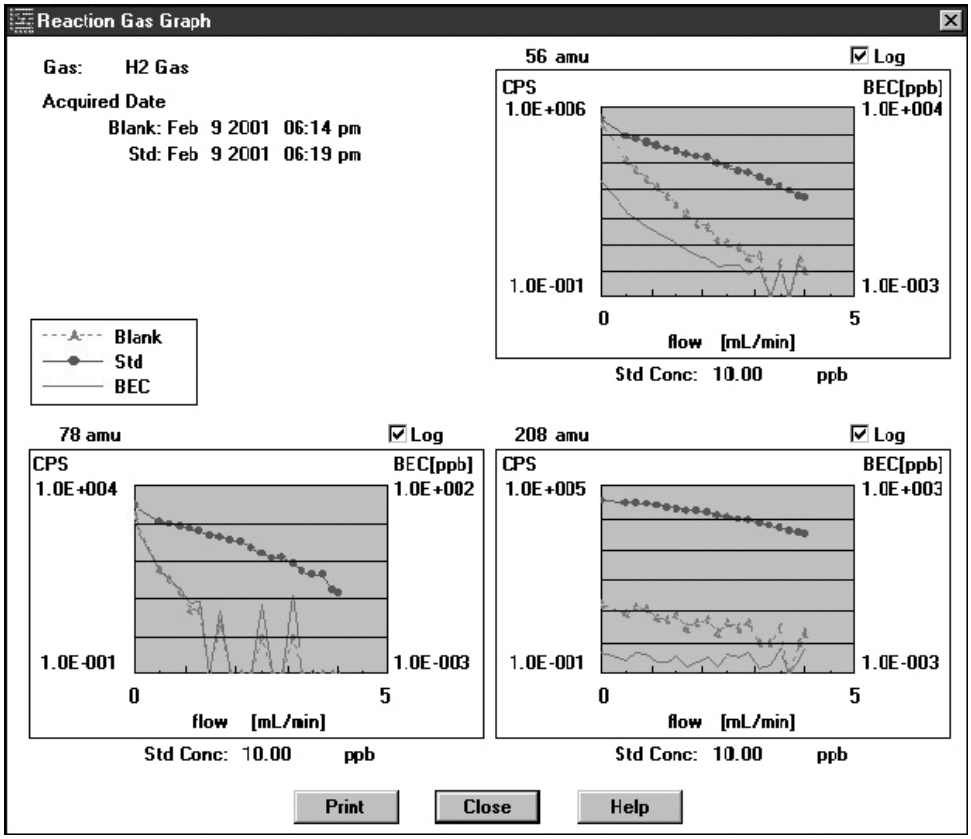



**Figure 12. Reaction gas autotuning panel.**

Figure 13 compares the practical dynamic range of various elemental techniques.

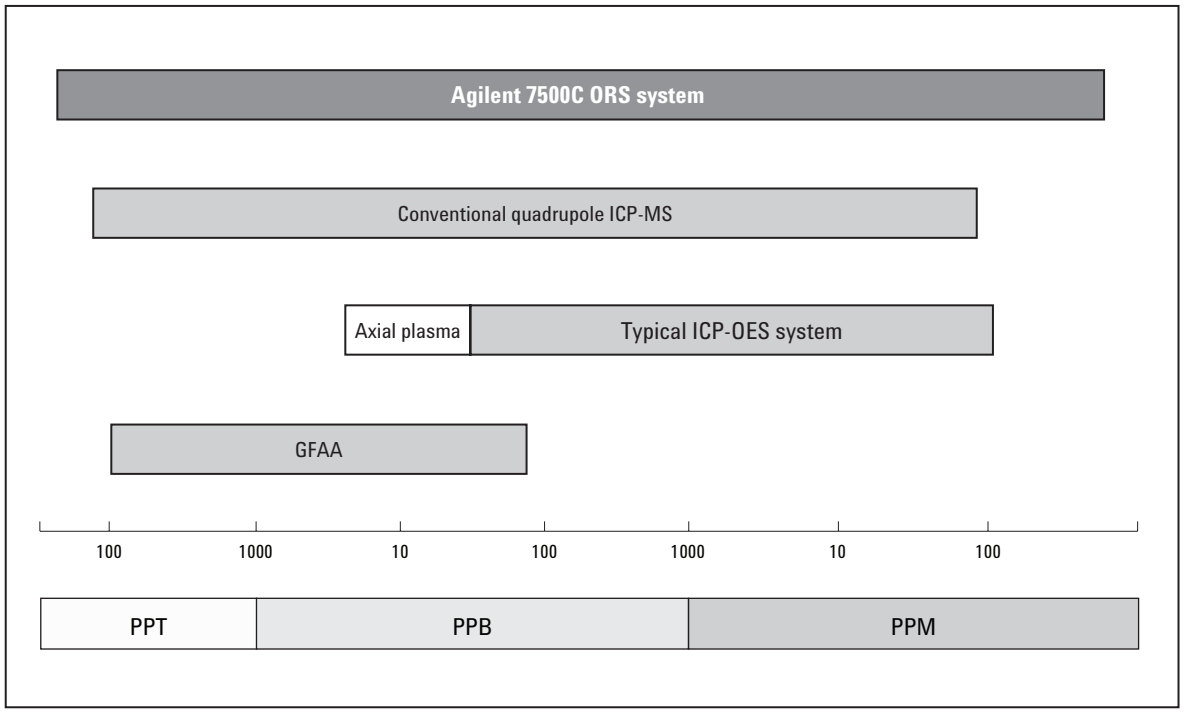



**Figure 13. Practical dynamic range in environmental samples comparing GFAA, ICP-OES, conventional ICP-MS and Agilent 7500C ORS ICP-MS.**

## Conclusions

Using the Agilent 7500c ICP-MS, it is now possible not only to meet, but to exceed the combined dynamic range of GFAA and ICP-OES for the analysis of typical environmental samples in a production laboratory environment. By taking advantage of the inherent matrix tolerance of the doubly off-axis octopole reaction system, hardware optimized for low sample flows, and ISIS, the 7500c can tolerate matrix levels previously restricted to optical ICP. Accurate quantitation of mineral elements at 100s or even 1000s of ppm is possible. At the same time, the high sensitivity, low background, and ability to eliminate or reduce spectral interferences by the Agilent 7500c allow the simultaneous measurement of ultratrace levels of previously difficult elements, such as arsenic, selenium, or mercury.

## For More Information

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