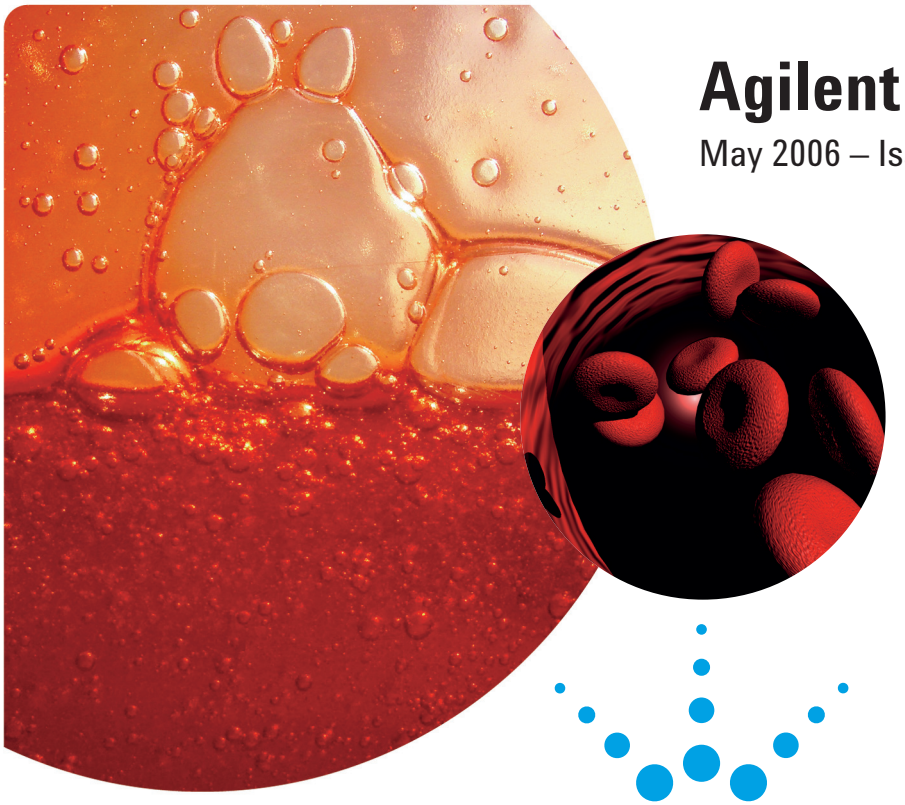

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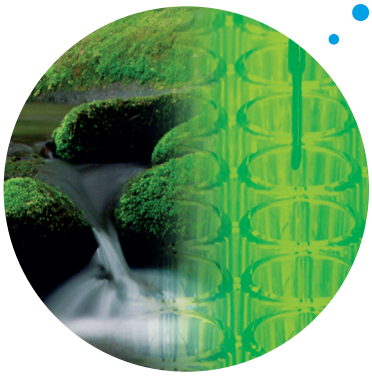

Inside this Issue

Agilent Technologies

Metal Profiling of Human Serum Using SEC-ICP-MS

Dominic Hare[1], Philip Doble[1], Michael Dawson[1], Anita R. Skandarajah[2], Robert L. Moritz[2], Richard J. Simpson[2], Rod Minett[3], Rudolf Grimm[4] and Val Spikmans[1]

1 University of Technology, Sydney, Australia
2 Joint Proteomics Laboratory, Ludwig Institute for Cancer Research & The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, VIC, Australia
3 Agilent Technologies Australia, VIC, Australia
4 Agilent Technologies Inc., Integrated Biology Solutions Unit, Santa Clara, CA, USA

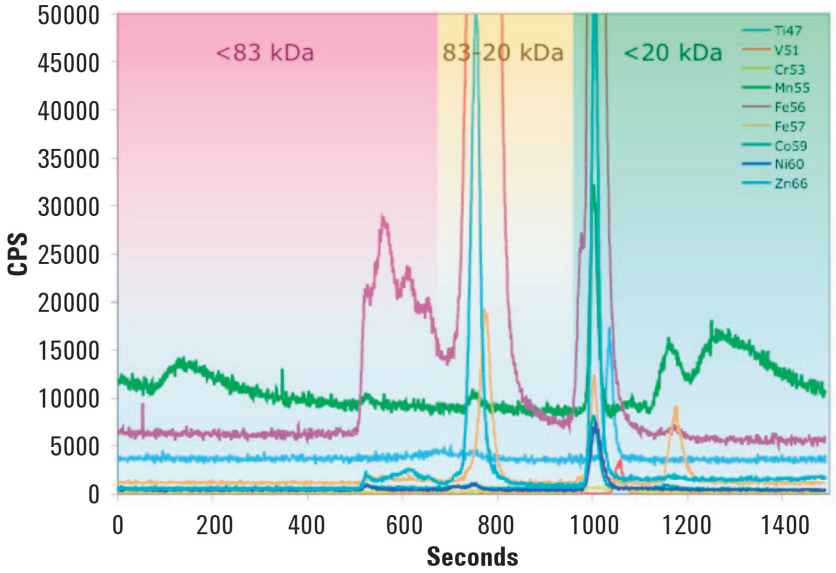

Figure 1: Multi-element SEC-ICP-MS chromatogram of pooled, undiluted Type O serum. Injection volume 10 µL. Flow rate 0.7 mL/min. Mobile phase 0.1 M $NaCH_3COO$.

Introduction

Metallomics is a new science at the forefront of modern protein research. It focuses on the analysis of the metallome, a term encompassing both metals bound to biomolecules within the cell membrane and metals either free or bound in interstitial fluids [1]. Analysis of metal ions bound to low abundant proteins requires an element specific detector of particularly high sensitivity. An ICP-MS is capable of detecting most elements important to biology (including P, S, Se, Si) at sub-ppb levels [2], making it the ideal analytical tool for the trace detection of metals bound to proteins. By connecting size exclusion chromatography (SEC) to ICP-MS, the proteins in the sample can be separated based on molecular size, allowing for the detection of elements in specific protein mass fractions. An analysis of this type will generate an elemental profile of the samples with information on the size of the proteins carrying these elements [3]. SEC-ICP-MS has the potential to be a powerful complementary technique to traditional proteomics analysis providing information on both elemental and protein profiles [4].

Approximately one third of all proteins within the human body are thought to contain metal ions [5], with roles in enzyme-based reactions, metabolism, storage and transport. SEC-ICP-MS has been used to analyze these proteins in both human tissues and fluids [6-8]. Specific metal binding proteins, such as metallothionein (MT) have been identified as possible biomarkers for disease, such as breast cancer [9], thyroid cancer [10] and Alzheimer's disease [11].

Setup of an SEC-ICP-MS system is relatively straightforward. The major advantage of this approach is the ability to analyze undiluted serum rapidly with minimal sample handling. Depending on the element measured, protein depletion may not always be necessary. Specially designed interfaces for higher resolution capillary chromatography have also been described [12]. This work attempts to develop a suitable system for elemental profiling of pooled human serum according to the mass of proteins present.

Materials and Methods

Pooled human serum samples were obtained from the Red Cross via the Ludwig Institute for Cancer Research, Melbourne. Tosoh Bioscience TSKGel® SEC Column A porous inorganic deactivated silica stationary phase was used for all separations. A high ionic strength 0.1 M $NaCH_3COO$ mobile phase (pH 7.0) ensured limited charge based retention of free and bound metals. The TSKGel 3000SWXL packing has a particle size of 5um and pore size of 25nm.

A standard 1100 Series LC system was used with a Peltier-controlled sample cooling unit. Mobile phase was delivered at 0.7 mL/min by a quaternary pump. UV signal was monitored using a post-column photo diode array detector (240nm).

The LC system was connected to the ICP-MS with 100um PEEK tubing. Both systems were controlled from a single computer operating Agilent ChemStation ICP-MS Top software. A 7500ce ICP-MS was used and was fitted with a Glass Expansion PFA OpalMist® concentric nebulizer.

Results

Figure 1 shows SEC-ICP-MS chromatograms obtained from the analysis of a 10uL sample of undiluted human serum. Three distinct binding fractions are observed, corresponding to large globular proteins (<83 kDa), high abundant transferrin and HSA (83-20 kDa) and low mass peptides (>20 kDa). All 9 isotopes analyzed (7 single elements plus ^{56}Fe and ^{57}Fe) can be identified, bound to at least one of these mass fractions.

Figure 2 shows the metallic content of peptides under 20 kDa in Type O serum, with all 9 isotopes analyzed present. Three resolvable mass fractions within this range are identified. Possible unresolved peaks are apparent in the ~14.7 kDa mass fraction, specifically ^{60}Ni and ^{66}Zn, though the peak capacity of the column used was unable to resolve them further.

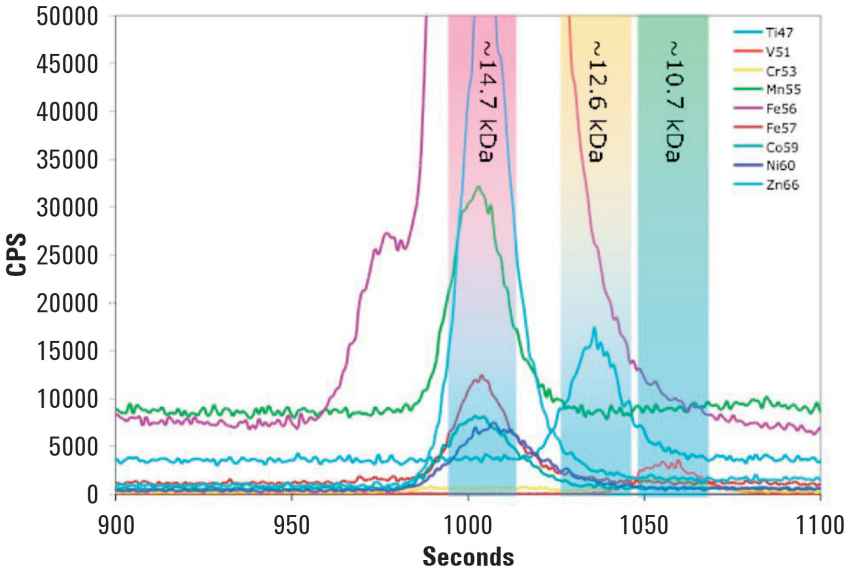

Figure 2: Elemental profile of peptides under 20 kDa in pooled Type O human serum.

Conclusions

SEC-ICP-MS has been shown to be a suitable screening technique for broad-scale analysis of elements bound to metal-binding proteins in human serum. High abundant metal binding proteins can potentially be identified according to metal content. Higher resolution separation techniques might be beneficial in providing higher resolution separation of low mass peptides. This study highlights the possibilities of using ICP-MS based detection in conjunction with structural elucidation techniques to obtain a more complete profile of the protein and elemental content of biological fluids.

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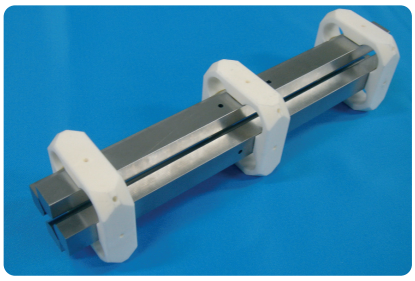
New Abundance Sensitivity Spec with Award Winning Quadrupole



Agilent has developed a new hyperbolic quadrupole - the Eagle Quad - with a stringent manufacturing tolerance of 1 micron. Agilent's Harvey Loucks, who invented the new quad, won the Bill Hewlett innovation award in recognition of his valuable contribution to improving the performance of Agilent's quad-based instrumentation.

After monitoring the quad's abundance sensitivity (AS) performance for some time, the ICP-MS manufacturing group have issued new AS specifications at both high mass and low mass for the 7500 Series as shown in Table 1. The 7500 Series now has the best AS specification of any ICP-MS ever produced.

	New 7500 AS Spec	Old 7500 AS Spec
Low	**$5x10^{-7}$ (Cs)**	$1x10^{-6}$ (Cs)
High	**$1x10^{-7}$ (Cs)**	$5x10^{-7}$ (Cs)

Table 1. Latest 7500 Series ICP-MS Abundance Sensitivity Specifications for high and low mass

All 7500 Series instruments now ship with a new Performance Certificate with the new AS specs.

Supporting Literature

- Agilent 7500 Series ICP-MS Specifications, 5989-2991EN

Optimizing Sample Throughput in ICP-MS

Steven Wilbur,
Agilent Technologies Inc., USA

Technological advances in ICP-MS hardware and software have resulted in unprecedented levels of performance and reliability. Sensitivity is routinely discussed at sub-ppt levels, interferences are all but eliminated, and the instruments are simple and reliable enough for routine use in high throughput commercial laboratories. However, these laboratories, under intense competitive pressure, also demand the highest possible throughput, while maintaining performance, simplicity and reliability. A number of factors contribute to overall throughput, including instrument uptime, time spent tuning and calibrating, etc. However, the most obvious factors are those that influence the actual analysis time, which will be discussed here.

Analysis time, or more accurately, average run-to-run time, is really a function of two components:
1. Sample wash-in and wash-out
2. Data acquisition

Both can be optimized, depending on sample types and analytical requirements.

Optimizing Sample Wash-in and Wash-out

Without resorting to discrete sampling systems, sample uptake and rinse out can be improved significantly using simple enhancements to the conventional peristaltic pumped sample introduction system, appropriate rinse solutions and intelligent software functions. Software functions are discussed on page 6.

Hardware

Optimizing wash-in wash-out involves minimizing the volume and internal surface area of the sample introduction components including the autosampler probe, peristaltic pump tubing, and sample transfer line. Also important is minimizing chemical interactions between analytes and the sample introduction system components. Reducing volume and surface area are as simple as using the narrowest and shortest lengths of tubing (including peripump tubing) that are consistent with the necessary flows. The standard 1.02 mm internal diameter (ID), 3-stop peristaltic pump tubing contributes unnecessary volume and surface area. Additionally Tygon, while being clean and mechanically suitable, is prone to interacting with certain elements under some conditions resulting in carryover. Therefore the length and diameter should be minimized. At a minimum, the excess tubing and third stop should be removed, leaving only about 1 cm tails beyond the 2 remaining stops. Reducing the diameter can reduce both the volume and surface area significantly, resulting in faster uptake and rinse out. If smaller diameter tubing is used, pump speed during acquisition must be adjusted to maintain the correct nebulizer flow (Table 1). Switching from 1.02 mm to 0.64 mm tubing can reduce the sample uptake and rinse out time by more than 50%.

Peripump tubing ID / mm	Correction factor
0.89	1.3
0.76	1.8
0.64	2.55

Table 1. Correction factors for various internal diameter (ID) peristaltic pump tubes used to maintain correct nebulizer flow. To use, multiply pump speed used for 1.02 mm tubing by the appropriate factor.

The following conditions have been shown to significantly reduce uptake and rinse out time.

Sample tubing ID	0.3 mm
Peripump tube ID	0.64
Pump Speed (analysis)	0.26 rps
Pump Speed (uptake)	0.5 rps

Chemistry

Dilute nitric acid has been the traditional diluent and rinse solution used in ICP-MS. This has been mainly because most metals are soluble in nitric acid, and it does not introduce additional matrix components such as Cl, S, C etc. that may cause polyatomic interferences. However, now that collision/reaction cells can eliminate polyatomic interferences, the analyst is free to use more appropriate chemistry for sample preparation and rinse solutions. At the very minimum, the addition of 0.5% HCl to the normal 1-2% HNO_3 will stabilize Ag and significantly improve the performance (linearity, washout) for Hg and the platinum group elements. Some elements (including Hg, Mo, Sb and Tl) exhibit better washout characteristics under alkaline conditions. An alkaline rinse solution composed of ammonium hydroxide, EDTA, Triton x-100 and hydrogen peroxide has long been used in the semiconductor industry for cleaning critical components.

This solution can be made up as follows and may improve washout in many circumstances. When using an alkaline rinse solution to rinse out samples prepared in acid solutions, it is advisable separate the acid samples and alkaline rinse by introducing a short rinse of DI water before and after the alkaline rinse.

Stock Solution: 2.5g EDTA (as the acid not Na salt); 0.2g Triton X-100; 15g NH_4OH; 20g H_2O_2 & make up to 250 mL with water. Dilute 1:10 in DI water for the final rinse solution.

Data Acquisition

Data acquisition time can be reduced in a number of ways, depending on the analytical requirements. The main contributors to excessive data acquisition time are:
1. Unnecessary isotopes acquired
2. Unnecessarily long integration time
3. Excessive stabilization time at acquisition start and at ORS mode switching
4. More replicate acquisitions than necessary

In many cases, data quality or regulatory requirements may mandate specific acquisition parameters including the isotopes acquired and the number of replicates. When not restricted by specific regulations, the use of collision/reaction cell ICP-MS such as the Agilent 7500 ORS, can reduce the number of necessary isotopes by eliminating the need for backup isotopes and interference correction masses. Other parameters can generally be optimized for faster analysis as well. While there may be some element of compromise between acquisition time and data quality, it is almost always

possible to improve analysis time while still meeting data quality objectives. The data in Table 2 shows the effects of various changes in the parameters listed above on the acquisition time for a typical environmental method for the analysis of 26 analytes in water samples.

Discussion

For many applications, depending on the sample type and analytical requirements, significant reduction in run-to-run time can be achieved by reducing acquisition time without adverse effects on performance. This is possible due to the high sensitivity and precision of the 7500 Series instruments. In most cases, detection limits are more limited by blank contamination than by instrument signal to noise. Reduction in integration time from 0.3 seconds per isotope (typical) to 0.1 seconds per isotope had no significant effect on calculated detection limits under typical environmental analysis conditions, but reduced the total acquisition time by 0.5 - 1 minute. In addition to reducing integration time, other techniques can also reduce the acquisition time. For example, while the use of the ORS to remove interferences will result in superior data for complex samples, in clean samples, the advantage may not justify the extra time required. Some tricks to improve throughput have no down side. For example, the ChemStation can be instructed to automatically return to the first ORS mode at the end of acquisition, thereby eliminating the need for additional gas stabilization before the next sample. This can save 10-15 seconds per run. Finally, though many regulations require multiple replicates, acceptable data can be had with a single replicate (as is the case for all time-resolved data). Reducing the number of replicates reduces the acquisition time proportionately, though at some cost in precision. Depending on the conditions, acquisition times for 26 elements can vary from as much as 166 seconds or more, to as little as 12.6 seconds, depending on requirements - see Table 3.

ORS Mode - optimized to remove all interferences using 3 modes (36 isotopes*)				
Conditions	15 sec stabilization time after all mode switches, 3 pts/pk, 3 reps	Reduce first stabilization time to 5 sec	Reduce integration pattern to 1 point/peak, 0.1 sec per mass*	Reduce to 1 replicate
Total acquisition time (sec)	166	156	89	53
Vial to vial run time including uptake and rinse (min)#	5.3	5.1	4.0	3.4
* 26 elements + 6 internal standards + correction masses for Li, In and Pb				

Using the standard rinse program allows for a reduction of 4 orders of magnitude after a running a 100 ppb standard

Table 2. Comparison of total acquisition times using ORS mode depending on acquisition parameters

No-gas Mode or no ORS - using correction equations to remove interferences (46 isotopes*)			
Conditions	3 pts/pk, total 0.3 sec integration time per mass	Reduce integration pattern to 1 point/peak, 0.1 sec per mass *	Reduce to 1 replicate
Total acquisition time (sec)	71	38	12.6
Vial to vial run time including uptake and rinse (min)#	3.7	3.1	2.7
* 26 elements (multiple isotopes for several) + 6 internal standards + multiple correction masses			

Using the standard rinse program allows for a reduction of 4 orders of magnitude after a running a 100 ppb standard

Table 3. Comparison of total acquisition times using non-ORS mode depending on acquisition parameters.

*Typical acquisition parameters include 3 points per peak at 0.1 second per point for most elements. By reducing the number of points to 1 pt per peak, the total integration time is effectively reduced by a factor of three.

Benefits of the New ICP-MS ChemStation – Version B.03.03 Intelligent Rinse

Steven Wilbur, Agilent Technologies Inc., USA

The newest version of the ICP-MS ChemStation includes more than 30 new features and enhancements designed to improve ease of use and productivity, including:

- Easier to use Tuning Window
- Pre-Run Monitor
- Batch View - data file viewer
- Offline Acquisition Editor
- New, more powerful Autotune for easier operation
- Higher sample throughput
  - Pre-emptive rinse and Intelligent Rinse
  - More flexible control of 2nd peri pump option

Of these, *Intelligent Rinse* is likely to be among the most useful for laboratories needing to analyze large numbers of highly variable samples in the shortest time possible.

Because of the high sensitivity and wide dynamic range of ICP-MS, rinsing back to blank levels after high samples is much more challenging than for less sensitive techniques like ICP-OES. The traditional approach has been to extend the program rinse times to insure that even after very high samples adequate rinsing is accomplished. However, in the case of very clean samples, or samples very similar to one another, this results in unnecessary time spent rinsing, which can add minutes to the total run time. A more efficient approach is to actively monitor the background while rinsing between samples and rinse only as long as necessary. This is exactly what *Intelligent Rinse* does.

Intelligent Rinse allows the user to specify up to 10 critical elements to monitor during rinsing. These can be monitored as raw Counts Per Second (CPS), internal standard corrected CPS (effectively concentration), or any other ratio desired. Then when the user defined thresholds have been achieved, rinsing stops and the next sample is introduced.

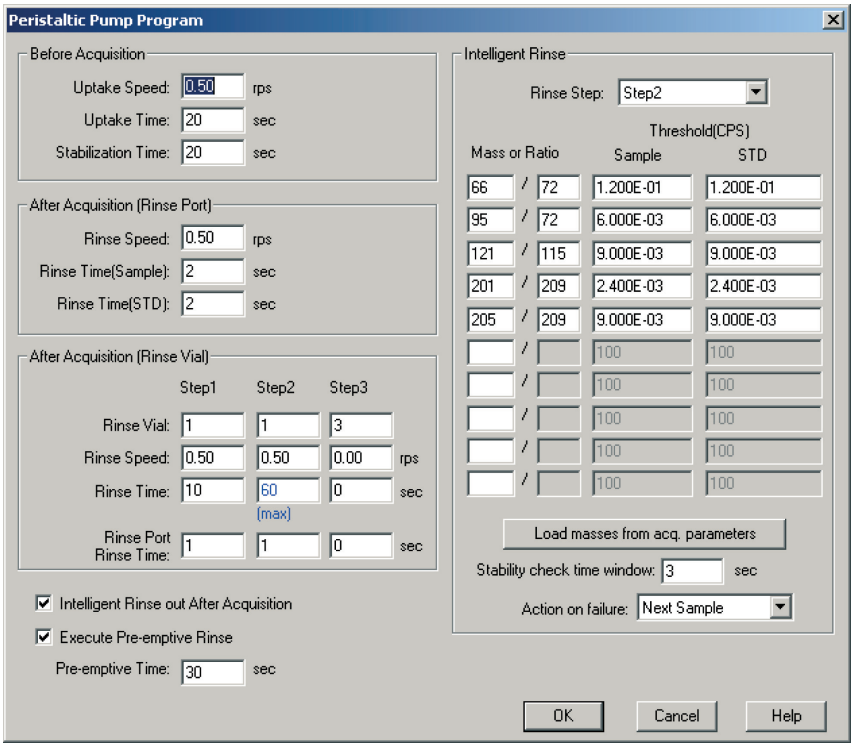


Figure 1. 7500 ICP-MS ChemStation screenshot of the Peristaltic Pump Control Panel set-up page where Intelligent Rinse is configured

Rinsing is always complete, but never any longer than necessary.

The ChemStation supports 4 independent autosampler positions to be used in any combination for rinsing as needed. Any of these 4 positions can be set as an *Intelligent Rinse* position. Figure 1 shows the Peristaltic Pump Control Panel where Intelligent Rinse is configured.

Intelligent Rinse even anticipates the case where it is not possible to achieve the specified rinse targets by utilizing a user-set maximum rinse time and action on failure. If, by the maximum time, the targets have not been achieved, rinsing will terminate and the specified action will be taken. This allows the user to either continue the sequence, as would be the case without *Intelligent Rinse*, or abort it. In the example above, *Intelligent Rinse* time after highly contaminated samples would be as long as 1 minute (the max specified), but rinse time after clean samples would be only a few seconds (the time required to determine that thresholds had been met). The net effect is that when running a sequence of highly varied samples, ranging for example from clean waters to highly contaminated soils, overall run-to run time can be reduced by as much as 30%.

Version B.03.03 Availability

Version B.03.02 is currently shipping with new instruments. The version described in this article (B.03.03) will start shipping with new instruments from mid July. Any user with B.03.xx software can download a free upgrade to B.03.03 from the Agilent website when available.

Free upgrades for version B.03.xx users will be available for download on the Agilent website from July.
http://www.chem.agilent.com/Scripts/cag_checkreg.asp?anch=ICP

The software is also upgradeable from earlier versions – see web site for more details.
http://www.chem.agilent.com/Scripts/cag_checkreg.asp?anch=ICP

Revised Maintenance Schedule for 7500 Series

Hidenori Koide, Agilent Technologies, Tokyo, Japan

It is important to maintain your Agilent 7500 regularly to extend the useful life of its components and optimize its analytical capabilities. Defining an appropriate maintenance schedule for your instrument depends on the number of samples/day, sample matrix type and the environment of the instrument. The 7500 Series user manuals are very conservative when stating typical recommended maintenance frequency, representing a worst-case sample load scenario. However, over the past two years since the 7500ce was introduced, Agilent has been carefully monitoring the performance of the 7500ce when used in very tough conditions in routine labs. Users report that the 7500ce is exceptionally rugged and can handle a high workload of high matrix samples with minimal maintenance. As a result, the recommended maintenance interval for the ion lenses and ORS has been significantly extended on the 7500ce.

The guidelines given in the table below apply to a typical environmental lab running a mix of sample matrices for 8 hours/day, 5 days/week. Under the new recommended maintenance schedule, 7500ce users typically will only need to break the vacuum system every 6 months to clean the cell lenses, and annually to clean or replace the octopole. The octopole can be cleaned if it becomes contaminated, but some users may prefer to simply install a new one to save a little time.

Refer to the 7500 Series Maintenance DVD (Agilent part # G3270-65100 – see Journal issue #26) for instructional videos on how to perform all maintenance tasks. It is easier to schedule maintenance if you keep a log of the instrument readings for each analysis. It is also strongly recommended to generate a tuning report each day, so any degradation in performance can be tracked. Table 1 lists the Agilent 7500 maintenance tasks by recommended task frequency.

Frequency	Component	Task/Action
Daily	Argon gas	Check argon gas pressure and volume
Daily	Drain vessel	Check, empty if required
Daily	Peristaltic pump tubing	Check for wear
Weekly	Torch, spray chamber, connector	Clean, replace when necessary
Weekly	Sampling cone, Skimmer connector	Check orifice and clean if required. Replace when necessary.
Monthly	Rotary pump	Check oil level and color. Check mist filter for presence of oil
Monthly	Nebulizer	Run "Neb test" and take appropriate action as indicated
Monthly	RF return strips and shield bar	Clean
Monthly	Cooling water	Check water level
Semi-Annually	Rotary pump	Change oil
Annually	Rotary pump oil mist filter	Replace mist filter
Annually	Penning gauge	Clean
Annually	Water strainer	Check and clean
Biannually	Argon gas filter	Replace
The components listed below should be checked periodically, at least on an annual basis, and appropriate action taken		
Periodically	Electron multiplier	Evaluate; replace when necessary
Periodically	Plasma gas, auxiliary gas tubing	Check; replace when necessary

Table 1. Maintenance schedule guidelines - general items

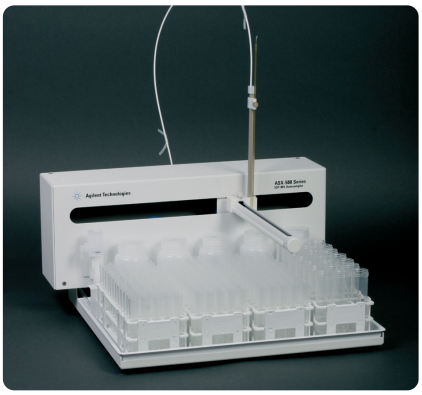
Ion Lenses and ORS

The frequency of maintenance required for these parts is more dependent on number of samples run per day and the sample matrix type. The guidelines given in the Table 2 apply to a typical environmental lab running a mix of sample matrices for 8 hours/day, 5 days/week. Labs with lighter workloads or cleaner sample matrices will be able to extend periods between cleaning. Running Intelligent Rinse and Pre-emptive Rinse software programs (ChemStation version B.03 onwards), or using ISIS will expose the interface to less sample matrix over time which will also extend periods between cleaning.

Frequency	Component	Task/Action
3 to 6 Months	7500a Extraction lenses	Clean
6 Months	7500a Einzel/Omega lenses	Clean
3 to 6 Months	7500ce/cs Extraction/Omega lenses	Clean
6 Months	7500ce/cs Entrance lens, Exit lens, Plate bias /Cell entrance, QP focus	Clean
Annually	Octopole	Clean or replace if preferred

Table 2. Maintenance schedule guidelines - ion lenses and ORS

New Agilent-Supported ASX Autosampler



Agilent has launched a new Agilent-branded autosampler which is manufactured by CETAC and is based on the ASX 520. The Agilent product number is G3286A.

The new Agilent branded autosampler is fully supported by Agilent engineers, which makes it much easier for users of the new systems to get autosampler support. A full range of Agilent service contracts and preventative maintenance programs are also offered. The standard support procedure is an exchange program: Agilent sends an exchange autosampler and the user sends their faulty autosampler to Agilent in the packaging materials provided. On site repair by Agilent engineers is also available in cases where the existing autosampler can not leave the lab - for example in some nuclear facilities.

Note: Agilent only supports the Agilent branded product at this time. Customers with CETAC branded autosamplers will still need to contact CETAC for support.

Trade Shows and Conferences

6th Int. Symposium on Speciation of Elements in Biological, Enviro and Toxicological Sciences
21-25 June 2006, Bialowieza, Poland
http://www.eurocongress.com.pl/issebets2006

Agilent ICP-MS UK & Ireland User Meeting
27 June 2006, Kingston University, Kingston-upon-Thames.

This information is subject to change without notice

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Welcoming New Agilent ICP-MS Users

A very warm welcome to all companies and institutions that have recently added an Agilent ICP-MS to their analytical facilities. Remember to join the Agilent web-based ICP-MS User Forum – the place where you can exchange information relating to your 7500.

To access the Forum, you will simply need to log-in to the Agilent web site, or register if you haven't already, and enter your instrument's serial number on your first visit only.

Look for the link to the ICP-MS User Forum from:
www.agilent.com/chem/icpms

Useful features include:

- e-Mail notification of any activity on the Forum
- Powerful search facility
- Agilent ICP-MS User Resource Library - a database specifically for user-relevant information

Contents of the Agilent ICP-MS User Resource Library:

- 7500ce Tuning Guide
- 7500a Tuning Guide
- 7500cs Tuning Guide
- Clinical Sample Prep Guide
- Guide to Analyzing Organic Solvents
- How to use FileView
- Technical Note: Configuration of Sample Introduction System for Rapid Measurement of 'Sticky' Analytes by ICP-MS
- New! Laser Ablation-ICP-MS Operation Guide

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- Unmatched Spectral Interference Removal in ICP-MS Using the Agilent Octopole Reaction System with Helium Collision Mode, 5989-4905EN
- Agilent 7500 Series ICP-MS Specifications, 5989-2991EN

Agilent ICP-MS Journal Editor

Karen Morton for Agilent Technologies
e-mail: editor@agilent.com

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