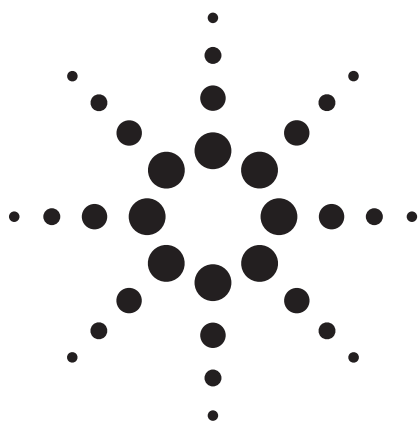


Improved Forensic Toxicology Screening Using A GC/MS/NPD System with a 725-Compound DRS Database



Application

Forensic Toxicology

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Abstract

Laboratories that perform toxicology screens are challenged by the requirement to look for large numbers of target compounds in samples that contain complex matrix interferences. GC/MS methods are widely used and accepted for this analysis. Full-scan EI methods offer many advantages for broad-range screening, such as unlimited numbers of targets, full-spectrum identity confirmation, and library searching for identification of nontargets. With recent advances in GC/MS technology, there are several opportunities to substantially increase the number of targets screened for and simultaneously reduce the time required per sample.

With the system described here, samples are screened for 725 compounds using Agilent's G1674AA Forensic Toxicology DBL. Data review time is substantially reduced using Agilent Deconvolution Reporting Software. Post-run bakeout of heavy-matrix compounds is replaced with column backflushing, which is faster and reduces system maintenance. Run time is reduced by using a fast GC run (9.75 min injection to injection) and simultaneously collecting scan, SIM, and NPD data. The scan data is deconvoluted and used to identify any of the 725 target compounds. SIM data is used to look for select low-level

compounds not detectable in scan mode. The nitrogen response of the NPD is used to highlight nontarget nitrogen compounds and identity confirmation and can be used for quantitation if needed. Using extracts of whole blood samples, the system finds all the compounds detected by the conventional method in significantly less time.

Introduction

GC/MS screening methods play an important role in the toxicology laboratory. With the continuing emergence of new drugs and toxins, the list of target compounds to be screened can easily number in the hundreds. For those compounds that are compatible with GC, GC/MS in full-scan mode with electron impact ionization (EI) is well suited for the task. The technique offers several advantages:

- It uses straightforward, reliable, and familiar instrumentation.
- Any number of targets can be monitored.
- The target list is not limited by the number of MRMs like MS/MS techniques.
- Years later, archived full-scan data can be examined for new targets.
- Identity confirmation is based on full spectra.
- Nontarget unknown compounds can be identified by searching spectra against NIST and other industry standard libraries.
- Ionization suppression due to matrix is much less of a problem than with LC/MS techniques.



Agilent Technologies

While GC/MS methods offer the above advantages, there are limitations with the conventional approach. As the number of target compounds in the screen increases, the size of tasks involved in the development, maintenance, and application of the methods grows very rapidly. These considerations often limit the scope of screening methods used in toxicology labs.

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 retention time 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. For methods that deal with only a few matrix types, the ions chosen for identification purposes can be selected such that they are minimized in the matrix. With a limited number of targets addressed by the method, recalibration of response factors, retention times, and qualifier ion abundance ratios can be accomplished with the injection of a few calibration mixtures.

Screening methods for very large numbers of targets in 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 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, retention times, and qualifier abundance ratios is difficult because of the large number of targets.
- The methods may be deployed in multiple laboratories without ready 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 important compounds not in the target list could be present in a sample.

In recent years, several techniques have become available to help address the above set of challenges. Retention time locking (RTL) produces retention times that precisely match from instrument to instrument and those in a database [1]. This eliminates the need for recalibration of the individual retention times and timed events like SIM groups. The introduction of reliable and inert Capillary Flow Technology (CFT) splitters allows for the simultaneous collection of mass spectral and nitrogen/phosphorus detector (NPD) data [2]. The NPD chromatogram highlights nitrogen-containing compounds, including those not in the MS target list. It is useful in confirming the presence of a nitrogen-containing target compound and can serve as an alternative means of quantitation.

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 (like fentanyl) down to very low levels.

One of the most significant tools developed for reducing the time required for data review is Agilent's Deconvolution Reporting Software (DRS) [4]. It uses advanced computational techniques (deconvolution) 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. If desired, hits can be confirmed by also searching against the main NIST MS reference library. The entire process is automated and provides a major time savings in data interpretation. The use of DRS also substantially reduces the number of both false positives and false negatives.

Since DRS uses 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. Also, since it uses the entire spectrum for identification instead of precise target/qualifier ion ratios, frequent updating of the ratios is not necessary. This is useful for targets that are rarely encountered but are still screened for.

This application describes the combination of the above techniques with a new database of 725 compounds, the Agilent G1674AA Forensic Toxicology DBL, to be used for screening purposes. The DBL contains:

- RTL methods for DB-5MS and DB-35MS columns

- Spectral libraries for DRS and the MSD ChemStation
- Preconfigured RTL methods for multiple speeds with run times of 30, 15, 10, 7, or 5 minutes, depending on hardware configuration
- Methods for both MSD direct connection (vacuum) and Capillary Flow Technology splitters (3.8 psig).
- Three quant databases included for each method:
 - Target and qualifiers are the biggest four ions.
 - Ions are optimized to give the best signal-to-noise ratio versus column bleed and background.
 - Ions are optimized to give the best signal-to-noise ratio versus common fatty acids found in blood.

The names of all the compounds in the database are listed in the appendix at the end of this application. Compounds in the DBL include drugs and select breakdown products, TMS derivatives, and acetyl derivatives. For those compounds entered as derivatives, in general, primary and secondary amino (including aliphatic and aromatic) compounds are acetylated. Hydroxyl groups (alcohols/phenols/carboxylic acids, etc.) are converted to TMS derivatives with BSTFA. Compounds having multiple functionalities (for example, phenylpropanolamine, which has a primary aliphatic amine and an alcohol) were acetylated with no further derivatization.

Methods are provided for two stationary phases to allow two-column confirmation and the ability to run other methods that require the same column on the same hardware. In general, the DB-5MS methods are preferred because the final oven temperature is lower.

The chromatographic conditions chosen for development of the database are general in nature and are compatible with the analysis of other compounds beyond those in the table. Since no one target list, no matter how large, can satisfy every lab's needs, new compounds can be added to the screen.

The retention times for compounds in the database are provided for both the column connected directly to the MSD and for the column outlet pressure at 3.8 psig using a CFT splitter. This was done to ensure that the retention times observed during sample analysis would closely match those in the database regardless of the instrument configuration.

The chromatographic conditions for the database were chosen to be compatible with Agilent's method translation technique. Constant-pressure mode was used in the GC inlet so that method translation could be used to precisely time-scale the methods for faster operation [5]. Provided with the Agilent Forensic Toxicology DBL are the files to run the analysis at precisely twofold (2x), threefold (3x), fourfold (4x), and sixfold (6x) faster than the primary database (1x). The choice of speed is determined by the degree of chromatographic resolution desired and the hardware capabilities of the GC/MSD system to be used.

For systems with a 120 V GC oven, an MSD with diffusion pump, and the column connected directly into the MSD, only 1x or 2x methods can be used. The 3x, 4x, and 6x methods require the fast oven (240 V) and performance turbopump because column flow rates exceed 2 mL per minute. Performance electronics are also preferred for the same methods. The 6x methods require both a 240 V oven and the oven "pillow" accessory to attain the 60 °C/min ramp rate. Note that use of the pillow requires that the MSD, inlet, and NPD (if used) be located in the back GC positions.

Three different versions of each method set are provided based upon the choice of ions used in the quant database. A method using the largest four ions in a compound's spectrum is supplied. The target ion is the ion with the largest abundance. The three qualifiers are the next three largest ions assigned in order of decreasing abundance. These method sets are provided for legacy reasons, and are used in some more advanced approaches.

The drawback of the largest four-ion approach is that, in some cases, the signal-to-noise performance suffers. For example, if the biggest ion for a compound is 207 and the stationary phase has its largest bleed ion at 207, the signal-to-noise ratio at that mass can be significantly reduced. The same problem is seen with low masses such as 44, where CO₂ and other background gases can result in interferences and increased noise. To reduce this problem, a second method set is provided where ions chosen for the quant database are selected to give best signal-to-noise ratios relative to column bleed and background gases. These are the methods that would normally be used, as they typically give best overall performance.

A third method type is provided where the choice of ions has been optimized for samples having large amounts of fatty acids typically seen in blood samples. These methods give the best signal-to-noise

ratios in high fatty-acid matrices. They are not the best choice for samples having low levels of interfering fatty acids.

Experimental

System Configuration

The system configuration used is shown in Figure 1. The GC is an Agilent 7890A (G3440A).

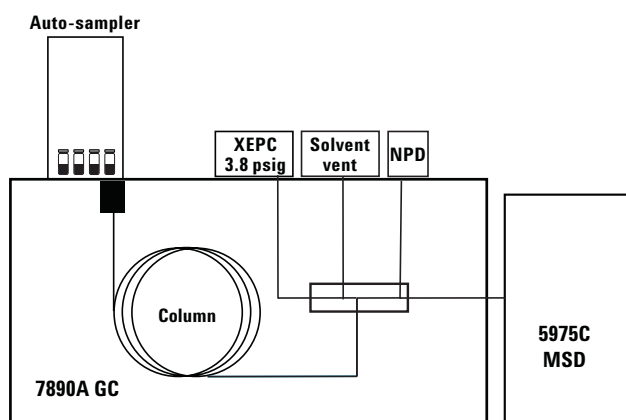


Figure 1. GC/MS/NPD system configuration used for screening blood extracts.

Key components are:

Fast Oven The primary 1x method uses a 30-m column with a 10 °C/min ramp rate and only requires the 120 V oven. With the 7890A 240 V oven (option 002), the screening method can be run up to 4 times faster using a 15-m column. If the 240 V GC is further equipped with options 199 and 202 (puts split/splitless injection port and MSD interface in the back of the oven) and uses the G2646-60500 oven insert accessory, the speed can be increased to 6 times faster (60 °C/min) with a custom length 10-m column. If an NPD is used with a splitter, option 299 places it in the back of the oven for use with the pillow.

NPD The 7890A Option 251 is a nitrogen phosphorus detector. The signal from the NPD is collected, stored, and processed by the MS ChemStation simultaneously with the MS data. NPD detectors are highly selective and exhibit very sensitive response to nitrogen and phosphorus compounds, with detection limits in the low picogram range. The NPD data can be used in several ways. Nontarget nitrogen (and phosphorus) compounds are highlighted for the data reviewer. The presence of a response at the retention time of an identified compound can be used to support confirmation of identity. The response on the NPD can be used for quantitative analysis, but only after calibration with a standard,

as the response factors are compound dependent and can vary with compound class. The NPD bead is incompatible with halogenated solvents and excess silanizing reagents. If these are to be used with an NPD, the splitter setup should have solvent venting capability.

Capillary Flow Technology Splitters Agilent offers two different column effluent splitters that can be used with the 7890A for this application. Option 889 is a two-way splitter that divides the effluent of the column between the MSD and the NPD. The 7890A Option SP1 (7890-0363) does the same, but adds solvent venting capability as well. The devices are based on diffusion bonded plate technology combined with metal column ferrules to make inert, easy-to-use, leak-free, high-temperature splitters. The splitters use Auxiliary EPC for constant pressure makeup (7890A 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 significantly reduces analysis times for samples that contain high-boiling matrix components and reduces both column head trimming and frequency of MSD source cleaning [6]. The Aux EPC also allows column changing and maintenance without venting the MSD.

For methods that use solvents compatible with the NPD and do not have silanizing reagent in the samples, the standard two-way splitter can be used. If halogenated (or other NPD incompatible) solvents or silanizing reagents are used, then the two-way splitter with solvent vent, 7890A Option SP1 (7890-0363), should be used to protect the NPD bead. This is the configuration used here.

MSD System The 5975C Inert MSD with performance turbo (G3243A) or 5973N Inert MSD with Performance Electronics and performance turbo (G2579A) EI 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 systems using splitters. It is also required for the faster versions (3x, 4x, and 6x) of the screening method with vacuum outlet (column connected directly to MSD). The standard turbo pump can be used for the slower 1x and 2x vacuum outlet versions of the method. Both the performance and standard turbos are compatible with backflushing. Backflushing cannot be done on systems with a diffusion pump.

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. As with conventional SIM methods, not all 725 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 fentanyl and phencyclidine.

DRS Software (G1716AA) Spectral deconvolution of the MS data enables identification of analytes in the presence of overlapped matrix peaks [4, 7]. 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 utilizes 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: (1) ChemStation, based on retention time and four-ion agreement; (2) AMDIS, based on “cleaned spectra” full ion matching and locked retention time; and (3) NIST05a search using a 163,000-compound library.

G1674AA Forensic Toxicology DBL This supplies the mass spectral library, method, and DRS files for the 725 compound screening methods.

Oven	
Voltage (VAC)	240*
Initial oven temperature	100 °C
Initial oven hold	0.25 min
Ramp rate	40 °C/min
Final temperature	325 °C
Final hold	1.25 min
Total run time	7.13 min
Equilibration time	0.5 min
Backflush time	0.5 min
Backflush temperature	325 °C

Column	
Type	DB-5MS
Agilent part number	Custom
Length	10 m
Diameter	0.25 mm
Film thickness	0.25 µm
Nominal initial flow	2.52 mL/min
Outlet pressure	3.8 psig

2-Way Splitter w/Solvent Vent	
7890A SP-1, num. 7890-0363	
MSD restrictor length	0.69 m
MSD restrictor diameter	0.15 mm
NPD restrictor length	0.36 m
NPD restrictor diameter	0.15 mm
Split ratio MSD:NPD	1.4:1
Solvent vent time range	0–0.75 min
Splitter pressure during run	3.8 psig
Splitter pressure during backflush	76 psig

NPD	
Hydrogen flow	3 mL/min
Air flow	60 mL/min
Nitrogen makeup flow	8 mL/min
Temperature	300 °C

MSD	
Agilent Technologies 5975 or 5973 inert with performance electronics	
Vacuum pump	Performance turbo
Tune file	Atune.U**
Mode	SIM/scan
Solvent delay	0.7 min
EM voltage	Atune voltage
Low mass	40 amu
High mass	570 amu
Threshold	0
TID	Off
Sampling	1
Quad temperature	180 °C
Source temperature	300 °C
Transfer line temperature	300 °C

*Injection port and MSD interface in back positions and G2646-60500 oven pillow

**Gain normalized, 1x

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	
Agilent Technologies 7890A with autoinjector and tray	
Inlet	EPC split/splitless
Mode	Constant pressure
Injection type	Splitless
Injection volume	1.0 µL
Inlet temperature	280 °C
Liner, Agilent dual-taper deactivated	P/N 5181-3315
Pressure, nominal	14.9 psig
RT locking compound	Proadifen (SKF-525a)
RT locking time	4.285 min
Purge flow	50 mL/min
Purge mode	Switched
Purge time	0.4 min
Gas type	Helium
Inlet backflush pressure	1 psig

Instrument Operating Parameters

Data Acquisition

The instrument operating parameters used (unless noted otherwise) are listed in Table 1.

DB-5MS was chosen as the stationary phase for the current system. The final temperature required to elute the last compound in the screen is 325 °C instead of 345 °C as required with DB-35MS. This results in shorter run times and longer column life.

The method parameters were chosen to give the best trade-off between chromatographic resolution and sample throughput. For the blood samples analyzed here, the 4x method gave adequate resolution with an relatively short run time. Although the 4x method can be run on a standard 15-m column, a 10-m column was chosen because it gives very similar resolution with a lower column flow rate.

Time was also saved by using backflushing instead of post-run column baking to remove heavy sample

matrix compounds. Backflushing is more effective, faster, and does not send the heavy materials and column bleed into the NPD and MSD source. With the current configuration, all heavy materials were removed from the column with a 0.5-minute back-flush. The shorter column length (10 m) results in a reduced backflushing time compared to the 15-m column.

The 4x method can be run with a 240 V oven without the pillow accessory. The pillow was used here because it somewhat decreases the cooldown time of the oven and reduces the amount of electricity consumed by the instrument.

Further reduction in the cycle time of the instrument was achieved by using the overlapped injection setting in the autoinjector. With this feature, the autoinjector prepares the next sample for injection and has the syringe ready while the oven is cooling down from the current injection. This feature can save approximately 1 minute in cycle time, depending on the injection parameters used.

The simultaneous acquisition of SIM, scan, and NPD

Table 2. SIM Groups Used in SIM/Scan Mode

SIM Group (number)	Start Time (min)	Compound	RT (min)	Target (amu)	Q1 (amu)	Q2 (amu)
1	0	Amphetamine	0.900	44	91	65
2	0.97	Methamphetamine	1.050	58	91	65
3	1.5	Methylenedioxymphetamine(MDA)	1.978	136	135	51
4	2.06	Methylenedioxymphetamine(MDMA)	2.147	58	135	77
4		Ecgonine methyl ester	2.222	94	82	96
4		Ethylecgonine	2.223	94	82	96
5	2.52	Meperidine	2.826	246	218	247
6	2.96	Ketamine	3.138	180	182	209
6		Phencyclidine	3.249	243	242	200
6		Tramadol	3.389	58	263	59
7	3.64	Methadone	3.866	72	57	165
7		Dextromethorphan	3.895	271	212	270
8	3.98	Cocaine	4.042	182	82	94
8		Cocaethylene	4.175	196	82	94
9	4.53	Diazepam	4.598	258	286	257
9		Tetrahydrocannabinol	4.666	299	300	231
9		6-Acetyl-morphine	4.773	268	327	328
10	4.85	Oxycodone	4.801	315	230	115
10		Temazepam	4.922	271	273	272
10		Diacetylmorphine	4.992	310	268	327
10		Fentanyl	5.177	245	146	189
11	5.25	Zolpidem	5.332	235	236	219
11		Clonazepam-M (amino-)	5.433	285	258	286
12	5.53	Alprazolam	5.630	308	279	280
12		Zaleplon	5.695	305	263	248
13	5.8	Zopiclone	5.905	112	99	139
13		Lysergide (LSD)	6.000	323	324	222

(all dwell times 5 msec)

data save a substantial amount of time compared to acquiring them in separate runs. The compounds and corresponding SIM groups monitored are listed in Table 2. Because the peaks in the 4x method are relatively narrow, the dwell times for SIM ions were set to 5 milliseconds.

By using the above time-saving steps, the cycle time from injection to injection is 9.6 minutes.

Data Analysis

Based on experience with analyzing 50 blood extracts, a data analysis scheme evolved that incorporated the DRS, SIM and NPD data.

The resulting data review scheme consisted of the following:

- Deconvolution results were generated with DRS and reviewed to determine compounds present. The AMDIS minimum match factor was set to 50. Any compounds with match factors less than 65 or retention time differences greater than 4 seconds were considered suspect (for example, not present unless other data like target/qualifier ratios supported presence). For suspect identifications, the NPD signal was inspected to see if there was a corresponding response of the same peak shape and retention time. If the suspect compound is nitrogen containing (as the vast majority of the compounds in the table are), NPD response provided evidence supporting the presence of the compound.
- Compounds identified by AMDIS but not found by the MSD ChemStation because of out-of-range qualifiers were manually inspected in QEdit. Quantitation was forced if AMDIS indicated an acceptable spectral and retention time match.
- A separate ChemStation data analysis method was used to review the SIM results for the 27 compounds listed in Table 2. Since SIM can detect compounds lower than can be confirmed with spectral data, identification relied on target/qualifier ion ratios and NPD data.
- The NPD trace was examined to find any larger peaks that did not correspond to identified targets. The deconvoluted spectra at the retention time of these peaks were searched against the NIST 05a library. As a practical matter, uncorrelated small NPD peaks were not pursued as they are numerous and the signal-to-noise ratio of the corresponding scan data is too small to be useful.

Except where otherwise indicated, the 4x method supplied with the ions optimized against column bleed was used for ChemStation data analysis. The approximate response factors supplied with the method were adjusted using a standard of 5 ng/ μ L of proadifen (the locking compound). The responses of all compounds in the quant database were multiplied by the factor required to make the calculated result for the proadifen standard equal 5 ng/ μ L. This allows the concentration of an identified target to be estimated if the compound has not been individually calibrated.

The approximate response factors supplied with the method are only intended to give a rough estimate of the concentration of uncalibrated analytes. Since valid quantitation requires recent recalibration of response factors on the specific instrument used for analysis, the estimated concentration should never be used to report quantitative results. The error in these values can easily be a factor of 10 or higher. The purpose of the estimated values is to give an approximate amount that can be used to guide standard preparation for quantitative calibration of the compound, if needed. Individual calibration should be used for all reported analytes.

The SIM data analysis method for the 27 compounds was constructed using the target and first two qualifier ions from the 4x fatty acid optimized method. This was to minimize interference from the matrix in the blood samples.

The peak recognition windows used in the MSD ChemStation were set to ± 0.150 minute for the scan data, ± 0.075 for the SIM data, and ± 6 seconds in AMDIS. These values were found to be sufficiently wide enough to allow for some retention time drift, yet narrow enough to minimize the number of false positives.

For comparison purposes, the data were also analyzed with two conventional data review approaches.

The first approach is the standard quantitation software, where the EIC of the target ion for each compound in the quant database is extracted and integrated. If a peak is detected within the peak recognition time window, the ratios of the qualifiers to the target are measured. Several optional forms of reporting are available. The reports used here were 1) report only compounds with a peak detected in the target ion EIC and that have all qualifiers within the acceptable range for ratios, and 2) report all compounds with a peak detected in the target ion EIC, regardless of qualifier status. The results of a report can then be reviewed in QEdit, where the EICs of the extracted target and qualifier

ions are overlaid for ease of inspection. The reference spectrum for the compound and the apex spectrum for the quant peak being examined are also displayed. Based on inspection of the EICs and spectra, the reviewer can include or exclude the compound from the report.

The second data review approach was to use the ChemStation Screener software. This is almost identical to QEdit, except that it also reports a cross-correlation value (XCR) of the apex spectrum for peak versus the reference library. The XCR value is an indication of spectral match quality and can be used as an additional parameter with which to locate targets. Screener has report options similar QEdit, and the same two types were used here. Note that Screener is a qualitative tool; compounds identified in Screener must then be quantified in QEdit.

Samples

Whole blood extracts prepared for GC/MS analysis were supplied by NMS Labs (Willow Grove, PA). The whole blood was prepared with a single step liquid/liquid extraction into a solvent, evaporated to dryness, and reconstituted in toluene at 1/10th volume.

Results and Discussion

Figure 2A shows the chromatographic results from one of the blood extracts, the simultaneously acquired scan, SIM, and NPD signals. The traces make the sample look deceptively simple. Figure 2B shows the same Scan TIC and NPD signals with the scales expanded. More than 400 individual compounds are in these chromatograms when low-level responses are included.

The data from the sample were reviewed with the conventional approaches. The first report with the standard quantitation software listed compounds where all qualifier-to-target ratios were within the rather generous 50% relative limits used here. Without manual review of the 28 compounds reported, 22 were false positives; that is, they were not really present. Of the 11 target compounds actually in the sample, this report only found six of them, leaving five as false negatives.

As this situation is not uncommon, it is usually necessary to have all compounds reported that have a response at the target ion, regardless of the qualifier ratio status. These “maybes” must then be manually reviewed in QEdit. Since the integrator must be set to capture very small peaks, there are large numbers of responses due to integration of baseline

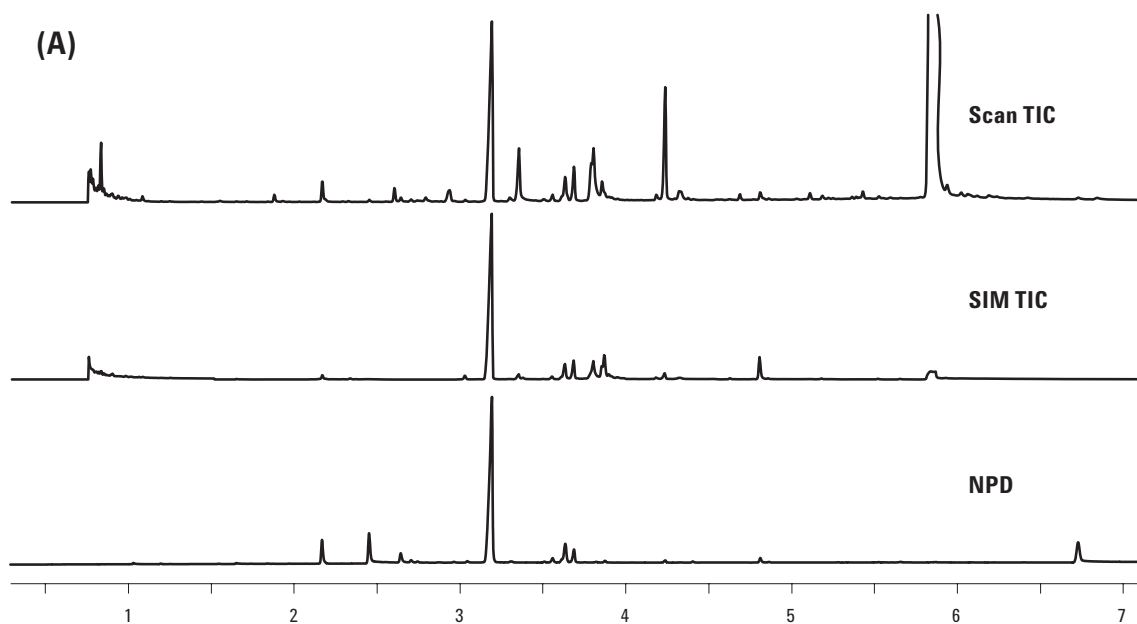


Figure 2A. Chromatograms of scan, SIM, and NPD signals from analysis of blood extract.

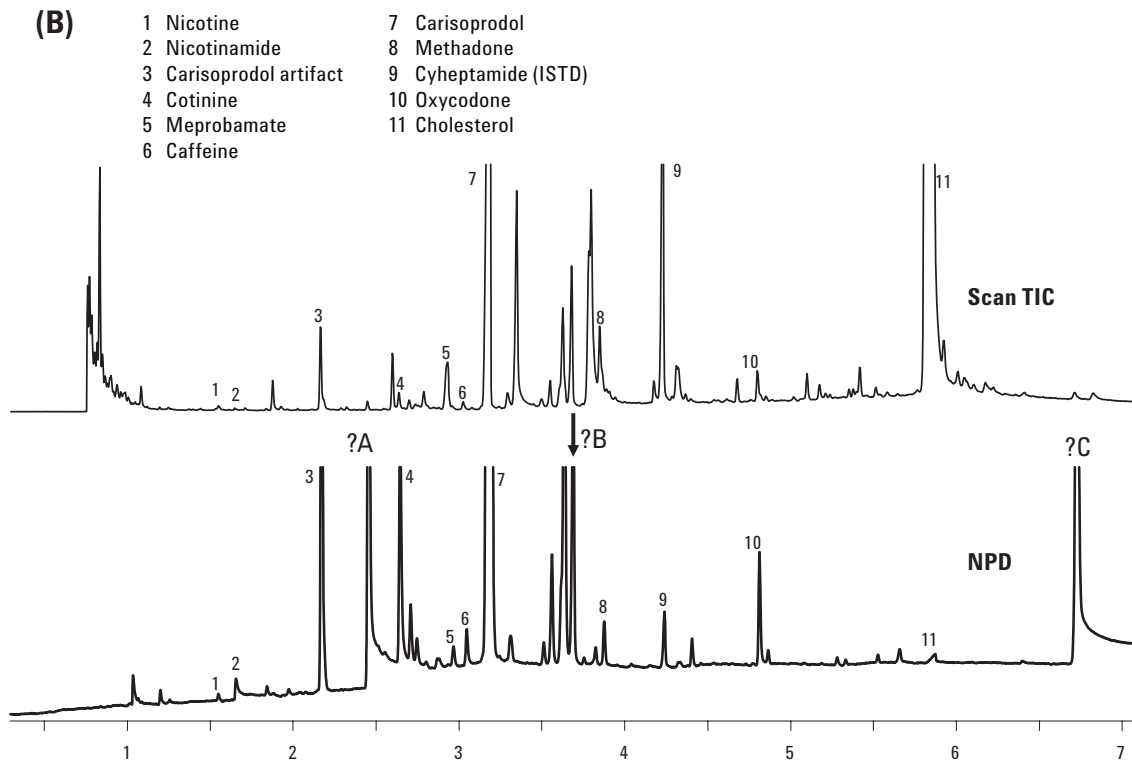


Figure 2B. Expanded scale chromatograms of scan TIC and NPD signals from analysis of blood extract. (continued)

noise. For the sample here, 367 compounds were reported found (that is, there was a response at the target ion). Of those, 356 were false positives. All 11 compounds actually present were found, so there were no false negatives. Thus, to avoid false negatives, the reviewer must manually evaluate 367 compounds to find the 11 present.

The data from the sample were then evaluated with the ChemStation Screener software. As expected, Screener reports based only on ion target/qualifier ion ratios gave very similar results to QEdit. The only way to avoid false negatives is to evaluate hundreds of target ion responses to find the 11 actually present.

In an attempt to reduce the number of false positives requiring evaluation, the Screener report listing all 273 compounds with a target ion response was sorted by the XCR in descending order. Several of the compounds actually present were clustered near the top of the list. However, the target actually present with the lowest XCR value was the 162nd compound in the list. This result suggests that XCR improves the likelihood of correctly locating target compounds, but will still result in false negatives

without close inspection of all of the compounds with a target ion response.

For the types of samples discussed here, correctly identifying the targets present with the conventional approach is one of the most time-consuming steps in the entire analytical process. This is why the use of deconvolution and DRS is so useful.

When this same sample was evaluated with the DRS software, 12 compounds were reported by AMDIS with a match factor for the deconvoluted spectrum greater than 50 and with retention times within 6 seconds of the locked retention time. After reviewing the 12 listed compounds, one was removed because its match factor was too low. All 11 compounds actually present were identified, with only one false positive included. The entire DRS and review process to correctly locate the targets actually present required about 5 minutes instead of more than an hour using either the QEdit target only or Screener approaches. With the compounds present in the sample identified by DRS, the final report was generated after using QEdit to quantify the targets.

MSD Deconvolution Report

Sample Name: CA5995

Date File: C:\msdchem\1\Apnote\FT5_4 x 10m_SamplesSimScan\CA5995_mss.D

Date/Time: 11:39 AM Wednesday, Apr 2 2008

The NIST library was not searched for the compounds that were found in the AMDIS target library.

R.T.	CAS #	Compound Name	Agilent	AMDIS	
			ChemStation Amount (~ng)	Match	R.T. Diff. sec.
1.539	54115	Nicotine	0.03	59	-0.5
1.6446	98920	Nicotinamide	0.27	93	-0.9
2.1631	999401024	Carisoprodol artifact	64.87	93	-0.5
2.6367	486566	Cotinine	1	96	-0.4
2.928	57534	Meprobamate	4.11	99	0.0
3.033	58082	Caffeine	0.04	82	-0.5
3.1832	78444	Carisoprodol	127.4	96	1.0
3.8653	76993	Methadone	0.39	74	-0.1
4.2279	7199293	Cyheptamide	22.5	98	0.1
4.8014	76426	Oxycodone	2.37	82	0.0
5.850	57885	Cholesterol	922.73	97	3.4

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

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

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 that 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 integrated) and all three qualifier ions are present in ratios that fall within the percent uncertainty values for that compound. The compound would also appear here if the data reviewer manually forced integration of the target ion.

The match value listed under the AMDIS column is the degree to which the extracted (deconvoluted) spectrum of the peak at that RT matched the spectrum in the AMDIS target library. The higher this number (out of a possible 100), 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.

An optional third feature of the report is the NIST search column (not shown). The NIST column lists the reverse match quality of the extracted spectrum compared with the NIST main library spectrum with the same CAS#. With the present setup, there are a large number of compounds for which a CAS# is not available. The Forensic Toxicology DBL contains some contrived CAS#s that would not be found in the NIST library. In the present analysis, the NIST search feature is therefore turned off.

Also shown in the NPD trace in Figure 2B are three peaks labeled ?A, ?B, and ?C. These three relatively large peaks are not in the target list of 725 compounds. The deconvoluted spectra corresponding to each of the three NPD responses were found in AMDIS and searched against the main NIST library. Peak ?A was identified as tributyl phosphate, a phosphorus compound commonly found as a sample handling artifact. Peak ?B was identified as 10,11-dihydrodibenz(b,f)(1,4)oxazepin-11-one. It was later found to be a second internal standard added during sample preparation. Peak ?C remains unidentified. It is not in the NIST 05a Library (the best hit was only a 38 match) and it appears in many samples.

It is instructive to go through the identification of some of the compounds in the report and look at

the details of the identifications made. Oxycodone was readily identified because it had a high match quality in the AMDIS column and a very small retention time difference. Figure 4A shows the extracted ion chromatograms (EIC) as seen in QEdit. All the ions are clearly visible without interference and the ratios of the qualifier ions to the target are within the acceptable range. Also shown are the SIM ion EICs. They also are clearly visible without interference and the ratios of the qualifier ions to the target are within the acceptable range. The bottom trace from the NPD in Figure 4A

shows a response with the same shape and at the same time as the oxycodone response in the mass traces. Figure 4B compares the deconvoluted spectrum found at the oxycodone retention time with the target library reference spectrum of oxycodone. The match is very good, with a match factor of 82. Oxycodone was an easy identification with all parameters clearly pointing to its presence.

Figure 5 shows a situation that is a bit more challenging. The compound here is methadone, whose spectrum has one large ion at 72; the remaining ones are very small. The EICs in Figure 5A are from

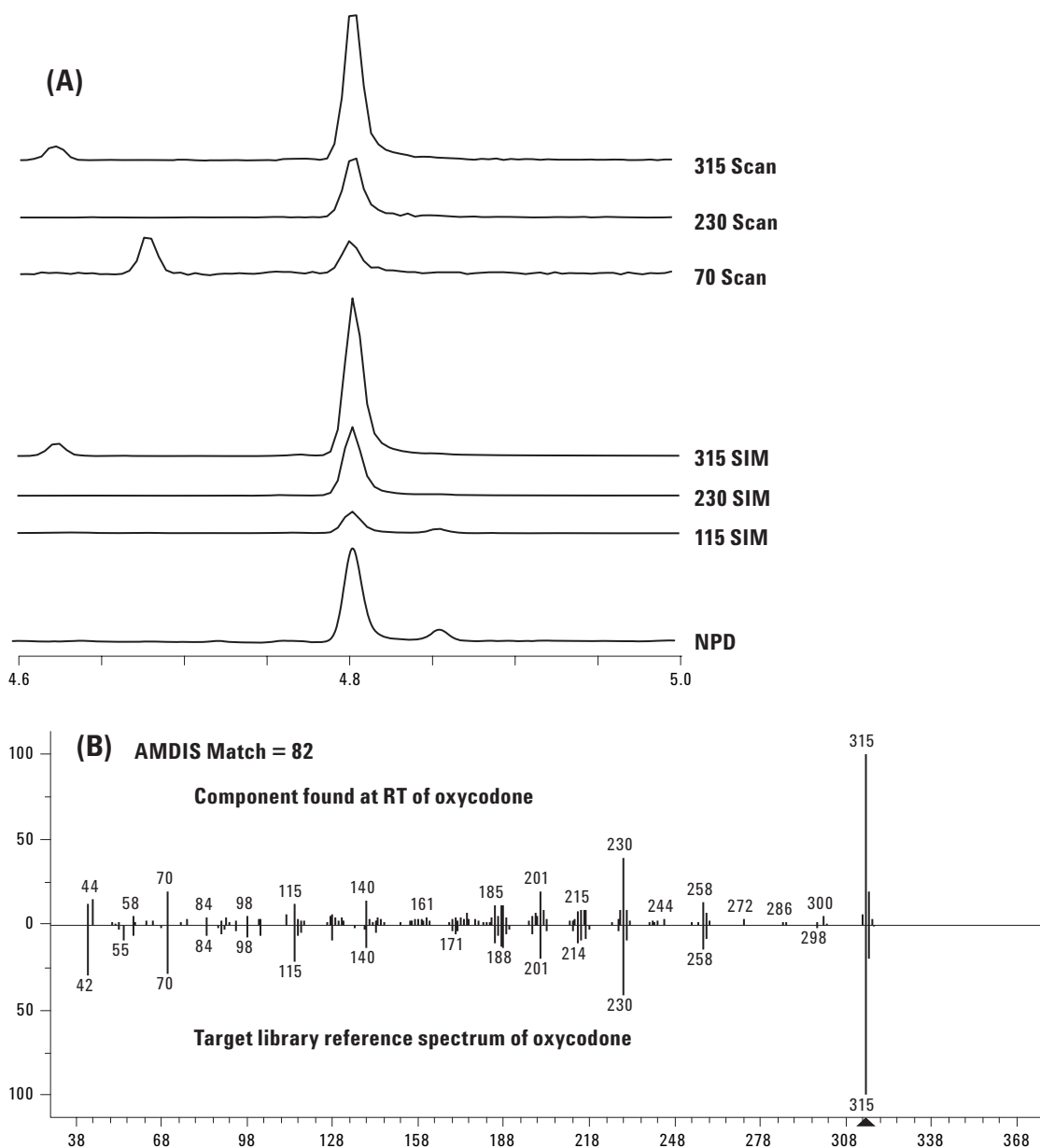


Figure 4. (A) Oxycodone response in SIM, scan, and NPD signals collected simultaneously. (B) Comparison of deconvoluted oxycodone spectrum with target library reference spectrum.

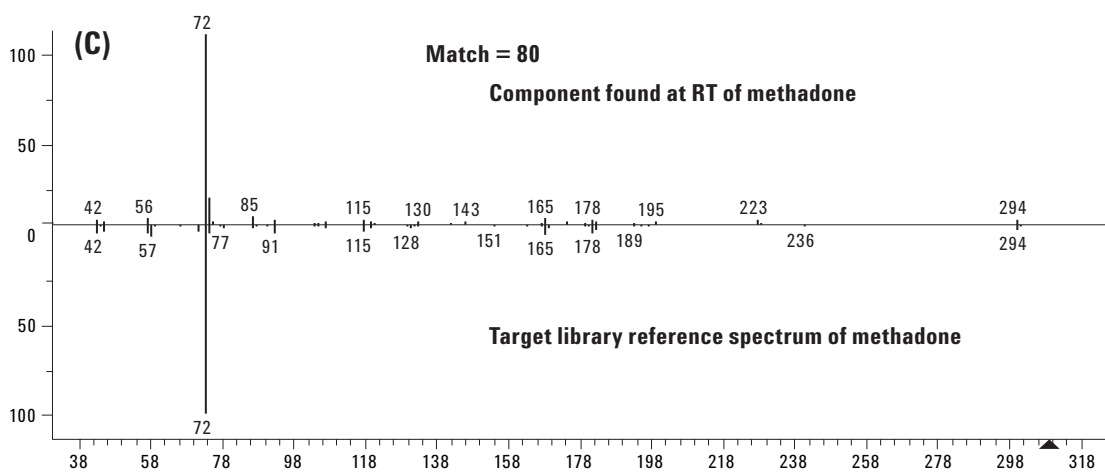
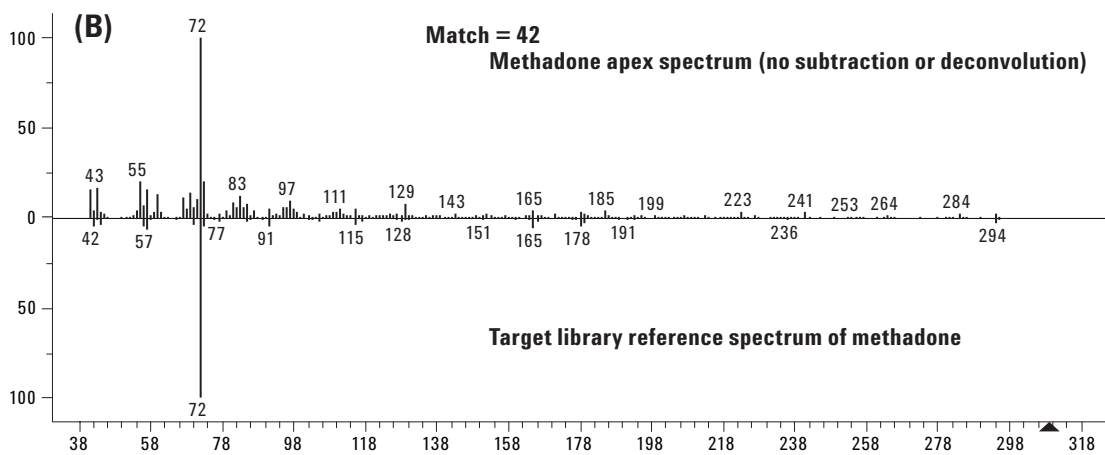
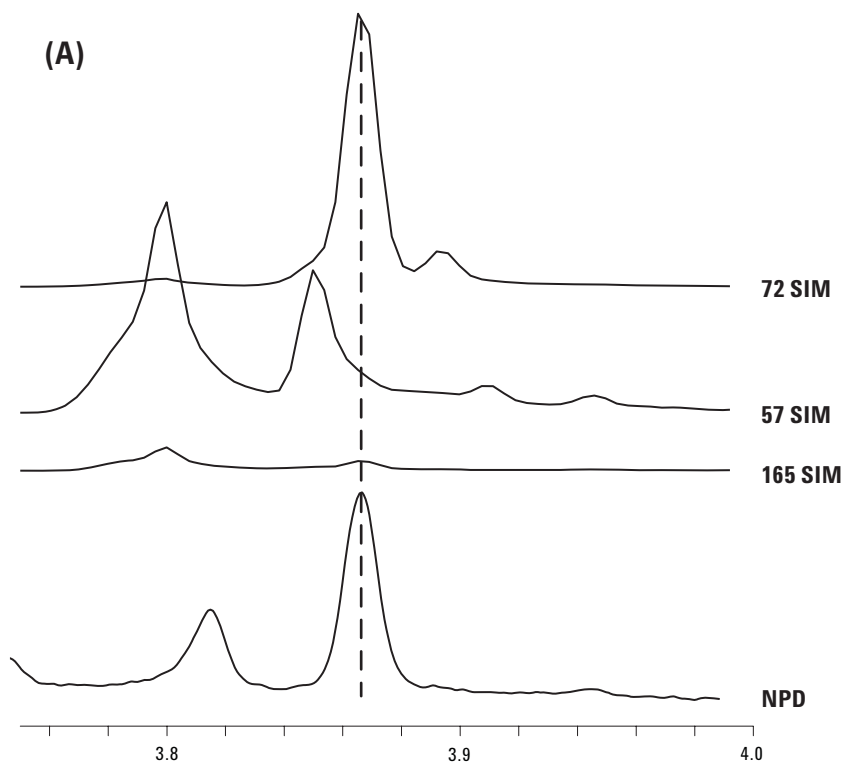


Figure 5. (A) Methadone SIM and NPD chromatograms.
(B) Comparison of reference spectrum with methadone spectrum without subtraction or deconvolution.
(C) Methadone deconvoluted spectrum searched against target library.

the SIM data. The traces from the scan data were identical (except of course with a lower signal-to-noise ratio). While there is a clear peak at the target ion, the middle qualifier (57) has a significant interference from the overlapping octadecanoic acid peak. With only the EIC data, the identification is questionable due to the loss of one of the qualifiers to interference. The NPD response shown below the SIM traces does support the fact that there is a nitrogen-containing compound at that retention time.

Figure 5B shows the apex spectrum at the methadone peak without subtraction or deconvolution compared with the target library reference spectrum. The match quality is unacceptably poor

at 42 due to the interference of the octadecanoic acid peak. While the 72 ion is clearly visible, the other methadone ions are obscured. In Figure 5C the deconvoluted spectrum from the methadone retention time is compared with the reference. Deconvolution successfully removed the octadecanoic acid interference, and now the match quality is 80, clearly indicating the presence of methadone in the sample. The indication of methadone is also supported by two of the three ions being clearly present and in the correct ratio as well as an NPD response with the same retention time and peak shape.

Although caffeine is not a particularly high-priority target compound, the example shown in Figure 6 is

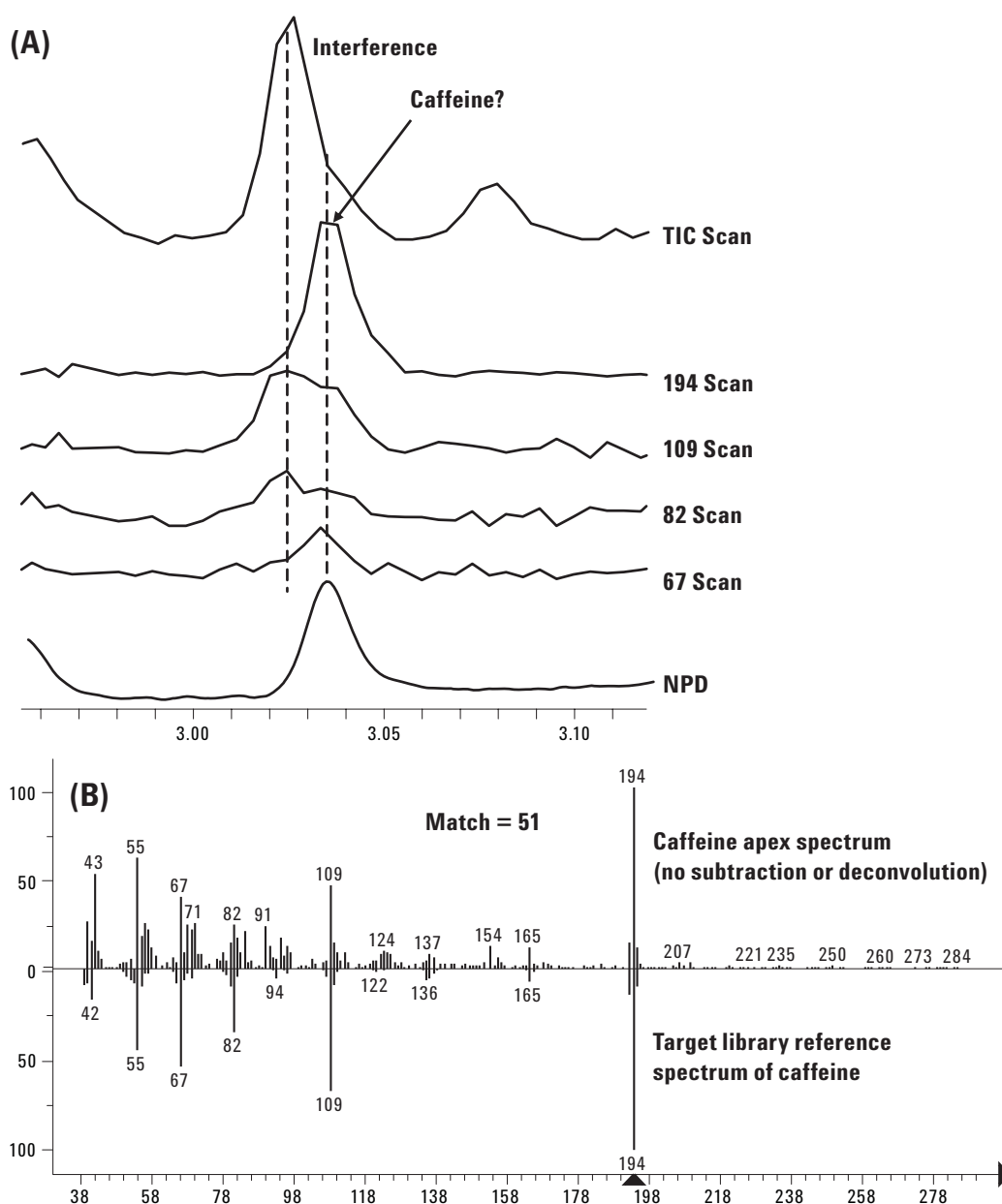


Figure 6. (A) TIC, scan EICs, and NPD signals for caffeine. (B) Caffeine spectrum without subtraction or deconvolution shows interference from matrix compound.

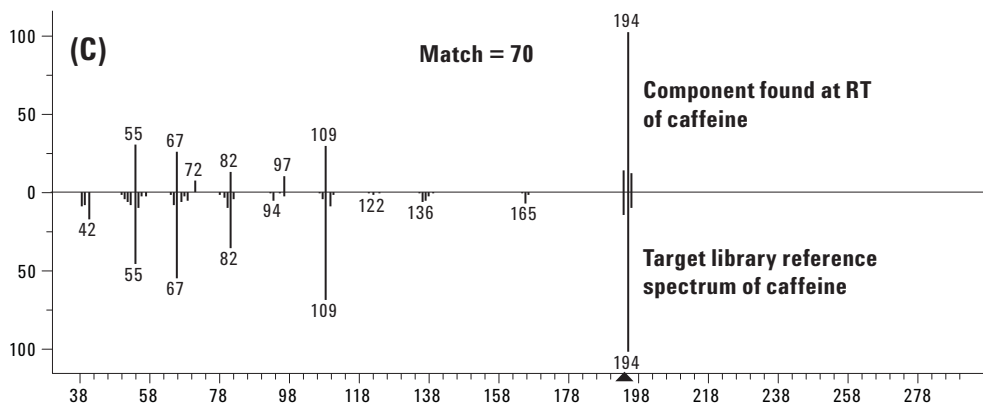


Figure 6C Caffeine deconvoluted spectrum searched against target library. (continued)

instructive. The caffeine, if present, is at a very low level as seen from the low signal-to-noise ratio of the four scan EICs shown in Figure 6A. Two ions, 109 and 82, also have interference problems from a large overlapping peak, as shown in the TIC trace at the top. The NPD trace does indicate a nitrogen-containing compound with the same peak shape and retention time as caffeine. The interfering peak was identified as 6,10,14-trimethyl-2-pentadecanone by searching the deconvoluted spectrum against the NIST main library. This compound also shares ions 109 and 82 with caffeine, resulting in the interference.

Figure 6B shows the apex spectrum of the caffeine peak without subtraction or deconvolution. When compared to the reference spectrum of caffeine, the match quality is poor, at only 51. Figure 6C shows the deconvoluted spectrum at the caffeine retention time compared to the reference spectrum and now the match quality is significantly improved to 70. This example demonstrates that the deconvolution process works even on small peaks with a low signal-to-noise ratio.

The example in Figure 7 is taken from a different sample and its purpose is to show the limits of deconvolution compared to the limits of the conventional approach. They are in fact similar because both approaches are limited by the same thing: signal-to-noise ratio. Figure 7A shows the scan and SIM EICs and the NPD trace for alprazolam. In the scan data, three of the four ions are barely visible and the fourth is lost in the noise. The SIM data clearly show a peak present at the alprazolam retention time and the ratios are in the correct range. The NPD also shows a response at the same retention time and with a similar shape. Figure 7B shows

the deconvoluted spectrum compared to the NIST 05a library spectrum of alprazolam. The match factor is only 57.5. The match is marginal because AMDIS could only find a fraction of the alprazolam ions due to the extremely low level of the compound. This again illustrates that the target/qualifier approach using scan data and deconvolution begin to fail at about the same signal-to-noise ratio. In this example, the SIM data and NPD data are very helpful. If only the scan data were available for this sample, the identification of alprazolam would be doubtful and probably not reported. Taken with the SIM data in the correct ratios and the supporting evidence of the NPD response, a much stronger case can be made that alprazolam is indeed present, although at a very low level.

The last example is from a sample containing extraordinarily high levels of fatty acid interferences. These are clearly visible in Figure 8A. In QEdit, the presence of meprobamate was indicated with the peak shown at 3.007 minutes in Figure 8B. Although the ratios of the qualifiers to the target ion were within the relatively wide windows used here, the identification was doubtful. Examination of the EICs shows what looks like multiple peaks at the retention time that QEdit found. The retention time was also farther away (+ 0.080 minute) from the expected retention time of 2.928 minutes than is typically seen with the method. Also, there is no clear peak shape evident in the four traces at the 3.007 retention time. Based on these results alone, meprobamate looks like a false positive.

The EIC traces shown were from the column bleed optimized method. The use of 83 as the target ion clearly has interference problems with the high-level of fatty acids in this sample. When the method with

fatty acid optimized ions was used, the picture became a bit clearer. In this method, ion 62 is used as the target because of its significantly lower degree of interference. Looking at the trace for ion 62 in Figure 8, the peak now appears at 2.948 and is much closer to the expected retention time at 2.928 minutes. While the response at ion 62 looks a bit more like a real peak, the other ions in the fatty acid optimized method were still questionable due to the degree of interference, suggesting that it still may be a false positive. The NPD trace (not shown) did not resolve the question, as there were NPD peaks near 2.928 and 3.007 minutes.

The question was easily settled using the new A.04 release of DRS software. This version allows you to import into QEdit the AMDIS extracted peak profile from the deconvolution data and overlay it with the QEdit EICs. It also imports the deconvoluted spectrum for comparison with the QEdit-subtracted spectrum and the library reference spectrum. These capabilities simplify the review process by showing the deconvolution information inside of QEdit. Inspection of the AMDIS extracted peak profile relative to the EICs of the scan data shows that in fact the response at the target found with the fatty acid optimized method is indeed meprobamate. The

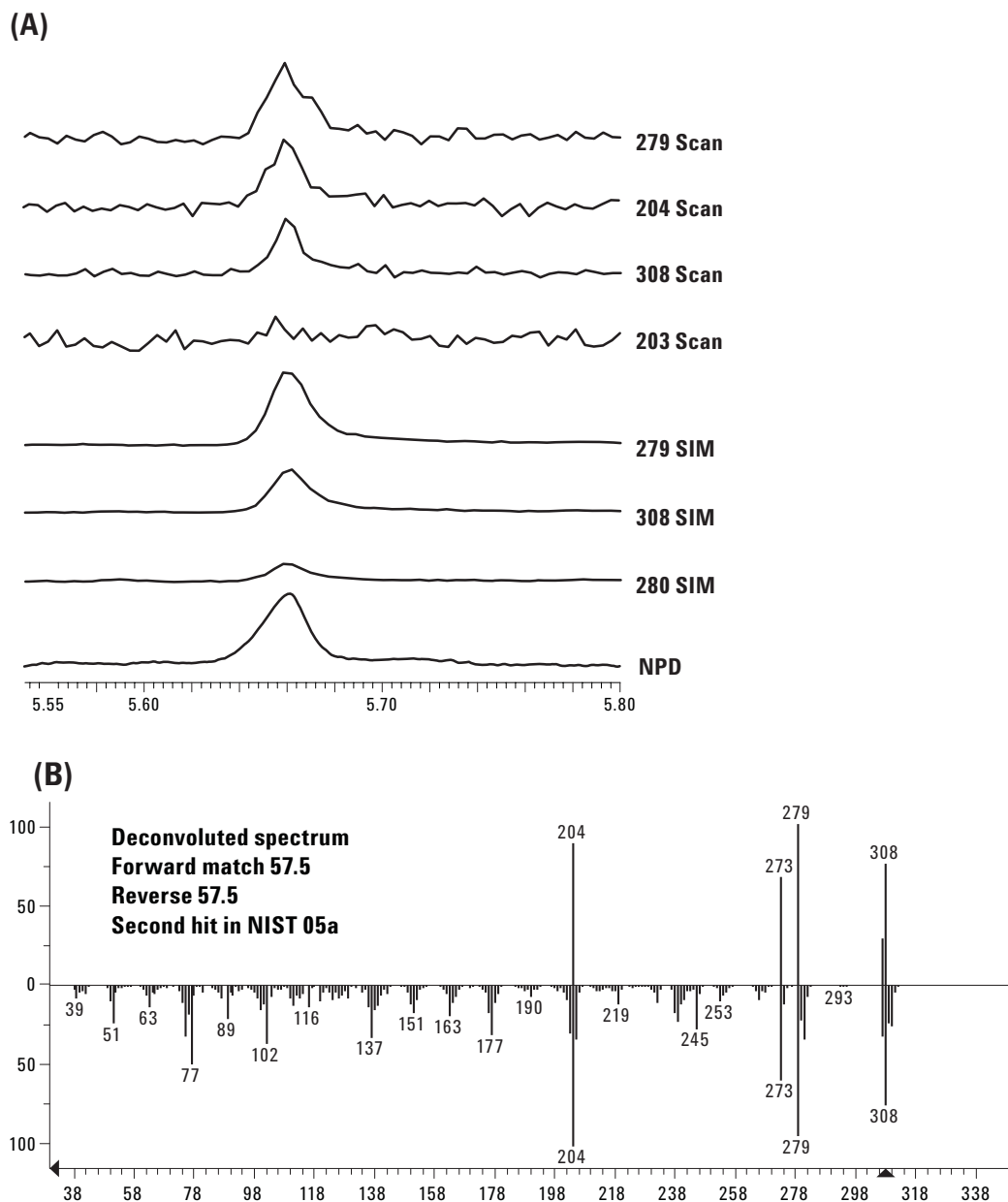


Figure 7. (A) Alprazolam response on SIM, scan, and NPD signals. (B) Alprazolam deconvoluted spectrum searched against NIST 05a library.

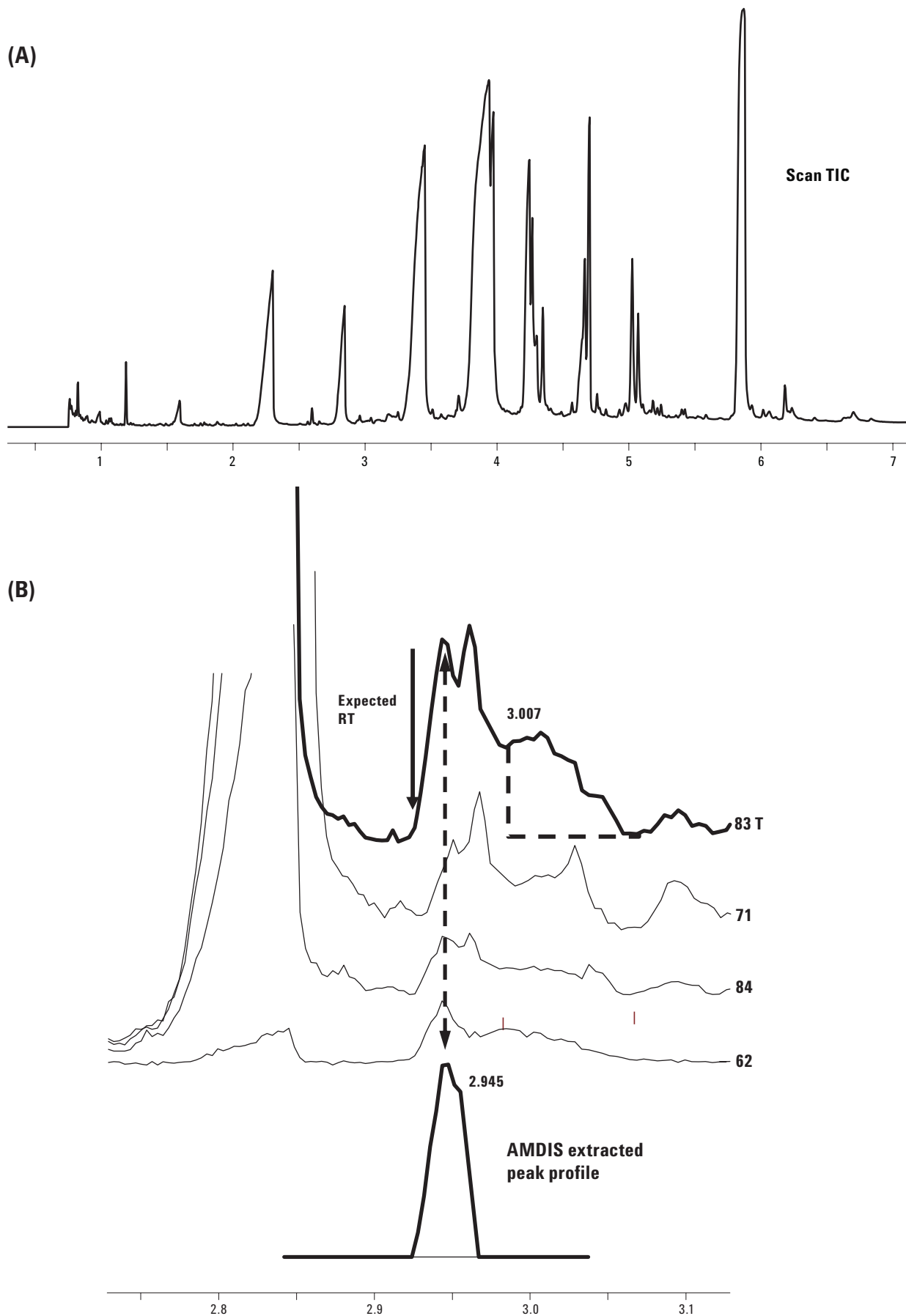


Figure 8. (A) Scan TIC chromatogram of sample with high levels of fatty acids.
 (B) Scan EICs from bleed optimized method overlaid with AMDIS extracted peak profile.

(C)

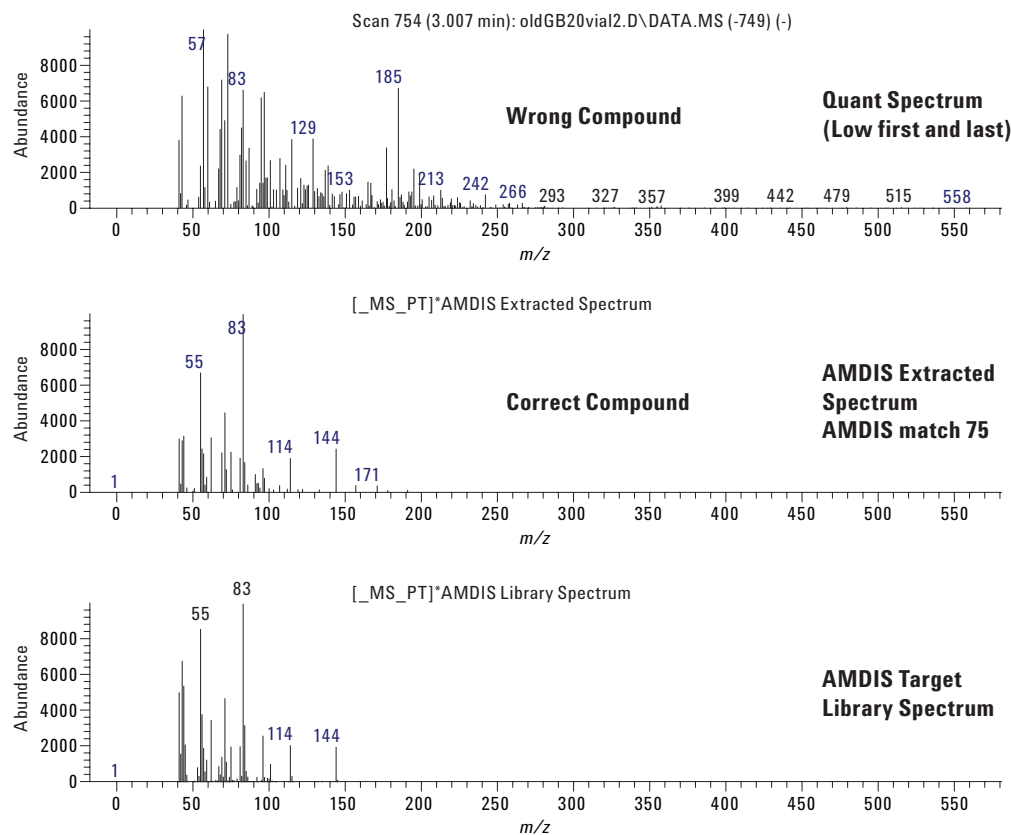


Figure 8C. Three meprobamate spectra presented in QEdit for comparison during data review using DRS A.04. (continued)

AMDIS extracted peak profile looks very similar to the peak profile in ion 62. If desired, the AMDIS extracted peak profile can be integrated for quantitation if the target ion has interference problems.

The best confirmation is provided by the deconvoluted spectrum. In Figure 8C are the three spectra presented in QEdit for comparison. The three spectra shown here were from the bleed optimized method. This method had incorrectly chosen the 3.007 peak as possibly being meprobamate, where the topmost spectrum is the spectrum at 3.007 minutes minus the spectrum five scans before, as the method uses “lowest first and last” as the subtraction method. Since the peak was found at the wrong retention time, the spectrum is of the wrong compound and of course does not match that of meprobamate. When searched against the NIST main library, meprobamate was not in the top 100 hits.

The middle spectrum is the deconvoluted component found by AMDIS. It has a match factor against the reference spectrum, shown in the bottom, of 75, confirming the presence of meprobamate. This example shows the utility of deconvolution in determining the presence of compounds that could easily be missed with the conventional approaches.

Conclusions

The system described here offers several advantages for screening toxicology samples. The advantages derive from a combination of techniques that result in both faster and more accurate screening results.

- Retention time locked target database of 725 compounds for screening with MS (G1674AA Forensic Toxicology DBL)

- CFT splitter – Use the NPD with MS data for added confirmation, find nontarget 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 increases accuracy of target identification, even in the most challenging matrices. The reduction of data interpretation from more than an hour to less than 10 minutes is especially useful.
 - Fast chromatography using shorter columns, faster ovens, and backflushing to greatly reduce run times.
4. Philip Wylie, Michael Szelewski, Chin-Kai Meng, and Christopher Sandy, “Comprehensive Pesticide Screening by GC/MSD Using Deconvolution Reporting Software,” Agilent Technologies publication 5989-1157EN
 5. B. D. Quimby, L. M. Blumberg, M. S. Klee, and P. L. Wylie, “Precise Time-Scaling of Gas Chromatographic Methods Using Method Translation and Retention Time Locking,” Agilent Technologies publication 5967-5820E
 6. Michael J. Szelewski and Bruce Quimby, “New Tools for Rapid Pesticide Analysis in High Matrix Samples,” Agilent Technologies publication 5989-1716EN
 7. Bruce D. Quimby and Michael J. Szelewski, “Screening for Hazardous Chemicals in Homeland Security and Environmental Samples Using a GC/MS/ECD/FPD with a 731 Compound DRS Database,” Agilent Technologies publication 5989-4834EN

There is considerable advantage to using a single system that combines all of the techniques discussed. However, adding any of the above separately or in different combinations can also provide advantages. The most significant improvement can be gained by using DRS. The time savings in the data review step easily justifies the effort required to implement it.

References

1. Vince Giarrocco, Bruce Quimby, and Matthew Klee, “Retention Time Locking: Concepts and Applications,” Agilent Technologies publication 5966-2469E
2. Chin Kai-Meng and Bruce Quimby, “Identifying Pesticides with Full Scan, SIM, uECD, and FPD from a Single Injection,” Agilent Technologies publication 5989-3299EN
3. Chin-Kai Meng, “Improving Productivity with Synchronous SIM/Scan,” Agilent Technologies publication 5989-3108EN

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Appendix

Compound name	CAS number*	Compound name	CAS number
10,11-Dihydro-10-hydroxycarbazepine	999402-02-7	Ampyrone-2AC	999240-02-7
10,11-Dihydro-10-hydroxycarbazepine TMS	999423-02-8	Anhydroecgonine methyl ester	043021-26-7
10,11-Dihydrocarbamazepin	003564-73-6	Anileridine	000144-14-9
5-Amino-2-chloropyridine	005350-93-6	Anisindione	000117-37-3
5-Methoxy-dipropyltryptamine	999001-02-4	Antazoline	000091-75-8
6-Acetyl-morphine	002784-73-8	Antazoline AC	999408-02-5
6-Acetyl-morphine TMS	999155-02-1	Antipyrine	000060-80-0
7-Aminoflunitrazepam	034084-50-9	Apomorphine 2TMS	074841-68-2
7-Aminoflunitrazepam TMS	999176-02-2	Aprobarbital	000077-02-1
7-Hydroxyamoxapine	037081-76-8	Aprobarbital 2TMS	999180-02-8
8-Methoxyloxapine	070020-54-1	Atenolol formyl artifact	999459-02-8
Acepromazine	000061-00-7	Atomoxetine	083015-26-3
Acetaminophen	000103-90-2	Atomoxetine AC	999257-02-2
Acetaminophen 2TMS	055530-61-5	Atovaquone	953233-18-4
Acetanilide	000103-84-4	Atovaquone TMS	999409-02-8
Adiphenine	000064-95-9	Atropine	000051-55-8
Adiphenine-M/artifact (ME)	003469-00-9	Atropine TMS	055334-03-7
Alfentanil	071195-58-9	Azacyclonol	000115-46-8
Allobarbital	000052-43-7	Azatadine	003964-81-6
Allopurinol TMS	999178-02-8	Barbital	000057-44-3
Alphaprodine	000077-20-3	BDMPEA	066142-81-2
Alphenal	000115-43-5	BDMPEA AC	999357-02-7
Alprazolam	028981-97-7	BDMPEA formyl artifact	999378-02-8
Alprenolol TMS	999381-02-1	Bemegride	000064-65-3
Alverine	000150-59-4	Benzocaine	000094-09-7
Amantadine	000768-94-5	Benzoyllecgonine	000519-09-5
Amantadine AC	999127-02-5	Benzoyllecgonine TMS	999462-02-1
Ambroxol	018683-91-5	Benzphetamine	000156-08-1
Ambroxol 2AC	999341-02-5	Benzquinamide	000063-12-7
Aminoglutethimide	000125-84-8	Benztropine	000086-13-5
Aminopyrine	000058-15-1	Benzylamine	000642-72-8
Amitriptyline	000050-48-6	Benzylpiperazine	002759-28-6
Amlodipine AC	999299-02-4	Benzylpiperazine AC	999129-02-1
Amobarbital	000057-43-2	Betahistine	005579-84-0
Amobarbital 2TMS	999179-02-1	Betahistine AC	999439-02-0
Amoxapine	014028-44-5	Betaxolol	063659-18-7
Amoxapine AC	999128-02-8	Betaxolol formyl artifact	999436-02-1
Amphetamine	000060-15-1	Biperiden	000514-65-8
Amphetamine AC	999107-02-7	Bisacodyl	000603-50-9
Ampyrone	000083-07-8	Bisoprolol	066722-44-9
Ampyrone AC	000083-15-8	Bromazepam	001812-30-2

* Compounds for which a real CAS number could not be found were given a contrived one beginning with 999. These are not real CAS numbers.

Compound name	CAS number	Compound name	CAS number
Bromazepam TMS	999158-02-0	Chlormezanone artifact	999245-02-2
Bromdiphenhydramine	000118-23-0	Chloroamphetamine	000064-12-0
Bromocriptine breakdown	025614-03-3	Chloroamphetamine AC	999414-02-7
Bromperidol	010457-90-6	Chlorophenylpiperazine	038212-33-8
Brompheniramine	000086-22-6	Chlorophenylpiperazine AC	999486-02-1
Brucine	000357-57-3	Chloroprocaine, 2-	000133-16-4
Buclizine	000082-95-1	Chloroquine	000054-05-7
Bupivacaine	002180-92-9	Chlorpheniramine	000132-22-9
Buprenorphine	052485-79-7	Chlorphenisn	000104-29-0
Buprenorphine TMS	999159-02-3	Chlorphentermine	000461-78-9
Bupropion	034911-55-2	Chlorphentermine AC	999130-02-8
Buspirone	036505-84-7	Chlorpropamide artifact-2	999246-02-5
Butabarbital	000125-40-6	Chlorprothixene	000113-59-7
Butabarbital 2TMS	052988-92-8	Chlorzoxazone	000095-25-0
Butacaine	000149-16-6	Cholesterol	000057-88-5
Butalbital	000077-26-9	Cholesterol TMS	001856-05-9
Butalbital 2TMS	052937-70-9	Cinnarizine	000298-57-7
Butethal	000077-28-1	Cisapride	081098-60-4
Butorphanol	042408-82-2	Citalopram	059729-33-8
Butorphanol TMS	100013-72-3	Clemastine	015686-51-8
Caffeine	000058-08-2	Clemizole	000442-52-4
Canrenone	000976-71-6	Clenbuterol	037148-27-9
Canrenone TMS	999413-02-4	Clenbuterol AC	999360-02-0
Cantharidin	000056-25-7	Clobazam	022316-47-8
Carbamazepine	000298-46-4	Clofibrate	000637-07-0
Carbamazepine-M (formyl-acridine)	999243-02-6	Clomipramine	000303-49-1
Carbinoxamine	000486-16-8	Clonazepam	001622-61-3
Carbromal-M/artifact	999196-02-0	Clonazepam TMS	999184-02-0
Carisoprodol	000078-44-4	Clonazepam-M (amino-)	004959-17-5
Carisoprodol artifact	999401-02-4	Clonazepam-M (amino) - TMS	999175-02-9
Cathinone AC	999485-02-8	Clonidine	004205-90-7
Celecoxib	169590-42-5	Clonidine 2AC	999131-02-1
Cetirizine methanol adduct	083881-46-3	Clonidine AC	999132-02-4
Cetirizine TMS	999183-02-7	Clopidogrel	113665-84-2
Chlophedianol	000791-35-5	Clozapine	005786-21-0
Chlophedianol TMS	999464-02-7	Clozapine AC	999133-02-7
Chloramphenicol 2TMS	021196-84-9	Cocaethylene	000529-38-4
Chlorcyclizine	000082-93-9	Cocaine	000050-36-2
Chlordiazepoxide	000058-25-3	Codeine	000076-57-3
Chlordiazepoxide artifact (desoxo)	999197-02-3	Codeine TMS	074367-14-9
Chlormezanone	000080-77-3	Colchicine	000064-86-8

Compound name	CAS number	Compound name	CAS number
Colchicine breakdown	999532-02-4	Diethyltryptamine	000061-51-8
Coniine	000458-88-8	Dihydrocodeine	000125-28-0
Coniine AC	999361-02-3	Dihydroxy-4-methylcoumarin, 7, 8 - TMS	999236-02-1
Cotinine	000486-56-6	Diiodohydroxyquin	000083-73-8
Cyclandelate	000456-59-7	Diltiazem	042399-41-7
Cyclandelate TMS	999442-02-3	Dimethadione	000695-53-4
Cyclizine	000082-92-8	Diphenadione	000082-66-6
Cyclobenzaprine	000303-53-7	Diphenhydramine	000058-73-1
Cyclophosphamide	000050-18-0	Diphenidol	000972-02-1
Cyclophosphamide -HCL	999379-02-1	Diphenidol TMS	999417-02-6
Cyheptamide	007199-29-3	Diphenoxylate	000915-30-0
Cyproheptadine	000129-03-3	Diphenylpyraline	000147-20-6
Dapsone	000080-08-0	Disopyramide	003737-09-5
Debrisoquine AC	999415-02-0	Donepezil	120014-06-4
Desalkylflurazepam AC	999298-02-1	Dothiepin	000113-53-1
Desethylidocaine (MegX)	999044-02-9	Doxapram	000309-29-5
Desethylidocaine AC (MegX)	999263-02-4	Doxepin (cis)	999515-02-5
Desipramine	000050-47-5	Doxepin (trans)	001668-19-5
Desipramine AC	999108-02-0	Doxylamine	000469-21-6
Desmethylclomipramine	000303-48-0	Dyphylline	000479-18-5
Desmethylclomipramine AC	999134-02-0	Dyphylline TMS	999446-02-5
Desmethylclozapine	006104-71-8	Ecgonine methyl ester	106293-60-1
Desmethyldoxepin (cis)	999516-02-8	Ecgonine methyl ester TMS	999162-02-6
Desmethyldoxepin (cis) AC	999517-02-1	Efavirenz	154598-52-4
Desmethyldoxepin (trans)	001225-56-5	Efavirenz AC	999489-02-0
Desmethyldoxepin (trans) AC	999443-02-6	Efavirenz TMS	999505-02-1
Desmethylselegiline	999072-02-5	Emetine	000483-18-1
Desmethylselegiline AC	999147-02-3	Encainide	999034-02-5
Desmethylsertraline	091797-58-9	Ephedrine	000299-42-3
Desmethyltramadol, O-	999018-02-9	Ephedrine 2AC	055133-90-9
Desmethyltramadol, O- 2TMS	999444-02-9	Epinephrine AC	999111-02-3
Desmethyltrimipramine	999019-02-2	Ergonovine AC	999447-02-8
Desmethyltrimipramine AC	999445-02-2	Estazolam	029975-16-4
Dextromethorphan	000125-71-3	Ethacrynic Acid TMS	999227-02-0
Diacetylmorphine	000561-27-3	Ethambutol AC	999261-02-8
Diazepam	000439-14-5	Ethamivan	000304-84-7
Dichlorophene	000097-23-4	Ethinamate	000126-52-3
Dichlorophene TMS	999237-02-4	Ethopropazine	000522-00-9
Diclofenac -H2O	999200-02-1	Ethosuximide	000077-67-8
Diclofenac TMS	999222-02-5	Ethotoin	000086-35-1
Dicyclomine	000077-19-0	Ethyl-2-malonamide, 2-	068692-83-1

Compound name	CAS number	Compound name	CAS number
Ethyl-2-malonamide, 2- TMS	999418-02-9	Flurazepam-M (desalkyl-)	002886-65-9
Ethylamphetamine	000457-87-4	Flurazepam-M (HO-ethyl-)	020971-53-3
Ethylamphetamine AC	999148-02-6	Flurbiprofen	005104-49-4
Ethylecgonine	999037-02-4	Flutamide	013311-84-7
Ethylecgonine TMS	999448-02-1	Flutamide TMS	999467-02-6
Ethylmorphine	000076-58-4	Fluvoxamine	054739-18-3
Ethylmorphine TMS	999221-02-2	Fluvoxamine AC	999262-02-1
Etodolac TMS	999212-02-1	Furazolidone	000067-45-8
Etofilline	000519-37-9	Furosemide 2TMS	999214-02-7
Etofilline TMS	077630-35-4	Gemfibrozil	025812-30-0
Etomidate	033125-97-2	Gemfibrozil AC	999389-02-5
Eucatropine Isomer 1	999038-02-7	Glutethimide	000077-21-4
Eucatropine Isomer 1 TMS	999278-02-3	Griseofulvin	000126-07-8
Eucatropine Isomer 2	999277-02-0	Guaifenesin	000093-14-1
Eucatropine Isomer 2 TMS	999518-02-4	Guaifenesin 2TMS	107966-19-8
Felbamate artifact 1	999250-02-1	Guanethidine	000055-65-2
Felbamate artifact 2	999251-02-4	Haloperidol	000052-86-8
Felbamate artifact 3	999252-02-7	Harmaline	000304-21-2
Felodipine	072509-76-3	Harmaline AC	999301-02-9
Felodipine-M/artifact (dehydro-)	999296-02-5	Harmine	000442-51-3
Fenfluramine	000458-24-2	Hexobarbital	000056-29-1
Fenfluramine AC	999139-02-5	Hexobarbital TMS	999469-02-2
Fenoprofen	031879-05-7	Hexylresorcinol	000136-77-6
Fenoprofen TMS	999310-02-0	Hexylresorcinol 3TMS	999422-02-5
Fentanyl	000437-38-7	Homatropine	000087-00-3
Finasteride	098319-26-7	Homatropine TMS	999282-02-9
Flavoxate	015301-69-6	Hydrastine	000118-08-1
Flavoxate-M/artifact (HOOC-) ME	999279-02-6	Hydrocodone	000125-29-1
Flecainide	054143-55-4	Hydromorphone	000466-99-9
Flecainide AC	999140-02-2	Hydromorphone enol 2TMS	999513-02-9
Flumazenil	078755-81-4	Hydromorphone TMS	221209-08-1
Flunarizine	052468-60-7	Hydroxychloroquine AC	999512-02-6
Flunitrazepam	001622-62-4	Hydroxyethylflurazepam TMS	999204-02-3
Fluoxetine	054910-89-3	Hydroxyloxapine, 8-	999053-02-0
Fluoxetine AC	999141-02-5	Hydroxyzine	000068-88-2
Flupenthixol	002709-56-0	Hydroxyzine AC	999113-02-9
Flupenthixol TMS	999387-02-9	Ibuprofen	015687-27-1
Fluphenazine	000069-23-8	Ibuprofen TMS	999165-02-5
Fluphenazine TMS	999280-02-3	Iminostilbene	000256-96-2
Fluphenazine-M (ring)	000092-30-8	Imipramine	000050-49-7
Flurazepam	017617-23-1	Indomethacin TMS	999318-02-4

Compound name	CAS number	Compound name	CAS number
Isocarboxazid	000059-63-2	Memantine	019982-08-2
Isometheptene AC	999265-02-0	Memantine AC	999115-02-5
Isoniazid	000054-85-3	Meperidine	000057-42-1
Isoniazid 2AC	999266-02-3	Mephenesin	000059-47-2
Isoniazid AC	999254-02-3	Mephenesin 2TMS	999325-02-9
Isoproterenol 2TMS	999424-02-1	Mephentermine	000100-92-5
Isoxsuprine	000395-28-8	Mephentermine AC	999143-02-1
Isoxsuprine TMS	999319-02-7	Mephenytoin	000050-12-4
Ketamine	006740-88-1	Mephobarbital	000115-38-8
Ketamine AC	999114-02-2	Mepivacaine	000096-88-8
Ketoprofen TMS	999320-02-4	Meprobamate	000057-53-4
Ketorolac TMS	999215-02-0	Mescaline	000054-04-6
Ketotifen	034580-13-7	Mescaline AC	999511-02-3
Lamotrigine	084057-84-1	Mescaline formyl artifact	999284-02-5
Lamotrigine 2AC	999255-02-6	Mesuximide-M (nor)	001497-17-2
Laudanosine	020412-65-1	Metaproterenol AC	999391-02-5
Levallorphan	000152-02-3	Metaxalone	001665-48-1
Levallorphan TMS	999321-02-7	Metaxalone AC	999116-02-8
Levetiracetam	102767-28-2	Methadone	000076-99-3
Levorphanol	000077-07-6	Methadone-M (EDDP)	999058-02-5
Levorphanol TMS	999223-02-8	Methamphetamine	000537-46-2
Lidocaine	000137-58-6	Methamphetamine AC	999117-02-1
Loratadine	079794-75-5	Methapyrilene	000091-80-5
Lorazepam	000846-49-1	Methaqualone	000072-44-6
Lorazepam 2TMS	999202-02-7	Metharbital	000050-11-3
Lorcainide	059729-31-6	Metharbital TMS	999186-02-6
Lormetazepam	000848-75-9	Methazolamide	000554-57-4
Loxapine	001977-10-2	Methcathinone AC	999300-02-6
Ly170222	999123-02-3	Methcathinone-M (HO-) 2AC	005650-44-2
Lysergide (LSD)	000050-37-3	Methdilazine	001982-37-2
Maprotiline	010262-69-8	Methimazole	000060-56-0
Maprotiline AC	999366-02-8	Methimazole AC	999368-02-4
Mazindol	022232-71-9	Methocarbamol 2TMS	999285-02-8
MBDB	100031-29-2	Methohexital	000151-83-7
MBDB AC	999142-02-8	Methohexital TMS	999425-02-4
Mecamylamine	000060-40-2	Methotrimpeprazine	000060-99-1
Meclizine	000569-65-3	Methoxyverapamil	016662-47-8
Meclofenamic acid TMS	999322-02-0	Methsuximide	000077-41-8
Medazepam	002898-12-6	Methylaminorex, 4-	029493-77-4
Mefenamic acid TMS	999324-02-6	Methylaminorex, 4- 2AC	999508-02-0
Mefloquine	053230-10-7	Methylaminorex, 4- AC	999510-02-0

Compound name	CAS number	Compound name	CAS number
Methylenedioxyamphetamine AC	999479-02-6	Nalorphine	000062-67-9
Methylenedioxyamphetamine (MDA)	004764-17-4	Nalorphine 2TMS	999473-02-8
Methylenedioxyethylamphetamine	014089-52-2	Naloxone	000465-65-6
Methylenedioxyethylamphetamine AC	999481-02-6	Naloxone TMS	999427-02-0
Methylenedioxymethamphetamine AC	999480-02-3	Naltrexol, beta-	999406-20-9
Methylenedioxymethamphetamine (MDMA)	042542-10-9	Naltrexol, beta- 2TMS	999405-02-6
Methylephedrine	000552-79-4	Naltrexol, beta- 3TMS	999520-02-4
Methylephedrine AC	999370-02-4	Naltrexone	016590-41-3
Methyl-nicotine	999065-02-0	Naltrexone 2TMS	999328-02-8
Methylphenidate	000113-45-1	Naltrexone 3TMS	999523-02-3
Methylphenidate AC	999144-02-4	Naltrexone TMS	999522-02-0
Methylphenobarbital	999509-02-3	Naproxen ME	999295-02-2
Methylprimidone	059026-32-3	Naproxen TMS	074793-83-2
Methylprimidone 2TMS	999286-02-1	Nevirapine	129618-40-2
Methypylon	000125-64-4	Nevirapine TMS	999451-02-4
Metoclopramide	000364-62-5	Niclosamide	000050-65-7
Metoclopramide AC	999145-02-7	Nicotinamide	000098-92-0
Metoprolol 2AC	999306-02-4	Nicotine	000054-11-5
Metronidazole	000443-48-1	Nifedipine	021829-25-4
Metronidazole TMS	999450-02-1	Nikethamide	000059-26-7
Mexiletine	031828-71-4	Nimodipine	066085-59-4
Mexiletine AC	999146-02-0	Nimodipine-M/artifact	999340-02-2
Mianserin	024219-97-4	Nitrazepam	000146-22-5
Mianserin-M (nor-)	999015-02-0	Nitrazepam TMS	999288-02-7
Mianserin-M (nor-) AC	999364-02-2	Nomifensine	024526-64-5
Midazolam	059467-70-8	Nomifensine AC	999371-02-7
Mirtazapine	061337-67-5	Noralfentanil	061086-18-8
Moclobemide	071320-77-9	Noralfentanil AC	999150-02-6
Molindone	007416-34-4	Norchlordiazepoxide	016300-25-7
Morphine	000057-27-2	Norchlordiazepoxide AC	999525-02-9
Morphine 2TMS	055449-66-6	Norchlordiazepoxide breakdown	999524-02-6
Muonic acid TMS	999166-02-8	Norchlordiazepoxide breakdown AC	999372-02-0
N,N-Dimethyl-5-methoxy-tryptamine	001019-45-0	Norclozapine 2AC	999135-02-3
N,N-Dimethyltryptamine	000061-50-7	Norclozapine AC	999136-02-6
Nabumetone	042924-53-8	Norcodeine	000467-15-2
N-Acetylprocainamide	999070-02-9	Norcodeine 2AC	999118-02-4
Nadolol 3TMS	999287-02-4	Nordiazepam	001088-11-5
Nalbuphine	020594-83-6	Nordiazepam TMS	999207-02-2
Nalbuphine 2TMS	999167-02-1	Norepinephrine 2AC	999119-02-7
Nalidixic acid	000389-08-2	Norepinephrine 3AC	999528-02-8
Nalidixic acid TMS	999238-02-7	Norfenfluramine	001886-26-6

Compound name	CAS number	Compound name	CAS number
Norfenfluramine AC	999120-02-4	Paramethadione	000115-67-3
Norfentanyl	999076-02-7	Pargyline	000555-57-7
Norfentanyl AC	999272-02-5	Paroxetine	061869-08-7
Norfluoxetine	999077-02-0	Paroxetine AC	999124-02-6
Norfluoxetine AC	999121-02-7	Pemoline	002152-34-3
Norketamine	999078-02-3	Pentachlorophenol	000087-86-5
Norketamine AC	999494-02-9	Pentazocine	000359-83-1
Normeperidine	000077-17-8	Pentazocine TMS	100013-72-2
Normeperidine AC	999122-02-0	Pentobarbital	000076-74-4
Normetanephrine AC	999373-02-3	Pentobarbital 2TMS	052937-68-5
Normethsuximide TMS	999429-02-6	Pentoxifylline	006493-05-6
Noroxycodone	057664-96-7	Pentylene-tetrazole	000054-95-5
Noroxycodone AC	999495-02-2	Pergolide	066104-22-1
Norpropoxyphene	999079-02-6	Perphenazine TMS	999291-02-0
Norpropoxyphene breakdown 1	999530-02-8	Phenacemide	000063-98-9
Norpropoxyphene breakdown 2	999531-02-1	Phenacetin	000062-44-2
Norpropoxypheneamide	999080-02-3	Phenacetin AC	999496-02-5
Norpseudoephedrine	000492-41-1	Phenacetin TMS	999504-02-8
Norpseudoephedrine AC	999081-02-6	Phenazopyridine	000094-78-0
Norpseudoephedrine artifact	999478-02-3	Phenazopyridine AC	999303-02-5
Nortriptyline	000072-69-5	Phencyclidine	000077-10-1
Nortriptyline AC	999151-02-9	Phencyclidine artifact	000771-98-2
Norvenlafaxine	130198-38-8	Phendimetrazine	000634-03-7
Norverapamil	067018-85-3	Phenelzine AC	999304-02-8
Norverapamil AC	999488-02-7	Phenindione	000083-12-5
Olanzapine	132539-06-1	Pheniramine	000086-21-5
Opipramol TMS	999226-02-7	Phenmetrazine	000134-49-6
Orphenadrine	000083-98-7	Phenmetrazine AC	999090-02-7
Ortho-cotinine	999083-02-2	Phenobarbital	000050-06-6
Oxazepam	000604-75-1	Phenobarbital 2TMS	052937-73-2
Oxazepam 2TMS	999168-02-4	Phenolphthalein	000077-09-8
Oxcarbamazepine	028721-07-5	Phenolphthalein 2TMS	999292-02-3
Oxprenolol 2AC	999374-02-6	Phenoxybenzamine	000059-96-1
Oxybutynin	005633-20-5	Phensuximide	000086-34-0
Oxycodone	000076-42-6	Phentermine	000122-09-8
Oxycodone enol 2TMS	999514-02-2	Phentermine AC	999152-02-2
Oxycodone TMS	221209-10-5	Phenylacetamide	000103-81-1
Oxymorphone	000076-41-5	Phenylbutazone	000050-33-9
Oxymorphone 2TMS	999521-02-7	Phenylbutazone artifact	999338-02-2
Oxymorphone TMS	999208-02-5	Phenylbutazone artifact TMS	999198-02-6
Papaverine	000058-74-2	Phenylbutazone TMS	074810-87-0

Compound name	CAS number	Compound name	CAS number
Phenylephrine 3AC	999091-02-0	Pyrilamine	000091-84-9
Phenylethylamine, beta-	000064-04-0	Pyrimethamine	000058-14-0
Phenylethylamine, beta AC	999343-02-1	Quetiapine	999097-02-8
Phenylpropanolamine	999498-02-1	Quetiapine TMS	999527-02-5
Phenylpropanolamine AC	999092-02-3	Quinacrine	000083-89-6
Phenyltoloxamine	000092-12-6	Quinidine	000056-54-2
Phenytolamine	000057-41-0	Quinine	000130-95-0
Phenytolamine 2TMS	063435-72-3	Ramelteon	999274-02-1
Pilocarpine	000092-13-7	Reboxetine	098769-81-4
Pindolol	013523-86-9	Ritodrine 3TMS	999218-02-9
Pindolol formyl artifact	999458-02-5	Rofecoxib	162011-90-7
PMA TMS	999172-02-0	Ropivacaine	132112-35-7
p-Methoxyamphetamine	000064-13-1	Salbutamol 3TMS	999394-02-4
Prazepam	002955-38-6	Salicylamide	000065-45-2
Prilocaine	000721-50-6	Salicylamide 2TMS	055887-58-6
Primidone	000125-33-7	Salicylic acid 2TMS	003789-85-3
Probenecid TMS	999294-02-9	Salicylic acid ethylester	000118-61-6
Procainamide	000051-06-9	Salicylic acid methylester	000119-36-8
Procaine	000059-46-1	Scopolamine	000051-34-3
Prochlorperazine	000058-38-8	Scopolamine TMS	999194-02-4
Procyclidine	000077-37-2	Secobarbital	000076-73-3
Procyclidine artifact (dehydro-)	999460-02-5	Secobarbital 2TMS	052937-71-0
Procyclidine TMS	999454-02-3	Selegiline	014611-51-9
Promazine	000058-40-2	Selegiline-M (HO-) AC	999482-02-9
Promethazine	000060-87-7	Sertraline	079617-96-2
Propantheline bromide	000050-34-0	Sertraline AC	999125-02-9
Propiomazine	000362-29-8	Sertraline-M (nor-) AC	999109-02-3
Propofol	002078-54-8	Sildenafil TMS	999213-02-4
Propoxur	000114-26-1	SKF-525a	000302-33-0
Propoxur-M/artifact	999393-02-1	Strychnine	000057-24-9
Propoxyphene	000469-62-5	Sufentanil	056030-54-7
Propylamphetamine	051799-32-7	Sulfadiazine	000068-35-9
Propylamphetamine AC	999302-02-2	Sulfadimethoxine	000122-11-2
Protriptyline	000438-60-8	Sulfamethazine	000057-68-1
Protriptyline AC	999273-02-8	Sulfamethazine AC	999501-02-9
Pseudoephedrine	000090-82-4	Sulfamethoxazole	000723-46-6
Pseudoephedrine 2AC	999500-02-6	Sulfanilamide	000063-74-1
Pseudoephedrine formyl artifact	999483-02-2	Sulfapyridine	000144-83-2
Psilocin 2TMS	999192-02-8	Sulfathiazole	000072-14-0
Psilocybin 3TMS	999193-02-1	Sulfinpyrazone	000057-96-5
Pyrazinamide	000098-96-4	Tacrine	000321-64-2

Compound name	CAS number	Compound name	CAS number
Talbutal	000115-44-6	Triazolam	028911-01-5
Tamoxifen	010540-29-1	Trifluoperazine	000117-89-5
Temazepam	000846-50-4	Triflupromazine	000146-54-3
Temazepam artifact-2	020927-53-1	Trihexyphenidyl	000144-11-6
Temazepam TMS	035147-95-6	Trimeprazine	000084-96-8
Terbinafine	091161-71-6	Trimethobenzamide	000138-56-7
Terfenadine TMS	999220-02-9	Trimethoprim	000738-70-5
Terflunomide AC	999502-02-2	Trimipramine	000739-71-9
Tetracaine	000094-24-6	Tripelenamine	000091-81-6
Tetrahydrocannabinol	001972-08-3	Tripolidine	000486-12-4
Tetrahydrocannabinol TMS	999529-02-1	Tropacocaine	000537-26-8
Tetrahydrozoline	000084-22-0	Tryptamine	000061-54-1
Tetrahydrozoline AC	999398-02-6	Tryptamine 2AC	999352-02-2
Thebaine	000115-37-7	Tryptamine AC	999353-02-5
Theobromine	000083-67-0	Tryptophan, D- AC	999519-02-7
Theophylline	000058-55-9	Valproic acid	000099-66-1
Thiamylal	000077-27-0	Venlafaxine	093413-69-5
Thiethylperazine	001420-55-9	Venlafaxine TMS	999173-02-3
Thiopental	000076-75-5	Verapamil	000052-53-9
Thioridazine	000050-52-2	Vigabatrin AC	999376-02-2
Thonzylamine	000091-85-0	Warfarin	000081-81-2
Ticlopidine	055142-85-3	Warfarin artifact	000122-57-6
Tiletamine	014176-49-9	Warfarin TMS	036307-79-6
Timolol TMS	999399-02-9	Xanthinol TMS	999239-02-0
Tocainide	041708-72-9	Xylazine	007361-61-7
Tocainide AC	999375-02-9	Yohimbine	000146-48-5
Tolazoline	000059-98-3	Yohimbine TMS	999457-02-2
Topiramate artifact (-SO ₂ NH)	020880-92-6	Zaleplon	151319-34-5
Topiramate breakdown	097240-79-4	Zolazepam	031352-82-6
Tramadol	027203-92-5	Zolpidem	082626-48-0
Tramadol TMS	999336-02-6	Zomepirac -CO ₂	999355-02-1
Tranlycypromine	000155-09-9	Zonisamide	068291-97-4
Tranlycypromine AC	999305-02-1	Zonisamide AC	999354-02-8
Trazodone	019794-93-5	Zopiclone	043200-80-2
Triamterene	000396-01-0	Zotepine	026615-21-4

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