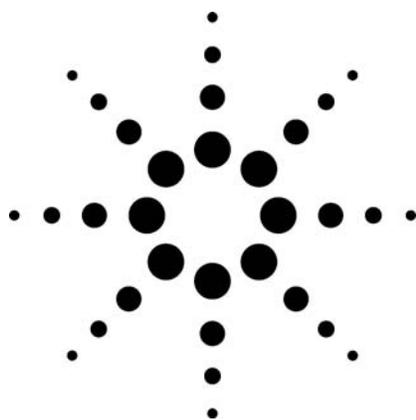


Parts-per-Trillion Level Calibration of Semivolatiles Using LVI-PTV-GC/MSD



Application

Environmental

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Abstract

The analysis of semivolatiles in the parts-per-trillion range presents challenges due to analyte activity, background contamination, and instrument sensitivity. Method requirements vary worldwide, with the least sensitive specifying 1- μ L injections and full scan data acquisition. Lower level calibrations can be achieved using large volume injection (LVI) with a programmable temperature vaporizing (PTV) inlet and the MSD operating in SIM mode. Decreased sample preparation can be used as a trade-off for these lower detection limits.

Introduction

Low-level semivolatiles analysis is used to concurrently measure a mixture of acids, bases, neutrals, and pesticides in drinking water or source water. Most laboratories analyze for > 100 compounds with a chromatographic run time of 25 to 40 minutes. Sample extraction is accomplished using liquid-solid extraction (LSE) with C_{18} disks or cartridges. Liquid-liquid extraction with a solvent such as dichloromethane is an alternative tech-

nique. Extract injection is typically 1 μ L hot splitless with the MSD operating in full scan mode, as specified in some commonly used methods, such as U.S. EPA Method 525.2 [1].

Sensitivity is an area where laboratories are seeking improved performance. Sensitivity can be affected by sample preparation, extract volume injected, instrument tuning, signal acquisition, and overall system activity.

A PTV inlet provides better sensitivity through large-volume injection. Instead of 1 μ L, 25 μ L of relatively clean sample extracts can be routinely injected. Active analyte degradation is minimized on a PTV, providing lower detection limits than using hot splitless injection.

Methods for semivolatiles usually require identification of analytes with retention time (RT) and ratios of qualifier ions to a target ion. Selected ion monitoring (SIM) acquisition can be used in place of full scan with a sensitivity, or signal-to-noise ratio, increase of 10 to 50x.

A typical calibration range for low-level semivolatiles is 0.1 to 10 ppm as is found in U.S. EPA Method 525. This application note will demonstrate a calibration 1,000x lower and 10x wider that is from 0.1 to 100 ppb. LVI-PTV with SIM data acquisition on a retention time locked (RTL) GC/MSD system was used to achieve this performance. This application is a follow-up note to reference 2, where additional background information can be found.



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Experimental

Instrument Operating Parameters

The recommended instrument operating parameters are listed in Table 1. These conditions may have to be optimized for use in another laboratory.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 7890A or 6890N			Front Injector	
Inlet	EPC PTV			Sample washes	0
Mode	Solvent vent			Sample pumps	2
Temp ramp	°C/min	Next °C	Hold min	Injection volume	25 microliters
Initial		20	0.60	Syringe size	50 microliters
Ramp 1	600	350	1.30	Preinj solv A washes	0
Ramp 2	10	250	0.00	Preinj solv B washes	1
Cryo	On			Postinj solv A washes	2
Cryo use temp	100 °C			Postinj solv B washes	2
Cryo timeout	10.00 min (On)			Viscosity delay	1 second
Cryo fault	On			Plunger speed	Variable
Pressure	11.77 psi (On)			Injection speed	50 microliters/minute
Vent time	0.60 min			Draw speed	600 microliters/minute
Vent flow	100.0 mL/min			Dispense speed	6,000 microliters/minute
Vent pressure	0.0 psi			Preinjection dwell	0 minutes
Purge flow	50.0 mL/min			Postinjection dwell	0 minutes
Purge time	2.50 min			MSD	Agilent Technologies 5975C, Trace Ion Detection
Total flow	53.9 mL/min			Drawout lens	6 mm large aperture drawout lens, part number G2589-20045
Gas saver	Off			Solvent delay	4 min
Gas type	Helium			Low mass	45 amu
PTV Liner	Agilent multi-baffle liner, no packing, part number 5183-2037			High mass	450 amu
Oven	240V			Threshold	0
Oven ramp	°C/min	Next °C	Hold min	Sampling	1
Initial		40	2.50	Quad temp	180 °C
Ramp 1	50	110	0.00	Source temp	300 °C
Ramp 2	10	320	1.10	Transfer line temp	280 °C
Total run time	26 min			Tune type	Autotune
Equilibration time	0.5 min			EM voltage	Tune voltage, 1,247 V
Oven max temp	325 °C			MSD-SIM	AutoSIM was used to pick ions, groups, and switching times
Column	Agilent Technologies HP 5 MSi, part number 19091S-433i			Number of groups	25
Length	30.0 m			Compounds/group	Varied 1 to 22
Diameter	0.25 mm			Ions/group	Varied 2 to 45
Film thickness	0.25 µm			Dwell time, msec	Varied 5 or 10
Mode	Constant flow			Cycles/peak	Minimum 10
Pressure	11.77 psi			Calibration Standards	
Nominal initial flow	1.5 mL/min			Ultra Scientific, North Kingstown, RI. Part number DWK-5252.	
Inlet	Front			Four mixtures, codiluted, resulting in 108 compounds at 4 concentration levels, spiked with 3 Internal Standards at 50 ppb and 4 surrogate standards at 50 ppb.	
Outlet	MSD				
Outlet pressure	Vacuum				
RTL	System retention time locked to phenanthrene-d10 at 12.700 min				
				Calibration standards made separately in both dichloromethane and ethyl acetate.	

The newer 7890A GC offers significant speed advantage over the older 6890N. Cooldown time from 320 °C to 40 °C is reduced from 7 minutes to 4.3 minutes. The MSD can optionally be mounted in the new rear position on a 7890A GC. With the PTV also installed in the back inlet position, the oven insert or “pillow” can be used to further reduce cooldown time to 3.3 minutes.

The PTV was operated in the Solvent Vent mode. Figure 1 shows the PTV temperature and flow programs together with the oven program. The PTV is held at 20 °C, a temperature below the boiling point of the solvent dichloromethane, 39.8 °C, during the injection period, 0.6 minute. The solvent is slowly evaporated through the vent line, held at 0 psi, with helium flow at 100 mL/minute. At the end of the injection period, the vent line is closed, inlet pressure is raised to 11.77 psi, and the PTV is rapidly heated to 350 °C. The vent line is reopened at the end of the splitless time, 1.3 minutes, and the inlet is purged at 50 mL/min. The PTV is allowed to cool during the run.

While the vent line is closed, the PTV is in the classical splitless mode with respect to flow. Because of the programmed temperature, compounds are vaporized and transferred onto the column at the lowest possible temperature. This

significantly reduces loss of active analytes, such as pesticides and bases, which are often specified in semivolatiles methods.

The PTV inlet liner, 5183-2037, is multibaffled and deactivated. It does not contain glass wool, which could contribute to active compound degradation. This liner has sufficient capacity to accommodate the 25- μ L injection volume at a correct injection speed.

The oven program relationship to the PTV parameters is shown in Figure 1. The oven starts at 40 °C and is held there during the injection/solvent vent cycle and splitless transfer of analytes onto the column. The oven then programs rapidly to 110 °C followed by a slower ramp for compound separation. The 240V oven was used but a 120V oven can also achieve the ramp rates found in Table 1.

The HP-5MSi column is designed for inertness and is well suited to this method. This is the latest version of the most popular column in environmental laboratories, the HP-5MS. The column was run in constant flow mode at 1.5 mL/min to maintain peak shape and sensitivity.

The system was retention time locked to phenanthrene-d10 at 12.700 minutes. The funda-

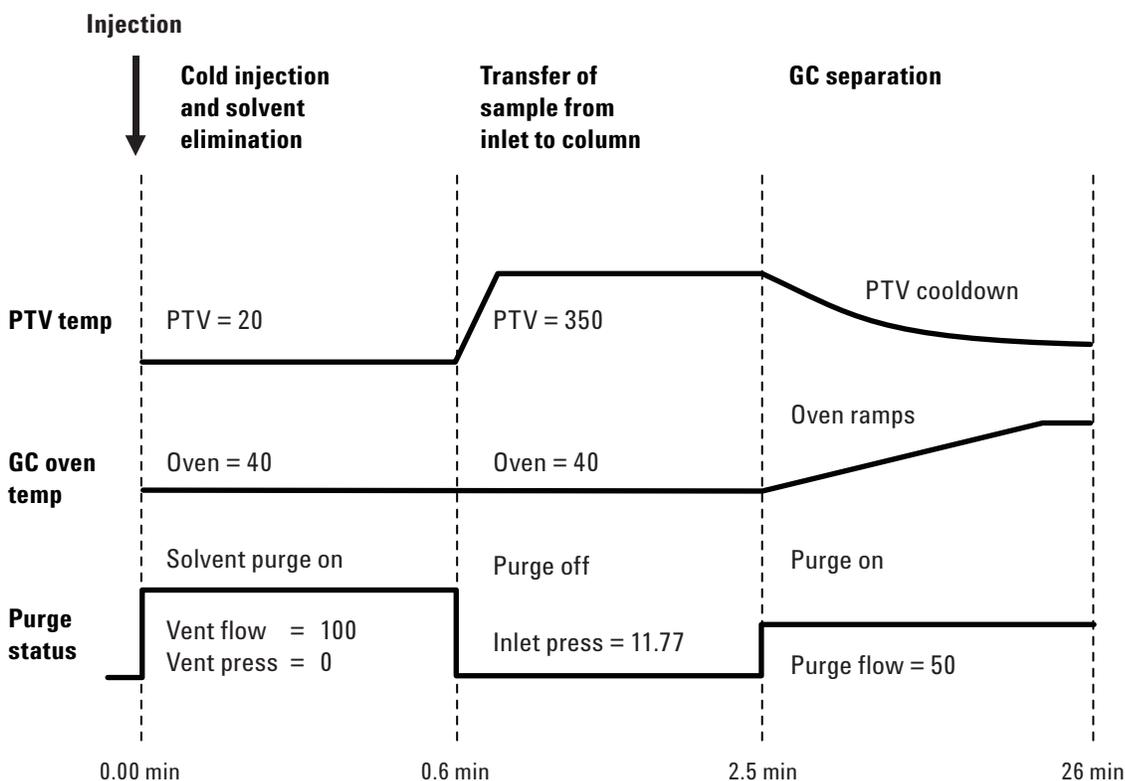


Figure 1. PTV temperature and flow programs.

mentals of RTL for GC/MSD systems can be found in reference 3. The primary benefit of RTL for this analysis is maintaining constant switching times for SIM groups. After clipping the column, a rerun and analysis of the locking standard is all that is needed to restore shifted peak times. Quantitation database and integration events times also do not have to be changed. Additional RTL application notes detailing the numerous benefits of RTL are available at www.agilent.com/chem. It is almost impossible to use a method with this many SIM groups, without RTL, in a productive laboratory.

Previous work has shown improved linearity across a wide calibration range using a 6 mm draw-out lens instead of the standard 3 mm lens [4]. Although this application uses a lower calibration range, the linearity improvement is still valid. The signal/noise loss using the 6 mm lens, even at low levels, was minimal compared to the linearity gain. The 6 mm lens is also included in Agilent Kit part number G2860A.

Scan parameters are listed even though the calibration was done using SIM data. All runs were made in synchronous SIM/scan mode, acquiring both SIM and scan data with a single injection. A sampling rate of 1, combined with the lower noise characteristics of the 5975C, was used to optimize signal/noise. This sampling rate, with a 45 to 450 mass range, resulted in approximately 10 scans across the peaks. The full scan data could be used to identify total unknowns by library searching, if present in sufficient amount. If full scan data is not needed, SIM/scan can be turned off and only SIM data collected. This will provide approximately 2x the number of data points across a peak.

AutoSIM setup was used in combination with the quantitation database to pick ions, groups, and switching times. Details of AutoSIM can be found in reference 5. The SIM acquisition table from AutoSIM was used directly with only two modifications. Tebuthiuron (ion 156) and tricyclazole (ion 189) are known for poor peak shape. Their target and qualifier ions were manually added to the groups across which the peaks eluted. A target ion plus one qualifier ion were used for all internal standards (ISTDs) and surrogate standards (SSs). A target ion plus two qualifier ions were used for all other analytes, if present in sufficient abundance in the spectra. The 10 SIM data points acquired across an average peak were used for calibration.

A source temperature of 300 °C was used instead of the typical 230 °C to 250 °C range. This higher temperature has been used to minimize peak tail-

ing, and therefore increase sensitivity, for PAHs [6] and to improve performance for semivolatiles [2].

Calibration standards were prepared in dichloromethane only for the single-component analytes. Standards were not prepared for toxaphene or the Aroclors. Disulfoton sulfoxide and disulfoton sulfone were not included in the commercially available mixture. A separate set of calibration standards was prepared in ethyl acetate.

Results and Discussion

The system was calibrated at four levels, 0.1, 1.0, 10, and 100 ppb, with the standards in dichloromethane. Tebuthiuron, known to be problematic, was the only analyte that showed insufficient response at the lowest level. The SIM total ion chromatogram (TIC) for the 1.0 ppb level run in SIM/scan mode is shown in Figure 2. Each calibration level contained 108 compounds plus three ISTDs and four SSs at 50 ppb. Intermediate calibration levels are specified by some methods but were not needed here to demonstrate system performance.

The best overall performance was accomplished using the PTV parameters in Table 1. Successful PTV injections are a balance of injection speed, temperature, vent flow rate, and vent time.

Injection speeds of 150, 100, and 50 $\mu\text{L}/\text{min}$ were tried. Faster injection rates showed decreased abundance for most analytes, regardless of RT. Sample passes through the liner, before solvent evaporation, and is swept out the vent line.

The initial PTV temperature was tested at 10, 20, 30, and 40 °C. Higher temperatures showed loss of the early eluters, those with volatility closer to that of the solvent. Lower temperatures preserve early eluters but hinder solvent venting.

The vent flow was tested at 50, 100, 200, and 300 mL/min . Increasing either the flow rate or vent time can decrease recovery of the early eluters. Decreasing the flow rate or vent time can result in excess solvent on the column and therefore poor chromatography. The minimum vent time must be matched to the injection time. In this case the injection takes 0.5 min (25 μL at 50 $\mu\text{L}/\text{min}$), so a vent time of 0.6 min was used.

Ethyl acetate is used in some methods as a solvent for solid phase extractions. Calibrations with standards in ethyl acetate showed worse performance

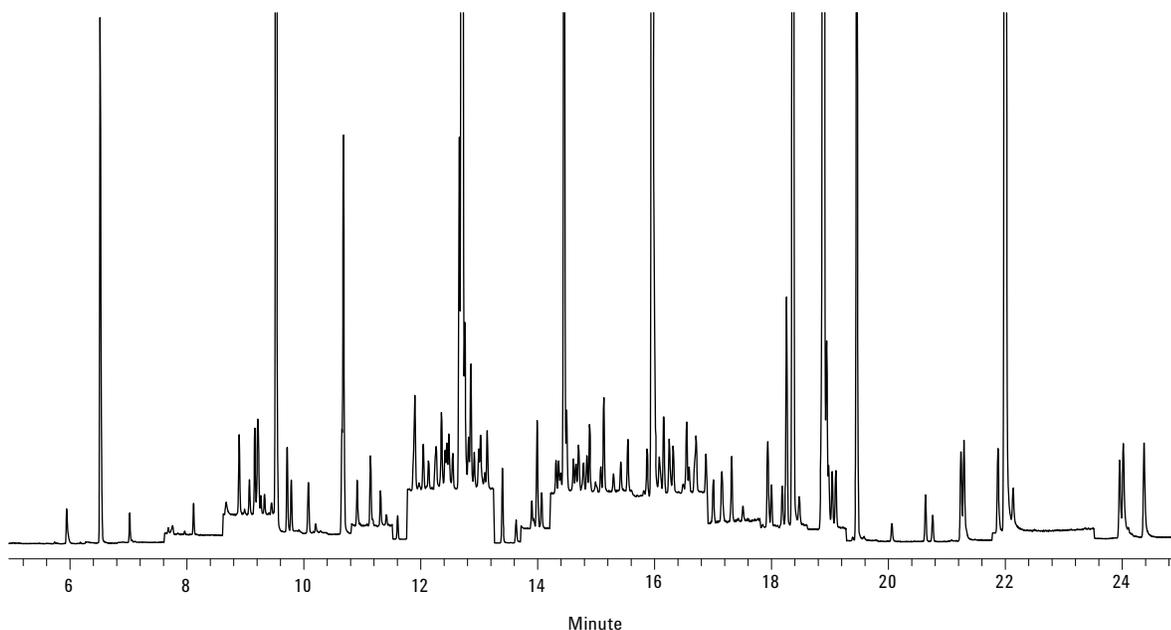


Figure 2. SIM TIC for the 1.0 ppb level run in SIM/scan mode.

than standards in dichloromethane. Ethyl acetate does not wet the stationary phase evenly, resulting in misshapen peaks, as the PTV did not eliminate 100% of the solvent. Adjusting the PTV parameters to account for the higher boiling point of ethyl acetate resulted in measurable losses of the early eluters. As a general rule, the earliest eluter for which quantitative recovery is required should have an elution temperature at least 100 °C greater than the solvent's boiling point. Ethyl acetate can be used successfully, but the lowest calibration point may be higher for some analytes.

Linearity can be determined by the percent relative standard deviation (%RSD) of the relative response factor (RRF) for each compound across the calibration range. The %RSD and the RRFs calculations are done automatically by the GC/MSD ChemStation software and can be reported in Excel. There is no correct %RSD as it is method dependent. As an example, U.S. EPA Method 525 has a criterion of < 30%RSD, but only for a subset of the compound list. The %RSDs of the RRFs for selected compounds are shown in Table 2.

Table 2. Signal-to-Noise and Linearity for Selected Analytes

Compound	RT	Target Ion	S/N 100 ppt	%RSD
Dichlorvos	7.01	109	6.5	4
Mevinphos	8.90	127	7.1	17
Simazine	12.24	201	4.8	6
Atrazine	12.35	200	20	6
Pentachlorophenol	12.48	266	22	24
Chlorpyrifos	14.78	197	2.7	12
2,2',3',4,6'-pentachlorobiphenyl	15.55	326	12	9
Phenamiphos	16.30	303	3.2	25
p,p'-DDT	18.00	235	13	9

At first glance some of the %RSD values appear high, such as pentachlorophenol (PCP) and the organophosphorus pesticides (OPPs). PCP is a known difficult compound and is commonly analyzed at significantly higher levels as in Method 525. The OPPs are very active and system inertness is critical to their successful analysis. Given this and the wide calibration range, the data shown here are excellent. As an additional overall measure of system linearity, the average of all %RSDs was 12% for SIM data in this study. The phthalates, easily detected at low levels, were excluded from this overall number due to common laboratory contamination. The %RSDs of the SSs ranged from 2% to 4%, demonstrating good repeatability.

As a further measure of system inertness, the %RSD for p,p'-DDT is 9%. The breakdown products

in an active system are p,p'-DDD and p,p'-DDE. Their %RSDs were 6% and 4%, respectively, indicating minimal breakdown. A separate mixture of p,p'-DDT and endrin was also analyzed for breakdown, using the classical U.S. EPA criteria. The p,p'-DDT % breakdown was 1.2 and Endrin was 1.9, well below the required 15%.

The signal-to-noise values are also shown in Table 2. Peak-to-peak noise was used, as this is what the analyst sees and has to work with. Atrazine and PCP values are sufficiently high that they could be calibrated and measured at a lower concentration. Chlorpyrifos and phenamiphos have S/N values below 5 and are near the limit of reproducible integration and hence quantitation. Extracted ions for PCP and chlorpyrifos are shown in Figure 3. In all cases the analytes exhibited sufficient S/N for successful calibration at the 100 ppt level.

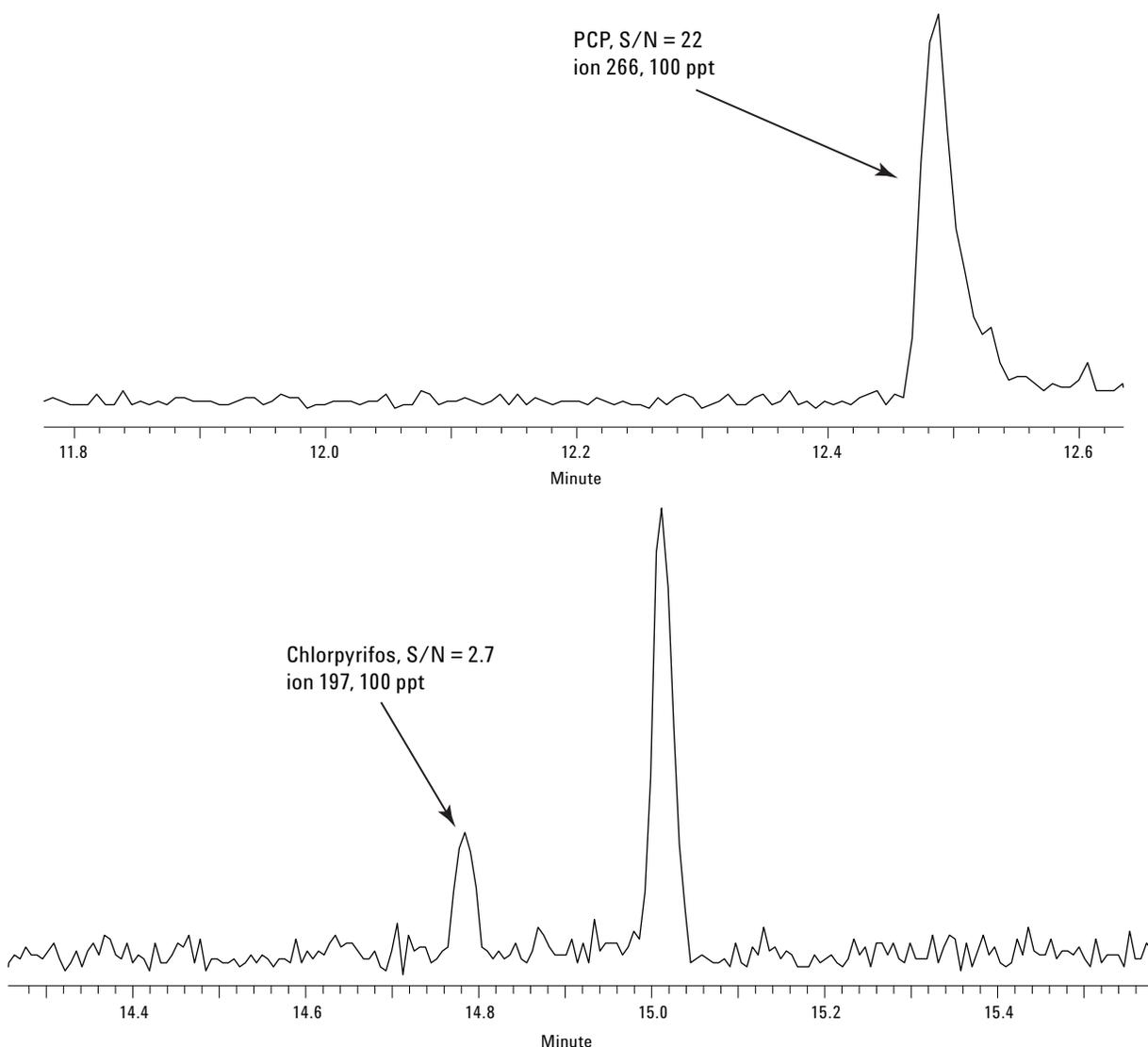


Figure 3. Extracted ions for PCP and chlorpyrifos.

As a trade-off to lower calibration levels and method detection limits, a laboratory could reduce sample preparation, shown in Table 3. The first column, “Traditional,” assumes 1 liter of water is extracted, concentrated to 1 mL, and 1 uL is injected. Methods using this approach have a lowest calibration level of 100 ppb (0.1 ppm) in scan mode. As described in this application, the “7890A-5975C” column maintains the same sample preparation but increases the sensitivity by a factor of 1,000, to the ppt level. The “Fast Prep” column shows extracting only 10 mL and still lowering the method limits by 10x compared to Traditional. Extracting 10 mL of sample is significantly easier and faster than 1 liter. Better recoveries may also be realized by needing less concentration of the extract. The Quick Screen extraction is accomplished directly in a 2-mL vial (Agilent p/n 5182-3454) with an integral pointed bottom. The dichloromethane extract is withdrawn from the bottom of the vial by the autosampler syringe. Variations of these examples can be used to maximize sensitivity and minimize sample preparation time.

Conclusions

Traditional semivolatiles methods can be altered to achieve better detection limits. Large volume injection-PTV coupled with SIM allows calibration to the 100-ppt level. Linearity is excellent for the wide calibration range used, even for active analytes. Using RTL saves the analyst time by preserv-

ing SIM group switching times. The 7890A reduces cycle times by rapid oven cooling. Laboratories can choose to lower method calibration limits and/or save time through reduced sample preparation.

References

1. U.S. EPA Method 525.2 is available from different sources listed on this Web site: www.epa.gov/OGWDW/methods/where.html
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6. M. Szelewski, “Synchronous SIM/Scan Low-Level PAH Analysis Using the Agilent Technologies 6890/5975 Inert GC/MSD,” Agilent Technologies publication 5989-4184EN.

Table 3. Sample Preparation and Calibration Limits

	Traditional	7890A-5975C	Fast Prep	Quick Screen
Sample concentration, ppb	0.1	0.0001	0.01	0.02
Lowest cal level, ppb	100	0.1	0.1	0.1
Injection volume, µL	1	25	25	25
Extract volume, mL	1	1	1	0.25
Sample size, mL	1000	1000	10	1.25

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