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Trusted Answers

Food Testing & Environmental Analysis

Application Compendium

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This application compendium contains a collection of environmental and food testing application notes demonstrating the low detection levels and sensitivity of the Agilent Triple Quadrupole GC/MS systems for the quantitation of pesticides, volatile and semi volatile compounds.

Application Note

Food Testing & Agriculture

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Estimation of Ethylene Oxide and Ethylene Chlorohydrin in Sesame Seeds Using Agilent 8890 GC and 7000D Triple Quadrupole MS System

Authors

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Abstract

This application note demonstrates the use of the Agilent 8890 GC system coupled with the Agilent 7000D GC/MS triple quadrupole mass spectrometer to detect and quantify ethylene oxide and ethylene chlorohydrin in sesame seed samples. The method provides the highest confidence in results for routine analysis for the food industry, whether involved in production, processing, storage, or commercial testing of sesame seed samples or for academic purposes. During sample preparation, the ethylene oxide residue in the sample is converted to ethylene chlorohydrin, which is followed by liquid-liquid extraction (LLE) with ethyl acetate, and cleaned up before injecting into GC/TQ. A limit of quantitation (LOQ) of 10 ppb has been demonstrated in matrix.

Ethylene oxide is used to sterilize oilseeds and spices during storage. Residues of ethylene oxide and its derivative, ethylene chlorohydrin (produced by reactions during storage) may be found in these foodstuffs.[1] Ethylene chlorohydrin can be used as a suitable marker to confirm the use of ethylene oxide for fumigation. The ethylene chlorohydrin can be evaluated in sesame seeds by a simple GC/TQ analytical method. This evaluation also provides the estimation of actual ethylene oxide present in the sample initially by using a conversion factor.

The method demonstrated in this work is useful for detecting ethylene chlorohydrin as a marker of fumigation of sesame seeds with ethylene oxide using the 8890 GC system coupled with the 7000D triple quadrupole MS (with 10 ng/g as the LOQ, which complies to MRL set by EU at 50 ng/g).[2]

Experimental

Chemicals required

- 0.1 N sulfuric acid (0.981 g sulfuric acid dissolved in 100 mL water)
- Saturated sodium chloride solution in water
- Water (Millipore, Milli-Q)
- Ethyl acetate (HPLC grade)
- Agilent QuEChERS dispersive cleanup kit (part number 5982-0028)
- Ethylene oxide reference standard (Sigma-Aldrich part number CRM48891)
- Ethylene chlorohydrin reference standard (Merck part number 8.00945.0100)

Apparatus required

An Agilent 8890 GC system equipped with an MMI inlet configured with postcolumn backflush option and a 7000D triple quadrupole MS, an ultrasonic bath, a water bath, a cold centrifuge, a table-top centrifuge, and a vortex mixer were used in this study. The procedure was as follows:

1. Weigh 2 g of sample into 50 mL centrifuge tube.
2. Add 2 mL of water, 2 mL of 0.1 N H_2SO_4, and 1 mL of saturated sodium chloride solution.
3. Sonicate for 20 minutes.
4. Rest sample in water bath at 50 °C for 1 hour.
5. Vortex and wait until the sample reaches room temperature.
6. Add 5 mL ethyl acetate and vortex for 10 minutes.
7. Centrifuge at 8,000 rpm for 5 minutes at 5 °C.
8. Take 1 mL of supernatant and add it to a dispersive QuEChERS cleanup tube (universal) (part number 5982-0028).
9. Shake and centrifuge at 5,000 rpm for 5 minutes.
10. Collect supernatant in a vial and inject into the GC/MS/MS.

Table 1. GC method.

GC Conditions	
Column	Agilent VF-624 ms, 60 m × 0.25 mm, 1.4 µm (p/n CP9103)
Inlet	Agilent Multimode Inlet 5190-2293, splitless liner Injection volume: 2 µL
Injection Mode	Pulsed Splitless, 25 psi until 0.8 min, purge flow of 40 mL/min at 1.25 min
Inlet Temperature	250 °C
Oven	60 °C for 2 min, at 10 °C/min to 150 °C, at 40 °C/min to 250 °C, hold 20 min
Carrier Gas	99.9995% Helium at 1.0 mL/ min, constant flow mode

Table 2. MS method MS conditions.

MSD Conditions	
Quadrupole Temperature	150 °C
Ion Source Temperature	EI 280 °C
Transfer Line Temperature	250 °C
MRM Transitions for Ethylene Chlorohydrin	82 → 31 (CE: 5) 80 → 43 (CE: 5) 80 → 31 (CE: 5)
EMV Mode	Gain factor: 10
Dwell Time for Each Transition	75
Solvent Delay	9.5

Results and discussion

With the above method, the LOQ was estimated to be at 10 ng/g for ethylene chlorohydrin in sesame seed samples as at this level, the peak is easily distinguished from baseline and matrix with signal to noise ratios >2.9. Figure 1 highlights the quantifier and qualifier EICs at LOQ level spiking. This LOQ satisfies the needs of customers and regulatory requirements of MRL set at 50 ng/g by EU. Figure 2 demonstrates the signal-to-noise for 10 ppb and 50 ppb level matrix standards.

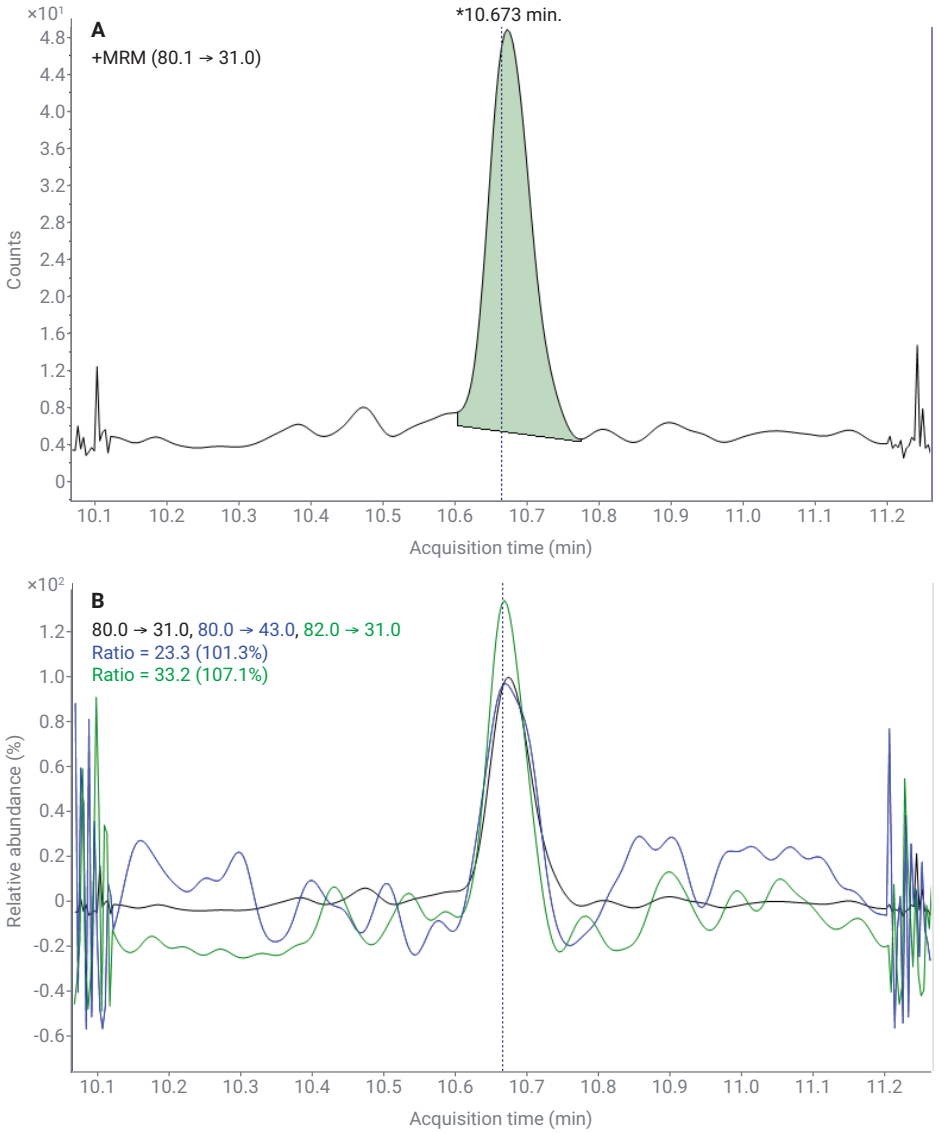

Figure 1. A quantifier and two qualifier peaks of ethylene chlorohydrin at the 10 ng/g spike level.

Calibration and linearity

A prespiked matrix linearity plot was generated for response (peak area) across concentration levels from 5 to 200 ng/g (Figure 4). Calibration was performed at six levels: 5, 10, 20, 50, 100, and 200 ng/g. Good linearity with R^2 >0.998 was observed.

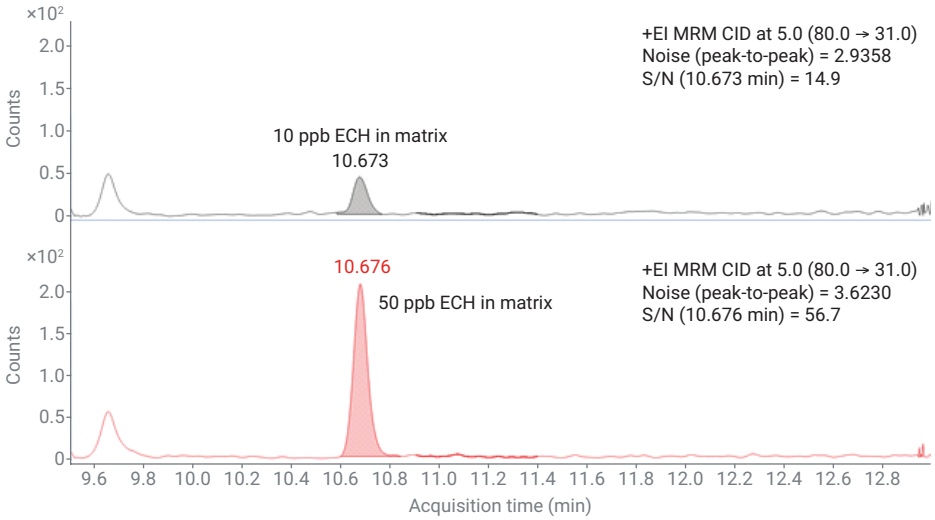

Figure 2. Sensitivity of ethylene chlorohydrin: MRM chromatograms of 10 ng/g spike and 50 ng/g spike.

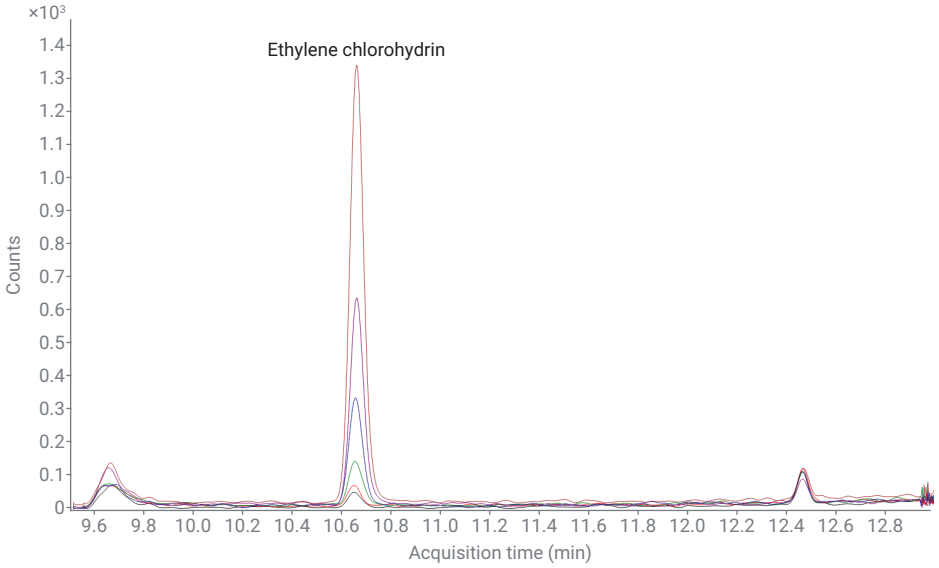

Figure 3. TIC MRM overlay of various concentrations of ethylene chlorohydrin in matrix ranging from 5 ppb to 200 ppb.

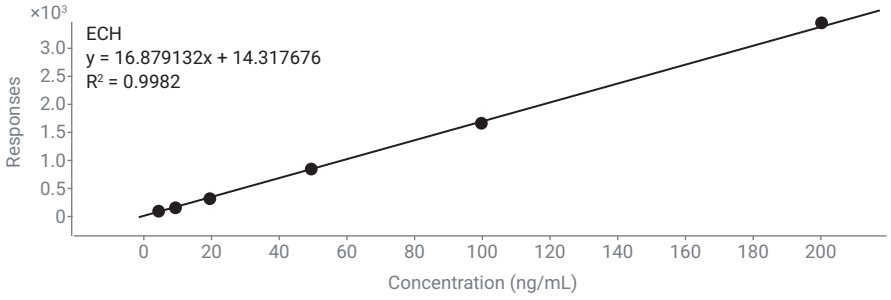

Figure 4. Calibration plot for ethylene chlorohydrin matrix-matched standards.

Repeatability

Repeatability of elution was demonstrated by injecting an ethylene chlorohydrin standard in matrix with a 50 ppb concentration. Relative standard deviation on peak areas of ethylene chlorohydrin calculated based on six replicate injections of 50 ppb matrix standard was 1.73%, as shown in Table 3.

Quantitation in sesame seed samples

The discussed method was extended to the sesame seed sample, which was purchased from a local market for the analysis and recovery study.

Recovery study

As shown in Table 4, no peak corresponding to ethylene chlorohydrin was found in the blank matrix of sesame seed. The recoveries of ethylene chlorohydrin and ethylene oxide from the real-world sesame seed sample were calculated using the spiking levels of 10 and 50 ppb for ethylene chlorohydrin and 10 ppb for ethylene oxide.

Three spike studies were performed as follows:

1. Ethylene chlorohydrin was spiked in the sesame seed sample at the 10 ppb level.
2. Ethylene chlorohydrin was spiked in the sesame seed sample at the 50 ppb level.
3. Ethylene oxide was spiked in the sesame seed sample at the 10 ppb level. This was done to check the applicability of the method for estimating the ethylene oxide content in sample. (A conversion factor of 0.55 was used as a multiplier to calculate the results for the ethylene oxide spiking sample).[2] The obtained results for percent recovery are discussed in Table 5.

Table 3. Percentage RSD (CV) for ethylene chlorohydrin for the 50 ppb matrix-matched standard.

Area Inj-1	Area Inj-2	Area Inj-3	Area Inj-4	Area Inj-5	Area Inj-6	%RSD
821	804	811	829	842	810	1.73

Table 4. Quantitation summary for calibration (5 to 200 ppb), spike recovery (10 and 50 ppb), and repeatability exercise (50 ppb).

Sample Name	Compound	Sample Type	RT	Response	Final Conc. (ng/g)
Matrix Blank_sesame seed	Ethylene Chlorohydrin	Sample			ND
Matrix calibration-1_5 ppb	Ethylene Chlorohydrin	Calibration	10.646	115	5.97
Matrix calibration-2_10 ppb	Ethylene Chlorohydrin	Calibration	10.650	165	8.9
Matrix calibration-3_20 ppb	Ethylene Chlorohydrin	Calibration	10.654	324	18.32
Matrix calibration-4_50 ppb	Ethylene Chlorohydrin	Calibration	10.654	856	49.85
Matrix calibration-5_100 ppb	Ethylene Chlorohydrin	Calibration	10.657	1,676	98.46
Matrix calibration-6_200 ppb	Ethylene Chlorohydrin	Calibration	10.657	3,449	203.5
Sesame_ECH SPK_10 ppb	Ethylene Chlorohydrin	Sample	10.673	184	10.08
Sesame_ECH SPK_50 ppb	Ethylene Chlorohydrin	Sample	10.676	859	50.04
Sesame_ETO SPK_10 ppb	Ethylene Chlorohydrin	Sample	10.673	267	8.23
50 ppb spk replicate-1	Ethylene Chlorohydrin	Sample	10.699	821	47.8
50 ppb spk replicate-2	Ethylene Chlorohydrin	Sample	10.707	804	46.79
50 ppb spk replicate-3	Ethylene Chlorohydrin	Sample	10.714	811	47.2
50 ppb spk replicate-4	Ethylene Chlorohydrin	Sample	10.718	829	48.29
50 ppb spk replicate-5	Ethylene Chlorohydrin	Sample	10.737	842	49.05
50 ppb spk replicate-6	Ethylene Chlorohydrin	Sample	10.733	810	47.14

Table 5. Recovery in sesame seed sample.

Compound Name	Spiking Amount (ng/g)	Observed Amount (ng/g)	Final Amount (ng/g)	Recovery (%)
Ethylene Chlorohydrin	10	10.078	10.078	100.8
	50	50.036	50.036	100.1
Ethylene Oxide	10	14.96	8.228	82.3

Conclusion

An accurate and rugged method was developed for analysis of ethylene oxide and ethylene chlorohydrin in sesame seeds. The sample preparation method uses easy and fewer time-consuming steps. The LOQ of the method is demonstrated at the 10 ng/g level in samples. Repeatable results were found for six replicates of spiked samples. Satisfactory recovery was found at the 10 ng/g spiked concentration of ethylene oxide and ethylene chlorohydrin in sesame seed samples. Thus, the method demonstrated in this study proves useful for the routine analysis of sesame seed samples fumigated with ethylene oxide under the established regulatory limits.

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1. Tateo, F.; Benoni, M. Determination of Ethylene Chlorohydrin as Marker of Spices Fumigation with Ethylene Oxide. *JFCA* **2006**, *19*, 83–87.
2. Procedure for Control of Contamination of Salmonella, Residues of Pesticides Including Ethylene Oxide (EO) in Sesame Seeds for Export to EU Countries. Indian Oil Seeds and Produce Export Promotion Council. http://www.iopepc.org/misc/2020/Procedure_1.pdf

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Low Calibration Limit Research for Multiresidue Pesticides in Milk Using the Agilent 8890/7010B and 7890B/7000C Triple Quadrupole GC/MS Systems

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Introduction

To ensure the safety of milk and dairy products, some countries have issued a series of regulations limiting pesticide residues. To meet these limits, reference methods have been defined in the regulations. The maximum allowed pesticide residues in milk are mostly much lower than those for fruits and vegetables in government regulations.[1] These lower levels require an advanced analytical platform to achieve the required high sensitivity. This application note describes two GC/MS/MS platforms: the Agilent 7890B/7000C and the Agilent 8890/7010B triple quadrupole GC/MS systems. Both are applicable for pesticide analysis in milk and their corresponding linearity ranges, respectively. The results demonstrate that the 8890/7010B system provides 1 ng/mL detection of almost 60% of pesticides, while 10% could be detected at 1 ng/mL on the 7890B/7000C system.

Experimental

Chemicals and reagents

All reagents and solvents were HPLC or analytical grade. Acetonitrile (ACN) was from Honeywell (Muskegon, MI, USA). The pesticide standards were purchased from Alta (Tianjin, China). Individual pesticide stock solutions (100 µg/mL) in ACN were stored at –20 °C, and the mixture solution (1 µg/mL) was prepared in ACN and stored at –20 °C.

Milk samples and calibration standard preparation

The samples were prepared following the method from the application note "Analysis of Multiclass Multiresidue Pesticides in Milk Using Agilent Captiva EMR—Lipid with LC/MS/MS and GC/MS/MS".[2] The details are as follows: 5 mL of milk was transferred into a 50 mL centrifuge tube. Two ceramic homogenizers, 10 mL of acetonitrile, and an Agilent QuEChERS extraction kit (part number 5982-5650) were added to each centrifuge tube. The samples were mechanically shaken with a Geno/Grinder at 1,000 rpm for five minutes, followed by centrifugation 4,000 rpm at 10 °C for an additional five minutes. A 4.8 mL aliquot of the extract was transferred to a new tube and 1.2 mL of water was added to mix gently. Next, the sample mixture was loaded onto an Agilent Captiva EMR—Lipid 6 mL cartridge. After finishing the gravity flow, 1.5 mL of solvent elution (80/20 ACN/H_2O) was added to the Captive EMR—Lipid and let gravity flow. 5 mL eluent was then transferred to a new 15 mL centrifuge tube, and 3.5 g of anhydrous $MgSO_4$ (EMR drying salt pouch, part number 5982-0102) was added to the tube for water removal. Samples were vortexed vigorously for three minutes, then centrifuged for five minutes at 8,000 rpm. The sample extracts were transferred to labeled autosampler vials for GC/MS/MS analysis.

Matrix-matched calibration standards were prepared by spiking pesticides in blank matrix extract. The blank matrix extract was from one of the milk samples that had none of the pesticides identified in the preliminary screening. The calibration solutions correspond to 1, 2, 5, 10, 20, 50, 100, 200, and 500 ng/mL of spiking concentration in milk. Since the entire sample preparation workflow introduced 2.5-fold dilution of the original sample concentration for GC/MS/MS, the final concentrations of calibration solutions in the labeled autosampler vials were 0.4, 0.8, 2, 4, 8, 20, 40, 80, and 200 ng/mL. For consistency, the concentrations mentioned in this study refer to the spiking concentration added before sample preparation.

Instrument conditions

Two GC/MS/MS platforms were used for the analysis of pesticides in milk. The 7890B/7000C system was configured with the extractor EI source, which delivers inertness and a wide calibration range. The 8890/7010B system was configured with the high efficiency source (HES), which can create up to twenty times more ions than the extractor and delivers confident analysis at ultra-trace levels.[3] The Agilent MassHunter Pesticide and the Environmental Pollutant MRM Database were used for building the acquisition method automatically and conveniently, including operating conditions such as MRM transitions, collision energy, and inlet pressure, etc. Retention time locking (RTL) was also used in the tests to ensure consistency of retention time between different instruments and consistency with the database. The GC/MS/MS instrument conditions are shown in Table 1.

Table 1. GC/MS/MS conditions for pesticide quantitation.

Parameter	Value
Injection Volume	1 µL
Inlet	Split/splitless; temperature: 280 °C; splitless mode, purge flow 30 mL/min at 0.75 min
Inlet Liner	Agilent Ultra Inert, splitless, single taper, glass wool (p/n 5190-2293)
Column	Agilent HP-5ms UI, 30 m × 0.25 mm, 0.25 µm (p/n 19091S-433UI)
Carrier Gas	Helium, ~1.019 mL/min, constant flow
Over Program	60 °C (1 min), 40 °C/min to 120 °C, then 5 °C/min to 310 °C
Transfer Line Temperature	280 °C
Collision Cell EPC	Quench gas He, 2.25 mL/min; collision gas N_2, 1.5 mL/min
Source Temperature (HES/Extractor)	280 °C
Quadrupole Temperature (MS1 and MS2)	150 °C
Acquisition Mode	dMRM
EM Voltage Gain Mode	10
Solvent Delay	3 min
Tune File	Atunes.eihs.tune.xml (HES for 7010B)/Atunes.eiex.tune.xml (Extractor for 7000C)

Results and discussion

Table 2 lists the linearity range and the R^2 values for both the 7890B/7000C and 8890/7010B systems. The calibration ranged from 1 to 500 ng/mL and was validated on both systems. This calibration range was for most analytes while some were not included at the lowest level because of their low response ability on GC/MS/MS systems. For example, novaluron has a linear range between 5 to 500 ng/mL on the 7010B system, but a range between 50 to 500 ng/mL on the 7000C system. Chlorantraniliprole has a linear range between 10 to 500 ng/mL on the 7010B system, but a range between 50 to 500 ng/mL on the 7000C system. The two systems are capable of meeting detection requirements, while the detection capacity of the 7010B system far exceeds the requirements of some regulations. The detailed linearity range for each compound is shown in Table 2. Figure 1 shows the low calibration limit achieved by the 7890B/7000C and the 8890/7010B triple quadrupole systems.

The low calibration limit is the smallest standard concentration within the linear range of the instrument. For most pesticides, the 8890/7010B system showed a much lower calibration limit, compared to the 7890B/7000C system. In theory, the 7010B system with HES can create up to 20 times more ions than the 7000C system with the extractor source and delivers confident analysis at ultra-trace levels. In practice, however, sensitivity is influenced by various factors, especially the compound itself.

Table 2. Linearity results for pesticides with the 8890/7010B and the 7890B/7000C triple quadrupole GC/MS systems.

Compound Name	RT (min)	Transitions		Linearity Range (ng/mL)		R^2	
		Quant	Qualifier	Agilent 7010B	Agilent 7000C	Agilent 7010B	Agilent 7000C
2,4,6-Trichlorophenol	7.726	131.8 → 97.0	96.9 → 62.0	1 to 500	1 to 500	0.9976	0.9952
Acetamiprid	27.873	126.0 → 73.0	152.0 → 116.1	5 to 500	100 to 500	0.9948	NA
Aldrin	19.569	262.9 → 192.9	254.9 → 220.0	1 to 500	10 to 500	0.9984	0.9985
Azinphos-ethyl	30.617	132.0 → 77.1	160.0 → 77.1	1 to 500	5 to 500	0.9930	0.9946
Azinphos-methyl	29.349	160.0 → 77.0	160.0 → 132.1	5 to 500	20 to 500	0.9950	0.9837
Azoxystrobin	37.058	344.1 → 329.0	344.1 → 171.9	5 to 500	10 to 500	0.9953	0.9986
Bentazone	20.364	119.0 → 92.0	198.0 → 119.0	10 to 500	20 to 500	0.9936	0.9995
Bifenthrin	28.326	181.2 → 165.2	181.2 → 166.2	1 to 500	1 to 500	0.9900	0.9998
Bitertanol	31.51	170.1 → 141.1	170.1 → 115.0	5 to 500	10 to 500	0.9979	0.9991
Boscalid	33.36	140.0 → 112.0	140.0 → 76.0	1 to 500	2 to 500	0.9940	0.9993
Buprofezin	23.764	104.0 → 51.0	104.0 → 77.0	10 to 500	10 to 500	0.9947	0.9993
Captan	21.419	151.0 → 80.0	149.0 → 79.1	50 to 500	100 to 500	0.9991	NA
Carbaryl	18.249	144.1 → 116.1	144.1 → 89.0	2 to 500	5 to 500	0.9909	0.9994
Chinomethionate (Oxythioquinox)	21.885	233.9 → 206.1	206.0 → 148.1	1 to 500	5 to 500	0.9950	0.9998
Chlorantraniliprole	28.337	277.8 → 215.0	277.8 → 248.8	10 to 500	50 to 500	0.9924	NA
Chlordane-cis	22.55	271.8 → 236.9	372.8 → 265.9	1 to 500	5 to 500	0.9978	0.9981
Chlordane-oxy	21.14	114.9 → 51.1	114.9 → 87.0	1 to 500	10 to 500	0.9976	0.9996
Chlordane-trans	21.986	271.7 → 236.9	372.8 → 265.8	1 to 500	10 to 500	0.9978	0.9998
Chlorfenvinphos	21.547	266.9 → 159.1	322.8 → 266.8	1 to 500	5 to 500	0.9941	0.9998
Chlorpropham	13.311	153.0 → 90.0	153.0 → 125.1	1 to 500	10 to 500	0.9996	0.9898
Chlorpyrifos	19.99	198.9 → 171.0	196.9 → 169.0	1 to 500	5 to 500	0.9931	0.9994
Chlorpyrifos-methyl	18.102	285.9 → 93.0	287.9 → 92.9	1 to 500	5 to 500	0.9900	0.9985
Clofentezine	5.28	136.7 → 102.0	138.7 → 102.0	1 to 500	1 to 500	1.0000	0.9993
Coumaphos	31.967	210.0 → 182.0	361.9 → 109.0	5 to 500	20 to 500	0.9925	0.9974
Cyfluthrin-1	32.788	226.0 → 206.0	198.9 → 170.1	2 to 500	5 to 500	0.9978	0.9977
Cyfluthrin-2	32.969	226.0 → 206.0	198.9 → 170.1	2 to 500	5 to 500	0.9962	0.9982
Cyfluthrin-3	33.118	226.0 → 206.0	198.9 → 170.1	5 to 500	10 to 500	0.9978	0.9965
Cyfluthrin-4	33.2	226.0 → 206.0	198.9 → 170.1	5 to 500	10 to 500	0.9960	0.9984
Cypermethrin-1	33.109	163.0 → 91.0	163.0 → 127.0	5 to 500	10 to 500	0.9972	0.9980

Compound Name	RT (min)	Transitions		Linearity Range (ng/mL)		R^2	
		Quant	Qualifier	Agilent 7010B	Agilent 7000C	Agilent 7010B	Agilent 7000C
Cypermethrin-2	33.197	163.0 → 91.0	163.0 → 127.0	5 to 500	10 to 500	0.9974	0.9977
Cypermethrin-3	33.371	163.0 → 127.0	163.0 → 91.0	2 to 500	10 to 500	0.9963	0.9981
Cypermethrin-4	33.564	163.0 → 91.0	163.0 → 127.0	2 to 500	10 to 500	0.9957	0.9968
Cyprodinil	20.899	225.2 → 224.3	224.2 → 208.2	1 to 500	1 to 500	0.9929	0.9996
Cyromazine	15.469	151.0 → 109.0	165.9 → 151.0	2 to 500	10 to 500	0.9900	0.9996
DDD-*o,p'*	23.715	235.0 → 165.2	237.0 → 165.2	1 to 500	20 to 500	0.9998	0.9981
DDD-*p,p'*	24.929	234.9 → 165.1	236.9 → 165.2	1 to 500	5 to 500	0.9998	0.9975
DDT-*o,p'*	25.037	235.0 → 165.2	237.0 → 165.2	1 to 500	1 to 500	0.9969	0.9998
DDT-*p,p'*	26.265	235.0 → 165.2	237.0 → 165.2	1 to 500	2 to 500	0.9963	0.9998
Deltamethrin	36.521	252.9 → 93.0	250.7 → 172.0	2 to 500	10 to 500	0.9934	0.9973
Demeton-S-methyl	12.7	88.0 → 60.0	142.0 → 78.9	5 to 500	10 to 500	0.9914	0.9979
Diazinon	16.415	137.1 → 84.0	137.1 → 54.0	1 to 500	5 to 500	0.9948	0.9984
Dichlofenthion	17.763	278.9 → 222.9	222.9 → 204.9	1 to 500	1 to 500	0.9933	0.9992
Dichloran	14.737	206.1 → 176.0	160.1 → 124.1	1 to 500	10 to 500	0.9953	0.9997
Dichlorvos	6.134	109.0 → 79.0	184.9 → 93.0	5 to 500	20 to 500	0.9978	0.9904
Dicrotofos	13.752	127.0 → 109.0	127.0 → 95.0	5 to 500	5 to 500	0.9965	0.9995
Dieldrin	23.382	262.9 → 193.0	277.0 → 241.0	2 to 500	10 to 500	0.9985	0.9983
Difenoconazole I	35.851	322.8 → 264.8	264.9 → 202.0	1 to 500	5 to 500	0.9942	0.9990
Difenoconazole II	35.979	322.8 → 264.8	264.9 → 202.0	1 to 500	2 to 500	0.9925	0.9992
Dimethipin	15.247	118.0 → 58.0	124.0 → 76.0	1 to 500	20 to 500	0.9974	0.9998
Dimethoate	14.846	87.0 → 46.0	142.9 → 111.0	2 to 500	10 to 500	0.9964	0.9996
Diphenylamine	12.696	169.0 → 168.2	168.0 → 167.2	1 to 500	1 to 500	0.9976	0.9977
Endosulfan I (*alpha* isomer)	22.42	194.9 → 159.0	194.9 → 125.0	2 to 500	20 to 500	0.9971	0.9947
Endosulfan II (*beta* isomer)	24.513	206.9 → 172.0	194.9 → 124.9	1 to 500	20 to 500	0.9967	0.9982
Endosulfan sulfate	26.03	271.9 → 237.0	273.8 → 238.9	1 to 500	1 to 500	0.9929	0.9998
Endrin	24.162	262.8 → 193.0	244.8 → 173.0	2 to 500	10 to 500	0.9932	0.9994
Ethion	25.192	230.9 → 129.0	230.9 → 175.0	1 to 500	5 to 500	0.9955	0.9987
Ethofenprox	33.918	163.0 → 107.1	163.0 → 135.1	1 to 500	1 to 500	0.9924	0.9999
Ethoprophos	12.985	157.9 → 97.0	157.9 → 114.0	1 to 500	5 to 500	0.9932	0.9982
Famoxadone	37.056	197.0 → 115.0	223.9 → 196.2	5 to 500	20 to 500	0.9957	0.9948
Fenamidone	28.623	238.0 → 237.2	268.0 → 180.2	1 to 500	5 to 500	0.9912	0.9996
Fenamiphos sulfone	27.887	319.8 → 292.0	171.0 → 107.0	10 to 500	10 to 500	0.9924	0.9999
Fenitrothion	19.165	277.0 → 260.1	277.0 → 109.0	1 to 500	10 to 500	0.9955	0.9970
Fenpropathrin	28.519	181.1 → 152.1	207.9 → 181.0	2 to 500	10 to 500	0.9931	0.9993
Fenpropimorph	19.979	128.1 → 70.1	128.1 → 110.1	1 to 500	5 to 500	0.9941	0.9992
Fensulfothion	24.771	291.8 → 156.0	291.8 → 108.8	2 to 500	5 to 500	0.9952	0.9957
Fenthion	19.899	278.0 → 109.0	278.0 → 169.0	1 to 500	5 to 500	0.9921	0.9991
Fenvalerate I	35.11	167.0 → 125.1	224.9 → 119.0	1 to 500	5 to 500	0.9945	0.9964
Fenvalerate II	35.512	167.0 → 125.1	224.9 → 119.0	1 to 500	5 to 500	0.9944	0.9965
Fipronil	21.642	366.8 → 212.8	368.8 → 214.8	1 to 500	10 to 500	0.9936	0.9997
Fipronil sulfide	21.379	351.0 → 254.9	420.0 → 350.9	1 to 500	5 to 500	0.9953	0.9998
Fipronil sulfone	23.961	382.8 → 254.9	384.8 → 256.8	1 to 500	5 to 500	0.9952	0.9992
Flusilazole	23.862	233.0 → 165.1	233.0 → 91.0	1 to 500	5 to 500	0.9908	0.9999
HCH-*alpha*	14.297	216.9 → 181.0	218.9 → 183.0	1 to 500	5 to 500	0.9992	0.9966

Compound Name	RT (min)	Transitions		Linearity Range (ng/mL)		R^2	
		Quant	Qualifier	Agilent 7010B	Agilent 7000C	Agilent 7010B	Agilent 7000C
HCH-*beta*	15.336	181.0 → 145.0	216.9 → 181.1	1 to 500	5 to 500	0.9990	0.9982
HCH-*delta*	16.495	181.1 → 145.1	217.0 → 181.1	1 to 500	5 to 500	0.9985	0.9987
HCH-*gamma*	15.562	181.0 → 145.0	216.9 → 181.0	1 to 500	5 to 500	0.9986	0.9958
Heptachlor	18.283	271.7 → 236.9	273.7 → 238.9	1 to 500	5 to 500	0.9960	0.9995
Heptachlor exo-epoxide	21.098	352.8 → 262.9	354.8 → 264.9	2 to 500	20 to 500	0.9938	1.0000
Hexachlorobenzene	14.561	283.8 → 213.9	283.8 → 248.8	2 to 500	100 to 500	0.9996	NA
Isopyrazam	31.01	159.0 → 42.1	159.0 → 139.0	2 to 500	5 to 500	0.9914	0.9996
Malathion	19.646	126.9 → 99.0	172.9 → 99.0	1 to 500	5 to 500	0.9950	0.9997
Mecarbam	21.625	158.9 → 131.0	130.9 → 74.0	5 to 500	20 to 500	0.9955	0.9997
Methacrifos	10.43	207.9 → 180.1	207.9 → 93.0	1 to 500	1 to 500	0.9979	0.9967
Methamidophos	5.839	141.0 → 95.0	141.0 → 79.0	1 to 500	10 to 500	0.9944	0.9964
Methidathion	22.09	144.9 → 85.0	144.9 → 58.1	1 to 500	2 to 500	0.9911	0.9992
Metrafenone	30.979	208.9 → 166.0	394.8 → 364.8	5 to 500	10 to 500	0.9945	0.9996
Novaluron	6.46	168.0 → 75.9	168.0 → 139.9	5 to 500	50 to 500	0.9926	NA
Oxamyl	11.015	162.0 → 114.9	98.0 → 58.0	5 to 500	20 to 500	0.9928	0.9999
Parathion	20.005	139.0 → 109.0	290.9 → 109.0	1 to 500	10 to 500	0.9904	0.9981
Pentachloronitrobenzene	15.761	295.0 → 237.0	236.9 → 142.9	1 to 500	10 to 500	0.9967	0.9992
Permethrin, (1R)-*cis*-	31.605	183.1 → 168.1	183.1 → 153.0	5 to 500	10 to 500	0.9959	0.9996
Permethrin, (1R)-*trans*-	31.854	183.1 → 168.1	183.1 → 153.0	5 to 500	10 to 500	0.9954	0.9995
Phenthoate	21.659	273.7 → 121.0	273.7 → 124.9	1 to 500	10 to 500	0.9962	0.9995
Phorate	14.199	260.0 → 75.0	230.9 → 128.9	2 to 500	10 to 500	0.9926	0.9985
Phorate sulfone	19.757	124.9 → 96.9	153.0 → 97.0	2 to 500	5 to 500	0.9919	0.9988
Phosalone	29.381	182.0 → 111.0	182.0 → 102.1	1 to 500	5 to 500	0.9915	0.9962
Phosmet	27.966	160.0 → 77.1	160.0 → 133.1	1 to 500	10 to 500	0.9951	0.9925
Pirimicarb	17.371	166.0 → 55.1	238.0 → 166.2	1 to 500	2 to 500	0.9930	0.9991
Pirimiphos-methyl	19.304	290.0 → 125.0	232.9 → 151.0	1 to 500	5 to 500	0.9941	0.9993
Prochloraz	32.089	195.9 → 96.9	180.0 → 138.0	2 to 500	20 to 500	0.9981	0.9987
Profenofos	23.298	207.9 → 63.0	338.8 → 268.7	1 to 500	10 to 500	0.9932	0.9998
Propanil	17.7	161.0 → 99.0	161.0 → 90.0	1 to 500	5 to 500	0.9935	0.9997
Propiconazole	26.158	172.9 → 145.0	172.9 → 74.0	5 to 500	5 to 500	0.9951	0.9997
Prothiofos	23.187	266.9 → 239.0	308.9 → 238.9	1 to 500	5 to 500	0.9910	0.9997
Pyraclostrobin	35.179	132.0 → 104.0	132.0 → 77.1	10 to 500	20 to 500	0.9913	0.9993
Pyrimethanil	16.152	198.0 → 118.1	198.0 → 183.1	1 to 500	5 to 500	0.9960	0.9984
Pyriproxyfen	29.613	136.1 → 78.1	136.1 → 96.0	1 to 500	1 to 500	0.9969	0.9997
Quinalphos	21.626	146.0 → 118.0	146.0 → 91.0	1 to 500	5 to 500	0.9953	0.9995
Quinoxyfen	26.03	271.9 → 237.1	237.0 → 208.1	1 to 500	1 to 500	0.9925	0.9998
Ronnel	18.642	285.0 → 269.9	286.9 → 272.0	1 to 500	2 to 500	0.9908	0.9992
Spirodiclofen	31.549	109.1 → 81.1	109.1 → 79.1	10 to 500	20 to 500	0.9975	0.9990
sulfoxaflor	12.695	173.7 → 104.1	173.7 → 154.0	2 to 500	20 to 500	0.9976	0.9933
Terbufos	15.855	230.9 → 175.0	230.9 → 129.0	1 to 500	5 to 500	0.9949	0.9988
Terbufos sulfone	21.215	153.0 → 97.0	198.9 → 96.9	1 to 500	5 to 500	0.9960	0.9994
Tetradifon	29.016	158.9 → 131.0	226.9 → 199.0	1 to 500	5 to 500	0.9970	0.9998
Thiabendazole	21.22	201.0 → 174.0	201.9 → 175.0	1 to 500	5 to 500	0.9943	0.9985
Triadimefon	20.098	208.0 → 181.1	208.0 → 111.0	1 to 500	5 to 500	0.9929	0.9996
Triazophos	25.643	161.2 → 134.2	161.2 → 106.1	2 to 500	5 to 500	0.9901	0.9993

NA: This compound has fewer than five calibration levels, so the R^2 values were not calculated.

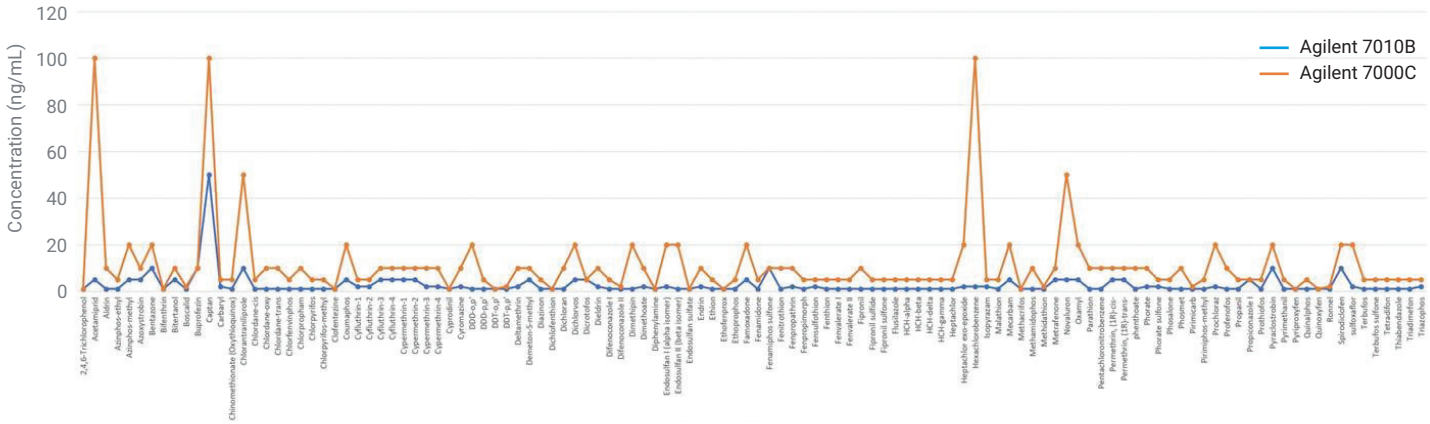

Figure 1. Low calibration limit achieved by the Agilent 7890B/7000C and Agilent 8890/7010B triple quadrupole systems.

Figure 2 demonstrates the statistical results of a low calibration limit by the two systems. Among the 118 pesticides, 13.5% of the compounds had the same low calibration limit on the 7010B and the 7000C; 28% had a low calibration limit 2 to 4 times lower on 7010B than on 7000C; 39.0% had a low calibration limit 5 times lower on 7010B than on 7000C; 15.3% of the compounds had a low calibration limit 10 times lower on 7010B than on 7000C; 4.2% of the compounds had a low calibration limit 20 times lower on 7010B than on 7000C.

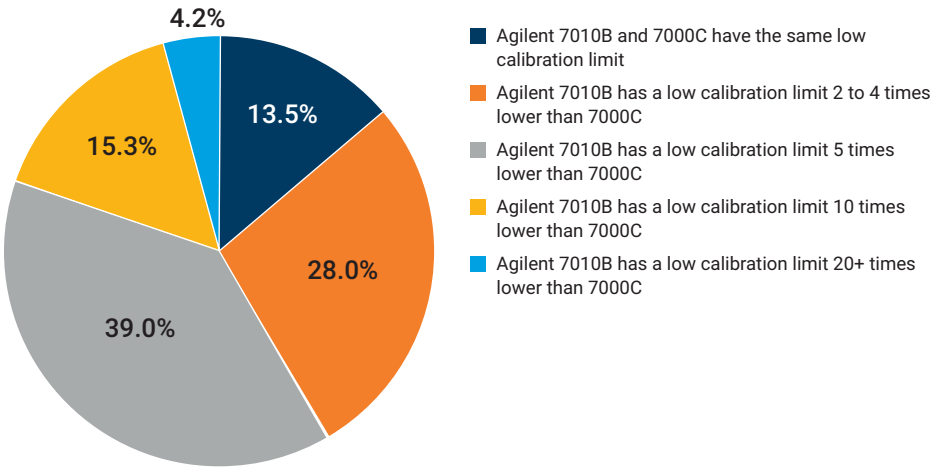

Figure 2. Statistical results of low calibration limit by the Agilent 7890B/7000C and the Agilent 8890/7010B triple quadrupole.

For most pesticides, the 7010 system achieved a lower calibration level with better peak shape and signal-to-noise ratio (S/N) at the low concentrations. As shown in Figures 3 and 4, for chlordane-oxy, S/N at 10 ng/mL was 32.0 with the HES source, and 7.4 with the extractor source. S/N for phosmet at 10 ng/mL was 23.1 with the HES source, and 5 with the extractor source. A good qualifier/quantifier ratio for the two compounds were maintained at the level of 10 ng/mL. Better peak shape and lower noise were observed on the HES source. Table 2 also lists the correlation coefficient for each pesticide on both 7010B and 7000C systems. Linearity across the range studied gave R^2 values of 0.99 or greater for all compounds on the two systems except for azinphos-methyl and chlorpropham on the 7000C system.

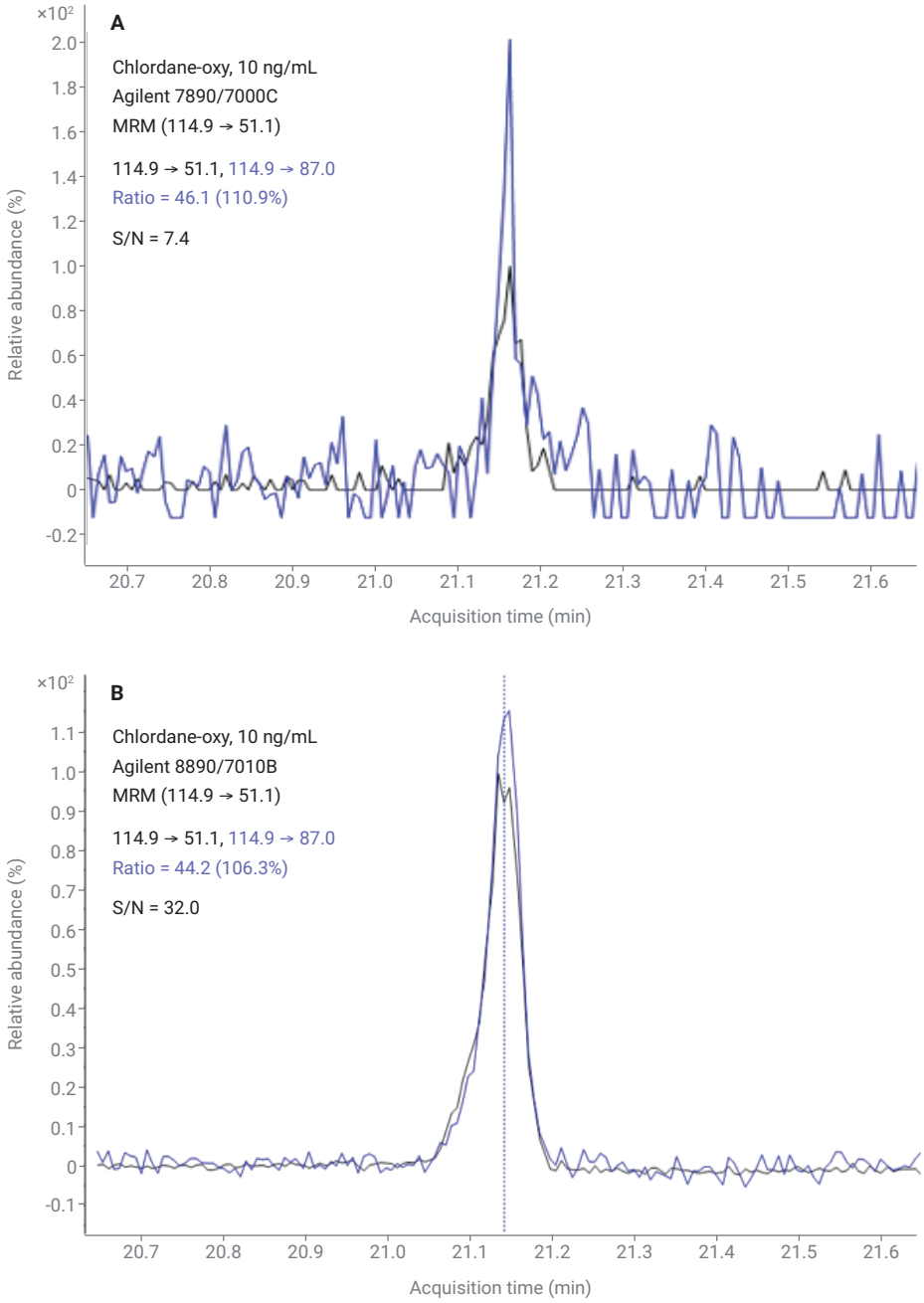

Figure 3. MRM chromatograms for quantifier and qualifier for chlordane-oxy.

Conclusion

The 7890B/7000C and 8890/7010B triple quadrupole GC/MS systems were investigated for response linearity ranges and detection limits of multiresidue pesticides in milk. For the 118 pesticides analyzed in this study the 8890/7010B triple quadrupole GC/MS system with the HES source showed the best performance for ultra-trace level analysis with detection of almost 60% of pesticides down to 1 ng/mL.

References

1. GB 2763-2019 National food safety standard—Maximum residue limits for pesticides in food: https://www.codeofchina.com/standard/GB2763-2019.html.
2. Yang, X.; Zhang, Y. J.; Zhang Z. M. Analysis of Multiclass Multiresidue Pesticides in Milk Using Agilent Captiva EMR— Lipid with LC/MS/MS and GC/MS/MS, *Agilent Technologies application note*, publication number 5994-2038EN, **2020**.
3. Andrianova, A. A.; Westland, J. L.; Quimby, B. D. Quantitation of Pesticides in Strawberries at Tolerance Levels Established by the US EPA. *Agilent Technologies application note*, publication number 5994-0799EN, **2019**.

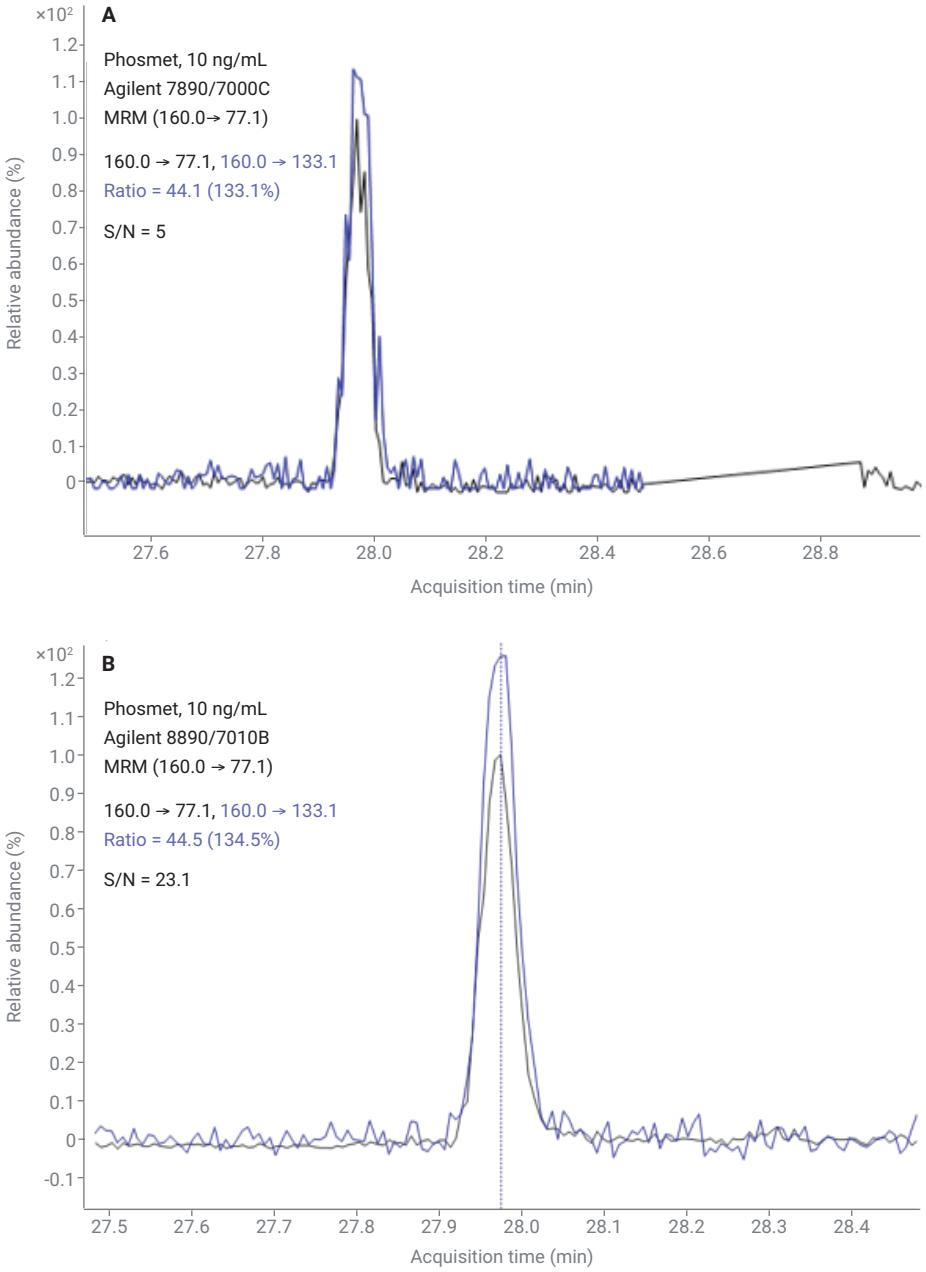

Figure 4. MRM chromatograms for quantifier and qualifier for phosmet.

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Application Brief

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Use of Salt to Increase Analyte Concentration in SPME Headspace Applications

Author

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Abstract

Static headspace gas chromatography is one of the most frequently used techniques for the analysis of flavor components in foods and beverages. Samples must be prepared to maximize the concentration of the volatile components in the headspace and minimize unwanted contamination from other compounds in the sample matrix. The use of solid phase microextraction (SPME) allows for a fast, solvent-less, selective analysis of the headspace compounds. The addition of salt to the sample matrix will often lower the partitioning coefficient (K) for some target analytes, thus increasing the concentration of analytes in the headspace, which is the key advantage of this methodology.

Experimental

Amount of salt

The magnitude of the salting-out effect on K is not the same for all compounds. Compounds with K values that are already relatively low will experience little change in partition coefficient after adding a salt to an aqueous sample matrix. The addition of salt, however, will assist by lowering the compounds with higher K values and increase their concentration in the headspace. Each application is different. As a rule, the amount of salt added should be enough to saturate the sample (20 to 40% wt/wt salt/sample ratio). Saturation will maintain the same ionic strength from sample-to-sample and ensure reproducibility.

For example, water salinity is 35 g/L, which equates to 3.5 g in 10 mL of sample. In this case, 4 g (±0.5 g) of salt to a 10 mL water-based sample will ensure that enough salt has been added to saturate the sample.

Type of salt

Sodium chloride (NaCl) is the most used salt to adjust ionic strength. However, other salts such as ammonium chloride (NH_4Cl), sodium sulfate (Na_2SO_4), or sodium hydroxide (NaOH) may have different salting out capabilities, particularly when dealing with complex matrices such as food. It is important to note that while salt may improve the SPME extraction of the desired analytes, it could also cause co-extraction of more matrix interferences or undesired compounds.

Method

Guaiacol and 4-methylguaiacol are main target compounds implicated in smoke-affected grapes and wines. The use of the DVB/carbon WR/PDMS SPME phase was chosen due to its selective extraction of odor and flavor compounds.

Sample preparation

- 20 mL headspace vial and cap (part numbers 5188-6537 and 5188-2759)
- 10 mL sample with 4 g of NaCl
- Samples (n = 5) spiked at 50 ppb
- Agilent SPME Arrow DVB/carbon WR/PDMS, 1.10 mm, 120 µm (part number 5191-5861)

Table 1. SPME headspace parameters.

Parameter	Setting
Predesorption Time	3 min
Predesorption Temperature	250 °C
Incubation Time	5 min
Heatex Stirrer Speed	1,000 rpm
Heatex Stirrer Temperature	40 °C
Sample Extract Time	10 min
Sample Desorption Time	3 min

An Agilent PAL3 autosampler with robotic tool change (RTC) was installed on an Agilent 8890 GC system with an Agilent 7000D triple quadrupole GC/MS. The SPME headspace parameters, GC method settings, and MS conditions are listed in Tables 1, 2, and 3, respectively.

Results and discussion

The increase of response of smoke impact volatiles is seen with the addition of 4 g of NaCl. Figure 1 shows the TIC scan of multiple smoke impact compounds when analyzed with and without the addition of NaCl. Figures 2 and 3 show the area differences of guaiacol and 4-methylguaiacol by analyzing their MRM transitions. Table 4 provides the area counts for both guaiacol and 4-methylguaiacol with and without the addition of NaCl.

Table 2. Agilent 8890 GC settings.

Parameter	Setting
Inlet Liner	Agilent Ultra Inert inlet liner, splitless, straight, 0.75 mm id, recommended for SPME injections (p/n 5190-4048)
Injection Mode, Temperature	Splitless, 250 °C
Control Mode	Constant flow (1.2 mL/min)
Column	Agilent J&W DB-HeavyWAX GC column, 30 m × 0.25 mm, 0.25 µm (p/n 122-7132)
Oven Program	120 °C (hold 1 min); 10 °C/min to 250 °C (hold 0 min); 60 °C/min to 280 °C (hold 0 min)

Table 3. Agilent 7000D triple quadrupole GC/MS conditions.

Parameter	Setting
Transfer Line	280 °C
Acquisition Mode	dMRM
Solvent Delay	3.0 min
Tune File	Atune.eiex
Gain	10
MS Source Temperature	280 °C
MS Quadrupole Temperature	150 °C

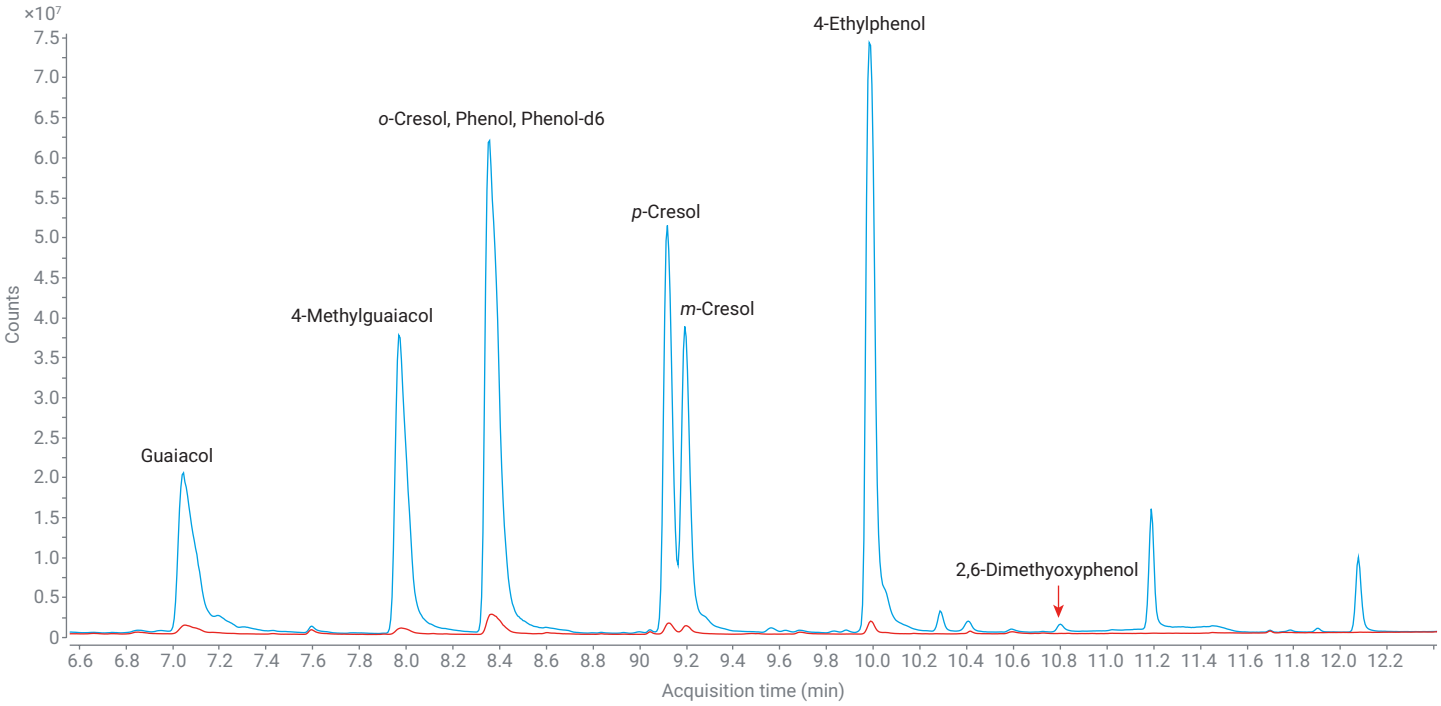
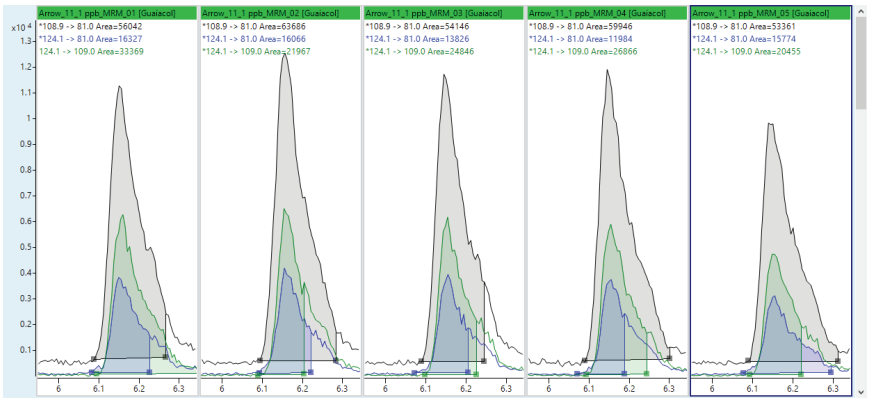


Figure 1. TIC scan of smoke impact compounds at 50 ppb extracted with the Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (p/n 5191-5861). The red trace indicates standards that were run without salt, and the blue trace indicates standards that were run with 4 g NaCl.

A) 1 ppb guaiacol with no NaCl



B) 1 ppb guaiacol with 4 g NaCl

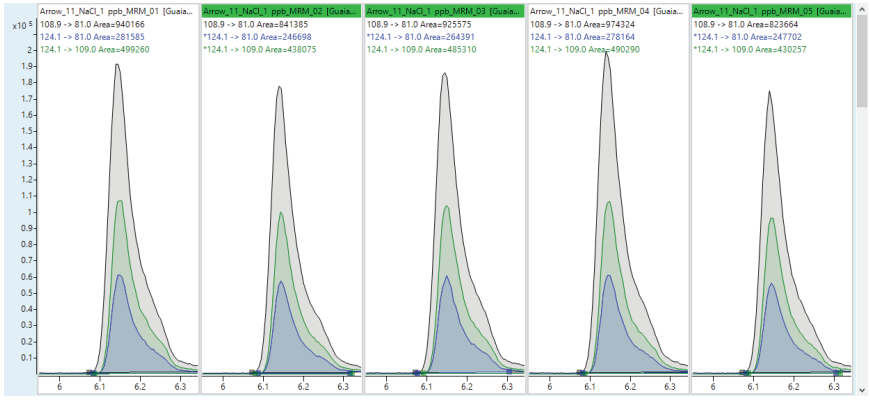
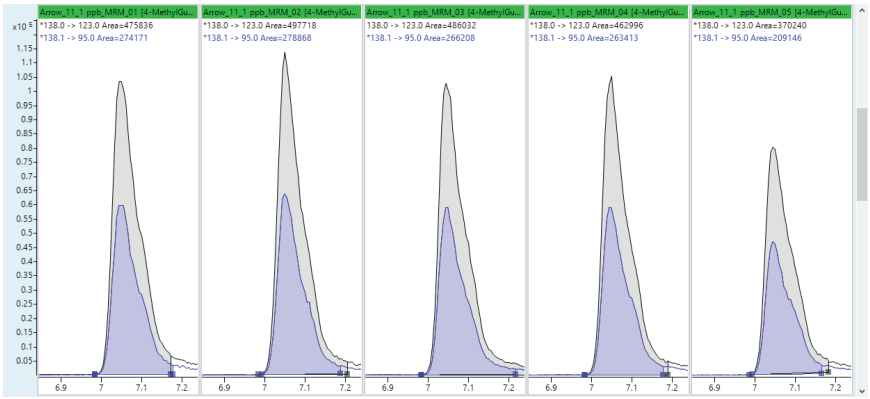


Figure 2. MRM comparison with area counts for 1 ppb guaiacol replicates with A) no addition of salt and B) 4 g NaCl. Extracted with the Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (p/n 5191-5861).

A) 1 ppb 4-methylguaiacol with no NaCl



B) 1 ppb 4-methylguaiacol with 4 g NaCl

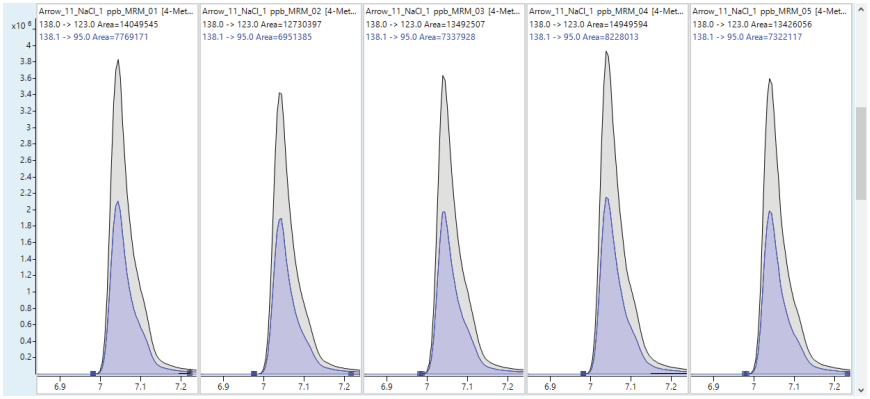
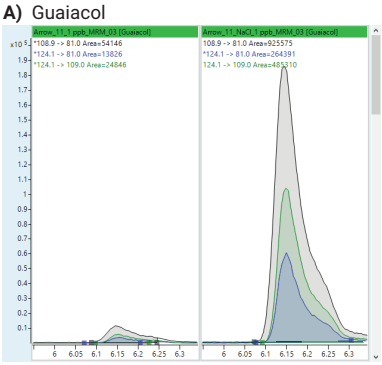


Figure 3. MRM comparison with area counts for 1 ppb 4-methylguaiacol replicates with A) no addition of salt and B) 4 g NaCl. Extracted with the Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (p/n 5191-5861).

A) Guaiacol



B) 4-Methylguaiacol

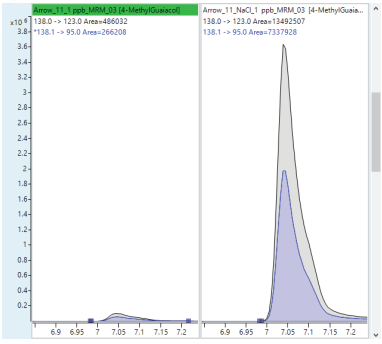


Figure 4. SPME comparison of wine impact compounds with and without NaCl for A) guaiacol and B) 4-methylguaiacol.

Table 4. Area counts of 1 ppb guaiacol and 4-methylguaiacol extracted with the Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (p/n 5191-5861).

Compound	Amount of NaCl	Replicate 01	Replicate 02	Replicate 03	Replicate 04	Replicate 05	% RSD
Guaiacol	0 g	56,042	63,686	54,146	59,946	53,361	7.04
	4 g	940,166	841,385	925,575	974,324	823,664	6.50
4-Methylguaiacol	0 g	475,836	497,718	486,032	462,996	370,240	10.67
	4 g	14,049,545	12,730,397	13,492,507	14,949,594	13,426,056	5.40

Conclusion

With the addition of NaCl to saturation, there is an average of 95% increase in response for the target compounds implicated in smoke-affected grapes and wines.

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Analysis of Free Volatile Phenols in Smoke-Impacted Wines by SPME

Authors

Jessica Westland and Vanessa Abercrombie

Agilent Technologies, Inc.

Abstract

Ever since the 2003 wildfires in Australia and British Columbia, smoke impact has been a global concern for wine production.[1] With the increase in wildfires over various regions around the globe, many growers and wineries continue to worry about smoke impact in grapes and their wine. Agilent has developed a solid phase microextraction (SPME) gas chromatography/mass spectrometry (GC/MS) method to analyze the free-form volatile phenols associated with smoke impact. The Agilent SPME-GC/MS/MS method for the analysis of free-form volatile phenols associated with smoke impact allows for confident identification and reliable quantitation.

Introduction

Research has shown that smoke compounds can be absorbed by vines and grapes causing off-flavors in wines. While there is strong evidence that these compounds are mostly present in grapes and juice as nonvolatile forms, analysis of their free fraction has been used as a tool for screening grapes and assessing impacts in wines.[2] In the wine making process, the growth and maturation of the grape is arguably the most important step. During the period of veraison, acid concentration decreases, and sugar concentration increases while aromatic and flavor compounds start to develop. There are many external factors, weather conditions being the most influential, that determine when grapes have matured and are ready for harvest. Other environmental conditions, unrelated to temperature, such as smoke from nearby fires, can have a large and negative impact on the sensory quality of the wine.[3]

Guaiacol and 4-methylguaiacol have been identified as the primary volatile aromatics that contribute to the undesirable smoke impact characteristic. While aging wine in oak barrels can also contribute to the concentration of guaiacol and 4-methylguaiacol, the ratio of these two compounds will differ. Smoke-impacted berries contain almost four times as much guaiacol as 4-methylguaiacol.[2] The aroma contributed by oak barrels will be perceived as smoke and char. In contrast, when the two compounds are present due to smoke impact, it will be more reminiscent of campfires and ashtrays, which is not desirable in wine.

Detection limits for the analysis of smoke impact compounds must be sensitive enough to detect below 1 ppb, which is why selected ion monitoring (SIM) or multiple reaction monitoring (MRM) are commonly used in GC/MS analyses. Direct analysis of wine can be challenging because of the sugars, organic acids, and other aromatic compounds with higher retentions. To simplify the extraction and analysis of these volatiles, SPME has become the extraction method of choice. Its popularity for use stems from its operational simplicity, suitability for automation, reduced use of organic solvents, and direct thermal desorption into a gas chromatograph.

Experimental

Target volatiles

The main volatile phenols in smoke, guaiacol and 4-methylguaiacol, are useful markers of smoke impact in wines. Their respective concentrations correlate with the degree of perceived smoke impact, particularly in wines not exposed to toasted oak. However, they are not the only two compounds that are found and analyzed in smoke-affected wines, Table 1 lists the target free form volatile phenols that were analyzed in this experiment.

Table 1. Target free form volatile phenols.

CAS Number	Compound
74495-69-5	Guaiacol-d3
90-05-1	Guaiacol
93-51-6	4-Methylguaiacol
95-48-7	o-Cresol
13127-88-3	Phenol-d6
108-95-2	Phenol
95-87-4	2,5-Xylenol
2785-89-9	4-Ethylguaiacol
90-00-6	2-Ethylphenol
108-68-9	3,5-Xylenol
106-44-5	p-Cresol
108-39-4	m-Cresol
123-07-9	4-Ethylphenol
91-10-1	2,6-Dimethoxyphenol

Method

Sample preparation:

- 20 mL headspace vial and cap (part numbers 5188-6537 and 5188-2759)
- 10 mL sample with 4 g NaCl (Figure 1)
  - Addition of NaCl to saturation increases response for target compounds in smoke-affected grapes and wine by an average of 95%[4]

Water Wine

Figure 1. 20 mL amber headspace vials with water and wine samples.

- Samples spiked with calibrators and/or internal standards (ISTDs)
  - ISTDs spiked in at 10 ppb
- Agilent SPME Arrow DVB/carbon WR/PDMS, 1.10 mm, 120 µm (part number 5191-5861)
  - DVB/carbon WR/PDMS SPME phase was chosen for its selective extraction of odor and flavor compounds
  - SPME Arrow was used because of its significant benefit in extraction efficiency due to its larger sorption phase volume, compared to a traditional SPME fiber[5]

An Agilent PAL3 autosampler with robotic tool change (RTC) was installed on an Agilent 8890 GC system with an Agilent 7000D triple quadrupole GC/MS. The SPME headspace parameters, GC method settings, and MS conditions are listed in Tables 2, 3, and 4, respectively. Table 5 provides the MRM transitions used for GC/MS/MS analysis.

Table 2. SPME headspace parameters.

Parameter	Setting
Predesorption Time	3 min
Predesorption Temperature	250 °C
Incubation Time	5 min
Heatex Stirrer Speed	1,000 rpm
Heatex Stirrer Temperature	40 °C
Sample Extract Time	10 min
Sample Desorption Time	3 min

Table 3. Agilent 8890 GC settings.

Parameter	Setting
Inlet Liner	Agilent Ultra Inert inlet liner, splitless, straight, 0.75 mm id, recommended for SPME injections (p/n 5190-4048)
Injection Mode, Temperature	Splitless, 250 °C
Control Mode	Constant flow (1.2 mL/min)
Column	Agilent J&W DB-HeavyWAX GC column, 30 m × 0.25 mm, 0.25 µm (p/n 122-7132)
Oven Program	120 °C (hold 1 min); 10 °C/min to 250 °C (hold 0 min); 60 °C/min to 280 °C (hold 0 min)

Table 4. Agilent 7000D triple quadrupole GC/MS conditions.

Parameter	Setting
Transfer Line	280 °C
Acquisition Mode	dMRM
Solvent Delay	3.0 min
Tune File	Atune.eiex
Gain	10
MS Source Temperature	280 °C
MS Quadrupole Temperature	150 °C

Table 5. MRM transitions for free form volatile phenols.

CAS Number	Compound	Precursor Ion (*m/z*)	Product Ion (*m/z*)	CE (V)
74495-69-5	Guaiacol-d3	124.1	109	15
		124.1	81	15
90-05-1	Guaiacol	127	109	15
		126.9	109	15
93-51-6	4-Methylguaiacol	138.1	95	15
		138	123	15
95-48-7	*o*-Cresol	108.1	107.1	15
		107.1	77	15
13127-88-3	Phenol-d6	99.1	71	10
		71	69	10
108-95-2	Phenol	94	66	10
		66	65	10
95-87-4	2,5-Xylenol	122	107	15
		122	94	15
2785-89-9	4-Ethylguaiacol	152	137	15
		137.1	122	15
90-00-6	2-Ethylphenol	122.1	107.1	15
		107.1	77	15
108-68-9	3,5-Xylenol	121.1	107.1	15
		121.1	77	15
106-44-5	*p*-Cresol	108.1	107.1	15
		107.1	77	15
108-39-4	*m*-Cresol	108.1	107.1	15
		107.1	77	15
123-07-9	4-Ethylphenol	122.1	107	15
		108.1	78	15
91-10-1	2,6-Dimethoxyphenol	154	139	15
		139.1	83	15

Results and discussion

Calibration

Blanks are important for quality control and robust quantitative analytical methods. In this experiment, Milli-Q (18.2 Ω) water was used as a blank to simulate a clean matrix without any interferences. However, since wine includes many components that can affect the measurement of the target analytes, white wine was used as a matrix blank.

Table 6 provides the calibration ranges and linearity values for the target free form volatiles when calibrated in Milli-Q water. Figure 2 shows guaiacol and 4-methylguaiacol Milli-Q water calibration curves together.

To account for matrix effects in quantitating guaiacol and 4-methylguaiacol, a bag-in-a-box white wine was chosen. The reasons this matrix was chosen were:

- The skins, where smoke impact compounds reside, are separated from the juice before the fermentation process.
- It is an unspecified blend, which represents a broader matrix.
- The packaging removes the exposure of oak and cork from the wine.

Table 6. Agilent 7000D triple quadrupole GC/MS calibration range and R^2 in Milli-Q water.

Compound	Calibration Range (ppb)	R^2
Guaiacol	0.2 to 50.3	0.999
4-Methylguaiacol	0.1 to 25	0.999
o-Cresol	0.2 to 50	0.996
Phenol	0.5 to 125.5	0.997
2,5-Xylenol	0.1 to 25	0.998
4-Ethylguaiacol	0.1 to 25	0.998
2-Ethylphenol	0.03 to 7.5	0.995
3,5-Xylenol	0.1 to 5	0.998
p-Cresol	0.1 to 25	0.997
m-Cresol	0.1 to 25	0.998
4-Ethylphenol	0.1 to 25	0.998
2,6-Dimethoxyphenol	0.1 to 25	0.998*

* Type = quadratic, origin = force; weight = 1/x.

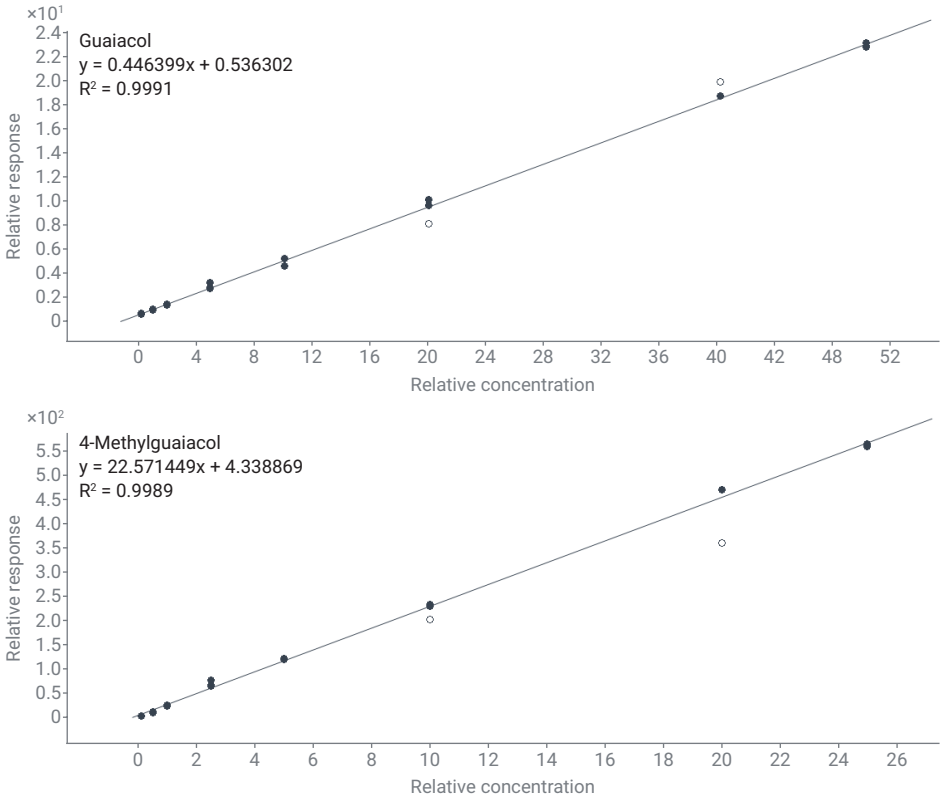

Figure 2. Calibration curves for guaiacol and 4-methylguaiacol in Milli-Q water.

Table 7 provides the calibration ranges and linearity values for the target free form volatiles when calibrated in the bag-in-a-box white wine. Figure 3 shows guaiacol and 4-methylguaiacol white wine calibration curves together.

Table 7. Agilent 7000D triple quadrupole GC/MS calibration range and R^2 in white wine.

Compound	Calibration Range (ppb)	R^2
Guaiacol	0.2 to 50.3	0.993
4-Methylguaiacol	0.1 to 25	0.996
o-Cresol	0.2 to 50	0.996
Phenol	0.5 to 125.5	0.997
2,5-Xylenol	0.1 to 25	0.996
4-Ethylguaiacol	0.1 to 25	0.996
2-Ethylphenol	0.03 to 7.5	0.995
3,5-Xylenol	0.1 to 5	0.998
p-Cresol	0.1 to 25	0.995
m-Cresol	0.1 to 25	0.995
4-Ethylphenol	0.1 to 25	0.996
2,6-Dimethoxyphenol	0.1 to 25	0.995

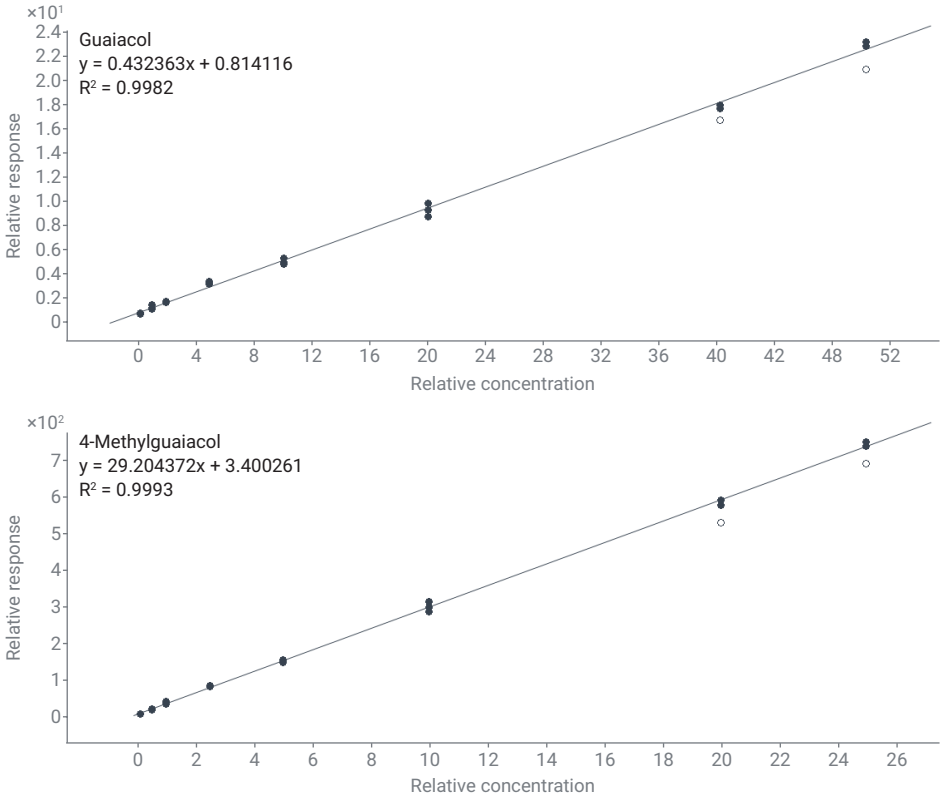

Figure 3. Calibration curves for guaiacol and 4-methylguaiacol in white wine.

Quantitation of smoke impact markers

In grapes not exposed to smoke, levels of 0.1 to 0.3 ppb for both guaiacol and 4-methylguaiacol can be observed. Guaiacol levels above 1 ppb could suggest exposure to smoke, and levels of guaiacol in smoke-exposed grapes have been as high as 55 ppb. On average, a ratio of 3.7/1 guaiacol/4-methylguaiacol is observed in undesirable smoke-impacted grapes and wine.[2]

Guaiacol and 4-methylguaiacol levels in all wine samples and the white wine blank, signals were quantitated based on the Milli-Q water calibration curve (Table 8). No sample had a quantitative level of 4-methylguaiacol.

Target free form volatile phenols were quantitated by white wine calibration from three replicates of each red wine sample (Table 9). Note that 4-methylguaiacol and 3,5-xylenol were below limit of quantitation (LOQ) for all samples, and therefore are not included in the table. The slight decrease in concentration of guaiacol from the Milli-Q water calibration to the white wine calibrations (standard deviation = 0.82 and RSD = 9.35%) indicates the matrix effects that wine has on the quantitation.

Conclusion

Consumers tend to respond negatively to smoke-affected wines. Since there are no effective ways to remove smoke compounds from grapes or wines, smoke impact can be a major problem for a vineyard. This contamination can be a significant financial impact for the grape-grower, as no harvest would mean no income. There is also a reputational risk, not only for the grape-grower but for the region.[6] The Agilent SPME-GC/MS/MS method for the analysis of free-form volatile phenols associated with smoke impact allows for confident identification and reliable quantitation.

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Table 8. Guaiacol levels identified in wine matrices.

Guaiacol	Franzia White Wine	Franzia Red Wine	CA Pinot Noir	OR Pinot Noir	Red Wine Sample
Average Concentration, n = 3 (ppb)	0.64	6.74	10.27	5.16	9.15
Standard Deviation	0.33	0.65	1.17	0.42	0.80
% RSD	51.80	9.57	11.40	8.13	8.72

Table 9. Average concentration (ppb) of targets identified in red wine samples.

Sample	Guaiacol	*o*-Cresol	Phenol	2,5-Xylenol	4-Ethylguaiacol	2-Ethylphenol	*p*-Cresol	*m*-Cresol	4-Ethylphenol	2,6-Dimethoxyphenol
Franzia Red Wine	6.32	0.41	2.73	<LOQ	0.09	<LOQ	1.61	0.38	< LOQ	0.77
CA Pinot Noir	9.97	1.90	5.58	0.23	0.22	0.01	0.75	0.68	0.08	1.05
OR Pinot Noir	4.68	2.05	6.20	16.23	10.81	<LOQ	1.73	1.44	24.81	0.60
Red Wine Sample	8.81	5.70	16.35	<LOQ	<LOQ	0.03	4.61	2.30	0.16	0.57

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Analysis of 1,4-dioxane in Water by Purge and Trap and Triple Quadrupole GC/MS

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Abstract

1,4-dioxane is a likely human carcinogen and has been found in groundwater at multiple sites throughout the United States. The physical and chemical properties and behavior of 1,4-dioxane create challenges for characterization and treatment. While it is relatively short-lived in the atmosphere (1 to 3-day half-life), 1,4-dioxane is highly mobile and may leach readily from soil to groundwater, where it has a long lifetime as it does not readily biodegrade in the environment.

This study uses a Teledyne Tekmar Atomx XYZ purge and trap system coupled to an Agilent 7010B Mass Spectrometer (MS) system in dynamic multiple reaction monitoring (dMRM) mode. Agilent MassHunter software created a working linear calibration curve and method detection limits (MDLs) for 1,4-dioxane.

The Agilent 7010B triple quadrupole GC/MS is the most sensitive version of the Agilent compact benchtop triple quadrupole (MS/MS) systems, providing attogram-level detection limits in electron ionization (EI) mode. The breakthrough in sensitivity allows for the optimization of sample preparation, reduces maintenance cycles by injecting less, and achieves new detection limits.

The Atomx XYZ is Teledyne Tekmar's most advanced purge and trap system and is based on the time-tested Atomx instrument platform. The concentrator's efficient trap cooling design reduces sample cycle time by as much as 14% over the previous model. Combined with its 84-position soil and water autosampler, the result is more samples tested per 12-hour period. An innovative moisture control system (MCS) improves water vapor removal by as much as 60%, thereby reducing peak interference and increasing GC column lifespan. In addition to other refinements, the Atomx XYZ incorporates a precision-machined valve manifold block to reduce potential leak sources and ensure that the system is both reliable and robust.

Current methodology for the analysis of 1,4-dioxane in water is limited by poor purging efficiency, which causes elevated detection limits. However, due to the low µg/L guidelines established across the country (Table 1), modifications to existing sample preparation procedures and more sensitive instrumentation is required to achieve faster turnaround times and lower levels of detection for 1,4-dioxane.

Introduction

1,4-dioxane is found in many locations due to its widespread use as a stabilizer in certain chlorinated solvents, paint strippers, greases, and waxes. Additionally, it is a byproduct present in many goods, including paint strippers, dyes, greases, antifreeze, aircraft deicing fluids, and in some consumer products. 1,4-Dioxane is also used as a purifying agent in the manufacture of pharmaceuticals and is a byproduct in the manufacture of polyethylene terephthalate (PET).

Because of the widespread prevalence of 1,4-dioxane as a contaminant in ground and drinking water and its potentially harmful effects therein, 1,4-dioxane is included on the fourth drinking water contaminant candidate list and is included in the Third Unregulated Contaminant Monitoring Rule (EPA 2009; EPA 2016a). EPA risk assessments indicate that the drinking water concentration representing a 1×10^{-6} cancer risk level for 1,4-dioxane is 0.35 µg/L (EPA IRIS 2013). While no federal maximum contaminant level (MCL) for drinking water has been established (EPA 2012), various states have established drinking water and ground water guidelines (Table 1).

Table 1. Drinking water and ground water guidelines established.

State	Guideline (µg/L)	Source
Alaska	77	AL DEC 2016
California	1.0	Cal/EPA 2011
Colorado	0.35	CDPHE 2017
Connecticut	3.0	CTDPH 2013
Delaware	6.0	DE DNR 1999
Florida	3.2	FDEP 2005
Indiana	7.8	IDEM 2015
Maine	4.0	MEDEP 2016
Massachusetts	0.3	MADEP 2004
Mississippi	6.09	MS DEQ 2002
New Hampshire	0.25	NH DES 2011
New Jersey	0.4	NJDEP 2015
North Carolina	3.0	NCDENR 2015
Pennsylvania	6.4	PADEP 2011
Texas	9.1	TCEQ 2016
Vermont	3.0	VTDEP 2016
Washington	0.438	WA ECY 2015
West Virginia	6.1	WV DEP 2009

1,4-Dioxane is a clear volatile liquid used primarily as a solvent and is subject to federal and state regulations and reporting requirements. 1,4-Dioxane has been reportable as a Toxics Release Inventory (TRI) chemical under Section 313 of the Emergency Planning and Community Right-to-Know Act (EPCRA) since 1987. It is designated as a Hazardous Air Pollutant (HAP) under the Clean Air Act (CAA), and is a hazardous substance under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). It was listed on the Safe Drinking Water (SDWA) Candidate Contaminant List (CCL) and identified in the third Unregulated Contaminant Monitoring Rule (UCMR3).

There have been several methods developed to test for 1,4-dioxane, primarily for soil and water. None of these methods meet the requirements to accurately detect low levels of 1,4-dioxane in water at ppt levels without extensive sample cleanup, requiring the need for the development of a testing method using purge and trap and GC/TQ technologies.

Drinking water analysis of Volatile Organic Compounds (VOCs) is performed using purge and trap concentration, following standard US EPA methods. This application modifies the purge and trap and gas chromatograph/mass spectrometer (GC/MS) parameters to create a robust method to detect 1,4-dioxane at the part-per-trillion level (ppt), despite its poor purge efficiency.

The quantitation of the target analyte 1,4-dioxane is performed by adding 1,4-dioxane-d_8 as an internal standard to all samples, controls, and calibrators. The deuterated analog of 1,4-dioxane behaves identically to 1,4-dioxane, both physically and chemically, allowing reproducible and accurate quantitation of 1,4-dioxane. This method has a linear quantitation range from 0.1 µg/L to 10 µg/L (ppb). A sample size of 10 mL (purge volume) is used to achieve low detection limits.

Experimental

Acquisition method

All analyses were performed on the Atomx XYZ system and the 7010B Mass Spectrometer. MS/MS was used to enhance sensitivity and selectivity. MassHunter software was used for all calculations.

As this is a triple quadrupole method, tuning is performed as per manufacturers recommendation using autotune. After initial full autotune, a passing Check Tune must be performed before the start of a batch and/or every 24 hours. If the check tune does not pass, corrective action must be performed, followed by a full autotune.

GC method parameters are shown in Table 2. Atomx XYZ method details are shown in Table 3. MS parameters are listed in Tables 4 and 5.

Materials

- Volumetric flasks, Class A, 1 mL, 10 mL, and 50 mL with ground glass stoppers
- Analytical balance
- Gas-Tight syringes, various volumes as appropriate
- 40 mL glass VOA vials
- Caps/bonded septa
- 1 mL mininert V-vials with lids
- Agilent 121-1324UI: DB-624 UI column, 20 m × 0.18 mm, 1.0 µm
- Agilent 60 µL (straight, UI) (part number 5190-4047)
- Ultrahigh purity helium
- Ultrahigh purity nitrogen
- Methanol, purge and trap grade
- DI Water

Table 2. Agilent 7010B triple quadrupole GC method parameters used for the analysis of 1,4-dioxane.

GC Inlet Parameters	
Temperature	200
Pressure	14.1 psi
Septum Purge Flow	3 mL/min
Inlet Mode	Split
Split Ratio	200:1
Liner	60 µL (straight) UI (p/n 5190-4047)
GC Oven Parameters	
Column	Agilent DB-624 UI, 20 m × 0.18 mm, 1.0 µm (p/n 121-1324UI)
Column flow	0.7 mL
Run Time	18 min
Initial Temperature	35 °C
Initial Hold Time	4 min
Column Ramp	15 °C/min
Ramp Final Temperature	240 °C
Hold Time	0.333 min

Table 3. Atomx XYZ method parameters used for the analysis of 1,4-dioxane.

Atomx XYZ Method	
Purge Settings	
Sample Equilibrate Time	0 min
Presweep Time	0.25 min
Prime Sample Fill Volume	3 mL
Sample Volume	10 mL
Sweep Sample Time	0.25 min
Sweep Sample Flow	100 mL/min
Sparge Vessel Heater	Yes
Sparge Vessel Temperature	80 °C
Prepurge Flow	0 mL/min
Prepurge Time	0 min
Purge Time	11.0 min
Purge Flow	40 mL/min
Purge Temperature	20 °C
MSC Purge Temperature	30 °C
Dry Purge Time	2 min
Dry Purge Flow	100 mL/min
Dry Purge Temperature	20 °C

Atomx XYZ Method	
Desorb Settings	
Water Needle Rinse Volume	12 mL
Sweep Needle Time	0.25 min
Desorb Preheat Temperature	245 °C
Desorb Time	2.0 min
Drain Flow	100 mL/min
Desorb Temperature	250 °C
GC Start Signal	Begin Desorb
Bake Settings	
Number of Water Bake Rinses	5
Water Bake Rinse Volume	12 mL
Bake Rinse Sweep Time	0.4 min
Bake Rinse Sweep Flow	100 mL/min
Bake Rinse Drain Time	0.6 min
Bake Time	6 min
Bake Flow	200 mL/min
Bake Temperature	260 °C
MSC Bake Temperature	180 °C

Table 4. Agilent 7010B triple quadrupole MS method parameters used for the analysis of 1,4-dioxane.

MS Parameters	
Tune File	atunes.eihs.tune.xml
MS Transfer Line Temperature	250 ˚C
Helium Quench Flow	2.25 mL/min
N_2 Collision Gas	1.5 mL/min
Source Temperature	250 ˚C
Gain Factor	20

Table 5. Agilent 7010B triple quadrupole MS compound-specific dMRM parameters for the analysis of 1,4-dioxane.

MS dMRM Parameters		Transition	Retention Time (RT)	Left RT Delta	Right RT Delta	Collision Energy
Target	1,4-dioxane	88 → 58.1 88 → 56.9	7.64 min	0.6 min	0.6 min	5 eV 5 eV
Internal Standard	1,4-dioxane-d_8	96 → 64.1 96 → 61.9	7.58 min	0.6 min	0.6 min	5 eV 5 eV
Wide/Wide quadrupole resolution windows						

Calibrator and ISTD preparation

Two stock solutions of 1,4-dioxane at 20 mg/L and 1,4-dioxane-d_8 at 4 mg/L were prepared in methanol. The 20 mg/L 1,4-dioxane solution may be transferred to a mininert vial and placed in a freezer for future use. The 4 mg/L 1,4-dioxane-d_8 solution was transferred to a vessel on the Atomx unit and added to every calibration level and sample automatically (10 µL).

Class A volumetric flasks and gas-tight syringes were used to make the calibrator solutions of 1,4-dioxane.

A series of calibration standards to encompass the desired calibration range were prepared (0.1 µg/L, 0.2 µg/L, 0.4 µg/L, 1 µg/L, 2 µg/L, 5 µg/L, and 10 µg/L). Calibration levels were created by adding specific volumes of the 20 mg/L solution to 50 mL volumetric flasks (partially filled with DI water) with a gas-tight syringe and then filling the flask to the line with DI water. Once prepared and thoroughly mixed, the calibration solutions were transferred into 40 mL VOA vials, ensuring zero-headspace when capped.

The linear calibration range for this analysis as validated was 0.1 to 10 µg/L.

Quality control checks require the average response factor for the calibration curve to have a relative standard deviation (RSD) of less than 20%. Each calibration point must have an accuracy of ±30% from the true value. When verifying the limit of quantitation, the accuracy must be within ±50% of the true value. The limit of quantitation must have a peak-to-peak signal-to-noise (height) value of greater than 3:1 to be classified as a peak.

Sample preparation

Aqueous samples were collected in 40 mL VOA vials with zero headspace, and analyzed as-is within seven days of the sampling date. If the concentration of 1,4-dioxane in the water sample is suspected to be high, or over the calibration range, the Atomx dilution feature may be used with a dilution of up to 1:100.

Quality control

Each batch of 20 samples includes a method blank (MB), a laboratory control sample (LCS), a laboratory control sample duplicate (LCSD), a matrix spike (MS), and a matrix spike duplicate (MSD). A sample duplicate is included for at least one sample in the batch.

Quality control for this method was monitored throughout data collection. Method blanks yielded nondetectable levels to ensure that there was no carryover.

The initial calibration (ICAL) was verified with the use of a certified reference material from a second source (ICV) and fell within 30% of the true value.

A continuing calibration verification (CCV) was prepared in the same manner as the calibration midpoint at 1 µg/L, was analyzed at the beginning of each analytical batch, and fell within 20% of the true value.

Results and discussion

Agilent 7010B GC/MS system equipped with an Atomx XYZ purge and trap system

Calibration data: Average of Response Factors, Ignore Origin, Weighting None

Seven levels used R^2 = 0.9997, Avg. RF RSD = 2.87% (Figure 1)

- Two continuing calibration checks: Accuracy 101% and 98%
- Multiple blanks were run throughout the batch
- Two LCS QCs: accuracy 98% and 99.6%

The MDL for 1,4-dioxane was calculated based on EPA methodology (EPA 821R16-006). The MDL was determined by spiking a sample (predetermined to contain nondetectable levels of 1,4-dioxane) at a concentration of 0.1 µg/L of 1,4-dioxane. Seven replicates of the spiked sample were injected, and an example chromatogram is shown in Figure 2.

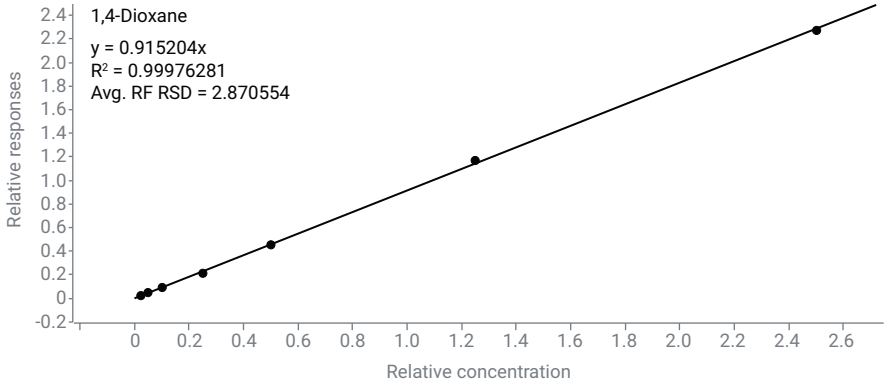

Figure 1. Calibration curve for 1,4-dioxane analysis on the Agilent 7010B GC/MS system with the Atomx XYZ purge and trap system.

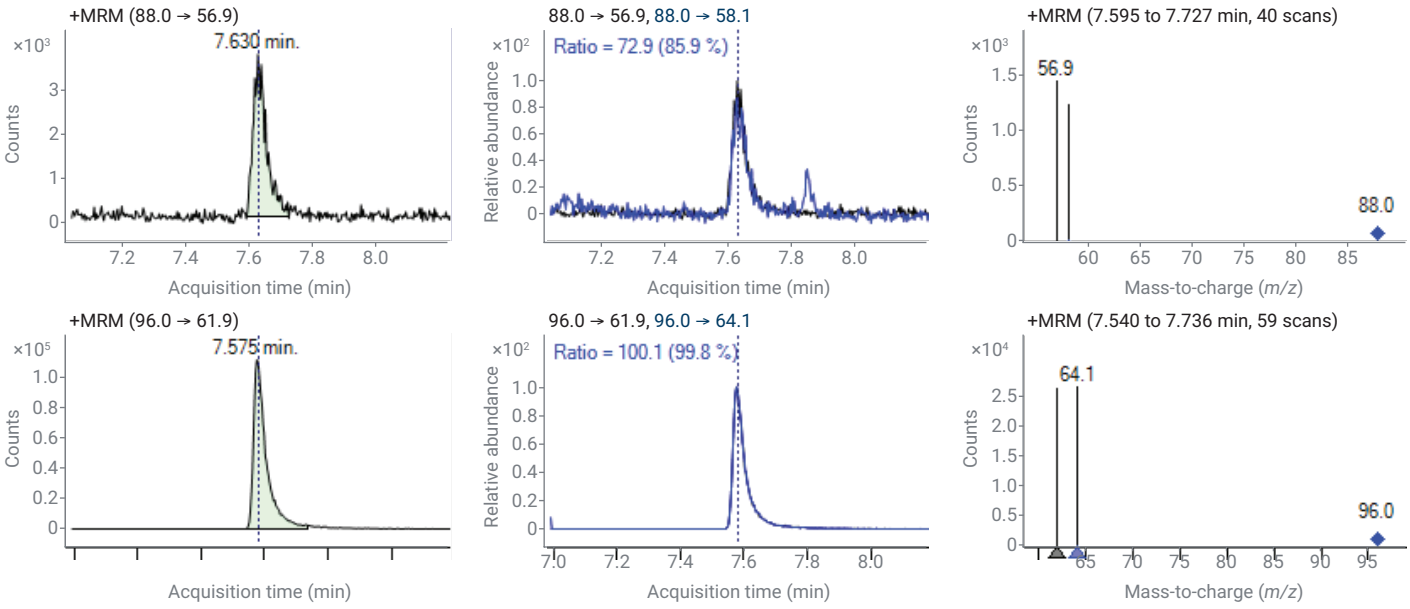

Figure 2. Example chromatography from one of the (0.1 µg/L) sample injections used for method detection limit (MDL) calculation of 1,4-dioxane on the Agilent 7010B GC/MS system with the Atomx XYZ purge and trap system.

The MDL was determined to be 0.0198 µg/L with a response RSD of 7.8%, as detailed in Table 6. A stability study was performed, as shown in Table 7.

Best practices

Best practices for the analysis of 1,4-dioxane in water by purge and trap with triple quadrupole GC/MS are listed in Table 8.

Table 6. Method detection limit was determined using 0.1 µg/L samples. Calculations were done automatically from Agilent MassHunter software with an average signal-to-noise ratio of 9.36.

Name	Retention Time (min)	Transition (*m/z*)	Concentration Average (µg/L)	Concentration RSD (%)	Method Detection Limit (µg/L)	Limit of Quantitation (µg/L)	S/N	Resp. RSD (%)
1,4-Dioxane	7.633	88.0 → 56.9	0.1288	4.9	0.0198	0.0629	9.36	7.8

Table 7. Stability study of continuing calibration and quality control samples using a 1.0 µg/L standard (calculations done automatically using Agilent MassHunter software).

Sample		1,4-Dioxane Results			
Type	Level	Retention Time (min)	Response	Calculated Concentration (µg/L)	Accuracy (%)
CC	4	7.64	129123	0.95	95.32
CC	4	7.64	123273	0.92	92.49
CC	4	7.63	81382	1.05	104.92
QC	4	7.64	124063	0.92	92.04
QC	4	7.64	122084	0.93	93.49

Table 8. Best practices for the analysis of 1,4-dioxane.

Instrument Measure	Frequency	Requirement	Correction
Check Tune	Before every batch/every 24 hours	Pass	Corrective action/perform full Autotune
Initial Calibration Verification (ICV)	Immediately after calibration	ICV ±30% true value	Reanalyze ICV, rerun calibration/corrective action
Continuing Calibration Verification (CCV)	Before each batch	CCV ±20% true value	Reanalyze CCV, rerun calibration/corrective action
Internal Standard (ISTD)	Added to every sample, QC, Calibration, and instrument check		
Retention Time (RT)	Evaluate in every sample	ISTD RT ±0.33 min Analyte RT <10 sec to midpoint ICAL or first CCV	Inspect and perform instrument maintenance
Matrix Blank (MB)	With every batch of 20 or fewer samples	Analyte <LOQ	Reprepare/Reanalyze/corrective action
Laboratory Control Spike and Duplicate (LCS, LCSD)	With every batch of 20 or fewer samples	RPD of LCS/LCSD <20%	Reanalyze/corrective action
Matrix Spike and Duplicate (MS, MSD)	With every batch of 20 or fewer samples	Spike Recovery ±30% RPD of MS/MSD <20%	Reanalyze/corrective action
Replace reference materials when responses do not pass criteria, are low compared to past calibrations, or reach their expiration date.			
Recalibrate when the CCV no longer passes within 20% of true value or maintenance has been performed.			

Conclusion

This method presents a sensitive, robust, and selective method to determine 1,4-dioxane in water using EPA 8260D purge and trap methodology. 1,4-Dioxane is notoriously difficult to analyze due to its solubility in water. Using elevated purge temperature along with MS/MS offers a reasonable analysis time along with the ability to detect very low concentrations of 1,4-dioxane without sacrificing laboratory throughput. This simple, yet reliable method demonstrates excellent sensitivity with low detection limits of 0.02 µg/L (20 ppt) being easily quantified and distinguishable from baseline. Furthermore, MDL and accuracy and precision for seven 0.1 µg/L standards showed no interference from excessive water. The benefits of using the Agilent triple quadrupole MS/MS capabilities and the Teledyne Tekmar Atomx XYZ purge and trap cannot be underestimated in lowering detection limits, reducing sample matrix interference, and improving signal-to-noise (S/N) ratio. The method described herein provides high selectivity and sensitivity with a more confidence-driven solution for the analysis of 1,4-dioxane in water.

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9. Modified SW-846 8270 SIM
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Full Scan Quantitative Analysis of Semivolatile Organic Compounds

Evaluating the performance of an Agilent 7000D GC/TQ in full scan data acquisition mode for SVOCs analysis

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Abstract

The Agilent 7000D triple quadrupole GC/MS system (GC/TQ) operating in full scan data acquisition mode was used for the quantitative analysis of semivolatile organic compounds (SVOCs) in environmental samples. Under appropriately selected operating conditions, the GC/TQ system was shown to provide excellent spectral library matching scores, high sensitivity, and linearity over a wide dynamic range. The retention time locking (RTL) functionality enabled the same retention times with the GC/TQ and the GC/MSD systems, hence, simplifying the review process. This application note provides the guidelines for data acquisition and processing with GC/TQ operating in full scan data acquisition mode. Following these guidelines, the full scan performance of the GC/TQ was comparable to that of the single quadrupole GC/MSD system when tested for the analysis of SVOCs over a working range of 0.4 to 100 ppm.

Introduction

The analysis of SVOCs by GC/MS is challenging due to the array of target analytes, including bases, neutrals, and acids that span broad molecular weight and boiling point ranges. EPA Method 8270D/E provides guidelines for conditions and quality control checks to facilitate successful analysis of SVOCs using GC/MS.[1] A previous application note[2] describes the use of the Agilent 5977 GC/MSD operated in full scan data acquisition mode, coupled to the Agilent 7890B GC, to meet the performance requirements and be in compliance with USEPA Method 8270D/E with calibration over a working range of 0.2 to 160 ppm in a single method. EPA 8270E revision 6 was the first version of the method to include use of GC/MS/MS (GC/TQ) for the analysis of SVOCs. GC/TQ operated in multiple reaction monitoring (MRM) mode delivers increased sensitivity, high selectivity afforded by MRM, robust data, and faster batch review due to the elimination of matrix interferences compared to GC/MSD as demonstrated in a previous application note.[3] If needed for a standard operating procedure (SOP), method validation, or sample screening, the GC/TQ can also be used in full scan data acquisition mode.

This study demonstrates that the 7000D GC/TQ system operating in full scan mode can be used to identify compounds using spectral library matching, with comparable performance to GC/MSD. This application note outlines the best practices for full scan data acquisition and processing using GC/TQ. The objective was to achieve excellent spectral library matching scores over 90, high sensitivity with limits of detection (LODs) at or below 50 ppb for most compounds, and linearity over a wide dynamic range of 0.4 to 100 ppm.

Experimental

The GC/TQ and GC/MSD systems used in this work were configured to achieve the best performance for the analysis of SVOCs as described in two previous studies.[2,3] The Agilent 7890B GC was coupled to either a 7000D GC/TQ or a 5977 Series GC/MSD, both equipped with an Inert Plus EI source, as shown in Figure 1A. The GC was equipped with a split/splitless (SSL) inlet, low pressure-drop (LPD) GC inlet liner (part number 5190-2295) shown in Figure 1B, and a 30 m × 0.25 mm, 0.25 µm 5 % phenyl (polysiloxane) column for best separation (part number DB-UI 8270D). The instrument operating parameters are listed in Table 1.

The 9 mm diameter extractor lens (part number G3870-20449) was used with both the GC/TQ and GC/MSD systems, as the lens was shown to greatly enhance method performance in SVOCs analysis.[3]

The injection volume was 1 µL in split mode, with a split ratio of 10:1 for GC/TQ, and pulsed split mode, with a split ratio of 5:1 for GC/MSD. The split ratio was optimized to meet the resolution requirement for benzo[b]fluoranthene and benzo[k]fluoranthene as specified in method 8270. The TQ was tuned with Atunes.eiex.tune.xml and the MSD was tuned with Atune.u. The electron multiplier gain setting was set to 1 for the GC/TQ and 0.3 for the GC/MSD. These settings ensured that the tallest peak in the base peak chromatogram (BPC) for the highest-level calibration standard used was in the range of 3 to 6 × 10^7 counts for GC/TQ and 3 to 6 ×10^6 counts for GC/MSD.

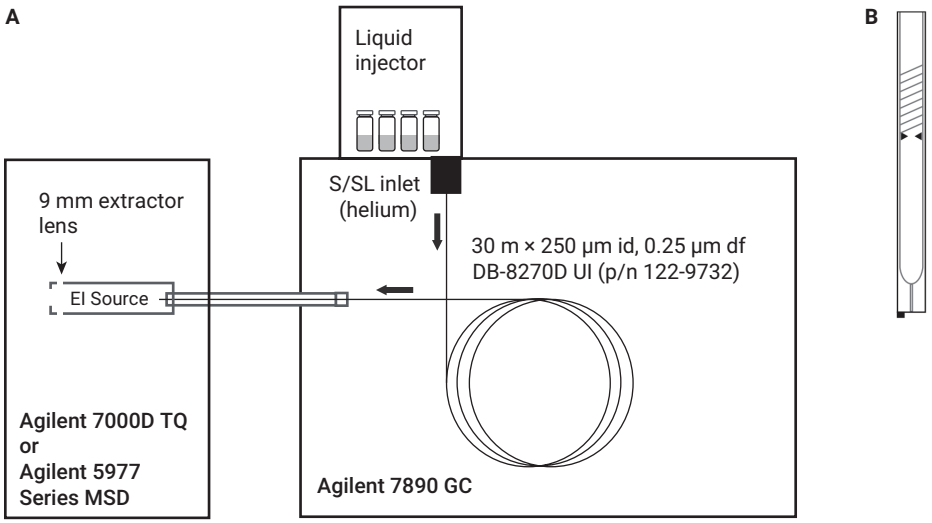

Figure 1. (A) Configuration of the Agilent 7890/7000D GC/TQ or Agilent 7890/5977 Series GC/MSD. (B) Ultra Inert (UI) Universal Low Pressure Drop Liner (part number 5190-2295).

Table 1. Gas chromatograph and mass spectrometer conditions for SVOCs analysis using GC/TQ and GC/MSD.

	GC/TQ	GC/MSD
GC		
Model	Agilent 7890 with fast oven, autoinjector, and tray	
Inlet	Split/splitless inlet (SSL)	
Mode	Split	Pulsed Split
Split Ratio	10:1	5:1
Injection Pulse Pressure	—	30 psi until 0.6 min
Septum Purge Flow	3 mL/min	
Injection Volume	1.0 µL	
Injection Type	Standard	
L1 Air Gap	0.2 µL	
Inlet Temperature	280 °C	
Carrier Gas	Helium	
Inlet Liner	Agilent universal low pressure drop liner, with glass wool (p/n 5190-2295)	
Oven		
Gradient	40 °C, hold 0.5 min, 10 °C/min to 100 °C, 25 °C/min to 260 °C, 5 °C/min to 280 °C	
Total Run Time	21.567 min	
Postrun Time	0 min	
Equilibration Time	0.5 min	
Column 1		
Type	Agilent DB-8270D UI, 30 m × 0.25 mm, 0.25 µm (p/n 122-9732)	
Control Mode	Constant flow	
Flow	0.992 mL/min	1.292 mL/min
Inlet Connection	Split/splitless inlet (SSL)	
Outlet Connection	MSD	
MS		
Model	Agilent 7000D TQ	Agilent 5977 Series MSD
Source	Agilent Inert Extractor Source with a 9 mm extractor lens	
Extraction Lens	9 mm (p/n G3870-20449)	
Vacuum Pump	Performance turbo	
Tune File	Atunes.eiex.tune.xml	Atune.u
Mode	MS1 Scan	Scan
Start Mass	35	
End Mass	500	
Scan Speed	220 ms	N = 2
Time Filter	On	—
Solvent Delay	2.5 min	
EM Voltage Gain Mode	1	0.3
Quad Temperature (MS1 and MS2)	150 °C	180 °C
Source Temperature	300 °C	
Transfer line Temperature	320 °C	
He Quench Gas	2.25 mL/min	—
N_2 Collision Gas	1.5 mL/min	—

For GC/TQ full scan acquisition mode, the following parameters were selected: MS1 Scan, 35 to 500 *m/z*, 220 ms scan speed, time filter – ON. These parameters were set in the TQ MS method editor of Agilent MassHunter workstation software, as shown in Figure 2. The default parameters were used for collision cell gases, i.e., 2.25 mL/min and 1.5 mL/min for He quench gas and N_2 collision gas, respectively.

For GC/TQ analysis in full scan data acquisition mode, 12 calibration levels were prepared from 0.4 to 100 ppm using a 68-compound mix and six internal standards (ISTDs). ISTD concentration was at the midpoint, at 20 ppm. LODs were calculated using a 0.5 ppm calibration standard injected in a split mode with a split ratio of 10:1, nine consecutive times. MassHunter workstation software was used for data acquisition and processing.

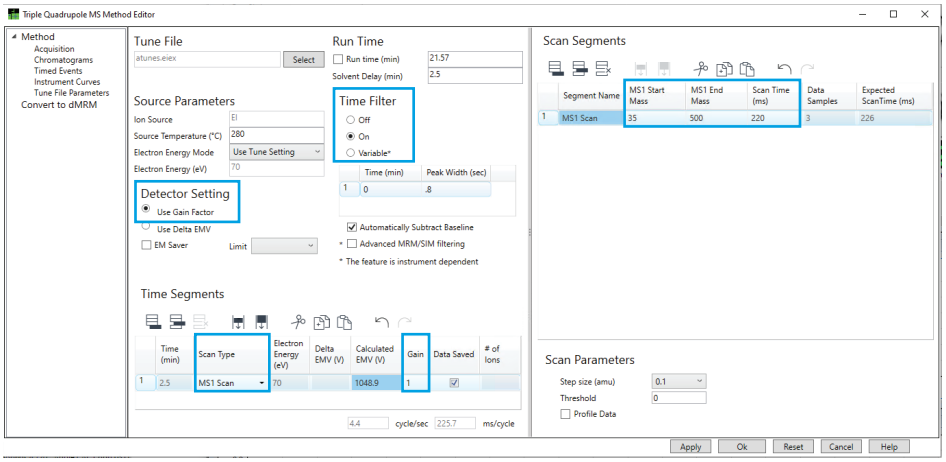

Figure 2. TQ MS Method Editor showing the full scan acquisition parameters used in this work.

Results and discussion

The use of GC/TQ operated in MRM for EPA 8270E SVOCs analysis is described in a previous application note.[3] The aim of this study was to show that the Agilent 7000D GC/TQ system operating in full scan mode can be used to identify compounds using spectral library matching and quantitate them, with comparable performance to GC/MSD.

This application note outlines the best practices for full scan data acquisition and processing using GC/TQ.

The performance of GC/TQ operated in full scan acquisition mode for SVOCs analysis was compared to that of GC/MSD operating in scan mode. Figure 3 shows a total ion chromatogram (TIC) for full scan data acquired with GC/TQ for a 1 ppm standard with a 10:1

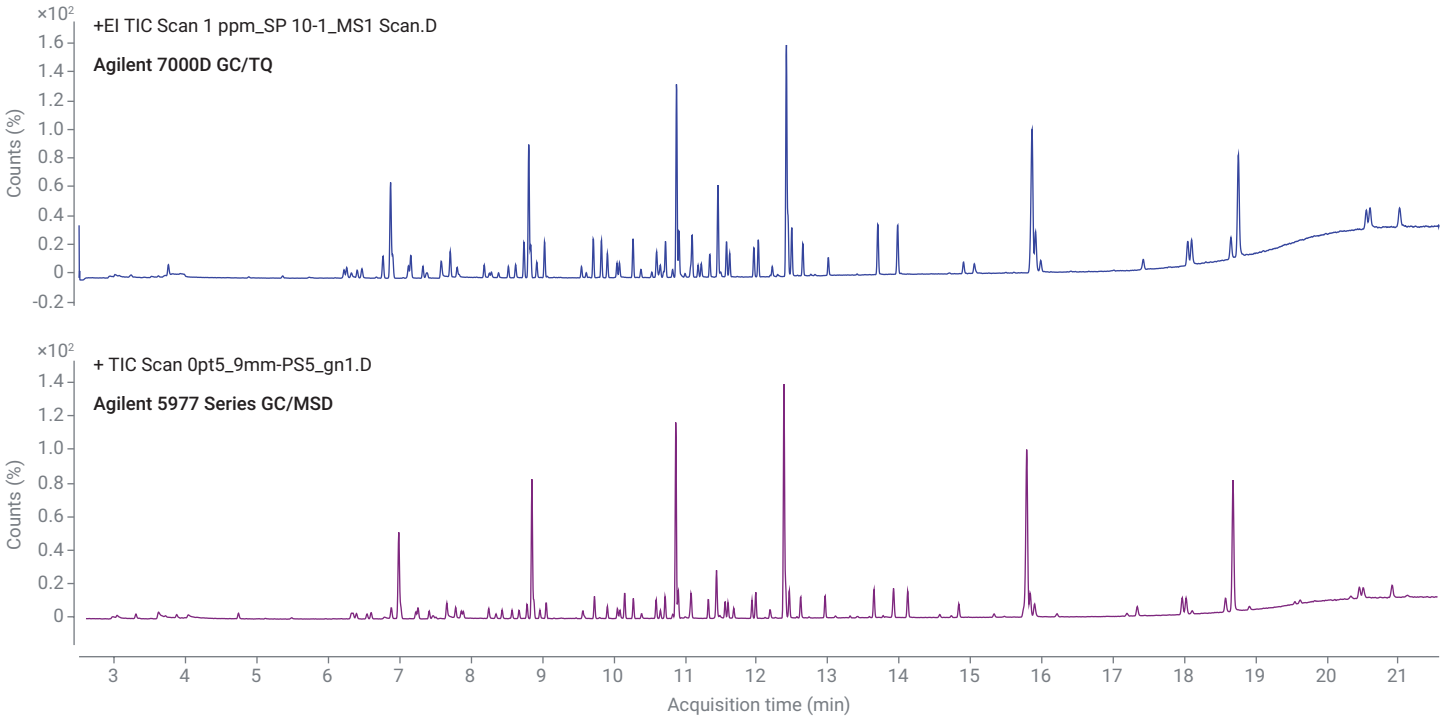

Figure 3. Scan TIC for a 1 ppm standard with a 10:1 split (0.1 ng on-column) analyzed with Agilent 7890/7000D GC/TQ (top). Scan TIC for a 0.5 ppm standard with a 5:1 pulsed split (0.1 ng on-column) analyzed with Agilent 7890/5977 Series GC/MSD (bottom).

split (0.1 ng on-column). A TIC acquired in scan with GC/MSD for a 0.5 ppm standard with a 5:1 pulsed split (0.1 ng on-column) is also shown in Figure 3.

Agilent RTL technology enables the same retention times for all target analytes between different Agilent GC/MS systems.[4] RTL is achieved by making an adjustment to column flow, so that the retention times on one system can be maintained after maintenance. RTL also allows close matching between instruments using the same nominal column, as shown in Figure 3.

Spectral fidelity with GC/TQ in full scan mode

Excellent spectral library match scores (LMS) for all SVOCs were observed with GC/TQ in full scan data acquisition mode against the NIST spectral library, as shown in Figure 4. To obtain the LMS values, a 10 ppm standard analyzed with a 10:1 GC inlet split was processed with MassHunter Unknowns Analysis software against the NIST spectral library. The observed LMS values are comparable to those obtained with GC/MS, with an average LMS of 95 for all 74 compounds. The results demonstrate that GC/TQ system can be used for sample screening to identify compounds using spectral library matching.

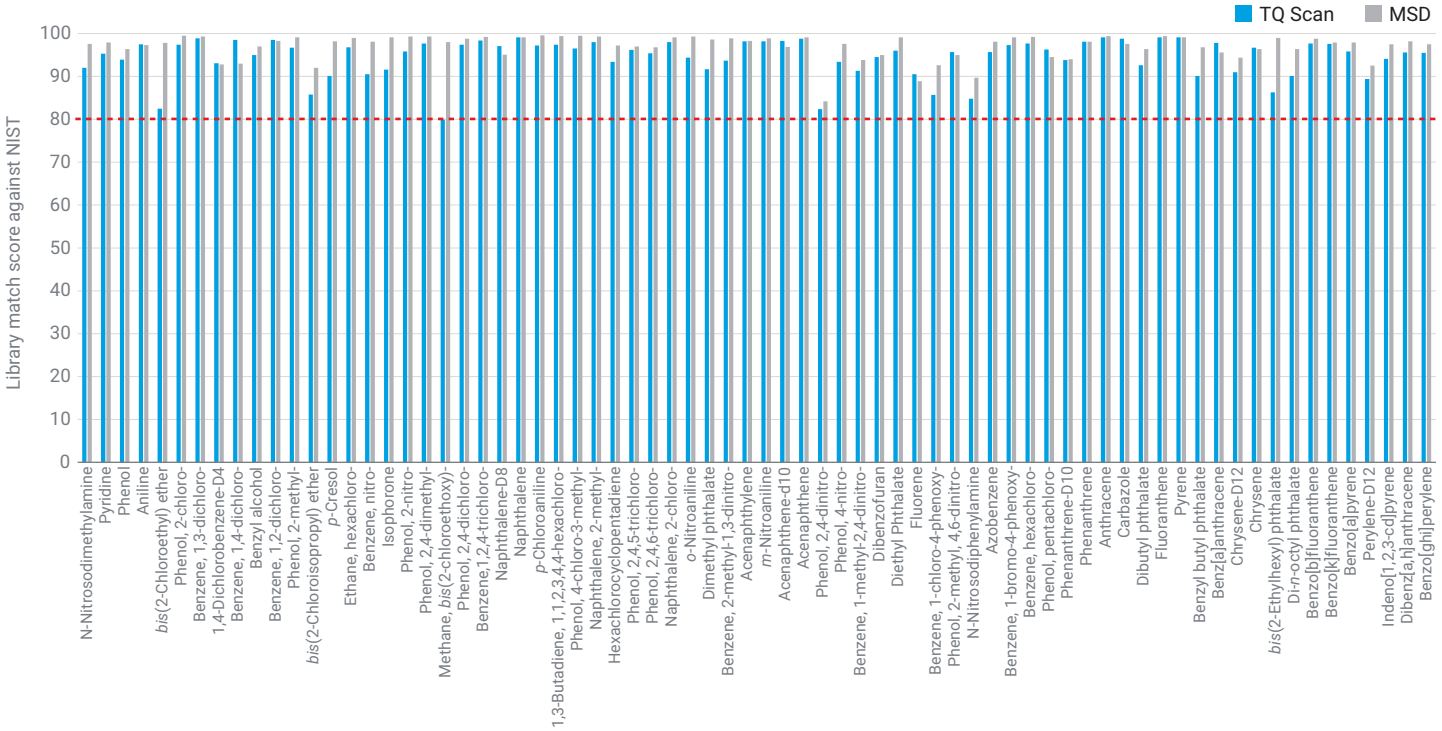

Figure 4. Library match score (LMS) against the NIST spectral library. Blue bars: results for a 10 ppm standard analyzed with Agilent 7890/7000D GC/TQ with a 10:1 split (1 ng of each component on-column). Gray bars: results for a 5 ppm standard analyzed with Agilent 7890/5977 Series GC/MSD with a 5:1 pulsed split (1 ng of each component on-column) in full scan data acquisition mode.

Analyzing GC/TQ full scan data against GC/MSD RTL library

The LMS values shown in Figure 4 were obtained when analyzing the sample against the NIST library. However, the sample can also be analyzed against the custom-built retention time-locked SVOCs library that was created using full scan data for an EPA 8270 SVOCs standard acquired with GC/MSD or GC/TQ. The advantage of analyzing the GC/TQ full scan sample against the custom library is that the compound hits can be filtered based on their retention times.[5] The RTL functionality provided the same retention times with the 7890/7000D GC/TQ and the 7890/5977 Series GC/MSD (Figure 3). Therefore, when the GC/TQ full scan sample was analyzed against the library built with the GC/MSD data, the compound hits could be filtered based on their retention times, simplifying the review process.

Figure 5 shows the Unknowns Analysis window for a sample analyzed with GC/TQ in full scan mode against a spectral library built in-house using GC/MSD SVOCs analysis results. The average LMS for all 74 compounds was 95, which is the same as the average LMS observed when searching the spectra acquired with GC/TQ against the NIST spectral library.

The components table in Figure 5 shows the identified components arranged in elution order, the match factor against the custom SVOCs library built with GC/MSD data, the component areas, and the delta RT. Delta RT is the difference between the observed retention time and the retention time for the target in the library. Small values of delta RT indicate a good alignment between the retention times observed with GC/TQ and GC/MSD. This workflow is useful when migrating the methods from GC/MSD to GC/TQ.

The GC/TQ chromatogram acquired in full scan data acquisition mode is shown on the top right of Figure 5, as a black trace. The identified components are highlighted using the green trace, and the selected component (benzyl alcohol at 7.121 minutes) is highlighted in red. The mirror plot (middle, right of Figure 5) shows the comparison between the deconvoluted mass spectrum of the highlighted component (benzyl alcohol) and the corresponding library spectrum. The spectrum below the mirror plot is the raw mass spectrum before deconvolution. The overlaid ions are shown under the Ion Peaks window to demonstrate that the ions that belong to the component have the same retention time apexes and chromatographic peak shapes.

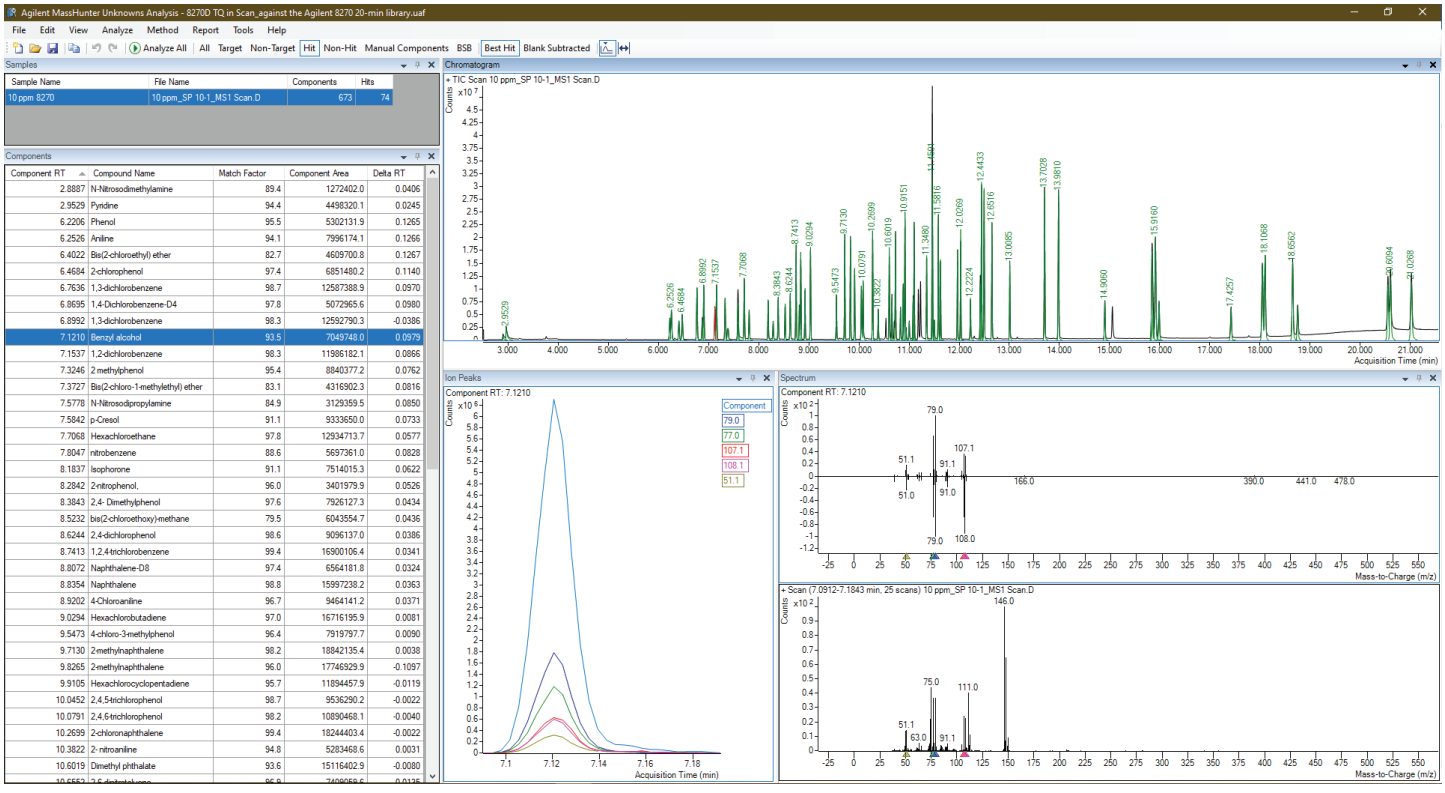

Figure 5. The Unknowns Analysis window featuring a 10 ppm SVOCs standard (10:1 GC inlet split) analyzed with GC/TQ in full scan data acquisition mode against a spectral library built in-house using GC/MSD SVOCs analysis results.

Sensitivity with GC/TQ in full scan mode

Figures 6A and 6B show the comparison of the extracted ion chromatograms (EICs) for hexachlorobenzene and acenaphthene analyzed with GC/TQ in full scan data acquisition mode (top) and GC/MSD (bottom). The loading on-column was 40 pg per analyte as a 0.4 ppm standard was analyzed in 10:1 GC inlet split with GC/TQ, and a 0.2 ppm standard was analyzed in 5:1 GC inlet pulsed split with GC/MSD. The signal-to-noise ratio for EICs achieved with GC/TQ in full scan mode operated under the conditions described in this work was comparable to that observed with GC/MSD in full scan data acquisition mode.

The LODs obtained with the 7890/7000D GC/TQ operated in full scan data acquisition mode are shown in Figure 7. The LODs for most compounds were under 50 ppb (pg/µL), comparable to LODs observed with GC/MSD. The compounds with higher observed LODs are known to be challenging for GC/MS analysis at low levels. These compounds include N-nitrosodimethylamine, 2-nitrophenol, 2,4 dinitrophenol, and 2-methyl-4,6-dinitrophenol.

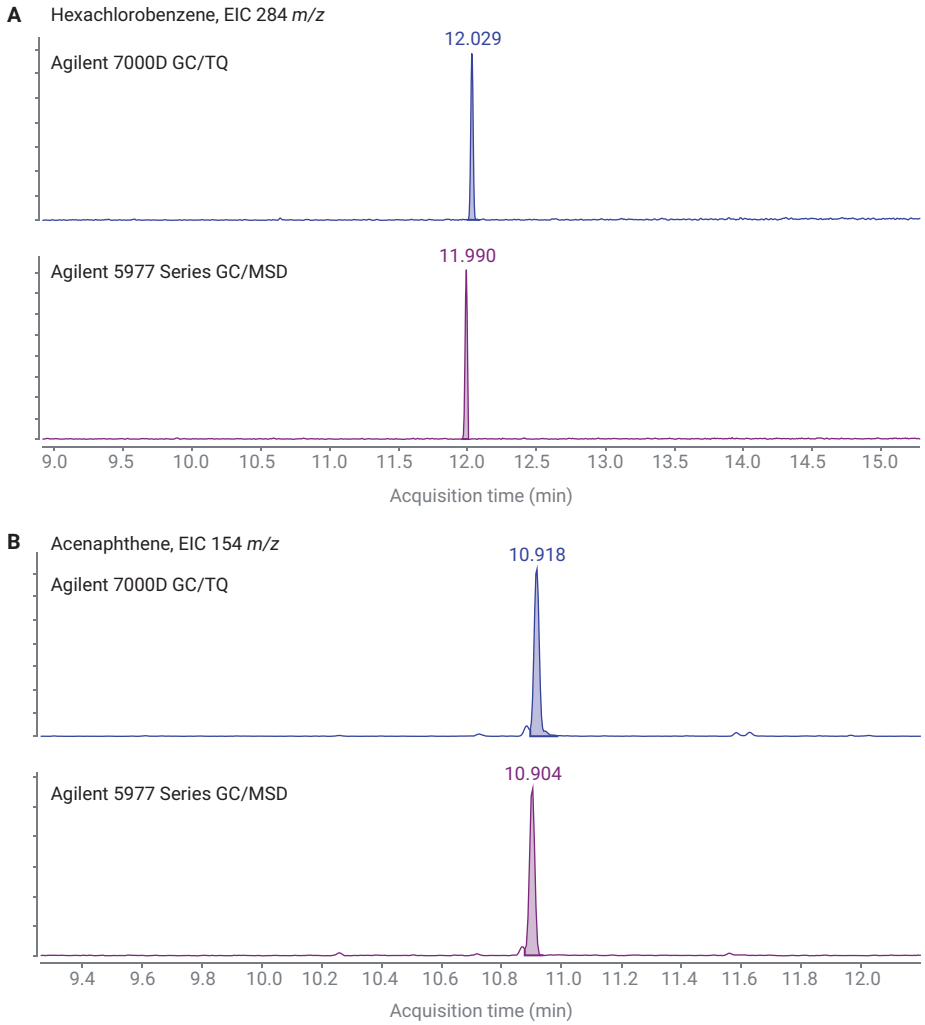

Figure 6. EICs acquired with GC/TQ in full scan data acquisition mode (top chromatograms in blue) and with GC/MSD in full scan mode (bottom chromatograms in purple) for: (A) 40 pg of hexachlorobenzene (*m/z* 284); (B) 40 pg of acenaphthene (*m/z* 154).

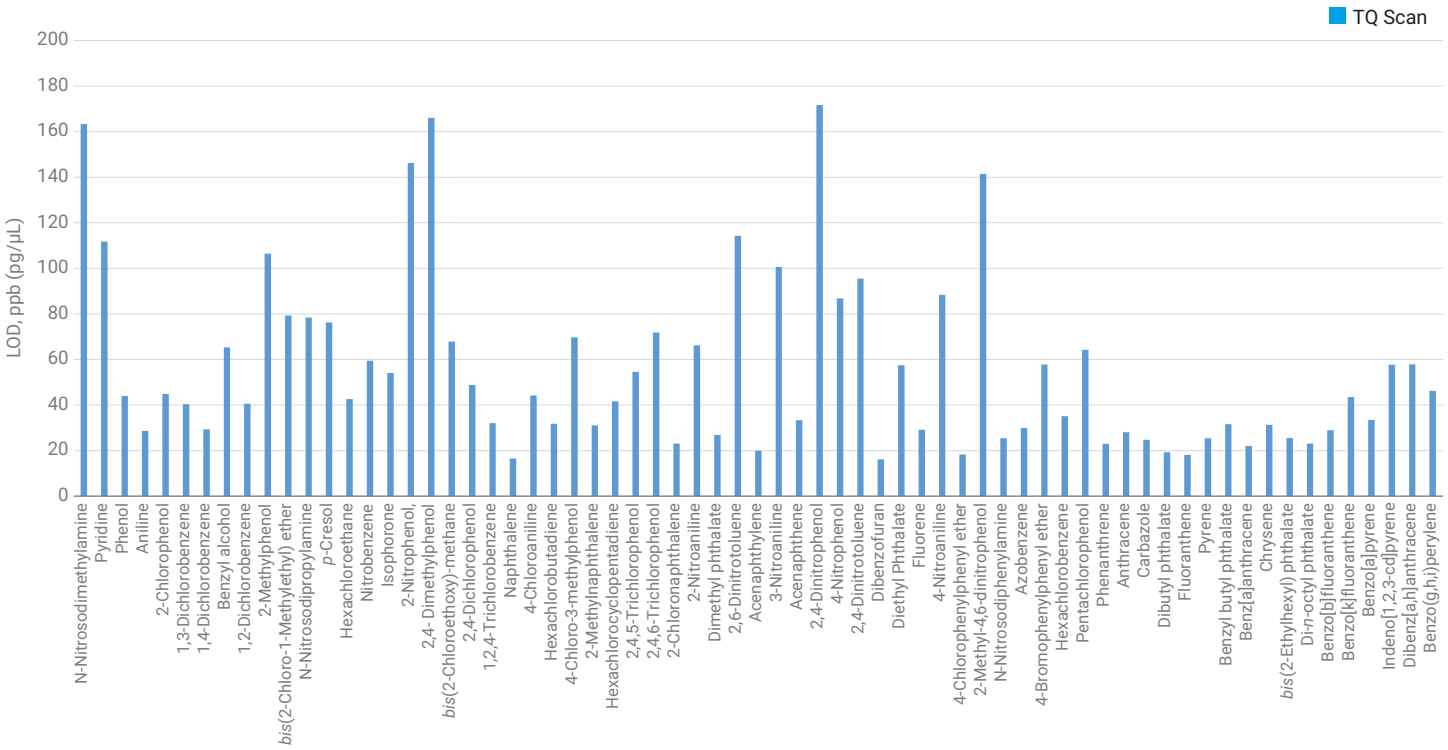

Figure 7. LODs with the 7890/7000D GC/TQ in full scan data acquisition mode obtained when performing nine sequential injections of a 0.5 ppm standard with a GC inlet split ratio of 10:1.

Resolution between benzo[b]- and benzo[k]fluoranthene

Chromatographic resolution between two isomer peaks for benzo[b] fluoranthene and benzo[k]fluoranthene was evaluated as this is commonly used as a marker of chromatographic performance in many standard methods. Figure 8 shows that the chromatographic resolution of the height of the valley between two isomer peaks for benzo[b] fluoranthene and benzo[k]fluoranthene was less than 50% of the average of the two peak heights at the midpoint concentration level with GC/TQ analysis in full scan mode.

Initial calibration performance with GC/TQ in full scan mode

To evaluate the calibration performance with GC/TQ in full scan mode, a 12-point calibration from 0.4 to 100 ppm using a 68-compound mix and six ISTDs was analyzed. Using MassHunter Quantitative Analysis, the relative response factor was determined for each component at each calibration level. The mean response factor was then calculated across the average relative response factors for the calibration curve of each compound, along with its relative standard deviation (RSD). Passing criteria state that the average response factor %RSD must be ≤20 (this is the preferred passing criteria). If this is not met, R^2 ≥0.990 is required for a linear curve fit. Finally, a quadratic fit with R^2 ≥0.990 that results

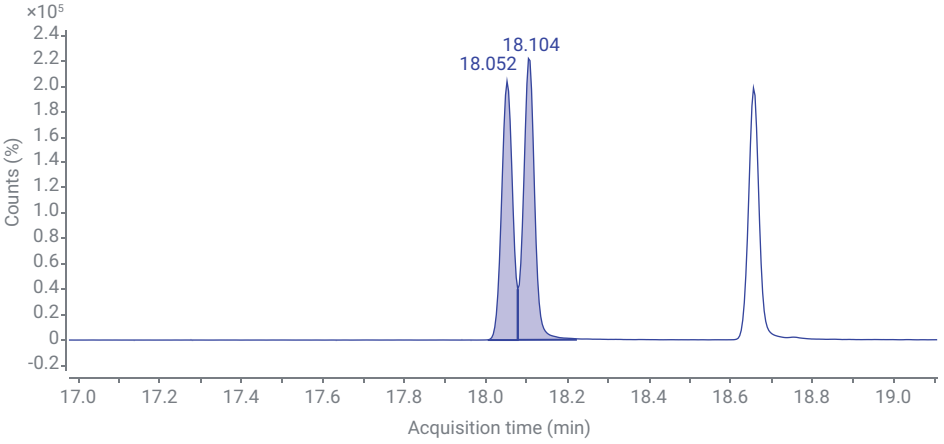

Figure 8. Benzo[b]- and benzo[k]fluoranthene resolution at 10 ppm with GC/TQ in full scan mode, EIC 252 *m/z*.

in the recalculated concentration of the low calibration point within ±30% of the standard's true concentration may be used. Accuracy for the lowest data point must be ±30%, and six points are needed when a curve fit is used. Relative standard error (RSE) was also calculated in MassHunter Quantitative Analysis to provide a measure of curve quality.

Table 2 summarizes the initial calibration performance for the SVOCs analysis achieved with GC/TQ in full scan mode over the evaluated concentration range of 0.4 to 100 ppm. The average response factor %RSD for 68 compounds was 16.1, with 47 out of 68 compounds meeting the average response factor %RSD passing criteria of ≤20. Either linear or quadratic calibration curve fit was used for the remaining 21 compounds.

The initial calibration curves for all 68 compounds had the RSE ≤20, with an average RSE of 11.0 across all the targets.

The calibration curves for N-nitrosodimethylamine and *bis*(2-chloro-1-methylethyl)ether are shown in Figure 9. The initial calibrations show excellent linearity, with the average response factor %RSD of 8.2 and 1.4, respectively, while maintaining accuracy at low calibration levels.

Table 2. The initial calibration performance for SVOCs analysis achieved with GC/TQ in full scan data acquisition over the evaluated concentration range of 0.4 to 100 ppm.

Number of Compounds with Average Response Factor %RSD <20	Average Response Factor %RSD for 68 Compounds	Number of Compounds with Relative Standard Error (RSE) <20	Average Relative Standard Error (RSE) for 68 Compounds	Number of Compounds with Linear Fit Passing R^2 >0.99 and Accuracy 30%	Number of Compounds with Quadratic Fit Passing R^2 >0.99 and Accuracy 30%
47	16.1	68	11.0	10	11

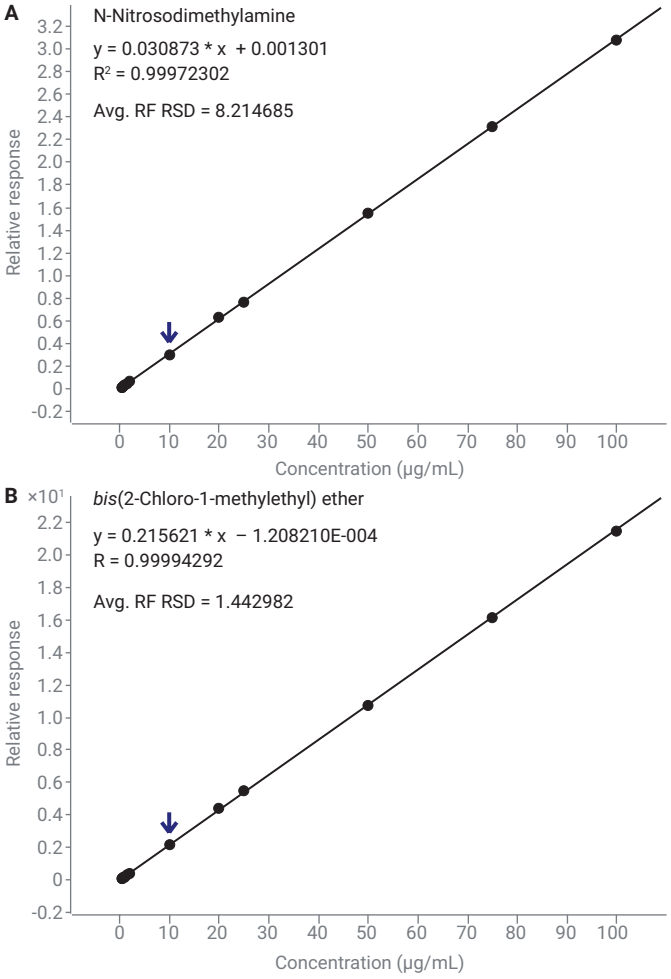

Figure 9. Example calibration curves for N-nitrosodimethylamine and *bis*(2-chloro-1-methylethyl)ether over the calibration range of 0.4 to 100 ppm acquired with GC/TQ in full scan mode using the GC inlet split ratio of 10:1.

Conclusion

The Agilent 7000D triple quadrupole GC/MS system was used for the analysis of semivolatile organic compounds (SVOCs) in full scan data acquisition mode. Using the operating conditions outlined in this application note, the 7000D GC/TQ system in full scan data acquisition mode enables excellent spectral library matching, high sensitivity, and linearity over a wide dynamic range of 0.4 to 100 ppm.

All the target compounds were identified against both the NIST library and the custom-built SVOCs spectral library, with high library match scores (average of 95) in both cases. The average response factor %RSD for 68 compounds was 10.96, with 47 out of 68 compounds meeting the average response factor %RSD passing criteria of ≤20. The LODs obtained with the GC/TQ for most of the compounds were under 50 ppb (pg/µL).

Following the best practices for data acquisition and processing, the full scan data acquisition performance of the GC/TQ was found to be comparable to that of the single quadrupole GC/MS system for SVOCs analysis. This performance enables laboratories to perform single quadrupole GC/MS workflows with GC/TQ when needed, extending the flexibility of GC/TQ for routine workflows, such as sample screening and compound identification in full scan mode.

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