The GC-APCI Interface for the Agilent Q-TOF LC/MS System Improves Sensitivity, Mass Accuracy, and Speed for a Wide Range of GC Applications

Technical Overview

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Introduction

The Agilent 3212 GC-APCI interface for use with the Agilent 6530, Agilent 6540, and Agilent 6550 Q-TOF LC/MS systems provides the highest GC chromatographic peak resolution and best mass accuracy in a robust and easy to use platform. The unique design of the GC-APCI ion source enables rapid switching between GC and LC/MS operation and the flexibility to handle a wide range of challenging applications. All of the Agilent 6500 series Q-TOF products offer fast acquisition speeds up to 50 spectra/sec to provide high quality data for very narrow GC peak widths.

The GC-APCI technology delivers:

- Very narrow chromatographic peak widths, especially for high boiling compounds, providing greater sensitivity and compound separation
- Improved flexibility and productivity for analysis of a broad variety of sample types
- Up to 3-fold faster analysis times due to higher gas flow rates
- Enhanced compound ionization, sensitivity and structural characterization for a wide range of analytes, due to easy access to a selection of makeup gases

This technical overview illustrates the advantages of the GC-APCI Interface for a wide range of applications, including environmental methods such as nitrosamine analysis and Method 8270 for semivolatile organic compounds. It can also provide superior performance for analysis of foods and natural products such as cooked meats and plant extracts. Superior sensitivity and linearity of quantitation are also demonstrated.

Agilent Technologies

Simple and secure coupling device provides fast switching between GC and LC/MS operation

The unique design of the GC-APCI interface enables switching between GC and LC operation within a few minutes. A short, heated transfer tube with an outer tube for extra protection connects the GC capillary to the APCI source, which provides the corona discharge for ion generation. This design helps ensure uniform transfer line heating and good resolution of compounds with high boiling points

The quick lock connection between the APCI interface housing and APCI source registers alignment with the ion sources, eliminating the need for adjustments to either column positioning or corona needle positioning, and providing very robust switching to GC-operation.

Combining both GC and LC capability with the TOF or Q-TOF mass analyzer provides the flexibility to handle a wide variety of sample types, from neutral to highly polar, and significantly increases the productivity of existing MS instrumentation.

The mass accuracy of the Q-TOF generates highly confident compound identification from the spectra of each compound. This mass accuracy advantage is also useful for providing identification of unknowns, when screening samples for compounds such as pesticides.

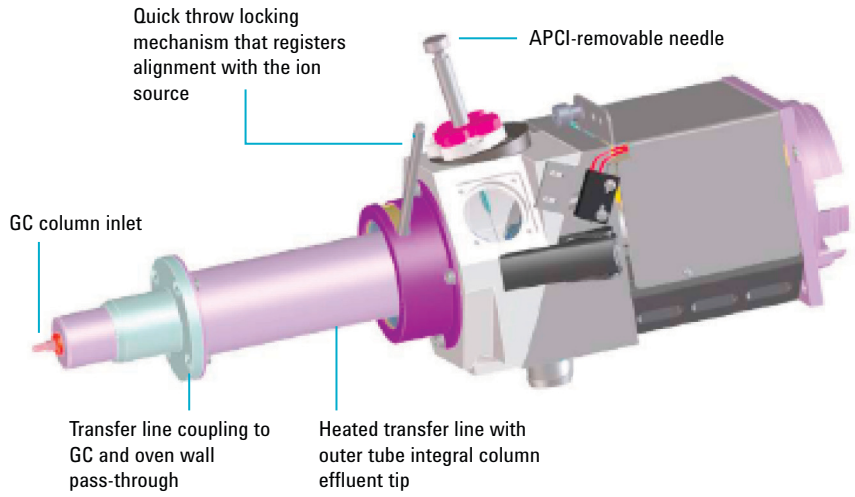


Figure 1. Agilent 3212 GC-APCI interface for the Agilent 6500 Series Q-TOF LC/MS systems.

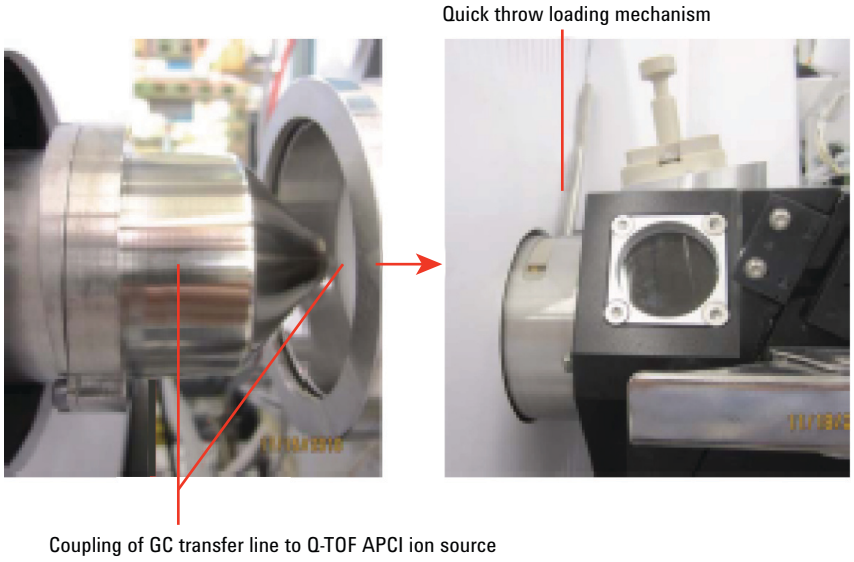


Figure 2. Illustration of the use of the Agilent 3212 GC-APCI interface to couple the Agilent 7890A GC to the Agilent 6540 Q-TOF LC/MS.

Excellent Sensitivity

Some compounds do not easily ionize using ESI, and APCI provides an excellent alternative for their detection and quantitation. Low picogram detection (on the column) can be achieved for many compounds, with excellent linearity of quantitation.

For example, a one picogram injection of octafluoronaphthalene (OFN) onto the GC column of the combined GC-APCI/6530 Q-TOF mass spectrometer (Figure 3) shows an approximate 10:1 or better signal-to-noise (S/N) ratio for the extracted ion chromatogram (EIC). Note that the lower panel shows the mass spectrum having primarily the radical ion [M]*+ at 271.9870 accurate mass.

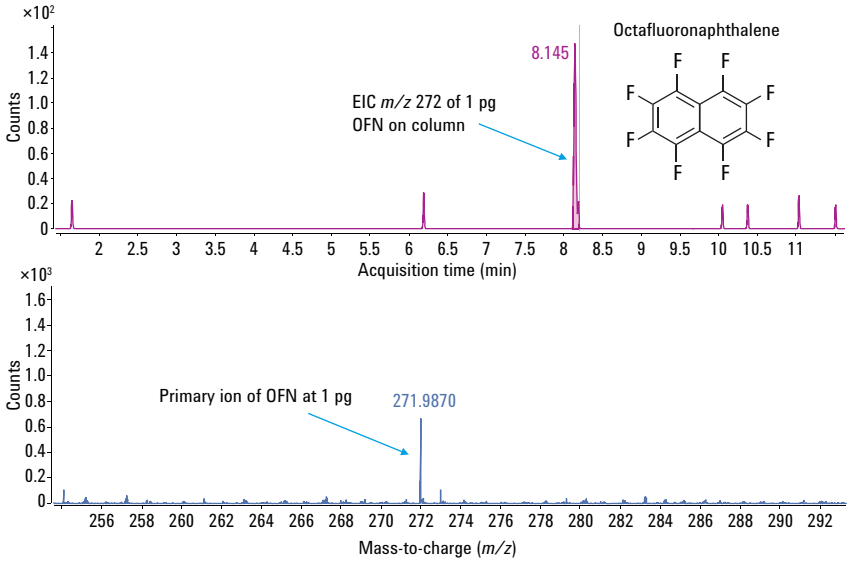


Figure 3. EIC (upper panel) and mass spectrum (lower panel) of 1 pg of octafluoronaphthalene (OFN) onto the GC column of the Agilent 3212 GC-APCI/Agilent Q-TOF mass spectrometer.

Reliable Quantitation

Linearity of quantitation across two orders of magnitude is good for several compounds that do not easily ionize by ESI, such as 2,6-dimethyl phenol and cholesterol (Figures 4 and 5), and for compounds that do not ionize at all in ESI, such as methyldecanoate (Figure 6). All of the coefficients of calibration (R^2 values) for these compounds were > 0.995. The rapid scan speed of the 6550 Q-TOF LC/MS helps ensure accurate quantitation for very narrow peaks.

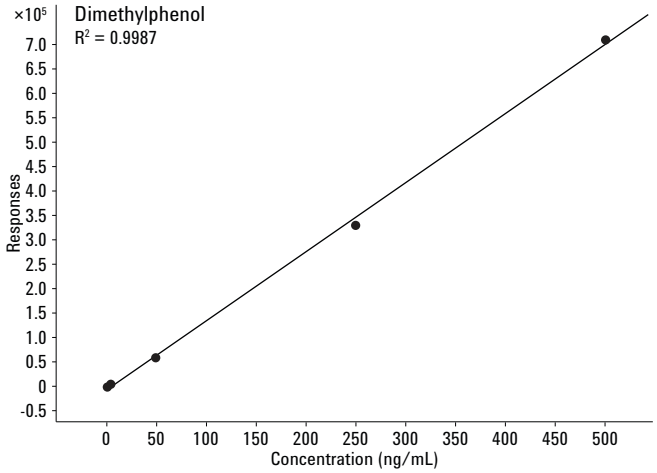


Figure 4. Calibration curve for 2,6-dimethylphenol, 1 to 500 pg on column.

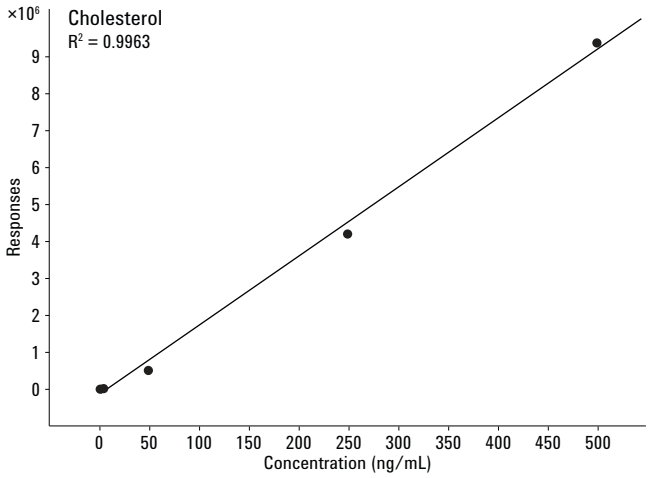


Figure 5. Calibration curve for cholesterol, 1 to 500 pg on column.

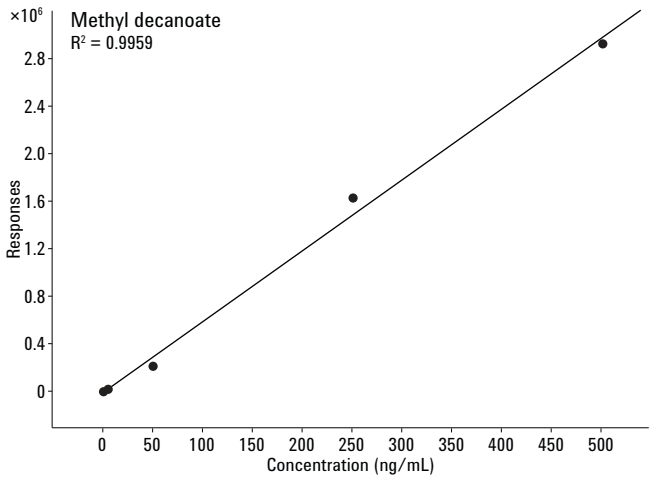


Figure 6. Calibration curve for methyl decanoate, 0.5 to 500 pg on column. This compound does not ionize using ESI.

Versatility

The Grob test mix is often used to characterize the performance of a GC/MS system, including signal response and mass accuracy, inertness of the flow path, and adequate transfer line heating. It is also a good test of the versatility of a system, since it contains a wide variety of small polar and nonpolar compounds, from alkanes to organic acids to aromatics.

The GC-APCI/6550 Q-TOF LC/MS system provides very narrow peaks for the Grob mix compounds, with widths < 2 seconds (Figure 7). The 6550 Q-TOF LC/MS system provides the fast scanning speed needed to generate such high quality data.

The system generates good separations for the wide variety of polarities represented in the mix. For example, butanediol is a highly polar nonvolatile compound, yet it exhibits minimal peak tailing (Figure 8).

The GC-APCI interface also enables analysis of compounds, such as methyldodecanoate, in the mix that do not ionize easily by ESI. The Q-TOF can provide high mass accuracy MS/MS spectra as well. (Figure 9).

Mass accuracy is also very good with the Grob test mix, ranging from –1.06 to +0.53 parts per million (ppm), across several of the compounds.

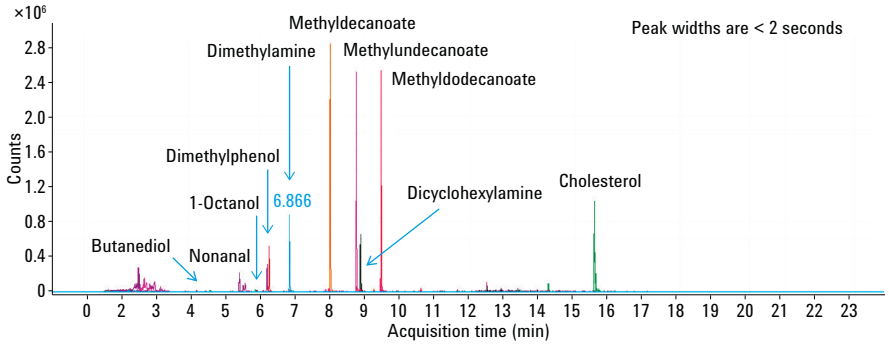


Figure 7. EIC Grob test mix analyzed on the Agilent 3212 GC-APCI/Agilent 6540 Q-TOF LC/MS.

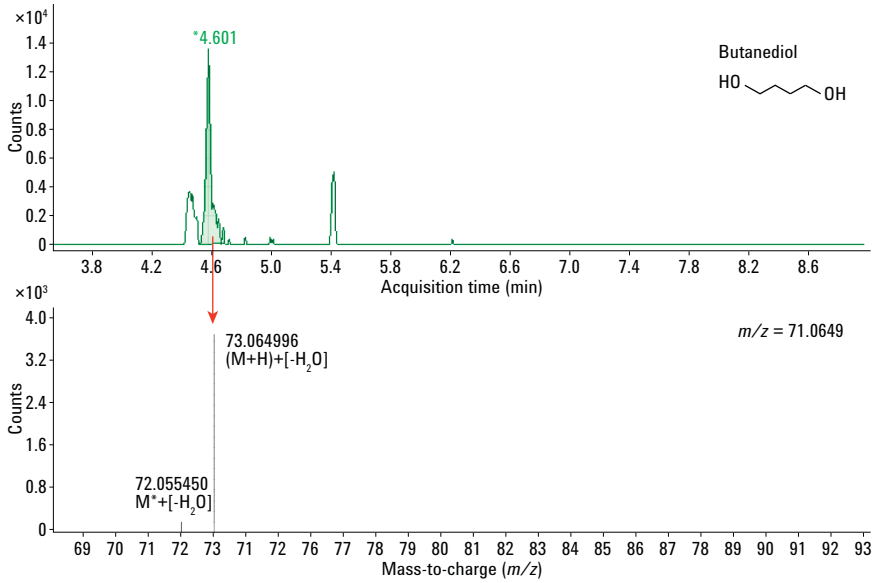


Figure 8. EIC and mass spectrum of highly polar and nonvolatile butanediol, illustrating minimal peak tailing.

Quantitation of PAHs in Food Matrices

Polycyclic aromatic hydrocarbons (PAHs) make up a class of more than 100 chemicals composed of up to six benzene rings fused together. PAHs are a human health concern because a number of studies have shown increased incidence of cancer (lung, skin, and urinary cancers) in humans exposed to PAH mixtures.

PAHs can occur in food either by uptake from the environment or as a result of food processing. While the list of priority PAHs varies in different countries, the United States Environmental Protection Agency (USEPA) and the European Union (EU) have identified 16 priority PAHs that require monitoring.

The GC-APCI/6540 Q-TOF LC/MS system was used to develop a method for detecting PAHs in cooked hamburgers, as the cooking process can generate these compounds. A sample of the cooked hamburger (10 g) was spiked with 100 pg of a mix of PAH standards. The sample was then extracted with organic solvents and cleaned up by passing it over a silica column.

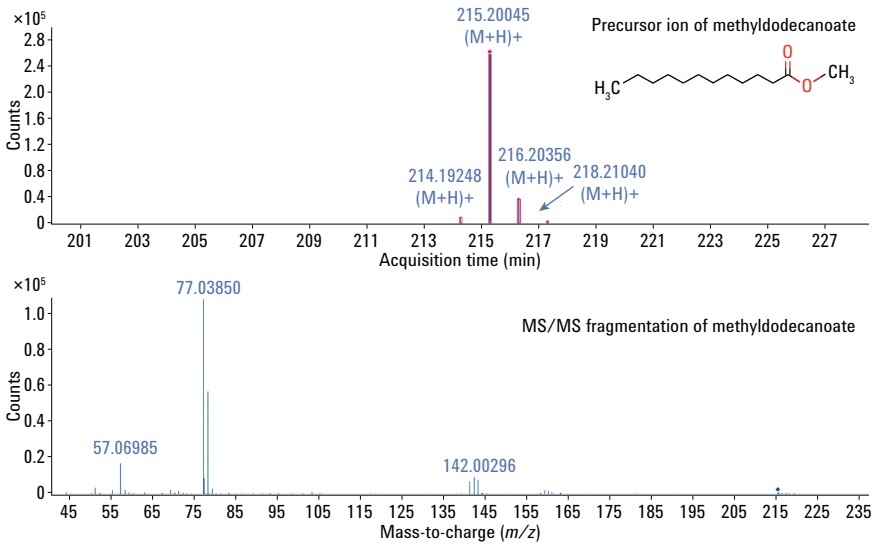


Figure 9. MS and MS/MS spectra of methyldodecanoate, which does not ionize easily by ESI.

Quantitation of PAHs in Food Matrices

An extracted ion chromatogram illustrates very narrow peaks, complete baseline resolution of the 16 compounds, identification of all of the compounds using accurate mass, and partial resolution of some of the isomers , (Figure 10). Expanding the region from 28.6 to 33.2 minutes illustrates the partial resolution and identification of the four isomers, with mass accuracy ≤ 0.8 ppm (Figure 11).

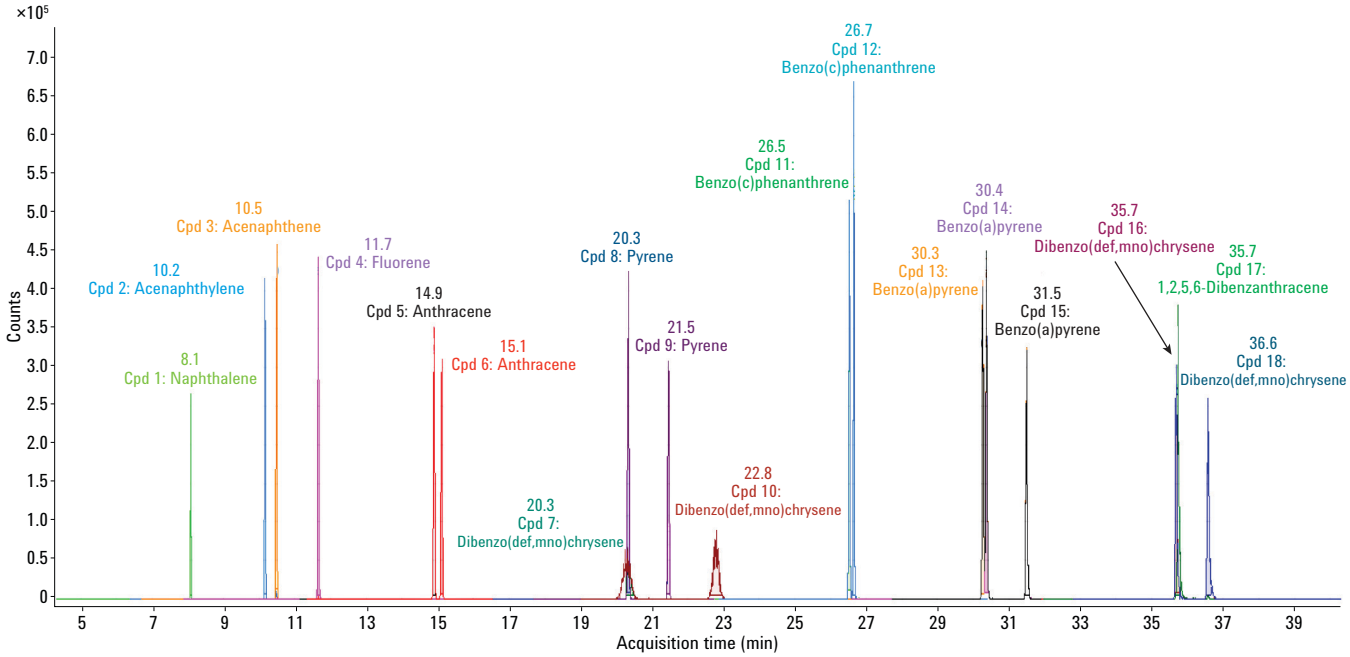


Figure 10. Extracted ion chromatogram of a standard mix of 16 PAH compounds identified using their accurate mass. The system was calibrated using the background ions from the GC column bleed.

Benzo(a)pyrene	$C_{20}H_{12}$	30.3	253.10101	252.09372	-0.72	3	334,291
Benzo(a)pyrene	$C_{20}H_{12}$	30.4	253.10111	252.09381	-0.37	2	375,124
Benzo(a)pyrene	$C_{20}H_{12}$	31.5	253.10125	252.09396	0.23	2	268,214

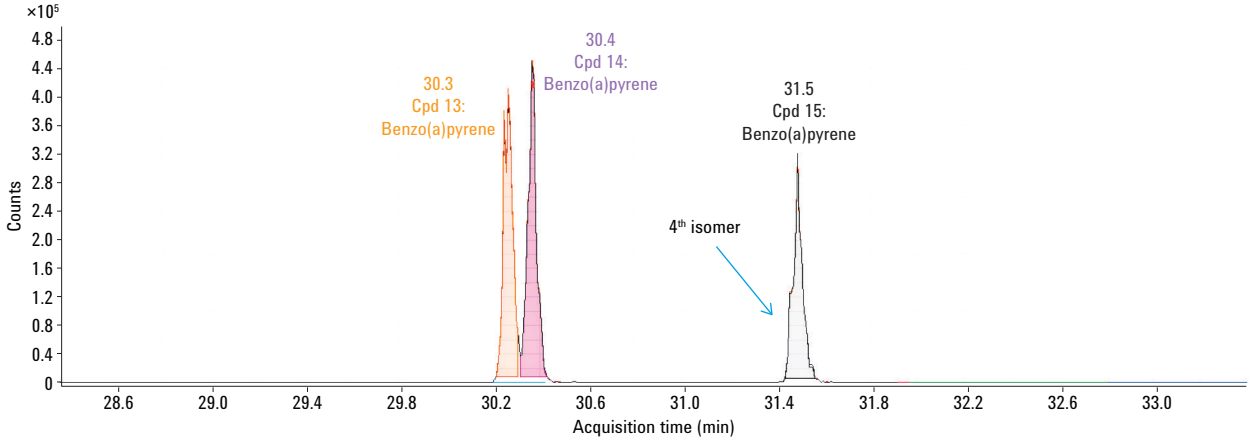


Figure 11. Expanded section of the extracted ion chromatogram shown in Figure 10 illustrating the partial separation and identification of the four isomers of benzo(a)pyrene.

Identification of the PAHS by mass only requires very accurate mass determinations. In this case, the mass accuracies ranged from as low as 0.02 ppm to no higher than 2 ppm, enabling confident compound identifications (Figure 12).

Compounds with very low proton affinity can be identified using the mass of the free radical cation $(M)^{*}+$, enabling confirmation (Figure 13).

This data was graciously provided by Agnieszka Olas and Karl Pettit of Marchwood Scientific.

Compound List

Automatically Show Columns

Show/Hide	Cpd	File	ID S	Name	Formula	RT	m/z	Mass	Score	RT Di	Diff (Tgt. ppm)	Ions	Height
☑	8	burger spiked...	FBF	Pyrene	C16 H10	21.1	203.0851	202.0781	98.38	-0.003	-0.92	4	31041
☑	6	burger spiked...	FBF	Phenanthrene	C14 H10	14.6	179.08506	178.0779	90.63	0	-1.97	3	27859
☑	1	burger spiked...	FBF	Naphthalene	C10 H8	7.9	128.06233	128.0631	64.09	0.006	3.69	2	36193
☑	14	burger spiked...	FBF	Indeno(1.2.3-C.D)pyrene	C22 H12	35.4	277.10126	276.0944	81.96	0	1.66	4	22244
☑	4	burger spiked...	FBF	Fluorene	C13 H10	11.4	167.08514	166.0781	98.77	0	-1.27	4	31510
☑	7	burger spiked...	FBF	Fluoranthene	C16 H10	20	203.08544	202.0784	99.74	0	0.61	4	22016
☑	16	burger spiked...	FBF	Dibenzo(a,h)anthracene	C22 H14	35.4	279.1168	278.1096	85.89	0	-0.11	3	21164
☑	9	burger spiked...	FBF	Chrysene	C18 H12	26.4	229.10117	228.094	99.2	0	0.62	4	35310
☑	11	burger spiked...	FBF	Benzo(k)fluoranthene	C20 H12	30.1	253.10121	252.0944	97.28	0	1.87	4	26868
☑	15	burger spiked...	FBF	Benzo(g,h,i)perylene	C22 H12	36.2	277.10127	276.0944	97.77	0	1.83	4	16127
☑	12	burger spiked...	FBF	Benzo(b)fluoranthene	C20 H12	30	253.1013	252.0945	98.84	-0.003	2.2	4	17692
☑	13	burger spiked...	FBF	Benzo(a)pyrene	C20 H12	31.2	253.10059	252.0939	95.64	-0.013	-0.1	4	19083
☑	10	burger spiked...	FBF	Benzo(a)anthracene	C18 H12	26.3	229.10083	228.0939	99.26	0	-0.02	4	28131
☑	5	burger spiked...	FBF	Anthracene	C14 H10	14.8	179.08533	178.0782	91.3	0.004	-0.38	3	27235
☑	2	burger spiked...	FBF	Acenaphthylene	C12 H8	9.9	153.06965	152.0625	99.21	0.004	-0.82	4	32986
☑	3	burger spiked...	FBF	Acenaphthene	C12 H10	10.2	155.08544	154.0781	99.38	-0.003	-1.3	4	32234

Figure 12. The mass accuracies obtained for the 16 PAHs spiked into the hamburgers.

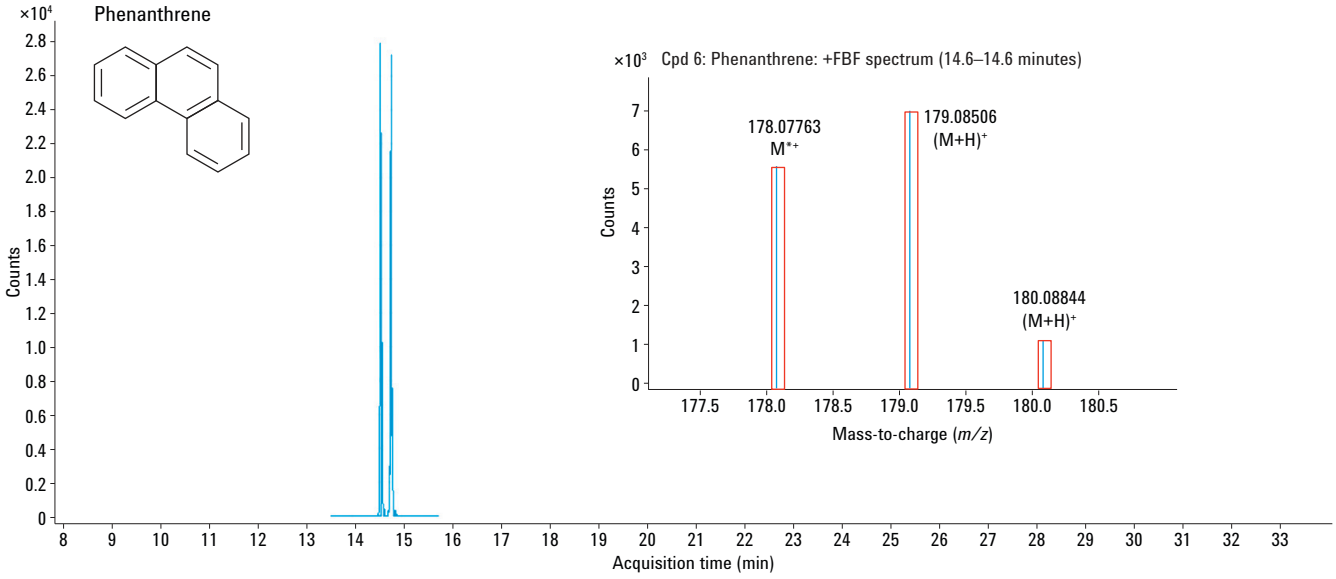


Figure 13. Phenanthrene is a low proton affinity compound, and it can be identified using both the $M^{*}+$ and (M+H)+ions.

Analysis of Semivolatile Compounds (EPA Method 8270)

EPA Method 8270 is widely used for the analysis of semivolatile compounds in environmental matrices, including water and soil. The list of compounds contains many pesticides, as well as PAHs.

The GC-APCI/6540 Q-TOF LC/MS system provides excellent results with Method 8270, using a 50 pg injection of an 8270 standard mix of 50 compounds (Figure 14). Many of the PAHs have similar molecular structures as well as similar and common molecular weights. Figure 14 demonstrates the benefits of having an isothermal transfer line, which results in very narrow peak widths and excellent resolution, particularly for the high boiling PAH compounds, such as those at the 1,120 second retention time (Figure 15). In addition, there is very little drift in the baseline.

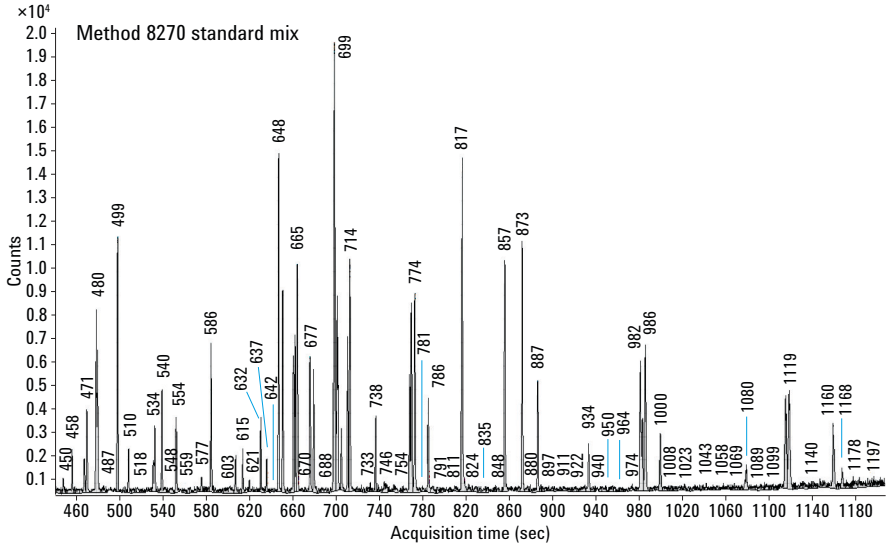


Figure 14. Base peak chromatogram of a 50 pg injection of an Method 8270 standard mix.

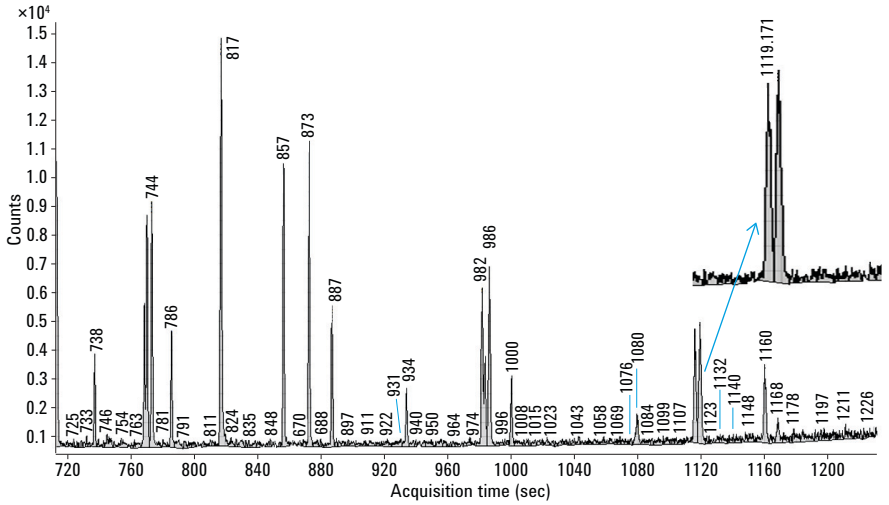


Figure 15. Expanded section of the base peak chromatogram shown in Figure 14 with an inset illustrating the excellent resolution of the late-eluting high boiling compounds, including two shown in the inset at 1,110 and 1,120 seconds, respectively.

In addition to narrow column widths, using the Q-TOF mass spectrometer provides the ability to identify compounds with very similar retention times based on very small mass differences, due to its excellent mass accuracy (Figure 16).

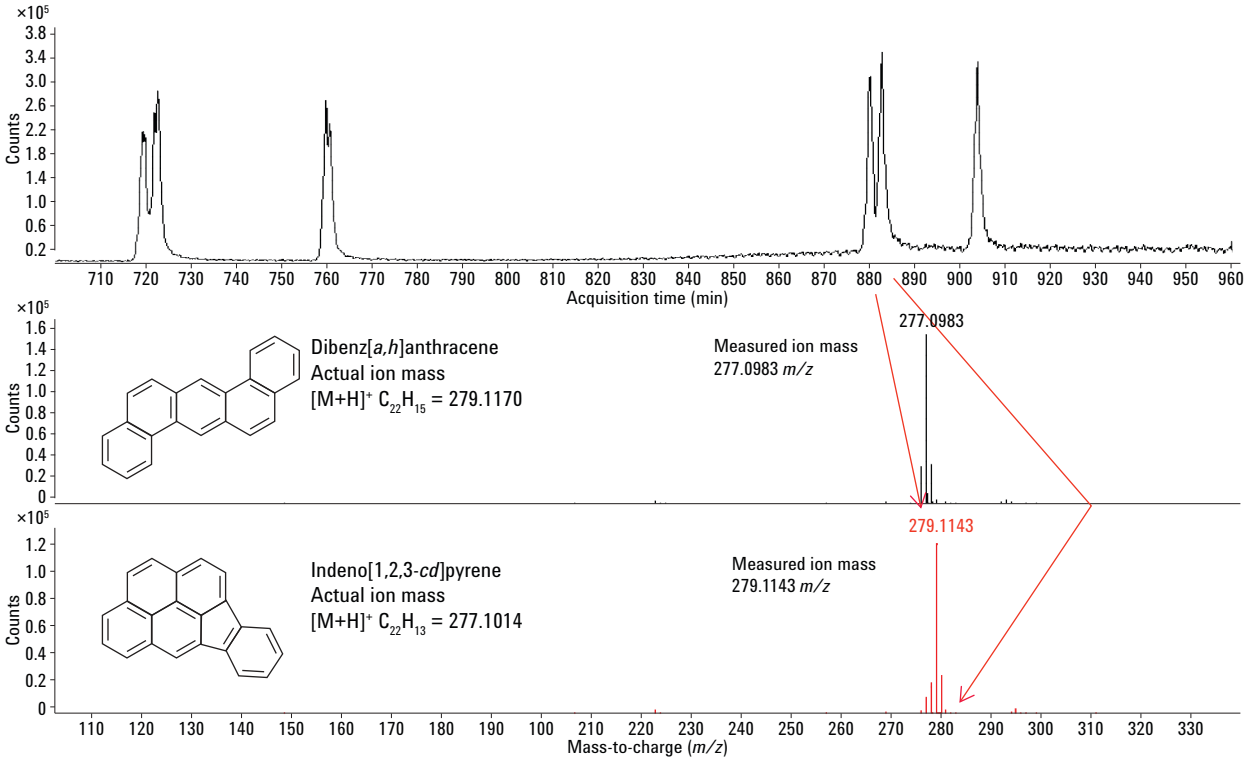


Figure 16. A base peak chromatogram and mass spectra illustrating the successful identification of two PAH compounds with small differences, due to the benefit of excellent chromatography and the accurate mass capability of the system.

Nitrosamines in Environmental Samples

Nitrosamines are classified as carcinogens by the USEPA and the Food and Drug Administration (FDA). Research studies have suggested that certain nitrosamines could be mutagens and could cause birth defects at very low concentrations.

Nitrosamines are found in in cured meat products, tobacco, rubber, cosmetics, and other consumer products. Concern has also grown regarding the health effects of naturally formed nitrosamines caused by added nitrites and amines in food.

Effective analytical techniques are needed by many industries to measure these toxic compounds. Gas chromatography combined with nitrogen and chemiluminescent detection offers reliable separation and sensitive detection, but not absolute molecular confirmation and identification. Gas chromatography combined with accurate mass measurements can provide a higher level of molecular structure confirmation.

Figure 17 illustrates the separation and detection of nitrosamine standards using the GC-APCI/6540 Q-TOF LC/MS system. The peak at 7.7 minutes appears to be a single chromatographic peak, but when ion extraction is performed using accurate masses, more peaks appear (Figure 18). The coelution of three nitrosamines is readily observed when using accurate mass to determine the compound.

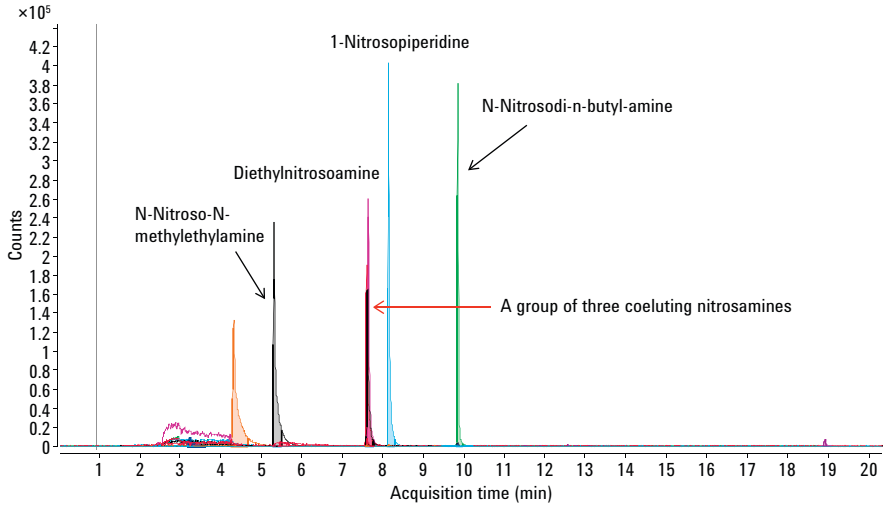


Figure 17. Extracted ion chromatogram of a standard mix of nitrosamines.

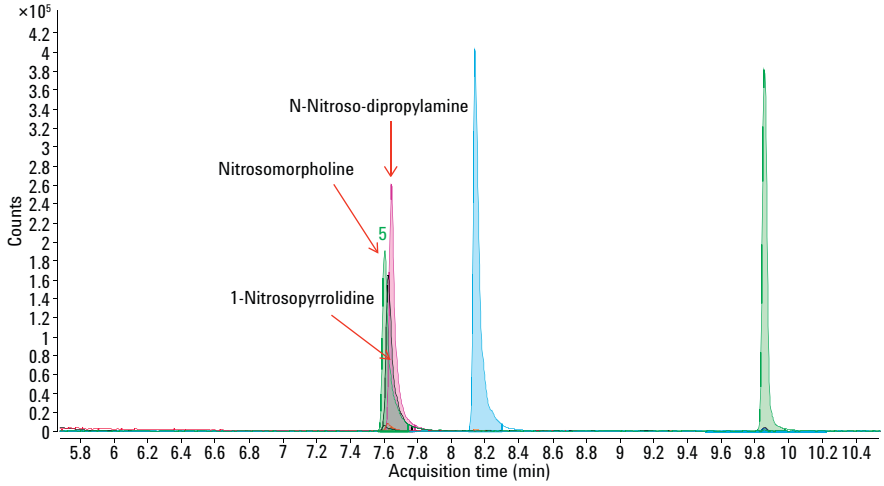


Figure 18. Expanded section of the extracted ion chromatogram shown in Figure 17 illustrating the ability of accurate mass to determine the identities of three coeluting compounds.

Accurate mass measurements can be used to identify two compounds with the same nominal mass, that differ by as little as 36 millimass units in accurate mass (Figure 19).

Natural Products Analysis

Curry leaf essential oil is used in Indian cooking as well as traditional medicine native to the Indian subcontinent. It is said to strengthen the gums and teeth, prevent nausea and cure stomach upsets, treat skin irritations and poisonous bites, and act as an antibacterial, antifungal, and astringent.

This commercially valuable oil has been shown to exhibit chemical diversity depending on the geographic location. This diversity impacts the aroma and flavor of the oil, as well as its potential health benefits.

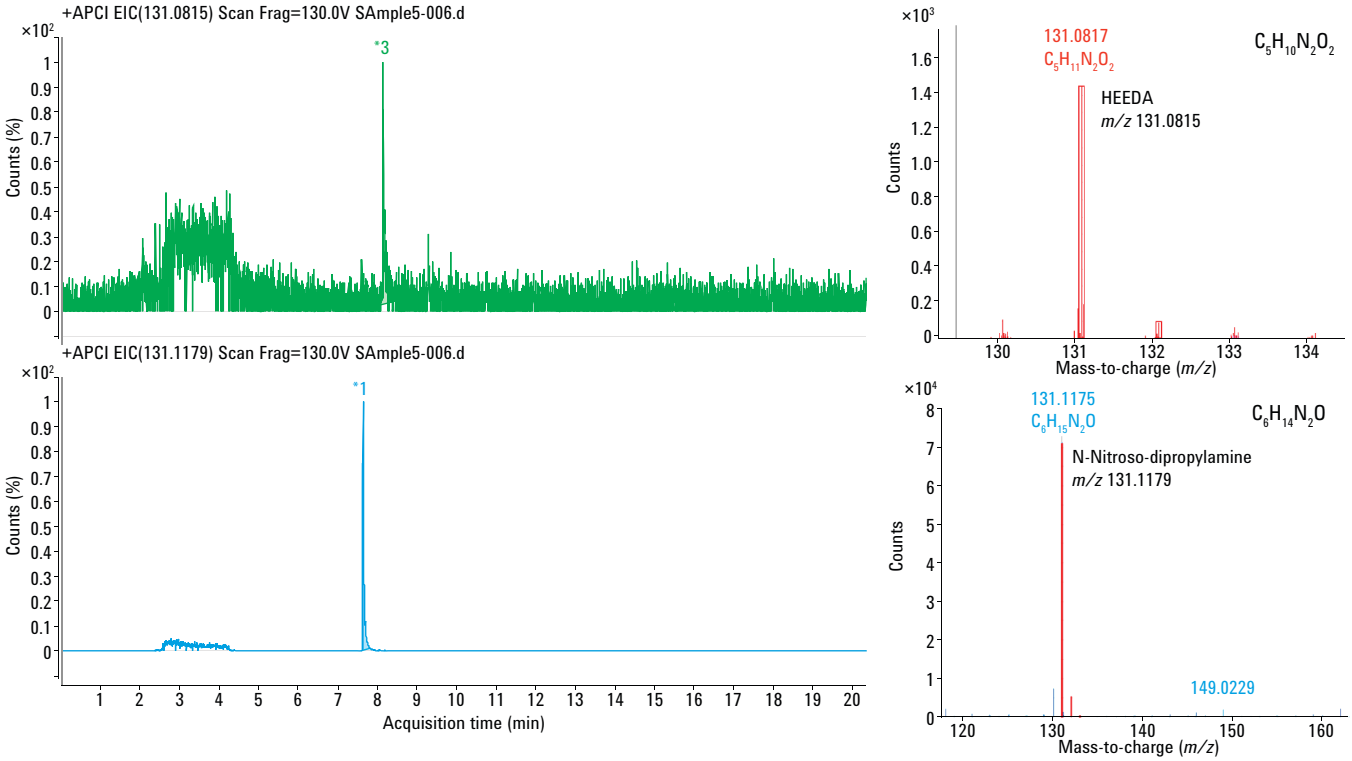


Figure 19. The advantages of accurate mass measurements for identifying two compounds with isobaric masses at nominal mass 131. The difference in accurate mass for the two identified coompounds is only 36 milimass units.

The GC-APCI/6550 Q-TOF LC/MS system and Mass Profiler Professional (MPP) software are excellent tools to study this chemical diversity. The retention time and accurate mass can be used to identify large numbers of compounds in the oil, and the analysis tools in MPP can be used to elicit differences in the compounds contained in oil sourced from different locations.

Once accurate masses have been determined for compounds, their retention times can be compared to those in the METLIN database using MPP, in order to confirm their identity. In this case, caryophyllene and cubenene have the same mass and retention time (Figure 20). Several other compounds identified using MPP and the METLIN database are shown in Figure 21.

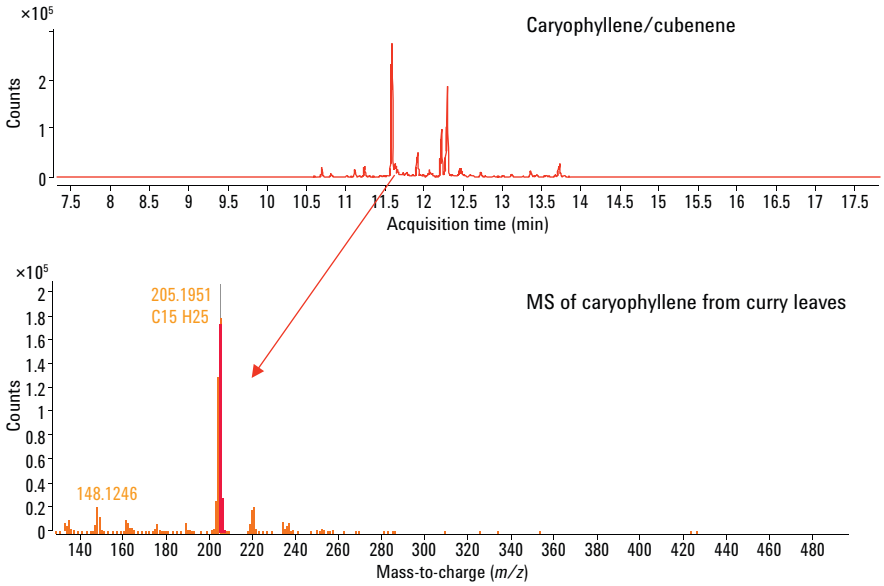
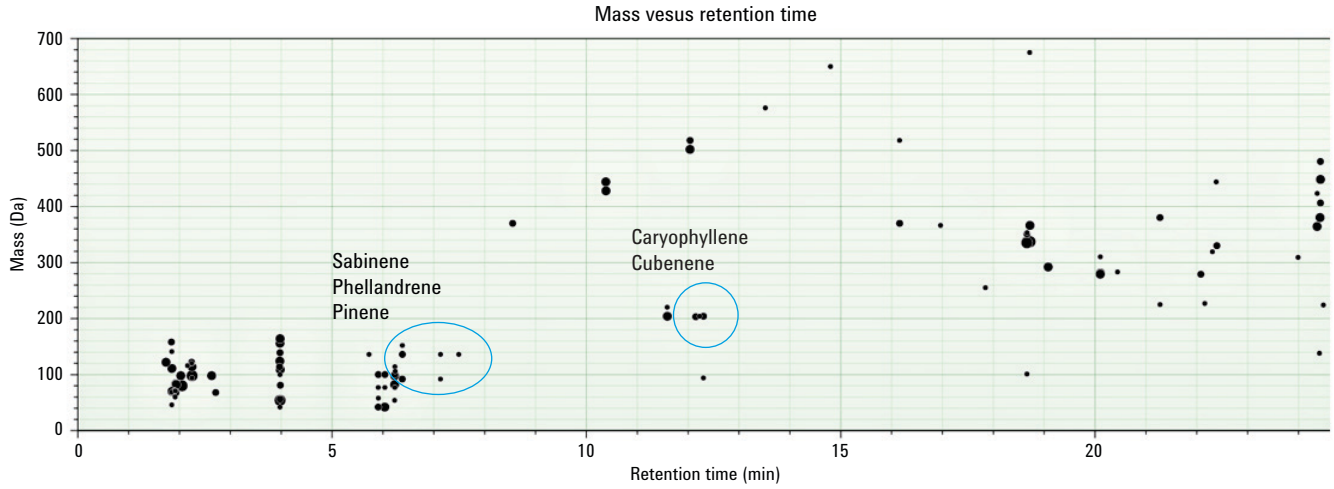


Figure 20. Extracted ion chromatogram and MS spectrum of components of curry oil.



Expression: Both Up Down **100 features**

Feature summary

	ID	Name	Formula	CAS	RT	SD	Mass	SD	Abundance	RSD	Freq.	Mark
1	72	Tropine	C8 H15 N O	120-29-6	1.849	0.000	141.1155	0.0000	91626	0.00	1	
2	12	Tranexamic acid	C8 H15 N O2	1197-18-8	3.974	0.000	156.1024	0.0000	905059	0.00	1	
3	84	Stearamide	C18 H37 N O	124-26-5	20.441	0.000	283.2874	0.0000	74850	0.00	1	
4	93	pentacosanal	C25 H50 O		16.961	0.000	366.3855	0.0000	61296	0.00	1	
5	15	Oleamide	C18 H35 N O	301-02-0	20.105	0.000	281.2719	0.0000	782030	0.00	1	
6	48	N-HISTIDYL-2-AMINONAPHTHAL...	C16 H16 N4 O	7424-15-9	22.079	0.000	279.1253	0.0000	212777	0.00	1	

Figure 21. A graph in MPP of mass versus retention time, also showing the identities of several of the compounds determined from RT and accurate mass.

Conclusion

The GC-APCI Interface for Agilent Q-TOF LC/MS systems improves productivity and reduces cost by adding GC capability to your laboratory. This cross-platform approach has attracted a lot of attention in several areas of analytical chemistry, especially metabolomics. For metabolic profiling, the traditional approach would use at least two different instruments: the GC/MS for the volatile components and LC/MS for the nonvolatile more polar species. This requires a lab to have two or more instruments to cover the range of polarity/volatility of compounds. With GC-APCI, the same mass spectrometer can be used for both GC and LC analyses.

Atmospheric pressure ionization enables as much as five times higher gas flow than dedicated GC systems. The end result is faster analysis times and larger sample loads. A wide range of makeup gases that can be readily attached to the interface assures ionization for a wide variety of samples. You gain the flexibility to handle a wide range of challenging applications, including those highlighted here. Very narrow GC peaks and the accurate mass capability of the Q-TOF LC/MS provide greater sensitivity, higher resolution and confident compound identifications.

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Published in the USA, April 22, 2013
5991-2210EN

Agilent Technologies