Screening for Hundreds of Pesticide Residues Using a GC/Q-TOF with an Exact Mass Pesticide Database in Food

Application Note

Food

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Abstract

The Agilent 7200 GC/Q-TOF, in concert with the Agilent MassHunter Qualitative Analysis All Ions workflow, and the first commercially available exact mass pesticide library, was used to rapidly screen, identify, and generate quantitative information for pesticide residues in five different food matrices. This technique helps eliminate false positives, and has the speed and accuracy to significantly improve the productivity of pesticide screening and quantitative work. A screening workflow using the new GC/Q-TOF Pesticide Library and the unique All Ions software tools enables the detection of pesticide levels as low as 10 ppb in complex matrices.

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Introduction

With increased international trade in food and food ingredients, there is even more emphasis on food safety. State-of-the-art pesticide screening requires the consideration of more than 1,000 pesticides and their metabolites. Of these, as many as 600 to 700 compounds can be included in routine monitoring programs. Testing approaches must be able to handle many compounds at a time, while being able to avoid matrix interferences coming from many different food matrices. The increasing global emphasis on pesticide screening is reflected in the implementation of European Union (EU) guideline SANCO/12571/2013 [1]. The most recent revision specifies criteria for qualitative screening supported by databases or libraries. An accurate-mass approach for pesticide screening using quadrupole time-of-flight mass spectrometry (Q-TOF) ensures reliable pesticide identification, and enables a virtually unlimited number of compounds to be screened simultaneously. For many of the most important compounds, gas chromatography (GC) coupled to a Q-TOF mass spectrometer is the ideal analytical tool for screening, confirmation, and quantification of both target and unexpected compounds at trace levels, even in complex matrices.

This application note introduces a workflow for the screening of pesticide residues in various foodstuffs using GC/Q-TOF and electron ionization (EI) in combination with a retention time locked GC method [2], midcolumn backflushing for increased method robustness [3], and a novel exact mass pesticide spectral library. Agilent MassHunter Software then automates the screening for more than 700 pesticides that are contained in a Personal Compound Database and Library (PCDL). The Agilent All Ions workflow chooses characteristic exact mass ions for each compound in the PCDL and extracts them from the chromatogram. To verify the hits, a coelution plot and coelution score are created to observe and express the covariance of the extracted accurate mass ions. The coelution score uses the retention time and the entire chromatographic peak information (including peak width and symmetry) to determine covariance of the characteristic ions.

This GC/Q-TOF screening approach complements GC/MS/MS target compound analysis. In addition, retrospective data analysis is also possible since chromatograms with full EI spectra are acquired. For any unexpected compounds, the user can quickly investigate the identities of such compounds with high resolution accurate mass data. If subsequent quantitative screening is considered important for future work, the critical ion information can easily be exported into a quantitative method. If necessary, hundreds of pesticides can be quantified in a single analysis.

Experimental

Reagents and standards

All high-purity pesticide standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany), Sigma-Aldrich (Steinheim, Germany) and Riedel-de Haën (Selze, Germany), and were stored at –30 °C. Individual pesticide stock solutions (1,000–2,000 mg/L) were prepared in acetonitrile and stored in amber screw-capped glass vials in the dark at –20 °C. Individual standard solutions, used for the optimization, and one 10 mg/L mix of all the standards in acetonitrile were prepared from the stock standards. The standard mix solution was used for the calibration by appropriate dilution in ethyl acetate. Ethyl acetate was obtained from Fluka Analytical Pestanal; acetonitrile was obtained from Sigma-Aldrich (Steinheim, Germany), and $MgSO_4$ was obtained from Panreac Quimica S.A. (Barcelona, Spain). Primary secondary amine (PSA) sorbent was obtained from Supelco (Bellefonte, Pennsylvania), and NaCl was from J.T. Baker (Deventer, The Netherlands).

Instruments

This study was performed using an Agilent 7890B GC system coupled to an Agilent 7200 Series GC/Q-TOF System. The instrument conditions are listed in Table 1, and the instrument system configuration is shown in Figure 1.

Sample preparation

Vegetable and fruit samples were obtained from local markets. Blank vegetable and fruit extracts were used to prepare the matrix-matched standards for validation purposes. In this way, five types of fruits and vegetables (apple, carrot, leek, tomato, and oranges) were extracted using the QuEChERS method, as previously described [4]. The vegetable extracts were spiked with the mix of standards at different concentrations (ranging from 10 to 200 µg/kg), and subsequently analyzed by GC/Q-TOF.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC conditions	
Columns	Agilent HP-5MS Ultra Inert, 15.0 m × 0.25 mm, 0.25 µm (p/n 19091S-431UI) Inlet multimode inlet, Outlet pressure controlled tee Agilent HP-5MSUI, 15.0 m × 0.15 mm, 0.15 µm (p/n 19091S-431UI) Inlet pressure controlled tee Outlet vacuum
Injection port	Multimode inlet
Injection mode	Splitless
Injection volume	1.0 µL
Injection port liner	Ultra inert liner split, straight, wool (5190-2293)
Carrier gas	Helium at 0.96 mL/min constant flow
Oven program	60 °C for 1 minute 40 °C/min to 120 °C for 0 minutes 5 °C/ min to 310 °C for 0 minutes
Retention time locking	Chlorpyrifos-methyl locked to 18.111 minutes
Backflush	Post run, 5 minutes, oven 300 °C 40 psi at pressure controlled tee, inlet 1 psi
Transfer line temperature	280 °C
Q-TOF MS conditions	
Instrument	Agilent 7200 Q-TOF
Ionization mode	EI
MS temperatures	Source 280 °C, Quadrupole 150 °C
Detection mode	45–550 *m/z* scan
Spectra acquisition rate	5 spectra/s

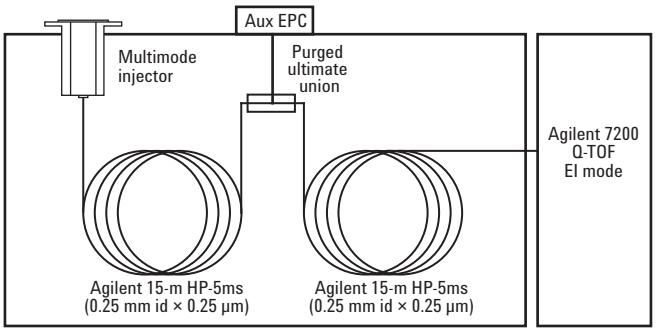

Figure 1. GC/Q-TOF configuration with midcolumn backflush.

Data acquisition and analysis

The data were acquired with the MassHunter Acquisition Software B.07.02. Data analysis for the pesticide screening was performed with the All Ions tool in MassHunter Qualitative Analysis Software (B.07.00) and the GC/Q-TOF Pesticide PCDL (p/n G3892A). Data analysis for pesticide quantitation was performed with the MassHunter Quantitative Analysis Software (B.07.01).

Results and Discussion

The All Ions tool

Data analysis was performed using the MassHunter Qualitative Analysis Software (B.07.00). Users can set up parameters for the All Ions MS technique in a new tab in the Find by Formula (FbF) area of MassHunter Qualitative Analysis called Fragment Confirmation (Figure 2). The tab allows the user to specify how many of the most specific ions to extract. Limits can also be set for fragment ion Extracted Ion Chromatograms (EICs) based on retention time (RT) difference, minimum signal-to-noise (S/N) ratio, and coelution score. The EICs of the most specific EI fragments in each PCDL spectrum are extracted and evaluated using a unique coelution score parameter. The coelution score was derived from a technique similar to UV chromatography's Peak Purity [1], in which the software calculates a number that takes into account multiple factors, such as abundance, peak shape (symmetry), peak width, and RT. Figure 3A provides an example of overlaid EICs for the ions derived from bupirimate in carrot extract. All of the ions have the same chromatographic apex and shape, suggesting that they originated from the same compound. The normalized ratios of the fragment ions to the reference ion intensity are plotted across the RT and made available to the user for inspection in a coelution plot (Figure 3B). If all ions exhibit a ratio of approximately 1 across the middle of the reference ion peak, as in this example, there is strong confirmation that the fragments belong to the same compound.

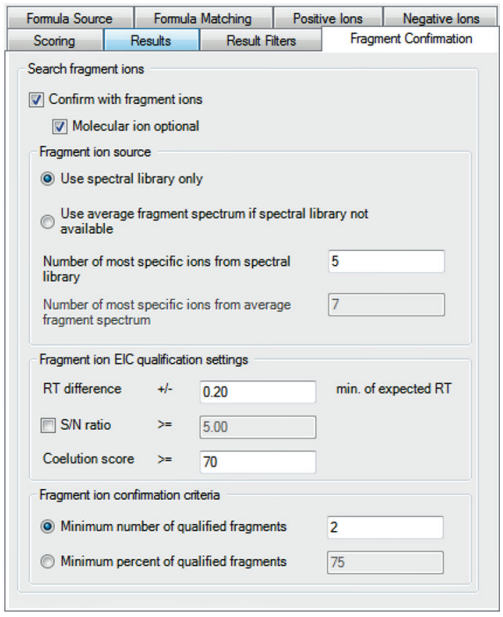

Figure 2. Fragment Confirmation tab from the Find by Formula (FbF) tool in Agilent MassHunter Qualitative Analysis.

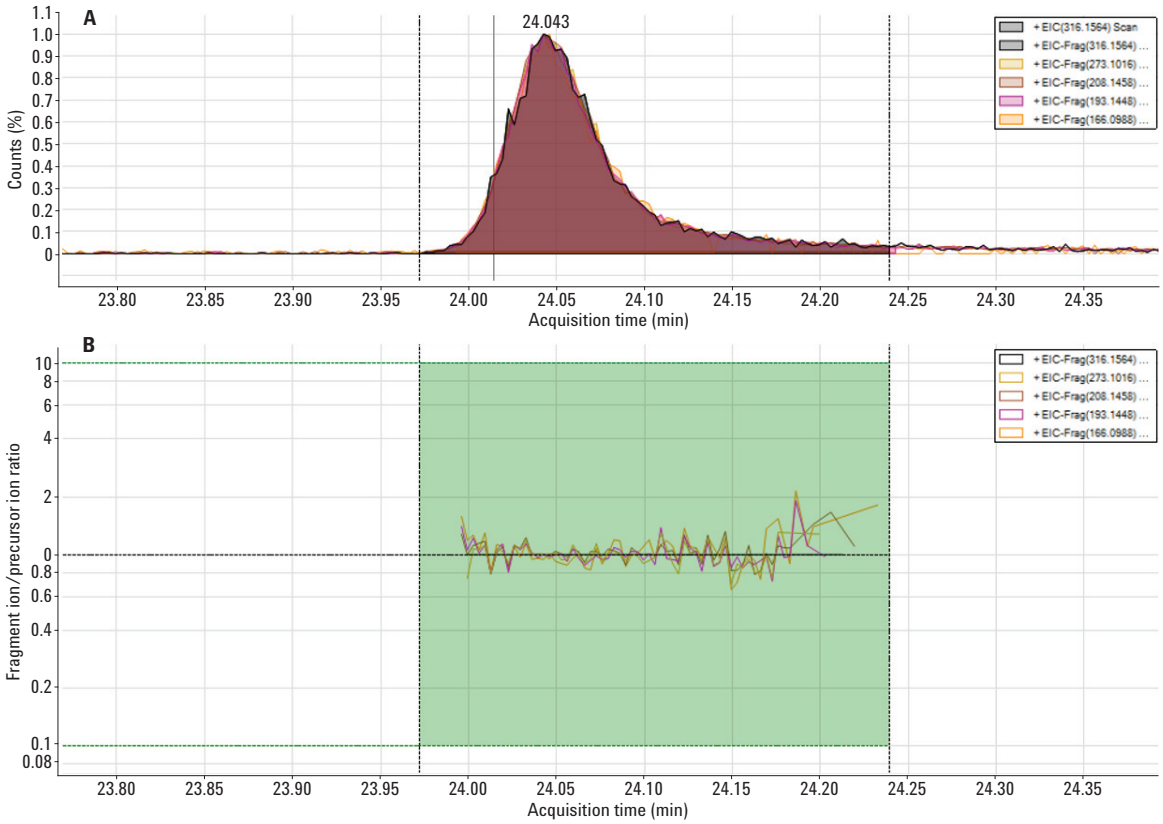

Figure 3. Overlaid EICs for bupirimate in carrot with the reference ion in grey and qualifying ions in other colors. All of the ions have the same chromatographic apex (A) and calculated coelution plot (B). All fragment ions exhibit ratios of approximately 1 across the middle of the reference peak, indicating strong coelution. This provided confirmation for the identification of bupirimate in the sample.

Viewing compound details

Users can easily inspect the results quickly, scrolling through all compounds in the screen and efficiently viewing overlaid EICs, the coelution plot, spectrum results for each compound, and the identification parameters, in this case for bupirimate (Figure 4). The identification parameters panel is shown by itself in Figure 5, displaying the measured mass and retention time, as well as the target, or reference values, and the identification scores. The flexibility of the All Ions tool settings enables the user to fine-tune it for the specific application by selecting the desired number of qualifying ions, coelution score, mass extraction window, and other parameters (Figure 2). In addition, RT locking ensures precise identification of the pesticides.

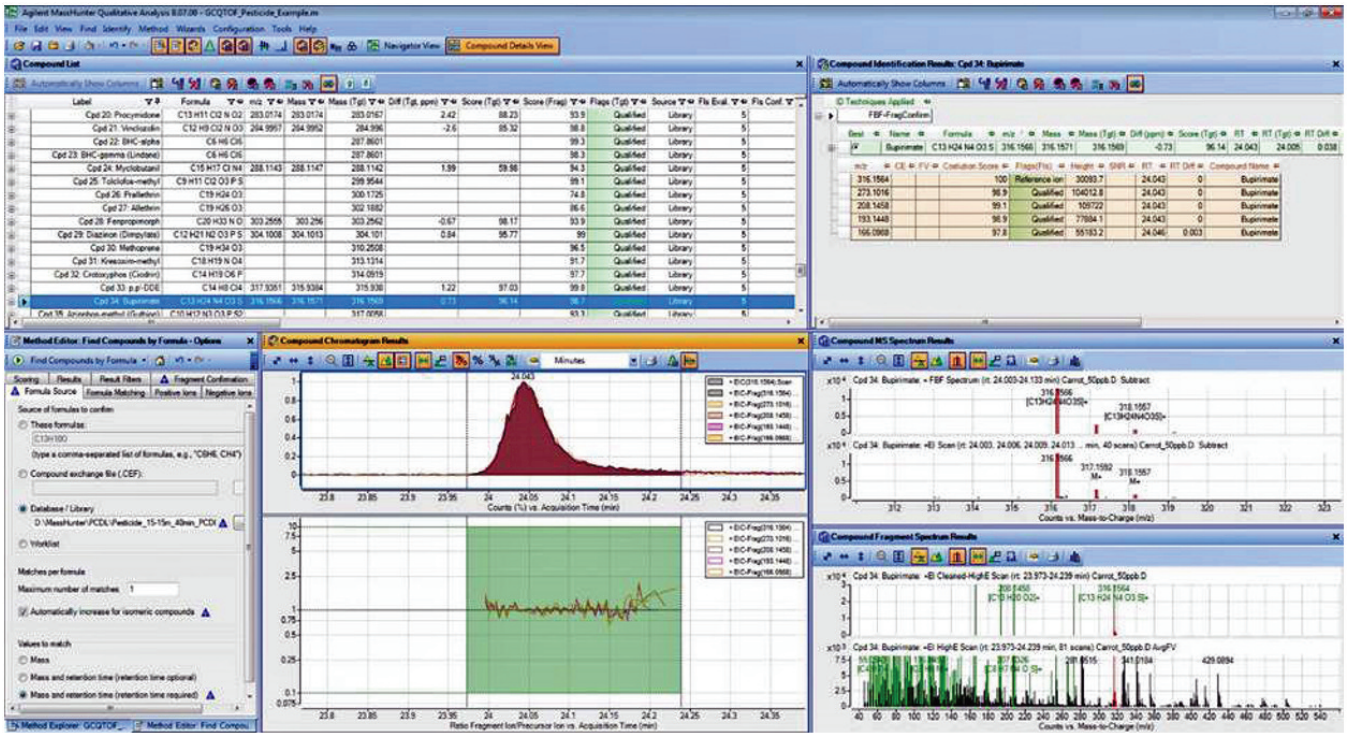

Figure 4. Agilent All Ions tool results overview for pesticides spiked into carrot extract.

ID Techniques Applied

FBF-FragConfirm

Best	Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (Tgt)	RT Diff	Score (RT)	Species	Flags	Notes
●	Bupirimate	C13 H24 N4 O3 S	316.1566	316.1571	316.1569	-0.73	96.14	24.043	24.005	0.038	89.78	M+		Pesticide; Fungicide; Veterinary drug

m/z	Coelution Score	Flags(Fls)	Height	RT	RT Diff	Compound Name
316.1564	100	Reference ion	30093.7	24.043	0	Bupirimate
273.1016	98.9	Qualified	104012.8	24.043	0	Bupirimate
208.1458	99.1	Qualified	109722	24.043	0	Bupirimate
193.1448	98.9	Qualified	77884.1	24.043	0	Bupirimate
166.0988	97.8	Qualified	55183.2	24.046	0.003	Bupirimate

Figure 5. Compound Identification Results pane from the Agilent All Ions tool.

As a validation study, 56 pesticides were spiked into five different matrices (apple, carrot, tomato, leek, and orange) at increasing concentration levels (Table 2). Most of the compounds were found at the lowest spiked level of 10 µg/mL (parts per billion, (ppb)) in all matrices, and their presence was verified by at least two additional fragment ions (as indicated by dark green cells) and their retention times.

Quantitative analysis

For unexpected compounds that are found, subsequent quantitative analysis may be considered necessary, and this can be set up simply by exporting the qualitative data to MassHunter Quantitative Analysis Software, using a Compound Exchange Format (CEF) file.

Table 2. Compound Screening Results in Four Matrices (10 to 200 ppb)

	Tomato				Carrot				Apple				Orange				Leek			
Compound	**10**	**50**	**100**	**200**	**10**	**50**	**100**	**200**	**10**	**50**	**100**	**200**	**10**	**50**	**100**	**200**	**10**	**50**	**100**	**200**
Dichlorvos																				
Biphenyl																				
Phenylphenol 2-																				
Chlorpropham																				
Trifluralin																				
HCH *alpha*																				
HCB																				
HCH *beta*																				
Propazine																				
HCH *gamma* (lindane)																				
Terbuthylazine																				
Pyrimethanil																				
Diazinon																				
Pirimicarb																				
Chlorpyrifos-methyl																				
Parathion-methyl																				
Vinclozolin																				
Tolclofos-methyl																				
Metalaxyl																				
Fenpropidin																				
Fenitrothion																				
Chlorpyrifos																				
Fenpropimorph																				
Pendimethalin																				
Fipronil																				
Procymidone																				
Endosulfan *alpha*																				
Dieldrin																				
DDE p,p'-																				
Myclobutanil																				
Bupirimate																				
Kresoxim-methyl																				
Endosulfan *beta*																				
Chlorobenzilate																				
DDD *p,p'*-																				
DDT *o,p'*-																				
Oxadixyl																				

White = not found; dark green = Found; light green = Found, qualifier used for quantitation

Table 2. Compound Screening Results in Four Matrices (10 to 200 ppb)(continued)

Compound	Tomato				Carrot				Apple				Orange				Leek			
	10	50	100	200	10	50	100	200	10	50	100	200	10	50	100	200	10	50	100	200
Endosulfan sulfate																				
DDT *p,p′*-																				
TPP																				
Iprodione																				
Tetramethrin I																				
Bromopropylate																				
Tetramethrin II																				
Bifenthrin																				
γ-Cyhalothrin																				
Acrinathrin																				
Bitertanol																				
Cypermethrin I																				
Cypermethrin II																				
Etofenprox																				
Esfenvalerate (SS,RR)																				
Azoxystrobin																				

White = not found; dark green = Found; light green = Found, qualifier used for quantitation

The CEF file contains information necessary to set up a quantitative method: compound name, retention time, reference ion, fragment ions (to create qualifiers), and relative abundances. The MassHunter Quantitative Analysis Software automatically selects the reference and qualifier ions, saving tedious manual processing. After the method has been set up, suspect samples can be run to acquire quantitative results. Turnkey automation allows MassHunter to both acquire and quantitate data, and provides a report for the targeted compounds.

MassHunter has long since provided a popular environment for reviewing quantitative results, and these software tools are available for GC/Q-TOF as well. In this case, Quantitative Analysis software allows viewing of quantifier and qualifier ions, but with an added level of confidence in the results provided by the scoring of the quality of identifications with accurate mass metrics. Figure 6 shows extracted ion chromatograms of bupirimate with two qualifier ions and their ratios plotted against the quantifier ion. During data processing, the MassHunter Quantitative Analysis software automatically flags qualifier ratios that are outside of user-specified limits.

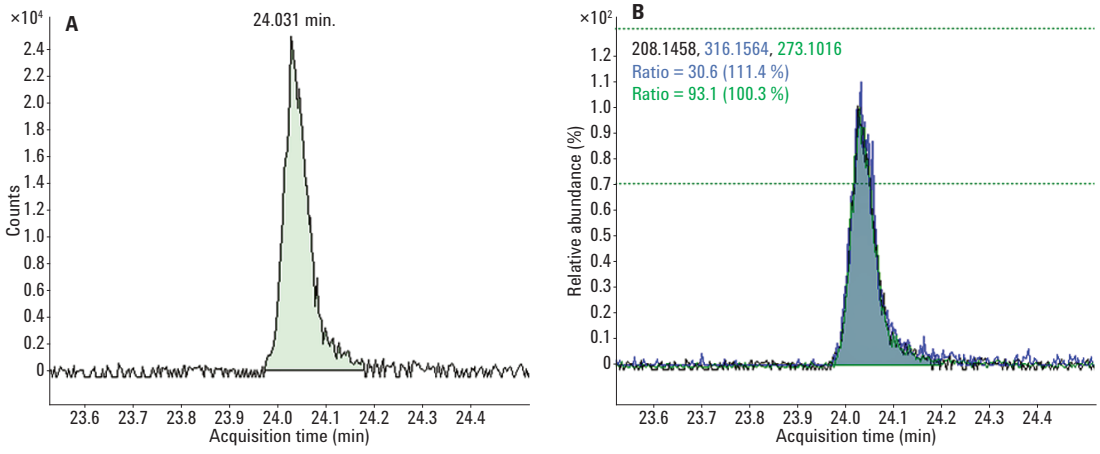

Figure 6. EICs of the quantifier (target) ion (A), as well as qualifier ions and the expected qualifier to quantifier ion ratio (B) for bupirimate in apple at 10 µg/mL (ppb).

The necessary calibration range for most compounds is usually from 10 to 200 ng/mL. When quantitating many compounds in one run, it is likely that some compounds will produce much higher responses than others. For such compounds, this can lead to saturation of the highest calibration standard of 200 µg/mL. Unifying response factors is done easily when using GC/Q-TOF, because EI often offers a range of candidate ions from which to choose. The resolution of the mass spectrometer also offers the use of carbon 13 isotope ions. The user can simply choose the ion that is optimal for the calibration range. This can even be changed retrospectively due to the untargeted nature of acquisition with a GC/Q-TOF. Retrospective analysis is exactly what is required when curating a quantitative method for the first time using spikes and standards. This was also part of the objective for this study, and the results will be shown in a separate report. For now, those compounds whose quantitation benefitted from adjustment away from the dominant ions are shown in Table 2 (light green cells).

Conclusions

The Agilent 7200 Series GC/Q-TOF, in combination with Agilent MassHunter Qualitative Analysis Software and the GC/Q-TOF Pesticide PCDL, can be used effectively to screen for pesticide residues in a variety of matrices at concentrations as low as 10 ppb. Accurate identification is assured by use of the unique Agilent All Ions tool. The advantages of using GC/Q-TOF include increased confidence in compound confirmation provided by accurate mass-high resolution data, the ability to perform retrospective analysis (particularly for unexpected peaks), and the ability to seamlessly go from qualitative to quantitative analysis.

These results are encouraging, because as new compounds appear on a laboratory's radar, not only can data collected in the past be re-interrogated, but a means is also available to create and expand optimized quantitative methods for the future.

References

1. SANCO/12571/2013. Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed, 2013.
2. V. Giarrocco, B. Quimby, M. Klee, Retention Time Locking: Concepts and Applications, *Agilent Technologies Application Note*, publication number 5966-2469EN, December **1997**.
3. H. Prest, Capillary Flow Technology for GC/MS: A Simple Tee Configuration for Analysis at Trace Concentrations with Rapid Backflushing for Matrix Elimination, *Agilent Technologies Application Note*, publication number 5979-8664EN, June **2008**.
4. L. Rajski, A. Lozano, N. Belmonte-Valles, A. Uclés, S. Uclés, M. Mezcua, and A. R. Fernandez-Alba. "Comparison of three multiresidue methods to analyse pesticides in green tea with liquid and gas chromatography/tandem mass spectrometry" *Analyst*, **138**, 921-931(2013).
5. H. P. Sievert, A. C. J. H. Drouen. "Spectral matching and peak purity in Diode Array Detection" in *High Performance Liquid Chromatography*, New York, Marcel Dekker, **51**, (1993).

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