Analysis of Pesticides in Vegetables Using the Agilent 1260 Infinity Analytical SFC System with Triple Quadrupole MS Detection

Application Note

Food Testing & Agriculture

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Abstract

This Application Note demonstrates the optimization of a separation method for the determination of pesticides in a complex food matrix by supercritical fluid chromatography (SFC) with triple quadrupole MS detection. Several gradients of different steepness are applied to the analysis of a vegetable matrix spiked with different concentrations of a multipesticide standard. The optimum separation conditions are determined by software-aided batch comparison to identify the gradient with the lowest matrix impact.

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Introduction

Pesticides are widely used in the production of all plant food products such as vegetables, fruits, corn, and grain to protect against various pests. Before plant-based food products enter the market, they have to be tested for possible pesticide residues, which must meet legal limits[1]. Therefore, samples of the complete plant food product have to be extracted and transferred into an analyzable form, typically a solution in organic solvent. This extraction is mostly done by the QuEChERS procedure[2]. The analysis of such samples by HPLC with triple quadrupole MS detection is state of the art. Unfortunately, during sample preparation, not only the pesticide residues are extracted, but also naturally inherent compounds, which make up the matrix. Pesticide and matrix compounds compete for ionization in the ion source of a mass spectrometer when they are eluted from the HPLC column at the same time. This hampers the accurate quantification of pesticides in complex food matrixes. If matrix compounds are present in a large excess, it is possible that they suppress the ionization of the pesticide completely.

Good separation of all compounds on the column can help to avoid this situation and have a strong influence on the mass spectrometric detection of the analytes. Careful optimization of the separation becomes as important as the adjustment of the MS parameters[3]. To compare several separation conditions, a batch analysis can be performed and the optimum conditions for best and broadest detection can be identified.

This Application Note demonstrates the detection of pesticides by supercritical fluid chromatography (SFC) with triple quadrupole mass spectrometry in a complex food matrix after optimization of the SFC separation and batch comparison of different separation conditions. The advantages of using SFC as a front end for the analysis of pesticides in plant food samples by means of mass spectrometry are separation speed, orthogonal selectivity, and tolerance of injections with organic solvents used during sample preparation.

Experimental

Instrumentation

All experiments were carried out on an Agilent 1260 Infinity Analytical SFC System (G4309A) comprising:

- Agilent 1260 Infinity SFC Control Module
- Agilent 1260 Infinity SFC Binary Pump
- Agilent 1260 Infinity High Performance Degasser
- Agilent 1260 Infinity SFC Autosampler
- Agilent 1290 Infinity Thermostatted Column Compartment
- Agilent 1260 Infinity Diode Array Detector with high pressure SFC flow cell
- Agilent 6460 Triple Quadrupole LC/MS System (G6460C)
- Agilent 1260 Infinity Isocratic Pump (G1310B)
- Splitter kit (G4309-68715)

Instrumental setup

The recommended configuration of the Agilent 1260 Infinity Analytical SFC System with the Agilent 6460 Triple Quadrupole LC/MS System is shown in Figure 1. The column is directly connected to a splitter assembly, which contains two combined splitters, an additional check valve to prevent of CO_2 flowing back into the make-up pump, and a solvent filter. At the first splitter the make-up flow coming from the isocratic pump is introduced into the flow path. This splitter is connected to the second splitter by a short 0.12-mm id capillary. Here, the flow is split into one part going to the MS and the other part going to the backpressure regulator (BPR) of the SFC module. The connection to the MS is made by a newly developed 50-µm id stainless steel capillary of 1-meter length, which is included in the splitter kit. The split ratio depends on the backpressure generated by this restriction capillary and the pressure set by the BPR. As a rule of thumb, an SFC backpressure of 120 bar diverts about 0.45 mL/min of the SFC flow to the ion source and 200-bar backpressure diverts about 0.6 mL/min to the ion source. Since electrospray MS is concentration-dependent, this has no influence on signal intensity.

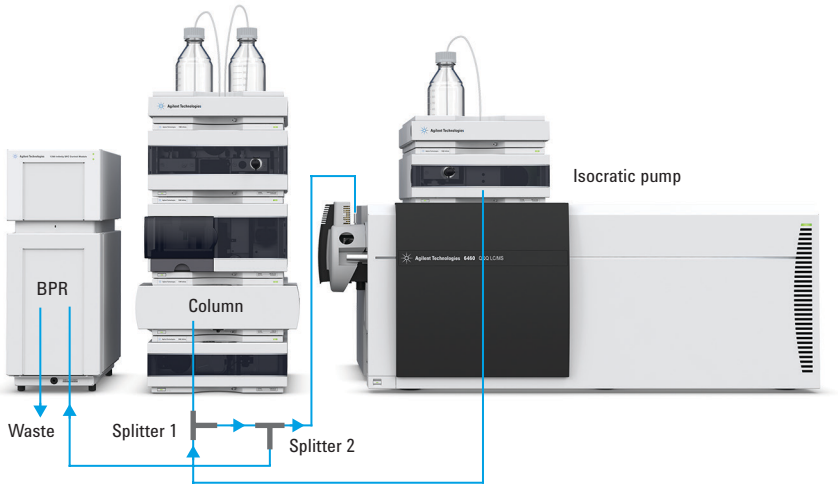

Figure 1. Configuration of the Agilent 1260 Infinity Analytical SFC System with the Agilent 6460 Triple Quadrupole LC/MS System. The column is connected directly to splitter 1 in the splitter assembly.

Column

Agilent ZORBAX NH2,
4.6 × 150 mm, 5 µm (p/n 883952-708)

Software

- Agilent MassHunter Data Scquisition Software for triple quadruple mass spectrometer, version 06.00. including SFC software add-on
- Agilent MassHunter Qualitative Software, version 06.00
- Agilent MassHunter Quantitative Software, version 07.00

Standards

A standard mixture containing 10 ng/µL of each of the 17 pesticides in acetonitrile solution was obtained from LGC Standards GmbH (Pesticide Mix 44, part no. 18000044) Mercatorstrasse 51, 46485 Wesel, Germany. The inherent pesticide degradation product atrazine desethyl was not investigated in this study because it is not relevant for vegetables and fruits – it is a degradation product from atrazine occurring in soil and water.

Chemicals

All solvents were LC/MS grade. Acetonitrile and methanol were purchased from J.T. Baker, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak).

SFC method

Parameter	Value
SFC flow	3 mL/min
SFC gradient 1	0 minutes, 2 % B; 5 minutes, 20 % B; 5.1 minutes, 50 % B Stop time 7 minutes Post time 2 minutes
SFC gradient 2	0 minutes, 2 % B; 10 minutes, 20 % B; 10.1 minutes, 50 % B Stop time 12 minutes Post time 2 minutes
SFC gradient 3	0 minutes, 2 % B; 15 minutes, 20 % B; 15.1 minutes, 50 % B Stop time 17 minutes Post time 2 minutes
SFC gradient 4	0 minutes, 2 % B; 8 minutes, 12 % B; 8.1 minutes, 50 % B Stop time 10 minutes Post time 2 minutes
Modifier	Methanol
BPR temperature	60 °C
BPR pressure	120 bar
Column temperature	40 °C
Injection volume	5 µL, 3 times loop overfill

Connection of SFC to MS by splitting and make-up flow

Parameter	Value
Make up composition	Acetonitrile + 0.2 % formic acid
Make-up flow	0.5 mL/min
Flow gradient	0 min, 0.5 mL/min to 5, 10, 15, or 8 minutes, 0.3 mL/min

MS method

Parameter	Value
Ionization mode	Positive
Capillary voltage	2,500 V
Nozzle voltage	2,000 V
Gas flow	8 L/min
Gas temperature	220 °C
Sheath gas flow	12 L/min
Sheath gas temperature	380 °C
Nebulizer pressure	25 psi
MRM conditions	See Table 1

Table 1. MRM conditions for pesticide compounds inherent in the used mixture obtained from MRM Optimizer (dwell time 10 ms, cell acceleration voltage 5 V).

	Precursor ion (*m/z*)	Fragmentor (V)	Product ion 1 (*m/z*)	Collision energy (eV)	Product ion 2 (*m/z*)	Collision energy (eV)
Metolachlor	284.1	90	252.1	12	176.1	24
Metazachlor	278.1	70	210.1	4	134.1	20
Metobromuron	259.0	85	170.0	16	148.1	12
Hexazinone	253.1	85	171.1	12	71.1	32
Linuron	249.0	85	181.1	12	159.9	16
Cyanazine	241.1	100	214.1	12	104.1	32
Diuron	233.1/235.1	95	72.1	20	72.1	20
Metoxuron	229.1/231.1	135	72.1	16	72.1	16
Terbuthylazine	230.1	55	174.1	12	104.1	32
Sebuthylazine	230.1	85	174.1	12	104.1	36
Methabenzthiazuron	222.1	65	165.1	12	150.0	36
Atrazine	216.1	85	174.0	16	104	28
Monolinuron	215.1	95	148.0	16	125.9	12
Chlorotoluron	213.1/215.1	65	72.1	20	72.1	20
Isoproturon	207.1	95	165.0	12	72.1	20
Simazine	202.1	105	132.1	16	124.1	16

Sample preparation

Rocket was obtained from a local greengrocer. Samples were extracted according to the official citrate buffered QuEChERS protocol using Agilent BondElut QuEChERS kits (p/n 5982-5650). 10 g homogenized rocket sample was weighed in a 50-mL polypropylene tube and extracted with 10 mL acetonitrile for 1 minute while shaking vigorously by hand. After the addition of an extraction salt packet containing 4 g of anhydrous $MgSO_4$, 1 g NaCl and 1.5 g buffering citrate salts, the mixture was again shaken for 1 minute, and then centrifuged at 3,000 rpm for 5 minutes.

After phase separation, a 6-mL aliquot of the upper acetonitrile phase was transferred to an Agilent BondElut QuEChERS EN dispersive SPE tube (p/n 5982-5256) containing 150 mg primary secondary amine (PSA) and 15 mg graphitized carbon black for sample cleanup and 900 mg anhydrous $MgSO_4$ to remove water. The tubes were closed and shaken for another minute. Afterwards, the tubes were centrifuged at 3,000 rpm for 5 minutes. A 4-mL amount of the final extract were transferred into a clean polypropylene vial. To improve the stability of the target pesticides, 40 µL formic acid was added to the final extract.

Results and Discussion

For evaluation of matrix effects, the final QuEChERS extract of the rocket sample was spiked with the pesticide solution to a concentration of 10, 20, and 100 ppb. The lowest spiking level of 10 ppb was chosen because a proper detection of all pesticides in a standard solution with 10 ppb each was possible with all applied gradients. The level of 10 ppb is also a typical performance requirement for the detection of pesticides in vegetables and fruits. The used rocket matrix is one of the more complex matrices occurring in the analysis of vegetables and fruits. Gradient 1 was used as the reference separation for this comparison. For comparison, two other gradients with lower steepness were generated by increasing the run time to 10 and 15 minutes while maintaining the final maximum content of organic modifier. In the shallowest gradient (gradient 3), the pesticide compound with the highest retention elutes at 7.5 minutes at approximately 10 % organic modifier. Therefore, in the optimized gradient 4, the organic content is increased to a final concentration of 12 % methanol in 8 minutes. A later increase to 50 % organic is used to clean the column from remaining matrix compounds. Figure 2 shows the separation of the 16 standard pesticide compounds under the conditions of gradient 4 in a standard mix at a concentration of 10 ppb.

For evaluation of matrix effects in the different gradients, a spiked rocket extract was compared to the separation of a calibration standard. In the MassHunter Quantitative Software, the standard solution with a concentration of 10 ppb was set to 100 %, and was used as a one-point calibration. In this way, changes in peak intensities for the spiked sample are flagged in the batch table for fast batch review. This table was transferred into the diagram shown in Figure 3, displaying the results at a glance. When comparing the pure standard solution analyzed by gradient 1 to a sample spiked in matrix analyzed by the same gradient, the intensity of the compounds typically decreases due to matrix suppression.

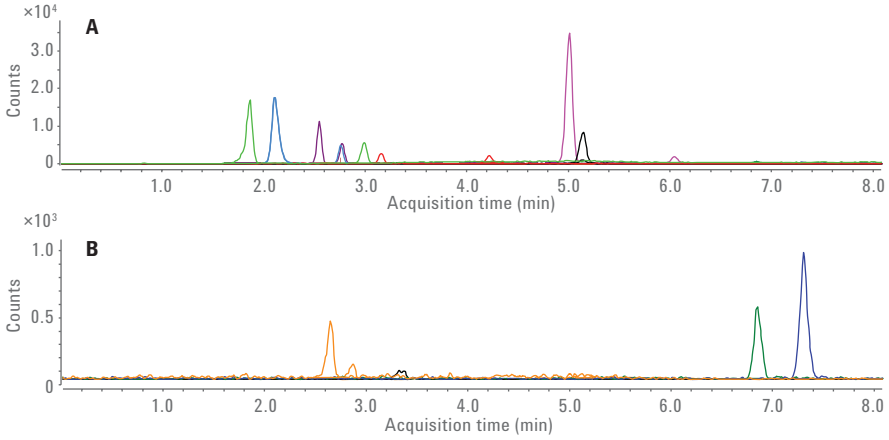

Figure 2. A) Separation of all 16 standard pesticide compounds at the 10 ppb level by means of gradient 4. B) Five pesticide compounds of lowest abundance at the 10 ppb level.

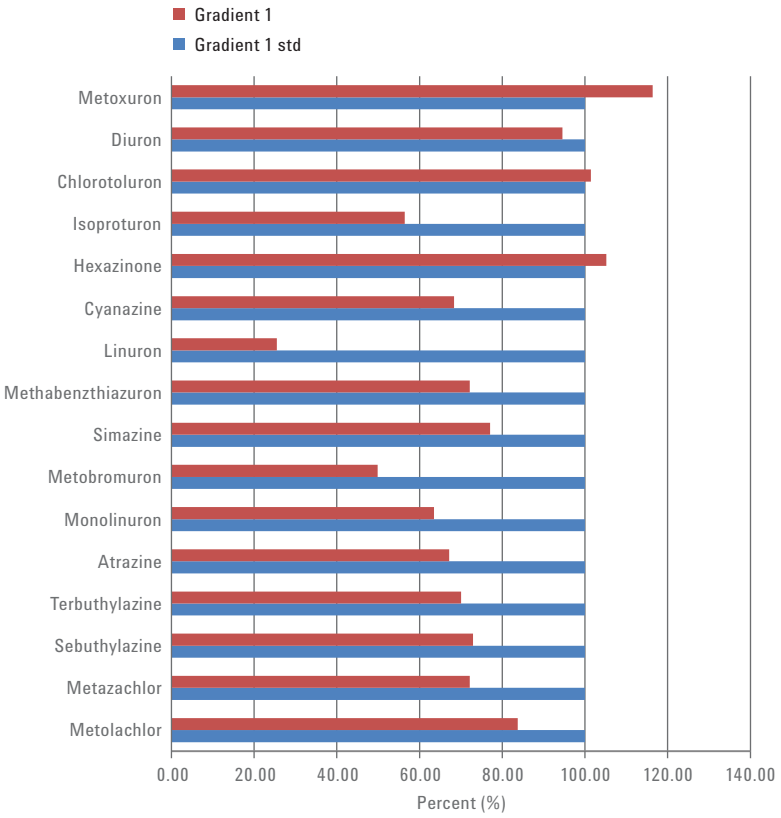

Figure 3. Comparison of matrix suppression of 16 pesticide compounds in rocket matrix (red) to a standard solution (blue).

A change in the gradient to a less steep increase in organic modifier possibly improves the separation of the compounds from the matrix compounds and results in higher signal intensities by less suppression due to high abundant matrix compounds.

To demonstrate this effect, gradients 2, 3, and 4 were also applied to the described spiked sample and the standard. The complete batch of samples and standards was compared by means of the MassHunter Qualitative Software. The area values obtained for the standards from each gradient were used as the basis in a one point calibration to compare to the spiked samples. The comparison of matrix effects is displayed in the graphical chart shown in Figure 4. It can be seen, that for most of the compounds the shallow gradients 2, 3, and 4 result in an improvement in signal intensity compared to the fastest gradient 1. Typically, the shallowest gradient 4 provides the highest signal intensities. For instance, the chromatograms of the pesticide compound isoproturon show an increase in response for the comparison of gradient 2 to 4 for the spiked matrix sample with the chromatogram of the initial gradient 1 (Figure 5). Presumably, the intensity increases due to the better separation from the matrix background and, thus, higher ionization yields. There are three exceptions, cyanazine, atrazine, and tertbuthylazine which gave higher intensities with gradient 1. However, due to the fact, that the majority of compounds produces higher intensities with gradient 4, and the majority of compounds have recoveries between 70 and 120% this one was used for the next experiments.

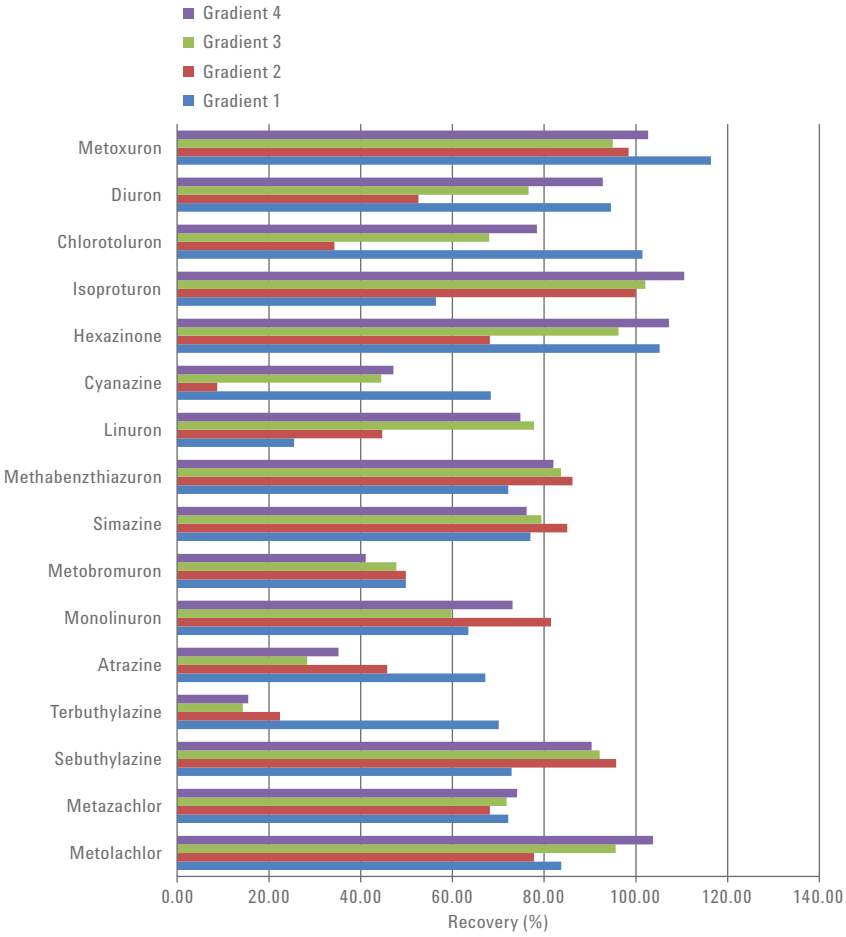

Figure 4. Comparison of matrix effects in different gradients of different steepness. The matrix effect is at its minimum for most of the compounds for gradient 4.

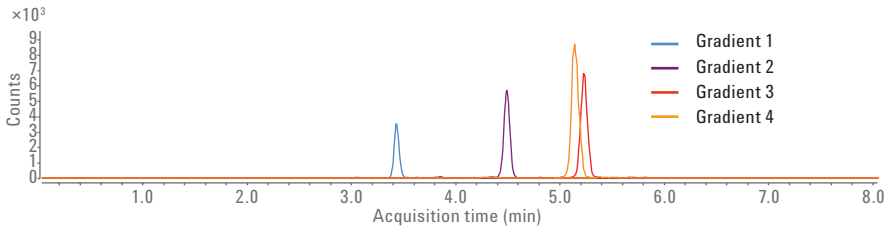

Figure 5. Signal intensities for isoproturon for the four applied gradients of different steepness.

Finally, quantification in rocket extract was done based on a solvent calibration using the 10, 20, and 100 ppb levels by means of gradient 4. The same concentration levels spiked in rocket matrix were used as samples. The comparison of the measured concentration shows the matrix effects in relative percentages (Figure 6). According to the SANCO guidelines SANCO/12571/2013, an apparent recovery of 70 to 120 % is acceptable[4]. The matrix effects per compound are very similar over the examined concentrating range (Figure 6). When compared to a solvent calibration, most of the tested compounds show recoveries within the acceptable range of 70 to 120 %. The two compounds atrazine and terbuthylazine were quantified with recoveries below 50 %. Dilution is an accepted way of minimizing matrix effects in complex samples. When diluting the QuEChERS extract spiked with pesticides to 100 ppb 1:10 with acetonitrile, recoveries for atrazine and terbuthylazine were 87 and 85 % respectively and, thus, within the acceptable range. The linearity of calibration for all compounds, calculated limits of quantitation (LOQ) and limits of detection (LOD) are summarized in Table 2.

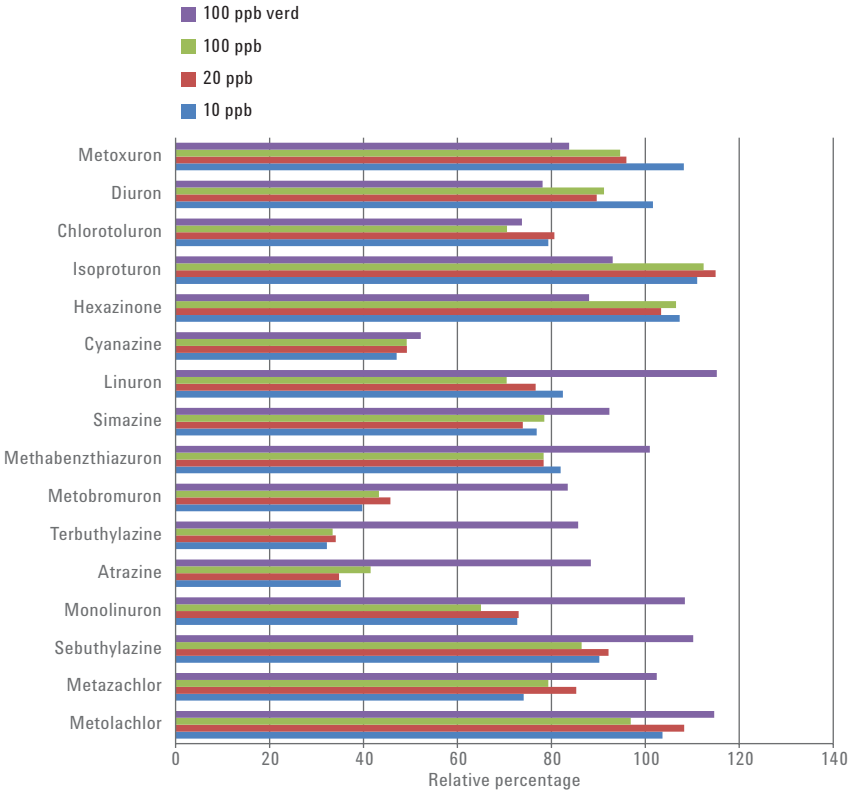

Figure 6. Comparison of spiked samples to a calibration in standard solution. Matrix effects are typically in a range of 70 to 120 %. Matrix effects could be additionally minimized by sample dilution.

Table 2. Summary of calibration, showing linearity of the individual compounds, LOQ, and LOD.

10 ppb	RT	LOD	LOQ	R^2
Metolachlor	1.869	0.08	0.25	0.9991
Metazachlor	2.117	0.12	0.40	0.9990
Sebuthylazine	2.554	0.55	1.83	0.9993
Monolinuron	2.647	1.77	5.90	0.9993
Atrazine	2.754	0.06	0.20	0.9993
Terbuthylazine	2.776	0.08	0.25	0.9995
Metobromuron	2.866	2.49	8.30	0.9991
Methabenzthiazuron	2.993	0.05	0.18	0.9994
Simazine	3.158	0.24	0.80	0.9995
Linuron	3.307	3.00	10.00	0.9990
Cyanazine	4.219	0.20	0.66	0.9992
Hexazinone	5.006	0.03	0.10	0.9995
Isoproturon	5.142	0.04	0.13	0.9998
Chlorotoluron	6.046	0.23	0.77	0.9991
Diuron	6.846	0.87	2.90	0.9992
Metoxuron	7.287	0.30	1.00	0.9992

Conclusion

This Application Note demonstrates the importance of optimizing the SFC separation on the influence of the sample matrix for the measurement of pesticides in vegetable and fruit samples by SFC with triple quadrupole MS. The optimization of the used gradient can improve the separation between analyte and high abundant matrix compounds and, thus, help to lower detection limits. For all tested pesticides, the required LOQ of 10 ppb could be met, and most of the compounds could be quantified in the required recovery range of 70 to 120 % based on a solvent calibration. In addition, the use of an SFC instrument brings the advantage of increased speed of the separation and the usability of samples dissolved in pure organic solvent directly from sample preparation by QuEChERS.

References

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Published in the USA, October 15, 2017
5991-5443EN

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