thermo scientific

APPLICATION NOTE 64971

Robustness, reproducibility, reliability with best-in-class sensitivity: Increased confidence in targeted quantitation of pesticides in food matrices

Authors

Katerina Bousova,[1] Michal Godula,[1] Claudia Martins,[2] Charles Yang,[2] Ed George,[2] Neloni Wijeratne[2]

[1]Thermo Fisher Scientific, Special Solution Center Europe, Dreieich, Germany

[2]Thermo Fisher Scientific, San Jose, California

Keywords

Pesticide Explorer Collection, European Regulation 396/2005, Commission Directive 2006/125/EC, European Commission 2002/657/EC, SANCO/12571/2013, European Commission 788/2012/EC, pesticide, food, QuEChERS, Vanquish Flex, TSQ Quantis MS, TraceFinder software

Goal

To present a fully tested LC-MS/MS methodology for rapid and robust quantitation of more than 250 pesticides below maximum residue limits (MRLs) with sensitivity, accuracy, and precision that meets stringent EU guidelines.

Introduction

Pesticides are chemicals used on crops to protect them from the negative activity of pests. As inappropriate application of a pesticide can result in serious health issues, determination of pesticide residues in foods and food products is an important part of routine food control. The European Union (EU) legislation (European Regulation 396/2005 and Commission Directive 2006/125/EC), currently the strictest regulations, sets maximum residue limits of pesticides in various products of plant and animal origin. These regulations present significant analytical challenges due to the low limits of quantitation (LOQ) required in certain food matrices.

ThermoFisher SCIENTIFIC

This application note presents a multi-residue analysis method that uses a comprehensive liquid chromatography—triple quadrupole mass spectrometry (LC-MS/MS) solution, the Thermo Scientific™ Pesticide Explorer Collection Standard Quantitation solution, for rapid and robust quantitation of more than 250 pesticides below their maximum residue limits. The solution includes the Thermo Scientific™ QuEChERS sample preparation kit, Thermo Scientific™ Vanquish™ Flex Binary UHPLC system, Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer, Thermo Scientific™ TraceFinder™ software, Thermo Scientific™ Accucore™ aQ C18 Polar Endcapped LC column, and method parameters to provide a start-to-finish workflow for pesticide analysis. The method results comply with the stringent EU guidelines concerning sensitivity, accuracy and precision.

Experimental

Overview

The workflow overview from sample preparation through LC-MS/MS analysis is shown in Figure 1. Samples were homogenized and extracted according to the European EN 15662 QuEChERS protocol prior to injection into the LC-MS/MS system.[1,2] The ready-to-use QuEChERS sample preparation kit containing extraction tubes and associated protocol was used for sample preparation. Identification of pesticide residues was based on retention time, the presence of a minimum of two product ions, and ion-ratio confirmation using selected reaction monitoring (SRM) of characteristic transition ions. Quantitation was calculated using matrix-matched calibration. All method performance criteria were established according to the relevant EU guidelines.[3–7]

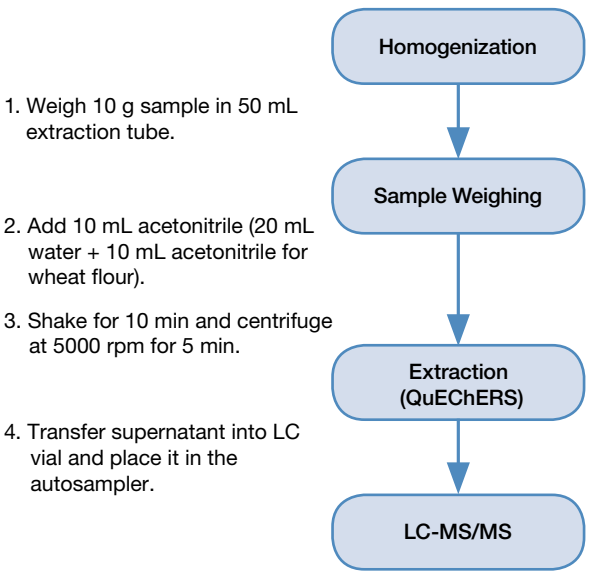

Figure 1. Workflow overview.

Method supplies

Tables 1A–1D lists the reagents, apparatus, consumables, and glassware used and their source, including Thermo Fisher Scientific part numbers where appropriate. With the exception of purified water, the reagents, apparatus, instruments, consumables, and glassware were all obtained from Thermo Fisher Scientific. The pesticides standards were purchased from ULTRA Scientific (USA).

Table 1A. Reagents.

Reagent	Part Number
Water UHPLC-MS grade	Fisher Chemical™ Optima™ W8-1
Methanol UHPLC-MS grade	Fisher Chemical Optima A458-1
Ammonium Formate >99%	A115-50
Acetonitrile UHPLC-MS grade	A956-1
Formic Acid >99%	28905

Table 1B. Apparatus/instruments.

Apparatus/Instruments	Thermo Fisher Scientific P/N or Source
Fisher precision balance	02225102
Sartorius analytical balance	14557812
Barnstead EASYpure II water	0905043
ULTRA-TURRAX® S 25 N - 25 G dispergation tool	1713300
ULTRA-TURRAX® T 25 digital homogenizer	3565000
Vortex shaker	14505141
Vortex universal cap	3205029
Accu-Jet® pipettor	3140246
Horizontal shaker	1069-3391
Horizontal shaker plate	1053-0102
Centrifuge, Thermo Scientific™ Heraeus™ Multifuge™ X3	75004500
TSQ Quantis triple quadrupole mass spectrometer	TSQ02-10001
Vanquish Flex Binary UHPLC system	5400.0225

Table 1C. Consumables.

Consumables	Thermo Fisher Scientific P/N or Source
LC vials	32051113
LC caps	3151266
Pipette, Thermo Scientific™ Finnpipette™ 100–1000 µL	3214535
Pipette, Finnpipette 20–200 µL	3214534
Pipette, Finnpipette 10–100 µL	3166472
Pipette, Finnpipette 500–5000 µL	3166473
Pipette, Finnpipette 1000–10,000 µL	3214536
Pipette holder	3651211
Pipette tips 0.5–250 µL, 500/box	3270399
Pipette tips 1–5 mL, 75/box	3270420
Pipette tips 100–1000 µL, 200/box	3270410
Pipette tips 20,000–10,000 µL, 40/box	3270425
Pipette, Pasteur soda lime glass, 150 mm	FB50251
Pipette suction device	3120891
Spatula, 18/10 steel	3458179
Spatula, nylon	3047217
Vial rack (2 mL)	12211001
Centrifuge tube rack	1066-3721
QuEChERS extraction tube, 50 mL, 250 pack	60105-216
Accucore aQ column 100 × 2.1 mm, 2.6 µm	17326-102130

Table 1D. Glassware.

Glassware	Thermo Fisher Scientific P/N or Source
Volumetric flask, 10 mL	FB50143
Volumetric flask, 25 mL	FB50147
40 mL screw cap vial	1054-1593
Caps for 40 mL screw cap vial	1009-0962
500 mL bottle	9653640
100 mL bottle	1006-8060
Beaker, 100 mL	FB-102-100
Beaker, 200 mL	FB-102-200

Sample preparation

Blank matrix samples (leek (LK)) used for validation experiments were purchased in local retail stores and were homogenized with an ULTRA-TURRAX homogenizer and extracted prior to fortified sample preparation. Matrix extracts were used as matrix blank samples and for preparation of matrix-matched calibration standards. Ready-to-use QuEChERS extraction kits used for sample preparation contained 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate, and 0.5 g sodium citrate for buffered extraction of target compounds. No cleanup has been used.

Homogenization of matrices was performed using the following steps:

1. A relatively large amount of leek matrix (~500 g) was placed into a beaker of appropriate size and labeled.
2. A 25 G dispergation tool was attached to the ULTRA-TURRAX homogenizer. (Note: For better recovery for some unstable compounds cryogenic homogenization is advised.[8])
3. Homogenization was performed at middle rotation speed (speed level 2–3) to create smooth homogenate.

Sample extraction was performed using the following steps:

1. Sample (10 g) was weighed into a 50 mL QuEChERS extraction tube.
2. Acetonitrile (10 mL) was added to the LK samples.
3. Samples were shaken for 10 min on a horizontal shaker and centrifuged at 5000 rpm for 5 min.
4. The supernatant was collected and 1 mL was transferred into a LC vial for instrumental analysis.

LC-MS/MS analysis

LC-MS/MS analysis was carried out using a Vanquish Flex Binary system coupled to a TSQ Quantis triple quadrupole mass spectrometer. TraceFinder software was used for instrument control, analysis, data review, and reporting. The LC conditions and gradient are shown in Tables 2 and 3. The LC gradient was optimized to reduce analysis time to 15 minutes, while maintaining good chromatographic separation.

Table 2. LC conditions.

Injection volume	1 µL
Column temperature	25 °C
Flow rate	300 µL/min
Analytical column	Accucore aQ column 100 × 2.1 mm, 2.6 µm
Run time	15 minutes
Tray temperature	5 °C
Needle-cleaning solvent	10% Methanol in water
Sample loop	25 µL
Mobile phases	A: 98% water with 2% methanol, 5 mM ammonium formate, and 0.1% formic acid B: 98% methanol with 2% water, 5 mM ammonium formate, and 0.1% formic acid

Table 3. LC gradient.

Time (min)	Flow (mL/min)	%A	%B
0	0.300	100	0
0.5	0.300	100	0
7	0.300	30	70
9	0.300	0	100
12	0.300	0	100
12.1	0.300	100	0
15	0.300	100	0

The TSQ Quantis triple quadrupole mass spectrometer was operated in timed-SRM mode. SRM conditions were optimized in an automated way using the new Compound Optimization Tool in Tune 3.0. The mass spectrometer settings are provided in Table 4.

Table 4. Optimized source and MS settings.

Ionization mode	Heated Electrospray (HESI)
Scan type	timed-SRM
Polarity	Positive/Negative switching
Spray Voltage for Positive mode	3700 V
Spray Voltage for Negative mode	2500 V
Sheath gas pressure	30 arbitrary units (Arb)
Aux gas pressure	6 Arb
Sweep gas pressure	1 Arb
Ion transfer tube temperature	325 °C
Vaporizer temperature	350 °C
CID gas pressure	2 mTorr
Cycle time	0.5 s
Q1 resolution (FWHM)	0.7
Q3 resolution (FWHM)	0.7

Results and discussion

Figure 2 shows the LC-MS/MS chromatogram of matrix spiked with more than 250 pesticides at a concentration of 100 µg/kg (1 µL injection). Despite the short chromatographic run time (15 minutes), good separation and detection of the pesticide compounds was achieved using the timed-SRM mode. With timed-SRM, data acquisition for a particular target compound is performed in a short retention time window around the known compound retention time. Timed-SRM significantly reduces the number of SRM transitions that are monitored in parallel within a certain retention time window. To achieve the expected limits of quantitation in a reproducible and precise way, good performance at short dwell times is necessary. Figure 3 highlights the performance of the TSQ Quantis mass spectrometer at low concentration levels in leek at short dwell times.

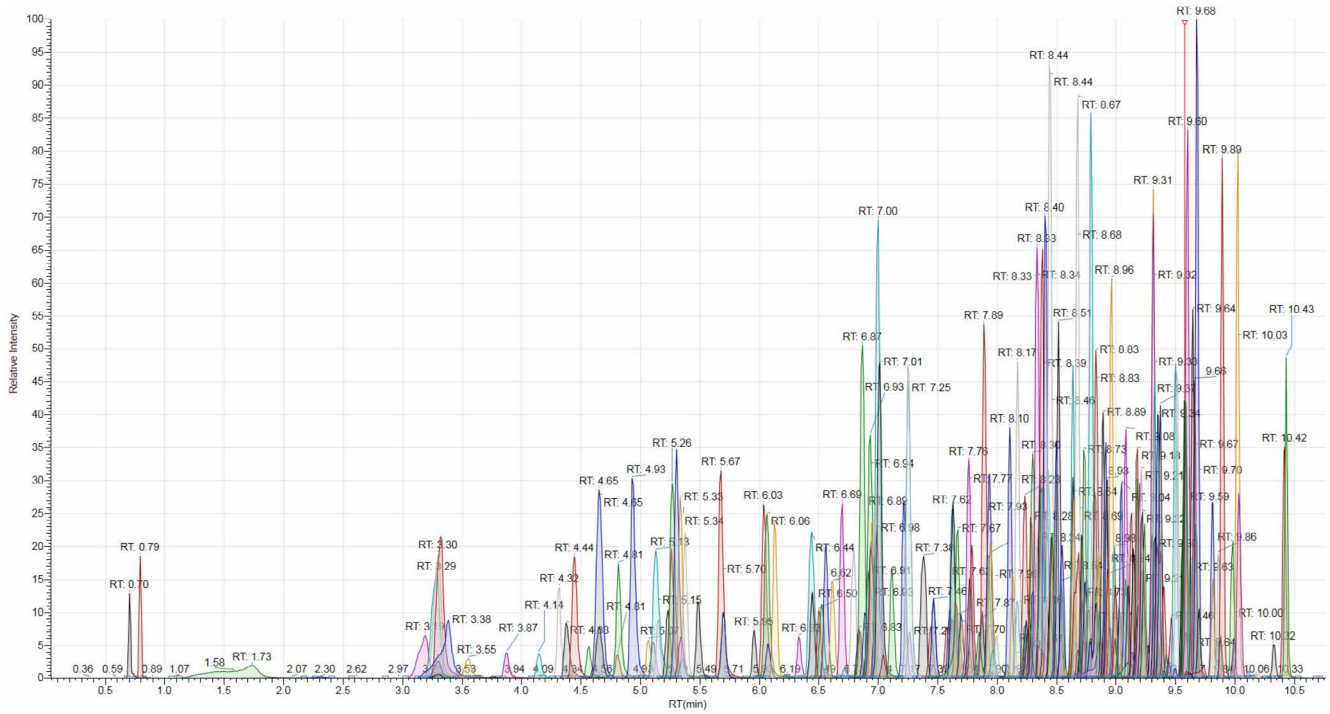

Figure 2. LC-MS/MS chromatogram of more than 250 pesticides in leek extract at 100 µg/kg.

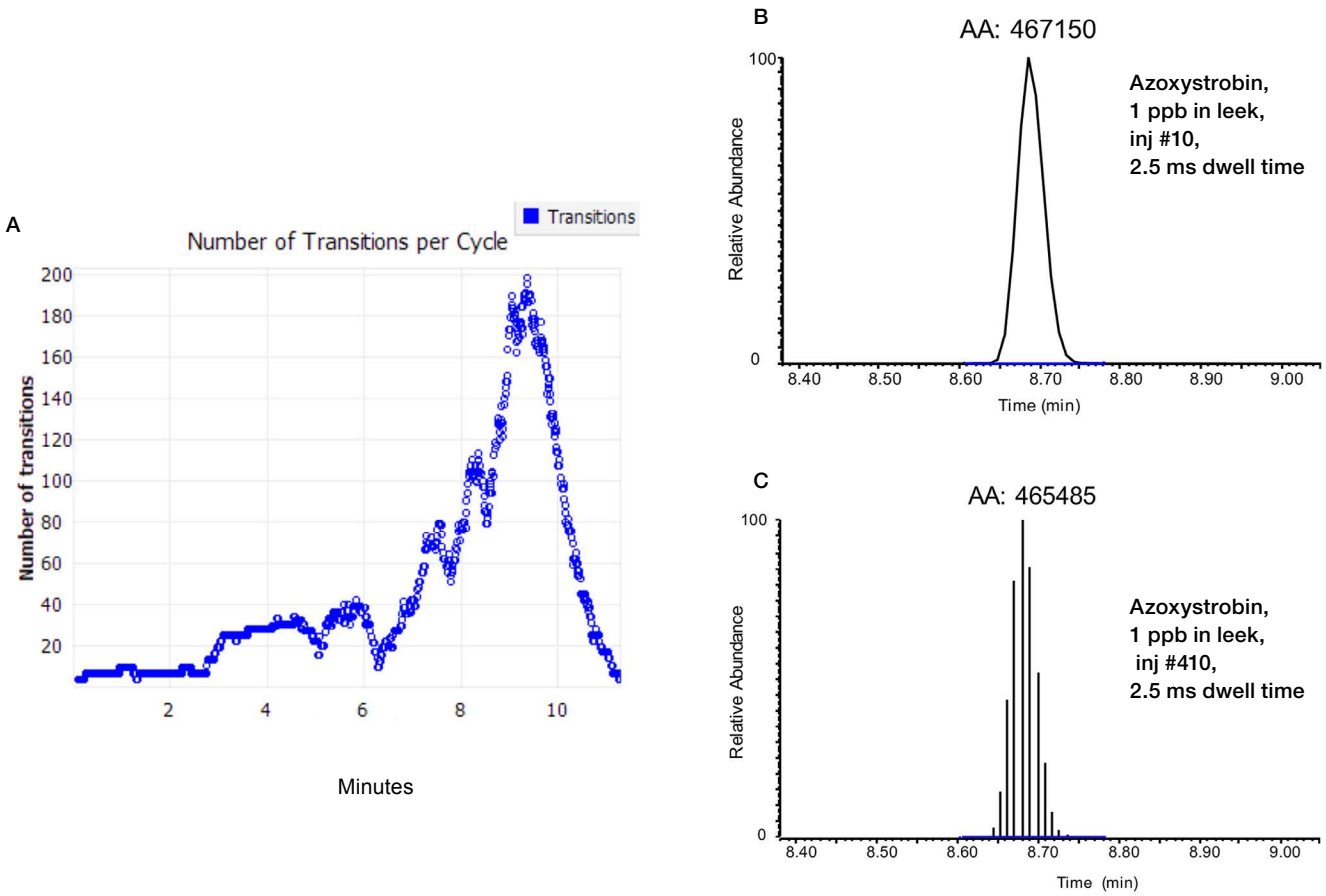

Figure 3. Demonstration of speed and reproducibility. Panel A depicts the number of transitions per unit time. Azoxystrobin elutes at 8.69 min and is acquired simultaneously with 130 other transitions. Panels B and C show the reproducibility of azoxystrobin comparing the 10th injection to 410th injection. Peak areas are consistent even at low dwell time (2.5 ms). Panel C also demonstrates an adequate number of data point across the peak in a complex region of the chromatogram.

LC-MS/MS workflow: Outstanding reproducibility, excellent robustness

Sensitivity, reproducibility, and accuracy is expected for this assay despite the complexity of the sample. The sensitivity demonstrated by the TSQ Quantis MS for the quantitation of pesticides in leek is superior to the previous generation triple quadrupole. Figure 4 summarizes a comparison between the TSQ Endura MS and TSQ Quantis MS for five pesticides, covering both ionization modes and a wide mass range.

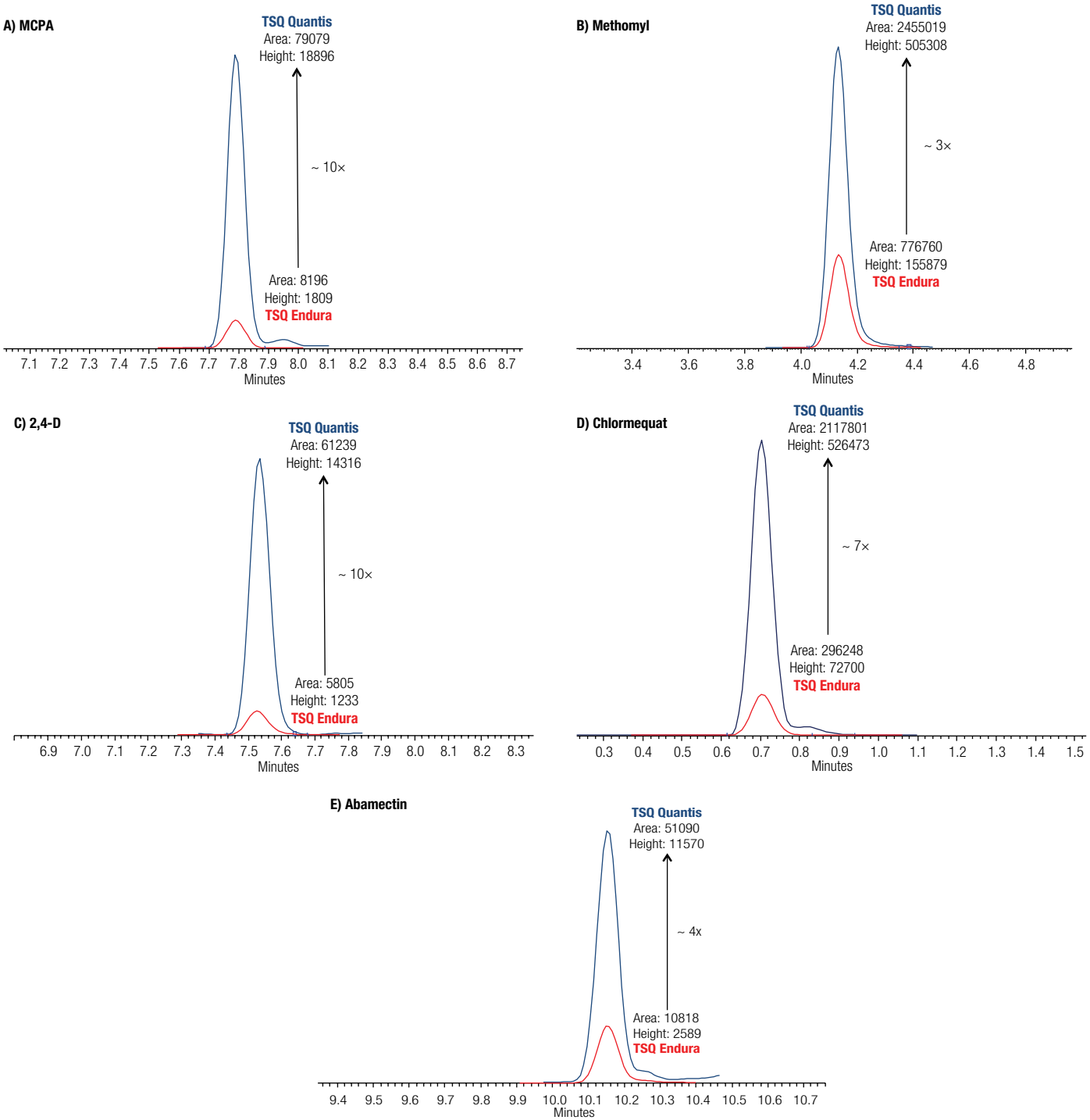

Figure 4. Chromatographic representation of five pesticides monitored with the TSQ Quantis MS (blue trace) vs. the TSQ Endura MS (red trace). Differences in performance are shown in peak area and peak height. A) MCPA – Negative Ionization Mode (198.9 → 105, 198.8 → 141) B) Methomyl – Positive Ionization Mode (163 → 88, 163 → 106) C) 2,4 D – Negative Ionization Mode (218.8 → 160.9, 218.8 → 125) D) Chlormequat – Positive Ionization Mode (122 → 63, 122 → 58) E) Abamectin – Positive Ionization Mode (890.5 → 305, 890.5 → 307.1, 890.5 → 567.1)

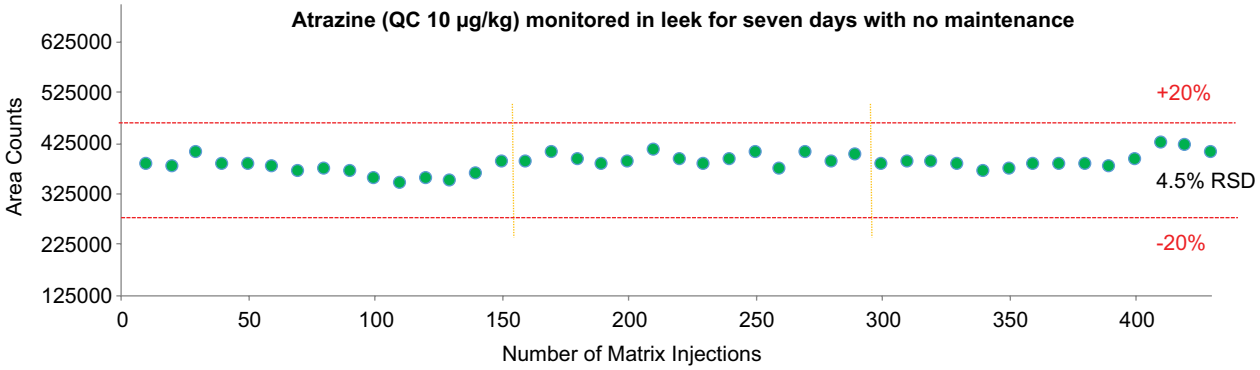

Figure 5. Atrazine QC monitored in leek for more than 400 injections with 4.5% RSD. Red lines represent ±20% of atrazine response at 10 µg/kg. Yellow lines show the exact moment the system was placed in standby mode for 12 h (no maintenance was performed).

Figure 5 shows the overall response of atrazine for more than 400 injections of matrix blanks and the 10 ppb QC in leek. The system was placed in standby mode for 24 h (2 × 12 h) to show reliable performance when starting from standby mode. The data shows that the response was within the expected ±20% range for at least 400 injections. No maintenance was performed between injections.

Conclusion

European Union regulations that set maximum residue limits of pesticides in foods are analytically challenging due to the low LOQs that must be achieved in complex matrices. This application note described a multi-residue LC-MS/MS method that uses the TSQ Quantis triple quadrupole mass spectrometer-based Pesticide Explorer Collection Standard Quantitation solution, for rapid and robust quantitation of more than 250 pesticides in leek at or below their respective MRLs.

The LC-MS/MS system selectivity and sensitivity enabled analysis of only 1 µL sample, without need for dispersive SPE sample cleanup or sample dilution, with increased robustness and throughput.

References

1. http://www.thermoscientific.com/content/tfs/en/product/hypersep-dispersive-spe-extraction-products-quechers.html
2. Anastassiades et al. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. Chromatogr. A.* **2003.** *1015,* 163–184.
3. Official Journal of the European Communities, COMMISSION DECISION of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. [Online] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32002D0657&from=EN (accessed April 20, 2017).
4. Official Journal of the European Union, COMMISSION DIRECTIVE 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children. [Online] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006L0125&from=EN (accessed April 20, 2017).
5. European Commission Directorate-General for Health and Food Safety, Safety of the Food Chain, Pesticides and biocides, Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. SANCO/11945/2015. [Online] https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_11945.pdf (accessed April 20, 2017).
6. Official Journal of the European Union, COMMISSION IMPLEMENTING REGULATION (EU) No 788/2012 of 31 August 2012 concerning a coordinated multiannual control programme of the Union for 2013, 2014 and 2015 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin. [Online] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32012R0788&from=EN (accessed April 20, 2017).
7. European Commission. Plants. EU Pesticides database. [Online] http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN (accessed April 20, 2017).
8. Fussell et al. *Food Additives & Contam.* **2007,** *24,* 1247–1256.

Find out more at **thermofisher.com/Altis-Quantis**

ThermoFisher SCIENTIFIC