

Fast Screening and Quantification of Pesticide Residues Using a Comprehensive LC-MS Solution: The Pesticide Explorer Collection – Standard Quantitation

Katerina Bousova¹, Ebru Sarikaya¹, Michal Godula¹, Claudia Martins², and Ed George²

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

²Thermo Fisher Scientific, San Jose, California

Key Words

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, UltiMate 3000, TSQ Endura, TraceFinder

Goal

To present a fully tested LC-MS/MS workflow for rapid and robust quantification 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. Inappropriate application of pesticides can have adverse effects on health; therefore, determination and quantification 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) requires an extensive and comprehensive study determining pesticides in various products of plant and animal origin. The requirements for low limits of quantification (LOQ) of pesticides pose significant analytical challenges, especially for some complicated food matrices.

This study presents a multi-residue analysis method enabled by Thermo Scientific™ Pesticide Explorer Collection Standard Quantitation Solution, comprising liquid chromatography–triple-stage mass spectrometry (LC-MS/MS), for rapid and robust quantitation of more than 250 pesticides below their required maximum residue limits (MRL). This comprehensive solution includes the Thermo Scientific™ QuEChERS sample preparation kit, Dionex™ UltiMate™ 3000 LC system, TSQ Endura™ triple quadrupole mass spectrometer, TraceFinder™ software, Accucore™ aQ column, and method parameters to provide a start-to-finish workflow for pesticide analysis. The method results address 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. Quantification was calculated using matrix-matched calibration. All method performance criteria were established according to the relevant EU guidelines.³⁻⁷

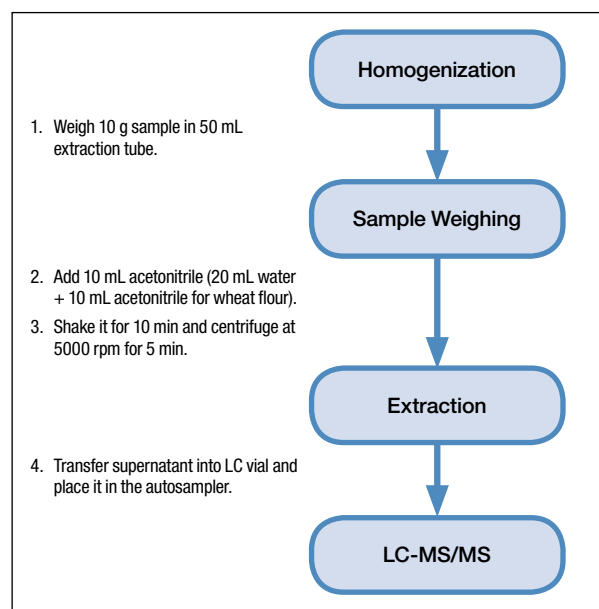


Figure 1. Workflow overview.

Method Supplies

Table 1 lists reagents, instruments, and consumables used.

The pesticides standards were purchased from Sigma-Aldrich® (Germany) and Laboratory Instruments Srl (CASTELLANA GROTTA, Italy). Quality control materials used were FAPAS #T19140 (lettuce puree), FAPAS #19110 (lettuce puree), FAPAS #T19142 (melon puree), and FAPAS #T0983 (wheat flour). FAPAS samples were selected primarily based on their content of target pesticides. However, due to limited availability, some of the matrices are different from the matrices spiked and analyzed (i.e. lettuce and melon puree versus strawberry and leek).

Table 1. Reagents, instruments, and consumables.

Method Supplies	Fisher Scientific Part Number/ Source
Reagents	
Acetonitrile (ACN), LC/MS grade	AC61514-0025
Ammonium formate, 99%	AC40115-2500
Formic acid, Optima™ LC/MS grade	A117-50
Methanol, Optima™ LC/MS grade	A456-212
Purified water	Obtained from Thermo Scientific™ Barnstead™ Easypure™ II water system
Water, LC-MS grade	AC61515-0025
Instruments	
TSQ Endura triple quadrupole mass spectrometer	
UltiMate 3000 RSLC	
Method Supplies	Thermo Fisher Scientific Part Number
Consumables	
QuEChERS extraction tube, 50 mL, 250 pack	60105-216
Accucore aQ column 100 x 2.1 mm, 2.6 µm	17326-102130

Sample Preparation

Blank matrix samples [strawberry (SB), wheat flour (WF) and 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 were used for sample preparation, and contained 4 g MgSO₄, 1 g NaCl, 1 g trisodium citrate dehydrate, and 0.5 g sodium citrate for buffered extraction of target compounds. The same QuEChERS sample preparation protocol was applied to all three of the matrices analyzed, however a modification was made for flour in which water was added to wet the matrix. No cleanup was used.

Homogenization of matrices was performed using the following steps:

1. A relatively large amount of each matrix (~500 g) was placed into an appropriately sized beaker and labeled.
2. A G25 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. 10 g sample was weighed into a 50 mL QuEChERS extraction tube.
2. 10 mL ACN was added to the SB and LK samples. For WF, 20 mL water was added to completely wet samples, and then 10 mL ACN was added.
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 an UltiMate 3000 RSLC system coupled to a TSQ Endura triple quadrupole mass spectrometer. TraceFinder software (revision 3.2 SP2) 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.

LC conditions	
Injection volume	1 μ L
Column temperature	25 $^{\circ}$ C
Flow rate	300 μ L/min
Analytical column	Accucore aQ column, 100 x 2.1 mm, 2.6 μ m
Run time	15 minutes
Tray temperature	10 $^{\circ}$ C
Needle-cleaning solvent	20% Methanol in water
Sample loop	100 μ L
Mobile phases	A: Water with 5 mM ammonium formate and 0.1% formic acid B: Methanol with 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 Endura triple quadrupole mass spectrometer was operated in timed-SRM mode. All SRM traces (parent, qualifier, quantifier ion) were individually tuned for each target analyte by direct infusion of each working standard solution. The mass spectrometer settings are provided in Table 4. For convenience and fast method implementation, the complete method including SRM settings is included with the Pesticide Explorer Collection Standard Quantitation Configuration.

Table 4. MS settings.

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 $^{\circ}$ C
Vaporizer temperature	350 $^{\circ}$ C
CID gas pressure	2 mTorr
Cycle time	0.5 s
Q1 resolution (FWHM)	0.7
Q3 resolution (FWHM)	0.7
Chrom filter	3 s

Results and Discussion

To evaluate method performance, three matrices, strawberry, leek (the most complex), and wheat flour, were analyzed. European Union guidelines for single laboratory validation and pesticide residue analysis were used to establish method performance criteria, including linearity, matrix effect, LOD, LOQ, precision, and trueness (bias). All method performance parameters were compared to the relevant legislative requirements and MRLs. For compounds containing more than one isoform, only one performance criterion was established.

Figure 2 shows the LC-MS/MS chromatogram of the strawberry extract spiked with more than 250 pesticides at a concentration of 100 µg/kg (1 µL injection). Despite the short chromatographic run time (15 min), good separation and detection of the pesticide compounds were 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. A longer measurement time (dwell time) is therefore available for each transition, resulting in higher sensitivity and lower quantitation limits, improved RSDs and more data points per chromatographic peak—in this case a minimum of 10 to 12 data points.

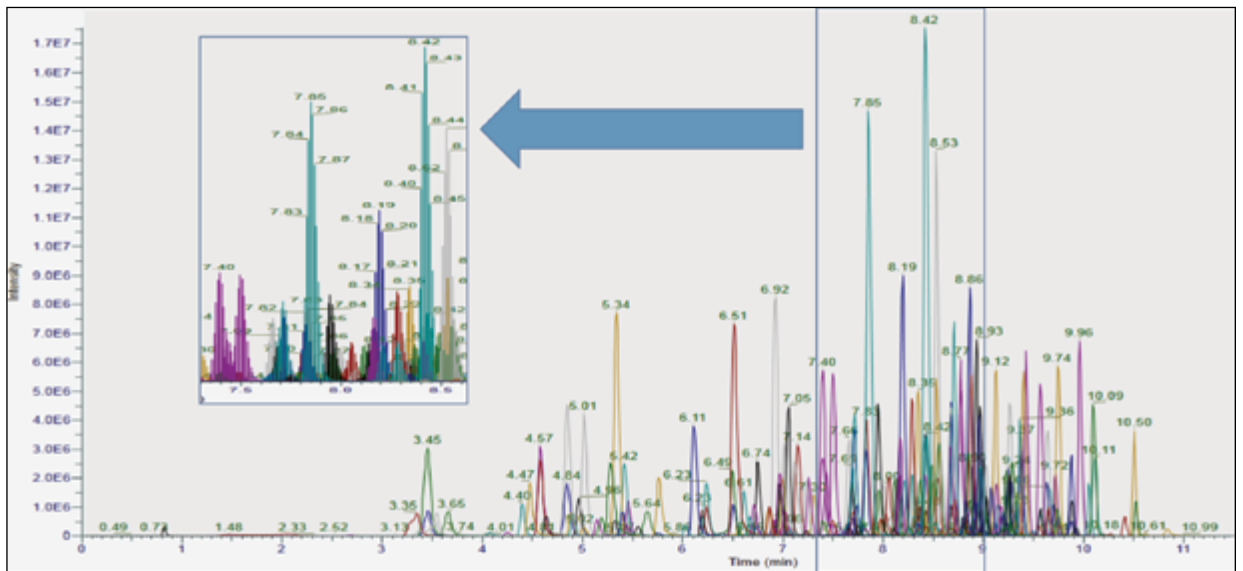


Figure 2. The LC-MS/MS chromatogram of more than 250 pesticides spiked into strawberry extract at 100 µg/kg shows good separation of compounds. Enough scans across the chromatographic peak were obtained throughout the chromatogram.

For the three matrices, including the very complex leek matrix, the LOD and LOQ values obtained demonstrated that the method enabled quantification of target pesticides below regulated MRLs. Table 5 presents the method LODs and LOQs for the target pesticides in the matrices tested. Table 6 compares the LOQ values obtained with the MRLs for selected pesticides. The pesticides selected in Table 6 represent different ionization modes and a range of retention times across the chromatogram. All compounds were detected and quantified below established MRLs.

Table 5. Method performance: LODs and LOQs ($\mu\text{g}/\text{kg}$) for target pesticides by matrix tested. LOQs were estimated taking into account reproducibility ($\text{RSDs} \leq 15\%$) and ion ratio criteria.

	Name	Polarity	RT	Strawberry		Leek		Flour	
				LOD	LOQ	LOD	LOQ	LOD	LOQ
1	2,4-D	-	7.6	5	10	5	10	2	5
2	Abamectin b1a (NH_4)	+	10.2	0.1	0.2	0.3	1	0.3	1
3	Acephate	+	2.9	0.5	1	0.5	1	1	3
4	Acetamiprid	+	5.6	0.1	0.5	0.3	1	0.1	0.3
5	Acibenzolar-S-methyl	+	8.8	1	2	2	5	0.1	0.3
6	Alachlor	+	8.9	1	5	1	5	1	3
7	Aldicarb sulfone	+	4.8	0.5	1	0.5	1	0.3	1
8	Allethrin	+	8.7	0.3	1	0.3	1	1	3
9	Ametryn	+	7.8	0.2	0.6	0.1	0.3	0.1	0.3
10	Aminocarb	+	3.5	0.05	0.1	0.05	0.1	0.03	0.1
11	Ancymidol	+	7.1	0.5	1	0.5	1	0.1	0.3
12	Anilofos	+	9.1	0.03	0.1	0.03	0.1	0.1	0.3
13	Aramite (NH_4)	+	9.7	0.03	0.1	0.03	0.1	0.03	0.1
14	Atrazine	+	7.7	0.1	0.5	0.1	0.5	0.03	0.1
15	Azaconazole	+	8.0	0.5	1	0.1	0.5	0.2	0.6
16	Azamethiphos	+	6.7	0.05	0.1	0.3	1	0.1	0.3
17	Azinphos-ethyl	+	8.8	5	10	5	10	0.3	1
18	Azinphos-methyl	+	8.2	0.5	1	1	5	1	5
19	Azoxystrobin	+	8.2	0.003	0.01	0.003	0.01	0.1	0.3
20	Bendiocarb	+	6.9	0.3	1	0.3	1	0.3	1
21	Benodanil	+	7.7	0.1	0.5	0.1	0.3	0.1	0.3
22	Benoxacor	+	8.1	0.3	1	0.3	1	0.3	1
23	Bensulfuron methyl	+	8.1	0.1	0.5	0.3	1	0.3	1
24	Bentazon	-	6.7	0.3	1	0.2	0.6	0.1	0.3
25	Benzoximate	+	9.3	0.1	0.5	0.3	1	0.3	1
26	Benzoylprop-ethyl	+	9.2	0.1	0.5	0.3	1	0.3	1
27	Bifenazate	+	8.7	0.3	1	2	5	2	5
28	Bitertanol	+	9.3	0.5	2	0.5	2	2	5
29	Boscalid	+	8.4	0.5	1	0.05	0.1	0.1	0.3
30	Brodifacoum	+	10.4	0.1	0.5	0.2	0.5	0.2	0.6
31	Bromacil	+	6.9	0.3	1	0.3	1	0.3	1
32	Bromoxynil	+	7.6	0.1	0.5	0.2	0.6	0.1	0.3
33	Bromuconazole	+	8.7	0.5	1	0.5	1	1	3
34	Bupirimate	+	8.8	0.5	1	0.5	1	0.3	1
35	Buprofezin	+	9.7	0.2	0.5	0.3	1	0.3	1
36	Butachlor	+	9.8	0.2	0.6	1	3	2	5
37	Butafenacil (NH_4)	+	8.7	0.1	0.3	0.1	0.3	0.03	0.1
38	Butocarboxim sulfoxide	+	3.5	0.5	1	1	3	1	3
39	Butoxycarboxim	+	4.8	0.1	0.3	0.1	0.3	0.3	1
40	Carbaryl	+	7.3	0.3	1	0.3	1	0.3	1
41	Carbendazim	+	4.6	0.2	0.5	0.2	0.5	0.1	0.3
42	Carbetamide	+	6.6	0.03	0.1	0.1	0.5	0.3	1
43	Carbofuran	+	6.9	0.03	0.1	0.03	0.1	0.03	0.1
44	Carbofuran-3-hydroxy	+	5.4	0.3	1	0.3	1	0.3	1
45	Carfentrazone-ethyl	+	9.0	0.3	1	0.03	0.1	0.1	0.3
46	Carpropamid	+	9.2	0.3	1	0.3	1	0.1	0.3
47	Chlorantraniliprole	+	8.0	0.3	1	0.3	1	0.3	1

	Name	Polarity	RT	Strawberry		Leek		Flour	
				LOD	LOQ	LOD	LOQ	LOD	LOQ
48	Chlorbromuron	+	8.6	0.2	0.5	0.3	1	0.3	1
49	Chlorfenvinphos	+	9.1	0.03	0.1	0.05	0.1	0.1	0.3
50	Chlorfluazuron	+	10.1	0.3	1	0.3	1	0.1	0.3
51	Chloridazon (pyrazone)	+	5.6	0.3	1	0.3	1	0.3	1
52	Chlormequat	+	0.7	0.2	0.5	0.03	0.1	0.01	0.03
53	Chlorotoluron	+	7.7	0.3	1	0.3	1	0.3	1
54	Chloroxuron	+	8.8	0.2	0.5	0.2	0.6	0.1	0.3
55	Chlorpyrifos	+	9.9	0.03	0.1	0.1	0.3	0.3	1
56	Cinosulfuron	+	6.7	0.03	0.1	0.2	0.6	0.001	0.005
57	Clethodim	+	9.5	0.3	1	2	6	2	5
58	Clomazone	+	8.2	0.03	0.1	0.03	0.1	0.1	0.3
59	Clothianidin	+	5.2	0.3	1	0.5	2	1	3
60	Coumaphos	+	9.2	0.3	1	0.3	1	0.3	1
61	Crotoxyphos (NH ₄)	+	8.4	0.03	0.1	0.1	0.3	0.3	1
62	Cumyluron	+	8.7	0.03	0.1	0.3	1	0.1	0.3
63	Cyanazine	+	6.7	0.03	0.1	0.3	1	0.1	0.3
64	Cyazofamid	+	8.9	0.03	0.1	0.3	1	0.3	1
65	Cycloate	+	9.5	0.3	1	0.3	1	0.1	0.3
66	Cycluron	+	7.9	0.3	1	0.3	1	0.1	0.3
67	Cyflufenamid	+	9.2	0.3	1	0.1	0.3	0.1	0.3
68	Cyromazine	+	1.7	2	5	2	5	5	10
69	Demeton-S-methyl sulfone	+	4.4	0.03	0.1	0.1	0.3	0.1	0.3
70	Desmedipham	+	8.0	10	30	2	5	10	20
71	Desmethyl-pirimicarb	+	5.0	0.1	0.5	0.2	0.6	0.3	1
72	Desmetryn	+	7.1	0.1	0.5	0.2	0.6	0.2	0.6
73	Diclobutrazol	+	8.8	0.3	1	1	3	0.1	0.3
74	Dicropthos	+	4.9	0.3	1	1	3	1	3
75	Diethofencarb	+	8.1	0.2	0.5	0.3	1	0.1	0.3
76	Difenacoum	+	10.1	0.03	0.1	0.2	0.6	0.1	0.3
77	Difenoconazole	+	9.4	0.2	0.5	0.03	0.1	0.03	0.1
78	Diflubenzuron	+	9.0	0.3	1	0.1	0.3	0.03	0.1
79	Dimefuron	+	8.0	0.1	0.3	0.3	1	0.3	1
80	Dimethametryn	+	8.9	0.1	0.3	0.1	0.3	0.2	0.6
81	Dimethenamid	+	8.4	0.3	1	0.1	0.3	0.1	0.3
82	Dimethoate	+	5.5	0.03	0.1	0.1	0.3	0.1	0.3
83	Dimethomorph	+	8.3	0.3	1	0.03	0.1	0.1	0.3
84	Dimoxystrobin	+	9.0	0.2	0.5	0.03	0.1	0.1	0.3
85	Diniconazole	+	9.4	0.3	1	0.3	1	0.3	1
86	Dinotefuran	+	3.7	0.3	1	0.3	1	0.3	1
87	Dithiopyr	+	9.5	0.3	1	2	5	0.3	1
88	Diuron	+	7.5	0.03	0.1	0.1	0.3	0.3	1
89	DNOC	-	7.7	0.3	1	1	3	0.3	1
90	Dodemorph	+	8.1	0.3	1	1	3	0.3	1
91	Epoxiconazole	+	8.9	0.3	1	0.03	0.1	0.1	0.3
92	Esprocarb	+	9.7	0.03	0.1	0.1	0.3	0.1	0.3
93	Etaconazol	+	8.8	0.3	1	0.3	1	0.1	0.3
94	Ethiofencarb	+	8.2	0.3	1	2	5	1	3
95	Ethiofencarb-sulfone	+	4.9	0.3	1	0.3	1	0.1	0.3

	Name	Polarity	RT	Strawberry		Leek		Flour	
				LOD	LOQ	LOD	LOQ	LOD	LOQ
96	Ethiofencarb-sulfoxide	+	5.0	0.03	0.1	0.03	0.1	0.1	0.3
97	Ethiprole	+	8.3	0.3	1	1	3	1	3
98	Ethirimol	+	6.2	0.3	1	0.1	0.5	0.1	0.3
99	Ethofumesate	+	8.3	0.3	1	5	20	5	20
100	Ethoxyquin	+	7.7	0.3	1	1	3	0.3	1
101	Etofenprox (NH ₂)	+	10.5	0.03	0.1	0.3	1	1	3
102	Etoxazole	+	10.0	0.02	0.05	0.03	0.1	0.1	0.3
103	Etrimfos	+	9.1	0.3	1	0.1	0.3	1	3
104	Fenamidone	+	8.3	0.03	0.1	0.3	1	0.3	1
105	Fenamiphos	+	8.9	0.03	0.1	0.03	0.1	0.03	0.1
106	Fenarimol	+	8.8	0.3	1	0.1	0.3	0.1	0.3
107	Fenazaquin	+	10.5	0.1	0.3	1	3	0.5	1.5
108	Fenbuconazole	+	8.9	0.3	1	0.3	1	2	5
109	Fenhexamid	+	8.7	0.003	0.01	1	3	0.3	1
110	Fenobucarb	+	8.2	0.03	0.1	0.1	0.3	0.1	0.3
111	Fenoxanil	+	9.4	0.1	0.3	0.03	0.1	0.1	0.3
112	Fenoxycarb	+	9.0	0.1	0.5	0.03	0.1	0.03	0.1
113	Fenpyroximat	+	10.1	0.003	0.01	0.01	0.03	0.03	0.1
114	Fensulfothion	+	7.8	0.03	0.1	0.03	1	0.3	1
115	Fenthion	+	9.2	0.3	1	1	3	0.3	1
116	Fenthion-sulfoxide	+	7.3	0.06	0.2	0.1	0.3	0.1	0.3
117	Fenuron	+	5.3	0.1	0.3	0.1	0.3	0.03	0.1
118	Flazasulfuron	+	8.1	0.1	0.3	0.3	1	0.03	0.1
119	Florasulam	+	6.2	0.1	0.3	0.03	0.1	0.1	0.3
120	Fluazifop	+	8.3	0.3	0.6	0.3	1	1	3
121	Fluazinam	-	9.7	0.03	0.1	0.3	1	0.1	0.3
122	Flubendiamide	+	9.0	1.5	5	1.5	5	5	10
123	Flufenacet	+	8.8	0.1	0.3	0.3	1	0.1	0.3
124	Flufenoxuron	+	9.9	0.3	1	0.1	0.3	0.3	1
125	Flumetsulam	+	5.3	0.1	0.3	0.3	1	0.3	1
126	Fluometuron	+	7.5	0.3	1	0.1	0.3	0.03	0.1
127	Fluopicolide	+	8.5	0.3	1	0.1	0.3	0.1	0.3
128	Fluopyram	+	8.7	0.03	0.1	0.03	0.1	0.1	0.3
129	Fluorochloridone	+	8.7	0.3	1	0.3	1	1	3
130	Fluoxastrobin	+	8.7	0.2	0.5	0.3	1	0.1	0.3
131	Fluquinconazole	+	8.7	0.3	1	0.5	1	0.3	1
132	Flusilazole	+	9.0	0.2	0.5	0.2	0.5	0.2	0.6
133	Flutriafol	+	7.7	0.1	0.3	0.3	1	0.3	1
134	Forchlorfenuron	+	8.0	0.1	0.5	0.1	0.3	0.1	0.3
135	Formetanate hydrochloride	+	3.4	0.3	1	0.3	1	0.1	0.3
136	Formothion	+	6.6	2	5	2	5	3	10
137	Fosthiazate	+	7.4	0.1	0.3	0.1	0.3	0.01	0.03
138	Fuberidazole	+	5.4	0.2	0.6	1	3	0.3	1
139	Furathiocarb	+	9.6	0.3	1	5	10	1	3
140	Griseofulvin	+	7.7	0.3	0.6	0.3	1	0.3	1
141	Halofenozide	-	8.4	0.3	0.6	0.3	1	0.01	0.03
142	Haloxyfop	+	8.9	0.3	1	0.1	0.3	0.03	0.1
143	Haloxyfop-methyl	+	9.4	0.02	0.05	0.3	1	0.1	0.3

	Name	Polarity	RT	Strawberry		Leek		Flour	
				LOD	LOQ	LOD	LOQ	LOD	LOQ
144	Heptenophos	+	7.9	0.3	1	1	3	0.3	1
145	Hexaconazole	+	9.2	0.3	1	0.5	1.5	0.3	1
146	Hexaflumuron	-	9.5	0.3	1	0.3	1	0.1	0.3
147	Hexazinone	+	7.0	0.03	0.1	0.1	0.3	0.1	0.3
148	Hexythiazox	+	9.9	0.03	0.1	0.1	0.3	0.1	0.3
149	Imazalil	+	7.7	0.3	1	0.1	0.3	0.3	1
150	Imazaquin	+	7.0	0.2	0.6	0.3	1	0.1	0.3
151	Imazethapyr	+	6.5	0.03	0.1	0.1	0.3	0.1	0.3
152	Imibenconazole	+	9.8	0.1	0.3	0.3	1	1	3
153	Imidacloprid	+	5.1	0.1	0.3	0.1	0.3	0.5	1.5
154	Indoxacarb	+	9.4	0.3	1	1	3	0.1	0.3
155	loxynil	-	8.1	1	3	0.3	1	1	3
156	lprovalicarb	+	8.7	0.3	1	1	3	1	3
157	Isocarbophos	+	7.8	0.3	1	0.1	0.3	2	5
158	Isoprocarb	+	7.7	0.2	0.6	0.3	1	0.3	1
159	Isoprothiolane	+	8.5	0.3	1	0.1	0.3	1	3
160	Isoproturon	+	7.8	0.1	0.3	0.1	0.3	0.03	0.1
161	Isoxaben	+	8.4	0.02	0.05	0.03	0.1	0.1	0.3
162	Isxadifen-ethyl	+	9.0	0.3	1	0.3	1	0.1	0.3
163	Kresoxim-methyl	+	9.0	0.3	1	1	3	2	5
164	Lenacil	+	7.7	0.03	0.1	0.3	1	0.3	1
165	Malaoxon	+	7.0	0.1	0.3	0.5	1.5	0.1	0.3
166	Mandipropamid	+	8.4	0.3	1	0.3	1	0.3	1
167	MCPA	-	7.8	0.5	2	1	3	2	5
168	Mefenacet	+	8.7	0.03	0.1	0.03	0.1	0.03	0.1
169	Mepiquat chloride	+	0.8	0.1	0.3	0.03	0.1	0.03	0.1
170	Mepronil	+	8.6	0.1	0.3	0.1	0.3	0.03	0.1
171	Metamitron	+	5.4	0.3	1	1	3	1	3
172	Metazachlor	+	7.7	0.3	1	0.1	0.3	0.1	0.3
173	Metconazole	+	7.2	0.1	0.3	0.1	0.3	0.1	0.3
174	Methabenzthiazuron	+	8.0	0.1	0.3	0.03	0.1	0.03	0.1
175	Methamidophos	+	2.1	0.3	1	1	3	1	3
176	Methiocarb	+	8.4	0.2	0.6	0.1	0.3	0.1	0.3
177	Methiocarb-sulfone	+	5.8	0.1	0.3	0.1	0.3	0.1	0.3
178	Methiocarb-sulfoxide	+	5.3	0.03	0.1	0.1	0.3	0.1	0.3
179	Methomyl	+	4.2	0.03	0.1	0.1	0.3	0.3	1
180	Methoprotryne	+	7.9	0.1	0.3	0.1	0.3	0.3	1
181	Methoxyfenozide	+	8.6	0.1	0.3	1	3	1	3
182	Metobromuron	+	7.8	0.1	0.3	1	3	0.3	1
183	Metolachlor	+	8.9	0.3	1	0.1	0.3	0.3	1
184	Metolcarb	+	6.6	0.2	0.6	0.3	1	0.3	1
185	Metosulam	+	7.1	0.1	0.3	0.3	1	0.1	0.3
186	Metoxuron	+	6.4	0.3	1	2	5	1	3
187	Metrafenone	+	9.3	0.1	0.3	0.3	1	0.3	1
188	Metsulfuron-methyl	+	7.0	0.3	1	0.1	0.3	0.1	0.3
189	Mevinphos	+	6.0	0.03	0.1	1	3	0.1	0.3
190	Mexacarbate	+	4.8	0.03	0.1	0.1	0.3	0.3	1
191	Monocrotophos	+	4.6	0.2	0.6	0.1	0.3	0.1	0.3

	Name	Polarity	RT	Strawberry		Leek		Flour	
				LOD	LOQ	LOD	LOQ	LOD	LOQ
192	Monolinuron	+	7.5	0.03	0.1	0.1	0.3	0.1	0.3
193	Napropamide	+	8.9	0.1	0.3	0.3	1	0.03	0.1
194	Neburon	+	9.1	0.3	1	0.1	0.3	2	5
195	Nicosulfuron	+	6.9	0.3	1	0.1	0.3	0.1	0.3
196	Nuarimol	+	8.3	0.3	1	0.3	1	1	3
197	Ofurace	+	7.0	0.3	1	0.3	1	0.3	1
198	Omethoate	+	3.3	0.1	0.3	0.1	0.3	0.1	0.3
199	Oxadixyl	+	6.5	0.3	1	0.3	1	0.3	1
200	Oxamyl (NH4)	+	4.0	0.1	0.3	0.1	0.3	0.01	0.05
201	Paclobutrazol	+	8.5	0.3	1	0.3	1	1	3
202	Penconazole	+	9.1	0.1	0.3	0.1	0.3	0.2	0.6
203	Pencycuron	+	9.4	0.1	0.3	0.1	0.3	0.1	0.3
204	Phenmedipham	+	8.0	2	5	2	5	5	10
205	Phenthoate	+	9.0	0.1	0.3	0.1	0.3	0.3	1
206	Phoxim	+	9.3	0.3	1	2	5	2	5
207	Picoxystrobin	+	9.0	0.03	0.1	0.3	1	0.3	1
208	Piperonyl butoxide	+	9.8	0.003	0.01	0.1	0.3	0.03	0.1
209	Piperophos	+	9.4	0.03	0.1	0.03	0.1	0.03	0.1
210	Pirimicarb	+	6.2	0.1	0.3	0.3	1	0.3	1
211	Pirimiphos-methyl	+	9.3	0.03	0.1	0.03	0.1	0.03	0.1
212	Primisulfuron-methyl	+	8.6	0.2	0.5	0.3	1	0.1	0.3
213	Prochloraz	+	9.3	0.1	0.3	0.1	0.3	0.3	1
214	Profenophos	+	9.6	0.03	0.1	0.01	0.03	0.03	0.1
215	Promecarb	+	8.5	0.1	0.3	0.3	1	0.1	0.3
216	Prometon	+	7.4	0.03	0.1	0.1	0.3	0.3	1
217	Prometryn	+	8.4	0.2	0.6	0.2	0.6	0.2	0.6
218	Propamocarb	+	3.5	0.03	0.1	0.03	0.1	0.03	0.1
219	Propazine	+	8.3	0.1	0.3	0.3	1	0.3	1
220	Propetamphos	+	8.6	0.3	1	1	3	0.03	0.1
221	Propiconazole	+	9.2	0.1	0.3	0.1	0.3	0.1	0.3
222	Propoxur	+	6.9	0.3	1	0.3	1	0.03	0.1
223	Propyzamide	+	8.6	0.3	1	0.3	1	1	3
224	Prosulfocarb	+	9.6	0.1	0.3	0.3	1	0.1	0.3
225	Pymetrozine	+	3.5	0.1	0.3	0.1	0.3	0.1	0.3
226	Pyraclostrobin	+	9.3	0.03	0.1	0.1	0.3	0.01	0.03
227	Pyrimethanil	+	8.3	0.3	1	0.3	1	0.3	1
228	Pyroxsulam	+	7.0	0.1	0.3	0.03	0.1	0.1	0.3
229	Quinoxifen	+	10.1	0.03	0.1	0.1	0.3	0.1	0.3
230	Quizalofop-ethyl	+	9.6	0.03	0.1	0.1	0.3	0.1	0.3
231	Quizalofop-p	+	8.9	0.3	1	2	5	2	5
232	Resmethrin	+	10.3	1	3	n	n	10	20
233	Rimsulfuron	+	7.4	0.03	0.1	0.3	1	0.3	1
234	Rotenone	+	8.9	0.3	1	0.3	1	0.3	1
235	Schradan	+	5.8	0.03	0.1	0.3	1	0.03	0.1
236	Sethoxydim	+	9.7	2	5	50	100	2	5
237	Simeconazole	+	8.8	0.3	1	5	10	5	10
238	Simetryn	+	7.1	0.3	1	0.3	1	0.3	1
239	Spinosad A	+	9.3	0.3	1	0.3	1	0.3	1

	Name	Polarity	RT	Strawberry		Leek		Flour	
				LOD	LOQ	LOD	LOQ	LOD	LOQ
240	Spiromesifen	+	9.9	0.3	1	1	3	1	3
241	Spirotetramat	+	8.7	0.03	0.1	0.1	0.3	0.3	1
242	Spiroxamine	+	8.6	0.1	0.3	0.3	1	0.3	1
243	Sulfotep	+	9.1	0.1	0.3	0.3	1	0.3	1
244	Sulprofos	+	9.9	1	3	1	3	2	5
245	Tebuconazole	+	9.1	0.3	1	0.03	0.1	0.1	0.3
246	Tebufenozide	+	9.0	0.3	1	1	3	2	5
247	Tebufenpyrad	+	9.7	0.03	0.1	0.3	1	0.1	0.3
248	Tebuthiuron	+	7.1	0.03	0.1	0.1	0.3	0.3	1
249	Teflubenzuron	+	9.4	1	3	2	5	5	10
250	Tepraloxydim	+	8.7	1	3	5	10	5	10
251	Terbumeton	+	7.5	0.1	0.3	0.1	0.3	0.1	0.3
252	Terbutylazine	+	8.4	0.1	0.3	0.1	0.3	0.1	0.3
253	Terbutryn	+	8.5	0.1	0.3	0.3	1	0.3	1
254	Tetraconazole	+	8.8	0.3	1	0.3	1	0.1	0.3
255	Tetramethrin	+	9.7	0.3	1	2	5	1	3
256	Thiabendazole	+	5.3	0.3	1	0.3	1	0.3	1
257	Thiacloprid	+	6.1	0.03	0.1	0.03	0.1	0.03	0.1
258	Thiamethoxam	+	4.5	0.03	0.1	0.1	0.3	0.1	0.3
259	Thidiazuron	+	7.1	0.3	1	0.3	1	0.3	1
260	Thiobencarb	+	9.4	0.1	0.3	0.1	0.3	0.1	0.3
261	Thiophanate-methyl	+	6.9	0.3	1	0.3	1	0.3	1
262	Tolfenpyrad	+	9.7	0.03	0.1	0.1	0.3	0.1	0.3
263	Tralkoxydim	+	9.9	0.1	0.3	0.03	0.1	0.03	0.1
264	Triadimefon	+	8.6	1	3	5	10	1	3
265	Triadimenol	+	8.5	0.3	1	0.3	1	1	3
266	Triazophos	+	8.7	0.01	0.05	0.1	0.3	0.1	0.3
267	Trichlorfon	+	5.2	1	3	1	3	1	3
268	Tricyclazole	+	6.5	0.03	0.1	0.1	0.3	0.1	0.3
269	Tridemorph	+	9.2	0.3	1	2	5	0.1	0.3
270	Trietazine	+	8.8	0.3	1	0.1	0.3	0.1	0.3
271	Trifloxystrobin	+	9.4	0.03	0.1	0.03	0.1	0.03	0.1
272	Triflumizole	+	9.6	0.03	0.1	0.3	1	0.3	1
273	Vamidothion	+	5.4	0.01	0.03	0.03	0.1	0.1	0.3
274	Zoxamide	+	9.2	0.1	0.3	0.3	1	0.3	1

Table 6. Comparison of the method LOQ to the MRL for selected pesticides.

Analyte	MRL ($\mu\text{g}/\text{kg}$)			LOQ ($\mu\text{g}/\text{kg}$)		
	Strawberry	Leek	Flour	Strawberry	Leek	Flour
Acephate	10	10	10	1	1	1
Azoxystrobin	50000	10000	300	0.01	0.01	0.3
Carbaryl	50	10	500	1	1	1
Dimethomorph (sum of isomers)	50	1500	10	1	0.1	0.3
Diniconazole	50	10	10	1	1	1
Oxamyl	50	10	10	0.3	0.3	0.05
Pencycurone	50	50	50	0.3	0.3	0.3
Pyraclostrobin	100	700	200	0.1	0.3	0.03
Spinosad A	50	500	1000	1	1	1
Zoxamide	50	20	20	0.3	1	1

The relative standard deviation (RSD) is an important qualitative parameter that can be used instead of signal-to-noise ratio to provide a better estimate of LODs and LOQs. For pesticide residue analysis, SANCO/12571/2013 specifies repeatability criteria of 20% RSD for all compounds within the method scope. For example, as shown in Figure 3, 2.9% RSD was obtained for seven replicate injections of bentazon in the leek matrix at 10 $\mu\text{g}/\text{kg}$. The method RSDs at the MRLs were below 15%, establishing method reproducibility.

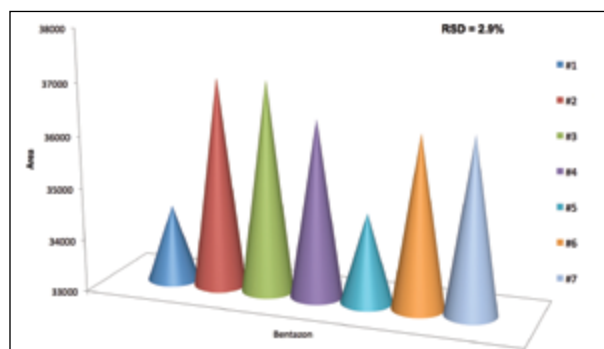


Figure 3. An RSD of 2.9% was obtained for seven replicate injections of bentazon in leek matrix at 10 $\mu\text{g}/\text{kg}$.

When using ESI, matrix effects can challenge accurate quantitation of pesticides. Though there are different strategies to compensate for these effects, the results presented in this application note are based on matrix-matched calibration. Figure 4 shows the effect of matrix on peak area. Although ion suppression is observed in the leek and wheat flour matrices, the method proved effective regardless of the matrix analyzed.

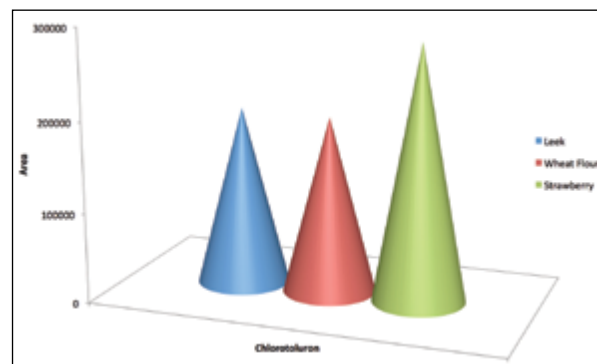


Figure 4. Matrix effects on peak area of chlorotoluron.

QuEChERS sample preparation offers a convenient and effective approach for extraction of pesticide residues in food matrices. The robust procedure has a number of compelling advantages: high recoveries, accurate results, high sample throughput, low solvent and glassware usage, reduced labor and bench space, and lower reagent costs. As shown in Figure 5, the percent recoveries achieved for selected pesticides at the 10 µg/kg level were acceptable and generally between 80 and 110% in the matrices analyzed. The pesticides selected represent results typical of all pesticides studied.

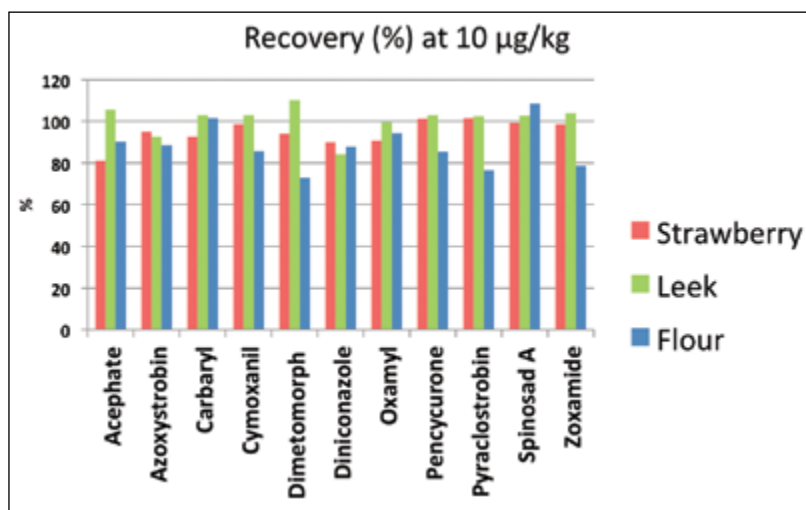


Figure 5. Recovery (%) of selected pesticides at the 10 µg/kg-level by matrix.

The quality control samples FAPAS #T19140 (lettuce puree), FAPAS #19110 (lettuce puree), FAPAS #T19142 (melon puree) and FAPAS #T0983 (wheat flour) were analyzed for their content of target pesticides to provide external quality control for method validation. As shown in Table 7, the measured target analyte values consistently fell within the acceptance range with acceptable %RSD values.

Table 7. External quality control (FAPAS) results for the relevant compounds.

Analyte	Fapas No.	Fapas Matrix	Assigned value (µg/kg)	Acceptance range (µg/kg)	Measured value (µg/kg)	RSD (%)
Carbaryl	T19142	Melon Puree	89.0	49.9–128.2	91.1	1.1
Diniconazol			52.3	29.3–75.3	59.7	9.0
Zoxamide			91.7	51.4–132.1	108.4	3.0
Pencycuron	T19140	Lettuce Puree	73.2	41.0–105.4	45.9	6.0
Thiamethoxam			48.8	27.3–70.3	36.3	9.1
Azoxystrobin	19110	Lettuce Puree	188.0	110–265	132.5	15.4
Dimetomorph (sum of isomers)			181.0	106–256	160.1	11.9
Propyzamide			197.0	116–277	195.1	16.5
Azoxystrobin	T0983	Wheat Flour	383.0	241–524	361.2	1.7
Fenhexamid			110.0	61–158	125.4	10.4
Imazalil			161.0	93–229	157.2	8.2
Thiabendazole				49.3–126.7	67.6	7.3

Conclusion

Regulations of the European Union pose some significant challenges to the analytical methods quantifying pesticide residues in complex matrices. This application note described a multi-residue LC-MS/MS method that uses the TSQ Endura triple quadrupole mass spectrometer-based Pesticide Explorer Collection Standard Quantitation solution for rapid and robust quantitation of more than 250 pesticides in fruit and vegetable matrices at their respective MRLs. For convenience and fast method implementation, the complete instrument and data processing method including SRM settings is included with the Pesticide Explorer Collection start-to-finish workflow solution.

The method results were shown to comply with the stringent guidelines set forth in SANCO/12571/2003 concerning sensitivity, accuracy, and precision. In 15 minutes, all target pesticides were detected and quantified in food matrices below established MRLs. Method RSDs at the MRLs were below 15%, establishing the method's reproducibility. Percent recoveries achieved at the 100 µg/kg-level using a standard QuEChERS sample preparation protocol were in general between 80 and 110%. The QuEChERS sample extraction procedure 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 September 18, 2015).
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 September 18, 2015).
5. EUROPEAN COMMISSION HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL, Safety of the food chain Chemicals, contaminants, pesticides, Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. SANCO/12571/2013. [Online] http://ec.europa.eu/food/plant/pesticides/guidance_documents/docs/qualcontrol_en.pdf (accessed September 18, 2015).
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 September 18, 2015).
7. European Commission. Plants. EU Pesticides database. [Online] <http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN> (accessed September 18, 2015).
8. Fussell et al. *Food Additives & Contam.* 2007, 24, 1247-1256.

To find a local representative, visit:

www.thermofisher.com/pesticides-LCMS