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Pesticide Multiresidue Analysis in Cereal Grains Using Modified QuEChERS Method Combined with Automated Direct Sample Introduction GC-TOFMS and UPLC-MS/MS Techniques†

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The QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation method was modified to accommodate various cereal grain matrices (corn, oat, rice, and wheat) and provide good analytical results (recoveries in the range of 70−120% and RSDs <20%) for the majority of the target pesticides (about 180 analytes). The method consists of a 1 h shaking of a milled sample (2.5−5 g) in 20 mL of 1:1 (v/v) water/acetonitrile (or 25 mL of 1.5:1 water/acetonitrile in the case of rice) to provide simultaneous matrix swelling and analyte extraction. Then, a $MgSO_4$/NaCl salt mixture (4:1, w/w) is added to the extract to induce phase separation and force the pesticides into the upper acetonitrile layer, a 1 mL aliquot of which is subsequently cleaned up using dispersive solid phase extraction with 150 mg of PSA, 50 mg of C_{18}, and 150 mg of $MgSO_4$. GC-amenable pesticides were analyzed using gas chromatography combined with time-of-flight mass spectrometry (GC-TOFMS) and the automated direct sample introduction technique for a large volume injection of the extracts. Ultraperformance liquid chromatography coupled to triple-quadrupole tandem mass spectrometry (UPLC-MS/MS) was employed for the analysis of LC-amenable pesticides. This method was implemented in a routine laboratory, providing about 3-fold increased sample throughput, 40−50% reduction in the cost of disposable materials and in the operation costs, 1:100 solvent waste reduction, and increased scope of the analysis versus the traditional approach based on the Luke method.

KEYWORDS: Pesticide analysis; sample preparation; QuEChERS; liquid chromatography−mass spectrometry; gas chromatography−mass spectrometry; direct sample introduction

INTRODUCTION

In most U.S. laboratories, the traditional approach to pesticide residue analysis in food has involved the so-called Luke procedure for sample preparation (*1*, *2*), followed by gas chromatographic (GC) analysis using multiple selective detectors, such as an electron capture detector (ECD), an electrolytic conductivity detector (ELCD), a flame photometric detector (FPD), or a nitrogen−phosphorus detector (NPD). GC with mass spectrometric (MS) detection has been typically employed only for confirmation of samples determined as positive by the element selective detectors. This GC analytical scheme has usually been complemented by high-performance liquid chromatography (HPLC) with postcolumn derivatization and fluorescence detection for thermolabile carbamate pesticides (*3*).

As the GC-MS and later also LC-MS technologies became more affordable and suitable for routine food testing, laboratories started using MS as the preferred primary tool for detection of a large number of pesticides independent of their elemental composition. The introduction of LC-MS with atmospheric pressure ionization opened the door to the analysis of more polar pesticides, which are not extracted well by using the Luke method. Moreover, the typical Luke procedure uses large solvent volumes (including dichloromethane) and, thus, is costly and generates a lot of waste per sample.

In 2003, Anastassiades et al. (*4*) introduced the QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation method for the analysis of pesticide residues in fruits and vegetables. Among other beneficial features, the QuEChERS procedure uses acetonitrile (MeCN), which enables extraction of more polar analytes and direct compatibility with both GC- and LC-MS analyses. The original QuEChERS method has been modified using acetate (*5*) or citrate (*6*) buffers to accommodate some of the difficult pesticides. After collaborative

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studies, these two modifications became AOAC International Official Method 2007.01 (*7*) and CEN standard method EN 15662 (*8*), respectively.

The aim of our work was to modernize a traditional pesticide residue methodology for cereal grains by adapting the QuEChERS method for important grain matrices and combining it with state-of-the-art GC- and LC-MS instrumentation. For GC-amenable pesticides, a time-of-flight (TOF) mass analyzer was used for analyte detection, providing fast acquisition of full mass spectra at high efficiency, thus enabling nontargeted pesticide residue analysis. The GC-TOFMS instrument was equipped with an automated direct sample introduction (DSI) system. The DSI technique is a form of a large volume injection (LVI), which uses disposable microvials for introduction of liquid (or solid) samples into the GC (*9*−*11*). The major advantage of DSI versus other LVI techniques is that nonvolatile matrix components remain in the microvial and are removed from the system after each GC run, resulting in a less frequent need for GC system maintenance. LC-amenable pesticides were analyzed using ultraperformance LC (UPLC) coupled to a triple-quadrupole tandem MS (MS/MS), providing fast chromatographic separation and sensitive and selective quantitation (*12*−*14*).

In this study, we optimized DSI-LVI-GC-TOFMS and UPLC-MS/MS conditions for the analysis of about 180 pesticides in cereal grain extracts. The QuEChERS method was modified to accommodate various cereal grain matrices (corn, oat, rice, and wheat) and provide good analytical results for the majority of the target pesticides in the method validation.

MATERIALS AND METHODS

Chemicals and Materials. Pesticide reference standards, all ≥95% purity, were obtained from Chemservice (West Chester, PA). Individual pesticide stock solutions (2000−5000 μg/mL) were prepared in ethyl acetate or MeCN and stored at −18 °C. Two composite pesticide stock solutions, MIX-1 and MIX-2 (for analytes, see **Tables 1** and **2**), were prepared at 10 μg/mL in MeCN with 0.1% acetic acid. The addition of 0.1% acetic acid prevents degradation of base-sensitive analytes in MeCN (*15*). Isotopically labeled internal standards (ISTD) of atrazine (ethylamine-d_5), carbofuran (ring-$^{13}C_6$), dimethoate (o,o-dimethyl-d_6), 2,4-DDT (ring-$^{13}C_6$), α-HCH ($^{13}C_6$), and parathion (diethyl-d_{10}) were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA). ISTD composite solution was prepared at 5 μg/mL in acetone. Triphenyl phosphate (TPP) from Sigma-Aldrich (Milwaukee, WI) was initially used as a QC standard but was later replaced by *trans*-permethrin (phenoxy-$^{13}C_6$) from Cambridge Isotope Laboratories, Inc. Working solutions of the QC standard were prepared at 1 and 5 μg/mL in acetone.

Acetone, ethyl acetate, and methanol (MeOH) were of high-purity grade for residue analysis obtained from Burdick & Jackson (Morristown, NJ). MeCN was of Optima grade obtained from Fisher Scientific (Pittsburgh, PA). Optima grade water (Fisher Scientific) was used for the first portion of the extraction process (to swell the matrix) and for the preparation the UPLC mobile phase. Formic acid (FA) was obtained as a 98% solution for mass spectrometry from Fluka (Buchs, Switzerland). Glacial acetic acid, NaCl, ammonium formate, anhydrous sodium acetate, and anhydrous $MgSO_4$ were purchased from Fisher Scientific (Fair Lawn, NJ). Sorbents for the dispersive solid-phase extraction (SPE) in the method development experiments included primary seconday amine (PSA) obtained from UCT, Inc. (Bristol, PA) and C_{18} from J. T. Baker (Phillipsburg, NJ). For method validation, prepacked minicentrifuge tubes (2 mL) with 150 mg of PSA, 50 mg of C_{18}, and 150 mg of anhydrous $MgSO_4$ and prepacked centrifuge tubes (50 mL) with 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were purchased from UCT.

Ultrahigh-purity helium and argon from Valley National Gases (New Brighton, MN) were employed as the carrier gas in GC-MS and as the collision gas in UPLC-MS/MS, respectively. Liquid headspace supplied nitrogen (Valley National Gases) served as desolvation and cone gases in UPLC-MS/MS.

Samples of milled corn, oat, rice, and wheat were provided by General Mills sample preparation facility. For routine analysis, most samples were milled to a flour consistency.

Optimized Sample Preparation Procedure. The optimized sample preparation procedure entailed the following steps: (1) weigh a milled, thoroughly homogenized corn (2.5 g), oat (3.5 g), rice (5.0 g), or wheat (5.0 g) sample into a 50 mL disposable polypropylene centrifuge tube (Corning, Lowell, MA); (2) add 10 mL of deionized water (15 mL in the case of rice) and 10 mL of MeCN using solvent dispensers; (3) add 200 μL of the ISTD standard solution; (4) vortex the tube to fully disperse the sample in the solvent and place it on a wrist action shaker for 1 h; (5) add 4 g of anhydrous $MgSO_4$ and 1 g of NaCl; (6) immediately seal the tube and shake vigorously by hand (or vortex) for 1 min to prevent formation of crystalline agglomerates during $MgSO_4$ hydration; (7) centrifuge the tube for 10 min at rcf > 3000 (in our case, 10000 rpm equivalent to 12857 rcf was employed, using a 5804 centrifuge from Eppendorf, Westbury, NY); (8) transfer 1 mL of the MeCN extract to a 2 mL minicentrifuge tube containing 150 mg of PSA, 50 mg of C_{18}, and 150 mg of anhydrous $MgSO_4$; (9) mix (vortex) the extract with the sorbent/desiccant for 30 s; (10) centrifuge the tube for 5 min; (11) transfer 300 μL of the supernatant into the chamber of a Mini-UniPrep syringeless filter vial (Whatman, Florham Park, NJ), add 30 μL of the 1 μg/mL QC solution, and mix thoroughly; (12) transfer 125 μL of the extract in the Mini-UniPrep vial into a deactivated glass insert (Agilent, Santa Clara, CA) placed in a GC autosampler vial and cap the vial with a heat-treated septum (overnight at 250 °C); and (13) compress the filter (polyvinylidene fluoride, PVDF, 0.2 μm) plunger of the Mini-UniPrep assembly to filter the extracts for the UPLC-MS/MS analysis.

Method Validation. The method was validated for each matrix in duplicate at three concentration levels: low, middle, and high, which were equivalent to 12.5, 50, and 125 ng/mL, respectively, for each pesticide in the final extract (assuming 100% recovery). This translates to 25, 100, and 250 ng/g for wheat and rice; 36, 143, and 357 ng/g for oat; and 50, 200, and 500 ng/g for corn. The cereal grain samples were fortified using 250 μL of spiking solutions containing 5000, 2000, and 500 ng/mL, respectively, of each pesticide in MeCN with 0.1% acetic acid. After fortification, the spiked samples were left at room temperature for 30 min prior to the addition of the extraction solvents. Matrix-matched calibration standards were used to calculate the analyte recoveries. Solvent-based standard solutions were also analyzed to assess the matrix effects.

Automated DSI-GC-TOF MS Analysis. GC-TOFMS analysis was performed using a Pegasus 4D (Leco Corp., St. Joseph, MI) TOF mass spectrometer combined with an Agilent 6890 GC instrument, which was equipped with a secondary oven and nonmoving quad-jet dual stage modulator for two-dimensional comprehensive GC×GC chromatography. Injection was conducted by a CombiPAL autosampler (Leap Technologies, Carrboro, NC) with an automated DSI accessory (LINEX) in combination with an Optic 3 programmable temperature vaporizer (PTV) inlet (both from ATAS-GL International, Veldhoven, The Netherlands). Leco ChromaTOF (version 3.22) software was used for GC-TOFMS control and data acquisition/processing, and CombiPAL Cycle Composer with macro editor (version 1.5.2) and ATAS Evolution software (version 1.2a) were used to control the automated DSI process and PTV (including column flow), respectively.

The automated DSI involved injection of 10 μL of sample extract from an autosampler vial into a disposable microvial (1.9 mm i.d., 2.5 mm o.d., 15 mm long; Scientific Instrument Services, Ringoes, NJ), which was Siltek deactivated by Restek (Bellefonte, PA), washed with acetone (heated at 250 °C), and placed in a LINEX DMI tapered liner. Using a LINEX gripper attached to the CombiPAL head, the liner is then transferred into the Optic inlet equipped with a pneumatically controlled LINEX head that can open and close automatically. A series of macros was designed using the Cycle Composer macro editor to create the DSI method and control the LINEX and CombiPAL mechanics.

The optimized Optic 3 PTV conditions involved solvent venting at an injector temperature of 100 °C for 4.5 min with an initial column flow of 0.8 mL/min and a split flow of 50 mL/min, followed by a splitless transfer of analytes for 4 min, for which the injector temperature was ramped to 280 °C (at 16 °C/s) and the column flow changed to 1.5 mL/min (kept constant for the entire GC run). After the splitless period, the split flow was kept at 50 mL/min for 6 min, at which point the split flow was reduced to 25 mL/min and the injector temperature was decreased to 250 °C.

The GC separation was conducted using a combination of a 20 m × 0.25 mm i.d. × 0.25 μm film thickness RTX-5 ms column and

Table 1. Retention Times (t_R) and MS Ions Used for Quantitation (Q *m*/*z*) in the DSI-LVI-GC-TOFMS Analysis

name	MIX	t_R (s)	Q *m*/*z*
acephate	1	596.9	94
alachlor	2	845.5	188
aldrin	2	895.2	263
ametryn	2	846.8	212
amitraz	1	1331.6	132
atrazine	1	743.1	200
azinphos-ethyl	1	1348.6	132
azinphos-methyl	1	1315.2	160
azoxystrobin	1	1488.0	344
BHC, α-	2	718.7	183
BHC, α-, $^{13}C_6$-	ISTD	718.3	225
BHC, β-	2	748.0	183
BHC, δ-	2	783.1	183
bifenox	2	1297.5	341
bitertanol I	2	1366.0	170
bitertanol II	2	1370.2	170
bromacil	2	888.0	205
bromophos	2	932.1	331
bromopropylate	2	1276.4	341
captan/captafol degradation product (*cis*-1,2,3,6-tetrahydrophthalimide)	2	614.1	151
carbaryl	1	841.7	144
carbaryl degradation product (1-naphthol)	1	625.6	144
carbofuran	1	738.2	164
carbofuran, 3-hydroxy-	1	604.0	180
carbofuran-7-phenol	1	539.8	164
carbophenothion	2	1180.8	342
chlordane, *cis*-	2	1020.5	373
chlordane, *trans*-	2	995.0	375
chlordimeform	2	693.4	181
chlorfenson	2	1032.7	175
chlorfenvinphos, *cis*-	1	952.6	267
chlorfenvinphos, *trans*-	1	971.7	269
chlorobenzilate	2	1117.1	251
chloroneb	2	625.0	191
chloropropylate	1	1117.6	251
chlorothalonil	2	789.6	266
chlorpropham	2	687.9	127
chlorpyrifos	2	905.3	197
chlorpyrifos-methyl	2	834.5	286
chlorthal-dimethyl	2	913.3	301
cinerin I	1	1083.7	123
coumaphos	1	1378.3	362
cyanazine	2	909.7	68
cyfluthrin I	2	1391.9	206
cyfluthrin II	2	1395.5	206
cyfluthrin III + IV	2	1399.5	206
cyhalothrin, γ-	2	1329.1	181
cyhalothrin, λ-	2	1338.8	181
cypermethrin I	2	1403.2	181
cypermethrin II−IV	2	1409.8	181
cyprodinil	1	945.5	224
DDD, *o*,*p*′-	2	1073.2	235
DDD, *p*,*p*′-	1	1132.8	235
DDE, *o*,*p*′-	1	1004.2	246
DDE, *p*,*p*′-	1	1059.0	246
DDT, *o*,*p*′-	2	1137.9	235
DDT, *o*,*p*′-, $^{13}C_6$-	ISTD	1136.3	247
DDT, *p*,*p*′-	1	1200.6	235
deltamethrin	2	1473.6	181
deltamethrin artifact	2	1463.7	181
demeton-O	2	670.0	88
demeton-S	2	729.4	88
diazinon	2	772.2	179
dichlobenil	2	560.7	171
dichlorvos	1	517.2	185
diclofop-methyl	2	1232.3	253
dicloran	2	731.0	176
dicofol degradation product (4,4′-dichlorobenzophenone)	2	908.7	250

Table 1. Continued

name	MIX	t_R (s)	Q *m/z*
dicrotophos	2	700.3	127
dieldrin	2	1059.6	261
dimethoate	1	732.7	93
dioxathion	2	754.4	270
diphenamid	2	934.3	167
diphenylamine	2	675.4	169
disulfoton	1	778.9	88
diuron degradation product (3,4-dichlorophenyl isocyanate)	1	544.1	187
endosulfan α	2	1015.3	243
endosulfan β	2	1113.6	195
endosulfan sulfate	2	1191.5	274
endrin	2	1096.7	263
endrin aldehyde	2	1168.4	345
endrin ketone	2	1262.4	317
EPN	1	1277.4	157
ethalfluralin	2	692.6	276
ethion	2	1144.2	231
ethoprophos	2	681.1	158
ethoxyquin	2	733.7	202
etridiazole	2	603.6	211
fenamiphos	1	1037.8	303
fenarimol	1	1341.4	251
fenchlorphos	2	854.7	285
fenitrothion	2	873.7	277
fenobucarb	2	668.9	150
fenobucarb degradation product	2	527.9	150
fenpropathrin	2	1289.8	181
fensulfothion	2	1125.9	293
fenthion	2	902.1	278
fenvalerate I	2	1439.8	167
fenvalerate II	2	1448.6	167
fluazifop-*p*-butyl	2	1107.3	282
fluvalinate, τ-	2	1414.5	250
folpet degradation product (phthalimide)	2	605.7	147
fonofos	2	763.9	246
heptachlor	1	845.4	272
heptachlor epoxide	1	956.5	353
hexachlorobenzene	2	727.3	284
imazalil	1	1048.2	215
iprodione	2	1266.9	316
iprodione degradation product	2	1130.9	187
isazophos	2	788.8	161
isofenphos	2	971.4	213
leptophos	2	1317.9	375
lindane	2	754.8	183
malathion	1	889.7	173
metalaxyl	2	853.0	206
methamidophos	1	510.0	94
methidathion	2	997.9	145
methiocarb	1	872.8	168
methiocarb degradation product	1	640.3	168
methoxychlor	2	1285.2	227
metolachlor	2	898.7	238
metribuzin	2	824.4	198
mevinphos	2	593.1	127
mirex	2	1323.3	272
monocrotophos	2	706.6	127
myclobutanil	2	1074.4	179
napropamide	2	1037.7	271
nitrapyrin	2	603.0	196
norflurazon	2	1197.7	303
omethoate	1	664.3	156
oxadiazon	1	1069.2	175
oxyfluorfen	1	1079.7	252
paraoxon	1	852.8	109
parathion	1	906.9	291
parathion, d_{10}-	ISTD	900.2	301
parathion-methyl	1	834.8	263

Table 1. Continued

name	MIX	t_R (s)	Q *m/z*
pendimethalin	2	956.4	252
pentachloronitrobenzene	2	760.6	237
permethrin, *cis*-	2	1369.9	183
permethrin, *trans*-	2	1374.3	183
permethrin, *trans*-, $^{13}C_6$-	QC	1381.8	189
perthane	2	1103.9	223
phorate	2	712.3	260
phosalone	2	1316.0	182
phosmet	1	1271.8	160
phosphamidon	2	823.5	264
piperalin	2	1214.3	314
piperonyl butoxide	1	1243.1	176
pirimicarb	2	805.9	166
pirimiphos-ethyl	2	940.3	318
pirimiphos-methyl	2	875.9	290
procymidone	2	985.9	283
profenofos	2	1053.0	339
profenofos degradation product (4-bromo-2-chlorophenol)	2	522.6	206
profluralin	2	761.1	318
promecarb	1	709.6	135
promecarb degr. product (5-isopropyl-3-methylphenol)	1	544.4	135
prometryn	2	851.9	184
propanil	1	820.9	161
propham	2	602.8	179
propiconazole I	1	1195.5	173
propiconazole II	1	1207.3	173
propoxur	1	670.3	110
propoxur degradation product	1	480.3	110
propyzamide	2	762.6	173
pyrethrin I	1	1176.6	123
quinalphos	1	975.0	146
quizalofop-ethyl	2	1408.5	299
resmethrin I	2	1238.3	171
resmethrin II	2	1247.0	171
simazine	2	736.5	201
simetryn	2	840.2	213
sulfallate	1	716.9	188
sulprofos	2	1164.6	322
terbacil	1	783.7	160
terbufos	2	758.9	231
tetrachlorvinphos	2	1017.0	329
tetradifon	2	1306.1	159
thiabendazole	1	963.6	201
thiobencarb	1	889.2	100
thionazin	2	667.7	248
tolylfluanid degradation product (DMST)	1	750.8	214
triadimefon	2	911.4	208
tribufos (DEF)	1	1059.0	169
trifloxystrobin	1	1211.1	116
trifluralin	2	700.3	264
trimethacarb	1	693.4	136
trimethacarb degradation product	1	526.0	136
vinclozolin	2	834.2	212

a 1 m × 0.1 mm i.d. × 0.1 μm film thickness RTX-pesticide2 column (both from Restek), which translated into a 1.68 m × 0.1 mm i.d. "virtual" column setting in the ATAS Evolution software. The oven temperature program (started after a 4.5 min solvent vent period) was as follows: 60 °C held for 4 min, ramped to 180 at 20 °C/min, then at 5 °C/min to 230 °C, 20 °C/min to 280 °C, and finally ramped to 300 at 40 °C/min, and held for 12 min. The total run time was 35 min.

The MS transfer line and ion source temperatures were held at 280 and 250 °C, respectively. The electron energy was 70 eV. The detector voltage was set at about +200 V above the value obtained in the tuning procedure (at 1750 V during the method validation). The TOF instrument acquired full scan spectra in the range of *m/z* 45−550 at a data acquisition rate of 10 spectra/s. Agilent's Pesticide and Endocrine Disruptor Database was converted into NIST format and used for MS library spectra searching and matching. **Table 1** gives retention times and MS ions used for the quantitation of the GC-amenable pesticides.

UPLC-MS/MS Analysis. UPLC-MS/MS analysis was performed using an Acquity UPLC system (integrated solvent and sample management system with a column heater module) interfaced to a Quattro Premier triple-quadrupole mass spectrometer (both from Waters Corp., Milford, MA). The MassLynx software (version 4.1) was used for instrument control and data acquisition/processing. Sample injection volume was 2 μL. An Acquity UPLC BEH C18 column (50 × 2.1 mm; 1.7 μm particle size, 130 Å pore size) from Waters was employed for the LC separation at 40 °C. A binary mobile phase was composed of (A) 10 mM ammonium formate in water (pH 3, adjusted using formic acid) and (B) 10 mM ammonium formate in MeOH. A linear mobile phase gradient started at 30% B (0−4 min) and

Table 2. Analyte-Specific UPLC-MS/MS Conditions, Including Retention Times (t_R)

analyte	MIX	t_R (min)	precursor ion (*m*/*z*)	quantitation product ion (*m*/*z*)	confirmation product ion (*m*/*z*)	cone voltage (V)	collision energy (V)	dwell time (ms)
acephate	1	0.38	184.1	142.9	49.0	17	16	12
acetamiprid	1	1.02	223.3	125.9	89.9	27	26	25
aldicarb	1	1.89	208.1	116.0	88.9	10	8	40
aldicarb sulfone	1	0.44	223.0	85.8	80.9	13	18	20
aldicarb sulfoxide	1	0.39	207.1	132.0	88.8	13	5	20
ametryn	2	7.08	228.3	186.1	95.9	32	19	30
amitraz	1	10.31	294.4	163.1	253.3	20	13	50
atrazine	1	5.80	216.5	174.1	104.5	20	22	30
atrazine, d_5-	ISTD	5.80	221.5	101.1	179.2	20	22	30
azinphos-ethyl	1	8.15	346.3	132.0	159.9	18	14	20
azinphos-methyl	1	6.91	318.1	132.1	261.1	14	7	10
azoxystrobin	1	7.59	404.4	372.2	344.1	21	18	15
bifenox	2	9.66	359.1	310.1	342.2	16	10	20
bitertanol	2	9.61	338.4	70.0	269.3	16	10	60
carbaryl	1	4.85	202.2	145.0	127.0	20	17	100
carbofuran	1	3.95	222.2	165.0	123.0	26	13	100
carbofuran, $^{13}C_6$-	ISTD	4.61	228.1	171.0	129.0	26	13	100
carbofuran, 3-hydroxy-	1	1.00	255.3	163.0	181.0	13	20	25
chloroxuron	1	8.05	291.3	72.0	164.1	34	16	20
cyanazine	2	3.00	241.4	214.3	96.0	35	19	70
cyprodinil	1	8.20	226.3	92.9	107.9	49	31	20
deltamethrin	2	10.38	523.2	281.0	N/A	20	12	30
dichlorvos	1	3.34	221.1	109.0	78.9	33	24	90
dicrotophos	2	0.65	238.3	193.0	193.0	21	11	60
dimethoate	1	1.00	230.2	199.0	171.0	20	9	25
dimethoate, d_6-	ISTD	1.33	236.0	204.9	87.8	20	9	25
diuron	1	6.23	233.2	71.9	159.9	26	25	20
fenobucarb	1	7.19	208.1	94.9	152.0	23	12	10
fensulfothion	2	6.78	309.2	281.1	253.1	29	14	30
fluvalinate, τ-	2	10.48	503.4	208.1	180.9	20	11	20
imazalil	1	6.60	297.3	158.9	172.9	29	25	20
imidacloprid	1	0.70	256.1	175.0	209.1	21	18	5
linuron	1	7.09	249.1	159.9	182.0	30	17	15
malathion	1	7.79	331.2	127.0	285.1	20	11	15
methamidophos	1	0.38	141.9	93.8	125.0	25	14	15
methidathion	2	6.67	303.3	144.9	84.9	19	9	30
methiocarb	1	7.39	226.3	121.0	169.0	20	16	15
methomyl	1	0.52	163.1	87.9	105.9	13	8	20
monocrotophos	2	0.58	224.2	193.0	97.9	21	8	70
myclobutanil	2	8.04	289.4	70.0	125.0	30	21	45
napropamide	2	8.37	272.4	129.1	171.0	23	15	40
norflurazon	2	6.72	304.2	284.2	160.1	45	22	30
omethoate	1	0.39	214.0	183.0	155.0	20	10	17
oxamyl	1	0.42	237.3	72.0	89.8	12	9	30
paraoxon	1	6.30	276.4	220.0	173.9	24	18	25
permethrin	2	10.56	408.3	183.0	355.3	15	9	20
permethrin, *trans*-, $^{13}C_6$-	QC	9.47	414.8	189.3	361.4	15	9	20
phosalone	2	9.52	368.3	182.0	322.2	22	11	30
phosmet	1	7.00	318.2	160.0	133.0	20	30	15
piperalin	2	6.41	331.4	173.0	231.2	40	27	40
profenofos	2	9.91	375.1	305.0	347.1	29	16	20
promecarb	1	7.64	208.3	109.0	151.1	21	14	15
prometryn	2	7.99	242.3	157.8	200.1	30	26	40
propanil	2	7.21	218.1	162.0	127.0	28	15	25
propham	1	5.71	180.2	138.1	120.0	13	9	30
propiconazole	1	9.34	342.4	159.0	122.9	36	49	30
propoxur	1	3.61	210.3	111.0	168.0	17	11	90
quizalofop-ethyl	2	9.89	373.4	299.3	271.2	31	19	20
resmethrin	2	10.48	339.4	171.1	91.0	23	16	30
simazine	2	3.31	202.2	132.0	124.1	32	18	70
spinosad A	1	9.76	732.7	142.1	N/A	40	21	30
spinosad D	1	9.95	746.7	142.2	N/A	40	21	30
tetrachlorvinphos	2	9.02	367.4	127.0	241.0	27	17	50
thiabendazole	1	0.95	202.2	175.0	131.0	47	24	40
thiobencarb	1	9.51	258.3	124.9	99.9	22	15	15
tolylfluanid	1	9.24	347.1	238.1	136.9	12	15	10
trifloxystrobin	1	9.79	409.4	186.0	206.1	23	18	15
trimethacarb	1	6.38	194.0	137.1	109.1	22	23	17

went to 60% B at 7.5 min (held until 8.5 min), followed by a gradient to 100% B at 10.5 min (held until 12.5 min), and concluded by column equilibration at initial conditions of 30% B (12.6−15 min). The flow rate of the mobile phase was 450 μL/min.

The MS determination was performed in electrospray (ESI) positive mode (using the optimized MS instrument parameters obtained by the tuning) combined with monitoring of the two most abundant MS/MS (precursor → product) ion transitions. **Table 2** gives analyte-specific MS/MS conditions and LC retention times for the LC-amenable analytes. The MS source conditions were as follows: capillary voltage of 1.7 kV, extractor voltage of 4.0 V, RF lens at 0.9 V, source temperature of 130 °C, desolvation temperature of 350 °C, collision gas (argon) pressure of 4.31×10^{-3} mbar, desolvation gas (N_2) flow of 600 L/h, and cone gas (N_2) flow of 100 L/h.

RESULTS AND DISCUSSION

As mentioned in the Introduction, our goal was to modernize a traditional methodology for the analysis of pesticide residues in cereal grains. We started with updating the target list of analytes to add mainly those frequently found in the Pesticide Data Program (PDP), which is a national pesticide residue database program administered by the USDA Agricultural Marketing Service (*16*). We were interested not only in residues found in cereal grains but also in fruit and vegetable commodities because the target analyte list and instrument methods were also intended for the analysis of pesticide residues in fruits and vegetables using the QuEChERS method with acetate buffer (AOAC International Official Method 2007.01). The target list of analytes for method development and validation is given in **Tables 1** and **2**. The instrument methods for the DSI-LVI-GC-TOFMS and UPLC-MS/MS analyses had to be developed and optimized first, followed by modification and optimization of the QuEChERS procedure for various cereal grain matrices (corn, oat, rice, and wheat).

DSI-LVI-GC-TOF MS Method Development and Optimization. The development of the automated DSI-LVI-GC-TOFMS method involved optimization of each individual component to obtain an overall working system. The GC column setup employed a combination of a 20 m × 0.25 mm i.d. × 0.25 μm film thickness RTX-5 ms column and a 1 m × 0.1 mm i.d. × 0.1 μm film thickness RTX-pesticide2 column, for first dimension (1D) and potential second dimension (2D) separations, respectively, if comprehensive two-dimensional GC×GC separation was desired or needed. Indisputably, the GC×GC analysis has several benefits, including mainly improved GC separation efficiency (thus method selectivity) and increased sensitivity due to the thermal focusing of the peaks eluting from the first dimension (*17*). However, a routine operation of a GC×GC system is rather demanding in terms of the relatively high liquid nitrogen consumption (for thermal modulation) and also when it comes to far more complex data processing as compared to a 1D analysis. Therefore, the optimized method used 1D GC separation for cereals, fruits, and vegetables, but the short secondary column was kept in place for an easy conversion to a 2D system for analysis of more complex samples, such as spices or tea. To facilitate a faster GC system equilibration, the secondary oven and modulator insulations were removed for the 1D operation. Also, the secondary oven and modulator were inactivated in the GC method.

As for the TOFMS part, the TOF mass analyzer enables fast acquisition of full mass spectra (up to 500 spectra/s is possible with the Pegasus system). In the MS method, we selected a mass range of m/z 45−550 to cover a typical mass range for pesticide spectra, including mirex with the highest monoisotopic molecular weight (540 g/mol) on our target list. A spectral acquisition rate of 10 spectra/s was used as a sufficient rate for peak characterization and deconvolution in 1D analysis (higher rates led to decreased sensitivity without any significant benefits). An ion source temperature of 250 °C was chosen as a compromise between sensitivity (ionization efficiency) and spectral quality (degree of fragmentation). Lower temperatures gave lower sensitivity (especially for the less volatile analytes), whereas higher temperatures might lead to overfragmented spectra with low abundance of higher ions and poor library match. To obtain deconvoluted reference spectra even for closely eluting peaks, analytes (in total 185 compounds monitored by GC-TOFMS, including important pesticide degradation products) were divided into two groups (designated MIX-1 and MIX-2 in **Table 1**) for two separate injections into the GC system.

The TOFMS instrument does not require presetting of analyte-specific conditions for each individual pesticide as opposed to, for example, single ion monitoring with quadrupole or tandem MS with triple-quadrupole or ion trap mass analyzers. Therefore, the analysis (data acquisition) is nontargeted. However, for routine pesticide residue analysis, it is difficult to process the data in a completely nontargeted fashion, relying only on spectral deconvolution, peak finding, and spectral matching algorithms provided by the data processing software. Instead, we preferred to create templates (in the calibration portion of the software) that enabled fast data review for pesticides on our target list by extracting traces of their quantitation ions in expected retention time windows and comparing their deconvoluted and raw MS spectra with library and reference spectra.

As mentioned in the Introduction, the DSI technique is a unique form of a LVI, which uses disposable microvials for introduction of samples into the GC system. As opposed to other LVI techniques (*18*), the nonvolatile matrix components remain in the microvial and are removed from the system after each GC run. Also, it is not necessary to trap the liquid sample in the liner at low temperatures because the microvial holds the liquid in the liner. Therefore, excessive inlet cooling is not required, and the initial inlet temperature can be set at a temperature suitable for effective solvent venting (100 °C in our case for MeCN injection). The solvent venting conditions (temperature, vent time, initial column flow, and split flow) were optimized to eliminate 80−90% of MeCN without losing early eluting analytes. It is advisible to leave 1−2 μL of the solvent in the microvial as a keeper (before ramping the injector temperature), but larger volumes should be avoided to prevent peak distortions and potential column bleed (*19*). Analyte transfer conditions (temperature programming rate, final inlet temperature, and column flow) were optimized to quantitatively transfer analytes (especially the late eluting ones). Different pressure pulses were tested for faster and more effective analyte transfer but did not result in significant improvements in analyte responses.

To improve injection reproducibility, the microvials were sent for Siltek deactivation. Also, it was important to rinse each microvial with acetone and heat it at 250 °C overnight prior to its use to remove serious background interferences in the GC-TOFMS analysis. Another source of interferences were siloxanes from septa in the autosampler vial caps, which were minimized by overnight heating of the septa at 250 °C.

UPLC-MS/MS Method Development and Optimization. Initially, the LC-MS/MS method development was focused on the conversion of the HPLC−fluorescence method for carbamate insecticides to a modern system that would not require post-column derivatization and would offer analyte identification based on MS/MS transitions. However, the list of LC-amenable analytes expanded when the original list of target analytes was updated with some modern, more polar pesticides, such as imidacloprid, acetamiprid, azoxystrobin, trifloxystrobin, or spinosads. Also, some pesticides traditionally analyzed by GC-MS but performing far better in LC-MS/MS were included in the LC-MS/MS method, such as more polar organophosphate

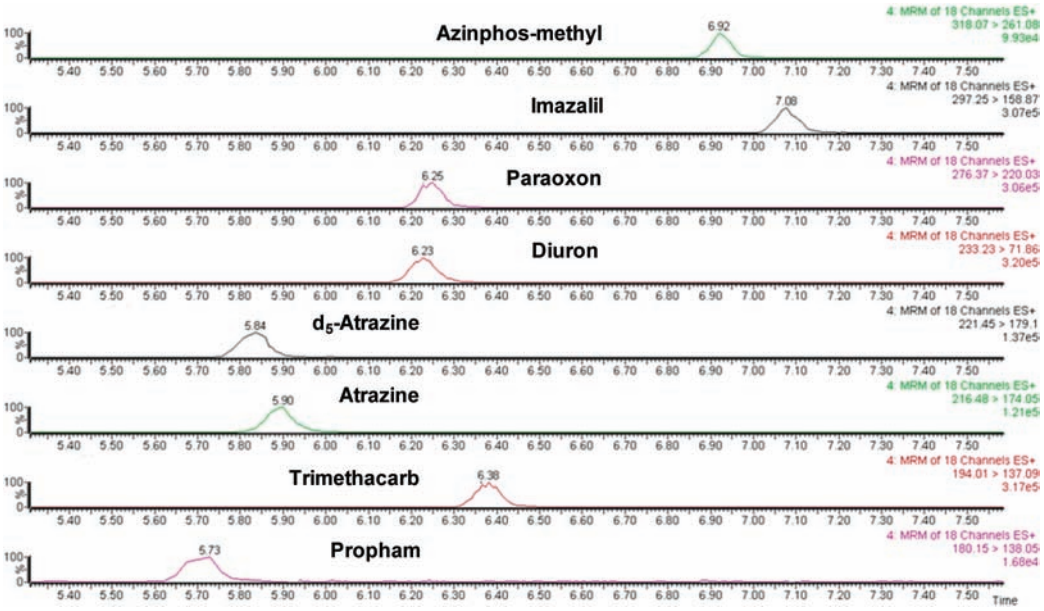

Figure 1. UPLC-MS/MS extracted ion chromatograms of selected pesticides spiked at 25 ng/g in wheat extract, which was obtained by the optimized sample preparation method.

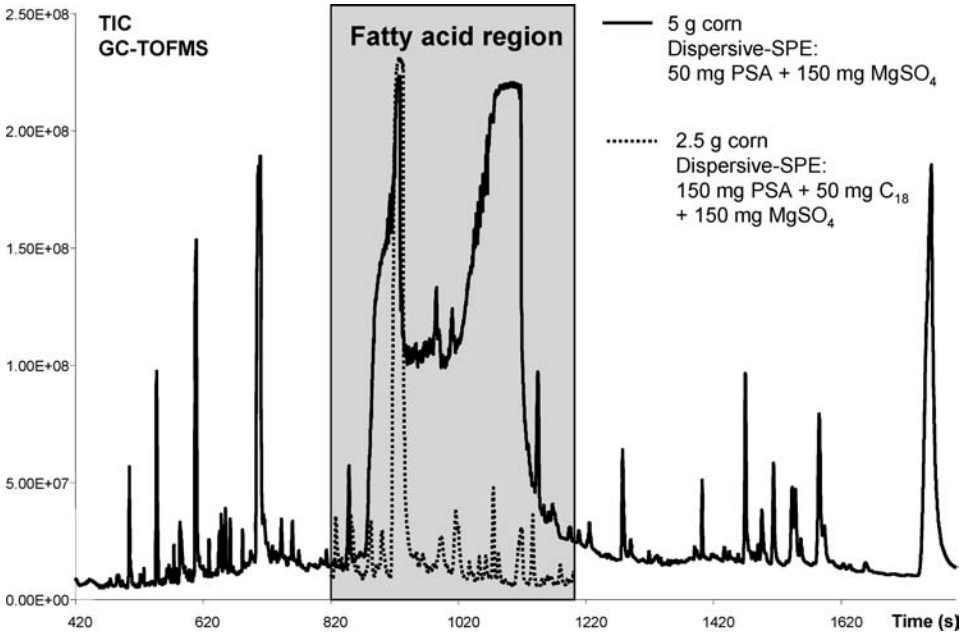

Figure 2. Total ion chromatogram obtained by a DSI-LVI-GC-TOFMS analysis of a corn extract prepared using 5 g of sample, original QuEChERS (with 10 mL of water addition for swelling), and 50 mg of PSA in the dispersive SPE step. The highlighted region of the chromatogram is saturated with fatty acids (mainly linoleic acid), with the dotted trace representing optimized analysis using 2.5 g of corn sample and conducting the dispersive SPE with 150 mg of PSA and 50 mg of C_{18}.

insecticides (e.g., acephate, methamidophos, omethoate, and dimethoate), imidazole fungicides (imazalil and thiabendazole), and even pyrethroid insecticides (e.g., permethrin, deltamethrin, resmethrin, and τ-fluvalinate). The latter group represents less volatile analytes that elute late in the GC analysis and are more problematic in terms of the transfer from the microvial into the GC column. The first two groups include compounds that are susceptible to matrix effects in GC (*20*, *21*).

At the time of the validation, the LC-MS/MS method included 64 analytes, 3 isotopically labeled internal standards, and 1 QC standard (see **Table 2**). Triphenyl phosphate (TPP) was initially chosen as the QC standard (added to the final extract before GC- and LC-MS analysis) because of its strong signal in both GC- and LC-MS and similarity to organophosporus pesticides. However, TPP was detected in all method and solvent blanks by LC-MS. The source of the contamination was not identified. However, given that TPP is a plasticizer, it is possible that the contamination is due to the use of disposable plastic consumables in the laboratory. We chose to replace TPP with [$^{13}C_6$]-*trans*-permethrin as the QC standard because of its availability as a labeled pesticide amenable to both GC- and LC-MS techniques.

The trend of including more analytes into the LC-MS/MS method is likely to continue because most pesticides (except for nonpolar, halogenated hydrocarbons) generally give better or

similar results in LC-MS/MS as compared to GC-MS (*22*). Furthermore, simultaneous analysis by GC-MS and LC-MS/MS provides confirmatory information for the analytes that are amenable to both of these techniques.

The LC-MS/MS analysis employed the UPLC technique using a short, narrow column with 1.7 μm particles for fast yet efficient separation. The method development involved tuning for analyte-specific MS/MS conditions (shown in **Table 2**) and optimization of analyte separation. **Figure 1** shows an example of extracted ion chromatograms of several pesticides spiked at 25 ng/g in a wheat extract, which was obtained by the optimized sample preparation procedure.

Sample Preparation Method Development and Optimization. For sample preparation of cereal grains, our goal was to adapt the QuEChERS method, which was originally developed for the analysis of fruits and vegetables. In brief, the original QuEChERS method (*4*) is based on extraction of a homogenized produce sample (10 g) with MeCN (10 mL). A combination of anhydrous $MgSO_4$ (4 g) and NaCl (1 g) is added to induce separation between the MeCN and aqueous layers (the water originates from the produce sample). The sample is shaken in a tube for 1 min and centrifuged. An aliquot (1 mL) of the upper MeCN layer is cleaned up using dispersive SPE with PSA (25 mg) and anhydrous $MgSO_4$ (150 mg). After brief vortexing/shaking (30 s) and centrifugation, the extract is ready for GC- and LC-MS(/MS) analyses. As opposed to the original method, the buffered AOAC method uses MeCN with 1% acetic acid for sample extraction and sodium acetate instead of NaCl in the salt mixture (*5*). Also, a double amount of PSA (50 mg) is added to the extract aliquot (1 mL) in the dispersive SPE, in part because the presence of acetic acid reduces the PSA capacity.

In comparison with fruits and vegetables, cereal grains represent dry matrices with a high content of fatty acids, which can interfere mainly in the GC-MS analysis. Therefore, several modifications had to be made to accommodate various cereal grain matrices and provide good analytical results for the majority of the target pesticides. These modifications involved mainly optimization of matrix swelling (addition of water), sample to solvent ratio, extraction time, and the sorbent combination and amount in the dispersive SPE cleanup.

As for the sample to solvent ratio, we started our optimization experiments with 5 g of sample (*23*), to which we added 10 mL of water and let the matrix swell for 1 h. After that, the original QuEChERS procedure was followed. For finely milled rice, 15 mL of water per 5 g of sample was necessary for effective swelling. Later, we optimized the sample amount for individual grains (see below) and also found that MeCN (10 mL) should be added to the sample at the same time as water, followed by shaking of the sample for 1 h to facilitate matrix swelling/extraction and improve analyte recoveries. The addition of MeCN can prevent enzymatic degradation of malathion and some other susceptible pesticides during the matrix swelling process (*24*).

The 5 g sample size seemed to be acceptable for wheat and rice, but it posed a problem for corn (and to a lesser extent for oat). **Figure 2** shows a total ion chromatogram (TIC) of a corn extract prepared using 5 g of sample, original QuEChERS (with 10 mL of water addition for swelling), and 50 mg of PSA in the dispersive SPE step. The middle, highlighted, region of the chromatogram is saturated with fatty acids (mainly linoleic acid but also oleic and palmitic acids). To improve the situation, we tested different amounts of PSA (50−200 mg) and also added 50 mg of C_{18}, which was previously demonstrated to be beneficial for samples with a higher fat content, such as milk, eggs, or avocado (*25*). Up to 150 mg per 1 mL of extract was acceptable in terms of analyte

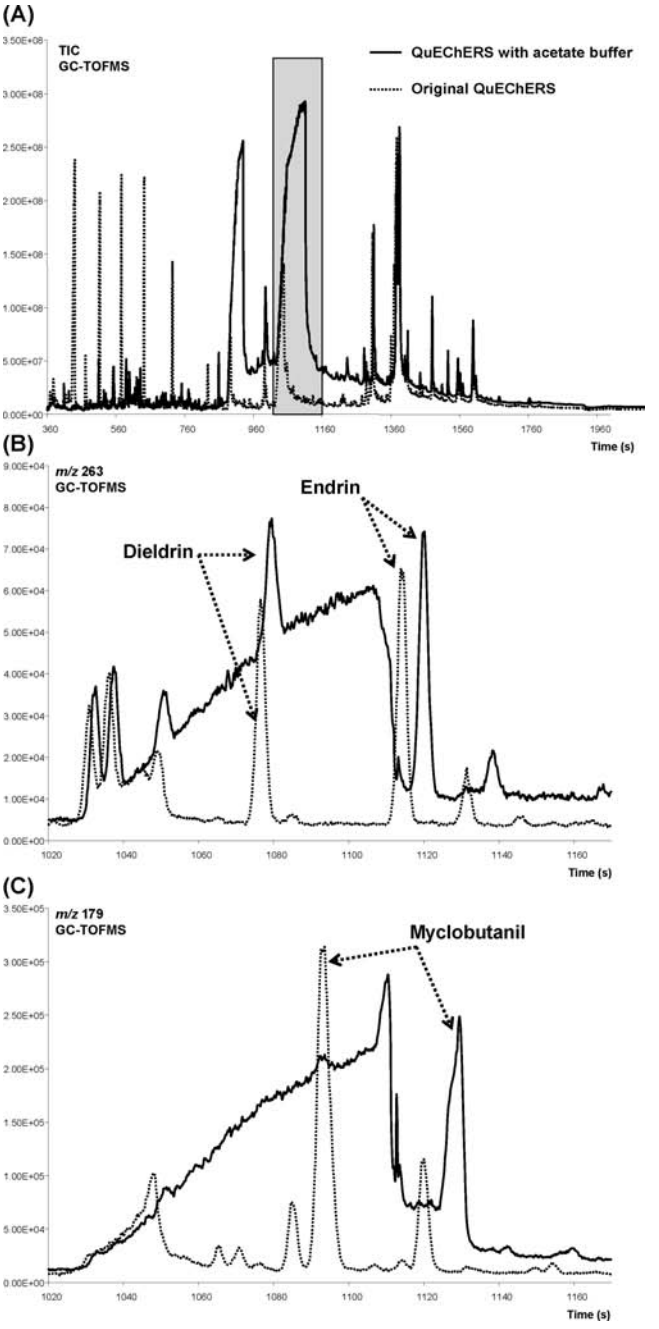

Figure 3. Overlays of (**A**) total ion GC-TOFMS chromatograms of spiked corn extracts obtained using 2.5 g of sample and original and buffered QuEChERS procedures, both with 150 mg of PSA and 50 mg of C_{18} in the dispersive SPE step. Chromatograms in **B** and **C** represent extracted ion chromatograms of *m/z* 263 and 179, respectively, showing selected pesticide peaks eluting in the region highlighted in (**A**).

recoveries and volume of the final extract available for the GC- and LC-MS analysis. For corn, however, we had to also decrease the sample size to 2.5 g (for oat to 3.5 g) to obtain good analyte peak shapes and rugged method performance. The dotted trace in **Figure 2** shows the TIC in the affected region after the original QuEChERS method was optimized for the corn analysis.

We also tried the buffered AOAC method but, even with the increased amount of PSA to 150 mg (and addition of 50 mg of C_{18}), the amount of fatty acids left in the final extract was overwhelming due to the reduced capacity of PSA in the presence of acetic acid. **Figure 3A** provides comparison of the TICs of spiked corn extracts obtained using 2.5 g of sample and original

Table 3. Pesticide Recoveries and RSDs in Cereal Grains Summarized (i) for the Four Cereal Grain Commodities Based on Different Concentration Levels (Expressed as Concentrations in the Cereal Grain Extracts) and (ii) for the Three Concentration Levels Based on Different Cereal Grain Commodities[a]

		% recovery (% RSD)													
		concentration level, n = 8 (all commodities)						commodity, n = 6 (all concentrations)							
		high		middle		low		wheat		rice		oat		corn	
analyte	method	(125 ng/mL)		(50 ng/mL)		(12.5 ng/mL)		(25−250 ng/g)		(25−250 ng/g)		(36−357 ng/g)		(50−500 ng/g)	
acephate	LC	82	(7)	**66**	(5)	**60**	(13)	**67**	(24)	71	(14)	73	(13)	**67**	(13)
acetamiprid	LC	112	(3)	93	(8)	84	(8)	93	(16)	103	(10)	97	(15)	92	(15)
alachlor	GC	99	(10)	99	(11)	108	(8)	109	(8)	97	(4)	105	(10)	96	(11)
aldicarb	LC	116	(5)	93	(8)	83	(9)	99	(20)	98	(20)	96	(16)	98	(11)
aldicarb sulfone	LC	112	(7)	90	(11)	79	(12)	97	(19)	96	(15)	90	(19)	91	(21)
aldicarb sulfoxide	LC	98	(6)	79	(5)	**69**	(7)	83	(22)	83	(16)	81	(16)	80	(13)
aldrin	GC	75	(5)	70	(14)	70	(24)	**64**	(21)	81	(13)	73	(12)	71	(9)
ametryn	LC	103	(4)	101	(8)	98	(5)	97	(5)	107	(3)	100	(7)	100	(4)
amitraz	LC	**69**	(16)	**59**	(14)	**51**	(18)	51	(15)	72	(15)	**56**	(17)	**58**	(15)
atrazine	GC	101	(13)	95	(15)	104	(17)	110	(15)	97	(19)	94	(9)	98	(9)
azinphos-ethyl	LC	117	(3)	95	(9)	84	(9)	96	(17)	108	(10)	96	(18)	95	(16)
azinphos-methyl	LC	116	(8)	93	(13)	87	(17)	96	(23)	109	(15)	87	(20)	102	(6)
azoxystrobin	LC	120	(8)	98	(7)	88	(11)	100	(19)	111	(11)	99	(18)	97	(15)
BHC, α-	GC	94	(14)	95	(13)	89	(21)	95	(18)	90	(12)	91	(21)	94	(14)
BHC, β-	GC	98	(6)	94	(8)	89	(19)	93	(15)	99	(9)	89	(17)	95	(8)
BHC, δ-	GC	89	(15)	88	(13)	93	(16)	91	(17)	87	(15)	91	(17)	90	(11)
bifenox	GC	99	(12)	89	(10)	89	(14)	87	(10)	105	(7)	87	(9)	97	(13)
bitertanol	LC	103	(5)	101	(7)	97	(9)	97	(5)	108	(6)	99	(6)	98	(8)
bromacil	GC	99	(12)	97	(9)	110	(20)	98	(13)	114	(19)	100	(6)	96	(14)
bromophos	GC	94	(18)	91	(20)	86	(16)	88	(19)	92	(14)	93	(20)	91	(20)
bromopropylate	GC	104	(10)	90	(9)	102	(13)	92	(11)	101	(12)	100	(2)	102	(15)
carbaryl	LC	116	(4)	95	(7)	87	(7)	98	(18)	106	(11)	97	(14)	96	(13)
carbofuran	LC	118	(5)	95	(6)	87	(7)	100	(19)	107	(12)	98	(14)	95	(14)
carbofuran, 3-hydroxy-	LC	114	(4)	91	(8)	84	(6)	96	(18)	101	(14)	95	(15)	92	(14)
carbophenothion	GC	97	(9)	89	(10)	93	(11)	88	(15)	100	(9)	92	(6)	93	(9)
chlordane, *cis*-	GC	88	(9)	82	(10)	84	(18)	78	(18)	92	(9)	84	(10)	85	(10)
chlordane, *trans*-	GC	85	(9)	81	(9)	81	(13)	76	(14)	89	(9)	81	(7)	83	(7)
chlordimeform	GC	99	(16)	118	(27)	100	39	118	(27)	97	(6)	108	(28)	100	(33)
chlorfenson	GC	101	(11)	98	(10)	101	(10)	99	(16)	103	(7)	99	(6)	99	(10)
chlorfenvinphos, *cis*-	GC	106	(13)	94	(11)	ND	ND	110	(16)	96	(15)	92	(11)	100	(7)
chlorfenvinphos, *trans*-	GC	110	(9)	94	(12)	109	(12)	110	(12)	96	(14)	102	(8)	109	(12)
chlorobenzilate	GC	108	(10)	96	(8)	100	(9)	98	(14)	107	(10)	104	(7)	98	(9)
chloroneb	GC	100	(13)	105	(19)	86	(20)	101	(20)	93	(13)	93	(29)	99	(11)
chloropropylate	GC	101	(12)	90	(12)	94	(9)	96	(13)	94	(16)	90	(10)	99	(7)
chlorothalonil	GC	**66**	(13)	ND	ND	ND	ND	**62**	(19)	**63**	(27)	**68**	(0)	**69**	(6)
chloroxuron	LC	112	(5)	92	(5)	84	(8)	96	(19)	98	(9)	96	(15)	94	(14)
chlorpropham	GC	99	(8)	111	(17)	87	(13)	108	(8)	100	(10)	98	(29)	96	(9)
chlorpyrifos	GC	96	(6)	90	(6)	91	(9)	89	(12)	94	(5)	97	(5)	90	(6)
chlorpyrifos-methyl	GC	98	(15)	97	(16)	91	(11)	99	(18)	96	(9)	95	(19)	93	(12)
chlorthal-dimethyl	GC	100	(5)	95	(8)	95	(10)	92	(10)	100	(7)	100	(5)	96	(7)
cinerin I	GC	90	(19)	101	(28)	ND	ND	100	(20)	108	(32)	79	(14)	93	(9)
coumaphos	GC	106	(11)	108	(19)	113	(10)	114	(7)	102	(22)	102	(15)	119	(10)
cyanazine	LC	108	(6)	101	(8)	102	(5)	100	(6)	108	(3)	103	(9)	103	(8)
cyfluthrin	GC	87	(27)	87	(14)	ND	ND	95	(21)	97	(27)	85	(21)	79	(16)
cyhalothrin	GC	101	(19)	93	(13)	87	(19)	97	(13)	97	(5)	100	(29)	91	(12)
cypermethrin	GC	93	(24)	91	(13)	ND	ND	108	(21)	92	(5)	91	(25)	82	(16)
cyprodinil	GC	91	(13)	90	(18)	90	(6)	88	(6)	94	(19)	85	(13)	94	(5)
DDD, *o,p′*-	GC	92	(6)	90	(12)	82	(13)	81	(16)	93	(9)	90	(4)	89	(11)
DDD, *p,p′*-	GC	83	(12)	83	(14)	77	(11)	76	(9)	83	(17)	81	(8)	85	(11)
DDE, *o,p′*-	GC	78	(18)	**69**	(20)	76	(7)	**67**	(15)	77	(22)	71	(13)	81	(4)
DDE, *p,p′*-	GC	81	(24)	**65**	(25)	70	(14)	**62**	(20)	77	(28)	67	(17)	78	(17)
DDT, *o,p′*-	GC	79	(9)	74	(10)	89	(25)	70	(12)	88	(9)	77	(4)	86	(25)
DDT, *p,p′*-	GC	75	(12)	73	(12)	75	(12)	71	(15)	76	(13)	73	(13)	77	(7)
deltamethrin	LC	99	(9)	90	(13)	100	(20)	90	(16)	103	(13)	92	(20)	101	(9)
demeton-O	GC	97	(16)	97	(27)	93	(22)	91	(18)	99	(15)	97	(32)	95	(19)
demeton-S	GC	111	(11)	100	(15)	101	(8)	109	(9)	101	(8)	107	(10)	101	(18)
diazinon	GC	100	(12)	93	(11)	89	(26)	94	(20)	108	(18)	91	(15)	88	(6)
dichlobenil	GC	94	(16)	93	(28)	88	(24)	89	(17)	94	(18)	94	37	90	(14)
dichlorobenzophenone	GC	97	(9)	86	(7)	87	(14)	87	(19)	97	(11)	89	(6)	90	(6)
dichlorvos	LC	97	(11)	86	(10)	81	(17)	84	(14)	102	(9)	84	(14)	82	(11)
diclofop-methyl	GC	102	(9)	97	(8)	94	(9)	96	(10)	100	(11)	100	(7)	97	(10)
dicloran	GC	106	(9)	105	(11)	102	(18)	110	(11)	108	(5)	101	(13)	103	(12)
dicrotophos	LC	98	(5)	94	(8)	94	(5)	92	(7)	98	(4)	97	(7)	95	(7)

Table 3. Continued

		% recovery (% RSD)													
		concentration level, n = 8 (all commodities)						commodity, n = 6 (all concentrations)							
		high		middle		low		wheat		rice		oat		corn	
analyte	method	(125 ng/mL)		(50 ng/mL)		(12.5 ng/mL)		(25−250 ng/g)		(25−250 ng/g)		(36−357 ng/g)		(50−500 ng/g)	
dieldrin	GC	86	(14)	86	(8)	86	(18)	79	(17)	92	(9)	83	(17)	89	(9)
dimethoate	LC	116	(4)	91	(5)	82	(9)	96	(20)	103	(14)	97	(14)	91	(18)
dioxathion	GC	99	(15)	104	(14)	94	(23)	104	(19)	87	(10)	101	(18)	103	(15)
diphenamid	GC	105	(10)	100	(9)	102	(9)	102	(13)	105	(9)	103	(6)	99	(9)
diphenylamine	GC	99	(12)	97	(13)	87	(20)	96	(20)	98	(8)	83	(19)	99	(10)
disulfoton	GC	97	(17)	87	(16)	95	(14)	93	(12)	89	(24)	89	(11)	101	(10)
diuron	LC	112	(4)	90	(5)	82	(8)	96	(17)	97	(16)	93	(15)	91	(15)
endosulfan α	GC	92	(15)	90	(8)	78	(23)	87	(12)	91	(8)	104	(16)	84	(6)
endosulfan β	GC	90	(14)	89	(9)	85	(5)	93	(15)	87	(7)	93	(11)	83	(8)
endosulfan sulfate	GC	81	(20)	88	(19)	92	43	81	(14)	73	(8)	90	(30)	90	(28)
endrin	GC	83	(23)	86	(12)	89	(17)	84	(20)	89	(11)	78	(18)	91	(20)
endrin ketone	GC	94	(22)	93	(10)	95	(10)	102	(12)	89	(12)	97	(19)	89	(13)
EPN	GC	88	(13)	100	(12)	94	(9)	89	(11)	100	(15)	93	(12)	95	(8)
ethalfluralin	GC	103	(10)	97	(11)	83	(10)	92	(9)	98	(12)	96	(22)	95	(10)
ethion	GC	105	(12)	95	(17)	96	(11)	97	(18)	103	(9)	104	(11)	94	(17)
ethoprophos	GC	104	(10)	98	(13)	97	(11)	103	(14)	104	(7)	98	(14)	96	(9)
ethoxyquin	GC	114	(37)	107	(28)	99	(16)	77	(24)	**146**	(27)	113	(31)	116	(12)
etridiazole	GC	93	(17)	92	(20)	101	(29)	94	(17)	98	(27)	93	(30)	93	(11)
fenamiphos	GC	121	(13)	96	(14)	104	(18)	116	(22)	102	(21)	106	(9)	103	(14)
fenarimol	GC	100	(13)	93	(13)	96	(8)	100	(7)	93	(15)	94	(14)	97	(11)
fenchlorphos	GC	95	(18)	93	(16)	87	(13)	89	(16)	93	(11)	93	(16)	93	(22)
fenitrothion	GC	109	(16)	105	(20)	104	(9)	110	(21)	100	(6)	113	(16)	103	(14)
fenobucarb	LC	117	(4)	96	(7)	86	(12)	98	(18)	106	(12)	98	(15)	95	(17)
fenpropathrin	GC	94	(10)	94	(8)	93	(11)	92	(14)	98	(7)	97	(6)	89	(8)
fensulfothion	LC	107	(7)	100	(7)	101	(5)	100	(6)	109	(5)	102	(7)	100	(6)
fenthion	GC	103	(12)	98	(12)	95	(9)	101	(16)	101	(6)	100	(8)	95	(13)
fenvalerate	GC	82	(26)	104	(10)	ND	ND	93	(25)	93	(7)	108	(24)	80	(20)
fluazifop-*p*-butyl	GC	109	(13)	98	(8)	101	(12)	100	(18)	109	(15)	106	(5)	99	(7)
fluvalinate, τ-	LC	100	(11)	97	(11)	89	(41)	76	(30)	118	(16)	98	(13)	91	(15)
fonofos	GC	99	(6)	97	(11)	92	(17)	96	(12)	99	(8)	92	(15)	97	(11)
heptachlor	GC	79	(18)	77	(23)	85	(11)	75	(3)	85	(26)	73	(13)	86	(9)
heptachlor epoxide	GC	87	(14)	86	(18)	89	(8)	84	(7)	91	(19)	80	(13)	93	(3)
hexachlorobenzene	GC	**62**	(16)	**62**	(19)	**58**	(26)	**51**	(16)	**66**	(17)	**57**	(26)	**67**	(13)
imazalil	LC	98	(8)	82	(9)	75	(15)	87	(16)	93	(14)	78	(17)	83	(11)
imidacloprid	LC	114	(8)	90	(15)	88	(15)	99	(23)	104	(12)	93	(19)	94	(15)
iprodione degradation product	GC	102	(18)	98	(13)	94	(13)	105	(13)	91	(9)	118	(6)	89	(13)
isazophos	GC	105	(9)	98	(8)	100	(10)	106	(11)	105	(8)	99	(9)	96	(6)
isofenphos	GC	107	(13)	99	(8)	104	(10)	104	(16)	108	(11)	105	(5)	99	(10)
leptophos	GC	78	(22)	83	(20)	72	(10)	75	(22)	74	(10)	80	(25)	79	(19)
lindane	GC	94	(11)	90	(10)	90	(18)	93	(14)	93	(17)	89	(16)	91	(8)
linuron	LC	116	(6)	94	(6)	83	(12)	94	(25)	103	(12)	97	(15)	98	(15)
malathion	LC	**122**	(7)	95	(7)	91	(8)	103	(17)	110	(12)	99	(19)	98	(15)
metalaxyl	GC	101	(13)	110	(18)	95	(14)	105	(15)	113	(12)	104	(14)	87	(12)
methamidophos	LC	79	(8)	**64**	(5)	**54**	(10)	**65**	(25)	**64**	(21)	**69**	(13)	**65**	(12)
methidathion	LC	107	(5)	103	(7)	107	(9)	103	(8)	112	(4)	103	(6)	105	(9)
methiocarb	LC	117	(6)	94	(8)	83	(14)	99	(19)	108	(10)	95	(18)	91	(20)
methomyl	LC	114	(6)	92	(8)	86	(5)	98	(18)	100	(10)	97	(16)	95	(15)
methoxychlor	GC	95	(9)	88	(11)	94	(13)	86	(16)	99	(9)	95	(6)	91	(8)
metolachlor	GC	103	(12)	98	(9)	104	(7)	104	(12)	105	(7)	107	(5)	95	(10)
metribuzin	GC	102	(9)	98	(8)	105	(10)	101	(8)	105	(9)	105	(7)	97	(12)
mevinphos	GC	99	(31)	107	(23)	ND	ND	107	(22)	99	(13)	97	51	106	(24)
mirex	GC	**62**	(10)	**59**	(12)	**60**	(17)	**52**	(15)	70	(6)	**60**	(4)	**60**	(9)
monocrotophos	LC	96	(5)	92	(6)	95	(5)	95	(6)	95	(5)	95	(6)	92	(6)
myclobutanil	LC	106	(6)	101	(8)	101	(6)	102	(6)	110	(6)	101	(4)	99	(8)
napropamide	LC	105	(5)	102	(7)	101	(4)	100	(5)	108	(4)	103	(4)	102	(8)
nitrapyrin	GC	97	(17)	95	(21)	102	(24)	93	(15)	101	(25)	97	(27)	100	(17)
norflurazon	LC	106	(5)	104	(9)	102	(7)	101	(5)	112	(5)	103	(5)	101	(8)
omethoate	LC	91	(6)	75	(5)	**64**	(8)	78	(21)	77	(15)	77	(16)	75	(15)
oxadiazon	GC	100	(13)	89	(15)	92	(9)	92	(12)	93	(17)	92	(12)	96	(13)
oxamyl	LC	113	(5)	91	(6)	80	(5)	96	(21)	98	(13)	92	(16)	93	(15)
oxyfluorfen	GC	94	(17)	91	(14)	87	(10)	83	(7)	91	(17)	89	(14)	98	(11)
paraoxon	LC	117	(5)	95	(6)	86	(7)	99	(18)	106	(13)	97	(16)	95	(13)
parathion	GC	100	(8)	101	(6)	99	(11)	102	(6)	100	(11)	100	(10)	99	(2)
parathion-methyl	GC	105	(14)	104	(14)	107	(19)	122	(12)	100	(17)	101	(10)	99	(11)

Table 3. Continued

		% recovery (% RSD)													
		concentration level, n = 8 (all commodities)						commodity, n = 6 (all concentrations)							
		high		middle		low		wheat		rice		oat		corn	
analyte	method	(125 ng/mL)		(50 ng/mL)		(12.5 ng/mL)		(25−250 ng/g)		(25−250 ng/g)		(36−357 ng/g)		(50−500 ng/g)	
pendimethalin	GC	104	(11)	91	(10)	93	(12)	90	(11)	112	(14)	94	(8)	97	(8)
pentachloronitrobenzene	GC	84	(5)	83	(15)	83	(16)	75	(8)	89	(12)	83	(15)	85	(8)
permethrin	LC	92	(9)	90	(12)	91	(20)	88	(17)	92	(16)	90	(14)	94	(9)
perthane	GC	95	(9)	88	(9)	90	(14)	84	(13)	98	(12)	91	(7)	91	(9)
phorate	GC	99	(10)	98	(11)	85	(23)	89	(28)	100	(7)	92	(18)	95	(8)
phosalone	LC	107	(7)	102	(8)	102	(7)	98	(6)	112	(4)	102	(6)	102	(6)
phosmet	LC	108	(9)	90	(9)	80	(10)	91	(21)	103	(12)	90	(12)	87	(12)
phosphamidon	GC	93	(13)	**125**	(30)	ND	ND	**131**	(30)	87	(8)	**127**	(24)	91	(17)
phthalimide	GC	86	(22)	79	(14)	80	(21)	84	(26)	88	(12)	**67**	(18)	85	(14)
piperalin	LC	**35**	(13)	**35**	(17)	**41**	(25)	**36**	(31)	**43**	(14)	**32**	(12)	**36**	(9)
piperonyl butoxide	GC	99	(12)	91	(21)	94	(9)	97	(7)	97	(23)	91	(13)	92	(14)
pirimicarb	GC	106	(10)	99	(10)	99	(8)	105	(13)	103	(10)	100	(8)	99	(9)
pirimiphos-ethyl	GC	107	(12)	92	(8)	97	(10)	100	(18)	101	(11)	102	(9)	96	(11)
pirimiphos-methyl	GC	103	(11)	99	(16)	100	(9)	101	(15)	105	(7)	104	(13)	95	(11)
procymidone	GC	105	(17)	104	(8)	103	(9)	109	(18)	108	(8)	101	(11)	100	(8)
profenofos	LC	97	(16)	95	(7)	96	(5)	91	(8)	104	(5)	93	(13)	97	(9)
profluralin	GC	108	(10)	93	(10)	89	(10)	100	(18)	98	(14)	99	(13)	94	(9)
promecarb	LC	117	(4)	96	(7)	87	(11)	98	(19)	109	(9)	98	(15)	96	(15)
prometryn	LC	105	(9)	99	(7)	98	(6)	97	(6)	110	(7)	98	(5)	99	(6)
propanil	GC	97	(12)	101	(8)	97	(7)	101	(12)	98	(6)	101	(6)	94	(11)
propham	LC	117	(5)	99	(8)	87	(15)	99	(13)	109	(10)	97	(20)	99	(18)
propiconazole	LC	112	(5)	91	(10)	84	(15)	90	(22)	106	(10)	91	(16)	96	(14)
propoxur	LC	118	(5)	95	(6)	89	(6)	99	(19)	107	(12)	99	(14)	97	(12)
propyzamide	GC	102	(16)	95	(15)	97	(11)	105	(13)	103	(9)	95	(12)	91	(21)
pyrethrin I	GC	104	(17)	92	(5)	ND	ND	98	(2)	110	(8)	90	(12)	107	(19)
quinalphos	GC	103	(8)	95	(10)	101	(18)	106	(18)	98	(12)	97	(10)	98	(9)
quizalofop-ethyl	LC	105	(11)	101	(8)	97	(7)	94	(6)	109	(6)	103	(12)	98	(7)
resmethrin	LC	98	(7)	89	(8)	94	(10)	85	(8)	98	(5)	92	(4)	99	(9)
simazine	LC	105	(6)	102	(7)	100	(4)	99	(3)	106	(4)	104	(7)	100	(6)
simetryn	GC	102	(9)	99	(9)	102	(14)	99	(14)	104	(10)	104	(8)	97	(10)
spinosad A	LC	87	(6)	71	(6)	**67**	(8)	73	(18)	81	(11)	74	(13)	73	(8)
spinosad D	LC	82	(5)	**67**	(5)	**64**	(8)	**68**	(17)	73	(13)	70	(14)	71	(8)
sulfallate	GC	96	(18)	86	(23)	91	(24)	79	(23)	95	(27)	85	(11)	101	(13)
sulprofos	GC	97	(9)	84	(8)	88	(15)	87	(14)	98	(11)	83	(15)	92	(5)
terbacil	GC	103	(10)	93	(14)	101	(16)	110	(12)	101	(8)	93	(14)	90	(14)
terbufos	GC	101	(11)	95	(10)	86	(15)	94	(20)	99	(11)	88	(15)	97	(7)
tetrachlorvinphos	LC	106	(9)	104	(9)	102	(5)	99	(6)	111	(8)	102	(8)	103	(6)
tetradifon	GC	93	(9)	91	(9)	89	(11)	89	(15)	94	(7)	92	(7)	91	(8)
tetrahydrophthalimide	GC	104	(20)	83	(10)	74	(33)	99	(32)	94	(15)	87	(9)	80	(28)
thiabendazole	LC	97	(7)	80	(6)	74	(8)	84	(16)	91	(12)	81	(12)	78	(14)
thiobencarb	LC	111	(6)	91	(10)	83	(7)	94	(15)	103	(14)	90	(14)	93	(17)
thionazin	GC	104	(12)	102	(13)	92	(14)	104	(15)	101	(3)	101	(17)	96	(13)
tolylfluanid	LC	**2**	(68)	**2**	(100)	**23**	(245)	**38**	(193)	**3**	(70)	**1**	(28)	**2**	(63)
triadimefon	GC	104	(14)	99	(9)	101	(11)	103	(17)	107	(10)	98	(10)	99	(7)
tribufos (DEF)	GC	92	(16)	79	(16)	81	(7)	80	(16)	84	(20)	82	(11)	88	(15)
trifloxystrobin	LC	120	(6)	96	(7)	89	(11)	99	(21)	110	(10)	99	(16)	98	(14)
trifluralin	GC	102	(6)	94	(12)	86	(14)	94	(10)	98	(10)	91	(21)	95	(9)
trimethacarb	LC	115	(6)	96	(11)	85	(11)	96	(18)	106	(14)	96	(15)	95	(17)
vinclozolin	GC	103	(12)	100	(10)	91	(7)	105	(13)	98	(11)	99	(11)	93	(7)

[a] Recoveries <70% or >120% are highlighted in bold. The method indicates which technique ("GC" for the DSI-LVI-GC-TOFMS and "LC" for the UPLC-MS/MS) was used for the determination ("ND" means not determined).

and buffered QuEChERS procedures, both with 150 mg of PSA and 50 mg of C_{18} in the dispersive SPE step. Panels **B** and **C** of **Figure 3** show comparison of selected pesticide peaks eluting in the region highlighted in **Figure 3A**, which mainly represents elution of a large amount of linoleic acid in the corn extract obtained with the buffered procedure. The peaks eluting in the affected region show apparent retention time shifts, peak shape distortions, and also reduced signal intensity due to the detector saturation with the large amount of fatty acids. For the above reasons, we do not recommend using the QuEChERS procedure with acetate buffer for cereal grains and other samples with a higher content of fatty acids.

The optimized procedure for cereals is summarized under Materials and Methods. After the dispersive SPE, centrifugation, and addition of a QC solution, an aliquot of the final extract can be directly injected into the GC-MS system. Filtration is not necessary because any potential particles will remain in the DSI microvial. For UPLC-MS/MS, filtration is highly recommended to prevent column clogging. For fast and convenient filtration, we employed Mini-UniPrep syringeless filters for the filtration of the

final extracts instead of syringe filters (*26*). The syringeless filters consist of two parts: a chamber and a filter plunger that together form an autosampler vial that can be used for sample storage and for sample introduction using common autosamplers.

Method Validation. The analyte recoveries and RSDs obtained in the method validation are summarized on the basis of concentration levels and cereal grain commodities in **Table 3**. **Figure 4** shows distribution of the recoveries and RSDs obtained in different cereal grains, demonstrating that recoveries in the range of 70−120% were obtained for 93−97% of the analytes, with RSDs being < 20% for 82−94% of the compounds in **Table 3**. The problematic analytes can be generally divided into four groups based on the following issues: degradation, high lipophilicity, high affinity to PSA, and low signal intensity.

The first group comprises pesticides that are susceptible to degradation, including mainly analytes that are well-known to degrade in MeCN at neutral/basic conditions, such as *N*-trihalomethylthio fungicides (captan, captafol, folpet, or tolylfluanid), chlorothalonil, and dicofol (*15*). Amitraz (recoveries of 51−72%) is another pesticide with degradation issues. The buffered procedure would probably provide somewhat better results for some of these analytes (*5*). We monitored degradation products for most of them as an indication of a potential application on crops. It should be noted that captan is the only pesticide from this group that has a tolerance set in cereal grains in the United States (*27*).

Highly lipophilic pesticides tend to give lower recoveries in the QuEChERS method applied to matrices with a higher amount of fat (*11*, *25*, *28*, *29*) because fat is practically not dissolved in MeCN and forms an additional layer. This leads to partition of lipophilic pesticides between MeCN and fat/oil layers, the extent of which depends mainly on the analyte lipophilicity and the amount of fat. In our case, the losses of lipophilic pesticides were not too significant because of the reduced sample to solvent ratio. The most lipophilic analyte on our list is hexachlorobenzene (HCB), which gave average recoveries in the range of 51−67%. Other lipophilic pesticides that gave recoveries of < 70% include mirex (52−70%), aldrin (64−81%), and DDE (62−81%).

The increase of the amount of PSA to 150 mg, which was necessary for effective removal of fatty acids, had a somewhat negative impact on acephate (recoveries in the range of 67−73%), methamidophos (64−69%), and spinosad (68−81%). Piperalin, a fungicide containing a carbonyl group, was the most affected analyte on our list, which gave significantly decreased recoveries (32−46%) due to the higher amount of PSA. Piperalin is applied exclusively on ornamentals; thus, we were willing to accept its lower recoveries.

In terms of sensitivity, the majority of the analytes could be quantified at or below our target lowest calibration level corresponding to 10 ng/g in the matrices. The most problematic analytes included on our original list were natural pyrethrins (cinerin, jasmolin, and pyrethrin), which did not give good responses in GC-TOFMS. Only results for cinerin I and pyrethrin I at higher concentration levels are provided in **Table 3**. To monitor a potential use of pyrethrins, we included piperonyl butoxide, a synergist of pyrethrins used in their formulations, which gave very good results in our method. Pyrethroid insecticides also did not yield best results at lower concentration levels in the GC-MS method. Therefore, several of them (deltamethrin, τ-fluvalinate, permethrin, and resmethrin) were included in the LC-MS/MS method before the method validation, and the rest of them are good candidates for future inclusion in an expanded UPLC-MS/MS method.

In summary, the developed method provided good results for the vast majority of the tested analytes and is currently being used for routine multiresidue analysis of pesticides in cereal grains and some other matrices with a higher content of fatty acids, such as flaxseed, peanuts, or various doughs (*30*). Furthermore, the optimized DSI-LVI-GC-TOFMS and UPLC-MS/MS conditions are also employed in the routine analysis of various fruit and vegetable extracts prepared by the buffered AOAC version of the QuEChERS method.

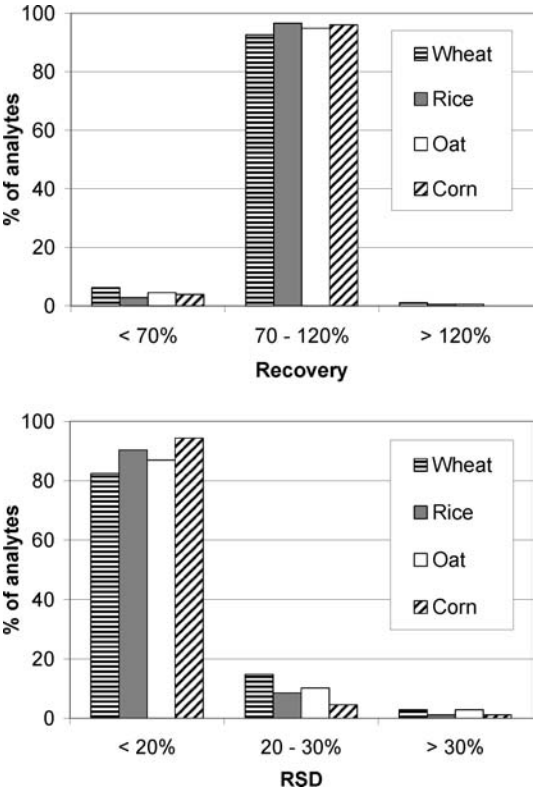

Figure 4. Distribution of recoveries and RSDs obtained in the method validation for the 176 tested pesticides in wheat, rice, oat, and corn.

Comparison of the New Method with the Traditional Approach. As compared to the traditional approach using the Luke method (combined with multiple GC runs, GC-MS confirmatory analysis of positive findings, and HPLC−fluorescence for carbamates), the new method based on the QuEChERS procedure combined with GC-TOFMS and UPLC-MS/MS has the following major benefits: (i) *reduced cost per sample* [about 40−50% reduction in the cost of disposable materials (solvents, SPE material, vials, etc.) and in the operational costs (instrument operation, service contracts, maintenance, data processing, etc.]; (ii) *reduced and less hazardous waste* (about 1:100 solvent waste reduction and no chlorinated solvents used); (iii) *increased sample throughput* (at least 3-fold increase in sample throughput); and (iv) *increased scope of analysis* (the new method enables analysis of many more pesticides, including mainly more polar analytes and compounds that cannot be detected by element-selective detectors in GC or by postcolumn derivatization in HPLC−fluorescence; furthermore, the GC-TOFMS analysis is not based on analyte-specific parameters; therefore, new analytes can be easily added to the list of target compounds and nontargeted screening with library matching is also possible).

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