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Determination of Multiresidues in Rapeseed, Rapeseed Oil, and Rapeseed Meal by Acetonitrile Extraction, Low-Temperature Cleanup, and Detection by Liquid Chromatography with Tandem Mass Spectrometry

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Supporting Information

ABSTRACT: A multiresidue method for determining pesticides in rapeseed, rapeseed oil, and rapeseed meal by use of liquid chromatography−tandem mass spectrometry is developed. Samples were extracted with acetonitrile or acidified acetonitrile and cleaned up by a 12 h freezing step. The recovery data were obtained by spiking blank samples at three concentration levels. The recoveries of 27 selected pesticides in rapeseed, rapeseed oil, and rapeseed meal were in the range of 70−118%, at the concentration level of 10 μg kg^{-1}, with intraday and interday precisions of lower than 22 and 27%, respectively. Linearity was studied between 2 and 500 μg L^{-1} with determination coefficients (R^2) of higher than 0.98 for all compounds in the three matrices. The limits of quantitation (LOQs) of pesticides in rapeseed, rapeseed oil, and rapeseed meal ranged from 0.3 to 18 μg kg^{-1}. The *n*-octanol−water partition coefficient showed more influence than water solubility in extracting pesticides by acetonitrile from matrices of high fat content. This method was successfully applied for routine analysis in commercial products.

KEYWORDS: *multiresidue, pesticide, rape, LC-MS/MS (QqQ)*

INTRODUCTION

Rape is one of the world's major oil crops. Rapeseed is a major oil commodity in China, where its production accounts for one-fourth of the world production.[1] The Yangtze Valley is the main producing area of rape in China, and the biggest rape planting belt in the world. The control of herbs, diseases, and pests in rape is a critical factor to increase the number and/or size of rapeseed and, thus, the resulting yield. In agricultural practice for rape fields, the use of insecticides, herbicides, and fungicides provides an unquestionable benefit for crop protection. However, pesticide residues may remain at the harvest stage, causing contamination of the rapeseeds used to produce rapeseed oil and rapeseed meal (as feed). Maximum pesticide residue levels (MRLs) have been set by the European Union (EU), the United States, Japan, and China for rapeseed. The MRLs of pesticides of interest in rapeseed[26−31] are listed in Table 1. However, only a few MRLs also have been set for rapeseed oil (crude and refined oil) and rapeseed meal. Therefore, it is necessary to monitor their residues regularly through multiresidue analytical methods that combine short analysis time, sufficient selectivity, and sensitivity. Pesticide residue determination in rapeseed and rapeseed oil is a very demanding task considering the inherent complexity of the matrix due to its high fat content. Methods applied to determine pesticide residues in fatty food often require a lot of steps and are very time-consuming. Also, crop varieties and different physicochemical properties of the target compounds make it difficult to develop analytical methodologies that could cover them in one method.

Many multiresidue procedures employing different cleanup techniques and a variety of detection methods have been reported for the determination of pesticide residues in oil, oilseeds, and oilfruits. The most common extraction technique used for oil was liquid−liquid extraction (LLE)[2,3] based on acetonitrile−hexane partitioning or oil−acetonitrile partitioning, followed by solid-phase extraction (SPE),[4,5] gel permeation chromatography (GPC),[8,9] or freezing[7,10−13] cleanup. Solid-phase microextraction (SPME),[14] matrix solid-phase dispersion (MSPD),[6,15−17] microwave-assisted extraction (MAE), atmospheric pressure microwave-assisted liquid−liquid extraction (APMAE),[18] and direct online reversed-phase liquid chromatography−gas chromatography analysis (RPLC-GC)[19] have also been proposed as extraction and/or cleanup. Recently, the use of Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) sample preparation has been validated and applied with success to diversified food types[16,20] including olives and olive oils.[21,22]

Capillary gas chromatography (GC) coupled with flame photometric (FPD)[3,5,12,18] electron capture (ECD),[9,11] thermoionic specific (TSD),[9] mass spectrometry (MSD),[7,9,10,14−16,21,23] and tandem mass spectrometry (MS/MS)[2,4,9,13,24] detections have been the technique for pesticide residue analysis in fruits and vegetables with high fat content. However, the number of polar and therefore non-GC-amenable

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Table 1. Partial Listing of the MRLs of Pesticides in Rapeseed

	MRL(mg/kg)					
pesticide	Codex	EU	China	USA	Japan	Australia
acetamiprid	–[a]	0.01[b]	–	–	–	–
acetochlor	–	0.2	0.2	–	–	–
atrazine	–	0.05[b]	–	–	–	0.02[b]
azoxystrobin	–	0.5	–	0.5	1	–
carbendazim	0.05[b]	0.1[b]	0.1	–	3	–
carboxin	–	0.1	–	–	0.03	–
chlorfenvinphos	–	0.05[b]	–	–	–	–
chlorpyrifos	–	0.05[b]	–	–	0.1	0.05[T,b,c,d]
clethodim	0.5	1	–	–	0.5	–
clomazone	–	0.02	–	–	0.02	–
diazinon	–	0.02[b]	–	–	0.1	–
dichlorvos	–	0.01[b]	–	–	0.1	0.1[T]
difenoconazole	0.05	0.5	–	–	0.02	–
diniconazole	–	0.05[b]	–	–	–	–
fenoxaprop-P-ehtyl	–	–	0.5	–	0.1	–
fluazifop-P-butyl	–	15	–	–	–	–
fluorochloridone	–	0.1[b]	–	–	–	–
haloxyfop-P-methyl	–	0.2	–	–	–	0.1
imidacloprid	0.05[b]	0.1	–	0.05	0.04	0.05[b]
metalaxy-M	–	0.1[b]	–	–	–	–
methidathion	0.1	0.05[b]	–	–	0.1	1[c]
metolachlor	–	0.1[b]	–	–	0.05	0.02[b]
oxadiazon	–	0.05[b]	–	–	–	–
paclobutrazol	–	0.02[b]	–	–	–	–
phoxim	–	0.02[b]	–	–	0.02	–
prochloraz	0.7	0.5	0.5	–	0.5	–
prometryn	–	–	–	–	–	–
propiconazole	0.02	0.1[b]	–	–	0.05	–
quizalofop-P-ethyl	–	–	–	–	1	0.02[b]
tebuconazole	0.5	0.5	–	–	0.05	0.3[T]
thiacloprid	–	0.3	–	–	–	–
thiamethoxam	–	0.05[b]	–	0.02	0.02	0.02[T,b]
triadimefon	–	0.2[b]	0.2	–	0.2	–
trichlorfon	–	0.1[b]	–	–	0.1	0.1[c]

[a] –, not mentioned. [b] At or about the limit of determination. [c] The MRL is set for oilseeds. [d] [T] indicates the MRL/EMRL is temporary, irrespective of the status of the ADI, until required information has been provided and evaluated.

pesticides used in oilseeds and oilfruits is increasing. This reason, along with requirements of sensitivity to detect lower pesticide residue concentrations, has prompted the need of using LC-MS for pesticide testing in fat matrices.[6,7,25] Moreover, LC coupled to tandem mass spectrometry (MS/MS)[2,15,16,20,21] is particularly useful for qualitative and quantitative purposes. Recently, the use of a triple-quadrupole (QqQ) analyzer[18,22] has been reported to determine multiclass pesticide residues in oil, oilseeds, and oilfruits with high acquisition speed, selectivity, and detectability.

In the current study, a simple extraction and analysis procedure for pesticide residues, which are registered in rape in China or have MRLs in China, the United States, the EU, Japan, and Australia, in rapeseed, rapeseed oil, and rapeseed meal was developed. In summary, a method was developed by using acetonitrile or acidified acetonitrile as the extract solvent to cover different matrices. The developed method involved simple solvent extraction followed by cleanup with a freezer at low temperature. The extract was then applied to LC-MS/MS (QqQ) for quantitative and qualitative analysis of 34 pesticides.

MATERIALS AND METHODS

Chemicals and Reagents. The standard pesticides were purchased from AEPI (Tianjin, China). The purity of the standard pesticides was from 93 to 99%. Stock standard solutions (1000 mg L^{-1}) for each pesticide were prepared in acetonitrle, except for carbendazim in (5% formic acid) methanol, and stored at −20 °C. Working standard solutions were prepared by dilution of the corresponding stock standard solution with acetonitrile and were stored at −20 °C.

Acetonitrile and methanol of HPLC grade were purchased from Fisher Chemicals (Fair Lawn, NJ, USA). HPLC-grade water was prepared by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Formic acid (88%), acetic acid (99.5%), sodium acetate (98%), magnesium sulfate (98%), and sodium chloride (99.5%) of analytical grade were purchased from Sino-pharm. Chemical Reagent (Beijing, China).

Apparatus. The chromatographic system was an Agilent 1200 series HPLC system consisting of a vacuum degasser, an autosampler, a column heater, a quaternary solvent delivery system, and a binary pump. The separations were performed using an Eclipse plus C18 analytical column of 2.1 × 50 mm and 3.5 μm particle size from Agilent Technologies. Column temperature was maintained at 30 °C. The injection volume was 5 μL, and to avoid carry-over, the autosampler was flushed by acetonitrile between analytical runs. Mobile phases A and B were water with 0.1% formic acid and acetonitrile, respectively. A gradient elution started at 30% of solvent B and was ramped linearly to 60% of solvent B in the first 3 min; the percentage of solvent B was linearly increased to 70% in 3 min, followed by a linear gradient to 100% of solvent B in 9 min, a hold for 1 min, and a ramp to the original composition in 15 min. The flow rate used was kept at 0.2 mL/min.

The HPLC system was connected to an Agilent 6410 triple-quadrupole LC-MS detector equipped with an electrospray interface operating in positive ion mode. The source parameters were as follows: capillary voltage, 4000 V; nebulizer pressure, 35 psi; drying gas flow, 8 L min^{-1}; gas temperature, 350 °C. Multiple reaction monitoring (MRM) experiments were conducted for all pesticides. The optimized settings for precursor and product ions monitored, fragmentor voltage, time windows/functions, and collision energies (CE) used were modified for each target analyte by using working standard solutions and the Pesticide DynamicMRM database.

Centrifugation was performed in two different instruments: an Anke TDL-40B centrifuge equipped with a bucket rotor (4 × 100 mL) (Shanghai, China) and a QL-901 Vortex (Kylin-bell Lab Instruments Co., Ltd., Jiangsu, China) were used for preparing the samples. Samples were stored in a Meiling B.CD-245W refrigerator freezer (Beijing, China).

Sample Preparation. Blank samples were used for validation studies and matrix-matched standard calibrations. Samples for recovery studies were spiked with a corresponding volume of the working solution and left for 30 min before the extraction.

Rapeseed and Rapeseed Oil. A portion (10 g) of ground rapeseeds or oil sample was weighed into a 50 mL centrifuge tube, and then 2 and 5 mL of ultrapure water was added for conditioning for rapeseeds and rapeseed oil, respectively. Then acetonitrile with 1% acetic acid (10 mL) was added, and the sample was shaken vigorously for 1 min with vortex mixer. Next, anhydrous sodium acetate (1 g) and anhydrous $MgSO_4$ (4 g) were added, and the sample was vortexed immediately for 1 min. The extract was then centrifuged for 5 min at 3800 rpm and frozen for 12 h at −18 °C. An aliquot of 1 mL of the upper layer was filtered through a 0.45 μm membrane and placed into a LC vial to carry out the direct LC-MS/MS analysis without further cleanup steps.

Rapeseed Meal. A portion (10 g) of ground rapeseed meal sample was weighed into a 50 mL centrifuge tube, and then 2 mL of ultrapure water was added for conditioning for rapeseed meal. Then acetonitrile

Table 2. Operational MRM Conditions and MRM Transitions Used for the Quantitation and Confirmation of Pesticides

			first transition (quantitation)		second transition (confirmation)		third transition (confirmation)			
pesticide	fragmentor	precursor ion	product ion (%, rel abundance)	collsion energy (eV)	product ion (%, rel abundance)	collsion energy (eV)	product ion (%, rel abundance)	collsion energy (eV)	Rt (min)	group
carbendazim	90	192	132 (12)	20	160 (100)	25			0.8	1
thiamethoxam	80	292	211 (100)	10	181 (51)	20			1.1	1
trichlorfon	120	257	109 (100)	20	221 (32)	10			1.2	1
imidacloprid	80	256	209 (100)	10	175 (77)	10			1.4	1
acetamiprid	80	223	126 (100)	15	56 (40)	10	90 (8)	20	1.6	1
thiacloprid	90	253	126 (100)	20	186 (8)	10			2.2	1
dichlorvos	120	221	109 (100)	15					3.4	2
prometryn	120	242	158 (100)	20	200 (67)	20			4.5	3
atrazine	120	216	174 (100)	15	104 (31)	25	132 (17)	20	5.4	3
carboxin	120	236	143 (100)	15	87 (33)	20			6.2	3
metalaxyl-M	120	280	192 (71)	15	220 (100)	10	160 (59)	25	6.3	3
clomazone	120	240	125 (100)	20	89 (4)	30			7.6	4
paclobutrazol	120	294	70 (100)	20	125 (7)	25			8	4
prochloraz	80	376	308 (100)	10	266 (18)	10	70 (33)	10	8.2	4
methidathion	80	303	145 (100)	5	85 (47)	10			8.4	4
azoxystrobin	120	404	372 (100)	10	344 (63)	15			8.9	5
triadimefon	120	294	197 (100)	10	69 (91)	15	225 (59)	10	8.9	5
tebuconazole	120	308	70 (100)	20	125 (9)	30			9.1	5
metolachlor	120	284	252 (100)	10	176 (7)	15			9.5	6
acetochlor	120	270	224 (100)	10	148 (35)	10			9.6	6
diniconazole	120	326	70 (100)	25	159 (7)	30			9.6	6
propiconazol	120	342	159 (100)	20	69 (93)	20			9.7	6
fluorochloridone	100	312	292 (100)	25	89 (46)	25			9.8	6
chlorfenvinphos	120	359	155 (100)	10	127 (65)	15	99 (99)	25	10.1	6
difenoconazole	160	406	251 (100)	20	337 (26)	15			10.3	6
diazinon	160	305	169 (100)	20	153 (56)	20	97 (26)	25	10.8	7
phoxim	80	299	129 (100)	10	77 (80)	20			11.4	8
haloxyfop-P-methyl	120	376	316 (100)	15	288 (14)	20			11.6	8
fenoxaprop-P-ethyl	120	362	288 (100)	20	121 (16)	25	244 (12)	20	12	8
quizalofop-P-ethyl	120	373	299 (100)	15	271 (20)	25			12	8
clethodim	120	360	164 (100)	20	268 (55)	10			12.3	8
fluazifop-P-butyl	120	384	328 (63)	15	282 (100)	20			13.1	9
oxadiazon	100	345	220 (100)	20	177 (30)	20			13.2	9
chlorpyrifos	100	350	198 (100)	20	97 (43)	15			13.3	9

(20 mL) was added, and the sample was shaken vigorously for 1 min with vortex mixer. Next, anhydrous NaCl (1 g) and anhydrous $MgSO_4$ (4 g) were added, and the sample was vortexed immediately for 1 min. The extract was then centrifuged for 5 min at 3800 rpm and frozen for 12 h at −18 °C. An aliquot of 1 mL of the upper layer was filtered through a 0.45 μm membrane and placed into a LC vial to carry out the direct LC-MS/MS analysis without further cleanup steps.

RESULTS AND DISCUSSION

LC-MS/MS Determination. The analysis was determined by LC-MS/MS MRM using at least one transition, in addition to their relative abundances, the retention time, and the assistance of the Pesticide DynamicMRM database. The precursor ion and product ion are summarized in Table 2, as well as the indicative retention times and time windows on the column.

Validation Procedure. To investigate the cleanup effect for the matrix by this method, the free-pesticide samples were used for preparation of a blank matrix. No interferences from the matrices were observed in MRM mode. The typical MRM chromatogram of blank and spiked rapeseed, rapeseed oil, and rapeseed meal samples are shown in Figure 1.

The linearity of rapeseed, rapeseed oil, and rapeseed meal samples was studied in the range of 2−500 μg L^{-1} with six calibration points (2, 5, 10, 50, 100, and 500 μg L^{-1}) by matrix-matched standard calibration, spiked with the corresponding volume of the working solution into the extract from blank samples with same extracting procedure. Linear calibration graphs were constructed by least-squares regression of concentration versus peak area of calibration standards. Linearity values of rapeseed, rapeseed oil, and rapeseed meal samples, calculated as determination coefficients (R^2), are shown in Table 3

Accuracy was evaluated in terms of the recovery. This study was performed at three concentration levels by spiking 5, 10, and 100 μg kg^{-1} for blank rapeseed and rapeseed oil and 10, 20, and 100 μg kg^{-1} for rapeseed meal samples with a corresponding volume of working solution. Five samples of each concentration were processed. The recoveries of selected pesticides in rapeseed, rapeseed oil, and rapeseed meal samples

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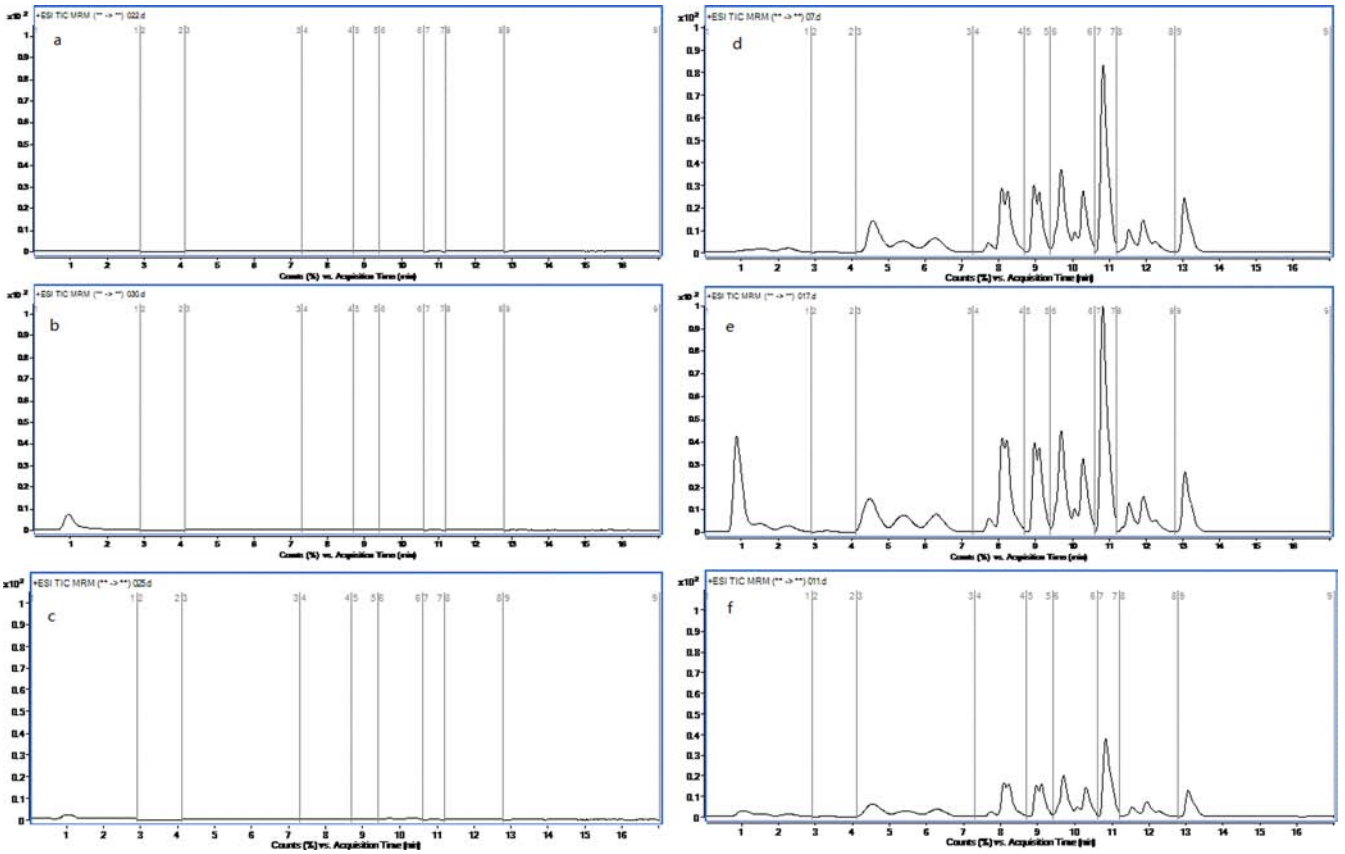

Figure 1. Typical MRM chromatograms of blank (a) rapeseed, (b) rapeseed oil, and (c) rapeseed meal samples and spiked (d) rapeseed, (e) rapeseed oil, and (f) rapeseed meal with 10 μg kg^{-1} of the target analytes.

are shown in Table 3. In addition, the use of spiked matrix standards has been shown to be essential for improving the quantification.

Precision was studied as intraday and interday precision. Intraday precision (%, RSD) was lower than 17, 19, and 18% for rapeseed, rapeseed oil, and rapeseed meal samples, respectively, at three concentration levels. The interday precision (%, RSD) was obtained by processing the same spiked samples on five different days at the mentioned three concentration levels (5, 10, and 100 μg kg^{-1}), the and RSD was lower than 30, 15, and 24% in the three matrices for 5, 10, and 100 μg kg^{-1}, respectively.

LOQs of the method were established at the lowest fortified level in each matrix, checking that this concentration yielded an S/N ratio equal to or slightly higher than 10, and were 0.6−15, 0.5−18, and 0.3−15 μg kg^{-1} for rapeseed, rapeseed oil, and rapeseed meal, respectively. By comparison of the result with those obtained using a cleanup step with GPC,[8,9] SPE,[4] and dispersive solid-phase extraction,[22] the proposed method showed lower detection limits for the pesticides in oil. However, the method in this study with a freezing step got much higher detection limits (methidathion, 3 mg kg^{-1}; paclobutrazole, 1 mg kg^{-1}; prochloraz, 0.9 mg kg^{-1}; tebuconazole, 1 mg kg^{-1}; and triadimefon, 2 mg kg^{-1}) than those in the Pizzutti et al.[22] study without a cleanup procedure (methidathion, 50 mg kg^{-1}; paclobutrazole, 100 mg kg^{-1}; prochloraz, 100 mg kg^{-1}; tebuconazole, 100 mg kg^{-1}; and triadimefon, 100 mg kg^{-1} The LOQs are shown in Table 3.

Optimization of the Extract Procedure. Experiments were carried out to optimize the extract procedure steps for rapeseed, rapeseed oil, and rapeseed meal samples.

Although lipids are not very soluble in acetonitrile, a small amount of fat may be coextracted, so further cleanup is still desirable. Due to the significant difference of melting points between fat (below 40 °C) and selected pesticides (normally above 250 °C), the coextracted fat can be separated from pesticides by freezing at −18 °C in the freezer, whereas pesticides are still dissolved in cold organic solvent. Thus, frozen coextract fat can be easily discarded from the extracts by centrifugation. The supernatant and the bottom layer were acetonitrile and frozen solid, respectively, for rapeseed and rapeseed meal. However, it was salt, saturated salt water, frozen oil, and acetonitrile from bottom to top for rapeseed oil.

For determining pesticide residues in food matrices of low water content or high fat content, a certain amount of water was added to get sufficient participation of the target analytes between matrix, water, and organic solvents such as acetonitrile. The estimation of the amount of water for conditioning the rapeseed, rapeseed oil, and rapeseed meal before extract procedure was performed by adding different amounts of water (0, 2, 5, 10, and 15 mL) to three matrices (10 g), respectively, at the concentration level of 100 μg kg^{-1}. After analysis in triplicate, the results showed that 2, 5, and 2 mL of water had the highest conditioning effect for rapeseed, rapeseed oil, and rapeseed meal samples, respectively.

The extract efficiencies of acetonitrile with anhydrous NaCl and acetonitrile containing 1% acetic acid with anhydrous sodium acetate were also estimated at the concentration level of

Table 3. Validation Parameters for Rapeseed, Rapeseed Oil, and Rapeseed Meal

Rapeseed											
		spike levels									
		5 μg/kg			10 μg/kg			100 μg/kg			
			RSD (%)			RSD (%)			RSD (%)		
pesticide	determination coefficient	av recovery (%)	intraday (n = 5)	interday (n = 25)	av recovery (%)	intraday (n = 5)	interday (n = 25)	av recovery (%)	intraday (n = 5)	interday (n = 25)	LOQ (μg/kg)
acetamiprid	0.9992	94	3	7	90	5	7	99	8	13	6
acetochlor	0.9991	106	15	20	97	6	13	93	13	17	5
atrazine	1.0000	79	1	4	74	3	7	91	7	8	0.7
azoxystrobin	1.0000	104	4	5	100	4	8	97	4	10	0.6
carbendazim	0.9979	77	8	13	79	17	25	81	11	21	10
carboxin	0.9998	93	4	5	96	7	12	86	6	8	8
chlorfenvinphos	0.9953	88	6	8	79	9	16	86	6	10	3
chlorpyrifos	0.9997	70	5	11	60	11	16	72	9	15	4
clethodim	0.9995	96	5	8	87	8	15	71	6	9	6
clomazone	1.0000	99	3	9	89	4	6	108	2	4	5
diazinon	0.9999	88	4	5	87	4	6	92	5	9	0.6
dichlorvos	1.0000	96	3	9	94	3	7	76	6	6	5
difenoconazole	0.9999	88	4	8	89	2	5	92	4	9	2
diniconazole	1.0000	90	6	11	84	3	9	88	8	11	3
fenoxaprop-P-ethyl	0.9996	95	2	7	92	3	8	92	4	6	3
fluazifop-P-butyl	0.9996	83	1	6	86	3	8	85	3	7	5
fluorochloridone	0.9990	91	14	19	98	5	10	97	3	5	5
haloxyfop-P-methyl	0.9999	96	4	10	95	3	5	79	7	11	1
imidacloprid	0.9943	80	5	6	76	7	8	95	4	7	5
metalaxyl-M	0.9997	107	3	6	106	3	8	77	3	8	7
methidathion	1.0000	93	10	17	95	6	9	84	3	7	3
metolachlor	0.9996	100	3	6	89	16	22	87	3	9	2
oxadiazon	1.0000	77	14	21	83	9	12	87	4	7	3
paclobutrazol	0.9999	72	4	8	70	2	5	95	3	5	1
phoxim	0.9999	54	5	9	57	8	12	95	10	16	10
prochloraz	1.0000	92	3	8	90	2	8	94	6	8	0.9
prometryn	0.9961	85	4	9	75	5	10	61	10	15	2
propiconazol	0.9995	78	5	7	85	3	6	81	1	4	2
quizalofop-P-ethyl	0.9987	82	3	9	81	7	13	90	11	15	4
tebuconazole	0.9997	98	4	9	90	2	5	79	6	4	1
thiacloprid	0.9993	105	1	5	97	4	9	79	3	6	10
thiamethoxam	0.9954	75	3	7	74	22	27	77	9	17	15
triadimefon	1.0000	94	3	7	96	5	8	97	9	11	2
trichlorfon	0.9887	123	7	10	102	7	11	88	5	9	5
Rapeseed Oil											
		spike levels									
		5 μg/kg			10 μg/kg			100 μg/kg			
			RSD (%)			RSD (%)			RSD (%)		
pesticide	determination coefficient	av recovery (%)	intraday (n = 5)	interday (n = 25)	av recovery (%)	intraday (n = 5)	interday (n = 25)	av recovery (%)	intraday (n = 5)	interday (n = 25)	LOQ (μg/kg)
acetamiprid	0.9978	99	9	11	101	3	9	94	3	9	15
acetochlor	0.9998	127	8	11	94	6	11	82	8	10	15
atrazine	0.9997	79	4	7	76	3	7	80	3	7	12
azoxystrobin	0.9993	115	7	8	110	4	6	88	10	16	18
carbendazim	0.9938	77	7	13	95	13	23	97	9	13	1
carboxin	0.9987	90	4	8	91	3	7	81	6	11	0.5
chlorfenvinphos	0.9986	47	9	12	73	4	9	85	2	7	7
chlorpyrifos	0.9996	71	10	13	42	19	32	63	15	24	5
clethodim	0.9995	75	6	15	63	4	9	71	2	8	7
clomazone	0.9990	94	4	9	89	3	9	85	3	10	3
diazinon	0.9997	92	5	8	84	3	9	76	4	9	3

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Table 3. continued

Rapeseed Oil											
		spike levels									
		5 μg/kg			10 μg/kg			100 μg/kg			
			RSD (%)			RSD (%)			RSD (%)		
pesticide	determination coefficient	av recovery (%)	intraday (n = 5)	interday (n = 25)	av recovery (%)	intraday (n = 5)	interday (n = 25)	av recovery (%)	intraday (n = 5)	interday (n = 25)	LOQ (μg/kg)
dichlorvos	0.9995	110	5	11	108	5	13	89	6	9	2
difenoconazole	0.9999	84	6	11	82	4	8	84	7	10	5
diniconazole	0.9997	75	5	6	72	4	9	82	4	9	1
fenoxaprop-P-ethyl	0.9994	77	6	11	72	4	9	75	7	9	3
fluazifop-P-butyl	1.0000	71	8	14	71	6	15	80	10	22	3
fluorochloridone	0.9990	89	4	7	98	8	11	82	11	19	2
haloxyfop-P-methyl	0.9995	91	6	14	86	1	7	84	2	5	10
imidacloprid	0.9986	123	6	9	93	1	7	89	1	6	1
metalaxyl-M	0.9994	107	7	10	107	2	8	86	4	7	15
methidathion	0.9981	93	11	13	93	4	7	85	3	9	10
metolachlor	0.9998	83	5	8	78	4	9	79	2	8	4
oxadiazon	0.9999	75	5	8	61	10	21	76	6	11	2
paclobutrazol	0.9997	94	7	12	102	3	7	85	5	9	5
phoxim	0.9994	96	11	18	84	14	22	80	10	19	1
prochloraz	0.9995	91	6	11	87	3	9	86	2	7	8
prometryn	0.9998	47	3	7	47	5	11	74	3	10	1
propiconazol	1.0000	74	2	7	73	4	10	85	3	7	2
quizalofop-P-ethyl	0.9998	25	14	20	44	3	10	77	4	11	4
tebuconazole	0.9989	101	4	6	91	4	10	86	8	12	4
thiacloprid	0.9998	106	4	8	104	4	10	92	2	7	1
thiamethoxam	0.9935	150	7	15	110	7	12	89	7	14	13
triadimefon	0.9997	96	7	10	88	2	8	87	1	5	3
trichlorfon	0.9818	109	5	9	139	3	10	90	4	9	10

Rapeseed Meal											
		spike levels									
		10 μg/kg			20 μg/kg			100 μg/kg			
			RSD (%)			RSD (%)			RSD (%)		
pesticide	determination coefficient	av recovery (%)	intraday (n = 5)	interday (n = 25)	av recovery (%)	intraday (n = 5)	interday (n = 25)	av recovery (%)	intraday (n = 5)	interday (n = 25)	LOQ (μg/kg)
acetamiprid	0.9998	91	11	20	96	6	9	97	5	11	15
acetochlor	0.9989	126	11	15	115	3	7	91	7	11	10
atrazine	0.9995	88	2	8	96	3	9	90	4	10	6
azoxystrobin	0.9993	100	3	7	110	2	8	90	1	8	1
carbendazim	0.9990	72	11	23	77	6	12	92	3	8	1
carboxin	0.9971	107	5	8	117	3	9	84	2	7	15
chlorfenvinphos	0.9990	76	10	17	83	5	9	95	7	12	5
chlorpyrifos	0.9983	118	18	30	112	4	9	88	2	11	5
clethodim	0.9996	100	6	8	103	2	8	84	1	6	5
clomazone	0.9983	97	4	9	111	3	10	95	4	18	3
diazinon	0.9999	114	3	9	117	3	12	94	5	11	0.3
dichlorvos	0.9989	118	4	10	115	2	10	90	5	11	8
difenoconazole	0.9999	96	4	12	100	2	8	91	3	11	3
diniconazole	0.9988	106	7	12	106	3	9	91	4	9	3
fenoxaprop-P-ethyl	0.9983	114	6	10	112	3	12	89	5	13	5
fluazifop-P-butyl	0.9999	86	6	11	90	4	10	89	5	10	2
fluorochloridone	0.9989	91	9	19	83	7	12	92	8	20	13
haloxyfop-P-methyl	0.9996	97	6	9	101	1	8	91	2	8	3
imidacloprid	0.9995	102	16	23	84	6	11	102	3	9	3
metalaxyl-M	0.9959	95	9	11	106	3	12	85	3	12	15
methidathion	0.9995	104	7	15	100	2	8	95	1	7	6

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Table 3. continued

		Rapeseed Meal									
		spike levels									
		10 μg/kg			20 μg/kg			100 μg/kg			
			RSD (%)			RSD (%)			RSD (%)		
pesticide	determination coefficient	av recovery (%)	intraday (n = 5)	interday (n = 25)	av recovery (%)	intraday (n = 5)	interday (n = 25)	av recovery (%)	intraday (n = 5)	interday (n = 25)	LOQ (μg/kg)
metolachlor	0.9993	108	2	9	110	3	9	86	2	8	2
oxadiazon	0.9993	79	7	10	87	8	15	86	5	12	3
paclobutrazol	0.9996	90	2	7	113	3	9	96	3	15	2
phoxim	0.9997	112	10	13	96	4	8	90	3	14	4
prochloraz	0.9993	105	5	11	104	2	7	87	3	9	1
prometryn	0.9995	87	8	15	87	3	11	88	2	8	5
propiconazol	0.9998	93	5	8	99	5	11	84	5	15	3
quizalofop-P-ethyl	0.9999	87	8	14	97	6	11	93	3	9	3
tebuconazole	0.9990	110	3	7	111	1	5	96	2	9	1
thiacloprid	0.9997	105	4	9	102	4	7	93	2	6	1
thiamethoxam	0.9988	106	6	12	96	10	19	110	4	10	5
triadimefon	0.9996	89	2	8	108	4	10	90	7	16	2
trichlorfon	0.9946	111	9	17	82	14	22	95	9	17	6

100 μg kg^{-1} for rapeseed, rapeseed oil, and rapeseed meal, respectively. Acetonitrile containing 1% acetic acid with anhydrous sodium acetate showed a higher extract effect for rapeseed and rapeseed oil, whereas acetonitrile with anhydrous NaCl had better extract efficiency for rapeseed meal.

Although acidified acetonitrile was used for extraction of pesticides in rapeseed and rapeseed oil but unmodified acetonitrile for rapeseed meal, the pH values, measured by pH-meter, of supernatant acetonitrile layers after centrifugation were all around 5.5 of the three matrices. The formic acid in acetonitrile might hydrolyze ester bonds of oil compound in rapeseed and rapeseed oil to promote extraction of target compounds, especially the pesticides with high n-octanol–water partition coefficient (kowlogP) values, from matrices.

The recoveries using the proposed extraction procedures described under Sample Preparation for rapeseed was between 70 and 110% (except prometryn, 61%), rapeseed oil ranged from 70 to 100% (except chlorpyrifos, 63%) and rapeseed meal from 70 to 110% for all target pesticides at the concentration level of 100 μg kg^{-1}.

Effect of Physical–Chemical Properties of Target Compounds on Pesticide Recoveries. Due to the fat content of the test matrices rapeseed oil (100%), rapeseed (35–40%), and rapeseed meal (<2%) and the physical–chemical properties of target pesticides, the tendency of the extraction efficiencies of 34 selected pesticides in three matrices and in different concentration levels of the same matrix were different, as presented in Figure 2.

As it can be seen in Figure 2, better extraction coefficients were obtained in the highest concentration level of 100 μg kg^{-1}, with recoveries in the range of 70–110% for 32 pesticides, except for chlorpyrifos (63%) in rapeseed oil and prometryn (61%) in rapeseed, compared with those obtained at 10 and 5 μg kg^{-1} concentration levels, with recoveries beyond the range of 70–120% for eight and nine of the selected pesticides, respectively. The compounds with the higher n-octanol–water partition coefficients (kowlogP > 3) and lower water solubilities (all values of kowlogP and water solubility from software e-Pesticide Manual 3.1, 2004–2005), such as oxadizan (kowlogP = 4.91; water solubility = 1.0 mg L^{-1} at 20 °C), chlorpyriphos (kowlogP = 4.7 and water solubility = 1.4 mg L^{-1} at 25 °C), quizalofop-P-ethyl (kowlogP = 4.61 at 23 ± 1 °C; water solubility = 9.08 mg L^{-1} at 25 °C), acetochlor (kowlogP = 4.14; water solubility = 223 mg L^{-1} at 25 °C), phoxim (4.104 in unbuffered water; water solubility = 3.4 mg L^{-1} at 25 °C), chlorfenvinphos (kowlogP = 3.85 and 4.22; water solubility = 121 and 7.3 at 23 °C for (Z)- and (E)-isomer, respectively), and prometryn (kowlogP = 3.1 un-ionized and water solubility = 33 mg L^{-1} at 25 °C) showed the lowest recoveries in lower levels of fortification.

To evaluate how the n-octanol–water partition coefficient and water solubility influence the extraction efficiencies in different matrices with fat content, the recovery trends of 34 pesticides in rapeseed, rapeseed oil, and rapeseed meal are presented in Figure 2. Obviously, the recoveries went down as the value of kowlogP rose in each spiking level, both in rapeseed and rapeseed oil. Nonpolar and polar pesticides were extracted from fatty matrices into a polar solvent. It is understandable that the recovery changed reversely along with liposolubility of compounds, as kowlogP can be considered to be an index reflecting liposolubility. Meanwhile, the recoveries went up as the value of water solubility rose in rapeseed in each spiking level. The same tendency was observed only at the high spiking level (100 μg kg^{-1}) of rapeseed oil. However, the recoveries decreased slightly when the value of water solubility rose in low spiking levels of 5 and 10 μg kg^{-1} in rapeseed oil. No obvious tendencies were observed in the matrix of rapeseed meal. It could be concluded that the n-octanol–water partition coefficient plays a more evident role than water solubility plays in extracting the pesticides with acetonitrile from the matrices of high fat content.

Real Sample Analysis. The method developed was applied to the analysis of pesticides in 20 commercial rapeseed, 20 rapeseed oil, and 12 rapeseed meal samples. The samples were analyzed following the preparation procedure. From the analytical results, the pesticide residues detected were clomazone in two rapeseed samples, with the concentration of 7 μg kg^{-1} and lower than LOQ, respectively. Phoxim (<LOQ), diazinon (<LOQ), atrazine (14–20 μg kg^{-1}), and

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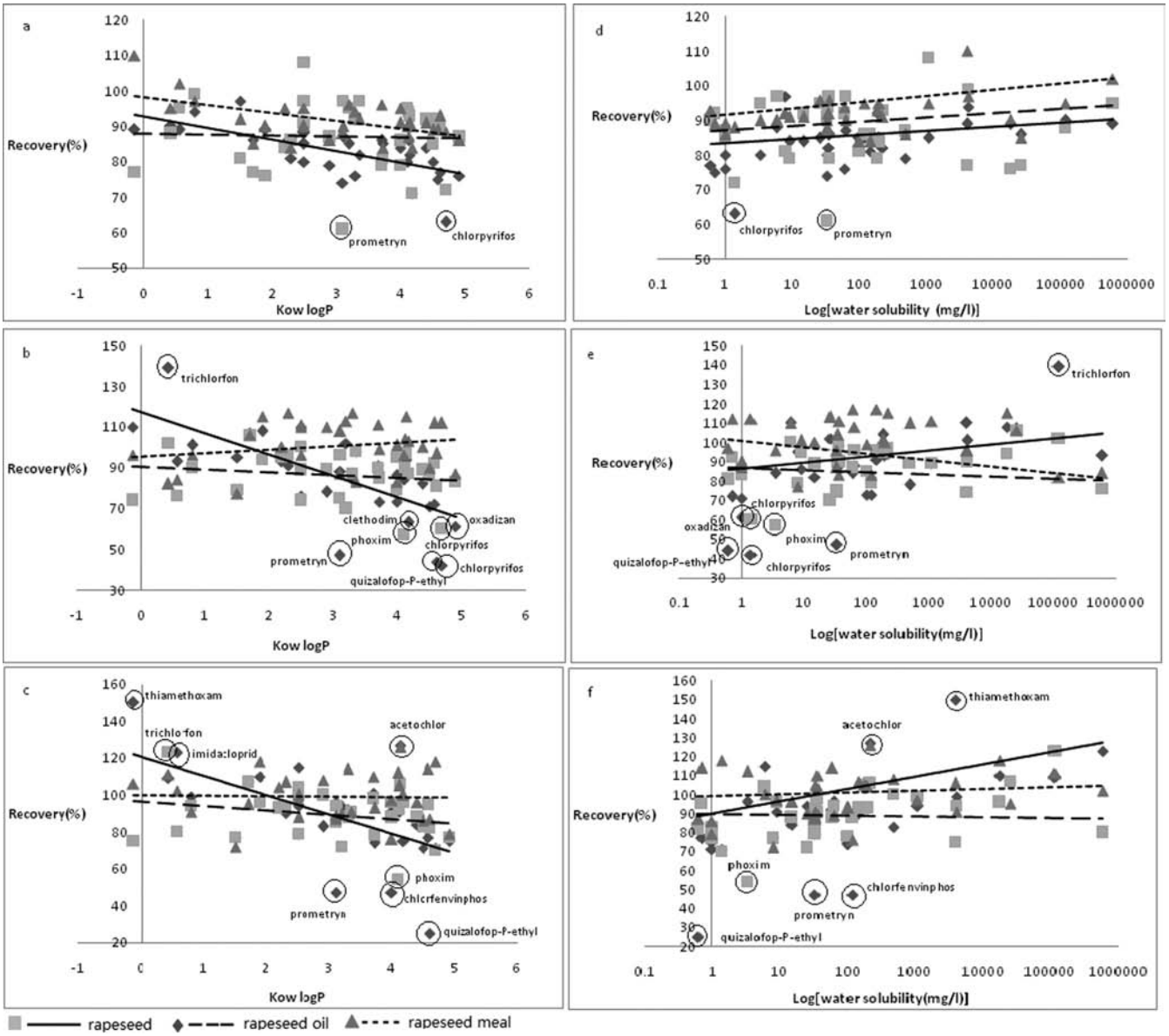

Figure 2. Influence of pesticides' *n*-octanol−water partition coefficients (kowlogP) and water solubility on their recovery.

triadimefon (17 μg kg^{-1}) were detected in 10 oil samples. Chlorpyrifos (7−13 μg kg^{-1}) was detected in five rapeseed meal samples, as shown in Table 4 in the Supporting Information.

The results confirm the feasibility of the proposed method, which can be easily implemented for routine testing and monitoring of pesticide residues in rapeseed, rapeseed oil, and rapeseed meal.

In this work, a simple, cheap, and environmentally friendly sample treatment has been evaluated for 34 pesticide multiresidues in rapeseed, rapeseed oil, and rapeseed meal. The linearity, precision, accuracy, and LOQs obtained illustrate the potential of LC-MS/MS (QqQ) for rapid screening of agrochemicals in three matrices of different fat content. Method validation results from this study showed that the established method was satisfied when applied in determination of samples for MRL compliance. For particular pesticides, poor recoveries were found especially in high-fat matrices and can be explained in accordance with their properties. The *n*-octanol−water partition coefficient and water solubility influence the extraction efficiencies with acetonitrile in matrices with different fat contents. Moreover, the proposed method is suitable for routine analysis. The results obtained in this study allow us to apply the developed method to further investigations and monitoring studies of pesticide residues in rapeseed, rapeseed oil, and rapeseed meal.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Table 4. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

QqQ, triple quadrupole; MRM, multiple reaction monitoring; R^2, determination coefficients; LOQ, limit of quantitation; LC-MS/MS, liquid chromatography−tandem mass spectrometry; MRL, maximum residue levels; EU, European Union; LLE, liquid−liquid extraction; SPE, solid-phase extraction; SPME, solid-phase microextraction; MSPD, matrix solid-phase dispersion; MAE, microwave-assisted extraction; APMAE, microwave-assisted liquid−liquid extraction; RPLC-GC, reversed-phase liquid chromatography−gas chromatography analysis; QuEChERS, quick, easy, cheap, effective, rugged, and safe; FPD, flame photometric; ECD, electron capture detector; TSD, thermoionic specific detector; MSD, mass spectrometry; CE, collision energies; RSD, relative standard deviation; LOD, limit of detection.

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