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Development of a new QuEChERS method based on dry ice for the determination of 168 pesticides in paprika using tandem mass spectrometry

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ABSTRACT

This study describes a new QuEChERS method referred to as the dry ice-partitioning QuEChERS method. This current method can be differentiated from the other QuEChERS methods in the sense that it uses dry ice rather than salts or buffers to extract and partition pesticides in the first extraction step. The dry ice-partitioning QuEChERS method consists of extraction method A (for detection of the acetonitrile layer) and extraction method B (for detection of both acetonitrile and aqueous layers). The extraction efficiency was then compared with the citrate-buffering QuEChERS method by means of recovery. Recovery values of the tested 168 pesticides were above 76%, with relative standard deviations of less than 20%. Certain problematic pesticides, including benfuracarb, carbosulfan, dichlofluanid, probenazole, pymetrozine, tolylfluanid, TFNA, and TFNG evidenced acceptable recoveries via the dry ice-partitioning QuEChERS method compared to the less than 70% recoveries of the citrate-buffering QuEChERS method examined herein. The matrix effect of paprika on the method developed herein was not significant, and matrix-matched calibration was performed well, with an $r^2 \geq 0.99$. The dry ice-partitioning QuEChERS method is capable of detecting the aqueous layer as well as the acetonitrile layer; this interesting feature makes it worth in application as an alternative QuEChERS method for the multiresidue analysis of pesticides within a broad polarity range in various matrices.

1. Introduction

The food safety of imported agricultural commodities has become a priority of consumers who demand good quality products, and is also important for the maintenance of a smooth international trade. Unfortunately, almost all of the agricultural commodities currently cultivated and distributed are exposed to contaminants. The primary components considered to harm food safety are pesticide residues, persistent organic pollutants, polycyclic aromatic hydrocarbons, food additives, derivatives from food processing (acrylamide, ethyl carbamate, formalin, furan, 3-MCPD, etc.), microbial toxins, and industrial chemicals (solvents, food container agents, heavy metals, and other inorganics) [1].

Since pesticides have been integrally applied during on-farm production and post-production processes; however, in efforts to improve the efficacy of global agricultural practices, some pesticides might carry the potential of inadmissible use and/or the presence of residues. Therefore, the distribution of food commodities treated with pesticides should be strictly controlled for the purpose of food safety, human health, and environmental conservation. In particular, pesticide residues in food must be thoroughly inspected, as they are related directly to human health. The primary standard for the control of pesticide residues in food is the maximum residue limit (MRL) or tolerance level, a measurement of regulatory power in the global food trade [2].

The Codex MRL of pesticide or veterinary drugs in food is one component of the Codex standards [3]. Although the Codex MRL has generally been used as a global reference, some countries continue to establish their own MRLs (Australia, Canada, China, EU, Japan, Republic of Korea, etc.) or tolerance levels (in the USA) [2,4]. It is, then, reasonable to establish appropriate MRLs to each country taking into consideration the differences in pesticides permitted for use on crops, cultural practices, regional climate, food intake, etc. Unlike their principal objective as a regulatory means for food safety and public health, the differences in pesticide MRLs between countries can present an obstacle to trade, and sometimes interferes in the international trade of agricultural commodities. Indeed, a trade conflict occurred between the Republic of Korea and Japan in 2009 centering around floni-

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camid (*N*-cyanomethyl-4-trifluoromethylnicotinamide) residues in paprika imported to Japan from the Republic of Korea. The MRL of flonicamid in paprika (sweet pepper) is 2.0 mg/kg in the Republic of Korea. When the Japanese Monitoring Agency tested paprika exported from the Republic of Korea, they detected levels of 0.7 mg/kg, which exceeded their acceptable limit (0.4 mg/kg) [5–7]. Therefore, Japan tightened its monitoring inspection on Korean paprika at that time, according to the Positive List System [7]. This MRL violation was basically caused by unsuitable pesticide use by of paprika-producing farmers aiming to export their products. Farmers cannot overlook the significant differences in flonicamid MRLs between the two countries, or ignore the residue definitions for enforcement applications in paprika. The MRL for flonicamid in the Republic of Korea is appointed only to the parent compound, flonicamid, as a target residue [5]. However, in Japan, its MRL is defined as the sum of a parent compound and its metabolites, TFNA (4-trifluoronicotinic acid) and TFNG (N-(4-trifluoronicotinoyl) glycine) [6]. Additionally, the official Korean analytical method was established to analyze the parent compound only using a GC-ECD in the KFDA Food Code [8].

A few analytical methods for flonicamid and its metabolites (TFNA and TFNG) have been previously reported [9,10]. To extract flonicamid, TFNA and TFNG, Hengel and Miller conducted liquid–liquid extractions with a mixture of acetonitrile and water (50/50, v/v) and a two-step solid-phase extraction cartridge cleanup followed by liquid–liquid partitioning until LC–MS/MS analysis [9], whereas Ricerca Bioscience used a pressurized liquid extraction method involving a mixture of methanol and water (30/70, v/v) and LC–MS/MS analysis [10]. To the best of our knowledge, the application of a QuEChERS method for the simultaneous determination of flonicamid, TFNA and TFNG in crops remains somewhat problematic.

The QuEChERS method is employed frequently as a sample preparation methodology for multiresidue pesticide analysis, and the method has been modified and validated for the detection of a broad range of pesticides, including acidic and basic ones in various matrices [11–34]. The original QuEChERS method consisted of an initial extraction with acetonitrile followed by partitioning after the addition of adequately mixed salts (anhydrous magnesium sulfate and sodium chloride), after which the extract was subjected to dispersive solid-phase extraction (DSPE) cleanup with primary secondary amine and anhydrous magnesium sulfate [11,12]. However, undesirable recoveries were obtained for certain pH-sensitive pesticides, such as captan, chlorothalonil, deltamethrin, dichlofluanid, dicofol, folpet, and pymetrozine when the original QuEChERS method was used [12,13]. To apply a QuEChERS method to overall pesticides containing pH-sensitive pesticides, the original QuEChERS method was modified with acid buffers. The acetate-buffering QuEChERS method was published by Lehotay et al. in 2005 and became "AOAC Official Method 2007.01" [13,16], and the citrate-buffering version in combination with accessorial graphite carbon black cleanup was entitled "European Standard EN 15662" [19,20]. Additionally, water was added to dry samples to obtain the necessary moisture [24–26,28–30], and a freeze-out and/or C_{18} sorbent to remove fatty components was introduced to the QuEChERS methodology [19,22,24–26].

Multiresidue methods for pesticide determination have traditionally involved gas chromatographs (GC) equipped with element/group selective detectors, such as electron capture detector (ECD), flame photometric detector (FPD), or nitrogen–phosphorus detector (NPD), or mass spectrometer only for the confirmation of positive samples [30,35].

A recent trend has involved the use of liquid chromatographic (LC) analysis in lieu of GC analysis because more polar, less volatile, and thermally labile pesticides have been increasingly introduced in recent years [36]. However, universal LC analysis does not always result in satisfactory selectivity or sensitivity, owing to the variety and complexity of matrices and the trace levels of detected pesticides [37]. In the past few years, LC coupled with tandem mass spectrometry (MS/MS) has been recognized as an innovative and effective approach in multiresidue pesticide analysis, owing to its marked prowess in the analysis of a wide range of pesticides, including pesticides amenable to GC [23,33]. LC–MS/MS is regarded as a highly sensitive, selective, and rapid means for both the quantification and identification of hundreds pesticides in a variety of complex matrices without the need for multiple complicated sample preparation steps [23,37]. Indeed, a combination of both the QuEChERS method and LC–MS/MS have recently been employed to determine multiresidue pesticides in many previous studies in the literatures [13,15–17,19–22].

In this paper, the dry ice-partitioning QuEChERS method was initially introduced by the authors to improve the recovery of some problematic highly polar pesticides in addition to flonicamid metabolites; TFNA and TFNG in paprika using LC–MS/MS. The method was then validated for a total of 168 including the above stated analytes. The superiority of the QuEChERS method developed herein was inspected with recovery tests of the citrate-buffered QuEChERS method described at http://www.quechers.com [34].

2. Experimental

2.1. Chemicals and reagents

Pesticide reference standards (purity > 96.0%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). HPLC-grade acetonitrile (MeCN), methanol, water, analytical-grade sodium chloride (NaCl), anhydrous magnesium sulfate ($MgSO_4$), formic acid, and acetic acid (HOAc) were supplied by Merck KGaA (Darmstadt, Germany). Sodium citrate tribasic dihydrate ($Na_3Cit{\cdot}2H_2O$) and disodium hydrogencitrate sesquihydrate ($Na_2HCit{\cdot}1.5H_2O$) were of analytical-grade and were provided by Sigma–Aldrich (Missouri, USA). Bondesil-primary secondary amine (PSA, 40 μm) and graphite carbon black (GCB) were purchased from Varian (California, USA). Ammonium acetate was of analytical-grade from Yakuri Pure Chemicals (Osaka, Japan), and dry ice and disposable syringe filters (PVDF, 0.2 μm) were purchased from Chang Shin Chemicals (Yeosu, Republic of Korea) and Woongki Science (Seoul, Republic of Korea), respectively.

2.2. Standard solutions

Individual stock solutions of pesticides were prepared in MeCN at 1000 μg/mL. A multicompound intermediate standard solution at a concentration of 5 μg/mL was prepared via appropriate dilutions of the stock solutions in MeCN containing 0.1% HOAc in order to prevent degradation of the analytes [14,37]. Serial dilutions of the intermediate standard solution were carried out to provide three multicompound working standard solutions (0.1, 0.5, and 1.0 μg/mL) in the solvent of the intermediate standard solution.

The individual stock solutions and the mixed intermediate and working standard solutions were stored at −20 °C in a dark amber bottle for a maximum period of 6 months.

Matrix-matched multi-level calibration standard solutions were prepared in sample extracts obtained from fresh organic paprika purchased from a local market (Gwangju, Republic of Korea). Aliquots (5 mL of a MeCN layer) of the blank samples, which were extracted via extraction method A (described below) were evaporated and reconstituted in 5.0 mL of a mixture of appropriate working standard solutions and 0.02% HOAc in MeCN to generate

final concentrations of 0.04, 0.06, 0.1, 0.2, 0.4, and 0.5 mg/kg for the matrix-matched calibration standards.

2.3. Instrumentation

2.3.1. LC–MS/MS

Liquid Chromatography was carried out using an Agilent 1200 Series Rapid Resolution LC System (California, USA), which consisted of a binary pump, autosampler, vacuum degasser, thermostated column compartment, and a diode array detector-coupled with an Agilent 6410 Triple Quadrupole LC/MS (QQQ). Tandem mass spectrometric (MS/MS) analysis was carried out via electrospray ionization (ESI) in positive or negative mode, and operated in multiple reaction monitoring mode (MRM). Table 1 summarizes the ESI parameters of MS/MS in both ionization modes and liquid chromatographic separation conditions. The optimization of the precursor ion, product ions, and collision energy (CE) was performed via direct injection of the individual pesticide standard solution (1 μg/mL) into the mass spectrometer. The most intense transition was used for quantitation, while the other was employed for confirmation. These optimization parameters are presented in Table 2. In the case of abamectin, mepanipyrim, mepronil, milbemectin, oxadixyl, and terbuthylazine, only one transition was generated and was, in turn, utilized for both quantitation and confirmation. A Mass Hunter Workstation Software (B.01.03) was used for instrument control, data acquisition, and processing.

2.4. Sample preparation

2.4.1. Sample processing

A representative portion of the fresh paprika sample was separated from the product, chopped, mixed well, and then homogenized with dry ice and stored at −40 °C pending analysis. The moisture content of the fresh paprika was 88% (w/w). No pesticide residues were ensured by the preliminary analysis for a blank sample.

2.4.2. Sample extraction by the QuEChERS method

2.4.2.1. Extraction by the dry ice-partitioning QuEChERS method. To extract 164 pesticides with the exception of flonicamid metabolites (TFNA and TFNG), propamocarb, and pymetrozine, 10 g of homogenized sample was withdrawn into a 125 mL polypropylene centrifuge tube. Thirty milliliters of MeCN and 10 mL of water were added to the tube, then subjected to 1 min of ultrasonic-assisted extraction. Approximately 10 g of dry ice granules were poured and maintained until layer separation. A 5 mL portion of the upper layer (MeCN) was transferred to a test tube, to which 62.5 mg of PSA, 375 mg of anhydrous $MgSO_4$, and 18.5 mg of GCB were added. The mixture was then vortex-mixed for 2 min. The final extract was filtered through a membrane filter (PVDF, 0.2 μm) and subsequently analyzed via LC–MS/MS.

For the extraction of TFNA, TFNG, propamocarb, and pymetrozine, the protocol described above was carried out, with the omission of the addition of anhydrous $MgSO_4$ to the MeCN layer. The final purified MeCN extract (1.0 mL) with the DSPE sorbents in the test tube and the aqueous layer separated in the centrifuge tube were mixed well in similar portions and filtered through a membrane filter (PVDF, 0.2 μm), and subsequently injected into LC–MS/MS. The dry ice-partitioning QuEChERS method for 164 pesticides and the method used for the other 4 pesticides are called extraction methods A and B, respectively, for brevity and clarity's sake.

2.4.2.2. Extraction by the citrate-buffering QuEChERS method, "QuEChERS, a mini-multiresidue method for the analysis of pesticide residues in low-fat products". Ten g of homogenous sample were weighed into a 50 mL centrifuge tube. Appropriate concentrations of the multicompound working standard solution were added to the tube and 10 mL of MeCN was poured. The tube was closed and shaken vigorously by hand for 1 min. A mixture of 4 g $MgSO_4$ anhydrous, 1 g NaCl, 1 g $Na_3Cit{\cdot}2H_2O$, and 0.5 g $Na_2HCit{\cdot}1.5H_2O$ was added and the tube was vigorously shaken for 1 min, followed by 5 min of centrifugation at 970 rcf. An aliquot of 5 mL of the supernatant MeCN phase was transferred to a 15 mL centrifuge tube containing 125 mg PSA, 750 mg anhydrous $MgSO_4$, and 15 mg of GCB, and the tube was shaken vigorously for 2 min and centrifuged for 5 min at 800 rcf. An aliquot of 2 mL of the cleaned extract was transferred into a screw cap vial and acidified via the addition of 20 μL of 5% formic acid solution in MeCN. Before the injection into the LC–MS/MS, the acidified extract was filtered through a membrane filter (PVDF, 0.2 μm).

Extraction methods A and B of the dry ice-partitioning QuEChERS as well as the citrate-buffering QuEChERS method are depicted in the diagram shown in Fig. 1.

2.5. Method performance and its comparative evaluation

2.5.1. Matrix effect and linearity

The matrix effect (ME) was evaluated by using a standard solution prepared in sample extract and pure solvent. The signal of the pesticide in matrix at 0.25 mg/kg was compared to that in solvent at the corresponding concentration. ME% was calculated via the following equation [38]:

$$\text{ME, \%} = \frac{(\text{peak area of matrix standard} - \text{peak area of solvent standard})}{\text{peak area of solvent standard}} \times 100$$

In view of the above equation, the positive and negative value of the ME% reflects the matrix-induced enhancement and suppression, respectively. No matrix effect is observed when ME% is equal to 0%.

Calibration curves of all the compounds in matrix were constructed by plotting the peak area against the concentration of the corresponding calibration standards at six concentration levels in a range of 0.04–0.5 mg/kg.

2.5.2. Recovery of the dry ice-partitioning QuEChERS method and comparative evaluation with recovery of the citrate-buffering QuEChERS method

The validity of the developed method was estimated by means of recovery experiments conducted at two fortification levels (0.025 and 0.25 mg/kg). All experiments were conducted in triplicate at each level.

To evaluate the acceptability of the developed method in routine pesticide multiresidue analysis, the mean recovery rates of the dry ice-partitioning QuEChERS method were compared with those resulting from the citrate-buffering QuEChERS method (QuEChERS-mini-multiresidue method for the analysis of pesticides), which was modified and validated for regulatory application in Germany as described at http://www.quechers.com [32,34] and was selected as the control method among the existing QuEChERS methods. The citrate-buffering QuEChERS method tested herein was modified at the amount of GCB. The recovery experiment conducted with 166 pesticides excluding TFNA and TFNG was carried out via the citrate-buffering QuEChERS method at 0.25 mg/kg in triplicate, and its procedure was briefly described in Section 2.4.2.2.

3. Results and discussion

3.1. Sample extraction

Carbon dioxide (CO_2) has been employed for chemical reactions, multi-industry (food, metal, nano, petroleum, pharmaceutical, plastic industries, etc.), and sample extractions, using diverse

Table 1
Electrospray ionization parameters of tandem mass spectrometry in positive or negative mode and liquid chromatographic separation conditions.

Parameter	Positive mode	Negative mode
ESI		
Capillary voltage	4000 V	3500 V
Gas temperature	350 °C	350 °C
Gas flow	10 L/min	10 L/min
Nebulizer gas	45 psi	45 psi
Nebulizer and collision gas	N_2	N_2
Chromatographic separation		
Column	YMC-Pack Pro C_8 (3.0 μm, 4.6 mm × 150 mm, YMC, Kyoto, Japan)	Eclipse Plus C_{18} (1.8 μm, 2.3 mm × 100 mm, Agilent, California, USA)
Column temp.	40 °C	40 °C
Flow rate	0.5 mL/min	0.2 mL/min
Injection volume	15 μL	5 μL
Mobile phase	A: 0.1% formic acid + 10 mmol ammonium acetate in water B: 0.1% formic acid in methanol–acetonitrile (7:3, v/v)	A: 0.1% formic acid in water B: acetonitrile

Gradient table	Positive mode			Negative mode		
	Time (min)	A	B	Time (min)	A	B
	0	95	5	0	98	2
	0.2	95	5	1	98	2
	2	40	60	4	50	50
	8	1	99	6	2	98
	11	1	99	11	2	98
	12	95	5	12	98	2
	16.5	95	5	16	98	2

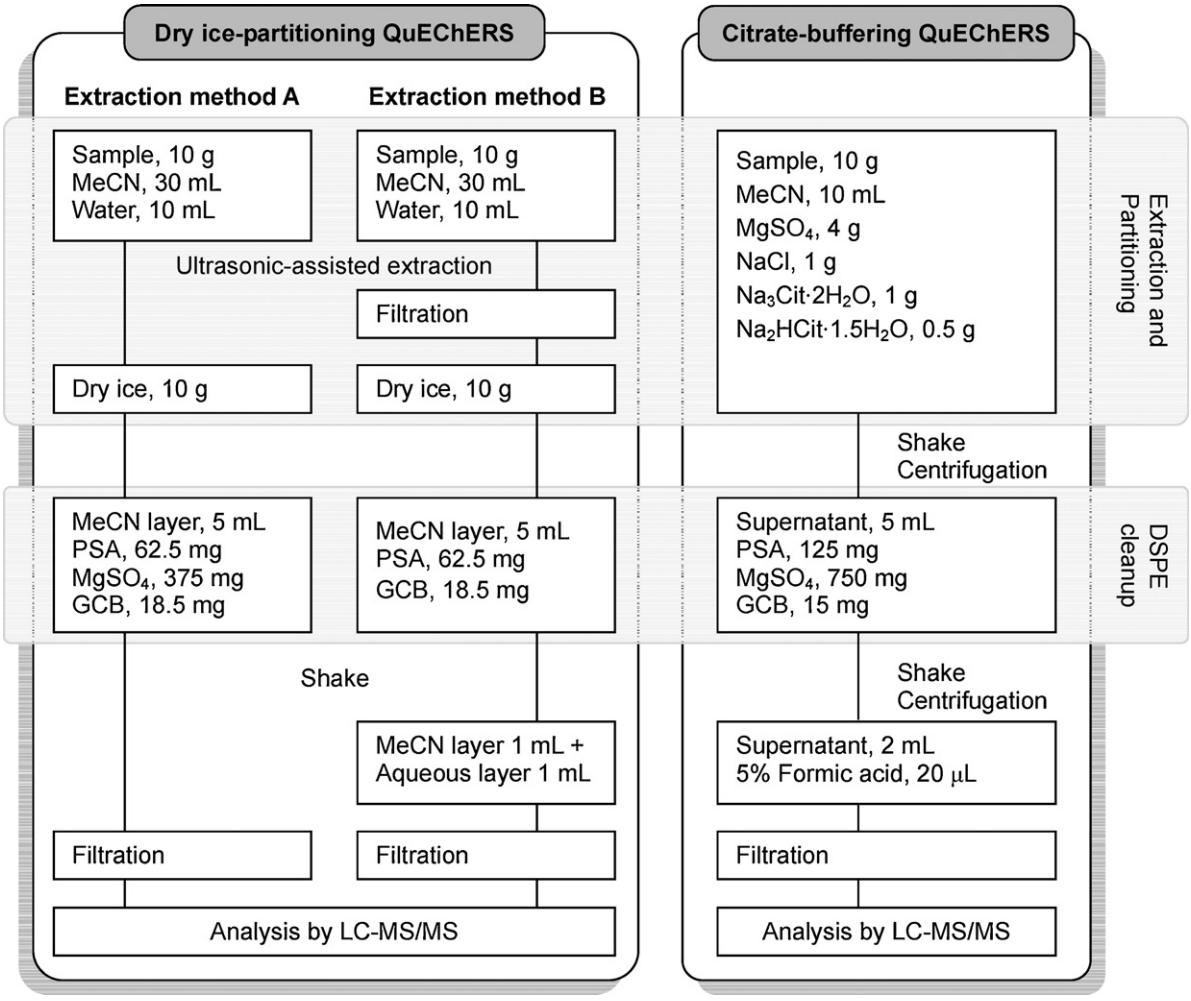

Fig. 1. The developed dry ice-partitioning QuEChERS method and the tested citrate-buffering QuEChERS method in this study.

Table 2
MRM data acquisition parameters of LC–MS/MS for the 168 pesticides selected and their lowest calibrated levels (LCLs) via the dry ice-partitioning QuEChERS method.

No.	Pesticide	t_R[a] (min)	Quantitation MRM 1	Quantitation MRM 2	Fragmentor (V)	CE 1 (V)	CE 2 (V)	LCL (mg/kg)
1	Abamectin	12.0	895 → 751	No	200	45	No	0.02
2	Acetamiprid	7.34	223 → 126	223 → 56	140	25	25	0.02
3	Acibenzolar-*S*-methyl	10.32	211 → 136	211 → 91	120	30	20	0.02
4	Acrinathrin	12.27	559 → 428	559 → 428	110	15	15	0.04
5	Alachlor	10.77	271 → 238	271 → 162	90	5	5	0.04
6	Aldicarb	8.35	213 → 89	213 → 116	110	15	15	0.02
7	Anilofos	11.09	368 → 199	368 → 171	110	10	10	0.02
8	Azinphos-methyl	9.70	318 → 125	318 → 167	90	15	15	0.02
9	Azoxystrobin	9.65	404 → 372	404 → 344	90	20	20	0.04
10	Benfuracarb	11.80	433 → 186	433 → 190	190	20	20	0.02
11	Benthiavalicarb	10.20	382 → 116	382 → 197	100	15	15	0.04
12	Bifenthrin	13.19	440 → 181	440 → 166	90	15	30	0.04
13	Bitertanol	11.03	338 → 99	338 → 70	90	10	10	0.04
14	Boscalid	10.10	343 → 307	343 → 140	110	20	20	0.02
15	Buprofezin	12.04	306 → 201	306 → 116	90	5	5	0.02
16	Butachlor	12.06	312 → 238	312 → 162	90	5	15	0.04
17	Cadusafos	11.08	271 → 159	271 → 131	90	10	10	0.04
18	Carbaryl	8.90	202 → 145	202 → 127	90	5	15	0.02
19	Carbendazim	6.70	192 → 160	192 → 132	120	20	20	0.02
20	Carbofuran	8.75	222 → 165	222 → 123	90	10	10	0.02
21	Carbosulfan	13.25	381 → 118	381 → 160	120	15	15	0.02
22	Chlorfluazuron	12.20	540 → 383	540 → 158	90	10	15	0.02
23	Chlorpyrifos-methyl	11.40	322 → 125	322 → 290	110	15	15	0.04
24	Chromafenozide	10.57	395 → 175	395 → 339	90	15	5	0.02
25	Clothianidin	7.19	250 → 169	250 → 132	70	10	10	0.02
26	Cyazofamid	10.60	325 → 108	325 → 261	90	10	10	0.02
27	Cyflufenamid	11.23	413 → 295	413 → 241	100	15	15	0.04
28	Cymoxanil	7.80	199 → 128	199 → 111	70	5	15	0.02
29	Cyproconazole	10.45	292 → 70	292 → 125	110	15	15	0.04
30	Cyprodinil	9.90	226 → 108	226 → 93	130	35	35	0.02
31	Deltamethrin	12.54	523 → 506	523 → 281	110	5	15	0.02
32	Diazinon	11.24	305 → 169	305 → 153	110	20	20	0.04
33	Dichlofluanid	10.75	333 → 224	333 → 123	110	20	20	0.02
34	Dichlorvos (DDVP)	8.66	221 → 109	221 → 145	110	15	10	0.02
35	Diethofencarb	9.85	268 → 226	268 → 180	90	10	10	0.04
36	Difenoconazole	11.30	406 → 251	406 → 337	130	20	20	0.04
37	Diflubenzuron	10.45	311 → 158	311 → 141	70	15	15	0.02
38	Dimepiperate	11.74	264 → 146	264 → 119	90	10	10	0.04
39	Dimethenamid	10.30	276 → 244	276 → 168	90	10	15	0.02
40	Dimethomorph	9.90	388 → 301	388 → 165	110	20	30	0.02
41	Dimethylvinphos	10.46	331 → 127	331 → 205	70	5	20	0.02
42	Dimethoate	7.52	230 → 199	230 → 171	90	5	5	0.02
43	Diniconazole	11.50	326 → 70	326 → 43	130	25	25	0.02
44	Dinotefuran	6.20	203 → 129	203 → 114	80	10	10	0.02
45	Diphenamid	9.70	240 → 134	240 → 167	110	20	20	0.04
46	Dithiopyr	11.56	402 → 354	402 → 334	110	15	15	0.04
47	Edifenphos	11.03	311 → 111	311 → 173	110	20	20	0.04
48	Emamectin	11.10	886 → 158	886 → 302	160	30	30	0.02
49	EPN	11.49	324 → 296	324 → 157	110	10	10	0.04
50	Esprocarb	12.10	266 → 91	266 → 71	110	20	20	0.04
51	Ethiofencarb	9.10	226 → 107	226 → 164	70	5	5	0.04
52	Ethoprophos	10.90	243 → 131	243 → 173	90	15	15	0.04
53	Etoxazole	12.27	360 → 141	360 → 177	130	20	20	0.02
54	Etrimfos	11.12	293 → 265	293 → 125	110	15	15	0.02
55	Fenamidone	10.00	312 → 236	312 → 103	110	10	10	0.02
56	Fenarimol	10.55	331 → 268	331 → 81	130	20	20	0.04
57	Fenazaquin	12.65	307 → 57	307 → 161	130	20	20	0.02
58	Fenbuconazole	10.50	337 → 70	337 → 125	110	15	15	0.04
59	Fenhexamid	10.65	302 → 97	302 → 55	130	25	25	0.04
60	Fenitrothion	10.30	278 → 125	278 → 109	110	20	20	0.02
61	Fenobucarb (BPMC)	10.05	208 → 95	208 → 57	90	15	15	0.02
62	Fenothiocarb	10.90	254 → 72	254 → 160	90	10	10	0.04
63	Fenoxanil	10.90	329 → 302	329 → 86	90	10	10	0.04
64	Fenpropathrin	12.30	350 → 125	350 → 97	90	10	30	0.04
65	Fenpyroximate	12.42	422 → 366	422 → 215	110	20	20	0.04
66	Fenthion	9.50	279 → 247	279 → 169	110	10	10	0.04
67	Ferimzone	10.92	255 → 132	255 → 117	110	10	20	0.02
68	Fipronil	10.80	437 → 368	437 → 255	150	15	30	0.02
69	**Flonicamid**	9.60	228 → 81	228 → 146	70	15	2	0.02
70	Fluacrypyrim	11.42	427 → 145	427 → 205	90	10	5	0.02
71	Flucythrinate	10.75	454 → 437	454 → 368	90	5	15	0.02
72	Flufenoxuron	12.00	489 → 158	489 → 141	110	20	25	0.04
73	Flumioxazin	9.60	355 → 299	355 → 176	90	10	20	0.04
74	Fluquinconazole	10.40	376 → 346	376 → 307	110	20	20	0.02

Table 2 (*Continued*)

No.	Pesticide	t_R[a] (min)	Quantitation MRM 1	Quantitation MRM 2	Fragmentor (V)	CE 1 (V)	CE 2 (V)	LCL (mg/kg)
75	Flusilazole	10.73	316 → 247	316 → 165	110	10	10	0.02
76	Flutolanil	10.20	324 → 262	324 → 242	110	15	15	0.02
77	Forchlorfenuron	9.20	248 → 129	248 → 155	90	15	15	0.02
78	Fosthiazate	9.20	284 → 104	284 → 228	90	15	15	0.02
79	Furathiocarb	12.00	383 → 195	383 → 167	110	15	15	0.04
80	Halfenprox	13.30	494 → 183	494 → 459	110	20	5	0.02
81	Hexaconazole	11.40	314 → 70	314 → 159	110	15	25	0.04
82	Imibenconazole	11.90	411 → 125	411 → 171	110	20	20	0.02
83	Imidacloprid	7.19	256 → 209	256 → 175	90	10	10	0.02
84	Indoxacarb	11.25	528 → 150	528 → 249	130	20	20	0.04
85	Iprobenfos (IBP)	10.87	289 → 91	289 → 205	90	15	5	0.04
86	Iprodione	10.58	330 → 245	330 → 288	110	10	10	0.02
87	Iprovalicarb	10.45	321 → 119	321 → 203	90	15	5	0.04
88	Isoprocarb	9.50	194 → 95	194 → 43	90	10	10	0.04
89	Isoprothiolane	10.47	291 → 231	291 → 189	90	5	5	0.02
90	Kresoxim-methyl	10.92	314 → 222	314 → 267	90	5	5	0.04
91	Lufenuron	11.70	511 → 158	511 → 141	110	20	20	0.04
92	Malathion	10.40	331 → 127	331 → 285	90	5	5	0.02
93	Mefenacet	10.30	299 → 148	299 → 192	90	10	10	0.02
94	Mepanipyrim	10.36	224 → 77	No	110	35	No	0.02
95	Mepronil	10.30	270 → 119	No	110	20	No	0.02
96	Metalaxyl-M	9.55	280 → 220	280 → 192	90	10	10	0.04
97	Metamifop	11.50	441 → 288	441 → 180	110	20	20	0.04
98	Metconazole	11.20	320 → 70	320 → 125	110	25	30	0.04
99	Methabenzthiazuron	9.12	222 → 165	222 → 150	90	10	20	0.02
100	Methidathion	9.80	303 → 145	303 → 85	90	5	5	0.02
101	Methiocarb	10.01	226 → 169	226 → 121	90	5	5	0.04
102	Methomyl	6.80	163 → 88	163 → 106	70	5	5	0.02
103	Methoxyfenozide	10.15	369 → 149	369 → 133	70	15	15	0.02
104	Milbemectin	12.89	551 → 337	No	250	34	No	0.02
		12.89	565 → 337	No	250	34	No	
105	Molinate	10.68	188 → 126	188 → 83	90	10	10	0.04
106	Myclobutanil	10.40	289 → 70	289 → 125	130	15	15	0.04
107	Napropamide	10.52	272 → 171	272 → 129	90	15	15	0.02
108	**Novaluron**	10.00	491 → 471	491 → 305	110	10	10	0.04
109	Nuarimol	9.80	315 → 252	315 → 81	130	20	20	0.04
110	Ofurace	8.85	282 → 254	282 → 236	110	5	5	0.04
111	Oxadiazon	12.01	345 → 303	345 → 220	130	10	10	0.04
112	Oxadixyl	8.10	279 → 219	No	100	5	No	0.02
113	Oxamyl	6.45	237 → 72	237 → 90	50	5	5	0.02
114	Paclobutrazol	10.10	294 → 70	294 → 125	110	15	30	0.02
115	Parathion-ethyl	10.95	292 → 236	292 → 264	90	10	10	0.04
116	Penconazole	11.15	284 → 70	284 → 159	90	15	15	0.02
117	Pencycuron	11.39	329 → 125	329 → 218	90	15	15	0.04
118	Pendimethalin	12.19	282 → 212	282 → 194	90	5	10	0.04
119	Pentoxazone	11.80	354 → 286	354 → 186	90	10	20	0.02
120	Phenthoate	10.96	321 → 247	321 → 163	90	5	5	0.04
121	Phorate	11.45	261 → 75	261 → 47	70	5	20	0.02
122	Phosalone	11.32	368 → 182	368 → 322	110	10	10	0.04
123	Piperophos	11.54	354 → 171	354 → 213	130	20	20	0.04
124	Pirimicarb	9.00	239 → 72	239 → 182	90	20	20	0.04
125	Pirimiphos-methyl	11.88	306 → 164	306 → 108	110	20	20	0.02
126	Probenazole	8.50	224 → 41	224 → 39	90	10	20	0.04
127	Prochloraz	11.15	376 → 308	376 → 266	90	5	15	0.02
128	Profenofos	11.90	375 → 305	375 → 347	130	10	10	0.04
129	Propamocarb	5.80	189 → 102	189 → 74	80	15	15	0.02
130	Propanil	10.14	218 → 162	218 → 127	110	15	15	0.04
131	Pymetrozine	5.91	218 → 105	218 → 79	90	15	30	0.02
132	Pyraclofos	11.11	361 → 257	361 → 195	130	15	15	0.02
133	Pyraclostrobin	11.00	388 → 194	388 → 296	90	10	10	0.04
134	Pyrazophos	11.10	374 → 222	374 → 238	130	15	15	0.02
135	Pyributicarb	12.00	331 → 133	331 → 108	120	25	25	0.02
136	Pyridaben	12.70	365 → 309	365 → 147	90	10	10	0.02
137	Pyridalyl	13.40	492 → 183	492 → 109	90	12	12	0.02
138	Pyrimethanil	9.98	200 → 82	200 → 107	130	35	35	0.02
139	Pyrimidifen	11.66	378 → 184	378 → 150	130	25	20	0.02
140	Pyriminobac-methyl	10.06	362 → 330	362 → 256	110	10	20	0.02
141	Pyriproxyfen	12.00	322 → 96	322 → 185	110	20	20	0.04
142	Pyroquilon	8.50	174 → 132	174 → 117	90	20	20	0.04
143	Quinoclamine	8.35	208 → 105	208 → 172	110	25	25	0.02
144	Simeconazole	10.56	294 → 70	294 → 135	110	15	15	0.02
145	Spinosyn A	8.80	732 → 142	732 → 98	150	20	40	0.02
	Spinosyn D	8.80	746 → 142	746 → 98	150	20	40	
146	Tebuconazole	10.97	308 → 70	308 → 125	130	20	25	0.02
147	Tebufenpyrad	11.86	334 → 145	334 → 171	130	25	25	0.04

Table 2 (*Continued*)

No.	Pesticide	t_R[a] (min)	Quantitation MRM 1	Quantitation MRM 2	Fragmentor (V)	CE 1 (V)	CE 2 (V)	LCL (mg/kg)
148	Tebupirimfos	12.25	319 → 277	319 → 153	110	10	10	0.04
149	Teflubenzuron	11.77	381 → 158	381 → 141	90	15	15	0.04
150	Terbuthylazine	10.24	230 → 174	No	100	15	No	0.02
151	Tetraconazole	10.34	372 → 159	372 → 70	150	25	25	0.02
152	Thiacloprid	7.55	253 → 126	253 → 90	110	25	25	0.02
153	Thiamethoxam	6.78	292 → 211	292 → 132	90	10	10	0.02
154	Thiazopyr	11.02	397 → 377	397 → 335	150	25	25	0.04
155	Thiodicarb	8.50	355 → 88	355 → 163	90	10	10	0.02
156	Thiophanate-methyl	8.30	343 → 151	343 → 311	100	15	15	0.02
157	Tolclofos-methyl	11.35	301 → 269	301 → 175	130	15	15	0.02
158	Tolylfluanid	11.09	347 → 137	347 → 238	50	15	15	0.02
159	**TFNA**	11.11	190 → 146	190 → 69	50	3	25	
160	**TFNG**	9.27	247 → 146	247 → 163	50	10	10	
161	Triadimefon	10.39	294 → 197	294 → 99	100	10	10	0.02
162	Triadimenol	10.40	296 → 70	296 → 99	70	20	20	0.06
163	Triazophos	10.30	314 → 162	314 → 178	100	15	15	0.02
164	Tricyclazole	7.72	190 → 163	190 → 136	90	20	20	0.02
165	Trifloxystrobin	11.47	409 → 186	409 → 116	90	20	20	0.04
166	Triflumizole	11.70	346 → 278	346 → 73	50	5	5	0.04
167	Triflumuron	11.00	359 → 156	359 → 139	100	15	15	0.04
168	Zoxamide	11.30	336 → 187	336 → 204	110	20	15	0.02

Bolds are detected in negative ionization mode.

[a] Retention time.

phases, including gaseous, liquid, solid, and supercritical phases. In the food industry, liquid, solid, and supercritical CO_2 are utilized for food distribution, storage, and processing, in particular, supercritical CO_2 is frequently employed for the extraction of phytochemicals [39] or organic contaminants, including pesticides [40], veterinary drugs [41], and persistent organic pollutants [42] from foods or environmental samples. In the monitoring of pesticide residues in foods, solid CO_2 (dry ice) is used as a sample processing step to produce cryogenic conditions, in order to prevent the denaturalization of samples and analytes; this is unlike the supercritical CO_2 extraction method [11,19,24]. In this study, however, dry ice was creatively employed to enhance partitioning for sample extraction.

This study was conducted as part of a broader effort to develop a determination method for flonicamid and its metabolites (TFNA and TFNG), thus helping to resolve the international trade issues regarding these compounds in paprika trade. Unfortunately, the extraction efficiencies of TFNA and TFNG did not satisfy our expectations in the citrate-buffering QuEChERS method, as they provided efficiencies of <20%. The remaining TFNA and TFNG residues were anticipated to be in the aqueous phase in a centrifuge tube. Herein, the introduction of dry ice into a miscible liquid of MeCN and water proved able to separate both layers from one another.

The separation of sample extract was induced via the sublimation of dry ice, which occurs at −78.5 °C at atmospheric pressure (1 atm) [43]. After 2–3 min of dry ice sublimation with the sample extract, the reduced temperatures of MeCN and water ranged from −4.0 to −5.0 °C and from −6.0 to −6.5 °C, respectively, and water was iced and super cooled in this study. The negative temperatures of the two solvents may reduce their entropies and allow them to separate. The density of ice and the supercooled water (from −10 to 0 °C) are 0.917 and 0.9981–0.9998 g/mL, which are heavier than that (0.8035 g/mL) of MeCN at 0 °C [44]. Therefore, water changed to ice and the supercooled water was separated with a MeCN layer from the mixed solution.

The citrate-buffering QuEChERS method uses 10 g of sample and 10 mL of MeCN in the literature [34]; however, in the present study, 30 mL of MeCN and an additional 10 mL of water was used for 10 g of sample since the phase separation of the sample extract with dry ice was not achieved with volumes of 10 or 20 mL of MeCN and the sample moisture adopted in the preliminary study. In order to induce sufficient sample extract separation via dry ice sublimation, 30 mL of MeCN and an additional 10 mL of water proved necessary.

Sorbents, such as 25 mg PSA, 150 mg $MgSO_4$, and 7.5 mg GCB per gram equivalent of sample were utilized for DSPE cleanup in the citrate-buffering QuEChERS method [34]. As a 5 mL portion of 30 mL sample extract from 10 g sample corresponds with 1.67 g of sample weight, our method should use 1.67-fold amounts of sorbents according to the citrate-buffering QuEChERS method. Anastassiades et al., the pioneer of the QuEChERS method, initially determined that 4-fold amounts of PSA per gram equivalent sample, along with $MgSO_4$ did not lead to a loss of the tested pesticides from the PSA capacity test in the DSPE cleanup [11]. Although GCB was also utilized at approximately 2.5-fold amounts in the tested dry ice-partitioning QuEChERS method, the 2.5-fold amounts of sorbents used in that QuEChERS method were proved adequate to produce good recoveries in the majority of cases.

Our dry ice-partitioning QuEChERS method described herein consisted of the previously elucidated extraction methods A and B. The key process in extraction method B was the withdrawal of the aqueous layer, and the filtration of the sample extract facilitated withdrawal without matrix interference. Anhydrous $MgSO_4$ was also absent in extraction method B, unlide extraction method A of the dry ice-partitioning QuEChERS method. In the preliminary trials, a portion of the MeCN extract purified with sorbents containing $MgSO_4$ could be mixed with the same portion of the aqueous extract at first. However, the mixed final paprika extract separated into two layers again 7–10 h later; that separation was prevented by the omission of $MgSO_4$. The salting-out effect of $MgSO_4$ is believed to be the cause of the subsequent separation.

In the dry ice-partitioning QuEChERS method, the use of anhydrous $MgSO_4$ and NaCl for salting-out was unnecessary in the extraction and partitioning steps, and the withdrawal and analysis of the aqueous layer was permitted following simple filtration with a membrane filter. The next sample preparations after the separation of the layers should be carried out within 30 min, during which time the layers remained separate (until reaching a temperature of 7–8 °C).

3.2. Method performance and its comparative evaluation

3.2.1. Matrix effect, linearity, and lowest calibrated level

The matrix effect is regarded as a signal suppression or enhancement of the analyte due to the co-elution of matrix components [35,36,45,46]. The matrix effect also depends strongly on the chem-

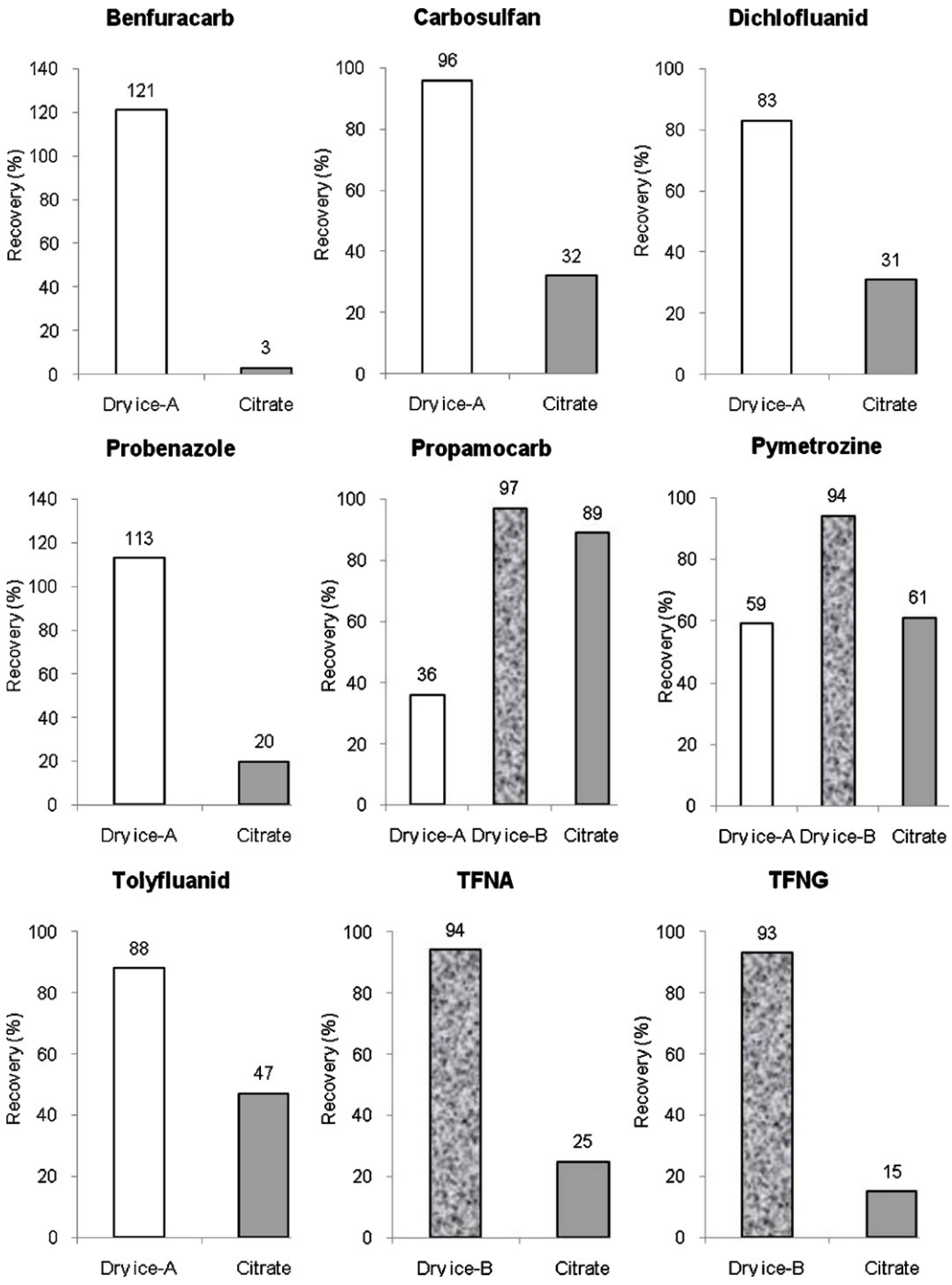

Fig. 2. Significant differences in recovery of the highlighted pesticides extracted via the dry ice-partitioning QuEChERS method or citrate-buffering QuEChERS method at 0.25 mg/kg; described as Dry ice-A for extraction method A of the dry ice-partitioning QuEChERS method and Dry ice-B for extraction method B; as Citrate for the citrate-buffering QuEChERS method.

ical nature of the analyte and the sample preparation procedure utilized. Certain instrumental parameters, such as the ionization source, ionization mode, flow rate, and mobile-phase composition have been reported to influence the matrix effect [35,36,45]. Since suppression or enhancement of the analyte response can vary considerably from matrix to matrix and differs substantially in pure solvent and in matrix, it is essential to take into account assessments of the matrix effect and/or the use of matrix-matched calibration standards in order to minimize quantitative errors of pesticide residues. The matrix effect was generally assessed by comparing not only each slope generated from calibration curve matching matrix [37,38,47], but also from each peak area detected at representative concentrations prepared in matrix extract or solvent [17,24,46]. In this study, the peak areas of the pesticides at a level of 0.25 mg/kg prepared in paprika extract or solvent were compared. The calculated matrix effects were less than ±20% in all pesticides. This effect was not considered to be significant and was rather mild, as previously demonstrated in the study of Kmellar et al. [47].

As another approach for quantitative analysis compensating for the matrix effect, matrix-matched calibration is frequently employed. The matrix-matched calibration curves for the pesti-

Table 3
List of pesticides grouped by their overall recoveries from two spiking levels (0.05 and 0.25 mg/kg, $n = 3$, respectively, carried out via the dry ice-partitioning QuEChERS method) or at 0.25 mg/kg ($n = 3$, carried out via the citrate-buffering QuEChERS method) in paprika.

Recovery	Pesticides
Dry ice-partitioning QuEChERS method	
>120%	Anilofos, azoxystrobin, benfuracarb, bitertanol, buprofezin, butachlor, chromafenozide, cyflufenamid, diethofencarb, dimepiperate, edifenphos, EPN, esprocarb, ethiofencarb, etoxazole, etrimfos, fenamidone, fenarimol, fenazaquin, fenbuconazole, fenhexamid, fenthion, flucythrinate, flusilazole, flutolanil, indoxacarb, isoprothiolane, kresoxim-methyl, mepronil, metalaxyl-M, methidathion, methoxyfenozide, nuarimol, ofurace, pendimethalin, pentoxazone, phenthoate, phorate, prochloraz, pyraclostrobin, pyributicarb, pyridaben, pyriminobac-methyl, tetraconazole, thiacloprid, thiazopyr, triazophos, trifloxystrobin, zoxamide
111–120%	Abamectin, acetamiprid, alachlor, aldicarb, azinphos-methyl, benthiavalicarb, bifenthrin, boscalid, cadusafos, carbaryl, carbofuran, carbosulfan, chlorfluazuron, chlorpyrifos-methyl, cyazofamid, cymoxanil, cyproconazole, cyprodinil, deltamethrin, diazinon, difenoconazole, dimethenamid, dimethylvinphos, dimethoate, diniconazole, diphenamid, dithiopyr, emamectin, fenitrothion, fenobucarb (BPMC), fenothiocarb, fenoxanil, fenpropathrin, fenpyroximate, ferimzone, fipronil, flonicamid, fluacrypyrim, flufenoxuron, flumioxazin, fluquinconazole, forchlorfenuron, fosthiazate, hexaconazole, iprobenfos (IBP), iprodione, iprovalicarb, isoprocarb, lufenuron, malathion, mefenacet, mepanipyrim, metamifop, metconazole, methabenzthiazuron, methiocarb, myclobutanil, napropamide, oxadiazon, paclobutrazol, parathion-ethyl, penconazole, pencycuron, phosalone, piperophos, pirimicarb, pirimiphos-methyl, probenazole, profenofos, pyraclofos, pyrazophos, pyrimethanil, pyriproxyfen, pyroquilon, quinoclamine, simeconazole, spinosad, tebuconazole, tebufenpyrad, tebupirimfos, terbuthylazine, thiophanate-methyl, tolclofos-methyl, triadimefon, triadimenol, triflumizole, triflumuron
91–110%	Acibenzolar-*S*-methyl, acrinathrin, carbendazim, clothianidin, diflubenzuron, dimethomorph, ethoprophos, furathiocarb, halfenprox, imibenconazole, imidacloprid, methomyl, milbemectin, molinate, novaluron, oxadixyl, oxamyl, propamocarb, propanil, pymetrozine, pyridalyl, pyrimidifen, teflubenzuron, thiamethoxam, thiodicarb, tolyfluanid, TFNA, TFNG, tricyclazole
70–90%	Dichlofluanid, dichlorvos (DDVP), dinotefuran
Citrate-buffering QuEChERS method	
>120%	Chlorfluazuron, deltamethrin, flonicamid, pentoxazone, profenofos
111–120%	Cyazofamid, flufenoxuron, milbemectin, novaluron, teflubenzuron, tolclofos-methyl
91–110%	Acetamiprid, acibenzolar-*S*-methyl, acrinathrin, alachlor, aldicarb, anilofos, azinphos-methyl, azoxystrobin, benthiavalicarb, bifenthrin, bitertanol, boscalid, buprofezin, butachlor, cadusafos, carbaryl, carbendazim, carbofuran, chlorpyrifos-methyl, chromafenozide, clothianidin, cyflufenamid, cymoxanil, cyproconazole, cyprodinil, diazinon, diethofencarb, difenoconazole, diflubenzuron, dimepiperate, dimethenamid, dimethomorph, dimethylvinphos, dimethoate, diniconazole, diphenamid, dithiopyr, edifenphos, emamectin, EPN, esprocarb, ethiofencarb, ethoprophos, etoxazole, etrimfos, fenamidone, fenarimol, fenbuconazole, fenitrothion, fenobucarb (BPMC), fenothiocarb, fenoxanil, fenthion, ferimzone, fipronil, fluacrypyrim, flucythrinate, flumioxazin, fluquinconazole, flusilazole, flutolanil, forchlorfenuron, fosthiazate, furathiocarb, halfenprox, hexaconazole, imibenconazole, imidacloprid, indoxacarb, iprobenfos (IBP), iprodione, iprovalicarb, isoprocarb, isoprothiolane, kresoxim-methyl, lufenuron, malathion, mefenacet, mepanipyrim, mepronil, metalaxyl-M, metamifop, metconazole, methabenzthiazuron, methidathion, methiocarb, methomyl, methoxyfenozide, myclobutanil, napropamide, nuarimol, ofurace, oxadiazon, oxadixyl, oxamyl, paclobutrazol, parathion-ethyl, penconazole, pencycuron, pendimethalin, phenthoate, phorate, phosalone, piperophos, pirimicarb, pirimiphos-methyl, prochloraz, propamocarb, propanil, pyraclofos, pyraclostrobin, pyrazophos, pyributicarb, pyridalyl, pyrimethanil, pyrimidifen, pyriminobac-methyl, pyroquilon, quinoclamine, simeconazole, spinosad, tebuconazole, tebufenpyrad, terbuthylazine, tetraconazole, thiacloprid, thiamethoxam, thiazopyr, thiodicarb, thiophanate-methyl, triadimefon, triadimenol, triazophos, tricyclazole, trifloxystrobin, triflumizole, triflumuron, zoxamide
70–90%	Abamectin, dichlorvos (DDVP), dinotefuran, fenazaquin, fenhexamid, fenpropathrin, fenpyroximate, molinate, pyridaben, pyriproxyfen, tebupirimfos
<70%	Benfuracarb, carbosulfan, dichlofluanid, probenazole, pymetrozine, tolyfluanid, TFNA, TFNG

Propamocarb, pymetrozine, TNFA, and TNFG were examined at 0.25 mg/kg via extraction method B of the dry ice-partitioning QuEChERS method.

cides fell within a range of 0.04–0.5 mg/kg, and good linearity was detected for most pesticides with correlation coefficients (r^2) better than 0.99.

To assess the sensitivity or detectability of the developed method, the lowest calibrated level (LCL) was examined using matrix-matched standard solutions, since a signal-to-noise ratio from the instrumental software is not necessarily appropriate for all pesticides and matrices [48]. The LCLs for each pesticide were 0.02 or 0.04 mg/kg, as illustrated in Table 2. Although the LCLs of many analytes were 0.02 mg/kg, the lowest levels of the matrix-matched calibration ranges for them were measured to be 0.04 mg/kg.

3.2.2. *Recovery of the dry ice-partitioning QuEChERS method and comparative evaluation with recovery of the citrate-buffering QuEChERS method*

It is recommended that an internal standard (IS) be used in order to implement and assure correct quantification and execution, according to AOAC Official Method 2007.01 [16] and European Standard EN 15662 [20], and Codex [49] and SANCO [50] guidelines. Since our study used relative single point matrix-matched external standard solutions to calculate the recovery percentages for the validity of the developed QuEChERS method, it should be mentioned that there was some divergence in the results of comparative analysis of recovery results from the dry ice-partitioning QuEChERS method and the citrate-buffering QuEChERS method. The dry

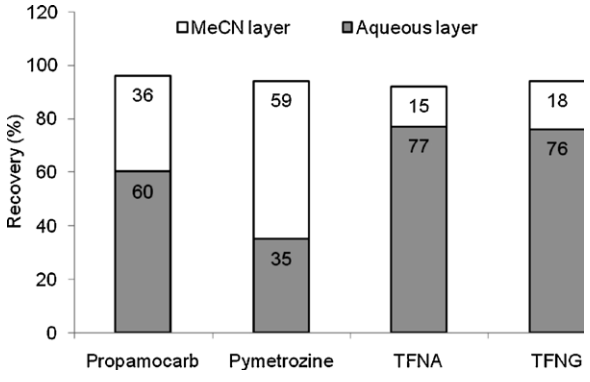

Fig. 3. Recovery of the analyte in each separated layer via extraction method B at 0.25 mg/kg.

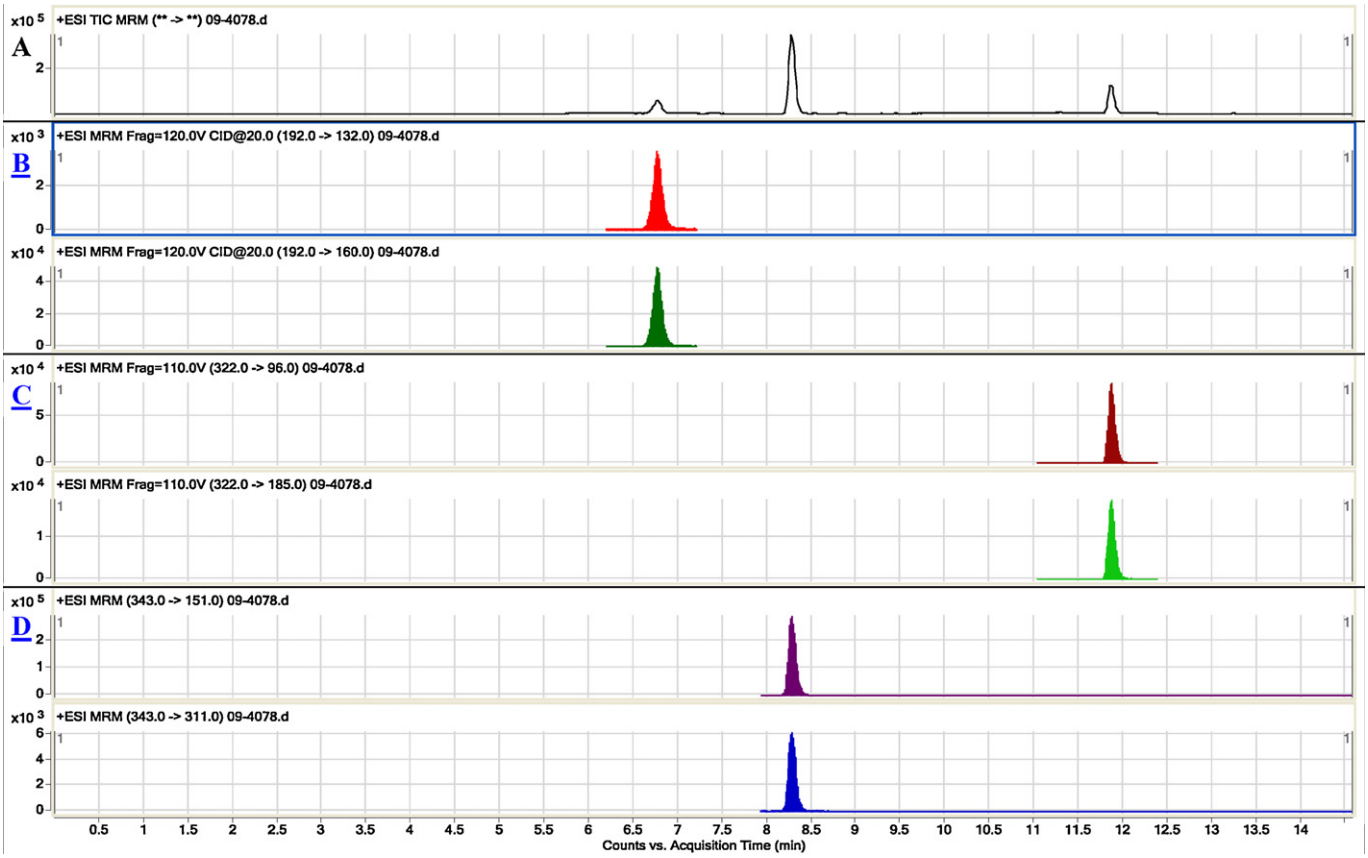

Fig. 4. A representative chromatogram of real sample—(A) TIC of detected pesticides; (B) first peak referred to carbendazim: 0.135 mg/kg; (C) pyriproxyfen: 0.039 mg/kg; (D) thiophanate-methyl: 0.218 mg/kg.

ice-partitioning QuEChERS method consisted of extraction method A and extraction method B, and the methods were employed to recover 168 pesticides from paprika samples fortified at 0.05 and 0.25 mg/kg, in triplicate. The recoveries of the citrate-buffering QuEChERS method at 0.25 mg/kg ($n = 3$) were also tested to produce a comparative rating with those of the developed method. Table 3 shows lists of pesticides in the given categories which are the range of overall recoveries of the analytes resulting from dry ice-partitioning or citrate-buffering QuEChERS. All RSDs were less than 20% in the tested two QuEChERS methods. Approximately 52% (87 pesticides) of the tested (168) pesticides yielded recoveries of 111–120%, 17% (29 pesticides) yielded recoveries of 91–110%, 2% (3 pesticides) yielded recoveries of 70–90%, and 29% (49 pesticides) yielded recoveries of more than 120% in the dry ice-partitioning QuEChERS method, whereas in the citrate-buffering QuEChERS method, approximately 3% (6 pesticides) yielded recoveries of 111–120%, 82% (138 pesticides) yielded recoveries of 91–110%, 7% (11 pesticides) yielded recoveries of 70–90%, 3% (5 pesticides) yielded recoveries of more than 120%, and 5% (8 pesticides) yielded recoveries of less than 70%. The majority of the pesticides tested via the dry ice-partitioning QuEChERS method were grouped into 111–120% and >120% recovery categories, while those tested by the citrate-buffering QuEChERS method all fell within 91–110% recovery, as shown in Table 3. The relatively high recovery in the dry ice-partitioning QuEChERS method may be attributable to the fact that MeCN extract (5 mL) after phase separation via dry ice is dehydrated by $MgSO_4$ during DSPE cleanup, and thus the concentrations of the pesticides are relatively increased. Additionally, the differences in the density of the MeCN extract by temperature and the consequential change in concentration of the analytes probably resulted in errors in the dry ice-partitioning QuEChERS method.

The pesticides for which recoveries of less than 70% were obtained in the citrate-buffering QuEChERS method evidenced precisely opposite results in the dry ice-partitioning QuEChERS method, as is shown in Fig. 2. At a fortification level of 0.25 mg/kg, the recovery values of benfuracarb (3%), carbosulfan (32%), dichlofluanid (31%), probenazole (20%), and tolylfluanid (47%) in the citrate-buffering QuEChERS method were increased dramatically to 121, 96, 83, 113, and 88%, respectively, via the dry ice-partitioning QuEChERS method (extraction method A). Dichlofluanid and tolylfluanid are representative *N*-trihalomethylthio fungicides, and are also well-known base-labile pesticides [12–14,16]. Despite neutral conditions, the reasonable recoveries of dichlofluanid (83%) and tolylfluanid (88%) from our dry ice-partitioning QuEChERS method probably led to a minimization of analyte degradation due to the cryogenic conditions afforded by dry ice during the sample processing and extraction steps [14,51]. Our neutral and cryogenic sample extraction conditions were also likely to contribute to good recoveries of benfuracarb and carbosulfan along with probenazole. In order to increase the recovery levels of benfuracarb and carbosulfan, the citrate-buffering QuEChERS method demands that samples be quickly analyzed, or that the pH adjustment of the cleaned extract via the addition of 5% formic acid be skipped [34], because these pesticides are readily hydrolyzed to carbofuran under acidic conditions [34,52]. However, Tsiplakou et al. obtained an acceptable recovery rate of carbosulfan from the pH adjustment in the citrate-buffering QuEChERS method, and the sample preparation in that study was decisively conducted under cryogenic conditions [22].

Meanwhile, the recoveries of propamocarb (36%) and pymetrozine (59%) were not unexpectedly unacceptable in the dry ice-partitioning QuEChERS method (extraction method A).

However, the application of extraction method B for propamocarb and pymetrozine raised the recovery to allowable values, 97 and 94%, respectively. Additionally, the recovery of TFNA was improved from 25% (resulted from the citrate-buffering QuEChERS method) to 94% via extraction method B of the dry ice-partitioning QuEChERS method; likewise, the recovery of TFNG was increased from 15% to 93%. Additionally, the residual concentrations of the four analytes in each separated layer (MeCN and aqueous layers) prior to being mixed, in extraction method B, were determined and aggregated (Fig. 3). Consequently, it has been proposed that extraction method B of the developed dry ice-partitioning QuEChERS method was able to increase the recoveries of propamocarb, pymetrozine, TFNA, and TFNG by withdrawing the aqueous layer containing the remainder of their residues.

Therefore, extraction method A proved suitable for 164 pesticides and extraction method B proved suitable for 4 compounds (propamocarb, pymetrozine, TFNA and TFNG) among the 168 analytes.

Although there are quantitative errors able to be resolved by using an IS, a new QuEChERS method using dry ice-partitioning was developed herein. It is somewhat difficult to evaluate with any discernment the dry ice-partitioning and citrate-buffering QuEChERS methods in terms of their ease and rapidity, but the dry ice-partitioning QuEChERS method is clearly cheaper and more eco-friendly, due to the fact that it requires no salting-out and buffering reagents during the extraction and partitioning steps.

3.3. Method application

The developed dry ice-partitioning QuEChERS method was applied to 50 samples collected from local paprika farmers in Hwasun and Gangjin, Republic of Korea, in April–June 2010. There were two positive samples containing residues of acetamiprid, carbendazim, imidacloprid, pyriproxyfen, and thiopanate-methyl, and all of the residues were lower their regulatory levels of the Republic of Korea. The representative chromatograms of the one positive sample are shown in Fig. 4.

4. Conclusion

A modified QuEChERS method coupled with LC–MS/MS was developed in the present study, and referred to as the dry ice-partitioning QuEChERS method. The dry ice-partitioning QuEChERS method uses dry ice to separate a sample extract into a MeCN layer and an aqueous layer without the need for salting-out and centrifugation. The method developed and described herein involved extraction method A – which detect the MeCN layer for general pesticides, and extraction method B – which combined the MeCN and the aqueous layer for propamocarb, pymetrozine, and flonicamid metabolites (TFNA and TFNG). Although only four pesticides were tested via extraction method B in this study, method B is likely to be appropriate for pesticides amenable to LC–MS/MS. Additionally, extraction method A can be readily applied to pesticides aimed at GC–MS (/MS) analysis. Partitioning with dry ice allowed for sample extraction steps under cryogenic conditions and determination of the aqueous layer. The cryogenic sample processing and the use of the aqueous layer improved the recovery rates to acceptable ranges for some interesting pesticides, including benfuracarb, carbosulfan, dichlofluanid, probenazole, tolylfluanid, and flonicamid metabolites (TFNA and TFNG). The dry ice-partitioning QuEChERS method can be employed to detect a broad range of pesticides and may be worth considering as a multiresidue analysis method for pesticides in foods.

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