Journal of Chromatography A, 1217 (2010) 6692–6703

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

High throughput analysis of 150 pesticides in fruits and vegetables using QuEChERS and low-pressure gas chromatography–time-of-flight mass spectrometry

Urairat Koesukwiwat[a,b], Steven J. Lehotay[a,*], Shui Miao[c], Natchanun Leepipatpiboon[b]

[a] US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA
[b] Chromatography and Separation Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
[c] Shanghai Institute for Food and Drug Control, 1500 Zhang-Heng Road, Shanghai 201203, China

ARTICLE INFO

Article history:
Available online 12 May 2010

Keywords:
QuEChERS
Sample throughput
Pesticide analysis
Vacuum-outlet gas chromatography
Time-of-flight mass spectrometry

ABSTRACT

A higher monitoring rate is highly desirable in the labs, but this goal is typically limited by sample throughput. In this study, we sought to assess the real-world applicability of fast, low-pressure GC–time-of-flight MS (LP-GC/TOFMS) for the identification and quantification of 150 pesticides in tomato, strawberry, potato, orange, and lettuce samples. Buffered and unbuffered versions of QuEChERS (which stands for "quick, easy, cheap, effective, rugged, and safe") using dispersive solid-phase extraction (d-SPE) and disposable pipette extraction (DPX) for clean-up were compared for sample preparation. For clean-up of all sample types, a combination of 150 mg $MgSO_4$, 50 mg primary secondary amine (PSA), 50 mg C_{18}, and 7.5 mg graphitized carbon black (GCB) per mL extract was used. No significant differences were observed in the results between the different sample preparation versions. QuEChERS took <10 min per individual sample, or <1 h for two chemists to prepare 32 pre-homogenized samples, and using LP-GC/TOFMS, <10 min run time and <15 min cycle time allowed >32 injections in 8 h. Overall, >126 analytes gave recoveries (3 spiking levels) in the range of 70–120% with <20% RSD. The results indicate that LP-GC/TOFMS for GC-amenable analytes matches UHPLC–MS/MS in terms of sample throughput and turnaround time for their routine, concurrent use in the analysis of a wide range of analytes in QuEChERS extracts to achieve reliable quantification and identification of pesticide residues in foods.

Published by Elsevier B.V.

1. Introduction

Hundreds of pesticides are widely used in current agricultural practices around the world, and it is not uncommon for residues of these pesticides to occur in food products, especially in fruits and vegetables. Many control authorities have established maximum residue limits (MRLs) or tolerances to protect the environment and consumer health [1,2]. Due to consumer awareness of potentially hazardous pesticide residues in foods, international trade issues, regulatory requirements, risk assessment and other reasons, monitoring of food items for pesticide residues is often conducted in government and private contract labs worldwide. To meet the demands of consumers, farmers, business interests, and regulators, the analytical methodology for pesticide residue determinations in complex matrices is continually improving as new technologies are being introduced. One of the longest held goals in the routine monitoring of pesticide residues by regulatory and private contract laboratories is to attain quick sample turnaround time and high sample throughput. In addition to being fast, useful methods must also achieve high quality results for a wide scope of analytes and matrices, have excellent robustness for routine use, meet low detection limits, and be affordable, simple to perform, environmentally friendly, and safe.

A major development in sample preparation involves the streamlined features of the QuEChERS (quick, easy, cheap, effective, rugged, and safe) approach for pesticide residue analysis of foods [4–16]. QuEChERS itself has evolved from the original unbuffered version [4] to a pair of multi-laboratory validated methods using acetate buffering (AOAC Official Method 2007.01) [5,6] or citrate buffering (CEN Standard Method EN 15662) [7,8]. These and other versions of QuEChERS have been adopted in many monitoring laboratories worldwide due to their beneficial features as described in its name. Currently, at least a dozen companies are marketing QuEChERS compatible products, including dispersive solid-phase extraction (d-SPE) and disposable pipette extraction (DPX) [17,18].

QuEChERS approaches typically use acetonitrile (MeCN) for extraction of a 10–15 g well-homogenized sample followed by salt-out partitioning of the water from the sample using anhydrous

* Corresponding author. Tel.: +1 215 233 6433; fax: +1 215 233 6642.
E-mail address: steven.lehotay@ars.usda.gov (S.J. Lehotay).

0021-9673/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.chroma.2010.05.012

$MgSO_4$, NaCl, and/or buffering agents, and further clean-up using d-SPE with anhydrous $MgSO_4$, primary secondary amine (PSA) and/or in combination with C_{18} and graphitized carbon black (GCB) sorbents. QuEChERS is a very flexible template and has been modified for different purposes depending on the analytes, matrices, analytical instruments, and analyst preferences. A recent comparison of main QuEChERS versions indicates that acetate buffering for extraction and use of 150 mg $MgSO_4$ + 50 mg PSA + 50 mg C_{18} + 7.5 mg GCB per mL extract for clean-up provides the overall most beneficial sample preparation method for pesticides analysis of fruits and vegetables [9].

In terms of analysis, a major advance in recent years entails the commercial introduction of ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) [18–20], which substantially increases the speed of analysis for hundreds of LC-amenable pesticides *vs*. previous LC methods. UHPLC–MS/MS methods have been demonstrated to reliably quantify and identify >100 pesticides in <10 min at ultratrace concentrations in QuEChERS-type extracts of food [19–21]. The QuEChERS approach works well for LC- and GC-amenable pesticides, and it is designed for concurrent analysis of split final extracts using LC and GC separations with MS determination and identification. Although many labs now use UHPLC–MS/MS to achieve <10 min analysis times for LC-amenable pesticides, common GC–MS (/MS) methods routinely used by monitoring labs are still 25–45 min long. This UHPLC *vs.* traditional GC time difference does not allow maximum sample throughput for the desired wide analytical scope of hundreds of pesticides. Preferably, results would be reported within the same working day so that nonviolative foods would be cleared for release to the markets for deliveries overnight.

There are several options to achieve faster GC–MS analysis times, as summarized in a thorough review article [22]. GC–MS using supersonic molecular beams, which allows up to 90 mL/min flow rates without loss of MS sensitivity, provides the fastest analysis times with least loss of separation efficiency [23,24], but this approach is not yet widely available at this time. Alternatively, low-pressure (LP) GC–MS (also called Rapid-MS, sub-ambient, or vacuum-outlet GC–MS) has key features of increased sample capacity, greater ruggedness and sensitivity, reduced analyte degradation, less peak tailing, and more ease-of-use to give it advantages over other competing fast GC–MS approaches for the analysis of GC-amenable residues in foods [22,25–33]. Its sacrifice of reduced separation efficiency for speed is partially compensated by highly selective MS detection.

Typically, LP-GC/MS involves use of a short, narrow (3 m × 0.15 mm i.d. or 0.1 m × 0.1 mm i.d.) uncoated restriction capillary connected between the inlet and a relatively short mega-bore analytical column (10 m × 0.53 mm i.d. × 1 μm film thickness). This column is maintained under vacuum conditions due to pumping from the MS system, which causes the helium carrier gas to have the viscosity and behavior more like hydrogen thereby shifting the optimal flow velocity (u_{opt}) from the van Deemter equation to greater flow rate. Meanwhile, the restriction capillary allows normal operating pressure at the inlet. In this way, no modifications of the injector system are needed and nearly any GC–MS instrument may be used in LP-GC/MS. The capillary restrictor also serves as a guard column, which gives a practical advantage to the use of the longer and wider restrictor dimensions of 3 m × 0.15 mm i.d. over the 0.1 m × 0.1 mm i.d. option [26,27].

Despite the many advantages and demonstrated feasibility of LP-GC/MS in the literature by several researchers [25–33], it has not been widely implemented for routine monitoring analysis. Commercial patent issues have been one reason for this, but the patent has not been renewed [34], which frees other vendors to market the approach. Secondly, there was no impetus to speed GC–MS analysis prior to the introduction of UHPLC–MS/MS because HPLC would still have been the rate limiting step. Now that UHPLC–MS/MS is more commonly employed, instrument vendors and the pesticide analysis community may take a renewed look at LP-GC/MS.

The objective of this study was to develop and evaluate a qualitative and quantitative method using LP-GC/TOFMS that achieves individual, pre-homogenized sample turnaround time of 20 min and sample throughput of 32 samples per 9 h with two chemists for 150 GC-amenable pesticides in fruits and vegetables. Sample preparation was based on QuEChERS, and in this study, we compared unbuffered and acetate-buffered versions coupled with d-SPE or DPX using the combination of 150 mg $MgSO_4$ + 50 mg PSA + 50 mg C_{18} + 7.5 mg GCB per mL extract for clean-up, with the goal to find the most efficient and effective approach. This paper mainly describes the quantitative and practical aspects, and a subsequent article will report the qualitative results from the blind analysis of pesticide-fortified sample extracts using this evaluated method [35].

2. Experimental

2.1. Chemicals and materials

The 150 pesticide analytes in this study are listed in Table 1 . All pesticide standards had purity ≥95% (typically >99%), and were obtained from the Environmental Protection Agency's National Pesticide Repository (Fort Meade, MD, USA), Dr. Ehrenstorfer GmbH (Augsburgm, Germany), and Chemservice (West Chester, PA, USA). Isotopically labeled internal standards (IS), atrazine-d_5 (ethyl-d_5) and fenthion-d_6 (*o*,*o*-dimethyl-d_6), were obtained from C/D/N Isotopes (Pointe-Claire, Quebec, Canada). Triphenylphosphate (TPP) was used as a quality control (QC) standard added to all final extracts, blanks, and calibration standards. Acetonitrile (MeCN) and ethyl acetate (EtOAc) were of HPLC-grade and obtained from J.T. Baker (Phillipsburg, NJ, USA), and toluene was from Sigma–Aldrich (St. Louis, MO, USA). Glacial acetic acid (HOAc) was of analytical grade obtained from J.T. Baker. For validation experiments, tomato, strawberry, potato, orange, and mixed lettuces were purchased from a local organic food store.

We used QuEChERS commercial products for sample preparation. For the initial extraction step, UCT (Bristol, PA, USA) provided 50 mL polypropylene tubes prepacked with 6 g anhydrous $MgSO_4$ plus 1.5 g NaCl, which were used in the unbuffered method, and Restek (Bellefonte, PA, USA) provided similar tubes containing 6 g anhydrous $MgSO_4$ plus 1.5 g anhydrous sodium acetate (NaOAc), which were used in the acetate-buffered method. For clean-up, two types of commercial products were also evaluated for all matrices. UCT provided 2 mL mini-centrifuge tubes for d-SPE, and DPX Labs (Columbia, SC, USA) provided 5 mL tips for DPX, both of which contained 150 mg anhydrous $MgSO_4$, plus 50 mg PSA, plus 50 mg C_{18}, plus 7.5 mg GCB.

2.2. Standard solutions preparation

Stock solutions of each pesticide, IS, and QC standard were prepared at ≈4000 ng/μL in toluene or EtOAc and stored in amber glass vials at −18 °C. A working standard pesticide mixture in MeCN (acidified with 0.1% HOAc) containing all 150 analytes at 20 ng/μL each was prepared from the stock solutions. This mixture also served as the high spiking solution in recovery experiments, and two additional spiking solutions of 1.25 ng/μL (low) and 5 ng/μL (mid) were prepared from appropriate dilutions in MeCN (acidified with 0.1% HOAc). A mixture of both IS compounds at 20 ng/μL in MeCN (acidified with 0.1% HOAc) and a 2 ng/μL TPP solution in MeCN (acidified with 0.1% HOAc) were also prepared.

Table 1
LP-GC/TOFMS retention times (t_R), MS ions (m/z), average regression values in matrix and matrix effects (%ME) (typical $n = 20$), and average overall %recoveries and %RSD (typical $n = 270$), for the pesticide analytes. Average $R^2 < 0.990$, average %ME > ±20, recovery <70%, and RSD > 20% are noted in bold text.

Pesticide	t_R (s)	Quant. ion	Qual. ions	Avg R^2	Avg ± SD %ME	Overall %Rec.	Overall %RSD
Mevinphos	134.0	127	192, 224	**0.984**	**−30 ± 34**	106	17
Propham	138.0	179	119, 137	0.991	−10 ± 24	101	17
Methacrifos	142.6	208	180, 240	0.998	−11 ± 28	102	10
Phthalimide	144.5	147	104	**0.973**	**125 ± 145**	89	26
o-Phenylphenol	147.9	170	115, 141	0.999	−1 ± 16	97	9
Heptenophos	153.4	250	124, 126	0.998	−3 ± 36	104	9
Tecnazene	157.6	261	203, 259	0.999	7 ± 16	91	9
Propoxur	158.6	110	152	0.997	7 ± 25	105	9
Propachlor	159.0	176	120, 211	0.998	−7 ± 28	103	9
Demeton-S-methyl	161.4	142	109, 142	0.999	1 ± 25	102	12
Diphenylamine	161.4	169	168	0.994	1 ± 14	92	14
Ethoprophos	162.0	242	158, 200	0.995	0 ± 19	101	10
Ethalfluralin	162.3	333	276, 316	0.998	−3 ± 13	100	11
Trifluralin	164.0	306	264, 335	0.999	−2 ± 13	99	6
Chlorpropham	164.9	213	171, 215	0.993	**24 ± 19**	100	9
Dicrotophos	168.4	127	193, 237	0.995	−7 ± 20	93	11
Cadusafos	168.9	159	213, 270	0.999	−3 ± 14	98	7
Phorate	170.3	260	121, 231	0.999	0 ± 12	100	8
α-BHC	173.0	219	217, 181	0.998	2 ± 18	99	9
Hexachlorobenzene	174.3	284	249, 286	**0.983**	−2 ± 11	**54**	**21**
Pentachloroanisole	175.3	265	267, 280	0.997	3 ± 12	81	10
Ethoxyquin	176.0	202	174, 217	0.993	2 ± 20	**64**	**61**
Carbofuran	177.6	164	131, 149	0.998	17 ± 44	109	12
Dicloran	178.1	176	178, 206	**0.970**	**27 ± 33**	88	13
Simazine	178.4	186	173, 201	0.998	−4 ± 22	95	7
Atrazine-d_5 (IS)	178.8	220	222, 205	n/a	n/a	n/a	n/a
Atrazine	179.0	200	202, 215	0.997	−7 ± 16	100	8
Terbuthylazine	179.4	214	216, 229	**0.954**	−9 ± 25	96	9
Propazine	179.6	229	231, 187	0.999	−8 ± 17	101	7
Propetamphos	181.0	194	222, 236	**0.988**	4 ± 16	101	8
Quintozene (PCNB)	181.6	295	249, 297	0.993	6 ± 14	82	11
β-BHC[a]	181.7	219	217, 181	**0.974**	−3 ± 15	103	11
Terbufos	181.9	231	153, 288	**0.962**	3 ± 16	100	8
Lindane (γ-BHC)[a]	182.2	219	217, 181	**0.974**	−3 ± 15	103	11
Dimethoate	182.8	229	143, 229	**nd**	nd	nd	nd
Diazinon	182.9	304	179, 276	**0.989**	3 ± 15	100	8
Cyanophos	183.2	243	109, 125	**0.975**	4 ± 21	105	9
Propyzamide	183.6	255	173, 257	0.995	8 ± 14	102	8
Fonofos	184.2	246	109, 137	0.990	1 ± 11	99	8
Pyrimethanil	185.7	198	199	0.997	7 ± ± 11	**67**	**33**
Disulfoton	189.0	274	153, 186	0.999	3 ± 13	98	9
Chlorothalonil	189.1	266	229, 268	**0.971**	−2 ± 29	78	**72**
δ-BHC	191.6	219	217, 181	0.997	−16 ± 57	104	12
Dichlofenthion	195.4	279	251, 281	0.999	7 ± 13	96	8
Phosphamidon	196.7	127	138, 264	**0.979**	−3 ± 26	109	17
Chlorpyrifos-methyl	197.8	286	288, 321	0.997	5 ± 16	96	8
Vinclozolin	198.9	212	214, 285	0.999	10 ± 12	100	7
Alachlor	200.0	160	188, 237	0.994	−1 ± 16	101	9
Tolclofos-methyl	201.0	265	250, 267	**0.977**	0 ± 16	100	8
Metribuzin	201.4	198	144, 214	**nd**	nd	nd	nd
Methyl parathion	201.5	125	109, 263	**0.988**	18 ± 13	102	10
Heptachlor	202.7	372	272, 274	0.998	1 ± 16	96	9
Fenchlorphos	203.3	285	167, 287	0.992	7 ± 16	94	8
Metalaxyl	203.6	206	220, 249	0.997	16 ± 20	100	12
Carbaryl	204.3	144	115	**0.976**	**81 ± 61**	104	11
Pirimiphos-methyl	205.3	290	276, 305	0.997	3 ± 17	98	7
Fenitrothion	207.4	277	247, 260	0.995	12 ± 29	102	9
Methiocarb	208.3	168	109, 153	0.993	**51 ± 61**	100	15
Malathion	208.9	173	127, 158	0.998	10 ± 27	102	7
Pentachlorothioanisole	210.2	296	263, 298	0.998	−2 ± 12	**54**	**25**
Chlorpyrifos	211.4	314	197, 316	0.998	−9 ± 18	92	9
Metolachlor	211.7	162	238, 240	0.994	−17 ± 23	101	10
Fenthion-d_6 (IS)	212.2	284	115, 131	n/a	n/a	n/a	n/a
Fenthion	212.8	278	153, 169	0.996	−11 ± 22	99	10
Demeton-S-methylsulfone	213.2	169	109, 142	**0.986**	**−32 ± 25**	98	16
Aldrin	213.5	263	265, 329	0.995	−17 ± 27	94	15
Parathion	214.1	291	155, 186	0.997	−6 ± 25	97	8
Triadimefon	215.1	208	181, 210	0.999	−1 ± 21	100	8
Fensulfothion	215.4	292	156, 308	0.999	**25 ± 25**	104	11
Tetraconazole	216.2	336	171, 338	0.992	5 ± 16	101	8
Pirimiphos-ethyl	216.3	304	318, 333	0.993	−2 ± 17	97	7
Dichlorobenzophenone	216.6	250	139, 252	0.999	3 ± 16	89	11
Bromophos	217.9	331	329, 333	0.998	2 ± 19	91	10
Pendimethalin	221.3	281	169, 252	**0.951**	−4 ± 24	92	9
Cyprodinil	222.6	224	225	0.997	5 ± 14	**59**	**43**
Isofenphos	223.6	213	185, 255	**0.983**	−2 ± 18	102	9
Chlorfenvinphos	224.6	267	269, 323	0.998	6 ± 25	103	9

Table 1 (*Continued*)

Pesticide	t_R (s)	Quant. ion	Qual. ions	Avg R^2	Avg ± SD %ME	Overall %Rec.	Overall %RSD
Heptachlor epoxide	225.2	353	355, 390	0.997	−5 ± 17	99	9
Penconazole	226.1	248	159, 250	0.997	−1 ± 29	97	9
Fipronil	227.9	367	351, 369	0.994	15 ± 29	105	10
Procymidone	228.3	283	255, 285	0.999	4 ± 17	101	7
Captan	229.4	151	149	**nd**	nd	nd	nd
Bromophos-ethyl	230.2	359	303, 357	0.997	8 ± 16	85	13
Folpet	231.1	260	262, 297	**nd**	nd	nd	nd
o,*p*′-DDE	231.9	246	248, 318	**0.978**	6 ± 14	94	10
trans-Chlordane	232.5	373	375, 410	**0.989**	4 ± 16	95	9
Methidathion	233.2	145	125	0.999	**23 ± 52**	105	10
Tetrachlorvinphos	233.4	329	240, 331	0.997	**27 ± 68**	102	10
Chinomethionat	234.7	234	174, 206	0.996	4 ± 28	**36**	**44**
cis-Chlordane	235.6	373	375, 410	0.995	−11 ± 15	97	15
α-Endosulfan	236.2	170	195, 339	0.994	−19 ± 19	102	**21**
trans-Nonachlor	237.5	409	407, 444	**0.989**	−12 ± 19	99	20
Disulfoton sulfone	237.9	213	125, 153	**0.975**	**40 ± 39**	106	12
Fenamiphos	239.4	288	217, 303	0.997	17 ± 30	98	9
Profenofos	240.9	374	337, 339	0.998	11 ± 44	99	16
p,*p*′-DDE	241.1	246	248, 318	**0.986**	−11 ± 19	94	18
Oxyfluorfen	242.2	252	361, 363	0.998	3 ± 22	101	13
Buprofezin	242.9	305	172, 190	**0.982**	16 ± 17	99	13
Kresoxim-methyl	243.6	116	131, 206	0.993	−6 ± 22	106	20
Bupirimate	243.8	273	208, 316	0.992	1 ± 20	101	9
Dieldrin	244.1	263	265, 380	0.990	−4 ± 19	99	13
o,*p*′-DDD	244.4	235	237, 320	**0.969**	−3 ± 20	98	13
Myclobutanil	245.9	179		0.992	14 ± 22	103	11
Endrin	250.1	317	319, 345	0.999	−5 ± 18	100	11
Ethion	252.2	384	153, 384	0.995	10 ± 20	102	8
p,*p*′-DDD[b]	253.1	235	237, 320	**0.957**	8 ± 19	97	9
o,*p*′-DDT[b]	253.4	235	237, 354	**0.957**	8 ± 19	97	9
β-Endosulfan	254.4	265	195, 339	0.994	3 ± 25	99	12
cis-Nonachlor	254.6	409	407, 444	0.994	2 ± 17	95	9
Fenthion sulfone	255.9	310	125, 136	0.999	**33 ± 22**	104	20
Sulprofos	256.8	322	156, 280	0.999	9 ± 13	95	8
Oxadixyl	258.3	163	132, 233	nd	nd	nd	nd
Triazophos	258.5	257	161, 313	0.999	**23 ± 35**	101	11
Carfentrazone ethyl	258.9	330	333, 411	0.999	10 ± 20	102	9
Kepone (chlordecone)	259.7	272	274, 355	0.995	3 ± 114	nd	nd
Carbophenothion	259.9	342	157, 344	0.999	12 ± 22	96	8
Famphur	261.1	218	125	0.996	13 ± 57	104	15
Propiconazole	261.7	259	173, 261	0.998	11 ± 15	96	10
p,*p*′-DDT	262.6	235	237, 354	0.995	8 ± 23	94	19
Endosulfan sulfate	264.3	387	272, 389	0.997	−3 ± 40	105	13
Propargite	265.7	135	231, 350	0.997	3 ± 19	101	8
Piperonyl butoxide	266.3	176	149, 338	0.998	10 ± 12	96	9
Resmethrin	266.8	123	143, 171	0.994	6 ± 13	89	16
TPP (QC)	268.0	326	215, 233	n/a	n/a	n/a	n/a
Tebuconazole	268.8	250	163, 252	0.996	**28 ± 21**	96	14
Captafol	271.0	151		**nd**	nd	nd	nd
Bifienthrin	272.4	181	165, 166	0.997	8 ± 13	91	9
Iprodione	274.0	314	186, 316	0.999	**55 ± 51**	99	**24**
Bromopropylate	275.0	341	183, 339	0.995	10 ± 13	99	9
EPN	275.4	157	169, 323	0.999	13 ± 21	96	10
Fenpropathrin	275.9	265	181, 349	0.998	10 ± 14	99	9
Methoxychlor	276.2	227	–	0.993	−4 ± 17	103	16
Endrin ketone	276.9	317	315, 345	0.996	−4 ± 18	101	14
Phosmet	277.4	160	317	0.994	**82 ± 127**	99	**24**
Fenoxycarb	277.9	255	116, 186	0.995	20 ± 41	100	11
Tetradifon	283.3	356	354, 229	0.998	12 ± 14	95	9
Leptophos	284.6	377	171, 375	0.998	16 ± 23	**65**	**32**
Phosalone	285.8	367	182, 184	0.996	**32 ± 46**	96	14
λ-Cyhalothrin	286.7	181	197, 208	0.998	15 ± 19	101	10
Azinphos-methyl	288.0	160	132	**nd**	nd	nd	nd
Mirex	291.7	272	274, 404	0.997	2 ± 15	80	16
Fenarimol	295.1	251	253, 330	0.995	19 ± 15	98	12
Azinphos-ethyl	296.7	132	160	0.992	**32 ± 49**	93	16
cis-Permethrin	301.3	183	163, 185	0.998	15 ± 12	91	10
trans-Permethrin	303.7	183	163, 185	0.998	17 ± 27	93	10
Dioxathion	307.3	271	125, 153	0.995	**46 ± 44**	99	15
Coumaphos	307.4	362	226, 364	0.992	**76 ± 95**	74	37
Cyfluthrin	312	206	199, 227	0.994	**25 ± 26**	96	14
Cypermethrin	322	163	165, 181	0.994	**33 ± 28**	90	18
Flucythrinate	326	199	157, 207	0.992	19 ± 23	104	16
Fenvalerate	345.1	167	169, 419	**0.989**	**30 ± 26**	99	15
Fluvalinate	348.8	250	181, 252	**0.982**	**24 ± 31**	99	13
Esfenvalerate	351.5	167	169, 252	**0.987**	16 ± 23	96	12
Deltamethrin	371.3	253	181, 251	**0.978**	**35 ± 43**	91	13

[a,b]Peak areas were combined for quantification; nd = not detected; n/a = not applicable.

For preparation of calibration standards, mixtures of 0.1, 0.25, 1, 4, and 10 ng/μL for the 150 analytes plus 2 ng/μL of each IS in each solution were prepared in MeCN (acidified with 0.1% HOAc). These solutions were used to prepare calibration standards equivalent to 10, 25, 100, 400, and 1000 ng/g for the analytes and 200 ng/g for the IS compounds by adding 50 μL of these solutions to 0.5 mL blank extracts (equivalent to 0.5 g sample) for matrix-matched standards, or to 0.5 mL MeCN for reagent-only standards. The presence of acid in the MeCN solutions, and use of brown glass vials, has been found to reduce degradation of base-sensitive pesticides [36].

2.3. Sample preparation method

Two versions of QuEChERS were evaluated in the study, which were based on the original version and AOAC Official Method 2007.01. Two different clean-up procedures using d-SPE and DPX were also evaluated for each QuEChERS method. In d-SPE, the extracts are mixed with loose sorbent(s) contained in centrifuge tubes, and in DPX, the sorbents are contained in a pipette tip fitted with a 2 μm pore-size metal screen or frit at the bottom, which allows the extract to mix with the sorbent(s) (further dispersed with air bubbles using a lever-arm manifold) and be dispensed, but does not allow the sorbent(s) to pass through.

The centrifuge tubes, commercial products, and autosampler GC vials needed for analyses were appropriately labeled in advance. Fruit and vegetable samples were cut into small portions with a knife, placed in a freezer until frozen, and then comminuted in a 2L chopper (Robotcoupe, Jackson, MS, USA) with dry ice. The homogenized samples were stored in the freezer before being thawed just prior to extraction.

The sample preparation procedure entailed the following steps: (1) weigh 15 g sample into a 50 mL polypropylene centrifuge tube; (2) add 300 μL of the spiking standard and 150 μL of IS solution and vortex for 1 min; (3) dispense 15 mL MeCN (for the unbuffered version) or 15 mL 1% HOAc in MeCN (for the buffered version) to the samples and shake the tubes vigorously by hand for 30 s; (4) pour the samples and extracts into the appropriate tubes containing 6 g anhydrous $MgSO_4$ and 1.5 g NaCl (unbuffered) or 6 g anhydrous $MgSO_4$ and 1.5 g anhydrous NaOAc (buffered); (5) shake the tubes vigorously by hand for 1 min (avoiding formation of oversized $MgSO_4$ agglomerates); (6) centrifuge the tubes at 3000 rcf for 2 min; (7) transfer 1 mL of MeCN extract (upper layer) to the d-SPE tubes containing 150 mg anhydrous $MgSO_4$, 50 mg PSA, 50 mg C_{18}, and 7.5 mg GCB; (8) vortex the d-SPE tubes for 30 s and centrifuge at 3000 rcf for 2 min; (9) transfer 0.5 mL of the final extracts into the appropriately labeled autosampler vials; (10) add 50 μL of QC standard solution, and for sample extracts, also add 50 μL of MeCN (acidified with 0.1% HOAc) to compensate for the addition of the pesticide standard solutions in the matrix-matched and reagent-only calibration standards.

For DPX, steps (7) and (8) in the protocol were substituted with the following: (a) place the 5 mL DPX tips containing 150 mg anhydrous $MgSO_4$, 50 mg PSA, 50 mg C_{18}, and 7.5 mg GCB in the DPX lever manifold (DPX Labs); (b) transfer 1 mL of MeCN extract from step (6) into a 15 mL centrifuge tube and place the tube in the rack; (c) use the lever arm to draw the 1 mL extract in and out from the bottom of the DPX tip twice, being sure to aspirate air into the tip for proper mixing of the sorbents with the extracts; and (d) dispense the final extract back into the same centrifuge tube. Continue with steps (9) and (10) as described already.

2.4. LP-GC/TOFMS conditions

The GC–MS analysis in this study was performed on an Agilent 6890 GC (Palo Alto, CA, USA) integrated with a Leco Pegasus 4D TOFMS instrument (St. Joseph, MI, USA). Injection was conducted by a Combi-PAL autosampler (Leap Technologies, Carrboro, NC, USA) in combination with an Optic-3 programmable temperature vaporizer (PTV) inlet (Atas-GL International, Veldhoven, The Netherlands). Ultra-high purity helium (Airgas, Radnor, PA, USA) was used as carrier gas at 20 psi (138 kPa) constant inlet pressure. Injection volume was 10 μL into liners containing sintered glass on the walls (Atas-GL International part # A100133). The PTV was programmed as follows: initial injector temperature 75 °C for 18 s (vent time 15 s) with split flow of 50 mL/min, followed by splitless transfer of analytes to the column for 2 min while the injector was ramped to 280 °C at 8 °C/min, then the split flow was reduced to 20 mL/min and the injector temperature was decreased to 250 °C until the end of the run.

The analytes were separated on a 10 m × 0.53 mm i.d. × 1 μm film thickness Rti-5ms analytical column coupled to a 3 m × 0.15 mm i.d. non-coated restriction capillary at the inlet (Restek). A GC column connector (Agilent part # 0101-0594) was used to couple the columns in which the restriction capillary fit inside the megabore column to make a zero dead volume connection. The combination of the columns corresponded to a 3.13 m × 0.15 mm i.d. virtual column setting if programmable flow programming were to be used. The GC oven was set at initial temperature 90 °C (held for 1 min), ramped to 180 °C at 80 °C/min, then 40 °C/min to 250 °C, and ramped to 290 °C at 70 °C/min and held for 4 min. An oven insert pad was used to reduce the oven size which enabled slightly faster heating and cooling. The total run time was 9.45 min (retention time of the last eluting peak, deltamethrin, was 6.18 min), and it took <3 min for the oven to re-equilibrate.

The transfer line and ion source temperature were set at 280 °C and 250 °C, respectively. The electron ionization energy was −70 eV. The detector voltage was 1800 V. A 130 s filament and multiplier delay was used. Spectral data acquisition rate was 10 spectra/s for collection of m/z 70–600 as previously described in sufficient for peak characterization and deconvolution in 1D analysis [10]. Leco ChromaTOF software version 3.22 was employed for the instrument control and data acquisition/processing. NIST 2005 mass spectral library software and Agilent's pesticide and endocrine disruptor database were used for mass spectral matching and peak identification. The analytical sequences consisted of 48 injections each day for 10 days. Before starting each new sequence, TOFMS was fully autotuned using the default parameters of the instrument. To reduce the accumulation of nonvolatile matrix components, maintenance consisted of replacing the sintered glass liner and cutting about 5 cm from the front of the restriction capillary after 96 injections (2 sequences).

2.5. Method validation

The recovery and reproducibility experiments were carried out for each sample matrix in 5 replicates each at 3 spiking levels (25, 100, and 400 ng/g) for each of the 4 sample preparation protocols. Sequences consisted of either unbuffered or buffered extracts with both d-SPE and DPX clean-up for each matrix. For example, the sequence on Day 1 consisted of tomatoes extracted with the unbuffered QuEChERS version cleaned-up with both d-SPE and DPX (30 spiked extracts at 3 levels plus 2 blanks), 10 matrix-matched calibration standards, 5 reagent-only calibration standards, and a reagent blank. This was repeated each day for 10 days until both extraction methods were used for tomato, potato, strawberry, orange, and lettuces. Atrazine-d_5 was used as the IS in all cases, and recoveries were calculated *vs*. the matrix-matched standard at the given spiking level for each concentration. The reagent-only calibration standards were used to assess matrix effects (ME). %ME is the %difference in the best-fit slope of the matrix-matched calibration standards *vs*. the best-fit slope from reagent-only standards,

which was calculated using peak areas without normalization to avoid inclusion of potential matrix effects from the IS in the calculation.

3. Results and discussion

3.1. Optimization of LP-GC/TOFMS conditions

The selected 150 pesticides were chosen from the GC-amenable monitoring target pesticides list of General Mills [10], which covered a wide range of pesticides to demonstrate the validity of the approach. The chosen pesticides are commonly monitored in many regulatory programs worldwide. Moreover, these pesticides are representative different pesticide classes often found at ultratrace levels in fruits and vegetables.

The goal of the study was to achieve <10 min analysis time for 150 pesticides using LP-GC/TOFMS, and instrumental conditions were devised to meet that goal. Faster analysis was possible, but increasing speed further compromises separation efficiency, which affects the ability of the MS to define the analyte peaks and obtain reliable integration among co-eluting analyte peaks and matrix interferences. We achieved 6.18 min separation of the 150 analytes and then maintained the high oven temperature of 290 °C for another 3.5 min to elute high boiling matrix components and avoid ghost peaks. Shorter hold time was also possible, but at the risk of worse performance and reduced ruggedness in routine applications.

Unless a time-consuming concentration step is performed, the QuEChERS method provides final extracts in MeCN of ≈1 g/mL sample equivalent. Large-volume injection (LVI) is required in this case to attain <25 ng/g detection limits in GC–MS, particularly when using full-spectral data collection. We chose to use 10 μL injection volume (≈10 mg sample equivalent) in the protocol, and by using the sintered glass wall-coated liner in the PTV, the solvent vent time was greatly reduced. The injection conditions used in the study were similar as our lab used in the AOAC International collaborative study on QuEChERS, in which we achieved exceptional results with the same injector in conventional GC–MS (quadrupole) using selected ion monitoring (SIM) mode [5]. Injection of the extracts at a temperature less than the boiling point helps to minimize degradation of thermally labile pesticides in the inlet.

The wall-coated sintered glass liners provided a large surface for absorbing the injected solvent without dripping to the bottom of the injector (which occurred with horizontal sintered glass liners). These liners were also found to be less active than glass wool placed in normal LVI liners. According to Agilent FlowCalc software, at 280 °C PTV temperature and 20 psi inlet pressure, the 10 μL MeCN vapor volume was 3.66 mL, but the vapor expansion was relatively gradual over the 26 s that it took for the PTV to reach the final temperature.

We also used the same LP-GC column configuration as Mastovska et al., which was found to provide exceptional ruggedness in a quadrupole GC–MS (SIM) instrument [26]. In an experiment to asses the TOFMS sensitivity *vs.* constant inlet pressure, we determined that 20 psi gave the greatest peak heights for the analytes, which also matched the previous finding with the quadrupole instrument [26]. Apparently, design characteristics for the MS pumping systems were similar, which is a major limitation in flow rate (and speed) that can be achieved in conventional GC–MS.

Based on Agilent FlowCalc software, the LP-GC/TOFMS settings gave 2.46 mL/min helium flow rate in the combination of columns (equivalent to 101 cm/s linear flow velocity in the 10 m, 0.53 mm i.d. analytical column) at the start of the chromatogram and 1.15 mL/min (73 cm/s) at the end of the run. For a megabore column, the u_{opt} under vacuum outlet conditions is ≈250 cm/s, with little loss of separation efficiency even at 350 cm/s [37]. Linear velocity of 250 cm/s in the analytical column corresponds to ≈15 mL/min flow rate in our system (and 72–105 psi inlet pressure), but unfortunately, the MS pumping system could not accommodate such a high flow rate. Even under the less than ideal chromatographic flow conditions in LP-GC, the higher than usual flow rate did not allow the instrument to meet air and water ion ratio criteria, and we had to reduce the flow rate during tuning until the ratios fell within acceptable limits in the software, despite that there were no leaks in the connections.

In addition to higher flow rate and reduced viscosity of the helium carrier gas under vacuum conditions, fast oven temperature programming was also helpful in yielding the short analysis time. Furthermore, the oven cool-down and re-equilibration time is just as important as chromatographic analysis time in achieving high sample throughput. The use of the oven pad reduced oven volume to help the oven better track its actual set temperature during the fast temperature ramp, and it reduced cool-down time from 290 °C to 90 °C from 2.44 min to 2.27 min (or just over 8 min in a sequence of 48 samples).

The TOFMS technique and instrument seemed highly suitable for this LP-GC/MS approach. The features of fast, low resolution TOFMS enabled the following: (i) full-scan mass spectra provided a greater amount of information for potential identification of targeted and non-targeted chemicals of interest than in SIM mode; (ii) improved sensitivity was obtained over common quadrupole instruments in full-scan mode; (iii) higher possible data acquisition rate yielded sufficient spectral information and enough points across the peaks for more reliable quantification and identification; and (iv) automated mass spectral deconvolution helped overcome overlapping of chromatographic peaks and gave background subtracted spectra. We anticipated that TOFMS would be very useful for simultaneous identification and quantification even for the many partially co-eluting analytes that occurred in the LP-GC technique.

However, just as higher flow rate reduced MS detection sensitivity, increasing data acquisition rate also reduced sensitivity [22,38]. Ideally, we would have preferred to use 50–100 spectra/s, to improve performance of the deconvolution software to better distinguish individual compound peaks without manual intervention, but we also wanted to achieve 10 ng/g limits of quantification (LOQ) for the pesticides. We had to make a compromise and used data acquisition rate of 10 spectra/s in this study, which enabled achievement of <25 ng/g LOQ and reasonable deconvolution capabilities for >125 pesticides. The extracted ions listed in Table 1 of individual analytes were used for quantification and qualitative purposes, and the full mass spectral matching factors of the deconvoluted spectra *vs.* the library spectra for each analyte was used for further identification. Qualitative aspects of this high throughput approach are presented in a separate article [35].

In terms of separation, the MS deconvolution feature at the final conditions was able to distinguish all 153 analytes (including the QC and 2 IS compounds) from mevinphos at 2.23 min to deltametrin at 6.18 min (see Table 1). The only analyte–analyte interference difficulties related to the similarity in the mass spectra of β-BHC and γ-BHC (lindane) and *o*,*p*′-DDT and *p*,*p*′-DDD, which could not be distinguished as two distinct peaks by the deconvolution software. The α-BHC and δ-BHC and *p*,*p*′-DDT and *o*,*p*′-DDD isomers also had the same mass spectra, but they were fully resolved chromatographically from the other BHC and DDD/DDT isomers. The retention times (t_R) of all the analytes were different, but the β-BHC and γ-BHC isomers were not fully resolved, and to do so would have extended the analysis time needlessly. In the case of *o*,*p*′-DDT and *p*,*p*′-DDD, not even traditional 30 m × 0.25 mm i.d. GC columns can typically resolve these peaks in 40 min methods. In our <10 min method, each could be determined from the other if one, the other,

Table 2
Lowest calibrated levels (LCLs) of the different pesticides depending on QuEChERS version and matrices (ND = not detected).

LCL	Pesticides
10 ng/g in all matrices and versions	Alachlor, aldrin, α-BHC, β-BHC + lindane, δ-BHC, bifenthrin, bromophos, bromophos-ethyl, bromopropylate, bupirimate, buprofezin, cadusafos, carbophenothion, carfentrazone-ethyl, *cis*-chlordane, *trans*-chlordane, chlorfenvinphos, chlorpropham, chlorpyrifos, chlorpyrifos-methyl, λ-cyhalothrin, *o,p′*-DDD, *p,p′*-DDD + *o,p′*-DDT, *o,p′*-DDE, *p,p′*-DDE, diazinon, dichlorfenthion, dichlorobenzophenone, dieldrin, diphenylamine, endosulfan sulfate, endrin, endrin ketone, EPN, esfenvalerate, ethafluralin, ethion, ethoprophos, famphur, fenarimol, fenchlorphos, fenitrothion, fenpropathrin, fenthion, fenvalerate, fipronil, flucythrinate, fluvalinate, fonofos, heptachlor, heptachlor-epoxide, hexachlorobenzene, isofenphos, leptophos, malathion, methacrifos, methoxychlor, metolachlor, mirex, myclobutanil, *cis*-nonachlor, *trans*-nonachlor, oxyfluorfen, parathion, penconazole, pendimethalin, pentachloroanisole, pentachlorothioanisole, *cis*-permethrin, *trans*-permethrin, *o*-phenylphenol, phorate, piperonyl butoxide, pirimiphos-ethyl, pirimiphos-methyl, procymidone, propachlor, propargite, propazine, propetamphos, propiconazole, propoxur, propyzamide, pyrimethanil, quintozene, resmethrin, sulprofos, tecnazene, terbufos, tetrachlorvinphos, tetraconazole, tetradifon, tolclofos-methyl, triadimifon, trifluralin, vinclozolin
10–25 ng/g in all matrices and versions	Atrazine, azinphos-ethyl, carbofuran, cyanophos, cypermethrin, cyprodinil, *p,p′*-DDT, disulfoton, α-endosulfan, β-endosulfan, ethoxyquin, fenamiphos, fensulfothion, heptenophos, iprodione, kresoxim-methyl, metalaxyl, methidathion, phosalone, profenofos, tebuconazole, terbuthylazine, triazophos
10–25 ng/g in some matrices and versions	Carbaryl, chinomethionat, chlorothalonil, chinomethionat, coumaphos, cyfluthrin, deltamethrin, demeton-S-methyl, demeton-S-methylsulfone, dicrotophos, dioxathion, disulfoton sulfone, fenthion sulfone, kepone, methiocarb, methyl parathion, mevinphos, phosmet, propham, simazine
100–1000 ng/g in some matrices and versions	Carbaryl, chinomethionat, chlorothalonil, coumaphos, cyfluthrin, deltamethrin, demeton-S-methyl, dicloran, dicrotophos, dimethoate, dioxathion, disulfoton sulfone, fenoxycarb, kepone, phosphamidon, cyfluthrin, fenoxycarb, methiocarb, methyl parathion, mevinphos, phosmet, propham, phthalimide, simazine
ND in some matrices and versions	Demeton-S-methyl, demeton-S-methylsulfone, dimethoate, fenthion sulfone, kepone, phosphamidon, phthalimide
ND in all matrices and versions	Azinphos-methyl, captafol, captan, folpet, metribuzin, oxadixyl

or both were present, but the quantification would be affected when both compounds were in the sample at different concentration ratios than in the standards. In our spiking experiments, we integrated and quantified the peaks together, which were kept at equal concentrations in the solutions.

3.2. Method performance

The optimization of conditions allowed us to meet our stated high sample throughput objectives of 32 samples prepared and analyzed per working day. However, this achievement would be meaningless if method performance and ruggedness were not acceptable for routine monitoring purposes. Method performance characteristics were evaluated and compared in terms of recovery (trueness), repeatability (within sequence precision), reproducibility (precision among sequences), detectability (lowest calibrated level), linearity (R^2), matrix effects (%ME), and ruggedness (number of analyses over multiple days). Aspects in terms of ease of use, speed, costs, and other practical factors were also assessed in a comparison of QuEChERS sample preparation versions conducted by two chemists. The individual results obtained in the method performance experiments for the determination of the 150 pesticides in tomato, strawberry, potato, orange, and lettuces using the different QuEChERS versions are summarized in the supplementary Excel spreadsheet available electronically. Table 1 lists the overall matrix-matched calibration R^2 values, matrix effects (%ME ± std. dev.), recoveries and reproducibilities (%RSD) for each pesticide over the 10 analytical sequences. Unfortunately, the Day 1 recoveries for tomato using the unbuffered extraction method were excluded due to mistakes in making some of the spikes, but we still included calibration results.

3.2.1. Linearity

The average linear regression R^2 values for each pesticide from the 20 matrix-matched calibration plots are shown in Table 1. Two sets of matrix-matched calibration standards of 10, 25, 100, 400, and 1,000 ng/g equivalents were prepared in each sequence, one each for d-SPE and DPX clean-up. Also, a set of calibration standards in solvent-only was also prepared at the same levels. The injection of the different calibration standards and levels were dispersed throughout the sequences in a consistent manner from day to day. The number pesticides (82 out of 150) with average linear R^2 values ≥0.995, and 110 overall gave $R^2 \geq 0.990$, which was remarkable considering the high speed of the method, number of injections made, and complexity of the matrices.

3.2.2. Lowest calibrated level

The signal/noise ratios from the instrument software were not necessarily accurate for all pesticides and matrices, thus we did not trust it to estimate LOQs. Therefore, we chose to use the lowest calibrated level (LCL) of matrix-matched standards to show the lowest concentration of each analyte at which the determination system was calibrated. The supplemental spreadsheet lists all the LCLs for each pesticide in each matrix/version, and Table 2 summarizes the results. Most of the pesticides (98) were able to be quantified at 10 ng/g, as listed in the top row of Table 2. Another large group of pesticides (43) gave LCLs of 10–25 ng/g depending on the matrix/method/day in the study. Other pesticides as listed were more problematic and gave LCLs 100–1000 ng/g, and finally, a few pesticides as listed in the table were not able to be detected even in the calibration standards in any of the sequences in the study.

3.2.3. Recoveries

This study was designed to be a test of the quantitative ability of LP-GC/TOFMS moreso than QuEChERS, but we took the opportunity to also make comparisons of different QuEChERS versions. Recovery validation experiments were conducted in each matrix at 3 spiking levels (25, 100, and 400 ng/g). Isotopically labeled internal standards were used to compensate for volumetric variations and water content differences among the matrices in sample preparation. Atrazine-d_5 and fenthion-d_6 were used as IS compounds due to their reasonable cost, no chance of occurring naturally in the samples, and amenability in both GC and LC analyses. Two were added in case a problem occurred with one of them and the other would be available as a backup. Peak areas of the analytes were divided by peak areas of atrazine-d_5 and/or fenthion-d_6 in the same sample or standard, which served as the signal used for quantification. Ultimately, atrazine-d_5, was used as the IS in all cases for purpose of consistency. Much care was taken in the protocol and experiments to ensure that volumes were consistent, and the results with or without the IS were similar in nearly all cases, but overall, the use of the IS gave slightly higher recoveries and greater precision in the study.

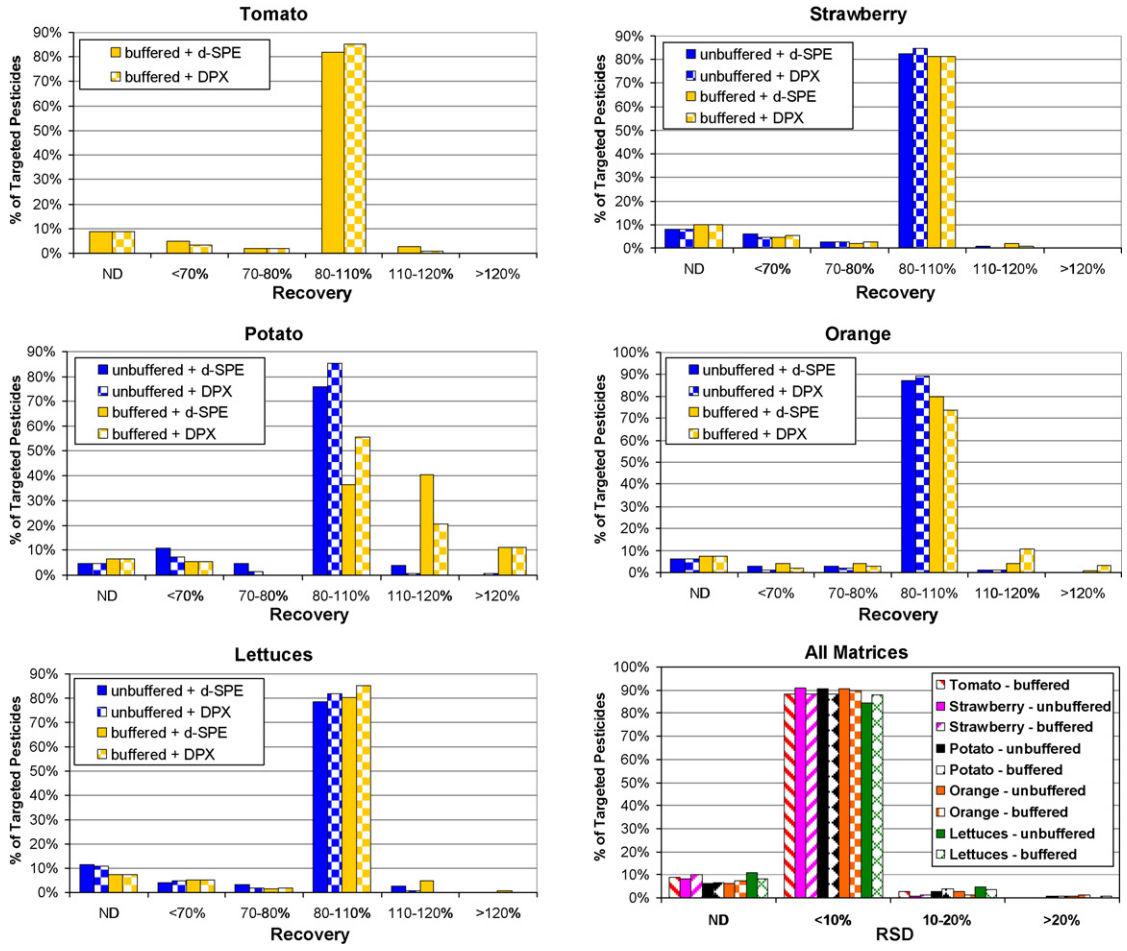

Fig. 1. The distribution of average recoveries and RSD (bottom right) of the 150 pesticide analytes in tomato, strawberry, potato, orange, and lettuces using the different QuEChERS extraction and clean-up versions and LP-GC/TOFMS analysis.

Table 1 lists the overall average recoveries of each pesticide in the study. The results include the combination of data from 5 different matrices spiked at 3 levels using 4 different QuEChERS versions over 9 days. Average recoveries <70% are given in bold text in the table, and these were all structurally planar pesticides that were affected by the use of 7.5 mg GCB in the clean-up step, which will be discussed later in Section 3.2.

Fig. 1 shows the distribution of recoveries for each matrix and sample preparation protocol. Typically 80% (≈120) of the analytes gave 80–110% recoveries. Recoveries within the range of 70–120% fall within the typical acceptance criteria for quantitative regulatory methods [3], and 126–136 (84–91%) of the 150 pesticides in the different matrices met that standard. These pesticides can be discerned from the results in Table 1 typically when reproducibility is <20% RSD (the supplemental information provides detailed results).

The buffered QuEChERS results in potatoes were the only exception, in which 113 (75% of the) pesticides fell within the 70–120% recovery range. As shown in Fig. 1, more pesticides gave recoveries >110% in the buffered extraction method for potato than in the other cases. On the surface, the normalization with the IS caused this bias because the average %recovery of the detected pesticides was 89 ± 16 without use of the IS and 106 ± 19 with the IS. However, this matrix also showed a greater degree of fatty acid co-extractives in those chromatograms, which is evident in Fig. 2(A).

Buffering caused a greater degree of co-extractives from potato than when buffering was not used. Although all the matrices contained roughly the same amount of fatty acids (0.063–0.213%), the differences in the amount of water (80% in potatoes *vs.* 87–95% in the other commodities) and large amount of starch (15%) [39], may have led to the greater amount of fatty acids in the acetate-buffered final extracts for potato. Potatoes range in pH from 5.5 to 6.2 [40], and the acetate buffering is done at pH ≈ 4.8 [6], which converts more of the fatty acids to the neutral state for greater partitioning into the MeCN at the lower pH. The 50 mg PSA per g equivalent of potato is overwhelmed by the greater amount of co-extractives and HOAc in the buffered QuEChERS version, which leads to less removal of the fatty acids in the final extracts. An increased amount of PSA can more effectively remove the fatty acids (*e.g.* Mastovska et al. used 150 mg PSA per mL extract for cereal and grains [10]), but it also somewhat lowers recoveries of relatively polar pesticides, such as acephate. In any case, the greater presence of fatty acids did not preclude the collection of high quality results overall or pose instrument troubles, but the unbuffered option did work better for LP-GC/TOFMS of potatoes than the buffered version.

As shown in Fig. 2(B), a similar finding was made in the case of orange extracts with respect to fatty acid co-extractives, but this only made a small difference in the results (Fig. 1). Buffering did not affect the co-extraction of terpenes (sesquiterpenes), which are substituents of citrus oils that co-eluted with analytes at the front of the chromatogram. More importantly, acetate buffering is needed to improve accuracy in the analysis of pymetrozine and other pH-dependent pesticides [6,9], which are registered for use in citrus.

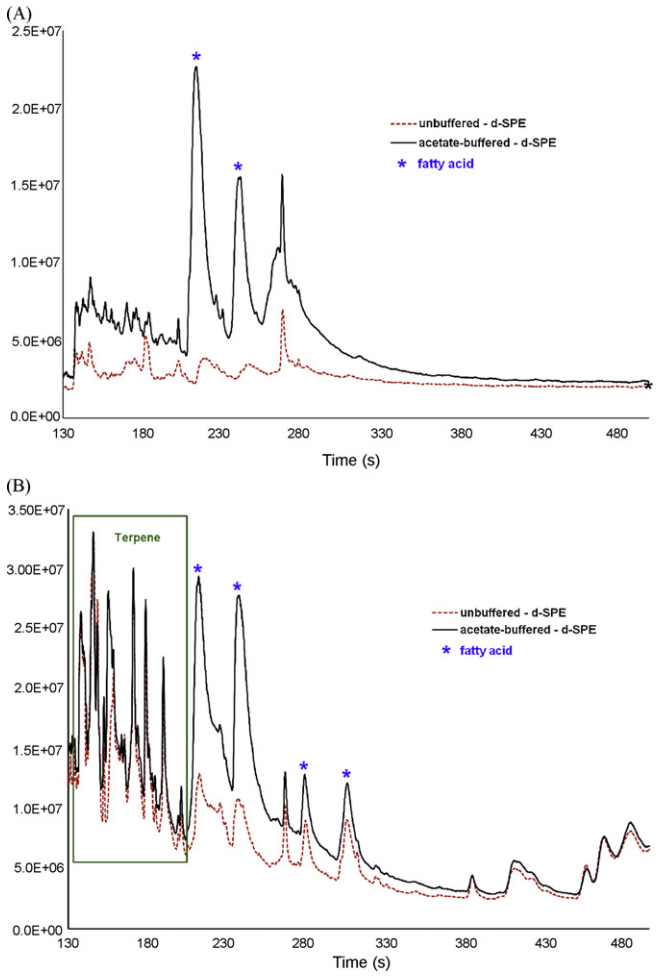

Fig. 2. Representative LP-GC/TOFMS total ion chromatograms of (A) potato and (B) orange extracts obtained using different QuEChERS unbuffered and buffered extraction versions with d-SPE clean-up.

In spiked samples, 11 of the 150 pesticides (8%) of them (azinphos-methyl, captafol, captan, demeton-S-methyl, demeton-S-methylsulfone, dimethoate, fenthion sulfone, folpet, kepone, metribuzin, and oxadixyl) were not detected due to degradation, low signal response, poor GC-amenability, and/or low mass quantification ions that were swamped by matrix interferences. These analytes are difficult by any multiresidue GC method in complex matrices. They are better analyzed by LC-MS/MS methods except captan, captafol, folpet, and possibly kepone (chlordecone), which degrade readily. These are pesticides known to be problematic and we were not surprised by the poor results for them. Other LC-amenable pesticides (atrazine, methiocarb, and phosmet) gave inconsistent results in LP-GC/TOFMS due to matrix interferences. They would still be covered when UHPLC–MS/MS is used concurrently with LP-GC/TOFMS, so these inconsistencies were not a concern.

Conversely, pyrethroid pesticides are known to pose difficulties in multiresidue analysis due their highly nonpolar nature and lower signal intensities. One would think that pyrethroids should give better results in GC, but some have had to resort to adding them to the list of analytes in reversed-phase LC-MS/MS in overall monitoring schemes [10]. In LP-GC/TOFMS, cyfluthrin and deltamethrin could not be detected at the 25 ng/g spiking level, but the other pyrethroids (cypermethrin, esfenvalerate, fenvalerate, flucythrinate, fluvalinate, permethrin, and resmethrin) provided high intensities and acceptable average recoveries of 74–120% in all matrices. LP-GC/TOFMS provides superior results for the pyrethroids due to the faster analysis times and narrower peaks (reduced diffusion) than in GC methods with $t_R > 30$ min for the nonvolatile analytes.

3.2.4. Choice and effect of sorbents in clean-up

Another aspect of this study was that we used the same set of sorbents in d-SPE and DPX clean-up for all matrices and both extraction versions. We found this combination to reduce matrix co-extractives [4,9], and even though C_{18} and GCB may not provide as much clean-up in some commodities in comparison with PSA [4], their use at the given amounts should not appreciably lower recoveries, and they can only help in clean-up. C_{18} retains trace amounts of lipid matrix components, but even a large amount of the hydrophobic sorbent does not lower pesticide recoveries in QuEChERS extracts.

GCB is notable for being able to retain chlorophyll from green vegetable extracts, and it also can remove sterols [7]. CEN Standard Method 15662 calls for the use of 0, 2.5, or 7.5 mg GCB per mL extract in d-SPE depending on the chlorophyll content of the sample matrix [7,8]. In the lettuces, 85–90% of the chlorophyll was removed by the 7.5 mg/mL GCB according to fluorescence and colorimetry measurements. We decided to simplify and standardize the clean-up to use 7.5 mg/mL GCB for all fruits and vegetables, and we expected that the recoveries would be consistently lower for structurally planar pesticides.

The recoveries and repeatabilities for several planar pesticides in the study are presented in Fig. 3. Despite that repeatability of the recoveries was quite good in the experiments, the reproducibility was worse than expected. We anticipated that all of the analytes would give results akin to those obtained for quintozene, with 65–90% recoveries overall, but coumaphos, pyrimethanil, cyprodinil, quinomethionate, and hexachlorobenzene showed greater variability without a consistent pattern in the 4 QuEChERS variations compared. We do not wish to speculate what is the cause of this inferior reproducibility, but the excellent repeatabilities eliminate that excessive variations occurred in the amount or type of GCB used in the commercial products. Recoveries were still acceptable (>70% with <20% RSD) for several planar analytes depending on matrix/version, and all of the results were acceptable for screening applications.

Chlorothalonil is another planar pesticide that is notoriously difficult in pesticide analysis, but it suffers from degradation as well as partial retention by GCB, thus it was not presented in Fig. 3. Its overall results appear in Table 1 and in the supplementary spreadsheet. As shown previously [6,9], buffering helped to improve results for chlorothalonil, especially in lettuce by lowering of pH and increasing stability of base-sensitive pesticides.

Recoveries higher than 120% were observed for a small number of analytes in tomato, strawberry, and lettuces, indicating the normal complications of analyte lability, and direct (interferences) and indirect matrix effects in the analysis.

3.2.5. Precision

The precision of the high throughput monitoring approach is indicated in Table 1 and in the lower right graph in Fig. 1, which displays the distribution of RSDs in all matrices and extraction versions. Repeatabilities were consistently <10% RSD (irrespective of recovery) for 84–91% of analytes in all matrices, and reproducibilities were often equally exceptional, as can be discerned from Table 1 (and supplementary information). RSDs between 10 and 20% were achieved for only 1–5% of the analytes, except slightly higher RSDs were obtained in the potato extracts (6% of analytes). These results demonstrate the high quality and ruggedness of the QuEChERS and LP-GC/TOFMS method and its capabilities for quantification in regulatory and other applications.

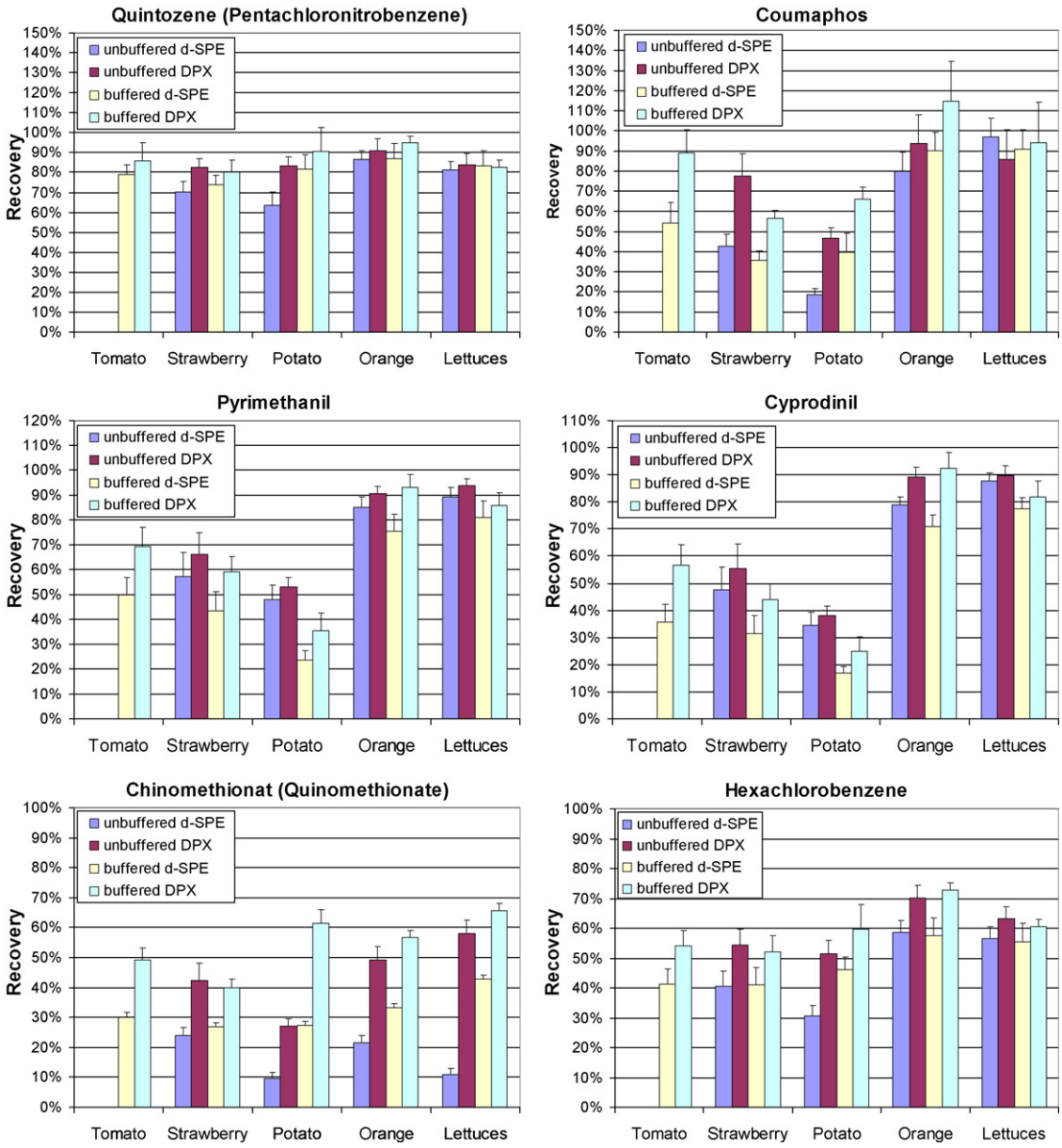

Fig. 3. Recoveries of the structurally planar pesticide analytes in the different matrices using the different QuEChERS extraction and clean-up versions (typical $n = 15$ of combined results from 3 spiking levels). The use of 7.5 mg GCB in the clean-up sometimes retained the planar pesticides to different extents.

3.3. Comparison of d-SPE and DPX for clean-up

In terms of recoveries and precision, no consistent differences occurred between d-SPE and DPX in the study. A few exceptions were found in potato and orange extracts for both unbuffered and buffered extractions, as already discussed, but any differences in the comparison were probably related more to the different chemists and matrix-matched calibration standards used than d-SPE or DPX. Independently, the DPX clean-up generally showed more fatty acids in the chromatograms than those from the d-SPE version. This was probably because different sorbent materials were used by the commercial vendors.

In terms of practical issues, the main advantage of using the DPX tips comes from the metal screen (2 μm) filters, which ensure that particles do not occur in the final extracts. In d-SPE, care must be taken to avoid particles from getting into the final extracts, and it is not possible to transfer the full available extract volume after centrifugation. In this way, slightly more final volume could be obtained from the DPX version than from d-SPE, but this volume was not highly reproducible, which was why we chose to take a 0.5 mL aliquot from both clean-up versions to yield known, consistent final volumes. We believe that this was important in achieving such good recoveries and RSDs in the method, but in routine practice, the IS should compensate for the small deviations in DPX final volumes. Our approach also extended the time needed to perform DPX *vs.* d-SPE (>5 min for DPX and <3 min for d-SPE), but both approaches achieve the same throughput if the DPX eluent is transferred directly into the autosampler vial. For high sample throughput, the DPX lever arm device could accommodate 20 samples at a time in a similar way as a centrifuge, but since a centrifuge is already necessary for initial QuEChERS extracts, the lever arm adds to the number of apparatus a lab needs to run the method routinely (we used a swinging bucket centrifuge in which 50 mL and 2 mL tube holders could be swapped quickly and easily).

The above-listed practical merits of each approach are debatable depending on analyst preference. Disadvantages of both d-SPE

and DPX *vs*. cartridge-based SPE are that ≈50% of the final extract volume is lost to the sorbents, and both dispersive partitioning approaches tend to sacrifice amount of clean-up for practical advantages (speed, ease, and cost).

3.4. Method ruggedness

QuEChERS is a portmanteau word that stands for "quick, easy, cheap, effective, rugged, and safe," and it has been shown to live up to its name in many studies, but as already described, it sacrifices degree of clean-up for increased analytical scope and improved practical factors. If QuEChERS is not rugged, its name would be "fast, easy, cheap, effective, and safe" (FECES). QuEChERS relies on the power of GC–MS(/MS) and LC-MS/MS instruments to achieve excellent performance and robustness in routine operations.

In addition to the multi-day, high-throughput analysis of calibration standards and spiked samples in this study, method ruggedness was evaluated from the signal responses of the IS and QC compounds added at 200 ng/g in all sample extracts. The GC/TOFMS instrument was maintained normally by changing the liner and cutting ~5 cm from the restriction capillary after every 96 injections (2 days). Automated MS tuning was conducted before starting each injection sequence. For the IS, consistent response and 25% RSD of the non-normalized peak areas were achieved from >430 injections over 9 days (tomato from day 1 was excluded). Greater variation was observed in the analysis of potato and orange extracts, presumably due to the greater chemical noise as already discussed, but response remained consistent and quality of results was still very good. Moreover, these results demonstrated ruggedness to a greater extent than described in other published methods using QuEChERS and LP-GC/MS. Our experience is that LC-GC/TOFMS showed more ruggedness than traditional GC–MS with columns of 30 m × 25 mm i.d × 25 μm film thickness, as would be expected using the thicker film megabore column.

3.5. Matrix effects

The amount of co-extracted matrix components in the final QuEChERS extracts is typically <0.2% according to previous studies [4,6,9], which corresponded to <20 μg injected in the 10 μL injection volume of 1 g/mL of equivalent extracts. Some of this material passed through the column along with the pesticide analytes, but much of the co-extractives remained on the sintered glass liner, at least based on visual observation after the analytical sequences. The liner itself typically contains a large number of active sites that interact with the more polar chemicals in the injected sample, and the nonvolatile matrix material also can make a layer of active surface that causes diminishment or enhancement in the response depending on the particular chemical interactions involved [41,42]. Matrix-matched standards are the most common way in routine practice to reduce indirect matrix effects in quantitative GC and LC pesticide residue analyses.

In our experience, matrix effects in GC–MS have been so high and variable that we could not adequately assess and compare different clean-up approaches designed to reduce or overcome the effects [9–11]. Due to full-spectra MS data collection by the TOFMS, we chose not to use analyte protectants [43,44] in this method, which would have added to the complexity of the chromatograms and software deconvolution process.

Table 1 and Fig. 4 (plus the supplemental file) also show that %ME was quite variable in this study among the 20 matrix-matched calibrations analyzed over 10 days in the different versions/matrices. However, we were pleasantly surprised that the matrix effects were as not as high and variable using this LP-GC/TOFMS approach as in traditional GC–MS. The increased sample capacity and shorter length of the megabore column was probably one factor that caused the improvement, and the use of LVI with the wall-coated sintered glass liners was probably another. Those pesticides that undergo the most severe matrix effects in GC are better detected by LC-MS/MS methods anyway, but it is important to have overlapping scope in the methods to improve identification and provide possible confirmation. Although 90–120 pesticides in the LP-GC/TOFMS method gave ±20% ME depending on matrix, it is still not advisable to quantify many of the analytes using reagent-only standards.

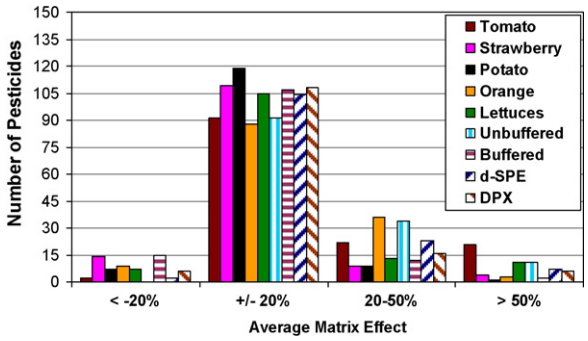

Fig. 4. Distribution of average matrix effects (%ME) of the 150 pesticide analytes in the different matrices using different QuEChERS versions and LP-GC/TOFMS analysis.

4. Conclusions

The results from this work demonstrate the potential for routine use of QuEChERS combined with LP-GC/TOFMS to achieve faster individual sample turnaround time and higher throughput than with common GC–MS methods, and LP-GC/TOFMS attains greater ruggedness than alternate fast GC–MS approaches. A simple, rapid, and reliable determination and identification for nearly 150 pesticide residues in fruits and vegetables was obtained in a single method, and concurrent analysis of the same final extract using UHPLC–MS/MS can more than double the analytical scope without reducing sample throughput. Approximately 36 samples per 9 h day can be performed with the method, which demonstrated acceptable performance and instrument ruggedness over time. Furthermore, the use of the acetate-buffered or no buffering for extraction with either d-SPE or DPX clean-up using a standardized combination of $MgSO_4$, PSA, C_{18}, and GCB powders was applicable to all matrices. We prefer to use acetate buffering with d-SPE as the final version due to practical advantages and greater overall scope to include pH-dependent pesticides in LC analysis [9].

The major limitation of the method so far was the time it took to process the results using the software. Although the signal of target analytes were automatically checked, assigned, and integrated compare to the reference file based on their mass spectra, the manual checking to better assign and identify peaks and correct integration errors was very time-consuming and onerous. The spiking of 150 pesticides to so many samples in this study contributed to this drawback. In a subsequent study, we used a different type of automated software to help address this problem in blind analysis of unknown analytes designed to better mimic real-world monitoring [35].

Disclaimer

Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture above others of a similar nature not mentioned.

Acknowledgments

The authors thank Katerina Mastovska for help with use of the instrument and software. This research was partially supported by The Royal Golden Jubilee Ph.D. Program (PHD0036/2550), Chulalongkorn University Graduate Scholarship to Commemorate the 72nd Anniversary of his Majesty King Bhumibol Adulyadej, and the Thailand Research Fund and Commission on Higher Education, Research Grant for Mid-Career University Faculty (TRF-CHE-RES-MR) (RMU518009). This work was also supported by the U.S.-Israel Binational Agricultural Research and Development Fund grant US 4273-09.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.05.012.

References

[1] USDA, Foreign Agricultural Service, Maximum Residue Limit Database (www.fas.usda.gov/htp/MRL.asp).
[2] Regulation (EC) No. 396/2005 of The European Parliament and of The Council of 23 February 2005 on Maximum Residue Levels of Pesticides in or on Food and Feed of Plant and Animal Origin and Amending Council Directive 91/414/EEC, Off. J. Eur. Communities L70/1, 16.3.2005 (http://ec.europa.eu/sanco_pesticides/public/index.cfm).
[3] Method validation and quality control procedures for pesticide residues analysis in food and feed, Document No. SANCO/10684/2009.
[4] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, J. AOAC Int. 86 (2003) 412.
[5] S.J. Lehotay, J. AOAC Int. 90 (2007) 485.
[6] S.J. Lehotay, K. Maštovská, A.R. Lightfield, J. AOAC Int. 88 (2005), 615 and 60A.
[7] P. Payá, M. Anastassiades, D. Mack, I. Sigalova, B. Tasdelen, J. Oliva, A. Barba, Anal. Bioanal. Chem. 389 (2007) 1697.
[8] CEN Standard Method EN 15662: Food of plant origin—determination of pesticide residues using GC–MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE—QuEChERS method (www.cen.eu).
[9] S.J. Lehotay, A.K. Son, H. Kwon, U. Koesukwiwat, W. Fu, K. Mastovska, E. Hoh, N. Leepipatpiboon, J. Chromatogr. A 1217 (2010) 2548.
[10] K. Mastovska, K.J. Dorweiler, S.J. Lehotay, J.S. Wegscheid, K.A. Szpylka, J. Agric. Food Chem. 58 (2010) 5959.
[11] U. Koesukwiwat, S.J. Lehotay, K. Mastovska, K.J. Dorweiler, N. Leepipatpiboon, J. Agric. Food Chem. 58 (2010) 5950.
[12] U. Koesukwiwat, K. Sanguankaew, N. Leepipatpiboon, Anal. Chim. Acta 626 (2008) 10.
[13] B. Kmellár, P. Fodor, L. Pareja, C. Ferrer, M.A. Martínez-Uroz, A. Valverde, A.R. Fernandez-Alba, J. Chromatogr. A 1215 (2008) 37.
[14] A. Garrido-Frenich, J.L. Martínez-Vidal, E. Pastor-Montoro, R. Romero-González, Anal. Bioanal. Chem. 390 (2008) 947.
[15] J. Wang, D. Leung, J. AOAC Int. 92 (2009) 279.
[16] T. Cajka, J. Hajslova, O. Lacina, K. Mastovska, S.J. Lehotay, J. Chromatogr. A 1186 (2008) 281.
[17] H. Guan, W.E. Brewer, S.T. Garris, S.L. Morgan, J. Chromatogr. A 1217 (2010) 1867.
[18] H. Guan, W.E. Brewer, S.L. Morgan, J. Agric. Food Chem. 57 (2009) 10531.
[19] L. Nováková, H. Vlcková, Anal. Chim. Acta 656 (2009) 8.
[20] J. Wang, W. Chow, D. Leung, Anal. Bioanal. Chem. 396 (2010) 1513.
[21] O. Lacina, J. Urbanova, J. Poustka, J. Hajslova, J. Chromatogr. A 1217 (2010) 648.
[22] K. Maštovská, S.J. Lehotay, J. Chromatogr. A 1000 (2003) 153.
[23] A. Amirav, A. Gordin, M. Poliak, A.B. Fialkov, J. Mass Spectrom. 43 (2008) 141.
[24] A.B. Fialkov, A. Gordin, A. Amirav, J. Chromatogr. A 991 (2003) 217.
[25] J. de Zeeuw, J. Peene, H.G. Janssen, X. Lou, J. High Resolut. Chromatogr. 23 (2000) 677.
[26] K. Maštovská, S.J. Lehotay, J. Hajšlová, J. Chromatogr. A 926 (2001) 291.
[27] K. Mastovská, J. Hajslová, S.J. Lehotay, J. Chromatogr. A 1054 (2004) 335.
[28] K. Ravindra, A.C. Dirtu, A. Covaci, Trends Anal. Chem. 27 (2008) 291.
[29] S.C. Cunha, J.O. Fernandes, A. Alves, M.B. Oliveira, J. Chromatogr. A 1216 (2009) 119.
[30] S.D.H. Poynter, R.A. Shellie, J. Chromatogr. A 1200 (2008) 28.
[31] J.W. Cochran, J. Chromatogr. Sci. 40 (2002) 254.
[32] J.L. Martínez Vidal, J.L. Fernández Moreno, F.J. Arrebola Liébanas, A. Garrido Frenich, J. AOAC Int. 90 (2007) 1146.
[33] F.J. Arrebola, J.L. Martínez Vidal, M.J. González-Rodríguez, A. Garrido-Frenich, N. Sánchez Morito, J. Chromatogr. A 1005 (2003) 131.
[34] J. de Zeeuw, J.A. Peene, R.C.M de Nijs, US Patent No. 6,301,952, October 16 2001.
[35] S.J. Lehotay, U. Koesukwiwat, H.G.H. Mol, H. van der Kamp, N. Leepipatpiboon, J. Agric. Food Chem., submitted for publication.
[36] K. Mastovská, S.J. Lehotay, J. Chromatogr. A 1040 (2004) 259.
[37] R.L. Grob, E.F. Barry, Modern Practice of Gas Chromatography, 4th ed., Wiley-Interscience, Hoboken, NJ, USA, 2004, p. 237.
[38] R.J.J. Vreuls, J. Dallüge, U.A.Th. Brinkman, J. Microcol. Sep. 11 (1999) 613.
[39] U.S. Department of Agriculture, Agricultural Research Service, USDA National Nutrient Database for Standard Reference, Release 22, 2009, Nutrient Data Laboratory Home Page: www.ars.usda.gov/ba/bhnrc/ndl.
[40] J. Bamberg, A. Kiszonas, Am. J. Potato Res. 8 (2010) 167.
[41] J. Hajslová, J. Zrostlíková, J. Chromatogr. A 1000 (2003) 181.
[42] D.R. Erney, A.M. Gillespie, D.M. Gilvydis, C.F. Poole, J. Chromatogr. 638 (1993) 57.
[43] M. Anastassiades, K. Maštovská, S.J. Lehotay, J. Chromatogr. A 1015 (2003) 163.
[44] K. Maštovská, S.J. Lehotay, Anal. Chem. 77 (2005) 8129.