JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Applications of Ultra-performance Liquid Chromatography Electrospray Ionization Quadrupole Time-of-Flight Mass Spectrometry on Analysis of 138 Pesticides in Fruit- and Vegetable-Based Infant Foods

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The applications of ultra-performance liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC QqTOF) in the determination of 138 pesticides in fruit- and vegetable-based infant foods were investigated. Pesticides were extracted from infant foods using a procedure known as the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method. UPLC QqTOF MS full-scan with a relatively high sensitivity proved to be an ideal tool for screening of a large number of pesticides in a single analysis. UPLC QqTOF MS/MS provided product ion spectra that allowed for unequivocal confirmation of pesticides. Quantification was achieved using matrix-matched standard calibration curves with isotopically labeled standards or a chemical analogue as internal standards. The method performance parameters that included overall recovery, intermediate precision, and measurement uncertainty were evaluated according to a designed experiment, that is, the nested design. Generally, about 90% of the pesticides studied had recoveries between 81 and 110%, 90% had intermediate precision of ≤25%, and 85% had measurement uncertainty of ≤50%. Compared to LC-ESI-MS/MS, UPLC QqTOF MS showed a relatively poor repeatability and large measurement uncertainty for quantification. In general, UPLC QqTOF can be used for screening, quantifying, and confirming pesticides in infant foods at 10 μg/kg.

KEYWORDS: UPLC QqTOF; pesticides; infant foods; measurement uncertainty

INTRODUCTION

There have been over 1100 pesticides (*1*) possibly used in various combinations and at different stages of cultivation and during postharvest storage to protect crops against a range of pests and fungi and/or to provide quality preservation. Pesticide residues, which might pose a potential risk for human health due to their subacute and chronic toxicity, could possibly end up in the final products of crops such as processed infant foods. European Union Commission Directive 96/5/EC (*2*) and its subsequent revisions, for example, 1999/39/EC (*3*), 2003/13/EC (*4*), and 2003/14/EC (*5*), have placed emphasis on the control of pesticides such that processed cereal-based foods and infant foods shall not contain residues of individual pesticides at levels exceeding regulatory maximum residue limits (MRLs), for example, 10 μg/kg. To determine the levels of pesticide residues and to screen for a large number of pesticides in various food commodities consistently remain a challenge for analytical chemists. Improved multiclass or multiresidue methodologies with high sensitivity and expanded scopes, which include as many pesticides and commodities as possible in a single method, are always required for checking compliance with MRLs and/or for risk assessment of consumer exposure to pesticides.

Pesticides can be analyzed by gas chromatography (GC) with electron capture detection, flame ionization detection, or nitrogen−phosphorus detection and/or liquid chromatography (LC) with ultraviolet, diode array, fluorescence, or electrochemical detection. However, these techniques may lack the selectivity and/or sensitivity required to meet the requirements at hand due to the complexity of food matrices. They have been largely replaced by GC− and LC−mass spectrometric techniques (*6*−*10*). Among LC-MS techniques, UPLC QqTOF has been recognized as an emerging technique to analyze chemical residues in food and environmental samples. It offers medium-range, high-resolution, accurate mass measurement, excellent full-scan sensitivity, and complete mass spectral information, therefore making QqTOF complementary to other quadrupole and ion trap mass spectrometers for identification and quantification. There are a few scientific papers that report on the analysis of pesticides in fruit- or vegetable-based infant foods using either GC- (*11*−*13*) or LC-MS (*14*−*17*). However, it has been found that there have been a limited number of applications of LC-

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10.1021/jf803419j CCC: $40.75 Published 2009 by the American Chemical Society
Published on Web 02/13/2009

MS for analysis of pesticides in infant foods (*8*), especially for the simultaneous determination of a large pool of pesticides in infant foods using UPLC QqTOF. In this paper, we present a study on the applications of UPLC QqTOF on the determination of 138 pesticides in five fruit- and five vegetable-based infant foods. The advantages and disadvantages of QqTOF MS (QqTOF operated in full-scan mode) and QqTOF MS/MS (QqTOF operated in MS/MS mode for product ion scan) were compared and discussed in terms of their applications for screening, quantification, and confirmation. The method was validated according to a designed experiment, that is, a nested design (*18*−*20*), to evaluate its performance characteristics including overall recovery, intermediate precision, and measurement uncertainty. The study proved that UPLC QqTOF was an important and practical tool to screen for pesticides in infant foods and to confirm their identities for regulatory purposes, particularly at 10 μg/kg.

MATERIALS AND METHODS

Materials and Reagents. Ten different types or brands of organic fruit- and vegetable-based infant foods, which included apples, apples and bananas, pears, bananas, apple juice, peas, sweet potatoes, creamed corn, squash, and carrots, were obtained from local markets. All of these infant foods were in the form of a purée (paste) except for apple juice and were packed or stored in small glass jars (128 mL per jar). All samples were free of pesticides tested. Ammonium acetate (reagent grade), [Glu^1]-fibrinopeptide B (F-3261), leucine enkephalin (L-9133), and magnesium sulfate anhydrous ($MgSO_4$) were purchased from Sigma-Aldrich Corp. (Canada). Acetic acid (glacial acetic acid, reagent grade, 99.7%), acetonitrile (distilled in glass), and methanol (distilled in glass) were obtained from Caledon Laboratories Ltd. (Canada). All water used was Milli-Q water, 18 MΩ·cm from a Milli-Q Reagent Water System (Millipore Corp.). Primary secondary amine (PSA, Bondesil PSA, 40 μm) was purchased from Varian Inc. (Canada). Sodium acetate anhydrous (ACS reagent) was from Thermo Fisher Scientific Inc. (Canada). Pesticide standards (**Table 1**, column 1) were obtained from EQ Laboratories Inc., Riedel-de Haen AG (Germany), or Chem Service. Carbendazim d_4 (100 μg/mL) and carbofuran d_3 (100 μg/mL) were purchased from EQ Laboratories Inc. Thiabendazole d_4 was from Chemical Synthesis Services (Northern Ireland). The standards obtained in solvent were penoxsulam 100 μg/mL in acetonitrile, flucarbazone (sodium) 10 μg/mL in water, carbendazim d_4 100 μg/mL in acetone, and carbofuran d_3 100 μg/mL in acetone.

Preparation of Reagents and Standard Solutions. Standard stock solution (2000 μg/mL) was prepared by weighing 20.0 mg of each individual pesticide (except carbendazim) into separate 10 mL volumetric flasks, dissolving in methanol, and making up to volume. Due to its poor solubility in methanol, carbendazim stock solution (200 μg/mL) was prepared by weighing 10.0 mg into a 50 mL volumetric flask, dissolving in methanol, and making up to volume. Internal standard stock solution (1000 μg/mL) was prepared by weighing 10.0 mg of thiabendazole d_4 into a 10 mL volumetric flask, dissolving in methanol, and making up to volume. Stock solutions were stored at −20 °C. Intermediate pesticide standard mix working solution (10.0 μg/mL, ppm) was made by transferring 500 μL of each stock standard solution (except carbendazim) and 5 mL of carbendazim stock solution into a 100 mL volumetric flask and diluting to volume with methanol. Benomyl, carbosulfan, formetanate, and thiophanate-methyl were not added to the mix due to their degradation during storage. The intermediate working solution was stored at −20 °C. A six-level pesticide standard mix working solution was prepared by transferring 0.1, 0.5, 2.0, 4.0, 6.0, and 10.0 mL of a 10 μg/mL intermediate working solution into six separate 50 mL volumetric flasks and making up to volume with methanol to prepare the 0.020, 0.1, 0.4, 0.8, 1.2, and 2.0 μg/mL six-level standard mix working solutions for construction of matrix-matched standard calibration curves. Three-level sample spike pesticide standard working solutions were made ready by transferring 1.0, 5.0, and 8.0 mL of 10 μg/mL intermediate working solution into 50 mL volumetric flasks and making up to volume with methanol to prepare the 0.2, 1.0, and 1.6 μg/mL three-level sample spike standard working solutions for sample spikes. An internal calibration standard working solution was prepared by transferring 100 μL of thiabendazole d_4 stock solution, 1 mL of carbendazim d_4 100 μg/mL stock solution, and 1 mL of carbofuran d_3 100 μg/mL stock solution into a 50 mL volumetric flask and making up to volume with acetonitrile. All working solutions were stored at 4 °C.

UPLC QqTOF Parameters. The UPLC QqTOF system utilized was a Waters Acquity ultra-performance liquid chromatograph (UPLC) coupled with Q-Tof Premier, that is, a quadrupole and orthogonal acceleration time-of-flight tandem mass spectrometer utilizing electrospray ionization interface (UPLC QqTOF) (Waters, Milford, MA). The system was operated under MassLynx 4.1 software.

(a) UPLC Profile. Mobile phase components were acetonitrile (solvent A) and 10 mM ammonium (solvent B). Gradient profile consisted of 0−9 min, 8−95% A; 9−11 min, 95−100% A; 11−12 min, 100% A; 12−14 min, 8% A. Flow rate was 0.4 mL/min. Injection volume was 10 μL. The UPLC column utilized was an Acquity UPLC BEH C_{18} column, 100 mm × 2.1 mm, i.d., 1.7 μm particle size (Waters). The column oven was set at 45 °C.

(b) QqTOF MS Conditions. Electrospray positive ion mode was utilized with the capillary voltage set at 3.20 kV. Source temperature was set at 120 °C, and desolvation temperature was 300 °C. Nebulizer nitrogen flow rate was regulated at 50 L/h, and desolvation nitrogen gas flow rate was set at 800 L/h. Collision gas argon pressure was regulated at 5.3×10^{-3} mbar, and collision energy was set 5 eV when QqTOF was operated in full-scan mode. Sampling cone voltage was 20 V. LM and HM resolutions were set at 4.7 and 15, respectively. Mass range was from *m*/*z* 50 to 950. TOF resolution was 15000 fwhm, which was measured with [Glu^1]-fibrinopeptide B at $[M + 2H]^{2+} = 785.8426$ in W-mode. Lock mass reference was leucine enkephalin ($[M + H]^+ = 556.2771$). Data were acquired in centroid format with programmable Dynamic Range Enhancement (pDRE) enabled for a dynamic range of 2 or 3 orders of magnitude for quantification under W-mode.

QqTOF MS/MS Conditions. QqTOF MS/MS parameters were the same as those of QqTOF MS except that the first quadrupole was used to serve as a mass filter. The collision energy was ramped from 5 to 70 eV, and data were acquired over a mass range from *m*/*z* 50 to 950. Therefore, a QqTOF MS/MS product ion spectrum was obtained for unequivocal confirmation of pesticides. Ideally, collision energy could have been optimized for each individual pesticide to achieve better sensitivity.

Sample Preparation and Extraction Procedure. Sample extraction and cleanup procedures followed the buffered QuEChERS method (*21*,*22*) or AOAC Official Method 2007.01 (*23*). Infant food samples (10 g/sample) were weighed into individual 50 mL polypropylene centrifuge tubes (VWR International, Canada). Five hundred microliters per three-level sample spike pesticide standard working solution was added into three centrifuge tubes to provide 10.0, 50.0, and 80.0 μg/kg of standards equivalent in samples, followed by adding 100 μL of the internal calibration standard working solution (20 μg/kg equivalent in samples). Then, 10 mL of acetonitrile/acetic acid (99 + 1, v/v) and 1 g of sodium acetate anhydrous were added to each sample, and after mixing, 4 g of magnesium sulfate anhydrous was added. The centrifuge tubes were capped and shaken for 45 s by hand, followed by centrifugation at 3000 rpm (∼2100*g*) for 2 min using an Allegra 6 centrifuge (Beckman Coulter Inc.). (Note: samples rich in starch such as sweet potatoes required a longer centrifugation time, such as 15 min.) Supernatants were transferred (6−8 mL/sample) into individual 15 mL polypropylene centrifuge tubes (VWR International, Canada) that contained 0.4 g of PSA and 1.2 g of $MgSO_4$ per tube. The centrifuge tubes were capped and shaken for 45 s and then centrifuged at 3000 rpm (∼2100*g*) for 2 min. Supernatants (5 mL/sample) were transferred into individual 5 mL Pyrex brand centrifuge tubes, precalibrated with 1 and 2 mL volume accuracy (VWR International, Canada). Each sample extract was evaporated to 0.2−0.3 mL using an N-EVAP nitrogen evaporator (Organomation Associates Inc.) at 30 °C under a stream of nitrogen. The extracts were than made up to 1 mL with methanol, vortexed for 30 s, then made up to 2 mL with 0.1 M ammonium acetate, and vortexed again for 30 s. Six hundred microliters of extract was

Table 1. Accurate Mass and UPLC QqTOF MS Method Performance Results

								fruits			vegetables		
(1) pesticide	(2) elemental composition	(3) ionization	(4) exact mass of $[M + H]^+$ or $[M + NH_4]^+$	(5) fragment ion	(6) exact mass of extracted ion for quantification	(7) retention time, min	(8) LCL S/N PtP[a]	(9) recovery, %	(10) precision, %	(11) measurement uncertainty, %	(12) recovery, %	(13) precision, %	(14) measurement uncertainty, %
acetochlor	$C_{14}H_{20}ClNO_2$	$[M + H]^+$	270.1261	$C_{12}H_{15}ClNO^+$	224.0842	6.19	3 (1)	100.1	10.5	24.9	100.9	11.7	24.7
aldicarb	$C_7H_{14}N_2O_2S$	$[M + H]^+$	191.0854	$C_5H_{10}NS^+$	116.0534	3.41	12 (5)	102.8	16.2	39.7	101.2	16.8	36.1
aldicarb sulfone	$C_7H_{14}N_2O_4S$	$[M + H]^+$	223.0753		223.0753	1.65	13 (5)	90.2	18.4	47.5	101.7	22.7	48.6
aldicarb sulfoxide	$C_7H_{14}N_2O_3S$	$[M + H]^+$	207.0803	$C_5H_{10}NOS^+$	132.0483	1.30	18 (5)	95.8	23.6	47.6	90.2	19.1	39.9
azaconazole	$C_{12}H_{11}C_{12}N_3O_2$	$[M + H]^+$	300.0307		300.0307	4.60	7 (1)	102.1	14.8	36.8	100.8	16.8	36.2
benomyl[b]	$C_{14}H_{18}N_4O_3$	$[M + H]^+$	291.1457		291.1457	6.04	n/a	n/a	n/a	n/a	n/a	n/a	n/a
benoxacor	$C_{11}H_{11}C_{12}NO_2$	$[M + H]^+$	260.0245		260.0245	5.49	7 (20)	105.3	20.6	41.6[j]	103.6	16.8	34.0[j]
bitertanol	$C_{20}H_{23}N_3O_2$	$[M + H]^+$	338.1869		338.1869	6.16	16 (5)	95.8	15.7	34.3	96.0	15.1	35.4
bromuconazole	$C_{13}H_{12}BrCl_2N_3O$	$[M + H]^+$	375.9619	ion with one Cl^{37}	377.9590	5.55	5 (1)	98.6	12.9	28.3	101.8	10.8	23.4
butafenacil	$C_{20}H_{18}ClF_3N_2O_6$	$[M + NH_4]^+$	492.1149		492.1149	6.36	15 (1)	98.2	18.3	44.0	90.5	20.8	53.1
butocarboxim sulfoxide	$C_7H_{14}N_2O_3S$	$[M + H]^+$	207.0803	$C_5H_{10}NOS^+$	132.0483	1.19	23 (5)	92.6	20.8	46.5	89.8	20.3	42.6
cadusafos	$C_{10}H_{23}O_2PS_2$	$[M + H]^+$	271.0955		271.0955	6.84	11 (1)	74.7	14.5	38.5	72.9	13.0	26.8
carbaryl	$C_{12}H_{11}NO_2$	$[M + H]^+$	202.0868	$C_{10}H_9O^+$	145.0653	4.35	28 (5)	103.3	10.4	25.1	100.4	10.7	22.6
carbendazim	$C_9H_9N_3O_2$	$[M + H]^+$	192.0773		192.0773	2.55	19 (1)	108.3	22.1	46.3	99.1	14.3	31.7
carbofuran	$C_{12}H_{15}NO_3$	$[M + H]^+$	222.1130		222.1130	4.14	20 (1)	102.7	15.0	31.3	103.5	14.8	32.0
carbosulfan[c]	$C_{20}H_{32}N_2O_3S$	$[M + H]^+$	381.2212		381.2212	9.23	44 (1)	39.5	14.7	n/a	n/a	n/a	n/a
carfentrazone-ethyl	$C_{15}H_{14}Cl_2F_3N_3O_3$	$[M + H]^+$	412.0443		412.0443	6.47	6 (1)	101.8	11.8	27.5	99.7	13.1	27.2
chlorimuron-ethyl	$C_{15}H_{15}ClN_4O_6S$	$[M + H]^+$	415.0479		415.0479	3.17	3 (1)	88.9	19.6	42.0	87.2	26.0	56.4
chloroxuron	$C_{15}H_{15}ClN_2O_2$	$[M + H]^+$	291.0900		291.0900	5.53	27 (1)	101.2	14.3	31.6	97.8	15.4	35.8
chlortoluron	$C_{10}H_{13}ClN_2O$	$[M + H]^+$	213.0795		213.0795	4.29	23 (1)	99.5	16.2	34.9	101.4	15.1	33.3
clodinafop-propargyl	$C_{17}H_{13}ClFNO_4$	$[M + H]^+$	350.0595		350.0595	6.69	5 (5)	75.0	17.7	54.4	69.2	14.7	32.3
cloquintocet-mexyl	$C_{18}H_{22}ClNO_3$	$[M + H]^+$	336.1366		336.1366	7.45	37 (1)	84.5	16.8	46.9	92.7	15.2	31.4
clothianidin	$C_6H_8ClN_5O_2S$	$[M + H]^+$	250.0165		250.0165	2.45	3 (5)	85.0	24.0	53.7	95.0	27.1	55.4
cycloxydim	$C_{17}H_{27}NO_3S$	$[M + H]^+$	326.1790		326.1790	5.05	6 (1)	106.7	15.2	31.9	109.7	11.8	24.4
cycluron	$C_{11}H_{22}N_2O$	$[M + H]^+$	199.1810		199.1810	4.42	5 (1)	101.8	12.6	25.9	101.1	17.8	35.9
demeton-*S*-methyl sulfone	$C_6H_{15}O_5PS_2$	$[M + H]^+$	263.0177		263.0177	2.02	25 (1)	96.0	18.3	37.0	98.7	17.8	36.8
demeton-*S*-methyl sulfoxide	$C_6H_{15}O_4PS_2$	$[M + H]^+$	247.0228		247.0228	1.63	27 (1)	93.9	14.4	29.3	94.6	13.7	27.5
desmedipham	$C_{16}H_{16}N_2O_4$	$[M + H]^+$	301.1188		301.1188	5.25	10 (1)	104.6	16.7	38.2	103.7	14.3	30.2
diethofencarb	$C_{14}H_{21}NO_4$	$[M + H]^+$	268.1549	$C_{11}H_{16}NO_4^+$	226.1079	5.45	24 (1)	105.7	14.1	28.4	107.5	18.0	38.2
difenoconazole	$C_{19}H_{17}Cl_2N_3O_3$	$[M + H]^+$	406.0725		406.0725	6.66	13 (1)	86.0	10.6	37.4	80.1	10.7	22.7
dimethametryn	$C_{11}H_{21}N_5S$	$[M + H]^+$	256.1596		256.1596	6.19	61 (1)	96.4	17.6	37.0	94.5	15.6	44.1
dimethomorph	$C_{21}H_{22}ClNO_4$	$[M + H]^+$	388.1316		388.1316	5.32	26 (1)	101.4	18.0	38.7	106.1	17.8	39.6
diniconazole	$C_{15}H_{17}Cl_2N_3O$	$[M + H]^+$	326.0827		326.0827	6.29	8 (1)	86.6	13.9	51.5	85.8	12.3	43.9
dioxacarb	$C_{11}H_{13}NO_4$	$[M + H]^+$	224.0923	$C_9H_{11}O_3^+$	167.0708	2.78	5 (1)	96.0	13.0	28.4	95.3	10.3	23.5
dipropetryn	$C_{11}H_{21}N_5S$	$[M + H]^+$	256.1596		256.1596	6.36	58 (1)	89.8	16.0	38.7	88.3	15.2	37.2
diuron	$C_9H_{10}Cl_2N_2O$	$[M + H]^+$	233.0248		233.0248	4.57	13 (1)	104.3	13.2	29.1	101.4	13.7	28.1
dodemorph	$C_{18}H_{35}NO$	$[M + H]^+$	282.2797		282.2797	10.33	110 (1)	101.6	11.1	26.5	96.1	11.9	24.2
emamectin B_{1a}	$C_{49}H_{75}NO_{13}$	$[M + H]^+$	886.5317		886.5317	7.92	32 (1)	101.4	14.3	31.5	100.2	17.4	35.2
epoxiconazole	$C_{17}H_{13}ClFN_3O$	$[M + H]^+$	330.0809		330.0809	5.69	26 (1)	103.9	15.1	36.8	102.1	15.0	39.6
ethiofencarb	$C_{11}H_{15}NO_2S$	$[M + H]^+$	226.0902	$C_7H_7O^+$	107.0497	4.51	14 (1)	101.7	12.6	29.3	103.6	12.4	29.8
ethiofencarb sulfone	$C_{11}H_{15}NO_4S$	$[M + H]^+$	258.0800	$C_9H_{13}O_3S^+$	201.0585	2.48	14 (1)	98.1	12.3	29.1	99.3	10.1	25.9
ethiofencarb sulfoxide	$C_{11}H_{15}NO_3S$	$[M + H]^+$	242.0851	$C_9H_{13}O_2S^+$	185.0636	2.18	10 (1)	98.4	12.9	28.8	97.9	13.5	27.3
ethirimol	$C_{11}H_{19}N_3O$	$[M + H]^+$	210.1606		210.1606	3.70	104 (1)	98.1	20.2	41.6	94.2	19.1	40.9
ethoprop	$C_8H_{19}O_2PS_2$	$[M + H]^+$	243.0642		243.0642	5.84	7 (1)	99.4	16.5	33.4	105.3	14.2	31.3
etofenprox	$C_{25}H_{28}O_3$	$[M + NH_4]^+$	394.2382		394.2382	8.98	18 (5)	88.8	45.9	94.6	80.6	47.3	96.9
etoxazole	$C_{21}H_{23}F_2NO_2$	$[M + H]^+$	360.1775		360.1775	8.13	115 (1)	97.0	25.9	56.3	89.4	27.1	57.7
fenamidone	$C_{17}H_{17}N_3OS$	$[M + H]^+$	312.1171		312.1171	5.69	24 (1)	97.8	15.2	34.7	102.4	19.4	39.7
fenazaquin	$C_{20}H_{22}N_2O$	$[M + H]^+$	307.1810		307.1810	7.97	44 (1)	99.7	20.8	49.0	93.9	21.2	45.7
fenhexamid	$C_{14}H_{17}Cl_2NO_2$	$[M + H]^+$	302.0715		302.0715	5.77	15 (5)	102.9	12.8	28.4	99.3	15.8	31.9
fenoxanil	$C_{15}H_{18}Cl_2N_2O_2$	$[M + H]^+$	329.0824		329.0824	6.51	14 (1)	98.0	11.2	31.4	101.3	8.5	22.2
fenpropidin	$C_{19}H_{31}N$	$[M + H]^+$	274.2535		274.2535	6.25	42 (1)	93.1	15.8	36.2	92.5	14.9	31.5
fenpropimorph	$C_{20}H_{33}NO$	$[M + H]^+$	304.2640		304.2640	9.09	47 (1)	99.9	15.7	31.8	97.7	17.8	37.2
fenpyroximate	$C_{24}H_{27}N_3O_4$	$[M + H]^+$	422.2080		422.2080	8.01	15 (1)	94.3	22.2	47.1	90.6	23.2	49.9
fentrazamide	$C_{16}H_{20}ClN_5O_2$	$[M + H]^+$	350.1384	$C_{10}H_{17}N_2O_2^+$	197.1290	6.84	12 (5)	65.7	23.5	82.6	62.3	20.9	42.0
fluazifop-butyl	$C_{19}H_{20}F_3NO_4$	$[M + H]^+$	384.1423		384.1423	7.74	24 (1)	94.0	14.3	31.9	99.8	20.6	44.1
flucarbazone[d]	$C_{12}H_{11}F_3N_4O_6S$	$[M + H]^+$	397.0430		397.0430	2.33	12 (5)	17.6	42.3	n/a	n/a	n/a	n/a
flutolanil	$C_{17}H_{16}F_3NO_2$	$[M + H]^+$	324.1211		324.1211	6.12	46 (1)	100.4	19.1	40.2	99.7	19.9	43.7
flutriafol	$C_{16}H_{13}F_2N_3O$	$[M + H]^+$	302.1105		302.1105	4.44	32 (1)	99.2	23.0	50.3	105.1	19.1	40.3
forchlorfenuron	$C_{12}H_{10}ClN_3O$	$[M + H]^+$	248.0591		248.0591	4.36	7 (1)	105.5	22.5	49.0	98.3	13.7	35.4
formetanate[e]	$C_{11}H_{15}N_3O_2$	$[M + H]^+$	222.1243		222.1243	1.81	38 (1)	113.9	30.1	n/a	n/a	n/a	n/a
fosthiazate	$C_9H_{18}NO_3PS_2$	$[M + H]^+$	284.0544		284.0544	4.46	14 (1)	102.4	15.0	31.1	105.2	16.4	32.9
fuberidazole	$C_{11}H_8N_2O$	$[M + H]^+$	185.0715		185.0715	3.05	35 (1)	96.6	20.8	41.8	97.1	19.8	45.0
furathiocarb	$C_{18}H_{26}N_2O_5S$	$[M + H]^+$	383.1641		383.1641	7.61	27 (1)	97.4	21.0	44.7	95.9	20.1	40.4
haloxyfop	$C_{15}H_{11}ClF_3NO_4$	$[M + H]^+$	362.0407		362.0407	3.94	3 (5)	55.0	30.5	61.5	49.6	30.2	67.7

Table 1. Continued

								fruits			vegetables		
(1) pesticide	(2) elemental composition	(3) ionization	(4) exact mass of $[M + H]^+$ or $[M + NH_4]^+$	(5) fragment ion	(6) exact mass of extracted ion for quantification	(7) retention time, min	(8) LCL S/N PtP[a]	(9) recovery, %	(10) precision, %	(11) measurement uncertainty, %	(12) recovery, %	(13) precision, %	(14) measurement uncertainty, %
3-hydroxycarbofuran	$C_{12}H_{15}NO_4$	$[M + H]^+$	238.1079	$C_{12}H_{14}NO_3^+$	220.0974	2.55	4 (1)	102.3	10.2	23.9	98.7	13.2	28.5
imazamethabenz-methyl	$C_{16}H_{20}N_2O_3$	$[M + H]^+$	289.1552		289.1552	3.76	74 (1)	101.6	13.4	27.0	99.5	14.4	29.1
imidacloprid	$C_9H_{10}ClN_5O_2$	$[M + H]^+$	256.0601		256.0601	2.61	6 (5)	104.4	13.7	28.2	107.5	14.0	30.2
indoxacarb	$C_{22}H_{17}ClF_3N_3O_7$	$[M + H]^+$	528.0785		528.0785	7.21	3 (1)	77.5	17.0	61.3	79.0	16.4	40.1
iprovalicarb	$C_{18}H_{28}N_2O_3$	$[M + H]^+$	321.2178		321.2178	5.60	15 (1)	106.7	19.9	41.3	108.0	17.2	38.3
isocarbamide	$C_6H_{15}N_3O_2$	$[M + H]^+$	186.1243		186.1243	2.55	14 (5)	100.0	15.2	30.7	96.8	16.4	38.3
isoprocarb	$C_{11}H_{15}NO_2$	$[M + H]^+$	194.1181		194.1181	4.75	3 (5)	99.8	10.0	21.6	103.3	10.2	22.0
isoxathion	$C_{13}H_{16}NO_4PS$	$[M + H]^+$	314.0616		314.0616	7.11	10 (1)	101.0	19.0	38.7	99.8	22.3	45.4
mepanipyrim	$C_{14}H_{13}N_3$	$[M + H]^+$	224.1188		224.1188	5.89	55 (1)	98.5	19.4	44.5	95.2	14.4	32.8
mephosfolan	$C_8H_{16}NO_3PS_2$	$[M + H]^+$	270.0388		270.0388	3.68	107 (1)	107.3	17.0	34.1	101.7	17.0	36.5
methabenzthiazuron	$C_{10}H_{11}N_3O_3$	$[M + H]^+$	222.0879		222.0879	4.20	25 (1)	96.8	22.5	45.5	93.1	22.0	44.3
methiocarb	$C_{11}H_{15}NO_2S$	$[M + H]^+$	226.0902	$C_9H_{13}OS^+$	169.0687	5.31	23 (5)	102.3	13.1	28.4	102.6	14.9	30.7
methiocarb sulfone	$C_{11}H_{15}NO_4S$	$[M + H]^+$	258.0800		258.0800	3.07	7 (5)	95.3	20.9	43.1	88.1	28.7	62.0
methiocarb sulfoxide	$C_{11}H_{15}NO_3S$	$[M + H]^+$	242.0851		242.0851	2.37	17 (1)	93.4	22.9	51.8	98.6	18.9	39.2
methomyl	$C_5H_{10}N_2O_2S$	$[M + H]^+$	163.0541	$C_3H_6NS^+$	88.0221	1.86	5 (5)	94.1	21.9	46.9	95.5	21.6	45.4
methoxyfenozide	$C_{22}H_{28}N_2O_3$	$[M + H]^+$	369.2178	$C_{18}H_{21}N_2O_3^+$	313.1552	5.97	11 (1)	100.9	23.8	55.4	99.2	21.0	46.8
metolcarb	$C_9H_{11}NO_2$	$[M + H]^+$	166.0868	$C_7H_9O^+$	109.0653	3.70	12 (20)	102.2	12.3	30.6[j]	105.7	15.1	34.8[j]
metoxuron	$C_{10}H_{13}ClN_2O_2$	$[M + H]^+$	229.0744		229.0744	3.39	35 (1)	102.0	18.3	36.7	99.8	18.9	39.2
molinate	$C_9H_{17}NOS$	$[M + H]^+$	188.1109		188.1109	5.67	4 (20)	96.9	14.6	30.6[j]	100.2	15.1	35.5[j]
napropamide	$C_{17}H_{21}NO_2$	$[M + H]^+$	272.1651		272.1651	5.92	101 (1)	101.7	23.0	47.0	102.9	17.8	35.8
naptalam	$C_{18}H_{13}NO_3$	$[M + H]^+$	292.0974	$C_{10}H_{10}N^+$	144.0813	2.61	11 (5)	34.1	19.5	70.8	28.7	41.6	84.1
neburon	$C_{12}H_{16}Cl_2N_2O$	$[M + H]^+$	275.0718		275.0718	6.26	17 (1)	85.9	22.8	51.5	82.7	14.2	38.4
ofurace	$C_{14}H_{16}ClNO_3$	$[M + H]^+$	282.0897		282.0897	4.51	22 (1)	106.8	20.2	40.8	103.4	16.5	36.0
oxamyl	$C_7H_{13}N_3O_3S$	$[M + H]^+$	220.0756	$C_3H_8NO_2^{+i}$	90.0549	1.69	4 (5)	93.8	20.1	40.7	92.0	22.1	44.3
oxamyl-oxime	$C_5H_{10}N_2O_2S$	$[M + H]^+$	163.0541		163.0541	1.32	3 (20)	105.1	24.2	48.6[j]	128.1	30.9	62.6[j]
paclobutrazol	$C_{15}H_{20}ClN_3O$	$[M + H]^+$	294.1373		294.1373	5.23	8 (1)	108.2	17.2	36.3	106.5	14.6	31.6
pencycuron	$C_{19}H_{21}ClN_2O$	$[M + H]^+$	329.1421		329.1421	7.00	21 (1)	89.4	17.0	39.4	92.4	13.9	29.1
penoxsulam[f]	$C_{16}H_{14}F_5N_5O_5S$	$[M + H]^+$	484.0714		484.0714	3.23	67 (1)	93.3	11.5	n/a	76.7	11.9	n/a
picolinafen	$C_{19}H_{12}F_4N_2O_2$	$[M + H]^+$	377.0913		377.0913	7.45	4 (1)	87.6	18.6	47.2	91.0	23.7	48.7
picoxystrobin	$C_{18}H_{16}F_3NO_4$	$[M + H]^+$	368.1110	$C_{12}H_{13}O_3^+$	205.0865	6.62	41 (1)	96.3	15.0	35.0	99.5	16.8	33.6
piperophos	$C_{14}H_{28}NO_3PS_2$	$[M + H]^+$	354.1327		354.1327	7.19	59 (1)	95.7	13.6	33.9	93.4	16.5	36.4
pretilachlor	$C_{17}H_{26}ClNO_2$	$[M + H]^+$	312.1730		312.1730	7.28	20 (1)	81.3	18.2	48.6	78.7	12.8	27.5
primisulfuron-methyl	$C_{15}H_{12}F_4N_4O_7S$	$[M + H]^+$	469.0441		469.0441	3.91	3 (1)	83.4	24.5	51.5	82.7	32.2	68.7
prodiamine	$C_{13}H_{17}F_3N_4O_4$	$[M + H]^+$	351.1280		351.1280	7.56	9 (20)	95.0	21.2	48.6[j]	94.3	27.7	57.5[j]
propoxur	$C_{11}H_{15}NO_3$	$[M + H]^+$	210.1130	$C_8H_{10}NO_3^+$	168.0661	4.07	8 (1)	102.1	9.5	20.1	104.3	9.9	20.9
pymetrozine	$C_{10}H_{11}N_5O$	$[M + H]^+$	218.1042		218.1042	1.54	5 (1)	81.4	12.4	27.6	90.7	16.3	47.6
pyraclostrobin	$C_{19}H_{18}ClN_3O_4$	$[M + H]^+$	388.1064		388.1064	6.84	3 (1)	66.5	18.5	77.5	56.2	19.5	39.3
pyraflufen-ethyl	$C_{15}H_{13}Cl_2F_3N_2O_4$	$[M + H]^+$	413.0283		413.0283	6.69	3 (5)	78.1	15.6	49.8	70.6	14.4	31.9
pyridalyl	$C_{18}H_{14}Cl_4F_3NO_3$	$[M + H]^+$	489.9758	ion with one Cl^{37}	491.9729	9.43	7 (5)	93.9	79.0	162.2	107.4	95.3	193.9
pyridaphenthion	$C_{14}H_{17}N_2O_4PS$	$[M + H]^+$	341.0725		341.0725	5.81	31 (1)	101.6	15.7	35.5	96.8	16.4	33.0
pyridate	$C_{19}H_{23}ClN_2O_2S$	$[M + H]^+$	379.1247		379.1247	9.03	10 (1)	66.4	30.6	78.9	57.4	47.3	95.0
pyrifenox	$C_{14}H_{12}Cl_2N_2O$	$[M + H]^+$	295.0405		295.0405	5.99	9 (1)	96.9	17.3	37.9	98.3	24.1	50.5
pyrimethanil	$C_{12}H_{13}N_3$	$[M + H]^+$	200.1188		200.1188	5.27	4 (1)	92.8	14.7	30.0	95.8	14.4	30.0
pyriproxyfen	$C_{20}H_{19}NO_3$	$[M + H]^+$	322.1443		322.1443	7.74	24 (1)	97.8	18.9	41.6	97.0	20.5	44.9
quinoxyfen	$C_{15}H_8Cl_2FNO$	$[M + H]^+$	308.0045		308.0045	7.44	4 (1)	79.0	16.5	69.7	78.0	17.8	37.5
quizalofop	$C_{17}H_{13}ClN_2O_4$	$[M + H]^+$	345.0642		345.0642	3.57	4 (5)	49.1	21.9	55.6	40.4	32.2	68.2
quizalofop-ethyl	$C_{19}H_{17}ClN_2O_4$	$[M + H]^+$	373.0955		373.0955	7.30	3 (1)	82.7	18.1	53.4	81.1	15.2	32.2
schradan	$C_8H_{24}N_4O_3P_2$	$[M + H]^+$	287.1402		287.1402	2.50	58 (1)	97.1	14.5	29.7	93.4	19.2	38.8
spinosad A[g]	$C_{41}H_{65}NO_{10}$	$[M + H]^+$	732.4687		732.4687	8.83	100 (1)	102.2	14.4	29.0	97.0	15.5	31.4
spinosad B	$C_{42}H_{67}NO_{10}$	$[M + H]^+$	746.4843		746.4843	9.26	35 (1)	99.1	14.8	29.9	93.8	15.8	36.0
spirodiclofen	$C_{21}H_{24}Cl_2O_4$	$[M + H]^+$	411.1130		411.1130	8.58	3 (1)	94.8	17.2	42.7	93.9	18.7	37.8
spiromesifen	$C_{23}H_{30}O_4$	$[M + H]^+$	371.2222	$C_{17}H_{21}O_3^+$	273.1491	8.48	64 (1)	94.8	15.4	37.4	96.1	21.2	42.7
spiroxamine	$C_{18}H_{35}NO_2$	$[M + H]^+$	298.2746		298.2746	7.12	73 (5)	99.5	15.4	35.0	95.8	16.0	33.9
sulfentrazone	$C_{11}H_{10}Cl_2F_2N_4O_3S$	$[M + NH_4]^+$	404.0162		404.0162	3.91	3 (1)	100.2	10.9	26.4	100.7	11.8	23.7
tebufenozide	$C_{22}H_{28}N_2O_2$	$[M + H]^+$	353.2229	$C_{18}H_{21}N_2O_2^+$	297.1603	6.43	43 (1)	98.0	16.6	36.8	100.5	17.5	36.7
tebufenpyrad	$C_{18}H_{24}ClN_3O$	$[M + H]^+$	334.1686		334.1686	7.39	41 (1)	92.8	15.9	43.2	98.4	15.4	34.8
tebupirimfos	$C_{13}H_{23}N_2O_3PS$	$[M + H]^+$	319.1245		319.1245	7.85	6 (1)	90.0	19.9	40.5	94.1	18.6	39.3
tepraloxydim	$C_{17}H_{24}ClNO_4$	$[M + H]^+$	342.1472		342.1472	3.05	8 (5)	139.9	14.8	33.9	135.2	15.3	34.0
tetraconazole	$C_{13}H_{11}Cl_2F_4N_3O$	$[M + H]^+$	372.0294		372.0294	5.84	7 (1)	102.7	17.0	37.7	101.7	19.0	39.3
thiabendazole	$C_{10}H_7N_3S$	$[M + H]^+$	202.0439		202.0439	2.85	124 (1)	99.4	19.0	39.2	101.3	16.9	37.6
thiacloprid	$C_{10}H_9ClN_4S$	$[M + H]^+$	253.0315		253.0315	3.27	3 (1)	96.2	19.2	42.9	99.5	13.6	27.5
thiamethoxam	$C_8H_{10}ClN_5O_3S$	$[M + H]^+$	292.0271		292.0271	2.11	11 (1)	87.0	10.5	25.7	93.5	18.7	38.6
thiazopyr	$C_{16}H_{17}F_5N_2O_2S$	$[M + H]^+$	397.1009		397.1009	6.88	4 (1)	77.3	16.6	43.1	70.8	18.2	36.7
thiodicarb	$C_{10}H_{18}N_4O_4S_3$	$[M + H]^+$	355.0568		355.0568	4.09	4 (1)	94.2	19.3	44.1	92.1	15.1	34.7
thiofanox sulfone	$C_9H_{18}N_2O_4S$	$[M + H]^+$	251.1066		251.1066	2.82	5 (5)	98.9	21.2	53.8	99.1	17.6	36.5
thiofanox sulfoxide	C9H18N2O3S	$[M + H]^+$	235.1116	$C_3H_6NOS^+$	104.0170	2.26	4 (1)	97.4	13.4	32.0	95.0	14.2	28.7

Table 1. Continued

(1) pesticide	(2) elemental composition	(3) ionization	(4) exact mass of $[M + H]^+$ or $[M + NH_4]^+$	(5) fragment ion	(6) exact mass of extracted ion for quantification	(7) retention time, min	(8) LCL S/N PtP[a]	fruits (9) recovery, %	fruits (10) precision, %	fruits (11) measurement uncertainty, %	vegetables (12) recovery, %	vegetables (13) precision, %	vegetables (14) measurement uncertainty, %
thiophanate-methyl[h]	$C_{12}H_{14}N_4O_4S_2$	$[M + H]^+$	343.0535		343.0535	3.90	46 (1)	99.1	6.7	n/a	n/a	n/a	n/a
tralkoxydim	C20H27NO3	$[M + H]^+$	330.2069		330.2069	5.58	9 (1)	109.1	17.3	37.4	112.0	16.5	36.3
trichlorfon	$C_4H_8Cl_3O_4P$	$[M + H]^+$	256.9304	ion with one Cl^{37}	258.9275	2.37	3 (5)	94.3	19.8	42.5	97.2	25.7	51.7
trietazine	$C_9H_{16}ClN_5$	$[M + H]^+$	230.1172		230.1172	5.96	16 (1)	98.3	17.7	40.8	100.5	18.5	37.2
trifloxysulfuron	$C_{14}H_{14}F_3N_5O_6S$	$[M + H]^+$	438.0695		438.0695	2.94	22 (1)	95.4	20.8	42.9	94.9	21.2	50.7
triforine	$C_{10}H_{14}Cl_6N_4O_2$	$[M + H]^+$	432.9326	$C_9H_{12}Cl_5{}^{35}Cl^{37}N_3O^+$	389.9082	4.68	4 (1)	100.1	13.6	27.4	99.6	12.8	25.7
trimethacarb	$C_{11}H_{15}NO_2$	$[M + H]^+$	194.1181	$C_9H_{13}O^+$	137.0966	4.77	8 (1)	102.9	9.8	22.8	104.9	10.6	22.7
zinophos	$C_8H_{13}N_2O_3PS$	$[M + H]^+$	249.0463		249.0463	5.05	3 (5)	96.7	16.4	36.1	99.6	15.4	32.0
zoxamide	$C_{14}H_{16}Cl_3NO_2$	$[M + H]^+$	336.0325	ion with one Cl^{37}	338.0295	6.75	4 (1)	82.0	12.6	37.7	83.2	10.2	25.2
internal standard													
carbendazim d_4	$C_9H_5D_4N_3O_2$	$[M + H]^+$	196.1024		196.1024	2.52							
carbofuran d_3	$C_{12}H_{12}D_3NO_3$	$[M + H]^+$	225.1318		225.1318	4.13							
thiabendazole d_4	$C_{10}H_3D_4N_3S$	$[M + H]^+$	206.0690		206.0690	2.83							

[a] Signal-to-noise (peak-to-peak) ratios at lowest concentration level (μg/kg), which are in bracket, in apple infant food matrix. [b] Benomyl degrades rapidly to carbendazim during the extraction. Any detected benomyl should be reported as carbendazim. Data were obtained from banana matrix spiked at 10 μg/kg. [c] Carbosulfan degrades rapidly to carbofuran during the extraction. Any detected carbosulfan should be reported as carbofuran. Data for recovery and repeatability were obtained from banana matrix spiked at 10 μg/kg. [d] Measurement uncertainty for flucarbazone is not available due to a lack of high concentration standard. Flucarbazone showed poor recovery and repeatability. Data were obtained from banana matrix spiked at 10 μg/kg. [e] Formetanate is not stable in working solution. To quantify formetanate, fresh standard working solution needs to be prepared. Data for recovery and repeatability were obtained from banana matrix spiked at 10 μg/kg. [f] Measurement uncertainty for penoxsulam is not available due to a lack of high-concentration standard. Recovery and relative standard deviation are estimated from apple and carrot matrices spiked at 10 μg/kg. [g] Spinosad is a mixture of spinosads A and B. Quantification can be based on either one. [h] Thiophanate-methyl degrades to carbendazim. Thiophanate-methyl can be reported as itself. Data for recovery and repeatability were obtained from banana matrix spiked at 10 μg/kg. [i] Elemental composition was determined by the MassLynx Elemental Composition calculator, but it was not to be confirmed by the chemical structure related to possible fragmentation. [j] Measurement uncertainties were estimated using two spike levels, i.e., 50 and 80 μg/kg.

transferred into a Mini-UniPrep syringeless filter device with polypropylene housing and PVDF 0.45 μm membrane (Whatman Inc.). Sample extracts were then ready to be injected into the UPLC QqTOF system.

Preparation of Matrix-Matched Calibration Standards and Calculation. Matrix-matched calibration standards were prepared by adding standards and internal standards to blank sample extracts after sample preparation. Blank infant food samples (10 g/sample) were weighed into six separate 50 mL centrifuge tubes, and these samples were processed through the extraction procedure as described above. Two hundred and fifty microliters of each six-level pesticide standard mix working solution was transferred into each of six blank sample extracts, providing 1.0, 5.0, 20.0, 40.0, 60.0, and 100.0 μg/kg of standard equivalent in samples. Then, 50 μL of internal calibration working solution was added to each sample (20 μg/kg equivalent in samples). The extracts were made up to 1 mL volume with methanol, vortexed for 30 s, made up to 2 mL volume with 0.1 M ammonium acetate, and vortexed again for 30 s. Six hundred microliters of extracts was transferred into a Mini-UniPrep vial. Sample extracts were ready to be injected into the UPLC QqTOF system.

Matrix-matched standard calibration curves for each individual pesticide were constructed using QuanLynx. Concentration, micrograms per kilogram (parts per billion, ppb), versus the ratio (analyte area/IS area) of each individual pesticide was plotted. Deuterium-labeled standards carbendazim d_4, carbofuran d_3, and thiabendazole d_4 were used as internal standards for their respective native compounds for quantification. Other pesticides used carbofuran d_3 as an internal standard for quantification. In general, a quadratic function was applied to the calibration curves based on the line of best fit. Occasionally, linear regression may be used for quantification. The $1/x$ weighting was used to improve the accuracy for quantification of pesticides at low concentration. Responses for the unknown concentration or fortified samples were compared to the curves to calculate the amount of pesticide residues, micrograms per kilogram (ppb), in samples. Matrix-matched calibration standards were prepared fresh for each batch of samples.

Experimental Design. The method was validated according to the nested experimental design, which was described elsewhere (*18*−*20*). In this study, there were a total of 10 infant food matrices, which included 5 fruit- and 5 vegetable-based infant foods. For each matrix, samples were spiked at three levels, that is, 10, 40, and 80 μg/kg, in triplicate. Spike experiments were repeated on two different days. Data were organized into two groups based on either fruit- or vegetable-type infant foods. Overall recovery, intermediate precision, and measurement uncertainty were calculated for each group.

Compiled Computer Program. A compiled computer program that consisted of SAS codes (SAS software release 9.1, SAS Institute Inc.) along with a Microsoft Excel (Microsoft Office 2002) workbook was utilized to calculate the overall recovery, intermediate precision, and measurement uncertainty from the nested design or data analysis (*18*). The compiled program using SAS EIS/OLAP Application Builder provides a semiautomated procedure for handling a large number of calculations in a few seconds.

RESULTS AND DISCUSSION

Extraction and Data Acquisition. Pesticides were extracted from infant food matrices (10 g/sample) following the buffered QuEChERS method (*21*, *22*) or AOAC Official Method 2007.01 (*23*). The QuEChERS method proved to be adequate and effective to extract pesticides and to remove interference compounds for UPLC QqTOF analysis of pesticides from infant food matrices as indicated by the method performance discussed below.

A quadrupole time-of-flight mass spectrometer can be operated as either a TOF mass analyzer (QqTOF MS, full-scan) or

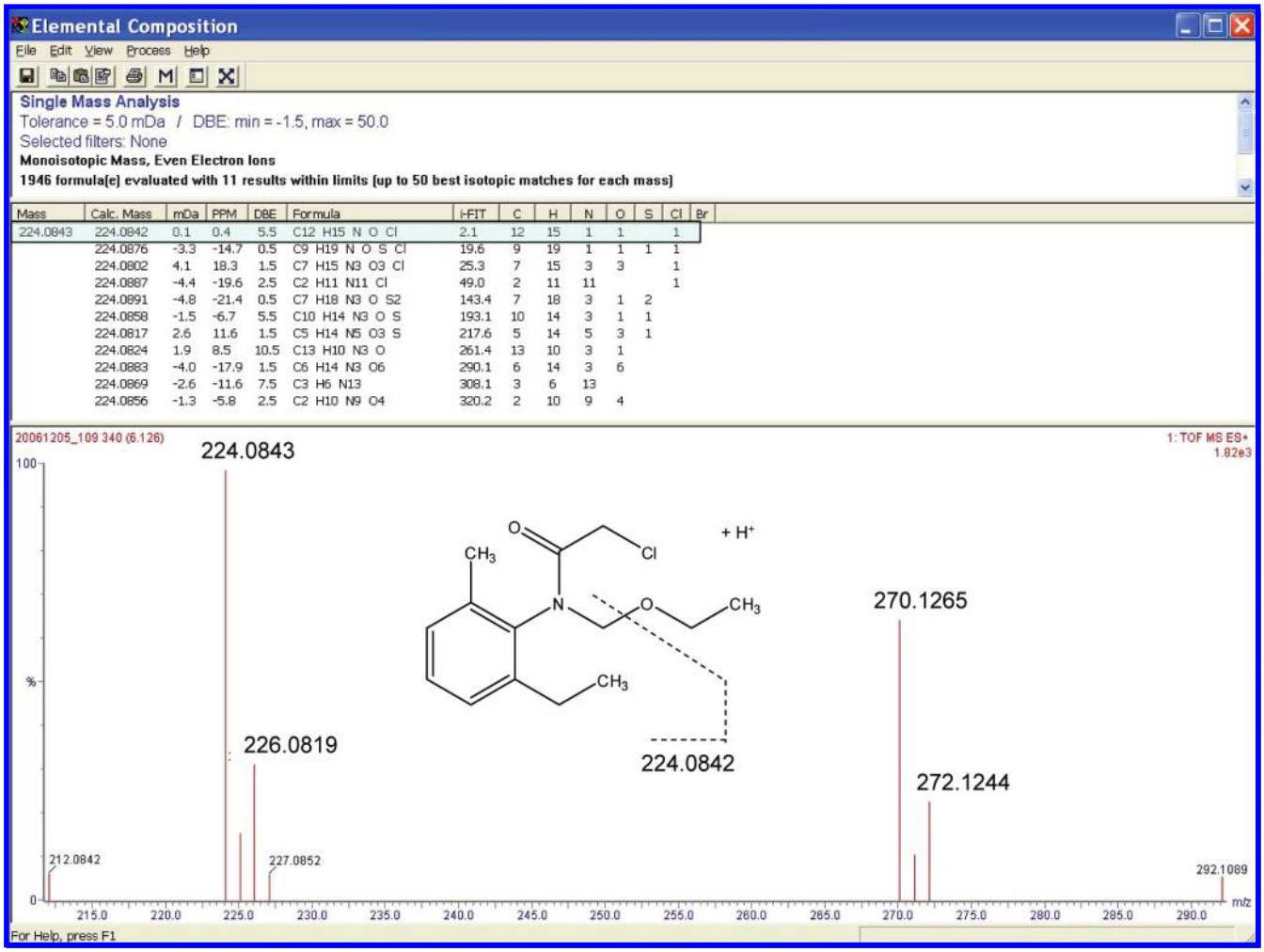

Figure 1. QqTOF mass spectrum and isotopic pattern of acetochlor and its possible fragmental pattern and fragment elemental composition generated by the MassLynx Elemental Composition tool. The i-FIT (isotopic fit) is a mathematical algorithm to compare the measured isotope ratios to those of the theoretical elemental composition. The lower the value, the better the fit.

a quadrupole TOF tandem mass spectrometer (QqTOF MS/MS, product ion scan). In routine practice, although a QqTOF MS/MS product ion spectrum provides more specificity for unequivocal confirmation of a pesticide, QqTOF MS is more practical than QqTOF MS/MS for targeted screening of a large number of pesticides or nontargeted screening of unknowns in a single analysis. Any incurred pesticides could be further confirmed using the QqTOF MS/MS. In the presence of ammonium acetate (10 mM) in the UPLC mobile phase, pesticides formed mainly $[M + H]^+$ and/or $[M + NH_4]^+$ (**Table 1**, column 3), which were used as target ions (**Table 1**, columns 4 and 6) for quantification. However, some of the pesticides experienced in-source decay or in-source collision-induced dissociation significantly, and consequently their fragment ions became the predominate ions that were chosen for quantification so as to lower the method detection limits. The possible elemental compositions of fragment ions (**Table 1**, column 5) were determined using the MassLynx Elemental Composition tool based on the accurate mass measurement, isotopical pattern (or i-FIT), and/or chemical structure (**Figure 1**).

One hundred and thirty-eight pesticides and three isotopically labeled standards (**Table 1**, column 1) were chromatographically separated within 12 min under a gradient profile using a UPLC BEH C_{18} column. **Figure 2** shows an example of a total ion current (TIC) chromatogram (**Figure 2A**) and extracted ion chromatograms (**Figure 2B**) of a few pesticides based on the exact masses. The UPLC demonstrated a satisfactory chromatographic performance given that the UPLC peaks were of Gaussian distribution, and retention times proved to be very reproducible under ±0.2 min shift within- and between-batches of analysis. Pesticides were eluted between 1.0 and 11.0 min with baseline peak-width between 5 and 10 s.

Matrix Effects and Calibration Curves. Matrix effects were one of the major challenges for UPLC QqTOF MS quantification when ESI was used as an interface. The matrix could either enhance or suppress ionization of pesticides; its effects might vary from sample-to-sample and ultimately affect the UPLC QqTOF MS quantitative performance. To evaluate matrix effects, the responses of pesticides in sample extracts were compared to those pesticide standards prepared in solvent buffer at the same concentration level, for example, 50 μg/kg equivalent in samples. As seen in fruit-based infant food matrices (**Figure 3A**), up to 39% of pesticides experienced ion suppression of ≥30% and up to 21% of pesticides had ion enhancement of >10%. Similar phenomena were observed in vegetable-based infant food matrices (**Figure 3B**). The matrix effects were considered to be significant because 49−68% of the pesticides studied had ion suppression of ≤30% or ion enhancement of ≤10%. As a comparison, 85−93% pesticides had ion suppression of ≤30% or ion enhancement of ≤10%, when the same samples (but sample extracts with additional three times dilution) were analyzed by LC-ESI-MS/MS (*17*). Compared to UPLC

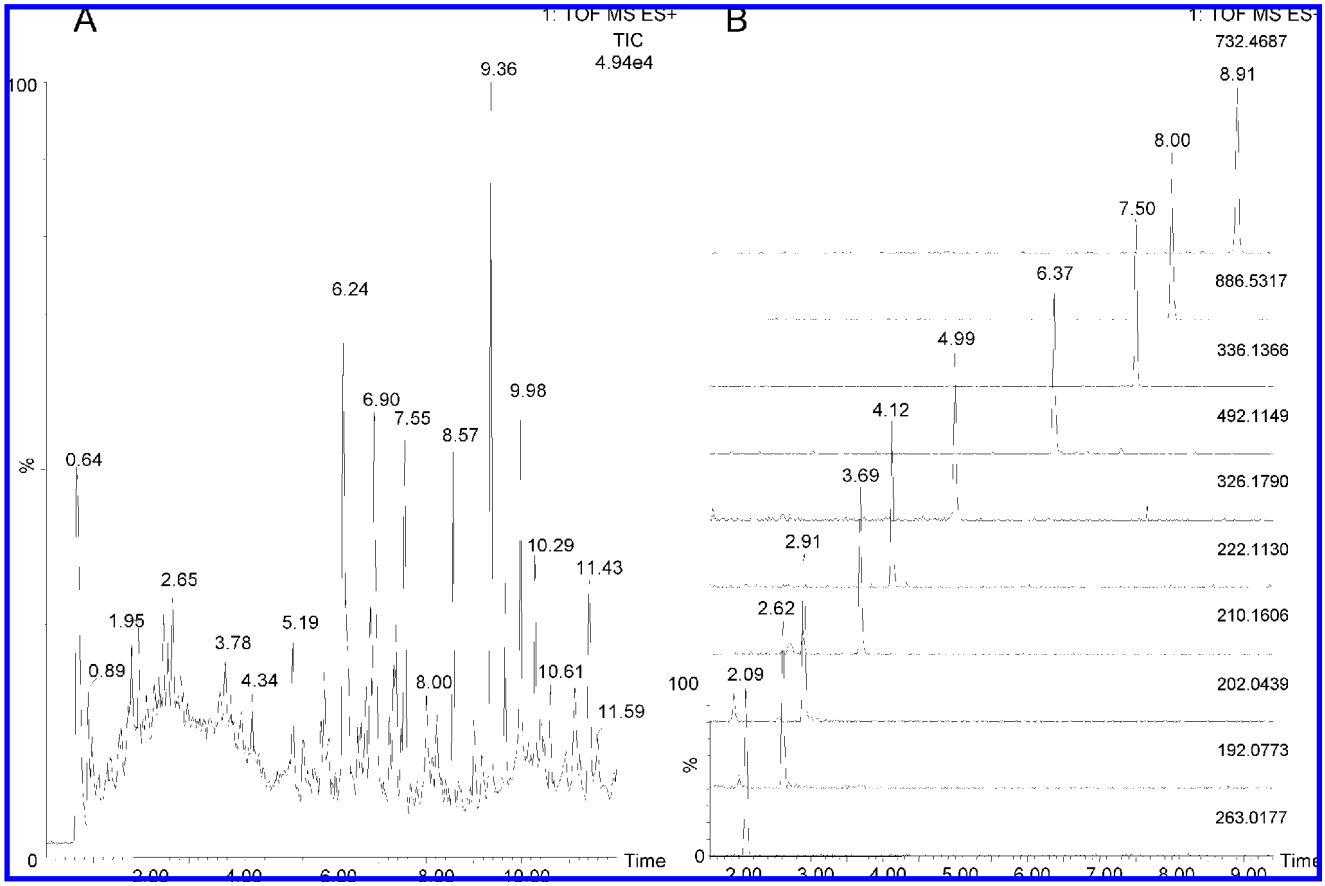

Figure 2. UPLC QqTOF MS chromatograms of pesticides (10 μg/kg) spiked in pear-based infant food: (**A**) total ion current chromatogram; (**B**) extracted ion chromatograms of pesticides with a mass error window of 50 mDa. From bottom to top: 1, demeton-*S*-methyl sulfone; 2, carbendazim; 3, thiabendazole; 4, ethirimol; 5, carbofuran; 6, cycloxydim; 7, butafenacil; 8, cloquintocet-mexyl; 9, emamectin B_{1a}; 10, spinosad A.

QqTOF MS, the LC-ESI-MS/MS demonstrated much fewer matrix effects, which might result from additional sample extracts dilution, small volume injection (i.e., 5 μL), and/or the different design in its mass spectrometric ion source (*17*).

Therefore, matrix-matched standard calibration curves and/or isotopically labeled standards were required to compensate for matrix effects so as to improve the UPLC QqTOF MS quantitative accuracy. Due to availability and cost, only three deuterium -labeled standards, that is, carbendazim d_4, carbofuran d_3, and thiabendazole d_4, were used as internal standards for their respective native compounds for quantification; other pesticides used carbofuran d_3 as an internal standard for quantification. The calibration curves were often observed to be significantly quadratic with coefficient of determinations (R^2) of $\geq$0.97 (**Figure 4**). Furthermore, due to the matrix effects, ion source contamination, or other unidentified factors, the responses of pesticides in the presence of matrices either decreased or increased over time depending on individual pesticides. Some exhibited small changes, whereas others changed dramatically as indicated by the differences between two injections of the same matrix-matched standard (**Figure 4**). Therefore, the matrix-matched standard calibration curves were constructed on the basis of the two injections, that is, before and after spike samples, of the calibration standards so as to average out the response changes during the course and, hence, to improve the method accuracy.

Method Validation. The UPLC QqTOF MS method was validated according to a designed experiment, that is, the nested design reported elsewhere (*18*−*20*), which allowed for studying and evaluating method performance parameters including accuracy expressed as overall recovery, intermediate precision, and measurement uncertainty (MU). Four factors including concentrations or spike levels of pesticides, matrix effects, day-to-day variation, and within-day variation were considered when the performance parameters were evaluated. First, three concentration levels were chosen, that is, fortified at 10, 50, and 80 μg/kg. Then, for each concentration, the overall recovery was estimated with five different matrices of either fruit- or vegetable-based infant foods. Third, for each matrix, the analysis was carried out on two different days, and fourth, each sample was prepared in triplicate per day, that is, three separate extractions. After completing all experiments, the performance parameters were calculated using a compiled SAS statistical program. Detailed equations and calculations were described elsewhere (*18*−*20*). Because benomyl, carbosulfan, formetanate, and thiophanate-methyl degraded rapidly or gradually and flucarbazone and penoxsulam were lacking in high concentration standards at the time of the study, 132 of 138 pesticides were able to be included in the nested design for method validation. The method performance results for the determination of 132 pesticides in 5 fruit- and 5 vegetable-based infant foods are summarized in **Table 1** (columns 9−14) and are illustrated in **Figure 5**. Generally, about 90% of pesticides had recoveries between 81 and 110%, 90% of pesticides had the intermediate precision of $\leq$25%, only 2% of pesticides had the intermediate precisions of $\leq$10%, and 85% of pesticides had MU $\leq$ 50% and 56−70% of pesticides had MU $\leq$ 40%. Results for butafenacil, chlorimuron-ethyl, clodinafop-propargyl, clothianidin, diniconazole, etofenprox, etoxazole, fentrazamide, haloxyfop, indoxacarb, methiocarb sulfone, methiocarb sulfoxide,

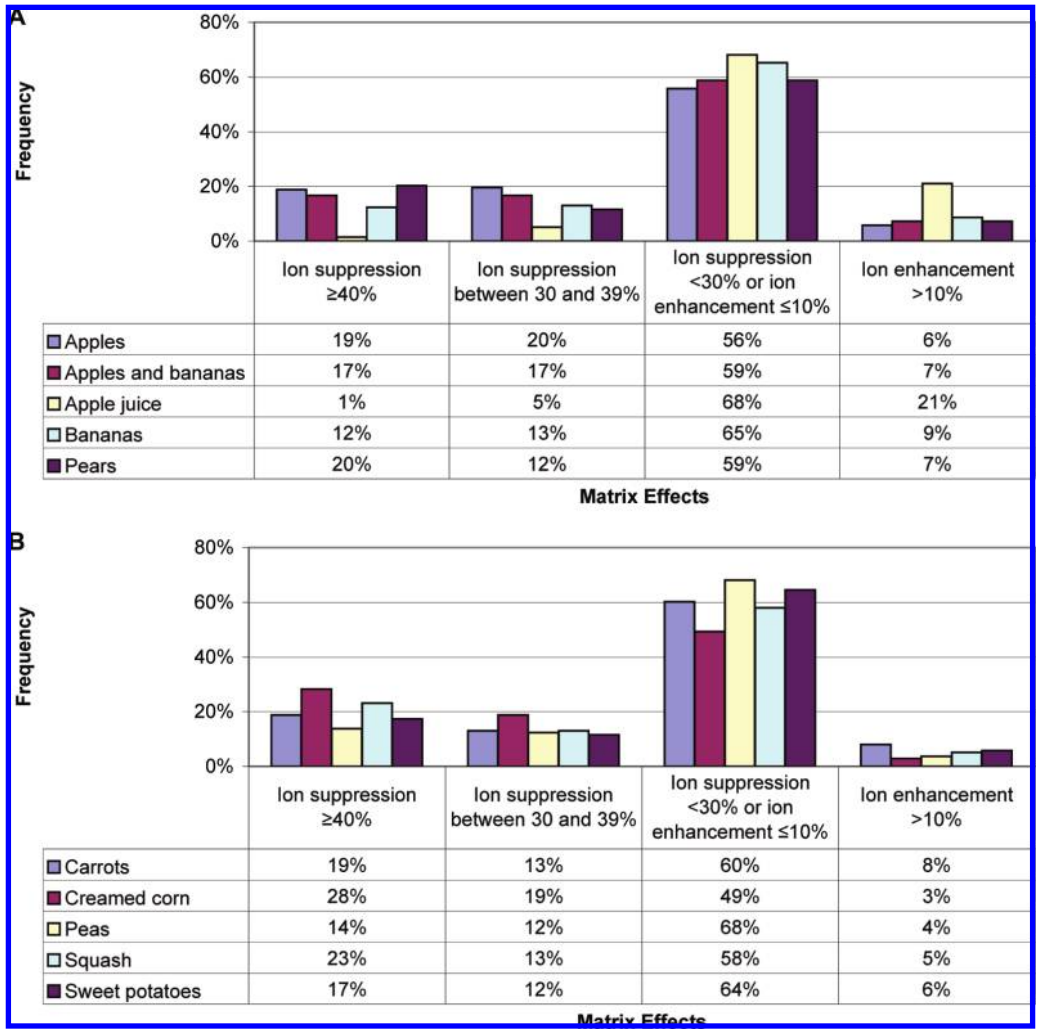

Figure 3. UPLC QqTOF MS matrix effects: (**A**) fruit-based infant foods; (**B**) vegetable-based infant foods. Data were from 135 pesticides plus 3 internal standards. Benomyl, carbosulfan, formetanate, and thiophanate-methyl were not included due to degradation. Comparison was done at 50 μg/kg equivalent in samples.

methoxyfenozide, naptalam, neburon, oxamyl-oxime, primisulfuron-methyl, prodiamine, pyraclostrobin, pyridalyl, pyridate, quinoxyfen, quizalofop, quizalofop-ethyl, thiofanox sulfone, and/or trichlorfon were considered to be semiquantitative due to their relatively large MU (>50%). In general, low recovery and/or poor repeatability contributed to the large MU (**Table 1**). The method's poor repeatability, which was observed as compound-dependent, could result from matrix effects. For example, trifloxysulfuron (**Figure 4B**) demonstrated much more variation than difenoconazole (**Figure 4A**) as indicated by the deviation of a triplicate. As another comparison, when the same samples (but sample extracts with additional 3 times dilution) were analyzed by LC-ESI-MS/MS (*17*), about 95% of pesticides had recoveries between 81 and 110%, 95% of pesticides had the intermediate precision of ≤20%, and 60−68% of pesticides had intermediate precisions of ≤10%; 94% of pesticides had MU ≤ 40% (*17*). Apparently, LC-ESI-MS/MS had smaller MU and better repeatability than UPLC QqTOF MS. These data support LC-ESI-MS/MS as a superior tool for quantification overall.

European Union (EU) Document SANCO/2007/3131, that is, "Method validation and quality control procedures for pesticide residues analysis in food and feed", is a guide document that is intended for the monitoring of pesticide residues in the EU (*24*). The document describes the method validation and analytical quality control requirements to support the validity of data used for checking compliance with MRLs, enforcement actions, or assessment of consumer exposure to pesticides. On the basis of the results obtained to date from EU proficiency tests, the document recommends a default MU, that is, 50%, be used by regulatory authorities in cases of enforcement decisions (MRL-exceedances) in the EU with a prerequisite that the laboratory proves its own calculated MU to be <50%. Therefore, UPLC QqTOF MS was able to successfully quantify up to 85% of the pesticides studied with MU ≤ 50%.

The method was also tested for its applicability for the detection or determination of benomyl, carbosulfan, formetanate, thiophanate-methyl, flucarbazone, and penoxsulam by performing a separate experiment for which standards were freshly prepared. Results indicated that the method was able to quantify formetanate, penoxsulam, and thiophanate-methyl (**Table 1**), and it could detect flucarbazone but with a very low recovery. Benomyl and carbosulfan degraded rapidly even during the extraction. Therefore, they should be reported as their respective degradation products, that is, carbendazim and carbofuran, if any incurred is detected.

The method sensitivity was evaluated on the basis of the signal-to-noise (S/N) ratios (peak-to-peak) at the lowest concentration level (**Table 1**, column 8). Most of pesticides could be detected and quantified below or at 5 μg/kg, except for

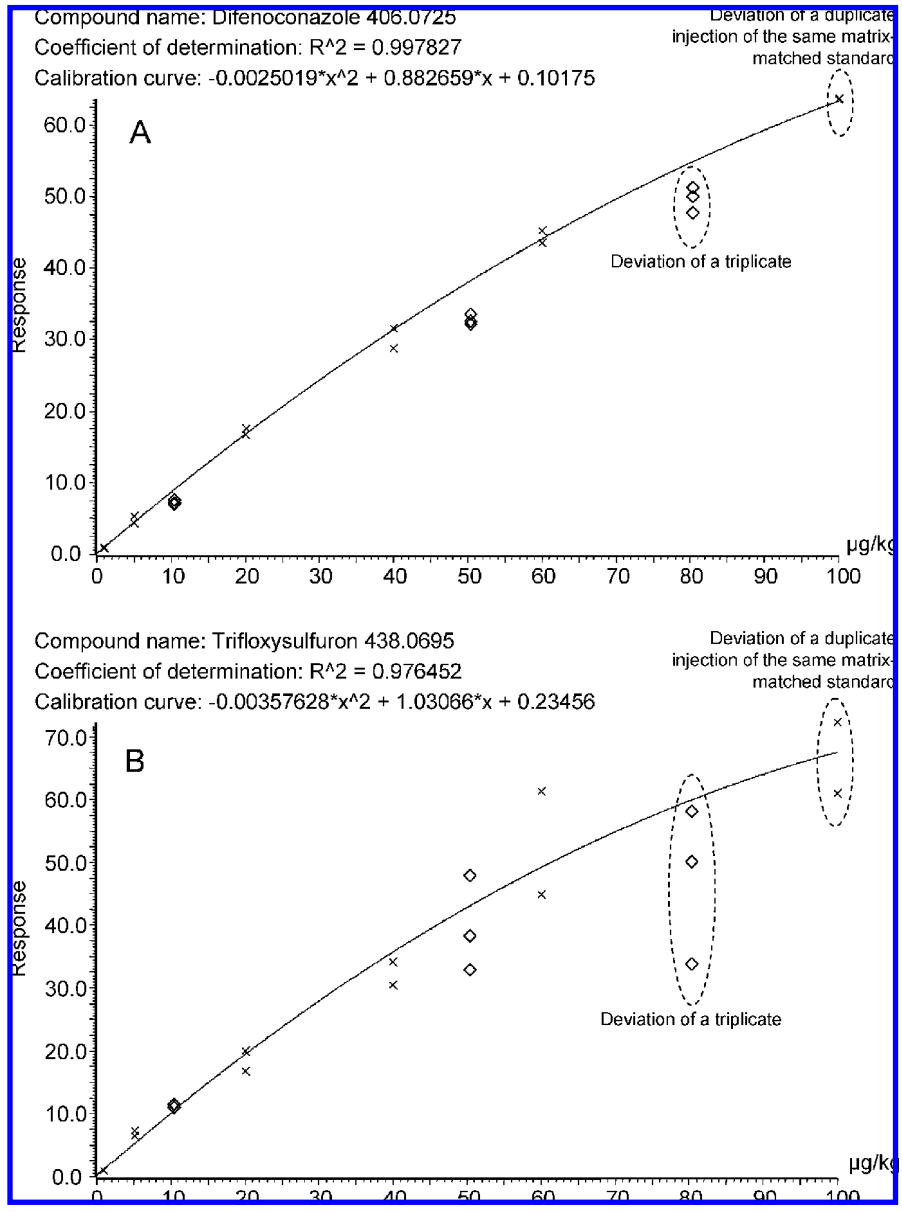

Figure 4. Matrix-matched standard calibration curves prepared from apple-based infant food: (×) responses that corresponded to individual calibration concentration levels, i.e., 1.0, 5.0, 20.0, 40.0, 60.0, and 100.0 μg/kg; (◇) responses that corresponded to individual three-spike concentration levels, i.e. 10, 50, and 80 μg/kg. Matrix-matched standards were injected twice, that is, before and after spike samples.

benoxacor, metolcarb, molinate, oxamyl-oxime, and prodiamine. Generally, UPLC QqTOF MS was about 1 or 2 orders of magnitude less sensitive than LC-ESI-MS/MS (*17*).

Confirmation. Due to its accurate mass measurement capability, UPLC QqTOF could be served as a powerful tool for confirmation of pesticides. The 2002/657/EC European Commission Decision (*25*) established an identification points (IPs) system to confirm organic residues and contaminants in live animals and animal products, but it does not consider TOF to be a high-resolution instrument and disadvantages its high accurate mass measurement capability. Therefore, the criterion, which used either absolute (*26*) or relative mass errors (*27*), for IPs assignment (**Table 2**) based on mass measurement accuracy rather than resolution power was proposed. The latter has an advantage that the IP rating criterion is consistent across a mass range or independent of *m*/*z* values. Therefore, when mass errors are between 2 and 10 ppm, one ion earns 1.5 IPs. QqTOF MS data can be acquired under low and high collision energies, that is, at 5 or 30 eV during the same analysis, and then both precursor and fragment ions could be possibly obtained that allowed additional IPs for confirmation. For example, when collision energy was set at 5 eV, emamectin B_{1a} was mainly detected as precursor ion (*m*/*z* 886.5324) (**Figure 6B2**). When relatively high collision energy such as 30 eV was applied, both precursor (*m*/*z* 886.5287) and fragment (*m*/*z* 158.1185) ions of emamectin B_{1a} were detected (**Figure 6C2**). In this case, a total of 3 IPs was assigned for confirmation. Furthermore, when QqTOF was operated in its MS/MS mode with the first quadrupole as a mass filter to select the precursor ion at *m*/*z* 886 to perform the tandem MS experiment, the product ion spectrum of emamectin B_{1a} was obtained. Both precursor (*m*/*z* 886.5280) and product (*m*/*z* 158.1175) ions were detected (**Figure 6A2**), and therefore, a total of 3.5 IPs was assigned. The resulting QqTOF MS/MS spectrum (**Figure 6A2**) was much more specific or "cleaner" than that of QqTOF MS (**Figure 6B2**,**C2**) because it was free of other ions, which were from coeluted compounds with emamectin B_{1a} at 8 min (**Figure 6**). Therefore, a QqTOF MS/MS should provide more specific

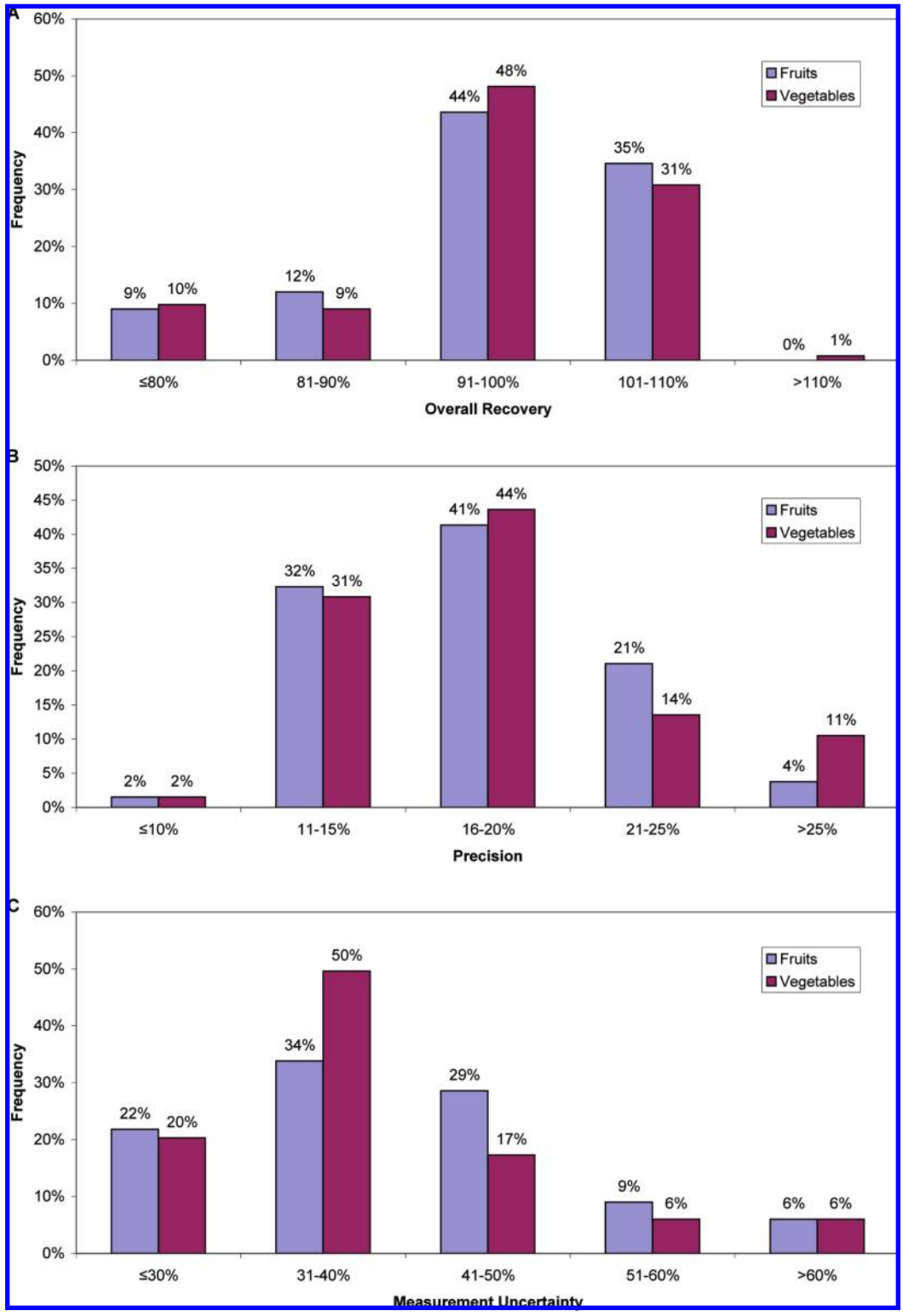

Figure 5. UPLC QqTOF MS method performance: (**A**) overall recovery; (**B**) precision; (**C**) measurement uncertainty.

data or spectrum than QqTOF MS for unambiguous identification of pesticides.

In conclusion, UPLC QqTOF was an important and powerful tool for the analysis of pesticide residues in infant foods due to its fast LC separation and TOF mass analyzer's medium-range high-resolution, accurate mass measurement, excellent full-scan sensitivity, and complete mass spectral information. Although LC-ESI-MS/MS was thought to be superior to UPLC QqTOF MS for quantification, UPLC QqTOF MS is ideal for screening of many pesticides as possible in a single analysis and confirming the identity of pesticides based on accurate mass measurement at trace level. Furthermore, QqTOF MS/MS provides additional specificity for unambiguous confirmation or identification of pesticides as a result of the selectivity of its first quadrupole. The UPLC QqTOF method reported in this paper was able to determine 138 pesticides in 5 fruit- and 5 vegetable-based infant foods with LODs as low as 1 μg/kg, particularly to quantify and confirm them at 10 μg/kg. Generally,

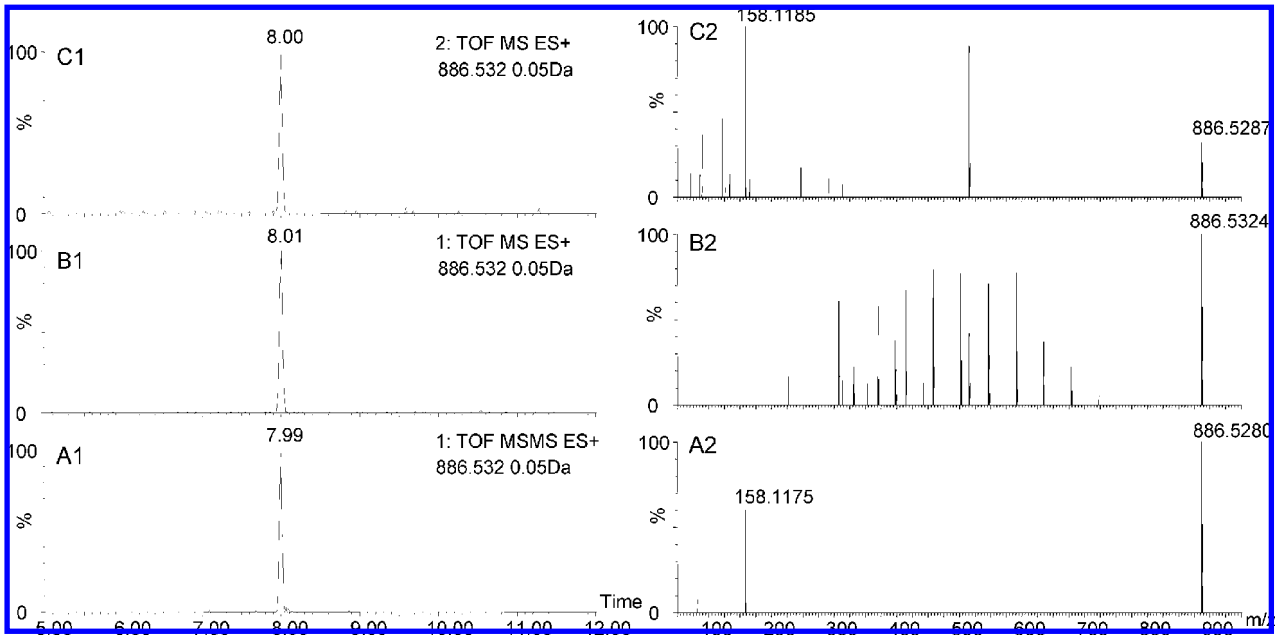

Figure 6. UPLC QqTOF MS/MS and UPLC QqTOF MS chromatograms (left) and mass spectra (right) of emamectin B_{1a} (10 μg/kg) spiked in pear-based infant food: (**A1**, **A2**) QqTOF MS/MS data with the precursor ion at *m*/*z* 886.53; (**B1**, **B2**) QqTOF MS data with collision energy set at 5 eV; (**C1**, **C2**) QqTOF MS data with collision energy set at 30 eV. Mass spectra were obtained by combining two to three scans across a UPLC peak with a number of background scans taken on each side of the peak subtracted.

Table 2. Relationship among MS Ions or Transitions, Mass Resolution, Mass Accuracy, and Identification Points (IPs)

MS technique	IPs obtained for each ion[a]
low-resolution mass spectrometry (LR)	1
LR-MS^n precursor ion	1
LR-MS^n product ion or transition products	1.5
HRMS	2
HR-MS^n precursor ion	2
HR-MS^n product ion or transition products	2.5

mass accuracy	IPs obtained for each ion[b]
error higher than 10 mDa[b] or ppm[c]	
single ion	1
precursor ion	1
product ion or transition products	1.5
error between 2 and 10 mDa or ppm	
single ion	1.5
precursor ion	1.5
product ion or transition products	2
error below 2 mDa or ppm	
single ion	2
precursor ion	2
product ion or transition products	2.5

[a] Criterion proposed by Commission Decision 2002/657/EC (*25*). [b] Criterion proposed by Hernandez et al. (*26*) and mass error in mDa. [c] Criterion proposed by Wang and Leung (*27*) and mass error in ppm.

about 90% of the pesticides studied had recoveries between 81 and 110%, 90% had the intermediate precision of ≤25%, and 85% had measurement uncertainty of ≤50%.

ABBREVIATIONS USED

LC-MS, general term for liquid chromatography coupled with any type of mass spectrometry or mass spectrometer; LC-ESI-MS/MS, liquid chromatography coupled with a triple-quadrupole mass spectrometer operated in multiple-reaction monitoring (MRM) mode with an electrospray ionization as LC-MS interface; QqTOF, general term for a quadrupole time-of-flight tandem mass spectrometry or mass spectrometer; QqTOF MS, QqTOF operated as a TOF mass analyzer in full-scan mode to acquire full-scan spectrum; QqTOF MS/MS, QqTOF operated in MS/MS mode with Q1 as a mass filter to acquire product ion scan spectrum.

ACKNOWLEDGMENT

We are grateful to Ralph Onciul and Donna Grant, Calgary Laboratory, Canadian Food Inspection Agency, for comments on this paper.

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Received for review October 31, 2008. Revised manuscript received December 23, 2008. Accepted December 29, 2008.

JF803419J