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Determination of Pesticides in Apple-Based Infant Foods Using Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry

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A liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) method was developed and validated to quantify and confirm trace levels of 13 pesticides including aldicarb sulfoxide, aldicarb sulfone, oxamyl, methomyl, formetanate, 3-hydroxycarbofuran, carbendazim, thiabendazole, aldicarb, propoxur, carbofuran, carbaryl, and methiocarb in apple-based infant foods such as apple sauces, apples and strawberries, apples and blueberries, and apples and plums. Data acquisition under MS/MS was achieved by applying multiple reaction monitoring of two fragment ion transitions to provide a high degree of sensitivity and selectivity for both quantification and confirmation. LC/ESI-MS/MS quantitative results were significantly affected by matrices, and thus, the standard addition was employed to compensate for the matrix effects to achieve the best accuracy of the method. Recoveries of 13 pesticides, spiked at 5.0, 25.0, and 45.0 μg/kg, were around 100% using the LC/ESI-MS/MS standard addition. The method detection limits (S/N $\geq$ 3:1) of 13 pesticides were less than 0.2 μg/kg.

KEYWORDS: Pesticides; carbamates; formetanate; carbendazim; thiabendazole; quantification; confirmation; standard addition; LC/ESI-MS/MS

INTRODUCTION

Pesticides are widely utilized at various stages of cultivation and during postharvest storage to protect fruit and vegetables against a range of pests and fungi and/or to provide quality preservation. Consequently, pesticides from a broad range of classes are used in various combinations and perhaps at different times to achieve the best control effects. Pesticides can be carried to the final products such as infant foods even following food processing. The European Union Directive 96/5/EC and its subsequent revisions, for example, 1999/39/EC, 2003/13/EC, and 2003/14/EC, have set the regulations that processed cereal-based foods and infant foods shall not contain residues of individual pesticides at levels exceeding 10 μg/kg or less. In Canada, pesticide residues in many foods have been tested under the Canadian National Chemical Residues Monitoring Program. The Canadian Food Inspection Agency was looking for improved methods for the testing of infant food commodities, to lower residue detection limits for future monitoring programs. Therefore, reliable confirmatory methods are required to monitor pesticide residues in infant foods and to ensure the safety of infant food supply.

Traditionally, many pesticides have been routinely analyzed using gas chromatography (GC) with various selective detection techniques from electron capture detection, flame ionization detection, and nitrogen−phosphorus detection to mass spectrometry (*1*−*3*). However, because of their physicochemical properties such as thermal instability and polarity, some pesticides such as *N*-methyl carbamates (*4*) are not amenable to GC; therefore, liquid chromatography (LC) has been used as an alternative technique to determine these compounds. Most LC methods use common ultraviolet, diode array, fluorescence, or electrochemical detection, which are occasionally combined with postcolumn derivatization. However, these techniques may not be sufficiently selective or sensitive because of the variety and complexity of food matrices and the trace levels of pesticide residues presented (*5*). Recently, LC/tandem mass spectrometry (MS/MS) has been found and proven to be a very practical technique with high sensitivity and selectivity to quantify many pesticides and confirm their identities in fruits and vegetables (*6*−*11*). Atmospheric pressure electrospray ionization (ESI) (*6*−*8*, *10*) and photoionization (PI) (*9*) are two common ionization techniques of LC/MS/MS. LC/MS/MS with ESI (LC/ESI-MS/MS) was able to detect pesticides as low as a few ppb (μg/kg), for example, 3 μg/kg as reported by Zrostlikova et al. (*7*), while LC/MS/MS with PI showed less matrix effects with detection limits as low as 0.3 μg/kg (*9*).

Applications of LC/ESI-MS/MS for analysis of pesticides in infant foods are rare in the literature. In this study, we present a validated LC/ESI-MS/MS method for quantification and confirmation of 13 pesticides in apple-based infant foods. A simple solid phase extraction (SPE) procedure was developed

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to extract and concentrate pesticides from matrices. Different calibration approaches were compared and discussed to address issues regarding extraction efficiency and matrix effects that determined the accuracy of the method. Ion ratios acquired under multiple reaction monitoring (MRM) of two fragment ion transitions were compared to provide the confirmatory criteria. Finally, a standard addition method was used to compensate for the matrix effects in order to obtain the best quantitative results.

MATERIALS AND METHODS

Materials and Reagents. Aldicarb sulfoxide, aldicarb sulfone, methomyl, formetanate, carbendazim, and propoxur were purchased from Riedel-de Haen AG (Seelze, Germany). Aldicarb, 3-hydroxycarbofuran, thiabendazole, carbofuran, carbaryl, methiocarb, and oxamyl were obtained from Chem Service (West Chester, PA). Acetonitrile and ammonium acetate were from Caledon Laboratories Ltd. (Georgetown, ON, Canada). Oasis HLB Plus 225 mg cartridges were from Waters Corp. (Milford, MA). Apple-based infant foods used in this study included two apple sauce samples (A and B), apples and strawberries, apples and blueberries, and apples and plums, which were obtained from a local market. Samples were kept at 4 °C. Because there were no blank samples available, for example, thiabendazole free samples, a matrix blank (free of any 13 pesticides) was prepared in our laboratory by homogenizing organic apples with peels and cores removed. This matrix blank was used to construct the "pseudo" matrix-matched standard calibration curves (MSCC) through the experiments. All water used was doubly deionized water (Milli-Q water purification system, Millipore Corp., Bedford, MA). Ammonium acetate stock solution (0.1 M) was prepared by dissolving 7.7 g of ammonium acetate in water and making up the volume to 1000 mL with Milli-Q water in a 1000 mL volumetric flask, and 0.01 M ammonium acetate was diluted 10 times from 0.1 M ammonium acetate. Solvent buffer, which was used to reconstitute the final sample extracts, was a mixture of acetonitrile and 0.01 M ammonium acetate (10:90).

Preparation of Standard Solutions. Individual standard stock solutions (1000.0 μg/mL) were prepared by weighing 10.0 mg of each individual pesticide (except carbendazim) into separate 10 mL volumetric flasks, dissolving in methanol, and making up to volume. Because of its poor solubility in methanol, carbendazim stock solution was prepared by weighing 10.0 mg into a 50 mL volumetric flask, dissolving in methanol, and making up to volume. Stock solutions were stored at −20 °C. Working standard solution (1) (1.0 μg/mL) was prepared by transferring 100 μL of each standard stock solution (except carbendazim) and 500 μL of carbendazim stock solution into a single 100 mL volumetric flask and diluting to volume with solvent buffer. Working standard solution (2) (0.1 μg/mL) was prepared by transferring 10 mL of working standard solution (1) into a 100 mL volumetric flask and diluting to volume with solvent buffer. All working solutions were stored at 4 °C and prepared weekly.

Extraction. A sample (5.00 ± 0.05 g) was weighed into 50 mL centrifuge tubes [polypropylene centrifuge tubes with screw caps (VWR International, Edmonton, AB, Canada)]. After 15 mL of acetonitrile was added, the centrifuge tube was capped and shaken for 15 min on an Eberbach shaker (Eberbach Corp., Ann Arbor, MI). Then, the sample was centrifuged [centrifuge: Allegra 6 centrifuge (Beckman Coulter, Inc., Fullerton, CA)] at 3210*g* for 15 min at room temperature. The supernatant was transferred into a Turbovap glass tube (200 mL, Zymark TurboVap II glassware, Zymark Center, MA) and evaporated to about 1 mL using TurboVap II nitrogen evaporator for which the temperature was controlled at 30 °C and the nitrogen flow rate was regulated at 100 kPa. The extract was then reconstituted with 20 mL of 0.01 M ammonium acetate. An Oasis HLB cartridge was preconditioned sequentially with 10 mL of methanol, 10 mL of water, and 2 mL of 0.01 M ammonium acetate. The reconstituted solution was loaded on the preconditioned Oasis HLB cartridge under vacuum at −2 to −3 inHg with a flow rate of ∼1 mL/min. The cartridge was then rinsed with 5 mL of 0.01 M ammonium acetate at a flow rate of ∼2 mL/min. The cartridge was evacuated continuously to "dryness" for 5 min under vacuum. Finally, pesticide residues were eluted with 5 mL of methanol at a flow rate of 1−2 mL/min under vacuum into a precalibrated 5 mL centrifuge tube [PYREX brand centrifuge tubes, precalibrated with 1 mL volume accuracy (VWR International)]. The eluate was evaporated to 0.2−0.3 mL using N-EVAP nitrogen evaporator (Organomation Associates Inc., Berlin, MA) at 30 °C under a stream of nitrogen and then was made up to 1 mL with solvent buffer. The mixture was vortexed for 30 s to dissolve the residues and transferred to LC vials [Mini-UniPrep syringeless filter device with polypropylene housing and PVDF 0.45 μm membrane (Whatman Inc., Clifton, NJ)] for LC/ESI-MS/MS analysis.

LC/ESI-MS/MS. The LC/ESI-MS/MS system used was an Alliance 2695 HPLC coupled with a Micromass Quattro Ultima Pt tandem mass spectrometer with electrospray interface (LC/ESI-MS/MS) and MassLynx 4.0 software (Waters).

LC Profile. Mobile phases were acetonitrile (solvent A), 0.1 M ammonium acetate with 20% acetonitrile in water (solvent B), and water (solvent C). A linear gradient profile consisted of 0−9 min, 8−90% A and 10% B; 9−13 min, 90% A and 10% B; 13−15 min, 100% A; and 15−20 min, 8% A and 10% B. Flow rates were 0−13 min, 0.2 mL/min; 13−19 min, 0.3 mL/min; and 19−20 min, 0.2 mL/min. The injection volume was 20 μL. Retention time windows for data acquisition are listed in **Table 1**. The LC column was YMC ODS-AQ S-3 120 Å 50 mm × 2 mm cartridge with 2.0 mm YMC Endfittings and YMC Direct Connect Endfitting (Waters). The guard column was YMC ODS-AQ S-3 120 Å 20 mm × 2 mm guard cartridge (Waters).

MS Conditions. MS parameters were set as follows. Ionization mode, electrospray positive ion mode; capillary voltage, 3.2 kV; source temperature, 120 °C; desolvation temperature, 280 °C; nebulizer nitrogen flow rate, 100 L/h; desolvation nitrogen gas flow rate, 600 L/h; collision gas argon pressure, 2.5×10^{-3} mbar; dwell times, 0.08 s; RF lens 1, 20; RF lens 2, 0.5; LM 1 resolution, 14.0; HM 1 resolution, 14.0; ion energy 1, 0.2 V; entrance voltage, −2 V; exit voltage, 1 V; LM 2 resolution, 14.0; HM 2 resolution, 14.0; ion energy 2, 0.5 V; and multiplier voltage, 650 V. The cone voltage, collision energy, and MRM are listed in **Table 1**. These settings were able to achieve unit mass resolution. The mass spectrometer was tuned to obtain reasonable responses and ion ratios under MRM for each individual pesticide using flow injection. For the flow injection, pesticides (1.0 μg/mL) were prepared in a mixture of acetonitrile and water (50:50) containing 0.01 M ammonium acetate. The flow rate of the syringe pump (Harvard Apparatus, Holliston, MA) was set at 20 μL/min.

Preparation of Calibration Curves and Calculation. LC/ESI-MS/MS MSCCs were utilized in this study for quantifying or screening pesticides in samples. A matrix blank (5.00 ± 0.05 g) was weighed into six separate 50 mL centrifuge tubes and then carried through the extraction procedure. The final sample extracts were added at 5, 50, 100, 150, 200, and 250 μL of working standard solution (1), respectively, and made up to 1 mL with solvent buffer to provide 1.0, 10.0, 20.0, 30.0, 40.0, and 50.0 μg/kg of pesticides equivalent in samples.

Concentration, μg/kg (ppb), vs the peak area of each individual pesticide was plotted to prepare the MSCCs for each individual pesticide using the QuanLynx of MassLynx 4.0. Linear or quadratic function was applied to the calibration curves based on the line of best fit or correlation coefficient (R^2). The fit weighting used was $1/x$ (*12*). MSCCs were prepared fresh for each day's samples.

Standard Addition. The sample preparation was the same as described in the Materials and Methods. After extraction, three portions, i.e., 300 μL each, of the sample extract were transferred into three separate LC vials labeled as a, b, and c. For samples containing pesticides between 5 and 50 μg/kg, 200 μL of solvent buffer was added to vial a; 40 μL of working standard solution (1) (26.7 μg/kg equivalent in samples) and 160 μL of solvent buffer were added to vial b; and 80 μL of working standard solution (1) (53.3 μg/kg equivalent in samples) and 120 μL of solvent buffer were added to vial c. For samples containing pesticides between 1 and 4 μg/kg, 200 μL of solvent buffer was added to vial a; 40 μL of working standard solution (2) (2.7 μg/kg equivalent in samples) and 160 μL of solvent buffer were added to vial b; and 80 μL of working standard solution (2) (5.3 μg/kg equivalent in samples) and 120 μL of solvent buffer were added to vial c. Then,

Table 1. LC/ESI-MS/MS Parameters for 13 Pesticides

compound	precursor ion	MRM transition *m/z*	cone voltage (V)	collision energy (eV)	retention time window (min)
aldicarb sulfoxide	$[M + H]^+$	207→132*[a]	35	7	1.9–6.0
		207→89	35	7	1.9–6.0
aldicarb sulfone	$[M + NH_4]^+$	240→148*	35	10	1.9–6.0
		240→76	35	10	1.9–6.0
oxamyl	$[M + NH_4]^+$	237→72*	35	7	1.9–6.0
		237→90	35	7	1.9–6.0
methomyl	$[M + H]^+$	163→106*	35	6	1.9–6.0
		163→88	35	6	1.9–6.0
formetanate	$[M]^+$	222→165*	35	15	5.5–8.9
		222→120	35	20	5.5–8.9
3-hydroxycarbofuran	$[M + NH_4]^+$	255→163*	35	15	5.5–10.3
		255→181	35	15	5.5–10.3
carbendazim	$[M + H]^+$	192→160*	50	17	5.5–10.3
		192→132	50	22	5.5–10.3
thiabendazole	$[M + H]^+$	202→175*	35	20	5.5–10.3
		202→131	35	20	5.5–10.3
aldicarb	fragment at *m/z* 116	116→89*	50	7	5.5–10.3
		116→70	50	7	5.5–10.3
propoxur	$[M + H]^+$	210→111*	35	9	6.7–11.0
		210→168	35	9	6.7–11.0
carbofuran	$[M + H]^+$	222→165*	50	14	6.7–11.0
		222→123	50	14	6.7–11.0
carbaryl	$[M + H]^+$	202→145*	35	5	8.9–11.0
		202→127	35	20	8.9–11.0
methiocarb	$[M + H]^+$	226→169*	50	9	8.9–13.0
		226→121	50	9	8.9–13.0

[a] Predominant ion defined as a base peak.

polypropylene inserts were put on to seal vials, taking care that the inserts were not pressed down at this step. After the mixtures were vortexed for 15 s, the inserts were pressed down to filter sample extracts. The samples were then ready for LC/ESI-MS/MS analysis.

The concentration of a pesticide added to the vial, expressed as equivalent in samples, was calculated as:

$$C_{\text{stdadd}} = \frac{V \times C_{\text{std}}}{W} \times \frac{1000}{300}$$

where C_{stdadd} is the concentration equivalent in samples (μg/kg), V is the volume of the working standard solution added (μL), C_{std} is the concentration of the working standard solution (1 or 2) (μg/mL), and W is the sample weight (g).

Accordingly, the C_{stdadd} of a pesticide in vial a was 0. The C_{stdadd} values of a pesticide in vial b and vial c of standard additions were 26.7 and 53.3 μg/kg, respectively, for example, when working standard solution (1) was used. Responses or peak areas (y-axis) were functions of concentrations (x-axis) of a pesticide in sample extracts. A plot of concentrations, for example, 0, 26.7, and 53.3 μg/kg, vs their responses was constructed using the function "LINEST" of Microsoft Excel 97 (Microsoft Office 97) to obtain the slope m and y-intercept n. Concentration or the amount of a pesticide in a sample, C_x (μg/kg), was calculated as: $C_x = n/m$ where C_x was the negative intercept on the x-axis that corresponds to the amount of a pesticide in a sample.

Statistics. Means and standard deviations were calculated using Microsoft Excel 97 (Microsoft Office 97). Linear regression and correlation coefficients (R^2) were generated using QuanLynx of MassLynx 4.0 software.

RESULTS AND DISCUSSION

MS/MS Data Acquisition. The ionization of 13 pesticides in the positive electrospray ion source was studied, and the pseudomolecular ions used for data acquisition are listed in **Table 1**. Pesticides can be ionized in forms of $[M]^+$, $[M + H]^+$, $[M + NH_4]^+$, and $[M + Na]^+$, depending on their chemical structures. Ammonium acetate was used as a modifier in the LC mobile phase so as to generate abundant ammonium adducts in the electrospray ion source. The presence of ammonium adducts suppressed the formation of sodium adducts, and thereafter, pesticides formed $[M]^+$, $[M + H]^+$, and/or $[M + NH_4]^+$, which showed high sensitivity and consistent responses. The intensity of aldicarb pseudomolecular ion was low due to its fragmentation with fragments at *m/z* 116, 143, and 199. To achieve the best sensitivity of the method for aldicarb, the fragment at *m/z* 116 was monitored and its transitions were used for both quantification and confirmation (**Table 1**).

Extraction. Thirteen pesticides in apple-based infant foods were extracted using acetonitrile and were further cleaned up and concentrated by Oasis HLB cartridges without the presence of sodium chloride during the extraction procedure. Preliminary experiments showed that poor recoveries and inconsistent repeatability of aldicarb sulfoxide, aldicarb sulfone, oxamyl, formetanate, thiabendazole, and carbofuran were observed when sodium chloride was used as in the traditional pesticide extraction procedure. Furthermore, a linear response, which is a prerequisite of the standard addition, with high correlation coefficient can only be achieved when the LC/ESI-MS/MS system is free of sodium ions. **Figure 1** shows typical LC/ESI-MS/MS chromatograms of an apple sauce sample fortified with the 13 pesticides after Oasis HLB cleanup and extraction. Pesticides were separated on a reverse phase LC column under the given gradient conditions within 13 min. The elution profile was in the following order with typical retention times (min) given in parentheses: aldicarb sulfoxide (2.75), aldicarb sulfone (3.95), oxamyl (4.10), methomyl (4.80), formetanate (6.21), 3-hydroxycarbofuran (7.14), carbendazim (7.32), thiabendazole (7.94), aldicarb (8.55), propoxur (9.51), carbofuran (9.59), carbaryl (9.92), and methiocarb (11.15). The tolerance of retention time matching did not exceed 5% relative to the retention time of standards. Although the retention time of formetanate might be affected by the matrix over long period of time, it was consistent within a batch of runs.

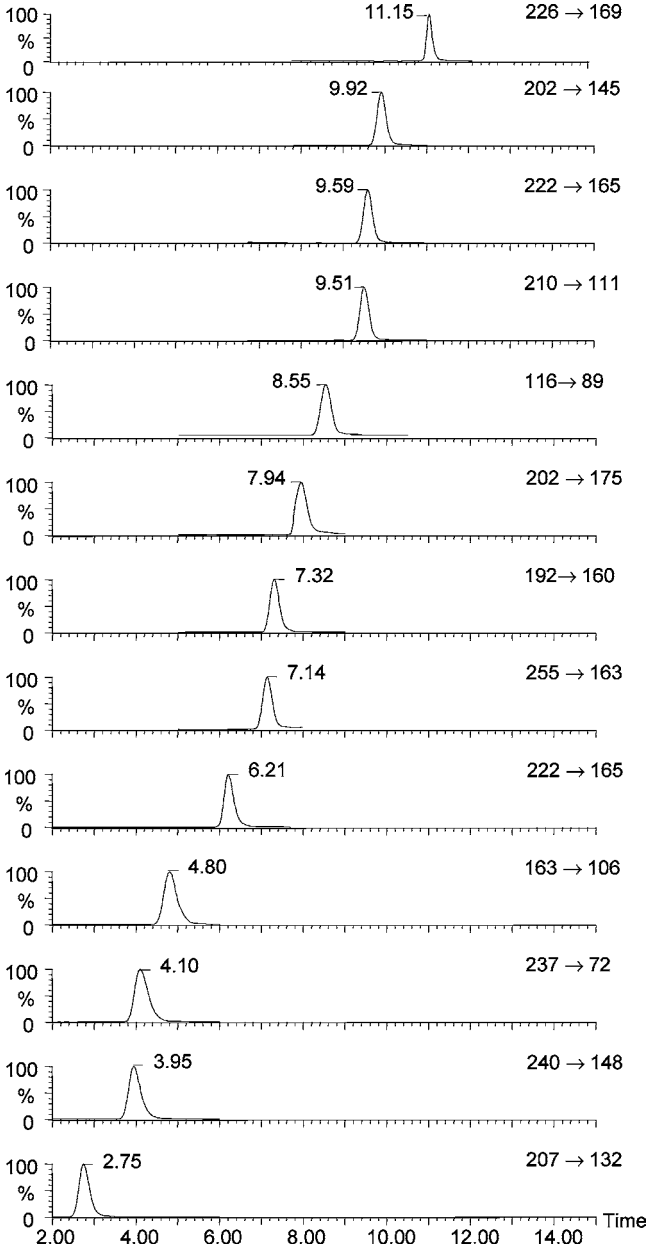

Figure 1. LC/ESI-MS/MS chromatograms of an apple sauce sample A fortified with 13 pesticides. From bottom to top: aldicarb sulfoxide (4.9 μg/kg), aldicarb sulfone (5.0 μg/kg), oxamyl (5.4 μg/kg), methomyl (5.7 μg/kg), formetanate (4.7 μg/kg), 3-hydroxycarbofuran (4.9 μg/kg), carbendazim (5.4 μg/kg), thiabendazole (5.5 μg/kg), aldicarb (5.8 μg/kg), propoxur (5.3 μg/kg), carbofuran (5.6 μg/kg), carbaryl (5.5 μg/kg), and methiocarb (5.0 μg/kg).

Calibration Curves. The accuracy of quantitative results of a method depends on the calibration. Three different calibration curves were constructed and compared, that is, standard calibration curves (SCC), MSCC, and method matrix-matched standard calibration curves (MMSCC). All calibration curves were prepared with six-points, and concentrations were converted as equivalent in samples, i.e., 1.0, 10.0, 20.0, 30.0, 40.0, and 50.0 μg/kg. MSCC was constructed as described in the Materials and Methods. SCC was prepared by diluting working standard solutions directly with solvent buffer to levels of interest. MMSCC was prepared by spiking working standard solution to the matrix blanks (5 g), which were then carried out through the extraction procedure. Calibration curves were plotted using linear or quadratic functions. The calibration curves for comparison are presented in **Table 2**, and **Figure 2** shows an example of calibration curves of thiabendazole plotted as linear regression. Calibration curves with the quadratic function ($R^2 > 0.999$) showed better fitting than those using the linear regression ($R^2 > 0.99$). However, either function can be used for constructing the calibration curves due to the high correlation coefficient or R^2. The differences among the calibration curves, i.e., SCC, MSCC, and MMSCC, of a pesticide were attributed to the extraction efficiency and/or matrix effects (**Figure 2** and **Table 2**). For example, the differences between SCC and MSCC reflected matrix effects; those between SCC and MMSCC showed both the extraction efficiency and the matrix effects; and those between MSCC and MMSCC were due to the extraction efficiency. In general, when there are no isotopically labeled standards available, the choice of calibration curves becomes critical to the accuracy of the method. Because significant differences can be seen between SCC and MSCC or MMSCC, either MSCC or MMSCC would be an option to overcome or compensate for the matrix effects. In this current study, because MSCC and MMSCC were close and similar, MSCC was chosen for the method to construct the calibration curves for quantitative studies, and thereafter, a recovery is an absolute recovery through the text, provided that the extraction efficiency of pesticides was not affected by different matrices.

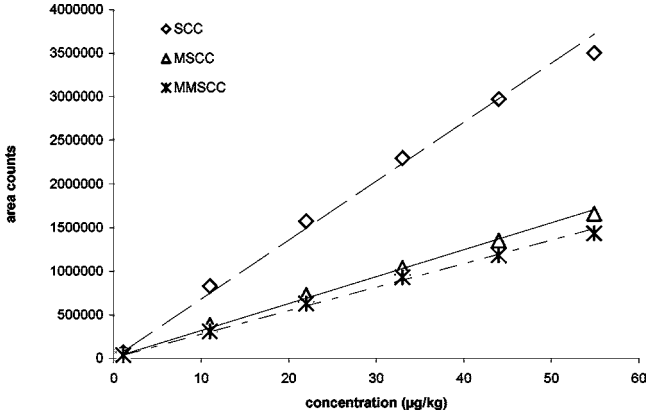

Figure 2. Calibration curves of thiabendazole. Fit weighting, 1/*x*.

Precision and Trueness. The LC/ESI-MS/MS method was first tested for its intraassay repeatability, i.e., precision within a day, and trueness, expressed as recovery, with the results shown in **Table 3**. Pesticides were fortified into the matrix blank at levels of 5, 25, and 45 μg/kg and then were extracted and analyzed using LC/ESI-MS/MS. The recoveries of pesticides of the intraassay ranged from 75.2 to 94.8% with relative standard deviations (RSDs) less than 13% except for formetanate. The large RSDs of formetanate, i.e., poor repeatability, indicated that this compound might not be stable or might decompose during the extraction. However, the method still was able to serve as a semiquantitative method to detect and confirm the presence of formetanate in samples.

The intermediate precision or interassay repeatability of the method was then studied. Pesticides were fortified into the apple sauce sample A at levels of 5, 25, and 45 μg/kg on four different days. Recoveries (**Table 3**) were low and varied with the pesticides. As discussed above, the matrix blank, prepared from organic apples, was used to construct the calibration curves. The low recoveries (**Table 3**) indicated that the matrix blank was not able to match the apple sauce samples in character with this further evidenced from the recovery variations of spiked apple sauces A and B (**Table 4**). Nevertheless, the RSDs (**Table 3**) of the interassay were generally less than 15%; therefore, it can be concluded that the method has a good intermediate

Table 2. Regressions of Calibration Curves

compound	concn range (μg/kg, equivalent in samples)	regression					
		SCC		MSCC		MMSCC	
		linear	quadratic	linear	quadratic	linear	quadratic
aldicarb sulfoxide	1.0–49.2	$23592.1x + 1467.6$ 0.9982^a	$-77.4x^2 + 26576.6x - 3732.5$ 0.9999	$16551.7x + 1716.2$ 0.9990	$-31.3x^2 + 17760.9x - 390.7$ 0.9996	$15055.1x + 2152.3$ 0.9981	$-42.5x^2 + 16695.3x - 705.5$ 0.9993
aldicarb sulfone	1.0–49.5	$18471.1x + 5034.8$ 0.9965	$-83.2x^2 + 21696.1x - 585.0$ 0.9998	$15943.0x + 5968.0$ 0.9958	$-75.8x^2 + 18881.6x + 847.2$ 0.9995	$14718.6x + 3891.9$ 0.9968	$-55.8x^2 + 16885.5x + 116.0$ 0.9992
oxamyl	1.1–54.5	$37281.2x + 1536.8$ 0.9987	$-84.4x^2 + 40884.1x - 5369.9$ 0.9998	$33963.4x + 6001.3$ 0.9978	$-98.5x^2 + 38166.8x - 2056.4$ 0.9995	$30836.4x + 3918.3$ 0.9982	$-62.8x^2 + 33520.2x - 1226.5$ 0.9991
methomyl	1.1–57.4	$17383.4x + 3111.2$ 0.9986	$-42.4x^2 + 19283.9x - 545.0$ 0.9999	$12642.6x + 2787.0$ 0.9981	$-37.0x^2 + 14302.7x - 406.8$ 0.9999	$10947.0x + 2051.6$ 0.9979	$-27.1x^2 + 12159.6x - 281.4$ 0.9993
formetanate	0.9–46.6	$154612.0x + 25382.5$ 0.9986	$-324.7x^2 + 166423.0x + 6771.54$ 0.9992	$92455.5x + 15917.2$ 0.9984	$-242.5x^2 + 101276.0x + 2017.2$ 0.9995	$72428.7x + 5129.5$ 0.9994	$-52.5x^2 + 74340.6x + 2116.7$ 0.9994
3-hydroxycarbofuran	1.0–49.5	$48072.5x + 10775.6$ 0.9962	$-231.7x^2 + 57055.8x - 4878.7$ 0.9999	$31147.9x + 5712.4$ 0.9979	$-108.1x^2 + 35338.5x - 1590.0$ 0.9999	$28178.1x + 3448.7$ 0.9989	$-61.6x^2 + 30568.8x - 717.4$ 0.9997
carbendazim	1.1–54.0	$133935.0x + 43242.6$ 0.9946	$-703.1x^2 + 163694.0x - 13758.3$ 0.9999	$34966.1x + 4625.7$ 0.9988	$-56.4x^2 + 37352.8x + 54.1$ 0.9992	$31490.7x + 986.2$ 0.9990	$-63.4x^2 + 34174.2x - 4153.9$ 0.9998
thiabendazole	1.1–54.9	$67576.8x + 3570.1$ 0.9968	$-267.3x^2 + 79075.6x - 18471.6$ 0.9999	$30858.9x + 11470.9$ 0.9983	$-84.4x^2 + 34491.1x + 4508.6$ 0.9980	$26921.6x + 9044.5$ 0.9987	$-59.8x^2 + 29497.3x + 4107.4$ 0.9997
aldicarb	1.2–57.9	$7986.6x + 1399.6$ 0.9952	$-36.9x^2 + 9663.5x - 2097.3$ 0.9999	$6010.5x + 930.1$ 0.9978	$-17.8x^2 + 6817.8x - 753.3$ 0.9997	$3993.9x + 871.9$ 0.9945	$-17.6x^2 + 4792.1x - 792.4$ 0.9987
propoxur	1.1–52.9	$63664.3x + 3010.8$ 0.9998	$-197.6x^2 + 71873.9x - 12673.5$ 0.9998	$51862.9x + 11051$ 0.9983	$-145.8x^2 + 57921.8x - 524.4$ 0.9998	$36461.2x + 5864.5$ 0.9967	$-129.8x^2 + 41854.2x - 4438.8$ 0.9991
carbofuran	1.1–56.4	$63203.9x + 882.3$ 0.9989	$-116.1x^2 + 68322.7x - 8950.9$ 0.9997	$53265.2x + 18420.4$ 0.9975	$-172.3x^2 + 60861.1x + 3828.8$ 0.9997	$44499.0x + 11687.7$ 0.9974	$-134.5x^2 + 50429.9x + 294.4$ 0.9993
carbaryl	1.1–55.2	$41292.2x + 3825.4$ 0.9972	$-145.9x^2 + 47600.3x - 8285.4$ 0.9997	$34498.5x + 10849.5$ 0.9980	$-102.5x^2 + 38930.3x + 2341.0$ 0.9998	$31122.0x + 7009.7$ 0.9965	$-108.5x^2 + 35811.2x - 1993.1$ 0.9989
methiocarb	1.0–49.5	$8824.2x - 1346.0$ 0.9988	$-14.9x^2 + 9404.7x - 2357.6$ 0.9993	$8290.41x + 865.1$ 0.9976	$-30.2x^2 + 9460.9x - 1174.6$ 0.9997	$7089.8x + 137.4$ 0.9976	$-18.2x^2 + 7795.9x - 1093.5$ 0.9987

[a] R^2 values.

Table 3. LC/ESI-MS/MS Repeatability of the Method for Determination of 13 Pesticides Spiked in Apple Sauce[a]

		intraassay[b] (matrix blank)				interassay[c] (sample A)			
				ion ratio[d]				ion ratio[d]	
compound	spike level (μg/kg)	recovery (%)	RSD (%)	mean (%)	RSD (%)	recovery (%)	RSD (%)	mean (%)	RSD (%)
aldicarb sulfoxide				(207→89)/ (207→132)				(207→89)/ (207→132)	
	4.9	94.0	2.3	66.1	5.7	81.0	6.1	59.9	5.0
	24.6	85.7	6.1	65.3	1.1	78.4	3.3	61.6	6.8
	44.3	85.9	1.2	64.5	1.4	81.1	6.5	61.4	7.5
aldicarb sulfone				(240→76)/ (240→148)				(240→76)/ (240→148)	
	5.0	93.4	4.2	61.0	1.4	69.8	8.2	33.4	9.6
	24.8	89.6	2.0	59.2	2.1	71.0	13.0	33.2	6.2
	44.6	89.6	3.6	59.3	2.2	71.9	9.6	32.1	4.6
oxamyl				(237→90)/ (237→72)				(237→90)/ (237→72)	
	5.4	91.2	2.7	25.5	1.9	57.4	7.0	21.1	5.0
	27.2	87.5	3.4	25.9	3.6	56.9	6.7	21.2	4.0
	49.0	87.2	4.9	26.5	0.7	56.4	4.7	20.7	4.5
methomyl				(163→88)/ (163→106)				(163→88)/ (163→106)	
	5.7	87.3	2.2	78.4	1.6	67.7	6.4	49.4	11.2
	28.7	84.9	3.0	78.6	0.3	68.9	4.4	49.0	7.4
	51.7	86.9	4.8	80.0	1.1	68.5	2.1	48.0	7.9
formetanate				(222→120)/ (222→165)				(222→120)/ (222→165)	
	4.7	71.6	7.1	4.2	4.1	34.8	13.8	4.4	8.9
	23.3	58.3	25.1	3.8	1.5	36.0	20.0	4.3	3.3
	41.9	59.0	3.8	3.8	1.5	33.0	8.2	4.4	1.1
3-hydroxycarbofuran				(255→181)/ (255→163)				(255→181)/ (255→163)	
	4.9	94.8	5.2	37.9	3.1	64.0	5.9	36.2	4.1
	24.7	87.9	0.9	37.5	1.1	65.8	7.0	36.5	1.4
	44.5	87.5	5.0	37.8	0.5	67.6	8.1	37.0	0.9
carbendazim				(192→132)/ (192→160)				(192→132)/ (192→160)	
	5.4	86.1	8.5	6.8	14.9	81.4	2.6	8.5	7.3
	27.0	89.8	2.6	6.2	5.2	85.6	5.9	7.9	6.9
	48.6	87.6	5.9	5.8	4.6	86.4	5.9	8.0	7.6
thiabendazole				(202→131)/ (202→175)				(202→131)/ (202→175)	
	5.5	84.4	8.1	37.0	1.8	70.4	4.1	25.9	48.7
	27.5	80.2	8.4	36.5	0.8	72.9	3.5	25.1	47.8
	49.5	82.5	12.7	37.1	1.4	72.4	3.9	25.2	46.6
aldicarb				(116→70)/ (116→89)				(116→70)/ (116→89)	
	5.8	79.8	3.4	67.2	4.2	66.1	15.9	67.8	3.3
	29.0	78.4	12.5	69.4	5.1	67.4	7.5	66.4	6.1
	52.1	79.5	7.5	67.9	1.0	64.4	9.2	66.7	7.0
propoxur				(210→168)/ (210→111)				(210→168)/ (210→111)	
	5.3	75.2	4.8	72.8	0.6	79.9	7.6	98.8	8.7
	26.5	76.9	9.4	73.3	1.0	82.2	4.0	100.0	10.2
	47.7	78.9	6.1	72.9	0.8	78.0	4.2	101.0	10.2
carbofuran				(222→123)/ (222→165)				(222→123)/ (222→165)	
	5.6	78.8	4.2	67.0	2.3	81.4	3.3	70.7	2.9
	28.2	81.1	6.6	64.2	0.8	82.8	7.1	66.3	4.8
	50.8	83.5	4.7	62.8	0.3	77.9	6.0	67.1	8.6
carbaryl				(202→127)/ (202→145)				(202→127)/ (202→145)	
	5.5	87.3	5.7	5.0	6.0	87.9	6.2	6.0	12.1
	27.6	84.4	8.5	4.5	2.2	90.0	8.2	6.1	9.6
	49.7	88.3	1.6	4.2	0.0	87.0	6.3	5.9	12.8
methiocarb				(226→121)/ (226→169)				(226→121)/ (226→169)	
	5.0	91.2	8.2	17.8	3.3	46.9	4.4	15.3	4.3
	24.8	89.4	8.9	17.4	2.0	48.2	3.2	15.8	6.1
	44.6	87.0	6.6	18.0	3.9	45.4	2.7	16.7	16.5

[a] MSCC as the calibration curves. [b] Means of triplicates ($n = 3$). [c] Means of four replicates [$n = 4$ (4 days)]. [d] Ion ratios of individual pesticides were expressed as percentages of the corresponding base peak.

Table 4. Matrix Effects on the Quantitative Results of LC/ESI-MS/MS Determination of 13 Pesticides Spiked in Different Apple or Apple-Based Samples[a]

		apple sauce A		apple sauce B		apples and strawberries		apples and bluberries		apples and plums	
compound	spike level (μg/kg)	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)
aldicarb sulfoxide	4.9	83.3	9.2	79.0	6.6	72.8	6.1	61.5	2.0	67.3	5.6
	24.6	80.5	1.8	81.5	0.5	66.1	4.2	56.4	3.1	54.7	10.9
	44.3	81.8	1.1	86.5	4.2	63.7	10.6	48.1	4.1	48.6	3.9
aldicarb sulfone	5.0	74.3	8.0	112.3	4.7	69.1	4.6	60.1	1.1	59.0	3.3
	24.8	68.8	2.2	112.8	5.2	65.9	3.2	61.3	0.7	58.0	5.4
	44.6	71.3	3.2	114.8	5.9	66.3	7.8	58.1	2.4	56.4	2.4
oxamyl	5.4	60.2	5.8	101.1	4.3	61.0	6.0	55.0	3.4	54.7	2.7
	27.2	55.2	2.2	98.7	2.3	58.5	3.2	56.4	2.0	51.2	5.2
	49.0	55.6	2.6	93.5	6.7	58.1	6.1	54.7	2.0	49.8	2.5
methomyl	5.7	73.6	2.7	67.1	1.5	46.6	6.6	43.2	5.1	35.9	4.7
	28.7	71.0	3.1	68.4	1.1	46.2	1.4	44.8	1.1	31.4	10.1
	51.7	69.5	1.4	69.7	1.8	44.5	5.8	40.2	2.3	28.2	3.8
formetanate	4.7	38.9	3.6	33.7	7.0	26.9	7.0	28.4	2.7	38.0	4.8
	23.3	37.9	7.8	32.9	5.0	25.1	6.1	25.9	5.5	33.3	3.4
	41.9	35.5	2.4	32.6	7.8	23.5	3.9	26.6	22.2	33.3	6.6
3-hydroxycarbofuran	4.9	68.6	2.7	37.7	2.0	45.1	3.2	48.4	3.8	65.7	2.8
	24.7	68.9	0.4	41.3	2.3	46.5	0.5	48.0	3.9	60.1	5.9
	44.5	70.9	1.0	42.5	4.9	46.9	4.8	48.7	1.3	58.1	1.2
carbendazim	5.4	86.1	3.3	69.1	3.7	47.3	2.3	44.9	2.3	62.5	5.0
	27.0	88.1	0.8	71.4	2.4	46.0	1.8	41.1	6.4	53.2	10.5
	48.6	92.6	2.9	71.6	1.0	44.0	4.0	37.4	2.1	47.4	5.2
thiabendazole	5.5	68.9	3.8	60.1	6.5	42.1	4.3	48.9	2.1	46.8	1.7
	27.5	68.6	2.0	60.1	3.8	39.4	0.4	47.0	4.4	42.1	9.4
	49.5	73.4	2.0	58.1	3.6	38.4	3.8	44.6	3.6	39.2	3.9
aldicarb	5.8	72.8	4.0	71.4	7.3	59.3	1.8	50.2	5.3	60.8	8.4
	29.0	73.0	0.8	69.4	5.2	54.8	3.1	44.3	4.0	50.4	10.6
	52.1	69.1	1.5	68.1	4.3	52.6	4.4	38.8	0.6	45.6	6.6
propoxur	5.3	87.7	4.2	82.1	5.7	71.9	5.1	69.5	1.8	76.8	6.3
	26.5	84.5	2.8	84.9	2.9	69.3	3.3	67.1	4.2	72.4	5.1
	47.7	80.3	1.4	82.1	2.1	69.1	2.6	60.6	3.8	69.0	4.2
carbofuran	5.6	88.7	7.7	78.0	6.6	70.8	6.7	68.8	2.1	74.8	7.5
	28.2	81.9	3.1	81.8	3.5	69.1	3.7	67.1	3.7	72.2	4.4
	50.8	79.3	2.5	80.9	4.5	68.1	2.0	62.7	3.4	69.0	4.5
carbaryl	5.5	94.8	6.4	86.0	5.4	70.6	6.5	73.9	1.9	77.4	5.6
	27.6	92.6	4.4	89.2	5.4	69.4	3.9	71.6	7.0	73.8	5.4
	49.7	95.2	4.1	89.0	2.6	68.4	6.5	61.1	3.7	68.8	7.1
methiocarb	5.0	46.5	7.4	58.9	2.1	67.4	6.4	37.4	13.5	56.0	9.0
	24.8	45.3	7.4	58.3	3.5	51.6	2.0	34.2	6.4	45.8	6.4
	44.6	46.4	1.6	57.7	3.6	49.3	6.6	27.8	8.2	42.0	5.8

[a] Data are means of triplicates ($n = 3$). MSCC as the calibration curves.

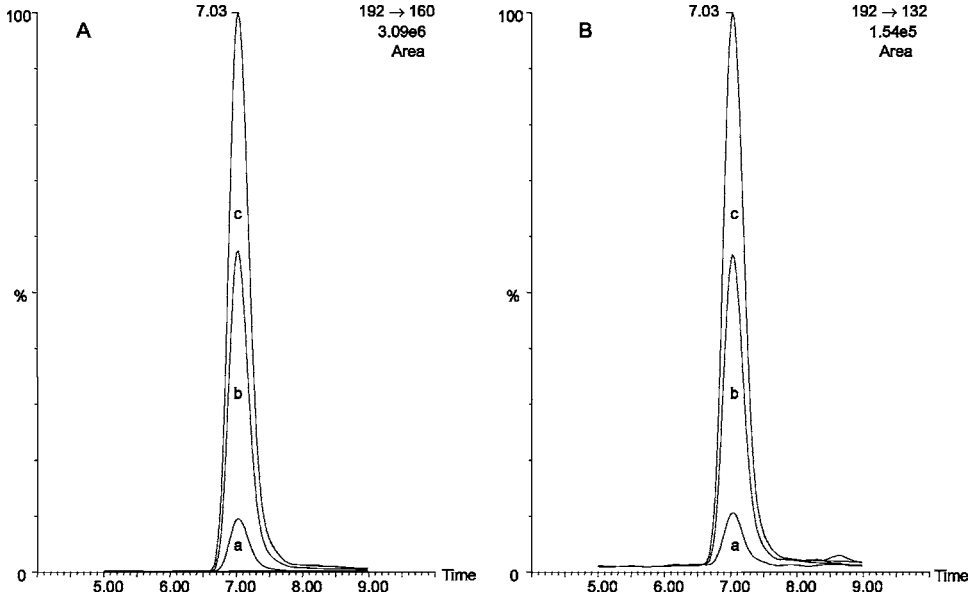

Figure 3. Standard addition LC/ESI-MS/MS chromatograms of carbendazim in an apple sauce sample B. Peak a: unknown concentration (fortified at 5.4 μg/kg equivalent in sample). Peak b: unknown + 28.8 μg/kg of carbendazim (in sample). Peak c: unknown + 57.6 μg/kg of carbendazim (in sample). (**A**) Transition at 190→160 for quantification. (**B**) Transition at 190→132 for confirmation. Ion ratios [(190→132)/(190→160)] for peaks a–c: 5.0, 4.9, and 5.0%, respectively.

Table 5. Quantitative Results of LC/ESI-MS/MS Determination of 13 Pesticides Spiked in Different Apple or Apple-Based Samples Using Standard Addition[a]

		apple sauce A		apple sauce B		apples and strawberries		apples and bluberries		apples and plums	
compound	spike level (μg/kg)	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)
aldicarb sulfoxide	4.9	102.8	10.8	104.1	9.2	99.9	6.3	105.4	13.9	114.2	3.7
	24.6	106.5	3.1	95.8	15.6	98.7	1.1	102.2	1.9	107.6	4.1
	44.3	95.1	3.3	105.3	6.7	100.6	4.7	107.3	11.6	110.2	6.2
aldicarb sulfone	5.0	100.5	4.3	111.8	7.1	115.8	8.5	127.4	8.9	136.1	4.2
	24.8	106.3	1.4	107.6	4.9	123.6	3.6	125.6	5.0	126.0	0.8
	44.6	96.4	4.5	117.3	1.0	131.2	3.5	144.8	6.8	126.7	4.4
oxamyl	5.4	96.6	8.4	104.8	4.6	97.3	3.4	114.6	13.5	116.9	13.7
	27.2	98.4	0.9	103.6	7.1	102.4	4.5	113.4	2.8	106.6	5.1
	49.0	90.1	2.0	102.7	4.6	112.3	5.8	124.8	8.3	106.4	5.2
methomyl	5.7	97.8	4.4	104.2	1.5	107.0	1.7	115.8	4.0	119.1	13.4
	28.7	101.4	2.8	106.3	6.7	104.9	2.3	109.2	2.0	107.0	5.1
	51.7	94.8	5.0	106.3	6.6	114.0	6.0	115.7	7.0	106.1	9.0
formetanate	4.7	60.6	5.6	84.0	6.5	73.5	4.5	70.7	7.7	83.2	5.6
	23.3	95.3	22.4	74.9	19.3	77.0	6.8	84.8	16.4	83.2	6.7
	41.9	81.0	11.3	77.1	11.9	78.0	12.9	84.1	10.0	83.4	11.0
3-hydroxycarbofuran	4.9	96.3	7.2	106.3	8.8	90.2	6.8	98.8	8.7	106.3	3.6
	24.7	103.3	3.2	106.2	5.5	100.0	3.3	96.1	5.7	103.3	3.5
	44.5	98.5	5.7	109.7	2.2	99.1	2.9	97.7	7.2	108.9	7.5
carbendazim	5.4	85.4	5.7	103.7	4.1	88.7	2.6	91.1	12.3	99.0	5.4
	27.0	95.0	0.8	97.6	1.8	93.1	3.2	92.1	3.7	96.0	3.1
	48.6	88.2	5.5	101.2	4.9	96.3	5.6	95.0	8.2	97.6	7.7
thiabendazole	5.5	87.8	6.4	103.4	2.2	91.0	3.7	101.9	9.2	98.2	0.5
	27.5	105.6	3.3	104.6	1.0	98.4	2.9	110.3	7.2	102.2	0.6
	49.5	97.3	6.2	110.8	9.8	101.4	5.7	113.4	8.5	105.5	11.2
aldicarb	5.8	89.5	12.6	104.9	5.3	102.6	1.7	100.1	8.3	106.8	11.4
	29.0	94.1	1.0	96.6	6.9	94.1	3.5	96.5	4.5	97.7	7.0
	52.1	90.1	7.2	105.0	8.0	97.2	3.9	95.0	5.1	101.1	10.8
propoxur	5.3	90.9	2.1	104.5	4.9	100.4	4.4	105.2	0.6	106.3	3.1
	26.5	96.9	3.7	99.4	5.4	100.5	1.9	100.9	2.7	104.6	3.3
	47.7	93.9	8.8	105.2	6.4	101.7	1.7	106.0	6.0	107.5	7.6
carbofuran	5.6	95.6	5.7	107.0	6.9	103.5	5.7	102.1	5.9	119.8	2.6
	28.2	103.0	4.0	103.4	3.0	106.0	3.5	104.4	7.0	108.1	3.4
	50.8	99.2	7.2	112.1	4.2	106.3	2.3	111.5	9.8	111.1	4.0
carbaryl	5.5	94.3	6.2	102.8	8.5	99.5	5.2	105.8	10.6	116.1	9.9
	27.6	108.6	2.7	102.7	10.2	92.6	2.9	101.1	8.5	104.1	10.4
	49.7	98.8	6.8	108.3	18.7	105.4	16.8	116.4	4.8	102.8	11.6
methiocarb	5.0	68.1	15.2	92.0	9.6	92.4	9.6	83.7	10.2	95.5	9.2
	24.8	93.9	12.2	97.9	12.6	92.7	10.0	89.7	4.1	95.0	1.4
	44.6	77.1	13.6	103.4	16.2	99.8	6.5	82.3	8.5	98.2	13.3

[a] Data are means of triplicates ($n = 3$).

precision. **Table 4** also shows the quantitative results of the spiked samples from different matrices including apples and strawberries, apples and blueberries, and apples and plums, for which calibration curves were prepared using the matrix blank. The low and variable recoveries of a pesticide from matrix to matrix (**Table 4**) were once again due to the mismatched matrix calibrations as discussed above. Although a further cleanup procedure was explored and different brands of analytical columns were tested, the matrix effects could not be overcome. MSCCs could not serve as a general approach, in this case, to achieve the best accuracy of the method. Therefore, alternative calibration, that is, the use of isotopically labeled standards or standard addition, has to be used in order to compensate for the matrix effects.

Standard Addition. Generally, it is very difficult and expensive to obtain every single isotopically labeled standard for each individual pesticide of interest. In contrast, standard addition is a relatively cheap and practical technique for samples when there is no blank matrix available, and it has been used to overcome the matrix effects, for example, in the LC/MS analysis of toxins in shellfish (*13*). Standard addition is a technique that introduces the standard of a target pesticide directly into samples or a procedure in which known amounts of an analyte are added to aliquots of sample extracts containing the analyte to produce new notional concentrations. The analyte responses generated by the spiked sample and original extracts are measured, and the analyte concentration in the original sample is determined from the slope and intercept of the response curve, from which a linear response, at least in a narrow range, is a prerequisite. The levels of known amounts of analyte added, which have to be determined by experiments, are also critical to obtaining an accurate result. The amount of analyte present in the sample can be determined in as little as three injections, i.e., the original sample (extract) and two spiked samples (extracts). **Figure 3** shows an example of standard addition for carbendazim in the apple sauce sample B. The sample was first fortified with carbendazim at 5.4 μg/kg equivalent. The amount of carbendazim fortified was defined as unknown, and its corresponding peak was labeled as peak a (**Figure 3**). The LC/ESI-MS/MS peaks b and c, therefore, were responses of the sum of the unknown and the standard added. Calculations of standard addition were described in the Materials and Methods. Quantitative results are presented in **Table 5**. The recoveries of standard addition method were around 100%, and the matrix effects were eliminated significantly. The method was tested further for its applicability to a low concentration

Table 6. Quantitative Results of LC/ESI-MS/MS Determination of 13 Pesticides Spiked in an Apple Sauce at Low Levels Using Standard Addition[a]

compound	spike level (μg/kg)	found (μg/kg)	recovery (%)	RSD (%)
aldicarb sulfoxide	1.0	0.9	86.4	14.0
	2.0	1.8	90.2	4.6
	3.0	2.6	89.3	11.5
	3.9	3.7	93.3	8.2
aldicarb sulfone	1.0	0.9	95.3	9.6
	2.0	2.0	100.6	4.2
	3.0	2.9	98.4	5.5
	4.0	3.9	98.2	0.4
oxamyl	1.1	1.0	88.2	2.3
	2.2	2.0	93.2	4.0
	3.3	2.9	89.7	3.5
	4.4	4.1	93.7	1.5
methomyl	1.1	0.9	81.7	1.0
	2.3	2.1	91.9	7.1
	3.4	3.1	89.8	2.2
	4.6	4.2	90.9	2.7
formetanate	0.9	0.6	64.8	24.5
	1.9	1.3	69.7	5.0
	2.8	2.1	76.5	10.2
	3.7	3.0	81.5	15.3
3-hydroxycarbofuran	1.0	0.7	66.9	24.2
	2.0	1.4	72.2	14.6
	3.0	2.4	81.2	2.9
	4.0	3.3	84.6	4.3
carbendazim	1.1	0.9	81.7	7.7
	2.2	1.9	87.5	12.5
	3.2	2.9	89.9	5.7
	4.3	3.9	91.3	6.1
thiabendazole	1.1	0.7	66.2	3.5
	2.2	1.4	62.9	14.2
	3.3	2.3	70.7	7.3
	4.4	3.4	78.2	7.6
aldicarb	1.2	0.8	67.9	5.4
	2.3	2.1	90.8	7.1
	3.5	3.0	85.8	2.4
	4.6	4.3	92.0	9.5
propoxur	1.1	0.8	76.1	17.1
	2.1	1.8	85.3	2.3
	3.2	2.8	88.8	4.2
	4.2	3.8	88.7	2.5
carbofuran	1.1	0.8	74.8	17.2
	2.3	2.0	89.1	3.8
	3.4	3.2	94.5	1.8
	4.5	4.4	97.2	12.2
carbaryl	1.1	0.9	84.9	18.6
	2.2	2.1	96.1	4.3
	3.3	3.3	100.4	6.0
	4.4	4.6	105.2	12.4
methiocarb	1.0	0.7	71.1	16.5
	2.0	1.9	94.8	8.5
	3.0	2.6	86.3	7.6
	4.0	3.6	91.9	4.1

[a] Apple sauce sample B was used. Data are means of triplicates ($n = 3$).

range, i.e., from 1 to 4 μg/kg, and results are shown in **Table 6**. The recoveries were reasonable and reflect the accuracy of standard addition although with large RSDs as expected at low concentrations. Therefore, it can be concluded that standard addition can serve as a very practical means to overcome the matrix effects for the LC/ESI-MS/MS analysis of pesticides in different matrices.

Confirmation. Ion ratios of some pesticides, for example, thiabendazole, may vary significantly from day-to-day (**Table 3**, under interassay column), whereas those obtained within the same day (**Table 3**, under intraassay column) remain very consistent. Therefore, ion ratios from the intraassay were used for confirmation (**Table 3**), and their RSDs were usually less than 15%. In general, the relative ion intensities of the detected

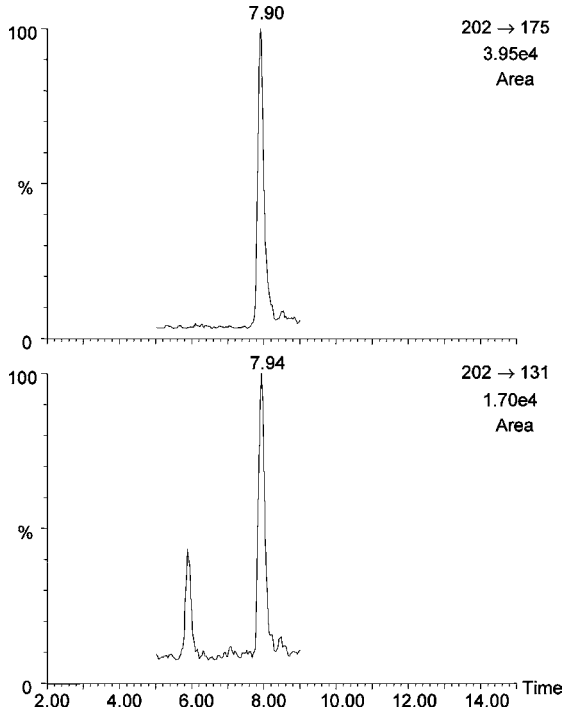

Figure 4. LC/ESI-MS/MS chromatograms of a sample containing 1.1 μg/kg (RSD = 3.9%, $n = 3$, determined by standard addition) of incurred thiabendazole. Ion ratios [(202→131)/(202→175)]: 12.2% (RSD = 5.7%, $n = 3$).

ions from incurred samples as shown, for example, in **Figure 4**, are essentially compared to those of corresponding calibration standards measured under the same conditions, i.e., from the same batch of runs, so as to confirm the identity of the pesticides. Furthermore, the ion ratios of a pesticide from incurred samples can be compared to the known pesticide spiked when the standard addition method is used as seen in **Figure 3**. In this study, two transitions were acquired for confirmation (**Tables 1** and **3**). Ion ratios acquired under the same conditions normally lay within tolerances as recommended by the European Commission Decision 2002/657/EC (*14*).

Method Limits of Detection (LOD). The method LOD (signal-to-noise, S/N ≥ 3) was determined by evaluating the MRM transition that provided to be the most intense analyte signal for the detection of a pesticide. Under the conditions specified in the method, the method LODs (μg/kg) were 0.1 for aldicarb sulfoxide, oxamyl, methomyl, formetanate, and thiabendazole; 0.2 for aldicarb, propoxur, carbofuran, and methiocarb; 0.06 for 3-hydroxycarbofuran; 0.08 for carbendazim; and 0.09 for carbaryl and aldicarb sulfone.

In conclusion, LC/ESI-MS/MS was found to be a sensitive method for determination of pesticides in apple-based infant foods at trace levels. Acetonitrile extraction and SPE with Oasis HLB cartridges served as a simple and rapid method to remove sugars and other substances in samples so as to extract and concentrate the pesticides from their matrix for further analysis. The LC/ESI-MS/MS method reported in this paper was able to quantify and confirm 13 pesticides in samples in a range from 1 to 50 μg/kg. The LC/ESI-MS/MS method LOD for 13 pesticides were 0.2 μg/kg or less. Standard addition can serve as a very promising and practical approach to overcome matrix effects and has a great potential to be applicable to other matrices where the LC/ESI-MS/MS technique is used. This validated LC/ESI-MS/MS method can thus be employed to determine 13 pesticides in apple-based infant foods for regulatory purposes,

particularly when confirmation of identities of incurred pesticides in samples is required.

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