

Determination of Formetanate Hydrochloride in Fruit Samples Using Liquid Chromatography–Mass Selective Detection or –Tandem Mass Spectrometry[†]

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A rapid multiresidue method that captures residues of the insecticide formetanate hydrochloride (FHCl) in selected fruits is described. The method was used to provide residue data for dietary exposure determinations of FHCl. Using an acetonitrile extraction with a dispersive cleanup based on AOAC International method 2007.01, also known as QuEChERS, which was further modified and streamlined, thousands of samples were successfully analyzed for FHCl residues. FHCl levels were determined both by liquid chromatography–single-stage mass spectrometry (LC-MS) and ultraperformance liquid chromatography (UPLC)–tandem mass spectrometry (LC-MS/MS). The target limit of detection (LOD) and the limit of quantitation (LOQ) achieved for FHCl were 3.33 and 10 ng/g, respectively, with LC-MS and 0.1 and 0.3 ng/g, respectively, with LC-MS/MS. Recoveries at these previously unpublished levels ranged from 95 to 109%. A set of 20–40 samples can be prepared in one working day by two chemists.

KEYWORDS: Formetanate hydrochloride; QuEChERS; LC-MS; LC-MS/MS; fruit; sub parts per billion; pesticide residues

INTRODUCTION

The miticide/insecticide formetanate hydrochloride (FHCl) (Figure 1) is applied to a variety of fruits including apple, pear, peach, nectarine, orange, grapefruit, lemon, lime, tangerine, and tangelo. It was registered for the first time in the United States in 1984, and subsequent food tolerances were set on the basis of estimated dietary exposure. Pesticide residue monitoring data are routinely provided to the U.S. Environmental Protection Agency (USEPA) for the purposes of risk assessment determinations and registration decisions. In 1996, the U.S. Food Quality Protection Act (1) mandated a complete reassessment of all existing pesticide tolerances in food. Accurate risk assessments require pesticide residue monitoring data, much of which is routinely provided to the USEPA by the U.S. Department of Agriculture's Pesticide Data Program (USDA-PDP). Residue data for the *N*-methyl carbamate, FHCl, {3-[(*EZ*)-dimethylaminomethyleneamino]phenyl methylcarbamate hydrochloride}, had not been reported to the EPA since 2001 (2). A single analyte method was originally used to provide the residue monitoring data. However, because it was expensive in terms of time and logistics and because its lowest achievable limit of detection (LOD) was 50 $\mu\text{g}/\text{kg}$, this method was not used after 2001. In 2007, the USEPA Office of Pesticide Programs (OPP) required residue data for FHCl in fruit commodities lower than a LOD of 10 $\mu\text{g}/\text{kg}$ for the purpose of determining dietary exposure, which would subsequently be used to make reregistration decisions for FHCl. A lower LOD was needed because the USEPA OPP uses a computational model for dietary exposure based on half the value of the LOD as an input in the risk assessment equation.

In addition to the single analyte method used in 2001, other methods exist for the analysis of FHCl. Those methods either require column-switching LC-UV with an acidic buffer (pH 3.0) and achieve a limit of quantitation (LOQ) of 0.05–0.06 $\mu\text{g}/\text{g}$ (3) or use LC-UV and achieve a lower LOD of 0.018 ($\mu\text{g}/\text{g}$) but require larger volumes of solvent (4). A multiresidue method using an acetone extraction and LC-FLD postcolumn derivatization yields poor recoveries and drifting retention times for FHCl (5).

The work presented in this paper is a multiresidue method that captures FHCl and is based on the analytical method of the AOAC International (2007.01) introduced by Anastassiades et al. in 2003 (6), also known as QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe), which was subsequently modified by Schenck et al. (7) and Wong et al. (8). The described method enables the accurate quantitation of FHCl using either LC-MS or LC-MS/MS, lowers the detection limits of FHCl, and has the added advantage of increasing the number of samples that can be extracted and analyzed in a day.

MATERIALS AND METHODS

Chemicals and Reagents. The FHCl standard and the surrogate standard, propoxur, were obtained from the USEPA National Pesticide Standard Repository (Environmental Science Center, Ft. George G. Meade, MD) and were of 99 and 99.9% purity, respectively. Acetonitrile and methanol were purchased from B&J (Morristown, NJ). ChlorAC buffer was purchased from Pickering Laboratories (Mountain View, CA).

Stock solutions of FHCl (1.0 mg/mL) were prepared by dissolving 0.025 g of the pesticide in 25.0 mL of a 5% H₂O/95% acetonitrile solution, sonicated briefly until in solution (approximately 30 s), and then stored at –5 °C. Stock solutions of propoxur (1.0 mg/mL) were prepared by

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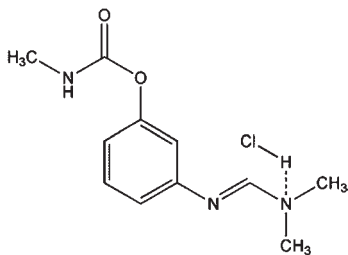


Figure 1. *N*-Methyl carbamate, FHCl: {3-[(*E*)-dimethylaminomethylene]phenyl methylcarbamate hydrochloride}.

dissolving 0.025 g of the pesticide in 25.0 mL of methanol and stored at -5°C .

A FHCl intermediate standard solution ($10.0\ \mu\text{g}/\text{mL}$) was prepared by transferring 1.0 mL from the stock solution to a 100.0 mL volumetric flask and diluting to volume with acetonitrile. A propoxur intermediate standard solution ($10.0\ \mu\text{g}/\text{mL}$) was prepared by transferring 0.500 mL from the stock solution to a 50.0 mL volumetric flask and diluting to volume with acetonitrile.

Solutions containing $10.0\ \mu\text{g}/\text{mL}$ of FHCl or propoxur were used to fortify samples for LC-MS analysis. Solutions containing $0.103\ \mu\text{g}/\text{mL}$ FHCl and $0.100\ \mu\text{g}/\text{mL}$ propoxur were used to fortify samples for LC-MS/MS analysis. Calibration standards for LC-MS were prepared in matrix with FHCl at $20.0\ \mu\text{g}/\text{L}$ (LOD), $60.0\ \mu\text{g}/\text{L}$ (LOQ), $120.0\ \mu\text{g}/\text{L}$ ($2 \times \text{LOQ}$), and $600.0\ \mu\text{g}/\text{L}$ ($10 \times \text{LOQ}$) and with propoxur at $24.0\ \mu\text{g}/\text{L}$ (LOD), $36.0\ \mu\text{g}/\text{L}$ (LOQ), $72.0\ \mu\text{g}/\text{L}$ ($2 \times \text{LOQ}$), and $360.0\ \mu\text{g}/\text{L}$ ($10 \times \text{LOQ}$). Calibration standards for LC-MS/MS were prepared in matrix with FHCl at $0.150\ \mu\text{g}/\text{L}$ (LOD), $0.450\ \mu\text{g}/\text{L}$ (LOQ), $0.900\ \mu\text{g}/\text{L}$ ($2 \times \text{LOQ}$), and $4.5\ \mu\text{g}/\text{L}$ ($10 \times \text{LOQ}$) and with propoxur at $0.050\ \mu\text{g}/\text{L}$ (LOD), $0.150\ \mu\text{g}/\text{L}$ (LOQ), $0.300\ \mu\text{g}/\text{L}$ ($2 \times \text{LOQ}$), and $1.5\ \mu\text{g}/\text{L}$ ($10 \times \text{LOQ}$). A dilution of 0.02% ChlorAC buffer (Pickering Laboratories) was used to prepare a final solution of 50:50 0.01% ChlorAC buffer and methanol, and this solution was used to reconstitute samples prior to LC analysis.

Sample Preparation. *Samples.* Frozen, organic peach slices and fresh nectarines, apples, oranges, and pears were purchased from a local market and used as control samples and were stored at -80°C until analyzed. When organic produce was not available, samples found to contain no FHCl residues were used as a supplemental source of controls. Incurred samples were collected by several U.S. states including California, Ohio, Washington, and New York. The samples were comminuted and shipped frozen by the USDA-PDP.

Procedure. Samples ($15.0 \pm 0.1\ \text{g}$) were weighed into 50 mL centrifuge tubes. To each sample (excluding the reagent blank, the matrix blank, and the matrix blank samples reserved for the matrix calibration standards) was added a suitable amount (0.075 mL of $0.100\ \mu\text{g}/\text{mL}$) of the surrogate standard, propoxur. Samples were extracted with 15 mL of acetonitrile. A bottle-top dispenser (BrandTech Scientific, Inc., Essex, CT) in a 4 L bottle of acetonitrile was used to add the solvent to each sample. Samples were then shaken for 1 min using a grinder (SPEX CertiPrep Inc., Metuchen, NJ) set at 1000 strokes per minute (spm). Magnesium sulfate (6 g) and NaCl (1.5 g), prepackaged in 50 mL centrifuge tubes (UCT, Bristol, PA), and a stainless steel grinding ball of $\frac{5}{32}$ in. diameter were added to each extract and shaken vigorously by hand for 5 s to sufficiently break up the crystalline agglomerates. When the $\text{MgSO}_4/\text{NaCl}$ was added to all tubes, a tray of tubes was shaken vigorously using the grinder set at 1200 spm for 2 min, ensuring that the solvent interacted well with the entire sample. Extracts were then centrifuged (Jouan Inc., Winchester, VA) for 5 min at 2500 rpm. To clean up the extract, the acetonitrile supernatant (9 mL) was transferred, using a repeating pipetor (Drummond Scientific Co., Broomall, PA) with glass pipets (Fisher Scientific), to a 15 mL polypropylene dispersive SPE tube containing prepackaged 150 mg of graphitized carbon black (GCB), 300 mg of primary–secondary amine (PSA), and 900 mg of MgSO_4 (UCT). To the supernatant in the dispersive SPE tubes was added 3 mL of toluene using a 1–5 mL pipet, (Fisher Scientific), and the tubes were capped and inverted to check for leakage. Tubes that leaked were uncapped, and the threads of the tube were wrapped with Teflon tape. A tray of SPE tubes was then placed in the grinder for 1 min at 1500 spm to ensure that all of the sorbent was “wetted” by the solvent. The

extracts were then centrifuged for 5 min at 2500 rpm. The supernatant was transferred to a glass culture tube (8 mL for LC-MS or 4 mL for LC-MS/MS). From the matrix blank samples, 2 mL of supernatant was transferred to each of four tubes for matrix-matched calibration standards. The sample extracts were evaporated to dryness using a *Turbovap* (Zymark, Hopkinton, MA) with a water bath set to 40°C . Excessive time (more than a few minutes after dryness) on the *Turbovap* resulted in reduced recoveries of FHCl. The fortification standard, FHCl, and the surrogate standard, propoxur, were then added to the four matrix standard tubes, and the four standards were again evaporated to dryness. For LC-MS analysis, all samples and standards were reconstituted to 1.0 mL with methanol/water (50:50) and vortex mixed for 20 s. For LC-MS/MS analysis, the four matrix standards were reconstituted to 1.0 mL with 0.01% ChlorAC buffer/methanol (50:50); and the matrix spike, matrix control, reagent blank, and samples were reconstituted to 2.0 mL with 0.01% ChlorAC buffer/methanol (50:50) and vortex mixed for 20 s. Samples and standards were filtered using $0.2\ \mu\text{m}$ PVDF syringe filters (Pall Life Sciences) prior to LC analysis.

Total milligrams injected for peach and nectarine samples using LC-MS was 60 mg with an injection volume of $10.0\ \mu\text{L}$. Total milligrams injected using LC-MS/MS was 7.5 mg with an injection volume of $5.0\ \mu\text{L}$. Sample sets consisted of 20–40 samples, 1 matrix blank, 1 matrix spike, 1 reagent blank, and 4 matrix standards. Samples were quantitated using a four-point calibration curve of matrix standards at concentrations equivalent to the LOD, LOQ, $2 \times \text{LOQ}$, and $10 \times \text{LOQ}$ of both FHCl and propoxur.

LC-MS Analysis. LC-MS was used for the analysis of peach and nectarine samples collected in 2007. LC-MS analysis was conducted with a Waters Alliance 2695 HPLC equipped with a Waters Micromass ZQ single-quadrupole mass spectrometer, model MM1 (Milford, MA) using a Waters Atlantis-T3 column (10 cm \times 2.1 mm) of $3.5\ \mu\text{m}$ particle size. Reverse phase operating conditions were as follows: an injection volume of $10\ \mu\text{L}$; a column temperature of 40°C ; a flow rate of $0.3\ \text{mL}/\text{min}$ using a mobile phase of methanol, H_2O , and 10 mM ammonium acetate prepared as two separate solutions, solvent A, 95% H_2O , 5% MeOH, and 10 mM ammonium acetate; and solvent B, 95% MeOH, 5% H_2O , and 10 mM ammonium acetate. The initial conditions were set at 95 + 5 (solvent A + solvent B), followed by a linear gradient in 19 min to 5 + 95 (solvent A + solvent B). The column was equilibrated at the initial conditions at the end of each run 95 + 5 (solvent A + solvent B) for 10 min. The total run time was 30 min.

The mass spectrometer was operated in the positive electrospray ionization (ESI+) mode. For FHCl, the ions monitored and cone voltages used were m/z 222.1 (28 V), 165.1 (45 V), and 120 (65 V). For propoxur, three ions were monitored at different cone voltages. These were m/z 210.1 (17), 168.0 (25), and 111 (40). The ion source temperature was 120°C , and the desolvation temperature was 325°C . The cone gas flow (nitrogen 99.9999% Roberts Oxygen, Rockville, MD) was $50\ \text{L}/\text{h}$, and the desolvation gas flow was $500\ \text{L}/\text{h}$ of nitrogen gas.

Analysis was performed in the selected ion mode using one target ion and two qualifier ions (meeting a 3:1 S/N ratio). The confidence limits of the relative abundance of structurally significant ions used for SIM and/or full scan identification/confirmation were within $\pm 20\%$ (absolute) when compared to the same relative abundances observed from a standard solution injection made during the same analytical run. The peak area of the target ion was used for quantitation and determined by the calibration curve. Standards were prepared in blank matrix extracts to counteract the matrix effect. Blank matrix extracts were made following the procedure for sample preparation described below using a blank sample without fortification. FHCl and propoxur were confirmed by their retention times, the identification of target and qualifier ions, and the determination of qualifier-to-target ion ratios. The retention times of FHCl and propoxur in the standards and samples were within ± 0.1 min of the expected times.

UPLC-MS/MS Analysis. LC-MS/MS analysis was conducted with a Waters Acquity ultraperformance liquid chromatograph (UPLC) equipped with a Quattro Premier tandem-quadrupole mass spectrometer using an Acquity HSS-T3 column (10 cm \times 2.1 mm) of $1.8\ \mu\text{m}$ particle size all supplied by Waters (Milford, MA). Reverse phase operating conditions were as follows: an injection volume of $5\ \mu\text{L}$; a column temperature of 40°C . The mobile phase used was the same as in the LC-MS analysis; however, the gradient changed as follows: after 1 min at the initial conditions of 95 + 5 (solvent A + solvent B) and a $0.3\ \text{mL}/\text{min}$ flow rate,

a linear gradient was programmed from 95 + 5 to 60 + 40 (solvent A + solvent B) in 8.0 min. At 8.1 min the flow rate was increased to 0.4 mL/min, followed by a second linear gradient over 4.9 min to 0 + 100% and then a third linear gradient in 1.0 min back to 95 + 5 (solvent A + solvent B). At 15.0 min the flow rate returned to 0.3 mL/min.

The mass spectrometer was operated in the positive electrospray ionization (ESI+) multiple reagent monitoring (MRM) mode. The ion source temperature was 130 °C and the desolvation temperature was 450 °C. The cone gas flow (nitrogen) was 50 L/h and the desolvation gas flow was 800 L/h of nitrogen gas. The collision cell gas was ultrahigh-purity argon at a flow of 0.3 mL/min. Two precursor/product ion transitions were monitored for each compound, the more abundant ion transition was used for quantitation, whereas the other ion transition was used for confirmation. Analytes were considered to be confirmed when MRM ion transition ratios were within $\pm 20\%$ (absolute) of the ratios in standards. The MRM transitions, cone voltages, and collision energies were as follows: for FHCi, 222.0 \rightarrow 92.80 (25 V, 35 eV) and 222.0 \rightarrow 164.9 (25 V, 15 eV); for propoxur, 210.0 \rightarrow 110.8 (17 V, 15 eV) and 210.0 \rightarrow 167.9 (17 V, 7 eV).

RESULTS AND DISCUSSION

Sample Extraction and Cleanup. Three different surveys of various fruit crops, collected from several states throughout the United States, were conducted specifically for FHCi over a period of three years. The first survey began with 580 peach and nectarine samples in 2007. Two follow-up surveys were conducted: one in 2008 of 1200 peach and nectarine samples and the other in 2009 of 2200 apple, pear, and orange samples. The results of the FHCi surveys are listed in the PDP Annual Summary Reports (9–11). The modified QuEChERS multiresidue extraction (7) was streamlined to increase sample production in order to analyze thousands of samples for FHCi. The modifications introduced to QuEChERS included simple substitutions for common laboratory tools, such as a bottle-top dispenser for a standard pipet or a graduated cylinder, and by using a motorized pipettor instead of a manual pipet bulb, the sample preparation time was efficiently reduced. A shaker/grinder capable of shaking large numbers of samples up to a speed of 1500 strokes/min was used to expedite large numbers of samples and reduce the analysts' fatigue in manually shaking samples. Each day 20–40 samples were analyzed simultaneously in an assembly line setup for overnight instrumental analysis.

The QuEChERS method comprises an extraction with acetonitrile followed by a cleanup using PSA and GCB with a few milliliters of toluene. Without the addition of toluene, the average recovery of FHCi was only approximately 70%. Toluene was added for the purpose of desorbing any FHCi bound to the GCB (8).

The final extracts were reconstituted in MeOH/H₂O (1:1) after a portion of the ACN/toluene extract had been dried using N₂. The benefit of this solvent exchange is that a substantial amount of coextractives are removed during filtration because they are insoluble in MeOH/H₂O, resulting in a cleaner extract without affecting the recovery of the analytes. Also, the type of filter membrane used and the extent of matrix dilution affected the recoveries of FHCi. Polyvinylidene fluoride (PVDF) membranes were found to retain FHCi in sample extracts when the matrix content was highly diluted with the MeOH/H₂O (1:1) solvent. This loss may be due to the fact that the PVDF membranes are hydrophobic and tend to retain organic compounds in a (1:1) aqueous solvent. Despite that, the filtrate from PVDF membranes was cleaner than other types of membranes such as nylon filter disks and resulted in less interference introduced to the mass spectrometer from the matrix. When further dilution of the sample extract was required to maintain peak area responses within the calibration curve, no additional filtration step was

added due to observed loss of analyte upon filtration of these matrix-diluted samples.

LC-MS and LC-MS/MS. LC-MS is a preferable alternative to the column-switching LC-UV FHCi method that uses an acidic buffer (pH 3.0) and achieves an LOQ of 0.06 $\mu\text{g/g}$ (5), and it is preferable to the single analyte method used in a 2001 survey, which achieved an LOD of 0.05 $\mu\text{g/g}$. The LC-MS method eliminates the need for an acidic buffer and column switching used in the LC-UV method. LC-MS is also a preferable alternative to LC-FLD postcolumn derivatization, which is only effective for sample screening because of inconsistent retention times and poor chromatography (3). Using LC-MS, an LOD of 3.33 ng/g or 20 ng/mL and an LOQ of 10 ng/g or 60 ng/mL (Figure 2) were achieved for all matrices. Although the QuEChERS dispersive sample cleanup is acceptable when rapid analyses are required, it is the minimum cleanup needed for LC-MS detection. Using the dispersive cleanup, the column needed to be regenerated, following the manufacturer's recommended procedure, after every 100 samples to maintain acceptable ion ratio criteria and peak resolution. If tandem mass spectrometry instrumentation is not available and sub parts per billion levels are not needed, LC-MS can be also be used as an effective and updated alternative to existing FHCi methods.

Using tandem mass spectrometry coupled with UPLC instead of LC-MS, the limits of detection were lowered 30 times to an LOD of 0.1 ng/g or 0.15 ng/mL and an LOQ of 0.3 ng/g or 0.45 ng/mL (Figure 3) for the following two surveys of FHCi. UPLC provided a reduced run time of 15 min, compared to 30 min when an HPLC system was used. Also, increased sensitivity and better peak resolution were obtained when using UPLC leading to more accurate quantitation of analytes. The T3 bonded phase used for LC-MS or LC-MS/MS analysis was adequate to overcome the early elution and peak broadening of FHCi in strongly aqueous mobile phases. T3 bonding utilizes a trifunctional C₁₈ alkyl phase bonded at a ligand density that promotes polar compound retention and aqueous mobile phase compatibility (12).

Significantly lowering the LOQ of FHCi to sub parts per billion levels not previously demonstrated by any laboratory presented challenges not encountered at the higher LOQs. The slow degradation of FHCi in aqueous solvents was more noticeable at the sub parts per billion LOQ. To overcome much of the loss of FHCi, samples were reconstituted with a preservative of 0.01% ChlorAC buffer/methanol solution instead of a water/methanol solution. The 0.01% concentration was chosen because at concentrations > 0.01%, the ChlorAC buffer adversely affected the chromatography of FHCi by causing peak broadening and peak asymmetry.

It was also observed that when the composites which were used to fortify samples with FHCi for spike recoveries became slightly oxidized, degradation of fortified FHCi resulted and adversely affected the recoveries of FHCi. Ascorbic acid was used during the preparation of control composites to slow oxidation of matrices such as apples. Finally, because of the increased sensitivity of the LC-MS/MS, 4 mL instead of 8 mL of extract supernatant was taken to dryness, halving the time needed for solvent exchange and further streamlining the method.

Recovery Studies. Control fruit samples of peach, nectarine, apple, pear, and orange were fortified before extraction at least in triplicate with FHCi at least at three of the four concentration levels of LOQ, 2 \times LOQ, 5 \times LOQ, and 10 \times LOQ. In addition, each sample was fortified with a surrogate/process control intended to ensure the integrity of a particular sample within an analytical system. The *N*-methyl carbamate, propoxur, was selected as the process control because it was not expected to be an incurred residue in the samples. With the exception of the

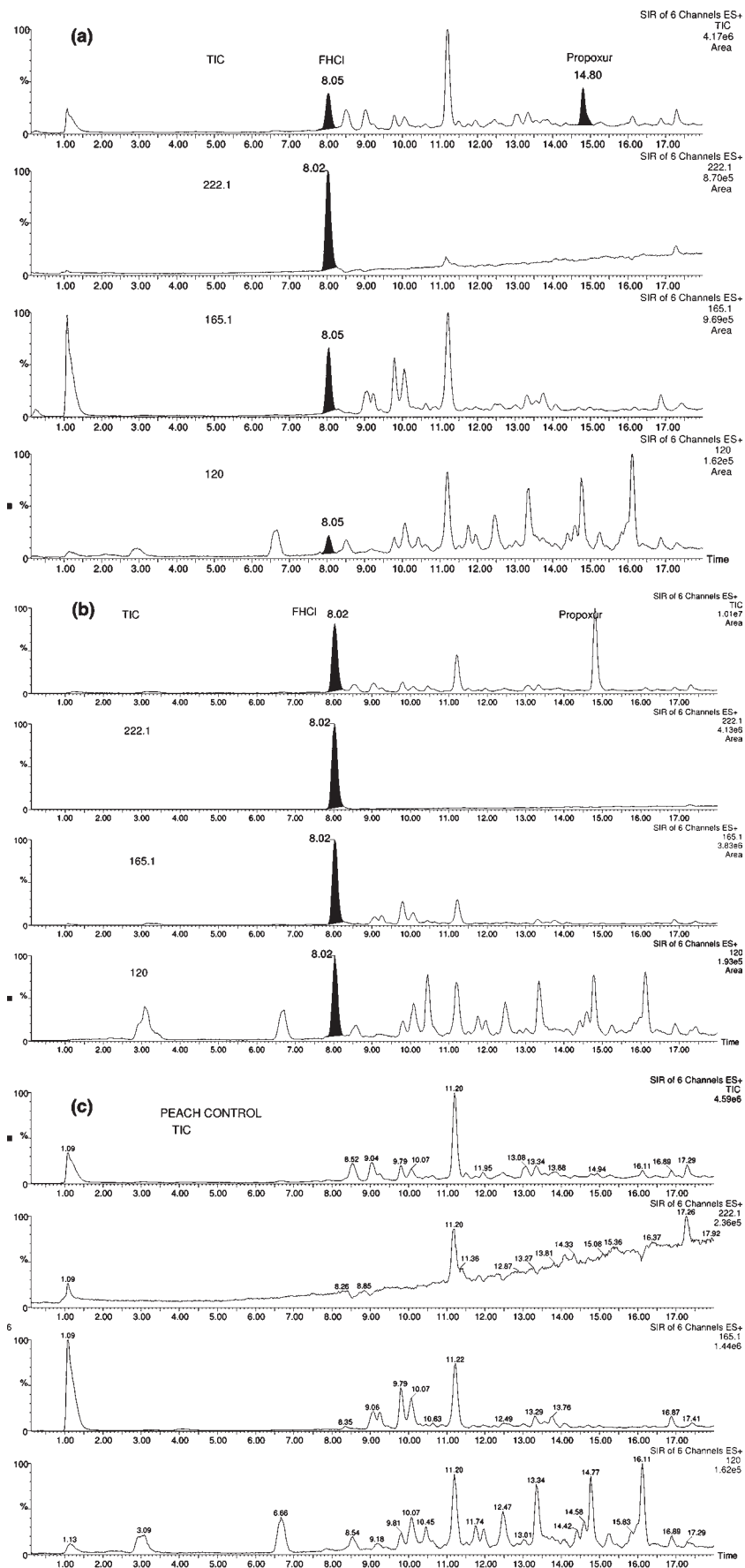


Figure 2. (a) LC-MS total and extracted ion chromatograms of a peach matrix standard at 20 ng/mL FHCl (equivalent to LOD of 3.33 ng/g) and propoxur. (b) LC-MS total and extracted ion chromatograms of a peach control sample spiked at $2 \times \text{LOQ}$ or 20 ng/g. (c) LC-MS total and extracted ion chromatograms of a peach control sample.

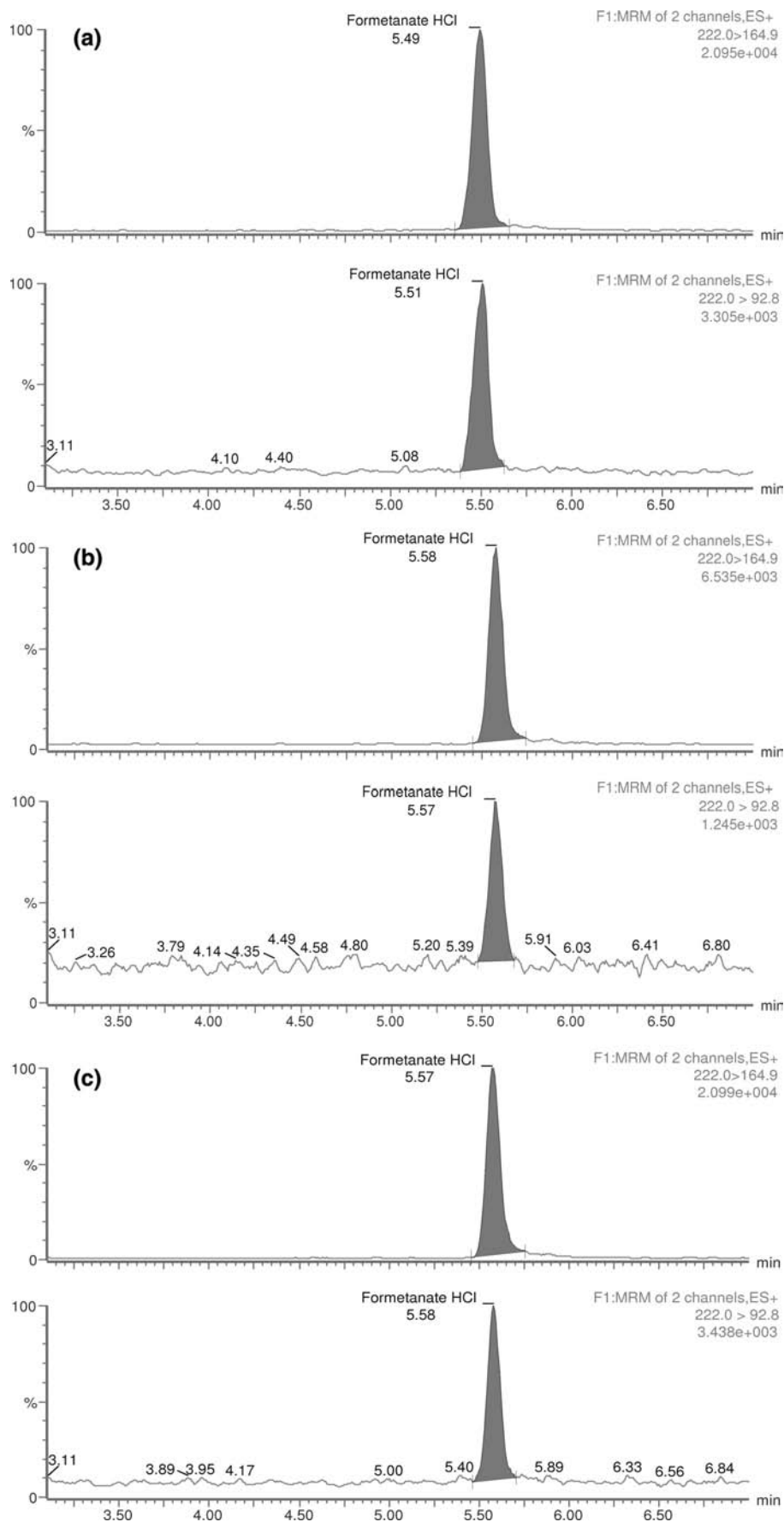


Figure 3. (a) LC-MS/MS ion transitions monitored for FHCI of a pear sample with 0.340 ng/g incurred FHCI. (b) LC-MS/MS ion transitions monitored for FHCI of a pear control sample fortified at the LOD of 0.1 ng/g. (c) LC-MS/MS ion transitions monitored for FHCI of an orange control sample fortified at the LOQ of 0.3 ng/g.

Table 1. Average Recovery Percentages and Relative Standard Deviations of FHCi Fortified at Different Levels on Fruit Commodities Using LC-MS/MS

	spike level (ng/g)	apples		pears		oranges		peaches		nectarines	
		av	%RSD	av	%RSD	av	%RSD	av	%RSD	av	%RSD
LOQ	0.3	96 (3) ^a	8.9	106 (3)	2.4	109 (3)	1.1	95 (4)	3.0	106 (2)	0.7
2 × LOQ	0.6	89 (7)	8.7	103 (7)	3.4	106 (7)	8.8	81 (8)	6.0	103 (7)	1.9
5 × LOQ	1.5	89 (3)	5.1	94 (3)	2.8	99 (3)	7.1	94 (3)	2.0	na ^b	na
10 × LOQ	3	90 (3)	1.3	93 (3)	3.5	95 (3)	5.3	79 (3)	9.0	na	na

^a Values within parentheses are number of replicates. ^b na, not analyzed.

Table 2. Average Recovery Percentages and Relative Standard Deviations of FHCi Fortified at Different Levels on Fruit Commodities Using LC-MS

	spike level (ng/g)	peaches		nectarines
		av	%RSD	av
LOQ	10	92 (3) ^a	12	na ^b
2 × LOQ	20	93 (7)	6.3	90 (2)
5 × LOQ	50	100 (3)	14	na
10 × LOQ	100	121 (3)	2.9	na

^a Values within parentheses are number of replicates. ^b na, not analyzed.

reagent and matrix blanks, each sample was fortified with propoxur at 30 ng/g or 5 × LOQ (propoxur LOQ = 6 ng/g).

The accuracy of the method (recovery percentages) and precision (relative standard deviation between replicates (%RSD)) of FHCi in all tested commodities using LC-MS/MS, demonstrating both intraday and interday precision, are listed in **Table 1**. Recoveries were consistent across the five matrices with averages in the range of 79–109% and relative standard deviations of <9.0%. **Table 2** shows the average recoveries and relative standard deviations for peach and nectarine fortified samples with averages in the range of 92–121 and relative standard deviations of <14.0% using LC-MS.

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