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Fast Extraction and Dilution Flow Injection Mass Spectrometry Method for Quantitative Chemical Residue Screening in Food

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Supporting Information

ABSTRACT: A prototype multiresidue method based on fast extraction and dilution of samples followed by flow injection mass spectrometric analysis is proposed here for high-throughput chemical screening in complex matrices. The method was tested for sulfonylurea herbicides (triflusulfuron methyl, azimsulfuron, chlorimuron ethyl, sulfometuron methyl, chlorsulfuron, and flupyrsulfuron methyl), carbamate insecticides (oxamyl and methomyl), pyrimidine carboxylic acid herbicides (aminocyclopyrachlor and aminocyclopyrachlor methyl), and anthranilic diamide insecticides (chlorantraniliprole and cyantraniliprole). Lemon and pecan were used as representative high-water and low-water content matrices, respectively, and a sample extraction procedure was designed for each commodity type. Matrix-matched external standards were used for calibration, yielding linear responses with correlation coefficients (r) consistently >0.99. The limits of detection (LOD) were estimated to be between 0.01 and 0.03 mg/kg for all analytes, allowing execution of recovery tests with samples fortified at ≥0.05 mg/kg. Average analyte recoveries obtained during method validation for lemon and pecan ranged from 75 to 118% with standard deviations between 3 and 21%. Representative food processed fractions were also tested, that is, soybean oil and corn meal, yielding individual analyte average recoveries ranging from 62 to 114% with standard deviations between 4 and 18%. An intralaboratory blind test was also performed; the method excelled with 0 false positives and 0 false negatives in 240 residue measurements (20 samples × 12 analytes). The daily throughput of the fast extraction and dilution (FED) procedure is estimated at 72 samples/chemist, whereas the flow injection mass spectrometry (FI-MS) throughput could be as high as 4.3 sample injections/min, making very efficient use of mass spectrometers with negligible instrumental analysis time compared to the sample homogenization, preparation, and data processing steps.

KEYWORDS: tandem mass spectrometry, flow injection analysis, chemical residue analysis, pesticide residue analysis, multiresidue methods, FED-FI-MS

INTRODUCTION

Chemical science research and products have significantly improved the quality of life. The responsible use of chemicals such as those found in pharmaceuticals, veterinary drugs, explosives, pesticides, and household and personal care products is necessary for environmental sustainability, public safety, and health. Routine chemical residue analysis in diverse matrices/media is one of many important tasks that ensure chemical products are used legally and responsibly. Chemical residue analysis also supports research and development efforts allowing the design of novel industrial products. Agriculture is just one exemplary field where chemicals are needed to sustain modern practices and fulfill societal needs (e.g., food supply). There are hundreds of pesticide active ingredients used by industry to manufacture thousands of crop protection product offerings for global agricultural markets. The number of agrochemicals on today's market and the composition of environmental and crop samples make it difficult to perform quantitative determinations. Multiresidue methods have been implemented over the past decades to streamline the quantitation of pesticide residues in food commodities. The capability of multiresidue methods has evolved from low-throughput procedures for gas chromatography that allowed quantitation of a few dozen chemicals[1,2] to ultralarge residue methods[3] that can measure hundreds of analytes more quickly and at lower levels.

Mass spectrometry (MS) is one of the most powerful analytical technologies employed by industry, universities, government agencies, and private laboratories, and quantitative residue analysis is an example application.[3−5] Advances in MS technology have significantly improved instruments. Mass spectrometers have become orders of magnitude more sensitive over the years, allowing the simplification of sample preparation methods because the need for extract preconcentration has significantly diminished. High-performance liquid chromatography (HPLC) is a preferred sample introduction technique for mass spectrometry, and HPLC systems have also improved. An example is the introduction of ultrahigh-pressure liquid chromatography (UHPLC),[6,7] which has provided improved resolution while lowering run times.[8−10] However, chromatography continues to be the sample throughput limiting step in methods for instrumental analysis to quantify chemical residues. On the other hand, the increased sensitivity, selectivity, and ruggedness of mass spectrometers have led to the development of ambient sampling/ionization techniques, such as desorption

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electrospray ionization (DESI),[11] direct analysis in real time (DART),[12] desorption atmospheric pressure chemical ionization (DAPCI),[13] and the atmospheric solid analysis probe (ASAP),[14,15] which allow direct MS analysis for chemical residue measurements.[16,17] Moreover, a recent study[18] demonstrated that it is also possible to conduct rugged chemical residue analysis by direct flow injection mass spectrometry (FI-MS) if samples are purified by solid-phase extraction (SPE) prior to instrumental analysis. FI-MS is mostly known for its application in qualitative high-throughput analysis in drug discovery;[19−21] the quantitative residue analysis capability reported in the recent proof-of-concept study[18] will likely expand the uses of this technique. Overall, direct mass spectrometric techniques, that is, flow injection[18] and ambient sampling/ionization,[16,17] can increase sample throughput with chemical residue instrumental analysis performed in seconds instead of minutes per sample. However, they do so by sacrificing selectivity due to the absence of chromatographic separation.[18] Consequently, direct MS methods should be rigorously tested to ensure interferences do not occur. The use of a confirmatory method to complement the high-throughput direct MS quantitative analysis may still be appropriate based on the application and/or intended use of the data. For example, measurement of multiple ion transitions, accurate masses, and/or highly specific gas-phase ion/molecule reactions[22,23] may represent sufficient qualitative confirmation of structural identity in academic or industrial research, particularly if the absence of interferences can be demonstrated by comparison to control samples of the exact source at each time point or variable investigated (e.g., control crop plot at the same farm in residue decline studies). On the other hand, a rigorous confirmatory test employing chromatographic separation could be mandatory when the reported result has legal implications and/or control samples of the source are not available.[24]

The most recent and notable advances in quantitative multiresidue methods have resulted from combining improvements in instrumental analytical technology together with novel sample preparation approaches. Examples include the original[25] and improved versions[26] of the QuEChERS method,[25,26] and the multiresidue extraction method recently proposed by Mol and co-workers;[27,28] both methods are the result of careful design of simplified sample preparation procedures for use with modern mass spectrometers. In this study, fast extraction and dilution flow injection mass spectrometry (FED-FI-MS)[29,30] is proposed as a methodology for high-throughput quantitative chemical residue screening. It optimizes steps from modern multiresidue analysis, such as QuEChERS[25,26] and generic extraction methods,[27,28] and utilizes throughput-improving equipment such as high-speed extractors[31] to expand the capability of FI-MS.[18] The study tested the hypothesis that dilution of extracts may allow direct flow injection MS multiresidue analysis, with the goal of reducing the time needed from sample extraction through instrumental analysis, thus increasing the throughput at several steps of the analytical process. The FED-FI-MS methodology has been designed for compatibility between sample preparation and direct MS analysis to allow satisfactory quantitative measurements and instrument performance.

MATERIALS AND METHODS

Reagents and Analytical Standard Materials. All reagents and solvents used were obtained from commercial sources, except the analytical standards. Acetonitrile, methanol, and water (HPLC grade) and ammonium hydroxide (28−30%) were obtained from EMD Chemicals (Gibbstown, NJ). Analytical standards of the active ingredients tested in this study (triflusulfuron methyl, azimsulfuron, chlorimuron ethyl, sulfometuron methyl, chlorsulfuron, flupyrsulfuron methyl, oxamyl, methomyl, aminocyclopyrachlor, aminocyclopyrachlor methyl, cyantraniliprole, and chlorantraniliprole) were synthesized by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Co. These compounds were selected because they are pesticide active ingredients and represent two well-established (sulfonylurea herbicides and carbamate insecticides) and two novel (pyrimidine carboxylic acid herbicides[32,33] and anthranilic diamide insecticides[34−37]) chemical classes. They also cover a wide range of physical and chemical properties such as solubility, volatility, polarity, and solution stability. Analyte structures are provided as Supporting Information (see Figure S1).

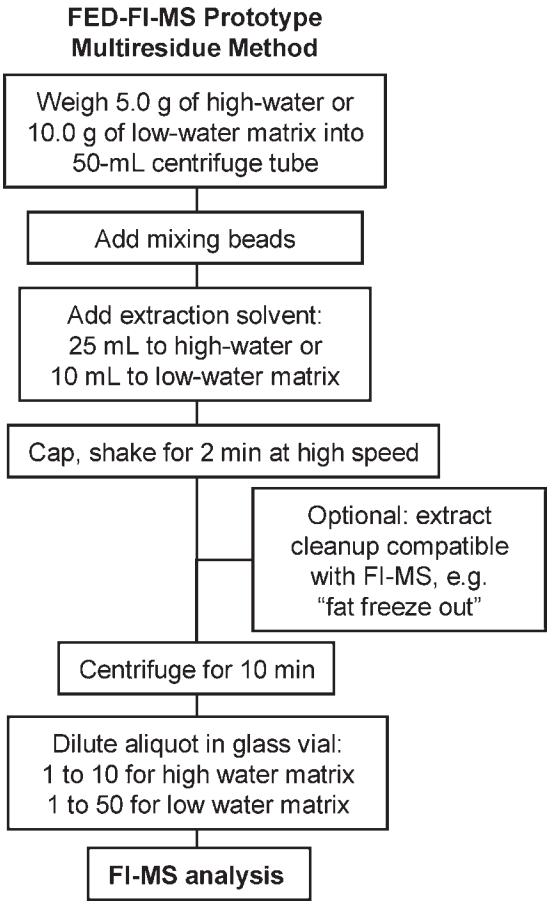

Figure 1. Schematic representation of the prototype fast extraction and dilution flow injection mass spectrometry (FED-FI-MS) multiresidue method developed for high-throughput chemical residue analysis.

Fortification and Calibration Solutions. Analytical standard stock solutions were individually prepared at a concentration of 200 μg/mL in acetonitrile for all analytes except aminocyclopyrachlor, which was dissolved in methanol because this compound is known to degrade in acetonitrile.[31] A 10 μg/mL multianalyte standard was prepared by combining aliquots of each individual stock standard solution into a common volumetric flask and diluting with methanol. A 1.0 μg/mL mixed standard was also prepared by dilution of the 10 μg/mL multianalyte standard using methanol as the solvent. The mixed standards were used for sample fortification during method development and validation. Matrix-matched external standards were used for analyte calibration in all cases. For each food type, a 1:10 dilution of the 10 μg/mL multianalyte standard was performed with matrix solvent to prepare

Table 1. Tandem Mass Spectrometry Instrument Parameters Employed for Quantitation of the Representative Compounds Used To Test the FED-FI-MS Method[a]

analyte	precursor ion type	Q1 isolated precursor ion (m/z)	Q3 scanned fragment ion (m/z)	DP (V)	CE (V)
triflusulfuron methyl	$(M + H)^+$	493	264	75	40
		493	268	75	40
azimsulfuron	$(M + H)^+$	425	182	75	40
		425	156	75	40
chlorimuron ethyl	$(M + H)^+$	415	186	75	40
		415	121	75	40
sulfometuron methyl	$(M + H)^+$	365	150	100	25
		365	199	100	25
chlorsulfuron	$(M + H)^+$	358	141	75	40
		358	167	75	40
flupyrsulfuron methyl	$(M + H)^+$	466	182	75	40
		466	156	75	40
aminocyclopyrachlor	$(M + H)^+$	214	68	110	34
		214	101	100	40
aminocyclopyrachlor methyl	$(M + H)^+$	228	68	90	40
		228	41	90	40
methomyl	$(M + H)^+$	163	88	50	10
		163	106	50	10
oxamyl	$(M + NH_4)^+$	237	72	85	30
		237	90	75	19
cyantraniliprole	$(M + H)^+$	475	286	110	34
		475	444	100	30
chlorantraniliprole	$(M + H)^+$	484	286	110	30
		484	453	110	26

[a] Abbreviations: Q1, quadrupole 1; Q3, quadrupole 3; DP, declustering potential; CE, collision energy.

a 1000 ng/mL intermediate standard. This solution was further diluted serially using matrix solvent to prepare calibration standards at six levels ranging from 0.70 to 100 ng/mL (see Table S1 in Supporting Information for the dilution chart followed for preparation of matrix-matched calibration standards). Adequate results were demonstrated with external calibration; hence, internal standards were not evaluated.

Control Samples. Lemon, pecan, corn meal, and soybean oil untreated control samples were available from previous DuPont studies. Solid samples were homogenized using a Hobart processor with copious amount of dry ice to obtain a representative sample. After homogenization, the dry ice was allowed to evaporate. All samples were maintained frozen at a target temperature of −20 °C. The samples were allowed to thaw prior to each use and returned to freezer storage immediately after.

Food Sample Extraction. A schematic summary of the method appears in Figure 1. Untreated pecan and lemon were used as representative low and high water content samples for method development and testing. High-water content samples were prepared by weighing 5.0 g of prehomogenized matrix into a 50 mL propylene centrifuge tube. Three steel balls were added to improve agitation and pulverization during extraction. A volume of 25 mL of extraction solvent (methanol) was added, and the samples were capped and extracted for 2 min using a 2000 Geno/Grinder high-speed extractor (SPEX CertiPrep, Inc., Metuchen, NJ) followed by centrifugation. A 10-fold dilution of the extracts (100 μL aliquot + 900 μL diluent) was performed in an autosampler vial prior to analysis. The diluent solvent used was 98.5% methanol/1.5% concentrated ammonium hydroxide (aq) v/v. The procedure for the low water content matrix was very similar, except that a 10 g sample size and a 10 mL extraction solvent volume were used, and extracts were diluted 50-fold (20 μL aliquot + 980 μL diluent) prior to analysis. The optional "fat freeze out" step (Figure 1) was performed for pecan by storing the extracts for 1 h in a freezer at approximately −20 °C to precipitate fats. Larger aliquots of control extracts were diluted

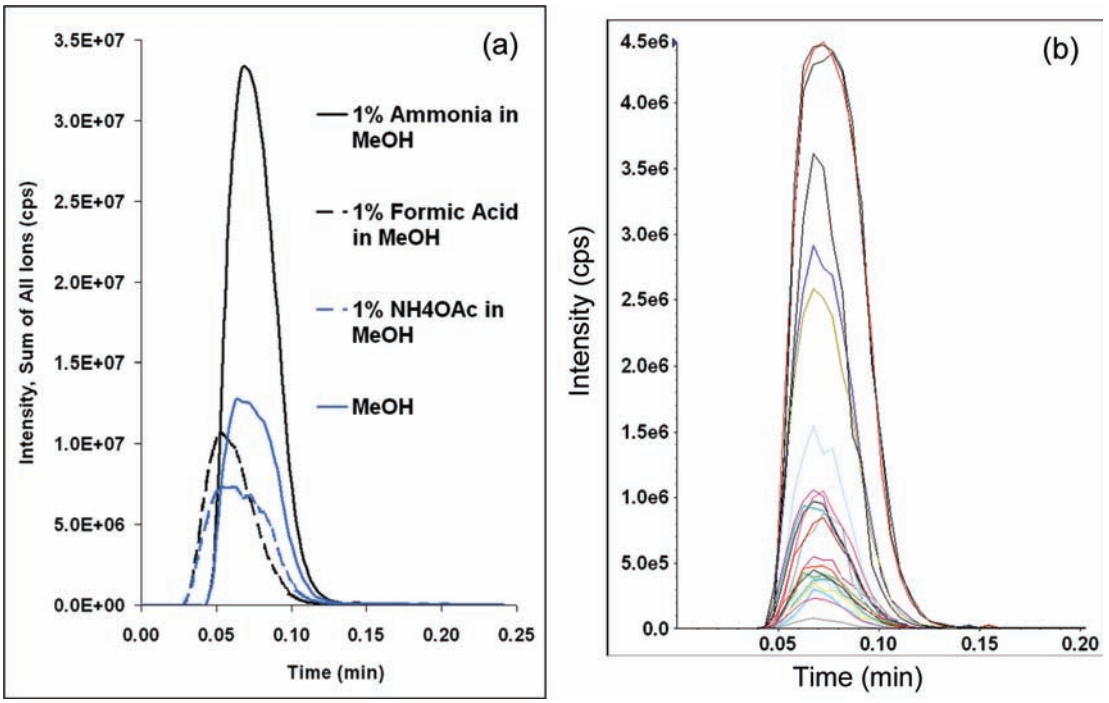

Figure 2. Ion chronograms[38] obtained during optimization of analyte ionization efficiency for diluent solvent selection. (a) Total intensities (sum of all MS/MS ion transitions) are displayed for 1.0 μL injections of 100 ng/mL mixed standards prepared in pure methanol and in methanol with formic acid 99:1 v/v, ammonium acetate 99:1 v/w, and concentrated ammonia (aq) 99:1 v/v. All 24 individual ion transitions (2 per analyte) are displayed in panel b for the 100 ng/mL mixed standard prepared in methanol/concentrated ammonia (aq) 99:1 v/v, which yielded the best instrument response.

with 98.5% methanol/1.5% concentrated ammonium hydroxide (aq) v/v, 10- and 50-fold for high and low water content matrices, respectively, to make 10 mL of diluted control matrix solvent used to prepare matrix-matched standards ranging from 0.7 to 100 ng/mL (see Table S1 in the Supporting Information for calibration standard preparation).

Instrumental Conditions. The instrumental analysis procedure was based on the FI-MS method recently published.[18] The method was optimized and expanded to include all analytes studied herein. Briefly, an Agilent 1100 series HPLC (Agilent Technologies, Wilmington, DE) coupled to an Applied Biosystems API-5000 triple-quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA) equipped with an electrospray ionization source was used for instrumental analysis of pecan and lemon samples. The HPLC system coupled to the mass spectrometer was upgraded during the study to an Agilent 1290 series, which was used for analysis of corn meal, soybean oil, and all solutions tested during the evaluation of matrix effects. Both systems were simply used for sample introduction in flow injection mode. A 1 m long PEEK capillary, $^{1}/_{16}$ in. outer diameter and 0.13 mm inner diameter (Part 0890-1915, Agilent Technologies), was used to connect the flow from the autosampler to the ion source. A 1 μL sample injection volume was used for samples and standards, whereas 10 μL injections of solvent blanks were performed between analytical sets for injector needle rinsing. Methanol was used as the carrier solvent at a flow rate of 400 μL/min. The flow was introduced directly and continuously into the ion source (no solvent flow splitting or divert valve before the ion source). Pictures of the instrumental setup appear in Figure S2 (Supporting Information). The Applied Biosystems API-5000 was operated in MS/MS positive ion mode with MRM detector output for quantitative analysis. The entire system and data acquisition were controlled by Analyst 1.4.1 software. Data output for all samples was processed using a smoothing factor = 5 and a bunching factor = 3 with the noise filter option available in the instrument software. A summary of optimized mass spectrometric conditions is provided in Table 1. Additional parameters were set as follows: resolution Q1 = unit, resolution Q3 = unit, ESI source voltage = 5.25 kV, dwell time = 7 ms for each ion transition, CUR = 28 psi, GS1 = 30 psi, GS2 = 80 psi, ion source temperature = 550 °C, and CAD pressure = 5.5 psi. For all experiments with the exception of the "method throughput potential" test, the Analyst 1.4.1 acquisition method consisted of a 15 s MRM data collection for all ion transitions immediately after injection. Following the acquisition, the system was allowed to flush for approximately 20 s prior to completion of the run. The method throughput potential test was performed by repeatedly injecting a soybean oil matrix-matched standard as quickly as allowed by the autosampler.

RESULTS AND DISCUSSION

Ionization Efficiency Optimization. The FI-MS acquisition method previously developed[18] was expanded to cover the 12 analytes tested in this study. In addition, the ionization efficiency was re-evaluated and improved by testing four types of sample diluent solvents and measuring the total ion current obtained from the MS/MS ion transitions of interest. Analyte-mixed standards were prepared at 100 ng/mL in pure methanol and in methanol with formic acid 99:1 v/v, ammonium acetate 99:1 v/w, and concentrated ammonia (aq) 99:1 v/v. Ion chronograms[38] recorded during this ionization efficiency optimization experiment are shown in Figure 2. The best instrument response was obtained when the compounds were dissolved in the methanol/ammonium hydroxide solvent mixture. Figure 2b displays the response obtained for the 24 ion transitions individually when the 100 ng/mL mixed standard prepared in that solvent was injected. Note that combinations of methanol, formic acid, and ammonium acetate were tested previously for

six compounds, but the effect of ammonium hydroxide was not evaluated in that study.[18] The results displayed in Figure 2a confirmed that for FI-MS analysis the use of pure methanol as sample solvent can yield better ionization efficiency for the tested compounds when compared to methanol with formic acid or ammonium acetate additives.[18] Moreover, the evaluation of methanol/ammonium hydroxide solvent mixture revealed a 3-fold improvement in ionization efficiency (see Figure 2a). The enhanced ionization efficiency could be the result of analyte/ammonium ion adducts formed during the electrospray process allowing a proton transfer reaction to yield MH^+ ions. A generic equation is shown below, where M = neutral analyte. In this reaction, a proton-bound dimer is formed, which subsequently dissociates, and the molecule with the greatest gas-phase proton affinity preferentially retains the charge. This gas-phase reaction is well-known and has been reported to occur during ionization of noncovalently bound clusters[39] and neutral molecules,[40,41] leading to signal enhancement of relatively basic species.[39−41]

$$M + NH_4^+ \rightarrow M\cdots H^+\cdots NH_3 \rightarrow MH^+ + NH_3$$

The ionization improvement was particularly important to allow analysis of extracts by simple dilution (instead of SPE cleanup[18]) because an increase in ionization suppression is expected for injection of samples that are not carefully purified. Consequently, a methanol/ammonium hydroxide combination was used as sample diluent in this study for FED-FI-MS analysis. Flow injection mass spectrometry does not suffer from the solvent limitations that affect HPLC methods. For example, high-pH solvents may shorten the lifetime of certain chromatographic columns, and achieving elution of all analytes in near 100% organic solvent to enhance ionization can be impractical in HPLC. The use of methanol as carrier solvent and methanol/ammonium hydroxide mixture as extract diluent significantly improves the ionization efficiency of analytes tested by FI-MS, counterbalancing the matrix suppression expected due to the lack of chromatographic separation. The experiment presented above provides a systematic approach to select the most appropriate diluent solvent when developing FED-FI-MS applications, especially for compounds not evaluated in this study. High-pH solvents are known to reduce analyte stability.[42] However, analyte stability is less critical when FI-MS is used because of the fast analysis.

Design of the Fast Extraction and Dilution (FED) Sample Preparation Method. Important steps in the widely known QuEChERS method[25,26] include the addition of salts, specifically $MgSO_4$ and NaCl, for extract dehydration and partition of acetonitrile/aqueous layers,[43] and a dispersive SPE purification. The liquid−liquid partition provides a purification step, and the extract dehydration makes the sample compatible with GC analysis.[43] These steps have been critical in the success of QuEChERS as a sample preparation method for both GC-MS and HPLC-MS analysis. However, the presence of significant levels of nonvolatile salts in extracts may not be compatible with flow injection MS for routine chemical residue analysis, because their ions are known to induce adduct formation and reduce the signal intensity of protonated molecular ions. Frequent injection of samples containing these salts at trace levels, without the possibility of separation/diversion prior to introduction into the mass spectrometer (as in HPLC-MS methods), may require more frequent ion source maintenance. The presence of water is not necessarily a problem in instrumental analysis performed by FI-MS. Atmospheric pressure ionization (API) sources, such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), function well with aqueous/organic solvent mixtures. Consequently, $MgSO_4$ and NaCl were excluded when the FED sample extraction/preparation procedure was designed. The use of a highly sensitive mass spectrometer allowed dilution of extracts as the main step to reduce the presence of matrix in the samples to be directly injected into the ion source.

Initial tests were performed for pecan and lemon following the FED method described in Figure 1, but both matrices were prepared according to the low-water sample preparation procedure (the "freeze out" step was not performed for lemon). Two 10 g control samples of each matrix were weighed into individual 50 mL centrifuge tubes; one was kept as control, and the other was fortified at 0.10 mg/kg to evaluate method performance. Analyte recoveries for this initial test were acceptable for pecan (average for all analytes = 91%, range = 82−103%); hence, the procedure for that matrix was kept unchanged in subsequent validation trials. On the other hand, lower recoveries were obtained for all analytes in lemon, with an average of 54% and a range of 43−79%. Visual inspection of the sample tubes revealed that the extract volume obtained for lemon was significantly higher than the 10 mL of extraction solvent added, and the increased volume was attributed to the release of water from the matrix. Three potential solutions were considered to develop a FED method satisfactory for high water content crops: (a) physical measurement of the final extract volume after sample extraction to use in residue calculation, (b) establishment of an experimental volume correction factor based on water content of each matrix, and (c) reduction of sample size while increasing extraction solvent volume to minimize the effect of matrix water release. Although points a and b may be appropriate for most applications, the latter approach (c) was selected to streamline the procedure (i.e., absence of final volume measurement) and design a single method with potential application to many high water content crops without the need to determine individual volume correction factors for each matrix. A second method test was performed for lemon using a sample size of 5.0 g, an extraction solvent volume of 25.0 mL, and a 10-fold dilution to compensate for the larger solvent-to-sample ratio. Acceptable recoveries, that is, 70−120% according to U.S. EPA guidelines,[44] were obtained for all analytes. Figure S3 (Supporting Information) shows an example picture of the extracted and diluted samples obtained after the FED method is followed exactly as described under Materials and Methods.

Method Selectivity Test and Validation Trials. The selectivity of the method was assessed prior to execution of validation trials. This was performed on the basis of a previously proposed selectivity testing protocol for FI-MS.[18] The selectivity test involved careful examination of ion chronograms obtained for each of the 24 MS/MS ion transitions (two per analyte) in lemon and pecan control samples. Baseline noise response was observed in all cases, confirming that matrix interferences were not present in the crops tested. High-level (100 ng/mL) standards of each analyte prepared in methanol/ammonium hydroxide solvent were also injected individually, and all 24 MS/MS transitions were monitored. In all cases, only the two transitions corresponding to the injected compound yielded a response, ruling out interferences between the analytes of interest, especially within each class (e.g., possible in-source fragmentation, MS/MS transition crosstalk, etc.). The inherent selectivity of FI-MS/MS is

Table 2. Analyte Recoveries Obtained during Validation of the FED-FI-MS Method for Lemon and Pecan Matrices (n = 8 Fortified Samples per Matrix) (Adapted by Permission of The Royal Society of Chemistry from Reference 30)

analyte	lemon % recovery (av ± σ)[a]	pecan % recovery (av ± σ)[a]
triflusulfuron methyl	92 ± 5	106 ± 4
azimsulfuron	97 ± 4	80 ± 5
chlorimuron ethyl	95 ± 4	98 ± 5
sulfometuron methyl	95 ± 5	101 ± 5
chlorsulfuron	85 ± 13	89 ± 11
flupyrsulfuron methyl	92 ± 5	98 ± 3
aminocyclopyrachlor	90 ± 7	90 ± 5
aminocyclopyrachlor methyl	91 ± 7	107 ± 5
methomyl	75 ± 14	107 ± 6
oxamyl	94 ± 21	108 ± 7
cyantraniliprole	96 ± 7	118 ± 18
chlorantraniliprole	93 ± 6	106 ± 9

[a] Abbreviations: av, average; σ, standard deviation.

lower than that of HPLC-MS/MS (i.e., lack of chromatographic separation). Consequently, selectivity testing is critical and should be performed to ensure reliable analyte identification, especially when the method is expanded to additional analytes and matrices. Further confirmatory tests may be appropriate depending on the intended application.

Validation sets consisting of two control samples, four samples fortified at the 0.05 mg/kg limit of quantitation (LOQ), and four samples fortified at 0.50 mg/kg (10 × LOQ) were analyzed for lemon and pecan. Validation data appear in Table 2. Average analyte recoveries obtained during method validation ranged from 75 to 118% with standard deviations between 3 and 21%, and the overall average recoveries (8 samples × 12 compounds = 96 measurements) were 91% for lemon and 101% for pecan. Note that average percent recoveries and precision results meet current guidelines for multiresidue screening methods. The lower (yet acceptable) analyte recoveries obtained with the lemon matrix can be attributed to the high water content of the matrix. The extract volume is defined as the extraction solvent added, that is, 25 mL for lemon, and used to calculate chemical residues. As previously discussed, the larger solvent-to-sample ratio used in the high-water version of the FED method minimizes the intrinsic error introduced by assuming a total extract volume of 25 mL during chemical residue calculation. However, matrix water is still released during extraction, increasing the total volume and slightly diluting the extracts, resulting in an overall average recovery approximately 10% lower than that obtained for pecan. Example ion chronograms of a solvent blank, control and fortified samples, and matrix-matched standards obtained for chlorantraniliprole in pecan matrix appear in Figure 3. The matrix-matched standard chronograms displayed (i.e., 1.0 ng/mL in Figure 3d and 10 ng/mL in Figure 3f) correspond to the expected concentration of samples fortified at 0.05 and 0.50 mg/kg, respectively (Figure 3c,e), and are shown together for comparison. Matrix-matched standard calibration yielded satisfactory linear responses for all analytes during method validation for both matrices, with correlation coefficient, r, consistently >0.99. The calibration curves obtained are provided as Supporting Information (Figures S4 and S5). The limits of detection (LOD) were estimated to be between 0.01 and 0.03 mg/kg for the analytes tested (see Limit of Detection for details).

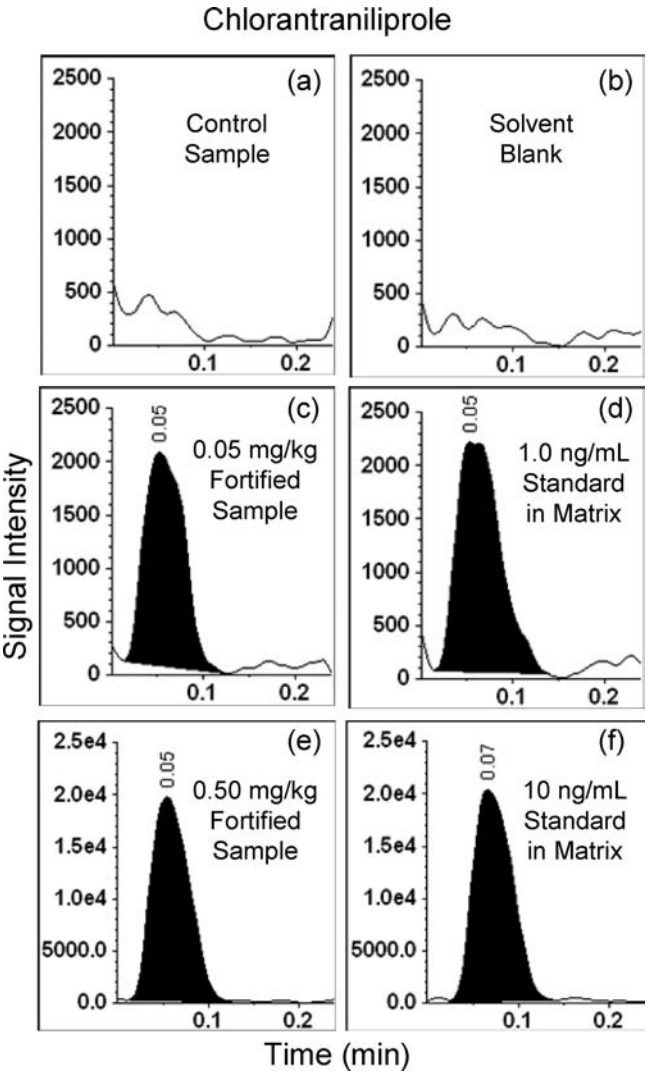

Figure 3. Chlorantraniliprole FED-FI-MS ion chronograms[38] obtained as part of the pecan validation set: (a) control; (b) solvent blank; (c) control sample fortified at 0.05 mg/kg; (d) 1.0 ng/mL standard in matrix, (e) control sample fortified at 0.50 mg/kg; (f) 10 ng/mL standard in matrix.

Intralaboratory Blind/Proficiency Test.[30]. An intralaboratory blind/proficiency test was conducted to further assess the method capability when measuring multiple analyte residues in produce samples. Ten pecan samples and 10 lemon samples were spiked by chemist A with analytes at various levels. The test was designed such that each sample contained between three and six analytes at levels within a 0.02−4.0 mg/kg range. Samples were then extracted and analyzed by chemist B following the FED-FI-MS method. Chemist B processed the data and reported results before the annotations from chemist A were revealed. A summarized report of all 240 residue measurements made (20 samples × 12 analytes) during the blind test is included as Supporting Information (see Table S2). Neither false positives nor false negatives occurred. Average recoveries were calculated for the spiked compounds overall. These were 91% for pecan (range = 68−127%, σ = 13%, n = 49) and 84% for lemon (range = 58−100%, σ = 9%, n = 53). These results positively highlight the capability of FED-FI-MS for quantitative multiresidue screening. However, the blind test was performed as an intralaboratory study,

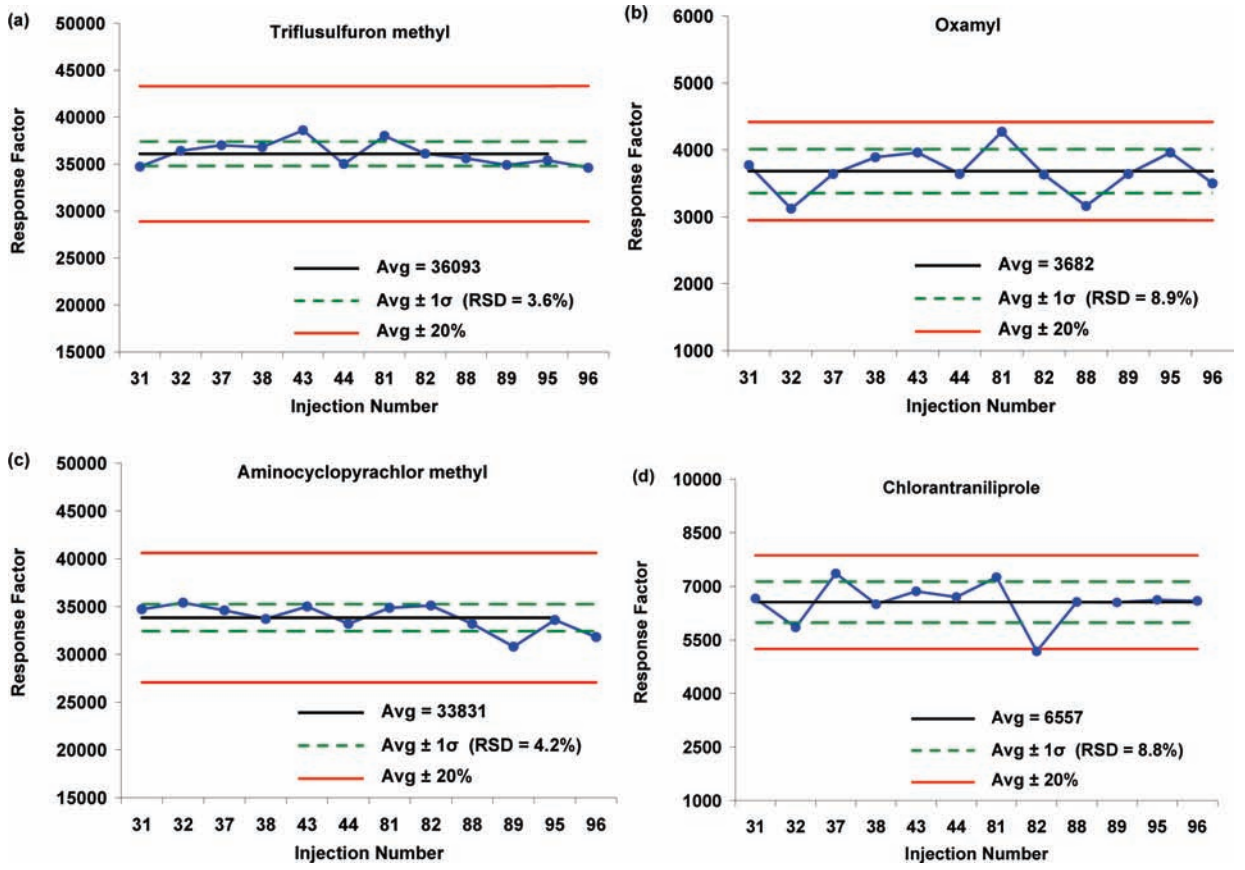

Figure 4. Representative analyte response factors (peak area/concentration) plotted for matrix-matched standards injected throughout the analysis of pecan validation and blind/proficiency sets: (a) sulfonylurea herbicide triflusulfuron methyl; (b) carbamate insecticide oxamyl; (c) pyrimidine carboxylic acid herbicide aminocyclopyrachlor methyl; (d) anthranilic diamide insecticide chlorantraniliprole. The graphs allow an assessment of consistency in instrument response over a span of approximately 1.5 h of uninterrupted use in FI-MS mode.

which measures variability resulting only from the personnel/operators because the same mass spectrometer, laboratory equipment, reagent suppliers, and analytical reference materials were used. The rate of false-positive and false-negative results is expected to be >0% for a methodology that is less selective than current HPLC-MS/MS methods.

Method Ruggedness − Consistency in Instrument Response. A total of 100 injections of solvent blanks, control/fortified samples, and matrix-matched standards were performed during the formal method evaluation, that is, 25 injections for each of the following analytical sets (in chronological order): lemon validation, pecan validation, lemon blind/proficiency, and pecan blind/proficiency. Instrumental analysis was performed without interruption. Therefore, the data allow the instrument response to be examined for consistency throughout the analysis of the sample sets. Figure 4 shows analyte response factors (peak area/concentration) plotted for matrix-matched standards injected throughout the analysis of pecan validation and blind sets for four representative analytes (one per chemical class). In addition, the average response factors (ARF) obtained for the validation set were compared to the blind set (100 × (blind set ARF − validation set ARF)/overall average ARF), allowing the consistency in instrument response to be evaluated. Overall, the set-to-set average response factor difference in pecan was −1.6%, with a range of −6.6 to 5.2% for the individual analytes. The differences are within the margin of error expected for electrospray ionization, although a decreasing trend is apparent in Figure 4c (aminocyclopyrachlor methyl). The decrease in response could be the result of analyte degradation in solution over time, because the diluent solvent is ammonium hydroxide in methanol, and the general trend for pesticide active ingredients is to be less stable in basic solvents.[42] This hypothesis is supported by the observation that the response factor did not vary equally for all compounds. Nevertheless, the fast analysis provided by FI-MS allows reliable measurements to be made.

The consistency obtained in instrument response for FED-FI-MS is significantly better than that obtained in the FI-MS proof-of-concept study, where samples were purified by SPE prior to analysis, targeting a lower LOD of approximately 0.003 mg/kg.[18] Therefore, the FED procedure seems to be adequate for the preparation of samples for FI-MS analysis, even more so than the previous SPE sample preparation method.[18] Higher throughput and better instrument performance are achieved at the expense of sensitivity, that is, LODs around 0.01−0.03 mg/kg instead of 0.003 mg/kg.

Processed Food Matrices. An experiment was conducted to test the applicability of FED-FI-MS for processed food analysis. Soybean oil and corn meal samples that originated from untreated crops were available at the time of the study. These commodities were ideal for the test because they cover oily/liquid and solid/starchy processed fractions. Seven 10 g samples were measured for each matrix; one was kept as a control for

Table 3. Average Analyte Recoveries Obtained during the Analysis of Corn Meal and Soybean Oil Samples Fortified at 0.05, 0.10, and 0.50 mg/kg (n = 6 Fortified Samples per Matrix)

analyte	corn meal % recovery (av ± σ)[a]	soybean oil % recovery (av ± σ)[a]
triflusulfuron methyl	101 ± 4	105 ± 8
azimsulfuron	83 ± 8	111 ± 7
chlorimuron ethyl	93 ± 7	103 ± 11
sulfometuron methyl	105 ± 6	100 ± 9
chlorsulfuron	76 ± 13	93 ± 18
flupyrsulfuron methyl	88 ± 5	109 ± 7
aminocyclopyrachlor	62 ± 4	105 ± 5
aminocyclopyrachlor methyl	104 ± 9	69 ± 13
methomyl	98 ± 12	72 ± 13
oxamyl	114 ± 16	80 ± 11
cyantraniliprole	108 ± 10	104 ± 13
chlorantraniliprole	103 ± 6	93 ± 5

[a] Abbreviations: av, average; σ, standard deviation.

selectivity testing (i.e., to rule out matrix interferences) and to prepare matrix-matched calibration standards, whereas the remaining 10 g samples were fortified at 0.05, 0.10, and 0.50 mg/kg (two at each level) to assess analyte recoveries. The soybean oil and corn meal samples were extracted and analyzed according to the FED-FI-MS method for low water content matrices. The "fat freeze out" step was not performed. Matrix interferences were not observed in control samples. Matrix-matched calibration yielded correlation coefficients (r) > 0.99 for all analytes. Recovery results appear in Table 3; a total of 22 of 24 average recoveries fell within the 70−120% acceptable range,[44] suggesting the FED-FI-MS method can be applied to the processed food commodities tested.

Method Performance for the Most Polar Analyte.[30] There are many pesticide active ingredients that are difficult to analyze, thus requiring single-analyte methods.[42] Highly polar compounds often fall into this category.[42] Aminocyclopyrachlor[33] is an amino acid (see structures in Figure S1, Supporting Information) that can exhibit ionic behavior depending on the pH of the system. The performance of FED-FI-MS obtained in this study for aminocyclopyrachlor highlights the potential of the methodology for analysis of highly polar compounds. FED-FI-MS benefits from a simple instrumental analysis procedure, whereas HPLC-MS methods can be affected by peak broadening and retention time shifts of polar analytes. In addition, the method performance obtained for aminocyclopyrachlor can be attributed to a simple extraction process and absence of purification steps such as aqueous/organic liquid/liquid partition, where polar compounds are often lost because they tend to prefer the aqueous layer.

Limit of Detection. LODs were defined as the analyte residue level in the food sample expected to yield a (peak-to-peak) signal-to-noise ratio = 3. Analysis of control and fortified samples allowed LODs to be estimated for each analyte by comparing the background response of controls (i.e., baseline noise) to peak signals obtained for samples spiked at 0.05 mg/kg. The FED-FI-MS prototype method LODs for the studied compounds in all four matrices tested (lemon, pecan, soybean oil, and corn meal) were estimated to be between 0.01 and 0.03 mg/kg. These LODs correspond to the FED-FI-MS method using the dilution factors and signal smoothing/noise filter specified under Materials and Methods. Smaller dilution factors were tested in attempts to achieve lower detection limits during method development, but matrix suppression increased significantly, making the method impractical.

Matrix Effects and Dilution Factors. An experiment was designed to evaluate the matrix effects as a function of the dilution factor. Matrix-matched standards at the 10 ng/mL concentration level were prepared as indicated under Materials and Methods, except that the dilution factor was varied, covering the ranges of 10−2000 for lemon and 50−10000 for pecan, corn meal, and soybean oil. A 10 ng/mL neat standard prepared in the 98.5% methanol/1.5% concentrated ammonium hydroxide (aq) v/v solvent was also made and used as reference. The analytical sets were arranged by matrix type, with the neat standard injected first followed by matrix-matched standards in order of increasing matrix content. A total of six solvent blank injections were performed between the analytical sets to minimize matrix carry-over effects. The analyte peak area obtained for each matrix-matched standard was then compared to analyte peak area of the neat standard by calculating relative responses, that is, (matrix-matched standard/neat standard) × 100.

The results (Table 4) follow the expected trend of weakening matrix effects as the dilution factor increases for the sulfonylurea herbicides, pyrimidine carboxylic acid herbicides, and anthranilic diamide insecticides in lemon, pecan, and corn meal. Most of the relative responses obtained for these compounds were between 80 and 120% for the 1000 and 2000 dilution factors applied to lemon extracts and for the 1000, 5000, and 10000 dilution factors applied to pecan and corn meal extracts. On the other hand, the carbamate insecticides experienced matrix enhancement. The relative responses obtained for methomyl were >100% in almost all cases (21 of 24), with significant matrix enhancement (i.e., relative response > 120%) in 15 of 24 scenarios tested. Oxamyl, the other carbamate insecticide tested, also experienced matrix enhancement, but to a lesser extent than methomyl. Relative responses were generally higher for soybean oil (see Table 4) compared to lemon, pecan, and corn meal. For example, 9 of 12 analytes experienced some matrix enhancement (relative response > 100%) in soybean oil for the dilution factor of 1000. A transition from matrix enhancement to matrix suppression was observed for various analytes in this matrix as the dilution factor was reduced from 1000 to 50. It seems that trace levels of certain compounds extracted from soybean oil improve ionization of the analytes, but as their concentration or that of other matrix components increases, the process/mechanism that leads to ion suppression dominates.

In general, matrix effects were not significant for most analytes when the larger dilution factors were applied to extracts. Pesticide maximum residue limits (MRLs) in food cover a wide range of concentrations depending on the chemical. The dilution factor used in FED-FI-MS can be adjusted to meet the application of interest. Higher LODs may be acceptable for certain applications. Therefore, larger dilution factors that lower matrix load and increase method performance should be considered and used as appropriate.

Method Throughput Potential. The throughput of the FED-FI-MS method was previously estimated at 72 samples/day for sample preparation by one chemist and 1300 injections/day for instrumental analysis by a single mass spectrometer.[29,30] An additional experiment was performed to test the instrumental analysis throughput potential of the technique by allowing the autosampler to operate as fast as mechanically allowed, while excluding the time previously allocated for flushing with carrier

Table 4. Matrix Effects as a Function of Dilution Factors in the FED-FI-MS Method

		relative response[a] (%)											
dilution factor[b]	matrix equivalent[c] (mg/mL)	triflusulfuron methyl	azimsulfuron	chlorimuron ethyl	sulfometuron methyl	chlorsulfuron	flupyrsulfuron methyl	aminocyclo-pyrachlor	aminocyclo-pyrachlor methyl	methomyl	oxamyl	cyantrani-liprole	chlorantrani-liprole
						Lemon Matrix							
2000	0.1	**87**	**92**	**89**	**88**	**80**	**91**	**93**	145	191	**119**	79	**85**
1000	0.2	**94**	**101**	**90**	**87**	**86**	**101**	**110**	156	305	163	**83**	**85**
200	1.0	71	**90**	78	61	70	72	**106**	151	290	**93**	49	54
100	2.0	73	**95**	**81**	69	**80**	77	**112**	167	344	**109**	44	52
20	10	37	56	45	36	45	39	61	**91**	186	65	22	23
10	20	20	34	24	20	29	21	35	53	**117**	33	10	14
						Pecan Matrix							
10000	0.1	**96**	**103**	**99**	**92**	**98**	**98**	**108**	**98**	128	135	**84**	**93**
5000	0.2	**103**	**107**	**107**	**96**	**101**	73	**97**	**105**	131	128	**85**	**98**
1000	1.0	**91**	**91**	**89**	**84**	**83**	55	**101**	**119**	**117**	123	58	**80**
500	2.0	76	**88**	72	71	77	48	**104**	**112**	156	**118**	45	63
100	10	43	59	48	43	47	52	**88**	75	**108**	**95**	18	25
50	20	40	55	40	40	46	38	**86**	69	**98**	**81**	12	20
						Corn Meal Matrix							
10000	0.1	**104**	**102**	**113**	**103**	**98**	78	**110**	**88**	**85**	**82**	**96**	**101**
5000	0.2	**97**	**95**	**111**	**97**	**95**	67	**103**	76	**87**	68	**89**	**95**
1000	1.0	**80**	**81**	**90**	78	**83**	60	**91**	**86**	**112**	62	72	**82**
500	2.0	73	78	79	70	71	53	**86**	**87**	148	65	63	66
100	10	44	59	52	43	56	51	**91**	75	150	50	33	35
50	20	36	49	44	36	44	37	70	73	161	41	27	28
						Soybean Oil Matrix							
10000	0.1	**106**	**96**	**101**	**107**	**95**	**82**	**85**	**96**	**101**	**97**	**89**	**96**
5000	0.2	**104**	**101**	**108**	**97**	**95**	**89**	**80**	**102**	**108**	**91**	**84**	**98**
1000	1.0	134	**118**	124	122	**103**	63	**87**	132	148	122	**86**	**111**
500	2.0	**117**	**97**	**107**	**113**	**92**	61	77	141	148	124	70	**88**
100	10	**110**	**82**	**86**	126	73	**85**	60	**95**	122	155	48	59
50	20	**91**	69	67	**120**	58	**82**	52	**99**	132	149	37	46

[a] Results expressed relative to the response obtained for a neat standard; bold text = 80−120% (matrix effect not significant); results <80% show significant matrix suppression; results >120% show significant matrix enhancement. [b] Dilution factor applied to the original extract prior to analysis. [c] Matrix equivalent is a measurement of how much matrix is contained in the injected sample, here defined as ME = (SW/EV) ÷ DF, where ME = matrix equivalent (mg/mL), SW = sample weight (mg), EV = extract volume (mL), and DF = dilution factor prior to injection.

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solvent between injections. A 100 ng/mL standard prepared in soybean oil matrix was selected to test the worst-case scenario in terms of potential for analyte carry-over, that is, highest standard concentration. The sample was injected repeatedly over a 6 min interval (ion chronogram provided as Supporting Information, Figure S6). The average time between injections calculated as the distance between peak maxima was 0.23 min (13.8 s), which corresponds to an instrumental analysis throughput of 4.33 injections/min. However, the software did not allow injections to be performed at high speed from multiple vials. Commands for execution of this experiment were made as part of the Analyst 1.4.1 acquisition method, and not the acquisition batch, bypassing the software limitation and thus allowing very fast injections to at least be performed for a single autosampler vial position. Note that carry-over was not significant in the test because a well-equilibrated baseline was obtained between injections for all ion transitions (see Figure S6 in the Supporting Information). The experiment suggests that more than 6000 injections/day may be possible during instrumental analysis following the proposed method. A different software application may be required to analyze entire sample sets with the fastest autosampler speed, and long-term method performance needs to be evaluated.

Limitations and Potential for Method Improvement.[45] The requirements that should be met for satisfactory performance of FED-FI-MS were identified throughout the study, together with potential method improvements.[45] For example, a limitation of FED-FI-MS is that samples of interest and calibration standards must be prepared with the same matrix composition to compensate for matrix effects. Control samples of the source tested were available during this study, allowing matrix-matched calibration standards to be prepared, but that is not always the case when chemical residue analysis is conducted. The use of internal standards[17] and other calibration approaches has been demonstrated to work well when matrix effects are encountered; thus, alternative standard calibration techniques should be tested for FED-FI-MS.

The mass spectrometer is the most expensive equipment used in residue analytical methods. The most significant throughput increase achieved with FED-FI-MS occurs at the instrumental analysis, making very efficient use of mass spectrometers with negligible instrumental analysis time compared to other steps of the method. The sample homogenization, preparation, and data processing clearly represent the throughput-limiting steps in FED-FI-MS and should be targeted in future method improvement efforts. The rapid instrumental analysis achieved with flow injection mass spectrometry also highlights an intrinsic limitation of triple-quadrupole mass spectrometers when employed for chemical residue analysis, which is that detection occurs for the specified compounds (targeted analysis) and individual ion transitions must be scanned/recorded for each analyte. Consequently, the scan cycle time increases with the number of analytes of interest, reducing the data points recorded (signal intensity vs time) for each ion transition. A total of 24 ion transitions were monitored in this study for 12 compounds. It may be possible to double the number of analytes with shorter dwell times. However, a multiresidue method based on FI-MS/MS would require multiple sample injections if hundreds of analytes are to be included. Time-of-flight mass spectrometers (TOF-MS) have been employed for nontargeted chemical residue analysis.[46] It has already been demonstrated that TOF-MS instruments can be a solution to the limitation of MS/MS targeted analysis; thus, evaluation of the FED-FI-MS method with high-resolution mass spectrometers is needed. The selectivity is dependent on mass accuracy;[46] thus, modern high-resolution mass spectrometers may be required to achieve adequate selectivity with FI-MS, that is, to compensate for the lack of chromatographic separation/retention time measurement. Increased selectivity and sensitivity could be obtained by reduction of matrix; thus, additional extract purification steps compatible with the FED-FI-MS method (see Figure 1, "optional extract cleanup") need to be designed and tested.

In summary, the FED-FI-MS methodology is applicable to pesticide multiresidue screening in food, and the advantages and limitations have been presented. Although agrochemicals were the model compounds tested in the analysis of food, the methodology is likely useful for quantitative screening of other organic chemicals (e.g., veterinary drugs, mycotoxins,[27,28,47] explosives,[48,49] and pharmaceuticals) at levels ranging between parts per billion (ng/g) and parts per million (μg/g) in a variety of matrices (e.g., body fluids, tissues, environmental samples, and fabric). Extended evaluations of FED-FI-MS may allow its uses in forensics, biomedical, environmental, and many other areas of science.

■ ASSOCIATED CONTENT

Supporting Information. Additional figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ DEDICATION

Dedicated to Prof. R. Graham Cooks on the occasion of his 70th birthday.

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(45) The authors recognize the need for research in the areas mentioned, and an objective of the detailed discussion of "Limitations and Potential for Method Improvement" presented in this paper is to encourage work for expanding the capabilities of FED-FI-MS.

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