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Determination of Trifloxystrobin and Its Metabolites in Hawaii Soils by ASE−LC-MS/MS

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Analytical methods for the determination of trifloxystrobin and four of its metabolites were developed in a leaching study conducted in Hawaii. To duplicate plots at each of five locations representing various agricultural areas in Hawaii, trifloxystrobin was applied at label rates and allowed to leach under normal rain and irrigation conditions. Soil samples were collected at weekly to monthly intervals and the residual concentrations of trifloxystrobin and metabolites measured. A quantitative analytical method for their determination in various soil samples was developed using accelerated solvent extraction (ASE), coupled with liquid chromatography−tandem mass spectrometry. Extraction solvent with various ratios of methanol to water, addition of $EDTANa_2$ to the extract solvent, and ASE cell temperature were adjusted to improve recovery. Deuterated (*E*,*E*)-trifloxystrobin was chosen as the internal standard of the analytical method. The limit of quantitation was 2.5 ppb in the soil for trifloxystrobin and its metabolites. Laboratory aerobic degradation studies with the soils from the five sites were also conducted to measure the same compounds.

KEYWORDS: Trifloxystrobin; trifloxystrobin acid; LC-MS/MS; ASE

INTRODUCTION

Trifloxystrobin is a mesostemic fungicide that interferes with the respiration of plant-pathogenic fungi. It is mostly used as a broad-spectrum foliar fungicide against many fungal pathogens within the Ascomycete, Deuteromycete, Basidiomycete, and Oomycete classes. In particular, it is used to treat powdery mildew, downy mildew, and anthracnose, which mostly attack fruit and vegetable crops (*1*). The compound has been recently introduced in Hawaii for fungus control in greenhouse and nursery crops and for field crops.

Trifloxystrobin (an ester) and its hydrolyzed acid metabolite (**Figure 1**) have four geometrical isomers based on the connected group arrangement of the two N═C double bonds. Their acronyms, CAS Registry Numbers, and chemical names are presented in **Table 1**. (*E*,*E*)-Trifloxystrobin is the most biologically active fungicide among the four isomers. Trifloxystrobin degrades rapidly in water and soil by mechanisms including metabolism, photolysis, and hydrolysis. Banerjee et al. (*2, 3*) showed that soil hydrolysis was the dominant pathway of degradation of trifloxystrobin and that the major metabolite was the *E*,*E* acid. Isomerization was of minor importance due to the absence of light. The same studies demonstrated that

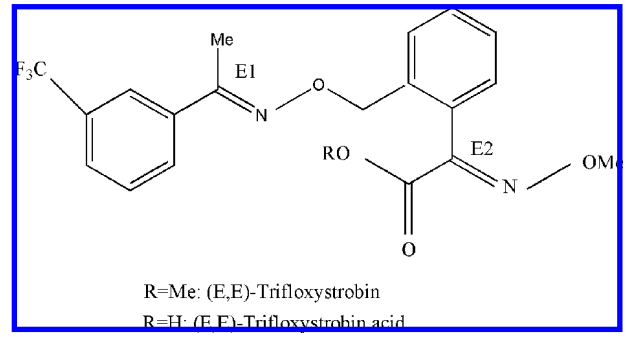

Figure 1. Chemical structures of (*E*,*E*)-trifloxystrobin and (*E*,*E*)-trifloxystrobin acid.

adsorption of both the parent compound and its metabolite acids follow linear and Freundlich isotherms; the extent of adsorption depends upon the binding sites available in organic matter and clay (*2, 3*).

Reported analytical methods for trifloxystrobin are primarily those for residue analysis from viniculture or other fruit and vegetable crops (*4–8*). Most literature reports the use of a variety of organic solvents for extraction; however, the literature reports limited use of supercritical fluid extraction (*9*). Solid-phase extraction (SPE) with various types of cartridges (*6, 10, 11*), matrix solid-phase dispersion (*5, 12*), gel permeation chromatography (*13*), and liquid–liquid extraction (*4, 8*) have all been used to purify crude extracts. Following purification, either liquid chromatography (LC) coupled with mass spectrometry (MS) (*11*) or with diode array detection (*4, 8*) or gas chromatography

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Table 1. Chemical Names, Acronyms, CAS Registry Numbers, and Codes of Trifloxystrobin and Its Metabolites

chemical name	acronym	CAS Registry No.	code
(*E*,*E*)-trifloxystrobin	EETF	141517-21-7	CGA279202
(*Z*,*Z*)-trifloxystrobin	ZZTF		CGA357262
(*Z*,*E*)-trifloxystrobin	ZETF	141493-03-0	CGA357261
(*E*,*Z*)-trifloxystrobin	EZTF		CGA331409
(*E*,*E*)-trifloxystrobin acid	EETFA	252913-85-2	CGA321113
(*Z*,*Z*)-trifloxystrobin acid	ZZTFA		
(*Z*,*E*)-trifloxystrobin acid	ZETFA		CGA373466
(*E*,*Z*)-trifloxystrobin acid	EZTFA		

using a nitrogen−phosphorus detector (*6, 8, 9*), electron capture detector (*6, 8*), and MS detector (*5, 8, 14*) has been used for analysis. The former owner and developer of the product, Novartis Crop Protection, established two methods to analyze trifloxystrobin and its metabolites from soil (*15, 16*). The most frequently used method involves extraction with 90% methanol (by shaking), followed by filtration and evaporation. The sample residue is further cleaned by passing it through a series of SPE cartridges (mixed-phase cation exchange disk cartridge, aminopropyl extraction disk cartridge, and a silica gel cartridge) before it is analyzed by LC-UV (*15*). The analytical method was subsequently improved using reversed phase LC coupled with a single-quadrupole mass detector (or LC-MS). The selectivity of the mass spectrometer eliminates the need to use SPE for sample cleanup. After the solvent is changed from 90% methanol to 50% acetonitrile, the solution is directly injected into the LC-MS. The limit of quantitation (LOQ, defined as the lowest fortification that still gave adequate recovery) is 10 parts per billion (ppb) in soil (*16*).

Following the modified method of the developer (*15, 16*), poor recovery (20–30%) was obtained for the trifloxystrobin acids, which might be due to the different physicochemical properties of the tropical soils of Hawaii. Finally, we successfully implemented accelerated solvent extraction (ASE) for extraction and high-performance liquid chromatography (HPLC) with tandem mass spectrometry (LC-MS/MS) for the analyses of trifloxystrobin and its metabolites. To our knowledge, this is the first time ASE has been employed to extract trifloxystrobin and its metabolites from soil.

MATERIALS AND METHODS

Reagents and Materials. Seven analytical standards obtained from Bayer CropScience (Kansas City, MO) included ZZTFA (95% purity), EETFA (99.1% purity), ZETFA (95% purity), ZETF (97.6% purity), EETF (98.9% purity), ZZTF (99.4% purity), and d3-EETF (1.34%, liquid solution). The parent trifloxystrobin (EETF) and four metabolites (ZZTFA, EETFA, ZETF, and ZZTF) were used in all of the analyses. Later, ZETFA was added to compare its behavior during LC-MS analysis. All analytes were dissolved in methanol as stock solution (1 mg mL^{-1}) and stored in a refrigerator at 4 °C, from which the low-level spike solution and the solutions for the calibration curve were prepared by appropriate dilution with 1:1 methanol/water. The spike solution for the high-level recovery test (300 μg mL^{-1}) was prepared by directly dissolving 3 mg of each five target compounds in 10 mL of methanol. The internal standard solution was prepared by diluting the stock solution of d3-EETF to 0.5 μg mL^{-1} with 1:1 methanol/water. To prepare the standard solutions for calibration curve (0.5–10 ng mL^{-1}), various volumes of the 1 μg/mL mixture (of five target compounds) standard solution were added into a 50 mL volumetric flask, followed by the addition of 25 mL of extraction solvent (50% methanol and 50% water, contained 0.5 g L^{-1} $EDTANa_2$), the volume of which was similar to the ASE extract volume. Subsequently, 1 mL of internal standard (0.5 μg mL^{-1}) was added to each flask. The final solution volumes were adjusted to 50 mL with 1:1 methanol/water.

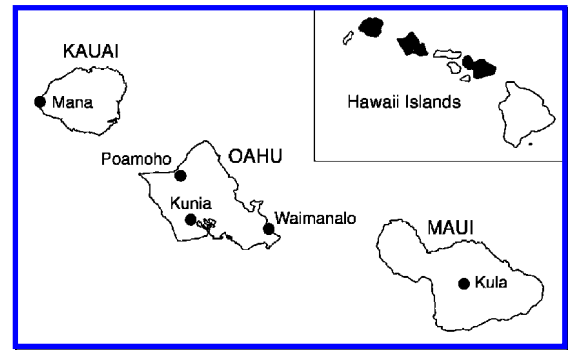

Figure 2. Experimental sites for measuring pesticide leaching evaluation in the soils of Hawaii.

HPLC-grade methanol was purchased from Burdick & Jackson (Muskegon, MI). Certified ACS-grade ethylenediaminetetraacetic acid disodium salt-2-hydrate ($EDTANa_2$) and ammonium formate were purchased from Fisher Scientific (Houston, TX). Deionized water was processed through a Millipore ultra pure system (Milli-Q, Millipore, Milford, MA). Diatomaceous earth as well as purified, washed, and ignited sand was purchased from J. T. Baker (Phillipsburg, NJ).

Sites and Soils. Poamoho, Waimanalo, and Kunia on the island of Oahu; Kula on the island of Maui; and Mana on the island of Kauai were selected for study because they offer differing soil, climatic, and topographic characteristics representative of agriculturally important soils in Hawaii. The locations of these five sites are presented in **Figure 2**. The soils chosen for this study differ in moisture and temperature regimens, mineralogy, and physical and chemical properties. The test plots are located on well-drained sites, flat or gentle slopes, or level terraces where erosion would be minimal.

The physicochemical properties of the soils from these five locations are shown in **Table 2**.

Accelerated Solvent Extraction. ASE 200 (Dionex, Sunnyvale, CA) was used for soil sample extraction. Preweighed samples (10 g) were stored in a freezer. Prior to analysis, the samples were brought to room temperature. Soil samples with various moisture contents were mixed with diatomaceous earth and then poured into 22-cm extraction cells. The extraction cell of ASE was filled to the top with sand. The extraction solution was a 50:50 mixture of methanol/water; the water contained 0.5 g L^{-1} of $EDTANa_2$. The oven temperature was set at 40 °C and cell pressure at 10.24 MPa. Solvent was introduced and heated to the set temperature in 5 min, followed by another 5 min of static time (solvent soaking time) of extraction. An amount of solvent equivalent to 60% of the cell volume was flushed through the cell. After each extraction, the system was purged with nitrogen for 60 s at 1.035 MPa. The extraction cycle per cell is 1. The extracts were transferred from the collection vials to 50-mL volumetric flasks. The collection vials were washed two times with 1 mL of methanol, and then the wash solutions were added to the volumetric flasks. One milliliter of internal standard (0.5 μg mL^{-1}) was added to each flask. The final solution volumes were adjusted to 50 mL with 1:1 methanol/water. Twenty microliters of each of these solutions was injected into LC-MS/MS for analysis.

Liquid Chromatography and Tandem Mass Spectrometry. A Thermo Finnigan Surveyor HPLC system (Waltham, MA) equipped with a Phenomenex (Torrance, CA) Gemini MS C18 column (2.0 mm × 150 mm, 5 μm) was employed in the analysis of trifloxystrobin and its metabolites. The mobile phase was a 30:70 (v/v) mixture of 10 mM aqueous ammonium formate and methanol. The flow rate was 0.2 mL min^{-1}, and the total run time was 15 min. The injection volume was 20 μL. The calibration range was 0.5–10 ng mL^{-1} for all compounds.

A Thermo Finnigan LCQ ion trap mass spectrometer equipped with an electrospray ionization (ESI) source was used as the HPLC detector. A metal needle system was installed in the ESI source. The capillary temperature was 250 °C, and the respective flow rates of the sheath gas and auxiliary gas were 80 and 15 (arbitrary units). The spray voltage was 4.5 kV.

The two-stage full scan mode and the positive ion (PI) mode were used. The $[M + H]^+$ ions of the five compounds were selected for

Table 2. Physicochemical Properties of Soils From Five Locations in Hawaii

	Poamoho, Oahu (P)	Waimanalo, Oahu (W)	Kunia, Oahu (K)	Kula, Maui (M)	Mana, Kauai (L)
soil type	silty clay Oxisol	gravelly clay Vertisol	silty clay Oxisol	loam Andisols	stony silty clay loam Vertisol
class	Rhodic Eutrustox	Vertic Haplustolls	Typic Eutrotorrox	Typic Eurtandepts	Typic Haplusterts
elevation (m)	213	30	85	911	3
annual rainfall (mm)	1000	1500	600	750	500
production	pineapple	floriculture and crops (banana, papaya, macadamia nuts, corn)	historically, sugar cane; now, crops (vegetables, seed corn, etc.)	pastureland, vegetable and protea floriculture	corn, sunflower, Cucurbitaceae family fruits such as melon or pumpkin
slope (%)	1–3	1–3	1–3	1–3	1–3
bulk density (g/cm^3)	1.2–1.3	1.0–1.3	1.2–1.3	0.7–0.9	1.2–1.5
field capacity water content[a] (% volume)	40–50	45–55	30–45	35–45	35–47
organic carbon[b] (%)	0.7 (0.5)	0.9 (0.9)	0.8 (0.4)	5.5 (0.5)	1.0 (0.6)
cation exchange capacity (CEC)[c]	low	moderate	low	moderate-high	
pH[b] (standard deviation)	6.2 (0.1)	6.1 (0.2)	6.7 (0.1)	7.0 (0.2)	8.4 0.1
mineralogy	kaolin/sesquioxide	montmorrilin with kaolin	kaolin/sesquioxide	montmorrilin	kaolin/amorphous

[a] Extrapolated from retention curves at −330 cm suction. [b] Based on seven samples to a depth of 2.10 m for P, W, and K; four samples to a depth of 0.9 m for M; and six samples to a depth of 1.80 m for L. [c] The CEC values are based on values from earlier studies.

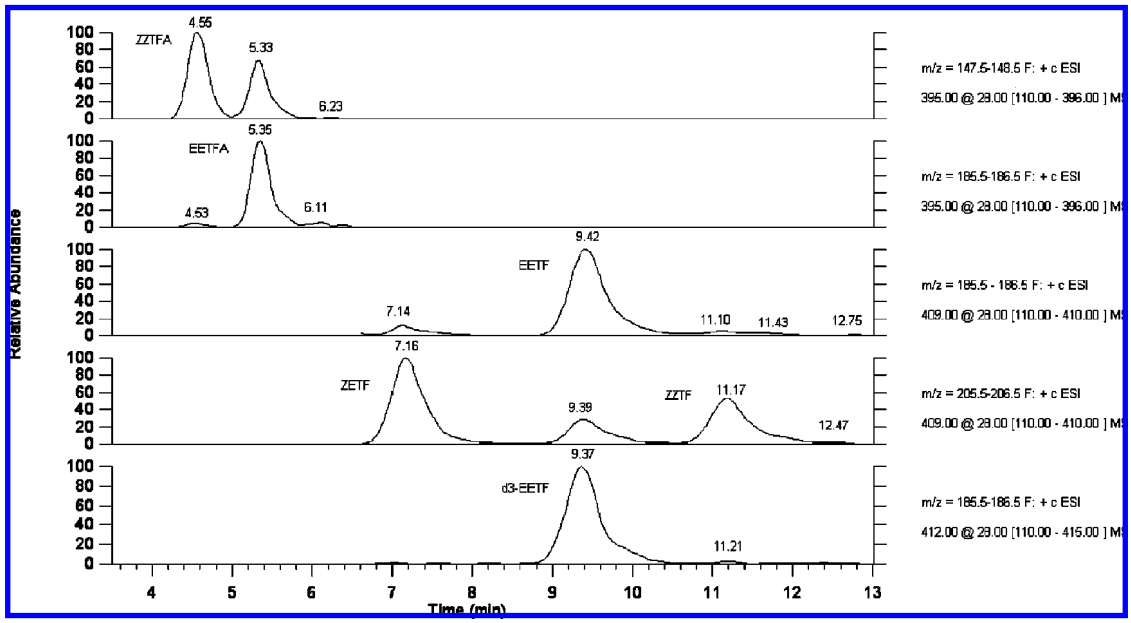

Figure 3. LC-ESI/MS chromatograms in positive ion mode (CID = 28%) of a combined solution (10 ng L^{-1}) of (*Z*,*Z*)-trifloxystrobin acid (ZZTFA) (scan range, *m*/*z* 110–396; transition, *m*/*z* 395–148), (*E*,*E*)-trifloxystrobin acid (EETFA) (scan range, *m*/*z* 110–396; transition, *m*/*z* 395–186), (*Z*,*E*)-trifloxystrobin (ZETF) (scan range, *m*/*z* 110–410; transition, *m*/*z* 409–206), (*E*,*E*)-trifloxystrobin (EETF) (scan range, *m*/*z* 110–410; transition, *m*/*z* 409–186), d3-(*E*,*E*)-trifloxystrobin (EETF) (scan range, *m*/*z* 110–415; transition, *m*/*z* 412–186), and (*Z*,*Z*)-trifloxystrobin (ZZTF) (scan range, *m*/*z* 110–410; transition, *m*/*z* 409–206).

monitoring. For all compounds, the capillary voltage was 20 V and the tube lens offset was −15 V. The normalized collision energy, activation Q, and activation time were tuned on the basis of the selected daughter ion current. For all analytes, the collision-induced dissociation (CID) was 28%, activation Q was 0.25, and activation time was 30 ms. Unlike the selected reaction monitoring (SRM), the two-stage full-scan mode is a two-step MS procedure monitoring the full product ions, which yield more information than that of SRM mode. However, in the software processing method, only the most prominent product ions of the studied compounds were chosen for quantitation in this study, which made it act as a normal SRM method in the quantitative analysis. **Figure 3** shows the chromatographs of the mixture of five compounds at concentrations of 10 μg L^{-1}.

The instrument's limit of detection (LOD, defined as S/N = 3) was 0.1 μg L^{-1} for all five compounds. All analyses were done in triplicate.

RESULTS AND DISCUSSION

Optimization of ASE Parameters. The composition and temperature of the ASE extract were tuned to maximize recovery from soil. A low-level spiking solution (1 μg mL^{-1} of the five compounds' mixture) was used for this series of analyses.

Extraction solvent with different ratios of methanol/water was tested to obtain the best recovery for trifloxystrobin (i.e., EETF) and two of its acid metabolites (i.e., EETFA and ZZTFA). The results in **Table 3** show that the higher the methanol/water ratio, the higher the recovery for EETF and the lower the recoveries for ZZTFA and EETFA. This result is consistent with the fact that the acid is more hydrophilic than the ester and thus more likely to be extracted by solutions with a higher water content. A mixture of 1:1 methanol/water was chosen as the extract solvent for all subsequent analyses to increase the recovery of the acids while not excessively reducing the recovery of the ester.

Using the 1:1 methanol/water mixture as the solvent, recovery of trifloxystrobin acid was improved for the W soil. However, it was still low for the K soil (**Table 4**). Because the K soil is rich in Fe^{3+} and Al^{3+}, it is assumed that a complex was formed

Table 3. Mean Recoveries and Relative Standard Deviations (RSD) of Trifloxystrobin (EETF) and Its Two Acid Metabolites, (*Z*,*Z*)-Trifloxystrobin Acid (ZZTFA) and (*E*,*E*)-Trifloxystrobin Acid (EETFA), at Spiked Level of 0.05 mg kg^{-1}, Obtained by ASE at 100 °C with Different Ratios of Methanol from Waimanalo (W) Soil[a]

% methanol		EETF	ZZTFA	EETFA
50	recovery (%)	69.7	79.1	87.6
	RSD	3.4	3.9	1.5
75	recovery (%)	78.9	53.8	60.7
	RSD	7.5	4.9	3.9
90	recovery (%)	90.6	39.5	47.1
	RSD	4.2	4.8	7.0

[a] Triplicate tests were done.

Table 4. Mean Recoveries and Relative Standard Deviations (RSD) of Trifloxystrobin (EETF) and Its Metabolites, ZZTFA, EETFA, ZETF, and ZZTF, at a Spiking Level of 0.05 mg kg^{-1}, Obtained by ASE at 40 °C without and with Addition of $EDTANa_2$ in the Extraction Solvent From Kunia (K) Soil[a]

extraction solvent		EETF	ZZTFA	EETFA	ZETF	ZZTF
1:1 MeOH/H_2O	recovery (%)	78.2	59.2	57.7	78.4	74.8
	RSD	5.6	7.0	10.5	2.6	4.0
1:1 MeOH/H_2O ($EDTANa_2$ 0.05%)	recovery (%)	74.8	78.9	91.8	80.5	75.2
	RSD	3.4	6.6	2.9	5.6	5.3

[a] Triplicate tests were done.

Table 5. Mean Recoveries and Relative Standard Deviations (RSD) of Trifloxystrobin and Its Metabolites, (*Z*,*Z*)-Trifloxystrobin Acid (ZZTFA), (*E*,*E*)-Trifloxystrobin Acid (EETFA), (*Z*,*E*)-Trifloxystrobin (ZETF), and (*Z*,*Z*)-Trifloxystrobin (ZZTF), at a Spiking Level of 0.05 mg kg^{-1}, Obtained by ASE with Different Oven Temperatures from Kula (M) and Mana (L) Soils Using 50% Methanol and 50% Water (with 0.5 g L^{-1} of $EDTANa_2$) as Extraction Solvent[a]

soil	ASE oven temp (°C)		EETF	ZZTFA	EETFA	ZETF	ZZTF
L	40	recovery (%)	72.3	62.5	69.5	73.5	68.6
		RSD	4.0	2.5	2.4	2.3	1.9
	70	recovery (%)	90.8	69.7	77.9	85.7	87.6
		RSD	2.0	2.2	7.9	1.0	4.2
	100	recovery (%)	95.7	70.4	83.9	89.2	93.9
		RSD	3.2	10.6	2.1	1.0	0.4
M	40	recovery (%)	90.8	77.3	68.5	90.0	82.5
		RSD	4.0	6.0	3.1	1.4	3.7
	70	recovery (%)	87.1	63.1	74.5	89.0	83.5
		RSD	5.4	14.0	6.2	1.1	2.0
	100	recovery (%)	97.8	67.0	77.2	96.0	97.4
		RSD	1.5	5.3	5.7	2.6	3.5

[a] Triplicate tests were done.

between the metal ion and the carboxylic and amine groups of the acid metabolites, which led to the strong adsorption of these compounds to the soil and low recovery during extraction. This hypothesis was further supported by the fact that recovery was increased by adding $EDTANa_2$ to the extraction solvent, a summary of which is presented in **Table 4**. Therefore, 1:1 methanol/water ($EDTANa_2$ 0.05%) was finally set as the extraction solvent for the analysis of all soil types.

Table 6. Base Peak Pattern of Trifloxystrobin Acid, Trifloxystrobin, and Their Isomers, (*Z*,*Z*)-Trifloxystrobin Acid (ZZTFA), (*Z*,*E*)-Trifloxystrobin Acid (ZZTFA), (*E*,*E*)-Trifloxystrobin Acid (EETFA), (*Z*,*E*)-Trifloxystrobin (ZETF), and (*Z*,*Z*)-Trifloxystrobin (ZZTF), after LC-ESI/MS

	m/*z* 148	*m*/*z* 186	*m*/*z* 206
trifloxystrobin acid	ZZTFA, ZETFA	EETFA	
trifloxystrobin		EETF	ZETF, ZZTF

The effect of ASE oven temperature on recovery is shown in **Table 5**. No significant difference in recovery under different temperatures was observed in the M soils. For the L soil, a higher temperature resulted in higher recovery; therefore, the ASE solvent should be heated to 100 °C for better recovery from this soil. The L soil is quite different from the other soils. It is quite heterogeneous and mixed with lacustrine clay and large pieces of coral. Possibly, it offers more binding sites. However, during the first several of batches of the sample analysis, instrument malfunction prevented heating to 100 °C and the tests were done under 40 °C. To keep the same extract condition, the whole extraction process for all soil samples in this project was conducted at 40 °C.

Chromatographic Separation. Under the HPLC conditions specified above, ZZTFA and ZETFA were not separable. Efforts to separate them failed, even after the proportion of H_2O in the mobile phase had been increased to elongate the retention time. The major fragments in the mass spectrum were also similar. Thus, these two isomers could not be differentiated by the technique employed in this study. Only ZZTFA was used to make a standard curve to quantitatively analyze the field samples. Thus, the calculated amount of ZZTFA should include ZETFA (which coelutes with ZZTFA). Because the MS responses of ZZTFA and ZETFA are similar, the results of ZZTFA are actually the sum of the amount of both ZZTFA and ZETFA, which were calculated on the basis of ZZTFA's calibration curve.

Mechanism of Fragmentation. The mass spectroscopic data of trifloxystrobin and its isomers have been discussed by Banerjee et al. (*17, 18*). **Figure 4** shows the mass spectra of trifloxystrobin, trifloxystrobin acid, and their isomers using the ESI source. **Table 6** summarizes the isomers' base peak pattern. Although the previous work stated that the double bond N═C connected to the meta-substituted benzene ring controls the type of base peak (*17, 18*), it is only consistent with the EI mass spectra. APCI/MS/MS spectra of acid metabolites showed the other pattern: the conformation on the other double bond −N═C connected to the ortho-substituted benzene ring played a more dominant role on the type of base peak, by which EZTFA and ZZTFA have *m*/*z* 148 as the base peak, whereas EETFA and ZETFA have 186 as the base peak (*18*). However, with our ESI source, the N═C connected to the meta-substituted aromatic system still controls the base peak type of both esters and acids. Both EETF and EETFA's base peak are *m*/*z* 186. ZETF and ZZTF have 206 as base peak; whereas their acids, ZETFA and ZZTFA, had less stability in *m*/*z* 192 (corresponding to their ester's *m*/*z* 206) and continued to lose CO_2 and *m*/*z* 148 (192 − CO_2) became their base peak. Ion *m*/*z* 148 could be further broken down to its daughter ion *m*/*z* 116 under MS/MS/MS fragmentation. From the above discussion, the fragmentation mechanism is illustrated in **Figure 5**.

Matrix Effect. It is a common perception that matrix-induced coelutes are likely to be responsible for the analytes' signal suppression (*19*). Blank (nonspiked) extract solutions of the five soils were used as a matrix to prepare a 10 μg

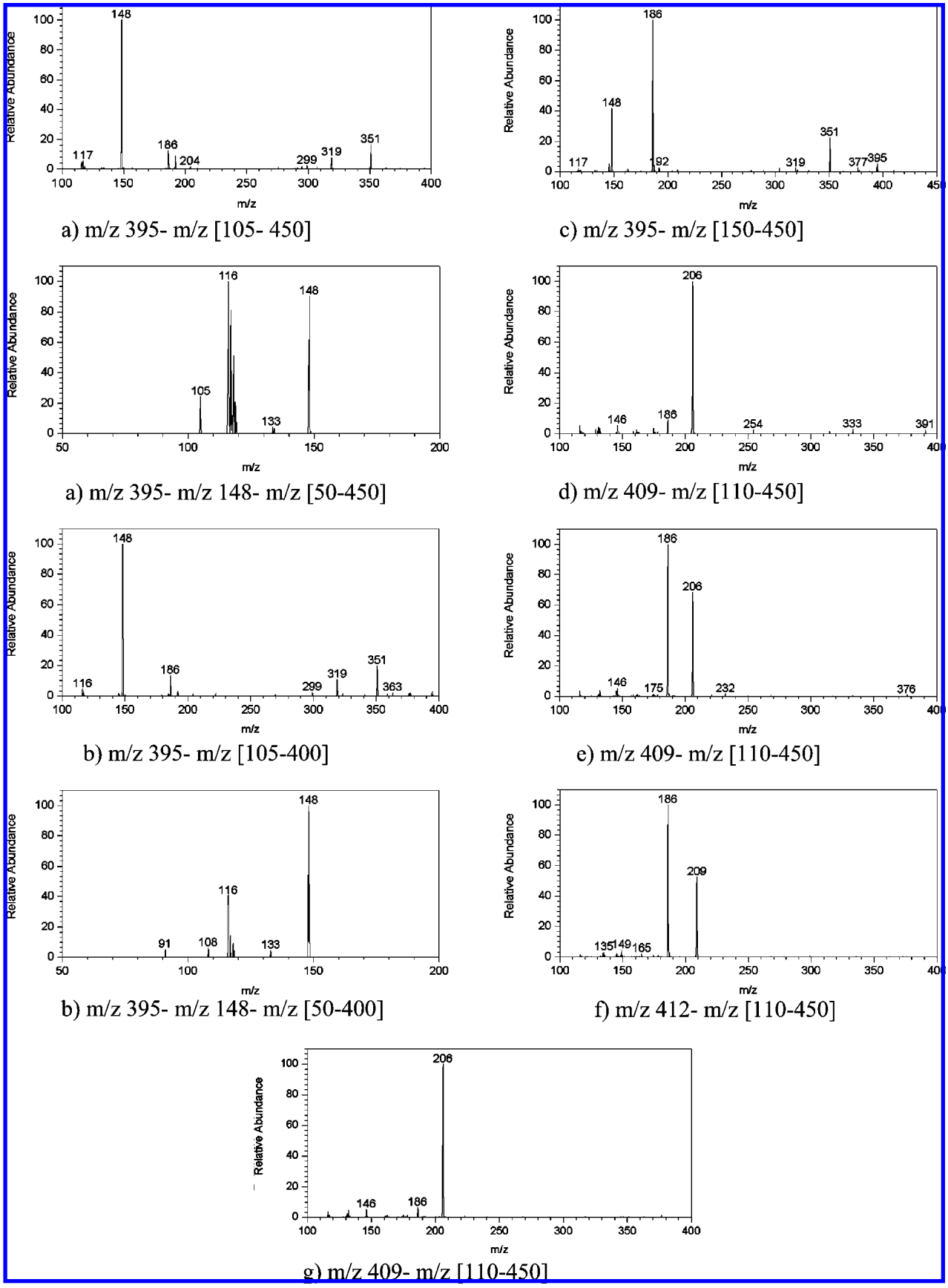

Figure 4. Mass spectrum under two-stage full-scan mode and three-stage full-scan mode by LC-MS/MS of (**a**) (*Z*,*Z*)-trifloxystrobin acid, (**b**) (*Z*,*E*)-trifloxystrobin acid, (**c**) (*E*,*E*)-trifloxystrobin acid, (**d**) (*Z*,*E*)-trifloxystrobin, (**e**) (*E*,*E*)-trifloxystrobin, (**f**) d3-(*E*,*E*)-trifloxystrobin, and (**g**) (*Z*,*Z*)-trifloxystrobin.

L^{-1} standard solution. The peak areas of trifloxystrobin and its metabolites in these solutions were compared to those in a standard solution prepared with deionized water. The results indicate that moderate ion suppression occurred, leading to

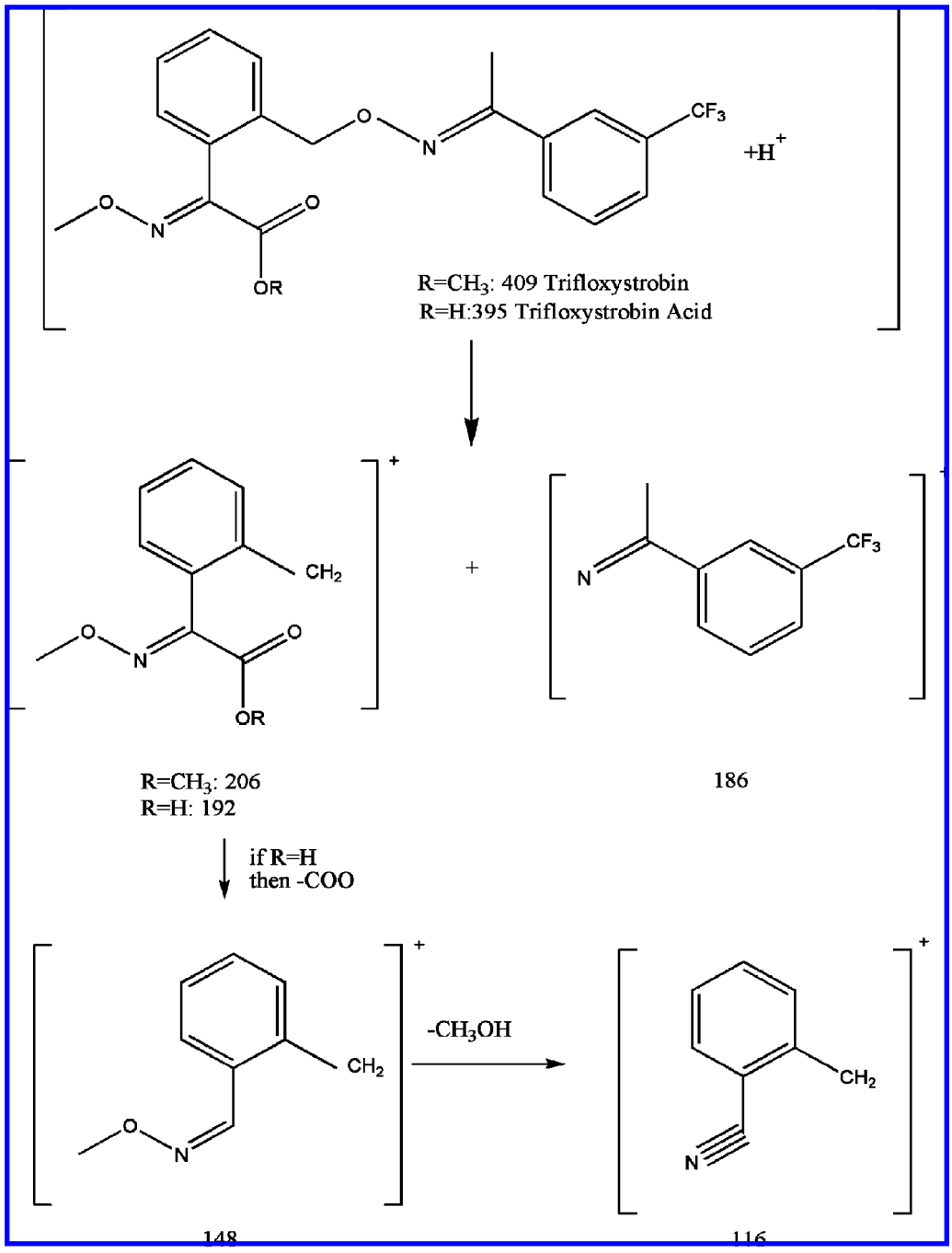

Figure 5. Fragmentation mechanism of trifloxystrobin and its acid.

Table 7. Mean Peak Area Ratios of Standard Solution Prepared by Matrix Compared to That Prepared with Deionized Water after LC-ESI/MS[a]

soil		ZZTFA	EETFA	ZETF	EETF	ZZTF	d3-EETF
K	peak area ratio (K/H_2O %)	88.6	71.7	93.3	73.5	93.1	88.6
	RSD (%)	1.7	6.8	5.8	2.2	1.5	3.4
L	peak area ratio (L/H_2O %)	89.0	85.5	92.9	74.3	90.1	80.7
	RSD (%)	5.0	5.7	8.3	3.8	9.4	4.4
M	peak area ratio (M/H_2O %)	83.2	76.9	92.4	72.4	96.0	88.1
	RSD (%)	5.5	5.7	4.3	1.5	5.4	1.0
P	peak area ratio (P/H_2O %)	80.7	76.5	93.5	72.2	95.6	82.9
	RSD (%)	1.2	8.7	8.1	2.0	0.5	2.3
W	peak area ratio (W/H_2O %)	89.4	88.4	90.6	83.9	90.6	95.9
	RSD (%)	4.5	2.9	4.2	3.8	2.4	4.9

[a] Triplicate tests were done.

Table 8. Mean Recoveries and Relative Standard Deviations (RSD) of Trifloxystrobin (EETF) and Its Metabolites, ZZTFA, EETFA, ZETF, and ZZTF, in the Recovery Experiments[a]

soil		EETF	ZZTFA	EETFA	ZETF	ZZTF
		Low Level (0.05 mg kg^{-1})				
L	recovery (%)	72.3	62.5	69.5	73.5	68.6
	RSD	4.0	2.5	2.4	2.3	1.9
W	recovery (%)	86.3	75.2	80.5	103.6	94.7
	RSD	9.2	8.3	5.7	3.1	2.9
M	recovery (%)	90.8	77.3	68.5	90.0	82.5
	RSD	4.0	6.0	3.1	1.4	3.7
K	recovery (%)	74.8	78.9	91.8	80.5	75.2
	RSD	3.4	6.6	2.9	5.6	5.3
P	recovery (%)	71.3	75.0	80.2	91.4	96.6
	RSD	6.0	7.2	7.1	5.1	3.5
		High Level (3 mg kg^{-1})				
L	recovery (%)	54.2	60.0	63.5	57.6	53.5
	RSD	2.0	7.0	8.0	1.0	4.0
W	recovery (%)	74.6	70.0	71.6	77.6	73.3
	RSD	4.0	6.0	4.0	3.0	3.0
M	recovery (%)	79.1	75.5	71.4	83.1	80.2
	RSD	4.0	3.0	4.0	2.0	4.0
K	recovery (%)	102.8	98.7	107.6	99.8	98.6
	RSD	1.0	5.0	6.0	4.0	2.0
P	recovery (%)	91.0	83.7	102.5	94.4	88.1
	RSD	6.0	2.0	5.0	3.0	2.0

[a] Triplicate tests were done.

Table 9. Calibration Curves' Linearity Information of (*Z*,*Z*)-Trifloxystrobin Acid, (*E*,*E*)-Trifloxystrobin Acid, (*Z*,*E*)-Trifloxystrobin, (*E*,*E*)-Trifloxystrobin, and (*Z*,*Z*)-Trifloxystrobin Generated by LC-ESI/MS/MS

	ZZTFA	EETFA	ZETF	EETF	ZZTF
slope	0.00897113	0.0102025	0.152802	0.0785677	0.0985367
correlation coefficient (r^2)	0.9967	0.9987	0.9986	0.9925	0.9983

Table 10. Concentration (Nanograms per Gram) Profile of EETF and Its Metabolites from the Two Test Plots on Week 16 at the Poamoho (P) Site

sampling depth (m)	EETF	ZZTFA + ZETFA	EETFA	ZETF	ZZTF
0–0.15 (1)[a]	6.7	ND[b]	19.5	ND	ND
0–0.15 (2)	4.3	ND	65.4	ND	ND
0.15–0.3 (1)	4.3	1.0	5.9	ND	ND
0.15–0.3 (2)	6.1	ND	2.0	ND	ND
0.3–0.6 (1)	4.1	ND	0.6	ND	ND
0.3–0.6 (2)	3.2	ND	ND	ND	ND
0.6–0.9 (1)	2.7	ND	ND	ND	ND
0.6–0.9 (2)	3.1	ND	ND	ND	ND
0.9–1.2 (1)	4.8	ND	15.3	ND	ND
0.9–1.2 (2)	4.7	4.6	ND	ND	ND
1.2–1.5 (1)	3.3	ND	7.9	ND	ND
1.2–1.5 (2)	4.0	1.9	1.5	ND	ND
1.5–1.8 (1)	5.1	ND	1.1	ND	ND
1.5–1.8 (2)	2.9	ND	ND	ND	ND
1.8–2.1 (1)	1.3	ND	ND	ND	ND
1.8–2.1 (2)	4.5	ND	ND	ND	ND

[a] Numbers 1 and 2 in parentheses imply duplicate samples. [b] Not detected.

the observation that the peak areas of the compounds prepared using the matrix solution decreased to 70–96% of those prepared using deionized water (**Table 7**). In the quantitative analysis, the peak area ratio of the target compounds to the internal standard was taken into calculation. Because both of them were suffering from ion suppression, the moderate matrix effect was even more compensated. For this reason, the matrix effect was considered to be negligible in this study and the value of peak area from the standard solution prepared without a matrix was used to calculate the standard curve in the quantitative analyses. The fact that the observed matrix effect was insignificant could be due to the absence of any sample concentration, so that the coelutes' interferences were not strong enough to cause serious matrix effects. No implementation of any concentration technique also simplified the whole sample preparation procedure compared to previous methods.

Table 11. Concentration (Nanograms per Gram) of EETF and Its Metabolites from Laboratory Degradation at Week 16 for Three Poamoho Soils Collected from Depth Intervals of 0–0.15, 0.15–0.3, and 0.6–0.9 m, Respectively

sampling depth (m)	EETF	ZZTFA + ZETFA	.	ZETF	ZZTF
0–0.15 (1)[a]	13.5	ND[b]	192.0	ND	ND
0–0.15 (2)	10.4	ND	142.0	ND	ND
0–0.15 (3)	10.5	ND	141.7	ND	ND
0.15–0.3 (1)	8.6	ND	50.3	ND	ND
0.15–0.3 (2)	9.9	ND	53.4	ND	ND
0.15–0.3 (3)	16.5	ND	183.8	ND	ND
0.6–0.9 (1)	23.9	ND	55.2	ND	ND
0.6–0.9 (2)	36.1	ND	54.2	ND	ND
0.6–0.9 (3)	45.1	ND	55.1	ND	ND

[a] Numbers 1−3 in parentheses imply triplicate samples. [b] Not detected.

Validation of the Method. The reliability of the method was confirmed by the spiked recovery test. The internal standard, d3-EETF, was tested first and confirmed that no EETF and its isomers were observed in the chromatography. The LOD, defined as signal-to-noise ratio (S/N) equal to 3, was 0.1 μg L^{-1}, whereas the developer's method reports it at 1 μg L^{-1} (*16*). The LOQ, defined as S/N = 10, was 0.5 μg L^{-1}. Considering the final extract volume was 50 mL and 10 g of soil was used in the analysis, the method LOQ should be 2.5 ppb in the soil. Two spike levels of recovery tests (0.05 and 3 mg kg^{-1}) were implemented. After spiking solution was fortified directly into each 10 g soil sample in the ASE cell, the cell was placed under the hood for at least 10 min and it was subjected to the procedure described in the earlier part of this paper. For the high-level spike recovery test, another 600 times dilution step (than the normal procedure) was done to bring the concentration down to the range of calibration curve. The results are presented in **Table 8**. The recovery was improved from the initial 20–30% to 68.5–107.6% for the W, M, K, and P soils of Hawaii and to 53.5–72.3% for L soil if the ASE temperature stayed at 40 °C (**Table 8**). No more effort was put into this work to lower the spike recovery levels, like the developer's method, because our method was used for overcoming the low recovery of Hawaii soil when applied to a leachability study, rather than the residue study. **Table 9** shows linearity information of the calibration curves, which include equation parameters and correlation coefficients (r^2) of each target compound.

To confirm that the ASE would not lead to degradation of EETF, pure EETF (1 mL of 0.5 μg mL^{-1} solution) was spiked to 10 g of soil and extracted. No isomers or acid metabolites were discovered in the extraction solution.

Analysis of Field Soil Samples and Degradation Soil Samples. Along with other insecticides tested, trifloxystrobin was applied at a rate of 3.12 g per plot (8 oz/acre application

rate). Each plot was 6.1 m by 9.1 m. Assuming a water penetration depth of 2.5 cm during pesticide application and a bulk density of 1.1 g cm^{-3} (typical of structured soils in Hawaii), the concentration in the top 2.5 cm soil would be 8.79 mg kg^{-1}. For a 5.0 cm water penetration depth, the concentration in the soil would be 4.4 mg kg^{-1}. On the basis of these estimated concentrations, which are expected to be encountered on the day of or 1 day after the application of trifloxystrobin, the high recovery level in this study was set at 3 mg kg^{-1}.

Leaching of trifloxystrobin and other compounds of interest was evaluated over a period of 16 weeks. After application, the plots were covered with straw mulch. The total amount of rain and irrigation water was similar to the evapotranspiration demand in the study areas. Details of the study design, sampling, and results can be found in Ray et al. (*20*). Soil samples were retrieved from the field in weekly, biweekly, and monthly intervals in the 16 week study period. At week 16, the residual EETF concentrations ranged from 10 to 20 ng g^{-1} in the sampled depths (0.75−2.1 m) depending on the site. The parent compound degraded within a few days of application and produced significant amount of EETFA, which steadily degraded over the 16 week sampling period to about $^1/_{10}$ of the initial values. **Table 10** shows the concentration profile of EETF and its metabolites for samples collected to a depth of 2.1 m at the two test plots of the Poamoho (P) site.

Laboratory aerobic degradation studies were conducted using soils from the depth intervals of 0–0.15, 0.15–0.3, and 0.6–0.9 m from the five sites over a period of 16 weeks. As the concentration of trifloxystrobin decreases with depth in the soil profile, the applied concentrations to the lower two soil layers were progressively less than that applied to the top soil layer. The moisture contents of the samples during incubation were kept similar to the field-capacity moisture contents of the soils. The soil samples were analyzed in 1−4 week intervals for EETF and its metabolites. Results showed rapid degradation of EETF (within 2 weeks) producing EETFA. **Table 11** presents the concentrations of EETF and its metabolite after 16 weeks of degradation for the P soils collected from the depth intervals of 0–0.15, 0.15–0.3, and 0.6–0.9 m, respectively. As can be found from this table (as well as **Table 10**), EETFA is the most prominent of the four metabolites in the field leaching and laboratory degradation studies. Additional results of the fate of these compounds will be discussed in a later paper.

The automated extraction (ASE) of trifloxystrobin and its metabolites from five soils of Hawaii followed by their LC-MS/MS analysis using our developed method was successful and optimal. The extraction solvent ratio was optimized to maximize the recovery of the compounds, and it contained 50% methanol and 50% water with 0.5 g L^{-1} of $EDTANa_2$. Addition of $EDTANa_2$ to the extract solvent was helpful in recovery of the analytes, which were assumed to form complexes with the metal ions present in some of the Hawaii soils. This was verified for those soils in which addition of $EDTANa_2$ enhanced extraction of the target compounds compared to the cases when no $EDTANa_2$ were added. For some of the clayey soils, extract temperature positively correlated with extraction efficiency. The method avoids the laborious and time-consuming sample preparation steps as presented in earlier literature, which also resulted in an insignificant matrix effect of this method. The ESI soft source fragmentation pattern was found to be different from previous work for some of the compounds.

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