JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Determination of Mesotrione Residues and Metabolites in Crops, Soil, and Water by Liquid Chromatography with Fluorescence Detection

PHILIP ALFERNESS* AND LAWRENCE WIEBE†

Zeneca Ag Products, Zeneca, Inc., Western Research Center, 1200 South 47th Street, Richmond, California 94804-0023

A method for the determination of residues of mesotrione and two metabolites in a variety of environmental matrixes has been developed. Mesotrione, a new selective herbicide for use in corn, is 2-(4-methylsulfonyl-2-nitrobenzoyl)-1,3-cyclohexanedione. The metabolite 4-methylsulfonyl-2-nitrobenzoic acid (MNBA) is determined with the parent compound in crops, whereas two metabolites, 2-amino-4-methylsulfonyl-benzoic acid (AMBA) and MNBA are determined with parent in soil and water. Crop samples are macerated with an acetonitrile/water mixture, and an aliquot is evaporated and acidified then centrifuged. Soil is shaken with an ammonium hydroxide solution, and an aliquot is acidified then centrifuged. For water analysis, an aliquot is acidified. Crop and soil extracts, and water, are cleaned up using reverse-phase high-performance liquid chromatography (RPHPLC) with mesotrione and MNBA isolated using a fraction collector. During this clean up, AMBA is determined in soil and water samples using fluorescence detection. The collected mesotrione and MNBA fractions are converted into AMBA via oxidation followed by reduction in the case of mesotrione, or by reduction alone in the case of MNBA. Both fractions are analyzed by RPHPLC with fluorescence detection using an AMBA external reference standard. The method was tested on corn grain, fodder, and forage, as well as on sugar cane. The limits of quantitation (LOQ) for each analyte are 0.01 mg/kg for crops, 0.005 mg/kg for soil, and 0.10 μg/L for water. Method fortification recoveries from all crop commodities averaged 79% (CV = 7%, n = 37 and 82% (CV = 5%, n = 37) for mesotrione and MNBA, respectively. Soil was fortified at 0.005 and 0.05 mg/kg. Recoveries were 79% (CV = 4%, n = 12), 96% (CV = 2%, n = 12), and 89% (CV = 2%, n = 12) for mesotrione, MNBA, and AMBA, respectively. Groundwater, drinking water, seawater, and river water were fortified at 0.1 and 1.0 μg/L. Recoveries for all waters were 80% (CV = 7%, n = 51), 94% (CV = 4%, n = 52), and 93% (CV = 9%, n = 51) for mesotrione, MNBA, and AMBA, respectively.

KEYWORDS: Mesotrione; ZA1296; metabolites; analysis; crops; soil; water; triketone; high-performance liquid chromatography; fluorescence detection

INTRODUCTION

Mesotrione [2-(4-methylsulfonyl-2-nitrobenzoyl)-1,3-cyclohexanedione], is a new selective, pre- and post-emergent herbicide for control of broad-leaved and some grass weeds in corn. The compound acts by competitive inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) which affects carotenoid biosynthesis (*1*). It was developed under the name ZA1296, one of a class of novel triketone compounds developed at Zeneca's Richmond, CA, research facility (*2*). The product, registered in Europe in 2000 and in the U.S. in 2001, is marketed under the name Callisto. The method described here was developed to determine the levels of mesotrione and metabolites that might be found after use in crops, soil, and water. In addition to corn commodities, data showing that the method is suitable for analysis of sugarcane are included here.

Metabolism studies in plants and soil show two degradates, MNBA and AMBA, can be formed from the parent mesotrione. As the U.S. Environmental Protection Agency (USEPA) has determined that AMBA is not an analyte of concern in crops, AMBA analysis is only for soil and water. The chemical structures of mesotrione, 4-methylsulfonyl-2-nitrobenzoic acid (MNBA), and 2-amino-4-methylsulfonyl-benzoic acid (AMBA) are shown in **Figure 1**.

Because of their recent development, little information exists in the literature concerning residue methodology of triketone herbicides. As a class, the triketones present the pesticide residue chemist with difficult problems to overcome. The compound

* To whom correspondence should be addressed: BioMarin Pharmaceuticals, Inc., 371 Bel Marin Keys, Novato, CA 94949. E-mail: palferness@biomarinpharm.com.

† Current address: Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608.

10.1021/jf011696y CCC: $22.00
Published on Web 06/01/2002

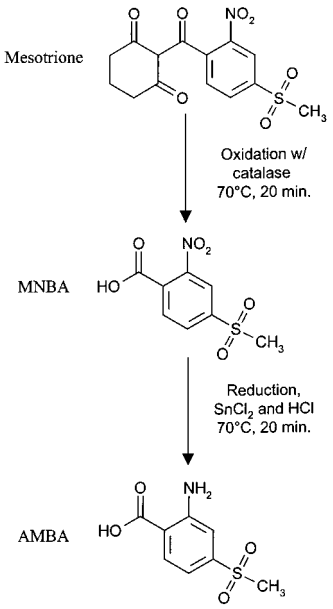

Figure 1. Conversion of mesotrione to MNBA and AMBA.

has multiple polar functionalities for which common derivatization techniques are not suitable. The lack of volatility requires derivatization prior to any GC analysis. An in-house (unpublished) Zeneca method for the determination of residues of a closely related triketone, sulcotrione [2-(2-chloro-4-methanesulfonylbenzoyl)cyclohane-1,3-dione] in soil relied on a derivatization of the three carbonyl groups with hydrazine or 2,-4 dintiro-phenylhydrazine. This procedure produced multiple hydrazone products and was determined to be a poor candidate for application to mesotrione. For the determination of sulcotrione residues in soil, the use of diazomethane for methylating carbonyl groups prior to GC analysis with electron capture detection has been reported (*3*, *4*). As with the hydrazine derivatization process, multiple derivatives (3 isomers) were obtained. An additional problem was the use of the highly carcinogenic diazomethane, making the procedure unacceptable to regulatory agencies. Sulcotrione has also been determined in soil using amino-bonded phase SPE cleanup prior to a reverse-phase HPLC analysis (*5*). No residue methods for mesotrione have been reported in the literature to date.

A closely related triketone, NTBC [2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione] has been used in the treatment of the disease tyrosinemia type II. A reverse-phase HPLC method for the determination of NTBC in plasma has been reported (*6*). A precolumn was used with restricted access to lipophilic pores, allowing retention of NTBC within the pores, while polar and large endogenous compounds were eluted in the void volume. The analyte was monitored by UV detection at 278 nm. With soil and crop extracts, low-molecular-weight interferences cannot be removed with such a procedure.

Initial soil degradation studies at Zeneca were supported with a direct assay for mesotrione alone by reverse-phase HPLC. In this method, soil was extracted with aqueous base, and the extract was dried and cleaned up over a silica solid-phase extraction column, eluting with a methanol/dichloromethane mixture. The eluate was evaporated to dryness and taken up in a water−acetonitrile mixture. Final determination was conducted on a ODS column with an acetonitrile−water gradient, monitoring absorbance at 270 nm. The detection limit was approximately 0.05 μg/g, but specificity was a difficulty that was encountered in many soils. When MNBA was identified as a soil metabolite, the simultaneous determination of this compound was impossible as the presence of the carboxylic acid group rendered MNBA too polar for retention under this system. An attempt was made using a similar method for residues in corn commodities, but extensive cleanup efforts failed to provide a reasonable chromatographic profile. The target limit of quantitation of 0.01 μg/g for crops could not be attained. When AMBA was identified as a second soil metabolite, its fluorescent properties were noted and eventually utilized to determine both AMBA and MNBA. A silica SPE cleanup of an aqueous soil extract was used to isolate AMBA from MNBA. This was followed by reduction (stannous chloride/HCl) of the nitro group of MNBA to form the fluorescent AMBA. A reverse-phase HPLC determination of the AMBA (reduced from the MNBA metabolite) accomplished the quantitation of MNBA, while a separate injection of the original aqueous extract allowed for quantitation of the AMBA metabolite. The reaction scheme is shown in **Figure 1**.

The initial success of this procedure for determination of the metabolite MNBA indirectly through the use of a fluorescent moiety, AMBA, was then taken one step further and applied to the parent as well. The silica SPE cleanup was replaced by a reverse-phase HPLC cleanup, and performed on an aqueous extract to isolate all three analytes of interest, the parent mesotrione, MNBA, and AMBA. AMBA was directly quantitated by fluorescence detection in this cleanup/isolation step (**Figure 2A**). As described above, isolated MNBA was converted to AMBA by reduction of the nitro group using a mixture of stannous chloride and concentrated HCl (**Figure 2B**). A second RP-HPLC injection determined the amount of AMBA present as a result of the reduction of MNBA (**Figure 2C**). The parent compound, mesotrione, then underwent oxidative cleavage (**Figure 2B**) using the enzyme catalase, resulting in the products MNBA and cyclohexane-1,3-dione. The MNBA (from mesotrione) was then reduced to AMBA, and a third RP-HPLC injection quantitated the amount of AMBA resulting from the mesotrione-to-MNBA-to-AMBA reaction scheme (**Figure 2D**).

This method was developed to determine low-level residues of mesotrione and its metabolites in a variety of environmental matrixes in support of tolerance petitions for registration in the United States and Europe. The method has been shown to provide selective, sensitive, and robust determinations of mesotrione, MNBA, and AMBA in soil and water; and for mesotrione and MNBA in corn commodities such as fodder, forage, and grain; in addition to sugar cane.

MATERIALS AND METHODS

Instrumentation. A Hewlett-Packard model 1090A liquid chromatograph, equipped in series with a model 1040A UV diode array detector (DAD), a model 1046 fluorescence detector (FLD), and a Waters Associates model 3500 fraction collector was used for both sample cleanup and final analysis. The fraction collector was equipped with a 3-way waste valve and vial rack for holding 4-mL vials, operation mode was no. 3, timed window, and the collections windows were determined as described in the HPLC cleanup section below. For the determination of residues in water, a manual fixed-loop injection valve (Valco C6U or equivalent) capable of making 5.0-mL injections is required. A 5.0-mL sample loop was made from a length of 0.02-in. i.d. PEEK tubing.

Reagents. Acetonitrile, methanol, water, and ammonium acetate were HPLC grade. Water used in the method other than for the HPLC mobile phase was distilled or deionized. Stannous chloride, 30% hydrogen peroxide, glacial acetic acid, concentrated hydrochloric acid, and 88% formic acid were ACS grade. Catalase enzyme of approximately 1×10^4 to 2×10^4 activity units per mg was obtained from Sigma (C40). Reagents were prepared by the following procedures. Stannous chloride reagent, 90 mg/mL $SnCl_2$ in 3 N HCl: Add 3.6 g of stannous

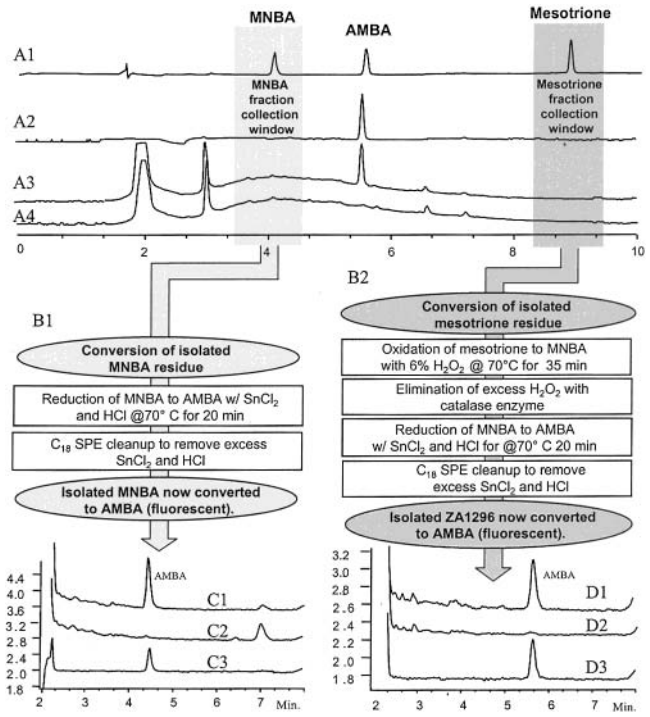

Figure 2. Method schematic: Residue isolation, conversion to AMBA, and final determination. (A) Determination of AMBA in soil extracts with concurrent isolation of MNBA and mesotrione residues: (A1) determination of fraction collection windows by injection of 0.2 μ/mL standard solution and monitoring at 254 nm; (A2) AMBA calibration standard at 1.0 ng/mL for quantitation of AMBA residues in soil extracts; (A3) extract of soil fortified at 0.005 mg/kg each analyte, with fractions for isolation of MNBA and mesotrione as indicated (concurrent recovery of AMBA was 86%); and (A4) control soil, with fractions collected as indicated. (B) Conversion of isolated residues to AMBA: (B1) conversion of isolated MNBA residues to AMBA; and (B2) conversion of isolated mesotrione residues to AMBA. (C) Final analytical determination of AMBA resulting from conversion from MNBA: (C1) quantitation of AMBA in MNBA fraction isolated from fortified soil extract in A3, 97% recovery; (C2) MNBA fraction isolated from control soil extract in A4; and (C3) AMBA calibration standard 0.1 ng/mL. (D) Final analytical determination of AMBA resulting from conversion from mesotrione: (D1) quantitation of AMBA in mesotrione fraction isolated from fortified soil extract in A3, 83% recovery; (D2) mesotrione fraction isolated from control soil extract in A4; and (D3) AMBA calibration standard 0.1 ng/mL. A1 monitored by UV at 254 nm, remaining chromatograms monitored by fluorescence as indicated in text. Final determination of MBNA in C1–C3 used initial 16% B elution; final determination of mesotrione in D1–D3 used initial 13% B elution. Final soil concentration in extracts in A3 and A4 was 0.2 g/mL; final extracts from C1, C2, D1, and D2 were 0.05 g soil/mL extract.

chloride to 10 mL of concentrated HCL, swirl to dissolve. Stannous chloride should dissolve easily in concentrated HCl. Dilute by adding 30 mL of deionized or distilled water. The solution should be clear, both before and after the addition of the water. Catalase reagent, about 2×10^4 units per mL: dissolve 10 mg (equivalent to 2×10^4 activity units/per mg) of catalase enzyme in 10 mL of deionized or distilled water. HPLC Mobile Phase A, 9 mM ammonium acetate and 0.25% formic acid: add 2.76 g of ammonium acetate to a 4-L bottle of HPLC grade water, add 10 mL of formic acid, shake well to dissolve. HPLC mobile phase B, acetonitrile/water (95:5): add 50 mL of HPLC grade water to 950 mL of HPLC grade acetonitrile. Acetate buffer, 0.1 M, pH 4.7: prepare by combining 0.77 g of ammonium acetate, 0.58 mL of glacial acetic acid, and 200 mL of deionized water.

Apparatus. An evaporation manifold with aluminum heating block to fit 4-mL and 8-mL vials was used (Techne-Dri Block DB-3, with sample concentrator unit). The HPLC cleanup column was an Inertsil ODS-2, 5-μm particle size, 250 × 4.6 mm i.d. (MetaChem 0296-250-X046 or Keystone 255-181), with a guard column (MetaChem 0296-CS). An equivalent column is the Phenomenex Prodigy ODS-2, 5-μm particle size, 250 mm × 4.6 mm i.d. (Phenomenex 00G-3300-EO) with a guard column (Phenomenex 03A-3300-EO). The HPLC column used for final determination was a Waters Spherisorb ODS-2, 250 mm × 3.0 mm i.d. (MetaChem 0184-250 × 030). An equivalent column is the MetaSil ODS, 250 mm × 3.0 mm i.d. (MetaChem 0380-250 × 30). C18 SPE columns were Varian Bond Elut LRC solid-phase extraction columns, C18, 500 mg, 10-mL reservoir volume (Phenomenex AHO-1210-3027, Chrom Tech 1211-3027). Bond Elut columns with 500 mg of packing material and a 2.8-mL reservoir volume may be substituted. Disposable syringe filters were Whatman Anotop inorganic membrane filters, 0.2-micron pore size with Luer hub, 25-mm diameter (Fisher 09-911-2, Whatman 6809-4022). Quantofix brand peroxide test strips, range 0−100 mg/L (Baxter P1127-10), were used. Syringes for standard aliquoting were 25-, 100-, and 250-μL capacity Gas-Tight syringes (Hamilton 1700 series) for aliquoting methanol calibration and fortification solutions. Sample vials for fraction collection were 4-mL screw-top vials, amber glass, silanized (Supelco 2-7216). For collection of post-conversion cleanup eluate, we used 4-mL screw-top vials, clear glass, silanized (Supelco 2-7220). Screw-top vials (8-mL; Fisher 06-412-3) were used for crude extract evaporation. PTFE-lined caps were used for 4-mL vials, 13-425 (Fisher 06-406-40); PTFE-lined caps used for 8-mL vials were 15-425 (Fisher 06-450-44). Vials for the autosampler were 2-mL crimp-top, silanized autosampler vials (Supelco 2-7061). A Teckmar model SDT-25 tissuemizer (Tekmar 10-0103-0180, equipped with a SDT-182EN probe, Tekmar 10-0104-000) was also used. An equivalent laboratory homogenizer may be substituted.

Reference Materials and Calibration Solutions. The reference standards were synthesized, characterized, and purified by Zeneca Ag Products; they are available from Syngenta Crop Protection, Jealott's Hill, Bracknell, Berkshire, England, RG12 6EY. Separate stock solutions of the three analytes were prepared at 1000 μg/mL in methanol. Separate working fortification solutions were prepared at appropriate levels by serially diluting the stock solutions using methanol. Separate solutions were maintained in order to increase flexibility in fortification schemes depending on whether one or more metabolites were determined. Separate AMBA calibration solutions are needed to determine the amount of AMBA conversion product present in the final extracts, as well as to determine AMBA, if needed, in the initial HPLC cleanup. Prepare intermediate AMBA calibration solutions by serially diluting the stock solution using water. Useful working calibration solution levels for the final analytical determination for crops and soil are 10, 1.0, and 0.10 ng/mL, and for water 50, 5.0, and 0.30 ng/mL. For quantitation of AMBA during the sample cleanup step, useful solution levels for soil and water are 10, 1.0, and 0.10 ng/mL. A retention time calibration solution is prepared to establish analyte retention times so that the appropriate eluate fractions are collected during the HPLC cleanup. Prepare an intermediate solution at 10 μg/mL in methanol by combining aliquots of the three stock solutions together. Transfer 1.0 mL of the intermediate solution to a 2-oz. narrow-mouthed bottle, and evaporate the methanol under a stream of nitrogen. Dilute with 50 mL of HPLC-grade water containing 0.2% formic acid to produce a working UV calibration solution with a concentration of 0.20 μg/mL.

Fortification. Analyze fortified and unfortified control samples with each sample set to demonstrate method recovery. For example, add 50 μL of a working fortification solution (1.0 μg/mL) to a control soil sample (10 g) to produce a fortification level of 0.005 mg/kg, add 100 μL of a working fortification solution (1.0 μg/mL) to a control crop sample (10 g) to produce a fortification level of 0.01 μg/g, and add 100 μL of a working fortification solution (0.01 μg/mL) to a control water sample (10 mL) to produce a fortification level of 0.10 μg/L. Extract as detailed below.

Extraction. *Crops.* Place a 10-g subsample of a homogeneous crop sample into an 8-oz., wide-mouthed jar. After taking into account the amount of water present in the sample, add an amount of the acetonitrile/water (1:1, v:v) extraction solution that brings the total aqueous volume to 125 mL. For example, a crop sample that contains

40% water (4 mL of water) would require that 121 mL of extraction solution be added. Add 1.0 g of NaCl. Macerate at a medium to high speed (>10 000 rpm) for three minutes using a tissuemizer. Centrifuge for 10 min at about 2000 rpm. The following steps constitute pre-cleanup evaporation: Transfer an 8.0-mL aliquot of the crop supernatant to an 8-mL glass vial. Evaporate, under a stream of nitrogen at a temperature of 60 °C, to approximately 2.3−2.7 mL volume, swirling the contents of the vial occasionally (2−3 times) during the evaporation. Add 2 mL of water containing 1% formic acid. Bring to a total volume of 8 mL. The 8 mL volume may be determined by preweighing the empty vial and recording its weight. After addition of the 2 mL of 1% formic acid, place the vial on the balance and add HPLC grade water until a net gain of 8 g over the initial weight of the vial is obtained. Cap the vials and place them on an aluminum heating block maintained at 65 °C for 10 min. Sonicate for 10 min. Centrifuge for 20 min at 3500 rpm. Transfer 1 mL to a silanized autosampler vial for the HPLC cleanup.

Soil. Place a 10-g subsample of a homogeneous soil sample into a 4-oz, wide-mouthed jar equipped with a PTFE liner. Add 50 mL of 0.05 M ammonium hydroxide extraction solution. Place on a mechanical shaker and shake for 30 min. Centrifuge for 15 min at about 2500 rpm. Transfer approximately 30 mL of the soil supernatant to a 50-mL polypropylene centrifuge tube. Adjust the pH of the aliquot to approximately 3.5−4.0 with formic acid. The amount of acid required may vary by soil type. Most soils require 50 μL, whereas some may require up to 100 μL. Add an initial amount, then replace the cap and shake the tube. Check the pH using the appropriate pH indicating strip. Add more formic acid if needed. Repeat the pH measurement. Centrifuge for 10 min at about 2500 rpm. Allow to stand for 15−30 min, then filter through a 0.2-μm filter. If the solution looks cloudy prior to the HPLC cleanup step, refilter. Reducing the pH to 3.0 or less may cause adsorption of AMBA onto precipitated material. Losses of AMBA may also occur in the filtration step at a pH of 3.0 or less. Transfer 1 mL to a silanized autosampler vial for the HPLC cleanup.

Water. Filter a 10-mL subsample of water through a 0.2-μm syringe filter into a 50-mL polypropylene, disposable test tube. Add 20 uL of formic acid, cap the tube, and shake.

HPLC Cleanup. During this part of the procedure, using an HPLC equipped with a postcolumn setup of, in series, a UV diode array detector (DAD), a fluorescence detector (FLD), and a fraction collector, AMBA is measured by FLD in water and soil, while fractions containing MNBA and mesotrione are collected for water, soil, and crops. To set accurate fraction collection windows, first determine the retention times of the three analytes as measured by the UV detector using the retention time calibration standard (0.20 μg/mL mesotrione, MNBA, and AMBA) while monitoring at an absorbance of 254 nm. Next, determine the fraction collector delay. This delay is defined as the amount to time required for an analyte to travel, via connecting tubing from the DAD, as measured by the DAD retention time, to the outlet of the fraction collector (FC). This parameter can be calculated by considering the flow rate and the volume of the connecting tubing. Note: to reduce band spreading due to laminar flow, the tubing should be as short as possible, 0.02-in. i.d. PTFE tubing is recommended for this connection. After the delay is determined, each fraction is collected starting at 0.6 min before, and going to 0.6 min after, the analyte elutes from the FC. This ± 0.6-min window should be widened if peak tailing is observed, or narrowed if interfering co-extractives are evident. A ±1.0-min window may be used for water analysis. Because the volume injected during the HPLC cleanup is used in calculation of the final sample-to-solvent ratio, this parameter should be confirmed. Fill vials with deionized water and weigh before and after injection to determine the actual volume injected. If this volume differs by more than 2% of the nominal injection volume find the cause of the problem or use the experimentally determined volume instead of the nominal volume.

The column used for the HPLC cleanup is an Inertsil ODS-2, 250 mm × 4.6 mm i.d., 5-μm particle size with appropriate guard column, all maintained at 50 °C. The mobile phases are as follows: reservoir (A) 9 mM ammonium acetate, 0.25% formic acid in water; reservoir (B) acetonitrile/water, 95:5, (v:v). The gradient is as follows: initial 7% B, linear gradient to 45% B at 5 min, hold at 45% B until 9 min, linear gradient to 90% B at 10 min, hold at 90% B until 12 min, return to 7% B% at 12.5 min, and 5 min reequilibration. The following conditions should be used: flow rate, 1.5 mL/min; FLD parameters, excitation 227 nm, emission 424 nm, response time 2.0 s; DAD parameters, 254 nm, peak width 0.02 min, sampling interval 0.32 s, autobalance on. Contact closure to start fraction collector, on at 0.01 min, off at 0.02 min. It is suggested that three individual instrument methods be used for control of the HPLC during the fraction collection run: (1) a UV- monitored method for analysis of the UV calibration standard, (2) a FLD-monitored method for analysis of the water blanks and AMBA standard, and (3) a FLD-monitored method with timed events for fraction collector control for analysis of sample extracts. All instrumental parameters, except for timed events and mode of detection, must be identical for each method.

Injection of Crop Extracts. Make a 250-μL injection (equal to 0.02 g of crop) of each crop extract into the HPLC system. Collect eluate fractions, corresponding to the fraction collection windows determined for MNBA (referred to as fraction A) and mesotrione (fraction B). Collect the eluate fractions in 4-mL silanized, amber glass vials (Be sure that these vials are devoid of any paper or cardboard fibers). Prior to injection of the extracts make a single injection of a water blank and collect fractions. Fractions resulting from this water blank are referred to as the HPLC reagent blank (HRB). At the end of the fraction collection run, inject the UV calibration solution to confirm that the retention times of the analytes have not drifted significantly.

Injection of Soil Extracts or Water. Make a 250-μL injection (equal to 0.05 g of soil) of each soil extract into the HPLC system, or for water make a 5.0-mL injection. Collect eluate fractions as described above for crops. In addition, inject 1.0 and 10.0 ng/mL AMBA calibration solutions for quantitation of AMBA residues in soil. For water, inject 0.1, 1.0, and 10 ng/mL AMBA calibration solutions for quantitation of AMBA residues. After every 4−6 extract injections reinject the AMBA calibration standard. Do not collect fractions during analysis of the AMBA standards. Monitoring the retention time of AMBA also helps to verify that the retention times remain constant and ensure that any residues elute in the collected fractions.

Evaporation of Isolated Extract Fractions. After collection, evaporate eluate fractions to dryness under a stream of nitrogen. Place vials in a heating block maintained at a temperature of 62−68 °C to facilitate evaporation. It is critical to completely dry the fractions, as small amounts of acetonitrile can reduce the effectiveness of the subsequent catalase reaction. However, care should be taken to prevent over-drying and potential loss of analyte. Therefore, remove each vial from the heating block as soon as the level of liquid approaches the bottom of the vial (approximately 100 μL remaining). Once all vials are removed, place them in a block maintained at room temperature and continue evaporation under a stream of nitrogen until dry.

Conversion of Mesotrione Residues to AMBA. *Oxidation of Mesotrione Residues to MNBA.* Add 300 μL of a 30% hydrogen peroxide solution to each of the vials (fraction B) containing mesotrione residues (add 750 μL to each vial for water samples). Heat at 83−87 °C for 20 min. Vortex every 5 min during heating. Remove from heating block and cool to room temperature. Centrifuge vial briefly (1−2 min) at 1000−1500 rpm prior to opening the vial in the next step in order to reduce small losses in the cap, from pressure release, or from sample transferred inadvertently from the vial mouth to the outside of the pipet tip. Losses from repeated opening of the vials can be reduced by centrifuging prior to each opening.

Elimination of Excess Hydrogen Peroxide. Using an Eppendorf pipet, add 650 μL of HPLC-grade water to each vial (add 750 μL for water samples). Cap the vials and swirl them gently to mix. Place all vials in an aluminum block. Place block in contact with dry ice to facilitate freezing of vials. Once frozen, uncap vials, and using an Eppendorf pipet, carefully add 50 μL of a solution containing ca. 20 000 units/mL of catalase enzyme. As the contents of the vial thaws the rate of the enzyme reaction will increase. (Addition of the enzyme solution to room temperature or unfrozen vials will cause vigorous bubbling, foaming, aerosol formation, and loss of sample.) After the solution thaws, monitor bubble formation. Sustained bubble formation (without swirling) indicates the reaction is not yet near completion. Tiny bubbles will form in the solution upon swirling of the vial contents. The amount of bubbles formed when the solution is swirled decreases as the reaction

is nearing completion. Check bubble formation every 3−5 min. Once bubble formation is at a minimum, the vial may be capped. Some reactions may occur very slowly. If the reaction is not complete within 30 min, add another 50-μL aliquot of catalase solution. Continue to monitor, by swirling, every 3−5 min. Excessively slow reactions may be sped up by placing the vial on a heating block for 5 min at 62−68 °C. Once all vials have been capped, place vials on a mechanical shaker for 20 min. Important: Capping the vials too early in the reaction process will lead to a build up of pressure in the vial and may cause loss of material upon opening. Opening of capped vials should be done only after centrifuging.

Determination of Residual Peroxide. The elimination of peroxide must be confirmed before proceeding with the following reduction reaction. Centrifuge vials prior to opening. Using an Eppendorf pipet, transfer 1−2 μL of the contents of each vial to a peroxide-indicating test strip. An immediate (3−5 s) blue color indicates the presence of peroxide. If any level of peroxide is indicated, cap the vial and shake for 10 min before retesting. If the test is still positive add an additional 50 μL of the catalase reagent, shake for 10 min, then retest. Do not dip the test strips into the solutions; excessive losses will occur.

Reduction of MNBA to AMBA (Mesotrione Residue). Add 500 uL of the stannous chloride reagent (750 uL for water samples) to each vial. Tightly cap the vial and heat at 63−67 °C for 20 min. Shake vials every 5 min. Remove and allow to cool to room temperature.

Conversion of MNBA Residues to AMBA. Add 1.0 mL of HPLC-grade water (1.5 mL for water samples) to each vial (fraction A) containing MBNA residues. Add 500 μL (750 μL for water samples) of the stannous chloride reagent to each vial. Tightly cap the vial and heat at 63−67 °C for 20 min. Shake vials every 5 min. Remove and allow to cool to room temperature.

Post-Conversion Cleanup of All Fractions. *SPE Column Preparation.* Condition a 500-mg, C18 solid-phase extraction column with 5 mL of acetonitrile containing 0.2% formic acid. Follow with 6 mL of HPLC-grade water containing 0.2% formic acid. Use a vacuum manifold to elute at a rate of 0.5−1.0 mL/min. This rate requires a low vacuum of about 0.5−1 in. Hg. Stop elution by closing the stopcock prior to the level of eluant reaching the column bed. When all columns have progressed to this point, continue using gravity elution until each vial's liquid level reaches the column bed, whereupon the gravity elution should stop without the column bed going dry. Place the stopcocks on the SPE manifold in the off position. Centrifuge sample vials prior to opening them.

SPE Cleanup. Using an Eppendorf pipet, transfer the contents of the first vial to the column reservoir, do not yet discard the pipet tip. Using a second pipet or other liquid-handling device, add 0.5 mL of HPLC-grade water to the sample vial, cap the vial, and swirl it. Using the first Eppendorf pipet, transfer the wash solution from the vial to the SPE column. Proceed with the remaining vials in the same manner. Once the contents of all the sample vials have been transferred, open the stopcocks and turn on the vacuum to allow elution at a rate of 0.5−1.0 mL/min. Stop elution of each column prior to the level of eluant reaching the column bed. Continue using gravity elution until the level of liquid reaches the column bed, whereupon the gravity elution should stop without the column bed going dry. Wash columns with a 1.5-mL aliquot of 0.2% formic acid in water. Use the same method of vacuum elution followed by gravity elution. Wash the column with a second 1.5-mL aliquot of 0.2% formic acid in water, but this time continue the vacuum elution until the column goes dry. Increase the vacuum to 5−10 in. Hg to further dry the column for an additional 30 s. Set up the elution rack outside of the SPE manifold. Use a top plate with 16-mm holes to hold the SPE columns. A second plate with 16-mm holes is used to hold the 4-mL, silanized, glass collection vials. The 4-mL vials should be preweighed to allow for final volume determination. Transfer the SPE columns from the manifold to the elution rack, and elute columns with 3.0 mL of acetonitrile containing 0.2% formic acid. Collect eluate in 4-mL, silanized glass vials. Start gravity flow by applying a small amount of positive pressure to the column reservoir using a small pipet bulb. Once gravity elution has completed, use the small pipet bulb to purge the column of eluant.

SPE Eluate Evaporation. Transfer vials to a heating block maintained at 63−67 °C. Start evaporation of eluate under a stream of nitrogen. After about 1 mL has been evaporated, add 0.6 mL of a 0.1 M, pH 4.7, acetate buffer. Continue evaporation under a stream of nitrogen. Remove each vial from the heating block when the volume reaches approximately 200−250 uL. Note: At this point the remaining extract is predominantly water. When all vials have been removed from the heating block, add acetate buffer to bring the total volume in each sample vial to 1.00 mL. On the basis of the initial weight of the vials obtained above, add the required volume of buffer to obtain a net weight gain of 1.00 g. Assume the density of the buffer is 1.0 g/mL. Place the capped vials in a heating block maintained at 63−67 °C for 2−3 min. Vortex for 20 s, then sonicate for 2−3 min. Transfer to silanized autosampler vials for analysis.

Final Analytical Determination Parameters. The analytical column is a Waters Spherisorb ODS-2, 250 mm × 3.0 mm i.d., 5-μm particle size maintained at 50 °C. The mobile phases are the same as for the HPLC cleanup. The gradient is as follows: initial 16% B for 5 min, linear gradient to 80% B at 7 min, hold at 80% B until 9 min, return to 16% B at 10 min, and 5 min reequilibration period. Flow rate is 0.75 mL/min, and injection volume is 200 μL. FLD parameters same as for the HPLC cleanup.

The suggested analytical scheme for AMBA during the final analysis could include injections in the following order: (1) One injection each of AMBA calibration standard (soil and crops: 0.10, 1.0, and 10 ng/mL; water: 0.30, 5.0, and 50 ng/mL), (2) reaction blank, reagent blank, and/or control extract (mesotrione, fraction B), (3) fraction B, mesotrione sample extracts (extracts equivalent to extractives from 0.05 g soil per mL, extractives from 5 mL of water per mL, or extractives from 0.02 g of crop per mL), (4) one injection of each AMBA calibration standard, (5) reaction blank, reagent blank, and/or control extract (MNBA, fraction A), (6) fraction A, MNBA sample extracts (same extract concentrations as for fraction B above), (7) one injection of each AMBA calibration standard.

Calculations. The concentration of the analyte in the original sample is calculated by using the external standard method. After determining an average response factor, RF = concentration of std (ng/mL)/peak area for AMBA, at each calibration level, an average response factor is determined. Next, the sample-to-solvent ratio (SS) is determined where SS (g/mL) = [(weight of sample)/(total volume of crude extract)] × [(volume injected on HPLC for fraction collection in mL)/(final volume, after dissolution of residuum from C18 SPE cleanup, nominally 1 mL)]. The concentration of AMBA (*A*), resulting from either mesotrione or MNBA, in the final extract is calculated, where *A* (ng/mL) = *R* × RF, and *R* is the AMBA response. To calculate residues in terms of mesotrione or MNBA, the molecular weight ratio of mesotrione to AMBA (*z*/*a*) and MNBA to AMBA (*m*/*a*), respectively, are calculated. The concentration of mesotrione (mg/kg) in the original sample is = *A* × (*z*/*a*)/SS/1000. Concentration of MNBA = *A* × (*m*/*a*) x SS/1000. The molecular weights of mesotrione, MNBA, and AMBA are 339, 245, and 215 daltons, respectively. For AMBA residues determined during the HPLC cleanup step of water, the concentration of AMBA in the original sample is determined where the concentration of AMBA (μg/L) = *R* × RF. For the determination of AMBA residues in the HPLC cleanup of soil, the concentration of AMBA (mg/kg) = *R* × RF/SS/1000, where SS = (weight soil extracted/total volume crude extract).

RESULTS AND DISCUSSION

Extraction Efficiency and Recovery. The extraction of the three analytes from soil with dilute aqueous base or from crops with an acetonitrile/water mixture proved to be effective. Because of the application rates, use patterns, and metabolism of mesotrione, incurred residues from actual field trials were rare. However, crop samples fortified with analyte contained in methanol solutions, and the solvent allowed to evaporate for 2−48 h, resulted in the levels of recoveries similar to those found in **Table 1**. For recovery from lab-fortified soil samples, fortification and drying prior to analysis resulted in levels of recovery similar to those found in **Table 2**. Data (unpublished) from the analysis of samples from soil dissipation studies

Table 1. Recovery of Mesotrione and MNBA from Crop Commodities

analyte	commodity	fortification level[a], (mg/kg)	recovery, % mean	CV[b]	N[c]	range[d]
mesotrione	corn forage	0.01	77	6.0	4	71–82
		0.10	79	6.5	4	74–86
	corn fodder	0.01	78	4.3	8	72–83
		0.10	81	3.4	5	83–84
	corn grain	0.01	85	14.3	4	74–99
		0.10	81	1.9	4	79–82
	sugar cane	0.01	75	4.8	4	70–78
		0.10	73	2.6	4	72–76
	all commodities	0.01	79	7.1	20	70–99
		0.10	79	4.3	17	72–83
		0.01–0.10	79	7.2	37	70–99
MNBA	corn forage	0.01	76	3.3	4	72–77
		0.10	86	4.6	4	83–92
	corn fodder	0.01	82	2.1	6	79–84
		0.10	86	1.5	5	85–88
	corn grain	0.01	82	1.5	4	81–84
		0.10	83	2.3	4	81–85
	sugar cane	0.01	79	8.3	4	69–83
		0.10	82	4.5	4	78–86
	all commodities	0.01	80	4.2	18	69–84
		0.10	85	3.4	17	78–92
		0.01–0.10	82	5.6	35	69–92

[a] Amount added, each analyte. [b] CV, coefficient of variation. [c] *N*, number of replicates. [d] Range of individual recovery values.

Table 2. Recovery of Mesotrione, MNBA, and AMBA from Soil (Sandy Loam and Loamy Sand)

analyte	fortification level[a], (mg/kg)	recovery, % mean	CV[b]	N[c]	range[d]
mesotrione	0.005	79	2.9	6	77–83
	0.050	78	4.6	6	72–81
	0.005–0.050	79	3.7	12	72–83
AMBA	0.005	89	2.5	6	86–92
	0.050	90	1.3	6	85–92
	0.005–0.050	89	2.2	12	85–92
MNBA	0.005	95	2.5	6	91–97
	0.050	97	1.3	6	96–99
	0.005–0.050	96	2.2	12	91–99

[a] Amount added, each analyte. [b] CV, coefficient of variation. [c] N, number of replicates. [d] Range of individual recovery values.

indicated that total analyte recovery was determined to be consistent with the amount of chemical applied and known degradation rates.

As a primary indication of method performance during routine analysis, corn grain, forage, fodder, and sugar cane were fortified at 0.01 and 0.10 mg/kg and analyzed to determine recovery. Recoveries from all commodities averaged 79% (CV = 7%, $n = 37$) and 82% (CV = 5%, $n = 37$) for mesotrione and MNBA, respectively (**Table 1**). Soil was fortified at 0.005 and 0.05 mg/kg. Recoveries were 79% (CV = 4%, $n = 12$), 96% (CV = 2%, $n = 12$), and 89% (CV = 2%, $n = 12$) for mesotrione, MNBA, and AMBA, respectively (**Table 2**). Groundwater, drinking water, seawater, and river water were fortified at 0.1 and 1.0 μg/L. Recoveries for all waters were 80% (CV = 7%, $n = 51$), 94% (CV = 4%, $n = 52$), and 93% (CV = 9%, $n = 51$) for mesotrione, MNBA, and AMBA, respectively (**Table 3**). Recovery data are corrected for the amount of background found in unfortified control samples.

Recovery of all analytes from all matrixes did not vary depending on fortification concentrations. Recovery of polar analytes is often subject to absorptive losses on surfaces, resulting in diminished recoveries at lower fortification rates.

Table 3. Recovery of Mesotrione, MNBA, and AMBA from Water

analyte	water type	fortification level[a], (μg/L)	recovery, % mean	CV[b]	N[c]	range[d]
mesotrione	drinking	0.1	84	8.0	6	76–92
	river	0.1	77	6.1	9	71–84
		1.0	81	5.2	10	73–88
	ground	0.1	85	1.5	4	83–86
		1.0	84	1.8	4	83–86
	sea	0.1	74	5.1	9	67–78
		1.0	81	4.3	9	74–85
	all waters	0.1	78	8.1	27	67–92
		1.0	82	4.4	24	73–88
		0.1–1.0	80	6.8	51	
AMBA	drinking	0.1	97	4.8	6	89–102
	river	0.1	95	4.5	9	89–102
		1.0	95	5.3	11	89–102
	ground	0.1	102	10.3	4	92–115
		1.0	91	0.6	4	90–91
	sea	0.1	88	5.6	8	80–95
		1.0	87	16.3	9	78–108
	all waters	0.1	94	7.7	26	80–115
		1.0	92	10.5	25	67–108
		0.1–1.0	93	9.1	51	67–115
MNBA	drinking	0.1	93	2.7	6	91–97
	river	0.1	91	3.9	9	84–97
		1.0	95	4.4	11	88–102
	ground	0.1	97	0.8	4	96–98
		1.0	97	2.0	4	94–98
	sea	0.1	92	4.3	9	87–99
		1.0	93	5.8	9	85–102
	all waters	0.1	93	3.9	27	74–99
		1.0	95	4.6	25	85–102
		0.1–1.0	94	4.3	52	84–102

[a] Amount added, each analyte. [b] CV, coefficient of variation. [c] N, number of replicates. [d] Range of individual recovery values.

This phenomenon was not in evidence here. There is, however, a noticeable and consistent drop in recovery of mesotrione, compared to the other two analytes, with recoveries of mesotrione averaging approximately 10−15% less than those of MNBA or AMBA. This is easily explained by the nature of the method. AMBA is efficiently extracted from soil and measured directly, or measured by direct aqueous injection in the case of the determination in water. MNBA is measured indirectly and recovery is in part based on the MNBA−AMBA reduction reaction. However, the determination of mesotrione relies on two reactions, the mesotrione-to-MNBA oxidation, and followed by the MNBA-AMBA reduction. As neither of these reactions are 100% efficient, recoveries of mesotrione can be expected to be lower.

Limits of Quantitation and Detection. The limits of quantitation (LOQ) for mesotrione and MNBA in crops is 0.01 mg/kg. The LOQ for all three analytes in soil was conservatively set at 0.005 mg/kg. The LOQ of the three analytes in water has been shown to be 0.1 μg/L. These LOQs were defined as the lowest analyte concentration at which the method was validated (eg., demonstrated good recovery with robust performance). The target LOQ values were based on expected or proposed regulatory tolerances and were not based on limitations of the method or instrumentation. An examination of the chromatograms in **Figures 2**, **3**, and **4** gives an indication that an LOQ based on method performance may, in fact, be lower than the values given above. Especially true for the soil and water analysis, a visual examination of the signal-to-noise ratio is indicative of this. In addition, the recovery data in **Tables 1**−**3** indicate that recoveries do not diminish with lower fortification rates, again pointing to the possibility that actual performance-based LOQ may be lower than these reported values.

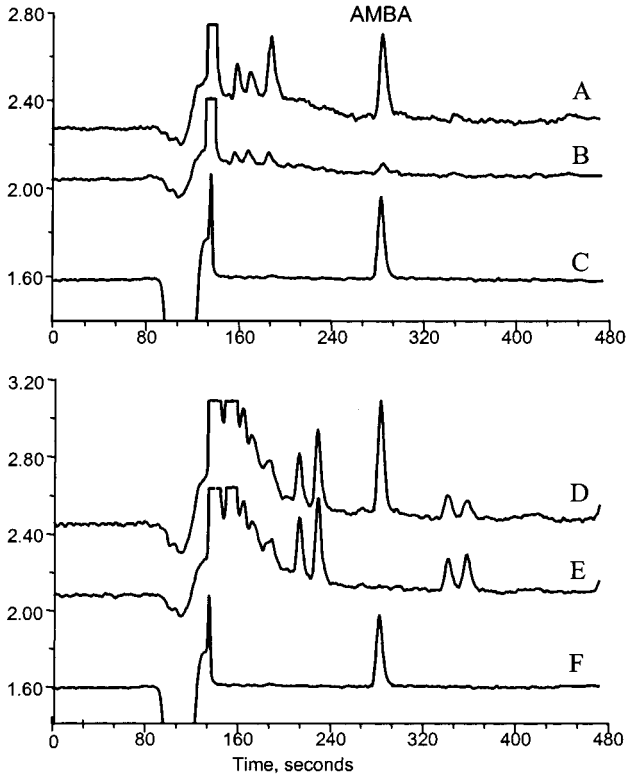

Figure 3. Final HPLC chromatograms of MNBA and mesotrione in corn fodder. (A) Determination of AMBA converted from mesotrione isolated from extract of corn fodder fortified at 0.01 mg/kg, recovery 79% (0.02 g crop/mL of extract). (B) Determination of AMBA converted from mesotrione fraction isolated from unfortified corn fodder control extract (0.02 g crop/mL of extract). (C) AMBA calibration standard 0.1 ng/mL (0.158 mesotrione equivalents/mL). (D) Determination of AMBA converted from MNBA isolated from extract of corn fodder fortified at 0.01 mg/kg, recovery 81% (0.02 g crop/mL of extract). (E) Determination of AMBA converted from MNBA fraction isolated from control corn fodder extract (0.02 g crop/mL) of extract. (F) AMBA calibration standard 0.1 ng/mL (0.114 MNBA equivalents/mL).

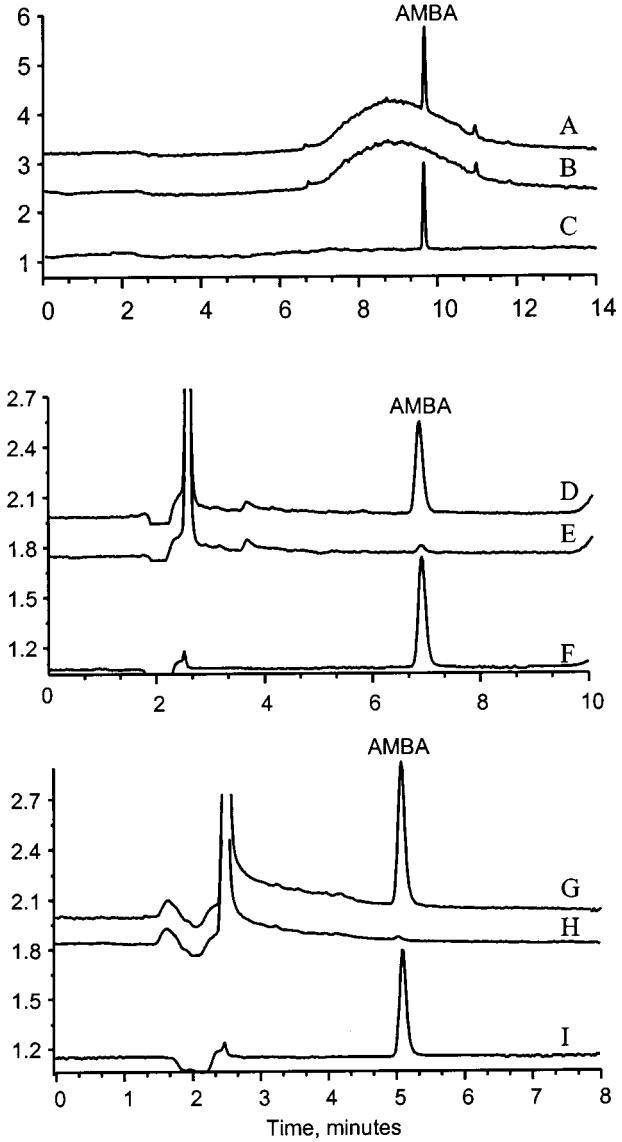

Figure 4. Final HPLC chromatograms of AMBA, MNBA, and mesotrione in river water. (A) Determination of AMBA by direct aqueous injection of 5.0 mL of river water fortified at 0.1 μg/L each analyte, recovery of AMBA 94%. (B) Direct injection of 5.0 mL of unfortified river water control. (C) AMBA calibration standard for quantitation of AMBA residue, 0.10 μg/L. (D) Determination of AMBA converted from mesotrione isolated from seawater fortified at 0.1 μg/L, 77% recovery. (E) Determination of AMBA converted from mesotrione fraction isolated from 5.0 mL of unfortified seawater control. (F) AMBA calibration standard 0.3 ng/mL (0.473 ng mesotrione equivalents/mL). Initial 13% B mobile phase. (G) Determination of AMBA converted from MNBA isolated from 5.0 mL of seawater fortified at 0.10 μg/L, 93% recovery. (H) Determination of AMBA converted from MNBA fraction isolated from unfortified seawater control. (I) AMBA calibration standard 0.3 ng/mL (0.342 ng MNBA equivalents/mL). Initial 16% B mobile. All chromatograms monitored using fluorescence detection as indicated in text. Final extracts in D, E, G, and H contained 5.0 g of water per mL of extract.

The limit of detection (LOD) for this method was not specifically determined. LODs are oftentimes subject to the performance of the instrumentation in an individual laboratory. However, estimation of LODs for this method may be made based on the signal-to-noise ratio in the chromatograms. On the basis of chromatograms from LOQ-level fortifications, LODs of 0.002 mg/kg for crops ($^1/_5$ the crop LOQ), 0.0005 mg/kg for soil ($^1/_{10}$ the soil LOQ), and 0.01 μg/L for water ($^1/_{10}$ the water LOQ) have been estimated.

Method Selectivity and Sources of Interference. A significant advantage to this method is the selectivity it provides for a variety of matrixes and at low concentrations. The ability of the method to selectively determine the analytes of interest over numerous other endogenous species is inherent to the design of the method and is due to a number of factors. In this method a crude extract is injected onto a highly efficient RPHPLC system and a small fraction of HPLC eluate is collected. The fraction is subjected to (in the case of mesotrione) two separate reactions and converted to a fluorescent species and rechromatographed a second time for the final determination. For an individual co-extractive to ultimately interfere with the final determination of AMBA, the following must take place. The species must (1) coelute in the collected fraction with the analyte during the initial HPLC cleanup, (2) survive the oxidation and/or reduction steps intact, or be created by the reactions, (3) be fluorescent or converted to a fluorescent species during the reactions, and (4) must coelute with AMBA during the final HPLC determination.

For the crop commodities, background ranged from 0 to 16% of the LOQ (LOQ = 0.01 mg/kg). However, the highest level of background observed was as likely to be due to reagent contamination as it was due to sample coextractives or analyte contamination. As a result, it is recommended that sample sets

include three types of controls: (a) unfortified sample, (b) reagent control, i.e., water injected into the HPLC during sample fractionation and then carried through as a sample (for both mesotrione and MNBA determination) to the end of the method, and (c) reaction blanks, i.e., one empty vial that is subjected to the mesotrione oxidation and reduction reactions, and one empty vial subjected to the MNBA reduction reaction; both vials carried through as a sample to the end of the method. In analyzing these three types of control samples, it is possible to determine the source of any excessive (>20% of the LOQ) background.

Demonstrating Analyte Isolation. This method involves the conversion to, and quantitation of, the common moiety AMBA. As a result, the isolation of mesotrione and MNBA from each other and from trace levels of AMBA that may be present in a sample is critical to the accuracy of the method. Upon initial use of the method it should be demonstrated that, in addition to adequate recovery of analyte fortified into control samples (method recovery), the presence of one analyte does not interfere (cause false positives) with the other. This can be accomplished by fortifying individual control extracts with mesotrione or MNBA. The mesotrione fraction (fraction B) of the sample fortified with MNBA can be analyzed for mesotrione, and shown to be free from interference. The MBNA fraction (fraction A) of the sample fortified with mesotrione, can be analyzed in a similar manner. Once isolation of the analytes and freedom from interferences is confirmed, method recovery can be demonstrated by the fortification of both analytes into a single unfortified control sample.

Determination of Conversion Efficiencies. Prior to running this method using actual samples, the analyst should determine the conversion efficiency using standards to perform the conversion reactions (mesotrione → MNBA → AMBA, and MNBA → AMBA), as neither reaction is 100% complete. In the authors' laboratory, conversion efficiency of mesotrione to AMBA was 85−90% and conversion efficiency of MNBA to AMBA was 90−95%. When starting this procedure, using an appropriate-sized syringe, transfer a 100-μL aliquot of a 0.10 μg/mL working fortification solution to a 4-mL, silanized vial. Prepare four replicates of mesotrione and four separate replicates of MNBA. Evaporate the aliquot to dryness under a stream of nitrogen**. Do not use heat for this evaporation. Do not over dry.** Remove from stream of nitrogen just after dryness is obtained. Proceed with the reactions as described in the method. After the determination of the mesotrione or MBNA equivalents resulting from the conversion of the resultant AMBA, calculate the conversion efficiency. For example, the conversion of 0.01 μg of mesotrione (100 μL × 0.10 μg/mL) at an 85% conversion rate should result in a final solution (1.0 mL) with an AMBA concentration of (0.01 × 215)/(339 × 0.85) or 0.0062 μg/mL. Conversion efficiencies should be in the ranges given above with CVs of 3−5%. The entire method should not be attempted until proficiency with this critical step is demonstrated.

Reagent Stability. The catalase reagent and the stannous chloride reagent should both be prepared fresh daily. All other reagents can be prepared on a monthly basis. The stannous chloride powder should dissolve easily in concentrated HCl. It should remain in solution after addition of the water. If at any point in its preparation or use the stannous chloride solution is cloudy it should be discarded, and a new supply of stannous chloride powder should be obtained. The catalase powder should be stored at a temperature of <0 °C. Once opened, the peroxide test strip container should be given an expiration date of one month. Mesotrione and MNBA standard solutions in methanol are stable for a period of one year, stored at <5 °C. AMBA standards in water are also stable for one year if stored at <5 °C.

Alternate Chromatographic Conditions. Two suggested mobile phase changes may help when there are interfering coextractives observed during the final analysis or there is poor resolution for the analytes during the HPLC cleanup. The HPLC parameters for the final analytical determination call for an isocratic elution of AMBA at a mobile phase composition of 16% B, followed by a rapid post-elution gradient to wash the column of co-extractives and reaction byproducts. During the method development process some samples were analyzed using a solvent program starting at an initial B of 13%, which provided an increase in resolution between AMBA and some co-extractives. As a result, 16% B is the recommended starting point for the gradient, but in the case of unresolved co-extractives, a lower initial % B value may be useful. To increase the resolution between MNBA and AMBA during the HPLC cleanup, increase the ionic strength of mobile phase A. Increasing the ammonium acetate concentration to 15−20 mM will decrease the retention time of MNBA, with the retention time of AMBA remaining virtually unchanged.

Selection of C18 Media. The use of the Inertsil C18 stationary phase was selected specifically for the initial HPLC cleanup step. When chromatographed with a number of other packing materials, mesotrione had a propensity to produce severely tailing peaks. This was attributed to the ability of mesotrione to chelate metals such as copper and zinc. Secondary mechanisms such as increased retention due to chelating with metal impurities present in the silica backbone may be responsible for the tailing. A switch to a column packing produced with extremely pure silica (>99.995%) such as the Inertsil, significantly reduced the amount of tailing. The elution of mesotrione in a narrow chromatographic band allows for a smaller collection window and inclusion of fewer co-extractives in the collected fraction. There was also some evidence during the method development process that the elimination of the peak tailing increased method recovery at low concentrations.

The choice of the Sperisorb packing material for the final AMBA determination was primarily based on the selectivity provided for the analysis of corn commodities. When a side-by-side comparison was made, comparing Inertsil to Spherisorb for the final determination of crop commodities, the Spherisorb provided greater resolution between AMBA and the co-extractive.

Elution Profile of MNBA During HPLC Cleanup. Of the three analytes, MNBA is the least hydrophobic, and is not easily retained on reverse-phase packings. During HPLC cleanup the MNBA peak is subject to distortions due to co-injected components of the extract or sample, including residual solvent (acetonitrile in crop extracts) and high concentrations of salt (injections of seawater). Large amounts of crop and soil co-extractives may also act to overload the column and contribute to band broadening of MNBA. An example of this behavior is shown in **Figure 5**. Here, seawater is spiked with levels of the three analytes that can be observed by UV detection. It is important to note that although the high salt content distorts the peak shape of MNBA, the retention time of MBNA is not altered significantly. In this case, only a small shift in the fraction collection window is suggested, but not required. In the authors' laboratory this distortion did not produce any adverse effects. However, when recoveries of MNBA are low, or when new sample matrixes are attempted, this spiking experiment may prove useful to a new user of this method.

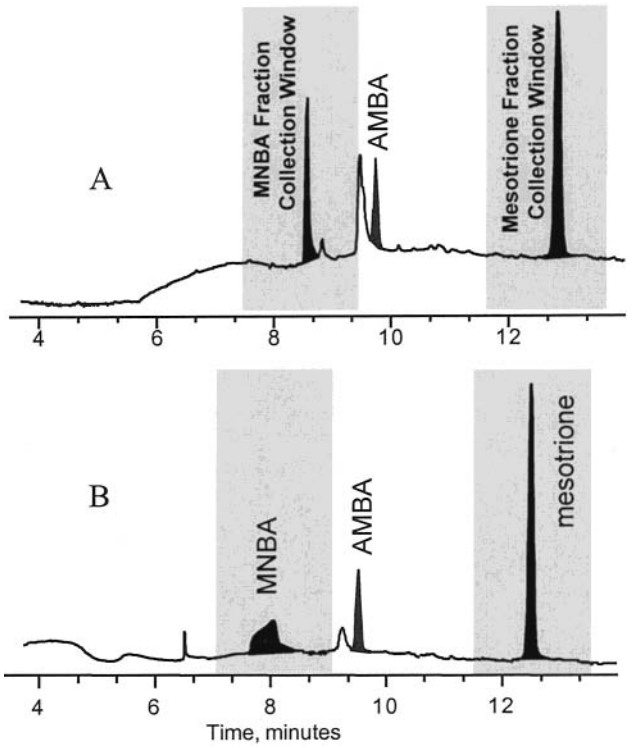

Figure 5. Determination of fraction collection windows and effect of large aqueous injections on HPLC cleanup chromatographic profile. (A) Direct aqueous injection (5.0 mL) of UV calibration solution containing 0.2 μg/mL of each analyte prepared as described in text for determination of analytes in water. (B) Direct aqueous injection (5.0 mL of seawater fortified with each analyte at 0.2 μg/mL) The longer retention times (compare to those of **Figure 1A**) are due to increased system dwell time after addition of a 5.0-mL injection loop on the manual injection valve. Analytes were monitored at 254 nm.

Sample Throughput and Logistics. This method is designed to be conducted over a two-day period. On day one, samples are extracted and an automated HPLC cleanup is conducted, usually overnight. Approximately 4−6 h are required for the analyst to perform the tasks leading up to the start of the overnight cleanup (and direct AMBA residue analysis in water and soil). On day two, 6−8 h are required to evaporate fractions, perform the conversion reactions, run the postreaction SPE cleanup, and set up the HPLC for the final determination of the AMBA reaction products, again usually an automated overnight analysis. Once an analyst is experienced with the method, determinations on 8−12 samples can be performed in this time frame. If only residues of mesotrione are to be determined, the time required will be less. Work on the method can be stopped at various points throughout the procedure for up to an hour. Stopping work at certain points should be avoided. These include (1) after evaporation of HPLC cleanup fraction, because after these fractions have been evaporated the conversion reactions should be carried out; and (2) after addition of the sample aliquot to the C18 SPE column, as the columns should be washed with the two 1.5-mL wash volumes as soon as possible.

Critical Success Factors. The following items are considered to be critical to the success of this residue method: (1) Understanding of the basic method structure and principles of this common moiety method. (2) Successful conduct of the conversion reactions, with conversion efficiencies and precision as outlined above in Demonstrating Conversion Efficiencies. Do not attempt the entire method until proficiency in these procedures are demonstrated. (3) Attempting individual portions of the method prior to determinations of all analytes at once. A reasonable approach would be to master the MBNA-to-AMBA conversion, followed by the mesotrione-to-MNBA-to-AMBA conversions. Then one can attempt an entire MBNA determination followed by the entire mesotrione determination.

SAFETY

Personnel untrained in the routine safe handling of chemicals and good laboratory practices should not attempt to use this procedure. Information of any specific chemical regarding physical properties, hazards, toxicity, and first-aid procedures can be found on the Material Safety Data Sheet accompanying the chemical (available from the supplier). In general, always wear safety glasses with side shields, work in a well-ventilated area, avoid inhaling vapors, and avoid contact of the chemicals with skin and clothing. Specific hazards include the use of hydrogen peroxide. The 30% reagent is a strong oxidizer and should be kept away from organic material or other sources of combustion. Flammable solvents used in the method include methanol and acetonitrile.

ACKNOWLEDGMENT

We acknowledge the efforts of Marilyn Ablang, Patrica Iwata, Charlotte Peyton, and Steven Schmidt who were instrumental in troubleshooting and refining this method, offering critically needed input under extremely tight timelines.

LITERATURE CITED

(1) Mitchell, G.; Bartlett, D. W.; Fraser, T. E.; Hawkes, T. R.; Holt, D. C.; Townson, J. K.; Wichert, R. A. Mesotrione: A new selective herbicide for use in maize. *Pest. Manage. Sci*. **2001**, *57*, 120−128.

(2) Lee, D. L.; Prisbylla, T. H.; Cromartie, T. H.; Dargarin, D. P.; Howard, S. W.; Provan, M. P.; Ellis, M. K.; Fraser T. E.; Mutter, L. C. The discovery and structural requirements of inhibitors of p-hydroxyphenylpyruvate dioxygenase. *Weed Sci*. **1997**, *45*, 601−609.

(3) Rouchaud, J.; Thirion, D.; Callens, D.; Bulcke, R. Soil dissipation of the post-emergence herbicide sulcotrione in maize crops treated with organic fertilizers. *Bull. Environ. Contam. Toxicol.* **1996**, *57*, 398−405.

(4) Rouchaud, J.; Neus, O.; Bulcke, R.; Cools, K.; Eelen, H. Sulcotrione soil metabolism in summer corn crops. *Bull. Environ. Contam. Toxicol.* **1998**, *61*, 669−676.

(5) Baer, U.; Calvet, R. Fate of soil applied herbicides: experimental data and prediction of dissipation kinetics. *J. Environ. Qual.* **1999**, 28, 1765−1777.

(6) Bielenstein, M.; Ekberg, S. Determination of 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione in plasma by direct injection into a coupled column liquid chromatographic system. *J Chromatogr. B, Biomed. Sci. Appl.* **1999**, *730*, 177−182.

Received for review December 21, 2001. Revised manuscript received March 31, 2002. Accepted April 1, 2002.

JF011696Y