

Determination of 22 Triazole Compounds Including Parent Fungicides and Metabolites in Apples, Peaches, Flour, and Water by Liquid Chromatography/Tandem Mass Spectrometry

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A liquid chromatography/tandem mass spectrometry (LC/MS/MS) method has been developed for the determination of 14 parent triazole fungicides and 8 of their metabolites found in apples, peaches, flour, raw water, and tap water. The triazole fungicides chosen for this multiresidue method development project included propiconazole, fenbuconazole and its RH-9129 and RH-9130 metabolites, cyproconazole, difenoconazole, tebuconazole and its HWG 2061 metabolite, hexaconazole, bromuconazole (both stereoisomers), epoxiconazole, tetraconazole, triticonazole and its RPA-404886 and RPA-406341 metabolites, triadimefon, triadimenol, and myclobutanil. Of special concern to the U.S. Environmental Protection Agency were the metabolites common to all triazole fungicides: free triazole, 1,2,4-triazole (T), and its 2-conjugates: triazolylalanine (TA) and triazolylacetic acid (TAA). These metabolites were the primary focus of this project. All samples were cleaned up by a combination of C18 solid-phase extraction (SPE), mixed-mode cationic SPE, and mixed-mode anionic SPE columns. A triple-stage quadrupole mass spectrometer, equipped with electrospray ionization in the positive-ion mode, was used to determine the compounds of interest. T, TA, and TAA were quantitated using isotopically labeled internal standards (IS), in which the 1,2,4-triazole ring had been synthesized by using ^{13}C and ^{15}N (IS_T, IS_TA, and IS_TAA). These isotopically labeled internal standards were necessary to correct for matrix effects. The T, TA, and TAA metabolites were quantitated at the 25–50 parts-per-billion (ppb) level in food commodities

and at 0.50 ppb in water. Recoveries were 70–101% from apples, 60–121% from peaches, 57–118% from flour, 75–99% from raw water, and 79–99% from tap water.

Congress passed the Food Quality Protection Act (FQPA) in August 1996. FQPA amended the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug and Cosmetic Act (FFDCA). The U.S. Environmental Protection Agency (EPA) registers pesticides for use in the United States under FIFRA and prescribes labeling and other regulatory requirements to prevent unreasonable adverse effects on the environment. Such effects are defined to include human dietary risk from pesticide residues. Under FFDCA, EPA establishes tolerances (maximum legally permissible levels) for pesticide residues in foods. In order to set tolerances, FQPA mandates a single health-based standard for all pesticides in all foods and provides special protections for infants and children. That standard is “a reasonable certainty that no harm will result from aggregate exposure to pesticide chemical residue, including all anticipated dietary exposures.” Under FQPA, both dietary exposure data and drinking water exposure data are needed for risk assessment analysis.

The triazole class of fungicides includes a variety of compounds that contain the 1,2,4-triazole moiety. The triazoles are an important class of systemic fungicides for the control and treatment of a wide range of fungal diseases on fruit, vegetable, nut, legume, and grain crops. There are 9 triazoles with U.S. crop tolerances and a number of others with import tolerances or pending import tolerances. Triazole fungicides have 3 common metabolites: 1,2,4-triazole (T), triazolylalanine (TA), and triazolylacetic acid (TAA); their structures are shown in Figure 1. Because the 3 common triazole metabolites had not been fully evaluated under FQPA, the EPA Office of Pesticide Programs determined that additional data would be needed for

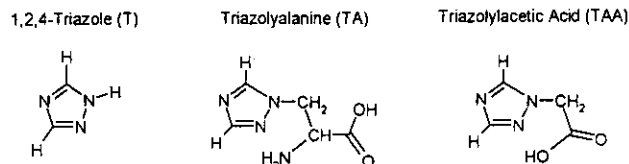


Figure 1. Chemical structures of the 3 common triazole metabolites of major concern.

re-registrations or new registrations of triazole fungicides. One data need identified by the EPA Health Effects Division (HED) was for dietary exposure monitoring of T, TA, and TAA in foods that are important in the diets of children.

In September 2002, the U.S. Triazole Task Force (USTTF) was formed to address the data needs of EPA. The founding members of the USTTF were Bayer CropScience LP, Dow AgroSciences, Sipcarn Agro USA, and Syngenta Crop Protection. BASF joined the USTTF in 2004. The goals of the USTTF were to provide the data needs identified by EPA, and to ensure that the triazole derivative fungicides remain available to U.S. product agriculture for the control of many crop diseases.

EPA needed a method capable of identifying and quantitating the 3 metabolites of major concern: T, TA, and TAA. Several methods appear in the literature for the quantitation of many parent triazoles (1–6), but there are none for the 3 common triazole metabolites. Five of the metabolites investigated in this study behaved so similarly to the parents (the fenbuconazole metabolites RH-9129 and RH-9130, the tebuconazole metabolite HWG-2061, and the triticonazole metabolites RPA-404886 and RPA-406341) that they were grouped together and processed in the same procedure used for the parents. The 3 common metabolites are polar compounds of low molecular weight and therefore behave very differently from all the other compounds. This difference led to additional chromatographic challenges both in the solid-phase extraction (SPE) cleanup procedures and in the instrumental analysis.

Experimental

Apparatus

(a) *Liquid chromatography (LC) system.*—Waters Alliance Separations Module 2695, P/N WAT270008; equipped with a Micromass Quattro Micro triple-stage quadrupole mass spectrometer, interfaced to Masslynx NT v 3.5 (Micromass) data processing software (Waters Corp., Milford, MA), or equivalent.

(b) *LC column.*—Waters Symmetry C18 (4.6 × 250 mm, 5.0 μm), P/N WAT054275 (Waters Corp.).

(c) *SPE Columns.*—Oasis[®] MCX, 6 cc, 500 mg, LP, extraction cartridges, P/N 186000776 (Waters Corp.); Oasis MAX, 6 cc, 500 mg, LP, extraction cartridges, P/N 186000865 (Waters Corp.); Supelclean[™] LC-18 (12 mL, 2000 mg) P/N 57117 (Supelco, Inc., Bellefonte, PA); SPE

vacuum manifold, Supelco Visiprep DL, P/N 5-7044 (Supelco), or equivalent; reservoirs and adaptors for SPE columns (Supelco), or equivalent.

(d) *Nitrogen evaporator, 12 sample.*—550-55EC, N-Evap-111 (Organomation Associates, Inc., Berlin, MA), or equivalent.

(e) *Nitrogen evaporator.*—TurboVap[®] LV concentrator (Zymark Corp., Hopkinton, MA).

(f) *Homogenizer.*—PowerGen 700 (polytron) equipped with a saw-toothed stainless steel rotor-stator generator (Fisher Scientific, Pittsburgh, PA), or equivalent.

(g) *Shaker.*—Orbital (Lab-Line Instruments, Inc., Melrose Park, IL), or equivalent.

(h) *Centrifuge.*—Jouan, Model C244 (Jouan Inc., Winchester, VA) or equivalent.

(i) *Food processor.*—Robot Coupe Model R 301 Ultra (Robot Coupe USA, Inc., Ridgeland, MS), or equivalent.

(j) *Vials.*—Screw-neck, bonded, pre-slit with silicone septa, PTFE, 12 × 32 mm, P/N 186000307 (Waters Corp.), or equivalent.

(k) *Gases.*—Liquid nitrogen dewar (for mass spectrometer) or nitrogen generator (Parker Balston, Model 75-A74, Tewksbury, MA, equipped with Atlas Copco stationary air compressor, Model SF4, Antwerp, Belgium), or equivalent, and argon (collision gas).

(l) *Büchner funnel.*—Coors, porcelain with fixed perforated plate, plate diameter 100 mm, 170 mm high, P/N 60243 (Fisher Scientific), or equivalent.

(m) *Filter paper.*—Glass microfiber filters GF/A, 90 mm, P/N 1820 090 (Whatman Inc., Clifton, NJ), or equivalent.

(n) *Culture tubes.*—Disposable, borosilicate glass, 20 × 150 mm, 20 mL, Kimax, P/N 60825-447 (VWR Scientific Products, San Francisco, CA), or equivalent.

(o) *Syringes.*—Disposable, plastic, 5 mL, Luer-Lok, P/N BD301027 (Becton Dickinson, Sparks, MD), or equivalent.

(p) *Syringe filter.*—Disposable, 13 mm, PVDF, 0.45 μm pore size, P/N 6777-1304 (Whatman, Clifton, NJ), with tube tip (optional), or equivalent.

(q) *Polypropylene wide-mouth bottles.*—Nalgene, 8 oz/250 mL capacity, polypropylene (pp), screw-cap, P/N 02-893B (Fisher Scientific), or equivalent.

(r) *Flasks.*—Erlenmeyer with side arm, 500 mL, Kimax, P/N 27060-500 (Fisher Scientific), or equivalent; and 250 mL Erlenmeyer flask, graduated, with screw-cap, Pyrex[®], P/N 4985-250 (Fisher Scientific), or equivalent.

(s) *Mixer.*—Vortex Touch Mixer, Model 232 (Fisher Scientific), or equivalent.

(t) *Centrifuge tubes.*—13 mL, graduated, glass, conical, with stopper, Kimble P/N 45176-13, or equivalent; 50 mL, graduated, glass, conical, with stopper, Kimble, P/N 45176-50, or equivalent; and 50 mL, graduated, polypropylene, disposable, P/N 430828 (Corning Inc., Corning, NY), or equivalent.

Reagents

(a) *Methanol (MeOH).*—OPTIMA grade (Fisher Scientific), or equivalent.

(b) *Water*.—LC grade (Fisher Scientific), or equivalent.

(c) *Acetonitrile (ACN)*.—LC grade (Fisher Scientific), or equivalent.

(d) *Acetic acid*.—Glacial (Fisher Scientific, Cat. No. A35-500), or equivalent.

(e) *Ammonium hydroxide (NH₄OH)*.—Reagent ACS (Fisher Scientific, Cat. No. A669-212), or equivalent.

(f) *Formic acid (FA)*.—88% (Fisher Scientific, Cat. No. A118P-500), or equivalent.

(g) *Reference standards*.—100 mg quantities of all analytical reference standards were obtained from the EPA National Pesticide Standard Repository (Environmental Science Center, 701 Mapes Rd, Ft. George G. Meade, MD) and from Sigma-Aldrich Chemical Co. (Milwaukee, WI).

(h) *Isotopically labeled internal standards*.—50 mg quantities of all isotopically labeled internal standards were obtained from Bayer Corp. (Stilwell, KS). Bayer Corp. has agreed to make the isotopically labeled internal standards (T, TA, and TAA) available to state or federal agencies for regulatory investigations via the EPA National Pesticide Standard Repository located at Fort Meade, MD. This arrangement is in support of Bayer Crop Science Registrations.

(i) *Stock standard solutions*.—(1) Stock standard solutions (500 µg/mL) prepared in MeOH, except for TA prepared in MeOH-water (40 + 10, v/v), 500 µg/mL. Accurately weigh 25 mg (corrected for purity) of each triazole standard separately into a separate 50 mL volumetric flask. Dilute to volume with MeOH, except for TA (see above). Store refrigerated. Solutions are stable for 1 year. (2) Stock internal standard (IS) solutions of isotopically labeled IS_T, IS_TA, and IS_TAA (500 µg/mL) prepared in water. Accurately weigh 25 mg (corrected for purity) of each isotopically labeled internal standard separately into a separate 50 mL volumetric flask. Dilute to volume with water. Store refrigerated. Solutions are stable for 1 year.

(j) *Fortification solutions*.—(1) 22 mixed standard fortification solution (5.0 µg/mL), prepared in MeOH. Pipet 1.0 mL of each stock standard (500 µg/mL) into a 100 mL volumetric flask. Dilute to volume with MeOH (5.0 µg/mL 22 mixed standard triazoles). Pipet 10 mL of the 5.0 µg/mL 22 mixed standard triazoles into a 50 mL volumetric flask, and dilute to volume with MeOH (1.0 µg/mL 22 mixed standard triazoles). Store refrigerated. Solutions are stable for 1 year. (2) Mixed fortification solutions of 3 isotopically labeled internal standards (3*_IS), prepared in water. Pipet 1.0 mL of each isotopically labeled stock internal standard (500 µg/mL) into a 100 mL volumetric flask. Dilute to volume with water [5.0 µg/mL (3*_IS) triazoles]. Pipet 10 mL of 5.0 µg/mL (3*_IS) into a 50 mL volumetric flask, and dilute to volume with water [1.0 µg/mL (3*_IS) triazoles]. Store refrigerated. Solutions are stable for 1 year.

(k) *Chromatographic calibration standards*.—Prepare calibration standards ranging from 0.001 to 0.1 µg/mL 22 mixed standard solution, each containing 0.10 µg/mL (3*_IS). Calibration standard, are prepared in MeOH-water (1 + 1, v/v). Store refrigerated. Solutions are stable for 1 year.

(l) *Mobile phase solutions*.—(1) 0.2% formic acid in water. Using a 1000 mL volumetric flask, fill half full with water, add 2 mL formic acid, and dilute to volume with water.

(2) 0.2% formic acid in MeOH. Using a 1000 mL volumetric flask, fill half full with MeOH, add 2 mL formic acid, and dilute to volume with MeOH. Solutions are stable for 1 year.

(m) *Elution solutions for MCX and MAX SPE columns*.—(1) 1% NH₄OH in MeOH. Using a 500 mL volumetric flask, partially fill with MeOH, add 5 mL NH₄OH, and dilute to volume with MeOH, for MCX elution. (2) 1% acetic acid in MeOH. Using a 500 mL volumetric flask, partially fill with MeOH, add 5 mL acetic acid, and dilute to volume with MeOH, for MAX elution.

(n) *Sample extraction solutions*.—(1) Apples: MeOH-water (1 + 1, v/v). A set of 6 samples requires approximately 2 L solution (mix 1 L MeOH with 1 L water). (2) Peaches: ACN and ACN-water (25 + 75, v/v) for SPE cleanup. (3) Flour: MeOH-water (80 + 20, v/v). A set of 6 samples requires approximately 2 L solution (mix 1600 mL MeOH with 400 mL of water).

Control Sample Acquisition and Preparation

Fresh crops were purchased from local organic food markets when available. When organic produce was not available, crops were purchased from other local supermarkets. Samples were prepared in accordance with the *Pesticide Analytical Manual (PAM)*, Volume I, Section 102 (7). Fruits were comminuted in a commercial processor. The composites were stored at temperatures of ≤60°C in capped 50 mL polypropylene centrifuge tubes. The samples were thawed to room temperature before usage.

The flour (all-purpose, enriched, bleached, and presifted) was purchased from a local grocery store.

The water was collected from a variety of sources. Raw, prechlorinated water (Patuxent Filtration Plant, Laurel, MD), tap water (Fort Meade, MD); and LC grade water were used for this development. Chlorinated water from the water treatment plant that contained chlorine levels of >1 parts per million (ppm) destroyed the TA. See the *Results and Discussion* section for proposed degradation pathways.

Apple Procedure

(a) *Extraction*.—Weigh 10.00 ± 0.05 g processed apples into a tared 250 mL Nalgene (pp) bottle. Fortify, for recovery studies, with 0.25 mL 1.0 µg/mL 22 mixed standard fortification solution. This is equivalent to 25 ppb in apples. Add 60 mL MeOH-water (1 + 1, v/v). Homogenize ca 1–2 min, using a polytron (speed control set between 3 and 4 on PowerGen Model 700); rinse the probe with MeOH-water (1 + 1, v/v) from a wash bottle. Centrifuge at 4000 rpm (3080 × g) for 10 min. Decant the supernatant through a glass microfiber GF/A filter into a 500 mL Erlenmeyer vacuum flask. Repeat the procedure by adding another 60 mL MeOH-water (1 + 1, v/v), centrifuge, and decant through the same glass microfiber GF/A filter, combining the supernatants in the 500 mL Erlenmeyer flask. (Note: Do not rinse the apple extract onto the filter paper. It

will not filter readily.) Transfer the extract to a 250 mL graduated cylinder equipped with stopper. Fortify all samples (include controls) using 1.0 mL of a 1.0 µg/mL (3*_ IS) internal standard fortification solution. Dilute to 200 mL with MeOH–water (1 + 1, v/v), stopper, and shake thoroughly to mix. Transfer a 20 mL aliquot of the apple extract onto a conditioned Supelclean LC-18 SPE column (*see* below). The internal standards are designed to correct for instrumental variation or signal suppression and not extraction efficiency in this case.

(b) *C18 SPE cleanup*.—Condition Supelclean LC-18 SPE column with 1 column volume of MeOH, followed by 1 column volume of apple extraction solvent, MeOH–water (1 + 1, v/v). Discard the conditioning solvents. Load 20 mL aliquot of apple extract. Collect the eluate in a clean disposable culture tube. Rinse the column with 5 mL MeOH–water (1 + 1, v/v), use vacuum to completely drain column, and combine eluates in the disposable culture tube. Save combined eluates for the next SPE step (*see* Oasis MCX and Oasis MAX procedures). The parent triazoles (and 5 of 8 metabolites) are retained on the LC-18 column. Elute parent triazoles and remaining metabolites with 20 mL MeOH into a clean disposable culture tube. Evaporate to dryness in TurboVap (T = 45°C). Redissolve the sample in 1.0 mL MeOH, mix on a Vortex mixer, and add 1.0 mL water; mix on a Vortex mixer (final volume of C18 fraction = 2.0 mL) and filter (0.45 µm, PVDF) if necessary. Assay by liquid chromatography/tandem mass spectrometry (LC/MS/MS).

(c) *MCX, MAX SPE cleanup*.—Condition Oasis MCX and Oasis MAX SPE columns with 2 column volumes of MeOH, followed by 2 column volumes of water. Discard conditioning solvents. “Piggyback” the conditioned SPE columns in tandem (MCX on top of MAX); use a reservoir to hold the sample. (*Note*: Fill each stacked MCX, MAX SPE column 3/4 full with water before loading the sample into the reservoir to aid flow.) Load the sample. Use a slight vacuum or gravity feed with positive pressure to drain the SPE columns at a flow of 1–2 drops/s. Discard the eluate. Rinse the columns with 2 column volumes of water followed by 2 column volumes of MeOH. (*Note*: A 1 column volume of MeOH improves the recovery of triadimefon.) Discard the eluate. Separate “piggybacked” columns. Use positive pressure to drain the MAX and MCX SPE columns for elution. T, IS_T, TA, and IS_TA are retained on the MCX column. Elute MCX column with 20 mL 1% NH₄OH in MeOH (v/v). Collect eluate in a clean disposable culture tube (cation fraction). TAA and IS_TAA are retained on the MAX column. Elute MAX column with 20 mL 1% acetic acid in MeOH (v/v). Collect eluate in a clean disposable culture tube (anion fraction). Evaporate each fraction (MAX, MCX) to ca 2–4 mL, using the TurboVap (T = 45°C). (*Note*: Do not allow the MCX, cation fraction, to go to dryness.) In contrast, the MAX (anion fraction) may go to dryness during this step. Redissolve the MAX (anion fraction) with 1.0 mL MeOH–water (1 + 1, v/v). Filter, if necessary (0.45 µm, PVDF), directly into an autosampler vial. Assay by LC/MS/MS. Transfer the sample from the MCX (cation fraction) disposable culture tube to a

clean 13 mL graduated centrifuge tube. Rinse the tube with 1–2 mL MeOH, and add rinsings to the 13 mL graduated centrifuge tube. Evaporate the sample, using N-Evap (T = 45°C) to <0.5 mL. Adjust the volume to 0.50 mL MeOH. Add water to bring the final volume to 1.0 mL. mix on a Vortex mixer and filter, if necessary, (0.45 µm, PVDF) directly into an autosampler vial. Assay by LC/MS/MS. (*Note*: The concentration of the bracketing standard, for recovery studies, should be equivalent to the expected concentration of the final solution of the fortified sample. Bracketing standard concentration = 0.025 µg/mL for 25 ppb-fortified MCX and MAX fractions, or = 0.0125 µg/mL for 25 ppb-fortified C18 fractions.) Refer to Figure 2 for a flowchart of the procedure.

Peach Procedure

(a) *Extraction*.—Weigh 10.00 ± 0.05 g processed peaches into a 50 mL polypropylene disposable centrifuge tube. Fortify, for recovery studies, with 0.5 mL 1.0 µg/mL 22 mixed standard fortification solution. This is equivalent to 50 ppb in peaches. Add 20 mL ACN, and cap tightly. Shake tube 10 min, using an orbital shaker (375–400 rpm). Centrifuge at 4000 rpm (3080 × g) for 10 min. Decant supernatant into a clean 50 mL graduated glass centrifuge tube and stopper. Repeat the procedure by adding another 20 mL ACN to the 50 mL polypropylene disposable centrifuge tube containing the peaches and cap tightly. Shake (break up peach sample from previous centrifugation), centrifuge, and decant,

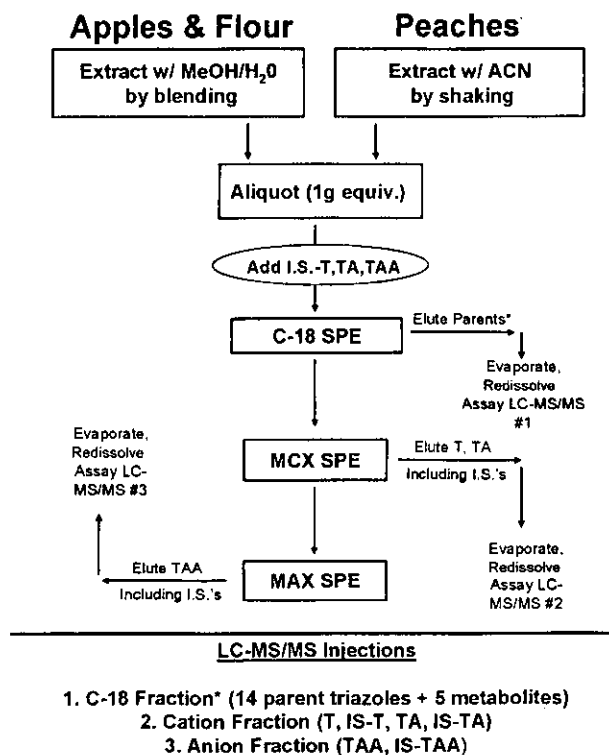


Figure 2. Flowchart for the extraction of apple, wheat flour, and peach commodities.

combining the supernatants in the 50 mL graduated glass centrifuge tube. Fortify all samples (include controls), using 1.0 mL of a 1.0 µg/mL (3*_IS) internal standard fortification solution. Dilute to 50 mL with ACN, stopper, and mix on a Vortex mixer or hand-shake. Transfer a 5 mL aliquot of the ACN peach extract to a clean 50 mL graduated glass centrifuge tube, add enough water to obtain a final volume of 20 mL, ACN-water (25 + 75, v/v), stopper, and mix on a Vortex mixer or handshake.

(b) *C18 SPE cleanup*.—Condition a Supelclean LC-18 SPE column with 1 column volume of MeOH, followed by 1 column volume of peach conditioning solvent, ACN-water (25 + 75, v/v). Discard the conditioning solvents. Load the 20 mL peach extract. Collect the eluate in a clean disposable culture tube. Rinse the sample glassware with 5 mL ACN-water (25 + 75, v/v), add to the column, use vacuum to completely drain, and combine eluates in the disposable culture tube. Save combined eluates for the next SPE step (see Oasis MCX and Oasis MAX procedures). The parent triazoles (and 5 of 8 metabolites) are retained on the LC-18 column. Elute parent triazoles and remaining metabolites with 20 mL MeOH into a clean disposable culture tube. Evaporate to dryness, using a TurboVap (T = 45°C). Redissolve the sample in 1.0 mL MeOH, mix on a Vortex mixer, add 1.0 mL water, mix on a Vortex mixer (final volume = 2.0 mL), and filter (0.45 µm, PVDF) if necessary. Assay by LC/MS/MS.

(c) *MCX, MAX SPE cleanup*.—See *Apple Procedure* for MCX, MAX SPE procedures. Assay by LC/MS/MS. (*Note*: The concentration of the bracketing standard, for recovery studies, should be equivalent to the expected concentration of the final solution of the fortified sample. Bracketing standard concentration = 0.05 µg/mL for 50 ppb-fortified MCX and MAX fractions, or = 0.025 µg/mL for 50 ppb-fortified C18 fractions.)

Flour Procedure

(a) *Extraction*.—Weigh 10.00 ± 0.05 g flour (all-purpose flour from grocery store) into a tared 250 mL Nalgene (pp) bottle. Fortify, for recovery studies, using 0.50 mL 1.0 µg/mL 22 mixed standard fortification solution. This is equivalent to 50 ppb in flour. Add 60 mL MeOH-water (80 + 20, v/v). Homogenize ca 1–2 min, using a polytron (speed control set between 3 and 4 on PowerGen Model 700); rinse the probe with MeOH-water (80 + 20, v/v) from a wash bottle. Centrifuge at 4000 rpm (3080 × g) for 10 min. Decant the supernatant through a glass microfiber GF/A filter into a 500 mL Erlenmeyer vacuum flask. Break up filter cake with a spatula. Repeat the procedure by adding another 60 mL MeOH-water (80 + 20, v/v), centrifuge, and decant through the same glass microfiber GF/A filter, combining the supernatants in the 500 mL Erlenmeyer flask. Rinse the Nalgene bottle and the filter cake with a few milliliters of MeOH-water (80 + 20, v/v) from a wash bottle. Transfer the extract to a 250 mL graduated cylinder equipped with stopper. Fortify all samples (include controls) using 1.0 mL of a 1.0 µg/mL (3*_IS) internal standard fortification solution. Dilute to 200 mL with MeOH-water (80 + 20, v/v), stopper,

and shake thoroughly to mix. Transfer a 20 mL aliquot of the flour extract to a clean 50 mL glass graduated centrifuge tube. Add 12 mL water. [*Note*: This will change the extract concentration to MeOH-water (1 + 1, v/v); the sample will become cloudy.] Stopper, and mix on a Vortex mixer or handshake. Load sample onto the conditioned SPE columns (see below).

(b) *C18 SPE cleanup*.—Condition Supelclean LC-18 SPE column with 1 column volume of MeOH, followed by 1 column volume of extraction solvent, MeOH-water (1 + 1, v/v). Discard the conditioning solvents. Load flour extract. Collect the eluate in a clean disposable culture tube. (*Note*: More than 1 culture tube may be required to collect all of the eluates.) Rinse the 50 mL sample glassware tube with 5 mL MeOH-water (1 + 1, v/v), add rinsings to column, use vacuum to completely drain column, and combine eluates in the disposable culture tube. Save combined eluates for the next SPE step (see Oasis MCX and Oasis MAX procedures). The parent triazoles (and 5 of 8 metabolites) are retained on the LC-18 column. Elute parent triazoles and remaining metabolites with 20 mL MeOH into a clean disposable culture tube. Evaporate to dryness in TurboVap (T = 45°C). Redissolve the sample in 1.0 mL MeOH, mix on a Vortex mixer, and add 1.0 mL water; mix on a Vortex mixer (final volume = 2.0 mL), and filter (0.45 µm, PVDF). Assay by LC/MS/MS.

(c) *MCX, MAX SPE cleanup*.—See *Apple Procedure* for MCX, MAX SPE procedures. Assay by LC/MS/MS. (*Note*: The concentration of the bracketing standard, for recovery studies, should be equivalent to the expected concentration of the final solution of the fortified sample. Bracketing standard concentration = 0.05 µg/mL for 50 ppb-fortified MCX and MAX fractions, or = 0.025 µg/mL for 50 ppb-fortified C18 fractions.)

Water (Raw Prechlorinated) Procedure

(a) *Extraction*.—Weigh 50.00 ± 0.05 g water (Raw prechlorinated water from the Patuxent Filtration Plant) into a tared 250 mL Erlenmeyer flask, graduated, with screw cap. Tap water may be fortified and substituted for raw water, provided the free Cl₂ is <1 ppm. Fortify, for recovery studies, using 0.025 mL 1.0 µg/mL 22 mixed standard fortification solution. This is equivalent to 0.5 ppb in water. Fortify all samples (include controls) using 0.10 mL of a 1.0 µg/mL (3*_IS) internal standard fortification solution. Cap flask and swirl contents to mix. Load aqueous sample onto the conditioned SPE columns (see below).

(b) *MCX, MAX SPE cleanup*.—Condition Oasis MCX and Oasis MAX SPE columns with 2 column volumes of MeOH, followed by 2 column volumes of water. Discard conditioning solvents. "Piggyback" the conditioned SPE columns in tandem (MCX on top of MAX); use a reservoir to hold the sample, or apply sample, using a Pasteur pipet. (*Note*: Fill each stacked MCX, MAX SPE column 3/4 full with water before loading the sample, if using a reservoir to aid flow. If a Pasteur pipet is used, there will be no need to fill the top MCX SPE column with water). Load the sample. Use a slight

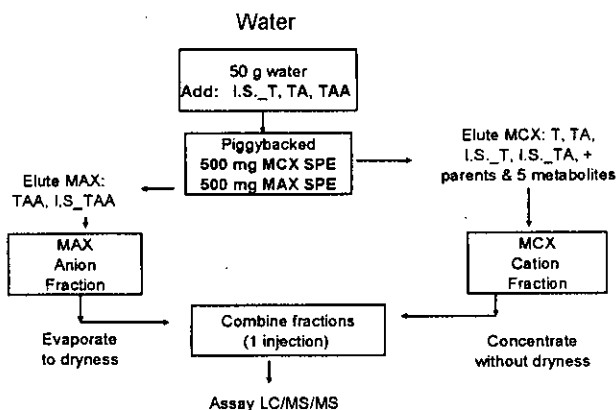


Figure 3. Flowchart for the extraction of water.

vacuum or gravity feed with positive pressure to drain the SPE columns at a flow of 1–2 drops/s. Discard the eluate. Rinse the sample glassware with 1–2 mL water if using a reservoir, and add rinsings to the reservoir. Rinse the columns with 2 column volumes of water followed by 2 column volumes of MeOH. If a Pasteur pipet is used, rinse the glassware with 1–2 mL MeOH, and add rinsings directly to the MCX SPE column. Add MeOH to the rinsings in the MCX SPE column to total 1 column volume. Discard the eluate. Separate “piggybacked” columns. Use positive pressure to drain the MAX and MCX SPE columns for elution. Parents, 5 metabolites, T, IS_T, TA, and IS_TA are retained on the MCX column. Elute MCX with 20 mL 1% NH₄OH in MeOH (v/v). Collect eluate in a clean disposable culture tube (cation fraction). TAA and IS_TAA are retained on the MAX column. Elute MAX column with 20 mL 1% acetic acid in MeOH (v/v). Collect eluate in a clean disposable culture tube (anion fraction). Evaporate each fraction (MAX, MCX) to ca 2–4 mL, using the TurboVap (T = 45°C). (Note: Do not allow the MCX, cation fraction, to go to dryness.) Transfer the sample from each disposable culture tube to a clean 13 mL glass graduated centrifuge tube. Rinse each tube with 1–2 mL MeOH, and combine rinsings in the 13 mL graduated centrifuge tube. Evaporate the sample, using N-Evap (T = 45°C). (Note: Evaporate the anion fraction to dryness at this step.) The final volume of the cation fraction should be ca 0.5–1 mL. Add 1–2 mL MeOH to the cation fraction tube, and transfer quantitatively to the dry anion fraction tube. Re-evaporate the combined sample to <0.5 mL, using N-Evap (T = 45°C) with a gentle flow of nitrogen. Adjust the volume to 0.5 mL MeOH. Add water to bring the final volume to 1.0 mL. Mix on a Vortex mixer and filter (13 mm, 0.45 μm, PVDF), if necessary, into an autosampler vial for LC/MS/MS analysis. (Note: The concentration of the bracketing standard, for recovery studies, should be equivalent to the expected concentration of the final solution of the fortified sample. Bracketing standard concentration = 0.025 μg/mL for 0.5 ppb-fortified combined MCX and MAX fractions.) Refer to Figure 3 for a flowchart of the water procedure.

Table 1. Timetable for LC gradient solutions^a

Time, min	A, %	B, %	C, %	D, %	Curve
0.00	0.0	0.0	50.0	50.0	1 ^b
10.0	0.0	0.0	50.0	50.0	6 ^c
11.0	0.0	0.0	30.0	70.0	6
19.0	0.0	0.0	30.0	70.0	6
20.0	0.0	0.0	25.0	75.0	6
30.0	0.0	0.0	25.0	75.0	6
40.0	0.0	0.0	10.0	90.0	6
49.0	0.0	0.0	10.0	90.0	6
50.0	0.0	0.0	50.0	50.0	6
62.0	0.0	0.0	50.0	50.0	6

^a At a flow rate of 0.30 mL/min.

^b Instantaneous composition curve.

^c Linear curve.

Instrumental Conditions

(a) The following LC conditions were used with a quaternary pump for all of the compounds. (1) Solvent A: MeOH (column flushing and needle wash); (2) Solvent B: water (column flushing and seal wash); (3) Solvent C: MeOH (0.2% FA)–water (0.2% FA) (10 + 90, v/v); (4) Solvent D: MeOH (0.2% FA)–water (0.2% FA) (90 + 10, v/v); (5) column: Waters Symmetry C18 (4.6 × 250 mm, 5.0 μm); (6) flow: 0.30 mL/min; (7) column temperature: 35°C; and (8) Injection volume: 50 μL. The timetable for the LC gradient elution of all compounds is shown in Table 1.

(b) MS/MS conditions for each compound.—The following MS/MS conditions were used for each compound: ion mode: electrospray (positive); capillary voltage: 3.20 kV; extractor voltage: 3 V; RF lens: 0.0 V; source temperature: 120°C; desolvation temperature: 300°C; nitrogen gas flow: 50 L/h cone, 500 L/h desolvation; LM 1, HM 1 resolution: 12.5 (see note below); LM 2, HM 2 resolution: 12.5 (see Note); gas cell Pirani pressure (argon collision gas on): 6.5 × 10⁻³ mbar; and multiple reaction monitoring (MRM) transitions: see Table 2.

[Note: If significant cross-talk issues are suspected, the mass resolution (peak widths) could be adjusted in an effort to strike a balance between sensitivity and selectivity. Although low resolution will increase instrument sensitivity, it may potentially decrease selectivity for ions having similar masses that elute together.]

Results and Discussion

The results of the validation data for recoveries are presented in Table 3. This table displays recovery data based on internal standards derived from the ratio of the native metabolites to the isotopically labeled internal standards (for T, TA, and TAA) and recovery data based on external standards for the other analytes investigated. Each sample set included control samples (negative unfortified samples, n = 3)

Table 2. MRM transitions for triazole compounds

Compound	Primary ion transition, m/z ^a	Secondary ion transition, m/z ^a	Cone voltage	Collision energy, eV	RT, min ^b	MRM Function No. ^c
TA	157.1→69.8	157.1→88.0	20	15	7.4	1
IS_TA	161.9→74.4	161.9→87.6	20	11	7.4	1
T	69.4→42.5		45	18	8.2	1
S_T	74.4→45.5		45	20	8.2	1
TAA	128→69.8	128→73	30	15	10.6	1
IS_TAA	132.7→74.4		30	16	10.6	1
RPA-406341	334→69.8		30	20	31.7	2
RPA-404886	334→69.8		30	20	32.2	2
HWG-2061	324.3→69.8		35	20	39.5	2
RH-9129	354.3→69.8	354.3→125.2	35	20	40.4	2
Triadimefon	294.3→69.8	294.3→57	30	20	41.7	2
Cyproconazole ^d	292.3→69.8	292.3→125.1	30	20	41.9	2
					44.7	
RH-9130	354.3→69.8	354.3→125.2	35	20	42.0	2
Myclobutanil	289.3→69.8	289.3→125.1	35	20	42.2	2
Bromuconazole 46	378.1→69.8	378.1→159.2	35	25	42.8	2
Triadimenol ^d	296.3→69.8	296.3→99.1	20	15	44.3	2
					45.0	
Triticonazole	318.3→69.8		25	15	45.5	2
Epoxiconazole	330.2→121.2	330.2→69.8	30	20	45.8	2
Tetraconazole	372.2→69.8	372.2→159.2	40	25	46.1	2
Fenbuconazole	337.3→69.8	337.3→125.2	35	20	47.1	2
Bromuconazole 47	378.1→69.8	378.1→159.2	35	25	50.2	2
Tebuconazole	308.4→69.8		35	20	51.2	2
Propiconazole ^d	342.2→68.9	342.2→159.2	35	20	51.7	2
					52.4	
Hexaconazole	314.3→69.8		35	20	53.8	2
Difenoconazole ^d	406.2→251.2		35	25	55.4	2
					56.1	

^a Masses were optimized during tuning. Fractional masses were selected from peak maxima during tuning.

^b RT = Observed retention time (min) of analyte.

^c MRM function window for primary ion transition; window 1 = 0–15 min; window 2 = 15–62 min.

and fortified samples (positive fortified samples, $n = 5$ or 6). Flour was the only commodity that had detectable residues in the control sample (see discussion below about endogenous TAA in flour).

Several analytical columns were evaluated for separation on the basis of the retention time of each analyte. Because the 3 common metabolites (T, TA, and TAA) eluted early in the separation, the Waters Symmetry C18 (4.6×250 mm) was the only column investigated that completely resolved these 3 compounds (see Figure 4). The remaining 19 compounds were separated both by mass and retention time when the elution gradient described in *Instrumental Conditions*, (a),

was used. Some of the fungicides chosen for this study differed by only 2 amu, and the potential for overlapping contributions or cross-talk was a concern (Figure 4). Further investigations were not carried out.

The apple procedure was the first to be turned over to the U.S. Department of Agriculture (USDA) Pesticide Data Program (PDP) to gather triazole data for dietary risk assessment (see chromatogram in Figure 5). Figure 4 shows the LC/MS/MS MRM chromatogram of each parent and metabolite.

The development of a tandem mixed-mode SPE extraction scheme was based on literature from Waters Corp. on their

Table 3. Percent recovery (RSD) data for triazole compounds

Compound	Apples fortified at 25 ppb ^a	Peaches fortified at 50 ppb ^a	Flour fortified at 50 ppb ^a	Raw water ^b fortified at 0.5 ppb ^a	Finished water ^{a, c} fortified at 0.5 ppb
Metabolites					
1,2,4 Triazole	89 (12)	83 (6.5) ^{d, e}	100 (3.7)	95 (9.3)	94 (4.0)
Triazole Alanine	98 (6.4)	79 (14) ^d	94 (7.7)	98 (8.0)	99 (6.2)
Triazole Acetic Acid	83 (11)	60 (12) ^d	118 (2.6)	99 (7.5)	97 (4.4)
Parents					
Myclobutanil	90 (7.0)	100 (5.0)	80 (8.5)	95 (3.3)	97 (6.0)
Cyproconazole	87 (3.2)	100 (4.2)	77 (6.1)	92 (4.3)	94 (6.2)
Triadimefon	70 (10)	88 (4.0)	78 (5.0)	75 (7.7)	79 (5.1)
Triadimenol	93 (8.1)	102 (9.3)	82 (6.2)	92 (3.0)	93 (4.9)
Tebuconazole	98 (5.8)	100 (8.0)	73 (5.5)	94 (5.0)	96 (5.9)
Hexaconazole	87 (8.9)	98 (9.3)	76 (20)	92 (3.0)	91 (4.7)
Triticonazole	98 (5.1)	105 (3.7)	78 (6.4)	90 (5.1)	93 (9.1)
HWG-2061	92 (8.0)	99 (2.4)	78 (6.5)	92 (4.0)	96 (6.7)
Epoxiconazole	91 (6.3)	103 (4.2)	74 (6.5)	92 (4.2)	88 (8.0)
RPA-406341	95 (4.4)	106 (5.8)	66 (5.8)	92 (4.0)	97 (6.3)
RPA-404886	95 (4.8)	104 (3.6)	68 (6.4)	90 (4.1)	93 (5.9)
Fenbuconazole	100 (7.2)	106 (6.5)	71 (10)	96 (5.1)	97 (7.1)
Propiconazole	89 (5.3)	98 (8.2)	69 (7.4)	88 (2.6)	87 (4.4)
RH-9129	92 (6.6)	103 (5.0)	75 (8.4)	92 (4.2)	95 (6.9)
RH-9130	101 (8.7)	104 (7.8)	79 (7.7)	95 (3.4)	95 (7.6)
Tetraconazole	83 (11)	97 (11)	75 (7.0)	93 (6.6)	90 (7.8)
Bromuconazole-46	88 (9.6)	104 (6.5)	74 (3.8)	89 (4.4)	94 (5.9)
Bromuconazole-47	92 (6.2)	104 (5.0)	73 (5.9)	93 (4.2)	92 (5.7)
Difenoconazole	98 (4.4)	121 (23)	57 (11)	92 (3.7)	93 (6.2)

^a *n* = 6.^b From Patuxant River; prechlorinated.^c Fort Meade, MD, tap water (Cl₂, <1 ppm).^d Results for fortification at 50 ppb; *n* = 5.^e Parent peaches were fortified at 25 ppb; *n* = 6.

Oasis SPE products, which can be found online. A mixed-mode system contains the respective ion-exchange sorbent as well as a reversed-phase sorbent. An MCX column is stacked on top of an MAX SPE column. All of the compounds, except for TAA, exhibit cationic properties and are therefore retained on the MCX SPE columns. However, because TAA is anionic, it will completely pass through the MCX, but will be retained on the MAX. The C18 SPE was added later to further clean up the fruit and grain matrices. These particular SPE columns minimized hazardous solvent usage (e.g., dichloromethane), liquid-liquid partitioning steps, and emulsion formations anticipated for the grain commodity. Other SPE schemes were investigated [e.g., Oasis Hydrophilic-Lipophilic-Balance (HLB) special columns for cleanup], but the C18/MCX/MAX combination yielded suitable results for all compounds. No further SPE schemes or products of alternative manufacturers were tested.

The water was collected from a variety of sources. Raw, prechlorinated water and finished chlorinated water were from the same source (Patuxent Filtration Plant). The tap water was from the laboratory at Fort Meade, MD, and bottles of LC-grade water were purchased for this project. When finished chlorinated water from Patuxent Filtration Plant was analyzed, the chromatograms indicated the absence of TA (Figure 6). If the finished water was found to contain >1.0 ppm free chlorine, then there was a loss of TA. If the finished water was quenched with sodium thiosulfate and then fortified with TA, the recoveries matched those obtained for the raw prechlorinated and normal tap waters (sodium thiosulfate is a dechlorinating agent). Previously several investigators, Bruchet et al. (8), Froese et al. (9), and Hruday, et al. (10), have examined the degradation of natural amino acids in chlorinated water as a possible source of odorous aldehydes in various treated water systems. It appears that the TA undergoes a similar fate. TA in chlorinated drinking water

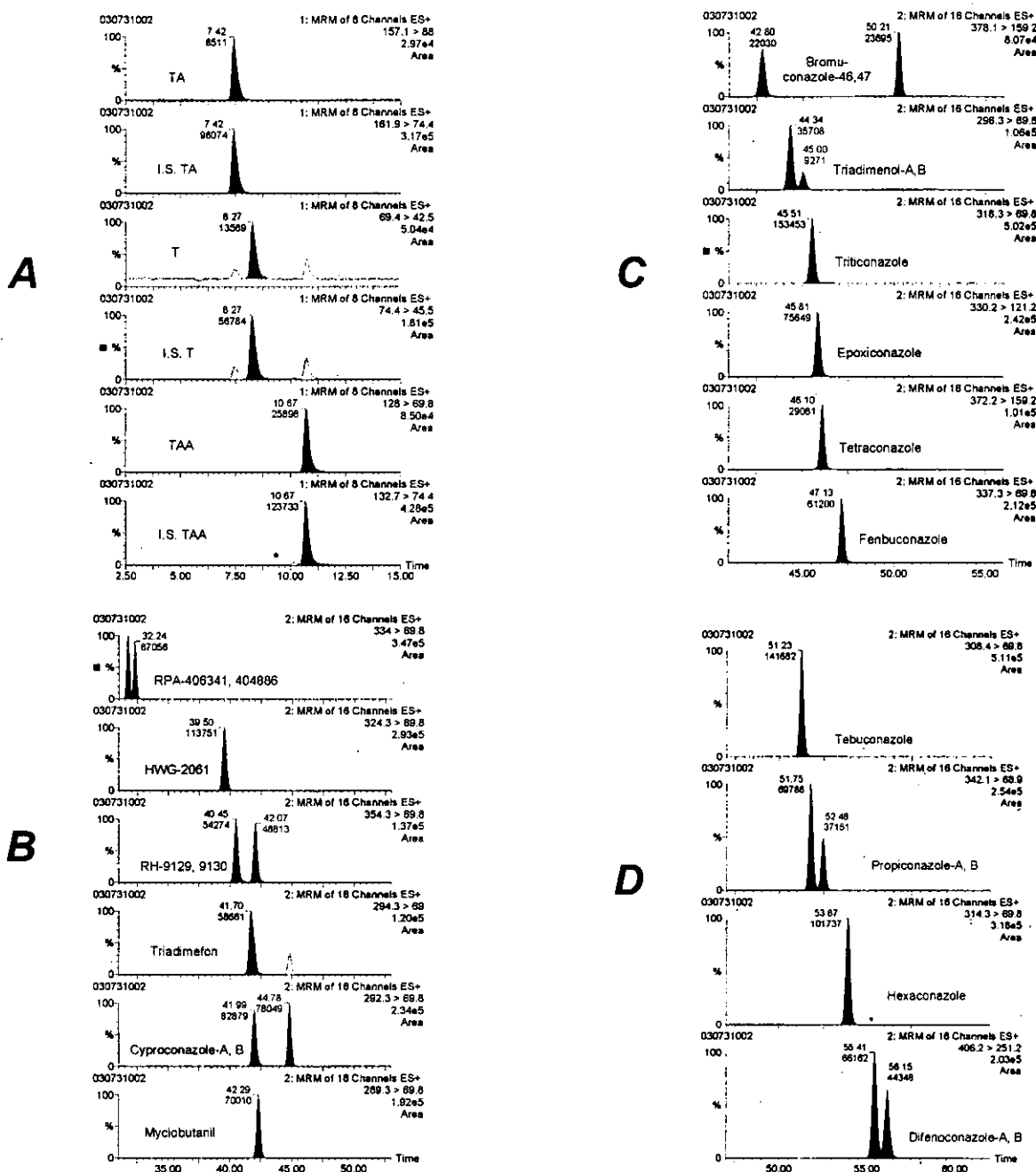


Figure 4. Calibration standards (A) LC/MS/MS MRM chromatograms of T, I.S. TA, I.S. TA, TAA, and I.S. TAA; (B) LC/MS/MS MRM chromatograms of RPA-406341, RPA 404886 (metabolites of Triticonazole), HWG-2061 (metabolite of Tebuconazole), RPA-404886, RPA-406341 (metabolites of Fenbuconazole), Triadimefon, Cyproconazole-A, B (chromatograph as 2 peaks, summed for quantitation), and Myclobutanil; (C) LC/MS/MS MRM chromatograms of Bromuconazole-46,47 (stereoisomers), Triadimenol-A, B (chromatograph as 2 peaks, summed for quantitation), Triticonazole, Epoxiconazole, Tetraconazole, and Fenbuconazole; (D) LC/MS/MS MRM chromatograms of Tebuconazole, Propiconazole-A, B (chromatograph as 2 peaks, summed for quantitation), Hexaconazole, Difenconazole-A, B (chromatograph as 2 peaks, summed for quantitation).

undergoes several steps via a chloramine intermediate to form triazole acetaldehyde (MW = 111; Figure 7). Triazole acetaldehyde is in equilibrium in acidic methanol-water solutions with triazole hydrate and triazole hemiacetal (Figure 7). Investigation of this process was performed with [^{14}C]triazolylalanine ([^{14}C]TA). An aliquot of [^{14}C]TA was

added to Stilwell, KS, tap water and allowed to incubate for 72 h. The sample was analyzed by LC/MS/MS by using a Zorbax R_x (150 \times 4.6 mm) column employing a 15 min linear gradient and a mobile phase of 0.1% formic acid and methanol. The mass spectrometer was operated in the positive-ion mode, scanning from 50 to 500 daltons (amu).

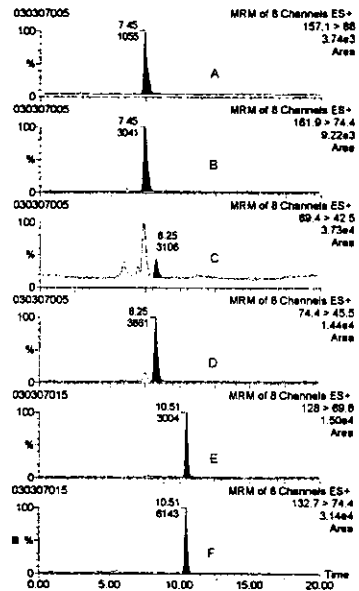


Figure 5. Fortification of apples at 25 ppb. (A) LC/MS/MS MRM chromatogram of TA; (B) LC/MS/MS MRM chromatogram of IS_TA; (C) LC/MS/MS MRM chromatogram of T; (D) LC/MS/MS MRM chromatogram of IS_T; (E) LC/MS/MS MRM chromatogram of TAA; (F) LC/MS/MS MRM chromatogram of IS_TAA. Chromatograms were normalized to the highest peak.

Seventy-five percent of the column effluent was split to a radioactivity flow monitor. Figure 8 shows that a significant amount of the original [¹⁴C]TA had indeed degraded after 72 h. The degradates were identified as triazole hydrate, triazole acetaldehyde, and triazole hemiacetal on the basis of mass spectral interpretation. On-column equilibration of the 3 species is responsible for the broadened peak shapes. Figure 9 shows that a standard of triazole acetaldehyde

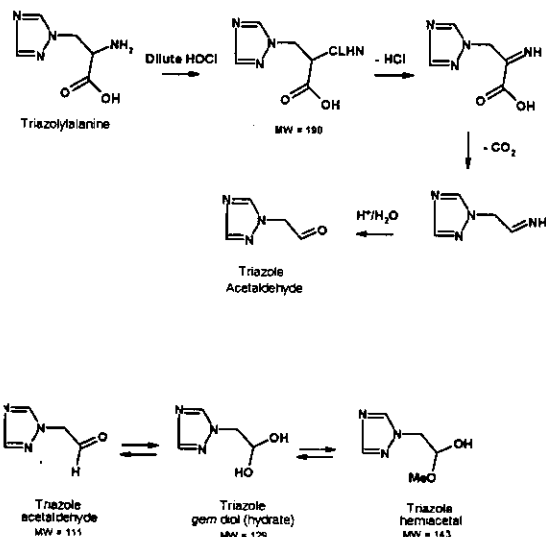


Figure 7. Degradation of TA to triazole acetaldehyde in chlorinated drinking water and illustration of equilibria in water-methanol solutions with the hydrate and hemiacetal.

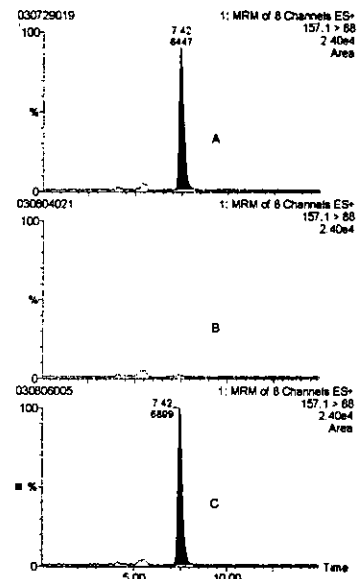


Figure 6. Absence of TA in finished chlorinated water. (A) LC/MS/MS MRM chromatogram of TA found in Fort Meade 0.5 ppb-fortified tap water; (B) LC/MS/MS MRM chromatogram showing absence of TA in finished chlorinated water from Patuxent River Treatment Plant (Cl₂ > 1 ppm) fortified at 0.5 ppb; (C) LC/MS/MS MRM chromatogram of TA in finished water, quenched with sodium thiosulfate, fortified at 0.5 ppb, and analyzed.

analyzed under the same conditions as the sample gives the same peak-broadening patterns.

The EPA funded a graduate student intern to gather pesticide residue data in peaches. The intern provided raw data in peaches from 4 growers for analyses. The growers had different pesticide-spray histories. The intern successfully used our method to determine the levels of incurred triazole

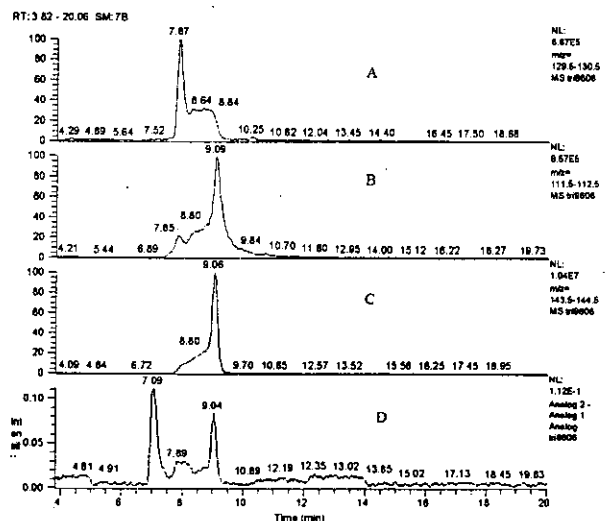


Figure 8. LC/MS/MS selected ion chromatograms of (A) triazole hydrate, (B) triazole acetaldehyde, and (C) triazole hemiacetal; (D) [¹⁴C] chromatogram illustrating breakdown of TA after 72 h of incubation in treated tap water.

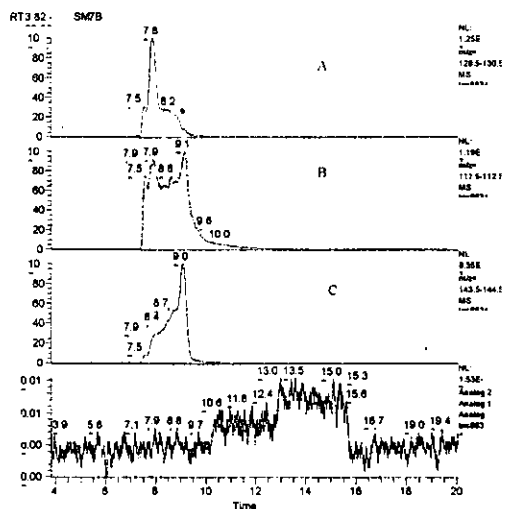


Figure 9. LC/MS/MS selected ion chromatograms of the triazole acetaldehyde standard analyzed under the same conditions as the incubated water sample (Figure 8). (A) Triazole hydrate, (B) triazole acetaldehyde, and (C) triazole hemiacetal.

fungicides in these field samples. This exercise demonstrated method ruggedness across several varieties of peaches. Positive and negative controls were run with each sample set to assess method performance.

Both the fortified peach samples and those with incurred residues showed some matrix interference in the region of the IS_TA (Figure 10). Two transitions of TA and IS_TA were monitored to determine which MRM would yield the best signal in a chromatogram for quantitation. By monitoring 2 MRM transitions, we were able to obtain confirmatory data for TA and IS_TA, although the primary focus of this project was determination and not confirmation.

There are many procedures in the literature that would have supported a second system (LC/MS, gas chromatography/MS) for analytical confirmation of the 14 parents and 5 other metabolites. The polar nature of T, TA, and TAA dictated the use of LC. The combination of MRM combined with the advantages of stable isotope for internal standards provided the specificity and instrumental ruggedness to measure these compounds by LC/MS/MS in a minimally purified matrix.

The USDA Grain Inspection Packers and Stockyard Administration (GIPSA) analyzed >600 flour samples for parent triazoles and metabolites, including TAA, as part of its monitoring work for the PDP. They found what appeared to be an endogenous peak for TAA in the flour (based on retention time and 2 transitions). They could not find true control flour. They tried to locate some flour sources before 1985, but none were located.

The flour used for this project was purchased from a local grocery store and contained what appeared to be approximately 25 ppb endogenous TAA (Figure 11). To confirm our findings, a sample of our control flour was sent to Bayer CropScience, which determined this same level, using a

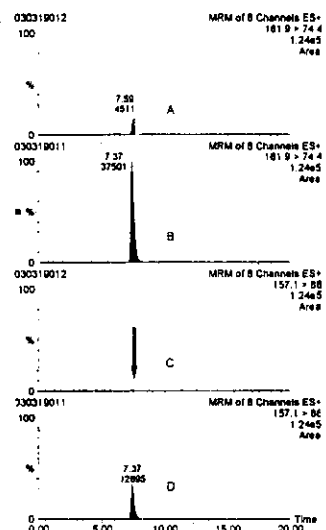


Figure 10. Control peach (TA in cation fraction). (A) LC/MS/MS MRM chromatogram of IS_TA showing matrix interference; (B) LC/MS/MS MRM chromatogram of standard IS_TA; (C) LC/MS/MS MRM chromatogram of TA; (D) LC/MS/MS MRM chromatogram of standard TA. Chromatograms are presented on the same scale.

different analytical method (derivatization procedure) and also a different instrument for analyses (TSQ 7000). In another effort to confirm that the peak was TAA and not an interference peak, 3 transitions of TAA were monitored: quantitation ion: 128>70 m/z ; confirmation ion #1: 128>73 m/z ; and confirmation ion #2: 128>101 m/z . The ratios of these ions were within 10% of those of a standard of TAA. These results were consistent with those of Bayer CropScience and the GIPSA laboratory in confirming TAA. This information corresponds to Bayer CropScience data generated from [^{14}C] conazole confined rotational crop studies which indicate that TAA and TA are significant metabolites generated from all conazole fungicides. Generally, the metabolites show a greater abundance in rotational crops than in the target crop.

A set of 6 samples of apples, peaches, or flour normally takes one analyst 8 h to prepare for unattended overnight analysis. Six water samples normally take 4 h to prepare for analysis. Each apple, peach, or flour sample required 3 injections on the LC/MS/MS instrument: (1) parents and 5 metabolites, (2) cation fraction, and (3) anion fraction. However, each water sample required only one injection because the fractions could be successfully combined without the loss of peak shape for the TAA.

Quantitation

All samples were initially quantitated by using an external standard technique for all analytes during the method development stage. The recoveries for T, TA, and TAA, however, were not acceptable (<70%) with the use of external standards. Isotopically labeled internal standards for these 3 compounds (IS_T, IS_TA, IS_TAA) then became available from the USTTF. Recoveries for T, TA, and TAA improved

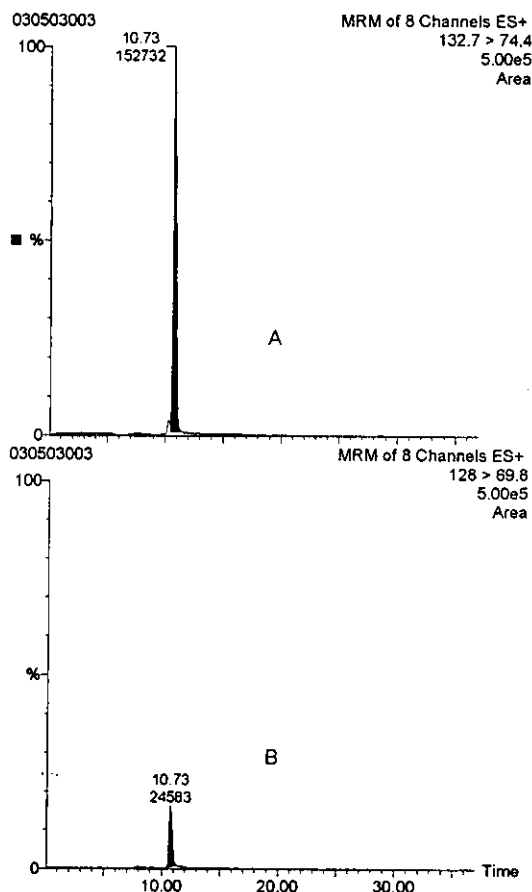


Figure 11. Control wheat flour (anion fraction).
 A) LC/MS/MS MRM chromatogram of IS_TAA;
 B) LC/MS/MS MRM chromatogram of endogenous TAA,
 approximately 25 ppb. Chromatograms are presented
 on the same scale.

markedly when an internal standard technique was used for quantitation. The T, TA, and TAA for all analyses were therefore determined by using the ratio of the native standard to the respective isotopically labeled internal standard, i.e., IS_T, IS_TA, and IS_TAA. The remaining analytes for all analyses were compared directly with the external standards. Apples were quantitated by using bracketing standards within the linear dynamic range. Standards and samples had the same injection volume. Six-point calibration curves for each compound were found to be linear from 1.0 to 100 ng/mL, without weighting and with correlation

coefficients (r^2) of >0.992. The limit of detection (LOD) and limit of quantitation (LOQ) were estimated from computer-generated software using a signal-to-noise ratio (S/N) program within a selected chromatogram of a positive fortified sample. LOD and LOQ estimates were calculated from the primary transitions for each compound. Three times the S/N was used to estimate the LOD, and 10 times the S/N was used to estimate the LOQ. For apples, the LODs ranged from 0.60 ppb (parents, TAA) to 6.0 ppb (T), and the LOQs ranged from 2.0 ppb (TAA) to 22 ppb (T). For peaches, the LODs ranged from 0.60 ppb (parents) to 8.0 ppb (T, TA), and the LOQs ranged from 2.0 ppb (parents) to 28 ppb (T). For flour, the LODs ranged from 0.20 (parents) to 9.0 ppb (TA), and the LOQs ranged from 0.70 ppb (parents) to 32 ppb (TA). For water, the LODs ranged from 0.0030 ppb (parents) to 0.10 ppb (T), and the LOQs ranged from 0.010 ppb (parents) to 0.30 ppb (T).

Acknowledgments

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