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Determination of Oryzalin in Water, Citrus Fruits, and Stone Fruits by Liquid Chromatography with Tandem Mass Spectrometry

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A new methodology is described for rapidly determining the herbicide oryzalin in water, citrus fruits, and stone fruits by liquid chromatography with negative ion electrospray ionization tandem mass spectrometry (LC/MS/MS). Oryzalin is extracted from water using a polymeric sorbent solid phase extraction (SPE) column and from fruit using methanol. The water samples require no further purification, but an aliquot of the fruit sample extracts is diluted with water and purified using a polymeric 96 well SPE plate. Purified extracts are concentrated prior to determination by LC/MS/MS at *m*/*z* 345 (Q1) and *m*/*z* 281 (Q3) using an external standard for calibration. The validated limits of quantitation were 0.05 μg/L in water (drinking water, surface water, and groundwater) and 0.01 μg/g in citrus fruits (oranges and lemons) and stone fruits (peaches and cherries). Recoveries averaged 102% for water samples and 85−89% for the various types of fruit samples. For all fortification levels combined, the relative standard deviations ranged from 4 to 6% for water and from 2 to 4% for fruit.

KEYWORDS: Oryzalin; water; citrus fruits; stone fruits; oranges; lemons; peaches; cherries; LC/MS/MS; 96 well solid phase plate

INTRODUCTION

Oryzalin [4-(dipropylamino)-3,5-dinitrobenzenesulfonamide] is the active ingredient in the herbicide Surflan (trademark of Dow AgroSciences LLC), which is registered for the preemergence control of many annual grasses and broad-leaved weeds in cotton, fruit trees, nut trees, vines, ornamentals, soya beans, ground nuts, oilseed rape, sunflowers, lucerne, peas, sweet potatoes, mint, and noncrop areas. Several toxicological properties, chemical and physical properties, and analytical methods for formulation analysis and residue analysis have been previously summarized (*1*). The structure of oryzalin is shown in **Figure 1**.

Reliable analytical methods are an important aspect of monitoring pesticide residue levels to ensure human and environmental safety. Residue methods utilizing gas chromatography with electron capture detection (GC-ECD) of a methylated derivative formed by reaction with methyl iodide have been described for determining oryzalin in soil, water, crops, crop processed commodities, meat, milk, and eggs (*1*−*4*). Oryzalin residues may also be determined and/or confirmed in these sample types as its methylated derivative by gas chromatography with mass spectrometric detection (GC-MS) (*1*, *5*). The determination of underivatized oryzalin in wine using a multiresidue approach with GC-MS has also been reported (*6*), although the recoveries were unacceptably low (0−50%).

The use of high-performance liquid chromatography (HPLC) instead of GC offers the advantage of not requiring derivatization to form a volatile derivative. Underivatized oryzalin may be determined by HPLC with ultraviolet (UV) detection in soil, water, meat, milk, and eggs (*1*, *7*).

In general, the published GC-ECD, GC-MS, and HPLC-UV methods involve the extraction of oryzalin using suitable organic solvents or C_{18} solid phase extraction (SPE). Initial purification of the extracts is accomplished using aqueous−organic partitioning and Florisil column chromatography (*7*) or SPE columns such as Florisil (*1*) or aminopropyl (*6*).

The advent of reliable instrumentation for liquid chromatography with tandem mass spectrometry (LC/MS/MS) offers an approach for determining oryzalin residues that is much more rapid, sensitive, and selective. The increased sensitivity also permits the use of smaller sample sizes and reduced volumes of solvents and reagents. The reduced sample volumes in turn permit the use of a 96 well SPE plate for purification of sample extracts, which greatly reduces time and cost by allowing the use of automated sample preparation and cleanup techniques (*8*). This paper describes the analytical methodology for LC/MS/MS methods and provides validation data for the determination of oryzalin in water, citrus fruits, and stone fruits.

EXPERIMENTAL PROCEDURES

LC/MS/MS System. The LC/MS/MS system was a MDS/SCIEX API 3000 with a MDS/SCIEX Analyst 1.1 data system (MDS/SCIEX,

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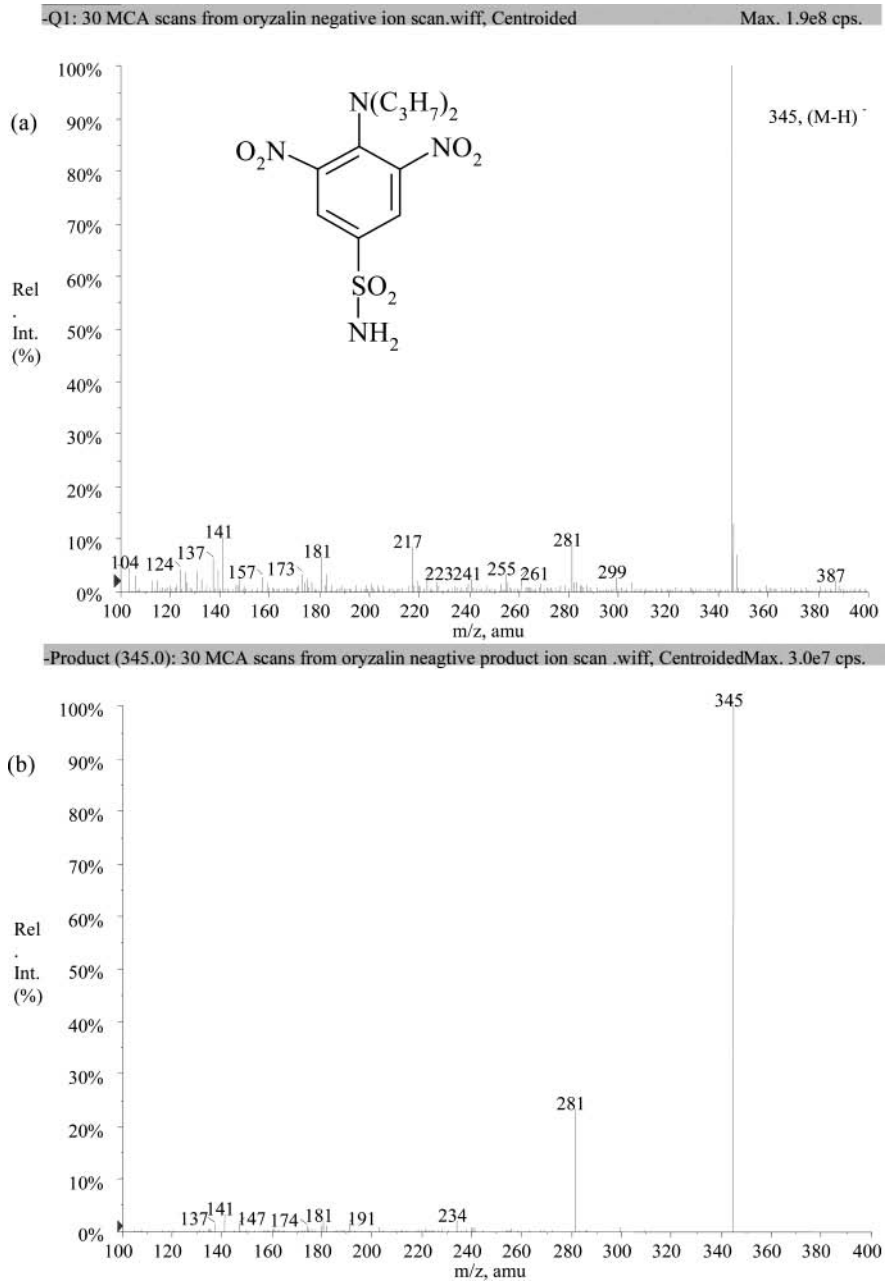

Figure 1. Mass spectra for oryzalin. (**a**) Mass spectrum for Q1 scan using electrospray negative ionization showing $(M - H)^-$ at *m*/*z* 345. (**b**) Product-ion mass spectrum of oryzalin showing major fragment ion at *m*/*z* 281 and (M − H) at *m*/*z* 345.

Foster City, CA) and an Agilent model Series 1100 binary pump, degasser, and autosampler (Agilent Technology, Wilmington, DE). The analytical column was a Zorbax SB-C8, 3.5 μm particle size, 75 mm × 4.6 mm i.d. (Agilent Technology), operated isothermally at 35 °C. The mobile phase consisted of methanol containing 0.1% acetic acid (reservoir A) and water containing 0.1% acetic acid (reservoir B). The mobile phase was run isocratically at 65% A/35% B for 5.0 min. The flow rate was 900 μL/min, with 200 μL split to the source after 3.0 min. The injection volume was 50 μL for water samples and 30 μL for fruit samples. An electrospray interface was utilized with negative polarity, and the scan type was multiple reaction monitoring (MRM). The pressure of nitrogen collision gas was set at 4.0 on the SCIEX instrument. The temperature of the nitrogen turbo gas heater was 425 °C, the acquisition delay was 3 min, the period duration was 2 min, and the ion spray voltage was set at −4200 V. The ion transition monitored was *m*/*z* 345 (Q1) to *m*/*z* 281 (Q3) with a collision energy of −26 V for 150 ms. The instrument was tuned on a monthly basis using polypropylene glycol SCIEX tuning solutions.

Reagents. Acetonitrile, methanol, and water were HPLC grade. Acetic acid was certified ACS grade. The analytical standard was obtained from the Test Substance Coordinator, Dow AgroSciences LLC (Indianapolis, IN).

Standard Preparation. *(a) Sample Fortification Standard Solutions.* Standard fortification solutions were prepared in methanol for generating method validation recovery data. For the fruit method, standard solutions were prepared at 10 and 1.0 μg/mL. For the water method, standard fortification solutions were prepared at concentrations of 1.0, 0.10, 0.010, and 0.0020 μg/mL.

(b) Standard Calibration Solutions. Standard calibration solutions were prepared in methanol/water/acetic acid (50:50:0.1) at concentrations of 0.1, 0.5, 1.0, 5.0, 10, 20, 35, and 50 ng/mL.

Initial Sample Preparation and Storage. *(a) Water.* Water samples required no initial sample preparation prior to analysis. The samples were stored in a refrigerator or freezer.

(b) Fruit. Fruit samples were prepared for analysis by freezing with liquid nitrogen or dry ice and then grinding or chopping with a hammer

mill having a 3/16 in. screen size (model 2001, Agvise Laboratories, Inc., Northwood, ND). After they were ground, the samples were manually mixed in a plastic bag and then transferred to high-density polyethylene freezer containers for storage at approximately −10 to −20 °C.

Fortification of Recovery Samples. *(a) Groundwater, Surface Water, and Drinking Water*. Untreated control samples (50.0 mL) were transferred into a series of 4 oz (120 mL) glass bottles with poly(tetrafluoroethylene) (PTFE)-lined caps (Fisher Scientific, Pittsburgh, PA). Fortified recovery samples were prepared for analysis by adding 250 μL of the appropriate fortification standard solution to the control samples to result in concentrations of 0.010, 0.050, 0.50, and 5.0 μg/L. [The fortified samples at 0.010 μg/L were prepared to demonstrate qualitative recovery at the validated limit of detection (LOD).] The analysis of the water samples was continued as described under Sample Extraction and Purification.

(b) Fruit. Untreated control samples (5.0 g) were weighed into a series of 4 oz (120 mL) glass bottles with PTFE-lined caps (Fisher Scientific). Fortified recovery samples were prepared for analysis by adding 50 μL of the appropriate fortification standard solution to the control samples to result in concentrations of 0.002, 0.010, 0.10, and 1.0 μg/g. (The fortified samples at 0.002 μg/g were prepared to demonstrate qualitative recovery at the validated LOD.)

Sample Extraction and Purification. *(a) Water*. Untreated control water samples (50.0 mL) in 120 mL bottles and the fortified water samples from above were extracted and purified by SPE. The following SPE procedure was used for the extraction and purification of oryzalin in water samples: A 33 μm, 60 mg Strata-X polymeric sorbent SPE column (Phenomenex, Torrance, CA) was placed on a vacuum manifold (model spe-12G, Mallinckrodt Baker, Inc., Phillipsburg, NJ). Using an adapter, a 75 mL SPE reservoir was attached to the SPE column. The SPE column was conditioned with 3 mL of methanol followed by 3 mL of water. The column was dried under full vacuum (−20 in. of Hg) for approximately 10 s between solvent additions. The water sample was added to the SPE column reservoir and was drawn through the column at approximately 5−10 mL/min with the aid of vacuum. The eluate was discarded, and the column was dried under full vacuum for 10 s. The sample bottle was rinsed with 3 mL of acetonitrile/water (30:70), and the rinse was added to the SPE reservoir. The rinse solution was drawn through the column at approximately 1 mL/min with the aid of vacuum. The eluate was discarded, and the column was dried under full vacuum for approximately 2 min. Oryzalin was then eluted from the SPE column at approximately 1 mL/min with two 1.5 mL aliquots of methanol, and the eluate was collected in a 12 mL culture tube. The sample was transferred to a 5 mL volumetric flask using a disposable polyethylene transfer pipet. The culture tube was rinsed with 2 mL of water, which was transferred to the volumetric flask. The solution was then diluted to volume (5.0 mL) by adding water. Analysis of the water samples was then continued as described under LC/MS/MS.

(b) Citrus Fruits and Stone Fruits. Untreated control fruit samples (5.0 g) in 120 mL bottles and the fortified fruit samples from above were extracted by adding 50 mL of methanol and blending the sample for approximately 1 min at 13000 rpm with a homogenizer (Omni-mixer model ES, Omni International, Inc., Warrenton, VA). The bottle was sealed with a PTFE-lined cap and shaken for 30 min on a flat-bed shaker (model 6000, Eberbach Corporation, Ann Arbor, MI) at approximately 180 excursions/min. The samples were placed in a centrifuge (model Centra GP-8, Thermo International Equipment Company, Needham Heights, MA) for 5 min at 2000 rpm.

A 1.0 mL aliquot of the extract was pipetted into a clean 12 mL culture tube, 2.0 mL of water was added, and the sample was mixed using a vortex mixer (model G-560, Scientific Industries, Inc., Bohemia, NY).

The sample extract was purified using a 96 well SPE plate. The following SPE procedure was used for purifying fruit sample extracts: A 33 μm, 30 mg Strata-X polymeric sorbent SPE plate (Phenomenex) was conditioned with 1.0 mL of methanol followed by 1.0 mL of water. The plate was dried under full vacuum (−20 in. of Hg) for approximately 10 s between solvent additions. A 1.5 mL aliquot of the 3.0 mL sample extract solution was added to the 96 well plate. The sample solution was drawn through the plate at approximately 1 mL/min with the aid of vacuum. The eluate was discarded, and the plate was dried under full vacuum for 10 s. The plate was rinsed with 1 mL of acetonitrile/water (30:70). The eluate was discarded, and the plate was dried under full vacuum for 5 min. Oryzalin was then eluted from the SPE plate at approximately 1 mL/min with two 0.75 mL aliquots of acetonitrile, and the eluate was collected in a 2 mL deep-well collection rack. Using a disposable polyethylene transfer pipet, the sample was transferred to a 40 mL vial with a PTFE-lined cap (National Scientific Co., Duluth, GA). The purified extract was taken just to dryness using an evaporator (model TurboVap LV, Zymark Corp., Hopkinton, MA) at 40 °C and 12 psi of nitrogen. (Note: To avoid reduced recoveries, it was necessary to remove the vials from the evaporator immediately after the solvent had evaporated.) The purified sample extract was then reconstituted in 1.0 mL of methanol/water/acetic acid (50:50:0.1). To aid dissolution, the sample solution was sonicated using an ultrasonic cleaner (model 1200, Branson Cleaning Equipment Co., Shelton, CT) and mixed using a vortex mixer (model G-560, Scientific Industries, Inc.) for 30 s. Analysis of the fruit samples was then continued as described under LC/MS/MS.

LC/MS/MS. The purified extracts for water, citrus fruit, or stone fruit samples were analyzed using the conditions described previously under LC/MS/MS System. The suitability of the chromatographic system was determined using the following performance criteria: (i) It was determined that the correlation coefficient (r) exceeded 0.995 for the quadratic equation that described the detector response as a function of the concentration of the standard calibration solutions. (ii) It was visually determined that sufficient resolution was achieved for the analytes relative to any background interferences. (iii) It was visually determined that a signal-to-noise ratio of at least 10:1 was achievable

Table 1. Recovery, Standard Deviation (s), and RSD of Oryzalin in Surface Water, Groundwater, and Drinking Water

	recovery[a]				
fortification level (μg/L)	average (%)	range (%)	s	RSD (%)	n
0.050	103	87–112	6.3	6.1	20
0.50	103	96–108	3.8	3.7	8
5.0	98	86–103	5.3	5.4	8
0.05–5.0	102	86–112	6.0	5.8	36

[a] Combined data for surface (stream and pond) water, ground (well) water, and drinking (tap) water.

Table 2. Recovery, Standard Deviation (s), and RSD of Oryzalin in Citrus Fruits and Stone Fruits

		recovery[a]				
matrix	fortification level (μg/g)	average (%)	range (%)	s	RSD (%)	n
whole citrus fruit[a]	0.010	83	82–85	1.0	1.2	5
	0.10	85	84–86	0.6	0.7	6
	1.00	88	85–90	1.9	2.2	5
	0.010–1.00	85	82–90	2.3	2.7	16
citrus pulp[a]	0.010	88	85–91	3.1	3.6	5
	0.10	89	79–95	5.6	6.3	6
	1.00	90	88–94	2.6	2.8	5
	0.010–1.00	89	79–95	3.9	4.4	16
citrus peel[a]	0.010	88	87–89	0.9	1.0	5
	0.10	88	81–92	3.9	4.4	6
	1.00	88	83–93	4.3	4.8	5
	0.010–1.00	88	81–93	3.2	3.6	16
stone fruit[b]	0.010	85	81–90	3.0	3.5	10
	0.10	86	82–91	2.9	3.4	6
	1.00	86	84–87	1.2	1.4	10
	0.010–1.00	86	81–91	2.3	2.7	26

[a] Combined data for oranges and lemons. [b] Combined data for peaches and cherries.

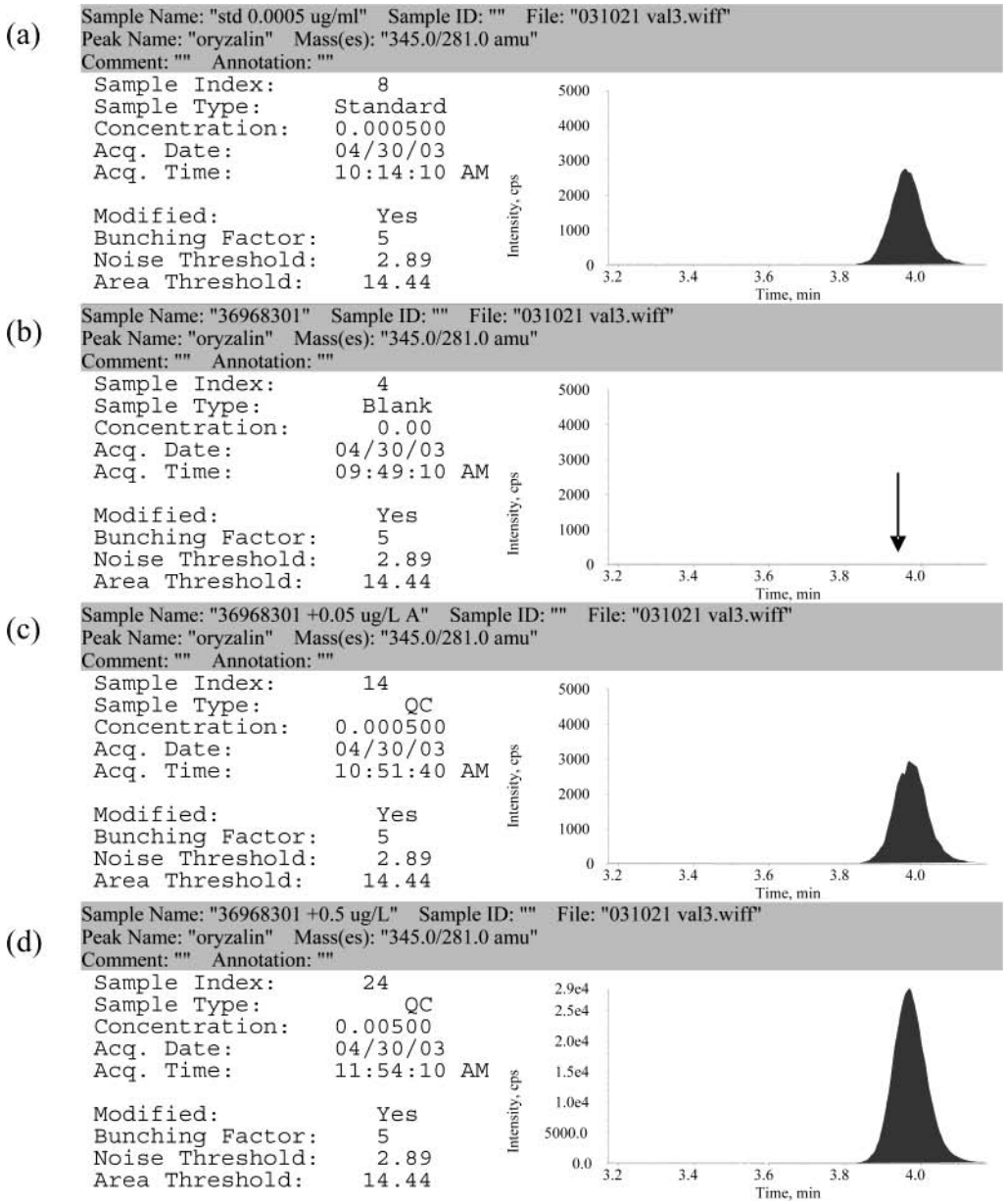

Figure 2. Typical MRM chromatograms for the determination of oryzalin in drinking water: (**a**) 0.0005 μg/mL standard, equivalent to an oryzalin water concentration of 0.05 μg/L; (**b**) control drinking water containing no detectable residue of oryzalin; (**c**) control drinking water fortified with oryzalin at 0.05 μg/L (LOQ), equivalent to a recovery of 107%; and (**d**) control drinking water fortified with oryzalin at 0.5 μg/L, equivalent to a recovery of 105%.

for the 0.5 ng/mL calibration standard, which was the concentration that was equivalent to the limit of quantitation (LOQ) of the method.

Sample solutions producing responses that exceeded the range of the standard calibration curve were diluted with methanol/water/acetic acid (50:50:1) to produce responses within the range of the standard curve. The equation for the standard curve was calculated using quadratic regression analysis with a $1/X$ weighting.

LODs and LOQs. Using a technique described previously (*9*), the LODs and LOQs for the methods were calculated from the standard deviation (s) of the μg/mL or μg/g results of fortified recovery samples. For water samples, the LOD and LOQ were calculated from the standard deviation of results from the 0.05 μg/L fortified recovery samples. For fruit samples, the LOD and LOQ were calculated from the results of the samples fortified at 0.010 μg/g. The LOD was calculated as three times the standard deviation (3s), and the LOQ was calculated as 10 times the standard deviation (10s).

RESULTS AND DISCUSSION

LC/MS/MS Sensitivity and Selectivity. LC/MS/MS produced the needed sensitivity and selectivity for determining residues of the herbicide oryzalin in water and fruit samples. The mass spectrometer (MDS/SCIEX API 3000) was automatically optimized by infusing oryzalin standard solutions and using the automated SCIEX algorithms to optimize the collision energy for producing the product ion. The atmospheric pressure chemical ionization mode was not suitable because oryzalin is unstable at high temperatures, thereby leading to less sensitivity. Positive ion electrospray ionization produced no usable signal. Fouling of the instrument during continual operation was avoided by diverting the flow to waste for the first 3 min.

Method Validation. The water method was validated over the range of 0.050−5.0 μg/L for drinking (tap) water, ground (well) water, and surface (stream and pond) water. The results are summarized in **Table 1**. The average recovery for all fortification levels combined (0.050−5.0 μg/L) was 102% with a relative standard deviation (RSD) of 5.8%.

The crop method was validated over the range of 0.010−1.0 μg/g for citrus fruits and stone fruits. Citrus crops included

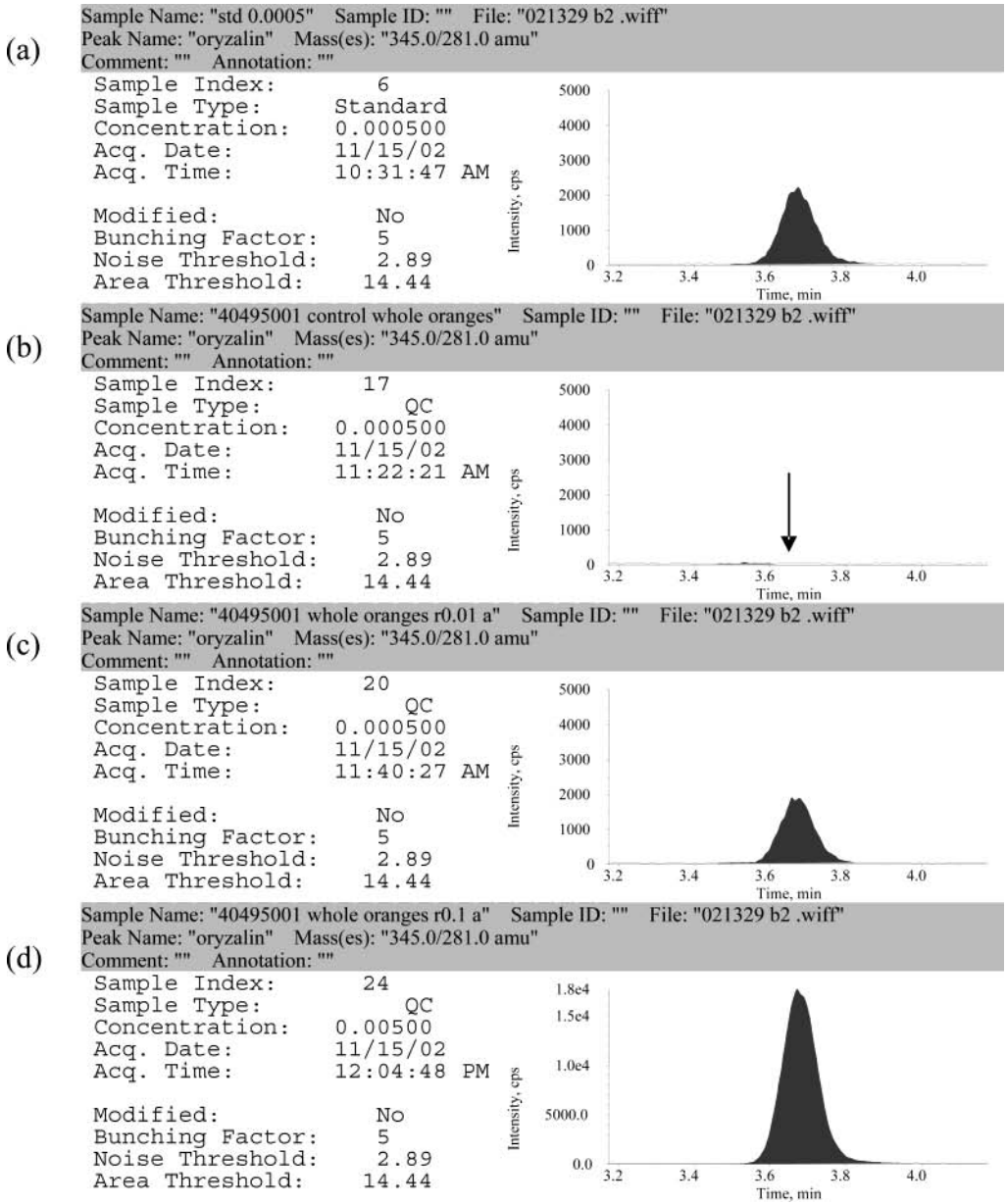

Figure 3. Typical MRM chromatograms for the determination of oryzalin in citrus fruit: (**a**) 0.0005 μg/mL standard, equivalent to a whole orange concentration of 0.01 μg/g; (**b**) control whole orange containing no detectable residue of oryzalin; (**c**) control whole orange sample fortified with oryzalin at 0.01 μg/g (LOQ), equivalent to a recovery of 83%; and (**d**) control whole orange sample fortified with oryzalin at 0.1 μg/g, equivalent to a recovery of 88%.

oranges and lemons (whole fruit, peel, and pulp), and stone fruit crops included peaches and cherries. The results are summarized in **Table 2**. The average recoveries for all fortification levels combined (0.010−1.0 μg/g) ranged from 85 to 89% for the various sample types. The corresponding RSDs ranged from 2.7 to 4.4%.

Lot-to-lot variation of SPE cartridges and plates has typically been negligible. However, if the recovery levels are unacceptably low and/or sample cleanup is inadequate, it might be necessary to standardize the elution profile of the SPE cartridges or the 96 well SPE plate. The rinse solvents and elution solvents may be divided into several smaller volumes that are collected and analyzed separately. The volumes of rinse solutions and elution solutions may then be modified as needed to ensure adequate recovery and cleanup.

Mass Spectra. A mass spectrum for oryzalin is contained in **Figure 1**.

Chromatograms. Typical chromatograms for the determination of oryzalin in water and fruit samples are contained in **Figures 2** and **3**. Chromatograms for the other sample types were essentially the same as those presented. The retention times for oryzalin differ in **Figures 2** and **3** because the samples were analyzed on two different HPLC columns that differed in age.

Linearity. The linearity of the detector was determined using eight calibration standards ranging in concentrations from 0.1 to 50 ng/mL. The correlation coefficient (r) describing the detector response as a function of concentration of the standard curve solutions was 0.9997 or greater during the validation of the methods.

LODs and LOQs. The calculated values for the LOD (3s) and LOQ (10s) are presented in **Table 3**. For the water method, the calculated LOD was 0.01 μg/L. The calculated value supported a method LOD of 0.01 of μg/L, which was included in the validation to demonstrate qualitative recovery. Likewise,

Table 3. Calculated LODs and LOQs for Determining Oryzalin in the Water and Fruit Methods Based on Standard Deviation (*s*)

analyte	average result[a]	*s*[a]	LOD (3*s*)[a]	LOQ (10*s*)[a]
water[b]	0.0515	0.0032	0.0095	0.0316
whole citrus fruit[c]	0.0083	0.0001	0.0003	0.0010
citrus pulp[d]	0.0088	0.0003	0.0010	0.0032
citrus peel[d]	0.0088	0.001	0.003	0.009
stone fruit[d]	0.0085	0.0003	0.0009	0.0030

[a] Units are μg/L for water samples and μg/g for fruit samples. [b] Combined data for surface (stream and pond) water, ground (well) water, and drinking (tap) water. [c] Combined data for oranges and lemons. [d] Combined data for peaches and cherries.

the calculated LOQ for water was 0.03 μg/L, which supported the validated method LOQ of 0.05 μg/L (**Table 1**).

For the fruit method, the calculated LOD ranged from 0.0003 to 0.0010 μg/g for whole citrus fruit, citrus peel, citrus pulp, and stone fruits. These calculated LODs supported a method LOD of 0.002 μg/g, which was included in the validation to demonstrate qualitative recovery. Likewise, the calculated LOQ ranged from 0.0001 to 0.0010 μg/g, which supported the validated LOQ of 0.010 μg/g (**Table 3**).

Assay Time. The assay time was greatly reduced by using the 96 well SPE plate. A typical sample set consisting of 30−35 water or fruit samples was prepared by one person in approximately 6 h, followed by LC/MS/MS analysis. The use of HPLC permits the determination of underivatized oryzalin, which yields efficiency gains as compared to derivatizing oryzalin for determination by GC-ECD or GC-MS (*1*−*5*). Another feature contributing to reduced analysis time is the sensitivity of the LC/MS/MS technique, which permits the processing of a small aliquot volume (1.0 mL) of the 50 mL sample extract; the small aliquot volume is amenable to use on the 96 well SPE plate so that a large number of samples can be processed efficiently. It is estimated that the LC/MS/MS approach improves sample throughput by a factor of at least 3−5-fold as compared to previously published methods for oryzalin (e.g., *1*−*4*). For methods that required an overnight derivatization for analysis by GC (*2*−*4*), the gain in efficiency is even greater.

Specificity and Confirmation. The presence of oryzalin was confirmed by comparing the liquid chromatographic retention times of the analyte in the calibration standards with those found in the samples while monitoring specific precursor ion/product ion transitions. Because of the highly specific nature of the MS/MS transitions monitored during detection, no further confirmation technique was required. Unlike HPLC-UV or GC-ECD, quantitation and confirmation can be achieved simultaneously and with greater specificity by LC/MS/MS.

In conclusion, residue methodology has been validated for the determination of oryzalin in surface water, drinking water, groundwater, stone fruit crops (peaches and cherries), and citrus crops (orange and lemon whole fruit, peel, and pulp). The sensitivity, selectivity, accuracy, and precision of the methodology make LC/MS/MS a valuable technique for environmental monitoring of residues. As compared to other published GC and HPLC methods of analysis, LC/MS/MS is a more sensitive and selective technique that substantially reduces the time required for quantitation and confirmation of residues.

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