Determination of Bromoxynil Octanoate in Soil and Corn by GC with Electron Capture and Mass Spectrometry Detection Under Field Conditions

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In this paper, a simple, and rapid analysis of bromoxynil octanoate is reported for soil, corn leaves, and corn seeds by utilizing one-step liquid-liquid extraction and partitioning, followed by GC with electron capture (ECD) and mass spectrometry (MS) detection. The method was validated by recovery experiments and assessment of matrix effects. Recoveries for GC-ECD and GC/MS were 82.3–110.7% with a relative standard deviation of <14% using matrix-matched calibration solutions for quantification. The limit of quantitation (LOQ) values were 0.005 and 0.2 mg/kg for the ECD and MS detector, respectively, depending on the sensitivity of the target compound. The concentration levels for bromoxynil octanoate residue found in soil, corn leaves, and corn seeds from field experiments were clearly below the LOQ of the ECD detector. The half-life times of bromoxynil octanoate were 2.2–4.2 days in soil and corn leaves. These results indicated that the developed method was appropriate for analysis of bromoxynil octanoate in soil and corn samples.

Pesticide use leads to increased crop yields and improvements to the quality of food produced, but pesticide residue levels in foods are of increasing concern to the public (1, 2). There are many broad-spectrum herbicides that have been designated as reduced-risk pesticides for humans, nontarget plants, and environmental resources. Bromoxynil octanoate, 2,6-dibromo-4-cyanophenyl octanoate, is widely used as a herbicide in the production of corn and other crops (3). There are some reports related to the use and efficacy of bromoxynil octanoate on plants to control their companion weeds (4–7).

In recent years, the development of sophisticated analytical methods that are capable of detecting trace quantities of pesticides has become increasingly important in the field of pesticide chemistry (8). And through 20 years of development, the technology of chromatography and mass spectrometry (MS) has improved rapidly. Unquestionably, MS gives a much higher degree of certainty in analyte identification than any chromatography technique (9). Thanks to this, the confirmation of target compounds can be achieved with a higher level of confidence. Gas chromatography (GC) coupled with MS has recently been proposed for the determination of pesticide residues in crops (10–15).

Although the use of bromoxynil octanoate is continuously increasing due to its attractive properties, there have been only a few analytical studies on its determination in plant tissue matrices by GC and by column liquid chromatography (LC) with UV detection (16–18). To the best of our knowledge, no work has been reported on the dissipation rate of bromoxynil octanoate in a complex matrix like corn. In general, for the determination of pesticide residues in complex matrices such as food, a liquid-liquid extraction (LLE) followed by multiple operation steps have been used as the conventional sample preparation method.

As reported in this paper, a simple, quick, and practical method for sensitive detection of bromoxynil octanoate from matrices like soil, corn leaf, and corn seed was developed using LLE, GC coupled with an electron capture detector (GC-ECD), and GC/MS for residue determination and confirmation. The technique described was applied to corn from 2 regions (Henan and Shandong) to monitor the fate of herbicide residues in the field and provide scientific evidence for both food monitoring and systematic determination of the environmental fate of bromoxynil octanoate.

Experimental

Reagents and Materials

Analytical standards of bromoxynil octanoate (99% technical) and its 25% emulsified concentrate (EC) formulation were obtained from Changqing Agrochemical Ltd Co. (Jiangsu, China). Anhydrous sodium sulfate and sodium chloride were analytical grade purchased from Beihua Fine-Chemicals Co. (Beijing, China). Hexane and acetone were GC grade purchased from Sigma-Aldrich (Steinheim, Germany).
Figure 1. Mass spectra of bromoxynil octanoate.

Figure 2. The selected peak of bromoxynil octanoate used for quantification, $m/z$ (a) 57, (b) 67, (c) 109 and 127, (d) 277.
Apparatus

(a) GC-ECD system.—The herbicide was separated and determined using a Varian model 3400 GC system fitted with an ECD and a DB-5 column (30 m × 0.25 mm id, 0.25 μm film thicknesses; J&W, Agilent Technologies, Wilmington, DE). The GC oven temperature program was started at 70°C for 1 min, increased to 200°C at a rate of 30°C/min, increased to 250°C at 15°C/min, and held for 10 min. The injector temperature was 250°C, and splitless injection was performed. Helium was used as the carrier gas, and nitrogen (30 mL/min) as the auxiliary gas for the ECD. The detector temperature was 300°C and the injection volume was 2 μL.

(b) GC/MS system.—A Trace Ultra gas chromatograph (Thermo Scientific, Madison, WI) fitted with a splitless injector and a TR-5ms capillary column (30 m × 0.25 mm id, 0.25 μm film thicknesses) was used to determine and confirm the identity of bromoxynil octanoate. It was coupled to a POLARIS Q ion-trap mass spectrometer and equipped with Xcalibur Version 1.4.1 software. The injector was operated at 250°C, and a pretreated sample volume of 1 μL was injected in the splitless mode. The oven was programmed as follows: 50°C (1 min), increased to 150°C (30°C/min), then to 250°C (10°C/min), and held for 10 min. The ion-trap mass spectrometer was operated in the electron ionization (EI) mode at 70 eV. The ion source was set at 250°C and the transfer line at 280°C. Helium was used as the carrier gas (1 mL/min). The target compound was identified with a full scan [mass-to-charge ratio (m/z) 50–420] and a selective ion monitoring (SIM) scan with confirmation ions m/z 109 (15%) and 127 (27%).

(c) Homogenizer.—Ultra-Turrax, Model T25-S1 (IKA-Werke, Staufen, Germany).

(d) Vacuum rotary evaporator.—Rotavapor Model RE2000A (Yarong Biochemical Instrument Co., Shanghai, China).

Standard Solutions

Stock solutions (1.00 mg/mL) of bromoxynil octanoate were prepared by adding 100 mg (adjusted for purity) of pure compound to separate 100 mL volumetric flasks and bringing up to volume with acetone. The stock solutions were stored at –20°C and were stable for 6 months. Calibration solutions were prepared in solvent (acetone) and in soil, corn leaf, and corn seed extracts from untreated samples in the 0.005–10 μg/mL concentration range. Matrix-matched standard solutions were prepared by evaporating to dryness portions of the final extract solution obtained from the uncontaminated matrices (soil, corn leaves, and corn seeds) under a gentle nitrogen stream and reconstituting the residue with the calibration solution.

Field Trials

The field trials were conducted in 2 experimental fields located in the Shandong and Henan provinces during the 2007 agricultural season using a manual sprayer to run off. The fields were divided into 30 m²-sized blocks for the control, as well as the dissipation rate study. The corn and bald soil were both sprayed, in 3 replications, with a 25% EC of bromoxynil octanoate diluted with water at a dosage of 56.3 mL of active ingredient of bromoxynil octanoate/hectare (1.5 times the recommended dosage). Soil
samples were collected from different depths ranging from 0 to 15 cm. The soil and corn leaf samples, which were collected at 0 (2 h after spraying), 1, 2, 4, 7, 14, 21, 28, and 60 days, were put into polyethylene bags and transported to the laboratory. The corn seeds were collected when harvested. All of the subsamples were kept deep-frozen (–20°C) until analysis. Control samples were obtained from the soil in the control plot. When they were analyzed, all the field samples were based on dry weight.

**Extraction and Purification**

(a) **Soil extraction.**—An aliquot (10 g) of sample was weighed into a 150 mL conical flask, and 50 mL extraction solvent acetone was added. The mixture was shaken vigorously for 45 min. After filtering, the final volume of filtrate was 50 mL, and 25 mL of the filtrate transferred to a 500 mL separatory funnel, to which 25 mL water, 25 mL saturated NaCl solution, and 50 mL hexane were added. The funnel was shaken

![GC/MS chromatograms of an untreated (a) and a fortified (b) corn leaf sample at 1 mg/kg.](image)

**Table 1. Calibration equation, LOD, and LOQ for bromoxynil octanoate in soil, corn leaves, and corn seeds**

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Conc range, mg/L</th>
<th>Calibration equation</th>
<th>Slope</th>
<th>RSD(^a), %</th>
<th>R(^2)</th>
<th>LOD, mg/kg</th>
<th>LOQ, mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>ECD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil</td>
<td>0.005–10</td>
<td>Y = 1031397C – 51808</td>
<td>3.5</td>
<td>0.9997</td>
<td>0.0015</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Corn leaves</td>
<td>0.005–10</td>
<td>Y = 921998C + 82982</td>
<td>3.6</td>
<td>0.9997</td>
<td>0.0015</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Corn seeds</td>
<td>0.005–10</td>
<td>Y = 863932C + 51551</td>
<td>1.2</td>
<td>0.9999</td>
<td>0.0015</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>MS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil</td>
<td>0.2–10</td>
<td>Y = 4218C – 828</td>
<td>7.2</td>
<td>0.9991</td>
<td>0.06</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Corn leaves</td>
<td>0.2–10</td>
<td>Y = 2959C – 362</td>
<td>3.1</td>
<td>0.9982</td>
<td>0.06</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Corn seeds</td>
<td>0.2–10</td>
<td>Y = 2894C + 7.4</td>
<td>1.2</td>
<td>0.9993</td>
<td>0.06</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) RSD = Relative standard deviation.

\(^b\) n = 3.
vigorously for 1 min, with venting. The lower layer was drained off, and the hexane phase was dried over anhydrous Na₂SO₄. The aqueous layer was partitioned 3 more times with 50 mL aliquots of hexane. All hexane fractions were pooled and concentrated to dryness with a rotary evaporator at 40°C. The residue was reconstituted with 5 mL acetone for chromatographic injection.

(b) Corn leaf and corn seed extraction.—An aliquot (10 g) of previously homogenized sample was weighed into a 150 mL conical flask and extracted with 50 mL acetone for 2 min using an Ultra-Turrax T25 mixer at 9500 rpm. The mixture was shaken vigorously for 30 min. After filtering, the procedure used was the same as described above for soil extraction.

Validation Studies

Calibration data, matrix effect assessment, precision (when repeated independent assays were performed), accuracy (when recovery assays were performed), sensitivity, and limit of quantitation (LOQ) were calculated for the analytical methodology developed. Recovery assays were performed by spiking uncontaminated soil, corn leaves, and corn seeds (10 g homogenized sample) at different levels with bromoxynil octanoate working solutions. The spiked samples were allowed to equilibrate for 1 h before extraction to allow the spiked solution to penetrate the test material. Replicated samples [number of determinations (n) = 5] were run, and the recovery values were calculated for each.

Results and Discussion

Analysis of Bromoxynil Octanoate by GC/MS

Figure 1 shows a mass spectrum and total ion chromatogram of the bromoxynil octanoate standard, in which the molecular ion was absent; the peak (m/z 275/277/279) corresponded to [CNC₆H₃Br₂O]⁺, from the bromoxynil moiety, and the base peak (m/z 127) corresponded to [C₈H₁₅O]⁺. This result confirmed that undecomposed bromoxynil octanoate eluted from the GC column because the negative ion detected was complementary to the ion detected in the EI positive spectrum (19). Figure 2 shows the selected ion chromatograms of bromoxynil octanoate added to the corn leaf sample. Ions other than m/z 109 and 127 were not detected in a low concentration of bromoxynil octanoate because many disturbances appeared around the bromoxynil octanoate fragment ion peak at m/z 57, and m/z 277 of the bromoxynil octanoate fragment ion was of low intensity. The fragment ions at m/z 109 and 127 were for chosen bromoxynil octanoate determination.

Method Validation

Chromatograms of bromoxynil octanoate samples are presented in Figure 3 (GC-ECD) and Figure 4 (GC/MS). The retention time of bromoxynil octanoate was 12.1 min for the ECD and 12.3 min for MS, and there were no interference peaks in this region of the chromatograms. Quantification was performed by the external standard procedure. In the studied ranges of concentration, from 0.005 to 10 mg/L for the ECD and 0.2 to 10 mg/L for MS, good linearity was achieved for bromoxynil octanoate [correlation coefficients (R²) > 0.999] for the 2 detectors and for 3 kinds of standard solutions (Table 1). The impact on the results of a difference in the column and the oven programmed temperature in both GC instruments was very little because of the external standard, and it was ignored in this study.

The limit of detection (LOD) and LOQ were defined as a signal-to-noise (S/N) ratio of 3:1 and 10:1, respectively. As shown in Table 1, the LOD values of bromoxynil octanoate were 0.0015 and 0.06 mg/kg for GC-ECD and GC/MS, respectively. The LOQ values were 0.005 and 0.2 mg/kg for...
the ECD and MS detectors, respectively, which were better than the LOQ values obtained for LC and GC (16, 17). Compared with the MS detector in this study, the ECD was more sensitive for different matrixes.

**Matrix Effect Assessment**

The response of the detector system to certain pesticides may be affected by the presence of coextractives from the sample (20, 21). These matrix effects may be observed as an increase or decrease in response, compared with those produced by solvent solutions of the target compound. The effect of the matrix can be variable and unpredictable in terms of measurable effects. The matrix effect on the detectors (ECD and MS) in the present method was investigated by comparing standards in solvent with matrix-matched standards. The relative responses (response matrix/response solvent) are shown for selected ratios in Figure 5. The quantification of the samples was performed for each analytical series using the means of 3 calibration curves. The calibration data obtained for this herbicide in matrix are shown in Table 1. The mean recoveries, calculated in triplicate by matrix-matched calibration standard of corn leaves and solvent at 0.05 mg/kg in corn leaf samples for GC-ECD were 95.3 and 102.4%, with relative standard deviation (RSD) values of 3.3 and 4.3%, respectively; the recoveries at 1 mg/kg were 98.4 and 109.7%, with RSD values of 8.2 and 7.8%, respectively. Thus, it may be concluded that matrix impact was not significant, and no significant difference between the different matrixes was observed (Table 1 and Figure 5). This might be important in routine control of pesticide residues, as only one matrix-matched calibration standard is necessary when analyzing several commodities within the same analytical batch.

**Recovery and Precision**

Recovery tests for soil, corn leaf, and seed samples were performed at fortification levels of 0.005, 0.05, and 1 mg/kg for GC-ECD and 0.5, 1, and 5 mg/kg for GC/MS on 3 different days. Good recoveries were obtained for bromoxynil octanoate at all fortification levels, as shown in Table 2. The mean recoveries of analyte were in range of 84.9 to 106.7% and 82.3...
to 110.7%, with repeatability (relative standard deviations, RSDr) values of 1.8–9.2 and 4.2–13.8%, and reproducibility (RSDR) values of 3.1–9.2 and 5.8–14.0% for ECD and MS, respectively. The recovery of bromoxynil octanoate was similar to that reported earlier for runoff water (75.4–100.2%) by LC (17) and in water samples (84.3–96%) by GC-ECD (18), and was better than reported for river sediment (44.6–91.1%; 18).

Field Sample Analysis

The developed GC methodology with ECD and MS detection was applied to determine the bromoxynil octanoate residues in soil, corn leaves, and seeds collected in the Shandong and Henan provinces in 2007, and to compare the results obtained with 2 chromatographic techniques (Table 3). The average levels of bromoxynil octanoate residue obtained after treatments for 60 days were both <0.005 mg/kg in corn seeds from Shandong and Henan.

The residues in soils determined by GC-ECD degraded from 0.495 to 0.011 mg/kg and from 0.605 to 0.011 mg/kg after treatment in Shandong and Henan, respectively, over a period of 28 days, and then decreased progressively until their complete disappearance after 60 days. The residues in corn leaves were higher and degraded from 20.7 to 0.345 mg/kg in 28 days in Shandong and Henan as determined by GC-ECD, and results were similar using GC/MS. The decline of bromoxynil octanoate residue in soil, corn leaves, and corn seeds can be explained in terms of the physicochemical properties of the active ingredients and the nature of the degradation process.

Bromoxynil octanoate’s half-life times in corn leaves were calculated as a pseudo first-order kinetic process with correlation coefficients greater than 0.91 and 0.98 with the ECD and MS detector. The half-life times of bromoxynil octanoate were 2.2–3.4 days by GC-ECD and GC/MS at 1.5× the recommended dosage. The dissipation trend of bromoxynil octanoate for the 2 detectors was also similar. The half-lives obtained by GC-ECD for bromoxynil octanoate in soil from Shandong and Henan were 3.6 and 4.2 days, respectively. The half-life times were slightly longer than those in the literature for subsurface water (0.3–1.0 day; 18). The analyte diminishes measurably with time, although the rate of loss may vary according to environmental conditions. Dilution due to the growth of the treated plants, physical loss, chemical degradation, and soil microorganism degradation are the main factors contributing to this diminution.

Conclusions

In conclusion, 2 GC systems (GC-ECD and GC/MS) provided simple and rapid determination of bromoxynil octanoate residues that is appropriate for routine analysis of different agricultural samples (soil, corn leaves, and corn seeds). The obtained linearity, recovery, precision, and LOQ values were found to be satisfactory, and the matrix did not significantly suppress or enhance the response of the instruments. The concentration levels found for analyte residue on corn seed samples at 1.5× the recommended dosage were below LOQs. A comparison of the results produced by the 2 GC systems showed that there was no significant difference for bromoxynil octanoate residues in corn leaf samples, except that the sensitivity of GC-ECD was much higher than that of GC/MS in the EI mode.

Bromoxynil is the active ingredient of bromoxynil octanoate, so the next step in our studies is to develop a multiresidue method of determination of bromoxynil, bromoxynil octanoate, and other bromoxynil esters.

Table 3. Average bromoxynil octanoate residues in soil and corn leaves at the 1.5 times the manufacturer-recommended dosage

<table>
<thead>
<tr>
<th>Days after treatment</th>
<th>Concentration ± SD, mg/kg (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soil</td>
</tr>
<tr>
<td></td>
<td>GC-ECD</td>
</tr>
<tr>
<td></td>
<td>Shandong</td>
</tr>
<tr>
<td>0</td>
<td>0.495 ± 0.03</td>
</tr>
<tr>
<td>1</td>
<td>0.411 ± 0.01</td>
</tr>
<tr>
<td>4</td>
<td>0.112 ± 0.02</td>
</tr>
<tr>
<td>7</td>
<td>0.108 ± 0.02</td>
</tr>
<tr>
<td>14</td>
<td>0.055 ± 0.008</td>
</tr>
<tr>
<td>28</td>
<td>0.011 ± 0.005</td>
</tr>
<tr>
<td>60</td>
<td>ND</td>
</tr>
</tbody>
</table>

a SD = Standard deviation.

b ND = Not detectable.
Acknowledgments

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