Determination of Triazine Herbicides in Aqueous Samples by Capillary HPLC-MS/MS

S.V. Calugaru, W.R. West, E.P. Gates, M.L. Lee

Abstract

The compact Axcend Focus LC® was coupled to a relatively small footprint Agilent Ultivo triple quadrupole mass spectrometer for the analysis of triazine pesticides in aqueous samples.

Components of a standard test mixture of seven triazine herbicides (alachlor, atrazine, cyanazine, metolachlor, metribuzin, prometryne, and simazine) were separated by HPLC on a C18 capillary column and quantified using multiple reaction monitoring mass spectrometry.

Introduction

Triazine herbicides are broadly used in agriculture and represent a potential source of environmental water contamination. Therefore, screening and quantification of these pesticide residues in aqueous samples (including drinking water) are important in environmental monitoring and food safety control.

Materials and Methods

Instrumentation

An Axcend Focus LC with 2.2.0 Axcend Drive™ software (Axcend, Provo, UT, USA) and an Agilent Ultivo triple quadrupole mass spectrometer (LC/TQ G6465B with Jet Stream Electrospray Ionization source and MassHunter software, Agilent, Santa Clara, CA, USA) were coupled together for this application. The MassHunter software (acquisition: v1.1, qualitative analysis: v10.0, and quantitative analysis: v10.0) was used for instrument control and data processing. A Model 22 syringe pump was from Harvard Apparatus (Holliston, MA, USA).

LC to MS Interface

To accommodate mobile phase microflow rates, the regular nebulizer of the ion source was replaced with a microflow nebulizer (Part No. G1946-67260, Agilent). The transfer line from the column cartridge to the microflow nebulizer (25 cm long, 360 µm OD, 25 µm ID PEEKsil tubing, Part No. 0624374, Trajan, Melbourne, Victoria, Australia)

was attached to the column using a zero-dead-volume 360 µm union with a 50 µm bore hole (Part No. C360UPK2, VICI Valco Instruments, Houston, TX, USA). The microflow nebulizer was attached to the end of the transfer line using a 1/16" to 360 µm zero-dead-volume reducing union with a 100 µm bore hole (Part No. C360RUS64, VICI Valco Instruments).

Chemicals and Solvents

Canadian Drinking Water Triazine Herbicides Mix containing seven components (alachlor, atrazine, cyanazine, metolachlor, metribuzin, prometryne, and simazine, 1 mg/mL each in methanol) was purchased from Restek (Bellefonte, PA, USA). Calibration standards were prepared by serial dilutions of this test mixture in water. Deuterated D5-cyanazine (100 µg/mL in acetone) and D7-prometryne (100 µg/mL in acetonitrile) were purchased from HPC Standards (Atlanta, GA, USA) and used as internal standards. Water and acetonitrile (both LC-MS grade) were obtained from Sigma-Aldrich (MilliporeSigma, St. Louis, MO, USA). Formic acid (LC-MS grade) was purchased from Thermo Fisher (Waltham, MA, USA).

HPLC Method

A C18 capillary column (10 cm x 150 µm i.d., 1.8 µm particle size, CoAnn, Richland, WA, USA) was used. UV absorption was monitored at 235 nm using an on-column detector (Axcend). A binary gradient was generated from Solvent A (97:3:0.1 water/acetonitrile/formic acid, v/v) and Solvent B (97:3:0.1 acetonitrile/water/formic acid, v/v). The mobile phase program was 3% B (0-1 min isocratic), 3-11% B (1-2 min linear gradient), 11-27% B (2-17 min linear gradient), 27-46% B (17-21 min linear gradient), 46% B (21-30 min isocratic), 46-97% B (30-32 min linear gradient), and finally 97% B (32-35 min isocratic). The flow rate was 1 µL/min and the injection volume was 250 nL (full loop).

MS Method

Specific molecular and fragment ions were identified for each analyte by infusing a diluted test mixture of triazine pesticides (each component at 10 µg/mL) using a syringe pump at a flow rate of 1 µL/min and performing the analysis in positive polarity product ion mode. Quantitative analysis was conducted in the multiple reaction monitoring (MRM) mode for selected ion transitions using the following instrument acquisition parameters: fragmentor voltage, 100 V; collision energy, 25 eV; capillary voltage, 3000 V; gas temperature, 200 oC; gas flow rate, 5 L/min; nebulizer pressure, 10 psi; dwell time, 30 ms. For quantitative analysis, D5-cyanazine and D7-prometrine were added as internal standards to all samples at a final concentration of 500 ng/mL and 50 ng/mL, respectively.

Results and Discussion

Analyte Identification

The chemical structures of the analyzed triazine herbicides are given in Figure 1, and the chromatographic separation of these compounds on a C18 capillary column with UV-absorption detection at 235 nm is shown in Figure 2. Each analyte in the chromatogram was identified based on MS data. Total ion current and extracted ion chromatograms for the analytes are shown in Figure 3.

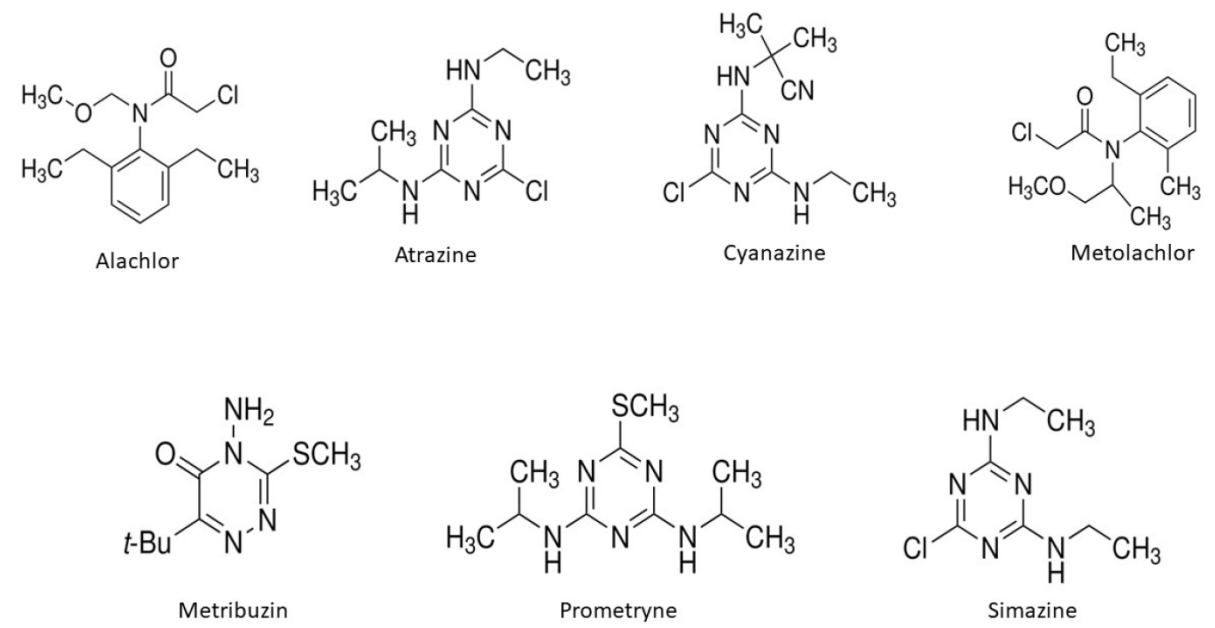

Figure 1. Chemical structures of triazine herbicides.

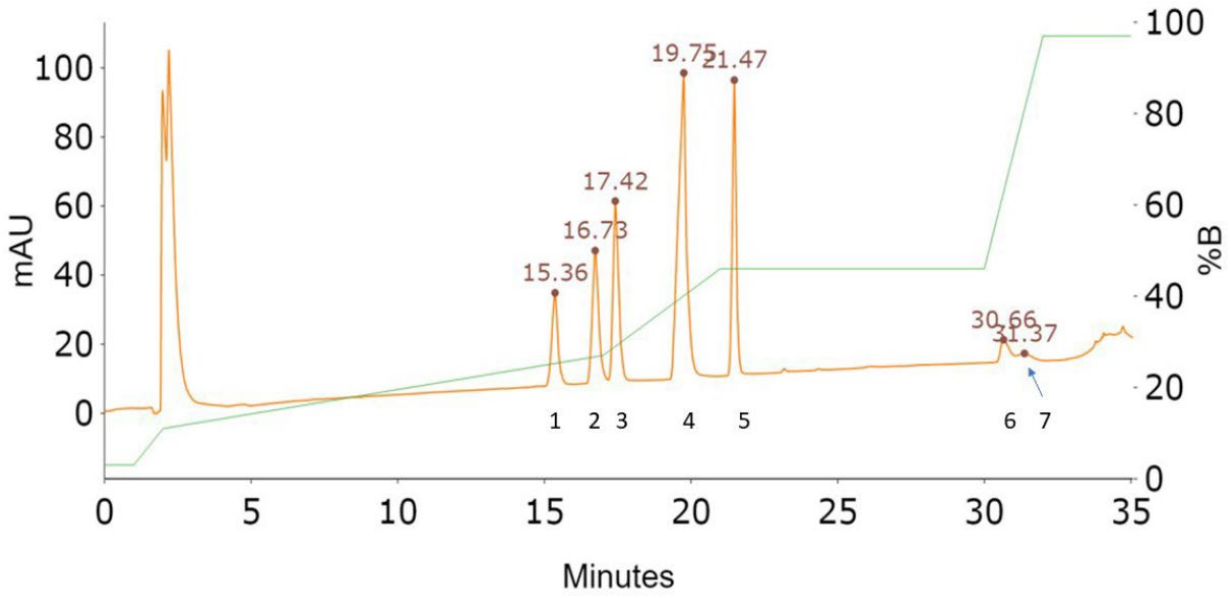

Figure 2. Chromatographic separation of a mixture of triazine herbicides (50 µg/mL each analyte, injection volume 250 nL) on a capillary C18 column with UV detection at 235 nm: (1) simazine, (2) metribuzin, (3) cyanazine, (4) prometryne, (5) atrazine, (6) metolachlor, (7) alachlor. The identifications are based on MS data (see Figure 3).

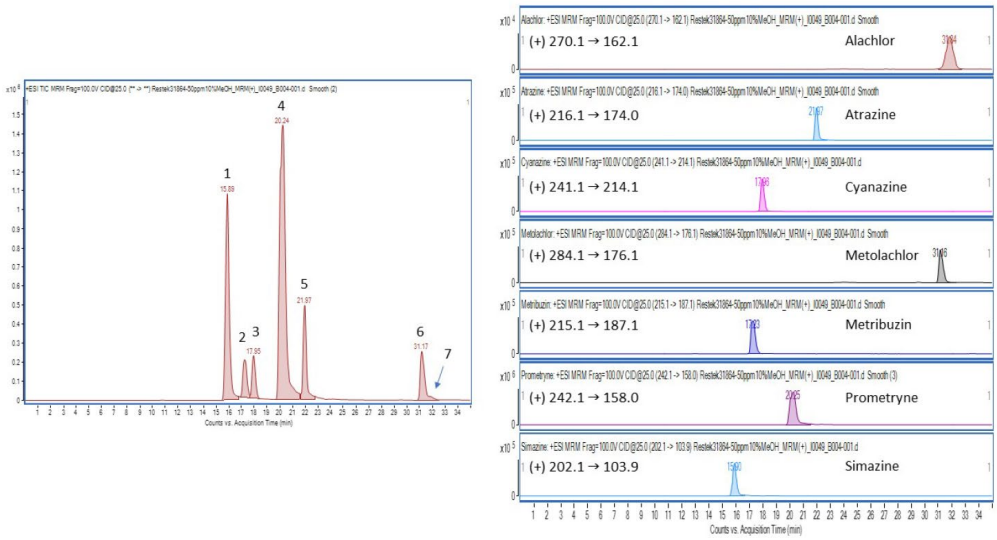

Figure 3. Chromatographic separation of a mixture of triazine herbicides (50 µg/mL each analyte, injection volume 250 nL) on a capillary C18 column: total ion current (left) and extracted ion chromatograms (right); (1) simazine, (2) metribuzin, (3) cyanazine, (4) prometryne, (5) atrazine, (6) metolachlor, (7) alachlor.

Analyte Quantification

The specific molecular and product ions identified by operating the MS in the positive polarity product ion mode are presented in Table 1. As mentioned above, selected ion transitions "molecular ion → product ion" were used to monitor the analytes in the MRM mode. Deuterated analogs of cyanazine and prometryne (D5-cyanazine and D7-prometrine) were added as internal standards at permanent concentrations of 500 ng/mL and 50 ng/mL, respectively, to normalize the intensities of the MS signals. The best calibration curves with regression coefficient R^2 above 0.99 for linear or quadratic regression were obtained with D5-cyanazine as an internal standard for cyanazine, atrazine, simazine, and metribuzin, and with D7-prometryne as an internal standard for prometryne, alachlor, and metolachlor. The calibration curves covered concentration ranges of 3-10000 ng/mL for metribuzin, and metolachlor; 3-3000 ng/mL for atrazine and prometryne; 10-3000 ng/mL for cyanazine; and 30-10000 ng/mL for alachlor and simazine. Representative calibration curves for cyanazine are shown in Figure 4. Additional optimization of the MS acquisition parameters could potentially improve the quantification limits.

Compound Name	Precursor Ion (*m/z*)	Quantifier Product Ion (*m/z*)	Qualifier Product Ion (*m/z*)
Alachlor	270.1	162.1	147.1
Atrazine	216.1	174.0	132.0
Cyanazine	241.1	103.9	214.1
Metolachlor	284.1	176.1	252.1
Metribuzin	215.1	187.1	84.1
Prometryne	242.1	158.0	200.0
Simazine	202.1	103.9	96.0
D5-Cyanazine (Internal Standard)	246.1	219.1	-
D7-Prometryne (Internal Standard)	249.1	159.0	-

Table 1. Detected Precursor and Product Ions of Atrazine Herbicides (Positive Polarity Mode)

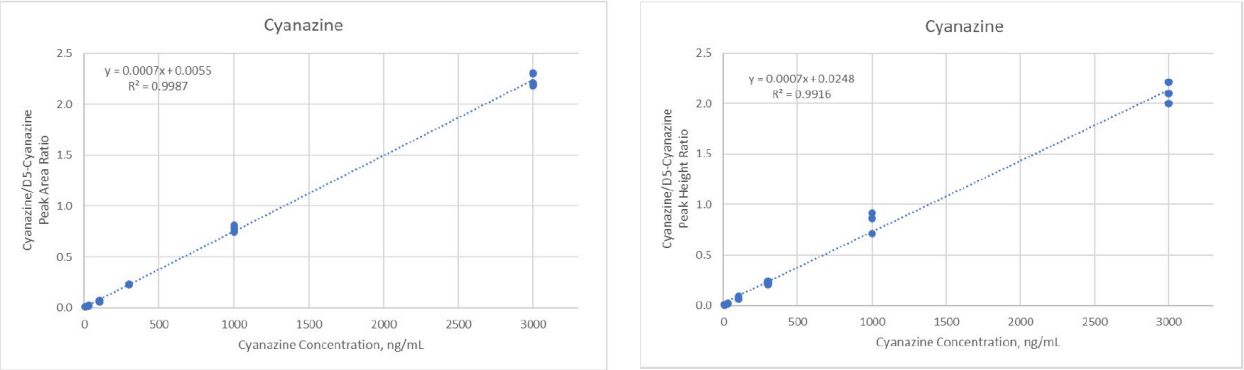

Figure 4. Calibration curves for cyanazine in the concentration range of 10-3000 ng/mL based on peak areas (left) and peak heights (right) for the ion transition (+) 241.1 → 103.9.

Conclusions

Detection and quantification of triazine herbicides using the compact Axcend Focus LC coupled to an Agilent Ultivo triple quadrupole mass spectrometer with microflow nebulizer was demonstrated. Components of a mixture of seven triazine herbicides (alachlor, atrazine, cyanazine, metolachlor, metribuzin, prometryne, and simazine) were identified and quantified by mass spectrometry using multiple reaction monitoring in the positive polarity mode. Further optimization of this method is likely possible.