Cantaurus, Vol. 6, 16-18, September, 08 © McPherson College Division of Science and Technology

Atrazine runoff from corn fields using high pressure liquid chromatography and solid phase extraction

Ryan Wenzel

ABSTRACT

The concentration of atrazine in the area surrounding the town of McPherson (mainly southwesterly around Elyria and Groveland) in the corn fields is determined using High Pressure Liquid Chromatography (HPLC), Solid Phase Extraction (SPE) tubes, and a UV-Vis detector. Samples were taken on April 1, 1998, from runoff and standing water following rainfall from areas surrounding corn fields and streams nearby where runoff could occur. The samples were processed using LC-18 SPE tubes (3 mL) and then run on the Shimadzu HPLC system equipped with a Supelco LC-8 column and the UV detector at 254 nanometers (nm). This process has produced significant, resolved and reproducible peaks with identifiable and quantitative size. It has been shown that most, but not all, of the atrazine is removed from the surface water after the winter months.

Keywords: *atrazine, HPLC, SPE tubes, surface-water runoff contamination, UV-Vis. detection*

INTRODUCTION

On the front page of the Sunday February 22, 1998 issue of *The Wichita Eagle* there was a article entitled "Poisoning the well: Rural water often tainted", which spanned three pages. This article dealt with the contamination of our water supply due to pesticides just such as atrazine. (Hays, 1998) Atrazine is a herbicide that is economical and effective in reducing crop losses due to weed interference. It is one of the most widely used herbicides in the United States and is used to control broadleaf weeds and certain grass weeds in fields of corn, wheat, soybean, and maize. (Steinheimer, 1990) Atrazine is a regulated chemical because of its toxicity, resulting in long term chronic damage. Yet, in 1990, it is estimated that 3.4 million kg of atrazine was used on the corn in Iowa alone, making it present (supposedly) year round in groundwater and streams in these areas. (Novak et al, 1997) It has also been found that although atrazine does somewhat leach into groundwater and soils, it has a tendency to remain in the upper part of the soil. (Juracek, 1997) In speaking with those whose job it is to monitor the atrazine spread onto the fields, it was said that there had not been any atrazine put on the fields for some time and it has all since been washed away.

The above mentioned lead to my research on the atrazine used in McPherson county on the corn fields. Samples were taken from local farms which produced corn in the previous growing season. These samples were removed two days after heavy rainfall from the surface water and runoff which had accumulated.

The HPLC was calibrated on standard atrazine solution prepared in HPLC grade methanol and was sensitive below 1 part per million (ppm).

MATERIALS AND METHODS

The first, and what has proven to be the most difficult part of the entire process was to determine if the available old HPLC would give accurate results. In order to do this it was first necessary to find out what we had and what we needed. Our system is composed of two LC-6A Shimadzu pumps, a SPD-6AV UV-Vis Spectrophotometer, and a RID-6A Refractive Index Spectrophotometer (which was not used). This was all controlled using a SCL-6A System Controller and outputs to a C-R6A Chromatopac which did the integration and calculation after peaks were obtained and printed. Samples were injected using a Dynateck Precision Sampling Syringe, 100 µL, RN-C-160 into a Rheodyne Syringe Loading Injector, Catalog No. 7125. The injector was fitted with a 20 µL sample tubing, and then samples were run through the Supelco LC-8 column which was protected by a Supelguard LC-8 Column guard. The atrazine samples were detected at 254 nm and the UV detector was also set at a 0.01 AUFS Absorbance setting. The system controller controls the injection and start time of the sample using a magnetic switch which is linked to it on the Rheodyne injector, this also cues the Chromatopac which begins charting and recording the chromatogram from the UV detector. The mobile phase was a 45:55 acetonitrile: water solution prepared using HPLC grade acetonitrile and water (Supelco cat. no. 27-071-7B and 27-073-3C respectively) and ran at a flow rate of 1.5 mm/min.

Standard preparation was performed by dissolving 5 mg of atrazine from 100 mg of standard packed in 1 mL of neat solvent (Supelco cat. no. 4-9085) in 50 mL of HPLC grade methyl alcohol (Supelco cat. no. 27-047-4B). This obtained a 1:10,000 relationship giving the initial 100 ppm standard. From this 1 ppm was made by dilution 1:99 mL and then 100 ppb using 1:9 mL dilutions. These were ran to obtain the basis to understand the unknown concentrations and our instruments sensitivity. (Steinheimer, 1990)

Samples were taken from the field at the areas

Table 1. Location of samples.

Sample	Which field was selected	Exact location in field
#1	Eisenhower Rd ≈1 block E of 12th Ave	Corn husks floating in ditch beside field
#2	1 block W of 13th Ave S. Side of Eisenhower Rd	In field still containing ≈ 1 ft high corn husks
#3	E of 14th Ave ≈2 blocks S. of Comanche Rd	Runoff Pit for irrigated corn field
#4	Between 14th-15th Ave on Comanche Rd	W. Side of Creek
#5	≈1 block E. of old 81 Highway on Comanche Rd	Under Irrigation System in field
#6	Between 9th and 10th S. of Dakota Ave	In field which still had few stocks standing

mentioned in Table 1. These areas were chosen because of there nearness to corn fields and because they spanned the southwestern portion of the county where the rainfall should have since washed off all the atrazine away. The procedure involved removing samples using a large syringe with bulb and screw top 70 mL bottles. These were then capped and labeled as well as marked on the map. (Maps of McPherson, 1994)

The samples were then prepared using Supelco LC-18 3 mL Solid Phase Extraction (SPE) tubes. This is done to help isolate the triazine compound in the runoff water samples as well as to help give sharper more accurate peaks. This process (if done correctly) should give ≈100% recovery of all atrazine as well as better response and resolution. (Supelco) The process begins by washing the tube 2 mL of methanol and then 2 mL of purified water to condition it. Next 3 mL of the sample is passed through the tube and washed with 1 mL of 4:3:1 water: acetonitrile: methanol solution. Then the atrazine is eluted using 1 mL of methanol. (Supelco) All standards and samples go through this process and each step took between 30 min and an hour depending on the viscosity of the sample.

As the samples were being prepared and eluted using the SPE tubes, the Chromatopac was calibrated using the 100 ppm and 1 ppm standards. Atrazine showed its peaks at a retention time of approximately 3.057 minutes with the tolerance set at 5%, as suggested as standard setting in the Chromatopac manual . Calibration was done using a two-point calibration curve method with response factors being calculated using the absolute calibration curve method (This is method "5" and mode "44" on the Chromatopac C-R6A series).

RESULTS

As mention above, once all the standards were prepared it was necessary to extract them with the same method as the samples would be extracted. This involved using the SPE tubes to maintain a standard method on all standards and samples. In running the standards without any extraction it was possible to get accurate results down to the 100 ppb range, but as just mentioned it was necessary to prepare the standards using the same method as the samples. In doing this it lost some of the concentration so it was necessary to calibrate my curve using 100 ppm and 1 ppm standards.

The machine was calibrated and gave response factors of F_1 = 1.71943 $\times$ 10^{-4} and F_2 = 0.1142 using the equations for an absolute calibration curve which are:

$$F_1 = \frac{C_1 \cdot Wsp_1 - C_2 \cdot Wsp_2}{100\left(A_1 - A_2\right)}$$

$$F_2 = \frac{C_2 \cdot Wsp_2}{100} - F_1 \cdot A_2$$

where:

A_1 : Area of standard sample 1 (580923)
A_2 : Area of standard sample 2 (5152)
C_1 : Concentration of standards sample 1 (100 ppm)
C_2 : Concentration of standards sample 2 (1 ppm)
Wsp_1 : Total weight of standard 1 (100)
Wsp_2 : Total weight of standard 2 (100)

using these values it was possible to obtain the concentration of atrazine in my unknown samples given only the area of the peak using the equation:

$$Content = \frac{F_i A_i + F_2}{Wsp} \cdot 100$$

After calibrating the instrument and all the samples had been eluded it was time to run the unknown samples. Each was run through exactly like the standards and none indicated atrazine. But sample #1 did show a bump that was not large enough to register with the current settings and appeared to possibly be in the retention time I was looking for. So I lowered my slope value from the Chromatopac calculated value of 6168 to half that (as the manual suggested) of 3000. This would force the Chromatopac to recognize much smaller peaks.

After running all the samples again a peak was found only on sample #1. This sample was repeated two more times for a total of three runs giving it values of

for atrazine concentrations of:
run 1 : 0.3732 ppm
run 2 : 0.4134 ppm
run 3 : 0.4572 ppm
and an average of 0.4146 ppm or 414.6 ppb.

DISCUSSION

The one peak detected was on sample #1. If one refers back to Table 1 it can be noticed that this was the sample that was taken from the side of a corn field in a area of water that was filled with floating corn stalks. This may have been why it seemed to retain the atrazine through the winter months and still show a small peak.

If I were to repeat this or similar research I would look for an alternative extraction technique that did not take so long for each phase, and did not cause a decline in concentrations, as the SPE tubes proved to. This would make it possible to detect concentrations much lower and possibly come up with more hits.

After running all the samples and coming up with only one peak it seems to show that nearly all the atrazine is washed off of the corn fields from the rains and snow over the non-growing seasons. This may lead one to wonder where does all this atrazine go? Does it go into the groundwater, or run down streams, or does it break down into something else? Any of these possibilities could pose definite environmental concerns for further research.

ACKNOWLEDGEMENTS

Jack Kelly, Supelco Technical Service, helped with selecting correct column, solutions, and standards. Richard Zerger, Professor of Chemistry, Bethel College, helped with product ordering. Leonard, Shimadzu Technical Service, helped with setting up old unused Shimadzu equipment. Levi Torrison, Student McPherson College, helped in obtaining samples

LITERATURE CITED

Hays, Jean and Erin Kennedy. 22 Feb 1998. Poisoning the well: rural water often tainted. Wichita Eagle. pp. 1-9A

Juracek, K.E. and E.M. Thurman. July-Aug 1997. Assessing aquifer contamination risk using immunoassay: trace analysis of atrazine in unsaturated zone sediments. Journal of Environmental Quality. 26:1080-89.

Maps of McPherson. 1994. Maps of McPherson pamphlet. McPherson College Library.

Novak, J.M., T.B. Moorman, and C.A. Cambardella. 1998. Atrazine sorption at the field scale in relation to soils and landscape position. Journal of Environmental Quality. 26:1271.

Steinheimer, Thomas R. and Martin G. Ondrus. 1990. Liquid chromatographic determination of atrazine and its degradation products in water. U.S. Geological Survey. Denver, CO.

Supelco. A cost effective way to monitor triazine herbicides in pond water. Supelco Pamphlet. Bellefonte, PA. pp. 8-9.