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UV detection of triazine herbicides and their hydroxylated and dealkylated degradation products in well water ☆

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Abstract

The aim of this work was to develop a simple method to confirm the presence of hydroxytriazine products (hydroxyatrazine, hydroxysimazine, hydroxyethylterbutyltriazine and hydroxydiaminotriazine) in water and to apply it to well water samples. The hydroxytriazines were concentrated on a Sep-Pak C_{18} cartridge. Analysis was performed by HPLC using an RP8-DB column with phosphate buffer (pH 4.7)–acetonitrile (72:28, v/v) as the mobile phase and photodiode-array detection at 233 nm. Hydroxyatrazine, hydroxysimazine and hydroxyethylterbutyltriazine were detected at ppb levels in samples from two shallow wells under a very sandy soil citrus orchard taken at three different times in a 1-year period.

1. Introduction

Triazines are used selectively in vineyards and on some fruit trees and also as non-selective herbicides for vegetation control on non-crop land. Atrazine, simazine, terbutylazine, terbumetone and terbutryne are citrus-selective herbicides widely recommended alone or in commercial mixtures in Spanish orchards. Accumulation of the herbicides in the soil normally does not occur, even with repeated application [1]. Khan and Marriage [2] found more hydroxyatrazine than atrazine or dealkylatrazine in samples taken more than 18 months after the last treatment from a peach orchard where atrazine was applied at 4.5 kg/ha during eight consecutive years. The results clearly indicated that hydroxyatrazine, a non-phytotoxic degradation triazine compound, has a long persistence. Hydroxyatrazine is adsorbed to a greater extent than the parent compound [3].

Pollution of water by pesticides, particularly herbicides, has been recognized in agricultural areas of the world for many years [4]. The EC Drinking Water Directive [5] sets a very low maximum admissible concentration (MAC) of 0.1 μg/l for any one pesticide and a MAC of 0.5 μg/l for total pesticides. Contamination of ground water in irrigation wells in citrus orchards in Spain has been reported [6]. Of the triazines

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tested for, only the dealkyl degradation compounds were found.

Hydroxytriazines (HTs), because of their high adsorption, are not found as well-water contaminants. However, HTs owing to their physico-chemical properties, require different analytical methods for routine analysis.

The purpose of this paper is to present a specific chromatographic method for the determination of HTs in water and for the analysis of water samples from shallow wells during different times of the year in a citrus orchard situated in a very sandy soil, with a long history of triazine treatment.

2. Experimental

The citrus orchard where well water samples were collected is located in the southern part of Valencia (Spain), between the Mediterranean area and the Albufera Lake. In that orchard, a weed control programme with a commercial triazine mixture involves two residual treatments per year, at the rate of 6 l/ha (terbutylazine 15% + terbumetone 15% + terbutryn 20% in the spring and terbutylazine 15% + terbumetone 15% + atrazine 20% in the autumn) and spot treatments with 10 l/ha of a commercial mixture composed of glyphosate 10% + simazine 28% to control the perennial weed species. The soil texture is classified as very sandy. Sampling was done by collecting two separate bottles of water on 30 June 1993, 27 July 1993, 13 April 1994 and 27 June 1994.

The method of analysis for the parent triazine compounds and the dealkylated products was described in a previous paper [7].

2.1. Method for determination of hydroxytriazines (HT)

Standard materials

All materials were of the highest purity available. Hydroxysimazine (HS) [2-hydroxy-4,6-(diethylamino)-5-triazine] was >97% pure, ahydroxyethylterbutyltriazine (HETT) [2-hydroxy-4-(ethylamino)-6-(terbutylamino)-5-triazine] was 97% pure and didealkylhydroxyatrazine (DDHT or ammeline) [2-hydroxy-4,6-diamino-5-triazine] and hydroxyatrazine (HA) [2-hydroxy-4-(ethylamino)-6-(isopropylamino)-5-triazine] were 95–99% pure, and all were provided by Ciba-Geigy (Basle, Switzerland). One stock standard solution of 100 mg/l was prepared in 0.02 *M* H_3PO_4–CH_3CN (65:35, v/v) and another of 10 mg/l in the mobile phase, 0.005 *M* K_2HPO_4–CH_3CN (72:28, v/v), and both were stored in a freezer at −18°C. Working standard solutions (1–1000 μg/l) were prepared in the mobile phase.

Reagents

H_3PO_4 and K_2HPO_4 were of analytical-reagent grade and CH_3CN and CH_3OH of HPLC grade, all from Merck (Darmstadt, Germany); water was of HPLC grade, obtained from a Nanopure II system (Barnstead, Dubuque, USA).

Apparatus

Samples were extracted using solid-phase extraction cartridges containing 1 g of Sep-Pak Vac 6 c.c. C_{18}-modified silica sorbent (Waters, Milford, MA, USA) on a twelve-port Sep-Pak vacuum manifold (Waters) with a Model XX5522050 vacuum pump (Millipore, Bedford, MA, USA).

HPLC was performed on a Model 1090 Series II liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) with a ternary gradient feature, an autoinjection system and a photodiode-array detector. Instrumental control was effected with a Model 9000 Series ChemStation using LC-Pascal software (Rev. 5.22).

Molar absorptivities were determined using a Shimadzu UV-160 spectrophotometer.

High-performance liquid chromatography

A Supelcosil LC-8-DB (5 μm) column (150 × 4.6 mm I.D.) from Supelco (Bellefonte, PA, USA) was used. Data were acquired at 233 nm having a 20 nm bandwidth with a reference signal at 330 nm, and a 60 nm bandwidth. The detector sensitivity was set at 15 mAU full-scale. Spectral data were acquired between 200 and 400

nm. The injection volume was 15 μl and the flow-rate was 1 ml/min.

The separation of HT was performed using isocratic conditions, but it was necessary to use an additional gradient to flush the less polar artifacts and other triazines (see Table 1). This is particularly important for a multiple sample sequence.

Procedure for water samples

The water sample was filtered through a 47 mm diameter Type HA HATF047000 filter (Millipore). HTs were extracted from water on a cartridge containing 1 g of C_{18}-modified silica sorbent previously rinsed with 10 ml of methanol and 10 ml of water. A 1000-ml water sample with 2 ml of methanol added and thoroughly mixed was applied to the cartridge at a flow-rate of 15–20 ml/min, connected to glass 1000-ml reservoirs and a vacuum pump. Following the extraction step, the cartridges were placed in a twelve-port Sep-Pak vacuum manifold and eluted with 2 ml of CH_3CN–0.005 *M* H_2KPO_4 (70:30, v/v) at a flow-rate of about 6 ml/min. The eluate was adjusted to 2 ml with the same solvent mixture, filtered through a 0.45-μm Miller-HV4 filter (Millipore), transferred to an autosampler vial and was then ready for HPLC analysis.

The identification of substances by comparison of their retention times (Fig. 1) and UV absorption spectra (Fig. 2) was carried out using a spectral library search. The wavelength was selected to optimize the reading for the four components simultaneously and the results obtained at 233 nm are shown in Fig. 2. For this wavelength, the molar absorptivities (ϵ) were

Table 1
Gradient separation conditions

Time (min)	Channel A (%)	Channel B (%)
0.10	72.0	28.0
5.00	72.0	28.0
8.00	20.0	80.0
10.00	72.0	28.0

Channel A, 0.005 *M* K_2HPO_4; channel B, CH_3CN

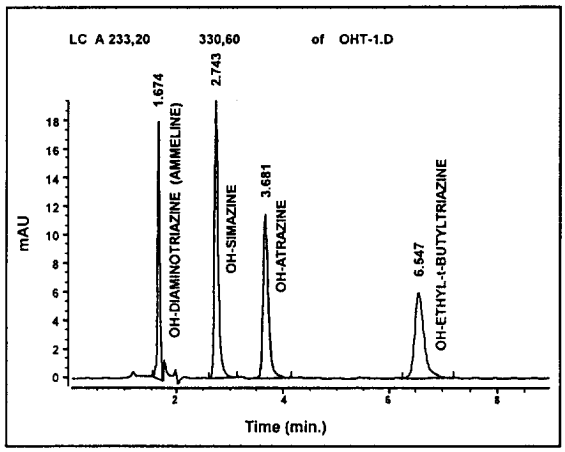

Fig. 1. HPLC of a standard mixture containing 15 ng of each HT on-column. Mobile phase, 0.005 *M* K_2HPO_4–CH_3CN (72:28, v/v); flow-rate, 1 ml/min; photodiode-array detection at 233 nm.

calculated according to $\epsilon_\lambda = A/cd$, where A is the absorbance measured at the given wavelength, c the concentration in mol/l, d the cell length in cm ($d = 1$ cm) and λ the wavelength ($\lambda = 233$ nm), using the mobile phase as reference. The results were of $\epsilon_{233} = 17 \cdot 10^3$ for DDHT, OHS and OHA and $15 \cdot 10^3$ for OHETT. Fig. 1 represents the response for 15 ng of each compound recorded at 233 nm and Fig. 2 the UV absorption spectra used with automated spectral library search obtained from the standard plotted in Fig. 1.

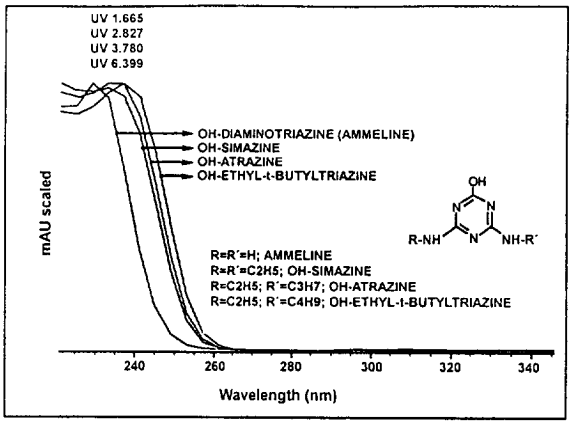

Fig. 2. UV spectra of ammeline, HS, HA and HETT in a standard mixture containing 15 ng of each on-column.

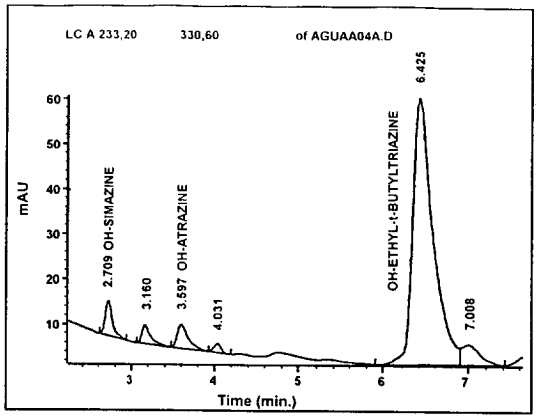

Fig. 3. Chromatogram obtained from a 1000-ml water sample from shallow wells in the citrus orchard, concentrated to 2 ml by the SPE procedure.

Fig. 3 illustrates a typical chromatogram obtained from a sample of 1000 ml of water extracted by solid-phase extraction (SPE) from the shallow well in the citrus orchard. Fig. 4 shows the results of the comparison of the UV spectra of chromatographic peaks at different times (2.7, 3.5 and 6.4 min) in Fig. 3 with an automated spectral library search.

Recovery

A recovery test was carried out by spiking samples with known amounts of standards and analysing the samples as described above. The results are given in Table 2 for a concentration of 1 μg/l.

Ammeline was not determined in the water sample because of the low recovery due to its high polarity for the C_{18}-modified silica adsorbent.

Detection limits

The detection limits with spectral identification were 0.05 μg/l for HS and HA and 0.1 μg/l for HETT.

3. Results

The recovery procedure is very simple. For all the water samples analysed the method provided

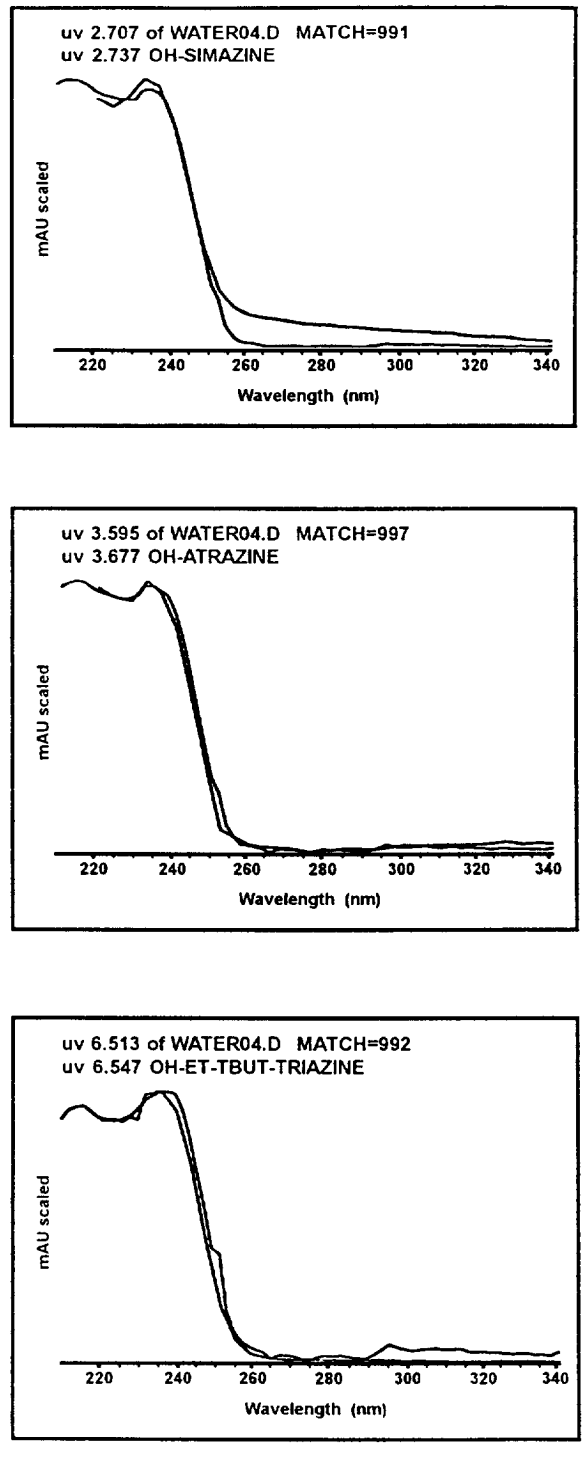

Fig. 4. Comparation of UV spectra of different chromatographic peaks (peak times 2.7, 3.5 and 6.4 min) in Fig. 3 with automated spectral library search.

Table 2
Recovery data for HTs in water (concentration 1 μg/l)

Compound	No. of measurements	Mean recovery ±S.D. (%)	R.S.D. (%)
Hydroxysimazine	8	37.4 ± 3.9	10.5
Hydroxyatrazine	8	121.0 ± 5.8	4.8
Hydroxyethylterbutyltriazine	8	107.1 ± 6.2	5.7

good results (extraction, clean-up and separation) for HS, HA and HETT (see Fig. 3) without co-elution problems.

The first analysis of water samples taken from the Mareny 1 well on 30 June 1993, before the hydroxytriazine method had been developed, gave terbutylazine 1.3, deethylterbutylazine 3.3, terbumetone 2.8 and deethylterbumetone 5.0 ppb.

Table 3 gives results for water samplings on three dates from the Mareny 1 and Mareny 2 wells. It is important to note that in all samples some triazine and its degradation compounds were found at concentrations above the EC limit. The temporal variation of the concentrations of the different chemicals follows approximately the herbicide orchard treatment programme during the year.

4. Discussion

In a previous survey [6] in another irrigation well (Rafelguaraf), far away from the present orchard, also situated in sandy soil but with a deeper water level (50 m), where triazines and dealkyldegradation products were also found, new samples were taken on three dates and hydroxy compounds were not detected. However, as shown in this paper, in shallow well waters under very sandy soils bearing triazine-treated orchards, it is possible to find not only the parent compounds and some dealkylated degradation products but also hydroxytriazines (HTs).

In Spain, terbumetone is always formulated as a mixture with terbutylazine, at the same concentration (25%). In both Mareny wells, terbumetone was found at higher concentrations than terbutylazine. This result is in agreement with an earlier survey on irrigation well water [6]. The source of hydroxyethylterbutyltriazine (HETT) is terbutylazine, terbumetone or terbutryne. In another study [7], terbutryne showed a very short persistence, being the least leachable of these compounds. Therefore, it is possible that the HETT found here comes only from terbutylazine and terbumetone.

The fact that the concentration of hydroxyatrazine is lower than that of hydroxy-

Table 3
Concentration of triazines and their degradation compounds in well water samples (μg/l)

Well	Date	A	Det-A	HA	S	HS	Tcl	Des-Tcl	To	Des-To	HETT
Mareny 1	27 July 1993	3.3	–	–	–	2.4	1.0	3.5	1.8	5.4	4.0
	13 April 1994	5.1	4.0	0.5	–	2.0	3.8	3.5	6.1	3.2	10.0
	27 June 1994	4.7	1.9	–	–	0.8	2.1	2.2	2.7	0.8	2.5
Mareny 2	27 July 1993	10.5	0.5	0.6	–	1.3	0.5	1.1	0.7	3.9	4.8
	13 April 1994	2.7	1.6	1.0	–	2.0	0.3	–	0.5	–	28.0
	27 June 1994	8.8	1.9	1.7	–	3.7	0.5	0.3	0.5	0.3	38.8

A = atrazine; Det-A = deethylatrazine; HA = hydroxyatrazine; S = simazine; HS = hydroxysimazine; Tcl = terbutylazine; Des-Tcl = dealkylterbutylazine; To = terbumetone; Des-To = dealkylterbumetone; HETT = hydroxyethylterbutyltriazine.

ethylterbutyltriazine does not mean that the former is less leachable and persistent than the latter because, as stated before, it comes at least from two sources of triazines. Further, considering the treatment programme during the year in this orchard, the atrazine level is lower than that of the total terbutyltriazines.

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