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Short communication

Development and validation of a high-performance liquid chromatographic method for the determination of clomazone residues in surface water

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Abstract

A method is described for the determination of clomazone residues in surface water by high-performance liquid chromatography with UV detection. The method involves solid-phase extraction with C_{18} extraction tubes. Clomazone was separated on a C_{18} column with a mobile phase of methanol–water (65:35, v/v) at pH 4.0 and a flow-rate of 1.0 ml/min. After optimization of the extraction and separation conditions, the method was validated. The method developed can be used for determination of clomazone in surface water, at the limit of 0.1 μg/l set by the European Union drinking water directive, with a 400-fold preconcentration. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Several hundred pesticides of different chemical structure are used worldwide in agriculture. Although these pesticides are considered to be essential for agricultural development, some of them can cause serious ambient contamination, principally in water [1–5].

The EEC Directive 80/778 concerning the quality of water designated for human consumption, establishes the maximum admissible concentration of each individual pesticide at 0.1 μg/l and the total amount of pesticides at 0.5 μg/l [1]. As regards surface water, the detection of many organic compounds is typically required at levels of 1–3 μg/l [2,3]. This rigorous standard for water purity requires the availability of suitable analytical methods with high sensitivity, selectivity, accuracy and precision.

According to the literature, a pesticide is able to contaminate ground water if its water solubility is higher than 30 mg/l, its K_{oc} (organic carbon partition coefficient) is less than 300 ml/g, its K_d (distribution adsorption constant) is less than 5 ml/g and its soil half-life is longer than 3 weeks [4,5]. Other relevant parameters are described in a review by Barceló [6].

Herbicides are potential contaminants of natural waters because they are directly applied to the soil and are leached into the surface water or transported into ground water [4].

The herbicide clomazone {2-[(2-chlorophenyl)-methyl]-4,4-dimethyl-3-isoxazolidinone} (Fig. 1) in particular is widely used against species of annual broadleaf weeds and grass. Clomazone is currently used for weed control in the cultivation of soybeans,

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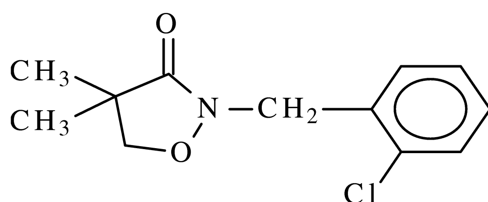

Fig. 1. Chemical structure of clomazone herbicide.

cotton, rice, sugar cane, corn, tobacco and various vegetable crops [7]. Clomazone is highly soluble in water (1100 mg/l), and has a K_{oc} of 150 ml/g, and a K_d value ranging from 0.47 to 5.30 ml/g. The field dissipation half-life of clomazone, determined from field studies in several soil types, ranged from 4 to 12 weeks [8]. Consequently, clomazone presents properties which indicate a groundwater contamination potential [4].

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are good options for herbicide monitoring in water [4,5,9–13]. A review covering both GC and HPLC methods used for herbicide monitoring in water has been published recently [9]. HPLC methods are preferred over GC methods in the case of more polar pesticides, with low volatilities and thermal instabilities because GC methods can only be used following a prior derivatization step [14]. Many HPLC methods have been developed for the determination of clomazone in soil [7,8,15–17] and in soybean [18,19]. However, no reference could be found to the determination of clomazone in water by HPLC.

Because of the rigorous limits for water purity, methods for extraction and preconcentration of the pesticides present in water have become necessary. To analyze pesticides at trace level in water samples, a preconcentration step is commonly carried out before analysis. For this purpose, solid-phase extraction (SPE) is replacing traditional methods [20] such as liquid–liquid extraction (LLE), and has been widely used for extraction of water samples prior to analysis. SPE reduces sample handling, labor and solvent consumption [9,13,21]. The most popular SPE sorbent for pesticides in water is octadecyl (C_{18}) bonded silica.

In this work, a simple, relatively fast and efficient HPLC–UV method was developed for the determination of clomazone in surface water. To obtain efficient preconcentration with good reproducibility and accuracy a C_{18} SPE system was applied. Finally, the proposed procedure was validated [22,23]. The parameters involved were calibration and linearity, limits of detection and quantification, precision (repeatability and reproducibility), and accuracy (recovery).

2. Experimental

2.1. Chemical and reagents

Clomazone standard (99.6%) was obtained from FMC (Uberaba, MG, Brazil). Methanol and acetonitrile of chromatographic grade were from Mallinckrodt (Phillipsburg, NJ, USA). Phosphoric acid of analytical grade was from Merck (Darmstadt, Germany). Water was purified with a Milli-Q water purification system (Millipore, Bedford, MA, USA).

The extraction tubes were Bond Elut C_{18} (size 3 ml, 200 mg) from Varian (Harbour City, CA, USA).

2.2. Instrumentation

The HPLC system consisted of a Shimadzu (Kyoto, Japan) Model LC-10AD pump associated with a 7125 Rheodyne (Cotati, CA, USA) six-port valve with a 20-μl loop, and a Shimadzu Model SPD-10AV UV–Vis absorbance detector connected to a Shimadzu Model C-R6A integrator for data acquisition. The pH of the mobile phase was adjusted with use of a Cole Parmer, series 500, pH meter. The analytical column was a Bondesil C_{18} 5 μm (250×4.6 mm I.D.) from Varian.

2.3. Procedure

The analytical column was operated at ambient temperature. The mobile phase was methanol–water (65:35, v/v), adjusted to pH 4.0 with phosphoric acid. It was prepared volumetrically from individually measured aliquots of methanol and water and was degassed for 15 min in an ultrasonic bath before use. The flow-rate was set at 1.0 ml/min and quantification was carried out with UV detection at 220 nm.

The HPLC system was conditioned by passing the mobile phase through it for 1 h at a flow-rate of 1.0 ml/min.

Stock standard solution (2000 mg/l) of clomazone was prepared by dissolution in methanol and was stored at −18°C. Calibration standards were prepared by appropriate dilution with the mobile phase and stored in the refrigerator (4°C).

A 200-ml volume of river water sample was fortified by addition of an established volume of stock solution (2.0 mg/l) of clomazone, resulting in five levels of fortification, 0.1, 0.5, 1.0, 3.0 and 5.0 μg/l. Before sample application, the SPE column was conditioned by passing consecutively 3 ml of methanol, 3 ml of Milli-Q water and 3 ml of Milli-Q water, pH 3. After adjusting the pH to 3 by addition of phosphoric acid, to increase the clomazone retention, the samples were mixed well and passed through the SPE column under vacuum at a rate of 3 ml/min. Just after the sample was passed through the column, it was washed with 3 ml of Milli-Q water, the eluate discarded and the sorbent bed dried under vacuum for 2 min. Then, the SPE column was washed with 500 μl of acetonitrile–water (50:50, v/v) to reducing the background interference due to co-extracted substances present in the water samples. Furthermore, a drying step for the sorbent after washing out water-soluble impurities contained in the samples was necessary with regard to recovery and reproducibility. Otherwise, if the sorbent is moist, extraction may occur inhomogeneously and more impurities may be eluted, disturbing the analysis. After that, the analyte was eluted with 1 ml (500+500 μl) of methanol. The solvent was evaporated to dryness under a gentle stream of nitrogen, the residue redissolved in 0.5 ml of mobile phase and injected into the chromatograph.

3. Results and discussion

Reversed-phase HPLC, with UV detection, has proven to be a good alternative for clomazone determination because no derivatization step is needed. Chromatographic separation in C_{18} type columns provides good results. The most intense absorption band of clomazone is assumed to belong to the $\pi \rightarrow \pi^*$ transition of the C=O group. The detection at 220 nm offers suitable chromatograms for the quantification of clomazone in real samples.

Under the chosen conditions, clomazone showed a retention time of 12.0±0.2 min, allowing a complete separation of its signal from those of foreign substances present in the water samples.

The different steps of validation of the method developed for the determination of clomazone in surface water are described. For the validation of a chromatographic method, typical analytical parameters have to be considered. These parameters are described below.

3.1. Calibration and linearity

For most chromatographic procedures a linear relation is observed between detector response (y) and analyte concentration (x). This can be expressed as a linear regression equation: $y=a+bx$. The parameters obtained by the selected chromatographic conditions for clomazone calibration correspond to:

$$y = -4261.5 + 164\,097x \; (r = 0.9998)$$

where y=peak area, x=clomazone concentration (mg/l), and r=correlation coefficient.

The linearity of a method is a measure of range within which the results are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range [23].

Throughout the analytical curve, obtained in the range of three orders of magnitude of concentration, the linearity was evaluated by means of the ratio between signal (S) and concentration (Q), defined by $(S/Q_i)=(S_i-a)/Q_i$, where the ratio signal/concentration for the ith point of the analytical curve, $(S/Q)_i$, is calculated from the corresponding measured signal S_i, of the corresponding concentration Q_i and the intercept of the analytical curve (a).

Points were considered to be in the linear range if their $(S/Q)_i$ values were in the interval $(1.00\pm0.05)b$, i.e., points whose signal/concentration ratios do not differ more than 5% from the slope. This tolerance interval is based on the IUPAC chromatography standards. For clomazone the linearity extended up to 10 mg/l.

3.2. Limit of detection and limit of quantification

The limit of detection (LOD) is the lowest concentration of analyte detectable by an analytical method and is expressed in concentration units. The limit of quantification (LOQ) is the lowest solute concentration that can be determined with acceptable precision and accuracy, under the stated experimental conditions. It is also expressed in concentration units. In this study the LOD and LOQ were determined according to the definition of Francotte et al. [23], and are defined as follows:

$$LOD = 2h_n C_s / h_s \quad (1)$$

$$LOQ = 6h_n C_s / h_s \quad (2)$$

where C_s is the amount of analyte injected; h_s is the peak height of the analyte; h_n is the largest deviation of detector signal from the average baseline level, measured at the retention time of the analyte.

To measure these parameters a series of diluted clomazone standard samples were used. From this series, the peak is selected whose height h_s is about 2–10-times larger than the signal-to-noise ratio h_n ($C_s = 0.05$ mg/l). The h_s value is the height of the analyte measured from the average baseline level to the top of the peak, while h_n is measured over 10 peak widths in the absence of analyte.

By comparison of the response with the baseline noise, the LOD was 0.012 mg/l, and the LOQ was 0.036 mg/l. Fig. 2 shows that the effective LOD and LOQ in the surface water samples, after 400-fold SPE preconcentration step, were 0.03 and 0.1 μg/l, respectively.

3.3. Precision (repeatability)

Precision (repeatability) determines the analysis deviation, and is an important criteria for evaluating analytical method performance. It is the degree of agreement among individual test results when the procedure is applied repeatedly. The precision is usually expressed as the relative standard deviation (RSD) [23].

Instrument precision was measured by comparing

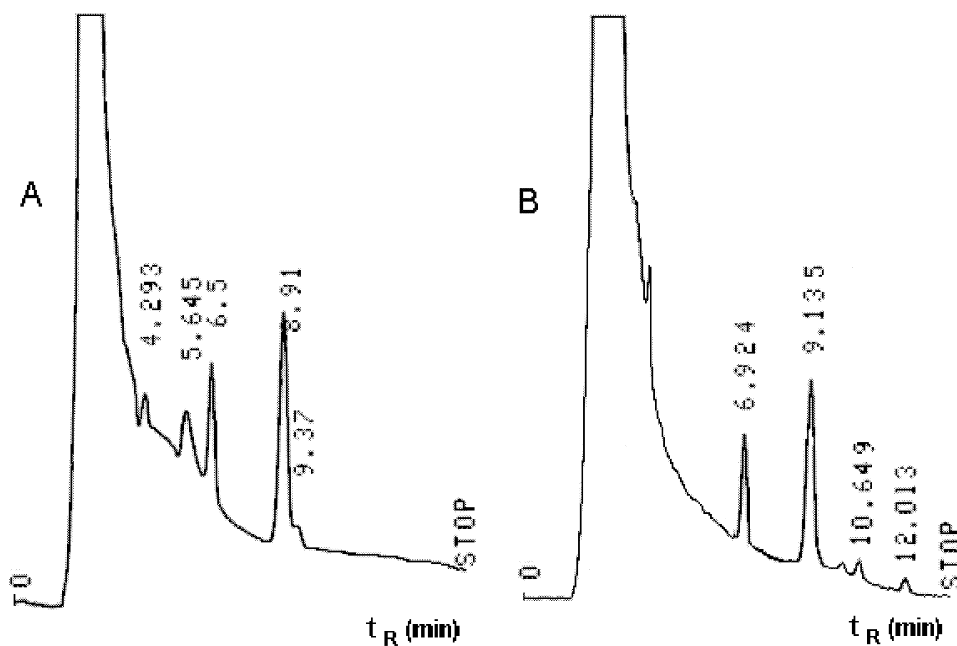

Fig. 2. (A) River water blank chromatogram and (B) chromatogram showing the separation of clomazone by HPLC–UV, after the extraction of 200 ml of river water spiked at 0.1 μg/l. Volume injected: 20 μl. Retention time: 12.0 min. Chromatographic conditions as described in the text.

standard deviation of the response from the injection in triplicate of 11 different calibration standard solutions (0.025 to 10.0 mg/l). The RSDs ranged between 0.6 and 4.5%, with a mean instrument precision of 2.1%.

Precision (repeatability) reflects the variation in results when repetitive analyses are made on the same conditions. The numerical value used is the relative standard deviation for repeatability (RSD_r).

Repeatability of the developed analysis method was determined by adding clomazone in four different concentrations to blank surface water. The within-batch recovery and repeatability (RSD_r) of spiked clomazone in surface water at the levels of 0.1, 0.5, 1.0, 3.0 and 5.0 μg/l are summarized in Table 1. The precision (repeatability) ranging from 1.0 to 10.1%, with a mean value of 6.4%. The results are fairly good for the concentration levels investigated.

3.4. Precision (reproducibility)

Precision (reproducibility) is the degree of agreement obtained by the analysis of the same sample under various test conditions. The usually numerical value used is the relative standard deviation for reproducibility (RSD_R). The reproducibility of this analytical method was determined by analyzing spiked surface water samples under various test conditions (different analysts, different instruments and different days). The between-batch recoveries and reproducibility (RSD_R) investigated at several levels are given in Table 2. The precision (reproducibility) ranged from 1.1 to 11.2%, with a mean value of 6.8%. The reproducibility (RSD_R) values are very good because all measurements should be within ±15% at all concentrations [22,24].

Table 1
Within-batch recovery and repeatability (RSD_r) for the herbicide clomazone in surface water spiked at four levels[a]

Clomazone concentration (μg/l)		Recovery (%)	RSD_r (%)
Spiked level	Found (mean±SD)		
0.1	0.089±0.008	89.1	8.9
0.5	0.524±0.042	104.8	8.1
1.0	0.975±0.097	97.5	10.1
3.0	2.691±0.105	89.7	3.9
5.0	4.875±0.049	97.5	1.0

[a] Number of replicates at each level (n)=9 (three extractions with three injections each). All made under the same conditions on the same day.

Table 2
Between-batch recovery and reproducibility (RSD_R) for the herbicide clomazone in surface water spiked at four levels[a]

Clomazone concentration (μg/l)		Recovery (%)	RSD_R (%)
Spiked level	Found (mean±SD)		
0.1	0.086±0.010	86.4	11.2
0.5	0.465±0.034	93.0	7.4
1.0	0.892±0.092	89.2	10.3
3.0	2.640±0.103	88.0	3.9
5.0	4.555±0.050	91.1	1.1

[a] Number of replicates at each spiked level (n)=9 (three extractions with three injections each). Each extraction and injection series was accomplished on 3 consecutive days.

3.5. Accuracy

The accuracy of an analytical method is the agreement between the true value of analyte in the sample and the value obtained by analysis. Accuracy is usually expressed as the recovery by the assay of known, added amounts of analyte [23].

Recovery is measured as the response of a processed spiked matrix standard, expressed as a percentage of the response of a pure standard, which has not been subjected to sample pretreatment. It indicates whether the method provides a response for the entire amount of analyte that is present in the sample. The recovery was calculated with Eq. (3) [22]:

$$\text{Recovery} = \frac{\text{response after extraction}}{\text{response of pure standard}} \cdot 100 \tag{3}$$

After defining the analytical conditions, tests were made on the recovery of clomazone with C_{18} SPE extraction tubes. Samples and blank assays were prepared and determined according to described procedure in this paper. The recovery tests were carried out on three replicates at each spike level. Results are the average from three injections.

All results other than those rejected for analytical reasons should be used in the calculation. The

accuracy of the method should be within 85 and 115% at all concentrations [22].

The average recovery obtained for clomazone at all concentrations and conditions investigated (see Tables 1 and 2) was determined as 92.7%, which is very satisfactory.

4. Conclusion

The mobile phase methanol–water (65:35, v/v), adjusted to pH 4.0 with phosphoric acid, is adequate for accurate analyses of clomazone. The wavelength utilized (220 nm) permits good detection of the herbicide.

The results obtained for calibration, linearity, precision and accuracy (recovery) show that this is a rapid and efficient method for the quantification of clomazone in surface water samples.

The LOD and LOQ were, respectively, 0.012 and 0.036 mg/l. The effective LOD and LOQ in the samples after SPE preconcentration step were 0.03 and 0.1 μg/l, respectively.

Thus, using appropriate SPE preconcentration, it is possible to determinate clomazone in water at concentration of 0.1 μg/l, satisfying the international limits for drinking water.

Acknowledgements

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