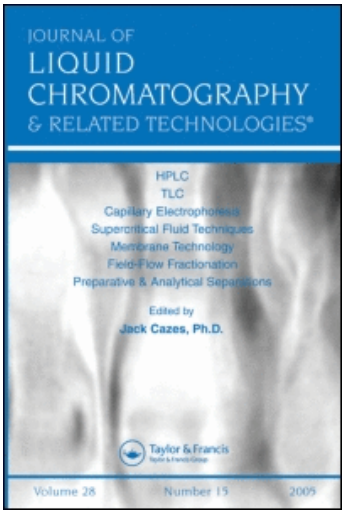

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DETERMINATION OF CARBETAMIDE IN WATER BY MICRO LIQUID-LIQUID EXTRACTION FOLLOWED BY HPLC

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ABSTRACT

The validation of analytical procedures using added samples as reference materials is proposed. The carbetamide extracted with dichloromethane is monitored by reverse-phase high performance liquid chromatography with a retention time of some 2.7 min. while Carbaryl is used as an internal standard. The accuracy of the method is checked analysing water samples previously spiked with different amounts of analyte. A method to obtain the signal associated to a chromatographic blank is presented, to include as another precaution, within the calibration procedures. The method was applied to the determination of carbetamide at very low concentration levels (2.50–10.0 $\mu g \cdot L^{-1}$) in different types of natural water samples. The detection limit (DL) was 0.02 $\mu g \cdot L^{-1}$. Around 100% recovery levels were customarily obtained in all cases.

INTRODUCTION

Carbetamide [(R)-1-(ethylcarbamoyl)ethyl carbanilate] is a selective herbicide which belongs to the carbamate group.

This herbicide, belonging to the family of amides, was introduced by Rhöne-Poulenc Agrochimie (Research Center–La Dargoire–France) and described by J. Desmoras et al. (1). It acts by radicular absorption and partially foliar absorption on yearly gramineas, and also on some species of dicotyledons. It stops the cellular multiplication of young tissues and roots (2). Its development, activities, mode of action, and effectiveness have been described by Rhöne–Poulenc Agrochimie, and it physical and toxicological properties have been summarized in Pesticide Manuals (3).

The analysis of carbetamide in water samples has usually been carried out with different chromatographic techniques (HPLC, GC). Hidalgo, C. et al. (4) developed a HPLC-DAD method on reverse phase using a solid phase C-18 preconcentration step. (DL $= 0.04\ \mu g \cdot L^{-1}$. Slobodník J. et al. (5) proposed a GC-MS method based on the use of a PTV injection system (DL $= 0.3\ ng \cdot ng^{-1}$).

Usually, analysis of trace amounts of organic compounds in water require an adequate preconcentration step, let us say solvent extraction or perhaps solid-phase extraction methods. Moreover, solvent phase extraction requires further concentration, which in many cases involves serious losses of compounds. Therefore, methods of analysis avoiding the solvent concentration step are more desirable for the recovery of organic traces in water (6–8).

The use of micro methods, whereby the amount of organic solvents is very small, also reduces the spilling to the environment and analysis times.

A HPLC method is presented here for the determination of carbetamide in water samples after previous micro liquid-liquid extraction. Simultaneously, a criterium to evaluate the detection limits in HPLC is presented, including a method to obtain the signal associated to a chromatographic blank as explained below.

It is well known that separation techniques, such as HPLC, GC, HPCE have had, until now, intrinsic problems associated with calibration because of difficulties in calculating signals pertaining to a blank when handling the data (14,15). Detection limits have been determined as 3σ or 2σ (9,10), where σ stands for standard deviation.

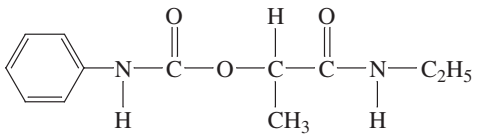

Figure 1. Structure of carbetamide.

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Other methods to overcome these difficulties have also been used, such as noise/signal ratio (S/N = 2 or S/N = 3), with the DL defined as that amount required to give a signal 2 or 3 times the background noise (11–13). In previous papers, we have been using criteria according to IUPAC recommendations applying it to transient techniques, such as Gas Chromatography (GC) (14), High Performance Capillary Electrophoresis (HPCE) (15), and Differential Pulse Voltammetry (DPV) (16). Namely, the proper blank signal is obtained by extrapolation of the analyte peak base width vs. analyte concentration to zero concentration.

EXPERIMENTAL

Apparatus and Measurements

All experiments were performed on a Beckman (Palo Alto, USA) system Gold HPLC 125 solvent module instrument (v. 8.1), equipped with a Beckman system Gold 168 diode array UV detector, and a Waters 717 plus autosampler (48-vial carousel).

The HPLC system was interfaced to an IBM PS/2 30-286 microcomputer. A Canon BJ-300 printer was used for graphical representation.

A Waters pico-tag column (C-18, 4 μm, 60 Å) for free amino acid analysis with an inner diameter of 3.9 mm and a total length of 30 cm was used. Detection was by direct UV absorbance at 239 nm for the carbetamide and at 275 nm for the carbaryl.

The micro liquid-liquid extraction procedure was carried out using the equipment shown in Figure 2. An IKA Labortechnik Eurostar basic stirrer was used to shake the samples mechanically in a separatory funnel. A home-made Na_2SO_4 micro column (3 mm internal diameter) was attached to the end of the funnel to dehydrate the samples.

Statistical Methods

Statgraphics (17) and Alamin (18) software packages were used for the statistical analysis of the data and regression analysis (Linear Model). The lack-of-fit test was applied to check the linearity of the calibration graphs in accordance with the analytical methods Committee (19).

Chromatography

All analysis were performed at room temperature (25°C). Isocratic elution was performed for carbetamide herbicide with a mobile phase of acetonitrile/water (50:50). The capacity factors (k′) of carbetamide and carbaryl estimated in

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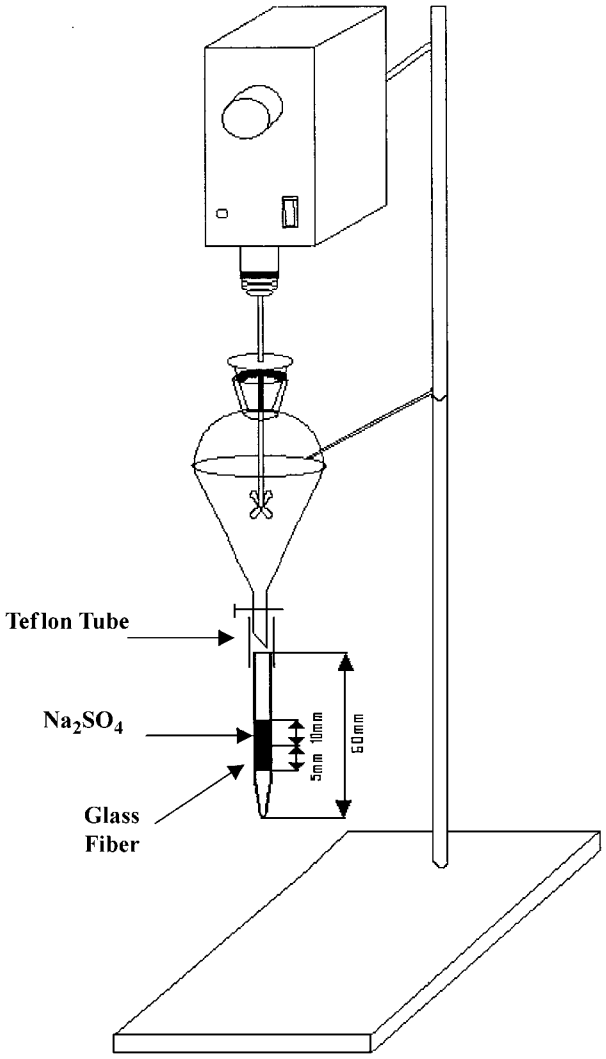

Figure 2. Equipment to extract the carbetamide from aqueous samples.

acetonitrile/water (50:50) were 2.0 and 3.6, respectively. The chromatogram was carried out in 5 minutes. The analytical column was rinsed after each run with 100% of solvent for 5 minutes. The flow rate was maintained at 1.0 mL · min^{-1}.

To prevent crystallisation of the inorganic salts, it was necessary to rinse the chromatographic system extensively with water at the end of each day. The

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column was subsequently rinsed with pure acetonitrile for regeneration. The solvent program was started simultaneously with the injection (50.0 μL).

Reagents

All electrolytes and samples were filtered through a 0.2 mm Nylon-66 membrane syringe filter immediately prior to use. Acetonitrile was HPLC grade. All other chemicals were of the analytical-reagent grade. Water was purified with a Millipore Q system. All other chemicals and solvents were purchased from Merck (Darmstadt, F.R.G.).

A stock solution of carbetamide [(R)-1-(ethylcarbamoyl)ethyl carbanilate] [CAS number: 16188-49-3] PESTANAL (Riedel-deHaën) containing 1.000 $g \cdot L^{-1}$ was prepared in a 100 mL volumetric flask, by dissolving 100.0 mg of the compound in ethanol 96%(v/v) (Panreac). This solution was used to spike the water samples.

A standard solution of 1.000 $g \cdot L^{-1}$ of carbaryl in ethanol 96%(v/v) (Panreac) was used as internal standard after adequate dilution with acetonitrile/water (50:50) to a final concentration of 50.0 $mg \cdot L^{-1}$.

Buffer solution at pH = 6 from 1 M sodium acetate and 1 M acetic acid were used.

Sodium chloride, sodium sulfate anhydre, and dichloromethane (Panreac) were used for the micro-extraction procedure.

Sample Treatment

Water samples were collected in amber glass bottles, previously cleaned with HCl and stored at 4°C until analysis. Water samples were fortified with different levels of carbetamide.

Analytical Procedure

To a 500 mL water sample containing 2.50–10.0 $\mu g \cdot L^{-1}$ of carbetamide, 150.0 g of NaCl is added. Then, 5.0 mL buffer solution (sodium acetate–acetic acid at pH 6) is added. The solution is transferred to a separation funnel and 6.0 mL of dichloromethane is added. The mixture is mechanically shaken for 1 min at 1500 rpm. The underlying organic phase is dried by passing through a home- made anhydrous sodium sulfate micro column and directly transferred to a 2-mL reaction vial.

After evaporation to dryness under Nitrogen, 0.20 mL solution of internal standard in acetonitrile/water (50:50) is added. The mixture is vortexed for

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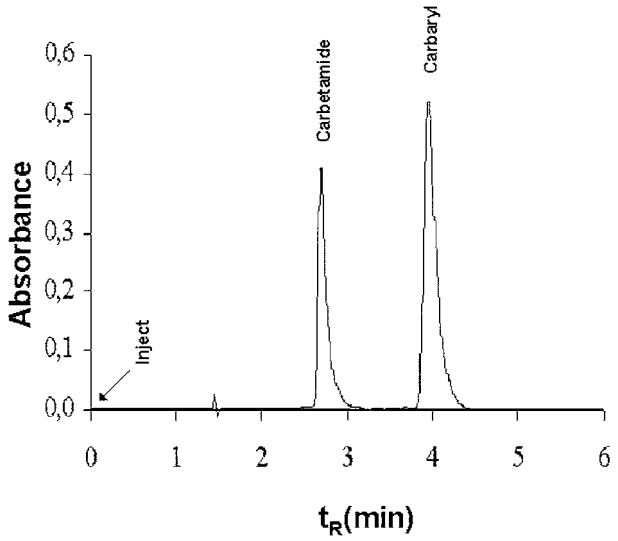

Figure 3. Typical chromatogram of carbetamide herbicide in ground water.

1 min. The samples are analysed by HPLC (50.0 μL). Linear analytical curves were obtained between 2.50 and 10.0 $\mu g \cdot L^{-1}$. A calibration graph was constructed in the same way, using solutions of carbetamide of known concentrations. Natural water samples containing an adequate amount of carbetamide are treated as described under the procedure.

The chromatogram obtained with the above-described conditions is shown in Figure 3. Only 10 min were necessary to complete the HPLC step.

RESULTS AND DISCUSSION

Carbetamide Preconcentration

Micro liquid-liquid extraction minimise the consumption of organic solvent. The organic solvent was dichloromethane because extraction recoveries were higher than with other solvents studied, i.e. n-hexane, iso-octane, diethyl ether, trichloromethane, and carbon tetrachloride. The average recovery obtained using dichloromethane was $99 \pm 3.1\%$.

The effect of ionic strength was studied using NaCl. An increase in the volume of organic phase recovered was found as ionic strength increased. Saturation of water samples with NaCl is then recommended before the dichloromethane is

added, so as to minimize the solubility of dichloromethane in water, thus reducing the volume of organic solvent used.

High reproducibility in the extractions was ensured using a mechanical mixing device (Fig. 2). Maximum efficiency was obtained for stirring speeds higher than 1500 rpm.

The organic phase volume selected was 6.0 mL and the pH adjusted to 6.0 with acetic acid-acetate buffer (20).

Analytical Parameters

Calibration graphs for the samples were made for the concentration range 2.50–10.0 $\mu g \cdot L^{-1}$ of carbetamide. To check the linearity of the calibration graph as recommended by the Analytical Methods Committee (19), the lack-of-fit test was applied to two replicates and three injections of each standard. Results for the intercept (a), slope (b), correlation coefficient (r), and probability level of the lack-of-fit test ($P_{lof}(\%)$) are summarized in Table 1.

The data show good linearity within the stated range. The precision determined as relative standard deviation (RSD) was measured for each concentration.

Detection and Quantification Limits

There is no agreement yet about how to obtain the ditection limits (DL) and quantification limits (QL) from the blank standard deviation in liquid chromatography. Frequently, the IUPAC recommendations are not strictly followed (21). We believe that the method we proposed to calculate DL (22) and QL is more in line with the IUPAC recommendations. It can be assumed that the chromatographic peak shape is a Gaussian-type (23); then, the estimation of base width $Wb = 6\sigma = 2.548\ W_{0,5h}$ (24), where $W_{0,5h}$ is the half-width of the peak. Extrapolation of the graph of W_b at different concentrations of analyte, can give us an

Table 1.

Analytical Parameters	
Intercept (a)	0.0052
Slope (b)	0.0969
Correlation coefficient (r)	0.9994
Lack-of-fit test (P-value)	0.4898
Linear dynamic range ($\mu g \cdot L^{-1}$)	2.50–10.0
Linearity [1-RSD (b)] (%)	98.53

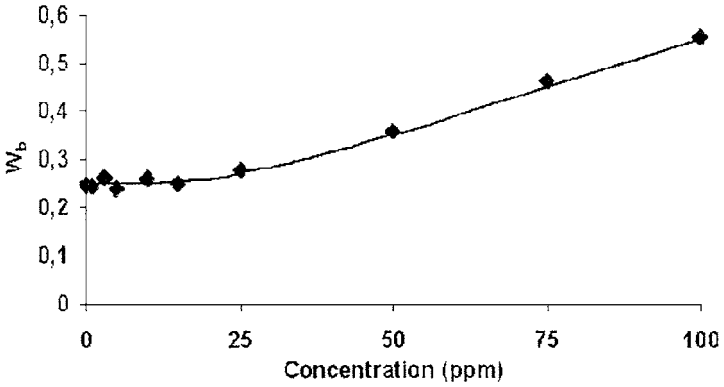

Figure 4. Base-width peak vs. analyte concentration.

adequate statistically significant idea of the width of the base for "zero concentration" (Fig. 4).

The blank signal for each analyte can be determined by integration over the baseline of the chromatograms taking a width $t_R \pm 0.5\ W_{b0}$, where t_R is the retention time of the analyte and W_{b0} has been evaluated as explained above. It relies on studying the blank standard deviation in a time interval corresponding to the peak width at its base, extrapolated to zero concentration. Detection limits which are better adjusted to a statistical evaluation, are thus implemented. DL and QL were calculated and the results obtained are also summarized in Table 2. This

Table 2. Detection Limits Calculated from Different Models

Model	DL* ($\mu g \cdot L^{-1}$)	QL** ($\mu g \cdot L^{-1}$)
Calibration[a]	0.08	0.27
Approximated[b]	0.17	0.56
S/Nc[c]	0.21	0.69
This paper[d]	0.02	0.08

[a]Calculated from Eq. $S_{c0} = \sqrt{(\frac{S_{yx}}{b}) \bullet (\frac{1}{n} + \frac{1}{m}) + (\frac{S_b}{b})^2 c^{-2}}$.

[b]Calculated from Eq. $S_{c0} = \sqrt{\frac{n-2}{n-1}} \bullet (\frac{S_{yx}}{b})$.

[c]Calculated from a signal 3 times background noise (DL).

[d]Calculated by extrapolation of the analyte peak base width vs. analyte concentration to zero concentration.

*$DL = 3 \bullet S_{c0}$.

**$QL = 10 \bullet S_{c0}$.

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table shows different values of the detection limit as determined from this work, compared with others calculated by different methods.

Validation and Applications of the Method

The proposed method was applied to the determination of carbetamide in ground water from Santa María farm (near Granada) and in tap water from the city of Granada. The validation of the proposed method was carried out by using Standard Addition Methodology (25), whereby three experiments are required to obtain the data set necessary to carry out the statistical protocol: a) Standard Calibration (SC) as described above; b) Standard Addition Calibration (AC) obtained by spiking five samples with carbetamide standard with final concentration of carbetamide in these samples: 0.00, 2.00, 4.00, 6.00 $\mu g \cdot L^{-1}$; c) Youden Calibration (YC).

A calibration curve was made with the Youden method, in which a calibration curve is established with continuous variations of sample volume (100, 200,

Table 3. Numerical Values of Parameters SC, AC, and YC

		AC		YC	
Parameter	SC	Tap Water	Ground Water	Tap Water	Ground Water
Calibration					
n	30	12	12	12	12
a	0.0052	0.5164	0.5208	0.0103	0.0117
b	0.0969	0.0971	0.0973	1.0325	1.0646
S_{yx}	0.0126	0.0117	0.0111	0.0120	0.015
S_p		0.0124	0.0122		
t(b)		0.1163	0.2547		
		P > 50%	P > 50%		
b_p		0.0969	0.0969		
a′	0.0049	0.5162	0.5219		
YB			—	—	
Analysis					
C ($\mu g \cdot L^{-1}$)	5.2098	5.2208	5.2638		
C_{sample} ($\mu g \cdot L^{-1}$)	10.4195	10.442	10.5276	10.655	10.984
t(c)		0,253	1.257		
		P > 50%	P > 20%		

Results of analyte content in spiked water sample; n: number of measurements; a: intercept; b: slope; S_{yx}: regression standard deviation; S_p: pooled standard deviation of SC and AC; t(b): statistic for slope ($\alpha = 0.01$); b_p: pooled slope of SC and AC; a′: corrected intercept; YB: Youden blank; C: solution analyte concentration; C_{sample}: sample analyte concentration; t(c): statistic for analyte concentration ($\alpha = 0.05$).

Table 4. Recovery Study of Carbetamide in Water

Water Sample	Added ($\mu g \cdot L^{-1}$)	Found* ($\mu g \cdot L^{-1}$)	Recovery (%)
Tap Water	5.00	4.79	95.8
	7.50	7.72	102.9
	10.0	10.4	104.0
Ground water	5.00	5.25	105.0
	7.50	7.32	97.6
	10.0	10.3	103.0

*Data based on the average obtained from three determinations.

300, 400 mL). The parameters obtained from these three checkings are shown in Table 3.

The P-value for the Student t test of the representative values of slope deduced for the Standard Calibration (SC) and Standard Addition Calibration (AC) methods show, in all cases (P-value > 0.05), the similarity of these slopes. The P-value for the Student t test to compare the concentrations of analyte deduced for the Standard Calibration (SC) and Standard Addition Calibration (AC) methods show, in all cases (P-value > 0.05), the similarity of these concentrations. We than concluded our method to be accurate. On the other hand, the non-existence of an intercept in the Youden Calibration (YC) implies the absence of matrix effect validating the method.

From this study it can be concluded, that the determination of carbetamide in water samples can be carried out directly by means of SC.

A recovery study on various types of water samples was carried out. The samples were analysed after adequate additions of carbetamide. The results are summarized in Table 4 and show that the recoveries are acceptable.

CONCLUSIONS

A method for the determination of carbetamide in water samples along the EU guidelines is proposed (DL $< 0.1\ \mu g \cdot L^{-1}$). A micro liquid-liquid extraction procedure is followed by the HPLC technique.

A criterium, according to IUPAC recommendations, based in the blank signal integration extrapolated to zero concentration is developed.

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