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Automated determination of phenylcarbamate herbicides in environmental waters by on-line trace enrichment and reversed-phase liquid chromatography–diode array detection

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Abstract

A fully automated liquid chromatographic method using on-line trace-enrichment, gradient elution and diode array detection is described for the trace-level determination of several phenylcarbamate herbicides, such as carbetamide, propham, desmedipham, phenmedipham, chlorbufam and chlorpropham, in environmental water samples. In this work, two different enrichment pre-columns have been assayed, a 5.8×4.6 mm I.D., 10 μm ODS Prelute cartridge and a 10×2 mm I.D. cartridge filled with 10 μm PRP-1 polymer, both coupled to a 150×4.6 mm I.D. analytical column filled with 5 μm ODS. Using the C_{18} pre-column, up to 50 ml of water sample could be percolated without peak broadening of any compound. However, a lack of reproducibility was observed in the case of carbetamide, the most polar analyte, after performing recovery experiments by percolating drinking and surface water samples spiked at several levels (0.5 and 4 μg l^{-1}). On the other hand, the PRP-1 pre-column allowed the enrichment up to 100 ml of water sample with satisfactory results for every compound, including carbetamide. The procedure was validated by recovery experiments in environmental water samples spiked at 0.2 and 1 μg l^{-1} yielding average recoveries between 84–108% with relative standard deviations in the range 2–12%. Detection limits as low as 0.04 μg l^{-1} were achieved. It was observed that desmedipham and phenmedipham degraded rapidly in the environmental water samples as showed the degradation studies performed along 24 h in drinking and surface waters spiked at 4 μg l^{-1}. Although the standard mixture, prepared in HPLC water, was stable for around one week, in the environmental water matrices more than 95% of each herbicide degraded after 6 h, and new chromatographic peaks corresponding to the degradation products were detected.

Keywords: Environmental analysis; Water analysis; Sample handling; Pesticides; Phenylcarbamates

1. Introduction

The widespread use of herbicides in agriculture leads to an increase in the presence of their residues in environmental samples. Phenylcarbamate herbicides are widely applied in agriculture for crop protection. Carbetamide is employed to control grasses and some broad-leaved weeds in chicory, red clover, endive, lucerne and oilseed rape. Phenmedipham is widely applied, sometimes together with desmedipham, as postemergent herbicide for broad-leaf weed control in beet crops, especially in sugar beet crops. Chlorpropham is frequently applied with propham as preemergent herbicides in different crops. They are also used for plant growth regulation especially in potatoes.

Some of these compounds, such as carbetamide, phenmedipham and propham, have been classified as probable or transient leachers used in Europe in amounts of over 50 tonnes per annum (500 tonnes in

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the case of carbetamide) in the so-called black list published in the 76/464/EEC Directive [1]. The majority of the high priority pesticides listed with some potential to leach are frequently found in environmental waters. This is the case for carbetamide, which has been detected in European ground and drinking water at concentrations greater than 0.1 μg l^{-1} [2]. However, some other compounds as phenmedipham and propham, which are also listed as priority pesticides, are apparently not found in environmental waters, and the availability of suitable analytical methods could be the cause [2].

The analytical determination of phenylcarbamate herbicides by gas chromatography (GC) is quite troublesome, requiring chemical derivatization. A multi-residue method for analysing herbicides in crops including propham, chlorpropham and phenmedipham by generation of anilines after alkaline hydrolysis was described [3]. The corresponding anilines were determined by GC with electron-capture detection (ECD) after bromation. Another procedure described the alkaline hydrolysis to *m*-toluidine, for determining phenmedipham in spinach, with direct determination of this compound (*m*-toluidine) by GC with flame ionization detection [4]. GC with mass spectrometry (MS) detection has also been used for the determination of thermolabile carbamates (phenmedipham) after derivatization with acetic anhydride [5].

Therefore, the use of liquid chromatography (LC) seems to be more appropriate since it is the technique of choice in the case of low volatile, polar and thermolabile compounds. LC followed by ultraviolet (UV) or MS detection after off-line solid-phase extraction (SPE) on different sorbents (C_{18}, graphitised carbon black and polymeric sorbents) has been used for developing multiresidual procedures in which several phenylcarbamate herbicides were enclosed [6–9]. However, in general, there is a lack of analytical methodology focusing on this family of herbicides, since the major part of reported procedures concerning carbamate analysis have been focused on methyl-carbamate insecticides [10].

According to the restrictive European Union regulations, which limit the maximum amount allowed for single pesticides in drinking water to 0.1 μg l^{-1} and for the sum of pesticides to 0.5 μg l^{-1} including toxic transformation products [11], very sensitive analytical methods for monitoring drinking water samples are required.

In this way, on- or off-line enrichment steps previously to the chromatographic determination are necessary. The main advantages of on-line methodology in front of off-line methods are well known [12] since it allows complete automation of the analytical processes avoiding sample manipulation. Besides, the whole amount of the analytes present in the sample, which are retained by the suitable sorbent, are introduced in the chromatographic system. In this way, smaller sample volumes than off-line methods are required to achieve similar detection limits.

On-line trace enrichment on short and usually disposable pre-columns joined to LC has been recently applied to the trace level determination of pesticide residues in environmental water samples [13–17]. The aim of the present work is the development of an on-line SPE procedure followed by LC with diode array detection (DAD) which allows the sensitive determination of carbetamide, propham, desmedipham, phenmedipham, chlorbufam and chlorpropham. For this purpose, an automatic sample processor originally designed for off-line SPE (ASPEC XL) has been adapted by performing hardware and software modifications of the commercial equipment.

2. Experimental

2.1. Chemicals

The phenylcarbamate herbicides were purchased from Dr. Ehrenstofer (Augsburg, Germany). LC-grade methanol and acetonitrile for pesticide residue analysis were purchased from Scharlau Sciences (Barcelona, Spain). LC-grade water was obtained by purifying demineralized water in a Nanopure II system (Barnstead, Newton, MA, USA).

Stock standard solutions of phenylcarbamate herbicides (400 and 40 μg l^{-1}) were prepared in methanol and stored at −20°C. Diluted standard mixtures were prepared in LC water and stored at 4°C.

2.2. Equipment

Experiments were performed using an automatic sample processor from Gilson (Villiers-le-Bel, France). This system included an ASPEC XL automatic processor equipped with two Rheodyne six-port valves, a Model 306 high-pressure preconcentration pump, a Model 402 low-pressure pump, a Model 817 eight-port valve actuator and a Gilson intelligent keypad. The LC system consisted of a 9012 ternary LC pump from Varian (CA, USA) and a HP 1100 Series diode array detector from Hewlett-Packard (Waldbronn, Germany).

As regards the LC pre-columns, a 5.8×4.6 mm I.D. Prelute cartridge packed with 10 μm Hypersil ODS was purchased from Gilson and a 10×2 mm I.D. cartridge filled with 10 μm PRP-1 sorbent was obtained from Teknokroma (Barcelona, Spain). The analytical column consisted of a 150×4.60 mm I.D. Res Elut packed with 5 μm C_{18} and was obtained from Varian.

Recording of chromatograms and quantitative measurements of peak areas were performed with a HP Chem Station for LC 3D System (software version A.05.03).

Surface water samples with suspended particles and important amounts of organic matter were previously filtered on 0.45-μm disposable nylon membranes purchased from Scharlau Sciences.

2.3. Procedure

Automation of on-line trace enrichment was performed using the ASPEC XL system (Fig. 1) in which a short column was placed in the sample loop position of a six-port valve. An eight-port valve actuator and a high-pressure pump were used for conditioning the short column and percolating samples.

The conditioning process was performed sequentially with 5 ml of methanol, 5 ml of acetonitrile and 5 ml of LC water at 4 ml min^{-1}; afterwards the water sample was percolated through the short column at 4 ml min^{-1} and, finally, the retained compounds were directly eluted by the suitable mobile phase, set at 1 ml min^{-1}, to the analytical column in the backflush mode during 20 min.

A laboratory-made Pascal software program was required in order to control the whole process, since the original software supplied with the equipment was not designed to perform this kind of work.

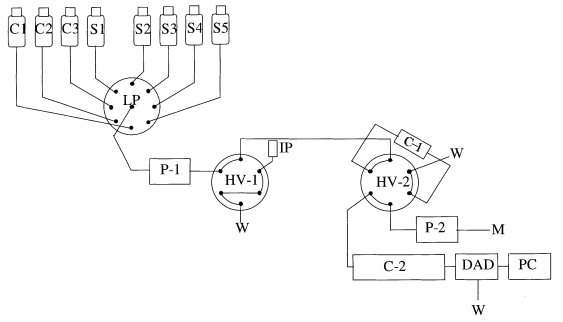


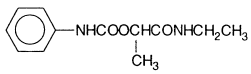
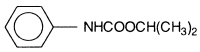
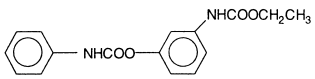
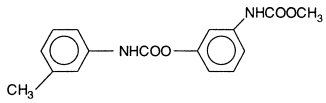
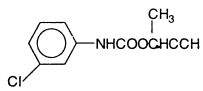
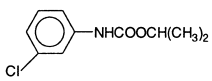
Fig. 1. Schematic representation of the on-line preconcentration system. C=Conditioning solvents; S=water samples; LP=low-pressure eight-port valve; HV-1 and HV-2=high-pressure six-port valves; P-1=preconcentration pump; IP=injection port for direct loop injections; C-1=enrichment column; P-2=eluent pump; M=mobile phase; C-2=analytical column; DAD=diode array detector; PC=personal computer; W=waste.

3. Results and discussion

The UV spectra of the studied phenylcarbamate herbicides were monitored in order to evaluate their UV maximum absorbance wavelength, which are shown in Table 1 together with some other physico–chemical parameters.

The chromatographic separation of analytes was studied by 100 μl loop injection of standard solutions on the analytical C_{18} column. Due to the wide range of polarity between the compounds, a gradient elution, which allowed correct resolution between chromatographic peaks, was optimised. Firstly, acetonitrile was preferred as organic modifier due to its lower viscosity and UV cut-off, however, after assaying different CH_3CN–water gradients, it was not possible to obtain good resolution between desmedipham and phenmedipham with suitable peak shape. This lack of resolution was solved by adding a constant percentage of methanol in the mobile phase, so that resolution between desmedipham and phenmedipham improved without significant increase of the background absorbance. The gradient elution programme, which was performed only modifying the amount of acetonitrile, is shown in Table 2. A

Table 1
Physical and chemical properties of studied phenylcarbamate herbicides shown in order of retention on a reversed-phase column

No.	Compound	Chemical structure	S_{H_2O}, 20°C (mg l^{-1})	λ_{max}
1	Carbetamide		3500	194, 236
2	Propham		32	196, 236
3	Desmedipham		7	200, 238
4	Phenmedipham		6	202, 238
5	Chlorbufam		540	206, 240
6	Chlorpropham		89[a]	208, 240

[a] S_{H_2O} at 25°C.

LC chromatogram of a 200 μg l^{-1} standard solution is shown in Fig. 2.

3.1. Preconcentration experiments

Once the LC separation was achieved, on-line trace enrichment of analytes was considered in order to decrease detection limits. The main parameter of the concentration procedure is the selection of the sorbent, which must allow a convenient breakthrough of the analytes, so that detection limits lower than 0.1 μg l^{-1} could be obtained.

Firstly, a 5.8×4.6 mm I.D. Prelute cartridge packed with 10 μm Hypersil ODS was selected, mainly due to the reported good reproducibility in retention, rapid equilibrium with mobile phase and unusual irreversible adsorption of solutes on this kind of silica sorbents [12]. Besides, a large number of organic compounds, from non-polar to moderately polar, are retained by *n*-octadecyl silicas, although

Table 2
Mobile phase used for the LC separation of phenylcarbamate herbicides

t (min)	MeOH (%)	Water (%)	CH_3CN (%)
0	30	50	20
11.5	30	47	23
20	30	35	35

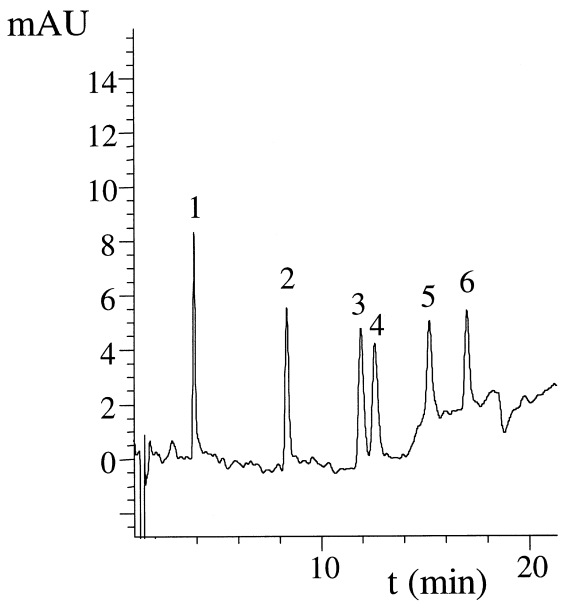


Fig. 2. Direct LC–DAD chromatogram (λ=236 nm) of a standard mixture solution (200 μg l^{-1}) obtained by 100 μl loop injection.

there is a limitation in the case of many polar to moderately polar analytes, which are not well retained [12]. So, in our case, the enrichment of carbetamide (the most polar analyte) could be troublesome.

Different sample volumes (10–100 ml) with the same amount of analytes (20 ng), were percolated through the C_{18} pre-column in order to evaluate the maximum sample volume which could be preconcentrated without breakthrough of analytes and avoiding peak broadening. Finally, 50 ml of water sample was selected as optimum. The response linearity of the procedure was studied with standard solutions ranging between 0.5–4 μg l^{-1} (n=5) achieving coefficients of regression larger than 0.999 in every case.

The former procedure was applied to drinking and surface water samples spiked at two levels of concentration (0.5 and 4 μg l^{-1}). The results, shown in Table 3 were satisfactory with regards to both recoveries and relative standard deviations (R.S.D.s) excepting for carbetamide, which presented lack of reproducibility at low levels of concentration. Fig. 3 shows a chromatogram of a surface water sample spiked at 0.5 μg l^{-1} together with its blank. According to this figure, detection limits down to 0.1 μg l^{-1} could be estimated.

However, from the later experiments it was also evident that both, desmedipham and phenmedipham degraded rapidly in the spiked water samples although standard solutions at similar levels of concentration were stable for around one week. In order to avoid the degradation during the performance of the later experiments, water samples were preconcentrated immediately after spiking but, obviously, additional degradation experiments, which are described in Section 3.2, were required.

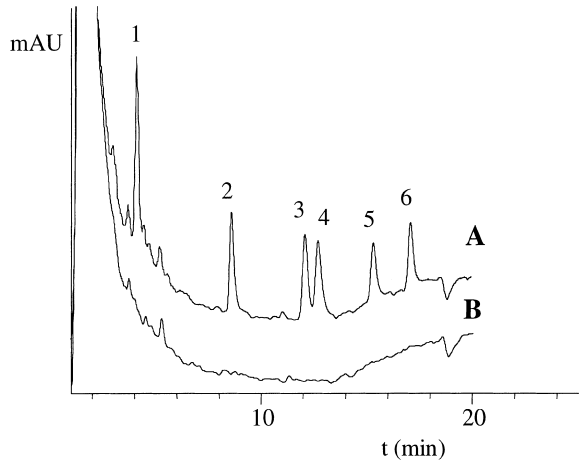


Fig. 3. On-line SPE–LC–DAD chromatograms (λ=236 nm) of (A) a 50 ml surface water sample spiked with selected herbicides at 0.5 μg l^{-1} and (B) the blank sample. Enrichment column: 5.8×4.6 mm I.D. C_{18} Prelute.

Attending to the problems with carbetamide, whose recoveries were not reproducible at low levels of concentration, a 10×2 mm I.D. cartridge filled with 10 μm PRP-1 sorbent was assayed as enrichment pre-column. In this case, the desorption was not possible with the proposed mobile phase in Table 2, mainly due to the presence of methanol (30%) which could not properly elute the retained analytes, although, on the other hand, was necessary for assessing peak resolution between phenmedipham and desmedipham. Therefore, the later analyte (de-

Table 3
Average recoveries and R.S.D.s (%) of the analytes by the proposed on-line SPE–LC–DAD procedures in environmental water samples spiked at different levels (n=5)

Compound	C_{18} pre-column				PRP-1 pre-column			
	Drinking water		Surface water		Drinking water		Surface water	
	0.5 μg l^{-1}	4 μg l^{-1}	0.5 μg l^{-1}	4 μg l^{-1}	0.2 μg l^{-1}	1 μg l^{-1}	0.2 μg l^{-1}	1 μg l^{-1}
Carbetamide	–	105 (3)	–	105 (4)	84 (12)	101 (3)	102 (8)	101 (3)
Propham	101 (2)	98 (3)	99 (3)	97 (3)	90 (5)	102 (5)	97 (6)	102 (3)
Desmedipham	84 (9)	86 (8)	94 (7)	98 (7)	–	–	–	–
Phenmedipham	87 (2)	97 (6)	98 (10)	108 (7)	87 (3)	101 (2)	93 (6)	104 (3)
Chlorbufam	105 (5)	99 (2)	102 (4)	97 (1)	106 (7)	101 (3)	99 (5)	105 (2)
Chlorpropham	103 (2)	99 (1)	108 (3)	106 (2)	105 (5)	101 (4)	99 (5)	108 (2)

Table 4
Mobile phase gradient used in the on-line trace-enrichment with PRP-1 pre-column

t (min)	Water (%)	CH_3CN (%)
0	50	50
7	40	60
10	35	65
20	35	65

smedipham) was discarded in further investigations assuming its lower use in European countries and its easy degradation in the environmental water samples. In this way, a new CH_3CN–water gradient elution, which allowed LC separation of the five analytes, was used (Table 4).

In this case, 100 ml of water sample were preconcentrated since it is well known that retention factors of analytes are between 25- and 40-times higher using polymeric sorbents instead of C_{18} silicas [18]. The response linearity of the method was studied for standard solutions ranging between 0.2–4 μg l^{-1} (n=6) and the results are shown in Table 5.

The method was validated by spiking drinking and surface water samples at 0.2 and 1 μg l^{-1} yielding satisfactory results (Table 3) regarding to recoveries and R.S.D.s, even for carbetamide. Fig. 4 shows the chromatograms of a surface water sample spiked at 0.2 μg l^{-1} and its blank. Detection limits between 0.04–0.1 μg l^{-1} could be estimated for the five phenylcarbamate herbicides studied.

3.2. Phenmedipham and desmedipham degradation

During the development of this work, an unexpected rapid degradation of desmedipham and phenmedipham in spiked environmental water samples was observed, meanwhile the standard solutions, prepared in LC water, were stable during several days. Degradation studies were performed in order to set up the degradation rate of these compounds as well as to obtain some information about degradation products.

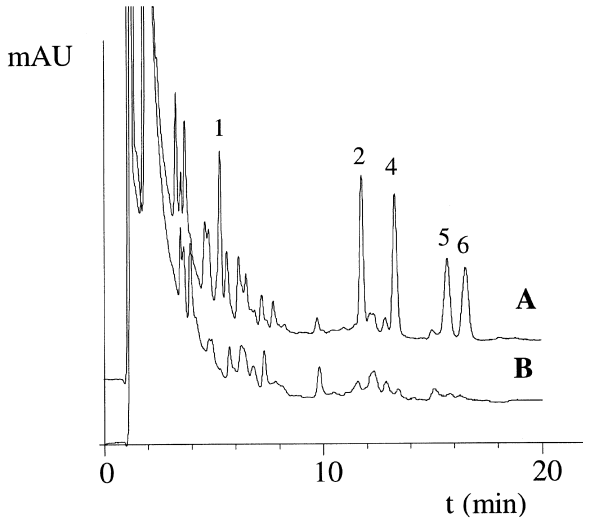


Fig. 4. On-line SPE–LC–DAD chromatograms (λ=236 nm) of (A) a 100 ml surface water sample spiked with selected herbicides at 0.2 μg l^{-1} and (B) the blank sample. Enrichment column: 10×2 mm I.D. PRP-1.

Five hundred ml of drinking and surface water samples were individually spiked with desmedipham and phenmedipham at 4 μg l^{-1}. The remaining amount of each analyte was estimated each hour, by on-line SPE of 50 ml of water sample using the C_{18} sorbent. The results, given in Table 6, show that after 6 h the presence of herbicides was insignificant.

With regard to phenmedipham, two different degradation products were detected (Fig. 5) while for desmedipham, one new compound was clearly found

Table 5
Calibration curves for phenylcarbamate herbicides (0.2–4 μg l^{-1}, n=6) in LC water samples

	Carbetamide	Propham	Phenmedipham	Chlorbufam	Chlorpropham
Standard error	11.75	19.48	16.00	7.686	15.00
R	0.9998	0.9997	0.9999	0.9999	0.9997
Intercept	−2.479	12.29	4.142	9.867	13.54
(S.D.[a]) Intercept	10.09	13.08	10.744	5.158	10.07
Slope	367.0	466.6	574.2	358.43	57.35
(S.D.) Slope	4.102	5.820	4.782	2.296	4.48

[a] Standard deviation.

Table 6
Amount not degraded and half-life times of desmedipham and phenmedipham in environmental water samples spiked at 4 μg l^{-1}

Time (h)	Drinking water		Surface water	
	Desmedipham (%)	Phenmedipham (%)	Desmedipham (%)	Phenmedipham (%)
0	100	100	100	100
1	70	65	65	72
2	40	38	34	47
3	22	21	18	30
4	10	11	9	20
5	4	4	5	10
6	2	2	2	5
24	0	0	0	0
$t_{1/2}$ (h)	1.01	1.01	1.05	1.39

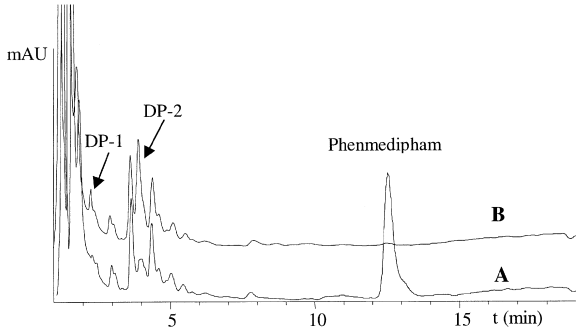


Fig. 5. On-line SPE–LC–DAD chromatograms (λ=236 nm) of (A) a 50 ml drinking water sample after spiking with phenmedipham at 4 μg l^{-1} and (B) 6 h later. DP-1 and DP-2= Degradation products.

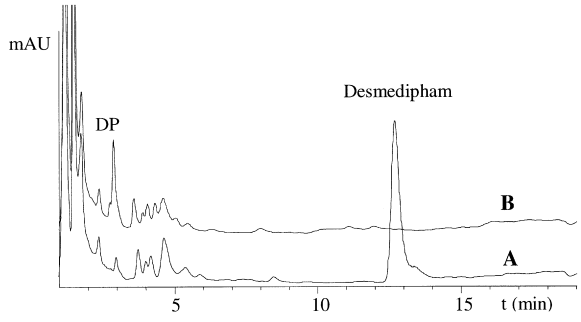


Fig. 6. On-line SPE–LC–DAD chromatograms (λ=236 nm) of (A) a 50 ml drinking water sample after spiking with desmedipham at 4 μg l^{-1} and (B) 6 h later. DP=Degradation product.

(Fig. 6). A decomposition of desmedipham was reported by Hogendoorn et al. [19,20] during the storage of standard solutions prepared in mixtures of methanol–water. They detected a degradation product whose identity was investigated by means of LC–MS using a ''frit-fast atom bombardment'' interface in the positive chemical ionisation mode proposing a structural formula of the degradation compound by interpretation of the mass spectrum.

Assuming that the degradation processes follows first-order kinetics, experimental data were fitted to exponential decay and the half-life times for desmedipham and phenmedipham were finally estimated (Table 6).

It is relevant to emphasise that although phenmedipham is included in the black list of pesticides, it seems to be difficult to found its residues in environmental waters, mainly due to its rapid degradation in these kind of matrices.

Obviously, in the near future, attention must be devoted to the chemical identification of the degradation products and, afterwards, to the development of new analytical procedures including these compounds. For this purpose, multidimensional LC with DAD or MS detection could be a very useful tool.

4. Conclusions

This paper has been focused on the on-line SPE–LC–DAD determination of several phenylcarbamate herbicides mainly due to their wide use in European countries and to the lack of available analytical

procedures. Two different sorbents were assayed in order to optimised the enrichment of analytes, and finally detection limits between 0.04–0.1 $\mu g\ l^{-1}$ were reached by preconcentration of 100 ml of water sample on a PRP-1 cartridge. The robustness of the procedures is also remarkable as the same enrichment cartridges and analytical column were used during all the experiments.

Finally, degradation studies of desmedipham and phenmedipham were included showing that these compounds are rapidly degraded in environmental waters, so that the determination of their residues in water samples should be focused on their transformation products.

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