

DETERMINATION OF PESTICIDES IN WATER BY LIQUID CHROMATOGRAPHY-(ELECTROSPRAY IONIZATION)-MASS SPECTROMETRY (LC-ESI-MS)

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A method for determination of a selected group of pesticides in water (tap and ground water) was developed using liquid chromatography-(electrospray ionization)-mass spectrometry (LC-ESI-MS). The pesticides dimethoate, carbaryl, simazine, atrazine, ametryne, tebuthiuron, diuron and linuron were isolated using liquid-liquid extraction with dichloromethane and analyzed on a reversed phase column, C-18, with gradient elution at flow rate of 1 mL. min⁻¹. Recoveries ranged from 89% to 112% (R.S.D. ≤ 10%) for tap water and from 76% to 98% (R.S.D ≤ 6%) for ground water. The enrichment procedure, followed by use of the selected ion monitoring (SIM) mode gave quantification limits low enough to reach the international legislation level of 0.1 µg.L⁻¹, with a 500-fold preconcentration. The method developed is practical, efficient and reliable for the determination of the selected group of pesticides in the water samples.

KEY-WORDS: MULTIRESIDUE; PESTICIDES-CONTAMINATION.

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1 INTRODUCTION

Pesticides having different structures and biological activities are widely used for agricultural and non-agricultural purposes throughout the world. Due to their widespread use, pesticides need to be determined in various environmental, such as soil, water and air. Thus, the development of reliable methods for systematic environmental analysis of pesticides residues is an important field of research.

A wide range of analytical techniques has been developed in order to identify the organic contaminants often present at trace levels in environmental samples. These compounds are then determined by gas chromatography (GC) or high performance liquid chromatography (HPLC) with a variety of detection systems. Since polar, non-volatile, thermally unstable or high molar mass compounds are unsuitable for gas chromatographic analysis, the use of high performance liquid chromatography to analyze pesticides that are not amenable for GC determination has become a robust and routinely applicable tool in environmental laboratories (BALTUSSEN *et al.*, 1998; FERRER & BARCELÓ, 1998; THURMAN, FERRER & BARCELÓ, 2001).

Nowadays, the on-line combination of liquid chromatography and mass spectrometry (LC-MS) occupies an important place in the analysis of pollutants since this method provides unequivocal identification of thermally labile and polar pesticides at trace levels (SLOBODNÍK, van BAAR & BRINKMAN, 1995; ABIAN, 1999). The newer interfaces for LC-MS, atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) have been important tools in environmental analytical chemistry, especially in the area of pesticide analyses in soil and water (HOGENDOORN & van ZONEN, 2000). The sensitivity, ruggedness, and ease of use of these newer interfaces have made method development rapid and reliable (NIESSEN & TINKE, 1995).

Most of chromatographic techniques cannot determine the low levels of contamination permitted in natural waters without preconcentration (QUEIROZ, MELO & JARDIM, 2002). Solid phase extraction and liquid-liquid extraction, among others, have found very wide applicability for the isolation from aqueous phases of semivolatile and nonvolatile compounds. Liquid-liquid extraction is frequently used for the isolation of pesticides from water samples and dichloromethane is the most common solvent because it is capable of extracting compounds having a wide range of polarities while its volatility makes sample concentration easy (PARRILLA & MARTINEZ VIDAL, 1997).

This work describes a multi-residue method for determining a selected group of pesticides in tap and ground water, at the 0.1 $\mu\text{g.L}^{-1}$ level or lower, using liquid-liquid extraction followed by LC-ESI-MS.

2 EXPERIMENTAL

2.1 CHEMICALS

The pesticides studied in this paper include several types of compounds widely used in Europe including organophosphorus, benzimidazole, carbamate, triazines and phenylurea. Many of the pesticides evaluated belong to the priority list of Europe, which considers pesticides used over 50.000 kg per annum and their capacity for probable or transient leaching (BARCELÓ, 1993). The characteristics of the pesticides studied are shown in Table 1. Atrazine, simazine and ametryne were obtained from Novartis. Tebuthiuron was donated by Dra. Vera Ferracini, from Embrapa Meio Ambiente. Carbendazim and carbaryl were obtained from Riedel-de-Häen. Diuron was obtained from DuPont while linuron was obtained from Hoescht. Dimethoate was donated by Dr. Eduardo Vicente, from Instituto de Tecnologia de Alimentos (ITAL) (Campinas, SP). HPLC grade methanol and formic acid were purchased from Acros (Geel, Belgium) and water was of Milli-Q grade (Millipore, Bedford, MA, USA). All other chemicals and solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Stock solutions of most of the pesticides were prepared by dissolving 1 mg.L^{-1} in methanol while

carbendazim was prepared in acetonitrile at 0.092 mg.L⁻¹. These stock solutions were stored at 4°C and diluted in 70:30 (v/v) methanol-water to spike ground water and tap water at the 0.1 µg.L⁻¹ level and for construction of the analytical curves.

2.2 INSTRUMENTATION

LC-MS was carried out on a HP 1100 series benchtop quadrupole LC-MSD (Hewlett-Packard, Waldbronn, Germany) with an electrospray interface (positive mode). The selected ions (selected ion monitoring - SIM) were: 125, 145, 160, 202, 216, 228, 229, 233 and 249 for analysis of the following pesticides dimethoate, carbaryl, carbendazim, simazine, atrazine, ametryne, tebuthiuron, diuron and linuron, respectively. The optimal settings for the MS operated in the positive ion electrospray mode were as follows: gas temperature: 340°C, nebulizing gas pressure: 0.38 MPa, drying gas flow rate: 12 L.min⁻¹, capillary voltage: 4000 V, quadrupole temperature: 100°C and CID voltage: 100 V. The separation was performed by using a 5 µm reversed phase column, C-18, 250 X 4.6 mm, from Phenomenex. Gradient elution was performed with methanol (solvent A) and water at pH 3.8 adjusted with formic acid (solvent B), at a flow rate of 1 mL.min⁻¹. The gradient program was from 45% A to 55% A in 20 min, then from 55% A to 75% A in 17 min, keeping % A constant during 7 min and, finally, decreasing to the initial condition in 3 min. The injection volume was 10 µL.

TABLE 1 – CHARACTERISTICS OF THE SELECTED GROUP OF PESTICIDES

Pesticide	Class	Type ^a	Solubility in water (mg.L ⁻¹)	Molecular weight
Dimethoate	Organophosphorus	I	25000	229.2
Carbendazim	Benzimidazole	F	28	191.2
Carbaryl	Carbamate	I	1000	201.2
Ametryne	Triazines	H	185	227.0
Atrazine	Triazines	H	70	215.7
Simazine	Triazines	H	5	201.6
Diuron	Phenylurea	H	42	232.1
Linuron	Phenylurea	H	75	248.0
Tebuthiuron	Phenylurea	H	2500	228.3

^a I = insecticide; F = fungicide; H = herbicide.

2.3 SAMPLE PREPARATION

The method of extraction was adapted from the literature (BALINOVA, 1996; PARRILA & MARTINEZ VIDAL, 1997). A volume of 0.5 L of tap water or ground water, at pH 2.5, adjusted with HCl, spiked with standards or not, was extracted with 3 portions of 50 mL of dichloromethane. Water was removed from the combined organic extract by addition of anhydrous sodium sulfate. Solvent evaporation was performed in a rotary evaporator until ± 3 mL, then this volume was transferred into a small vial and the remaining solvent was evaporated using a stream of nitrogen gas. A volume of 1 mL of methanol/water 6:4 (v/v) was added and this solution was filtered through a 0.45 µm membrane before injection into the chromatograph.

3 RESULTS AND DISCUSSION

3.1 SELECTION OF PESTICIDES

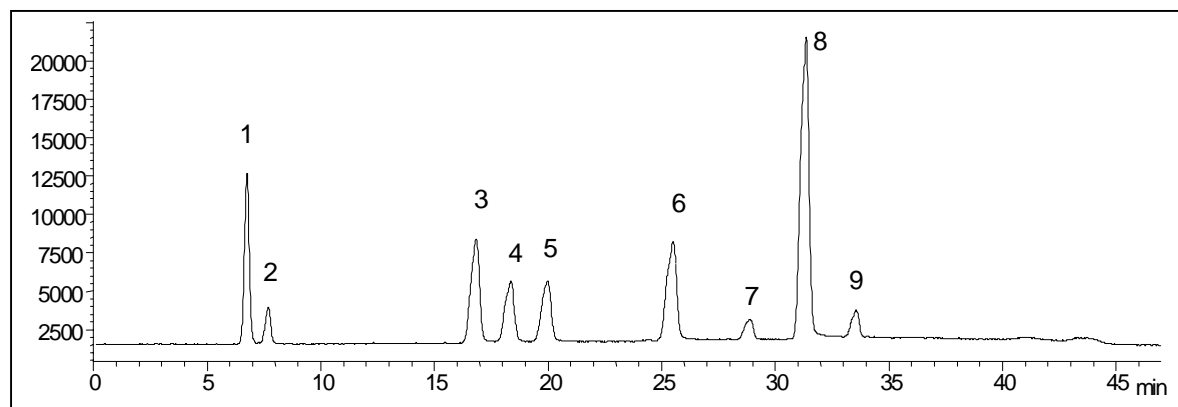
Monitoring of pesticides in ground water has been a topic of increasing importance over the last few years in the world. In some important agricultural areas, where pesticides have caused contamination in the hydrological system or where the vulnerability is high, especially where ground water is the primary source of drinking water, water monitoring must be developed to assess and evaluate the pesticide concentration level (VIGHI & FUNARI, 1995).

Although a single residue method is often used for analysis of water samples, when nothing is known about the nature of possible contaminants, multi-residue methods are needed. Ideally, multi-residue methods should provide rapid identification and quantification of as many different pesticides as possible at the required sensitivity limit. For this study, a limited number of analytes were selected, taking into consideration those pesticides that are poorly amenable to analysis by standard GC methods, while being representative of classes of pesticides having wide use in agriculture and thus being possible contaminants of ground water. Table 1 lists the selected pesticides.

3.2 OPTIMIZATION OF LC-MS CONDITIONS

The selected group of pesticides, carbendazim, dimethoate, carbaryl, simazine, atrazine, ametryne, tebuthiuron, diuron and linuron belong to diversified classes of pesticides and gradient elution is necessary for the chromatographic separation since these compounds have very different polarities. To identify the mobile phase composition that yields maximum detectability for the combined LC-ESI-MS set-up, various compositions of the eluent, at different pH values, were tested. Formic acid was added to the mobile phase to increase the molecular ionization of the compounds and to improve the detection in the MS analysis. After testing different conditions for the separation of the pesticides, the preferable mobile phase was methanol (solvent A) and water, pH 3.8 adjusted with formic acid (solvent B), with the gradient already described. After the optimization of LC-MS conditions, baseline resolution was obtained for the selected compounds. Figure 1 shows a chromatogram obtained using a mass spectrometry detector (MS) with electrospray interface, in full scan mode.

FIGURE 1 - CHROMATOGRAM OBTAINED FOR THE SEPARATION OF PESTICIDE STANDARDS: 1 = CARBENDAZIM; 2 = DIMETHOATE; 3 = SIMAZINE; 4 = TEBUTHIURON; 5 = CARBARYL; 6 = ATRAZINE; 7 = DIURON; 8 = AMETRYNE; 9 = LINURON



Mass detector mode "Full Scan". Experimental conditions: analytical column 250 X 4.6 mm, C-18, 5 μ m. Gradient elution with methanol and water (pH 3.8).

Selected ion monitoring (SIM) was used for quantification of the tested pesticides in order to increase the detectability. The most intense ions were chosen. Most of these ions correspond to $[M + H]^+$, since the positive mode was employed, except for the ions 125, 145 and 160, used to monitor dimethoate, carbaryl and carbendazim, respectively. Figure 2 presents the mass spectrum for dimethoate, carbaryl and carbendazim.

3.3 SAMPLE PREPARATION

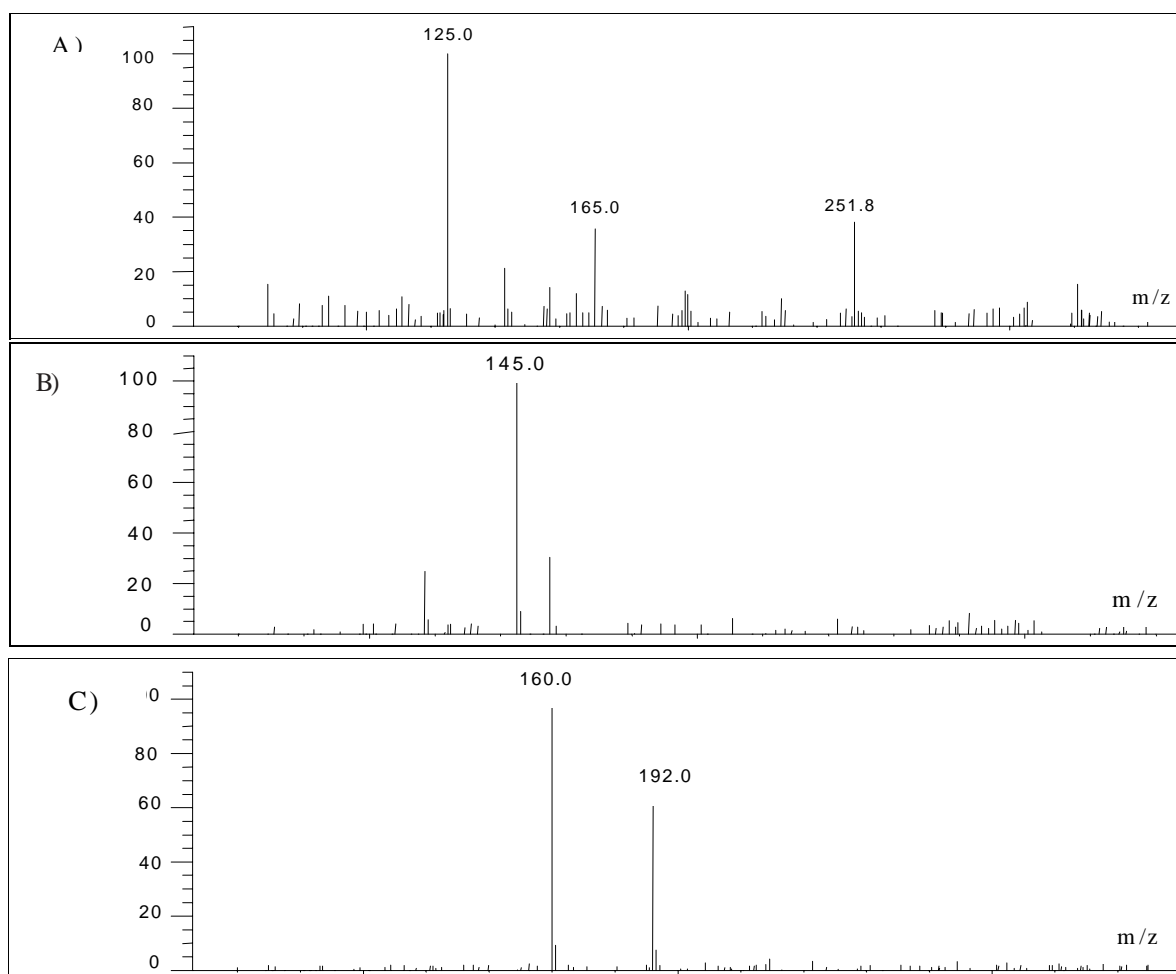
LLE with dichloromethane was used to isolate pesticides from tap water and ground water.

Water samples were spiked with the standards and the recovery values are shown in Table 2. Recoveries were calculated by comparison to a direct injection of standard (analytical curve). The average results obtained for pesticide recoveries ranged from 89% to 112% for tap water and from 76% to 98% for ground water. These values are considered acceptable since a 70-130% recoveries are approved. The results for precision ranged from 0% to 11.1% for tap water and 0.5% to 5.1% for ground water, indicating good repeatability.

LLE is not suitable for analysis of carbendazim due to its loss during the evaporation process. Analytical curves were obtained using standard solutions of the pesticides and showed good linearity in the range from LOQ to $500 \mu\text{g.L}^{-1}$, with correlation coefficient >0.999 .

The limits of detection (LOD) and quantification (LOQ) were calculated at signal-to-noise ratios of 3 and 10, respectively. The results obtained are shown in Table 2.

FIGURE 2 – MASS SPECTRUM: A) DIMETHOATE, B) CARBARYL AND C) CARBENDAZIM



3.4 APPLICATION

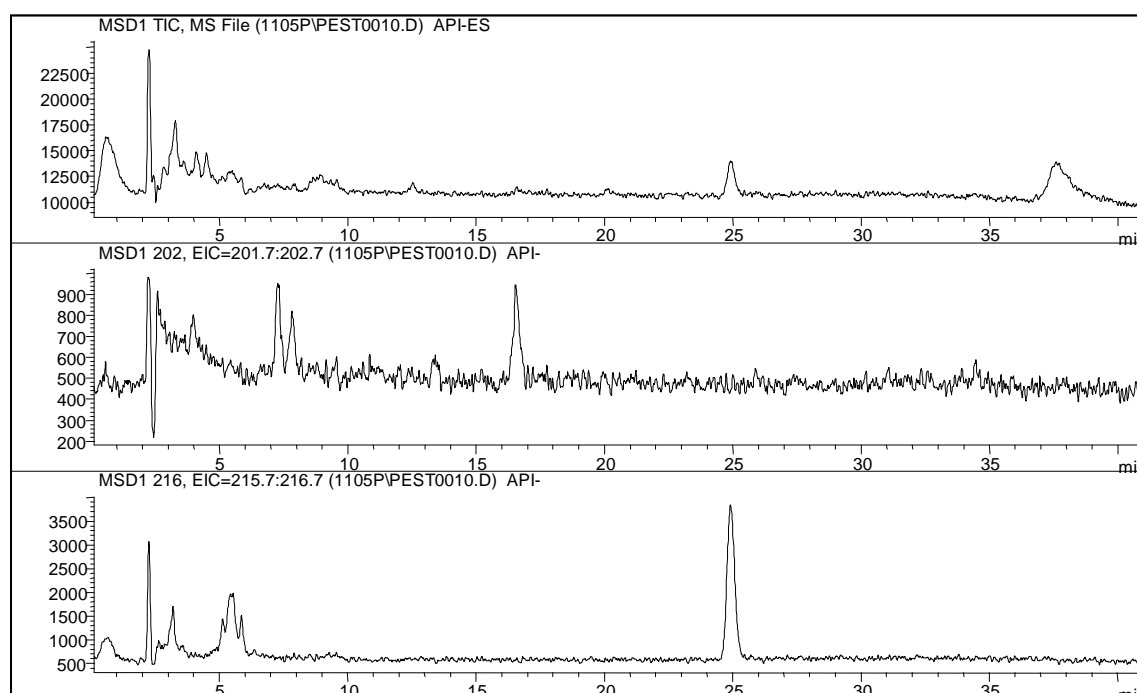
The method was applied to real samples. Tap water was collected at the laboratory in Ghent, Belgium. Ground water was collected from an artesian well, also in Belgium. Pesticides that belong to the triazine class (simazine and atrazine) were detected in tap water (Figure 3) but no pesticides were found in the ground water. The concentration of simazine was below the LOQ and the atrazine concentration was $0.03 \mu\text{g.L}^{-1}$. These concentrations are lower than the acceptance limit of EEC Drinking Water Directive (EEC 80/778), which have legal tolerance levels of $0.1 \mu\text{g.L}^{-1}$ for individual substances and $0.5 \mu\text{g.L}^{-1}$, for the sum of pesticides (including their main metabolites). The method was shown to be sensitive enough to detect these pesticides, even at this sub- $\mu\text{g.L}^{-1}$ level.

TABLE 2 – RECOVERY, PRECISION (R.S.D, %), LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ) FOR PESTICIDES IN WATER SAMPLES AFTER EXTRACTING WITH DICHLOROMETHANE (LLE)

Pesticide name	Tap water		Ground water		LOD $\mu\text{g.L}^{-1}$	LOQ $\mu\text{g.L}^{-1}$
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)		
Dimethoate	97.1	1.1	80.7	0.7	0.01	0.05
Carbaryl	89.0	0.0	81.4	2.2	0.01	0.03
Simazine	112.4	9.9	82.7	2.1	0.006	0.02
Atrazine	95.4	1.0	97.9	1.3	0.006	0.02
Ametrine	93.5	1.5	94.5	1.2	0.006	0.02
Tebuthiuron	101.1	0.9	90.6	3.3	0.03	0.1
Diuron	100.6	1.4	86.5	5.9	0.03	0.1
Linuron	102.0	3.9	75.5	0.7	0.03	0.1

Samples spiked with a $0.1 \mu\text{g/L}$ concentration. Number of replicates = 3.

FIGURE 3 - CHROMATOGRAMS OF TAP WATER. A) FULL SCAN; B) DETECTION OF SIMAZINE AT T_R 16.8 MIN (SIM MODE) E C) DETECTION OF ATRAZINE AT T_R 25.0 MIN (SIM MODE)



4 CONCLUSION

Although the LC-ESI-MS is expensive, the benchtop model used is a robust instrument and can be used in routine analyses.

Methanol–water mobile phase having the pH adjusted with formic acid is adequate for accurate confirmation of the pesticide identities using MS scan detection while the SIM mode permits their quantification.

The results obtained for calibration, recovery and linearity showed that the method is practical, efficient and reliable for determination of the selected group of pesticides in water samples (tap and ground water), except for carbendazim, which is not recovered due to losses during the evaporation process. The LOD and LOQ, considering the 500-fold pre-concentration step, satisfy international legislation ($0.1 \mu\text{g.L}^{-1}$).

RESUMO

DETERMINAÇÃO DE PESTICIDAS EM ÁGUA POR CROMATOGRÁFIA EM FASE LÍQUIDA (IONIZAÇÃO DO TIPO ELECTROSPRAY)-ESPECTROMETRIA DE MASSAS (CL-IE-EM)

Desenvolveu-se método para a determinação de selecionado grupo de pesticidas em água (de torneira e subterrânea), usando cromatografia em fase líquida (ionização do tipo electrospray) espectrometria de massas (CL-IE-EM). Os pesticidas dimetoato, carbaril, simazina, atrazina, ametrina, tebutiuron, diuron e linuron foram isolados por meio de extração líquido-líquido com diclorometano e analisados em coluna de fase reversa, tipo C-18, com eluição por gradiente e vazão de 1 mL min^{-1} . Foram obtidas recuperações nas faixas de 89% a 112% (R.S.D. $\leq 10\%$) para água de torneira e de 76 a 98% (R.S.D. $\leq 6\%$) para água subterrânea. O procedimento de enriquecimento, que pré-concentra amostras em 500 vezes, seguido do uso de monitoramento seletivo de íon (modo SIM) forneceu limite de quantificação suficientemente baixo para atender a legislação internacional ($0,1 \mu\text{g.L}^{-1}$). O método desenvolvido mostrou-se prático, eficiente e confiável para a determinação dos pesticidas selecionados em amostras de água.

PALAVRAS-CHAVE: MULTIRRESÍDUO; PESTICIDAS-CONTAMINAÇÃO.

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