

# Multi-residue analysis of polar pesticides in surface water and sediment by high performance liquid chromatography

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## ABSTRACT

We have successfully studied the analytical method of polar pesticides like carbofuran, pirimicarb, thiodicarb, atrazine, simazine, carbaryl, diuron, isoprocarb in surface water and sediment by HPLC-UV. The method could be applied to HPLC- MS. The stable recoveries ranged from

79 – 110 % with surface water and sediment samples. Especially, a cleanup procedure combined QuEChERS method and solid phase extraction has been developed to analyse these compounds in sediment, a very complex matrix.

**Key words:** QuEChERS, sediment, pesticides, surface water, HPLC – UV.

## INTRODUCTION

The polar pesticides ( $\log K_{ow}$  1.6 – 2.8) as simazine, atrazine (triazine herbicides), thiodicarb, pyrimicarb, carbofuran, carbaryl, isoprocarb (carbamate insecticide) and diuron (phenylurea herbicides) have been widely used due to their properties. They strongly dissolve in water and persist in the environment. Hence, according to the European Union directive on water quality (98/83/EC) the maximum concentration admissible for levels of pesticide residues in drinking and surface water is 0.10  $\mu\text{g L}^{-1}$  for individual and 0.50  $\mu\text{g L}^{-1}$  for the sum of pesticides [1]. The analysis of sediments should be included in environmental studies because they are the result of the integration of all processes (biological, physical and chemical) that occur in an aquatic ecosystem, influencing the metabolism

of the whole system. Sediments are very different in composition forms and processes and can provide valuable information about water quality [2]. Trace analysis of organic contaminants such as pesticides in environmental samples typically consist of following consecutive steps: isolation of analytes from the sample matrix, removal of bulk co-extracts from crude extract, identification and quantification of target analytes and examination to make sure that there have been no false positive results [3].

Many innovations have occurred in analytical methods for the extraction of pesticides from different matrices (e.g. food, biological and environmental) that reduce the analysis time,

minimize the number of analytical steps, use fewer reagents in smaller amounts and provide

high recovery. Recently, *Anastassiades et al.* [4] developed an approach called “quick, easy, cheap, effective, rugged and safe” (QuEChERS), which involves extraction with acetonitrile (ACN) partitioned from the aqueous matrix using anhydrous  $MgSO_4$  and NaCl followed by a dispersive-SPE cleanup with  $MgSO_4$  and primary secondary amine (PSA). The QuEChERS method commonly uses GC-MS and LC-MS/MS to cover the wide range of pesticides for analysis (*Cunha et al.*) [5]. In this paper, we adopted its principle for cleaning up the sediment sample in combination with Oasis HLB SPE prior to analysis by LC-UV.

## EXPERIMENTAL

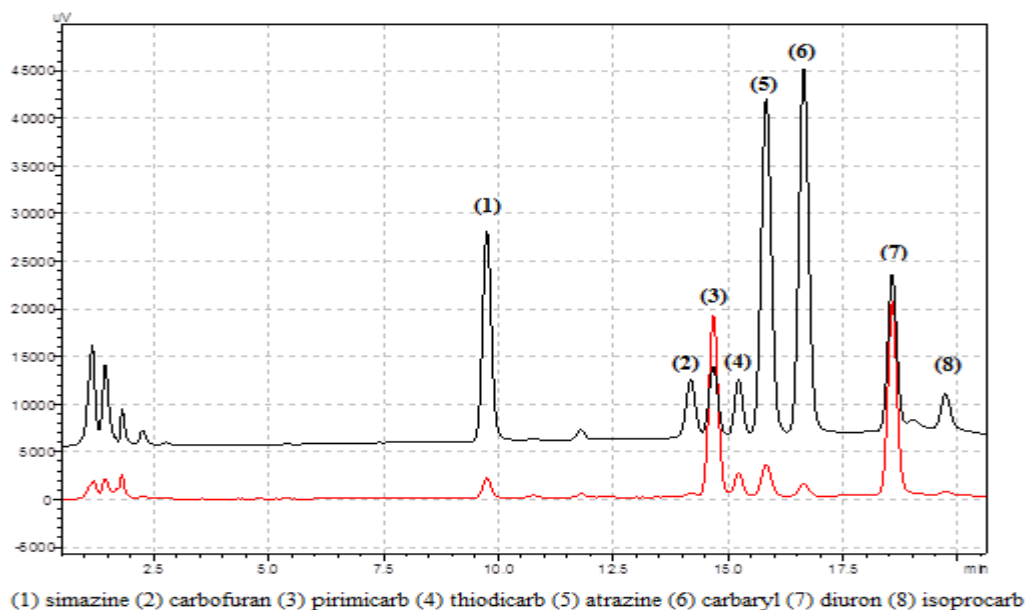
### Chemicals and materials

The standard pesticides were obtained with 99 % purity from TechLab (France). Individual standard solutions were prepared at  $1000\text{ mg L}^{-1}$  in methanol and stored at  $-4\text{ }^\circ\text{C}$ . Working standard solutions were prepared by diluting with mobile phase solution (acetonitrile and ultrapure water (20/80, v/v) mixture) at suitable concentrations. All working standard solutions were stored in dark at  $4\text{ }^\circ\text{C}$ . Acetonitrile (ACN) and methanol (MeOH) (HPLC grade  $\geq 99.9\%$ ) were purchased from Scharlau (Spain). Dispersive – SPE sorbents included PSA, obtained from Varian (USA) and C18 (50  $\mu\text{m}$ ) obtained from J.T.Baker (USA). NaCl and  $MgSO_4$  were obtained from Merck (Germany). The SPE procedure was performed

using a VacElut vacuum manifold from Agilent. The Oasis HLB sorbent was purchased (60  $\mu\text{m}$ ) from Waters (Ireland).

### High performance liquid chromatography-UV determination of pesticides.

A HPLC-UV system (Shimadzu, Japan) consisted of a LC-20AD pump and a UV SPD-20A detector was performed with a C18 X – bridge (3.0  $\times$  100 mm, 3.5  $\mu\text{m}$ ). The injection volume was 20  $\mu\text{L}$  and the analysis was carried out at a flow rate of  $0.4\text{ mL min}^{-1}$ . Chromatographic separations were operated at  $30\text{ }^\circ\text{C}$  with a flow rate of  $0.4\text{ mL/min}$ . Guard columns (50 mm  $\times$  2.1 mm i.d) of phenomenex with the respective phases were used. The mobile phase composition was made up of A: acetonitrile (ACN) and B: ultrapure water (UPW). The elution started at 20 % A for 0.5 min. From 0.5 to 13.0 min a linear gradient from 20 % A to 30 % A was applied and then from 13.0 to 18.0 min a linear gradient from 30 % A to 35 % A. The composition of 35 % A was held for 1.0 min and then returned 20 % A. Afterwards, the mobile phase composition was maintained at 20 % A for 3.0 min to elute the remaining interferences and re-equilibrate the column. The detection wavelengths were set at 220 nm for simazine, pyrimicarb, carbaryl, thiodicarb and 254 nm for diuron, carbofuran, atrazine, isoprocab after investigating absorption wavelengths of the analytes. Data acquisition and processing were performed using LC solution software (Shimadzu).



**Figure 1.** Chromatograms of eight pesticide standard solution at 220 nm wavelength (black-upper) and 254 nm wavelength (red-lower)

### Sample collection and Treatment

#### *Surface water samples*

Surface water samples were collected by directly filling the 2 L plastic container from the surface water body being sampled [6]. Samples were kept away from sunlight and stored at ambient temperature for transportation. The sample was filtered with GFF (0.45  $\mu\text{m}$  x 47 mm, Supelco) or GF/F (0.7  $\mu\text{m}$  x 47 mm, Whatman) membrane and stored at 4 °C for one month.

A volume of 200 mL filtered surface water sample was loaded through 200 mg SPE Oasis HLB cartridge that was previously conditioned with 3 mL of MeOH and 3 mL of ultrapure water. The cartridge was then rinsed with 10 mL of MeOH and ultrapure water (5/95, v/v) mixture to remove impurities, dried with argon and eluted with three volumes of 1 mL MeOH. The eluent was dried by argon to less than 0.5 mL and

reconstituted to 1 mL with mixture of MeOH and ultrapure water (20/80, v/v).

This later step gave a more compatible solution with HPLC mobile phase. Finally, this solution was filtered with 0.22  $\mu\text{m}$  PTFE/L filter (Chrompure) prior to analysis on HPLC-UV system. Sample extracts were stored in the dark at 4 °C until analysis.

#### *Sediment samples*

Sediment samples were taken at Cá Trê bridge, Sai Gon river, district 2. Air-dried sediment samples were homogenized and 2.0 g dry sediment was transferred to centrifuge tubes 50 mL. Samples were extracted by 10 mL of ACN, 4 g of  $\text{MgSO}_4$  and 1 g of NaCl in each tube and centrifuging it at 3.000 rpm for 1 min; transferring 5 mL of ACN extract to a commercial SPE cartridge containing 330 mg PSA. 330 mg C18 and a 1 cm layer of  $\text{MgSO}_4$  activated with 3 mL of ACN. This extract was passed through a

preconditioned SPE cartridge. Then, the solid phase extraction was carried out in the same way as described for the surface samples treatment.

## RESULTS AND DISCUSSION.

Two sample matrices, surface water and sediment were spiked with eight pesticides extracted by the methods presented and analyzed by RP – HPLC. Both methods were found to be relatively quick and easy to use.

The single operator precision and accuracy for the water extraction method are shown in Table 1. The accuracy of each pesticides extracted from both the spiked Evian drinking water and from the spiked river water is expressed as the mean of the percent recovery for a given number of tests. The precision of each pesticide extraction is expressed as the standard deviation of the corresponding percent recoveries.

**Table 1. Average recoveries (R) (n = 3), the relative standard deviations (RSD %) (n=3) and MDL in water extraction method**

Compounds	Standard concentration ( $\mu\text{g L}^{-1}$ )	Drinking water EVIAN			River water		
		R (%)	RSD (%)	MDL ( $\mu\text{g L}^{-1}$ )	R (%)	RSD (%) n = 3	MDL ( $\mu\text{g L}^{-1}$ )
Simazine	0.51	95.9	3.0	0.012	86.3	1.7	0.078
Carbofuran	1.00	87.9	4.8	0.061	79.6	3.5	0.21
Pirimicarb	1.00	95.8	4.5	0.072	91.6	4.2	0.25
Thiodicarb	1.00	61.1	2.8	0.72	<b>34.9</b>	<b>8.2</b>	2.5
Atrazine	0.99	98.5	3.4	0.054	92.1	3.0	0.19
Carbaryl	0.50	84.1	7.3	0.038	81.0	4.4	0.11
Diuron	1.01	92.5	2.0	0.013	89.7	4.0	0.073
Isoprocab	1.99	83.8	3.7	0.38	78.5	8.2	1.1

With the drinking water EVIAN, we obtained good recoveries (> 80 %) for all except for thiodicarb (61.1 %) that might loss due to sample filtration. The experiments on filtration step with 0.7  $\mu\text{m}$  membrane were realised on river water sample. The obtained results showed that the recoveries were less than 3 to 9 % in comparison with drinking water EVIAN sample, especially for thiodicarb (26.2 %). Thus, these polar pesticides are seemly absorbed on solid particles in river water and retained on membrane.

The QuEChERS method was applied to sample preparation in this study, because it has several advantages over most of the traditional extraction techniques. According to *Lehotay* [7] high recoveries for a wide polarity and volatility

range of pesticides, very accurate results, low solvent usage and waste, and high sample throughput. Besides these advantages, a single person can perform the method without much training or technical skill. The method is quite rugged, relatively inexpensive and few materials and glassware are needed. This method is nowadays the most applied extraction method for the determination of pesticide residues in food samples, providing acceptable recoveries for acidic, neutral and basic pesticides (*Prestes et al.*) [8] such as fruits and vegetables (*Anastassiades et al.* [4]; *Aysal et al.* [9]), rice (*Koesukwiwat et al.* [10]) milk, eggs and avocados (*Lehotay et al.*[11]) olives and olive oil (*Cunha et al.* [12]) and soil (*Lesueur et al.*[13]).

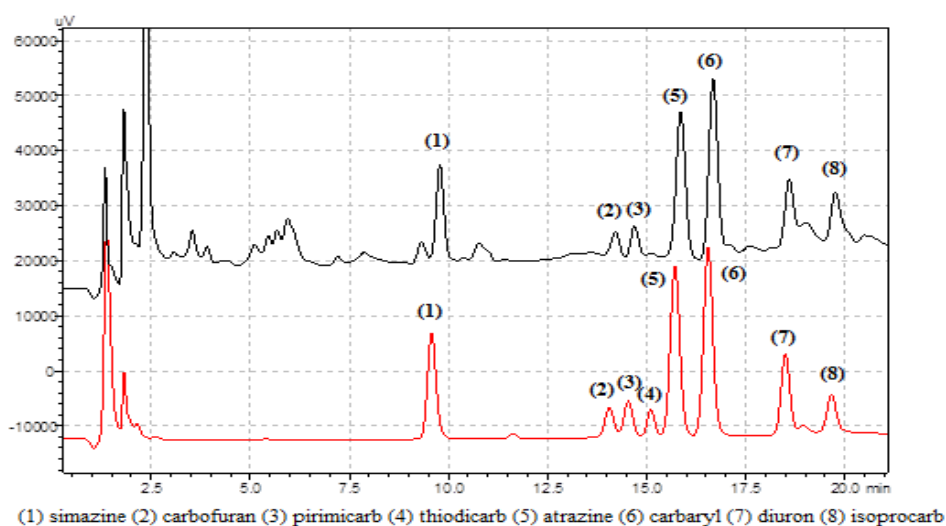
To our knowledge, this is the first application of the method to sediments.

The representative chromatograms obtained from extracts of pesticide-fortified in sediment ( $0.5 \text{ mg kg}^{-1}$ ) after the application of QuEChERS method are shown in Fig. 2. The QuEChERS method resulted in extracts that contained the target analyte, with high recovery and free from interferences in the region of the chromatogram near the retention time of the pesticides. The

experiments were performed by spiking the sediment samples with the pesticides being studied. The recoveries obtained for all pesticides in sediment at different concentrations ranged from 79 % to 116% for all except for thiodicarb, with relative standard deviations below 8.3 %. (Table 2). These values are within the range stipulated by the U.S. Environmental Protection Agency (Tolosa *et al.* [14]), which is from 70 % to 110 % with relative standard deviations below 30 %.

**Table 2. Average recoveries (R) (n = 3), the relative standard deviations (RSD %) (n=3) and MDL in sediment extraction method**

Compounds	Standard concentration ( $\mu\text{g Kg}^{-1}$ )	R (%)	RSD (%)	MDL ( $\mu\text{g L}^{-1}$ )
Simazine	0.40	79.1	2.3	5.7
Carbofuran	0.78	99.3	6.5	15.3
Pirimicarb	0.78	86.3	1.3	18.2
Thiodicarb	0.79	-	-	-
Atrazine	0.77	87.3	1.4	13.9
Carbaryl	0.39	94.7	0.33	8.0
Diuron	0.98	97.3	5.4	5.3
Isoprocab	1.57	110.8	8.3	80.3



**Figure 2.** Chromatograms of 8 pesticides standard solution (*red-lower*) and spiked sediment sample (*black-upper*)

Method detection limits (MDLs) were determined at  $S/N = 3$  and method quantification limits (MQLs) were at  $S/N = 10$ . The MQLs (Table 2) were higher than those reported in the recently published LC-MS/MS methods but MDLs were all low enough to detect these pesticides in surface water according to the requirement of Council Directive 98/83/EC. Otherwise, in order to get better sensibility we could increase the injection volume up to 100  $\mu\text{L}$  (instead of 20  $\mu\text{L}$  as presented in this paper).

## CONCLUSION

In this study, we developed a QuEChERS method in combination with Oasis HLB solid

phase extraction (SPE) process for the determination of polar pesticides in sediment by HPLC – UV with high sensitivity, stability and reliability. Recovery of analytical method is from 79 – 92 % (for sample water) and 79 – 110 % (for sample sediment), which is satisfactory for the field of analyzing pesticides substances at trace levels in environmental samples. Our method is adaptable for LC – MS.

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# Phân tích đa dư lượng các hợp chất bảo vệ thực vật phân cực trong nước bề mặt và bùn lắng bằng phương pháp sắc ký lỏng hiệu năng cao

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## TÓM TẮT

Chúng tôi đã nghiên cứu thành công phương pháp phân tích các chất BTV phân cực mạnh như carbofuran, pirimicarb, thiodicarb, atrazine, simazine, carbaryl, diuron, và isoprocarb trong nước bề mặt và trong bùn lắng bằng HPLC-UV và có thể áp dụng cho HPLC-MS. Hiệu suất thu hồi ổn

định từ 79 – 110 % cho cả hai nền mẫu. Đặc biệt một quy trình chiết làm sạch vận dụng kết hợp nguyên lý của phương pháp QuEChERS và chiết pha rắn đã được phát triển để phân tích những hợp chất này trong bùn lắng, một nền mẫu rất phức tạp.

**Từ khóa:** QuEChERS, bùn lắng, thuốc bảo vệ thực vật, nước bề mặt, HPLC – UV.

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