SPME-GC-MS determination of selected aniline pesticides in surface water

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Abstract

An analytical methodology to determine Alachlor, Butralin, and Metolachlor herbicides, and Metalaxyl fungicide, in surface water was developed using Solid Phase Microextraction (SPME) and Gas Chromatography coupled to Mass Spectrometry (GC-MS). The developed method was validated, and the quantification limits were found to be between 0.2 to 1.8 μg.L^{-1}, depending upon the compound. The precision, as measured by the relative standard deviations (RSD), was in the range 3.5% to 27%. The higher RSD was found for Butralin due to the occurrence of adsorption of this compound in the extraction fiber. After validation the developed methodology was employed to the determination of these four pesticides in water samples that supplies Araraquara and São Carlos cities (São Paulo - Brazil). Samples from Feijão Creek, Anhumas Creek, Cruzes Creek and Paiol Brook, as well as samples from after-treatment at SAAE (Water and Sewer Supply Service – São Carlos, SP, Brazil) and DAAE (Water and Sewer Supply Department – Araraquara, SP, Brazil) were evaluated. Samples from both winter and summer seasons were collected and analysed. The results show that the samples analyzed using the in-house validated methodology were not contaminated with these pesticides within the investigated concentrations.

Keywords: water, anilines, pesticides, SPME, GC-MS, herbicides.

"Dedicated to Professor Harold McNair on the occasion of his 80th birthday".

1. Introduction

Water is extremely important to the Earth ecosystem. The relevance of water quality preservation is constantly increasing. The intensification of agricultural production, the monoculture cultivation to the human nourishment, and the industrial process have been requiring a proper regulation of pesticide uses.

The Economic Commission for Europe established 0.1 µg.L^{-1} as the maximum admissible concentration of any pesticide in human consume destination water and 0.5 g.L^{-1} to the total residues. However, is not clear if the transformation products are also included in this directive[1]. This limit have been polemic because it did not consider the toxicity of each product, and for some compounds, the available analytical methodologies do not achieves detection limits in this order. On the other hand, the USA Environmental Protection Agency (US-EPA) and the Health World Organization (HWO) established maximal levels to individual pesticides in human consume destination water, based on toxicological and epidemiological studies. EPA methods used to determine Alachlor (methods 505, 525.1, 525.2, 507 and 551.1) and Metolachlor (Methods 507 and 551.1) in water, among other compounds, are described in the literature[2,3] but robust, quick and cheap methods to determine Butralin and Metalaxyl were not found.

In Brazil, CONAMA Resolution 357[4], established contaminants maximal limits in water to various destinations, which include the evaluation of some Organochlorine, Organophosphorous and Carbamates pesticides. Resolution 36/GM from the Health Ministry established limits for pesticides in human consume destination water. However, the majority of pesticides nowadays used, such as Pyrethroids insecticides and several herbicides, are not included in these regulation.

Since beginnings of 70´s, the Brazilian agriculture, in particular the agriculture performed in São Paulo state, have been suffering major technological changes, including the replacement of workers by machines and, especially, a large increase in the use of chemical products. At that time, Rodrigues and coworkers[5] related that the use of pesticides was higher than 78000 ton/year of applied active principle, or 2.5 kg/ha harvest.

Sanchezbrunete and coworkers[6], gives one account that the control of noxious weeds in corn plantation is done using herbicides such as Atrazine and Chloroatrazine in binary mixtures with Chloroacetamide herbicides as Alachlor and Metolachlor. The environmental contamination power of theses herbicides comes from their physical-chemistry properties and the culturally used practices. Higher concentrations of theses herbicides generally are detected in surface and underground water, in different countries (Economic Commission for Europe, 1992).

Cholotriazin, Choloroacetamides and Nitroanilines analysis usually have been done by gas chromatography (GC) with nitrogen-phosphorous detector (NPD) or electron-capture detector (ECD) and, more recently, by reversed phase liquid chromatography[7-10]. Residues confirmation have been done, in most cases, by GC-MS and less frequently by LC-MS(MS). Solid Phase Microextraction (SPME) has been used for pesticide determination in water[11-14] coupled with both GC or LC. SPME is a fast and simple technique that minimizes sample preparation steps, reducing the analysis time consuming, systematic errors and sample contaminations, as earlier shown by Pawlyszyn and coworkers[15].

In this work, an analytical methodology to determine Alachlor, Butralin, and Metolachlor herbicides, and Metalaxyl fungicide (Figure 1) in surface water has been developed, validated and applied to the analysis of these pesticides in water samples.

Metolachlor, *2-ethyl-6-methylaniline* is an organic compound widely used as a herbicide. It is a derivative of aniline and is a member of the chloroacetanilide herbicides. It has been detected in ground and surface waters and various concentrations throughout the U.S. It is classified as a Category C pesticide by the United States Environmental Protection
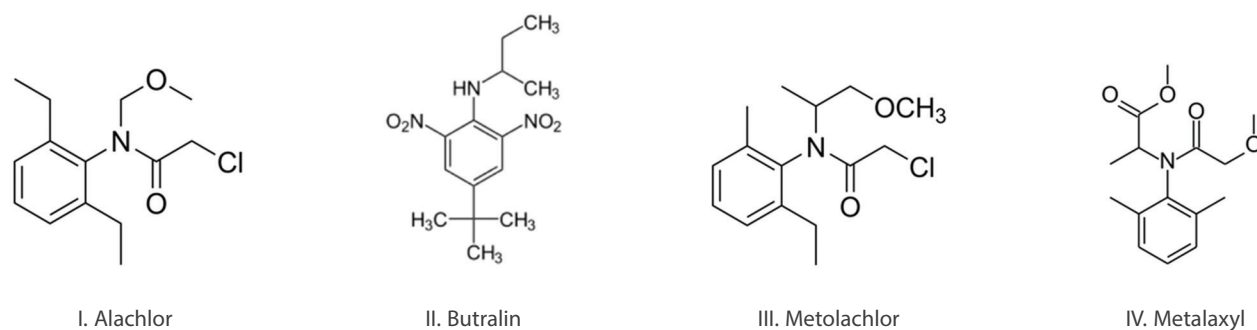
I. Alachlor   II. Butralin   III. Metolachlor   IV. Metalaxyl

Figure 1. Chemical structure of the selected pesticides evaluated in this study.

Agency (US EPA) which indicates limited evidence of carcinogenicity. *Alachlor, 2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl) acetamide*, is a herbicide used for weed control on corn, soybeans, sorghum, peanuts, and beans. It has been evaluated for carcinogenic activity in rats and mice. In accordance with the EPA proposed Guidelines for Carcinogen Risk Assessment, Alachlor was classified as "likely" to be a human carcinogen at high doses, but "not likely" at low doses. *Metalaxyl, methyl N-(methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate*, is a phenylamide fungicide with systemic function, used to control Pythium in a number of vegetable crops, and Phytophthora in peas among other applications. The oral and dermal LD50 in rats indicates slight toxicity by ingestion and dermal application. No information was available regarding the inhalation toxicity of metalaxyl. *Butralin, (RS)-N-sec-butyl-2-6-dinitroaniline*, is a weak oxidizer herbicide, stable to hydrolysis and somewhat to photolysis. It is very toxic in contact with skin, causing sensitization by skin contact, and irritating to eyes, respiratory system and skin. It is considered very toxic to aquatic organisms may cause long-term adverse effects in the aquatic environment. As a consequence of the toxic characteristics to humans and the environment presented by these compounds, and the large use of pesticides in Brazil at the presente time, their analysis in water samples is imperative.

The methodology in this work, based on SPME as the extracting, clean-up and concentration technique, followed by GC-MS, was validated and the analytical determination of these four pesticides in water samples that supplies Araraquara and São Carlos cities (São Paulo - Brazil) from both winter and summer seasons, was done. Samples from after-treatment at SAAE (Water and Sewer Supply Service – São Carlos, Brazil) and DAAE (Water and Sewer Supply Department – Araraquara, Brazil), from both winter and summer seasons, were also collected and analysed.

2. Materials and Methods

Pesticide analytical standards were purchased from Supelco (Bellefonte, PA, USA), with purity grade higher than 97.1%. To prepare the standard solutions acetone P.A. from Carlo Erba (Milano, Italy) and Milli-Q grade water from Millipore (Bedford, MA, USA) were used. In order to study the ionic strength effect on the extraction, sodium chloride P.A. from Merck (Apartado, Darmstadt, Germany) was used. For the SPME experiments, a holder and poliacrilate fibers (85 µm film thickness), both purchased from Supelco were used. Stir bars and 5 mL conical botton glasses both from Kimax (Kansas City, MO, USA), and a magnetic stirring Corning (São Paulo, SP, Brazil), as well as a thermostatic bath Quimis (Diadema, São Paulo, Brazil). The glass materials were silanized according to Doong and coworkers[16] procedure, using methanol, acetone, hexane and toluene from Mallinckrodt (Phillipsburg, NJ, USA), a 10% (v/v) mixture of dichlorodimethylsilane (Supelco) in toluene and an oven from Fanem (São Paulo, SP, Brazil). Analyses were performed on a

Shimadzu QP5050A (Shimadzu, Kyoto, Japan) gas chromatograph-mass spectrometry system. Pesticides were separated on a DB5 column from J & W Scentific (Agilent, São Paulo, Brazil) having the following dimensions: 30 m × 0.25 mm I.D. × 0.30 μm film. The split-splitless injector was operated in the splitless mode. The injector port and detector temperatures were 523 K and 553 K, respectively; Helium was used as carrier gas at 30 cm/sec. The column temperature was held at 343 K for 2 min, increased to 493 K at rate of 10 K.min^{-1}, increased to 543 K at rate of 2 K.min^{-1}, increased to 553 K at rate of 5 K.min^{-1}, with the final temperature held for 4 min. The detector was operated in the Single Ion Monitoring (SIM) mode, with a detector energy of 1.5 eV and electronic impact of 70 eV. The monitored ions were of m/z 132, 160, 162, 188, 206, 238 and 266.

To develop the methodology a 100 mg/L stock solution of the four pesticides was used. The optimized parameters were extraction time of the compounds to the fiber, dessorption time on the GC injector, temperature extraction and salting out effect. Direct extractions were done, and the position of the fiber in the solution, stirring rate and position of the fiber on the injector were all maintained constant.

3. Results and Discussion

3.1. Method development

To determine the ideal extraction time, the temperature of extraction was fixed on 303 K and the fiber was maintained on the injector port during the whole chromatographic run. The immersion time should be sufficient to allow the fiber to absorb a significant quantify of analyte. The pesticide Butralin did not reach the equilibrium until 60 min, as shown in Figure 2, being the only compound evaluated that did not reach the equilibrium until 25 min. As Butralin presents a good detector response, a good compromisse between the extraction time and area counts was obtained after 20 minutes of exposure time. The minimum analyte carryover was obtained with 5 min of desorption time.
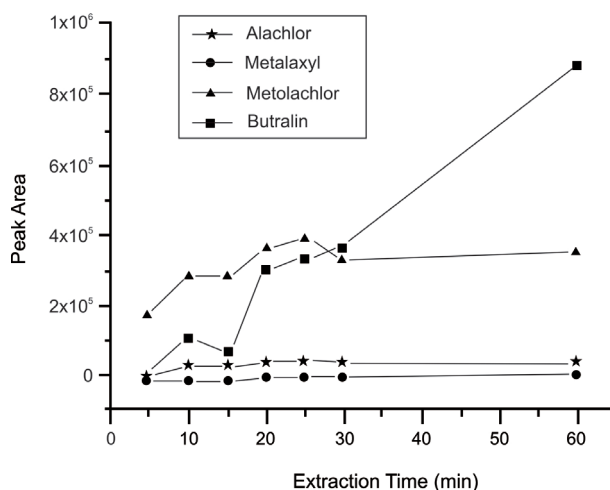

Figure 2. Variation of the area of the extracted analyte as a function of the extraction time using SPME.

As shown in Figure 3, the best temperature extraction for Alachlor, Metalaxyl and Metolachor was 303 K. Butralin presets a different performance again, being 303 K selected as the best extraction temperature for all compounds.
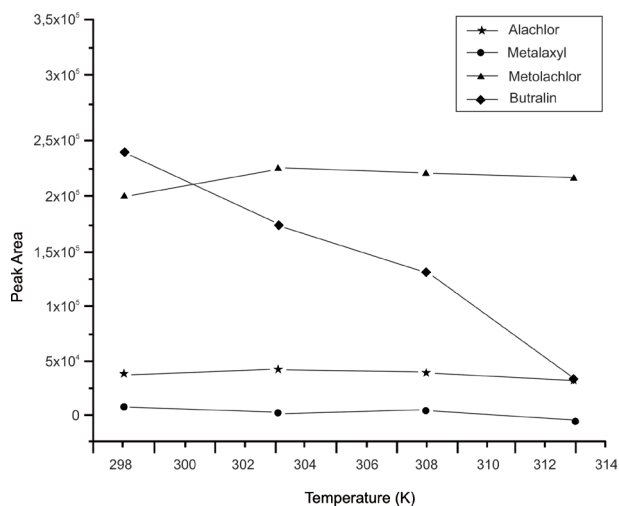

Figure 3. Effect of the temperature extraction on the pesticides recovery.

In order to study the salting out effect, NaCl was added to the standard solutions in concentrations of 20, 35, 60 mg.mL^{-1}, and saturated. As shown in Figure 4, Butralin presented a divergent behavior compared to the other compounds when salt was added. This

effect occurs probably because the two nitro groups could present a week ionic interaction that increases the matrix affinity when the ionic concentration in the solution increase. However, when the ionic concentration increase, the solvating power decrease, and the interaction between fiber and the other tree compounds increase. So, the average better salt concentration found was 60 mg.mL^{-1}.
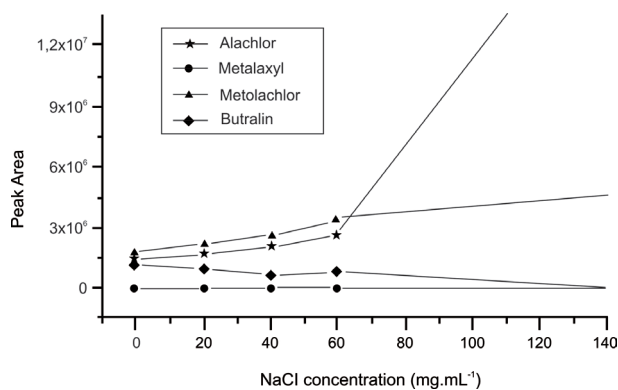

Figure 4. Salting out effect.

The final methodology assumed as the best condition amongst the ones investigated in this work has been as follow. Using 3 mL of sample and 180 mg of NaCl into a conical glass, the stir bar was inserted and the glass was closed with an aluminum paper film before the lid. This glass was inserted in the stirring and heating system, being under stirring (400 rpm) and constant temperature (303 K) for 10 min to achieve the equilibration. Then, the SPME system was inserted into the vial and the fiber was displayed inside the solution for 20 minutes, under constant stirring and temperature.

The fiber was retracted from the solution and inserted in the GC injection port for 5 min after which the chromatographic run began.

3.2. Method Validation

To obtain the calibration curve, standard concentration of solutions containing the 4 pesticides in concentrations ranging from 0.1 µg.L^{-1} to 100.0 µg.L^{-1} were used. The methodology was applied tree times for each curve point, in order to generate an average value for each point. The straight lines equations obtained from calibration curve, the Linear Range (r), the precision (obtained with 12 analyses of 100µg.L^{-1} solutions containing the 4 pesticides) as measured by the Relative Standard Deviation (RDS), the Detection Limit (LOD) and Quantitation Limits (LOQ) for each pesticide are shown on Table I.

As shown in Table 1, the Linear Range obtained for Metalaxyl is extremely poor, probably because this compound was retained in the stir bar, as confirmed in a separate experiment using blank (Milli-Q) water. Every glass material was silanized, but this compound did not disappear even in the blank extractions. The only one material that was not silanized at this step was the stir bar. When a blank experiment as done using the same apparatus, except that the stir bar was changed to a new bar, the Metalaxyl peak disappeared from the solution. A similar observation gave origen to a new extraction technique, named Stir Bar Sorptive Extraction (SBSE)[17], in which pesticides were observed to be retained in a stir bar coated using a polydimethylsiloxane film. To overcome this problem, which could also happen with other analytes, the use of silanyzed glass stir bar is higly recommended.

Table 1. Straight lines equations, Linear Range (r), Relative Standard Deviation (RDS), Detection Limit (LOD) and Quantitation Limits (LOQ) from each pesticide.

	Straight Line Equations	**r**	**RSD (%)**	**LOD (µg.L^{-1})**	**LOQ (µg.L^{-1})**
Alachlor	-693,8516 + 1302,95657 X	0.99752	3.86	0.00732	0.2196
Metalaxyl	9365,17892 + 19,85094 X	0.20391	15.66	0.4584	1.3753
Metolachlor	-1935,43355 + 2112,47939 X	0.99667	3.15	0.0942	0.2826
Butralin	-34206,10069 + 6691,86406 X	0.98751	27.22	0.6267	1.8801

3.3. Surface Water Analysis

Typical chromatograms obtained from the determination of theses pesticides in water samples that supplies Araraquara and São Carlos cities (São Paulo - Brazil) are shown on Figure 5.

The analysis were done with samples collected from Feijão Creek (I), Anhumas Creek (II), Cruzes Creek (III) and Paiol Brook (IV). Samples from after-treatment at SAAE (Water and Sewer Supply Service – São Carlos) (V) and DAAE (Water and Sewer Supply Department – Araraquara) (VI)

are also analyzed. Samples from both winter and summer seasons were collected. A blank obtained with Mili-Q grade water (A), a crude sample (C), and spiked sample (D) containing a 10 $\mu g.L^{-1}$ pesticides solution, as well as a 10 $\mu g.L^{-1}$ standard solution containing the four pesticides studied (B) were analyzed for each sample collected. Each sample was analyzed in triplicate. As shown on Figure 5, in case they were present any one of these pesticides could be detected in the collected samples, using the developed methodology.
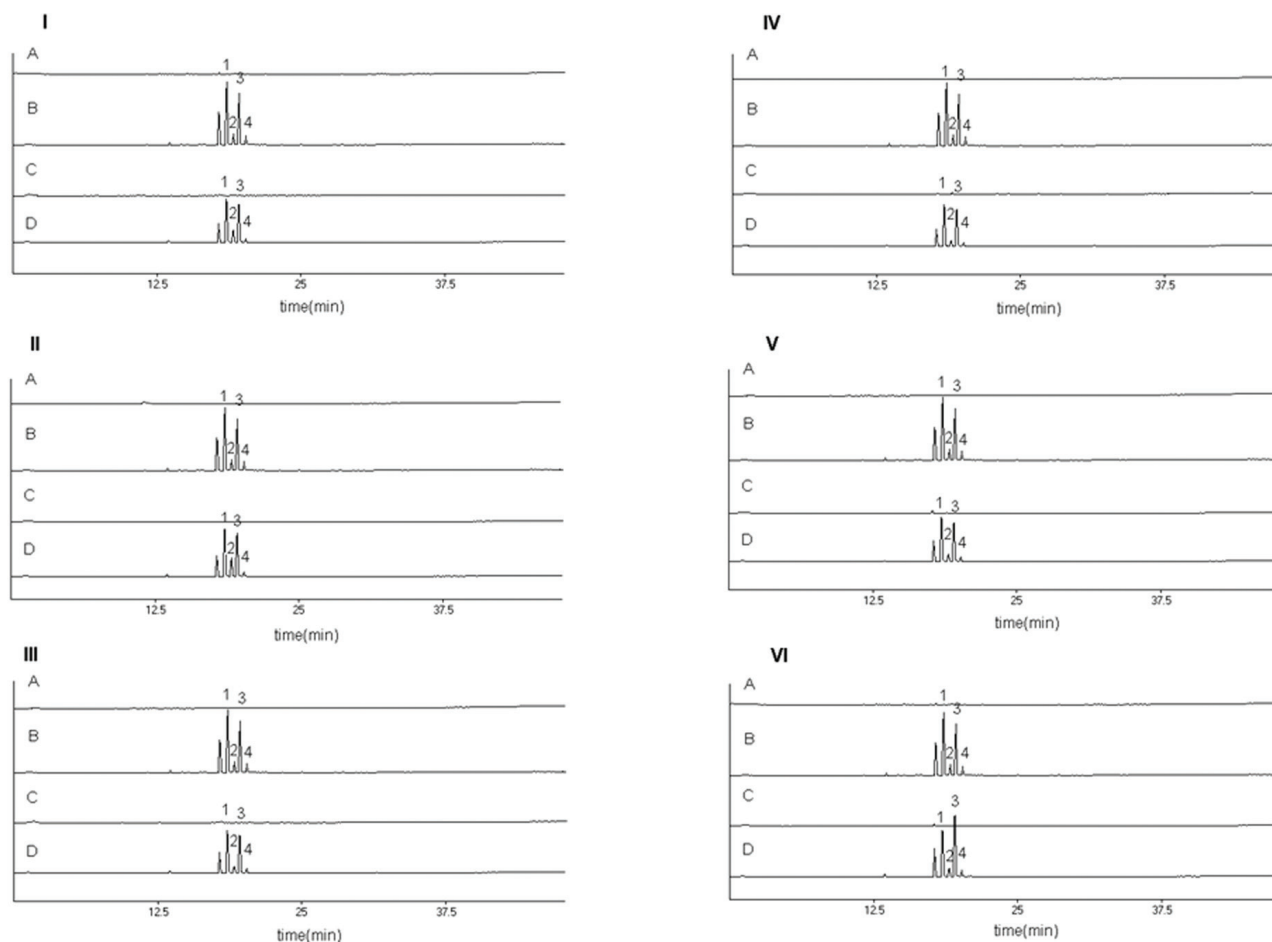

Figure 5. Chromatograms obtained for typical water samples. I - Feijão Creek; II - Anhumas Creek; III - Cruzes Creek; IV - Paiol Brook; V - after-treatment water sample from SAAE; and VI - after-treatment water sample from DAAE. A - blank (Milli-Q) water; B – (Milli-Q) water spiked with a 10 $\mu g.L^{-1}$ pesticides standard solution; C - water sample; D - water sample fortified with a 10 $\mu g.L^{-1}$ pesticides standard solution. Peaks: 1 = Alachlor; 2 = Metalaxyl; 3 = Metolachlor; 4 = Butralin.

4. Conclusion

The developed and validated methodology to determine Alachlor, Butralin, and Metolachlor herbicides, and Metalaxyl fungicide in surface water using SPME technology presented good precision for Alachlor and Metolachlor pesticides, and the detection limits obtained to these compounds are under the maximum limits established by the Economic Commission for Europe (0.1 µg.L^{-1}). Metalaxyl presented low precision due to its retention on the stir bar, as discussed. To solve this problem, silanized glass stir bar should be used. The presented results shows that the water samples both before and after-treatment at the Municipal Water Treatment Service at Araraquara and São Carlos cities (São Paulo - Brazil), sampled during both summer and winter, were not contaminated with these pesticides in the detectable concentrations achieved by the employed analytical methodology.

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