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EVALUATION OF STIR BAR SORPTIVE EXTRACTION FOR DETERMINATION OF CHLOROPHENOLS IN WATER SAMPLES USING GC-MS

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Abstract

The paper shows possible applications of a mobile sorptive unit that consisted of a polydimethylsiloxane (PDMS) stir bar to the determination of chlorophenols in water. Due to the high costs of thermal desorption devices, the release of chlorophenols from the sorptive unit into organic solvent was carried out in an ultrasonic field. Both the extraction time and the type of organic solvent were selected experimentally. The qualitative composition of the extract was studied by gas chromatography – mass spectrometry (GC – MS). The following optimum conditions for chlorophenols assays in water by SBSE – LD – GC/MS were determined: extraction time – 120 min., desorption with ethyl acetate – 15 min. The Stir Bar Sorptive Extraction (SBSE) used in the study is competitive with commonly used extraction techniques such as Solid Phase Extraction (SPE), Solid Phase Microextraction (SPME) or Liquid Phase Microextraction (LPME).

Streszczenie

Przedstawiono możliwości wykorzystania ruchomego elementu sorpcyjnego, który stanowił pręt z polidimetylosiloksanu PDMS, w procesie oznaczania chlorofenoli w wodzie. Z uwagi na wysoki koszt urządzeń do desorpcji termicznej, uwolnienie chlorofenoli z elementu sorpcyjnego do rozpuszczalnika organicznego dokonano, w polu ultradźwiękowym. Czas ekstrakcji jak i rodzaj rozpuszczalnika organicznego dobrano doświadczalnie. Jakościowy skład ekstraktu badano za pomocą chromatografii gazowej sprzężonej ze spektrometrią mas (GC – MS). Określono następujące optymalne warunki oznaczania chlorofenoli w wodzie metodą SBSE – LD – GC/MS: czas ekstrakcji 120 min. oraz desorpcji – 15 min. z użyciem octanu etylu. Przedstawiona w pracy metoda ekstrakcji z wykorzystaniem ruchomego elementu sorpcyjnego SBSE (Stir Bar Sorptive Extraction) jest konkurencyjna do powszechnie wykorzystywanych metod ekstrakcji takich jak ekstrakcja ciecz-ciecz, ekstrakcja do fazy stałej SPE (Solid Phase Extraction), mikroekstrakcja do fazy stacjonarnej SPME (Solid Phase Microextraction), jak i mikroekstrakcja do fazy ciekłej (Liquid Phase Microextraction LPME).

Keywords: **Stir bar sorptive extraction; Determination of chlorophenols; Water.**

1. INTRODUCTION

Chlorophenols are impurities recognized as a priority by both EU and US regulations [1-3]. All compounds in that group exhibit toxic properties, and 2,3,4,6 – tetrachlorophenol and pentachlorophenol are also carcinogenic [4]. Furthermore, 2,4 – dichlorophenol and pentachlorophenol are regarded as compounds of estrogenic characteristics and have been classified as Endocrine Disruptors [5]. EU regulations specify that the maximum total concentration of chlorophenols in drinking water must not exceed 0.5 μg/l [6].

Some of the chlorophenols, e.g. 4 – chlorophenol, are components of disinfectants. Others, those with at least two atoms of chlorine, are used as pesticides or their half – finished products [7]. Chlorophenols are also formed during water disinfection as a result of humic matter chlorination [8]. Some occur in the environment as a result of photo – or biodegradation of

other chemical substances, e.g. 2,4 – dichlorophenoxyacetic acid [9]. Chlorophenols penetrate into the environment primarily from agricultural sources (pesticides) and most of them enter surface waters. Their emission into the atmosphere is limited by their relatively low volatility [9]. In the surface waters, chlorophenols undergo photochemical changes that cause the formation of compounds of various toxicity and durability, e.g. the photodegradation of pentachlorophenol PCP produces about 30 new chemical compounds – derivatives of phenols, catechols, alcohols and carboxylic acids [10-11]. All chlorophenols are characterized by a very strong smell detected in water even at the ng/l level [9].

The analytical procedures used to assay chlorophenols in water are based on the following steps: (i) extraction of a compound from water matrix, (ii) purification, (iii) assays by GC or HPLC, frequently preceded by derivatization of tested compounds, and finally (iv) detection using a number of techniques i.e. spectrophotometry, amperometry, electron capture and mass spectrometry [4]. Due to the occurrence of low concentrations of chlorophenols in water, the extraction step must be carried out prior to assays. Usually, chlorophenols are separated by liquid – liquid extraction (LLE) [12] and solid phase extraction (SPE) [13]. However, the necessity to use large quantities of organic solvents in those techniques is a considerable disadvantage. Also, the evaporation of excess solvent often becomes a necessity to reach the required enrichment factor. Those techniques are time-consuming as well. Solid phase microextraction (SPME) and liquid phase microextraction (LPME) are modern solvent-free (or limited use of organic solvents) techniques used to separate chlorophenols from water matrix [14]. Unfortunately, they are characterized by low sensitivity. Currently, stir bar sorptive extraction (SBSE) is taken into consideration in terms of enrichment and isolation of chlorophenols from water. In this technique, compounds are extracted to the magnetic stir bar covered with a layer of extraction medium. The most common extraction agent is polydimethylsiloxane (PDMS), volume range of 55-219 µl. The extraction involves an intensive shaking of a water sample (10-250 ml) with the sorptive unit followed by the release of adsorbed analytes from the sorptive medium during desorption [15]. SBSE is employed in combination with thermal desorption (TD) of compounds making use of automatic desorption devices coupled with a chromatograph [16]. One of the techniques which are not well-known is desorption of compounds to a small quantity of organic solvent (Liquid Desorption LD). A review of the techniques for sample preparation used in chlorophenols assays have been presented by Quintana and Ramos [17]. Chlorophenols assays by gas chromatography necessitate their prior derivatization to silyl [18] and acyl [16] derivatives. Acylation is carried out directly in the water environment. Figure 1 shows a schematic of 2,4 – dichlorophenol acylation.

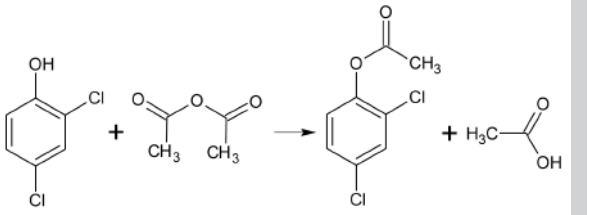

Figure 1.
Diagram of 2,4 – dichlorophenol acylation

Benito et al. [18] investigated 46 polar chemical compounds from the group of pharmaceuticals and phenols employing SBSE. The detection limits in this technique for 37 of the compounds tested fell within the range of 1-800 ng/l, which proves the technique to be attractive in contemporary environmental analytical research.

This paper is aimed at showing possible applications of a mobile sorptive unit that consisted of a polydimethylsiloxane (PDMS) stir bar to the determination of chlorophenols in water. The compounds were derivatized in water to acyl derivatives using acetic anhydride. Due to the high costs of thermal desorption devices, the release of chlorophenols from the sorptive unit into organic solvent was carried out in an ultrasonic field. Both the extraction and desorption times in the ultrasonic field and the type of organic solvent were selected experimentally. The paper also presents the conditions for chlorophenols assays using GC – MS.

2. METHOD AND MATERIALS

2.1. Materials and chemicals

Organic solvents i.e. analytically pure dichloromethane, acetonitrile, ethyl acetate, acetic anhydride and sodium carbonate (Na_2CO_3) were manufactured by POCH (Poland). The sorptive material – polydimethylsiloxane (PDMS) bar, length 2 cm, diameter 0.2 cm and weight 0.07 g – was supplied by GoodFellow (Germany). The ultrasonic IS – 1K bath was used, working frequency of 35 kHz,

was produced by Intersonic (Poland). During extraction, the samples were stirred mechanically using a Labor System shaker (Poland).

The chlorophenol standards i.e. 2,4 – dichlorophenol (2,4 – DCP), 2,4,6 – trichlorophenol (2,4,6 – TrCP), 2,3,4,6 – tetrachlorophenol (2,3,4,6 – TeCP) and pentachlorophenol (PCP) were supplied by Sigma – Aldrich (Poland). The 1.0 mg/ml base solution of chlorophenols were prepared in acetonitrile. Working solution of chlorophenols were prepared at 100 ng/µl in methanol. The internal standard used was mirex obtained from Sigma-Aldrich (Poland). The mirex concentration was equal to 10 ng/ml and was prepared in an organic solvent which was used to compounds desorption.

2.2. Stir bar sorptive extraction

The extraction of phenols in water samples was carried out as follows:

- acylation of the compounds in water and sorptive extraction using a mobile sorptive stir bar (Stir Bar Sorptive Extractions – SBSE),
- desorption of the analytes in an ultrasonic field.

In order to derivatize the chlorophenols, 250 µl of acetic acid anhydride was poured into a water sample (25 ml, pH = 11-12, adjustment by adding sodium carbonate) and intensively stirred for 2 min. Next, the sorptive material was inserted (PDMS bar), the vessel was closed and mechanically shaken at 300 r.p.m. for 120 minutes. After extraction, the sorptive material was transferred into organic solvent (200 µl of ethyl acetate). The next step was analytes desporption in an ultrasonic bath for 15 minutes. Extraction and desorption times in the ultrasonic field as well as the type of organic solvent were selected experimentally.

The recovery and precision of the method were assessed by replicate analysis (n = 6) of tap and surface water samples with standard concentrations equal 100, 400 and 1000 ng/ml.

2.3. GC – MS analysis

The extracts obtained were analyzed by GC – MS using a Varian Saturn 2100 T gas chromatograph equipped with a ion trap mass detector. The extracts were separated in a VF – 5 ms column employing the following temperature program of the oven: 60°C (2 min.), 15°C/min. to 270°C (14 min.). The other parameters are given in Table 1. The quantitative analysis was made by the technique of internal standard adding mirex into the sample (IS). Each calibration curves for all chlorophenols as acetyl derivatives were plotted for 6 points. These points were taken from the range of concentration in deionized water equal to: in case of 2,4 – DCP c = 100 – 2000 ng/ml, in case of 2,4,6 – TrCP and 2,3,4,6 – TeCP c = 20-2000 ng/ml and in case of PCP c = 100-4000 ng/ml.

Table 1.
Experimental parameters for measurements

GC	Column	Varian VF – 5 ms (0.25 mm i.d. x 30 m, df: 0.25 µm)
	Temperature	60°C (2 min.) – 15°C/min. 270°C (14 min.)
	Injection volume Injector	3 µl 280°C, splitless
	Carrier gas / Flow	helium / 1.2 ml/min.
MS	Ionization / Ionization energy	EI / 70 eV
	Ion source temperature	200°C

2.4. Theoretical recovery of SBSE

The theory of SBSE is similar to that of solid phase microextraction (SPME) and assumes that the partition coefficients between the extracting medium (PDMS phase) and water ($K_{PDMS/W}$) are proportional to those between n – octanol and water phase ($K_{O/W}$). This can take the following form:

$$K_{O/W} = K_{PDMS/W} = \frac{C_{SBSE}}{C_W} = \frac{m_{SBSE}}{m_W} \cdot \frac{V_W}{V_{SBSE}} \qquad (1)$$

where:

C_{SBSE} – concentration of the substance assayed in PDMS phase, ng/ml

C_W – concentration of the substance assayed in water, ng/ml

m_{SBSE} – mass of analyte in PDMS phase, ng

m_W – mass of analyte in water phase, ng

V_{SBSE} – volume of SBSE phase, ml

V_W – water volume, ml

It is assumed that $\frac{V_W}{V_{SBSE}} = \beta$ (volume coefficient).

Equation (1) can take the form of equation (2):

$$\frac{K_{O/W}}{\beta} = \frac{m_{SBSE}}{m_W} = \frac{m_{SBSE}}{m_0 - m_{SBSE}} \qquad (2)$$

where m_0 is the initial amount of a given compound in water (ng).

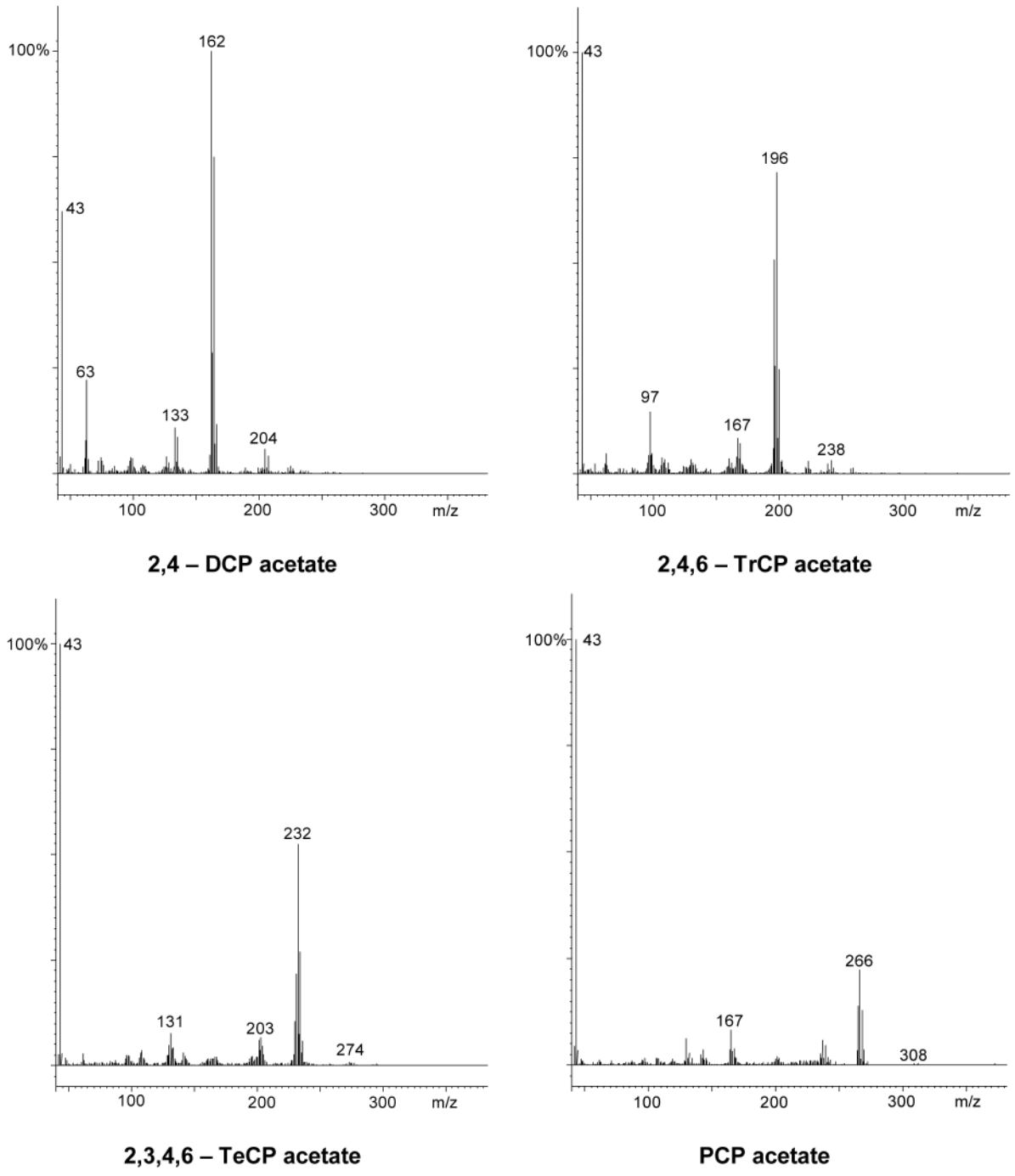

Figure 2.
Mass spectra of acyl derivatives of chlorophenols

After conversion, equation (2) looks as follows:

$$\frac{m_{ext}}{m_0} = \frac{\frac{K_{O/W}}{\beta}}{1+\left(\frac{K_{O/W}}{\beta}\right)} \cdot \text{recovery} \qquad (3)$$

Extraction effectiveness depends on the ratio between partition coefficient $K_{O/W}$ and volume coefficient β. Table 2 shows the theoretical recovery of the compounds calculated by means of equation 3.

Table 2.
Log$K_{O/W}$ and theoretical recovery of phenolic xenoestrogens

Compound	Log$K_{O/W}$[a]	Theoretical recovery, %	
		Sample volume (phase ratio)	
		10 ml (β = 159)	25 ml (β = 398)
2,4 – DCP	2.80	79.9	61.3
2,4 – DCP acetate	2.88	82.7	65.6
2,4,6 – TrCP	3.45	94.7	87.6
2,4,6 – TrCP acetate	3.52	95.4	89.3
2,3,4,6 – TeCP	4.09	98.7	96.9
2,3,4,6 – TeCP acetate	4.17	98.9	97.4
PCP	4.74	99.7	99.3
PCP acetate	4.81	99.8	99.4

[a] the log$K_{O/W}$ values as calculated from "SRC $K_{O/W}$ WIN"

The theoretical recovery increases with increasing log$K_{O/W}$ of the compound, therefore, the separation of chlorophenols from water in the form of acyl derivatives is justified. Except for 2, 4 – DCP, no considerable differences in recovery for the extraction of 10 ml and 25 ml water samples were found. The volume of a sample accepted in the assays was 25 ml.

3. RESULTS AND DISCUSSION

3.1. Derivatization

The mass spectra of the acyl derivatives of the chlorophenols were recorded over a mass range of 40-350 g/mol and shown in Fig. 2.

The main peaks of 2,4 – DCP, 2,4,6 – TrCP, 2,3,4,6 – TeCP and PCP acyl derivatives were determined for m/z 162, 196, 232 and 266. Due to their intensive signals, those ions can be used to identify compounds in a mixture and selected ions monitoring (SIM) in a GC – MS qualitative analysis.

3.2. Validation of the method

The research was preceded by a selection of organic solvent to desorb chlorophenols from the sorptive material. Three solvents were of interest: dichloromethane, acetonitrile and ethyl acetate. Fig. 3 reveals that ethyl acetate is the best solvent to desorb all the chlorophenols tested, hence its selection for the research. Various periods of desorption time (5-30 min.) were tested and the amount of analytes extracted did not increase at times higher than 15 min. so this desorption time was selected for further experiments.

The need to find the optimum extraction time resulted in the tests on the extract obtained after the sorptive element was exposed to a deionized water sample (25 ml) for 30, 60, 90, 120, 150, 180 and 210 minutes. The concentration of the phenols was 1000 ng/ml. Equilibrium was noticed after the extraction time of 120 min. (Fig. 4) which was selected for the tests.

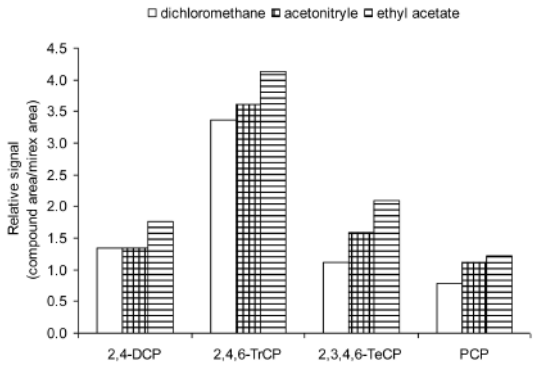

Figure 3.
Comparison of different desorption solvents (c = 1000 ng/ml, V_p = 25 ml, extraction 120 min. and 300 r.p.m., desorption time 15 min.)

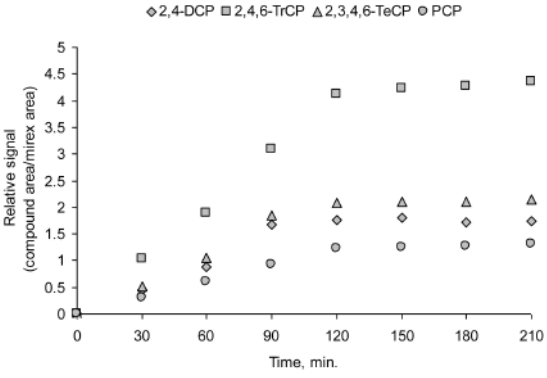

Figure 4.
Extraction time profil

The detection limit of SBSE – LD – GC/MS technique for 2, 4- DCP and PCP reached 50 ng/ml, and 10 ng/ml for 2, 4, 6 – TrCP and 2, 3, 4, 6 – TeCP. The limit of quantification was 150 ng/ml for 2,4 – DCP and PCP and 30 ng/ml for 2,4,6 – TrCP and 2,3,4,6 – TeCP. The linear ranges for the responses of the mass detector were found to be 100-2000 ng/ml for 2,4 – DCP, 20-2000 ng/ml for 2,4,6 – TrCP and 2,3,4,6 – TeCP, and 100-4000 ng/ml for PCP. The linear correlation coefficient (R^2) was higher than 0.972. The validation results of chlorophenol determination technique are collated in Table 3.

Table 3.
Validation of SBSE with in situ derivatization and LD – GC/MS technique

Compound	LOD[a] (ng/ml)	LOQ[b] (ng/ml)	Correlation coefficient (R^2)[c]
2,4 – DCP	50	150	0.972 (100 – 2000)
2,4,6 – TrCP	10	30	0.998 (20 – 2000)
2,3,4,6 – TeCP	10	30	0.997 (20 – 2000)
PCP	50	150	0.999 (100 – 4000)

[a] Limit of detection (S/N = 3); [b] Limit of quantification (S/N > 10); [c] values in parentheses are the linear ranges of the calibration curves (ng/ml)

The recovery and precision of quantitative assays of chlorophenols for tap and surface waters, concentrations of the compounds of interest being 100, 400 and 1000 ng/ml, are shown in Table 4. The water samples were analyzed repeating each assay six times and using standard curves prepared for calculations. Originally, the waters tested did not contain chlorophenols. The recovery of the compounds of interest reached more than 63% for chlorophenol concentration of 10 ng/ml and more than 72% for the concentrations of 400 and 1000 ng/ml. The precision of the quantitative assays expressed as the mean standard deviation was less than 17% for the concentration of 100 ng/ml and less than 10% for the concentration of 300 and 1000 ng/ml.

Table 4.
Recoveries of chlorophenols in spiked tap water and surface water

Compound	Sample	Amount spiked (ng/ml)					
		100		400		1000	
		Recovery (%)[a]	R.S.D. (%)[a]	Recovery (%)[a]	R.S.D. (%)[a]	Recovery (%)[a]	R.S.D. (%)[a]
2,4 – DCP	Tap water	63	12	4	6.3	81	9.6
	Surface water	93	9.4	80	5.3	79	4.5
2,4,6 – TrCP	Tap water	73	15	85	6.0	86	4.9
	Surface water	81	15	77	4.7	87	4.1
2,3,4,6 – TeCP	Tap water	67	14	81	8.8	80	8.7
	Surface water	69	17	104	5.4	94	3.7
PCP	Tap water	65	4.8	72	5.3	72	5.5
	Surface water	86	8.6	85	4.8	72	9.0

[a] The recoveries and precision were also examined by replicate analysis (n = 6)

4. CONCLUSIONS

The determinations of chlorophenols in water samples using SBSE with in situ derivatization followed by LD – GC/MS was investigated. Advantages of the new extraction method over established enrichment techniques such as liquid-liquid and solid phase extraction are the small amounts of organic solvents required and the low sample consumption.

The derivatization of the compounds in water proposed herein – as an essential step of the chromatographic preparation – does not interfere with the determination step as by – products of derivatization stay in the water environment.

The SBSE and LD – GC/MS technique enables the quantitative determination of chlorophenols in waters in the range of 10-50 ng/ml. The assays carried out for two types of water that contained 100, 400 and 1000 ng/ml of the compounds tested were characterized by a satisfactory precision from 3.7 to 17%. Thus, the above mentioned techniques may be used to monitor the occurrence of chlorophenols in water and during their removal by membrane filtration.

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