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Solid-phase microextraction–gas chromatography–mass spectrometry for the analysis of selective serotonin reuptake inhibitors in environmental water☆

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Abstract

The continuous contamination of surface waters by pharmaceuticals is of most environmental concern. Selective serotonin reuptake inhibitors (SSRIs) are drugs currently prescribed for the treatment of depressions and other psychiatric disorders and then, they are among the pharmaceuticals that can occur in environmental waters. Solid-phase microextraction (SPME) coupled to gas chromatography–mass spectrometry has been applied to the extraction of five SSRIs—venlafaxine, fluvoxamine, fluoxetine, citalopram and sertraline—from water samples. Some of the analytes were not efficiently extracted as underivatized compounds and so, an in situ acetylation step was introduced in the sample preparation procedure. Different parameters affecting extraction efficiency such as extraction mode, fiber coating and temperature were studied. A mixed-level fractional factorial design was also performed to simultaneously study the influence of other five experimental factors. Finally, a method based on direct SPME at 100 °C using polydimethylsiloxane–divinylbenzene fibers is proposed. The performance of the method was evaluated, showing good linearity and precision. The detection limits were in the sub-ng/mL level. Practical applicability was demonstrated through the analysis of real samples. Recoveries obtained for river water and wastewater samples were satisfactory in all cases. An important aspect of the proposed method is that no matrix effects were observed. Two of the target compounds, venlafaxine and citalopram, were detected and quantified in a sewage water sample.

Keywords: Water analysis; Environmental analysis; Solid-phase microextraction; Experimental design; Serotonin reuptake inhibitors

1. Introduction

In recent years, the growing environmental presence of pharmaceuticals and personal care products (PPCPs) has attracted the attention of several authors [1–12]. Up to very recently, these compounds, which are included in products of a wide spectrum of therapeutic and consumer uses, have not been considered as pollutants and so, their long-term toxicity has not been fully evaluated. There are several reasons for the environmental concern of human pharmaceuticals. They are prescribed in enormous quantities and, although there are no data on their quantities, these compounds are continuously introduced in the environment via excretion. They are excreted without being metabolized, slightly transformed or as conjugates. Most of these residues are polar structures and they are not well retained onto solids in the process of sewage treatment. The consequence is the continuous contamination

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of the surface waters [9–11], and even groundwaters [12]. Whether pharmaceuticals, at the concentrations found in the environment, pose an important risk to humans or wildlife need still further investigation [3,6].

Selective serotonin reuptake inhibitors (SSRIs) are recently developed drugs indicated for the treatment of depressions and other psychiatric disorders. Although there are not enough data on the quantities of these compounds released in the environment, in the last few years there has been a dramatic increase in the prescription of antidepressants. Recently, Fong [13] has extensively studied the effects of SSRIs on aquatic life.

In consequence, SSRIs have the potential for long-term aquatic effects and then, they are now being considered as emerging environmental pollutants [6,14].

Fluvoxamine {5-methoxy-1-[4-(trifluoromethyl)phenyl]-*O*-(2-aminoethyl) oxime, (1*E*)-(9Cl) 1-pentanone}, fluoxetine {*N*-methyl-γ-[4-(trifluoromethyl)phenoxy]-(9Cl) benzenepropanamine)}, citalopram {1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-(9Cl) 5-isobenzofurancarbonitrile} and sertraline {4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-(1*S*,4*S*)-(9Cl) 1-naphthalenamine} are highly selective serotonin reuptake inhibitors and venlafaxine {1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl)]-(9Cl) cyclohexanol} is an antidepressant which inhibits both serotonin and norepinephrine reuptake.

Several analytical methods were developed for the analysis of these antidepressants in biological matrices, some of them dealing with high-performance liquid chromatography [15] and others with gas chromatography, in this last case using a mass spectrometer as detector [16]. The low concentrations of pharmaceuticals found in environmental samples require analytical methods with high sensitivity and selectivity [2,17]. Solid-phase microextraction (SPME) is a technique that was successfully applied to determine a wide range of drugs in biological and environmental samples [18–22], but there are no references on its application to determine SSRIs in environmental water samples.

In this paper, a rapid and sensitive method for quantification of five SSRIs (venlafaxine, fluvoxamine, fluoxetine, citalopram and sertraline) using solid-phase microextraction coupled to gas chromatography–mass spectrometry (GC–MS) is proposed. The optimization of the method is fully discussed and the validation parameters are presented. The optimized method has been applied to different real water samples (river water, and influent and effluent waters of sewage treatment plants), and results demonstrate that these compounds occur in urban sewage waters.

2. Experimental

2.1. Reagents and materials

Venlafaxine and citalopram were from Almirall Prodesfarma (Barcelona, Spain), fluvoxamine from Solvay-Pharma (Barcelona, Spain), fluoxetine from Dista (Madrid, Spain) and sertraline from Pfizer (Madrid, Spain). Acetic anhydride, acetone, and sodium chloride were supplied from Merck (Mollet del Vallés, Barcelona, Spain), sodium sulfate anhydrous was purchased from Scharlau (Barcelona, Spain), and potassium bicarbonate was obtained from Aldrich (Steinheim, Germany).

Commercially available 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane–divinylbenzene (PDMS–DVB), 85 μm polyacrylate (PA), 74 μm Carboxen–polydimethylsiloxane (CAR–PDMS) and 65 μm Carbowax–divinylbenzene (CW–DVB) fibers housed in manual SPME holders were obtained from Supelco (Bellefonte, PA, USA).

Different real water samples were analyzed: a river water; the influent and effluent from a sewage treatment plant (STP), corresponding to a population of approximately 100 000 inhabitants located in Galicia (NW Spain); and the influent waters from two STP located in Catalonia (NE Spain), one for a population of approximately 10 000 inhabitants, and the other for a population of 120 000.

2.2. Gas chromatography–mass spectrometry

Analyses were carried out on a Varian 3400 GC, equipped with a split/splitless injector, coupled to a Varian Saturn 3 ion trap mass spectrometer (Varian Chromatography Systems, Walnut Creek, CA, USA). Experimental parameters were as follows: column, CP-SIL 8 CB 30 m, 0.25 mm i.d., 0.25 μm film; temperature program, 60 °C for 2 min, heated to 250 °C at 25 °C/min, heated to 280 °C at 10 °C/min, and finally heated to 292 °C at 1.5 °C/min (total analysis time, 20.6 min). Helium was employed as carrier gas at an initial head column pressure of 8 psi. Injector was programmed to return to the split mode after 2 min from the beginning of a run. Injector temperature was held constant at 270 °C. Trap and transfer line temperatures were 220 and 292 °C, respectively. The mass spectrometer was used in the positive electron impact mode at 70 eV with automatic gain control. A mass range of *m*/*z* 43–420 was scanned, and the detector was turned off for the first 11 min of the run.

2.3. Extraction procedure

Water samples previously filtered through Millipore (Madrid, Spain) glass fiber filters, were placed in 22-mL headspace vials. To improve the extraction of some of the target compounds, a derivatization process was carried out. A detailed description has been described elsewhere [23,24]. After addition of sodium chloride and the reagents required for the acetylation process (potassium hydrogen carbonate and acetic anhydride), the vial was sealed with an aluminum cap and a Teflon-faced septum. In the experiments run at 50 and 100 °C, the vial was immersed in a water bath and let to equilibrate for 5–15 min before SPME. The fiber was exposed to the sample magnetically stirred during 30 min. The fiber

was then immediately inserted into the GC injection port and analysis was carried out. Desorption time was set at 3 min.

3. Results and discussion

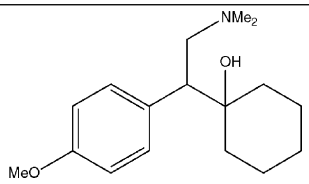
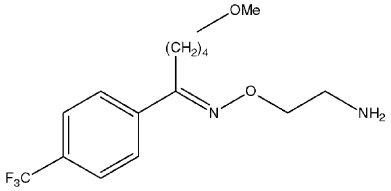
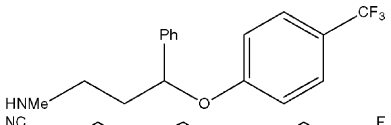
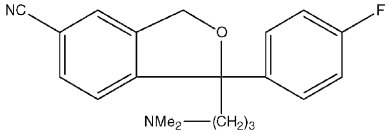
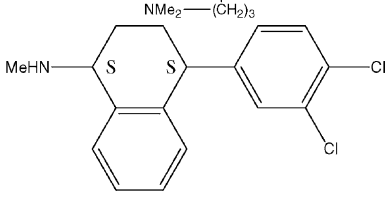
3.1. Development of a derivatization–SPME GC–MS method

Initial experiments were conducted to optimize the chromatographic temperature program and, thus, to achieve an adequate resolution of venlafaxine, fluvoxamine, fluoxetine, citalopram and sertraline. As it will be shown later in this paper, three of the analytes—fluvoxamine, fluoxetine and sertraline—will be determined as their corresponding acetyl derivatives after the introduction of an in situ derivatization step. The final chromatographic conditions selected as well as the mass spectrometry parameters and the quantification and identification ions for each compound are summarized in Table 1.

SPME is a relatively new extraction technique that has not been previously applied to the analysis of the target analytes. Therefore, experiments were performed in order to study the possibility of carrying out SPME for the analysis of the five target compounds. Aliquots of 10 mL of water spiked at the ng/mL level were magnetically stirred and SPME was performed using a PDMS–DVB fiber. The extraction time was 30 min. In these experiments, the sample mode [direct extraction SPME and headspace mode (HS)-SPME], the introduction of an in situ acetylation step to transform the analytes in less polar compounds, and the extraction temperature (25 and 100 °C) were studied. When the samples were not derivatized, only three of the target analytes (venlafaxine, citalopram and sertraline) could be identified by SPME–GC–MS analysis. On the other hand, when a derivatization step was introduced by adding acetic anhydride and sodium bicarbonate to the samples, all the compounds were adequately determined; three of them—fluvoxamine, fluoxetine and sertraline—as their acetyl derivatives. In addition, the responses obtained for all the target analytes at 25 °C were considerably lower than the responses obtained at 100 °C. In fact, one of them, venlafaxine, could not be detected at 25 °C. These results are shown in Table 2. The influence of sampling mode in the response achieved for the target analytes was also studied. Most of the analytes were not even detected demonstrating that HS-SPME is not suitable for this group of compounds (see also Table 2).

The performance of five different coatings, PDMS, PDMS–DVB, CW–DVB, PA and CAR–PDMS, for the extraction of SSRIs was also studied and compared. Three of the fibers CAR–PDMS, PDMS and PA had responses considerably lower than the other two CW–DVB and PDMS–DVB;

Table 1
Retention times and selected ions for the analysis of the target compounds

	Retention time (min)	Identification ions (*m*/*z*)	Quantification ions (*m*/*z*)	
Venlafaxine	10.69	58, 134, 278	58	
Fluvoxamine[a]	11.32	60, 86, 102, 361	102	
Fluoxetine[a]	11.48	44, 86, 117, 190, 352	44	
Citalopram	12.43	58, 238, 324	58	
Sertraline[a]	15.64	239, 290, 274, 347	274 + 290	

[a] Compounds determined as acetyl derivatives.

Table 2
Influence of temperature and extraction mode on the response, expressed as a percentage of the maximum (obtained by direct sampling at 100 °C)

	Venlafaxine	Fluvoxamine	Fluoxetine	Citalopram	Sertraline
HS–SPME (25 °C)	–	–	–	–	–
HS–SPME (100 °C)	–	–	–	2	0.4
SPME (25 °C)	–	66	28	3	22
SPME (100 °C)	100	100	100	100	100

Table 3
Factor levels considered for the mixed-level fractional design and optimal values found after experimentation

Key	Factor	Level		
		Low (−)	High (+)	Optimum
A	Salt addition (%)	0	30	0–15
B	Extraction temperature (°C)	50	100	100
C	Derivatization reagent (μL)	40	80	40
D	Thermostatization time (min)	5	15	5
E	Fiber	PDMS–DVB	CW–DVB	PDMS–DVB

so, only these two last fibers were included in the factorial design that we go on to describe.

A factorial design was performed with the purpose of selecting the best extraction conditions affecting the derivatization–SPME process. For this study, a spiked water sample with individual SSRI concentrations of 10 ng/mL was employed. The extraction time was 30 min. The following five experimental factors were studied: addition of salt, extraction temperature, amount of derivatization reagent, thermostatization time before SPME and fiber coating.

We chose a mixed-level fraction $3 \times 2^{4-2}$ type IV resolution design, which involved 12 runs [25]. All parameters were studied at two levels with the exception of the amount of salt added, which was studied at three levels. Table 3 lists the upper and lower levels assigned to each factor and Table 4 shows the design matrix.

An analysis of the results obtained, after running the 12 experiments, produced the standardized first-order Pareto charts shown in Fig. 1. Fig. 2 shows the main effect plots for some of the compounds (fluoxetine, citalopram and sertraline). As can be seen, temperature was the most important factor for almost all the analytes and it was significant for all of them. This factor has a positive effect on the response obtained as can be seen in Fig. 2 where the slope of the line corresponding to this factor is positive. This means that SPME improves when the temperature increases from 50 to 100 °C. Also, the kind of fiber was a significant factor for the extraction of the SSRIs and all the analytes were better extracted when PDMS–DVB was used. This fiber had arbitrarily been assigned to the low level of the factor fiber and, therefore, the slope of the corresponding line in the main effects charts is negative (higher

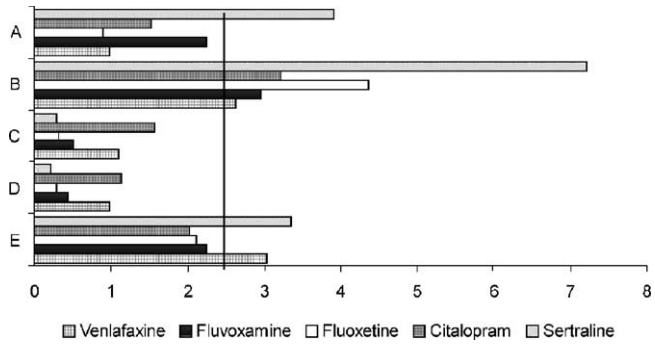


Fig. 1. Standardized first-order Pareto charts. Vertical line indicates the statistical significance bound for the effects. See Table 3 for factor keys.

Table 4
Matrix of the mixed-level fractional design

Experiment	NaCl (%)	Extraction temperature (°C)	Derivatization reagent (μL)	Thermostatization time (min)	Fiber coating
1	0	50	40	5	PDMS–DVB
2	0	50	80	15	PDMS–DVB
3	0	100	40	5	CW–DVB
4	0	100	80	15	CW–DVB
5	15	50	40	15	CW–DVB
6	15	50	80	5	CW–DVB
7	15	100	40	15	PDMS–DVB
8	15	100	80	5	PDMS–DVB
9	30	50	40	5	PDMS–DVB
10	30	50	80	15	PDMS–DVB
11	30	100	40	5	CW–DVB
12	30	100	80	15	CW–DVB

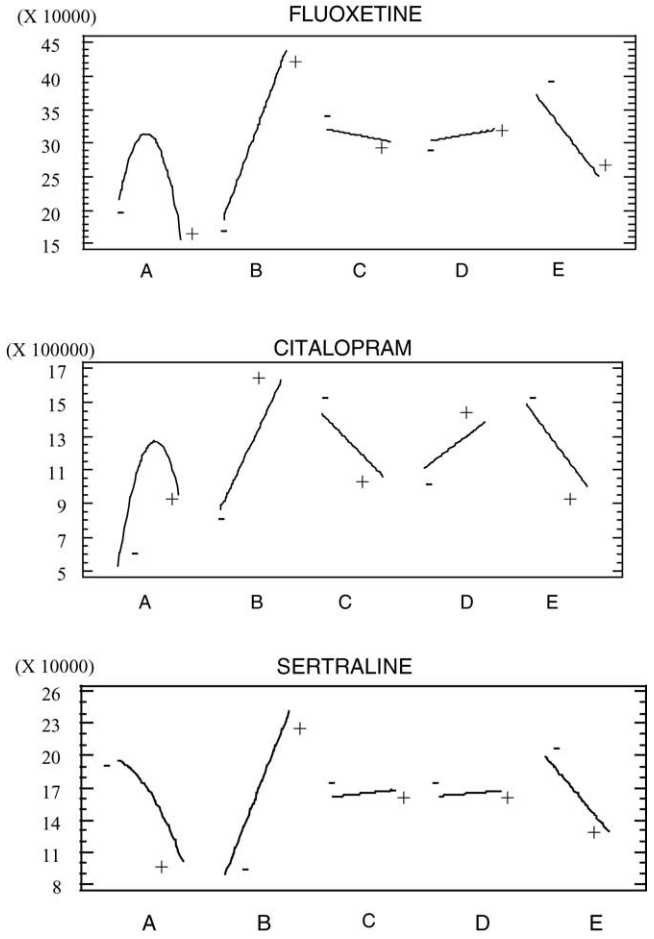


Fig. 2. Main effect plots for selected compounds (fluoxetine, citalopram and sertraline). See Table 3 for factor keys.

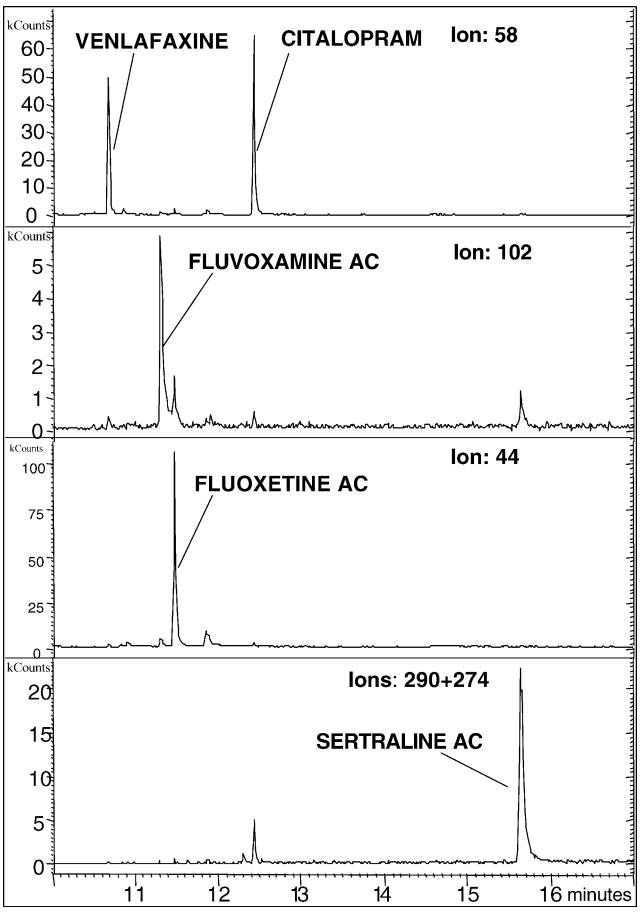


Fig. 3. SPME–GC–MS selected ion chromatograms for a spiked water sample containing 5 ng/mL of each analyte.

responses for the low factor level). Regarding the lifetime of these two coatings, PDMS–DVB showed to be more stable than CW–DVB, maintaining its performance above 50 extractions in most cases.

The amount of NaCl is initially only significant for sertraline when the first-order Pareto charts are considered (Fig. 1), that is, when the low and the high level of this factor are compared. However, this factor was studied at three levels and as can be seen in the main effects graphs, the corresponding line to this factor is curved, which indicates that its optimal level lies between 0 and 30% of NaCl for all compounds excluding sertraline. The optimal value given for the experimental design is about 15% for venlafaxine, fluvoxamine, fluoxetine and citalopram. On the other hand, the addition of any amount of NaCl has a negative effect on response for sertraline.

Finally, as can be seen in the Pareto charts as well as in the main effects charts (Figs. 1 and 2) the other two factors studied, the volume of derivatization reagent and the thermostatization time before SPME are not significant. The slope in the main effect charts for the amount of derivatization reagent was negative and the low level of this factor (40 μL) was therefore chosen as optimum, although the level of this factor does not significantly affect the extraction of the target analytes. The thermostatization time was also not significant and, thus, this factor was set at 5 min for next studies.

The last column of Table 3 summarizes the optimal conditions for the SPME of SSRIs. These optimal values were the same for all the compounds with the exception of percentage of NaCl, as has been mentioned previously. Thus, in later studies, the amount of NaCl was set at 15%, which does not favor the extraction of sertraline but benefits the extraction of the other compounds.

Fig. 3 shows the selected ion chromatograms obtained for a spiked water sample (5 ng/mL of each analyte) extracted using the optimal conditions.

3.2. Performance study and application of the SPME method

To evaluate the linearity of the SPME method, a calibration study was performed using the optimal conditions indicated earlier. The concentration ranged from 0.1 to 10 ng/mL. The method exhibited a directly proportional relationship between the extracted amount of each SSRI and its initial concentration in the sample. The correlation coefficients (R^2) were 0.993–0.999 for the target analytes (see Table 5).

Table 5
Linearity, limit of detection and repeatability of the proposed method

	Correlation coefficient (R^2)	Detection limit (ng/mL)	Repeatability (R.S.D., %)	
			10 ng/mL	1 ng/mL
Venlafaxine	0.995	0.027	5.6	1.9
Fluvoxamine	0.994	0.075	3.6	14.7
Fluoxetine	0.999	0.017	3.0	9.6
Citalopram	0.994	0.015	11.1	17.0
Sertraline	0.993	0.017	9.8	13.2

The precision of the experimental procedure was also evaluated at two concentration levels: 1 and 10 ng/mL. The results, expressed as relative standard deviations (R.S.D.s), are shown in Table 5. Detection limits (S/N = 3) are under 0.1 ng/mL for all the target analytes (see Table 5).

The recoveries of the method as well as the matrix effects were evaluated using three real samples: river water, and effluent wastewater and influent wastewater samples from an urban water treatment plant. These samples did not show initial detectable concentration of SSRIs making them suitable for recovery studies. The river water sample and the effluent wastewater were spiked with the target analytes to obtain the concentrations summarized in Table 6. Direct comparison of the responses obtained for the same spike levels in Milli-Q water allowed to confirm the absence of matrix effects in the studied samples. Then, the samples were quantified using external standard calibration. Recoveries obtained for each sample are given in this table. As can be seen, recoveries were satisfactory for the target compounds in both samples studied.

The influent wastewater sample was contaminated with 0.02% of urine from two patients treated with fluoxetine and citalopram, respectively. In addition, Milli-Q water was also contaminated with the same urine samples. The results obtained for these samples are compared in Table 7 where the absence of matrix effects for the influent wastewater sample can be seen.

Table 6
Extraction recoveries (%) in real water samples from Galicia (NW Spain)

	Recovery (%)	
	River	Effluent
Venlafaxine	94 ± 4	120 ± 16
Fluvoxamine	105 ± 4	102 ± 1
Fluoxetine	100 ± 1	103 ± 3
Citalopram	105 ± 4	102 ± 1
Sertraline	88 ± 8	98 ± 1

River and effluent waters were spiked with 0.25 and 1 ng/mL, respectively. See text for more details.

Table 7
Comparison of the responses obtained (response ± S.D.) for an influent sewage water sample and Milli-Q water, both spiked with identical amounts of urine from two patients treated with fluoxetine and citalopram, respectively

	Fluoxetine	Citalopram
Influent water	77672 ± 1980	371334 ± 60036
Milli-Q water	73354 ± 5349	407423 ± 27610

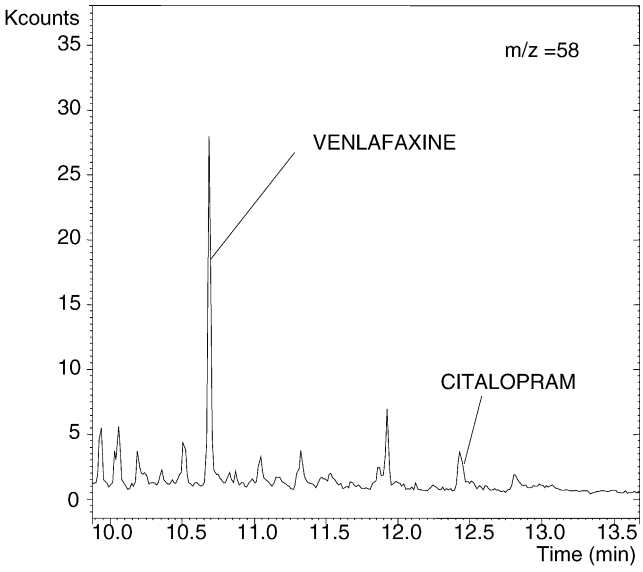


Fig. 4. SPME–GC–MS selected ion chromatogram (*m*/*z* 58) of a real contaminated sewage water.

The method was also applied to other two sewage water samples from Catalonia. In one of the samples obtained from a STP corresponding to a little population, two of the target analytes, venlafaxine and citalopram, were detected and quantified. The concentrations found were 2.01 ± 0.05 and 0.34 ± 0.04 ng/mL, respectively. Fig. 4 shows a chromatogram obtained for this sample.

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