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Determination of benzene, toluene, ethylbenzene and xylenes in soils by multiple headspace solid-phase microextraction

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Abstract

Multiple headspace-solid phase microextraction (MHS-SPME) is a recently developed technique for the quantification of analytes in solid samples that avoids the matrix effect. This method implies several consecutive extractions from the same sample. In this way, the total area corresponding to complete extraction can be directly calculated as the sum of the areas of each individual extraction when the extraction is exhaustive, or through a mathematical equation when it is not exhaustive. In this paper, the quantitative determination of benzene, toluene, ethylbenzene and xylene isomers (BTEX) in a certified soil (RTC-CRM304, LGC Promochem) and in a contaminated soil by multiple HS-SPME coupled to a gas chromatography-flame ionisation detector (GC-FID) is presented. BTEX extraction was carried out using soil suspensions in water at 30 °C with a 75 μm carboxen-polydimethylsiloxane (CAR-PDMS) fibre and calibration was carried out using aqueous BTEX solutions at 30 °C for 30 min with the same fibre. BTEX concentration was calculated by interpolating the total peak area found for the soils in the calibration graphs obtained from aqueous solutions. The toluene, ethylbenzene, *o*-xylene and *m*,*p*-xylene concentrations obtained were statistically equal to the certified values.

Keywords: Soil; Multiple headspace-solid phase microextraction; Benzene; Toluene; Ethylbenzene; Xylene

1. Introduction

Soils can be easily contaminated by organic pollutants as consequence of uncontrolled spills, accidents, industrial wastes or the abuse of pesticides and herbicides. Contamination by benzene, toluene, ethylbenzene and xylene isomers (BTEX) is associated to petroleum products such as fuel-oil or gasoline, and human exposition to these compounds can have serious health consequences like neurological diseases or cancer.

Solid-phase microextraction (SPME) [1–4] is a rapid, selective, easily automated and solvent-free technique that simplifies the analysis of volatile and semivolatile compounds in environmental matrixes such as air [5], water [6] or soil [7–16]. SPME has been reported for the analysis of different volatile organic compounds (VOCs) in soils such as BTEX [7], chlorobenzenes [7], PAHs [7,8], chlorophenols [9], herbicides [10,11], pesticides [12,13], aromatic acids [14], or even chemical warfare agents (CWAs) such as sulfur mustard [15] or the nerve agent VX [16].

Calibration by SPME is usually carried out by external standard or standard addition in liquid samples. However, in complex samples such as soils it is really difficult to find the same kind of matrix, and the matrix effect appears. Calibration by direct spiking of soil samples with analytes has been reported [8,12,15,16], but the differences in the behaviour of the native analytes and the spiked analytes has not been considered, and this method requires ageing the soils for a long time [7] to remove these differences. Other reports describe the use of an extraction step previous to the analysis by SPME, such as extraction with pressurized solvents [9], solvent extraction [10], microwave extraction [11] or ultrasonic extraction [13].

In this paper, the quantitative determination of BTEX in soils is performed by multiple HS-SPME [4,17–19]. This is a method for the quantification of analytes in solid samples that avoids the matrix effect [20], reduces the manipulation time of the sample and avoids analyte losses by evaporation. This method implies several consecutive extractions from the same sample. In this way, the total area corresponding to the

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complete extraction of the analyte can be directly calculated as the sum of the areas of each individual extraction when the extraction is exhaustive, or using the following mathematical equation when it is not exhaustive:

$$A_T = \frac{A_1}{1-\beta}$$

where A_1 is the peak area in the first extraction and β is calculated from the linear regression of the logarithms of the individual peak areas [17]:

$$\ln A_i = (i-1)\ln\beta + \ln A_1$$

and where A_i is the peak area obtained in the ith extraction.

BTEX extraction in soils was carried out using soil suspensions in water. The parameters that affect the extraction by multiple HS-SPME such as the type of fibre, amount of soil, addition of water, temperature and extraction time were studied.

Since multiple HS-SPME provides a value of total area independent of the kind of matrix, the calibration was performed using a matrix different from the soil: aqueous BTEX solutions. Water was selected as solvent because this is the simplest system, it is compatible with the fibre coating, the fibre–water distribution constant (K_{fs}) for the target analytes is high and the extraction time is short [18]. After the optimisation of the SPME variables, the features of the method were established. Finally, the method was applied to the analysis of a certified and a contaminated soil. The BTEX concentrations found in the certified soil were statistically compared with reference values.

2. Experimental

2.1. Samples

Two different samples of soils were analysed, a certified soil: RTC-CRM304 (Laramie, Wyoming, USA) distributed by LGC Promochem (Barcelona, Spain), and a 2-month aged spiked soil.

2.2. Chemicals

The following chemicals were used to prepare stock solutions in methanol: benzene (≥99.9%), ethylbenzene (≥99.5%), toluene (≥99.9%), *o*-xylene (≥99.5%), *m*-xylene (≥99.5%), *p*-xylene (≥99.5%) from Supelco (Bellefonte, PA). Dilutions in water of 0.16–17 μg/ml (with 0.5% of methanol in all the solutions) were used for calibration. In order to reduce losses by evaporation, the aqueous BTEX solutions were stored at 4 °C in sealed vials without free headspace since BTEX migrate easily from the aqueous solution to the headspace, and were introduced in the vials just before the analysis. Moreover 3.0 mm thick septa were used for the caps in the analyses.

2.3. Instruments and materials

A Varian 3800 gas chromatograph (Walnut Creek, California, USA) with a Flame Ionisation Detector (FID) and a Combipal Autosampler (CTC Analytics), which allows automated HS-SPME injections, were used. The GC-FID was equipped with a WCOT fused silica column with a stationary phase CP-Select 624 CB (30 m × 0.25 mm i.d. with 1.4 μm phase) from Varian (Walnut Creek, California, USA).

2.4. Spiking procedure

A non-polluted sieved soil was dried in an oven at 120 °C for 3 days in order to remove any organic trace and humidity. Then, 2 ml of a BTEX solution in methanol were homogeneously added to 30 g of soil and the mixture was hermetically sealed. A BTEX solution containing 92.0, 87.0, 388.0, 159.5, 222.0 and 217.0 μg/ml of benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene and *p*-xylene, respectively, was used. The spiked soil was shaken using an orbital agitator for 2 days and aged at 4 °C for 2 months.

2.5. Sampling procedure

Suspensions of 15–20 mg of soil in 600 μl of ultrapure Milli-Q water were placed in 20 ml headspace glass vials sealed with steel caps with 3.0 mm thick Teflon/silicone septa. Before the extraction, the samples were incubated at 30 °C and agitated at 400 rpm for 10 min to help BTEX to migrate from the matrix to the gas phase. BTEX extraction by multiple HS-SPME was carried out at 30 °C, with a 75 μm carboxen-polydimethylsiloxane (CAR-PDMS) fibre in the headspace of the vial above the samples for 20 min in three consecutive extractions. Desorption time was 10 min.

Calibration was carried out in the same way using 25 μl of aqueous BTEX solutions. Extraction was performed at 30 °C with a 75 μm CAR-PDMS fibre for 30 min after 10 min of incubation at 30 °C and agitation at 400 rpm. The number of extractions ranged from 2 to 4 (until the complete extraction of the analytes).

2.6. Chromatographic conditions

The carrier gas was helium at 1.7 ml/min. The temperature of the detector was set at 300 °C with a make-up flow of helium at 25 ml/min, a H_2 flow of 30 ml/min and an air flow of 300 ml/min. The column oven temperature program began with an initial temperature of 35 °C for 5 min, and then temperature increased at a rate of 10 °C/min up to 225 °C, and finally this temperature was held for 1 min. The run time was 25 min. An insert of 0.8 mm was used, and the injector was maintained at 280 °C for the 75 μm CAR-PDMS fibre and at 250 °C for the 100 μm PDMS fibre, with splitless mode at initial time followed by a 1:50 split ratio at 0.5 min.

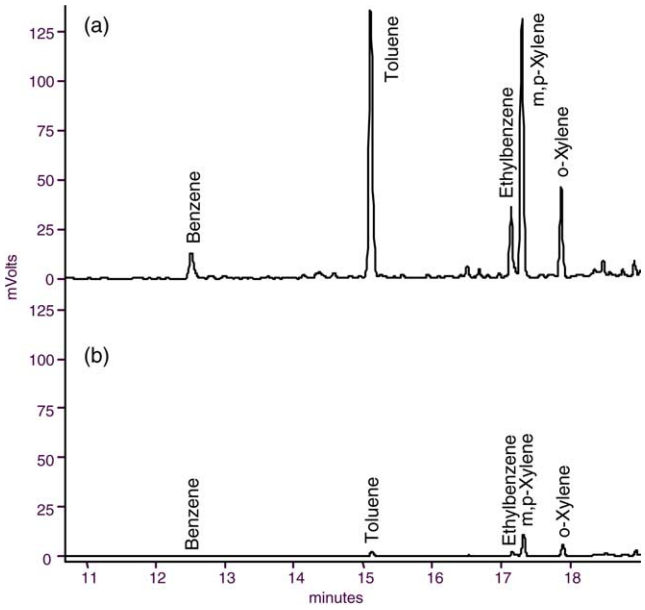

Fig. 1. Chromatograms obtained by HS-SPME for the BTEX determination in the certified soil at 30 °C using (a) a 75 μm CAR-PDMS fibre and (b) a 100 μm PDMS fibre.

3. Results and discussion

3.1. Selection of HS-SPME conditions in soils

3.1.1. Type of fibre

One hundred micrometer polydimethylsiloxane fibre has been reported [7] for the analysis of BTEX in soils, however, we selected a 75 μm CAR-PDMS fibre because it provided better sensitivity in spite of its shorter linear ranges for these compounds and the fact that in multiple HS-SPME the amount of extracted analyte must be large to observe variations in the peak area with the number of extractions. Fig. 1 shows the chromatograms obtained by HS-SPME for BTEX determination in the certified soil using a 75 μm CAR-PDMS fibre and a 100 μm PDMS fibre (HS-SPME conditions are described under Experimental). Higher chromatographic signals were obtained using the 75 μm CAR-PDMS fibre.

Table 1
Correlation coefficients (R^2) of $\ln A_i$ vs. $(i-1)$ found for BTEX using different masses of certified soil

Soil mass (mg)	Benzene	Toluene	Ethylbenzene	*m,p*-Xylene	*o*-Xylene
88.0	–	–	–	0.95	–
70.8	–	0.91	0.92	0.98	0.96
53.6	–	0.83	0.88	0.97	0.93
26.8	–	0.95	0.97	0.987	0.98
18.8	0.995	0.9998	0.9997	0.9997	0.9998
15.2	0.998	0.9995	0.9992	0.997	0.996
14.2	1	0.9999	0.9990	0.9992	1

(–): non linear.

3.1.2. Amount of soil

The mass of soil placed in the vial must be appropriate to observe an exponential decay of the peak area with the number of extractions. If the mass is too low, sensitivity problems (due to small chromatographic signals) and reproducibility problems (if the sample is not very homogeneous) can occur. If the mass is too large, bad correlation coefficients of the area logarithm versus the number of extraction are found and some analytes do not show an exponential decay of the peak area. Table 1 shows the correlation coefficients (R^2) of $\ln A_i$ versus $(i-1)$ found for BTEX using different masses of certified soil. 15–20 mg was the selected range of masses.

3.1.3. Temperature and addition of water

The addition of water to the soil sample causes higher extraction yields and a significant increase in the chromatographic signals [7]. Moreover, water displaces the analytes from the active sites in the soil, they are desorbed from the soil into the solvent for solvation, and then they migrate to the headspace. Table 2 shows the relative BTEX areas obtained by HS-SPME in the certified soil using different temperatures for soil in suspension, soil in suspension with salt-saturated water, and dry soil (the areas are related to the values obtained at 30 °C with soil in suspension and expressed as a percentage). An increase in temperature caused a decrease in the peak areas, and, as expected, better results were obtained when water was added to the soil sample although the addition of salt was unfavourable for solvation. Therefore, the selected conditions were suspensions of soils in 600 μl of water at 30 °C.

Table 2
Relative areas[a] obtained in the HS-SPME determination of BTEX in the certified soil using a 75 μm CAR-PDMS fibre at different temperatures for soil in suspension, soil in suspension with salt-saturated water, and dry soil

Compound	Soil in suspension			Soil in suspension with saturated water			Dry soil		
	30 °C	60 °C	90 °C	30 °C	60 °C	90 °C	30 °C	60 °C	90 °C
Benzene	100	61	17	70	46	24	63	48	34
Toluene	100	72	40	76	46	35	60	58	47
Ethylbenzene	100	66	34	78	52	42	57	65	59
m,p-Xylene	100	70	36	76	53	43	59	61	59
o-Xylene	100	74	41	77	56	50	63	69	66

[a] Mean of three replicates.

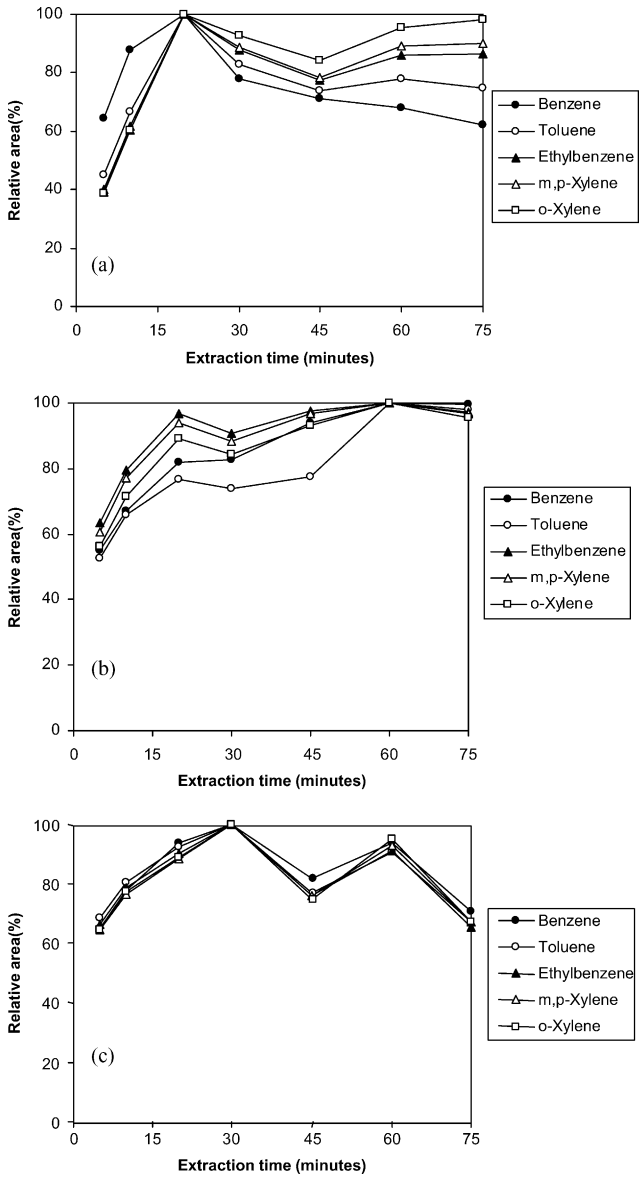

Fig. 2. Influence of the extraction time on the HS-SPME of BTEX at 30 °C using a 75 μm CAR-PDMS fibre for (a) a certified soil for (b) a contaminated soil and for (c) an aqueous solution. See the text for the HS-SPME, GC-FID conditions and BTEX concentrations in the aqueous solution.

3.1.4. Extraction time

Extraction time was the last SPME variable studied in the soils. Fig. 2 shows the extraction curves obtained for BTEX in the certified soil and the contaminated soil. A maximum was observed at 20 min, but equilibrium was reached at 60 min.

The theory of multiple HS-SPME [17] presumes sampling under equilibrium conditions, however, sampling under non-equilibrium conditions is possible provided that the waiting time and temperature between the extractions are also kept constant. In order to check that the total peak area values obtained at 20 min were the same that the ones obtained at 75 min, statistical tests were applied. Table 3 shows the total peak areas of BTEX obtained by multiple HS-SPME at 20 and 75 min. Total peak areas were calculated by the linear regression of the logarithms of the individual areas of three consecutive 20 min extractions, and as sum of the areas of four consecutive 75 min extractions.

Table 3
Total peak area[a] per milligram of soil found by multiple HS-SPME for 20 and 75 min, and statistical parameters F_0 and t_0

Compound	20 min	75 min	Statistical parameters	
	$\bar{x}_1 \pm s_1$ (mV s/mg) ($\times 10^3$)	$\bar{x}_2 \pm s_2$ (mV s/mg) ($\times 10^3$)	F_0	t_0
Benzene	13 ± 3	12 ± 5	3.44	0.42
Toluene	32 ± 5	28 ± 7	2.13	0.86
Ethylbenzene	6.5 ± 0.8	6.49 ± 0.12	49.60	0.04
m,p-Xylene	26 ± 4	26 ± 3	1.60	0.01
o-Xylene	10.3 ± 1.5	10.0 ± 0.9	2.69	0.27

[a] Mean of three replicates.

The first test applied was a statistical test of homogeneity of variances. The variances are homogeneous ($s_1^2 = s_2^2$) when the calculated F_0 value is lower than the tabulated F_C value, and for $n_1 = 3$, $n_2 = 3$ and $\alpha_C = 0.05$, the F_C value is 39.00. Variances were homogeneous, except for ethylbenzene.

The second test was a statistical test for homogeneous samples to determine whether the mean values obtained by multiple HS-SPME with the different duration steps were the same ($\bar{x}_1 = \bar{x}_2$). The mean values are equal when the calculated t_0 value is lower than the tabulated t_C value, and for $n_1 = 3$, $n_2 = 3$ and $\alpha_C = 0.05$ the t_C value is 2.776.

The third test was a statistical test to compare two mean values in heterogeneous samples (for ethylbenzene). For $n_1 = 3$, $n_2 = 3$ and $\alpha_C = 0.05$, the t_C value is 4.303.

Table 3 shows the calculated F_0 and t_0 values. The BTEX total peak areas obtained using 20 and 75 min were statistically equal ($t_0 < t_C$). Therefore, multiple HS-SPME can be performed by non-equilibrium steps in order to reduce the analysis time.

Fig. 3 shows the HS-SPME-GC-FID chromatograms obtained for three consecutive 20 min extractions from the certified soil.

3.2. Standard solutions

Water was selected to prepare calibration solutions of BTEX from the stock standard solutions in methanol. The influence of the extraction time was also studied for aqueous BTEX standard solutions. Extraction times ranged from 1 to 75 min, the concentration of BTEX ranged from 2.6 μg/ml for benzene and ethylbenzene to 6.9 μg/ml for toluene, and three replicates were performed. The variation of the peak areas versus the extraction time for 25 μl of an aqueous BTEX solution is shown in Fig. 2. A value of 100 was assigned to the maximum peak area for each compound and the rest of the areas were correlated to this value. An extraction time of 30 min was selected as extraction time for calibration.

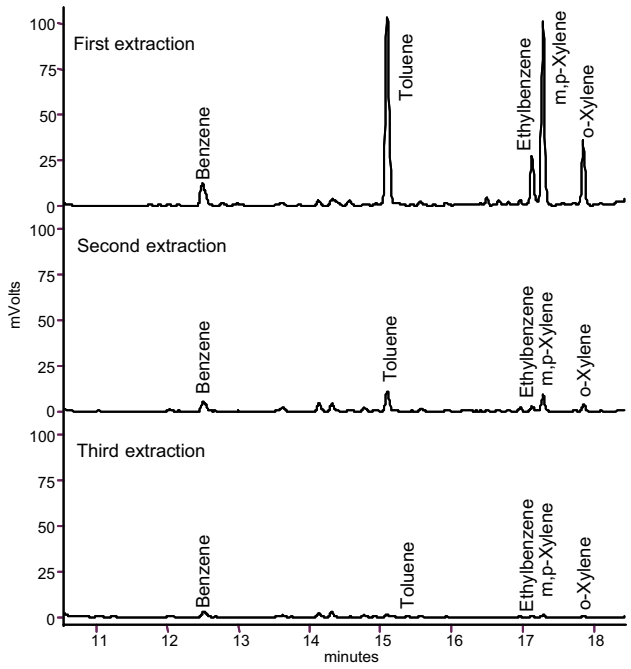

Fig. 3. Chromatograms of three consecutive HS-SPME extractions of BTEX from the certified soil.

3.3. Features of the method

The linearity study of the total peak area versus the BTEX mass was performed with a 75 μm carboxen-polydimethylsiloxane fibre at 30 °C for 30 min using 25 μl of aqueous BTEX standard solutions. The number of HS-SPME extractions ranged from 2 (for the most diluted solution) to 4 (for the most concentrated one). In this way, all the analytes were completely extracted from the vial and the total peak area was calculated as the sum of the individual peak areas.

The ranges of the BTEX masses studied, the linear ranges, the limits of detection (LOD), the slope and intercept with their standard deviations, the correlation coefficients (R^2), and the relative standard deviation obtained can be found in Table 4. The total area was linear (R^2 between 0.994 and 0.996) in the studied range (0–160 ng for benzene and ethylbenzene, 0–210 ng for xylenes, and 0–416 ng for toluene) and reproducibility was 3–7% expressed as a relative standard deviation. For soil samples, this value was around 15% (calculated from Table 3).

Table 5
Analysis of the reference soil (RTC-CRM304) by MHS-SPME-GC-FID. Results of the *F*- and *t*-tests

Compound	Concentration ± S.D. (mg analyte/kg soil)		*F*-test	*t*-test
	Certified values ($n = 26$)	Found values ($n = 6$)	F_0	t_0
Benzene	4.58 ± 1.36	3.2 ± 1.0	1.77	2.34
Toluene	19.3 ± 3.26	18 ± 6	3.22	0.70
Ethylbenzene	5.02 ± 0.85	5.6 ± 1.5	3.03	1.33
m,p-Xylene	21.8 ± 4.13	23 ± 6	2.33	0.43
o-Xylene	7.51 ± 1.01	8.0 ± 2.4	5.39	0.80

3.4. Validation and application of the method

BTEX concentration in soil samples was calculated by interpolating the total peak area obtained for the soils in the calibration graphs shown in Table 4. The accuracy of the MHS-SPME-GC-FID method was checked by analysing a certified reference soil containing BTEX. Table 5 shows the BTEX concentrations found by MHS-SPME and the certified concentration values with their standard deviations.

F- and *t*-tests were applied to verify that the proposed method gave the same BTEX concentration than the reference values. The results of the statistical tests are shown in Table 5.

First, a *F*-test of variance homogeneity was applied. For $n = 6$ (MHS-SPME), $n = 26$ (reference value) and $\alpha_C = 0.05$, the F_C value is 6.27, except for benzene, with a F_C value of 3.13. All the calculated F_0 values were lower than the F_C values, and thus it can be claimed that the variances are homogeneous.

Then, a *t*-test for homogeneous samples was applied to compare the mean values obtained by MHS-SPME with the reference values. For $n = 6$, $n = 26$ and $\alpha_C = 0.05$, the t_C value is 2.042, whereas for $\alpha_C = 0.02$, the t_C value is 2.457. Table 5 shows the calculated F_0 and t_0 values. Toluene, ethylbenzene, *o*-xylene and *m,p*-xylene concentrations were statistically equal to the certified ones ($t_0 < t_C$). The concentration obtained for benzene was lower than the certified one when a α_C value of 0.05 was used (however, the mean value was equal to the reference value when α_C was reduced to 0.02), probably due to evaporation losses in the soil on account of its high volatility. These results prove that multiple HS-SPME removes the matrix effect.

Table 4
Features of the MHS-SPME method

Compound	Studied range (ng)	Linear range (ng)	Slope ± s_m (mV s/ng)	Intercept ± s_b (mV s) ($\times 10^3$)	LOD (ng)	R^2	R.S.D.[a] (%) (mass level, ng)
Benzene	0–158	0.44–158	1585 ± 52	−7 ± 4	0.2	0.994	3.9 (66)
Toluene	0–416	1.25–416	894 ± 22	−5 ± 5	1.0	0.996	6.9 (260)
Ethylbenzene	0–161	0.36–161	636 ± 17	−2.5 ± 1.4	0.2	0.996	3.2 (67)
m,p-Xylene	0–420	1.83–420	600 ± 17	−7 ± 4	1.0	0.995	6.2 (260)
o-Xylene	0–211	0.90–211	590 ± 15	−2.7 ± 1.7	0.4	0.996	6.0 (132)

s_m: standard deviation of the slope. s_b: standard deviation of the intercept.
[a] Calculated from three replicates.

Table 6
BTEX concentrations in a spiked soil found by MHS-SPME-GC-FID

Compound	Concentration ± S.D. (mg analyte/kg soil)
Benzene	0.055 ± 0.011
Toluene	0.15 ± 0.04
Ethylbenzene	2.8 ± 0.3
m,*p*-Xylene	3.0 ± 0.3
o-Xylene	1.97 ± 0.18

Once the method had been validated, it was applied for determining the BTEX concentration in a spiked soil. The results are shown in Table 6.

4. Conclusions

Multiple HS-SPME is a suitable method to remove the matrix effect from BTEX determinations in contaminated soils. It is a simple and inexpensive alternative to other extraction techniques such as microwave assisted extraction, accelerated solvent extraction, etc. and avoids the use of organic solvents.

The total area results obtained under non-equilibrium conditions were statistically equal to the ones obtained under equilibrium conditions. Therefore, it was not necessary to reach equilibrium and the analysis time was significantly reduced.

BTEX concentration values in the certified soil were statistically equal to the reference ones (except for benzene). This fact proved that the matrix effect had been removed.

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