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Use of experimental design for the purge-and-trap-gas chromatography–mass spectrometry determination of methyl *tert.*-butyl ether, *tert.*-butyl alcohol and BTEX in groundwater at trace level

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Abstract

An efficient method for the simultaneous determination of methyl *tert.*-butyl ether, *tert.*-butyl alcohol, benzene, toluene, ethylbenzene and xylene isomers in groundwater by purge-and-trap-gas chromatography–mass spectrometry was developed and validated. Experimental design was used to investigate the effects of temperature of extraction, time of extraction and percentage of salt added to the water samples. Regression models and desirability functions were applied to find the experimental conditions providing the highest global extraction yield. Validation was carried out in terms of limits of detection (LOD), limits of quantitation (LOQ), linearity and precision. LOD values ranging from 2.6 to 23 ng l⁻¹ were achieved, whereas linearity was statistically verified over two orders of magnitude for each compound. Precision was evaluated testing two concentration levels. Good results were obtained both in terms of intra-day repeatability and intermediate precision: RSD% lower than 4.5% at the highest concentration and lower than 13% at the lowest one were calculated for intra-day repeatability. A groundwater sample suspected of contamination by leaking underground petroleum storage tanks was analysed and some of the analytes were detected and quantitated.

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1. Introduction

The environmental contamination of groundwater

by leaking underground petroleum storage tanks is an issue of renewed interest.

Oxygenate compounds such as methyl *tert.*-butyl ether (MTBE) and its degradation products, i.e., *tert.*-butyl alcohol (TBA) and *tert.*-butyl formate (TBF) [1–3] and volatile aromatic compounds such as benzene, toluene, ethylbenzene, *o*-, *m*-, *p*-xylene (BTEX) are well known fuel components commonly found in groundwater contamination [4,5].

Methyl *tert.*-butyl ether is one of the most widely

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used oxygenate compounds added to gasoline at up to 10% (v/v) not only as octane enhancer, but also to reduce hydrocarbon emissions and ozone levels according to the mandate of the Clean Air Act Amendments of 1990.

Unlike BTEX, MTBE is a persistent water contaminant owing to its solubility and its slow rate of degradation [6,7]: it is considered a possible human carcinogen by the US Environmental Protection Agency (USEPA) and values between 20 and 40 $\mu\text{g l}^{-1}$ represent the current EPA consumer acceptability threshold for drinking water [8]. Among BTEX, benzene is the most dangerous compound: being a recognised carcinogenic agent, it is a substance with a high research priority by international organisations and 1 $\mu\text{g l}^{-1}$ is the fixed maximum concentration level in drinking water.

According to various EPA methods, the analyses for MTBE, MTBE degradation products or BTEX in water require preconcentration of the analytes: this is usually achieved by purge-and-trap [9], but more recently direct aqueous injection techniques [10] and solid-phase microextraction [11,12] have been employed using gas chromatography with flame ionisation detector (FID) or gas chromatography–mass spectrometry. Currently, no EPA method for the simultaneous determination of MTBE, BTEX and MTBE degradation products has been validated.

The aim of this study was to develop and validate an efficient analytical method for the simultaneous determination of these analytes at trace levels in groundwater by purge-and-trap–gas chromatography–mass spectrometry (PTI-GC–MS). Experimental design was employed to study the effects of three parameters on the purge-and-trap extraction: temperature of extraction, time of extraction and percentage of sodium chloride added to the water samples (salting-out effect). The calculated regression models were used to estimate the responses (extraction recoveries) performing the search for the optimal extraction conditions (highest global PTI-GC–MS recovery) by the multicriteria decision method of the desirability functions.

The method was validated in terms of limits of detection, limits of quantitation, linearity and precision allowing the determination of all the analytes at ng l^{-1} levels in groundwater. The method developed was then applied to determine MTBE, BTEX and

MTBE degradation products in groundwater suspected of contamination by leaking underground petroleum storage tanks.

2. Experimental

2.1. Chemicals

Methyl *tert.*-butyl ether (99.7% purity) and *tert.*-butyl alcohol (99.3%) were purchased from Sigma–Aldrich (Milan, Italy); benzene, toluene, ethylbenzene, *o*-, *m*-, *p*-xylene, styrene, 1,4-dioxane methanol and sodium chloride (99.5% purity) were from Carlo Erba Reagents (Milan, Italy).

Standard and working solutions, prepared from the 1000 mg l^{-1} stock solution by dilution in methanol and groundwater, respectively, were stored in 12-ml completely filled vials at 4 °C until analysis.

Experimental design and method validation were carried out on groundwater samples taken from Savona (Italy).

2.2. Purge-and-trap extraction

A PTI 4560 Sample Concentrator (O.I. Analytical, TX, USA) on-line coupled with the GC–MS system and equipped with a multibed TENAX/SIGEL/CMS 4560 adsorbent trap (O.I. Analytical) was used to extract the analytes from 5-ml aqueous samples.

Helium was used as “purging” gas at a flow-rate of 30 ml min^{-1} ; the adsorbent trap was thermally desorbed at the temperature of 180 °C for 4 min.

2.3. GC–MS analysis

A HP 6890 Series Plus gas chromatograph (Agilent Technologies, Milan, Italy) equipped with MSD 5973 mass spectrometer (Agilent Technologies) was used for GC–MS analysis. Helium was used as the carrier gas at a flow-rate of 1.2 ml min^{-1} ; the gas chromatograph was operated in splitless mode with the PTV injector (Agilent Technologies) maintained at the temperature of 260 °C and equipped with a PTV multi-baffled liner (I.D. 1.5 mm, Agilent Technologies). Chromatographic separation was performed on a 60-m \times 0.25-mm, d_f 1.0 μm HP-1 capillary column (Agilent Technologies). The fol-

lowing GC oven temperature program was applied: 50 °C for 1 min, 10 °C min⁻¹ to 140 °C, 140 °C for 5 min, 10 °C min⁻¹ to 180 °C. Transfer line and source were maintained at the temperature of 280 and 150 °C, respectively. The mass spectrometer was operated in time scheduled single-ion monitoring mode (SIM) by recording the current of the following ions: *m/z* 59 ion for TBA until 5.5 min, *m/z* 73 for MTBE from 5.5 to 6.3 min, *m/z* 78 for benzene from 7 to 8.5 min, *m/z* 91 for toluene from 8.5 to 9.8 min, *m/z* 88 for 1,4-dioxane from 9.8 to 10.8 min, *m/z* 91 and *m/z* 106 for ethylbenzene, *o*-, *m*-, *p*-xylene, *m/z* 104 for styrene from 10.8 min. A dwell time of 100 ms was used for all the ions. Preliminarily, full scan EI data were acquired to determine appropriate masses for SIM under the following conditions: ionisation energy, 70 eV; mass range, 35–300 amu; scan time, 3 scan/s. All the analyses were performed setting the electron multiplier voltage at 1000 V.

Signal acquisition and elaboration were performed using the HP Chemstation (Agilent Technologies).

Photon correlation spectroscopy (PCS) was employed in order to determine aggregate dimension using a Kontron N4.MD instrument at a fixed angle of 90° and at a temperature of 25 °C. Each value was the average of 10 measurements.

2.4. Experimental design and optimisation procedure

The experiments were carried out on samples of blank groundwater samples (5 ml) spiked with 2 µg l⁻¹ of TBA and 1 µg l⁻¹ of MTBE and BTEX.

A 2³ two-levels full factorial design (FFD) was performed [13] to investigate the effects of temperature of extraction (*T*), time of extraction (*t*) and percentage of sodium chloride (NaCl) added to the water samples: low and high levels were *T*=30–60 °C, *t*=10–30 min, NaCl=0–25% (w/v). This experimental plan allows the evaluation of the effects of the main factors and of their interactions.

The order of experiments was randomised in order to avoid possible memory effect of the analytical apparatus.

An *F*-test comparing the experimental and calculated responses at the centre of the experimental

domain was performed to evaluate the existence of relevant quadratic effects [14]:

$$F_{\text{calc}} = \frac{(y_f - y_o)^2}{s_o^2(1/N_f + 1/N_o)}$$

where y_f is the mean of the estimated values obtained for the experiments of the FFD, i.e., the calculated response at the centre of the experimental domain; y_o is the mean of the replicated measurements at the centre of the experimental domain; N_f is the number of factorial experiments; N_o is the number of experiments at the centre of the experimental domain and s_o is the experimental error estimated through the replicated measurements at the centre point. A 95% confidence level was chosen.

A star design [14] was added to the factorial design experiments since some analytes showed relevant quadratic effects.

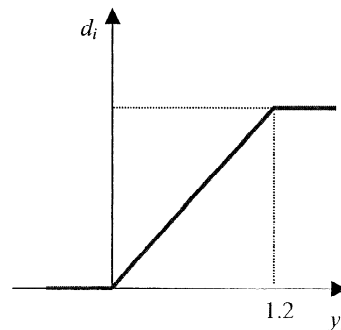
The final regression models were then calculated using the central composite design (CCD) experiments obtained both from the full factorial design and the star design. The best regression models were obtained by a forward search step-wise variable selection algorithm with a $F_{\text{to enter}} = 2$.

The optimal conditions were evaluated by the global desirability *D* [15]:

$$D = \left[\prod_{i=1, q} d_i \right]^{1/q}$$

where *q* is the number of responses included in the multicriteria optimisation and d_i is the desirability of the *i*th response defined as follows:

$$\begin{aligned} \text{if } y_i < 0 & \quad \text{then } d_i = 0 \\ \text{if } 0 < y_i < y_{i\text{MAX}}^* & \quad \text{then } d_i = y_i / (\max y_i + 20\% \max y_i) \\ \text{if } d_i > y_{i\text{MAX}}^* & \quad \text{then } d_i = 1 \end{aligned}$$



being y_i the value of the i th response estimated by the corresponding regression model.

Each desirability function d_i is maximum in the optimal conditions for the corresponding analyte. The maximum of D corresponds to the best possible compromise providing contemporary optimal values for each response.

The maximum of D was determined by a grid search algorithm [16], estimating the responses by mean of the regression models.

All statistical analyses were carried out by using the statistical package SPSS 10.0 for Windows (SPSS, Bologna, Italy).

2.5. Validation procedure

Validation process of the PTI-GC–MS method was performed according to EURACHEM guidelines [17,18].

Detection (y_D) and quantitation (y_Q) limits were expressed as signals based on the mean blank (\bar{x}_b) and the standard deviation of blank responses (s_b) as follows:

$$y_D = \bar{x}_b + 2t s_b \quad y_Q = \bar{x}_b + 10 s_b$$

where t is the constant of the t -Student distribution (one-tailed) depending on the confidence level and degrees of freedom (df). A 95% confidence level was chosen. \bar{x}_b and s_b were calculated performing 10 blank measurements.

The concentration values of the detection limit (LOD) and quantitation limit (LOQ) were obtained by projection of the corresponding signals y_D and y_Q through a calibration plot $y = f(x)$ onto the concentration axis.

Linearity was established over two orders of magnitude in the following ranges: 5–500 ng l⁻¹ for TBA, 6–600 ng l⁻¹ for MTBE, 12–1200 ng l⁻¹ for benzene, 27–2700 ng l⁻¹ for toluene, 40–4000 ng l⁻¹ for ethylbenzene, 6.5–650 ng l⁻¹ for *m*- and *p*-xylene, respectively, and 6–600 ng l⁻¹ for *o*-xylene.

Styrene was used as internal standard for BTEX quantitation at the concentration of 1 μg l⁻¹, whereas 1,4-dioxane at the concentration of 0.2 μg l⁻¹ was used for TBA and MTBE quantitation. Six con-

centration levels were analysed performing three measurements at each concentration level.

Homoscedasticity was verified by applying the Bartlett test; lack-of-fit and Mandel's [18] fitting test were also performed to check the goodness of fit and linearity. The significance of the intercept (significance level 5%) was established running a F -test.

Intra-day repeatability and intermediate precision [19] were calculated in terms of RSD (%) on two concentration levels for all the analytes, performing three replicates at each level. In addition, intermediate precision was estimated over 3 days verifying homoscedasticity of data and performing analysis of variance (ANOVA) at the confidence level of 95%.

3. Results and discussion

The aim of this work was to find the extraction conditions providing the highest PTI-GC–MS yields of MTBE, TBA and BTEX from groundwater and to validate the method for the simultaneous determination of all the analytes.

The optimisation of the extraction conditions was a critical step in the method development because of the different physical and chemical properties of the compounds analysed in this study.

3.1. Experimental design

A 2³ two-level factorial design was preliminarily used to evaluate the significance of the main and interaction effects of the parameters investigated. The experimental domain was defined taking into account instrumental and operative limits, namely: temperature values lower than 30 °C could not be maintained over long time; temperature values higher than 80 °C caused lower breakthrough volumes of the adsorbent material utilised to trap the analytes; extraction times greater than 60 min would determine long analysis times.

In order to evaluate the repeatability of the measurements over the time, four replicates at the centre of the experimental domain were added before and after performing the eight factorial design experiments. For each compound the main and interaction effects were calculated.

The salt effect was significant for all the analytes,

but, with the exception of TBA, it always showed a negative effect.

The presence of curvature was tested by the F -test described in Section 2. Only TBA and MTBE showed a significant curvature, F_{calc} values being 133.41 and 27.25, respectively ($F_{\text{tab}} = 10.1$), indicating that a quadratic model had to be used. Additional measurements corresponding to a star design were then added in order to investigate which variables were responsible for the quadratic effects.

The regression models (Table 1), calculated by step-wise regression analysis, were then used to depict the response surfaces (Fig. 1) and to search for the highest global PTI-GC-MS recovery within the explored domain. This was obtained using the grid search algorithm to explore the experimental domain, evaluating the goodness of the results by means of the desirability functions calculated on the basis of the responses predicted by the regression models. A global desirability $D = 0.63$ was calculated and the optimal extraction conditions (Fig. 2) were found in correspondence to an extraction temperature of 60 °C, an extraction time of 30 min and no NaCl added to the solutions. The obtained value of the global desirability showed that the optimal extraction conditions were good for all the compounds with the exception of TBA: in fact, this analyte showed a

single desirability value lower than those calculated for the other compounds ($d_{\text{TBA}} = 0.43$, whereas values for the other compounds ranged from 0.60 to 0.77). This behaviour could be explained taking into account the possibility of stronger interactions (hydrogen bonds) between this analyte and water, thus requiring different extraction conditions than those utilised for the other compounds.

As a general result, it has to be mentioned that the addition of NaCl to the water samples did not produce the expected salting-out effect, causing by contrast the maintenance of a higher concentration of the compounds in the aqueous phase [20,21]. As a consequence, extraction recoveries were reduced by the salt addition for all the analytes but TBA. As previously described, the possible formation of hydrogen bonds between TBA and water could explain the different behaviour: in this case, the addition of NaCl could produce the salting-out effect improving TBA extraction. In order to elucidate the global obtained negative salt effect, light scattering measurements were performed on aqueous samples. Samples containing 25% (w/v) of added salt showed the formation of 4-nm aggregates, whereas the same solutions in which sodium chloride was not added did not show these aggregations. The less effective extraction of the analytes, as resulted by the ex-

Table 1
Regression coefficients of the polynomial functions calculated using the forward method (standard error in parentheses)

TBA	$y = 96\,000 (\pm 7600) + 33\,400 T (\pm 6800) + 37\,100 \text{NaCl} (\pm 6800) - 17\,200 t \times \text{NaCl} (\pm 7600) + 19\,000 T \times t (\pm 7600) - 25\,000 T \times t \times \text{NaCl} (\pm 7600) - 26\,000 t^2 (\pm 10\,000)$ $R^2 = 0.88$
MTBE	$y = 75\,100 (\pm 3000) + 6200 T (\pm 2700) + 9300 t (\pm 2700) - 15\,500 \text{NaCl} (\pm 2700) - 7900 t^2 (\pm 4000)$ $R^2 = 0.81$
Benzene	$y = 81\,000 (\pm 3200) + 2900 T (\pm 580) + 8600 t (\pm 2900) - 38\,900 \text{NaCl} (\pm 2900) + 6900 t \times \text{NaCl} (\pm 3200)$ $R^2 = 0.94$
Toluene	$y = 19\,400 (\pm 750) + 1500 t (\pm 570) - 9900 \text{NaCl} (\pm 1000) - 1700 t \times \text{NaCl} (\pm 720)$ $R^2 = 0.87$
Ethylbenzene	$y = 16\,200 (\pm 600) - 7700 \text{NaCl} (\pm 740) + 500 t (\pm 97) - 1000 t \times \text{NaCl} (\pm 430)$ $R^2 = 0.91$
<i>m</i> - + <i>p</i> -xylene	$y = 29\,800 (\pm 490) + 1300 t (\pm 590) - 14\,600 \text{NaCl} (\pm 590) - 2100 t \times \text{NaCl} (\pm 590)$ $R^2 = 0.98$
<i>o</i> -xylene	$y = 16\,900 (\pm 280) - 7900 \text{NaCl} (\pm 350) - 900 t \times \text{NaCl} (\pm 300)$ $R^2 = 0.98$

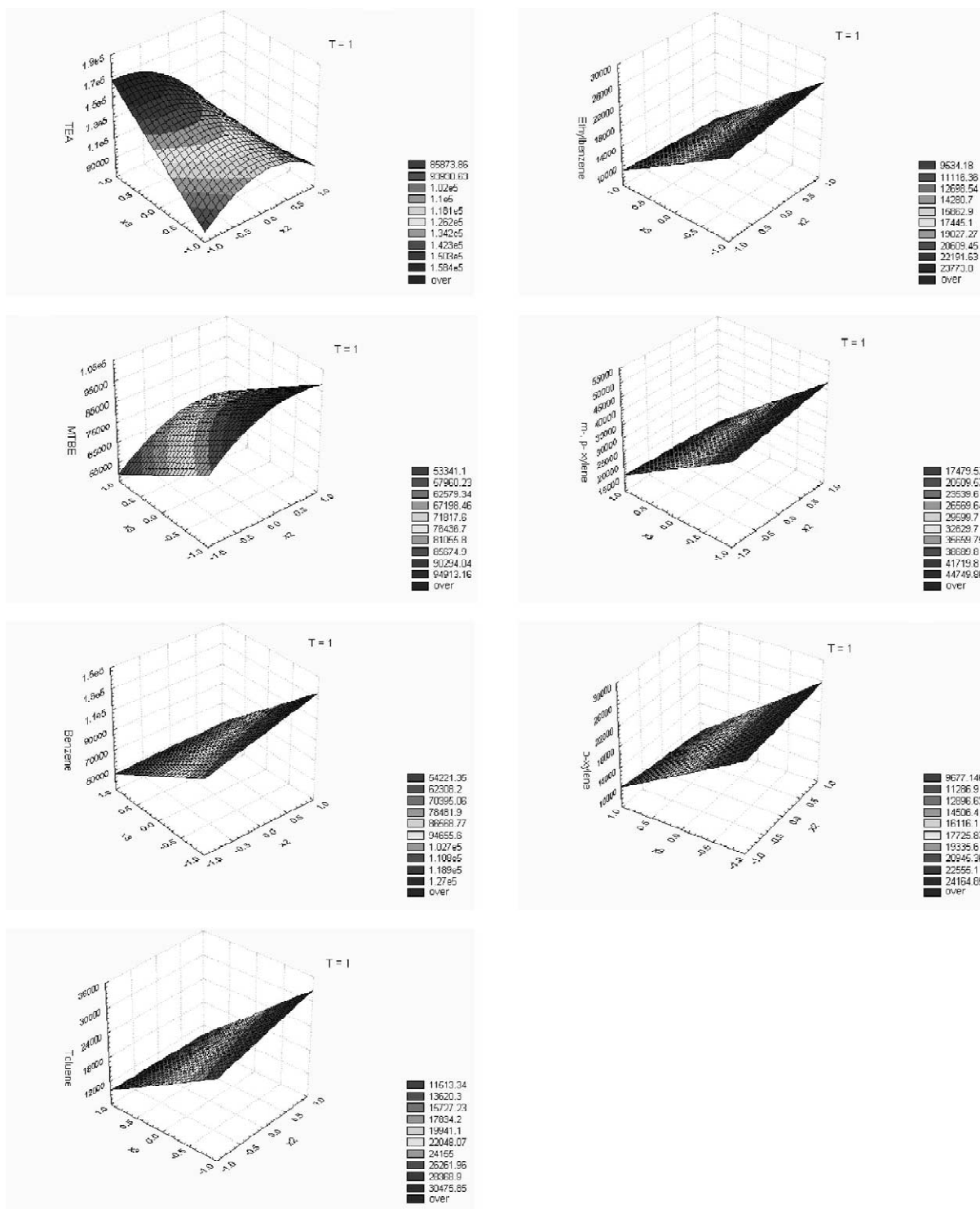


Fig. 1. Response surfaces depicted from the regression models calculated by step-wise regression analysis.

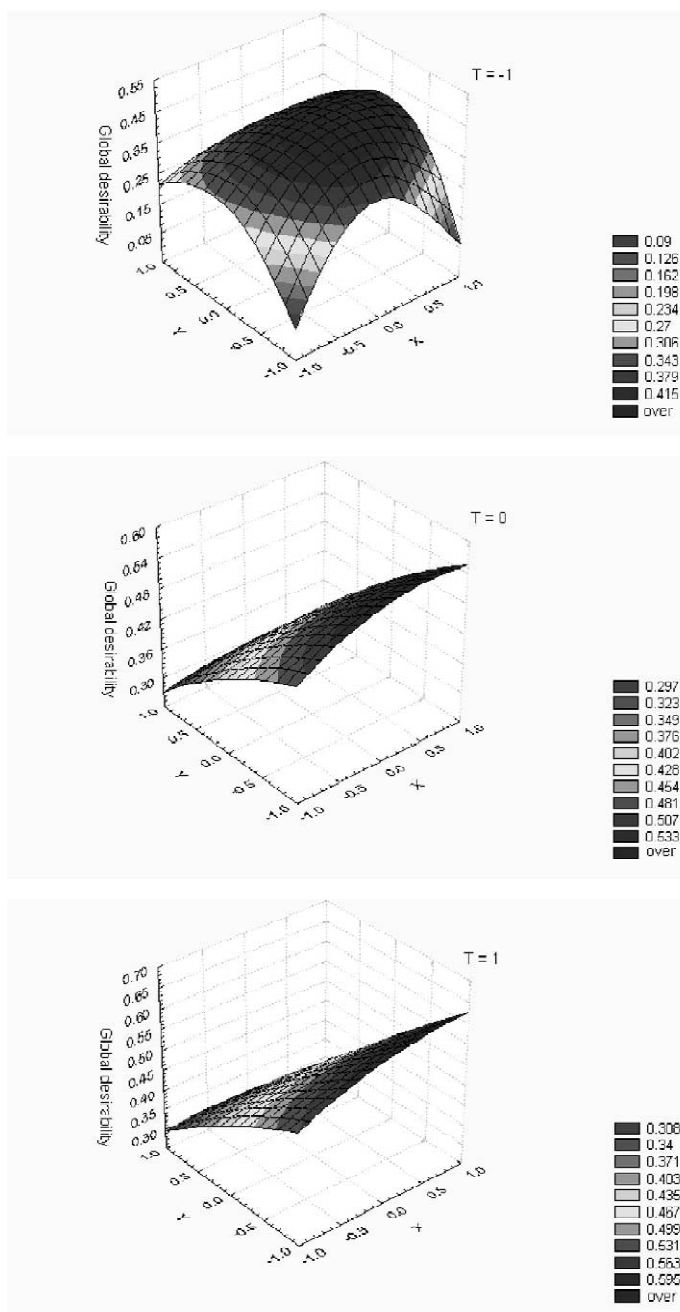


Fig. 2. Global desirability.

perimental design findings referred to aqueous solutions added with NaCl, could be ascribed to the formation of aggregates. To explain this behaviour, we supposed that TBA could act as surfactant agent

improving solubility of the low polar analytes in water. Experiments performed in the optimised conditions showed high recoveries, always higher than those obtained in the central composite design. In a

further step, the method was validated operating under these conditions.

3.2. Method validation and applications

The method was validated in terms of detection limits, quantitation limits, linearity and precision by using the experimental setting providing the optimised conditions.

LOD and LOQ values were calculated by constructing an appropriate calibration curve in a range of one order of magnitude and satisfying the $0.5 x_1 \leq \text{LOD} < x_1$ condition (where x_1 is the first concentration level). As shown in Table 2, LOD values ranging from 2.6 to 23 ng l⁻¹ were obtained, whereas LOQ values were typically twice as high. As shown in the same table, the method showed good linearity over two orders of magnitude for all the analytes. The mathematical verification of linearity (Table 2) was performed by applying Mandel's fitting test.

Method precision was evaluated testing two concentration levels. Good results were obtained both in terms of intra-day repeatability and intermediate precision (Table 3): RSD% lower than 4.4% at the highest concentration and lower than 12% at the lowest one were calculated for intra-day repeatability,

Table 3

Intra-day (RSD%) and intermediate precision (ANOVA) for PTI-GC-MS analysis ($n=3$) of MTBE, TBA and BTEX

Compound	Concentration (ng l ⁻¹)	RSD (%)	ANOVA P ^a
TBA	5	9.1	0.097
	500	1.5	0.059
MTBE	6	1.9	0.365
	600	1.1	0.469
Benzene	12	3.0	0.095
	1200	1.8	0.418
Toluene	27	7.2	0.142
	2700	1.7	0.327
Ethylbenzene	40	8.9	0.897
	4000	2.1	0.752
<i>m</i> -+ <i>p</i> -Xylene	6.5/6.5	11.6	0.666
	650/650	1.9	0.313
<i>o</i> -Xylene	6	4.9	0.077
	600	4.4	0.135

^a Confidence interval = 95%.

ty, whereas intermediate precision was evaluated by performing ANOVA on the data acquired over 3 days. ANOVA showed that mean values were not significantly different ($P > 0.05$) at 95% confidence level. The method validated was then applied to analyse a groundwater sample suspected of contamination by leaking underground petroleum storage tanks. Full scan EI chromatograms were recorded for

Table 2
PTI-GC-MS LOD values, linearity and Mandel's fitting test

Compound	LOD (ng l ⁻¹)	Range (ng l ⁻¹) Mandel's fitting test, F_{calc}^a	$a (\pm \sigma_a)$	$b (\pm \sigma_b)$	r^2 ($n=18$)
TBA	2.5	5–500 0.27	0.065 ± 0.004	0.57 ± 0.03	0.996
MTBE	3.3	6–600 1.98	0.15 ± 0.01	4.61 ± 0.07	0.994
Benzene	6.0	12–1200 6.43	0.33 ± 0.06	7.16 ± 0.04	0.998
Toluene	12.9	27–2700 7.24	0.15 ± 0.04	4.54 ± 0.12	0.999
Ethylbenzene	22.6	40–4000 5.37	— ^b	1.435 ± 0.09	0.997
<i>m</i> -+ <i>p</i> -Xylene	5.9	6.5–650/6.5–650 3.0	0.018 ± 0.002	1.36 ± 0.08	0.998
<i>o</i> -Xylene	2.8	6–600 0.63	4.787 ± 0.011	1.56 ± 0.06	0.996

Calibration fitting: $y = a + bx$.

^a $F_{\text{tab}}(1,15) = 8.68$, confidence interval = 99%.

^b Intercept not significant.

identification and confirmation purposes. Operating under SIM conditions, MTBE and TBA were detected and quantitated at 136 (± 5) and 1.29 (± 0.05) $\mu\text{g l}^{-1}$, respectively.

4. Conclusions

An efficient PTI-GC–MS method for the simultaneous determination of MTBE, TBA and BTEX at trace levels in water was developed and validated. By applying a chemometric approach based on the use of experimental design methods, the optimal extraction conditions corresponding to the highest global PTI-GC–MS recovery were calculated by the multicriteria decision method of the desirability functions. These conditions were found in correspondence with a temperature of extraction of 60 °C, a time of extraction of 30 min and no salt added. With this experimental setting, the method was then validated in terms of limits of detection, limits of quantitation, linearity and precision allowing the determination of all the analytes at low ng l^{-1} levels. The method developed was successfully applied to a groundwater sample suspected of contamination by leaking underground petroleum storage tanks. MTBE and its degradation product TBA were univocally identified and determined.

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