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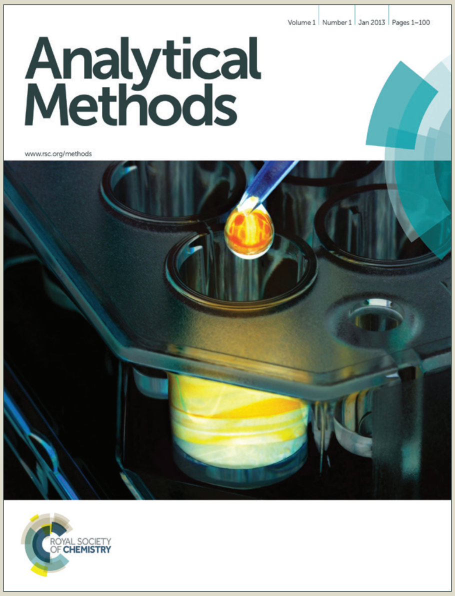

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SPME determination of low concentration levels of monoaromatic chemical markers in soils after remediation by supercritical fluid extraction

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Abstract

Soil contamination by organic compounds has been a serious concern over the years. Among the various forms of pollutant release, gas station contamination has been widely emphasized. The presence of simple aromatic compounds in soils sampled near gas stations may indicate contamination, considering that such compounds are frequently found in petroleum derived products. The present paper reports on the development of a process that uses pressurized fluids for the remediation of contaminated soils. A supercritical fluid extraction (SFE) employing CO_2 as solvent and an 11.0 g capacity extraction cell were utilized. In order to demonstrate the efficiency of the process, an analytical procedure employing headspace–solid phase micro extraction (HS-SPME) as the sample preparation method and GC-MS as the separation technique was optimized and validated. Limits of quantification and detection obtained were in agreement with the values established by national and international regulatory agencies. The linear range obtained was from 10 to 260 µg kg^{-1}, with determination coefficients $r^2 > 0.98$ for all analyses. Different removal rates for the contaminants studied were observed, being the best results achieved employing CO_2 in supercritical state.

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1. Introduction

Soil contamination has been a worldwide concern due to the different pollutants that can be found in this matrix. PAHs (polycyclic aromatic hydrocarbons), PCBs (polychlorinated biphenyls), pesticides, pharmaceuticals and monoaromatic hydrocarbons (benzene, toluene, ethylbenzene and three isomers of xylene) are frequently found in soil.[1–6] The latter are often indicators of contamination by compounds derived from petroleum, such as gasoline, which are frequently found in soils near gas stations or oil refineries.[7]

Some of these compounds can be easily degraded or leached, although recalcitrant compounds may be adsorbed and found at low concentrations.[8]

In Brazil, the Sao Paulo state environmental company (CETESB) reported approximately 2,900 contaminated areas in the state, of which 80% correspond to contamination by gas stations.[9] As a consequence, the development of technologies for the remediation of contaminated soils has become crucial. However, the difficulty in finding appropriate forms of treatment is a current topic, especially when an in-situ treatment is required, since the transport and storage of contaminated soils may increase the remediation process costs.[10]

Supercritical fluid extraction (SFE) has been long used for the development of analytical methodologies for solid matrices, as it enables analyses in shorter periods, in comparison to classical techniques, such as Soxhlet, as well as a considerable reduction in the amount of solvents used.

This technique has also been used as a remediation process for contaminated environmental sites, as it completely removes contaminants from environmental

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matrices.[11–13] The contaminants removed after a remediation process by SFE are easily destroyed by different methods, including incineration and chemical oxidation.

CO_2 is usually the best fluid of choice for SFE systems, as it is easy to obtain, is relatively of low cost, non-flammable, non-toxic and has mild supercritical parameters (31 ºC and 73 bar for temperature and pressure, respectively).[14] Furthermore, it is a non-destructive method, which maintains the structural and physical properties of the soil.[15]

Although many groups have reported on the remediation of soils by using SFE systems, with almost 100% efficiency, most of them use very high initial concentrations of contaminants, which results in alarming remaining concentrations.

Zhou and coworkers (2004)[16] successfully treated soils and sediments contaminated by PCBs. After 40-60 minutes of extraction using CO_2/5 mol % MeOH, almost 100% of the concentration had been decreased. However, the final concentration was approximately 5 mg kg $^{-1}$ PCB in soil, which is still a relatively high value.

This paper proposes a remediation process for soils contaminated with petroleum derived products employing supercritical CO_2. For that purpose a lab-made system was used, after an optimization by experimental design process of the variables (factors), which included the effects of temperature, pressure and extraction time operating in both static and dynamic modes.

The use of experimental design is extremely important when there is interest in optimizing a system with more than one variable (factor) that exerts an effect on the response variable studied. With this opmitization step it is possible to verify the effects caused by the individual factors and by the interaction between them, generated on the response variable, often being applied to optimize SFE methods aiming the removal of organic pollutants in environmental matrices.[17-19]

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The efficiency of the remediation process was monitored by SPME (solid phase micro extraction), a technique developed by Arthur and Pawliszyn (1990).[20] This technique has already proved to be fast, robust and efficient for the analysis of volatile compounds in solid and complex matrices, including the analysis of monoaromatic compounds such as benzene, toluene and xylene isomers.[21-24] The method was developed and validated to ensure the presence of low concentration levels of contaminants in the treated soil.

2. Material and Methods

2.1. Chemical Reagents

Benzene, ethylbenzene and xylene isomers (*m,o,p*-xylene) were obtained from Alltech (State College, USA). Methanol analytical grade was purchased from Tedia (Fairfield, USA). Isopropylbenzene, used as internal standard, was purchased from Supelco (Bellefonte, USA). A standard solution of four contaminants was prepared in methanol at 500 mg L^{-1}; solutions used for the SPME validation experiments were obtained from this one by proper dilution. The internal standard solution was prepared in methanol at 600 mg L^{-1}. Polydimethylsiloxane (PDMS) fibers (L =1.0 cm; d_f = 100 μm) and appropriate holders were supplied by Supelco (Bellefonte, PA) and conditioned according to the manufacturer's instructions.

2.2. Sampling

Two types of soil were used in this work: one soil sample free of contaminants was used for both SPME and SFE optimization (termed uncontaminated soil or blank

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sample) and two soil samples (termed contaminated soil), one sampled nearby a washing vehicle systems and the other in an underground fuel storage tanks, were used to confirm the efficiency of the remediation process. All soil samples analyzed contained approximately 4.0% of organic matter. The soil used as uncontaminated was first evaluated by SPME to verify the possible presence of the target compounds. It was not found any evidence of the presence of the investigated contaminants.

The uncontaminated soil sample was collected at a depth 15-20 cm at the University of Sao Paulo, USP - São Carlos campus (− 22.004797 longitude and − 47.898991 latitude) and sieved to 2 mm to maintain their homogeneity during the tests. For the SPME validation experiments, blank samples were spiked with amounts of 10 to 260 µg kg^{-1} of contaminants (in six different concentration levels), enclosed in sample vials and cooled at – 20 °C for 48 h, in order to promote the analyte-soil interaction.[25]

The soil samples used for the SFE procedures were spiked at 2.0 mg kg^{-1} and stored for 48 h at – 20 °C. After the tests, the soil remaining in the extraction chamber was submitted to SPME analysis in the headspace mode.

2.3. Solid phase micro extraction (SPME) procedures

The chemical markers were extracted in 22-mL sample vials and temperature was controlled by a thermostatic bath model Q214 M2 (Quimis, Diadema, Brazil). The PDMS fibers (100 µm) were exposed to the headspace for 15 to 45 minutes. The amount of sample (1.0 and 2.0 g) and temperature of exposure (30 and 70 ºC) were also evaluated. A 2^3 factorial design (3 independent variables in 2 different levels), generated by Statistica 12.0 software, was performed with triplicate experiments in the center

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point, resulting in a total of 11 experiments. The absolute area of the chromatographic peaks was used as the response variable. The analytes were desorbed from the fiber by direct exposure in a GC injection port for 2.0 minutes.

Linearity, intra-day precision, limit of quantification and limit of detection were determined before the chemical markers analysis by HS-SPME/GC-MS.

2.4. Supercritical Fluid Extraction

The original concept of the system used for the SFE procedures was developed before in our research group by Sargenti and Lanças (1994).[26] For the current experiments the modification / replacement of some pre-existing components was necessary. The restrictor, previously used, was replaced by 2 valves at the outlet of the hot water bath to ensure control of the CO_2 flow rate. The first valve, an on/off valve, controls the flow of the system and enables extractions in both static and dynamic modes. As it does not have a fine adjustment of the gas flow rate, a micrometric valve was installed in sequence. Both valves were purchased from Swagelok (Solon, USA).

For the evaluation of the sub critical and supercritical conditions, regarding the removal of contaminants (response variable), four independent variables were evaluated in 2 different levels, according to a fractional factorial design 2^{4-1}: time ratio of static / dynamic extraction (¼ and ½ of the total extraction time), pressure (55 and 95 bar), temperature (25 and 45 °C), and total extraction time (10 and 30 minutes).

2.5. Chromatographic Conditions

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All analyses were performed in a QP 5050A GC-MS (Shimadzu, Japan) fitted with a 30 m x 0.25 mm x 0.25 µm SLB – 5MS column. The split/splitless injector was operated in splitless mode at 250 ºC for 2.0 minutes, fitted with a proper liner for the SPME analysis, with 0.75 mm ID and deactivated by the manufacturer (Supelco, Bellefonte, USA). The oven temperature program was set to 40 ºC for 1 minute, then heat at 12 ºC min $^{-1}$ up to 150 ºC. Helium was the carrier gas at 1.4 mL min^{-1}. The detector operated at 300 ºC and a selected ion monitoring (SIM) mode was chosen to enhance the detectability. Electron ionization mode at 70 eV was used.

3. Results and Discussion

3.1. SPME optimization and validation

An SPME method was developed and validated for the evaluation of the soil remediation process by SFE. According to the experiments performed by the 2^3 factorial design, only the temperature and the amount of soil used for extraction were significant (at *p*-values of 0.05) variables to the further optimization.

From the 2^3 factorial design results, it was possible to perform a central composite design, fixing the exposure time of the fiber to 45 minutes, since this parameter was not significant for the process efficiency, but exerted a positive effect on the response variable. The amount of sample in the vial and the temperature were evaluated in 5 different levels. The real and coded values for each variable are shown in Table 1. Twelve experiments with the absolute chromatographic peak areas as the response variable for each contaminant were conducted (Table 2).

TABLE 1

TABLE 2

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An increase in the temperature caused a decrease in the absolute chromatographic peak areas. This result is similar to that found by Esquerro and co-workers (2004),[27] who performed successive extractions of BTEX in a soil solution by HS-SPME at 30, 60 and 90 ºC and obtained the best results at 30 ºC. However, their study did not evaluate the SPME extraction at temperatures below 30 ºC.

The best extraction conditions were obtained for 4.5 g of soil at 15 ºC.

Table 3 shows data regarding HS-SPME method validation for the determination of benzene, ethylbenzene, *m*-xylene*, p*-xylene and *o*-xylene. For the best data fit of benzene, was necessary to use the weighted linear regression method, using the x^{-1} as weight factor.[28]

Although toluene was also included as one of the markers of interest in the early steps of this work, it was later excluded due to the frequent use of this solvent in the laboratory that prevents us from obtaining blank samples with contamination in low concentration levels.

TABLE 3

The LD (3-6 μg kg^{-1}) and LQ (10-20 μg kg^{-1}) values are satisfactory, considering that the Canadian Soil Quality Guide Line for the Protection of Environmental and Human Health (2004)[29] established that benzene values must be lower than 9.5 μg kg^{-1} for residential areas.

Although other study used SPME for determination of BTEX in Brazilian soil, with successful extraction in just 4 minutes, the LQ achieved was 16 μg kg^{-1}. For this process the use of saturated NaCl solution was necessary in order to improve the extraction of the evaluated compounds.[30]

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3.2. SFE Optimization

Initially, an experiment using only half capacity of the extraction cell (5.0 g of contaminated soil) was conducted. However, in the experimental design 2^{4-1}, no variable showed to be significant for the process, which can be explained by the presence of interstitial space in the extraction chamber, as it reduces the contact between the contaminated sample and the fluid extractor and decreases the efficiency of the remediation process.

To evaluate this effect, a second experimental design 2^{4-1} was performed under the same conditions and with the maximum cell capacity, i.e. approximately 11.0 g of soil, spiked with 2.0 mg kg^{-1} of all contaminants.

Table 4 shows the results expressed as the percentage of the removed evaluated contaminants.

TABLE 4

A high removal rate (recovery > 73%) was observed in all experiments and for all contaminants studied, especially benzene and ethylbenzene. There was an increased variation in the efficiency removal for xylene isomers in comparison to other contaminants, and being the best results achieved when the extraction was performed under CO_2 above critical conditions.

The data displayed in Table 4 enabled the estimative of the significant effects of all variables studied, as shown in the Pareto charts for the four contaminants (Figure 1). No variable showed to be significant for the benzene extraction, probably because this is the most volatile compound in comparison to the other three, which facilitates its transport into the extraction fluid.

FIGURE 1

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However, for the other three evaluated compounds, the pressure became a significant variable with a positive effect, i.e. the higher the pressure, the better the removal of contaminants was. Due to the fractional factorial design used, the effect values regarding the interactions between the variables could not be calculated.

Other important information extracted from the Pareto charts is the lower effect values for the variables time ratio of static / dynamic extraction and total time extraction. Similar results were found by Gonçalves *et al.* (2006)[31] in their studies on the extraction of pesticide residues from soil samples. A central composite design was used to estimate the effect of extraction temperature and pressure, extraction time and amount of modifier. The efficiency was affected predominantly by pressure and temperature, and the least important variable was the extraction time.

A new statistical analysis of the same data was conducted taking into account only pressure and temperature. A 2^2 factorial design was obtained and performed in duplicate, as shown in Table 5.

TABLE 5

Pareto charts for the dependent variables were developed; however, as this is now a full factorial design, the effect of the interaction between pressure and temperature could be analyzed (Figure 2).

FIGURE 2

As expected, the behavior of benzene did not change, as there was no change in the extraction parameters utilized in the experiments. However, for ethylbenzene and xylene isomers the interaction between pressure and temperature was significant. Temperature was also significant for the efficiency of the process.

The study of the influence of the temperature in the extraction process is extremely important, since the properties of the fluid can be modified during the

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experiments. A variation in temperature may cause a change in both volatility of the contaminant and matrix structure.[32]

Although temperature exerted a negative effect, the effect of the interaction between pressure and temperature was positive and higher than the isolated effect caused by just the temperature. A simultaneous increase in both temperature and pressure favored the removal of those contaminants.

At lower pressures ($P < 150$ bar) CO_2 has lower solvent strength as the temperature is increased, probably due to the drastic reduction in the CO_2 density under this condition. The solubility of some compounds in CO_2 can decrease, which compromises the efficiency of extraction and explains the negative effect exerted by the temperature on the extraction of xylene isomers.[33,34] The correlation coefficient (r^2) for the model proposed was higher than 0.98, except for benzene, whose r^2 was 0.58.

The efficiency of SFE for removing monoaromatic organic compounds has been published in a pioneering work of Eckert-Tilotta et al.[35] Using a commercial SFE system, with 2,4 g of loam, their recoveries were about 42, 86 e 87% for benzene, ethylbenzene and xylene isomers, respectively, employing CO_2 at 400 bar and 65 ºC. In the present work almost 100% efficiency was reached for all contaminants evaluated, using 11 g of soil and CO_2 at 90 bar and 45 ºC. The SFE system used in this work for remediation purposes, employed a combination of both static and dynamic extraction modes, which may explain the superior results obtained, since in this way the fluid-contaminant interaction is improved when compared with a single extraction mode.[27] Another relevant issue used in this work was the optimization approach using an experimental design that allowed to evaluate the effect of each variable studied, as well as the effect generated by their interaction. Thus, better remediation condition could be reached.

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Due to the high removal rates found, no additional experiments were performed considering that at 90 bar (pressure) and 45 °C (temperature) the removal efficiency for all contaminants was close to 100%.

4. Application to Real Sample

SPME analyses, which preceded the SFE remediation process, revealed the presence of all contaminants studied in both samples, at concentrations far above the higher limit of quantification of the proposed analytical method.

In the samples collected near the fuel storage tanks, other fragments of m/z similar to those of benzene, ethylbenzene, xylene and their isomers were found. This identification was possible because the mass analysis was carried out in the selected ion monitoring (SIM) with the masses monitored according to the typical fragments of aromatic compounds (m/z 77, 78, 91), according to chromatogram presented in Figure 3.

FIGURE 3

After the remediation process by supercritical fluid, the soil samples remaining in the extraction cell were again analyzed by HS-SPME/GC-MS in order to determine the markers concentration. The contaminants found in the remaining soil were below the lower limit of quantification and detection of the proposed analytical method, thus confirming the efficiency of the remediation process.

5. Conclusion

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The proposed analytical method for the determination of the chemical markers benzene, ethylbenzene and xylene isomers in soil samples follows the current trend in sample preparation, towards the concept of green chemistry. Indeed, no organic solvent was used in the whole process, including both the extraction and desorption steps. The HS-SPME method showed appropriate limits of quantification proposed by both international (EPA) and national (CETESB) regulatory agencies, with a linear range of 10-260 μg kg $^{-1}$ for most analytes, with $r^2 > 0.98$.

SFE showed to be a viable alternative for the remediation of contaminated soil achieving near 100% efficiency for all contaminants evaluated. The influence of temperature and pressure depends on the type of contaminants investigated and becomes more prominent for those analytes of higher molecular weight.

This study also demonstrates the importance of avoiding the use of univariate optimizations for extractions with supercritical fluid, since this form of optimization would not reveal the effects generated by the interactions between the variables; in this case temperature and pressure. This interaction effect was evident as found through the use of a factorial experimental design.

In summary, the evaluated remediation process showed to be appropriate to remove the investigated aromatic markers from contaminated soil samples. The validated analytical method employed as the quality control procedure of the remediation process, based upon SPME, demonstrated to be also adequate. Both SFE and SPME utilized are techniques environmentally friend and analytical tools adhering the relevant concept of green chemistry.

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Table 1- Coded values and actual values for each variable in the central composite design for SPME optimization.

Variables	**Coded variables**				
	–1.41	**–1**	**0**	**+1**	**+1.41**
Amout of soil (g)	2.59	3.0	4.0	5.0	6.41
Temperature (ºC)	8.0	10.0	15.0	20.0	22.0

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Table 2 - Central composite design matrix for SPME optimization and results obtained for the response variables expressed in absolute area.

Runs	Independent variables		Benzene	Ethylbenzene	*m,p*-Xylene	*o*-Xylene
	x_1	x_2				
1	-1.0	-1.0	78024	288994	455122	238692
2	-1.0	1.0	83552	334877	543438	289650
3	1.0	-1.0	144583	501361	608421	430274
4	1.0	1.0	170670	417478	679449	357523
5	-1.41	0.0	31798	262147	390584	212210
6	1.41	0.0	130849	392949	632442	331367
7	0.0	-1.41	154096	357132	572312	301439
8	0.0	1.41	105235	324941	527454	277979
9 (C)	0.0	0.0	225662	767931	1000283	555312
10 (C)	0.0	0.0	185737	611594	899232	456203
11 (C)	0.0	0.0	226001	762961	1121791	572080

Note: x_1 refers to the independent variable amount of sample and x_2 to temperature

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Table 3 – Parameters evaluated during the validation of the HS-SPME method utilized for the determination of monoaromatic compounds in soil by GC-MS

Chemical Markers	Linear Range (μg kg^{-1})	Analytical curve (a, b, r^2)	LD (μg kg^{-1})	LQ (μg kg^{-1})	Inter-day precision	
					Level	DPR (%)
Benzene	10-260	0.0038 - 0.0101 0.981	3	10	Low Medium High	4.8 4.2 5.8
Ethylbenzene	10-260	0.0060 - 0.0348 0.990	3	10	Low Medium High	1.3 1.6 1.1
***m,p*-Xylene**	10-260	0.0054 - 0.0252 0.990	3	10	Low Medium High	1.9 1.2 0.7
***o*-Xylene**	20-260	0.0024 - 0.0209 0.992	6	20	Low Medium High	2.6 1.0 0.1

Note: Considering $y = ax + b$ as an equation of the curve and r^2 the determination coefficient.

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Table 4: Results of the fractional factorial design 2^{4-1} for benzene, ethylbenzene, *m, p*-xylene and *o*-xylene expressed as extraction efficiency.

Runs	**Independent Variables**				**Extraction Efficiency (%)**			
	x_1	x_2	x_3	x_4	**Benzene**	**Ethyl-benzene**	***m,p*-Xylene**	***o*-Xylene**
1	-1	-1	-1	-1	99.09	99.34	89.81	84.65
2	1	-1	-1	1	98.81	99.53	92.71	88.04
3	-1	1	-1	1	99.10	99.88	98.64	97.03
4	1	1	-1	-1	99.10	99.87	98.48	97.58
5	-1	-1	1	1	99.14	99.09	84.18	75.61
6	1	-1	1	-1	99.15	99.08	82.92	72.78
7	-1	1	1	-1	99.14	99.98	100.21	99.84
8	1	1	1	1	99.17	99.99	100.35	100.01

x_1, x_2, x_3 and x_4 refer, respectively, the ratio between the time of dynamic and static extraction, pressure, temperature and extraction time.

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Table 5: 2^2 factorial design results as the extractions efficiency, obtained in duplicate, during the evaluation of the pressure and temperature effects.

Runs	**Independent Variables**		**Extraction Efficiency (%)**			
	x_2	x_3	**Benzene**	**Ethyl-benzene**	***m,p*-Xylene**	***o*-Xylene**
1.1	-1	-1	99.09	99.34	89.81	84.65
1.2	-1	-1	98.81	99.53	92.71	88.04
2.1	1	-1	99.10	99.88	98.64	97.03
2.2	1	-1	99.10	99.87	98.48	97.58
3.1	-1	1	99.14	99.09	84.18	75.61
3.2	-1	1	99.15	99.08	82.92	72.78
4.2	1	1	99.14	99.98	100.21	99.84
4.2	1	1	99.17	99.99	100.35	100.01

x_2 and x_3 refers, respectively, to pressure and temperature

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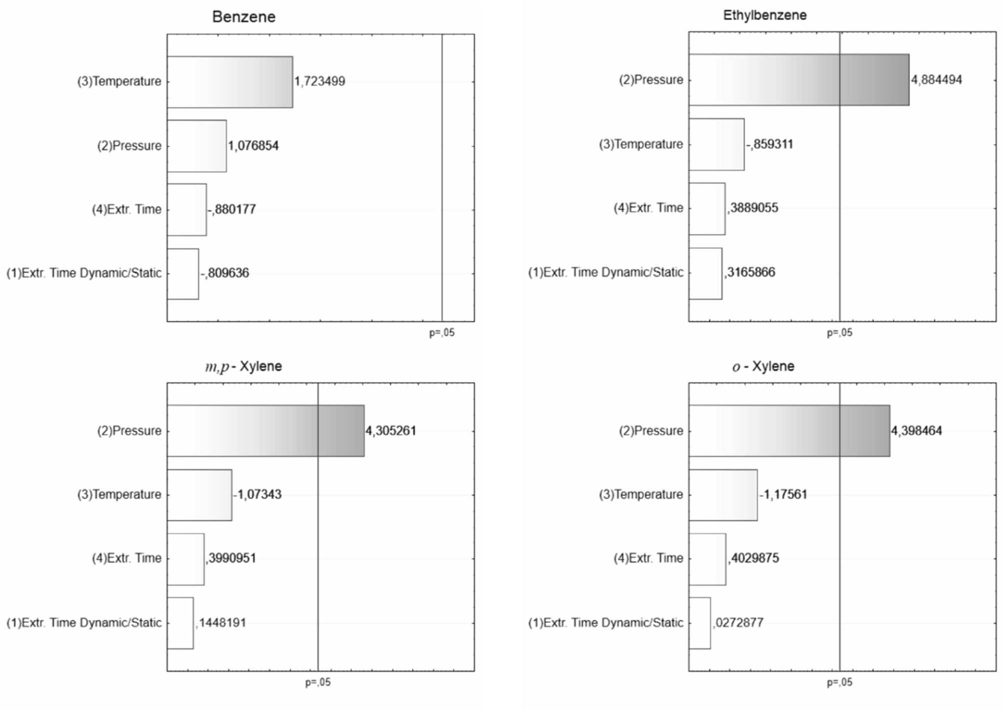

Figure 1

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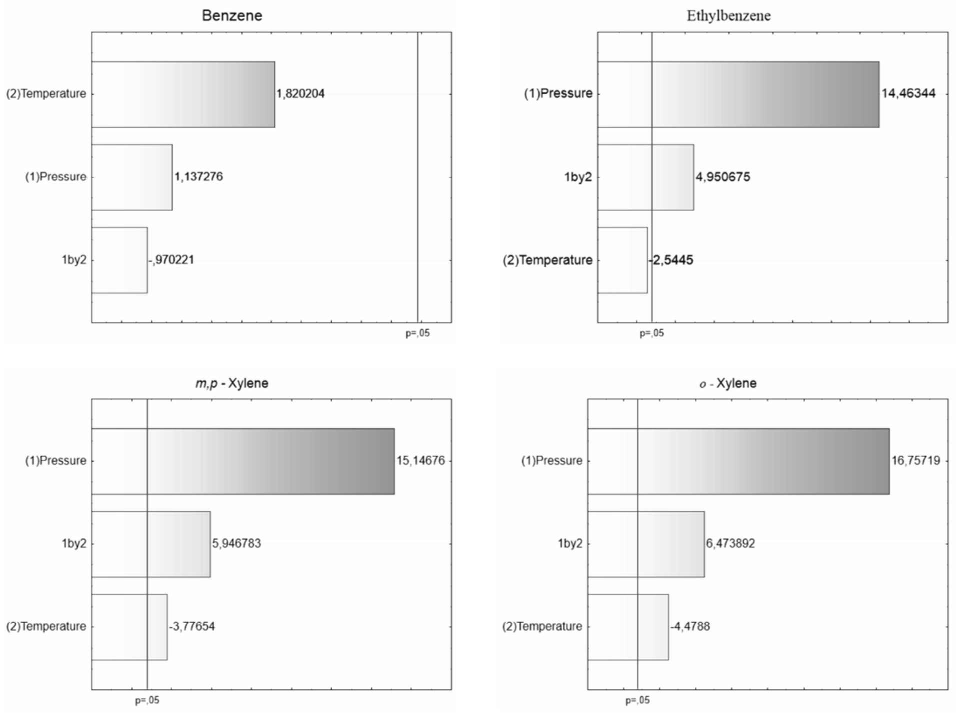

Figure 2

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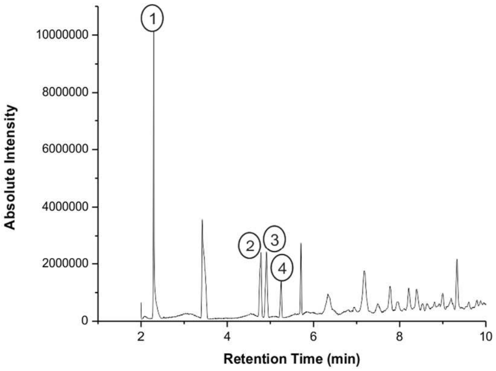

Figure 3

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