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Sample preparation procedure using extraction and derivatization of carboxylic acids from aqueous samples by means of deep eutectic solvents for gas chromatographic-mass spectrometric analysis

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Abstract:

The paper presents a new procedure for the determination of organic acids in a complex aqueous matrix using ultrasound-assisted dispersive liquid-liquid microextraction followed by injection port derivatization and GC-MS analysis. A deep eutectic solvent (choline chloride : 4-methylphenol in a 1:2 mole ratio) was used both as an extracting solvent and as a derivatizing agent to yield ion pairs which were next converted to methyl esters of organic acids in a hot GC injection port. The procedure was optimized in terms of selection of a deep eutectic solvent, disperser solvent, and the ratio of their volumes, pH, salting out effect, extraction time, injection port temperature and time of opening the split valve. The developed procedure is characterized by low LOD (1.7 – 8.3 µg/L) and LOQ (5.1 – 25 µg/L) values, good repeatability (RSD ranging from 4.0 to 6.7%), good analyte recoveries (68.8 – 106%) and a wide linear range. The procedure was used for the determination of carboxylic acids in real effluents from the production of petroleum bitumens. A total of ten analytes at concentrations ranging from 0.33 to 43.3 µg/mL were identified and determined in the effluents before and after chemical treatment. The study revealed that in effluents treated by hydrodynamic cavitation an increase in concentration of benzoic acid and related compounds was observed.

Keywords: carboxylic acids, deep eutectic solvents, gas chromatography, industrial effluents, injection port derivatization, ion pairs.

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1. Introduction

Carboxylic acids, such as volatile fatty acids, benzoic and hydroxybenzoic acids occur commonly in municipal wastewater [1-2], and also at relatively high concentrations in industrial effluents, including paper [3-4], pharmaceutical [5], textile, tanning [6], or petroleum industries [7]. As a result of malodorous nature of the most volatile acids, potential toxicity of individual compounds and a negative impact on the aqueous environment, carboxylic acids have been the subject of numerous studies concerning the technology of their removal [8-10], and the development of procedures for their determination at low concentration levels.

At present, the determination of carboxylic acids in samples with aqueous matrices involves chromatographic techniques, primarily gas chromatography. However, due to specific properties of carboxylic acids, *i.e.* high polarity, high boiling point and low volatility of a large number of the acids, only a small fraction of them (including volatile fatty acids from C_1 to C_{12}) can be subjected to direct GC analysis [11-12]. Hence, it is often necessary to derivatize analytes through esterification, alkylation or silylation. The majority of available derivatization procedures is based on application of alkylsilane derivatizing agents which form unstable derivatives and the time of reaction can be as long as 24 hours [13]. Other commonly used derivatization methods require strongly toxic reagents [14-15] or reagents characterized by a low derivatization yield [16]. Among the available procedures, only a few can be considered environmentally friendly and free from the above shortcomings. Examples include the use of alkylchloroformates in esterification [17] or, recently popular, derivatization resulting in formation of ion pairs making use of nontoxic quarternary ammonium salts, such as tetramethylammonium chloride (TMA-Cl), tetramethylammonium acetate (TMAAc), tetrabutylammonium hydrogensulfate (TBA-HSO_4), tetrabutylammonium chloride (TBA-Cl), tetrabutylammonium bromide (TBA-Br) or tetrabutylammonium iodide (TBA-I) [7,18]. The latter procedure has a number of advantages due to its simplicity of derivatization, which involves addition of a derivatizing agent and a buffer solution. Thus formed ion pairs are converted to esters in the hot GC injection port.

Determination of low concentrations of organic acids in wastewater samples requires an appropriate extraction technique which, in accordance with principles of green chemistry, should be simple, rapid, automatable and using only small volumes of organic solvents. One of the sample preparation techniques meeting the above criteria is dispersive liquid-liquid microextraction (DLLME) introduced in 2006 [19]. However, since the inception of this technique it was pointed out that it makes use of mostly hazardous chlorinated organic solvents with a density higher than water, which facilitates separation of phases and transfer of the extract to the GC injection port or to autosampler vials. Solvents having density lower than water can also be used but collection of the extract is more difficult as it requires an additional step of solidification of organic drop or the use of special vials which are not yet available commercially [20-22].

An alternative to toxic chlorinated solvents are deep eutectic solvents (DESs). These are liquids which are formed upon mixing two solids (at ambient temperature), typically quarternary ammonium salts being hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD), whose melting points are much lower than those of individual components [23]. In addition, DESs have physico-chemical properties similar to ionic liquids; however, the synthesis of DESs is much simpler and cheaper and they are less toxic and easier to biodegrade than ionic liquids [24]. The majority of papers refer to synthesis and use of hydrophilic DESs [23,25-27], and only a few mention hydrophobic DESs which can be potentially used as extracting solvents for samples with an aqueous matrix [28-33].

The paper describes a new procedure for the determination of carboxylic acids in aqueous samples which was used for the analysis of effluents from the production of bitumens. The procedure is based on ultrasound-assisted dispersive liquid-liquid microextraction combined with GC injection port derivatization and mass spectrometric detection (IP-USA-DLLME-GC-MS), in which the same deep eutectic solvent is used as an extracting solvent and a reagent for the formation of ion pairs (IPR).

2. Experimental

2.1. Reagents

Solvents (purity >99.9 %), including isopropanol (IPA), methanol (MeOH), and acetone (AC) were purchased from POCH (Poland). Choline chloride (ChCl), 4-chlorophenol (4CPh), 4-ethylphenol (4EtPh), phenol (Ph), 2-methylphenol (2MPh), 3-methylphenol (3MPh), 4-methylphenol (4MPh), and 2,6-dimethylphenol (2,6DMPh) were obtained from Sigma-Aldrich (USA), while sodium chloride (NaCl), sodium hydroxide (NaOH) and hydrochloric acid 35-38% were purchased from POCH (Poland). High-purity standards (benzoic acid, 2-methylbenzoic acid, 4-methylbenzoic acid, 2,4-dimethylbenzoic acid, 4-*tert*-butylbenzoic acid, 2-hydroxybenzoic acid, octanoic acid, nonanoic acid, decanoic acid, 10-undecylenic acid, dodecanoic acid) and internal standard: 2-chlorobenzoic acid were obtained from Merck (Germany). Compressed gases: hydrogen (purity N 5.5) generated by a PGXH2 500 Hydrogen Generator (PerkinElmer, USA), air (purity N 5.0) generated by a DK50 compressor with a membrane dryer (Ekom, Poland) and further purified by a GC3000 zero air generator (PerkinElmer, USA), and nitrogen (purity N 5.0) (Linde Gas, Poland).

2.2. Real samples

Effluents from the production of bitumen 20/30 from vacuum bottom of crude oil Rebco:Kirkuk 65:35 m/m (mixture of Russian and Iraqi crude oils) were collected behind a plate separator which separated condensed organic phase from aqueous phase. The aqueous phase of raw effluents was investigated along with the effluents subjected to various chemical treatments, including hydrodynamic cavitation (HC) as well as hydrodynamic cavitation combined with oxidation by hydrogen peroxide (HC/H_2O_2). A detailed characteristic of the effluents was provided in previous papers [34-36]. In the study, 300 mL of raw effluents and effluents after treatment were collected in 350-mL glass bottles.

2.3. Apparatus

A model QP2010 GC–MS SE gas chromatograph-mass spectrometer (Shimadzu, Japan) equipped with acombi-PAL AOC 5000 autosampler (Shimadzu, Japan) and an HP-5 MS (30 m x 0.25 mm x 0.25 µm) capillary column (Agilent, USA) were used in the investigations. A PerkinElmer Autosystem XL gas chromatograph with flame ionization detector (PerkinElmer, USA) and an HP-1 (30 m x 0.53 mm x 1.5 µm) capillary column (Agilent, USA) were employed in optimization of operational parameters. LabSolutions software (Shimadzu, Japan) with NIST 14 mass spectra library and TurboChrom 6.1 (PerkinElmer, USA) were used for data management. An RK 156 BH ultrasonic bath (BANDELIN electronic GmbH & Co. KG, Germany) was used for extraction and an EBA 8S centrifuge (Hettich, Germany) was used for the separation of extracts from samples. FT-IR spectra were recorded using a Bruker Tensor 27 spectrometer (Bruker, USA) with KBr pellet holder and OPUS software (Bruker, USA).

2.4. Synthesis and characteristics of deep eutectic solvents

Deep eutectic solvents were synthesized as follows: 1.39 g ChCl (HBA) and an appropriate amount of HBD (including Ph, 2MPh, 3MPh, 4MPh, 2,6DMPh, 4EtPh, and 4CPh) so as to obtain mixtures with a mole ratio (HBA : HBD) of 1:0.5, 1:1, 1:2, 1:3, 1.4 and 1:5 were added to a 20-mL beaker. The mixture was stirred magnetically at 50°C until a homogeneous liquid was obtained.

2.5. Derivatization and extraction procedure

A sample of effluent (9 mL) was transferred to a 12-mL vial followed by addition of 5 µL of a 5000 µg/mL solution of an internal standard (2-chlorobenzoic acid). Next, the solution pH was adjusted using a 7% HCl solution. 1.0 mL of a mixture consisting of DES (ChCl : 4MPh (1 : 2)) serving as an extracting solvent/derivatizing agent and MeOH as a disperser solvent in a 1:1 volume ratio was then added to the vial. The vial was closed tightly and placed in an ultrasonic bath at room temperature (RT) for 10 min, followed by centrifugation for 5 min at 4000 rpm. Subsequently, 200 µL of the organic phase was collected with an autopipette and transferred to 2-mL vials equipped with 300-µL micro-inserts. The volume of the extract subjected to GC-MS analysis was 0.5 µL.

2.6. Chromatographic conditions

Temperature program: 50°C (5 min) – ramped at 5°C/min to 250°C (5 min); injection port temperature 300°C; purge off time 2 min (splitless mode); 1 µL of the extract was injected into the GC system; detector temperature 300°C; ion source temperature (EI, 70 eV) 200°C; GC/MS transfer line temperature 300°C; the carrier gas was hydrogen (1 mL/min).

2.7. FT-IR analysis

FT-IR spectra were taken using attenuated total reflectance (ATR) using the following operating parameters: spectral range 4000-550 cm^{-1}, resolution: 4 cm^{-1}, number of sample scans: 256, number of background scans: 256, slit width: 0.5 cm, 500 µL of DES was used for IR scan.

3. Results and discussion

3.1. Synthesis of DES

Deep eutectic solvents used for extraction must meet several requirements, including:

- high affinity for analytes,
- liquid state at RT,
- density significantly different from that of water,
- low solubility and high stability in aqueous phase,
- good solubility in disperser solvents and ease of dispersion in aqueous samples,
- separability from analytes during chromatographic process.

In addition, if a DES is used as a derivatizing agent, the following properties must be considered:

- selectivity and yield of derivatization reaction,
- lack of formation of byproducts that could interfere with analytes or be adsorbed in the liner or GC injection port or inside GC capillary column.

Derivatization reaction using ChCl is shown in Figure 1.

Based on the above considerations, various DESs were synthesized by combining ChCl (HBA) with phenolic compounds (HBD) in various mole ratios. Combination of HBA and HBD in mole ratios 1:0.5 and 1:1 did not yield clear liquids even after long stirring, which indicated that no deep eutectic solvents were formed. In contrast, homogeneous liquid were obtained after combination of ChCl with phenols in 1:2, 1:3, 1:4 and 1:5 mole ratios. In the next step, the DESs containing Ph and 2,6DMPh were rejected due to their high solubility and instability in water. FT-IR analyses were carried out to confirm the formation of DESs. A characteristic feature in the IR spectrum of deep eutectic solvents is a shift of the band corresponding to the presence of O–H group for a pure HBD toward a lower wavenumber. Bands corresponding to stretching vibrations of the O-H group in all pure HBDs can be observed in the 3285 – 3371 cm^{-1} range. On the other hand, DESs are characterized by bands in the 3139–3200 cm^{-1} range, which correspond to the N-H stretching vibrations characteristic of ChCl, which overlap with the O-H stretching vibrations, thus demonstrating formation of a hydrogen bond between HBD and HBA. An example of FT-IR spectra of DES (ChCl : 4MPh, mole ratio 1:2) and pure 4MPh, in which a noticeable shift in the wavenumber from 3285 to 3200 cm^{-1} can be observed, is shown in Figure 2. FT-IR spectra of the remaining DESs are depicted in Figure S1. The same shifts are observed for DESs in the other mole ratios: 1:3, 1:4 and 1:5.

3.2. Optimization of derivatization and extraction conditions

Optimization of extraction and derivatization conditions was carried out for the analytes, *i.e.*, dodecanoic, decanoic, nonanoic, 10-undecylenic, and 2,4-dimethylbenzoic acids by comparison of peak areas of individual peaks. The process was optimized in terms of kind and volume of DES and disperser solvent, salting out, pH, extraction time, injection port temperature and purge off time.

3.2.1. Kind of DES and its volume

The kind of deep DES and its volume have a major effect on the efficiency of both extraction and derivatization of organic acids, since DES plays a dual role: it is both an extraction solvent and a derivatizing agent. It forms ion pairs with the analytes which are then converted to methyl esters in the GC injection port. Several deep eutectic solvents consisting of ChCl (HBA) and various HBDs, including 2MPh, 3MPh, 4MPh, 4EtPh and 4CPh in mole ratios (HBD : HBA) 1:2, 1:3, 1:4 and 1:5 were tested. Structures of the investigated HBA and HBDs are shown in Figure S2.

Among the DESs studied, the highest extraction and derivatization yield for the majority of analytes was obtained for ChCl : 4MPh in 1:2 mole ratio. Very similar results were also obtained for the DESs composed of ChCl and the other two cresol isomers, *i.e.*, 2MPh and 3MPh. Only for 2,4-dimethylbenzoic acid the highest yield of extraction and derivatization were obtained with ChCl : 4CPh (1:2). In each case, the effectiveness of extraction and derivatization gradually decreased with an increase of the amount of HBD in DES (Figure 3). On the one hand, the increase in HBD content should improve extraction yield as it results in reduction of density and viscosity of DESs [37-38], which in turn increases the effectiveness of emulsification of the extraction solvent in the aqueous phase– this effect was also observed in another paper [39]. On the other, however, the increase in HBD content brings about a decrease in concentration of ChCl responsible for the formation of ion pairs, which requires excess ChCl, thus lowering the yield of derivatization [7].

The next optimization step involved the effect of DES (ChCl : 4MPh 1:2) volume in the 400 to 800 µL range (Figure 4), which was mixed with 500 µL of MeOH and added to 9 mL of a sample. When using 400 µL of the DES, only a few microliters of the organic phase were obtained following extraction, which precluded the use of an autosampler, thus deteriorating precision of the results. Consequently, this volume was excluded from further considerations. An increase in DES volume in the investigated range resulted in a decrease in peak areas of the analytes due to a greater volume of the sedimented phase. Ultimately, 500 µL of ChCl : 4MPh (1 : 2) were used in further investigations.

3.2.2. Disperser solvent and its volume

Several disperser solvents were tested in the investigations: AC, ACN, IPA and MeOH; 500 µL of each of the solvents were mixed with 500 µL of DES (ChCl : 4MPH 1:2) and added to 9 mL of a sample. The selected DES was insoluble in AC, so this solvent was excluded from subsequent experiments. Among the disperser solvents studied, the highest extraction yield for all the analytes was obtained with methanol (Figure 5).

The effect of volume of the disperser solvent (MeOH) was studied next. The following volumes were used: 0, 300, 500, 700 and 900 µL. The obtained results, shown in Figure 6, reveal that the extraction yield increases with an increase in disperser solvent volume from 0 to 500 µL, followed by a decrease of the yield with a further increase in the volume of MeOH. This decrease of extraction yield can be attributed to increases in the volume of organic phase and solubility of the analytes in the aqueous phase of samples. On the other hand, too small a volume or lack of the disperser solvent resulted in a low effectiveness of extraction.

3.2.3. pH

One of the more important parameters affecting the yield of extraction and derivatization is pH which should be higher than the pK_a of all of the investigated analytes (pK_a range of 4.2 – 5.3) in order to form their deprotonated forms. Analytes in their ionized form are obtained by raising the pH (+ 2 pK_a) [[40]-[41]]. Accordingly, the following pH values were tested 3, 5, 7, 9, 11 and 13 (Figure 7). As expected, a gradual increase in the extraction and derivatization yield was observed when the pH was raised from 3 to 9 while further increase resulted in a drop in the effectiveness of extraction and derivatization. Consequently, subsequent investigations were carried out at pH 9.

3.2.4. Salting out effect

The salting out effect was examined by adding NaCl at the following concentrations: 0, 5, 10 and 20 [% w/v]. The presence of salts can theoretically improve extraction yield by decreasing the solubility of analytes in the aqueous phase, thus facilitating their transfer to the organic phase. On the other hand, however, it can increase density and viscosity of samples, thus reducing the effectiveness of dispersion of the extraction solvent. The investigations revealed that the addition of even a small amount of salt reduced the extraction yield (Figure S3). Consequently, subsequent studies were carried out without salt addition.

3.2.5. GC injection port temperature and kind of liner

Theoretically, the injection port temperature should have a significant effect on conversion of ion pairs into methyl esters. Higher temperature can improve the yield of derivatization reaction. However, an excessively high temperature can cause decomposition of the derivatives, appearance of extraneous peaks of decomposition products in the chromatogram and lowered derivatization yield [42]. Thus, the injection port temperatures studied were 280, 300 and 320°C. The obtained results (Figure S4) reveal that the injection port temperature has a minor effect on the yield of derivatization in the investigated range which is consistent with a previous work [7]. Therefore, subsequent experiments were carried out at 300°C, which allows formation of methyl esters while preventing the effect of high temperature on the septum resulting in its decomposition and appearance of ghost peaks in the chromatogram.

An inlet liner for splitless injections packed with silanized glass wool was used in the investigations. In the analysis of effluents with a complex matrix the wool plays an important role. It ensures faster evaporation of the sample and prevents transfer of nonvolatile components of the effluent onto the GC column. The glass wool did not affect the derivatization process. Obtained results for pure standards using liner with and without wool were comparable.

3.2.6. Duration of splitless injection

Duration of splitless injection should be long enough to introduce all the derivatized analytes onto the column; however, a too long time can result in peak tailing thus adversely affecting limits of detection and quantitation [43-44]. Thus, the following duration of splitless injection were examined: 0.5, 1, 2 and 3 min. The results shown in Figure 8 reveal a significant increase in detector response from 0.5 to 2 min followed by a gradual decrease in peak areas after 3 min. Furthermore, with a duration of splitless injection of 3 min, peak tailing was observed. Consequently, in subsequent studies a duration of splitless injection of 2 min was used.

3.2.7. Sonication time

In this study, two methods of intensification of extraction were compared: manual agitation and sonication. The time of manual agitation ranged from 20 to 120 s. The obtained results revealed an increase in the extraction yield after 60 s. Similar results were obtained in other studies [7,45]. Further extension of time of manual agitation did not improve the extraction yield. The analyte response factors after 60 s of both manual agitation and sonication were very similar. However, extending sonication time in the 1 min to 15 min range resulted in an increase of the extraction yield, which was not observed for manual agitation. Further extension of sonication time to 20 min did not significantly improve analyte enrichment (Figure S5). Due to favorable effect of sonication on the formation of dispersion in samples, subsequent extractions were carried aided by 15-min sonication.

3.3. Validation of procedure

Quantitative analysis was carried out by the internal standard method using 2-chlorobenzoic acid as the internal standard. To this end, a 10-point calibration curves were determined over the 0.005-50 µg/mL concentration range. Two characteristic mass-to-charge (m/z) ratios were selected for each analyte, including Target Ion which was used for calibration and determination of analyte concentrations in real samples, and Qualifier Ion, which was used to confirm identification of derivatives of the acids by comparison of the areas of Target Ion to Qualifier Ion (Table 1).

The limits of detection and quantitation ranged from 1.7 to 8.3 µg/L and from 5.1 to 25 µg/L, respectively. A wide linear range and coefficents of determination (R^2) ranging from 0.9988 to 0.9999 were obtained. Analytical characteristics of the developed procedure is compiled in Table 1.

The analyte recovery (R) from deionized water and from a sample of real effluent for all the investigated carboxylic acids was determined by spiking the samples with 10 µg/mL of the analytes. Satisfactory recoveries were obtained in both cases, ranging from 68.8 to 105% and from 72.2 to 106%, respectively. In addition, the precision of the developed procedure was determined by calculating relative standard deviation (RSD) values from the results of analyses performed on the same day (Intra-day RSD) as well as those performed during three consecutive days (Inter-day RSD). The obtained results of RSD, ranging from 2.5 to 6.0% and from 4.5 to 6.7%, demonstrate good precision of the developed procedure. All the R and RSD values are compiled in Table 2.

A comparison of the developed procedure with other procedures for the determination of carboxylic acids in samples of effluents revealed that the proposed procedure offers lower detection limits and a wider linear range than the existing procedures while being competitive with respect to the precision (Table 3).

3.4. Analysis of real effluents

The developed procedure was used for the analysis of samples of real effluents, both raw and subjected to treatment by hydrodynamic cavitation alone or combined with oxidation using hydrogen peroxide. The analytes were identified based on the ratio of intensities of characteristic ions listed in Table 2, taking the confidence interval of ± 15%, and also based on the values of retention times ± 0.2 min.

In samples of raw effluents (I) and (II), 11 carboxylic acids were identified at concentrations ranging from 0.446 to 37.3 µg/mL, the most abundant being benzoic, octanoic, nonanoicand decanoic acids (Figure 9). In samples of effluents treated solely by hydrodynamic cavitation, fatty acids were oxidized to a small extent with the percent degradation not exceeding 16.2%. On the other hand, a substantial increase in concentration of benzoic acids was observed. The increase in content of benzoic, 2-methylbenzoic and 4-methylbenzoic acids is likely due to oxidation of toluene and xylene isomers [48] whereas the increase in concentration of 2-hydroxybenzoic acid can be explained by oxidation of phenol [49], *i.e.,* the compounds which occur in substantial amounts in effluents from the production of bitumens [45,52-54]. Increased concentrations of benzoic and 4-methylbenzoic acids can also be observed in samples of effluents treated by HC/H_2O_2. For the remaining benzoic acids as well as fatty acids, degradation ranging from 13.6 to 44.9% was observed. The obtained results reveal a poor effectiveness of HC treatment in degrading carboxylic acids. To improve the rate of removal of carboxylic acids, application of additional oxidants, such as hydrogen peroxide, is required.

The identified carboxylic acids along with their concentrations are listed in Table 4.

4. Summary

The paper presents a novel procedure for the determination of carboxylic acids in aqueous matrices and demonstrates its usefulness in the analysis of industrial effluents having a complex matrix by IP-USA-DLLME-GC-MS. This is the first report presenting the procedure based on the use of a deep eutectic solvent (ChCh:4MPh 1:2) for both extraction of the analytes and their derivatization. Such an approach greatly simplifies the entire sample preparation step and reduces the time of analysis, which makes it a viable alternative to time-consuming procedures commonly used for the determination of carboxylic acids in aqueous samples. The use of inexpensive and simple to synthesize deep eutectic solvents, considered to be green organic solvents of a new generation, to extract analytes eliminates consumption of popular but toxic chlorinated solvents. At the same time, it demonstrates that the developed procedure is environmentally friendly. The determined validation parameters, including low LOD and LOQ values, a wide linear range, high recovery and RSD values within an acceptable range confirm the usefulness of the procedure to determine low concentrations of carboxylic acids in samples with a complex matrix.

Most of the recent reviews on effluent treatment processes do not provide details on the removal of specific contaminants from real effluents, instead offering total parameters, such as COD and BOD or TOC. On the other hand, the studies of model effluents usually involve changes in concentration of model contaminants [55-56]. Consequntly, characterization of effluents can be incomplete especially in terms of formation of secondary contaminants. This can be attributed to poor recognition of advantages of modern chromatographic techniques among scientists dealing with effluent treatment technologies as well as to the lack of procedures for analysis of a wide variety of contaminants in real effluents. The present paper is intended to partially this void. Effluents from the production of bitumens contain substantial amounts of fatty and benzoic acids. The analysis of these effluents treated by hydrodynamic cavitation reveals an increase in concentration of benzoic acids as a result of oxidation of other organic compounds while the effectiveness of degradation of fatty acids is low. To improve the effectiveness of treatment, the use of additional oxidants, such as hydrogen peroxide, is necessary.

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Table 1 Analytical characteristics of the developed procedure

Compound	t_R [min]	Target ion	Qualifier ion	Calibration curve	R^2	LOD[a] [µg/L]	LOQ[b] [µg/L]	Linear range[c] [µg/L]
Benzoic acid	14.21	105	136	$y = 0.6496x + 0.0161$	0.9988	8.3	25	25 ± 48000
Octanoic acid	14.44	74	87	$y = 1.2780x + 0.0940$	0.9988	5.1	15	15± 57000
2-methylbenzoic acid	15.21	119	150	$y = 6.8927x + 0.0506$	0.9991	3.2	9.6	9.6 ± 70000
2-hydroxybenzoic acid	15.29	120	152	$y = 0.4988x + 0.0078$	0.9994	5.4	16	16 ± 42000
4-methylbenzoic acid	15.35	119	150	$y = 7.4330x + 0.0456$	0.9999	3.2	9.6	9.6 ± 71000
Nonanoicacid	15.57	74	87	$y = 4.3579x + 0.0208$	0.9999	1.7	5.1	5.01 ± 49000
2-chlorobenzoic acid (IS)	16.46	139	170	-	-	-	-	-
2,4-dimethylbenzoic acid	17.12	133	164	$y = 5.7719x + 0.0061$	0.9999	2.2	6.6	6.6 ± 52000
Decanoicacid	17.90	74	87	$y = 3.4120x + 0.0535$	0.9999	2.3	6.9	6.9 ± 46000
10-undecylenic acid	19.45	74	87	$y = 1.4223x + 0.0071$	0.9989	7.3	22	22 ± 50000
4-tert-butylbenzoic acid	20.95	177	192	$y = 4.0612x + 0.0036$	0.9998	2.4	7.2	7.2 ± 42000
Dodecanoicacid	22.99	74	87	$y = 3.2876x + 0.0025$	0.9997	2.1	6.3	6.3 ± 43000

[a] *The limit of detection (LOD) was calculated from: LOD = 3·S/N (S - signal, N – noise).*

[b] *The limit of quantitation (LOQ) was calculated from: LOQ = 3·LOD.*

[c] *The linearity of calibration curve was estimated using the correlation coefficient (r). In order to confirm an appropriate selection of the linear range, a standard residual analysis was performed [46].*

Table 2 Recovery of analytes and relative standard deviation of determinations

Compound	R [%] [a]	R [%] [b]	Intra-day RSD[c] [%]	Inter-day RSD [d] [%]
Benzoic acid	68.8	72.2	5.4	6.0
Octanoic acid	81.5	82.1	5.1	6.7
2-methylbenzoic acid	98.7	99.1	3.3	4.5
2-hydroxybenzoic acid	105.3	106.1	6.0	6.4
4-methylbenzoic acid	93.7	95.1	4.0	5.6
Nonanoic acid	88.4	92.2	6.0	6.3
2,4-dimethylbenzoic acid	98.4	99.3	2.5	4.9
Decanoic acid	87.8	91.7	4.4	6.0
10-undecylenic acid	96.8	97.2	4.4	6.4
4-*tert*-butylbenzoic acid	97.2	100.0	5.2	5.7
Dodecanoicacid	90.8	93.2	5.3	6.1

[a] *Recovery (R) determined after addition of 10 µg/mL of analyte to deionized water. (Recovery (R) was calculated from:* $R[\%] = \frac{C_{quant}-C_0}{C_{expect}} \cdot 100\%$*, where:* $C_{quant.}$*– found analyte concentration in spiked sample [µg/mL],* $C_{expect.}$ *– analyte concentration added as spike [µg/mL],* C_0*– found analyte concentration in non-spiked samples [µg/mL]).*

[b] *Recovery (R) determined after addition of 10 µg/mL of analyte to real effluent.*

[c] *Intraday RDS determined after addition of 10 µg/mL of analyte to deionized water and 4 analyses on the same day.*

[d] *Interday RDS determined after addition of 10 µg/mL of analyte to deionized water and 6 analyses in 3 consecutive days.*

Table 3 Comparison of the developed procedure with other procedures found in the literature

Analytes	**Matrix**	**Method**	**Derivatizing agent**	**LOD [µg/L]**	**RSD [%]**	**Linear range [µg/L]**	**Ref.**
Aliphatic acids C_2-C_8	Municipal wastewater	DI/GC-FID	-	16 – 85	<9	0.1 – 500000	[47]
Aliphatic acids C_3 – C_{12}, Benzoic acids	Industrial effluents	IP-DLLME/ GC-MS	TBA-HSO_4	6.9 -1120	< 6.42	50 - 50000	[7]
Aliphatic acids C_2-C_6	Municipal wastewater	DI/GC-FID	-	600 - 3100	< 5.1	10000 - 300000	[12]
Aliphatic acids C_2-C_6	Municipal wastewater	TD/GC-FID	-	400 - 2600	< 2.8	10000 - 300000	[12]
Aliphatic acids C_2-C_7	Urban wastewater	HS-SPME/ GC-NCI-MS a)	-	2 - 150	<16	10 - 3000	[50]
Aliphatic acids C_2-C_7	Urban wastewater	HS-SPME/ GC-PCI-MS b)	-	10 -120	< 37	16 - 2700	[50]
Aliphatic acids C_2-C_7	Urban wastewater	HS-SPME/ GC-FID	-	6 - 680	< 16	5 - 45000	[50]
2-hydroxybenzoic acid	Urban wastewater	SPE/LC-ESI-MS	-	0.015	13	0.5 - 25	[51]
Aliphatic acids C_8 – C_{12}, Benzoic acids	Industrial effluents	IP-USA- DLLME/ GC-MS	DES (ChCl : 4MPh)	1.7 – 8.3	<6.68	5 –50000	This work

a) *NCI- Negative Ion Chemical Ionisation*

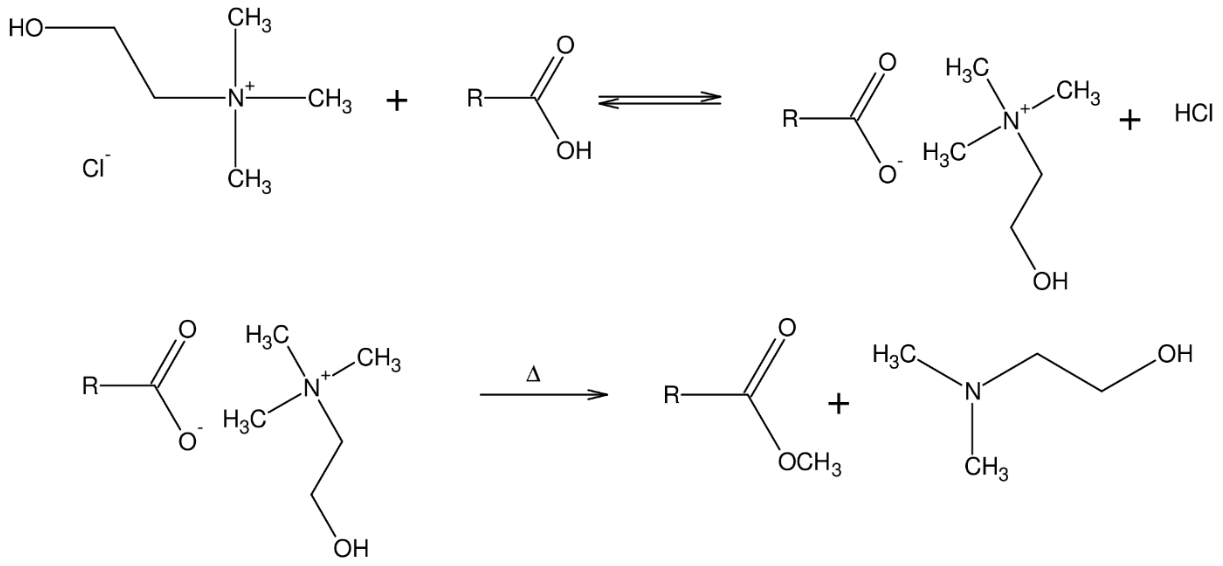
b) *PCI- Positive Ion Chemical Ionisation*

Table 4 Concentrations of identified analytes along with percent degradation after various treatment methods. Numbers in bold indicate increase in concentration following treatment

Compound	**Concentration ± SD a) [µg/mL]**					
	Raw effluent (I)	**HC/H_2O_2**	**Degradation [%]**	**Raw effluent (II)**	**HC**	**Degradation [%]**
Benzoicacid	36.6 ± 2.0	**43.3 ± 2.4**	- **18.12**	37.3 ± 2.2	**41.27 ± 2.34**	- **10.76**
Octanoicacid	30.1 ± 1.7	29.1 ± 1.7	3.45	30.2 ± 2.1	28.22 ± 1.81	6.62
2-methylbenzoic acid	2.63 ± 0.11	1.878 ± 0.080	28.52	3.11 ± 0.14	**4.23 ± 0.17**	- **36.01**
2-hydroxybenzoic acid	3.39 ± 0.21	2.93 ± 0.18	13.57	3.26 ± 0.18	**6.40 ± 0.36**	- **96.32**
4-methylbenzoic acid	7.19 ± 0.32	**8.48 ± 0.39**	- **17.94**	8.03 ± 0.39	**10.21 ± 0.51**	- **27.15**
Nonanoicacid	21.0 ± 1.3	14.53 ± 0.90	30.78	21.9 ± 1.3	20.6 ± 1.2	6.02
2,4-dimethylbenzoic acid	5.87 ± 0.22	3.81 ± 0.15	35.12	5.65 ± 0.24	4.11 ± 0.21	27.26
Decanoicacid	17.47 ± 0.90	9.63 ± 0.52	44.88	17.2 ± 1.0	15.98 ± 0.91	7.36
10-undecylenic acid	<LOD	<LOD	-	<LOD	<LOD	-
4-*tert*-butylbenzoic acid	0.446 ± 0.023	0.329 ± 0.019	26.67	0.573 ± 0.033	0.484 ± 0.025	16.23
Dodecanoicacid	11.83 ± 0.68	6.82 ± 0.40	42.35	11.78 ± 0.69	11.65 ± 0.70	1.10

a) *SD – standard deviation (n = 3).*

515 **Figures**



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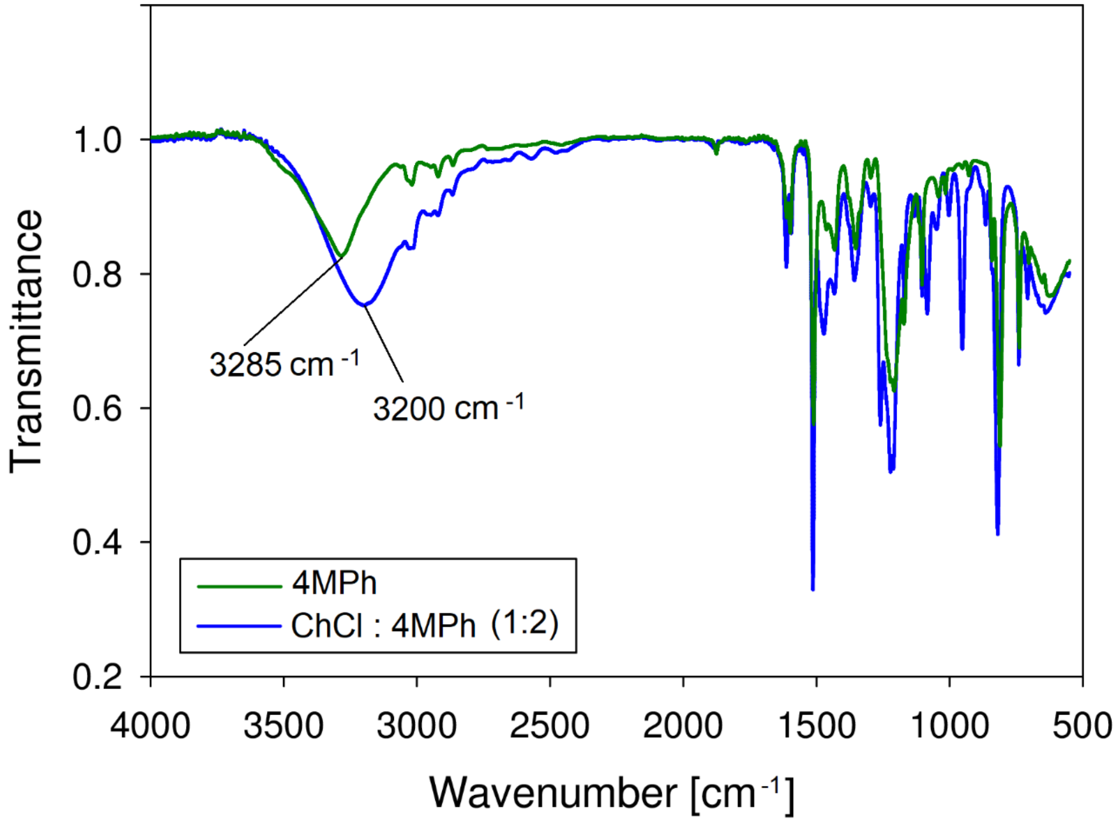
517 **Figure 1** Derivatization of carboxylic acids



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519 **Figure 2** FT-IR spectra of 4MPh and ChCl:4MPh (1:2)

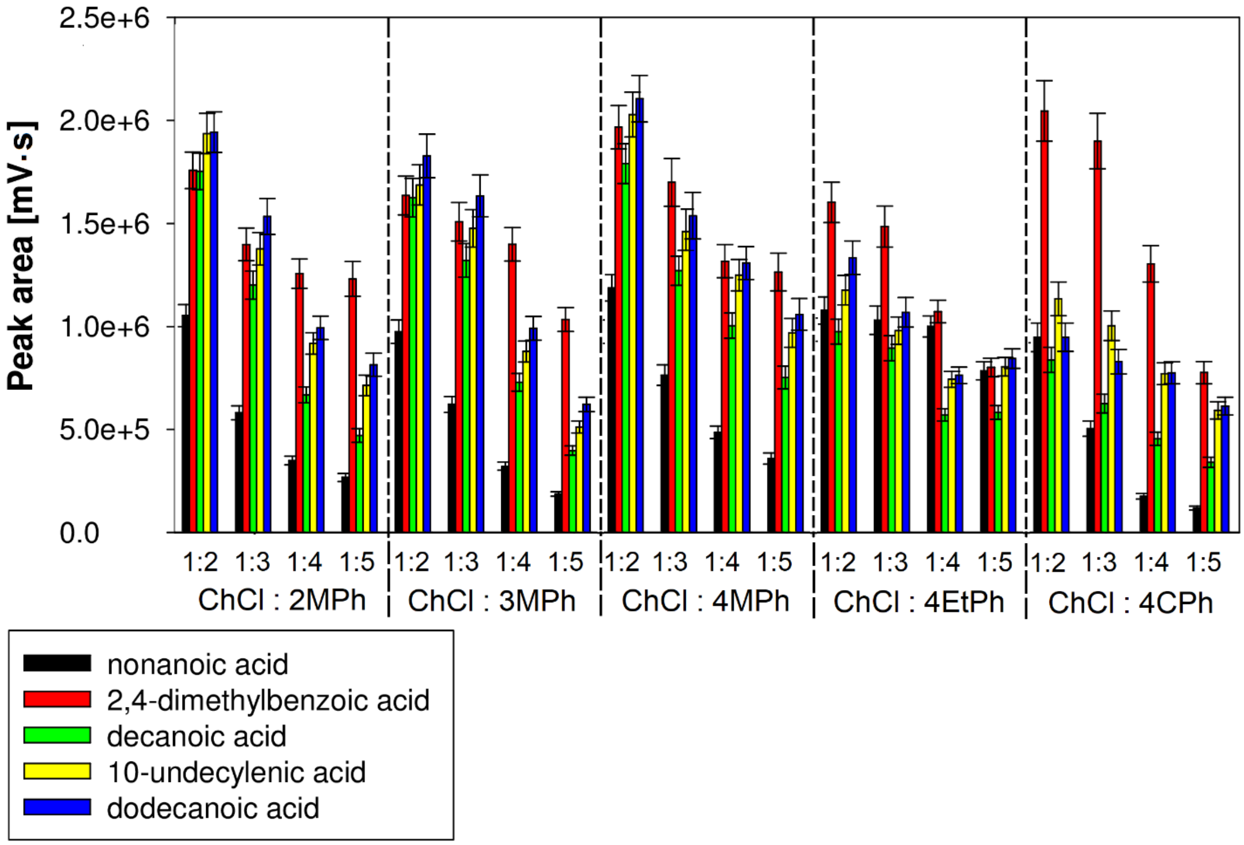

Figure 3 The effect of various deep eutectic solvents on the yield of extraction and derivatization

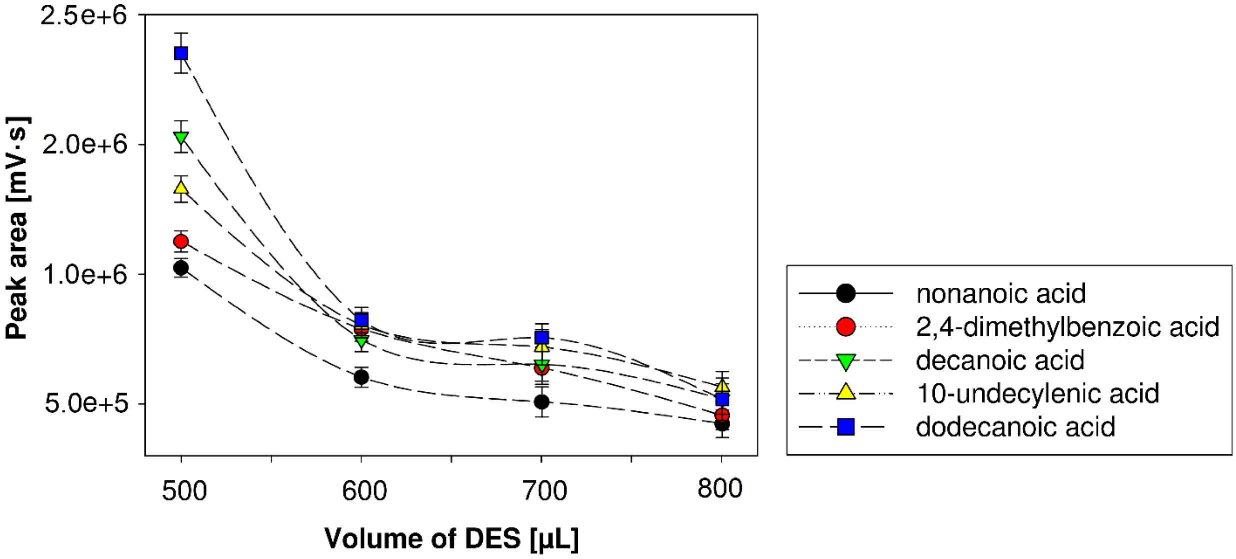

Figure 4 The effect of DES volume on the yield of extraction and derivatization

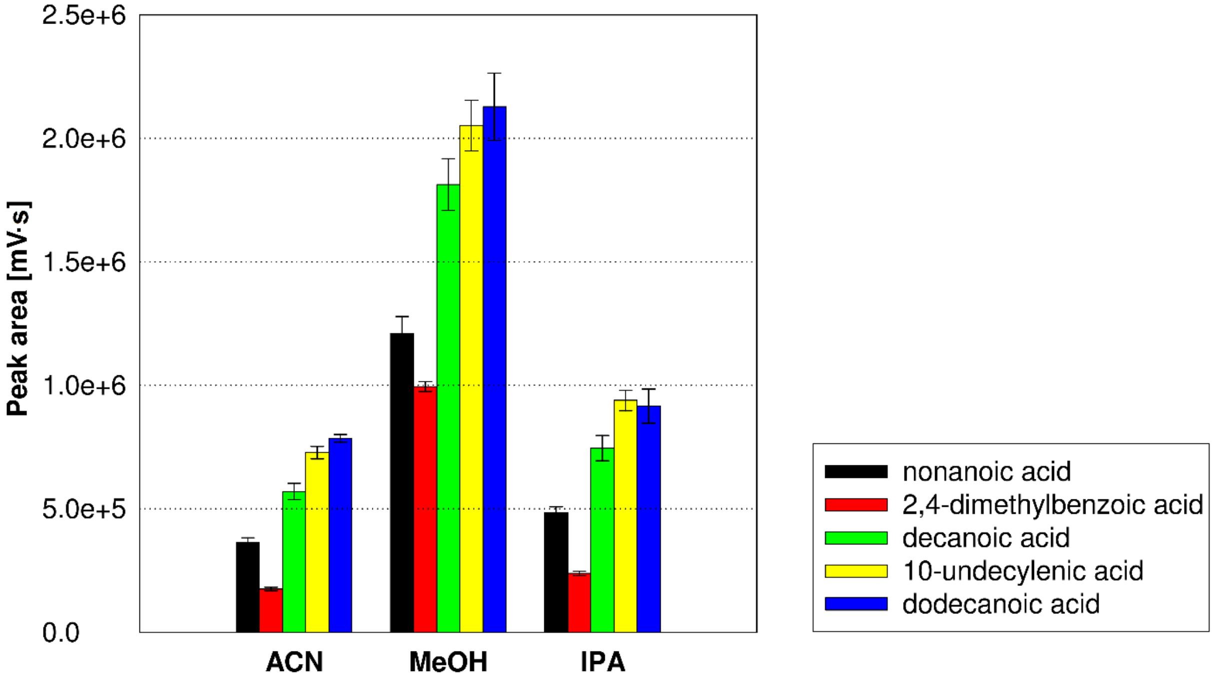

Figure 5 The effect of various disperser solvents on the extraction yield

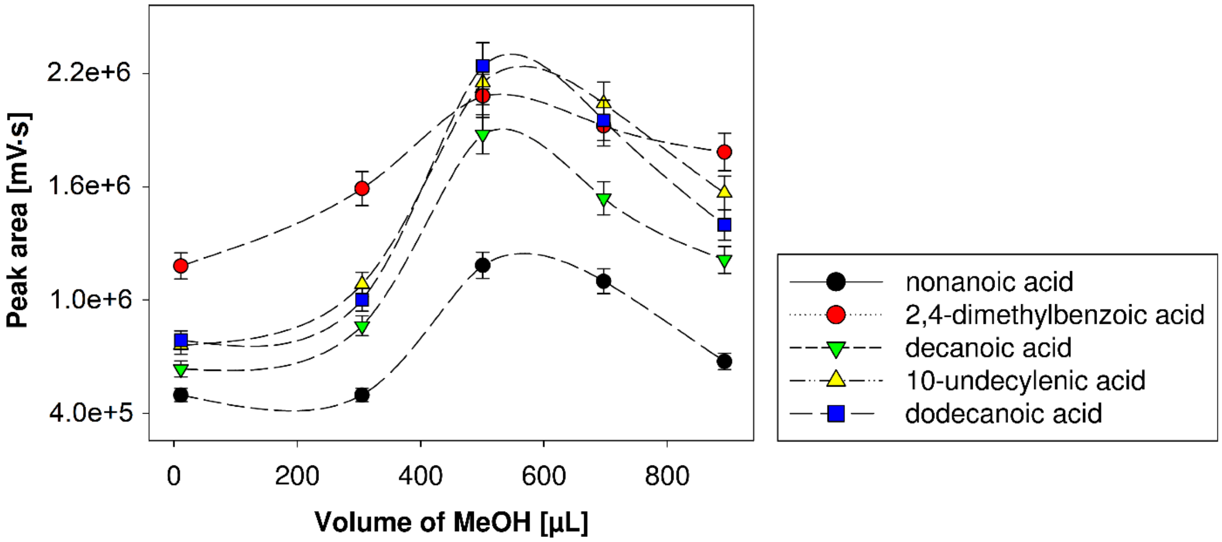

Figure 6 The effect of disperser solvent volume on the extraction yield

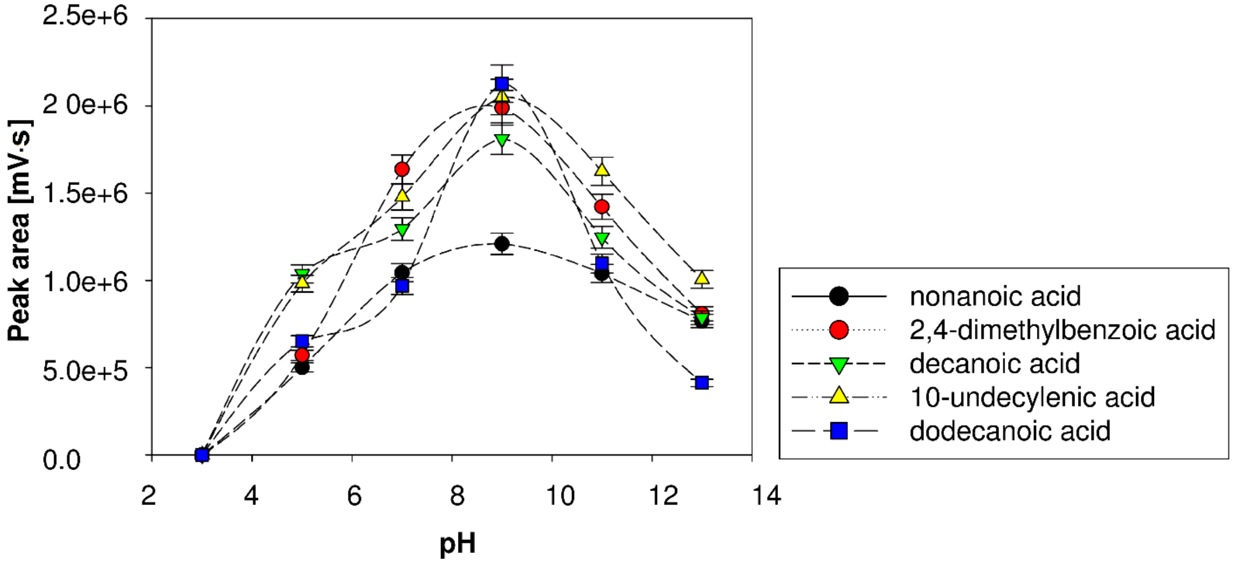

Figure 7 The effect of pH on the yield of extraction and derivatization

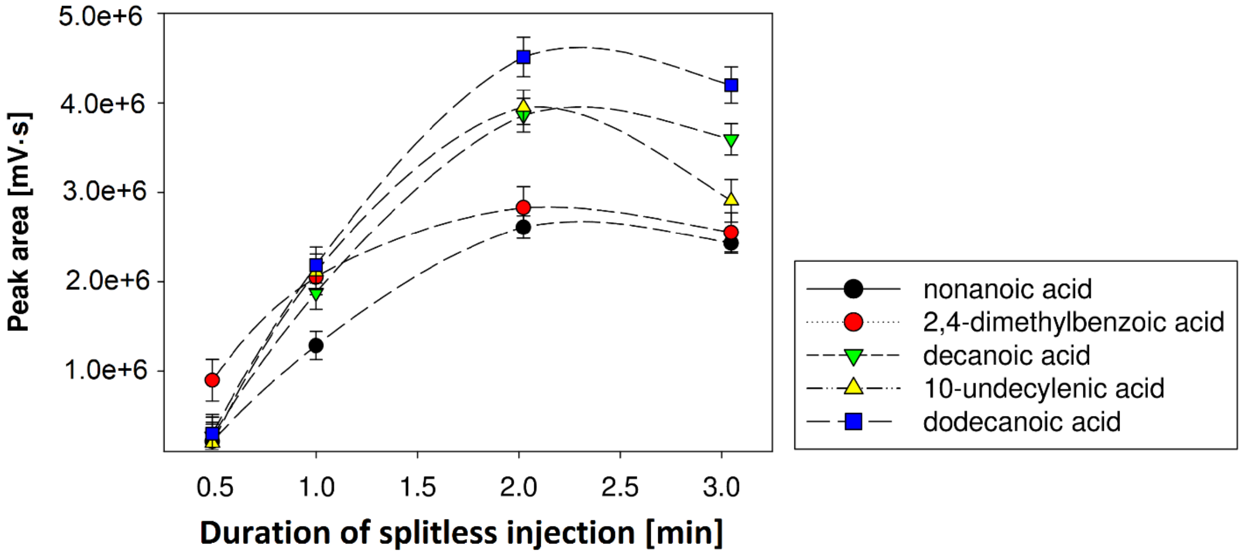

Figure 8 The effect of duration of splitless injection on the yield of derivatization

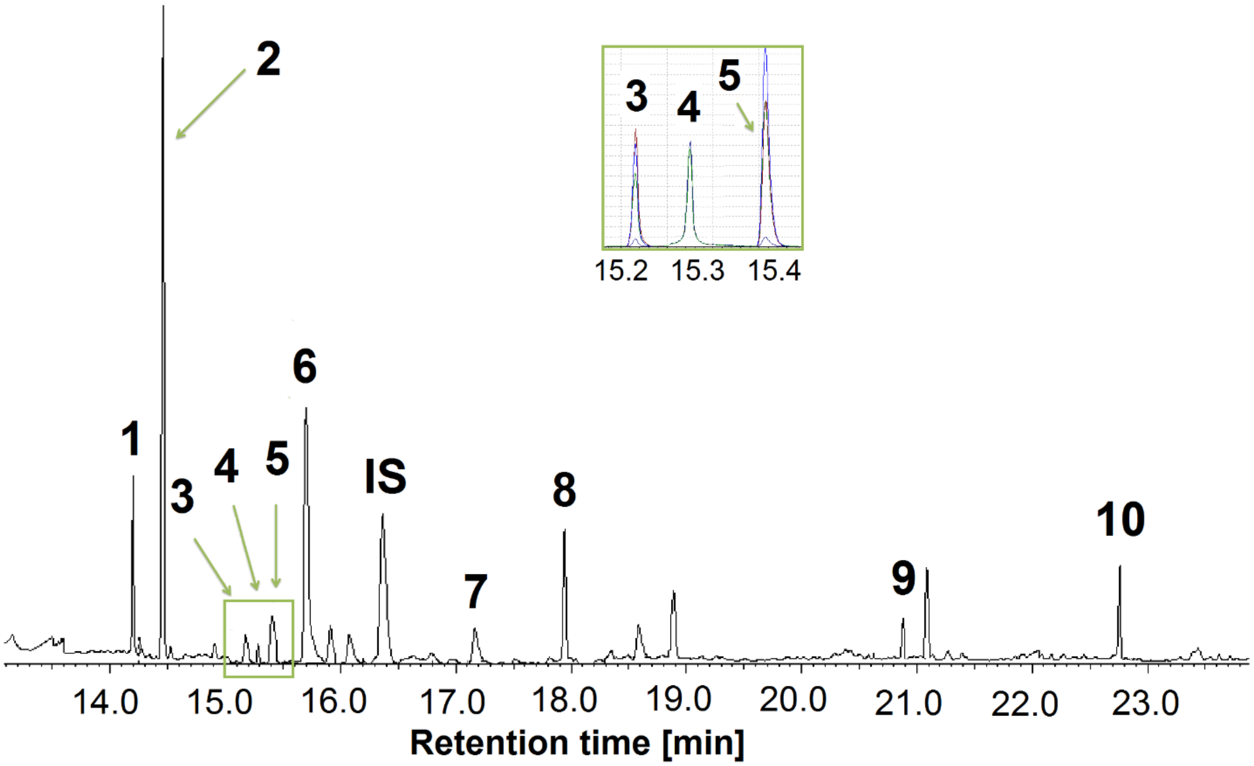

Figure 9 Chromatogram of real raw effluents. Identified compounds: 1) benzoic acid, 2) octanoic acid, 3) 2-methylbenzoic acid, 4) 2-hydroxybenzoic acid, 5) 4-methylbenzoic acid, 6) nonanoic acid, 7) 2,4-dimethylbenzoic acid, 8) decanoic acid, 9) 4-tert-butylbenzoic acid, 10) dodecanoic acid