

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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33 1. Introduction

34
35 Carboxylic acids, such as volatile fatty acids, benzoic and hydroxybenzoic acids occur commonly in
36 municipal wastewater [1-2], and also at relatively high concentrations in industrial effluents, including
37 paper [3-4], pharmaceutical [5], textile, tanning [6], or petroleum industries [7]. As a result of
38 malodorous nature of the most volatile acids, potential toxicity of individual compounds and a negative
39 impact on the aqueous environment, carboxylic acids have been the subject of numerous studies
40 concerning the technology of their removal [8-10], and the development of procedures for their
41 determination at low concentration levels.

42 At present, the determination of carboxylic acids in samples with aqueous matrices involves
43 chromatographic techniques, primarily gas chromatography. However, due to specific properties of
44 carboxylic acids, *i.e.* high polarity, high boiling point and low volatility of a large number of the acids, only
45 a small fraction of them (including volatile fatty acids from C₁ to C₁₂) can be subjected to direct GC
46 analysis [11-12]. Hence, it is often necessary to derivatize analytes through esterification, alkylation or
47 silylation. The majority of available derivatization procedures is based on application of alkylsilane
48 derivatizing agents which form unstable derivatives and the time of reaction can be as long as 24 hours
49 [13]. Other commonly used derivatization methods require strongly toxic reagents [14-15] or reagents
50 characterized by a low derivatization yield [16]. Among the available procedures, only a few can be
51 considered environmentally friendly and free from the above shortcomings. Examples include the use of
52 alkylchloroformates in esterification [17] or, recently popular, derivatization resulting in formation of ion
53 pairs making use of nontoxic quarternary ammonium salts, such as tetramethylammonium chloride
54 (TMA-Cl), tetramethylammonium acetate (TMAAc), tetrabutylammonium hydrogensulfate (TBA-HSO₄),
55 tetrabutylammonium chloride (TBA-Cl), tetrabutylammonium bromide (TBA-Br) or tetrabutylammonium
56 iodide (TBA-I) [7,18]. The latter procedure has a number of advantages due to its simplicity of
57 derivatization, which involves addition of a derivatizing agent and a buffer solution. Thus formed ion
58 pairs are converted to esters in the hot GC injection port.

59 Determination of low concentrations of organic acids in wastewater samples requires an appropriate
60 extraction technique which, in accordance with principles of green chemistry, should be simple, rapid,
61 automatable and using only small volumes of organic solvents. One of the sample preparation
62 techniques meeting the above criteria is dispersive liquid-liquid microextraction (DLLME) introduced in
63 2006 [19]. However, since the inception of this technique it was pointed out that it makes use of mostly
64 hazardous chlorinated organic solvents with a density higher than water, which facilitates separation of
65 phases and transfer of the extract to the GC injection port or to autosampler vials. Solvents having
66 density lower than water can also be used but collection of the extract is more difficult as it requires an
67 additional step of solidification of organic drop or the use of special vials which are not yet available
68 commercially [20-22].

69 An alternative to toxic chlorinated solvents are deep eutectic solvents (DESs). These are liquids which
70 are formed upon mixing two solids (at ambient temperature), typically quarternary ammonium salts
71 being hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD), whose melting points are much
72 lower than those of individual components [23]. In addition, DESs have physico-chemical properties
73 similar to ionic liquids; however, the synthesis of DESs is much simpler and cheaper and they are less
74 toxic and easier to biodegrade than ionic liquids [24]. The majority of papers refer to synthesis and use of



75 hydrophilic DESs [23,25-27], and only a few mention hydrophobic DESs which can be potentially used as
76 extracting solvents for samples with an aqueous matrix [28-33].

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

82

83 **2. Experimental**

84 **2.1. Reagents**

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

97 **2.2. Real samples**

98 Effluents from the production of bitumen 20/30 from vacuum bottom of crude oil Rebc0:Kirkuk
99 65:35 m/m (mixture of Russian and Iraqi crude oils) were collected behind a plate separator which
100 separated condensed organic phase from aqueous phase. The aqueous phase of raw effluents was
101 investigated along with the effluents subjected to various chemical treatments, including hydrodynamic
102 cavitation (HC) as well as hydrodynamic cavitation combined with oxidation by hydrogen peroxide
103 (HC/H₂O₂). A detailed characteristic of the effluents was provided in previous papers [34-36]. In the
104 study, 300 mL of raw effluents and effluents after treatment were collected in 350-mL glass bottles.

105 **2.3. Apparatus**

106 A model QP2010 GC-MS SE gas chromatograph-mass spectrometer (Shimadzu, Japan) equipped with
107 a combi-PAL AOC 5000 autosampler (Shimadzu, Japan) and an HP-5 MS (30 m x 0.25 mm x 0.25 μm)
108 capillary column (Agilent, USA) were used in the investigations. A PerkinElmer Autosystem XL gas
109 chromatograph with flame ionization detector (PerkinElmer, USA) and an HP-1 (30 m x 0.53 mm x 1.5
110 μm) capillary column (Agilent, USA) were employed in optimization of operational parameters.
111 LabSolutions software (Shimadzu, Japan) with NIST 14 mass spectra library and TurboChrom 6.1
112 (PerkinElmer, USA) were used for data management. An RK 156 BH ultrasonic bath (BANDELIN electronic
113 GmbH & Co. KG, Germany) was used for extraction and an EBA 8S centrifuge (Hettich, Germany) was



114 used for the separation of extracts from samples. FT-IR spectra were recorded using a Bruker Tensor 27
115 spectrometer (Bruker, USA) with KBr pellet holder and OPUS software (Bruker, USA).

116 **2.4. Synthesis and characteristics of deep eutectic solvents**

117 Deep eutectic solvents were synthesized as follows: 1.39 g ChCl (HBA) and an appropriate amount of
118 HBD (including Ph, 2MPH, 3MPH, 4MPH, 2,6DMPH, 4EtPh, and 4CPh) so as to obtain mixtures with a mole
119 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
120 stirred magnetically at 50°C until a homogeneous liquid was obtained.

121 **2.5. Derivatization and extraction procedure**

122 A sample of effluent (9 mL) was transferred to a 12-mL vial followed by addition of 5 µL of a 5000
123 µg/mL solution of an internal standard (2-chlorobenzoic acid). Next, the solution pH was adjusted using a
124 7% HCl solution. 1.0 mL of a mixture consisting of DES (ChCl : 4MPH (1 : 2)) serving as an extracting
125 solvent/derivatizing agent and MeOH as a disperser solvent in a 1:1 volume ratio was then added to the
126 vial. The vial was closed tightly and placed in an ultrasonic bath at room temperature (RT) for 10 min,
127 followed by centrifugation for 5 min at 4000 rpm. Subsequently, 200 µL of the organic phase was
128 collected with an autopipette and transferred to 2-mL vials equipped with 300-µL micro-inserts. The
129 volume of the extract subjected to GC-MS analysis was 0.5 µL.

130 **2.6. Chromatographic conditions**

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

135 **2.7. FT-IR analysis**

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

140 **3. Results and discussion**

141 **3.1. Synthesis of DES**

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

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

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

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

153 Derivatization reaction using ChCl is shown in Figure 1.

154 Based on the above considerations, various DESs were synthesized by combining ChCl (HBA) with
155 phenolic compounds (HBD) in various mole ratios. Combination of HBA and HBD in mole ratios 1:0.5 and
156 1:1 did not yield clear liquids even after long stirring, which indicated that no deep eutectic solvents
157 were formed. In contrast, homogeneous liquid were obtained after combination of ChCl with phenols in
158 1:2, 1:3, 1:4 and 1:5 mole ratios. In the next step, the DESs containing Ph and 2,6DMPH were rejected
159 due to their high solubility and instability in water. FT-IR analyses were carried out to confirm the
160 formation of DESs. A characteristic feature in the IR spectrum of deep eutectic solvents is a shift of the
161 band corresponding to the presence of O–H group for a pure HBD toward a lower wavenumber. Bands
162 corresponding to stretching vibrations of the O-H group in all pure HBDs can be observed in the 3285 –
163 3371 cm^{-1} range. On the other hand, DESs are characterized by bands in the 3139–3200 cm^{-1} range,
164 which correspond to the N-H stretching vibrations characteristic of ChCl, which overlap with the O-H
165 stretching vibrations, thus demonstrating formation of a hydrogen bond between HBD and HBA. An
166 example of FT-IR spectra of DES (ChCl : 4MPH, mole ratio 1:2) and pure 4MPH, in which a noticeable shift
167 in the wavenumber from 3285 to 3200 cm^{-1} can be observed, is shown in Figure 2. FT-IR spectra of the
168 remaining DESs are depicted in Figure S1. The same shifts are observed for DESs in the other mole ratios:
169 1:3, 1:4 and 1:5.

170

171 3.2. Optimization of derivatization and extraction conditions

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

176 3.2.1. Kind of DES and its volume

177 The kind of deep DES and its volume have a major effect on the efficiency of both extraction and
178 derivatization of organic acids, since DES plays a dual role: it is both an extraction solvent and a
179 derivatizing agent. It forms ion pairs with the analytes which are then converted to methyl esters in the
180 GC injection port. Several deep eutectic solvents consisting of ChCl (HBA) and various HBDs, including
181 2MPH, 3MPH, 4MPH, 4EtPh and 4CPh in mole ratios (HBD : HBA) 1:2, 1:3, 1:4 and 1:5 were tested.
182 Structures of the investigated HBA and HBDs are shown in Figure S2.

183 Among the DESs studied, the highest extraction and derivatization yield for the majority of analytes
184 was obtained for ChCl : 4MPH in 1:2 mole ratio. Very similar results were also obtained for the DESs
185 composed of ChCl and the other two cresol isomers, *i.e.*, 2MPH and 3MPH. Only for 2,4-dimethylbenzoic
186 acid the highest yield of extraction and derivatization were obtained with ChCl : 4CPh (1:2). In each case,
187 the effectiveness of extraction and derivatization gradually decreased with an increase of the amount of
188 HBD in DES (Figure 3). On the one hand, the increase in HBD content should improve extraction yield as
189 it results in reduction of density and viscosity of DESs [37-38], which in turn increases the effectiveness

190 of emulsification of the extraction solvent in the aqueous phase– this effect was also observed in
191 another paper [39]. On the other, however, the increase in HBD content brings about a decrease in
192 concentration of ChCl responsible for the formation of ion pairs, which requires excess ChCl, thus
193 lowering the yield of derivatization [7].

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

201 **3.2.2. Disperser solvent and its volume**

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

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

214 **3.2.3. pH**

215 One of the more important parameters affecting the yield of extraction and derivatization is pH
216 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
217 form their deprotonated forms. Analytes in their ionized form are obtained by raising the pH (+ 2 pK_a)
218 [[40]-[41]]. Accordingly, the following pH values were tested 3, 5, 7, 9, 11 and 13 (Figure 7). As expected,
219 a gradual increase in the extraction and derivatization yield was observed when the pH was raised from 3
220 to 9 while further increase resulted in a drop in the effectiveness of extraction and derivatization.
221 Consequently, subsequent investigations were carried out at pH 9.

222 **3.2.4. Salting out effect**

223 The salting out effect was examined by adding NaCl at the following concentrations: 0, 5, 10 and 20
224 [% w/v]. The presence of salts can theoretically improve extraction yield by decreasing the solubility of
225 analytes in the aqueous phase, thus facilitating their transfer to the organic phase. On the other hand,
226 however, it can increase density and viscosity of samples, thus reducing the effectiveness of dispersion
227 of the extraction solvent. The investigations revealed that the addition of even a small amount of salt

228 reduced the extraction yield (Figure S3). Consequently, subsequent studies were carried out without salt
229 addition.

230 **3.2.5. GC injection port temperature and kind of liner**

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

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

245 **3.2.6. Duration of splitless injection**

246 Duration of splitless injection should be long enough to introduce all the derivatized analytes onto
247 the column; however, a too long time can result in peak tailing thus adversely affecting limits of
248 detection and quantitation [43-44]. Thus, the following duration of splitless injection were examined:
249 0.5, 1, 2 and 3 min. The results shown in Figure 8 reveal a significant increase in detector response from
250 0.5 to 2 min followed by a gradual decrease in peak areas after 3 min. Furthermore, with a duration of
251 splitless injection of 3 min, peak tailing was observed. Consequently, in subsequent studies a duration of
252 splitless injection of 2 min was used.

253 **3.2.7. Sonication time**

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

263 **3.3. Validation of procedure**

264 Quantitative analysis was carried out by the internal standard method using 2-chlorobenzoic acid as
265 the internal standard. To this end, a 10-point calibration curves were determined over the 0.005-50
266 $\mu\text{g/mL}$ concentration range. Two characteristic mass-to-charge (m/z) ratios were selected for each
267 analyte, including Target Ion which was used for calibration and determination of analyte concentrations
268 in real samples, and Qualifier Ion, which was used to confirm identification of derivatives of the acids by
269 comparison of the areas of Target Ion to Qualifier Ion (Table 1).

270 The limits of detection and quantitation ranged from 1.7 to 8.3 $\mu\text{g/L}$ and from 5.1 to 25 $\mu\text{g/L}$,
271 respectively. A wide linear range and coefficients of determination (R^2) ranging from 0.9988 to 0.9999
272 were obtained. Analytical characteristics of the developed procedure is compiled in Table 1.

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

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

285

286 **3.4. Analysis of real effluents**

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

292 In samples of raw effluents (I) and (II), 11 carboxylic acids were identified at concentrations ranging
293 from 0.446 to 37.3 $\mu\text{g/mL}$, the most abundant being benzoic, octanoic, nonanoic and decanoic acids
294 (Figure 9). In samples of effluents treated solely by hydrodynamic cavitation, fatty acids were oxidized to
295 a small extent with the percent degradation not exceeding 16.2%. On the other hand, a substantial
296 increase in concentration of benzoic acids was observed. The increase in content of benzoic, 2-
297 methylbenzoic and 4-methylbenzoic acids is likely due to oxidation of toluene and xylene isomers [48]
298 whereas the increase in concentration of 2-hydroxybenzoic acid can be explained by oxidation of phenol
299 [49], *i.e.*, the compounds which occur in substantial amounts in effluents from the production of
300 bitumens [45,52-54]. Increased concentrations of benzoic and 4-methylbenzoic acids can also be
301 observed in samples of effluents treated by HC/ H_2O_2 . For the remaining benzoic acids as well as fatty
302 acids, degradation ranging from 13.6 to 44.9% was observed. The obtained results reveal a poor
303 effectiveness of HC treatment in degrading carboxylic acids. To improve the rate of removal of carboxylic
304 acids, application of additional oxidants, such as hydrogen peroxide, is required.



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

306 **4. Summary**

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

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

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486 **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
Nonanoic acid	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
Decanoic acid	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
Dodecanoic acid	22.99	74	87	y = 3.2876x + 0.0025	0.9997	2.1	6.3	6.3 ± 43000

487 ^{a)} The limit of detection (LOD) was calculated from: $LOD = 3 \cdot S/N$ (S – signal, N – noise).

488 ^{b)} The limit of quantitation (LOQ) was calculated from: $LOQ = 3 \cdot LOD$.

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

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493 **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
Dodecanoic acid	90.8	93.2	5.3	6.1

494 ^{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
495 [µg/mL], C_0 – found analyte concentration in non-spiked samples [µg/mL]).

496 ^{b)} Recovery (R) determined after addition of 10 µg/mL of analyte to real effluent.

497 ^{c)} Intraday RDS determined after addition of 10 µg/mL of analyte to deionized water and 4 analyses on the same day.

498 ^{d)} Interday RDS determined after addition of 10 µg/mL of analyte to deionized water and 6 analyses in 3 consecutive days.

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

Analytes	Matrix	Method	Derivatizing agent	LOD [$\mu\text{g/L}$]	RSD [%]	Linear range [$\mu\text{g/L}$]	Ref.
Aliphatic acids $\text{C}_2\text{-C}_8$	Municipal wastewater	DI/GC-FID	-	16 – 85	<9	0.1 – 500000	[47]
Aliphatic acids $\text{C}_3\text{ – C}_{12}$, Benzoic acids	Industrial effluents	IP-DLLME/ GC-MS	TBA-HSO ₄	6.9 -1120	< 6.42	50 - 50000	[7]
Aliphatic acids $\text{C}_2\text{-C}_6$	Municipal wastewater	DI/GC-FID	-	600 - 3100	< 5.1	10000 - 300000	[12]
Aliphatic acids $\text{C}_2\text{-C}_6$	Municipal wastewater	TD/GC-FID	-	400 - 2600	< 2.8	10000 - 300000	[12]
Aliphatic acids $\text{C}_2\text{-C}_7$	Urban wastewater	HS-SPME/ GC-NCI-MS ^{a)}	-	2 - 150	<16	10 - 3000	[50]
Aliphatic acids $\text{C}_2\text{-C}_7$	Urban wastewater	HS-SPME/ GC-PCI-MS ^{b)}	-	10 -120	< 37	16 - 2700	[50]
Aliphatic acids $\text{C}_2\text{-C}_7$	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 $\text{C}_8\text{ – C}_{12}$, Benzoic acids	Industrial effluents	IP-USA- DLLME/ GC-MS	DES (ChCl : 4MPh)	1.7 – 8.3	<6.68	5 –50000	This work

504 ^{a)} NCI- Negative Ion Chemical Ionisation

505 ^{b)} PCI- Positive Ion Chemical Ionisation

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

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

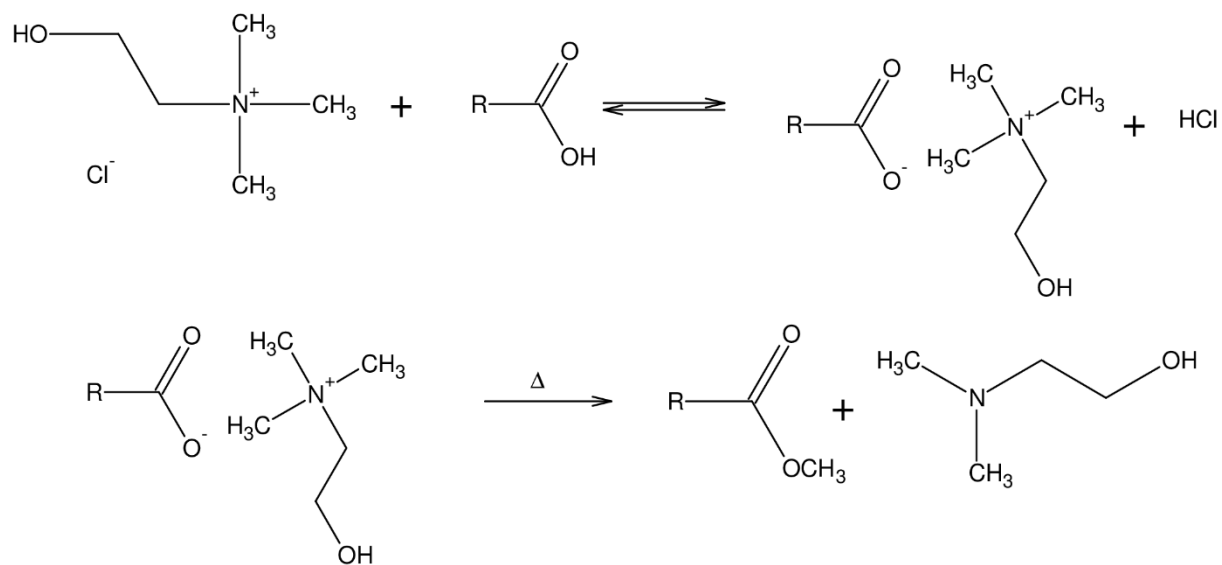
511 ^{a)} SD – standard deviation (n = 3).

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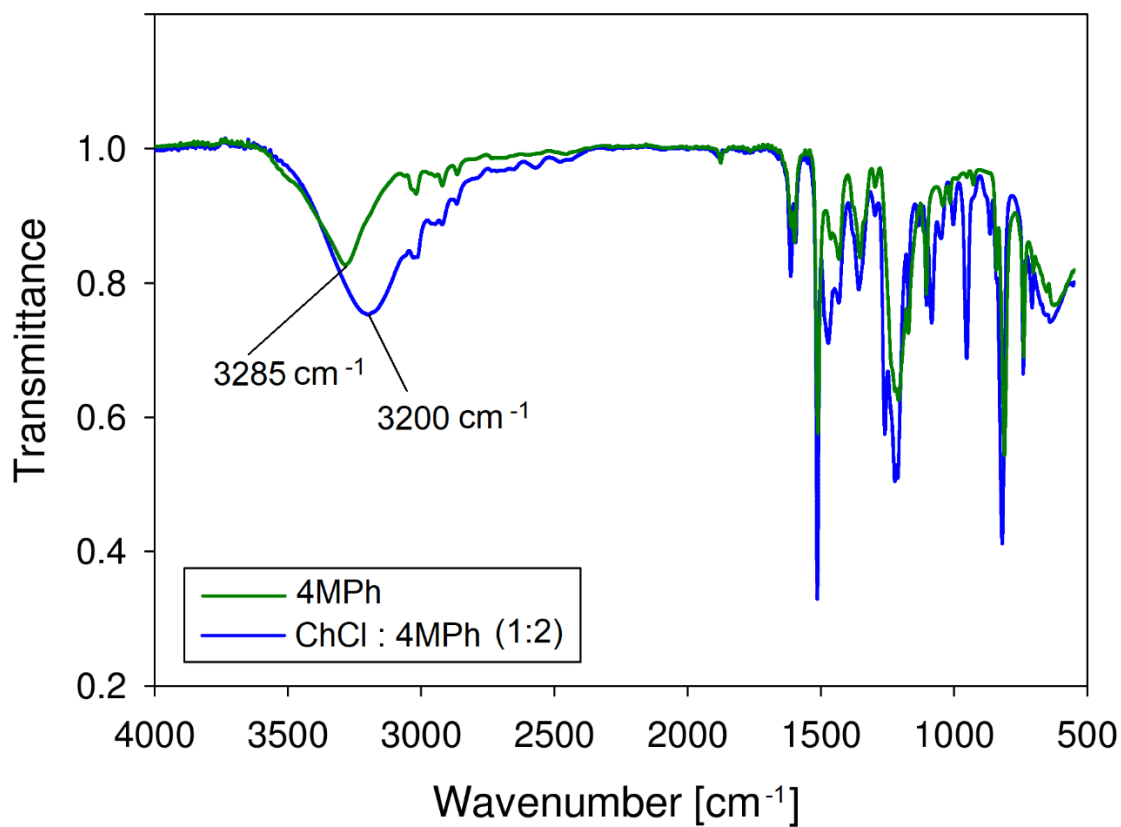
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515 **Figures**



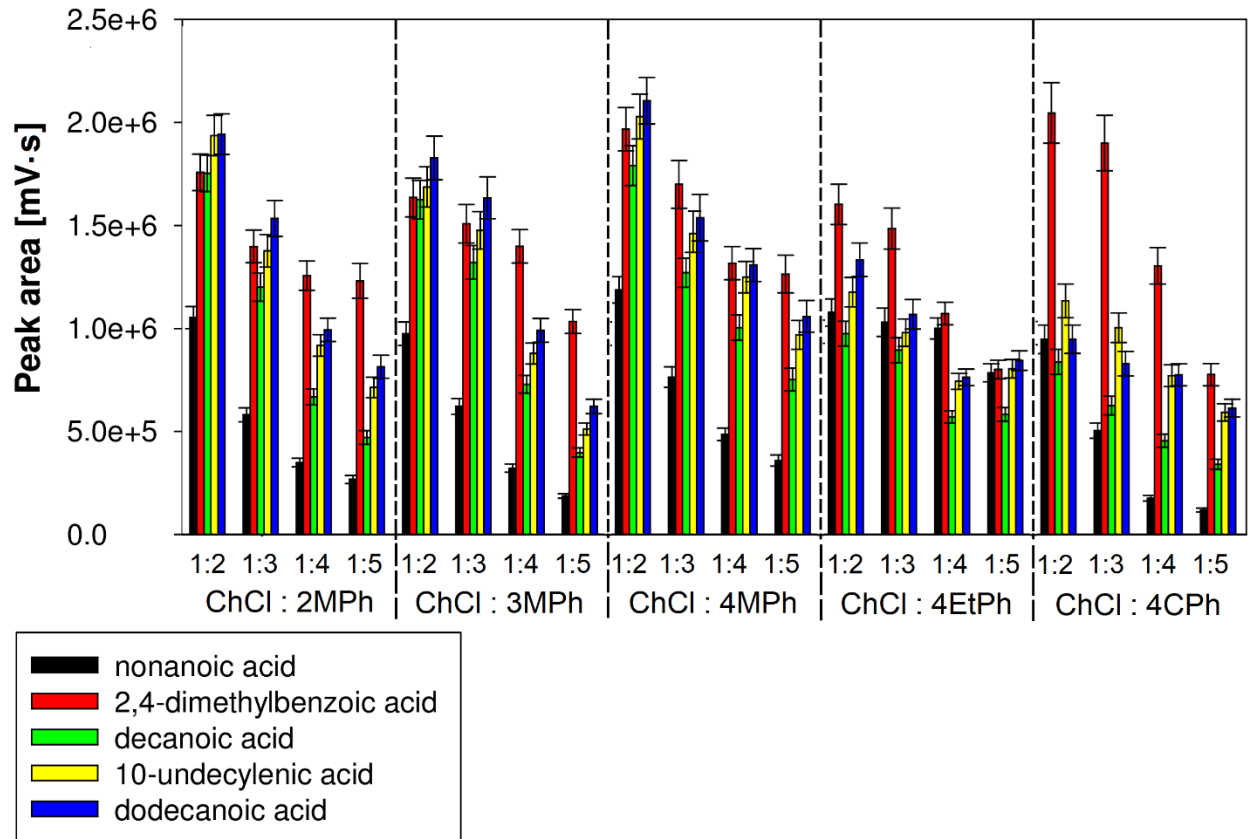
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517 **Figure 1** Derivatization of carboxylic acids



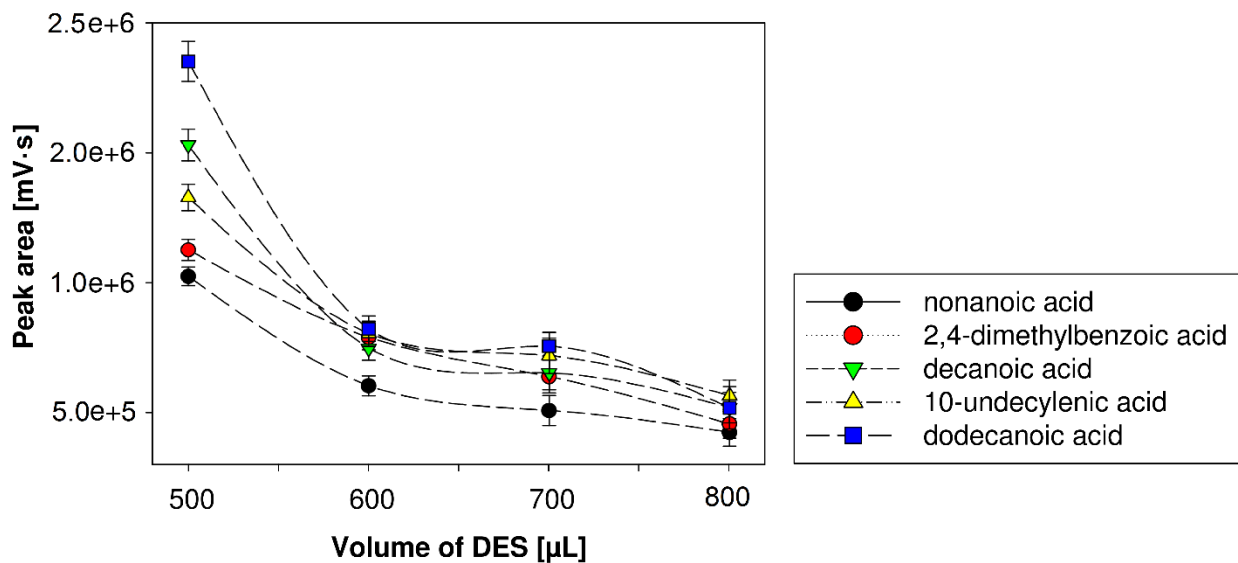
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519 **Figure 2** FT-IR spectra of 4MPH and ChCl:4MPH (1:2)



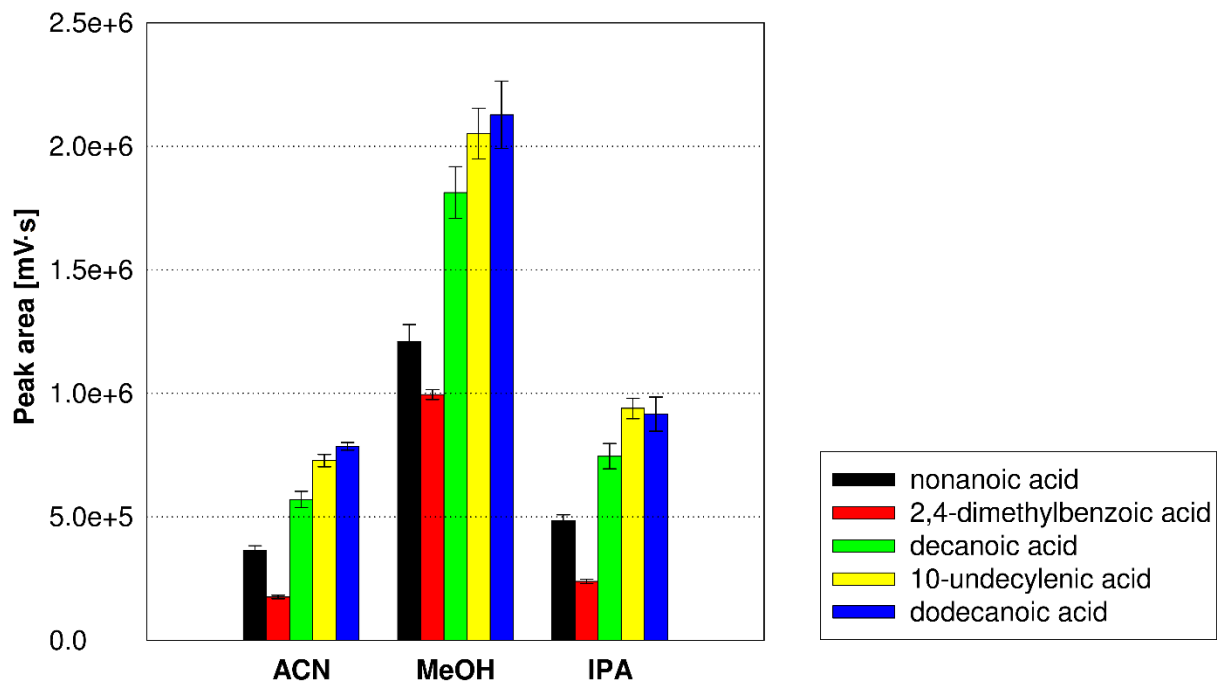
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521 **Figure 3** The effect of various deep eutectic solvents on the yield of extraction and derivatization



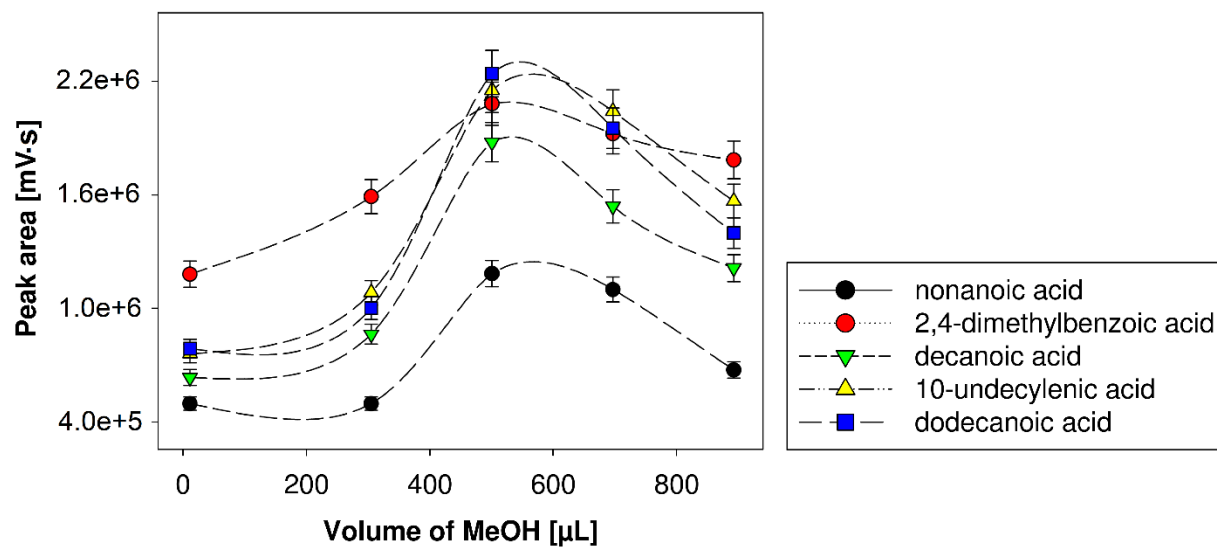
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523 **Figure 4** The effect of DES volume on the yield of extraction and derivatization



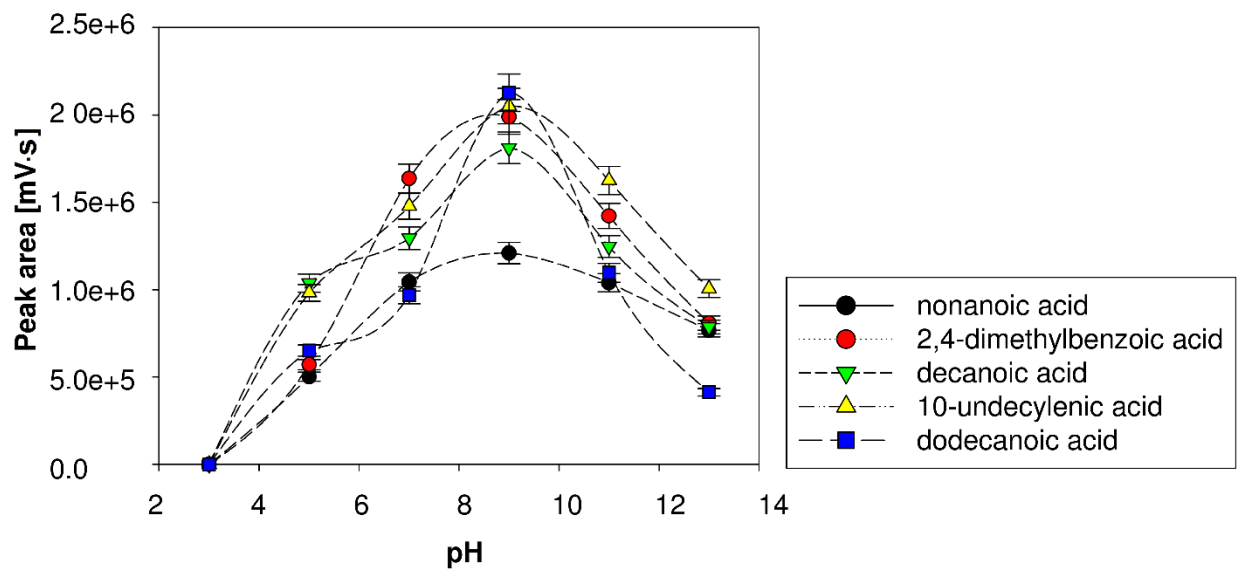
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525 **Figure 5** The effect of various disperser solvents on the extraction yield



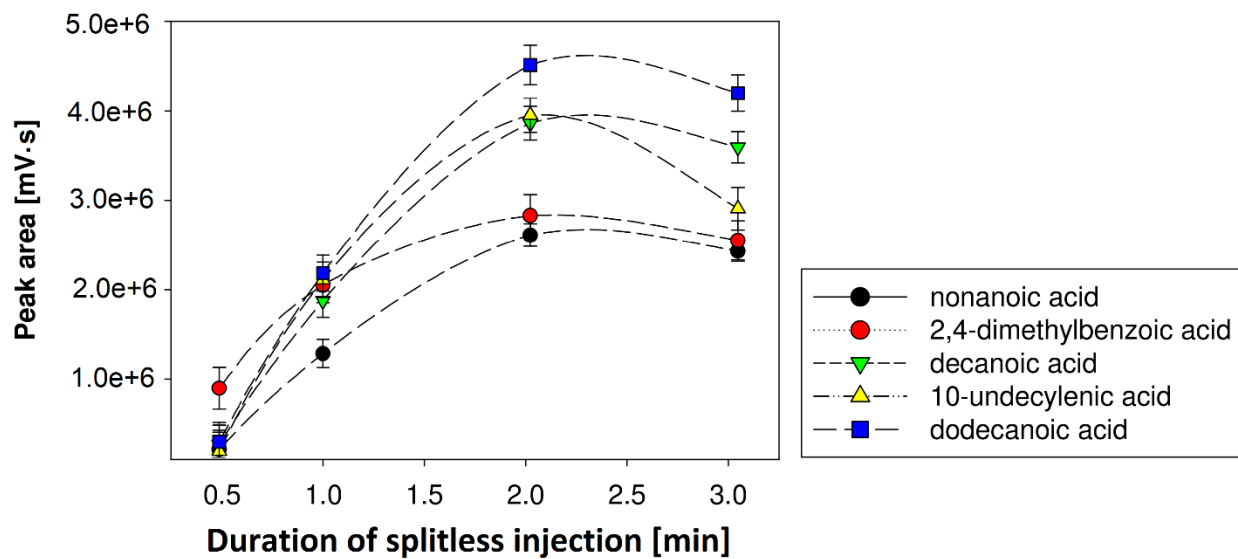
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527 **Figure 6** The effect of disperser solvent volume on the extraction yield



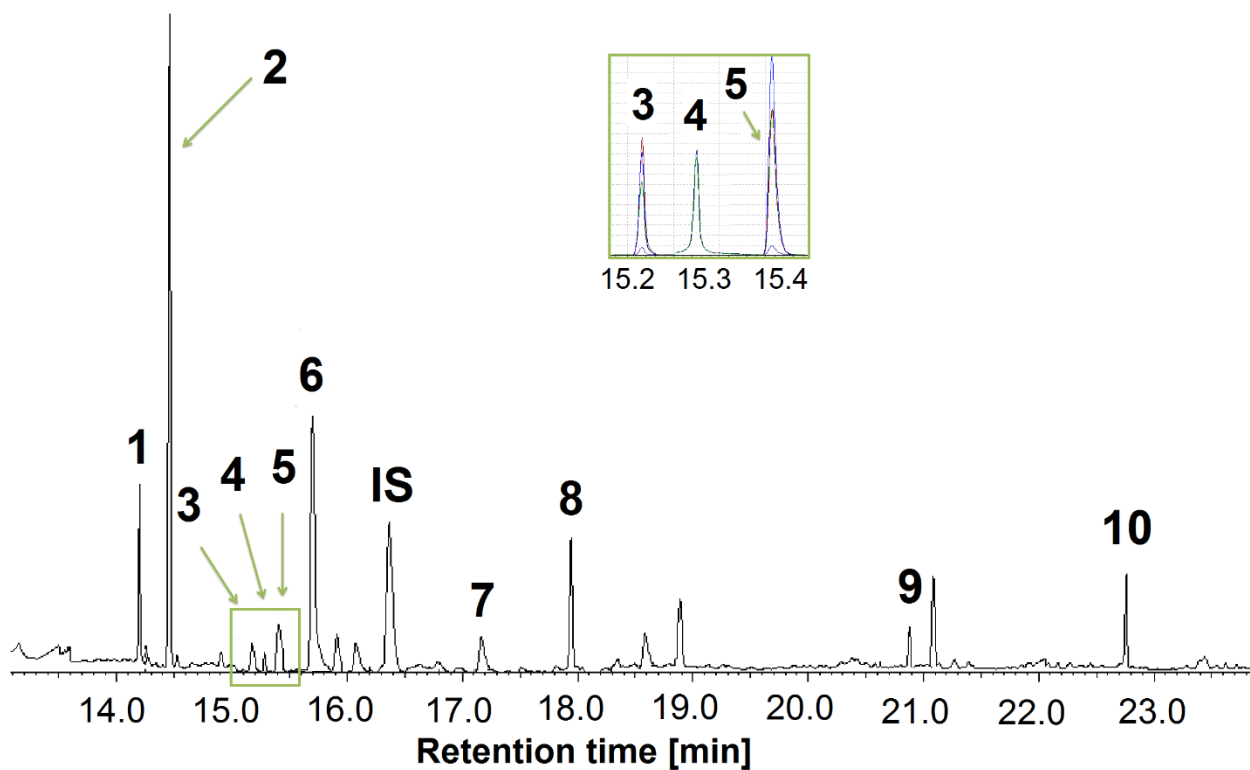
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529 **Figure 7** The effect of pH on the yield of extraction and derivatization



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



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533 **Figure 9** Chromatogram of real raw effluents. Identified compounds: 1) benzoic acid, 2) octanoic acid, 3)
 534 2-methylbenzoic acid, 4) 2-hydroxybenzoic acid, 5) 4-methylbenzoic acid, 6) nonanoic acid, 7) 2,4-
 535 dimethylbenzoic acid, 8) decanoic acid, 9) 4-tert-butylbenzoic acid, 10) dodecanoic acid

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