Method for the determination of carboxylic acids in industrial effluents using dispersive liquid-liquid microextraction with injection port derivatization gas chromatography–mass spectrometry

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abstract

The paper presents a new method for the determination of 15 carboxylic acids in samples of postox-idative effluents from the production of petroleum bitumens using ion-pair dispersive liquid-liquid microextraction and gas chromatography coupled to mass spectrometry with injection port derivati-zation. Several parameters related to the extraction and derivatization efficiency were optimized. Under optimized experimental conditions, the obtained limit of detection and quantification ranged from 0.0069 to 1.12 μg/mL and 0.014 to 2.24 μg/mL, respectively. The precision (RSD ranged 1.29–6.42%) and recovery (69.43–125.79%) were satisfactory. Nine carboxylic acids at concentrations ranging from 0.10 μg/mL to 15.06 μg/mL were determined in the raw wastewater and in samples of effluents treated by various oxi-dation methods. The studies revealed a substantial increase of concentration of benzoic acids, in samples of wastewater after treatment, which confirms the need of carboxylic acids monitoring during industrial effluent treatment processes.

Keywords: Carboxylic acids, Dispersive liquid-liquid microextraction, Gas chromatography, Injection port derivatization, Mass spectrometry, Wastewater

1. Introduction

Carboxylic acids, including fatty acids and benzoic acids, are often present in both municipal and industrial effluents [1–5]. A number of papers devoted to the most effective methods of degradation of the organic acids in wastewater have been recently published [2,6–9]. Also some analytical methods of control of the treatment efficiency of these group of compounds are available in the literature [10]. This particular interest on carboxylic acids results from their potential influence on the aquatic ecosystem, human health as well as odorous character of the most volatile compounds. The ever-growing restrictions concerning quality of wastewater, discharged from wastewater treatment plants is also an important driving force for studies on this group of compounds related to the environmental protection issues.

At present, the most common techniques for the determination of the organic acids are the chromatographic techniques, including liquid chromatography, and capillary electrophoresis [11]. However predominant role and leading in this field is gas chromatography [4,10]. Nevertheless, only a small fraction of carboxylic acids, including volatile fatty acids (C_1–C_{12}) can be determined, directly, using GC [12–14]. Those compounds that are not volatile and are characterized by high polarity and boiling point must be derivatized prior to the chromatographic analysis. To that point, compounds containing carboxyl group are subjected to various reactions, including esterification, silanization or alkylation. Among the available derivatization reagents, the most popular that block polar groups which are proton donors, are the alkylsilane reagents, such as *N*,*O*-*Bis*(trimethylsilyl)trifluoroacetamide, trimethylchlorosilane [15], *N*,*O*-*bis*(trimethylsilyl) acetamide, that introduce a trimethylsilane group into an acid molecule. Despite many advantages, silane derivatives are highly unstable and the sample analysis must be carried out within 12–24 h, which constraints the possibility of using this type of reagents in routine analyses [15]. Many compounds used in derivatization, such as dimethyl sulfate, diethyl sulfate [16], or diazomethane [17] are highly toxic. Despite their high reaction yield in a short time, such reagents are substituted with those more environmentally friendly ("green"), which in turn give lower reaction yield in much longer time *i.e.* pentafluorobenzyl bromide [18]. Another group of derivatization reagents are alkyl chloroformates. The reaction of carboxylic acids esterification using these reagents takes place in a few seconds, and alkyl esters could be easily extracted from water to non-toxic solvents [19].

In recent years reagents producing ion-pairs *i.e.* tetramethylammonium chloride (TBA-Cl), tetrabutylammonium hydrogen sulfate

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($TBA\text{-}HSO_4$), tetrabutylammonium bromide (TBA-Br) or tetrabutylammonium iodide (TBA-I), tetramethylammonium acetate (TMAAc) [20] have been used for derivatization of carboxylic compounds. The short time of a single derivatization reaction is a considerable advantage of such procedure, because it limits only to addition of reagent and the buffer solution to the sample resulting in formation of ion-pairs. The main process of derivatization *i.e.* ion-pair transformation into ester derivatives takes place in a hot injector of gas chromatograph.

Additionally, to improve the sensitivity of the method, a proper extraction stage should be used. A preferred extraction technique is easy to use, allow the automation of procedure and is less time-consuming. Important criterion is also small consumption of organic solvents. Among popular extraction techniques *i.e.* liquid-liquid extraction [10,21], liquid-phase microextraction [3] solid phase extraction [22], solid phase microextraction [23], used for isolation and enrichment of the carboxylic compounds, a technique that meets all of the aforementioned requirements is dispersive liquid-liquid microextraction [24].

This paper describes a novel method for the determination of carboxylic acids in samples of postoxidative effluents from the production of petroleum bitumens using ion-pair dispersive liquid-liquid microextraction coupled to gas chromatography–mass spectrometry with injection port derivatization (IP-DLLME-GC–MS).

2. Materials and methods

2.1. Materials

Except for carbon disulfide purchased from Sigma Aldrich (USA), all solvents (dichloromethane (DCM), chlorobenzene (CB), chloroform (CF), carbon disulfide (CDS) isopropanol (IPA), methanol (MeOH), acetone (AC)) were purchased from POCH (Poland). Purity of all solvents was greater 99.9%. Sodium chloride (NaCl) and sodium hydroxide (NaOH) were from POCH (Poland), $TBA\text{-}HSO_4$ from Sigma-Aldrich (USA).

All high quality standards (benzoic acid, 2-methylbenzoic acid, 4-methylbenzoic acid, 2,4-dimethylbenzoic acid, 4-*tert*-butylbenzoic acid, 2-hydroxybenzoic acid, propanoic acid, butyric acid, 2-ethylhexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, decanoic acid, 10-undecylenic acid, dodecanoic acid) and internal standard: 2-chlorobenzoic acid were obtained from Merck (Germany).

2.2. Real samples

Samples of postoxidative effluents from the production of petroleum bitumen 20/30 from the vacuum residue of REBCO:Kirkuk (88:12 w/w), a blend of Russian and Iraqi crude mixture, were collected from a plate separator which separated the condensed organic phase from the aqueous phase. The investigated samples were the aqueous phase of raw wastewater, as well as chemically treated wastewater through oxidation. The studied advanced oxidation processes (AOPs) were: titanium dioxide in combination with ozone and UV radiation (TiO_2/O_3/UV); titanium dioxide in combination with ozone, hydrogen peroxide and UV radiation ($TiO_2/O_3/H_2O_2$/UV); hydrodynamic cavitation in combination with the ozone (HC/O_3) and Sonocavitation in combination with the ozone (SC/O_3). Detailed information on the characteristics of the postoxidative effluents and phenomena occurring during the bitumen oxidation were described in our previous papers [25–27].

2.3. Apparatus

Separation was performed on GC instrument coupled with Mass Spectrometer model QP2010 GC–MS SE (Shimadzu, Japan) equipped with an autosampler combi-PAL AOC 5000 and auto injector (Shimadzu, Japan), and a Clarus 580 gas chromatograph (Perkin Elmer, USA) with a flame ionization detector (FID). The capillary columns used were Rxi-624Sil MS (60 m × 0.25 mm × 1.40 μm) (Restek, USA) and DB-624 (60 m × 0.32 mm × 1.80 μm) (Agilent, Santa Clara, CA, USA). Data were processed using LabSolutions software (Shimadzu, Japan) with NIST 14 mass spectra library; TurboChrom 6.1 software (Perkin Elmer, USA) and an EBA 8S centrifuge (Hettich, Germany).

2.4. Procedures

2.4.1. Sample preparation

The samples of aqueous phase of the raw effluent (9 mL) was removed to a 12-mL vials and spiked with 10 μL of a 5% solution of internal standard, 2-chlorobenzoic acid, in AC and derivatized by adding 0.61 g $TBA\text{-}HSO_4$ (final concentration of $TBA\text{-}HSO_4$ was 0.2 M). The pH was adjusted to 7.0 using 5 M NaOH. Subsequently, 300 μL of the extraction solvent–CF and 900 μL of disperser solvent–IPA was added. The sample was vigorously shaken for 60 s, followed by centrifugation at 4000 rpm for 5 min at room temperature. A volume of 250 μL of the sedimented organic phase was removed and analyzed by GC–MS.

2.4.2. Chromatographic conditions

The following conditions were used in the investigations: oven temperature program: 60 °C (5 min) – ramped at 7 °C/min to 260 °C (20 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). Column: Rxi-624Sil MS (60 m × 0.25 mm × 1.40 μm) (Restek, USA).

2.4.3. Method validation

The limit of detection (LOD) was calculated from Eq. (1):

$$\mathrm{LOD} = \frac{3.3 \cdot \mathrm{Sa}}{\mathrm{b}} \tag{1}$$

where: Sa – standard deviation of the intercept of calibration curve, b – the slope of calibration curve.

The limit of quantitation (LOQ) was calculated from Eq. (2):

$$\mathrm{LOQ} = 2\mathrm{LOD} \tag{2}$$

Linear range: The linearity of calibration curve carried out using an internal standard calibration was estimated using the correlation coefficient (r). In order to confirm an appropriate selection of the linear range, a standard residual analysis was performed [28].

Recovery (R) was calculated from Eq. (3).

$$R\,[\%] = \frac{C_{quant} - C_0}{C_{expect}} \cdot 100\% \tag{3}$$

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].

3. Results and discussion

3.1. Optimization of dispersive liquid-liquid microextraction and derivatization

The IP-DLLME was optimised using 40 μg/mL mixture of nine carboxylic acids (2-ethylhexanoic acid, octanoic acid, nonanoic acid, decanoic acid, 10-undecylenic acid, dodecanoic acid, benzoic acid, 2-methylbenzoic acid and 2-hydroxybenzoic acid). The effect of variation of individual parameters *i.e.* derivatization agents and extraction parameters was evaluated by comparing peak areas for the selected compounds. The optimization process was performed using GC-FID.

3.1.1. Selection of type and volume of extraction solvent

The selection of extraction solvent is particularly important in dispersive liquid-liquid microextraction. Four organic solvents of density greater than water were tested, including DCM, CF, CB, CDS (Fig. 1). From practical reasons only solvents of density greater than water have been investigated. Solvents of densities lower than water, regarded as eco-friendly, cause significant challenges during extract collection that requires solidifying sample by freezing or using in house-made vials [29] and therefore are not applicable as a routine method in industrial laboratories. Among tested solvents, CF, of the greatest density (1.49 g/cm^3), provides comparatively high analytical signal. Similar results have also been observed for perfluorocarboxylic acids [30].

In the first experiment, the following pre-selected extraction and derivatization conditions were used: 300 μL of extraction solvent, 700 μL of disperser solvent–AC, pH 11, 0.05 M TBA-HSO$_4$, injection port temperature 300 °C, splitless for 1 min, extraction time 60 s and 0% of NaCl.

Following our recent investigations AC was used as a disperser solvent, the extraction time and salt concentration in the sample were selected as reported in our previous works [31–33]. The derivatization conditions were selected on the basis of the results reported before [34].

To investigate the effect of volume of extraction solvent on the extraction yield, a series of experiments were performed with different CF volumes *i.e.* 200, 300, 500, 700 and 900 μL (Fig. S1). The experimental results indicated that peak areas of all tested compounds increased by diminishing volume of extraction solvent. The volume of 200 μL of CF (9 mL sample) did not provided sufficient volume of sedimented phase, which strongly influenced the reproducibility of the results. Consequently, 300 μL of CF was used in further studies.

3.1.2. Selection of type and volume of disperser solvent

The extraction efficiency of three disperser solvents *i.e.* AC, MeOH and IPA were compared (Fig. 2). IPA was found to provide the best efficiency for all tested compounds except for 2-hydroxybenzoic acid, for which MeOH produced better efficiency.

In order to achieve the optimized conditions, different experiments were carried out within the volume of disperser solvent range of 0–1100 μL (Fig. S2). The results indicated that the peak areas increased with increasing volume of disperser solvent from 0 to 900 μL and start to decrease with volume of disperser solvent from 900 to 1100 μL. Therefore, a volume the 900 μL of IPA was chosen for further studies.

3.1.3. Concentration of ion-pair reagent

Derivatization of compounds containing a carboxylic group, with ion-pair generation, is usually achieved using tetrabutylammonium salts such as TBA-Cl, TBA-HSO$_4$, TBA-Br or TBA-I [35]. However, most of papers related to this topic indicate the lack of significant influence of the type of conter-ion (anion) in ion-pairing reagent (IPR) on the efficiency of derivatization [35]. Thus, the least costly reagent, TBA-HSO$_4$, was used in the research. The concentration tested was ranging between 0.0015 M and 1 M. It is worth to mention, that in the case of this method, the ion-paring effect affects two steps of sample preparation procedure *i.e.* the extraction and derivatization in hot GC injector. The amount of IPR must be sufficient to form ion-pair moieties with all molecules of analytes, which allows their effective extraction to organic phase. This stage is followed by derivatization taking place in-situ in the hot GC injector. It has been found, that in general, the increased concentration of the added ion-pair produce more effective analytes derivatization. Such dependence was observed for the majority of analytes while increasing the concentration of TBA-HSO$_4$ from 0.0015 M to 0.5 M. For the majority of aliphatic carboxylic acids, further increase of concentration had no significant improvements. On the other hand in the case of benzoic acids, including benzoic acid and 2-methylbenzoic acid the decrease in extraction efficiency over the concentration range was reported. The same effect is observed for 2-hydroxybenzoic acid after increasing concentration of TBA-HSO$_4$ from 0.4 M to 0.5 M. It is most probably due to the phenomenon of Nernst's diffusion layer present on the interface of the aqueous phase and the organic phase. The increase of the salt concentration in the water phase makes the mass transfer of ion-pair to organic phase less effective [36]. Moreover, different influence of TBA-HSO$_4$ reagent concentration on various organic acid groups was observed.

The derivatization efficiency increases for all compounds with concentration of TBA-HSO$_4$ up to 0.2 M. However further increase of concentration cause considerable increase in effectiveness only for the benzoic acids (Fig. 3), while it has only minor effect on the derivatization efficiency of aliphatic acids and 2-hydroxybenzoic acid.

Additionally, the increase in TBA-HSO$_4$ concentration has a relevant influence on the decrease of organic phase volume which was observed over 0.2 M. Almost absence of organic phase was reported for 1 M concentration. Due to the insufficient volume of the organic phase for TBA-HSO$_4$ concentrations over 0.2 M, required for sufficient repeatability of the results, concentration level of 0.2 M of the derivatization reagent was used in further studies. It provides satisfactory efficiency of extraction resulting in acceptable sensitivity of the method.

It is worthwhile to mention that in the case of changes in the extraction system, such as the extraction or disperser solvents, the ion-pair reagent concentration should be re-optimized, since ion-pair-assisted liquid-liquid extraction is very sensitive to these parameters. Apart from the polarity of the organic phase on extraction yields, other phenomena such as ion-pair formation may also play an important role in relation to the latter.

3.1.4. pH

The pH of the solution is also a key parameter and should be higher than pK$_a$ of any tested acid (pK$_a$ between 4.2 and 5.3), in order to get deprotonated forms of analytes [35]. The ionic form of acids can be obtained by pH adjustment towards more alkaline (pK$_a$ + 2), therefore pH of 7, 9, 11 and 13 were tested (Fig. S3). The results indicate that pH in the range of 7–11 had no significant effect on the extraction and derivatization efficiency, while increase to value from 11 to 13 causes gradual decrease of the process effectiveness. Following the tests, further investigation was performed using pH adjustment to 7, achieved by adding 0.61 g of TBA-HSO$_4$ to the real wastewater samples.

3.1.5. Temperature of GC injector and type of liner

The gas chromatograph injector's temperature is an important parameter in the terms of the derivatization efficiency [37]. Gener-

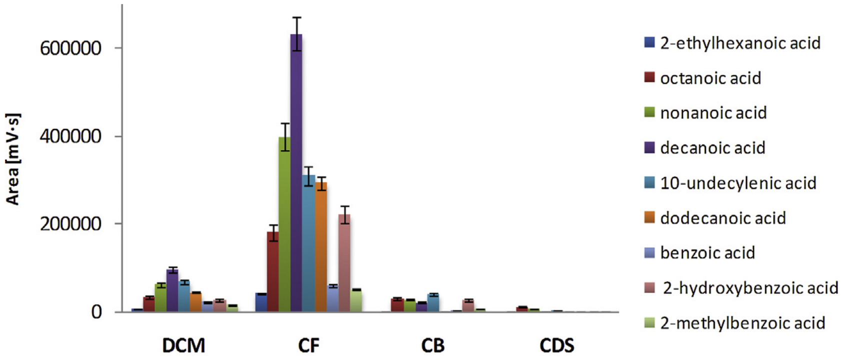

Fig. 1. Effect of kind of extraction solvent on extraction yield.

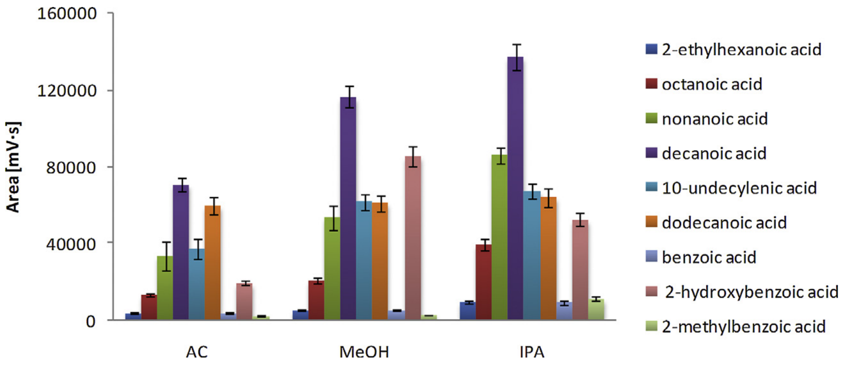

Fig. 2. Effect of kind of disperser solvent on extraction yield.

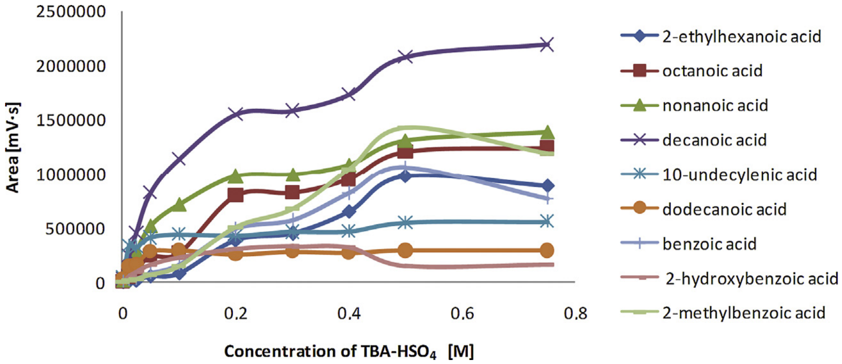

Fig. 3. Effect of concentration of TBA-HSO$_4$ on extraction yield.

ally, a higher temperature may increase the rate of the reaction for most of the compounds, but too high temperature may cause that other pathways of reaction will take place as well as decomposition of the derivatives [28]. The effect of injection port's temperature on the derivatization performance was studied in the range of 280–320 °C and the results are shown in Fig. S4. The results indicated that the injection port's temperature in the studied range had no significant effect on the derivatization and extraction efficiency. Thus, a typical temperature of injection *i.e.* 300 °C was used. The overheating of the injector can cause a problems with ghost peaks resulting from septum bleeding. The selected temperature provides good peak shape and eliminates problems with discrimination of higher boiling analytes. Furthermore, analysis of blank runs revealed absence of problems related to phenomenon of deposits formation/impurities adsorption in the injector, thus any additional peaks were not found on the chromatograms from analysis of pure solvent.

The injector was equipped with a splitless liner (packed with deactivated by silanization glass wool). The glass wool increases the surface area for vaporization of the sample and minimizes the risk of the contamination of chromatographic column by non-volatile components of the sample. The developed method is designed for analysis of crude ("dirty") wastewater samples, thus it is highly important to prevent undesirable interactions of the components from the effluent matrix with the chromatographic system. A presence of contamination on the wool surface, during routine maintenance, was observed for each chromatographic system used in our GC laboratory for analysis of the DLLME extracts (without derivatization) of this type of samples. Any additional interferences weren't observed after analysis batches with derivatization step.

3.1.6. *Time of splitless mode*

The time of splitless mode injection is a important parameter that affects the results of the derivatization procedure. The time of splitless mode after injection should be long enough for intro-

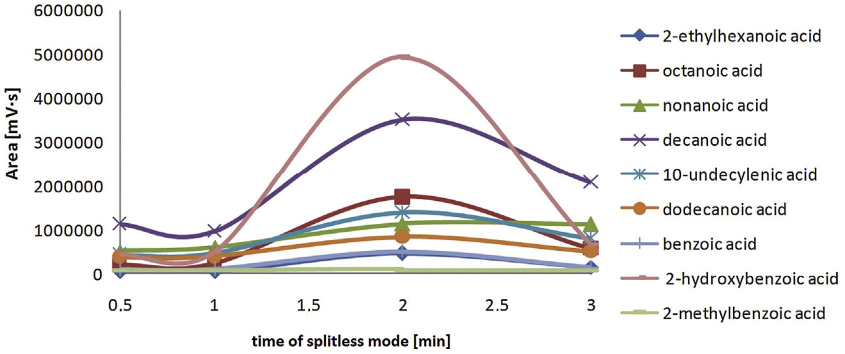

Fig. 4. Effect of purge off time on derivatization yield.

Table 1
Compilation of calibration parameters for butylated derivatives.

Compound	t_R [min]	Target ion	Qualifier ion	Calibration curve	R^2	LOD [μg/mL]	LOQ [μg/mL]	Linear range [μg/mL]
propanoic acid	18.09	75	87	y = 0.0010x – 0.0001	0.9999	0.98	1.96	1.96–71.25
butanoic acid	19.14	71	89	y = 0.00081x – 0.00003	0.9999	1.12	2.24	2.24–68.25
heptanoic acid	26.90	131	113	y = 0.2674x – 0.0027	0.9999	0.27	0.54	0.54–71.85
2-ethylhexanoic acid	26.94	145	127	y = 0.1101x – 0.0007	0.9991	0.41	0.82	0.82–75.19
octanoic acid	28.86	145	127	y = 0.2462x – 0.0014	0.9996	0.15	0.30	0.30–62.35
benzoic acid	29.32	105	123	y = 0.6971x – 0.0022	0.9998	0.065	0.13	0.13–57.91
nonanoic acid	30.68	159	141	y = 0.5213x – 0.0020	0.9997	0.11	0.22	0. 22–77.12
2-methylbenzoic acid	30.78	119	136	y = 0.7726x – 0.0006	0.9990	0.041	0.082	0.082–22.78
2-hydroxybenzoic acid	31.38	120	194	y = 1.7063x + 0.0018	0.9996	0.034	0.068	0.068–56.38
4-methylbenzoic acid	31.64	119	136	y = 0.7305x – 0.0002	0.9995	0.029	0.058	0.058–21.84
decanoic acid	32.40	173	155	y = 0.4439x – 0.0014	0.9992	0.10	0.20	0.20–57.53
2-chlorobenzoic acid (IS)	32.66	139	111	–	–	–	–	–
2,4-dimethylbenzoic acid	33.01	132	150	y = 1.2028x + 0.0007	0.9998	0.016	0.032	0.032–25.31
10-undecylenic acid	34.01	124	149	y = 0.1740x + 0.0008	0.9997	0.16	0.32	0.32–37.70
4-*tert*-butylbenzoic acid	35.50	219	163	y = 3.2644x + 0.0020	0.9978	0.0069	0.014	0.014–19.06
dodecanoic acid	35.68	201	183	y = 0.2693x + 0.0022	0.9990	0.10	0.20	0.20–51.12

duction all analytes into the GC column, but too long time may cause peaks tailing [38,39]. The influence of purge off time on the derivatization efficiency was studied in the range of 0.5–3 min in splitless mode with an injection port temperature of 300 °C (Fig. 4). The results revealed that the maximum derivatization efficiency was obtained for 2.0 min purge-off for all studied analytes. Consequently, a 2-min time of splitless was selected as the optimal.

3.1.7. *Salting out of analytes*

Usually, the addition of a salt causes improvement in the extraction effectiveness through the decrease in solubility of tested compounds in the aqueous phase. The effect of addition of NaCl on the extraction efficiency was studied in the range of 0–20% (w/w). It has been found that even small amounts of sodium chloride added to the sample (5% w/w) effectively prevent extraction of aliphatic acids, but at the same time the increase of NaCl concentration (0–20%) had a positive effect on an extraction efficiency of benzoic acids (Fig. S5). Similar effect was previously reported [20]. To explain this behavior, the extraction system can be compared to ion-pair reversed phase HPLC, the acceptor phase in DLLME extraction is less polar same as stationary phase comparing to eluent in RP-HPLC. The commonly known rule is the decrease of retention factor of solutes forming ion-pairs with increasing concentration of salt [40] *i.e.* decreasing extraction yield from water to organic phase. This phenomenon in IP-RP-HPLC is further explained by reduced formation of ion-pairs with increased salt concentration (decreasing retention/extraction yield). This effect takes place only in case of some groups of substances. Due to unambiguous results for investigated groups of compounds it was decided not to add any NaCl to wastewater samples. If the application of the procedure would be limited to analysis of benzoic acid and its alkyl-substituted derivatives the conditions of the extraction should include the addition of salt to obtain the salting out effect and improve extraction effectiveness.

3.1.8. *Extraction time*

The extraction time (the time of shaking a sample with a mixture of the disperser and extraction solvents) was tested *i.e.* 20, 40, 60 and 120 s. (Fig. S6). The results indicate a gradual growth of the extraction efficiency from 20 to 60 s. Extractions longer than 1 min had no significant effect on the extraction effectiveness. This result varies considerably from those obtained in previous studies, in which the extraction time had no significant effect on the analyte recovery [31–33].

While developing new methods, based on implementation of the DLLME technique, usually parameters like sample volume, time and centrifugation speed are also optimized. However, as demonstrated in references [31–33], both sample centrifugation speed and time of centrifugation have no significant effect on the extraction efficiency, thus 5 min of the centrifugation at 4000 rpm was applied in all the tests. Furthermore, it is commonly known that sample volume increase, while using the same volume of an extraction solvent affects positively the enrichment factor. This allows to further “tune” this method if the purpose would be to control analytes at trace concentration level. A 9 mL sample volume has been used in tests due to practical feasibility of sampling wastewater treatment without causing any significant disruption to the treatment process, however, if necessary this volume can be increased to allow for control of analytes at trace concentration level.

3.2. *Evaluation of the method*

Based on the internal standard method, the calibration of 15 carboxylic acids was achieved using 2-chlorobenzoic acid as an

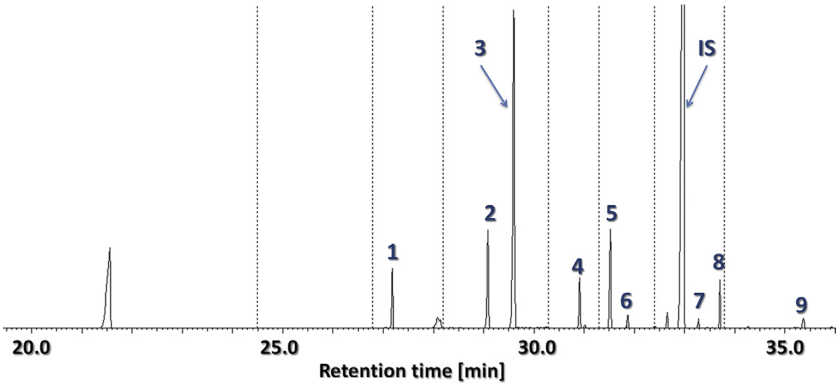

Fig. 5. Chromatogram of raw postoxidative effluents. Identified compounds: (1) heptanoic acid, (2) octanoic acid, (3) benzoic acid, (4) nonanoic acid, (5) 2-methylbenzoic acid, (6) 4-methylbenzoic acid, (7) 2,4-dimethylbenzoic acid, (8) decanoic acid, (9) dodecanoic acid.

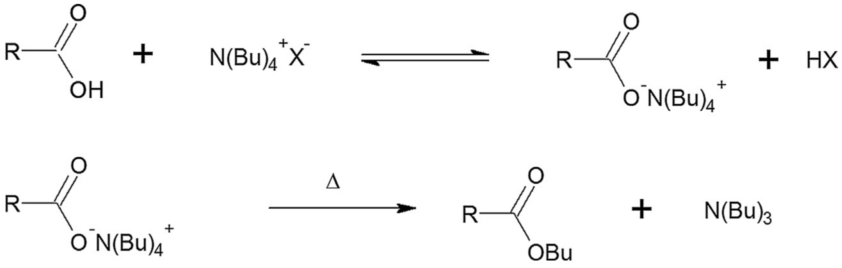

Fig. 6. Reaction mechanism of the in-liner derivatization.

internal standard. A 7-point calibration curves with concentrations ranging from 0.05 to 50 μg/mL were used for quantification. For each derivative two representative values of mass-to-charge (*m*/*z*) ratio were observed, including target ion, used for quantitative analysis, and qualifier ion used for confirmation of the compound identification (on the basis of comparison of target ion to qualifier ion ratio). The same approach was used in our previous paper [41]. The values of the selected *m*/*z* ratios for standard carboxylic acids are presented in Table 1.

The developed method has low detection limits ranging from 0.0069–0.065 μg/mL for benzoic acids. In the case of volatile fatty acids the method have lower sensitivity, and decreases along with molecular mass decrease. It is evident for propionic and butanoic acids, for which the LOD values are 0.98 and 1.12 μg/mL respectively. For compounds containing over nine atoms of carbon per molecule, those values are between 0.10 and 0.41 μg/mL. The linear response in investigated range of concentration was found with determination coefficient (R^2) higher than 0.998 for all analytes.

Analytical characteristics of the method for all studied analytes are compiled in Table 1.

The recovery of the analytes has been evaluated at two concentration levels: 0.5 and 10 μg/mL for water samples. Additional series of experiments were performed using wastewater samples at primary level of concentration of analytes. The results are presented in Table 2. Due to relatively high LOD and LOQ values of propanoic, butanoic, 2-ethylhexanoic and heptanoic acids, the recovery was determined only for 10 μg/mL spike. Recovery values obtained for the water and wastewater samples are in the range 69–122% and 70.45–125.79% respectively, in turn, the RSD values ranging from 1.29–6.21% and 2.10–6.42% respectively. Thus, the analyte recovery and method repeatability at the two tested concentrations provide satisfactory results also for real wastewater matrix.

3.3. *Application of the procedure to the real samples*

The developed procedure was applied for the determination of the content of carboxylic acids in samples of effluents from the production of petroleum bitumens and in effluents samples after treatment with various oxidation methods.

An example of the chromatogram of raw post-oxidative effluent is presented on Fig. 5. The identification of the organic acid derivatives was performed based on comparison of the intensity of characteristic ions presented in Table 3 with values determined during the method development and on retention time of each compound, for which adopted tolerance intervals respectively ±15% for intensity and ± 0.2% t_R [min] for retention time. The empty chromatogram fragment between 23.5 and 28 min results from turning off the detector in this period of time, because of elution of a very intensive tributylamine peak, which is a by-product of derivatization process. The derivatization reaction is presented on Fig. 6.

This method was designed for industrial effluents that can differ in composition from batch to batch, thus to maintain in each case a satisfactory level of separation, a 60.0 m column was used to provide some “buffer of resolution” and allow for flexibility of this procedure. For simpler matrix and specific applications of this procedure time of the GC separation could be shortened by using shorter columns with thicker film.

In the samples of raw wastewater, 9 compounds have been identified at concentrations ranging from 0.13 to 15.1 μg/mL, including heptanoic acid having the highest concentration level (15.1 μg/mL) as well as benzoic acid (7.5 μg/mL). Slightly lower concentration levels have been determined for octanoic acid, 2-methylbenzoic acid and 4-methylbenzoic acid. Remaining compounds were determined in concentrations below 0.58 μg/mL.

In the effluent samples treated with ozone and UV radiation with addition of titanium dioxide as a catalyst only slight partial reduction in concentration of most carboxylic acids was observed

Table 2
Recoveries of carboxylic acids.

Compound	Recovery [%][a] (0.5 µg/mL)	RSD [%][a] (n = 4)	Recovery [%][a] (10 µg/mL)	RSD [%][a] (n = 4)	Recovery [%][b] (10 µg/mL)	RSD [%][b] (n = 4)
propanoic acid	–	–	104.19	5.59	100.81	5.66
butanoic acid	–	–	98.95	6.21	93.16	6.42
heptanoic acid	–	–	78.21	2.29	72.45	2.21
2-ethylhexanoic acid	–	–	77.21	3.97	70.69	4.18
octanoic acid	69.43	4.11	92.48	2.90	98.18	3.01
benzoic acid	79.45	5.25	95.02	2.78	113.19	2.94
nonanoic acid	79.26	5.03	111.32	1.71	95.16	3.26
2-methylbenzoic acid	72.31	4.20	99.91	2.29	82.91	2.86
2-hydroxybenzoic acid	84.87	4.07	102.16	2.73	105.11	3.87
4-methylbenzoic acid	87.54	3.54	100.25	3.06	90.80	3.41
decanoic acid	77.79	5.21	111.24	3.58	114.26	3.97
2,4-dimethylbenzoic acid	116.31	3.02	121.24	2.07	125.79	2.41
10-undecylenic acid	99.64	1.59	103.64	1.29	90.07	2.10
4-butylbenzoic-*tert*-butylbenzoic acid	110.88	5.50	115.84	1.72	120.21	3.45
dodecanoic acid	79.67	5.60	117.92	3.29	121.79	3.35

[a] Extraction recoveries and relative standard deviations in deionized water sample at spiked levels.

[b] Extraction recoveries and relative standard deviations in raw wastewater sample at spiked levels.

Table 3
Concentrations of carboxylic acids in raw wastewater and in wastewater treated with $TiO_2/O_3/UV$, $TiO_2/O_3/H_2O_2/UV$, hydrodynamic cavitation/O_3 (HC/O_3) and sonocavitation/O_3 (SC/O_3).

Compound	Concentration ± SD[a] [µg/mL]								
	Raw wastewater	$TiO_2/O_3/UV$	Reduction (−)/**increase (+)** [%]	$TiO_2/O_3/H_2O_2/UV$	Reduction (−)/**increase (+)** [%]	HC/O_3	Reduction (−)/**increase** (+) [%]	SC/O_3	Reduction (−)/**increase (+)** [%]
heptanoic acid	15.06 ± 0.38	15.01 ± 0.41	−0.33	14.37 ± 0.44	−4.56	1.56 ± 0.21	−89.62	3.04 ± 0.10	−79.82
octanoic acid	1.390 ± 0.077	1.250 ± 0.071	−10.07	1.120 ± 0.064	−19.42	0.751 ± 0.042	−45.97	0.830 ± 0.035	−40.29
benzoic acid	7.52 ± 0.28	7.51 ± 0.28	−0.13	7.49 ± 0.27	−0.40	3.84 ± 0.15	−48.94	8.43 ± 0.29	**+12.10**
nonanoic acid	0.420 ± 0.018	0.370 ± 0.012	−11.9	0.390 ± 0.022	−7.17	0.351 ± 0.014	−16.50	0.280 ± 0.019	−33.33
2-methylbenzoic acid	2.36 ± 0.10	2.36 ± 0.11	0.00	2.210 ± 0.055	−6.35	2.014 ± 0.071	−14.66	3.76 ± 0.13	**+59.32**
4-methylbenzoic acid	1.110 ± 0.041	1.104 ± 0.044	−0.90	1.024 ± 0.039	−7.73	0.861 ± 0.040	−22.43	2.18 ± 0.11	**+96.58**
2,4-dimethylbenzoic acid	0.130 ± 0.011	0.1100 ± 0.0035	−15.38	0.1002 ± 0.0029	−23.00	0.0587 ± 0.0019	−54.85	0.0610 ± 0.0031	−53.08
decanoic acid	0.310 ± 0.020	0.300 ± 0.018	−3.23	0.286 ± 0.013	−7.67	<LOQ (~0.14)	−54.84	<LOD	−«99.99
dodecanoic acid	0.58 1 ± 0.034	<LOQ (~0.17)	−70.74	<LOQ (~0.15)	−74.18	0.271 ± 0.014	−53.36	0.282 ± 0.015	−51.46

The bold values correspond to the increase (+) of concentration.

[a] SD – standard deviation (n = 3).

(<15%) except for dodecanoic acid whose concentration decreased by 71%. Improvement of the wastewater treatment efficiency was obtained after addition of the hydrogen peroxide (H_2O_2). In treated samples the reduction of the concentration of volatile fatty acids was below 74% and the reduction of benzoic acids was below 23%. In wastewater samples that have been subjected to treatment by hydrodynamic cavitation combined with oxidation by ozone, all carboxylic acids were partially degraded. The highest effectiveness of oxidation was reported for heptanoic acid. Degradation of the remaining acids was below 55%. Different results were obtained for sonocavitation combined with ozonation. Like in the abovementioned processes, all volatile fatty acids were effectively degraded. At the same time the concentration of benzoic acids, including 4-methylbenzoic acid and 2-methylbenzoic acid was increasing during the treatment. This indicates that these compounds are by-products, probably of toluene and xylene isomers oxidation [42]. These compounds were recently identified in such type of effluents [26].

The summary of the results for identified analytes in real samples is compiled in Table 3. The reported increase of selected compounds proves, that such method is useful for evaluation of industrial wastewater treatment processes.

4. Conclusions

The paper presents a novel method for the determination of carboxylic acids in post-oxidative elluents using IP-DLLME-GC–MS. It is the first paper describing a method dedicated to detail process control of carboxylic acids for industrial wastewater treatment processes. The developed method allows obtaining a cumulative positive effects of two steps of sample preparation, simplified to single-step analytical procedure, gaining on the sensitivity and selectivity in respect to carboxylic acids in complex aqueous matrix. Presented ion-pair dispersive liquid-liquid microextraction and derivatization method is simple and feasible to perform in several minutes, which makes it advantageous to usually used methods, in which time of a single extraction and derivatization can reach up to a few hours. Additional benefit of presented method is minimal consumption of organic solvents and use of a non-toxic derivatization reagent making it more environmentally friendly, than standard methods. Furthermore, formation of butyl esters, ensures higher and more specific values of m/z ratio on the mass spectrum used for quantitative analysis.

Obtained validation parameters proved that the developed method allows determination of benzoic acids and less volatile fatty acids in wastewater samples at low concentration levels with good repeatability and satisfactory recovery allowing for process control of industrial wastewater treatment processes. For effluents subjected to sonocavitation combined with ozonation an increase of concentration of benzoic acids during treatment was reported.

Conflicts of interest

The authors have declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2017.08.045.

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