Determination of short-chain carboxylic acids and non-targeted analysis of water samples treated by wet air oxidation using gas chromatography-mass spectrometry

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Abstract

A method based on gas chromatography (GC) coupled with electron ionization mass spectrometry (EI-MS) employing N,O-bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (BSTFA + 1% TMCS) as derivatization agent was developed to quantify short-chain carboxylic acids (C_1-C_6) in hospital wastewater treated by wet air oxidation, an advanced oxidation process. Extraction from water and derivatization of volatile and semi-volatile short chain carboxylic acids were optimized and validated and limits of quantification (LOQ = 0.049 mg L^{-1} - 4.15 mg L^{-1}), repeatability (RSD = 1.7 % – 12.8 %), recovery (31 - 119 %) and trueness (relative bias = -19.0 % – 3.4%) were acceptable. The validated method was successfully applied to monitor the concentration of organic acids formed after wet air oxidation of water samples. Results showed that the method described herein allowed to identify 38 % and up to 46 % of the final COD chemical composition after wet air oxidation of acetaminophen spiked in deionised water and hospital wastewater samples, respectively. The developed method also allowed to perform qualitative non-targeted analysis in hospital wastewater samples after treatment. Results demonstrated that glycerol, methenamine, and benzoic acid were also present in the samples and their presence was confirmed with reference standards.

Keywords: advanced oxidation process, transformation products, hospital wastewater, method optimization, non-targeted screening

1. Introduction

Advanced oxidation processes (AOPs) are among the most promising solutions to eliminate toxic chemicals such as pharmaceuticals, personal care products, illicit drugs, and other trace organic contaminants (TrOCs) from municipal wastewater. These techniques employ reactive species such as hydroxyl radicals ($HO^{\bullet}$), hydrogen peroxide, ozone and superoxide anion radicals ($O_2^{\bullet-}$) with low selectivity towards organic compounds and generate CO_2, H_2O, short-chain organic acids and inorganic ions as end products [1]. AOPs include electrochemical oxidation, Fenton processes, ozonation, photolysis, and wet air oxidation (WAO) among others.

AOPs show high removal rates for a wide range of organic compounds and the efficiency of these technologies is generally measured by the elimination of target compounds and the reduction of chemical oxygen demand (COD) [2-4]. However, these processes also generate various transformation products which may be associated with toxic effects on biota [5,6] or activated sludge [7]. During treatment by AOPs, TrOCs are usually degraded first into compounds closely related to their parent molecules with the loss or addition of a few functional groups [8-11]. These transformation products are in turn degraded further into low molecular weight compounds and since TrOCs such as pharmaceuticals are generally composed of aromatic rings, short-chain carboxylic acids are formed following aromatic ring opening if the advanced oxidation conditions allow it [12]. These acids can be produced at higher proportions compared to other transformation products since they are resistant to further oxidation [13].

Among the diverse AOPs available, WAO which uses water below its critical point and an oxidant such as air remains sparsely studied for municipal wastewater treatment despite showing high removal efficiency for organic compounds [14-18]. Previous studies have demonstrated that organic compounds are degraded mostly into acetic acid. However, other carboxylic acids are observed such as formic, propionic, oxalic, succinic, and *p*-coumaric [19,20]. Therefore, to ensure that WAO treatment conditions are optimal and that TrOCs are completely mineralized, monitoring of short-chain carboxylic acids is of interest, especially since they may accumulate during treatment. Additionally, monitoring the formation of transformation products in WAO is critical since in some conditions, treated effluents show toxicity towards a crustacean (*Daphnia magna*) and a bacterium (*Aliivibrio fischeri*) [14].

Transformation products of organic compounds, specifically carboxylic acids have been analysed by various techniques [21], such as reversed phase liquid chromatography coupled to UV detection to quantify oxidation products of interest such as phenolic compounds and muconic acid [22], oxalic and oxamic acids [23] or formic and acetic acids [24]. Ion-exclusion chromatography coupled with electroconductivity detection has been also employed to quantify formic, acetic, propionic, lactic, succinic, malic and citric acids [13]. Methods based on gas chromatography coupled with flame ionization detection have been developed to determine diverse carboxylic acids such as acetic, formic, propionic, butyric, isobutyric, valeric, isovaleric, hexanoic and heptanoic [25-27]. Methods using UV, electroconductivity or flame ionization detection are good choices for quantification of targeted compounds, but somehow limit the information that can be obtained from samples by not allowing non-targeted analyses.

Gas chromatography coupled with mass spectrometry (GC-MS) has been used with continuous solid-phase extraction (SPE) and microwave assisted derivatization to analyse acetic, propionic, oxalic, glycolic, succinic, fumaric acids and other small fatty acids [28]. However, methods using SPE or headspace solid-phase microextraction (HS-SPME) [25] present a risk of compound loss due to the selectivity of the extraction medium as well as during the evaporation step done before analysis in the case of SPE. This is especially critical in the case where targeted and non-targeted analyses are performed with the same method to quantify and identify known and unknown transformation products. In such acases a less selective sample preparation method than SPE or HS-SPME should be used to limit potential loss of analytes. Also, to adequately quantify volatile compounds, evaporation should be avoided.

All the methods mentioned above were able to meet certain specific objectives but are often limited in the number of analytes analysed which may restrict the monitoring of transformation products formed during advanced oxidation processes of specific TrOCs such as pharmaceuticals. For example, GC-MS methods for the quantification of selected volatile fatty acids in wastewater have been developed [29,30], however in the case of pharmaceuticals, only small carboxylic acids (< 6 carbons) are monitored to understand their transformation pathways, since they generally do not contain long carbon chains. Also, in some cases, previous analysis methods do not allow the identification of unknown transformation products of pharmaceuticals in the samples, which is important in monitoring of fate of these TrOCs after water treatment.

Therefore, a simple method requiring minimal sample preparation, capable of analysing semi-volatile and volatile carboxylic acids while also allowing the identification of unknown compounds in water is still lacking. For those reasons, the main objective of this study was to develop and validate a sensitive, rapid, and robust GC-MS quantitative method to monitor the formation of volatile (formic, acetic and propionic acids) and semi-volatile (lactic, glycolic, oxalic, malonic, glyoxylic, maleic, succinic, fumaric, malic, muconic and tartaric acids) carboxylic acids formed during treatment of water containing pharmaceutical residues by WAO or other AOPs. Additionally, the validated method was used to screen non-targeted transformation products. As a model case, the developed method was applied to study the fate of pharmaceuticals in hospital wastewater treated by WAO.

2. Material and methods

2.1 Chemicals and reagents

Molecular structures of target volatile and semi-volatile carboxylic acids are shown in Figure 1. Glacial acetic acid (purity 99.7 %), propionic acid (99.87 %), L-(+)-lactic acid (≥ 99.99 %), glycolic acid (99.5 %), oxalic acid (99.3 %), malonic acid (99.9 %), glyoxylic acid monohydrate (> 99.99 %), maleic acid (99%), succinic acid (99.8 %), fumaric acid (99.8 %), DL-malic acid (99.1 %), *trans,trans*-muconic acid (97.9 %) and L-(+)-tartaric acid (99.92 %) and acetaminophen (> 99.0 %) were purchased from Sigma-Aldrich Canada (Oakville, ON). Formic acid (> 99.0 %) was bought from Fisher Scientific Canada (Ottawa, ON). Dimethyl fumarate, acetic acid-d_4 (purity 99.27 %) and succinic acid-2,2,3,3-d_4 (purity 99 %) were used as internal standards and were also purchased from Sigma-Aldrich Canada.

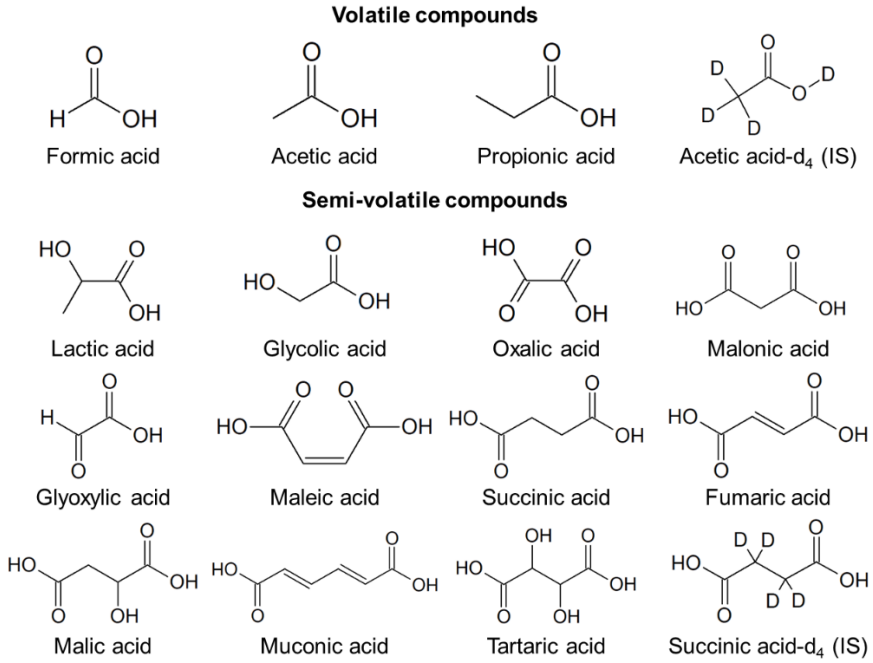

Figure 1. Molecular structures of the target analytes used in this study.

Acetonitrile (ACN) Optima LC/MS grade, methanol (MeOH) Optima LC/MS grade, methyl *tert*-butyl ether (MTBE) HPLC grade and water LC-MS grade were bought from Fisher Scientific Canada. N,O-bis(trimethylsilyl)trifluoroacetamide with 1% of trimethylchlorosilane (BSTFA with 1% TMCS) made by Cerilliant, dichloromethane (DCM) HPLC Plus grade and sodium chloride (NaCl) were bought from Sigma-Aldrich Canada. Diethyl ether anhydrous ACS reagent, anhydrous sodium sulfate ACS grade (Na_2SO_4) and hydrochloric acid 12 N (HCl) were purchased from VWR International (Mississauga, ON).

Fresh stock solutions of analytical standards of formic, acetic, propionic and acetic acid-d_4 standards were individually prepared before every analysis in LC-MS grade water at 1 g L^{-1} and were stored at 4 °C if they were used on the same day. Working solutions of volatile compounds and acetic acid-d_4 were prepared at 100 mg L^{-1} in LC-MS grade water. Stock solutions of semi-volatile acids and succinic acid-d_4 were prepared in MeOH at 1 g L^{-1} and stocked at -20 °C. Semi-volatile compound working solutions were prepared in LC-MS grade water at 5 mg L^{-1} and succinic acid-d_4 working solution were prepared in MeOH at 46.7 mg L^{-1} before every analysis. Dimethyl fumarate solution was prepared in MTBE-ACN 1:1 (*v/v*) at 1 g L^{-1}. BSTFA with 1% TMCS was kept at -20 °C.

2.2 Sample preparation

2.2.1 Volatile acids

Formic, acetic and propionic acids are in liquid state at room temperature and are also highly volatile compounds. Therefore, a liquid-liquid extraction was done to separate them from the matrix (water) to avoid an evaporation step. First, samples were diluted to be in the linearity range and were extracted as follows: 200 µL of diluted samples were filtered on 0.20 µm Millex PTFE syringe filters made by MilliporeSigma (Burlington, MA) and were then transferred to a 2 mL microcentrifuge tube. Then, a volume of 25 µL of acetic acid-d_4 internal standard at 100 mg L^{-1} in water was added. To acidify samples, 25 µL of HCl 1.2 N were added. The sample was vortexed for 20 s and 600 µL of MTBE were added for the first extraction. The solution was mixed for 1 min and centrifuged at 6000 rpm (2160 × *g*) for 4 min using a Fisherbrand High-Speed Mini-Centrifuge. Next, 400 µL of the organic phase were transferred in another microcentrifuge tube. A volume of 400 µL of MTBE was added in the first microcentrifuge tube to extract a second time. Then, the solution was mixed 1 min and centrifuged for 4 min again in the same conditions. Then, 400 µL were extracted and combined with the previous extract. The extracted portion was dried over with Na_2SO_4. A 500 µL volume of dried extract was filtered on 0.20 µm PTFE syringe filter and then transferred in a 2 mL vial and 100 µL of BSTFA with 1% TMCS were added for derivatization. Finally, the derivatized sample extract was injected for analysis by gas chromatography-quadrupole mass spectrometry (GC-MS).

2.2.2 Semi-volatile compounds

Samples that contain semi-volatile (i.e., in solid state at room temperature) compounds in water were evaporated and derivatized as follows: 500 µL of sample, diluted to be in the linearity range if necessary, and filtered on 0.20 µm Millex PTFE filter were added in a glass tube. Then, a volume of 30 µL of succinic acid-d_4 at 46.6 mg L^{-1} was added as internal standard. The solution was evaporated under a N_2 stream at 60 °C until dryness. A 620 µL volume of MTBE-ACN 1:1 *(v/v)* and 80 µL of BSTFA with 1% TMCS were added. The solution was vortexed for 1 min and heated for 30 min at 60°C in a heating block. Finally, the solution was transferred to a 2 mL vial and a volume of 1 µL was injected and analysed by GC-MS.

2.3 Gas chromatography-quadrupole mass spectrometry

All analyses were performed on a GC 2010 gas chromatograph coupled to a QP2010S quadrupole mass spectrometer made by Shimadzu (Kyoto, Japan). The chromatographic separation was carried out with an HP-5MS capillary column (stationary phase 5% diphenyl-95% dimethylpolysiloxane) of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness made by Agilent Technologies. The injector, ion source and the GC-MS interface temperature were set respectively to 250 °C, 250 °C and 300 °C. The flow rate of helium carrier gas was kept at 1 mL min^{-1} with a linear velocity of 36.1 cm s^{-1}. The injection volume of the samples was 1 µL.

For volatile organic compounds analysis, samples were injected with a solvent delay time of 2.2 min and a split ratio of 50:1. Since formic, acetic and propionic acids elute in the same window as the derivatization agent and its by-products, a 50:1 split ratio of was used to limit the introduction of these compounds in the column and to preserve the detector. The initial column temperature was 40 °C and it was held for 2 min, ramped to 120 °C at a rate of 15 °C min^{-1} and then finally ramped to 290 °C at a rate of 35 °C min^{-1} and held 0.5 min. For analysis of semi-volatile acids, samples were injected with a 5 min solvent delay time and the splitless mode was used with a sampling time of 0.35 min. This delay avoids the unnecessary detection of BSTFA and its by-products. The initial column temperature was 70 °C and held 1 min, ramped to 200 °C with a rate of 10 °C min^{-1} and finally ramped to 290 °C with a 35 °C min^{-1} rate.

For the two methods, electron ionization (EI) at 70 eV was employed. MS data were acquired in selected ion monitoring (SIM) mode using the target ion $[M\text{-}CH_3]^{\bullet+}$ and confirmed by other ions that had a significant response in the standard scans (Table 1).

Table 1. SIM parameters for compounds of interest.

	Compounds	**Retention time (min)**	**Quantitative ion (*m/z*)**	**Confirmation ions (*m/z*)**
Volatile compounds	Formic acid	2.31	103	75
	Acetic acid-d_4 (IS)	3.10	120	76
	Acetic acid	3.13	117	75
	Propionic acid	4.30	131	75
Semi-volatile compounds	Lactic acid	5.46	219	191, 147
	Glycolic acid	5.66	205	177, 147
	Oxalic acid	6.47	219	190, 147
	Malonic acid	7.51	233	248, 147
	Glyoxylic acid [a]	8.22	265	191, 147
	Maleic acid	8.91	245	147
	Succinic acid-d_4 (IS)	8.97	251	147
	Succinic acid	9.01	247	147
	Fumaric acid	9.44	245	147, 73
	Malic acid	11.38	335	233, 147
	Muconic acid	12.87	271	286, 147
	Tartaric acid	13.27	423	292, 147

[a] Glyoxylic acid in water is rapidly converted to a geminal diol (2,2-dihydroxyacetic acid). It is therefore in this form that glyoxylic acid was analysed.

Volatile compounds had only one confirmation ion because of their small size which does not allow further fragmentation. Maleic, succinic-d_4 and succinic acid also had one confirmation ion to maximize the chromatographic resolution and the dwell time since they were analysed in the same SIM segment.

2.4 Data processing

2.4.1 GC-MS acquisition and data treatment

GC-MS data were acquired by Shimadzu GCMS Real Time Analysis and processed using Shimadzu GCMS Postrun Analysis from GCMS Solution Version 2.50. Chromatogram peaks were integrated manually. Internal calibration with isotope-labelled compounds (acetic acid-d_4 and succinic acid-d_4) was employed and calibration curves using the least squares method were determined by Microsoft Excel 365. To determine the influence of heating time during derivatization, the peak area differences of each analyte were considered directly without any correction with the internal standards (acetic acid-d_4 and succinic acid-d_4), which themselves could also be influenced by heating. For semi-volatile compounds, dimethyl fumarate was used as internal standard to correct any variability in the peak areas due to injection in the GC-MS since peak area changes were rather subtle. For volatile compounds, peak area changes due to heating were reproducible, so internal standard was not necessary for those tests. Dimethyl fumarate was selected because it has a similar structure to the target compounds and was not affected by the derivatization step.

Statistical analyses were carried out with OriginPro 2020. One-way analyses of variance (ANOVA) tests were performed with a significance level $\alpha = 0.05$ and equal sample size for each condition ($n = 3$). Homogeneity of variance tests were done with Levene's test. The significative differences between means were determined by Tukey's posthoc test. In some cases, the assumption of homogeneity of variances was not respected. However, since ANOVA is a robust test towards variance heterogeneity, results were considered valid [31].

2.4.2 Method validation and quality control

Limits of detection and quantification (LOD and LOQ) were determined using the selected quantitative ions *m/z* listed in Table 1. The LOD of each analyte were calculated from calibration curves using the formula $LOD = 3 \times s_B/a$ and for $LOQ = 10 \times s_B/a$, where s_B is the standard error of the y-intercept and a the slope of the calibration curve [32]. Linearity was verified with correlation coefficients (R^2) higher than 0.99 and the linearity range was determined between the LOQ and the highest concentration tested. Trueness was expressed as relative bias and acceptable values were between -20 % and +10 % according to the European Commission decision 2002/657/EC [33]. Intra-day and inter-day precision were determined with the relative standard deviation (RSD

%). Recovery was determined by comparing the analytical response for extracted/evaporated samples at low and high concentration levels with unextracted standards (spiked solvent). Carryover was determined with the signal of a blank after analysis of the highest concentration compared to the smallest concentration of the calibration curve for each compound. This last parameter was studied to ensure that there are no cross-contamination and backflash in the injector to avoid ghost peaks and to ensure that the running parameters were adequate to keep the system clean.

2.5 Wet air oxidation conditions

2.5.1 Acetaminophen spiked deionized water sample

A volume of 40 mL of a concentrated solution of ACT (10.2 g L^{-1}) was introduced in a 170 mL horizontal batch reactor model Cellule 2646.0000 made by TOP Industrie (Vaux-le-Pénil, France) (Figure 2). The final desired volume was completed with 33 mL of deionized water added by a dosage pump. Air was purged with nitrogen to create an inert atmosphere while increasing temperature with a fast-heating ramp until 60°C and then with a ramp of 3 °C min^{-1} until reaching the desired temperature value. Based on previous study, 250 °C was chosen as working temperature because in those conditions, WAO exhibited good elimination rates for ACT [14]. Once 250 °C was reached, air in excess at 160 bar was injected to initiate the oxidation process. Samples were taken at 20 °C, 250 °C and after 10, 20, 30, 60 and 90 minutes after air injection. After completing the test, samples were preserved at -20 °C until GC-MS analysis.

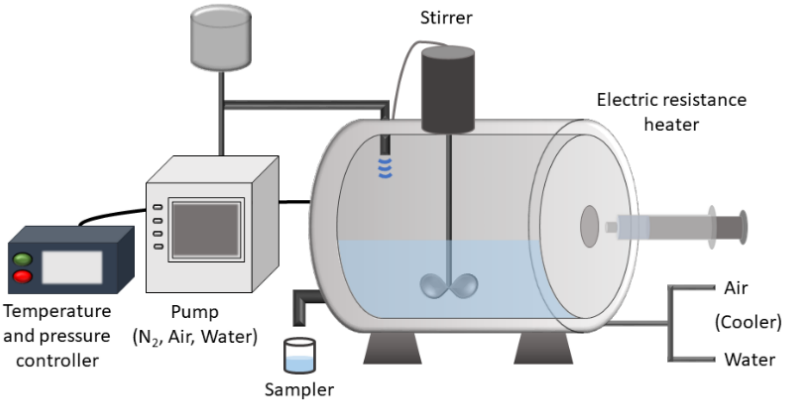

Figure 2. Simplified diagram of a WAO reactor

2.5.2 Hospital wastewater sample

A wastewater sample was collected at a local hospital and was treated with a HA1001 model batch reactor made by TOP Industrie. Four tests with different residence times were done. For each test,

a sample volume of 150 mL was introduced in the reactor and air was purged with nitrogen. Reactor was heated with a fast ramp until 60°C and then at a pace of 3 °C min^{-1} until reaching the temperature previously optimized for this type of sample (i.e., 290 °C) [14]. Air at 160 bar was injected in the reactor to start oxidation. WAO of hospital wastewater was performed at residence times of 10, 15, 20 and 25 min and at the end of each test, samples were collected and stored at -20 °C until GC-MS analysis.

3. Results and discussion

3.1 Method optimization for volatile compounds

Since some short-chain carboxylic acids are thermolabile or do not have adequate volatility or polarity to be analysed by gas chromatography, derivatization of analytes was required. Silyl derivatives are widely used for quantification or identification and many derivatives are listed in NIST library. N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) is generally used as silylating agent with trimethylchlorosilane (TMCS) as a catalyst to enhance derivatization efficiency. However, optimal conditions may vary from one analyte to another, such as temperature, heating time and solvent. Therefore, optimization tests were necessary [34,35].

3.1.1 Liquid-liquid extraction

Liquid-liquid extraction of volatile acids was carried out in MTBE because it is required to have an aprotic solvent not miscible in water to be able to derivatize with BSTFA right after extraction. Ethyl acetate was tested, but it created interferences with acetic acid because of solvent impurities. DCM was also tested but was too volatile and evaporated during the derivatization step due to heating.

To optimize liquid-liquid extraction, the salting-out effect was tested by adding NaCl to the saturation concentration (90 mg of NaCl in 250 µL aqueous sample). For each carboxylic acid, the addition of salt had no significant impact on signal improvement compared to no addition (Figure 3). Adding salt to the sample can improve liquid-liquid extraction by decreasing the solubility of analytes in the water phase and facilitate their transfer to the organic phase. Salt can also increase the density and viscosity of the aqueous phase, which reduces the efficiency of the dispersion of the extraction solvent, as observed by Makoś et al. (2018) [36]. The second condition tested was the acidification of samples by adding HCl so that most analytes could be in their undissociated form. Samples were under pH 2, about two pH units below the analytes' pK_a (formic: 3.75, acetic: 4.76 and propionic: 4.87). A significant signal increase was only observed for formic acid which has the lowest pK_a (Figure 3). The third condition tested was to combine salted and acidified

conditions by adding NaCl and HCl. Again, only formic acid showed significant differences compared to no addition (Figure 3). Considering these findings, subsequent optimization was carried out by adding only HCl to reduce the preparation steps. After extraction, samples were dried over with anhydrous Na_2SO_4 to improve the overall signal since silylation derivatization is affected by moisture [36,37].

3.1.2 Heating time

Heating can improve the derivatization rate by increasing analytes' solubility. For many compounds derivatization will be instantaneous while others require heating to be effective [34]. To test this condition, a 60 °C temperature was chosen to be near the boiling point of MTBE (55 °C) (Figure 4).

For each target volatile acid, heating caused a signal decrease that could be due to partial evaporation of compounds given their boiling points (acetic: 118 °C, formic: 101 °C, propionic: 141 °C). It was observed that all signals decreased significantly after 60 min of heating. Also, there were significant differences between 0 min and 30 min for formic and acetic acids and between 30 min and 60 min for acetic and propionic acids. For these reasons, the following analyses were carried out without heating.

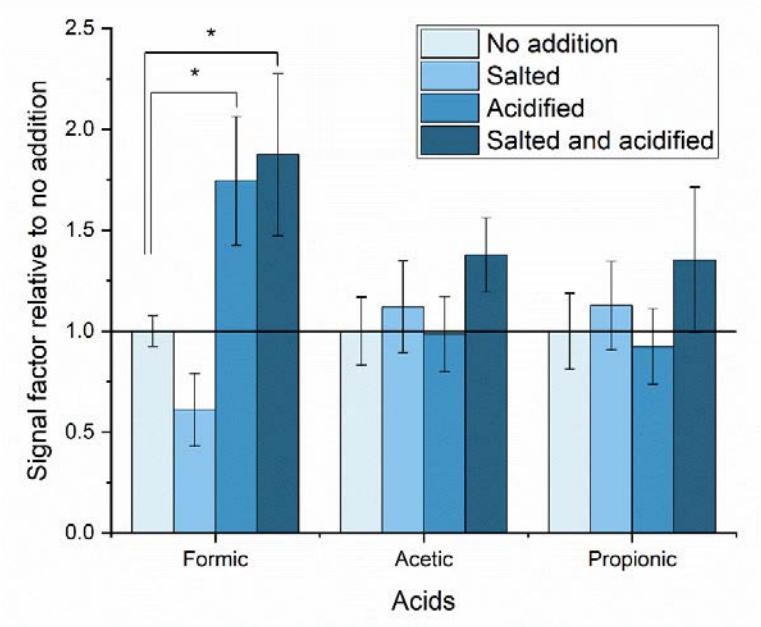

Figure 3. Signal improvement factor of liquid-liquid extraction optimization relative to unsalted and unacidified samples. Length of error bars represents ± 1 standard deviation (n = 3). Each compound was added at a concentration of 12.5 mg L^{-1}. The horizontal line indicates signal enhancement threshold = 1 (i.e., no change compared to unsalted and unacidified samples). Asterisks indicate statistically significant differences (p<0.05) according to the ANOVA and Tukey post hoc tests.

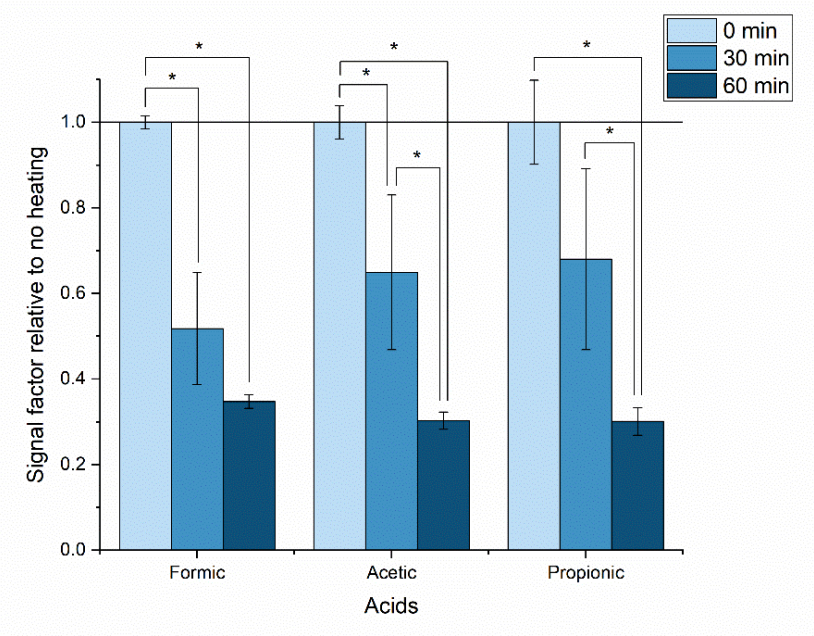

Figure 4. Signal decrease factor with heating time at 60 °C relative to room temperature for target volatile acids after adding BSTFA. Length of error bars represent ± 1 standard deviation (n = 3). Each compound was added at a concentration of 12.5 mg L^{-1}. The horizontal line indicates signal enhancement threshold = 1 (i.e., no change compared to room temperature samples). Asterisks indicate statistically significant differences ($p < 0.05$) according to the ANOVA and Tukey post hoc tests.

3.2 Method optimization for semi-volatile compounds

3.2.1 Derivatization solvent

Although BSTFA can be used alone as solvent, other organic solvents, which should not contain active hydrogen atoms, can be added during derivatization because aprotic organic solvents can enhance the dissolution of analytes and derivatives [34]. Less polar organic solvents such as ether are excellent solvents for the silylation reaction but do not accelerate the reaction rate unlike more polar solvents such as acetonitrile [38]. For these tests, low polarity solvents like ether and MTBE and high polarity solvents like ACN and DCM were chosen. A mixture of MTBE-ACN (1:1, *v/v*) was also tested. Results are shown in Figure 5.

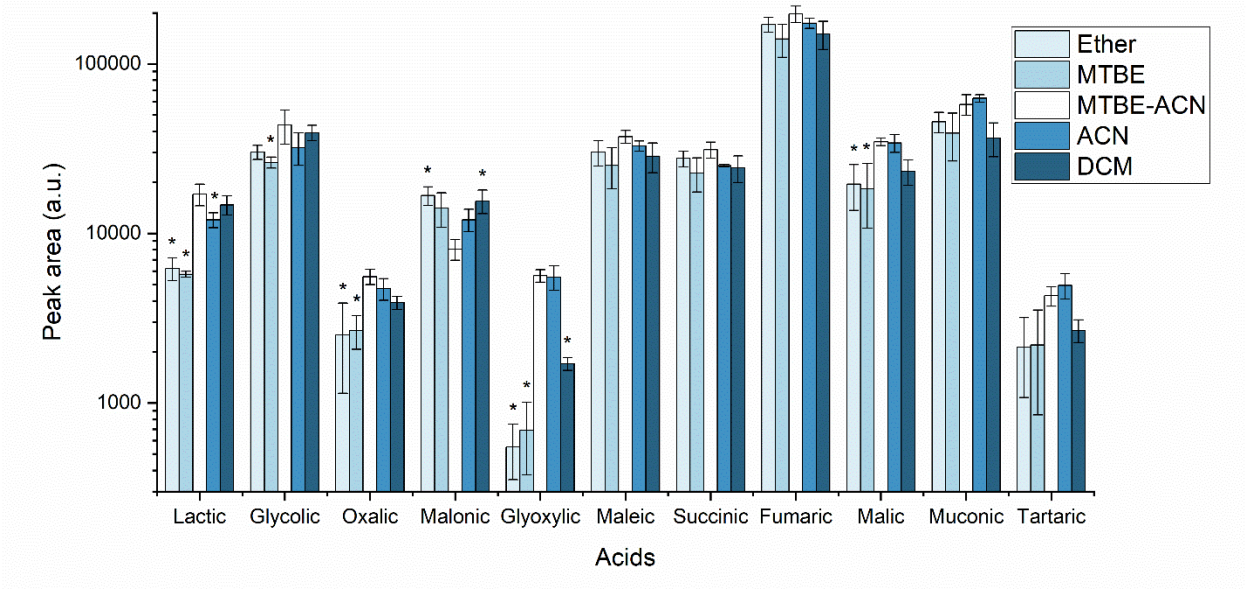

Figure 5. Effect of organic solvents added to BSTFA during derivatization of target semi-volatile acids. Length of error bars represent ± 1 standard deviation (n = 3). Each compound was added at a concentration of 1 mg L^{-1}. Asterisks indicate statistically significant differences with MTBE-ACN ($p < 0.05$) according to the ANOVA and Tukey post hoc tests.

According to statistical analysis, peak areas of maleic, succinic, fumaric, muconic and tartaric acids were statistically similar for all solvents. MTBE-ACN (1:1, *v/v*), ACN and DCM gave the best signal, especially for glycolic, oxalic, and malic acids. For lactic acid, MTBE-ACN and DCM were the best solvent choices. For malonic acid, it was DCM and ether and for glyoxylic acid it was MTBE-ACN and ACN. For the following experiments, MTBE-ACN (1:1, *v/v*) mixture was chosen as solvent to increase the solubility of analytes, facilitate the silylation reaction but also to limit the solvent evaporation during heating.

3.2.2 Heating time

Heating at 60 °C was also tested for semi-volatile acids (Figure 6). In this test, analyte peak areas varied but no trend with significant drops was observed for volatile acids.

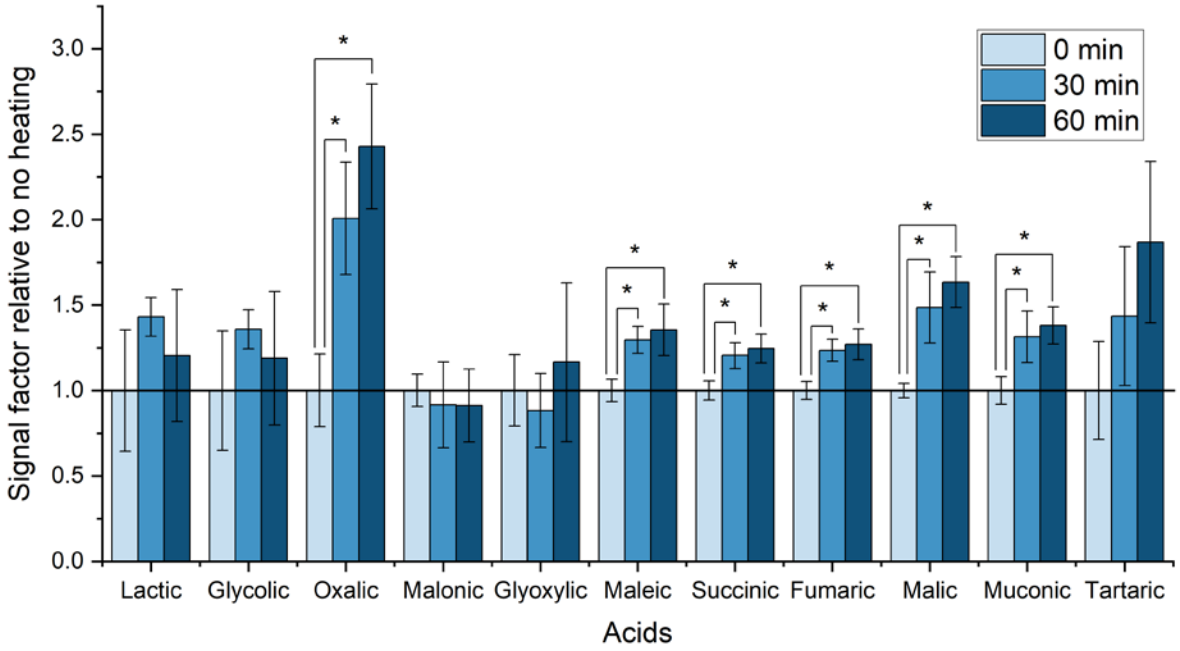

Figure 6. Signal factor with heating time at 60 °C relative to room temperature for target semi-volatile acids after adding BSTFA. Length of error bars represent ± 1 standard deviation (n = 3). Each compound was added at a concentration of 1 mg L^{-1}. The horizontal line indicates signal enhancement threshold = 1 (i.e., no change compared to room temperature samples). Asterisks indicate statistically significant differences ($p < 0.05$) according to the ANOVA and Tukey post hoc tests.

For six semi-volatile acids, heating caused a signal enhancement factor. Compared to volatile acids, this increase can be explained by the fact that semi-volatile acids have at least two functions to derive and require warming to improve solubility or drive the reaction to completion [38]. In all cases, there was no significant difference between 30 and 60 minutes, therefore 30 minutes of heating was chosen for further analyses.

3.3 Analytical performance of the optimized methods

Analytical performance parameters of the optimized methods were determined according to the procedures mentioned in section 2.4.2 and are shown on Table 2.

All compounds showed excellent linearity with correlation coefficients higher than 0.99 and an acceptable linearity range. Relative bias values right after derivatization were between -18.7 % and 3.4 %, which is largely within acceptable values. Stability of derivatized analytes was evaluated by relative bias at 9 h and 24 h after silylation. Samples were kept at room temperature in the auto-sampler rack. For all compounds, relative bias values after 9h were between -13 and

+7.2 %, which are also acceptable values. However, most compounds, excluding formic, acetic, maleic, succinic and fumaric acids, could not be determined with acceptable relative bias values 24 h after derivatization.

Intra and inter-day RSD% were between 1.7 % and 15.9 % excluding glyoxylic acid which were 25.0 % and 26.5 % respectively. This variation can be explained by the fact that glyoxylic acid is converted to its geminal diol form in water, thus analysed in this form. Therefore, the conversion may differ very likely from sample to sample and with temperature. Recovery rates were evaluated at two concentration levels (low and high) and acceptable values between 71 and 119 % were obtained, except for two compounds, lactic acid and glycolic acid, where values of 31 and 39 % were obtained at high concentration level. In agreement with the relative bias and correlation coefficient results, the use of internal standards allows the correction for lactic and glycolic acids, which allows quantification of these carboxylic acids. It was not possible to determine the recovery rates of glyoxylic acid since the diol form could only be formed in presence of water. Indeed, very low signals were observed for spiked MTBE-ACN samples, but higher and constant signals were obtained with evaporated spiked water samples, which confirms the need of water in samples and can explain the variability in the relative bias. Carryover values were between 0.1 and 6.4 % and no significant peaks were detected in blank, which confirms that there is no cross-contamination.

Table 2. Method performance for volatile and semi-volatile compounds.

	Acid	LOD (mg/L)	R^2	Linearity range (mg/L)	Relative bias (%) [a]			Relative standard deviation (RSD%, *n*=3)		Recovery (%) [b]		Carryover (%)
					At derivatization	9h after derivatization	24h after derivatization	Intra-day	Inter-day	Low level	High level	
Volatile compounds	Formic	1	0.9996	4-100	-5.0	-4.7	-2.2	8.6	16	92 ± 1	92 ± 14	0.8
	Acetic	1	0.9996	3-100	1.4	1.4	3.9	3.4	8.3	100 ± 7	96 ± 13	0.7
	Propionic	1	0.9995	4-100	3.4	-1.4	28	2.2	8.8	97 ± 2	119 ± 16	0.7
Semi-volatile compounds	Lactic	0.03	0.9972	0.1-5.00	3.4	-11	-37	6.4	9.1	96 ± 5	31 ± 4	1.1
	Glycolic	0.05	0.9936	0.2-5.00	1.5	-11	-38	1.7	5.6	80 ± 5	39 ± 6	0.9
	Oxalic	0.05	0.9997	0.1-5.00	-4.9	-2.2	-41	5.9	13	98 ± 10	118 ± 3	5.7
	Malonic	0.06	0.9993	0.2-5.00	-9.0	-19	-44	13	13	82 ± 9	71 ± 7	3.1
	Glyoxylic	0.2	0.9943	0.6-5.00	-17	-13	-44	25	26	N.D.	N.D.	5.5
	Maleic	0.07	0.9974	0.2-5.00	0.9	7.2	4.8	13	14	102 ± 12	112 ± 8	1.1
	Succinic	0.01	0.9999	0.05-5.00	-0.3	3.9	-1.2	2.4	4.3	101 ± 6	102 ± 6	1.0
	Fumaric	0.04	0.9985	0.1-5.00	-6.5	-11	-7.4	3.0	2.9	85 ± 3	93 ± 7	0.1
	Malic	0.1	0.9954	0.4-5.00	-12	-6.4	-39	9.0	14	92 ± 7	107 ± 5	5.4
	Muconic	0.1	0.9967	0.4-5.00	-19	-12	-38	6.0	10	91 ± 7	108 ± 10	0.6
	Tartaric	0.1	0.9953	0.4-5.00	-3.7	-6.0	-36	4.2	7.1	101 ± 14	90 ± 2	6.4

[a] Relative bias, intra-day and inter-day RSD % were determined at 45 mg L^{-1} for volatile acids and 0.75 mg L^{-1} for semi-volatile acids.
[b] Recovery ratios were determined at 5 and 50 mg L^{-1} for volatile acids and 0.75 and 4 mg L^{-1} for semi-volatile acids.
N.D. : not determined

3.4 Applications to monitor transformation products generated by wet air oxidation

3.4.1 Spiked acetaminophen in water

Optimized analytical methods were used to monitor transformation products formed during WAO treatment of a water sample spiked with an organic compound. Common concentrations of pharmaceuticals in hospital wastewater are in the range of micrograms-per-liter [39,40]. To carry out an analytical proof of concept, a deionized water sample containing initially about 5 000 mg L^{-1} of ACT, which has a theoretical COD of 1.8 g O_2 per g of ACT (according to Reaction 1), was treated by wet air oxidation at 250 °C for 90 minutes as described in section 2.5.1. ACT quantification was carried out using the method for semi-volatile compounds. A validation was done beforehand (R^2=0.9983, LOD = 0.1 mg L^{-1}, linearity range from 0.5 to 5 mg L^{-1}, relative bias = -0.41 % , relative standard deviation = -2.2% at 4 mg L^{-1} (n = 3) were obtained. Analytical methods developed previously are therefore suitable for expected concentrations under these conditions and dilutions were necessary.

Monitoring of transformation products over treatment time was possible (Figure 7) and the major product formed was acetic acid, reaching a concentration around 900 mg L^{-1} after 60 minutes. In these conditions, succinic and glycolic acid were also formed with maximal concentrations of 161 and 113 mg L^{-1} respectively after 30 minutes. Maleic acid reached a concentration of 53 mg L^{-1} at 10 minutes before undergoing further transformation. Oxalic, glyoxylic, fumaric and malic acids were also formed with maximal concentrations ranging from 2 to 11 mg L^{-1} at 10 minutes. Propionic, malonic, muconic and tartaric acids were < LOD. These results are consistent with other AOPs treatment of acetaminophen where the formation of similar compounds was documented [41,42].

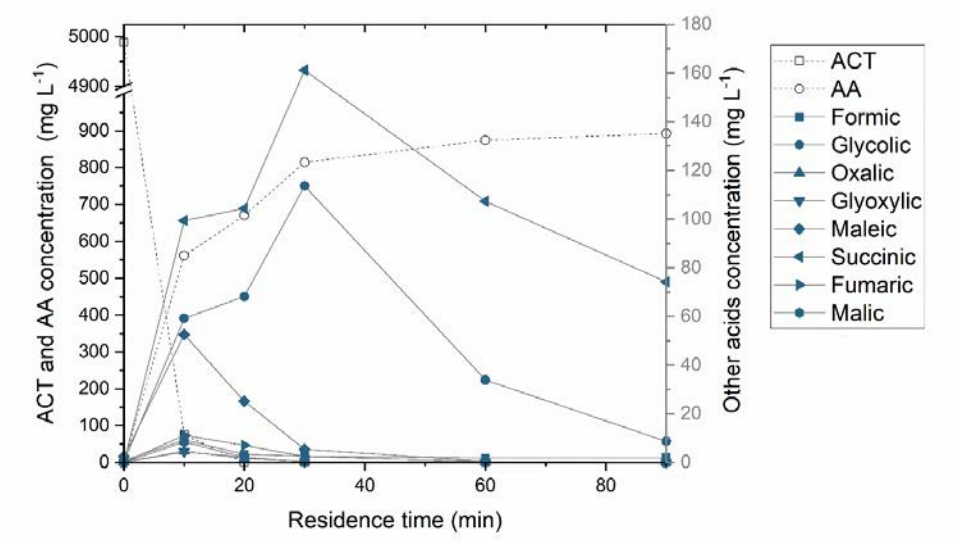

Figure 7. Concentration profile of carboxylic acids formed during wet air oxidation of ACT in deionized water. Left y-axis represents acetaminophen (ACT) and acetic acid (AA) concentrations and right y-axis represents concentrations of other acids.

Actual COD was also determined by the dichromate method [43] before WAO and after 90 min of treatment, however this measure only provides a global value of the organic load in those samples. Thus, to determine the COD distribution, individual loads were estimated based on the measured concentrations of each acid in both samples by using the balance equation for a complete oxidation process [44] (Reaction 1):

$$C_mH_nO_kCl_wN_xS_yP_z + [m + 0.25\,(n - 3x) - 0.5k + 2\,(y + z)]\,O_2 \rightarrow mCO_2 + 0.5(n - 3x)\,H_2O + xNH_3 + wCl^- + ySO_4^{-2} + zPO_4^{3-} + \text{heat} \quad \text{(Reaction 1)}$$

From this equation, the contribution of ACT and each acid to the global COD was determined. Initially, acetaminophen contributed to 100 % of the COD, but after 90 min of WAO treatment, acetic acid represented 35 % of the COD, succinic acid 2.8 % and the other target acids 0.3 %. About 61.9 % of the COD was still unknown from this WAO experience. In comparison with other AOPs like UV-LED-based advanced oxidation processes [45], electro-Fenton process [5] or UV/H_2O_2[41], other intermediates including amino compounds, hydroquinone, benzoquinone, benzaldehyde and benzoic acid were monitored, hence the usefulness of a non-targeted analysis coupled to SIM methods since the intermediates produced by WAO treatment are not yet well known.

3.4.2 Qualitative and quantitative analysis of hospital wastewater

Developed methods were also used to monitor transformation products generated after WAO of wastewater sampled from a local hospital. First, wastewater samples were treated by WAO with residence time of 10 min, 15 min, 20 min and 25 min after reaching 290 °C. Untreated and treated samples were then prepared and derivatized using optimized methods. First, non-targeted (Figure 8) GC-MS analyses were carried out to identify a maximum of compounds present in all samples before treatment and ensure a thorough monitoring over time of transformation products. The preparation method employed for semi-volatile carboxylic acids was used for non-targeted analyses to limit the loss of compounds during water extraction.

Identification of non-targeted compounds was made according to the NIST 08 database with a match probability > 90 %. Glycerol, glycolic acid, methenamine, benzoic acid and succinic acid were confirmed with standards. Non-targeted analysis of untreated hospital wastewater revealed the presence of amino acids, urea, glycerol and creatinine, which are metabolic products commonly found in urine [46,47]. However, these results compared with the non-targeted analysis of 10 min treated wastewater showed a completely different chromatogram. Glycolic and succinic acids, both of which can be quantified by the developed method, as well as methenamine, benzoic acid and glycerol could be observed. A decrease in the signal of glycerol of 85% was observed and transformation of initial compounds seemed complete. Non-targeted analysis was also performed on 15 min, 20 min and 25 min treated samples and similar chromatograms to the sample treated for 10 min were obtained. Short-chain carboxylic acids were also quantified by methods developed herein in the same samples (Table 3).

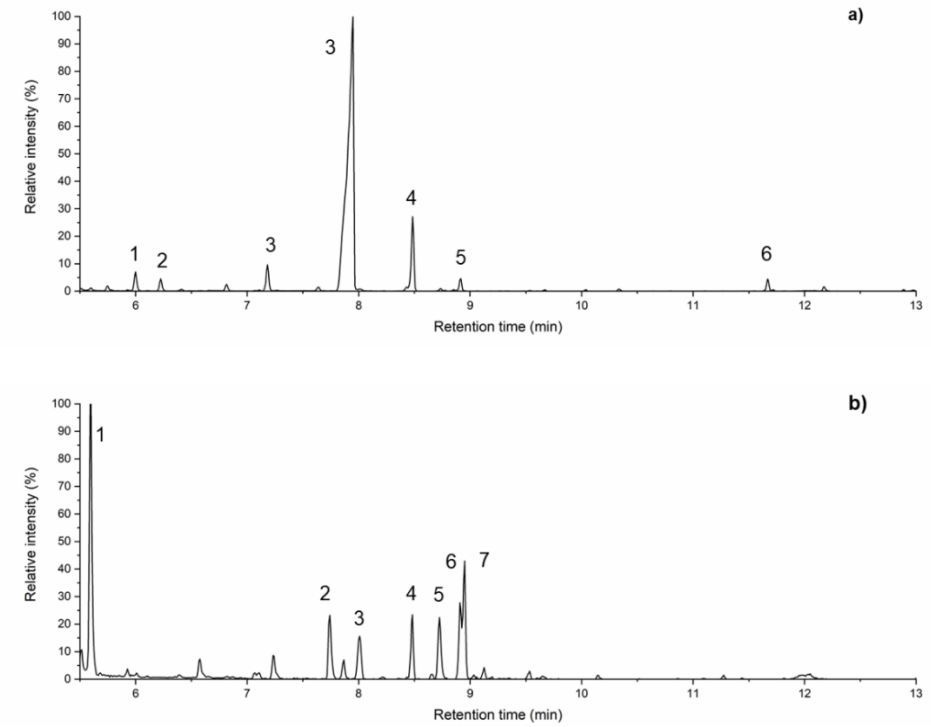

Figure 8. Non-targeted analyzes of a) untreated hospital wastewater (1. alanine, 2. glycine, 3. urea, 4. glycerol, 5. succinic acid-d_4 [internal standard] 6. creatinine) and b) hospital wastewater treated for 10 min (1. glycolic acid, 2. methenamine, 3. benzoic acid, 4. glycerol, 5. unknown compound (database match < 90%), 6. succinic acid-d_4, 7. succinic acid).

Table 3. Quantification of short-chain carboxylic acids in untreated and treated hospital wastewater by wet air oxidation.

Compounds	Untreated wastewater (mg L^{-1})	Wastewater treated by wet air oxidation			
		10 min (mg L^{-1})	15 min (mg L^{-1})	20 min (mg L^{-1})	25 min (mg L^{-1})
Formic acid	-	11.79	16.53	12.66	12.71
Acetic acid	> LOD, < LOQ	38.71	42.80	38.44	39.17
Propionic acid	-	-	-	-	-
Lactic acid	-	-	-	-	-
Glycolic acid	-	3.90	3.71	1.46	1.34
Oxalic acid	-	-	-	-	-
Malonic	-	-	-	-	-
Glyoxylic	-	-	-	-	-
Maleic	-	-	-	-	-
Succinic	-	5.44	6.49	3.21	2.61
Fumaric	-	-	-	-	-
Malic	-	-	-	-	-
Muconic	-	-	-	-	-
Tartaric	-	-	-	-	-

- : concentration < LOD

For untreated hospital wastewater, all acids were below their LOD. Only acetic acid was above the LOD, but was not quantified because it was below its LOQ. In hospital wastewater treated by WAO, formic, acetic, glycolic and succinic acids were formed and quantified. Formic and acetic acids were mainly formed among the targeted compounds which agrees with ACT previous results (Figure 7). However, unlike the ACT treatment, other acids were not quantifiable probably due to the lower initial COD (573 mg O_2 L^{-1} vs. 10 000 mg O_2 L^{-1} for wastewater and ACT samples respectively) and the higher treatment temperature (290 °C vs. 250 °C). Using Reaction 1, the COD distribution of each treated sample was determined.

No carboxylic acid was quantified in the untreated wastewater sample. Therefore, the totality of measured COD was considered as unidentified (Figure 9). For the treated wastewater sample, the quantified acids represented 35 %, 46 %, 39% and 38% of the actual COD respectively for 10, 15, 20 and 25 min of residence time. This means that 54 % to 65 % of the COD remains to be identified in the wastewater samples treated by WAO.

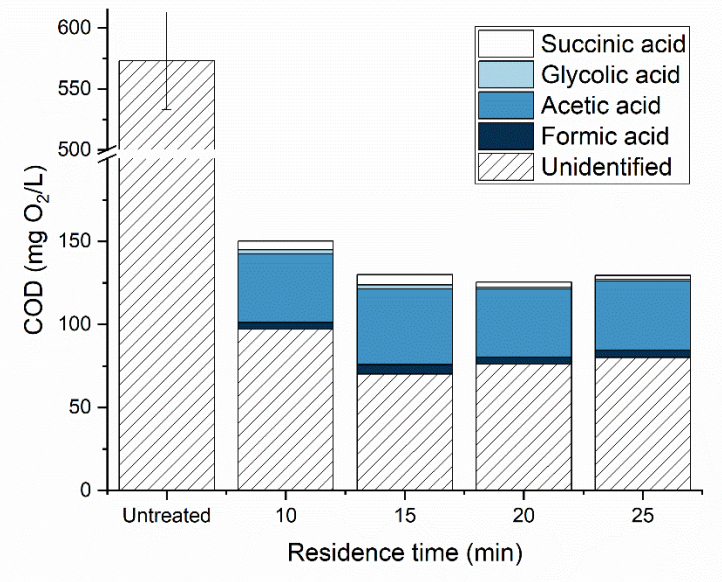

Figure 9. COD distribution of wastewater samples

4. Conclusion

The present study showed the development of a efficient analytical GC-MS method to quantify 3 volatile and 11 semi-volatile carboxylic acids in water samples. The validated method showed satisfactory linearity, trueness, recovery and precision suitable to analyse (C_1-C_6) volatile and semi-volatile carboxylic acids in water at concentrations between 3.4 mg L^{-1} and 100 mg L^{-1} and 0.05 mg L^{-1} to 5 mg L^{-1} respectively. Analysis of water samples containing acetaminophen demonstrated that the methods allowed to monitor the formation of short-chain organic acids during WAO treatment that represented 38 % of the final COD. Formic, acetic, glycolic and succinic acids could also be monitored in hospital wastewater according to treatment time, which made it possible to follow the transformation of organic compounds by identifying 35 % to 46 %

of the final COD. The developed method also allowed to perform non-targeted analysis and identified glycerol, glycolic acid, methenamine and benzoic acid in hospital wastewater samples treated by wet air oxidation. This method could be applied to monitor degradation of other TrOCs and the evaluate performance of other AOPs.

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