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Characterization of carboxylic acids in atmospheric aerosols using hydrophilic interaction liquid chromatography tandem mass spectrometry

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ABSTRACT

A sensitive hydrophilic interaction liquid chromatography/electrospray ionization mass spectrometry (HILIC/ESI-MS/MS) method was developed for determination of selected aliphatic (i.e. malonic, succinic, glutaric, adipic, pimelic, suberic, azelaic, maleic, fumaric, glycolic and pyruvic acid), alicyclic (i.e. cis-pinonic and pinic acid) and aromatic (i.e. trimesic, phthalic acid and its isomers) carboxylic acids. Analytes were separated on an amide column using a gradient elution with a 10 mM constant ionic strength mobile phase containing acetonitrile and aqueous ammonium acetate buffer (pH 5.0). The influence of the buffer type, pH, polar modifier and temperature on analyte retention under HILIC was studied. Static sonication-assisted solvent extraction was optimized for sample preparation prior to analysis. The recoveries obtained were higher than 90% for most analytes. The method was proven to be sensitive with limits of detection ranged from 0.03 to 16.0 μg/L in selected reaction monitoring mode (SRM). The repeatability and intermediate precision of the method, expressed as RSD (%) of the peak area ratio between analytes and their internal standards were generally lower than 5%. The method was successfully applied for determination of the studied acids in samples of ambient aerosol particles. A big advantage of the new method is also its ability to detect and separate the isobaric compounds of the selected carboxylic acids. Our results demonstrate that the method is specific and sensitive for the determination of a wider range of polar carboxylic acids at low concentrations in complex samples of aerosol particles.

1. Introduction

Aerosol particles are ubiquitous in the troposphere and exert an important influence on the global climate and environment. Organic material represents a substantial fraction of ambient aerosols; hence it is an important contributor to the earth's climate, dictating air quality and the adverse human health effects of atmospheric aerosols. Until now around 10,000–100,000 different organic compounds have been measured in the atmosphere [1]. Special attention is given to so called secondary organic aerosols (SOA), whose formation mechanisms and chemical composition, in general, are very poorly understood; therefore, this can be a major source of uncertainty in the prediction of aerosol concentrations and properties [2,3].

SOA is formed when the atmospheric oxidation products of volatile organic compounds (VOCs) undergo gas-to-particle transfer. The initial oxidation of VOCs in the atmosphere generates less volatile and more water soluble oxygenated products which may contain different functional groups (e.g. carboxylic, aldehyde, ketone, etc.) and also can be further oxidized. All these oxidized products comprise an important fraction of the organic mass found in SOA, known as water-soluble organic compounds (WSOC). WSOC play an important role in the ability of aerosol particles to act as cloud condensation nuclei (CCN) [4] and in the complex and not yet well known liquid-phase chemistry of clouds and fog [5]. Despite intensive scientific work and progress in recent years WSOC are still not well chemically characterized [6]. Up to now, several groups of organic compounds are identified in WSOC, such as: mono-, di- and poly-carboxylic acids, their keto, hydroxyl and oligomer products, ketones, aldehydes, polyols, amines, organosulfates and organonitrates (e.g. [7–12]).

For determination of carboxylic acids, which are very common organic constituents in atmospheric aerosols, different separation techniques have been used, such as ion chromatography (IC) [13–16], liquid chromatography–mass spectrometry (LC–MS) [9,17–20], two-dimensional liquid chromatography – time-of-flight mass spectrometry [21], capillary electrophoresis [22–24] and gas chromatography–mass spectrometry [25–30]. Hydrophilic interaction liquid chromatography (HILIC) tandem mass spectrometry has been recently used to characterize functional groups present in high-molecular weight, water-soluble organic components in atmospheric aerosols [7].

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HILIC is a type of normal-phase liquid chromatography technique which uses common reversed-phase solvents in the mobile phase and is mainly used for separation of polar and ionized compounds [31,32]. Polar organic acids usually have no retention under reversed-phase (RP) conditions or sometimes can only be retained using 100% aqueous mobile phases. On the other hand, HILIC uses organic (usually acetonitrile)/aqueous mobile phases with high organic-to-water ratios (higher than 65% of organic). The electrospray ionization (ESI) process is significantly enhanced when using such mobile phases, therefore providing lower detection limits [33].

The retention mechanisms in HILIC are complex including partitioning, hydrogen bonding, electrostatic, dipole–dipole interactions, ion-exchange, but also adsorption of the analytes between the water-enriched layer on the polar stationary phase and the bulk mobile phase [31,34,35]. Amide stationary phase is less reactive compared to amino or bare silica phases and is said to be less prone to adsorption and mobile phase pH effects [32,35–37]. In comparison to bare silica and diol columns, a wider applicability of the amide column for separation of different acidic and basic compounds was recently reported [38]. Using a proper buffer pH is essential for keeping analytes ionized and more retentive throughout the separation [33,39].

The goal of the present work was to develop a sensitive and comprehensive method for characterization (separation, identification and quantification) of different carboxylic acids in atmospheric aerosols using a hydrophilic interaction liquid chromatography–electrospray ionization tandem mass spectrometry (HILIC/ESI-MS/MS). A new method was applied for the quantitative determination of the following classes of acids: aliphatic mono- and dicarboxylic (C_2–C_9), alicyclic mono- and dicarboxylic, and aromatic di- and tricarboxylic acids in atmospheric aerosol particles. The influence of the chromatographic parameters on HILIC retention of these acids was also studied.

2. Experimental

2.1. Reagents, standards and standard solutions

Acetonitrile and methanol (Chromasolv gradient grade, for HPLC, ≥99.9%, Sigma Aldrich, St. Louis, USA), 2-propanol (LiChrosolv gradient grade, for HPLC, ≥99.9%, Merck KGaA, Darmstadt, Germany), ethanol (absolute, ACS reagent, ≥99.5%, Sigma Aldrich), tetrahydrofuran (Chromasolv plus, for HPLC, ≥99.9%, inhibitor-free, Sigma Aldrich) and high purity water (18 MΩ), supplied by a Milli-Q water purification system (Millipore, Bedford, MA, USA) were used for the mobile phase preparation. Methanol and acetonitrile were also used for preparation of carboxylic acid standard solutions and for extraction of filter deposits. Glacial acetic acid (100% Suprapur), ammonium acetate (Fractopur), formic acid (98–100% GR for analysis) and ammonia solution (25%, Suprapur) all from Merck as well as ammonium formate (Puriss p.a., eluent additive for LC) and ammonium bicarbonate (eluent additive for LC–MS) both from Fluka (Buchs (SG), Switzerland) were used for buffer preparation.

The following standards (Table A.1 in Appendix A) were used for preparation of standard solutions: maleic (Agros, Geel, Belgium); oxalic, malonic, malic and glycolic acid (all from Fluka, Buchs (SG) Switzerland); succinic, glutaric, adipic, pimelic, suberic, azelaic, pyruvic, benzoic, phthalic, trimesic and fumaric acid (all from Merck KGaA, Darmstadt, Germany); isophthalic, terephthalic, cis-pinonic, pinic, phthalic-3,4,5,6-d_4 and succinic-2,2,3,3-d_4 acid (all from Sigma Aldrich). Except trimesic acid with the purity of 98.7%, all other standards had purity higher than 99% and were used without further purification.

Individual standard stock solutions of the studied natural and deuterated acids (as internal standards) were prepared at concentrations of 100 mg/L in methanol. From the stock solutions composite standard solutions were made in acetonitrile at concentrations of 10, 250 and 1000 μg/L for the natural acids and of 500 μg/L for the deuterated acids. These composite standard solutions were further diluted using 10 mM ammonium acetate buffer (pH 5.0) in acetonitrile–water mixture 90:10 (v/v) (in the following text as initial mobile phase – iMP) to prepare final calibration standards ranging from 0.01 to 500 μg/L (all containing both internal standards at fixed concentration of 50 μg/L). Before the injection into the LC–MS/MS system, the standard mixture was filtered through a PTFE membrane filter (pore size 0.2 μm, Iso-Disk, Supelco, Bellefonte, PA, USA). All standard solutions were stored at 4 °C and were stable for at least 2 months.

2.2. Instrumentation and optimization of ESI-MS/MS conditions

In our study an Agilent 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany, equipped with degasser, quaternary pump, autosampler and diode-array UV/Vis detector-DAD) coupled to a triple quadrupole – linear ion trap hybrid mass spectrometer (4000 QTRAP LC/MS/MS System, Applied Biosystems/MSD Sciex, Ontario, Canada), equipped with a TurboIonSpray (TIS) source (a variation of electrospray ionization – ESI source), was used. Central supply of high purity nitrogen was used as nebulizer, drying and collision gas (for the MS). Gradient delay (dwell) volume of the HPLC system is 1085 μL and was determined by the procedure given elsewhere [40]. Analyst 1.5 Software (Applied Biosystems/MDS Analytical Technologies Instruments) was used for acquisition and analysis of the LC–MS/MS data.

A negative polarity ESI-MS/MS was used for detection of the target acids. The triple quadrupole (QqQ) scanning mode, selected reaction monitoring (SRM), was used for specific detection and sensitive quantification of the analytes. Compound dependent MS parameters were optimized for all analytes. For that purpose, a mixed standard solution (200 μg/L) was syringe infused directly into the TIS (ESI) source at a flow rate of 10 μL/min. For all acids the $[M-H]^-$ deprotonated molecular ions were the most intensive and were chosen for further optimization of the SRM conditions. Under the same conditions product ion spectra were obtained and for each acid the highest intensity product ion was chosen for further optimization. The optimized compound dependent MS/MS parameters, such as declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP) are given in Table A.1 in Appendix A. Unit resolution was set for both mass scanning quadrupoles Q1 and Q3. The ion source dependent parameters were optimized using flow injection analysis (FIA) at a flow rate of 0.5 mL/min, by coupling the HPLC system (without column) with the MS instrument and injecting 50 μL of 10 μg/L mixed standard. The MS dwell time was set to 75 ms. Two isocratic mobile phase compositions were used during FIA-MS/MS, i.e. ACN: water: 100 mM ammonium acetate buffer pH 5 = 81:9:10 (v/v/v) and ACN:water:100 mM ammonium acetate buffer pH 5 = 72:18:10 (v/v/v). The final ion source parameters were chosen from both FIA-MS/MS experiments in such a way to maximize the peak response (sensitivity) for all acids throughout the whole gradient. These conditions were tested later with LC–MS/MS and proved to be optimal for balanced sensitivity between early and late eluting acids. The curtain, nebulizer (Gas 1) and auxiliary gas (Gas 2) were set to 12.0, 55.0 and 60.0 psi, respectively. Ion spray voltage was −4500 V and source temperature was held at 650 °C. Collision gas (CAD) was set high (vacuum: $4.5–5.0 \times 10^{-5}$ torr, base vacuum – with collision gas turned off: 0.9×10^{-5} torr).

Table 1
Gradient elution program for the optimized HILIC–ESI/MS/MS method.

Time (min)	A (%)	B (%)	C (%)
0	10	90	0
1	10	90	0
3	10	81	9
13	10	81	9
18	10	72	18
19	10	72	18
20	10	63	27
25	10	63	27
26	10	90	0
30	10	90	0

Solvent A: 100 mM ammonium acetate buffer pH 5.0.
Solvent B: acetonitrile.
Solvent C: MilliQ water.

2.3. Chromatographic conditions

The chromatography was performed on an XBridge Amide column (100 mm × 3.0 mm I.D., 3.5 μm particle size, Waters, Milford, MA, USA). The ternary mobile phase consisted of acetonitrile (ACN), water and 100 mM ammonium acetate aqueous buffer at pH 5.0. Gradient elution was employed during which an effective final buffer concentration of 10 mM was maintained at a flow rate of 0.5 mL/min (Table 1). The column temperature was kept constant at 25 °C by means of thermostated water bath.

2.3.1. HILIC retention of analytes as a function of percent of acetonitrile

A constant ionic strength (10 mM) of mobile phase containing ammonium acetate buffer (pH 5.0) in ACN – water mixture was used isocratically at a flow rate of 0.5 mL/min. ACN content in the final mobile phase was varied from 65 to 90% in 5% increments. The test standard solution was a mixture of selected acids, each at concentration of 12.5 mg/L. An injection volume of 50 μL, column temperature of 25 °C and the detection at 210 and 220 nm (20 Hz acquisition rate) were used.

2.3.2. Influence of buffer type on the HILIC retention

An optimized gradient elution with a constant ionic strength of mobile phase (10 mM) containing different buffers in ACN–water mixture was used (Table 1). For this purpose ammonium acetate, ammonium formate and ammonium bicarbonate buffers at pH 9.0 and ammonia solution at pH 10.9 were used. A flow rate of 0.5 mL/min, column temperature of 25 °C, injection volume of 50 μL and MS detection (in SRM) were employed. The concentration of the selected acids in the test standard mixture was 500 μg/L.

2.3.3. Influence of buffer pH on the HILIC retention and on ESI-MS/MS sensitivity

Buffers containing ammonium acetate, ammonium formate and ammonium bicarbonate with different pH values were examined. The chromatographic and detection conditions as well as the test standard mixture used were the same as those described in Section 2.3.2. The signal-to-noise (S/N) ratio of the carboxylic acids was determined using the S-To-N script in the Analyst 1.5 Software.

2.3.4. Influence of polar modifier on the HILIC retention

A mobile phase with a fixed concentration of 10 mM ammonium acetate buffer with a pH 5.0 in a quaternary mixture consisted of ACN–water–100 mM ammonium acetate buffer-polar modifier was used under the optimized gradient elution conditions. Five percent of the aqueous content in the mobile phase was replaced with methanol, ethanol, 2-propanol or tetrahydrofuran to study the change in analyte retention. The test standard mixture and the other chromatographic and detection conditions were the same as in Section 2.3.2.

2.3.5. Influence of column temperature on the HILIC retention

Column temperature was varied from 25 to 45 °C in 5 °C increments. A mobile phase containing 10 mM ammonium acetate buffer with pH 5.0 in ACN–water mixture was employed under optimized gradient conditions. Other chromatographic and detection parameters were the same as in Section 2.3.2.

2.4. Sample collection and preparation

The samples with particulate matter PM_{10} (particles with size below 10 μm) and control blank filters were provided by the Environmental Agency of the Republic of Slovenia. PM_{10} samples were collected on quartz fiber filters (QMA 47 mm diameter, Whatman) at semi-urban location in Ljubljana using a low volume reference sampler Leckel. The air flow through the sampler was 2.3 m^3/h and the sampling time was 24 h. Filters were heated at 500 °C for 3 h before sampling. The weighing of the filters were done according to the standards: EN 12341:2000 and EN 14907:2005; they were weighed before and after sampling, after conditioning for 48 h at RH of 50 ± 5% and temperature of 20 ± 1 °C. The samples from winter (February) and summer (August) 2010 were chosen for the quantitative determination of the studied acids. Filters with deposits and blank filters were stored at −18 °C until analysis. Before extraction, they were equilibrated to room temperature under controlled ambient conditions (in Cleansphere) and afterwards spiked with the standard solution of succinic-d_4 and phthalic-d_4 acid (absolute mass of 0.1 μg). The spiked filters were extracted using the adapted static sonication-assisted solvent extraction procedure [17,21]. They were extracted three times with 15 mL of methanol in an ice ultrasonic bath for 15 min. The total extract was subsequently evaporated to dryness at 25 °C under a gentle stream of nitrogen. The residue was dissolved in 2 mL of iMP. All information concerning the optimization and influence of the real aerosol particles on the extraction recovery is given in Appendix A.

3. Results and discussion

3.1. Optimization of ESI-MS/MS conditions

The SRM is very selective tandem MS scan mode that provides sensitive detection of trace compounds by reducing the chemical noise. Although the SRM transitions of oxalic, benzoic and malic acids gave some response during the direct syringe infusion – MS/MS and FIA-MS/MS, these acids were not detected by HILIC–ESI/MS/MS at any concentration used in this study. They were separated on column and detected using LC-UV/Vis DAD, which confirms their retention under HILIC conditions. The inability to detect oxalic acid with the similar LC–MS/MS system has been recently reported [41]. We observed the same difficulties with malic acid also, while benzoic acid eluted next to dead time and its ESI-MS/MS response was diminished by the enhanced ion suppression near dead time. Thus, these three acids were excluded from further LC–MS/MS experiments.

3.1.1. Investigation of the ion suppression effects during HILIC–ESI/MS/MS

It is well known that the method performance is directly influenced by the matrix effects on ionization (such as ion suppression or ion enhancement of the analyte signal) in LC–MS [42]. Therefore, careful examination of these effects is very important during method development for trace analysis in complex samples. We initially address the possible problems arising from the matrix effects

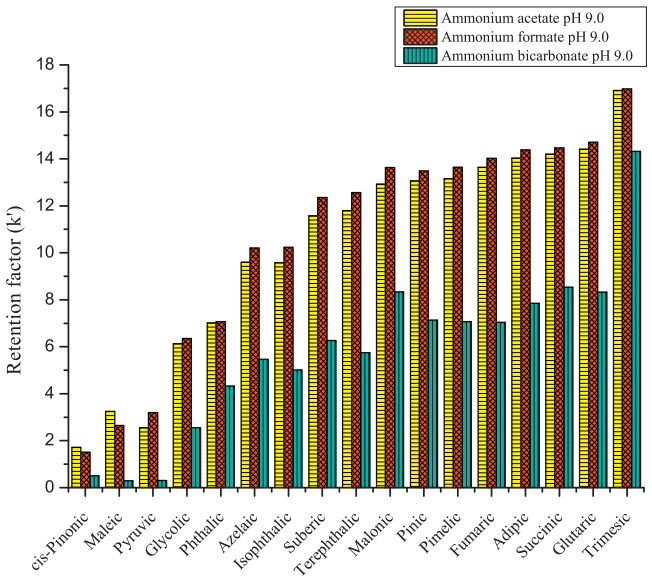

Fig. 1. Influence of buffer type on the retention factors of selected organic acids under HILIC. All buffer salts were at 10 mM concentration and pH 9 in the final mobile phase.

by using internal standards for quantification and by achieving suitable separation for all of the analytes studied. The investigation of the ion suppression effects was done by simultaneous introduction (via T-splitter) of syringe infused solution containing 500 μg/L of deuterated internal standard (succinic-d_4 or phthalic-d_4 acid) at 5 μl/min flow rate together with 0.5 mL/min of LC flow, into the TIS interface (for schematic view see Fig. 1 in Ref. [42]). The SRM signal of the internal standard was monitored during analysis of iMP and real sample, under the optimized HILIC-ESI/MS/MS conditions. The suppression regions were recorded by observing the steep changes in the ion current of the internal standards during the analysis. Strong ion suppression (approx. by a factor of 10 max.) was observed around the dead time, before elution of the first analytes (cis-pinonic and maleic acid) and at the end of the gradient, after the elution of the last analyte (trimesic acid). Moderate suppression (approx. by a factor of 2.5 max.) was also observed in the region were phthalic acid elutes and was found that this suppression is a result mainly of the initial steep change in the gradient (from 1 to 3 min, see Table 1). Using the phthalic-d_4 internal standard for quantification of phthalic acid compensates this effect and ensures proper precision and accuracy for its analysis. However, it was found that when phthalic-d_4 was used for the quantification of the other studied acids, their concentrations were overestimated. Better precision and accuracy of analysis for these acids were obtained using the succinic-d_4 acid as internal standard.

3.2. Optimization of chromatographic conditions

Proper separation and resolution among analytes that would provide sufficient retention factor, k' ($1 < k' < 20$) are very important considerations when matrix effects on ionization in LC/MS should be minimized. For that purpose, efforts were made to optimize the chromatography and test the matrix effects on sample ionization afterwards. The study of the influence of acetonitrile content on the retention of acids revealed their diverse chromatographic properties under HILIC that makes isocratic elution not favorable approach for analysis. Using the data from the isocratic runs a gradient elution was optimized in order to obtain reasonable separation and retention of the acids on column. The final, optimized gradient elution program using ternary mobile phase is given in Table 1. The column was equilibrated with 15 column volumes of iMP before next injection.

3.2.1. Influence of buffer type on the retention

The LC/MS calls for selection of volatile buffer salts in order to improve sensitivity, avoid ion suppression and lessen the maintenance needs of the atmospheric pressure ionization sources. Also HILIC itself demands use of buffers that are soluble in a mobile phase containing higher percentage (up to 90–95%) of organic modifier (ACN). For our experiments, ammonia solution and three buffer salts: ammonium acetate, ammonium formate and bicarbonate met the demands stated before. When ammonia solution was used in the mobile phase, the retention for all acids was very low with asymmetric peak shapes and coelutions. In contrast, the retention and the peak shapes of the analytes were better when ammonium salts were used as buffers. Strege [43] stressed the influence of the buffer anion type on the retention of acidic and basic compounds, using TSKgel Amide-80 column. He suggested that the influence of the pH on solute retention is less important than the influence of the buffer anion character when amide stationary phase is used for HILIC. Comparing the analyte retention factors (k') obtained with different ammonium salt in mobile phase (Fig. 1) gives an insight into the influence of buffer type. The retention characteristics of all analytes are very similar when ammonium formate or acetate is used. Guo and Gaiki came to the same conclusions for acidic compounds as salicylic acid and aspirin on TSKgel Amide-80 column [44]. Retention factors were significantly smaller when ammonium bicarbonate was used and for some acids asymmetrical (tailing) peaks were observed. These effects were already observed in some previous studies [43,44]. When non-buffered ammonium salts mobile phases were used under the same elution conditions, the same conclusions can generally be made (Fig. A1 in Appendix A).

With an increase in the concentration of the ammonium acetate buffer (pH 5.0) in the mobile phase, a higher retention for all acids was observed. This can be explained with the fact that with the increased concentration the migration of the salt from the organic-rich bulk mobile phase into the partially immobilized water-enriched layer is enhanced. This process leads to attraction of additional water molecules into the layer, making it more hydrophilic. As a final result, stronger retention on column is observed [44]. The concentration of ammonium acetate buffer was optimized in the range from 4 to 12 mM, to give suitable retention and the best column efficiency for all acids, while keeping it as low as possible in order to avoid suppression effects during ESI. A 10 mM concentration has completely fit the requirements.

The influence of ionic strength gradient (from 4 to 12 mM) versus constant concentration of 10 mM (ammonium acetate buffer pH 5.0), along with the solvent gradient, on the retention of acids was also studied. More symmetrical peaks, higher column efficiency and greater retention for the early eluting as well as shorter retention for the late eluting peaks were observed during the elution with the constant ionic strength of the mobile phase (data not shown). So, for further optimization steps the constant (10 mM) ionic strength mobile phase was used.

3.2.2. Influence of buffer pH on the retention and ESI-MS/MS sensitivity

The retention of studied acids was examined under elution with mobile phases of different pH values. From Fig. 2 can be seen that all acids have shorter or no retention under acidic (pH 3.1) conditions, except for pyruvic acid which has more or less constant retention factor at all pH values studied. The reason for this behavior is probably its low pK_a value of 2.39 [45], meaning that pyruvic

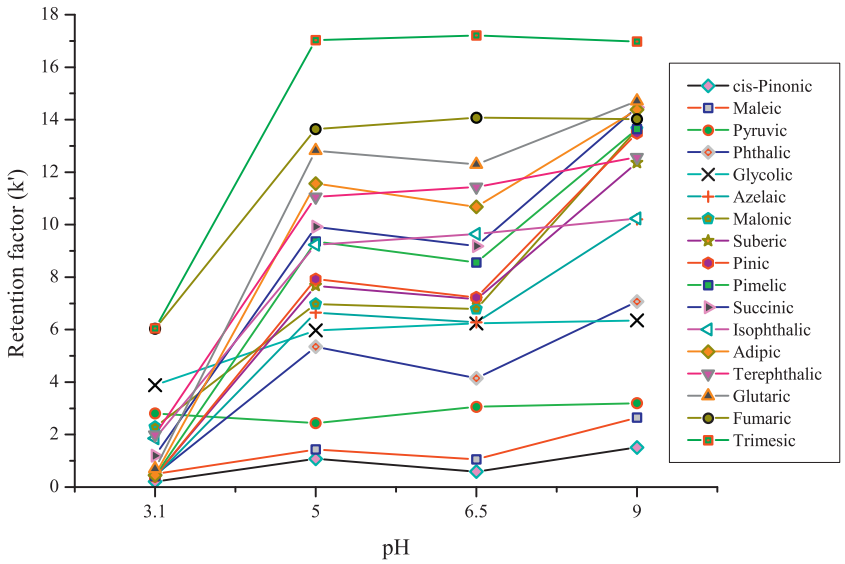

Fig. 2. Influence of pH on retention. Buffer salts used: ammonium acetate for pH 5.0 buffer and ammonium formate for pH 3.1, pH 6.5 and pH 9 buffers. Experimental details are given in Section 2.3.3.

acid is ionized and have similar retention characteristics at all pH values. For the other acids the retention increases with the pH. However, at pH 5.0 and 6.5 there is no significant difference among the retention factors for all acids. For several acids, such as maleic, phthalic, azelaic, suberic, pinic, pimelic, succinic, adipic and glutaric the retention is even higher at pH 9.0. The best resolution and the highest peak capacity were obtained at pH 5.0.

The influence of pH on ESI-MS/MS sensitivity was investigated at three different pH values (5.0, 6.8 and 9.0) with ammonium acetate buffer (10 mM in final mobile phase). The ESI-MS/MS sensitivity was evaluated through the absolute analyte peak area and analyte peak signal-to-noise ratio (S/N). The sensitivity at pH 5.0 and 6.8 was the same (except for terephthalic, azelaic and succinic acid, which have higher peak areas at pH 6.8). At pH 9.0, analyte peak areas are similar to that at lower pH values, except for benzene di- and tricarboxylic acids for which the areas are significantly higher (Fig. A2 in Appendix A). At higher pH these acids are more ionized which in turn enhances the deprotonated molecular ion $[M-H]^-$ formation during ESI that leads to higher sensitivity. In contrast, the ESI-MS/MS sensitivity for maleic acid is significantly lower at pH 9.0 compared to that at pH 5.0 and 6.8. These results are generally in a good agreement with a recent HILIC study, which reports higher MS signal intensity (as peak areas) for acidic aromatic compounds at pH 9 compared to pH 3, when amide column was used [38]. However, when the peak S/N ratios at different pH values are compared (Fig. 3), better sensitivity is found at pH 5.0 for all acids, except for trimesic. The higher peak S/N ratios at pH 5.0 are a result of two important observations, i.e. narrower and more symmetric peaks and lower background noise. If we assume that equal column efficiency (peak capacity) can be achieved at pH 5.0 and pH 9.0, then the peak S/N ratios at pH 9.0 would be much higher that those observed at pH 5.0. As a final result, a mobile phase containing 10 mM ammonium acetate buffer at pH 5.0 was chosen for the further method optimization.

3.2.3. Influence of polar modifier on the HILIC retention

As previously reported [46], the retention of the polar analytes enhances when part of the strongest eluting solvent, i.e. water is replaced with weaker eluting solvents in HILIC. From our observations, the retention of the acids increased in the following order: methanol < ethanol < 2-propanol < tetrahydrofuran (Fig. A.3 in Appendix A). Not only the retention, but also the analyte elution order, resolution and column efficiency were changed when polar modifier was added. These effects were mainly observed for the middle and later eluting acids which had the tendency to elute toward the end of the chromatogram (and gradient) when the polar modifier was changed from methanol to tetrahydrofuran. In the same direction, the resolution among peaks was lost, resulting in several coelutions. However, comparing the analyte peak areas obtained with and without a polar modifier, the higher values were found when polar modifier was present in the mobile phase. This effect was also observed in the previous studies [33,38]. With methanol and 2-propanol as polar modifiers, analyte peak areas, especially for later eluting compounds, were up to 50% higher. Thus, adding a polar modifier into the mobile phase gives a possibility to fine tune the separation and sensitivity under HILIC-ESI/MS/MS.

3.2.4. Influence of column temperature on the HILIC retention

It is known that column temperature has an important influence on the separation under HILIC conditions [36,44,47]. In our

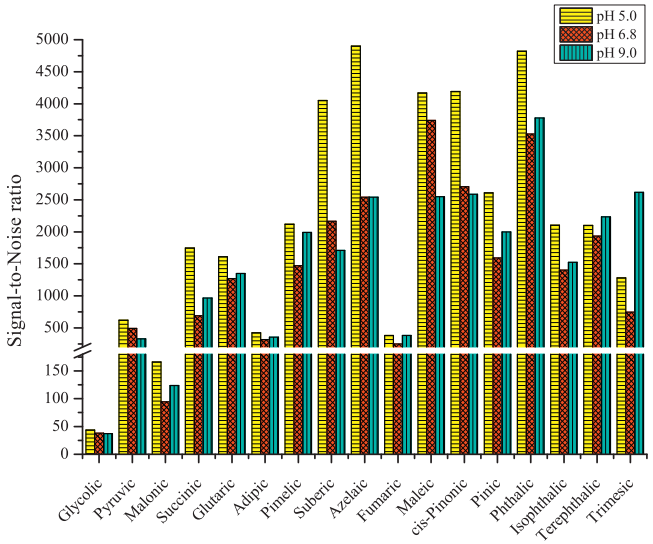

Fig. 3. Influence of pH on peak S/N ratio. Buffer used: 10 mM (final mobile phase concentration) ammonium acetate at pH 5.0, pH 6.8 and pH 9.0. Other experimental details are given in Section 2.3.3.

Table 2
Analytical properties of the HILIC–ESI/MS/MS method.

Acid	Retention time (t_R) (min)	Internal standard	Concentration range (μg/L)	LOD (μg/L)	LOQ (μg/L)	Repeatability RSD (%)[a]			Intermediate precision RSD (%)[a]			Coeff. of determination[c]
						Spiking level (μg/L)			Spiking level (μg/L)			R^2
						50	100	250	50	100	250[b]	
Malonic	10.37	Succinic-d_4 acid	10.6–265[d]	3.00	10.6	8.9	3.5	2.8	6.6	5.4	4.4	0.9983
Succinic	14.20	Succinic-d_4 acid	0.5–273	0.10	0.32	2.2	1.8	0.9	3.8	5.1	2.4	0.9998
Glutaric	17.96	Succinic-d_4 acid	0.3–275[d]	0.09	0.30	3.2	3.1	6.3	4.5	2.4	4.9	0.9948
Adipic	16.34	Succinic-d_4 acid	5.4–269[d]	1.70	5.40	1.5	1.9	1.9	7.1	3.5	2.4	0.9988
Pimelic	13.47	Succinic-d_4 acid	0.3–262[d]	0.09	0.30	1.9	2.6	2.8	5.4	4.0	4.0	0.9993
Suberic	11.27	Succinic-d_4 acid	0.1–251[d]	0.03	0.10	2.0	1.6	4.1	6.7	3.1	7.1	0.9986
Azelaic	9.94	Succinic-d_4 acid	0.1–264[d]	0.03	0.10	1.9	1.5	5.0	9.2	3.8	8.2	0.9980
Fumaric	19.04	Succinic-d_4 acid	5.4–271[d]	1.70	5.40	2.7	1.8	1.3	4.2	4.1	1.9	0.9985
Maleic	3.17	Succinic-d_4 acid	0.1–256[d]	0.03	0.10	1.9	1.6	8.2	10.1	2.7	6.7	0.9913
Pyruvic	4.48	Succinic-d_4 acid	5.0–250	1.00	3.30	2.7	3.2	7.7	12.1	5.2	8.9	0.9941
Glycolic	9.06	Succinic-d_4 acid	52.0–260[d]	16.0	52.0	4.4	2.9	1.5	3.8	3.7	1.6	0.9988
cis-Pinonic	2.70	Succinic-d_4 acid	0.3–277[d]	0.09	0.30	1.9	2.6	5.5	14.2	2.3	9.4	0.9975
Pinic	11.60	Succinic-d_4 acid	0.25–250[d]	0.08	0.25	1.5	1.3	3.3	6.2	1.5	6.4	0.9991
Phthalic	8.25	Phthalic-d_4 acid	0.1–260[d]	0.03	0.10	2.3	1.1	1.4	5.2	4.2	3.8	0.9997
Isophthalic	13.3	Succinic-d_4 acid	0.5–277[d]	0.15	0.50	3.0	2.0	3.2	4.2	2.7	2.5	0.9989
Terephthalic	15.68	Succinic-d_4 acid	0.3–265[d]	0.09	0.30	1.4	1.6	3.3	4.8	2.7	3.7	0.9984
Trimesic	23.44	Succinic-d_4 acid	5.2–259[d]	1.60	5.20	3.3	2.0	5.1	4.1	7.8	4.8	0.9953

[a] $n = 5$.
[b] $n = 4$.
[c] Coefficient of determination of the calibration curve.
[d] lower limits in the concentration range studied are also LOQs for the corresponding acids.

study with the increase of column temperature, a decrease in the retention time was observed for all compounds, except for trimesic acid (Fig. A4 in Appendix A). The same trend was previously reported for other acidic compounds on TSK-gel Amide-80 column [36]. However, the column efficiency, peak area and peak height were not improved by higher column temperature. Moreover, with shorter retention the resolution was lost and the early eluting compounds (i.e. cis-pinonic, maleic and pyruvic acid) eluted close to the dead time region (where the biggest ESI suppression effects are observed). In addition, at temperatures higher than 35 °C peak splitting was observed for azelaic and pinic acid. Hence, a column temperature of 25 °C was chosen as optimal.

Finally, the injection volume was also optimized for best sensitivity. The highest volume that does not deteriorate the efficiency was 100 μL and subsequently was used during the method validation and application.

3.3. Analytical method performance

The analytical performance and suitability of the optimized method (Fig. 4A) were assessed by the method validation, which includes testing the linearity, limit of detection (LOD), limit of quantification (LOQ), precision (repeatability and intermediate precision) and accuracy (expressed as recoveries). The calibration curves for the linearity test were constructed by plotting the peak area ratio between the analyte of known concentration and its internal standard on the ordinate and analyte concentration (as μg/L) on the abscissa. The number of concentration points in the calibration curves varied from 6 to 11, depending on the concentration range studied. Five replicates of the standard solutions were analyzed at each concentration. The experimental data were fitted to a quadratic equation. For all analytes, the coefficients of determination (R^2) were > 0.99 (Table 2).

The LODs and LOQs were determined as concentrations that give signal-to-noise of 3 and 10, respectively (Table 2). LODs for most of the analytes range from 0.03 to 1.70 μg/L. The highest LODs were obtained for malonic and glycolic acid, 3.0 and 16.0 μg/L respectively. The LOQs mostly range from 0.10 to 5.4 μg/L, except for malonic and glycolic acid (10.6 and 52.0 μg/L, respectively). The LODs of the studied acids are generally much lower that those reported in the previous studies using different analytical techniques (Table A.2 in Appendix A). Repeatability (intra-day precision) and intermediate precision (inter-day) of the peak area ratio between analytes and their internal standards were evaluated using standard solutions at three concentration levels (Table 2). Repeatability is good and lower than 5% for most of the acids, except in a few cases, mainly at 250 μg/L when it is between 5 and 9%. Intermediate precision was evaluated for each analyte over three nonsuccessive days. It is generally below 10% for almost all acids at all concentrations, except for maleic, pyruvic and cis-pinonic acid at 50 μg/L.

For recovery studies, three blank filters were spiked with the standard solutions containing all analytes at three concentrations, i.e. 50, 100 and 250 μg/L, as well as internal standards at 50 μg/L. The recoveries were determined by comparing the peak area ratios between acids and their internal standards of the spiked samples with those of the corresponding liquid standards (Table 3). Peak areas of analytes from the spiked filters were corrected with their corresponding peak areas from the non-spiked blank filter. Recoveries of analytes at 50 μg/L were in the range of 73–118%, 85–111% at 100 μg/L and 73–109% at 250 μg/L, except for pyruvic acid. Repeatability (as relative standard deviation – RSD) of the analytical procedure is better than 5%. Some higher RSD values were observed for the lower concentrations of malonic, pyruvic and glycolic acid (Table 3). The recoveries for pyruvic acid were much lower (4–14%). This observation might be a consequence of less effective extraction of pyruvic acid from the filters and/or influence of matrix effects on its ionization efficiency during ESI. Although the ion suppression test did not reveal region of suppression at the retention time of pyruvic acid, there still might be some inevitable suppression in negative ESI which is more deleterious for smaller and more polar ions (like pyruvate ion) than for larger mono- and dicarboxylic ions [48,49]. This statement can be supported also by the lower recovery efficiencies (around 75%) for glycolic acid, which elutes later than pyruvic acid. However, our results show a very satisfactory extraction efficiencies and reproducibilities for all other studied acids.

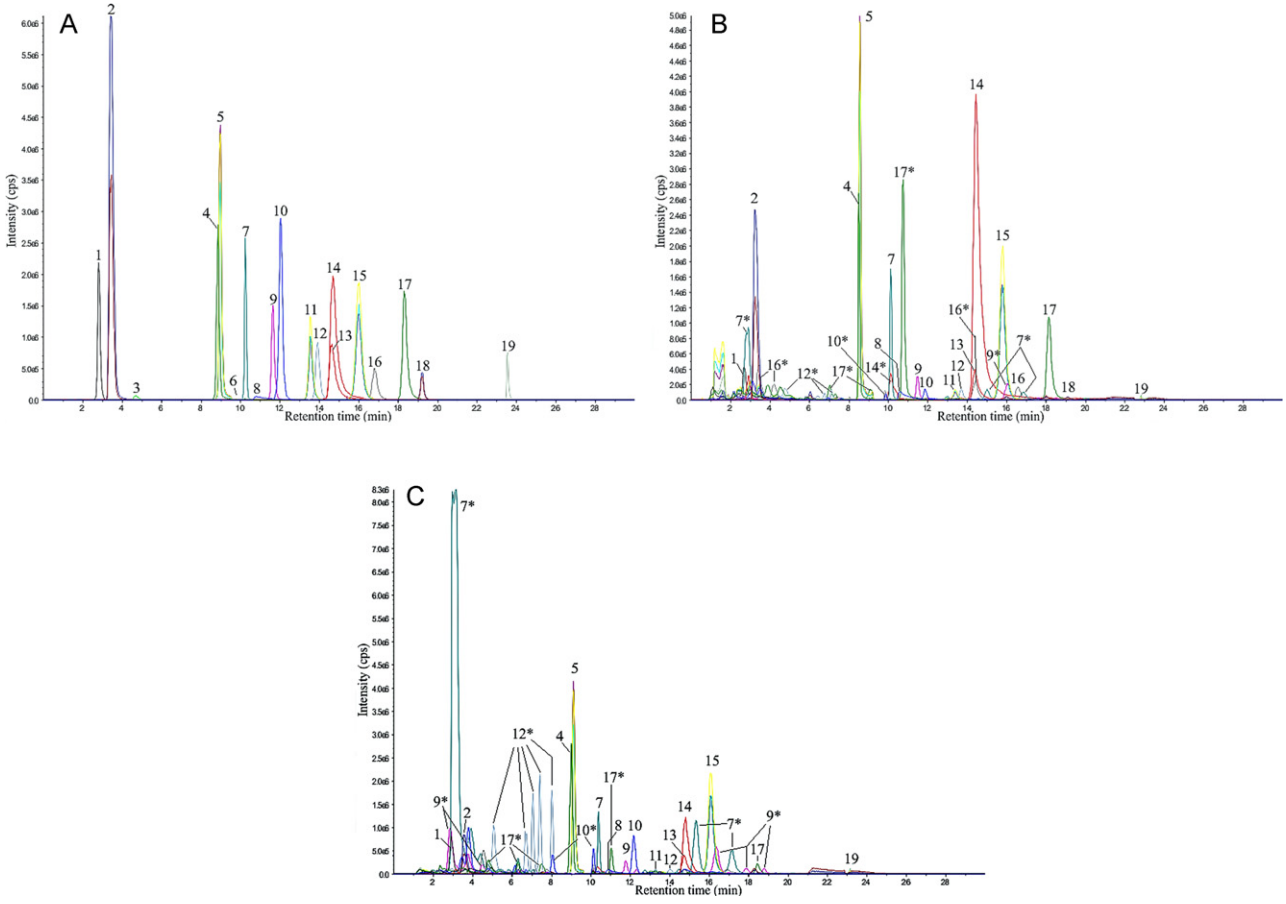

Fig. 4. Extracted ion chromatogram (XIC) for the standard solution containing 17 carboxylic acid (at conc. of 100 μg/L) and 2 internal standards (at conc. of 50 μg/L) (A); for winter 2010 (B) and summer 2010 PM_{10} samples from Ljubljana, Slovenia (C). Compounds are (1) cis-pinonic acid, (2) maleic acid, (3) pyruvic acid, (4) phthalic-d_4 acid, (5) phthalic acid, (6) glycolic acid, (7) azelaic acid, (8) malonic acid, (9) suberic acid, (10) pinic acid, (11) isophthalic acid, (12) pimelic acid, (13) succinic-d_4 acid, (14) succinic acid, (15) terephthalic acid, (16) adipic acid, (17) glutaric acid, (18) fumaric acid, (19) trimesic acid, (7*) azelaic acid isobaric compound, (9*) suberic acid isobaric compound, (10*) pinic acid isobaric compound, (12*) pimelic acid isobaric compound, (14*) succinic acid isobaric compound, (16*) adipic acid isobaric compound, and (17*) glutaric acid isobaric compound.

3.4. Application of the analytical method in analysis of real atmospheric aerosol

The optimized and validated method was used for determination of the target compounds in PM_{10} aerosol samples collected during winter and summer 2010 in Ljubljana, Slovenia. Typical chromatograms of aerosol samples are shown in Fig. 4B and C. The most abundant acids in the winter samples are succinic acid (mean concentration of 32.2 ng/m^3), followed by trimesic (13.8 ng/m^3), malonic (12.4 ng/m^3) and glutaric acid (11.5 ng/m^3) (Table 4). In

Table 3
Recoveries and injection repeatability of the spiked extracts.

Acid	Spiked concentration – 50 μg/L		Spiked concentration – 100 μg/L		Spiked concentration – 250 μg/L	
	Recovery (%)[a]	RSD (%) (n = 6)[b]	Recovery (%)[a]	RSD (%) (n = 6)[b]	Recovery (%)[a]	RSD (%) (n = 6)[b]
Malonic	92 ± 3.3	6.8	92 ± 1.3	5.2	95 ± 8.7	1.8
Succinic	104 ± 1.5	1.7	101 ± 3.1	1.1	101 ± 0.8	0.6
Glutaric	106 ± 0.8	2.3	104 ± 5.4	2.4	97 ± 5.6	0.7
Adipic	107 ± 1.3	1.4	102 ± 5.2	1.1	94 ± 7.5	1.5
Pimelic	109 ± 2.3	0.7	102.6 ± 5.4	2.2	96 ± 7.5	0.6
Suberic	108 ± 2.5	1.2	103 ± 5.5	1.8	97 ± 7.6	1.0
Azelaic	109 ± 2.3	1.2	100 ± 5.5	2.8	93 ± 7.9	1.0
Fumaric	106 ± 2.0	0.8	99 ± 4.6	1.7	96 ± 7.4	0.6
Maleic	117 ± 1.2	1.3	109 ± 3.9	2.4	109 ± 2.1	0.7
Pyruvic	4 ± 1.2	12.6	8 ± 6.4	2.9	14 ± 6.0	1.2
Glycolic	73 ± 1.6	4.2	85 ± 6.1	6.1	73 ± 2.6	1.1
cis-Pinonic	118 ± 1.3	1.4	110 ± 6.0	3.0	102 ± 8.8	1.0
Pinic	114 ± 2.1	1.0	108 ± 4.8	1.8	103 ± 1.2	0.7
Phthalic	118 ± 14.9	0.9	111 ± 6.3	1.8	105 ± 2.3	0.8
Isophthalic	110 ± 2.3	0.0	104 ± 4.6	2.2	95 ± 7.5	0.7
Terephthalic	113 ± 1.6	1.8	110 ± 3.4	1.1	100 ± 4.6	0.8
Trimesic	96 ± 5.9	1.6	96 ± 11.6	1.8	107 ± 8.7	1.22

[a] Average recovery of 3 analyzed spiked filters.
[b] Repeatability of peak area ratio between the acid and its internal standard from six replicate injections of spiked filter extracts.

Table 4
Concentrations of the studied carboxylic acids in PM_{10} samples from winter and summer 2010 from Ljubljana, Slovenia.

Acid	Aerosol concentration (ng/m^3)			
	Winter samples (n = 5)		Summer samples (n = 5)	
	Mean	Range	Mean	Range
Malonic	12.4	5.9–19.2	14.1	6.5–23.0
Succinic	32.2	20.9–40.7	10.2	4.8–12.2
Glutaric	11.5	6.0–20.3	2.0	1.2–2.3
Adipic	5.8	3.1–9.2	2.0	1.7–2.5
Pimelic	2.9	1.3–5.1	1.1	0.64–1.5
Suberic	2.3	1.2–4.0	1.0	0.30–1.5
Azelaic	4.4	1.6–9.4	3.1	0.32–5.5
Fumaric	0.55	0.31–0.78	0.54	0.32–0.70
Maleic	4.6	2.9–6.9	1.4	1.0–1.7
Pyruvic[a]	2.1	1.0–3.6	2.1	1.1–3.2
Glycolic	4.2	1.2–8.4	5.9	1.7–11.4
cis-Pinonic	1.9	1.6–2.4	5.4	4.6–7.0
Pinic	0.40	0.12–0.81	1.4	0.24–2.8
Phthalic	5.6	3.2–10.1	2.6	2.2–2.9
Isophthalic	2.4	1.0–3.9	0.26	0.10–0.38
Terephthalic	8.9	2.5–15.1	2.3	0.70–12.3
Trimesic	13.8	1.2–20.8	5.1	2.4–8.4

[a] Measured values that are not actual concentrations in atmospheric aerosols due to the lower extraction recovery.

the summer samples, malonic acid predominates (mean concentration: 14.1 ng/m^3) and is followed by succinic (10.2 ng/m^3) and glycolic acid (5.9 ng/m^3). The total concentration of the determined acids is higher in winter. The same trend is observed for the saturated aliphatic and aromatic dicarboxylic acids as well as for trimesic acid. Their primary emission (e.g. terephthalic acid) and the emission of their precursors, as well as their formation reactions are enhanced in winter, mainly due to the stronger anthropogenic influence (from industry, traffic, households) [3]. In contrast, pinic and cis-pinonic acids are more abundant in the summer aerosols, as a result of the higher emission of their volatile precursors (such as terpenes) from the biosphere and their enhanced secondary formation.

Using the characteristic SRM transitions for detection of the target analytes together with their characteristic retention times in HILIC–ESI/MS/MS ensures their accurate and sensitive determination. Sample chromatograms (Figs. 4B and C) show several peaks at different retention times, but with the same SRM transition as the studied acids. These isobaric peaks correspond to the compounds that give ions with *m/z* values equal to the *m/z* of the studied acid ions, indicating the presence of isomeric compounds. For example, the SRM signal for pimelic acid in the chromatogram obtained from the summer aerosol sample shows nicely separated peaks at lower retention times (Fig. 4C). These peaks can be identified by using other tandem MS scanning modes, such as neutral loss, precursor or product ion scans. The evidence for the good separation and detection of the studied acids together with their isobaric compounds increases the method's significance and broadens its applicability in the characterization of complex atmospheric aerosols.

4. Conclusions

A new HILIC–ESI/MS/MS method was developed and validated for determination of selected aliphatic, alicyclic and aromatic carboxylic acids in atmospheric aerosols (except for oxalic, malic and benzoic acid). The influence of the chromatographic parameters on the acids retention under HILIC was studied. The optimal conditions for both retention and sensitive tandem MS detection of acids were chosen after systematic method optimization. All acids were eluted within 24 min of a 30 min gradient program. The final method allows determination in the parts-per-trillion (ng/L) range for most of the carboxylic acids studied and is generally more sensitive than previously published methods. The sample preparation was optimized both to maximize the analyte recovery and to minimize the operational time. In comparison with the widely accepted GC–MS methods for determination of acidic compounds in atmospheric aerosols, the proposed method lessens the extent of analyte loss and tedious work associated with sample derivatization, characteristic for GC–MS methods. All selected acids were determined in atmospheric PM_{10} samples; however, the measured values for pyruvic acid are not accurate due to its lower extraction recovery. In addition, many isobaric compounds to the studied acids were detected as well. Other tandem MS scans such as neutral loss, precursor or product ion scan together with their retention under HILIC will be used for future elucidation of their structure, which can give more information about the complex chemical composition of the atmospheric aerosols.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.05.020.

Supplementary data include additional figures and tables associated with this article and can be found in the online version

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