Talanta 97 (2012) 55–62

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Aliphatic and aromatic amines in atmospheric aerosol particles: Comparison of three ionization techniques in liquid chromatography-mass spectrometry and method development

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ARTICLE INFO

Article history:
Received 13 January 2012
Received in revised form
11 March 2012
Accepted 24 March 2012
Available online 24 April 2012

Keywords:
Aliphatic and aromatic amines
Liquid chromatography — tandem mass spectrometry
Ultrafine aerosol particles
SMEAR II station

ABSTRACT

A complete methodology was developed for the determination of ten aliphatic and nine aromatic amines in atmospheric aerosol particles. Before the liquid chromatography — tandem mass spectrometric separation and determination, the derivatization reaction of the analytes using dansyl chloride was accelerated by ultrasounds. From three different ionization techniques studied electrospray ionization was superior in terms of sensitivity, linearity, repeatability and reproducibility over atmospheric pressure chemical ionization and photoionization for the target analytes. The method developed was validated for the gas phase, 30 nm and total suspended atmospheric aerosol particles. The method quantification limits ranged between 1.8 and 71.7 pg. The accuracy and the potential matrix effects were evaluated using a standard addition methodology. Recoveries from 92.1% to 109.1%, the repeatability from 0.6% to 8.4% and the reproducibility from 2.3% to 9.8% were obtained. The reliability of the methodology was proved by the statistical evaluation. Finally, the developed methodology was applied to the determination of the target analytes in eight size separated ultrafine particulate ($Dp=30\pm4$ nm) samples and in eight total suspended particulate samples collected at the SMEAR II station. The mean concentrations for aliphatic amines were between 0.01 and 42.67 ng m^{-3} and for aromatic amines between 0.02 and 1.70 ng m^{-3}. Thirteen amines were quantified for the first time in 30 nm aerosol particles.

1. Introduction

The chemical composition of atmospheric aerosols has been studied extensively during the last decades and reviewed in the literature [1]. However, only a few papers have been dedicated to the determination of chemical composition of ambient nanometer sized particles. Recent studies, focused on the composition of these particles, have demonstrated the relevance of the nitrogen compounds for the aerosol chemistry and their participation in particle growing processes [1–5].

The most common and abundant nitrogen containing organic compounds found in the atmosphere are the low-molecular weight aliphatic amines with one to six carbon numbers, especially aniline from aromatic amines, nitro aromatic compounds from nitro-organic compounds, amino acids and amides [5–8] with biogenic and anthropogenic sources. The most common biogenic sources are the degraded biomolecules and biopolymers, and the cellular metabolism. On the other hand, waste combustion, sewage treatment, automobile exhaust, vulcanization fumes, fish processing plants, fish stands of city markets and industrial animal farms can be regarded as the main anthropogenic sources [6].

The sampling of the size segregated ultrafine particles is a highly challenging task [9]. Two different devices such as, impactors and differential mobility analyzers (DMA) have been recently utilized [10–12], but unfortunately the collection efficiency of the former tends to be poor for particles significantly smaller than 100 nm due to evaporation processes [10]. The size-separated particles can then be collected on the impactor plate or on a suitable filter, extracted and analysed with chromatographic techniques utilizing mass spectrometric detection or directly transferred to a mass spectrometer [12,13].

It is well known that a significant amount of gaseous compounds can be adsorbed on the filter, causing overestimation of particulate concentration of compounds, especially in the case of nanometer-size aerosols [14]. New sampling systems based on

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http://dx.doi.org/10.1016/j.talanta.2012.03.062

particle size separation using a DMA and simultaneous collection of gas-phase zero samples allow the quantification of these artifacts [15].

The determination of nitrogen containing compounds in aerosol particles is mainly based on chromatographic techniques such as gas chromatography (GC) [16], ion chromatography (IC) [17] and high performance liquid chromatography (HPLC) [18], although other separation techniques such as capillary electrophoresis (CE) has been also employed [19]. CE allows the determination of the target analytes, including tertiary amines without derivatization but the mismatch between the low concentration of the analytes in the samples and the relatively high detection limits provided by CE hinders its use for the analysis of real aerosol samples. On the other hand, the derivatization is generally mandatory in the case of GC and LC to improve the separation efficiency and the detection sensitivity of the analytes [20,21].

o-Phthaldialdehyde, fluorenylmethylchloroformate, dansyl chloride, dabsyl chloride and 1,2-naphthoquinone-4-sulfonate are typical derivatization reagents in liquid chromatography. An adequate optimization procedure and/or the use of an auxiliary energy such as, ultrasound or microwaves can minimize the time needed for the derivatization, the amount of expensive reagents, and analyte losses [22,23]. Unfortunately the derivatizations reagents are suitable only for primary and secondary amines but not for tertiary amines [22].

Fortunately the hyphenation of LC to mass spectrometry (MS) allows the analysis of the underivatized analytes, although most of the methods reported in the literature include a derivatization step in order to facilitate the separation and improve the sensitivity [18,24]. The selection of the most suitable ion source in terms of sensitivity, linearity, repeatability and reproducibility is a key factor in the method development. From the three different atmospheric pressure ionization sources, electrospray (ESI) [24], atmospheric pressure chemical ionization (APCI) [25] and atmospheric pressure photoionization (APPI) [26], ESI has been the most widely used in LC-MS studies, but the best results achieved are greatly depended on the target analytes in the question.

The aim of this study was to develop a reliable LC-MS method for the determination of amines in ultrafine and total suspended atmospheric aerosol particles. The special goal was to select the best ionization technique for ten aliphatic amines and nine aromatic amines studied in aerosol particles. Then the methodology developed was validated by evaluating the accuracy and the potential matrix effects using a standard addition methodology and by exploiting the analytical results obtained for natural samples by the new methodology and by our previously validated method [18].

2. Experimental

2.1. Reagents

Diethylamine, dipropylamine, *iso*-butylamine, ethylendiamine, 3-propylphenylamine, *iso*-propylphenylamine, 2-methylphenylamine, 4-methylphenylamine, *p*-aminophenol, 2-amino-1-butanol, 2-aminobutyric acid, and *N*-methylformamide from Fluka Chemie GmbH (Buchs, Switzerland), tripropylamine, dimethylamine, *sec*-butylamine, phenylamine, *N*-methylphenylamine, and 4-ethylphenylamine were from Sigma-Aldrich (St. Louis, MO, USA); and 4-aminobenzoic acid from Merck (Darmstadt, Germany). 2-Pyridylbenzoimidazole and triphenylphosphate were used as internal standard [18,27].

Stock standard solutions of all the analytes, at concentrations of 1000 $\mu g\,mL^{-1}$, were prepared in methanol (Sigma-Aldrich). Standard working solutions containing all the analytes at 1 $\mu g\,mL^{-1}$ were weekly prepared in methanol. The internal standard solution was prepared at 10 $\mu g\,mL^{-1}$ in methanol. All solutions were stored at $-20\,^{\circ}C$ in glass vials and kept in the dark until use.

Dansyl chloride (Sigma-Aldrich) was used as derivatization reagent. A 50 mM reagent working solution was weekly prepared in acetone (VWR, Leuven, Belgium). This solution was stored in the dark at 5 °C until use.

Different borate buffer solutions with a concentration ranged between 25 and 50 mM, and pH ranged between 8.1 and 10.7 were used in the optimization of the derivatization reaction. These solutions were prepared from disodium-tetraborate 10-hydrate (Merck). Toluene from Lab-Scan (Gliwice, Poland) was added to avoid losses of volatile compounds during the drying step after ultrasound-assisted extraction.

Acetonitrile (VWR), water (DirectQ-UV, Millipore Corp., Billerica, USA), acetic acid (Fluka) and ammonia solution 25% (VWR) were employed for the preparation of the different LC mobile phases.

2.2. Instruments

The manifold used for the collection of aerosol particles has been described earlier [15]. Briefly, 0.1-m Vienna type differential mobility analyzer (DMA) was used for the size segregation of the 30 ± 4 nm particles. The DMA was operated in a closed-loop flow arrangement. The size segregated particles were collected onto a filter placed downstream from the DMA. A timer was connected to the HV supply and to a three-way valve that turned off the HV supply and, simultaneously, switched the three-way valve to another direction. Thus, ultrafine particles and particle-free air were collected onto two different filters. This measurement cycle was maintained for the full collection period. Before size segregation, the sampled particles were brought to a known charge distribution with an Am-241 alpha-source (60 MBq). A separate filter holder connected to a vacuum system controlled by a restrictor was used for the collection of TS particles. The air flow used for the collection of TS particles was the same than in the case of the ultrafine particles. The scheme of the sampling manifold can be seen as supplementary information (Scheme-S1). Fluoropore, type FALP, 1 μm Teflon filters (Millipore, Cork, Ireland) were used for sample collection.

Ultrasound irradiation was applied with a Branson Sonifier S-250A (60 Hz and 200 W) (Branson, Danbury, CT, USA) equipped with a titanium alloy micro tip (3 mm diameter). The extraction chamber consisting of a PEEK cylinder (5 cm length and 7.5 mm i.d.), was closed with screw caps and equipped with filters to allow pumping of the extraction solvent through the chamber while maintaining the solid sample inside.

An Agilent 1100 series liquid chromatograph (Palo Alto CA, USA) was furnished with an Waters Sunfire C18 column (150×2.1 mm i.d., 3.5 μm particle size) (Milford, MA, Ireland), coupled to an Esquire 3000 plus ion trap mass spectrometer (Bruker Daltonics, USA) for the detection. Three different ion sources were tested and optimized in this research, electrospray and atmospheric pressure chemical ionization sources from Bruker; and atmospheric pressure photoionization source from Agilent. The compatibility of the Agilent ion source with the Bruker mass spectrometer was ensured by the manufacturer. Vaporizer heater and lamp were controlled through Bruker software and the HyperTerminal, respectively.

2.3. Samples

Sixteen atmospheric aerosol samples, eight 30 ± 4 nm particles and eight total suspended particles (TSP), were simultaneously collected from March 14 to May 16, 2011 at the Station for Measuring Forest Ecosystem-Atmosphere Relations (SMEAR II) in Hyytiälä, Finland (61°51′N, 24°17′E, 180 m above sea level) [28]. Additional five 30 ± 4 nm samples were collected also at the SMEAR II station and analyzed with the method developed in this study and with our earlier validated method [18].

2.4. Method development

2.4.1. Aerosol particle sampling

Total suspended and 30 ± 4 nm aerosol particles were simultaneously collected onto filters with the sampling device described in Scheme-S1. The air flow-rate for the collection of the aerosol particles was set to 4 L min^{-1}. The collection time, was ranged from 118 to 331 h. Gas-phase adsorption samples for particle-free air (no voltages on DMA) were simultaneously collected with particle samples to offset the retention onto the FALP filters of the target analytes present in the gas phase. Filters were kept in dark in a freezer at −18 °C until analysis.

2.4.2. Sample preparation

The validated sample pretreatment method has been described in detail earlier [18]. Briefly, the filter samples were submitted to a dynamic ultrasound-assisted extraction for 20 min (water bath temperature 25 °C maintained during the extraction, flow rate was 1 mL min^{-1}, ultrasonic irradiation duty cycle was 0.5 s, output amplitude was 50% (100 W), and probe tip located 2 cm above the extraction chamber) using methanol as extraction solvent. A few drops of toluene were added to the extract as a trapping agent for volatile compound. The extract volume was readjusted to 5 mL using rotary evaporator and a small addition of fresh methanol. Methanolic aerosol extracts containing the target analytes were stored in the freezer until analysis.

2.4.3. Derivatization step

Aliquots of 50 μL of either the standard solutions or the extracts were adjusted to pH 9.1 by adding 50 μL borate buffer. The resulted solutions were diluted with a 3:1;v:v mixture of water:acetone to a final volume of 150 μL. Additional 10 μL of the internal standard solution and 40 μL dansyl chloride in acetone were added to the mixture. The final sample volume was 200 μL. After a vigorous agitation in a vortex for 1 min, the reaction mixture was subjected to ultrasound irradiation (output amplitude was 50% of the converter applied power (100 W), duty cycle was 0.5 s with the probe tip placed at 3 cm above the bottom surface of the water bath and 2.0 cm on the side of the reaction vial) for 15 min at 35 °C to favor the reaction. The reaction vials were kept in dark while not in use.

2.4.4. Determination method

The sample was directly analyzed after the derivatization step. The initial mobile phase was a mixture of 80% A (water acidified with 1% acetic acid) and 20% B (acetonitrile). After injection (25 μL), the initial mobile phase was maintained under isocratic conditions for 2 min. Then, an initial linear gradient elution from 20% to 40% B in 3 min was followed by other period of isocratic conditions for 2 min. Then, four consecutive linear elution gradients were applied: from 40% to 60% B in 9 min, from 60% to 67.5% B in 6 min, from 67.5% to 80% B in 11 min; and from 80% to 100% B in 2 min. Finally, the instrument was kept under isocratic conditions (100% B) for 5 min. The flow rate was 0.3 mL min^{-1} during the whole chromatographic process. The total analysis time was 30 min, with 7 min being required to re-establish and equilibrate the initial conditions.

The excess of the derivatization reagent and the non-volatile salts were diverted to the waste using a valve connected to the ion trap mass spectrometer. The valve was turned into the mass spectrometer position after 1.5 min. The rest of the eluate, containing the target analytes, was electrosprayed in the positive mode and monitored by MS–MS detection. Four different segments of the chromatographic elution were needed in order to allocate all the analytes in this study. The flow and temperature of the drying gas (nitrogen) were 8 L min^{-1} and 300 °C, respectively. The nebulizer pressure was 2.75 bar (40 psi). The mass spectrometer parameters used to drive and trap the different precursor ions were optimized for different segments and the optimum values can be seen in Table S-1. The dwell time was set to 20 ms.

2.5. Statistical analysis

Statgraphics Centurion XV, from Statpoint Technologies, Inc. (Warrenton, VA, USA) was used for the optimization of the derivatization procedure and for the data treatment of the results.

3. Results and discussion

3.1. Optimization of the liquid chromatographic method

The experimental chromatographic variables were optimized, by utilizing the standard solution derivatized by a conventional procedure [29], for the best separation in the shortest time and with the highest sensitivity. The variables studied were the injection volume, composition and flow-rate of the mobile phase (Table S-1). In this step, the mass spectrometer, furnished with the electrospray ion source, was operated in full scan mode detecting ions within a limited mass range (from 50 to 600 m/z). The scan time was fixed at 500 ms. In addition, in this study it was possible to select the precursor ions for analytes and internal standards and to estimate the number of dansyl chloride moieties in the molecule. The peak area obtained for the individual compounds from the total ion current (TIC) chromatogram was used as response variable in this study. A chromatogram obtained for the standards under the optimal conditions is shown in Fig. 1(A).

Non-derivatized analytes and 2-(2-pyridyl)-benzimidazole, used as an internal standard for these compounds, were eluted within the first 5 min of the chromatogram. The rest of the analytes were eluted according to the following order: amino-substituted carboxylic acids, amide and derivatized amines. The retention order of the hydroxylamines was based on the substituent, but 2-amino-1-butanol was surprisingly eluted between the non-derivatized analytes and the amino-substituted carboxylic acids; and *p*-aminophenol overlapped with the derivatized amines. Triphenylphospate was used as internal standard for the derivatized compounds.

The duration of the chromatographic run was similar to that found in the literature for the analysis of the target analytes using other liquid chromatographic techniques [17]. Shorter retention times can be achieved by using chromatographic columns packed with smaller particles, which can improve, at the same time, the resolution [30]. Although no baseline separation was achieved for all the target analytes, the developed method allowed the determination of the target analytes, using the mass spectrometer as a detector without ion suppression problems (Fig. 1(B)).

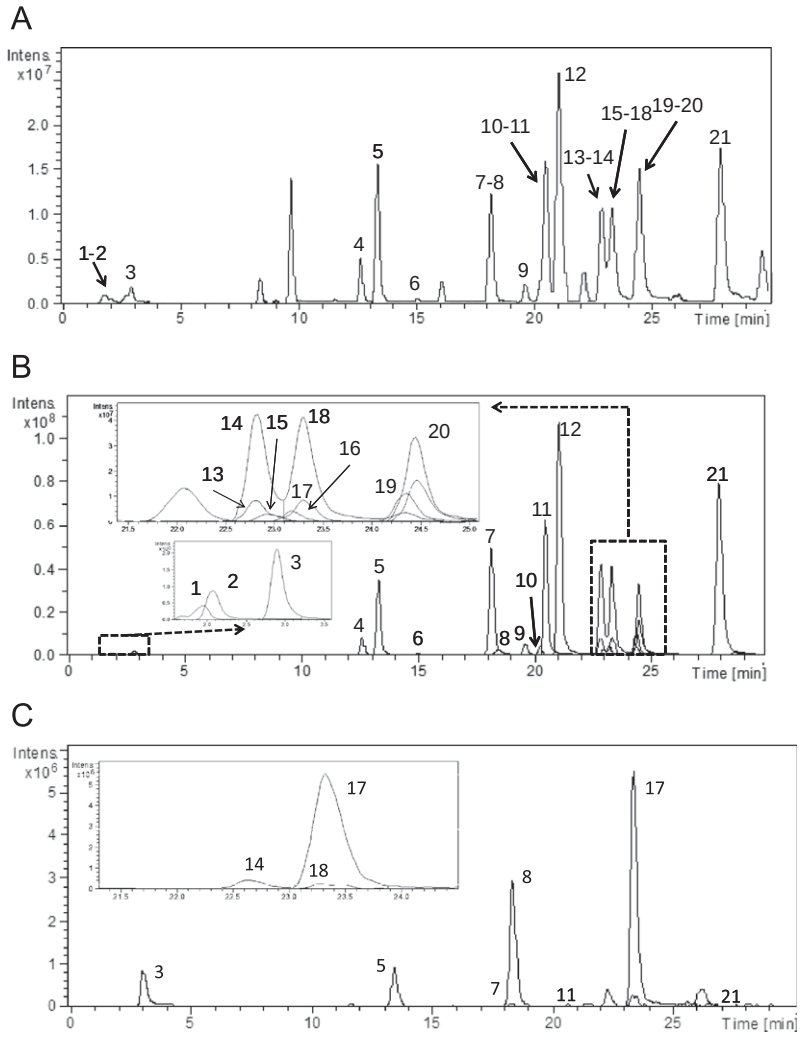

Fig. 1. Typical chromatograms obtained for the analysis of standard solutions and ambient aerosol samples. (A) Total ion current chromatogram obtained for a standard solution; (B) extracted ion chromatogram obtained for a standard solution; and (C) extracted ion chromatogram obtained for an ambient aerosol sample. (1) Tripropylamine; (2) *iso*-propylphenylamine; (3) 2-(2-pyridyl)-benzimidazole (IS); (4) 2-amino-1-butanol; (5) 2-aminobutyric acid; (6) 4-aminobenzoic acid; (7) *N*-methylformamide; (8) dimethylamine; (9) *p*-aminophenol; (10) phenylamine; (11) *sec*-butylamine; (12) *iso*-butylamine; (13) *N*-methylphenylamine; (14) 2-methylphenylamine; (15) ethylenediamine; (16) diethylamine; (17) triphenyl phosphate (IS), (18) 4-methylphenylamine; (19) 4-ethylphenylamine; (20) 3-propylphenylamine; (21) dipropylamine.

3.2. Optimization of the detection method

The optimization of the mass spectrometric detection was divided into two steps. In the first one the conditions for the isolation and fragmentation of the precursor ion for each analyte with the highest efficiency were optimized. The second step was focused on the selection of the optimal ion source. In that way, three different ion sources, ESI, APCI and APPI operating under optimal conditions, were evaluated for the analysis of the target analytes in terms of sensitivity, linearity, repeatability and reproducibility.

3.2.1. Mass spectrometric conditions

The precursor ion was selected, for the individual analytes, according to the information obtained from the optimization of the chromatographic step. It should be emphasized that $[M+H]^+$ was selected as precursor ion in all the cases. Therefore, the use of a specific ion source is not relevant for the optimization of the precursor ion isolation and fragmentation conditions. In this case, the mass spectrometer was furnished with the ESI ion source.

The different voltages and radiofrequencies needed for the isolation of the precursor ions were optimized for the individual compound but intermediate conditions were selected taking into account that some of the compounds had similar retention times. The ion trap mass spectrometer allowed the simultaneous analysis of ten ions, and all the ions present in the same segment had to be isolated under the same conditions. Due to this, the chromatogram was divided into four segments. The optimal values of different parameters for each segment can be found in Table S-2A.

Finally, the fragmentation of the precursor ions was also optimized. The optimum voltage was considered when the intensity of the precursor ion falled to 5% of the main product ion intensity. The influence of the dwell time on the sensitivity tested resulted in the best value of 20 ms. Molecular weight, number of dansyl chloride moieties in the reaction product, precursor ion, fragmentation voltage, segment number and product ions for the target analytes are listed in Table S-2B.

3.2.2. Selection of the ion source

Three different ion sources ESI, APCI and APPI, were evaluated for the analysis of the target analytes, in terms of sensitivity,

linearity, repeatability and reproducibility. The optimization was developed using standard solutions.

The different parameters which can affect the ionization of the target compounds were optimized for the different ion sources. The optimized variables, the tested ranges and the optimum values are shown in Table S-2C. It can be concluded that the common parameters such as nebulizer pressure, drying gas flow rate or drying temperature had the same optimal values regardless of the ion source selected.

The sensitivity was evaluated using instrumental detection (IDL) and quantification (IQL) limits, which were estimated with the standard solutions. The peak height to averaged background noise was calculated with the background noise taken as the peak-to-peak baseline near the analyte peak. The IDL and IQL values were calculated on the basis of minimum acceptable values of the signal-to-noise (S/N) ratio of 3 and 10, respectively (Table S-3A). The IDL values ranged between 0.1 and 17.4 pg, between 0.1 and 18.2 pg; and between 0.1 and 19.7 pg for ESI, APCI and APPI, respectively. In addition, the calculated IQLs ranged between 0.3 and 57.4 pg, between 0.4 and 60 pg; and between 0.2 and 65.0 pg for ESI, APCI and APPI, respectively. It should be emphasized that no single ionization technique provided the best sensitivity for all the analytes under the study. ESI gave the best results for the non-derivatized aromatic amines, amine substituted carboxylic acids and derivatized amines. On the other hand, APCI was the best ionization technique for non-derivatized aliphatic amines, hydroxylamines and the amide. The results achieved APPI were quite similar to those obtained by APCI.

The linearity was evaluated using different parameters provided by the calibration curves such as, intercept, correlation coefficient, number of concentration levels used in the calibration and linear range. The calibration curves were calculated as the ratio of standard peak area to internal standard peak area as a function of the standard compound concentration, expressed as an absolute amount. The calibration curves were performed with multistandard solutions at ten concentration levels. From all the models fitted using Statgraphics Centurion XV linear regression provided in all the cases the highest r^2 values. The statistical significance of the deviation of the intercept from zero value was evaluated for all the analytes and the ion sources utilized in this study. No statistical differences were established as the obtained p-values for the t-test were in all the cases greater than 0.05. The correlation coefficients for all the target analytes and for three ion sources used in this study were over 0.993 but the curves provided by the ESI contained the highest number of points. At least six concentration levels, eight for the most of the analytes, were used in the development of the calibration curves. One exception should be emphasized; namely the curve developed for p-aminophenol using the APCI had the highest number of datapoints. The results provided by the APPI were once again similar to those obtained by the APCI.

In addition, the linear range was also evaluated. The largest linear ranges (between IQL and 25 ng) were found for the most of the analytes when the ESI was used. The smallest linear range was achieved for p-aminophenol (between IQL and 18.7 ng), but its linear range could be enlarged (between IQL and 25.0 ng) by using atmospheric pressure chemical ionization or photoionization sources, that gave smaller linear ranges than the ESI for the rest of the analytes. The values for the correlation coefficients, the number of calibration levels used for the calculation of the individual equations and the linear range are found in Table S-3B.

Finally, the ionization repeatability and reproducibility was studied using standards at two different concentration levels. One of them was selected close to the IQL and the second one near the middle point of the dynamic range. The ionization repeatability and reproducibility were calculated based on a set of five sample replicates in three different days. These values are presented in Table S-3C. The best results, in terms of the ionization repeatability and reproducibility, were obtained for the APCI in the most of the cases. The repeatability ranged between 0.5% and 6.7% and the reproducibility ranged between 1.2% and 8.2%. On the other hand, the values provided by the ESI were under 10% in all the cases. In addition, ESI provided the best repeatability and reproducibility values for 2-amino-1-butanol, 4-aminobenzoic acid, butylamine and 3-phenylpropylamine. As expected, the worst results were obtained by the APPI ion source. In this case, the ionization repeatability and reproducibility, expressed as relative standard deviation, was ranged from 10% to 20% for the most of the analytes. The deviation is caused by the high sensitivity of the APPI source to the dopant agent and the low repeatability of the device used for post-column addition of dopant (a syringe pump connected to the outlet of the column with T-piece).

As a summary, although the ESI was not the most sensitive ion source for all the analytes under the study, it was the most sensitive for the majority of them. In addition, the ESI provided the best linearity, and the reasonably good ionization repeatability and reproducibility compared to the APCI and APPI making it the best option for the analysis of the target analytes.

3.3. Optimization of the derivatization procedure

It is well known that ultrasound energy is able to accelerate nucleophilic addition/elimination reactions between dansylchloride and amino group of the analytes [31]. In this case, the chemical variables (pH, concentration of the derivatization reagent and concentration of buffer) were optimized simultaneously to the ultrasound variables (irradiation amplitude, probe position, duty cycle), temperature and reaction time using a multivariate approach. Standard solutions containing all the analytes at 0.5 $\mu g\,mL^{-1}$ were used as a sample. The descriptive variable was a response factor calculated using the following equation $RF_i=(A_i/A_{IS})$, where A_i is the peak area of the analyte and A_{IS} is the peak area of the internal standard.

In the first step of the optimization, a Plackett–Burman design ($2^8 \times 3/64$, type III resolution, 3 degree of freedom and involving 12 randomized runs plus three center points) was built for the screening of the variables potentially affecting the derivatization procedure. Both the tested and the optimum values of the variables are shown in Table S-4. It can be concluded that buffer concentration, probe position and duty cycle were not statistically influential factors within the ranges applied in this study. However, the results showed that the most intensive responses were obtained with the highest values tested (50 mM, 2 cm on the side of the vial and 0.5 s for buffer concentration, probe position and duty cycle, respectively). Therefore, these values were selected for the further experiments. The other variables were influential factors, within the ranges under study; and therefore, they were subsequently optimized.

Lower pH values and higher values for derivatization reagent concentration, irradiation amplitude, temperature and reaction time were tested in the second screening design (Table S-4). In this case, a half fraction design (2^{5-1}, type IV resolution, 3 degree of freedom and involving 16 randomized runs plus three center points) was employed. The results of this screening show that temperature was a non-statistically influential factor within the range under the study. However, the best results were obtained at the highest value (35 °C) tested, which was selected for the further experiments. Concentration of the derivatization reagent, irradiation amplitude, pH and reaction time were influential factors within the range under the study. The irradiation amplitude, which had positive statistical influence in the first experimental design, showed a negative statistical influence in this second design.

Therefore, this variable was fixed to 50% of the output converter (100 W). Higher values for the concentration of the derivatization reagent and the reaction time; and lower pH values were tested in the third design (Table S-4).

A response surface based on a central composite design (2^3+star, 9 degree of freedom and involving 16 randomized runs) was selected for the final optimization of the last three variables. The lack of fit test, used to evaluate the reliability of the model, determined that the developed model was adequate to describe the observed data. The optimal values for pH, derivatization reagent concentration and reaction time were 9.1, 50 mM and 15 min, respectively. Although an optimum value was used for the derivatization time, the reaction kinetic was studied using a univariate approach. Six different reaction times, from 5 to 30 min, were tested using the optimal values for the rest of the variables. Fifteen minutes were enough to ensure the complete derivatization of the target analytes.

The chemical variables (pH, buffer and derivatization reagent concentrations) have the same optimal values in the classical derivatization procedure [29] and the ultrasound assisted method. However, the reaction temperature was substantially lower in the case of the ultrasound assisted method (35 *vs* 60 °C); avoiding the most of the losses due to the evaporation and decomposition of the analytes [29,32]. The ultrasound assisted method shortened the derivatization time to the third (15 *vs* 60 min) in comparison with the classical method [29].

3.4. Characterization and validation of the developed methodology

Three different sample matrices such as gas phase collected onto the filter, ultrafine (30 nm) and total suspended particles (TSP), were used for the validation of the developed methodology. Pool samples from the different matrices were prepared by combining aliquots of extracts provided by samples with the same matrix. These sample pools were used in almost all the steps of the validation with the exception of the calibration curve development.

The method quantification limits (MQL) were calculated from the chromatograms obtained for the natural samples. Once again, the ratio of the peak height to the averaged background noise was used for the calculation. The MDLs were between 1.8 and 68.8 pg in the case of the gas phase collected on the filters, between 3.4 and 70.9 pg for ultrafine particles; and between 3.6 and 71.7 pg for the TSP. Detailed information about MQL obtained for the individual compounds in the three sample matrices is found in Table 1.

The on-table stability of the derivatized samples was also evaluated. Real aerosol samples with different matrices, were analyzed once every 4 h for a period of 36 h. No statistical variations were found in the concentration of the target analytes within the first 24 h. After that, the concentration of the analytes in the samples clearly decreased. In all the cases, the samples were stored in the dark until analysed and between analyses.

Repeatability and reproducibility were evaluated for the developed analysis method in a single experimental setup with duplicates using real samples. Two measurements from each sample matrix were conducted each day for seven days. Eq. (1) was used to determine the between-day variance:

$$s^2_{\text{between}} = (\text{MS}_{\text{between}} - \text{MS}_{\text{within}})/n_j \quad (1)$$

where MS is the mean square (residual sum of squares rated by the freedom degrees) and n_j is the number of replicates per day. The day to day laboratory reproducibility, s^2_{WR}, was calculated by Eq. (2).

$$s^2_{\text{WR}} = s^2_r + s^2_{\text{between}} \quad (2)$$

where s^2_r is the residual mean squares within-days and s^2_{between} is the variance due to the between-day effect.

Table 1
Detection and quantification limits obtained by LC-MS for the individual compounds in gas phase, 30 nm and total suspended ambient aerosol samples. Results are expressed as pg (injection volume 25 μL).

Analyte	Gas phase	30 nm	TSP
p-Aminophenol	32.1	35.3	36.7
2-Amino-1-butanol	8.1	8.3	8.4
N-Methylformamide	63.4	65.2	66.3
Dipropylamine	10.1	11.4	12.3
Tripropylamine	64.1	66.8	71.7
iso-Butylamine	5.5	5.6	5.8
sec-Butylamine	8.8	18.2	14.6
Dimethylamine	68.8	70.9	71.5
Diethylamine	8.7	14.5	12.4
Ethylenediamine	3.6	5.2	5.4
2-Aminobutyric acid	4.0	6.6	5.2
4-Aminobenzoic acid	6.4	6.5	6.7
Phenylamine	58.9	59.3	62.3
4-Ethylphenylamine	1.8	6.7	6.1
N-Methylphenylamine	5.9	6.4	6.1
iso-Propylphenylamine	3.3	3.4	3.6
2-Methylphenylamine	7.8	9.7	10.2
4-Methylphenylamine	6.3	6.5	6.8
3-Propylphenylamine	12.2	16.3	24.2

The within day repeatability, expressed as a relative standard deviation (RSD), ranged from 0.6% to 8.3% for gas phase collected on filters, from 0.6% to 8.4% for the ultrafine particles and from 1.3% to 7.6%, for the TSP. A day to day laboratory reproducibility, also expressed as the RSD, ranged from 2.5% to 9.1% for the gas phase collected on filters, from 2.3% to 9.1% for the ultrafine particles and from 6.2% to 9.8% for the TSP (Table S-5A).

The validation of the developed methodology was completed by a two step procedure: 1) standard addition to establish the accuracy and the potential matrix effect; and 2) a comparison between the results obtained with the developed methodology and those acquired using a standard reference method. It should be emphasized that it is not possible to use any of the commercially available standard reference materials because the target analytes have neither been analyzed nor certified.

Pool samples from the different matrices, spiked at two different concentration levels (0.25 and 10 ng of each analyte), were used to establish the accuracy of the method and the potential matrix effects. Recoveries ranged from 92.5% to 108.1% for the gas phase collected on filters, from 92.8% to 108.7% for the ultrafine particles and from 92.1% to 109.1% for the TSP (Table S-5B). An absence of significant differences between the concentrations added and those found in terms of mean, range, variance, and median, were established by different statistical tests such as ANOVA, multiple ranges, Levene and Kruskal-Wallis, and Mood's median.

The final step of the validation was the comparison of the results obtained by the developed methodology with those provided by our earlier validated method [18]. The latter is also based on LC-MS but the analytes were analyzed without the derivatization. The main limitation of this method was that only ethylendiamine, diethylamine, dipropylamine, *p*-aminophenol, *iso*-propylphenylamine and tripropylamine could be compared. Aerosol samples containing ultrafine particles, without a correction of the gas phase contribution, were used in this study. The results are assembled in Table S-5C1. The same statistical tests used to establish the differences between the added concentrations and those found in the samples were used in this case. No significant differences were found in terms of mean, range, variance, and media (Table S-5C2).

3.5. Application of the methodology to ambient aerosol samples

The new method was applied to 16 aerosol particle samples collected at the SMEAR II station [28], eight contained the TS

Table 2
Analysis of amines in 30 nm and total suspended atmospheric aerosol particles using the developed methodology. Mean concentration and concentration range are expressed as ng m^{-3}. Number of analyzed samples in brackets (five repetitions were analyzed from each sample).

Analyte	30-nm (8)		TSP (8)	
	Mean	Range	Mean	Range
p-Aminophenol	0.09	ND–0.52	0.12	ND–0.37
2-Amino-1-butanol	0.01	ND–0.04	0.18	ND–0.59
N-Methylformamide	14.10	ND–30.69	4.33	ND–9.82
Dipropylamine	0.33	ND–1.51	0.79	ND–2.65
Tripropylamine	0.39	ND–1.21	2.15	ND–6.21
iso-Butylamine	ND	–	ND	–
sec-Butylamine	0.18	ND–0.38	0.34	ND–1.06
Dimethylamine	0.85	ND–4.15	7.20	ND–17.94
Diethylamine	0.36	0.04–0.78	0.61	0.20–0.90
Ethylenediamine	0.05	ND–0.25	0.39	ND–1.64
2-Aminobutyric acid	29.5	4.17–65.17	42.67	ND–79.54
4-Aminobenzoic acid	ND	–	1.14	ND–3.24
Phenylamine	0.64	ND–1.37	1.70	ND–3.76
4-Ethylphenylamine	0.04	ND–0.08	0.10	ND–0.34
N-Methylphenylamine	0.11	ND–0.14	0.14	ND–0.21
iso-Propylphenylamine	0.04	ND–0.27	0.09	ND–0.17
2-Methylphenylamine	0.02	ND–0.08	0.04	ND–0.11
4-Methylphenylamine	0.04	ND–0.07	0.08	ND–0.20
3-Propylphenylamine	0.07	ND–0.17	0.54	ND–3.69

ND: Not detectable.

particles and eight 30 nm particles. The contribution of the gas phase to the particle composition was corrected in all the cases. Mean concentrations and concentration ranges of the target analytes for the different particle sizes are shown in Table 2. Fig. 1(C) demonstrated the typical chromatogram obtained from a natural sample.

In general terms, the results provided by the developed methodology are in agreement with those found in the literature for some of the target compounds [18]. Differences, up to one order of magnitude, can be explained by the different methodology used for the correction of the gas phase contribution to the particle composition [12]. It should be emphasized that a more truthful approach was used in the present research.

The evaluation of the results, as a function of the different families of analyzed compounds, revealed that the mean concentrations of aliphatic amines, aromatic amines, amino-substituted carboxylic acids and hydroxylamines were smaller in 30 nm particles than in TSP. The opposite trend was found for the *N*-methylformamide, most probably because *N*-methylformamide can be easily transformed during the particle growing by reaction with OH radicals [33]. In addition, the ratio between aliphatic and aromatic amines, associated to natural or industrial emissions, is in agreement with the sampling place [5].

The concentrations of *N*-methylformamide and 2-aminobutyric acid in ultrafine particles are surprisingly high. The adsorption of these analytes on the particle surface or their formation during the long sampling periods could explain these results. It should be emphasized that the method used for the correction of the gas phase contribution to the particle composition is able to offset the adsorption of the gas phase on the filter but no other artifacts which can affect the concentration of the target analytes on the particles during the sampling step.

4. Conclusions

A reliable method based on LC-MS for the determination of aliphatic and aromatic amines in ultrafine and total suspended atmospheric aerosol particles was developed in this research. The new method was applied for the determination of 19 amines in 16 ambient aerosol particle samples collected at the SMEAR II station. To the best of our knowledge 13 amines were quantified for the first time in 30 nm aerosol particles where the contribution of gas-phase was taken into consideration.

The use of ultrasounds as an auxiliary energy substantially reduced the reaction temperature and time needed in comparison with the classical derivatization procedures. The optimal values of 15 min and 35 °C for the reaction time and temperature avoided the analyte losses due to the evaporation and also hindered the formation of reaction by-products.

The use of MS–MS detection allowed the reliable identification of the target analytes and the reduction of the liquid chromatographic noise. The ESI, selected as the best ionization technique, provided a good performance in terms of sensitivity, linearity and ionization repeatability and reproducibility.

The developed method was fully characterized and validated using three different sample matrices such as gas phase collected on filters, ultrafine 30 nm particles and TSP. Finally, the results obtained from the application of the method to atmospheric air samples were in a good agreement with the data found in the literature.

Acknowledgement

Financial support was provided by the Academy of Finland Centre of Excellence program (project no 1118615). Vesa-Pekka Vilja is thanked for the assistance is some LC-MS experiments.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.03.062.

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