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Trace analysis of free and combined amino acids in atmospheric aerosols by gas chromatography–mass spectrometry

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ABSTRACT

The analysis of amino acids by gas chromatography mass spectrometry (GC–MS) after their derivatization with N-(*t*-butyldimethylsilyl)-N-methyltrifluoroacetamide was investigated as an alternative approach for the determination of free (FAA) and combined amino acids (CAA) in aerosols. This technique showed excellent linearity with r^2 values ranging from 0.9029 to 0.9995 and instrumental limits of detection ranging from 0.3 to 46 pg for the different amino acids. The quality of water used for sample extraction was found to be of utmost importance for achieving low blank levels of FAA and CAA. The addition of isopropanol during the extraction of aerosols was also shown to minimize the coextraction of inorganic salts that interfered with the analysis of FAA, Moreover, the ascorbic acid was found to be the most effective reagent for preventing the oxidative destruction of CAA during the hydrolysis process. By the analysis of spiked aerosol samples, the average recoveries determined for FAA and CAA were higher than 60% and the associated relative standard deviation was lower than 10% for the majority of amino acids. The application of the adopted method in background aerosols of the eastern Mediterranean enabled the unambiguous identification and quantification of 20 amino acids. The total concentration of FAA and CAA in aerosols ranged from 13 to 34 ng m^{-3} and from 29 to 79 ng m^{-3}, respectively. The GC–MS based method is proposed to overcome several analytical difficulties usually encountered with the conventional HPLC-fluoresence technique.

1. Introduction

Over the last decade, there is a growing interest about the role of amino acids and proteinaceous material in aerosols. Amino acids were initially hypothesized to contribute significantly to the water-soluble organic carbon in atmospheric particles [1], while significant amounts of these compounds have been detected in marine aerosols [2], continental aerosols [3], rain [4], and fog water [3,5]. Due to their high abundance and hygroscopicity [6], amino acids and proteinaceous material can potentially affect the hygroscopic growth and cloud formation activity of aerosols. In addition, some amino acids, such as L-leucine, were found to possess ice-nucleating ability [7], whereas the oxidation of L-methionine has been hypothesized to produce ultra-fine particles in the Arctic [8]. Recently, the reaction between amino acids and small aldehydes (e.g. glyoxal) in cloud droplets was identified as sources of aerosols [9]. As reported, amino acids might be implied in the formation of light absorbing oligomers via aldol reactions with aldehydes in the atmosphere. Furthermore, considering that most airborne allergens are proteins or glycoproteins [10], the interest for atmospheric proteinaceous material also relates to the possible health-effects that they may induce. For example, several proteins of airborne pollen (e.g. cedar, olive and birch pollen) are known to cause allergies and their amino acid sequence has been determined [11]. Because of the multiple roles amino acids may play in the atmosphere, there is a necessity for monitoring and assessment of their levels in aerosols.

The levels of free amino acids (FAA) in ambient particles have been measured in several areas around the world [3,5,12–14], while measurements of combined amino acids (CAA), which are supposed to represent proteins, peptides, amino acid–humic acid complexes or other combined forms, are still very sparse [3,5,12]. In all previous studies, the identification/quantitation of amino acids was based on the derivatization of the target compounds with *o*-phthalaldehyde (OPA) and the analysis of the derivatives by means of HPLC with fluorescence detection. The widespread use of the OPA/HPLC method may give the impression that major analytical problems associated with its application on environmental samples have been resolved. However, this method is not free of substantial drawbacks. The fluorimetric assay reagent is sensitive to both light and oxygen and has to be prepared regularly, while the derivatization process is not efficient for all different amino acids (OPA does not react with secondary amino acids and sulfur amino acids)

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[15]. In addition, the amino acid derivatives are not very stable [15] and they have to be analyzed immediately after derivatization, while coelution problems with interfering substances cannot be eliminated.

The derivatization of amino acids with N-(*t*-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) causes the simultaneous silylation of the amino- and carboxyl groups in a single step. The products from this simple and robust derivatization procedure exhibit much better stability and they are amenable to gas chromatographic analysis. Up to now, numerous publications have appeared on the optimization of derivatization conditions, the gas chromatographic conditions and the mass spectrometric identification of amino acid derivatives [16–19]. However, the majority of these studies were focused on the analysis of pure amino acid standards rather than complex environmental matrices. Only a few studies have demonstrated the applicability of this approach for the analysis of amino acids in biological samples [20,21], while the applications for inorganic environmental matrices are limited to Mars analog soils [22].

Silylation by MTBSTFA in conjunction with gas chromatography–mass spectrometry (GC–MS) could be an alternative method for trace analysis of free and combined amino acids in aerosols. In comparison to HPLC with fluorescence detection, the GC–MS based technique was considered to provide better chromatographic resolution, unambiguous and accurate identification of amino acids in atmospheric particles. More importantly, due to its simplicity and the limited use of chemical reagents, this method was deemed to exhibit low blank levels, which is a prerequisite for the efficient analysis of amino acids in low-volume aerosol samples.

The aim of the present study was to develop a reliable GC–MS based method for the analysis of amino acids in atmospheric samples. For this reason we endeavored to diminish the specific inorganic matrix complications in the extraction, derivatization, identification and quantification procedures. In addition, we applied and evaluated the performance of the established method to determine free and combined amino acids in marine background aerosols from the eastern Mediterranean Sea. To the best of our knowledge, this is the first time that a GC–MS based method has been used for the analysis of free and combined amino acids in atmospheric samples.

2. Experimental

2.1. Materials

MTBSTFA with 1% *tert*-butyldimethylchlorosilane, water (Chromasolv Plus for HPLC), concentrated hydrochloric acid (37%, v/v; Molecular Biology Grade), isopropanol, acetonitrile, dimethylformamide and antioxidants (ascorbic acid, thioglycolic acid, mercaptoethanol and dithiothreitol) were purchased from Sigma–Aldrich. For each antioxidant, a solution was prepared at 25 $\mu g\,\mu l^{-1}$. Hydrochloric acid solutions of 0.1 and 6 M, required for the preparation of amino acid standard solutions and acid hydrolysis of aerosol extracts, respectively, were obtained by diluting concentrated HCl (Molecular Biology Grade) with an appropriate volume of water (Chromasolv Plus for HPLC). All solvents and reagents were of highest purity and were regularly tested for amino acid contamination to ensure low blanks. Ultra-pure water applied for preliminary extraction experiments was obtained by a PURELAB Ultra purification system (Elga, UK).

Individual L-amino acids (≥99.0% purity) in crystalline form were also purchased from Sigma–Aldrich (Steinheim, Germany). A stock solution containing 80 $\mu g\,ml^{-1}$ of each amino acid was prepared in 0.1 M HCl and stored frozen at −10 °C. Working standards of amino acids (20 $\mu g\,ml^{-1}$) were regularly prepared by diluting stock solution with 0.1 M HCl. Separate stock (80 $\mu g\,ml^{-1}$) and working solutions (20 $\mu g\,ml^{-1}$) were prepared for recovery (2-aminobutyric acid) and internal standards (norvaline and 2-aminopimelic acid). Polypropylene tubes (Sigma–Aldrich) were used for the preparation of all standards to minimize losses from adsorption to surfaces.

2.2. Aerosols sampling

The aerosol samples used for evaluating the performance of the analytical method (modification of extraction solvent, addition of antioxidants and recovery experiments) were collected from the marine background sampling station of Finokalia (35°19′N, 25°40′E), a coastal site 70 km eastward of Heraklion (Crete, Greece). For the final analysis of FAA and CAA in aerosols, another six samples were collected between 25 and 30 June 2007. Sampling was conducted using a high-volume air sampler that pumped ambient air through a 20 cm × 25 cm glass fiber filter (GFF). In order to collect sufficient amount of suspended particles, the system was operated for 24 h at a flow rate of 30 $m^3\,h^{-1}$ (~720 m^3 per sample). Before sampling, GFFs were heated at 450 °C for 5 h to remove any traces of amino acids, wrapped in pre-combusted aluminium foil and sealed in plastic bags. After each deployment, the GFFs were collected, resealed, and stored at −18 °C until analysis.

2.3. Sample preparation and extraction

For the analysis of FAA, a portion of each particle-loaded GFF (30% of the filter, corresponding to 220 m^3 of air) was cut in narrow strips and placed into a 15 ml Falcon tube. The filter was spiked with 75 μl of recovery standard working solution (1500 ng) and the tube was filled up to 12 ml with a mixture of water:isopropanol (1:1). Water-soluble amino acids were extracted by sonication at 60 °C for 20 min. After sonication, the extract was placed in a separate 15 ml Falcon tube and centrifuged at 5000 rpm (2800 × *g*) for 20 min to remove suspended debris and filter particles. The clear supernatant was carefully decanted into a clean 50 ml Falcon tube and the extraction procedure was repeated once more. The pooled supernatants were evaporated to 0.5 ml using a Martin Christ (Osterode, Germany) rotational vacuum concentrator (RVC) operated at 60 °C, 1300 rpm and 1500 Pa. If filter particles were visible after concentration, the extract was placed in a 1.5 ml Eppendorf vial and centrifuged at 5000 rpm (2800 × *g*) for 20 min. The concentrated clear extract was placed into a 0.9 ml glass vial and evaporated to dryness by RVC.

A slightly modified procedure was applied for the analysis of combined amino acids. Due to the higher atmospheric concentrations of these components, a smaller portion of the filter (about 8% of GFF corresponding to 60 m^3 of air) was used. The filter strips were extracted twice with 6 ml of water and the extract was evaporated to 0.2 ml. The latter was transferred into a Pyrex glass hydrolysis tube and evaporated to dryness by RVC. Subsequently, 25 μl of freshly prepared ascorbic acid solution (20 $\mu g\,\mu l^{-1}$) and 250 μl of HCl 6 M were added into the hydrolysis tube using a syringe. The hydrolysis experiments using other types of antioxidants (Section 3.3) were performed using the same procedure. The tube was then evacuated (1000 Pa), flushed with argon 5 times, tightly sealed under vacuum with a Teflon stopcock and the sample was hydrolyzed for 24 h at 110 °C. After cooling at room temperature, the hydrolysate was transferred into a vial and evaporated to dryness.

2.4. Derivatization

Several investigations have shown that MTBSTFA reagent enables a very efficient, simple and rapid silylation of pure amino

Table 1
Analytical characteristics for amino acid derivatives using GC–MS (selected ion monitoring) method.

Amino acid	Retention time (min)	Quantitation ion[a] (*m*/*z*)	Confirmation ion[a] (*m*/*z*)	Correlation coefficient[b] (r^2)	LOD[c] (pg)	LOQ[d] (pg)
Alanine	2.90	158 (100)	232 (58)	0.9950	0.7	2.3
Glycine	3.20	218 (76)	246 (72)	0.9919	2.4	7.8
2-Aminobutyric acid	3.74	246 (49)	172 (100)	0.9901	1.3	4.2
Valine	4.51	186 (100)	288 (34)	0.9823	0.5	1.8
Norvaline	4.75	186 (100)	288 (37)	0.9666	0.5	1.7
Leucine	5.27	200 (100)	302 (34)	0.9619	0.3	1.2
Isoleucine	5.84	200 (100)	302 (50)	0.9624	0.4	1.3
Proline	6.49	184 (100)	258 (24)	0.9955	1.7	5.5
Methionine	10.06	292 (83)	218 (100)	0.9563	4.2	14
Serine	10.43	288 (100)	390 (95)	0.9995	0.8	2.8
Threonine	10.82	303 (100)	404 (42)	0.9801	1.2	4.1
Phenylalanine	11.83	302 (100)	234 (68)	0.9583	0.6	1.9
Aspartic acid	12.87	302 (100)	418 (79)	0.9901	1.8	6.0
4-Hydroxyproline	13.26	314 (100)	416 (28)	0.9908	1.4	4.7
Glutamic acid	14.47	432 (100)	330 (66)	0.9879	2.0	6.7
Asparagine	14.78	417 (100)	302 (32)	0.9926	5.3	18
Lysine	15.82	300 (79)	198 (53)	0.9795	10	34
Glutamine	16.30	431 (100)	198 (15)	0.9879	17	56
2-Aminopimelic acid	17.02	460 (82)	358 (100)	0.9859	2.8	9.3
Arginine	17.10	442 (100)	340 (85)	0.9029	15	52
Histidine	18.06	196 (100)	440 (74)	0.9842	46	154
Tyrosine	18.60	302 (100)	466 (14)	0.9686	0.5	1.6
Tryptophan	18.86	302 (100)	375 (34)	0.9045	20	65

[a] Numbers in parentheses indicate % relative intensity of ions.
[b] Correlation coefficients obtained from linear regression analysis of calibration curves.
[c] Instrumental limit of detection based on a signal-to-noise ratio of 3.
[d] Instrumental limit of quantification based on a signal-to-noise ratio of 10.

acid standards [16–19]. The derivatization procedure described by Buch et al. [22] was applied in the present study with minor modifications. Each sample was spiked with 75 μl of the internal standard working solution (1500 ng) and evaporated to complete dryness. Then, 10 μl of dimethylformamide and 60 μl of MTBSTFA were added and the vial was sealed with a PTFE-lined cap. Finally, the sample was heated at 70 °C for 20 min to achieve the chemical derivatization of amino acids and the derivatives were analyzed by GC–MS.

2.5. GC–MS analysis

The analysis of amino acid derivatives was conducted on an Agilent 6890 gas chromatograph equipped with an on-column injector and interfaced with an Agilent 5973 mass spectrometer operating in electron impact ionization (70 eV electron energy) and selected ion monitoring mode. The confirmation and quantification ions were carefully selected to ensure high signal for amino acids and low interference from aerosol matrix (Table 1). Analytes were separated on a 15 m DB5-MS capillary column (Agilent, phenyl arylene polymer, 0.25 mm i.d, 0.25 μm film thickness) operating with helium carrier gas (constant velocity 46 cm s^{-1}) under the following temperature program: from 120 to 150 °C at 120 °C min^{-1} (5 min hold), to 240 °C at 7 °C min^{-1} and finally to 295 °C at 20 °C min^{-1} (16 min hold). A deactivated retention gap (5 m, 0.32 mm i.d.) was attached in the front of the analytical column and it was regularly trimmed to prevent column contamination. The temperature of the injector, transfer line, ion source and quadrupole filter was kept constant at 260, 300, 230 and 150 °C, respectively. The identification of amino acids derivatives was based on comparison of their MS data and retention times with those of authentic reference standards. The concentrations of combined amino acids in aerosol samples were calculated by subtracting the quantities of free amino acids from those measured after acid hydrolysis. The presented results for both free and combined amino acids were blank-subtracted and recovery corrected.

3. Results and discussion

3.1. Alleviation of blank levels in amino acids analysis

Regardless of the sensitivity of an analytical method, consistently low blank values are crucial to achieve low method detection limits and to ensure that the amounts of the analytes recovered from the samples are sufficiently higher than background contamination levels. Considering that the present method aimed to be used for the quantification of amino acids in small aerosol samples, the attainment of as low blank values as possible was of utmost importance.

In a previous study investigating residues of amino acids in lunar fines, it was reported that the contact of samples with hands, laboratory dust, glassware, utensils and reagents can be a source of contamination [23]. As a consequence, special care was taken during the treatment of aerosol samples. To minimize the effect of laboratory contamination, sample handling was performed using solvent-extracted tools and powder-free gloves, while all analyses were undertaken in a laminar-flow hood. Despite these precautions, preliminary results from the analysis of blank samples indicated high levels of FAA and CAA.

A step-by-step investigation of the experimental procedure indicated that the ultra-pure water which was initially used for sample extraction was by far the main source of contamination. The analysis of ultra-pure water directly obtained from the purification system exhibited a concentration of 31 ± 3 and 441 ± 37 ng ml^{-1}, for total FAA and CAA, respectively. These high levels of amino acids were probably the result of a contamination at the outlet of the system and water storage reservoirs [23]. In order to reduce blank levels, distilled and double-distilled water was subsequently prepared using a Kuderna-Danish apparatus. Single distillation yielded a significant improvement in water quality as the total concentrations of both free (1.6 ± 0.2 ng ml^{-1}) and combined amino acids (30 ± 2 ng ml^{-1}) decreased by one order of magnitude (Fig. 1). A further purification was not achieved after

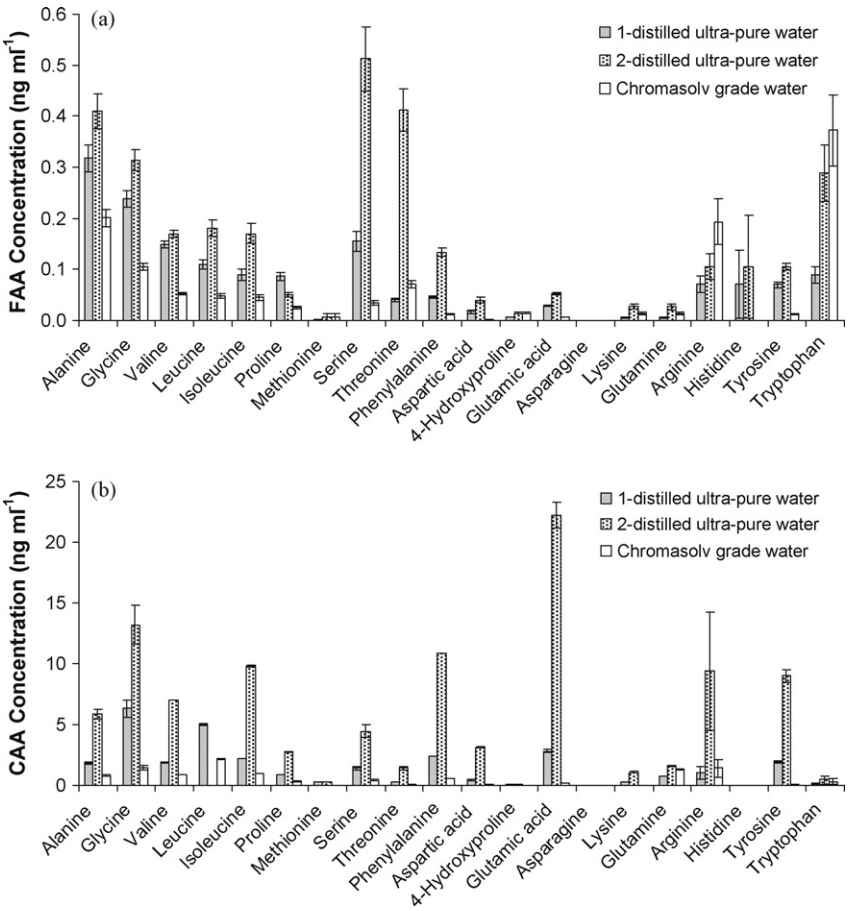

Fig. 1. Concentrations of (a) free (FAA) and (b) combined amino acids (CAA) in single-distilled, double-distilled and Chromasolv grade water.

a second stage of distillation. In fact, higher concentrations of amino acids were measured in double-distilled water (3.1 ± 0.4 and 100 ± 10 ng ml^{-1} for FAA and CAA, respectively), possibly reflecting a contamination from the prolonged contact of the distillate with glassware and air. Finally, the analysis of Chromasolv grade water provided the optimum results (Fig. 1). This reagent contained the minimum concentrations of free (1.2 ± 0.2 ng ml^{-1}) and combined amino acids (11 ± 1 ng ml^{-1}) and thus it was selected for the extraction of filter samples in the present study. Considering that purified water can be easily contaminated with actively multiplying microorganisms during short periods of storage [23], special care was taken to ensure a stable quality of water throughout experiments. For this reason, a freshly opened bottle of Chromasolv grade water was split in 50 ml portions, transferred to Falcon tubes and stored frozen at −18 °C until use.

3.2. Attenuation of matrix effect on the derivatization process

Although the use of MTBSTFA reagent enables a very efficient, simple and rapid silylation of amino acids [16–19], the application of this procedure to water extracts from environmental samples (e.g. soils, aerosols, sediments) can be problematic because soluble inorganic salts can interfere with the derivatization of amino acids with MTBSTFA and restrain their dissolution in the injection solvent [22]. Furthermore, Buch et al. [22] proposed that a mixture of isopropanol and water can be a better choice for sample extraction, because it enables the extraction of amino acids from soils, while it reduces the amount of coextracted inorganic salts.

Sea salts and mineral dust particles are also major components of natural aerosols [24] and thus they can hinder the derivatization of amino acids. To determine if the addition of isopropanol can improve amino acid analysis, portions of an aerosol sample were spiked with amino acids and extracted with water or an isopropanol:water mixture (1:1). From the comparison of the GC–MS chromatograms, it was observed that the extraction of pre-spiked aerosols with water resulted in much lower peak areas for all amino acids. The majority of analytes indicated 2–3 times higher signal in isopropanol:water than in pure water extracts (Fig. 2). This difference was more pronounced for methionine and histidine, which exhibited 40 and 30 times higher signal when isopropanol:water mixture was used. The lower signal observed for amino acids in water extracts is in line with the suppressive role of coextracted matrix salts and minerals during the derivatization process, while the increase of signal after isopropanol addition presumably reflects the limited extraction of salts from aerosols and the alleviation of their effect.

Besides the increase in absolute signal intensities, the addition of isopropanol did not affect the composition of amino acids. With the exception of methionine and histidine, the relative intensity of amino acids in water and isopropanol:water extracts was quite similar, implying that the addition of isopropanol does not cause a significant qualitative effect in the results. It should be also clarified that the observed differences in absolute signal intensities do not affect the quantitative analysis of FAA, as this is based on the internal standard technique. For the case of combined amino acids, the use of isopropanol was avoided because this could cause protein precipitation [25] and a possible underestimation of proteinaceous content in aerosols.

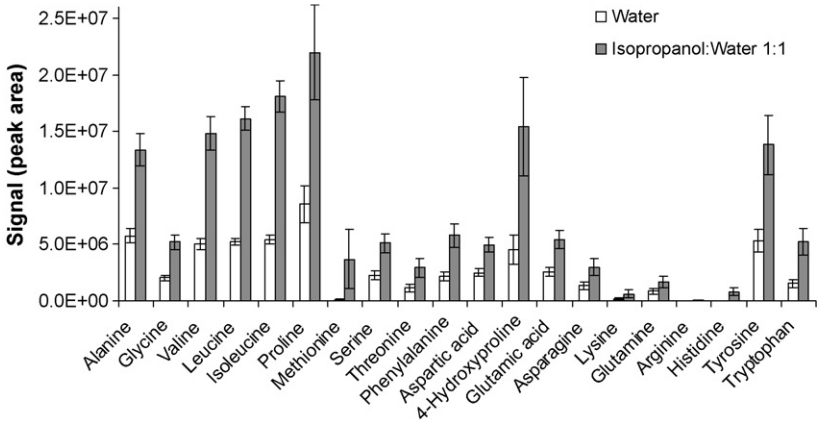

Fig. 2. Signal intensity of free amino acids in spiked aerosol samples extracted with water and an isopropanol:water (1:1) mixture.

3.3. Attenuation of matrix effect on the hydrolysis efficiency of combined amino acids

The analysis of combined amino acids requires the evaporation of the aqueous extract and the hydrolysis of proteinaceous material with HCl 6 M. This method has been shown to be very efficient for the hydrolysis of pure proteins [16] and peptides [17], but its application in environmental samples can be less effective due to the ubiquitous presence of nitrates. Under the strongly acidic hydrolysis conditions, nitrates are converted to nitric acid, which in turn reacts with amino acids and cause their nitration or their oxidative degradation [26,27]. The severity of these reactions depends on the concentration levels of nitrate and it has been shown to induce very low yields of combined amino acids in seawater [27]. However, the addition of an excess of ascorbic acid or other antioxidants before hydrolysis has been suggested as an effective means to prevent degradation and achieve higher recoveries of amino acids [26,27].

Considering that nitrate is usually an important component of aerosols [24], the analysis of combined amino acids in air samples could be also problematic. Indeed, preliminary experiments with aerosol samples (without the use of antioxidants) demonstrated very low recoveries (<10%) or a nearly complete loss of amino acids during hydrolysis (Fig. 3). To enhance method performance, the protective effect of several antioxidants was evaluated using aerosol extracts pre-spiked with amino acids and hydrolyzed under an excess of each antioxidant. Fig. 3 demonstrates that the addition of mercaptoethanol did not exert any appreciable effect. Furthermore, the use of dithiothreitol caused only a modest improvement and the recoveries of most amino acids remained below 40%. In contrast, significantly better results were obtained when 500 μg of thioglycolic acid or ascorbic acid were incorporated during hydrolysis. With the use of thioglycolic acid, the recoveries of most amino acids varied from 60 to 80%, while relatively higher values were obtained with ascorbic acid. The utilization of a larger quantity of ascorbic acid (1000 μg) did not lead to any further improvement, implying that the antioxidant was already in excess. Consequently, the addition of 500 μg ascorbic acid was considered sufficient to compensate for the detrimental effect of nitrates and to achieve the highest recoveries of combined amino acids in aerosol samples.

3.4. Analytical performance

To evaluate the linearity of the GC–MS method, variable volumes of an amino acids standard mixture (200 pg μl^{-1}) were separately evaporated, derivatized, and the dilution series of amino acids derivatives was subsequently analyzed (10–400 pg of each analyte injected). The calibration plots constructed from peak area versus injected amount showed excellent linearity for all compounds tested in the range 10–400 pg (Table 1). The linear correlation coefficients (r^2) for the standard curves of all derivatives ranged between 0.9029 and 0.9995 and they were considered adequate for the purpose of amino acid analysis (Table 1).

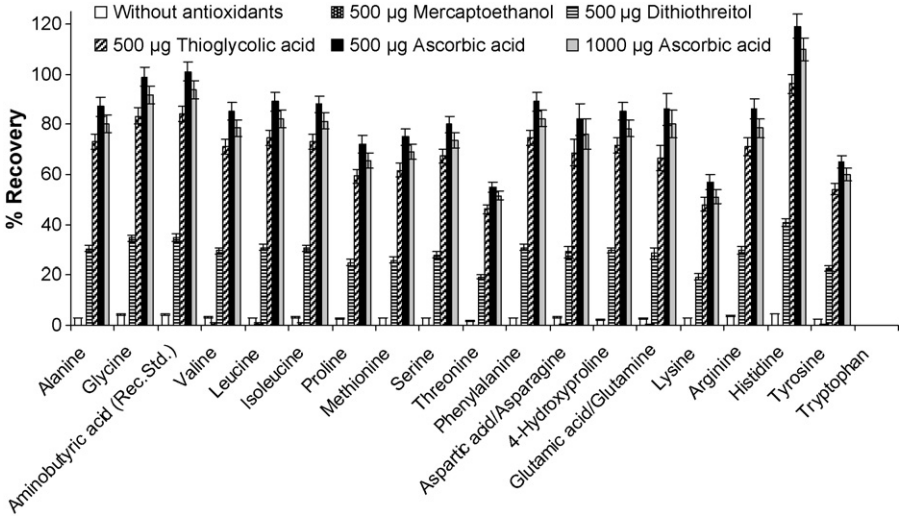

Fig. 3. Recovery of combined amino acids from spiked aerosol samples hydrolyzed under an excess of different antioxidants.

The instrumental limit of detection (LOD) and limit of quantification (LOQ) of each amino acid was determined as the minimum amount of analyte producing a chromatographic peak with a signal-to-noise ratio equal to 3 and 10, respectively (at the chosen quantification ion). In general, the LODs of amino acids were in the high picogram to low nanogram range as the majority of them varied from 0.3 to 10 pg (Table 1). Only five analytes (glutamine, arginine, tryptophan, and histidine) exhibited LODs above 10 pg, while histidine presented the highest value (46 pg). The GC–MS detection of derivatized amino acids in SIM mode provided excellent sensitivity and enhanced selectivity. As shown in Fig. 4, the peaks of amino acids in aerosol extracts were narrow, symmetrical, baseline resolved and easily identifiable. Tailing and peak distortion was observed only for histidine, arginine and tryptophan (not shown in Fig. 4), leading to lower sensitivity and higher inaccuracy in the quantification of these compounds.

The recoveries of FAA and CAA were estimated by applying the analytical method to aerosol samples that were spiked with individual amino acids (Table 2). For the majority of FAA, the analytical recovery was higher than 60% and showed high reproducibility (<10%). Lower recoveries were observed for the basic amino acids (lysine, histidine, arginine) and threonine (16–32%). Low recoveries of basic amino acids have also been reported in previous studies and have been attributed to the adsorption on glassware [28] or other surfaces. In addition, it is possible that the sample matrix might inhibit the derivatization of the specific compounds.

The recoveries estimated for the majority of CAA were also higher than 60% and presented excellent reproducibility. However, tryptophan, asparagine and glutamine could not be detected in hydrolysates. This observation is not unexpected, since acid hydrolysis causes the complete destruction of tryptophan and promotes the deamination of glutamine and asparagine to glutamic acid and aspartic acid, respectively [29]. Due to this conversion, the recoveries shown in Table 2 for glutamic and aspartic acid include glutamine and asparagine, respectively. Overall, the recoveries of free and combined amino acids are considered satisfactory, while the use of aminobutyric acid as recovery standard allows a correction for any analyte losses during sample treatment. It should be also pointed out that the analysis of amino acids by GC–MS enables the use of isotope labeled analogs as recovery standards, which could further facilitate quantification.

Table 2 presents the results from blank sample analysis. For individual FAA, the method blank levels varied from 6 to 88 ng, while six analytes were not detectable. The method detection limits (MDL), defined as average blank plus 3 standard deviations, were lower than 60 ng for the majority of amino acids (equivalent to an air concentration of <0.3 ng m^{-3} for a 220 m^3 air sample). Higher MDLs were obtained only for glycine, aspartic acid, arginine and tryptophan (107–196 ng). The method blank levels of individual CAA ranged from 1 to 20 ng, while the corresponding MDLs were lower than 70 ng for all analytes (equivalent to an air concentration of <1.1 ng m^{-3} for a 60 m^3 air sample).

With regard to the conventional HPLC-fluorescence determination of amino acid using OPA, instrumental detections limit as low as 50 fmol have been previously reported [30]. Though, other studies suggest that the practical limit of analysis is approximately 1 pmol [31], which translates to about 100–150 pg per amino acid. In either case, the HPLC-fluorescence method is deemed to be more sensitive that the GC–MS based method but the detection limits of the latter are considered adequate for the detection of amino acids in atmospheric aerosols. Moreover, the blank levels achieved in the present study were low enough and enabled the trace analysis of airborne amino acids (Section 3.5). The HPLC-fluorescence method has been used in all previous studies investigating FAA and CAA in aerosols, but detailed information about blank levels were not available for comparison. The influence of coextracted

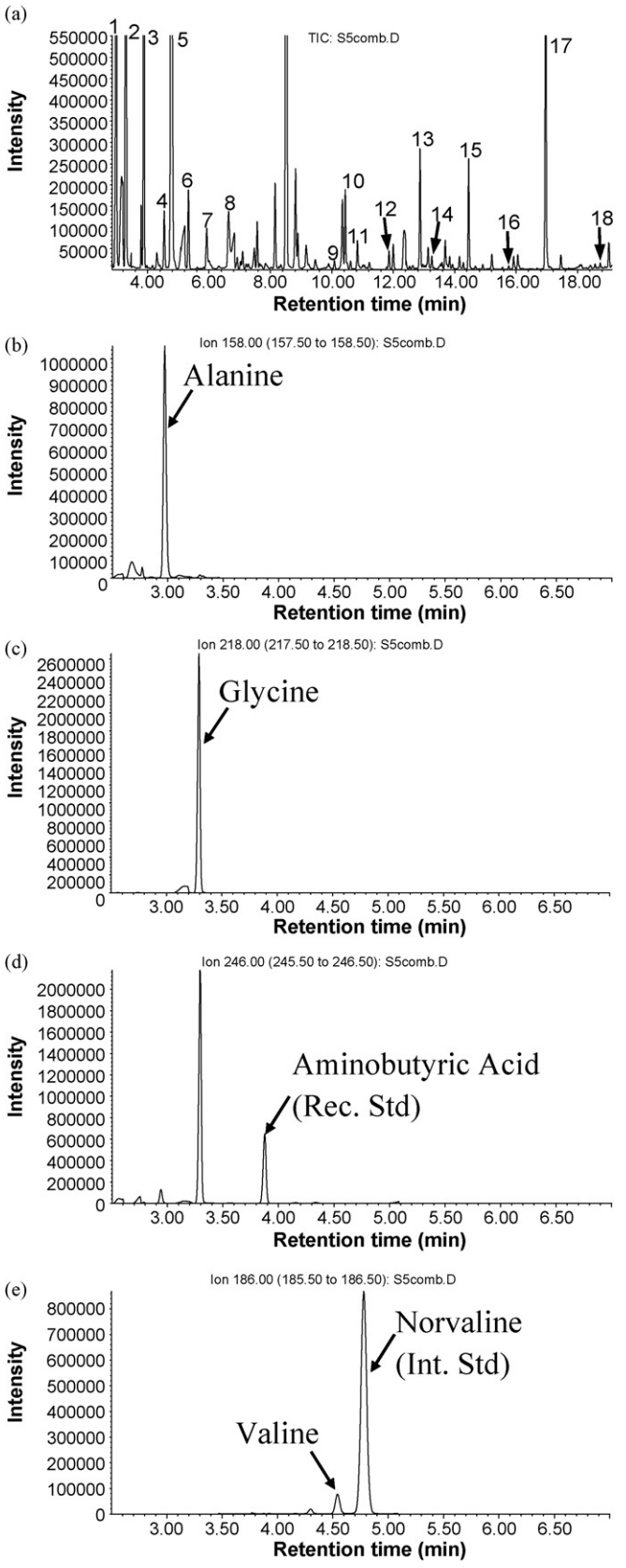

Fig. 4. Representative (a) total ion chromatogram and (b–e) selected ion monitoring chromatograms from GC–MS analysis of combined amino acids in aerosols. Peaks: 1 = alanine, 2 = glycine, 3 = 2-aminobutyric acid, 4 = valine, 5 = norvaline, 6 = leucine, 7 = isoleucine, 8 = proline, 9 = methionine, 10 = serine, 11 = threonine, 12 = phenylalanine, 13 = aspartic acid, 14 = hydroxyproline, 15 = glutamic acid, 16 = lysine, 17 = 2-aminopimelic acid, 18 = tyrosine.

Table 2
Spike recovery data, blank levels and estimated method detection limits (MDL) of free (FAA) and combined amino acids (CAA).

Amino acid	% Recovery		Blank (ng)		MDL (ng)	
	FAA	CAA	FAA	CAA	FAA	CAA
Alanine	105 ± 9	87 ± 4	28 ± 5	14 ± 6	43	32
Glycine	47 ± 3	99 ± 9	66 ± 14	20 ± 10	108	50
2-Aminobutyric acid[a]	100 ± 5	101 ± 2	–	–	–	–
Valine	97 ± 4	85 ± 1	10 ± 2	9 ± 3	16	18
Leucine	90 ± 8	89 ± 1	11 ± 2	15 ± 7	17	36
Isoleucine	96 ± 11	88 ± 1	10 ± 3	8 ± 3	19	17
Proline	114 ± 10	72 ± 1	6 ± 1	4 ± 2	9	10
Methionine	87 ± 7	75 ± 1	N.D.	3 ± 1	4.2[d]	6
Serine	66 ± 8	80 ± 6	30 ± 9	18 ± 9	57	45
Threonine	32 ± 3	55 ± 5	13 ± 7	13 ± 5	34	28
Phenylalanine	77 ± 5	89 ± 1	12 ± 3	8 ± 4	21	20
Aspartic acid	84 ± 13	82 ± 1[b]	88 ± 36	8 ± 5[b]	196	23[b]
4-Hydroxyproline	103 ± 34	85 ± 4	N.D.	3 ± 2	1.4[d]	9
Glutamic acid	72 ± 3	71 ± 5[c]	15 ± 5	18 ± 10[c]	30	48[c]
Asparagine	64 ± 4	–	N.D.	–	5.3[d]	–
Lysine	16 ± 2	57 ± 1	N.D.	15 ± 16	10[d]	63
Glutamine	51 ± 10	–	N.D.	–	17[d]	–
Arginine	29 ± 1	86 ± 37	45 ± 31	13 ± 4	138	25
Histidine	27 ± 16	119 ± 5	N.D.	1 ± 1	46[d]	4
Tyrosine	83 ± 6	65 ± 1	12 ± 3	8 ± 3	21	17
Tryptophan	118 ± 29	–	65 ± 14	–	107	–

[a] Recovery standard.
[b] Aspartic acid results include asparagine.
[c] Glutamic acid results include glutamine.
[d] Method detection limit determined as the instrumental detection limit.

ions, abundant in aerosols (e.g. calcium), which lower the response of amino acid OPA derivatives [15], was never studied and reported for aerosols. Considering that gas chromatography offers inherently higher resolution than HPLC, the present method minimizes coelution problems between amino acids and alleviates the effect from interfering compounds. Additionally, mass spectrometry is more selective than fluorescence systems and allows unambiguous identification of amino acid by monitoring their characteristic fragment ions. Other problems that have been reported for the conventional HPLC-fluorescence method [15], such as the presence of fluorescent contaminants in buffers or OPA reagent solution and the degradation of the column packing material, are not encountered with GC–MS analyses. More importantly, the MTBSTFA derivatives of amino acids are considerably more stable (for more than 5 days; data not shown) than those formed with OPA, and they do not have to be analyzed immediately upon preparation. Based on these enhanced features, the GC–MS based method established in the present study is considered to be a valuable improvement for the analysis of amino acids in aerosols.

3.5. Determination of free and combined amino acids in marine background aerosols

To evaluate the efficiency of the established method, amino acids were analyzed in six aerosol samples collected from a marine background area of eastern Mediterranean (Table 3). With the exception of methionine and tyrosine, all FAA were detected in at least one sample. The mean concentrations of individual compounds varied from 0.1 to 6.9 ng m^{-3}, with the highest levels observed for tryptophan, glycine and glutamine. The total FAA concentration ranged between 13 and 34 ng m^{-3} providing an average of 23 ± 7 ng m^{-3}. In addition to glutamine, asparagine and tryptophan, which were lost during acid hydrolysis, only arginine and histidine could not be detected in aerosol hydrolysates. The concentrations of individual CAA varied from 0.1 to 17 ng m^{-3}, while glycine, alanine, aspartic acid and glutamic acid were the most abundant compounds. Moreover, the total CAA concentration ranged from 29 to 79 ng m^{-3} and the average value (53 ± 20 ng m^{-3}) was about 2 times higher compared to that of FAA.

The levels of total FAA in background aerosols of eastern Mediterranean were generally lower than those previously measured in northern California (63–1720 pmol m^{-3}; equivalent to 9–235 ng m^{-3}) [3] and in urban aerosols of Hong Kong (84–192 ng m^{-3}) [12] and Nanjing, China (39–396 ng m^{-3}) [13]. In addition, substantially higher levels of combined amino acids were observed in northern California (1020–3210 pmol m^{-3}, which are equivalent to 140–439 ng m^{-3}) [3] and Hong Kong (51–229 ng m^{-3})

Table 3
Concentrations of free (FAA) and combined amino acids (CAA) measured in background marine aerosols of eastern Mediterranean (N = 6).

Amino acid	FAA (ng m^{-3})		CAA (ng m^{-3})	
	Average	Range	Average	Range
Alanine	0.9 ± 1.3	N.D.–3.3	12 ± 4	6.7–18
Glycine	3.6 ± 1.0	2.2–4.4	17 ± 14	6.8–44
Valine	0.2 ± 0.3	N.D.–0.9	2.1 ± 0.9	0.9–3.5
Leucine	0.2 ± 0.5	N.D.–1.2	1.6 ± 0.9	0.3–2.8
Isoleucine	0.1 ± 0.3	N.D.–0.7	1.2 ± 0.6	0.5–2
Proline	1.4 ± 0.9	0.7–2.9	1 ± 0.6	0.7–1.6
Methionine	N.D.	–	0.2 ± 0.2	N.D.–0.4
Serine	0.8 ± 0.3	0.5–1.3	1.2 ± 0.8	0.6–2.7
Threonine	0.1 ± 0.1	N.D.–0.3	2.1 ± 0.9	0.9–3.7
Phenylalanine	0.3 ± 0.7	N.D.–1.8	1.0 ± 0.7	0.4–1.7
Aspartic acid	0.8 ± 0.8	N.D.–2.1	5.6 ± 3.0[a]	2.3–11[a]
4-Hydroxyproline	0.7 ± 0.8	N.D.–2.2	0.1 ± 0.2	0–0.4
Glutamic acid	1.3 ± 0.4	0.7–1.9	6.7 ± 4.1[b]	2.8–14[b]
Asparagine[c]	1.1 ± 2.3	N.D.–5.6	–	–
Lysine	0.2 ± 0.5	N.D.–1.3	0.1 ± 0.3	N.D.–0.7
Glutamine[c]	3.4 ± 1.9	1.7–7.0	–	–
Arginine	0.4 ± 1.0	N.D.–2.5	N.D.	–
Histidine	0.2 ± 0.2	0.1–0.6	N.D.	–
Tyrosine	N.D.	–	0.6 ± 0.6	N.D.–1.7
Tryptophan[c]	6.9 ± 6.3	N.D.–16	–	–
SUM	23 ± 7	13–34	53 ± 20	29–79

[a] Aspartic acid results include asparagine.
[b] Glutamic acid results include glutamine.
[c] Asparagine, glutamine and tryptophan were destroyed during acid hydrolysis.

[12]. It is interesting to point out that the levels of CAA in northern California were substantially higher than those of FAA, which is in line with the findings of the present study.

4. Conclusions

This study shows that derivatization with MTBSTFA in conjunction with GC–MS detection offers a suitable alternative technique for the analysis of free and combined amino acids in aerosols. The derivatization is accomplished in a single step and, due to the limited use of reagents, the contamination risk is minimized and easily controlled. This approach provides excellent linearity and sufficiently low detection limits. Sample extraction was found to be a potential source of contamination and the purity of water should be as high a possible in order to achieve low blanks. The coextraction of inorganic salts from aerosols can interfere with the derivatization of free amino acids, but this effect can be alleviated by the addition of isopronanol during sample extraction. Moreover, the addition of ascorbic acid during hydrolysis was proved to be of utmost importance because it prevents the degradation of combined amino acids by nitrates ubiquitously present in aerosol extracts. The high selectivity and sensitivity of the GC–MS based technique in conjunction with the low blank levels enabled the determination of amino acids in aerosol samples from the background atmosphere of the eastern Mediterranean. The total concentration of combined amino acids varied from 29 to 79 ng m^{-3} and it was substantially higher than the levels of free amino acids (13 and 34 ng m^{-3}).

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