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Development of a method for the analysis of underivatized amino acids by liquid chromatography/tandem mass spectrometry: Application on Standard Reference Material 1649a (urban dust)

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ABSTRACT

A liquid chromatography–tandem mass spectrometry analytical procedure has been developed for the detection and quantitative determination of underivatized amino acids at low concentrations in a Standard Reference Material—urban dust. In order to minimize interferences of other compounds, an accelerated solvent extraction followed by a solid phase extraction on two different cartridges was applied prior to LC–MS–MS.

Fourteen amino acids were separated by high resolution liquid chromatography, detected and quantified by multiple reaction monitoring on a triple quadrupole. The proposed methodology has been applied for the first time on Standard Reference Material 1649a (urban dust) from the National Institute of Standards and Technology, that does not report certification values for these compounds.

This methodology avoids the derivatization step and allows the amino acid quantification in a complex matrix, such as that of atmospheric particulate matter, and represent a good method suitable to analyze this class of compounds in atmospheric aerosol.

The selected strategy demonstrated to be fit-for-purpose, by applying it to a real atmospheric sample with the aim to verify the efficacy of the study and to provide information about the organic matter content.

1. Introduction

Free amino acid (aa) contribution to ambient inhalable airborne is due to direct emission from plants and animals or may be issued by peptides and proteins through enzymatic or photo-catalytic reactions in atmosphere [1,2]. They are a key class of atmospheric ON compounds and can contribute to secondary aerosol formation.

In 1998, Mondal studied free amino acids in crushed and homogenized pollen from four plants that had been shown to play a significant role in causing various allergic disorders in sensitive patients and he found a percentage higher than 6% in weight [3].

Significant amounts of free amino acids were found in the marine aerosol [4,5], in the continental aerosol [6,7], as well as in the rain [1] and in the fog [6,8].

As for their analytical determination, the high polarity of these compounds, their low volatility and the lack of strong chromophore groups make their separation and determination difficult. In order to solve these problems, pre- and post-column derivatization reactions have been developed, for either increasing the volatility of the analytes for analysis by gas chromatography [5,9], or introducing chromophore/fluorophores groups in their structure for analysis by liquid chromatography with UV or fluorescence.

In most studies [1,4,6,8,10], the identification and quantitation of amino acids in particulate matter were performed by HPLC analysis with a fluorescence detector, after derivatization of compounds with o-phthalaldehyde/mercaptoethanol (OPA).

In addition, in the same studies, the extraction of amino acids from aerosols was performed in an ultrasonic bath by hydroalcoholic solutions, and, after centrifugation and filtration, the samples were directly analyzed [6,7] by chromatography. Concerning the purification of aa from complex matrices (plasma, and various aqueous root exudation samples, honey) recent papers [11–13] consider mixed-mode stationary or polymeric strong cation exchange sorbents suitable. All of the existing derivatization methods present various analytical problems: derivative instability, reagent interference, long preparation times, inability to derivatize the secondary amino groups and difficulties in

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derivatization toward specific amino acids [14]. Therefore the ability to investigate amino acids, without derivatization, by liquid chromatography–tandem mass spectrometry (HPLC/MS–MS), reduces sample preparation times and eliminates reagent associated interferences and the possible side reactions that may occur by performing derivatization in complex sample matrix [15].

Since, at present, in literature, few data are available of such compounds in atmosphere, we focused their determination on particulate matter. In particular, first of all, as there are no urban dusts with certified and reference values for amino acids, we optimized their extraction, purification and analysis in a Standard Reference Material (SRM) from National Institute of Standards and Technology (NIST), SRM 1649a—urban dust and then we applied the optimized method to a real atmospheric sample.

We used high pressure and temperature extraction followed by SPE purification on two cartridges in series and then we detected underivatized amino acids by high resolution liquid chromatography coupled to tandem mass spectrometry (HPLC–MS/MS) in multiple reaction monitoring (MRM) mode on a triple quadrupole.

Since particulate matter samples are very "dirty", we gave great importance to the step of purification both in order to guarantee the analysis of trace amounts of compounds, in case of small available quantities of particulate matter and in order to reduce matrix and ion suppression effects. Although other authors used, for different matrices, simply one SPE cartridge for the purification [11–13], we obtained an interference-free determination and an increase in signal-to-noise ratios (S/N), only thanks to a double purification step on two different cartridges and MS–MS detection. Moreover, since it was not possible to prepare calibration solutions that mimed the environment in which the analytes were found and the interactions between the analytes and other materials in the matrix (altering the observed response), the calibration curves were prepared with the standard addition method. Results obtained in this study can also represent an important starting point to future inter-comparison studies.

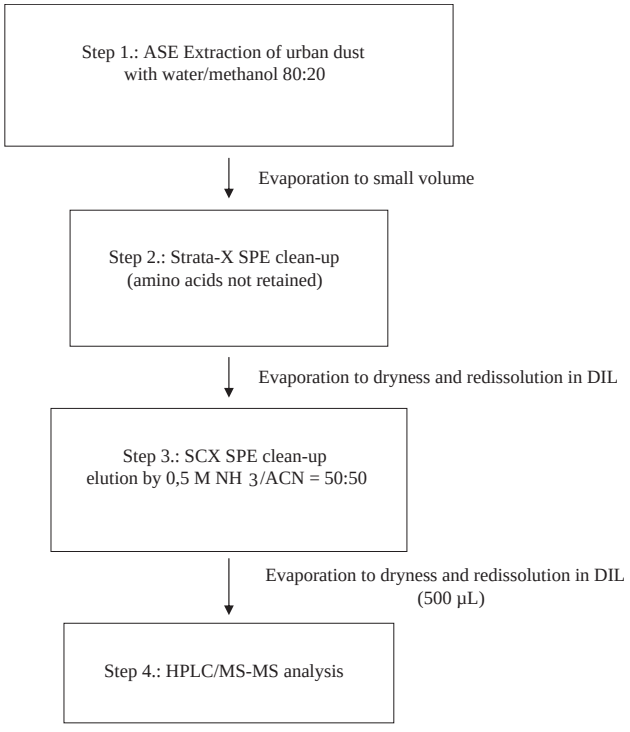

Fig. 1. Block diagram of the complete procedure for urban dust analyses.

2. Experimental

2.1. Chemicals and reagents

Ultra gradient acetonitrile (ACN), methanol (MeOH) and water (H_2O) were obtained by ROMIL LDT (Cambridge, UK). Formic acid (HCOOH) 99%, ammonia (NH_3) solution 30% and ammonium acetate (CH_3COONH_4), ammonium formate ($HCOONH_4$), and acetic acid glacial (CH_3COOH) were purchased from Carlo Erba Reagents S.p.A. (Arese, Milan, Italy).

L-methionine, L-serine, L-threonine were supplied by Sigma Chemicals; DL-alanine, DL-asparagine, DL-glutamine, DL-glycine, DL-isoleucine, DL-leucine, DL-phenylalanine, DL-proline, DL-tyrosine, DL-tryptophan and DL-valine were obtained from Fluka (Sigma-Aldrich, Milan, Italy).

Isotopically-labeled ^2H amino acids (L-isoleucine-2-[$^2H_1$], DL-serine-2,3,3-[$^2H_3$] and L-threonine-2,3-[$^2H_2$], purity $>98\%$) were obtained from C/D/N Isotopes Inc. (Pointe-Claire, Quebec, Canada) and the other two internal standards (DL-proline-2,3,3,4,4,5,5-[$^2H_7$], L-tryptophan-[$^2H_8$], purity of 98%) were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA).

Stock standard solutions of all analytes were prepared by dissolving each compound in water/methanol 50:50 (1 mg/mL) and storing them at $-20\ ^\circ C$. Working solutions were prepared by successive dilution of the stock standard solutions.

In order to redissolve the samples after evaporation, a diluent solution (DIL) was prepared as follows: 5% 5 mM CH_3COONH_4 solution, adjusted to pH 4 with HCOOH and 95% mixed solution of 90% ACN, 10% H_2O acidified with 0.1% HCOOH.

RM 1649a—urban dust was supplied by NIST (Gaithersburg, MD, USA). This urban dust was collected in 1976–1977 in the Washington, D.C. area over a period in excess of twelve months. The material was then sieved through a fine-mesh sieve ($<125\ \mu m$) and issued as SRM 1649 in1982 as a standard for polycyclic aromatic hydrocarbon analyses in aerosols. This material was re-certified in 1999 for additional organic and inorganic contaminants as well as radiocarbon and total organic carbon content and issued as SRM 1649a.

Table 1
Retention times, capacity factor of amino acids in HPLC by column 3 under isocratic condition with: Pump A: 15% (mobile phase A: 5 mM CH_3COONH_4, adjusted to pH 4 with HCOOH). Pump B: 85% (mobile phase B: ACN/H_2O acidified with 0.1% HCOOH=90:10). Flow: 0.250 mL/min.

Compound	Retention time (min)	Capacity factor	Compound	Retention time (min)	Capacity factor
Phenylalanine (Phe)	2.40	2.00	**Tyrosine (Tyr)**	2.87	2.59
Proline (Pro)	2.42	2.03	**Alanine (Ala)**	3.38	3.23
Leucine (Leu)	2.54	2.18	**Threonine (Thr)**	3.47	3.34
Isoleucine (Ile)	2.54	2.18	**Glutamine (Gln)**	3.65	3.56
Methionine (Met)	2.62	2.23	**Glycine (Gly)**	3.85	3.81
Valine (Val)	2.75	2.44	**Serine (Ser)**	3.93	3.91
Tryptophan (Trp)	2.80	2.50	**Asparagine (Asn)**	4.03	4.04

2.2. Sampling

Urban particulates PM_{10} (fine suspended particulates less than 10 μm in aerodynamic diameter) were enriched from air over a 48-h period by means of a HYDRA Dual Sampler (FAI Instruments, Rome, Italy) onto PTFE filters (47 mm of diameter and 0.2 mm of porosity) (Pall Corporation, Port Washington, NY, USA).

The final volume of sampled air was equal to about 110 N m^3, and the amount of PM_{10}, collected on PTFE filter with the dual channel sampler, was equal to 2.72 mg. Before and after collection, filters were conditioned for 24 h in a chamber maintained at 50% relative humidity and 20 °C, weighted and then processed through the entire analytical procedure.

2.3. Materials and instrumentation

An Accelerated Solvent Extractor ASE200-Dionex (Thermo Scientific, Rodano, Milan, Italy), operating at high pressure and temperature, allowed to achieve an effective extraction with only one static cycle. The extracts were evaporated by a SE 500-Dionex solvent evaporator (Glas-Col, Terre Haute, IN, USA) under a nitrogen stream. A vacuum manifold 12-Port model SPE (Alltech,

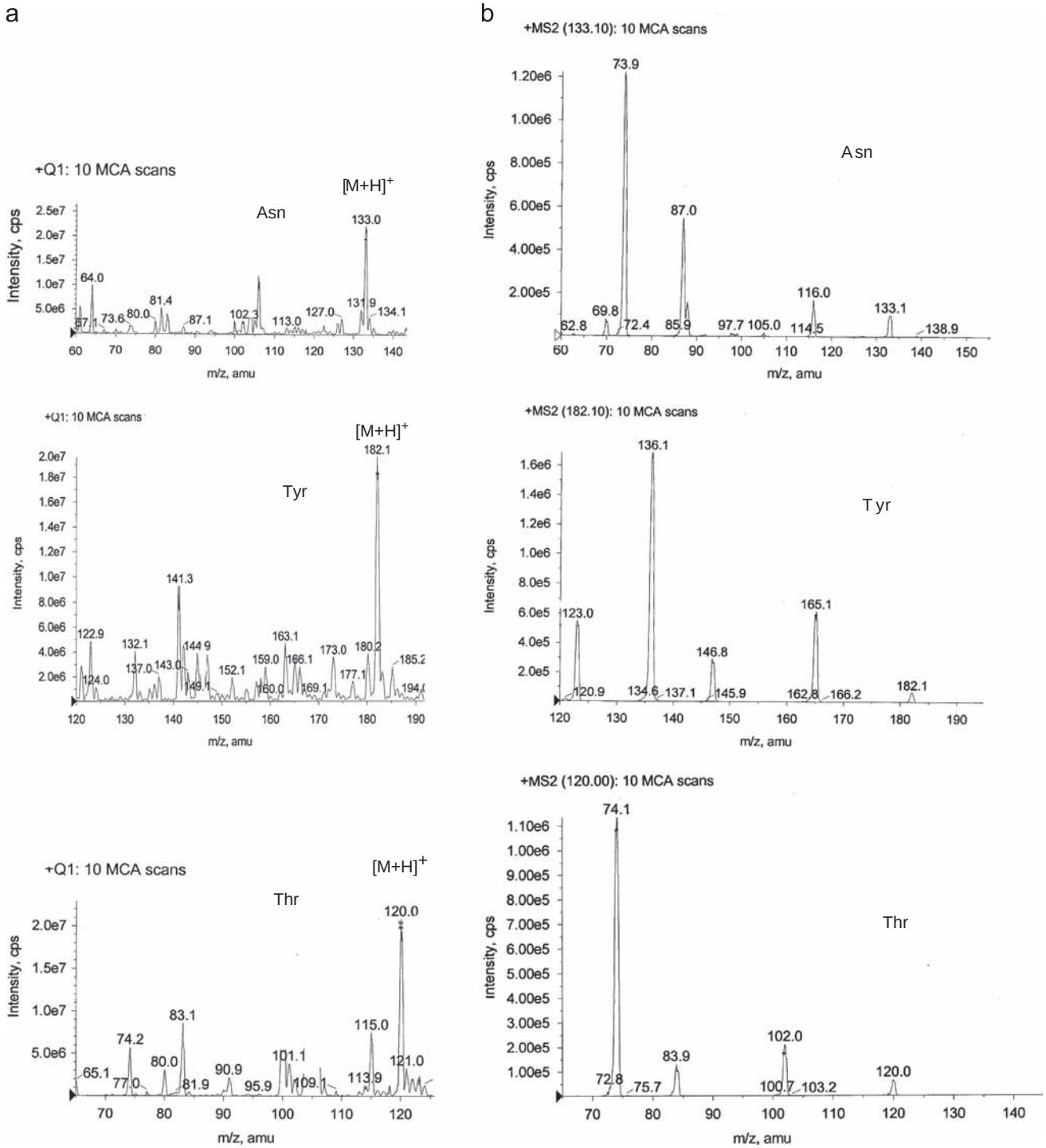

Fig. 2. (a) Full scan Q1 spectra from Asn, Tyr, and Thr. Infusion (10 ng/μL) at 10 μL/min. (b) MS–MS spectra of Asn, Tyr and Thr chosen as representative according to their characteristic fragmentations. Precursor ions Asn *m*/*z* 133, Tyr *m*/*z* 182, Thr *m*/*z* 120. Conditions as in Table 2.

Casalecchio di Reno, Bologna, Italy) was used to quickly elute the analytes from the SPE cartridges at a constant flow. Strata-X cartridges (200 mg/6 mL), Strata-X-C cartridges (30 mg/1 mL) and Strata SCX (500 mg/6 mL) were obtained from Phenomenex (Castel Maggiore, Bologna, Italy); SCX Alltech® Extract-Clean™ cartridges (500 mg/4 mL) on Styrene-DVB base were purchased from Grace (Alltech, Casalecchio di Reno, Bologna, Italy).

Three different columns were evaluated. Column 1 was a C_{18} (250 mm × 2.1 mm) slurry packed in our lab with size particles of 5 μm (Spherisorb, Deeside Ind. Est., Queensferry, UK); column 2 was a LUNA HILIC 50 mm × 2.0 mm I.D, 3 μm (Phenomenex, Castel Maggiore, Bologna, Italy); column 3 was an Acclaim® Trinity™ Analytical P1 2.1 mm × 100 mm I.D, 3 μm (Thermo Scientific, Rodano, Milan, Italy).

The amino acids were analyzed by liquid chromatography/positive ion electrospray ionization–tandem mass spectrometry (HPLC/(+) ESI–MS/MS) at a flow rate of 250 μL/min and the injection was of 20 μL.

An Agilent 1100 series binary HPLC pump system (Agilent Technologies, Santa Clara, CA, USA) fitted with an autosampler Agilent G1313A (Agilent Technologies, Santa Clara, CA, USA) was coupled to an API 2000 triple quadrupole mass spectrometer (AB SCIEX S.r.l., Forster City, CA) with an IonSpray source.

2.4. Analytical procedure protocol

SRM 1649a was ASE extracted using H_2O/MeOH 80:20, at a temperature of 100 °C and a pressure of 1500 psi.

As regards extract purification, two subsequent solid phase extractions were performed prior to analysis by HPLC–MS/MS.

The ASE extracts (about 12 mL) were evaporated to 6 mL, using a nitrogen flow at a temperature of 80 °C and loaded onto the first cartridges, Strata-X SPE, linked to a vacuum system. Strata-X cartridges were previously conditioned with 6 ml of MeOH and equilibrated with 6 ml of H_2O. The amino acids were not retained, whereas non-polar and low-polar interferences were held by the stationary phase. The amino acid solutions were evaporated to dryness and redissolved in DIL, at a pH suitable to convert them into cationic form. The solution was then loaded on a strong cation-exchange column (Alltech SCX cartridges) previously conditioned by 3 mL of 0.25 M HCOOH (pH~2).

Six milliliter of 5 mM HCOOH/MeOH, 95:5 were used as washing solution. Finally amino acids were eluted by 20 mL of 0.5 M NH_3 solution (pH~11) containing 50% ACN.

The sample was evaporated to dryness, in order to concentrate amino acids as well as to volatilize NH_3, and redissolved in 500 μL of DIL solution prior to HPLC/MS–MS analysis.

The whole procedure is illustrated in Fig. 1.

2.5. Calibration curves

Three sets of calibration curves ("A", "B" and "C") were prepared in order to evaluate the method linearity, estimate the matrix effect and determine amino acid concentrations in SRM—urban dust and in real samples.

Type "A" consisted of six standard solutions with increasing analyte concentrations, and constant concentrations of the ^2H-labeled amino acids added as internal standard (IS).

The multistandard solutions contained amino acids at different concentrations, depending on instrument sensitivity and the expected concentration in urban dust. Thus, for example, tyrosine concentration ranged from 44.5 to 430.1 ng/mL, while glycine concentration ranged from 495 to 940.5 ng/mL.

In order to build "B" curves, six aliquots of about 0.2 mg of SRM 1649a each were previously subjected to the procedure of Fig. 1, and then spiked with the same standard solutions of curve "A" immediately prior to LC injection.

The standard addition method was applied to build curve "C". Six aliquots of about 0.2 mg of SRM 1649a were spiked with standard solutions of increasing concentration and ISTD solutions, before applying the whole procedure of Fig. 1. The first SRM aliquot was only IS spiked. A linear plot of the peak area ratios (ordinate) against the amount of standard added (abscissa) estimated the analyte concentrations in SRM 1649a by directly finding the intercept on abscissa.

For all the curves, each solution was injected three times in HPLC/MS–MS and the regression model was applied to the calibration data set.

Leu, Ile, Val, Met and Phe were quantified with $^2H_1$-Ile; Tyr and Trp with $^2H_8$-Trp; Ser, Gln, Gly and Asn with $^2H_3$-Ser; Ala and Thr with $^2H_2$-Thr and Pro with $^2H_7$-Pro.

2.6. Recovery

Amino acid recovery was determined after each step of the procedure (ASE extraction, first SPE purification, second SPE purification), using amino acid standard solutions and adding the IS just before HPLC injection.

Total recovery was instead calculated on SRM 1649a spiked with different amounts of amino acids before the extraction and adding the IS prior to LC–MS/MS analysis.

Table 2
Optimized values of ISV, DP, FP, CE parameters, monitored precursor (Q1) and product (Q3) ions for each amino acid and IS ordered according to their elution time.

Compound	Ion source voltage (ISV)	Declustering potential (DP)	Focusing potential (FP)	Collision energy (CE)	Q1 (*m*/*z*)	Q3 (*m*/*z*)
Phe	5500	20	400	30	166	120
Pro	5500	20	400	20	116	70
Leu	5500	10	400	15	132	86
Ile	5500	10	400	15	132	86
Met	5500	20	400	20	150	104–133
Val	5500	15	400	15	118	72
Trp	5500	10	400	20	205	146–188
Tyr	5500	15	380	20	182	136–165
Ala	5500	15	350	20	90	44
Thr	5500	20	400	20	120	74–102
Gln	5500	20	400	30	147	84
Gly	5500	20	400	20	76	30
Ser	5500	10	400	20	106	60–88
Asn	5500	15	400	20	133	74–87
$^2H_7$-Pro	5500	20	400	20	123	77
$^2H_1$-Ile	5500	10	400	15	133	87
$^2H_8$-Trp	5500	10	400	20	213	196
$^2H_2$-Thr	5500	20	400	20	122	76
$^2H_3$-Ser	5500	10	400	20	109	63

3. Results and discussion

3.1. Chromatographic conditions

Recently some authors used hydrophilic interaction ultra-performance liquid chromatography in order to effectively separate underivatized amino acids [12,16].

Our preliminary experiments were performed in order to find the most suitable column for the separation of amino acids. Columns 1 and 2 (based on hydrophilic interaction ultra-performance liquid chromatography), with different compositions of mobile phase (H_2O/MeOH and H_2O/ACN), added by HCOOH, CH_3COOH or CH_3COONH_4 and $HCOONH_4$ (in order to increase chromatographic separation) did not give satisfactory results due to poor retention or tailed peaks.

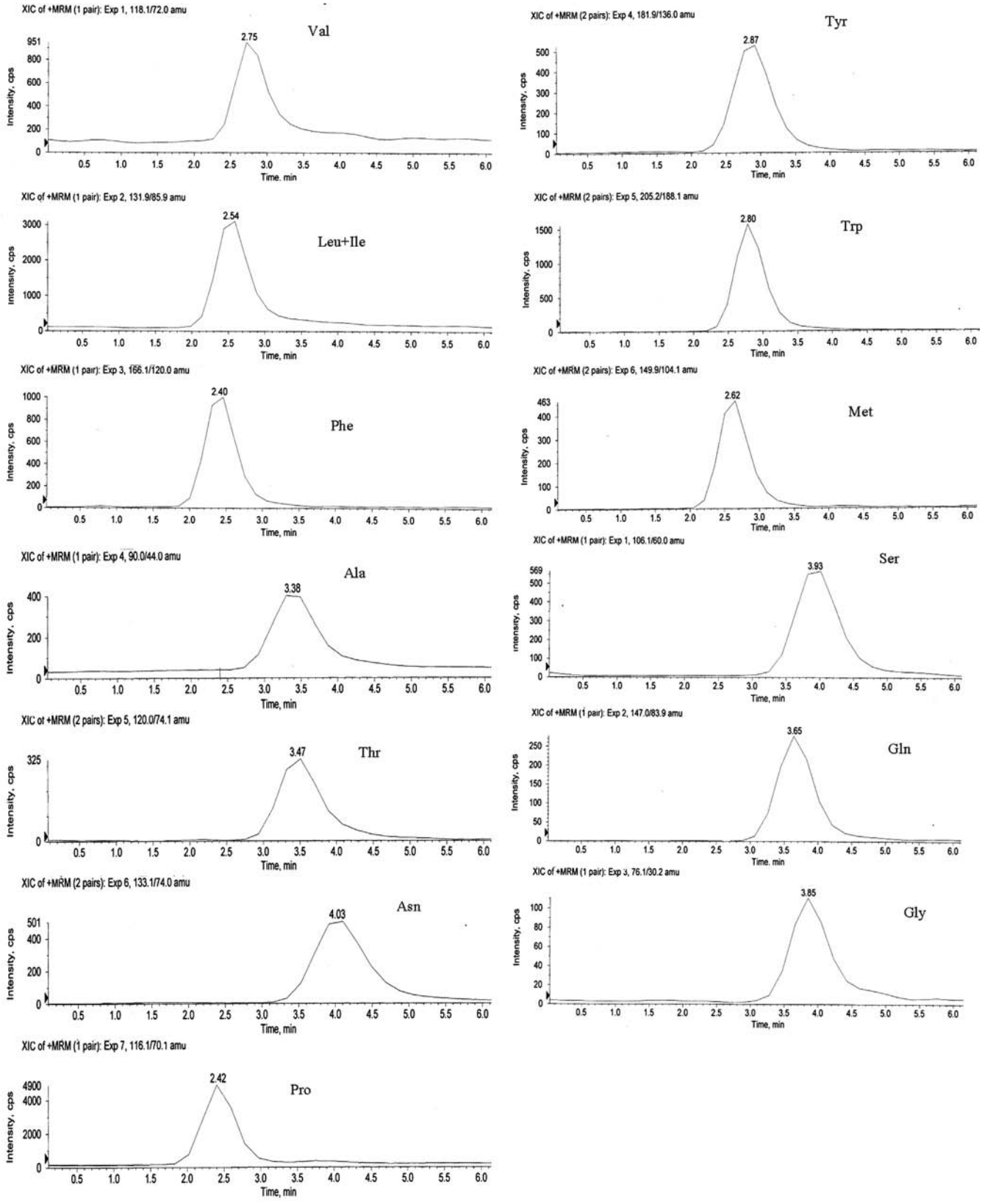

Fig. 3. HPLC–MS–MS in MRM mode of an aa standard mixture. Extracted ion currents (XICs) are shown for each aa. The XICs refer to the most intense chosen transitions for each of the analytes (see Table 2).

In order to solve these problems column 3 was used. The new column, based on Nanopolymer Silica Hybrid (NSH) technology, provides multiple retention mechanisms including reversed-phase, anion-exchange and cation-exchange.

The best separation and chromatographic run-time results were obtained by eluting amino acids in isocratic condition with 15% phase A (5 mM CH_3COONH_4, adjusted to pH 4 with HCOOH), and 85% phase B (ACN/H_2O acidified with 0.1% HCOOH, 90:10). All the compounds and IS were eluted in less than 5 min. Table 1 shows retention times and capacity factors for each amino acid.

3.2. LC/MS/MS analysis

In order to achieve the highest possible sensitivity and to find the specific MS–MS transitions, the instrument parameters were optimized by direct continuous pump infusion of standard solutions of the analytes at 10 ng/μL, at a flow rate of 10 μL/min, both in full and product ion scan. Experiments were carried out under positive and negative polarity with different additives to improve ionization efficiency. The best results were obtained operating in positive ion mode using formic acid for all compounds. All amino acid spectra showed an abundant $[M+H]^+$ ion on Q1, which was then chosen as precursor ion for MS–MS analysis. Full scan Q1 spectra of some analytes and their respective MS–MS spectra are shown in Fig. 2a and b. Amino acid fragmentations were already studied elsewhere [15,17–19].

Table 2 shows ISV, DP, FP and CE parameters and the most intense transitions monitored for each amino acid.

Finally, all the analyses were carried out by LC/MS/MS in multiple reaction monitoring (MRM) mode to obtain high specificity and sensitivity. Fig. 3 shows the MRM chromatogram (extracted ion currents XICs) of a standard mixture. The XICs refer to the most intense transitions for each amino acid.

3.3. Linearity, LOD and LOQ

Good linearity was obtained in the investigated concentration range for each aa, as demonstrated by R^2 values between 0.980 and 0.999 of calibration curves reported in Table 3.

Limits of detection (LODs) were calculated from the confidence interval of the calibration curve "C" according to the guidelines of Agenzia Regionale Prevenzione e Ambiente (ARPA) [20]. LOD was found to range between 9.5 ng/mL for Tyr and 26 ng/mL for Thr. The LOD is typically matrix, method, and analyte specific. Comparison of the detection limits obtained in this work with previously published data showed similar detection limits to those obtained by Samy [15] and Barbaro [7], although our values were obtained from matrix calibration curves.

3.4. Matrix effect

Urban dust is a very difficult matrix, and the effects were evaluated thanks to type "A" and type "C" curves. To this aim, we evaluated the slope ratios of the calibration curves obtained from matrix ("C") and from standard solutions ("A").

Both positive and negative matrix effects were observed for most of the amino acids, except for five of them: Gln, Val, Thr, Asn and Pro.

Phe, Trp, Met, Ser and Tyr showed a signal suppression in the matrix-matched calibration curve. Leu/Ile, Gly and Ala exhibited signal enhancement in the same curves. Therefore matrix-matched calibration curves represent the best way to quantify amino acids in real environmental samples.

3.5. Recovery

Amino acid recovery was determined after each step of the procedure of Fig. 1, through the corresponding linear calibration curve "A"; and after the whole procedure, using calibration curve "B". Each sample was analyzed in triplicate by LC–MS/MS in MRM mode.

As for step 1, multistandards of amino acids were added to ASE cells, and, after extraction, evaporated and analyzed by HPLC/MS–MS, adding the IS solution just before injection. The best results were obtained using H_2O/MeOH 80:20, at a temperature of 100 °C and a pressure of 1500 psi. ASE extraction gave recoveries between 70% and 103%, depending on the amino acid.

Table 4
Average recovery (over three concentrations)±RSD (relative standard deviation) for ASE extraction, for the second clean-up (SPE SCX) step, and average recovery (over five concentrations) for the whole procedure for each investigated aa.

Compound	ASE recovery % ± RSD	SPE SCX recovery % ± RSD	Whole procedure recovery %
Phe	79 ± 8	75 ± 15	70–79
Pro	83 ± 5	102 ± 4	64–83
Ile	89 ± 1	91 ± 11	62–95
Leu	89 ± 1	91 ± 11	62–95
Met	71 ± 5	77 ± 9	62–74
Val	92 ± 4	93 ± 13	72–95
Trp	70 ± 6	82 ± 16	60–75
Tyr	102 ± 10	72 ± 10	67–88
Ala	103 ± 12	84 ± 11	85–95
Thr	102 ± 6	81 ± 7	61–93
Gln	71 ± 11	78 ± 12	62–77
Gly	73 ± 4	79 ± 16	65–90
Ser	103 ± 13	98 ± 11	78–90
Asn	101 ± 9	81 ± 9	66–93

Table 3
Equations and R^2 of the three calibration curves ("A", "B", "C") for each amino acid.

Analyte	Equations of calibration curve type "A"	R^2	Equations of calibration curve type "B"	R^2	Equations of calibration curve type "C"	R^2
Phe	$Y=0.408X+0.041$	0.993	$Y=0.329X+0.078$	0.993	$Y=0.206X+0.092$	0.988
Pro	$Y=1.670X+0.021$	0.997	$Y=1.479X+0.143$	0.999	$Y=1.580X+0.476$	0.999
Leu+Ile	$Y=0.585X+0.676$	0.996	$Y=0.802X+0.373$	0.998	$Y=0.637X+0.862$	0.993
Met	$Y=0.168X+0.015$	0.992	$Y=0.175X+0.009$	0.997	$Y=0.114X-0.006$	0.993
Val	$Y=0.566X-0.011$	0.999	$Y=0.485X+0.112$	0.994	$Y=0.431X+0.308$	0.995
Trp	$Y=0.586X-0.021$	0.994	$Y=0.393X+0.041$	0.998	$Y=0.046X+0.019$	0.992
Tyr	$Y=0.259X+0.025$	0.994	$Y=0.293X+0.042$	0.995	$Y=0.166X+0.091$	0.996
Ala	$Y=1.758X+0.080$	0.993	$Y=1.481X+0.486$	0.998	$Y=0.943X+2.150$	0.992
Thr	$Y=1.341X+0.043$	0.994	$Y=1.187X+0.111$	0.994	$Y=1.088X+0.474$	0.980
Gln	$Y=0.576X-0.007$	0.992	$Y=0.591X-0.020$	0.992	$Y=0.587X-0.016$	0.996
Gly	$Y=0.126X-0.001$	0.994	$Y=0.129X+0.002$	0.997	$Y=0.198X+0.005$	0.998
Ser	$Y=0.804X+0.008$	0.997	$Y=0.769X+0.227$	0.995	$Y=0.352X+0.609$	0.999
Asn	$Y=1.424X+0.039$	0.995	$Y=1.369X+0.132$	0.996	$Y=1.502X+0.015$	0.999

Table 5
Amino acid concentrations in urban dust SRM 1649a, and in a real sample, expressed in ng of compound per mg of dust. SD=standard deviation.

Compound	ng aa/mg SRM 1649a	SD	ng aa/mg real sample	SD
Phe	213.0	± 15.5	84.3	± 8.8
Pro	145.5	± 2.0	43.2	± 3.6
Leu/Ile	641.0	± 11.0	102	± 12.2
Met	26.0	± 1.5	–	
Val	338.5	± 7.5	88.2	± 16.0
Trp	190.5	± 7.0	47	± 7.8
Tyr	261.0	± 2.5	80.7	± 13.5
Ala	1339.5	± 33.5	514.9	± 2.8
Thr	256.0	± 6.5	111.8	± 8.3
Gln	42.5	± 2.0	100.3	± 10.3
Gly	293.5	± 8.0	318.8	± 47.2
Ser	2644.5	± 37.5	496.4	± 24.6
Asn	15.5	± 1.0	37.1	± 4.1

As for step 2, the same multistandard solutions were loaded on Strata-X cartridges. Each analyte showed a recovery higher than 95%.

As for step 3, three types of strong cation exchange cartridges were tested. The silica based sorbent, having a limited pH work range, showed to be unsuitable. Therefore, polymer based columns were selected and the best results were obtained by using Alltech SCX cartridges, eluting with 20 mL of a mixture 0.5 M NH_3 (pH~11)/ACN, 50:50. We obtained recoveries of over 70% with an average of 85%.

In order to calculate the total recovery, SRM 1649a aliquots were spiked with five concentration levels of aa and submitted to the whole procedure, adding the IS prior to LC–MS/MS analysis. The recovery results ranged between 60% and 95%.

Table 4 summarizes the partial and the total recoveries.

3.6. Amino acids in urban dust

In SRM 1649a, concentrations of amino acids were calculated using the *x*-intercept of the standard addition method calibration curve (type "C"). After translation to 0, curve "C" was also used to determine amino acid concentrations in real atmospheric samples.

In Table 5, the amino acid concentrations in urban dust SRM 1649a (first column) and in real sample (third column), expressed in ng of compound per mg of dust, are shown. Each value is associated to its standard deviation.

As expected, the two samples are essentially different (due to different sampling site, emission source, sampling period, particle sizes etc.) but both the urban dusts resulted richer in serine and alanine, according to their high natural abundance, while asparagine and methionine were the less abundant amino acids. In both cases total amino acid content was less than 1% by weight of urban dust.

4. Conclusion

Trace amount of aa were determined in standard reference material NIST SRM 1649a and in a real particulate matter sample thanks to a fit-for-purpose method optimized in the present work. Due to the complexity of the matrix and to ion suppression effect the sample preparation was more complex compared to others matrices. An extraction at high pressure and temperature with H_2O/MeOH 80:20, a purification by two SPE cartridges in series and the analysis in HPLC/MS–MS in MRM mode provided good results for the determination of underivatized amino acids in a complex matrix such as that of urban particulate. Up to now there are not previously reported literature values for aa in this SRM and, despite the large differences of concentration ranges (one to two orders of magnitude) among different amino acids, this method is easy to apply and allows a routine quantification of aa in real atmospheric samples within a single analysis.

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