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Simultaneous analysis of amino acid and biogenic polyamines by high-performance liquid chromatography after pre-column derivatization with *N*-(9-fluorenylmethoxycarbonyloxy)succinimide

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Abstract

A high-performance liquid chromatography method for the simultaneous analysis of amino acids and biogenic polyamines, using a new procedure for pre-column derivatization of amino groups with *N*-(9-fluorenylmethoxycarbonyloxy)succinimide is described. The separation of 20 amino acids and 4 biogenic polyamines was achieved within 32 min on a sequence of three short (50 mm) reversed-phase C_{18}, 5 μm columns by elution buffers based on dibutylamine phosphate. The method linearity, calculated for each amino acid and polyamine, has a correlation coefficient higher than 0.991, in concentrations ranging from 0.2 to 50 μM, except for spermine and methionine, where the correlation coefficients were $r = 0.984$ and $r = 0.979$, respectively. The stability of derivatives in acidified samples at 4 °C and room temperature was demonstrated. The limit of quantitation was estimated to be around 50 pM in 50 μl sample injection. The repeatability of the method, expressed as R.S.D., ranged from 1.1 to 6.7%. The presented method was applied for the quantitation of amino acid and polyamine contents in beer, wine, and cell culture samples, using 2-aminoheptanoic acid or 1,7-diaminoheptane as internal standard.
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1. Introduction

Natural polyamines putrescine (Put), cadaverine (Cad), spermine (Spm), and spermidine (Spd) are found ubiquitously in all living cells. They have multiple functions in living organisms, such as growth factors, antioxidants, stabilizers of DNA and RNA, metabolic regulators, nutrients and second messengers [1–3]. The precise roles of polyamines in cellular physiology are not well defined yet. The polyamines and their biological functions are under vigorous investigation by a diverse set of disciplines. Multiple pathways such as biosynthesis, catabolism, uptake, and excretion tightly regulate polyamine intracellular concentrations. It is widely accepted that under most circumstances, the major sources of cellular polyamines come from their synthesis from amino acid precursors. The principal precursors of polyamines are the amino acids L-ornithine and L-methionine. The initial hydrolysis of L-arginine to L-ornithine and urea may also be considered [4]. For these reasons, the availability of precise analytical methods for the simultaneous determination of amino acids and polyamines in some matrices are of primary importance from practical point of view. Various methods have been introduced. HPLC analysis, utilizing pre-column derivatization with fluorescent labels seems to be the method of choice. The methods in a current use have been reviewed recently, accenting on their advantages and drawbacks [5,6].

The 9-fluorenylmethoxycarbonyl (Fmoc) group is widely used for protection of amino groups in peptide synthesis [7]. The Fmoc group was originally introduced in the analytical practice by Einarsson et al. [8] as a fluorescent label in amino acid analysis. Then the Fmoc-group has been widespread for analysis of amino acids and amino function possessing compounds [9]. Derivatization and analytical procedures have been carefully studied, modified, optimized, discussed and criticized in details, but to our knowledge, the attention has been focused on 9-fluorenylmethoxycarbonyl chloride (FMOC-Cl) in all cases [5,10–12].

We report here a new procedure for the introduction of Fmoc group as a fluorescent label with *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (Fmoc-OSu) (Fig. 1) [13]. This procedure, in conjunction with the mobile phase and the

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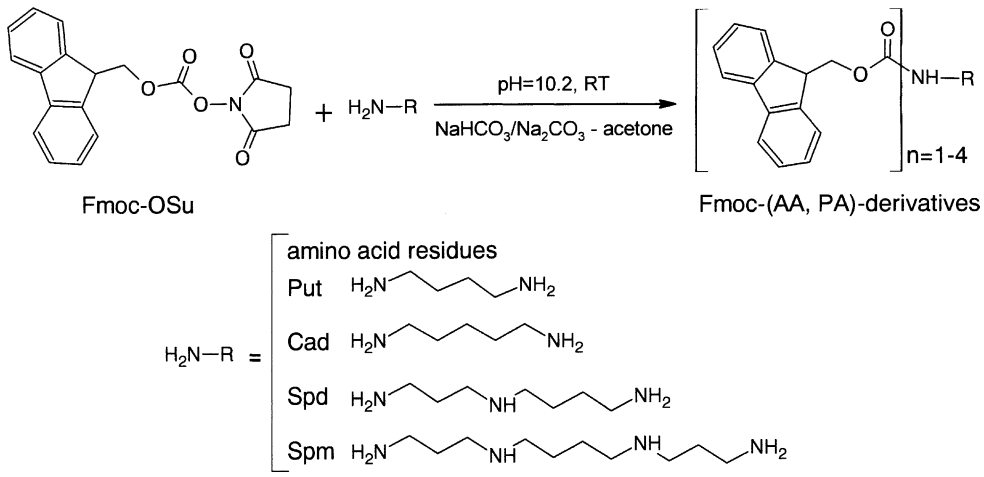

Fig. 1. Derivatization reaction and chemical structure of biogenic polyamines. RT: room temperature; AA: amino acid; PA: polyamine.

column system presented here, allows the simultaneous analysis of 20 amino acids and 4 polyamines. The analysis based on this pre-column derivatization with Fmoc-OSu is reproducible and linear. We applied the presented procedure for the simultaneous quantitative analysis of amino acids and polyamines in beer, wine, and cell culture.

2. Experimental

2.1. Materials

Standard analytical kit, containing 21 of L-amino acid + glycine and 2-aminoheptanoic acid were obtained from Fluka (Buchs, Switzerland), Putrescine, cadaverine, spermine, spermidine and 1,7-diaminoheptane were purchased from Sigma (Germany), Fmoc-OSu and FMOC-Cl were from Bachem (Bubendorf, Switzerland) and used after two times recrystalization. The acetonitrile of super gradient grade was obtained from Lab-Scan (Dublin, Ireland). Dibutylamine was purchased from Merck (Germany). The water used for buffer preparations was purified on a Purelab UHQ II system (ELGA, Vivendi Water Systems, Netherlands). All other reagents were of the highest purity available.

Beer and wine samples were purchased from food store. The popular Bulgarian market beer Kamenitza light, Zagorka lager, Ledenika light, Pirinsko pivo light and Kamenitza hard were termed, in order of listing, as beers 1–5. A Bulgarian white wine "Shardonnay–Khan Krum" and two types of "Kabernet Sauvignon"—a red Bulgarian wine were termed, as white, red 1 and red 2, respectively.

2.2. Standards

The stock standard solutions in 1.0 mM concentration of each amino acid and polyamine were prepared in 0.1 M hydrochloric acid and kept at 4 °C. All calibrations and working solutions were prepared from stock standards by diluting with 0.1 M hydrochloric acid.

2.3. Sample preparation

2.3.1. Beer and wine

The beer and wine samples were analysed after filtration through 0.22 μm membrane (Alltech, Lokeren, Belgium) without any other pretreatment.

2.3.2. Cell cultures

Cultured human keratinocytes and tumor cell lines (A431, Nb2 and MCF-7) were analysed. Cells were washed twice with ice-cold phosphate buffer saline and lysed with 1.0 ml of 0.2 M perchloric acid, containing 15 nM of internal standard (IS), in ultrasonic bath for 30 min. Then they were centrifuged at 12 000 × *g* for 15 min at 4 °C (Labofuge 400R, Hereus, Germany). The supernatants were used for analysis, after filtration through 0.22 μm membrane. The precipitates were solubilized in 0.1 M NaOH and used for determination of the protein contents.

2.4. Derivatization buffer

An amount of 5.3 g (0.5 M) of sodium carbonate and 1.46 g (5 mM) of EDTA were added to 100 ml of a 0.5 M solution of sodium hydrogencarbonate. The buffer solution pH was adjusted to 10.20 ± 0.05 with 5 M NaOH on a Model CG 842 pH meter (Schott, Mainz, Germany), which was equipped with BluLine pH 14 pH electrode (Schott). The buffer was filtered through 0.22 μm membrane, degassed in ultrasonic bath under vacuum and stored at 4 °C. During the monthly utilization no precipitation and pH changes were observed.

2.5. Derivatization

A 100 μl sample and 15 μl, containing 15 nM, of the internal standard (1.0 mM 2-aminoheptanoic acid in 0.1 M hydrochloric acid) were added to 200 μl derivatization buffer in 1.5 ml well-capped reaction vessels and mixed for 20 s. Then 300 μl of 3 mM Fmoc-OSu solution in acetone were added.

The resulted reaction mixture was incubated at room temperature for 20 min on a test-tube rotator (Snijders, Tilburg, The Netherlands). The derivatization was stopped by addition of 30 μl of conc. hydrochloric acid. The derivatized samples were kept at 4 °C. 50 μl of each acidified sample were used for analysis.

2.6. HPLC equipment and chromatographic conditions

2.6.1. Equipment

The analyses were carried out with Model 1050 Hewlett-Packard (Germany) chromatography system equipped with quaternary pump Model 1050 and 50 μl Rheodyne injector loop (Rheodyne, Germany). Detection of Fmoc derivatives of amino acid and polyamines was performed on a Model 1046A fluorescent detector (Hewlett-Packard) operating at excitation and emission wavelengths of 262 nm and 630 nm, respectively. Data acquisition and processing were carried out with CSW 1.7 chromatography software (DataApex, Czech Republic). A sequence of three columns: Nucleodur C_{18} bonded phase, end capped, 50 mm × 4.6 mm i.d., 5 μm particle size, 100 Å pore size (Macherey–Nagel, Germany) was used for simultaneous separation of amino acid and polyamines.

2.6.2. Chromatographic conditions

The separation of Fmoc-derivatized amino acids and polyamines was achieved by binary gradient with a flow-rate of 1.5 ml min^{-1}. Eluent A was 5 mM dibutylamine containing 5% (v/v) acetonitrile, adjusted to pH 2.20 with orthophosphoric acid. Eluent B was mixture of 95/5% (v/v) of acetonitrile and eluent A. The resulting pH was corrected to pH 2.20 with orthophosphoric acid. Both eluents were filtered through 0.45 μm nylon membrane (Hewlett-Packard) and degassed in ultrasonic bath under vacuum. The utilised mobile phase elution gradient is shown in Table 1. All changes in the composition of the mobile phase were linear. The analyses were carried out at room temperature.

Table 1
Chromatographic gradient conditions for simultaneous analysis of amino acids and polyamines

Time (min)	Eluent A (%)	Eluent B (%)
0	72	28
2	72	28
5	62	38
7	62	38
13	57	43
15	49	51
18	49	51
21	37	63
25	20	80
26	0	100
31	0	100
32	72	28

3. Results and discussion

3.1. Derivatization

Initially, derivatization of the standard amino acid and polyamines mixture was performed by FMOC-Cl in commonly used boric buffer (0.2 M sodium borate, pH 10.4). As derivatization of Spm and Spd requires the highest excess of reagent [12], the first experiments were carried out with 5 mM solution of FMOC-Cl in acetone and incubated for up to 30 min in attempt to achieve full hydrolysis of FMOC-Cl. Because of the different nature of the analysed components the extraction step could not be performed.

Unfortunately, the peak of excess FMOC-Cl was always present on chromatograms, interfering with the resolution of Orn and Lys. In addition, many unknown peaks appeared in the area of polyamines on chromatograms. We found that His, Tyr, Orn, Lys and polyamines on chromatograms were represented by more than one peak. Contrary to the expected, the peaks of Spm and Spd were less intensive than those of Put and Cad. In disagreement with previous reports [14,15], low polyamines peaks were observed when derivatization was performed using cancer cell extracts in 0.2 M perchloric acid. This was attributed to the low buffer capacity, failing to maintain pH above 8, required for the successful derivatization.

To overcome the problems described above, two main changes were performed. Briefly, we used stronger capacity buffer, replacing boric buffer with carbonate buffer. As a result, a much higher yield of polyamines, and a decreased FMOC-Cl peak were achieved (Fig. 2A), unaffecting the separation. Further, we performed derivatization using Fmoc-OSu instead of FMOC-Cl. The advantages of Fmoc-OSu are that it reacts rapidly with the primary and secondary amines, without reacting with hydroxyl groups. A small amount of or no oligopeptides are formed when amino acid derivatives are prepared with this reagent [13]. In our chromatographic conditions, the excess Fmoc-OSu peak appears in area, where it does not interfere with the resolution of amino acid and polyamine peaks on chromatograms (Fig. 2B).

To optimise the conditions for simultaneous derivatization of amino acid and polyamine by Fmoc-OSu a series of experiments were carried out using: (i) different buffer compositions (sodium and/or potassium salts of hydroxycarbonate and carbonate, with or without EDTA); (ii) buffer pH (ranging from 8.6 to 10.4 in steps of 0.2); (iii) Fmoc-OSu concentrations (1.0, 2.0, 3.0, 5.0 and 10.0 mM) and (iv) reaction time (5, 10, 20, 30 and 60 min). The apparently optimal conditions are described in the Section 2. The derivatization reaction was stopped by acidification with conc. HCl acid to pH of around 2.0, where Fmoc derivatives are stable [7,11,16].

The stability of acidified Fmoc-derivatized samples was studied by daily re-analysis of individual standard and cell culture samples. They were analysed immediately

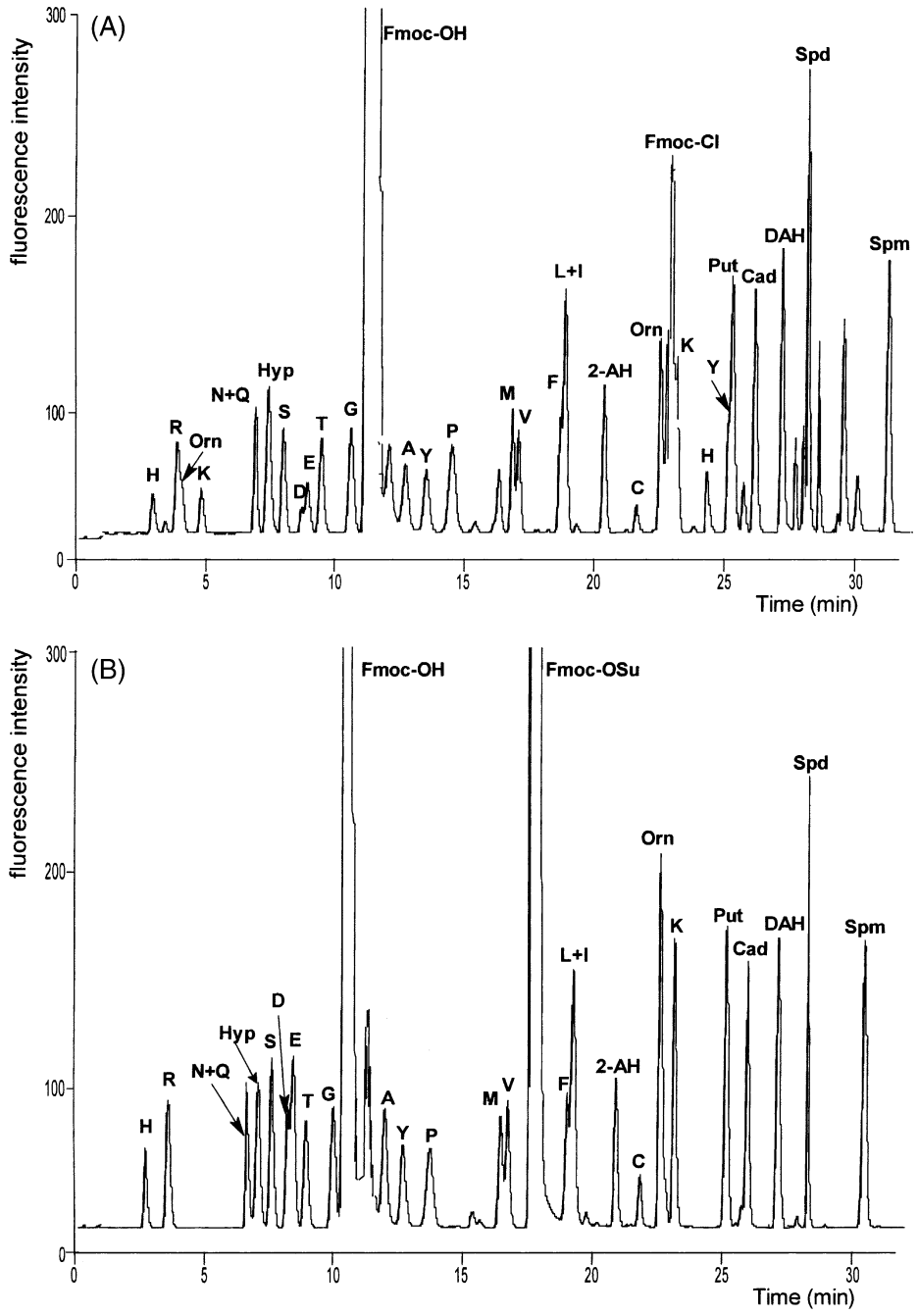

Fig. 2. HPLC separation of 5 μM standard solution of amino acids and polyamines, after derivatization with (A) FMOC-Cl, (B) Fmoc-OSu. The derivatization and chromatographic conditions are given in the text. Amino acid peaks are labeled with one letter abbreviations—Hyp: hydroxyproline; Orn: ornithine, 2-AH: 2-aminoheptanoic acid; DAH: 1,7-diaminoheptane.

after derivatization and aliquots of them were stored at 4 °C and room temperature. Samples stored at 4 °C displayed decrease in the fluorescent yield of less than 3% for amino acids and around 5% for polyamines after a week. We ascribed this to either absorption to the tube walls or micro-precipitation, as no additional peaks were observed on chromatograms. Samples stored at room temperature showed negligible decrease (less than 3%) in peak areas when stored up to 48 h. Storage for more than 48 h results in the presence of additional peaks and significant decrease in His, Arg, Lys and polyamine peak areas on chromatograms (Table 2).

The repeatability of derivatization procedure was established by analysis of 10 different derivatizations of the same sample. Two standards with amino acids and polyamines in concentration of 10 and 1 μM, respectively, and one cell culture sample were tested. Table 3 shows the relative standard deviations of peak areas. Relatively high

Table 2
Standard deviations of peak area (%) of sample daily re-analyses

amino acid/polyamine	Standard deviations of peak area (%)											
	1 Day[a]		2 Days		3 Days		4 Days		5 Days		8 Days	
	4 °C	*RT*	4 °C	*RT*	4 °C	*RT*	4 °C	*RT*	4 °C	*RT*	4 °C	*RT*
His	1.37[b]	1.71	1.87	3.07	2.24	8.15	2.36		2.87		6.02	
	1.46[c]	2.31	2.96	2.62	2.65	15.67	2.87		3.13		9.48	
Arg	2.30	0.99	1.90	2.60	2.67	7.42	2.75		3.05		5.43	
	1.51	1.24	1.59	2.95	1.93	9.10	2.54		3.20		5.66	
Asn + Gln	1.87	1.37	2.60	3.00	2.56	6.76	2.63		2.78		9.24	
	2.62	1.80	2.95	2.87	2.84	8.36	2.80		3.11		10.64	
Hyp	0.76	1.14	1.25	1.51	1.65	2.75	1.71		1.86		2.52	
	1.25	1.10	1.59	1.83	1.79	4.01	2.32		2.34		2.70	
Ser	1.21	0.88	2.01	1.46	2.32	2.42	2.48		2.53		2.73	
	1.37	1.57	2.27	1.96	2.49	3.53	2.61		2.61		2.90	
Asp	1.82	1.62	2.12	2.87	2.58	5.89	2.64		2.98		3.51	
	2.38	2.58	2.23	2.52	2.64	8.50	2.83		3.15		5.03	
Glu	2.68	2.61	2.54	2.98	2.75	10.18	2.71		2.85		3.92	
	2.15	2.40	2.36	2.12	2.86	9.48	2.92		3.49		5.48	
Thr	1.21	1.63	1.36	1.86	1.42	1.95	1.40		1.52		2.18	
	1.65	1.61	1.98	1.75	1.45	1.86	2.06		1.75		2.86	
Gly	2.32	1.95	2.35	2.21	2.49	2.25	2.39		2.68		2.56	
	2.12	1.75	2.65	2.32	2.58	2.46	2.76		2.65		3.68	
Ala	1.58	1.69	1.62	1.72	1.41	1.75	1.65		1.75		2.32	
	1.72	1.68	1.63	1.64	1.86	1.84	1.93		2.02		2.23	
Pro	1.78	1.96	1.76	2.32	1.78	2.80	1.82		1.86		2.65	
	1.93	2.25	1.98	2.68	2.18	3.05	2.25		2.21		2.85	
Tyr	2.12	1.36	2.21	2.73	2.52	2.98	2.50		2.68		2.87	
	2.65	1.64	2.54	2.18	2.60	2.92	2.78		2.82		3.12	
Met	1.68	1.63	2.65	2.80	2.70	5.56	2.85		3.15		4.85	
	1.86	1.86	2.46	3.11	2.85	6.23	2.90		3.21		4.25	
Val	1.56	1.65	2.36	1.89	2.31	2.15	2.30		2.58		2.63	
	1.98	1.44	2.68	1.78	2.46	1.96	2.67		2.78		2.78	
Phe	2.36	1.83	2.38	1.80	2.42	2.23	2.56		2.68		2.66	
	2.59	2.14	2.65	2.53	2.70	2.69	2.86		3.11		3.02	
Leu + Ile	1.46	1.35	2.26	1.40	2.31	1.56	2.30		2.58		2.63	
	1.98	1.65	2.08	2.45	2.46	2.26	2.77		2.78		2.78	
2-AH	1.26	1.55	1.31	1.60	1.31	1.66	1.59		1.58		1.63	
	1.48	1.75	1.58	1.79	1.56	2.06	1.77		1.78		1.78	
Orn	2.56	2.80	2.46	3.14	2.81	3.89	2.80		2.98		3.63	
	2.98	2.74	3.08	3.54	2.96	4.05	3.07		3.78		3.78	
Lys	2.46	2.65	2.46	3.63	3.61	4.84	3.80		3.98		4.13	
	2.18	2.87	2.38	3.90	3.36	4.54	3.57		3.78		4.32	
Put	2.35	2.43	2.46	2.65	2.81	3.57	3.80		3.98		4.63	
	1.98	2.78	2.08	2.80	2.96	4.12	3.07		3.78		3.82	
Cad	2.65	2.54	2.95	2.65	3.54	2.70	3.52		4.65		6.84	
	–	–	–	–	–	–	–		–		–	
Spd	2.56	3.01	3.46	3.10	4.81	3.58	4.80		4.88		7.63	
	2.98	3.23	3.08	3.20	3.96	3.76	4.57		4.78		8.78	
Spm	3.56	3.32	3.46	3.43	4.81	4.13	4.64		5.12		9.13	
	2.98	3.56	4.28	4.43	4.65	5.65	5.07		5.78		14.78	

[a] The elapsed time between the daily sample runs was 19–23 h.
[b] Standard solution with 10 μM concentration of each amino acid and polyamine.
[c] Cell culture extract.

variations (R.S.D. between 3.3 and 4.5%) were obtained for not well-separated components. High variation for Spm (R.S.D. = 6.7%) at low concentration was also observed. The relative standard deviation of the peak areas demonstrates good reproducibility of the proposed derivatization procedure.

3.2. Chromatographic analysis

The simultaneous analysis of 20 amino acids and 4 biogenic polyamines in samples of different origin was achieved in only 32 min gradient elution by utilization of dibutylamine phosphate as ion-pairing reagent. A modifier for the mobile

Table 3
Relative standard deviation (%) of peak area for Fmoc amino acid and polyamines derivatives

Amino acid/polyamine	Standard and sample		
	10 μM ($n = 10$)	1 μM ($n = 10$)	Cell extract ($n = 10$)
Histidine	1.2	1.5	1.5
Arginine	1.1	1.9	2.3
Asparagine + glutamine	2.0	2.6	2.3
Hydroxyproline	1.1	1.4	1.5
Serine	1.5	1.7	1.6
Aspartic acid	3.4	3.8	3.9
Glutamic acid	3.6	3.5	3.3
Threonine	1.6	1.7	2.0
Glycine	2.2	2.3	2.1
Alanine	2.4	2.6	2.6
Proline	1.5	1.5	1.6
Tyrosine	1.7	1.6	2.3
Methionine	3.3	4.0	3.8
Valine	3.3	3.4	3.6
Phenylalanine	4.2	4.5	4.2
Leucine + isoleucine	3.9	4.2	4.0
2-Aminoheptanoic acid	1.2	1.4	1.4
Cysteine	3.5	3.9	–
Ornithine	1.3	1.3	2.0
Lysine	1.3	1.4	1.6
Putrescine	1.2	1.3	1.3
Cadaverine	1.1	1.3	–
Spermidine	1.6	2.0	2.1
1,7-Diaminoheptane	1.4	1.8	1.7
Spermine	3.1	6.7	3.0

phase was chosen taking in account the different nature of the analysed components. As Fmoc derivatives of polyamines have extremely hydrophobic properties and branched shape we were interested in finding a suitable reagent capable to reduce the retention time and tailing of respective peaks on chromatograms. In this respect, the dibutylamine seems to be the additive of choice [17,18]. Based on our experience with dibutylamine phosphate buffers in separation and purification of synthetic peptides (unpublished observations), we prepared elution buffers as described in the Section 2. pH of organic buffer was corrected to the initial value to overcome the formation of pH gradient during the analysis.

The simultaneous analysis of amino acids and polyamines was carried out on sequence of three Nucleodur C_{18}, 50 mm × 4.6 mm i.d., 5 μm particle size, 100 Å pore size columns. This system was found superior to the tested ones: Hypersil ODS 150 mm × 4.6 mm, 5 μm (Hewlett-Packard) and Adsorbosphere C_{18}, 150 mm × 4.6 mm, 3 μm (Alltech, USA) single columns in terms of amino acids and polyamines resolution and separation of Fmoc peaks from analysed components (data not shown). In addition, the utilization of three continuously attached columns simplified the cleaning-up procedures. Most of the sample contaminations were retained in the first column. The cleaning-up procedures were carried out usually after at least 100 injections. The loss of resolution between the peaks of glycine and Fmoc-OH was used as an indicator for necessary cleaning. Columns were disconnected and washed separately with the appropriate solvent composition. Generally, washing was performed by passing through the column 90% (v/v) aqueous acetonitrile, containing 0.1% (v/v) trifluoroacetic acid (TFA), in isocratic mode. The sequence positions of the columns were changed during reassembly. This mode of work enables significant extension of the column life without any significant loss of peak resolution.

The advantages of the herein described chromatographic conditions, compared to previously reported methods [10–12] are: (i) short analysis time (the length of a single run is only 35 min, including 3 min for column re-equilibration with at least nine blank volumes (∼0.45 ml) for each column); (ii) most of the analysed components are baseline separated; (iii) all components elute as narrow, well-defined and nicely-shaped peaks; (iv) significant reduction of organic solvent consumption; (v) amino acids separate well from polyamines on chromatograms, thus allowing an easy switch to different modes of analysis—amino acids or polyamines; and (vi) extended column life.

Unfortunately, no separation of two amino acid pairs was achieved under these conditions. All attempts to resolve the peaks of Asn/Gln and Leu/Ile pairs were unsuccessful, as the separation of these amino acids results in the loss of resolution for other analysed components.

Linear calibration curves were obtained with known amounts of amino acids and polyamines ranging from 0.2 to 50 μM (0.2, 0.5, 1, 2, 5, 10, 15, 25, and 50 μM, 7 replication of each level). The correlation coefficients (r) were found to be higher than 0.991 with exception for Spm and Met with $r = 0.984$ and $r = 0.979$, respectively. In the case of Spm, this may be a result of the relatively low repeatability of the derivatization reaction, as previously mentioned. We suppose that the relatively low r for Met is a result of oxidation during the derivatization and/or the storage of the samples.

The use of 1,6-diaminohexane, 1,7-diaminoheptane (DAH) and 2-aminoheptanoic acid (2-AH) as internal standards was tested. As 1,6-diaminohexane has similar retention time with some of the unknown peaks, present in the samples, it was abandoned. Both 1,7-diaminoheptane and 2-aminoheptanoic acid were found suitable for use as internal standards.

2-AH was preferred as an internal standard due to: (i) its higher linear regression correlation coefficients for most of the amino acids and polyamines; (ii) its linear calibration curve ranging between 0.2 and 50 nmol ml^{-1} has higher correlation coefficient ($r = 0.995$) compared to DAH; and (iii) 2-AH standard solution is easily prepared and more stable than DAH for prolonged storage.

It is worth mentioning that in the case of cubic regression, DAH has excellent correlation coefficients, regarding polyamines ($r = 0.998$ Put, $r = 0.997$ Cad, $r = 0.998$ Spd, $r = 0.995$ Spm) when used as internal standard in concentrations ranging between 1 and 20 μM (1, 2, 5, 10, and 20 μM, 5 replication of each level).

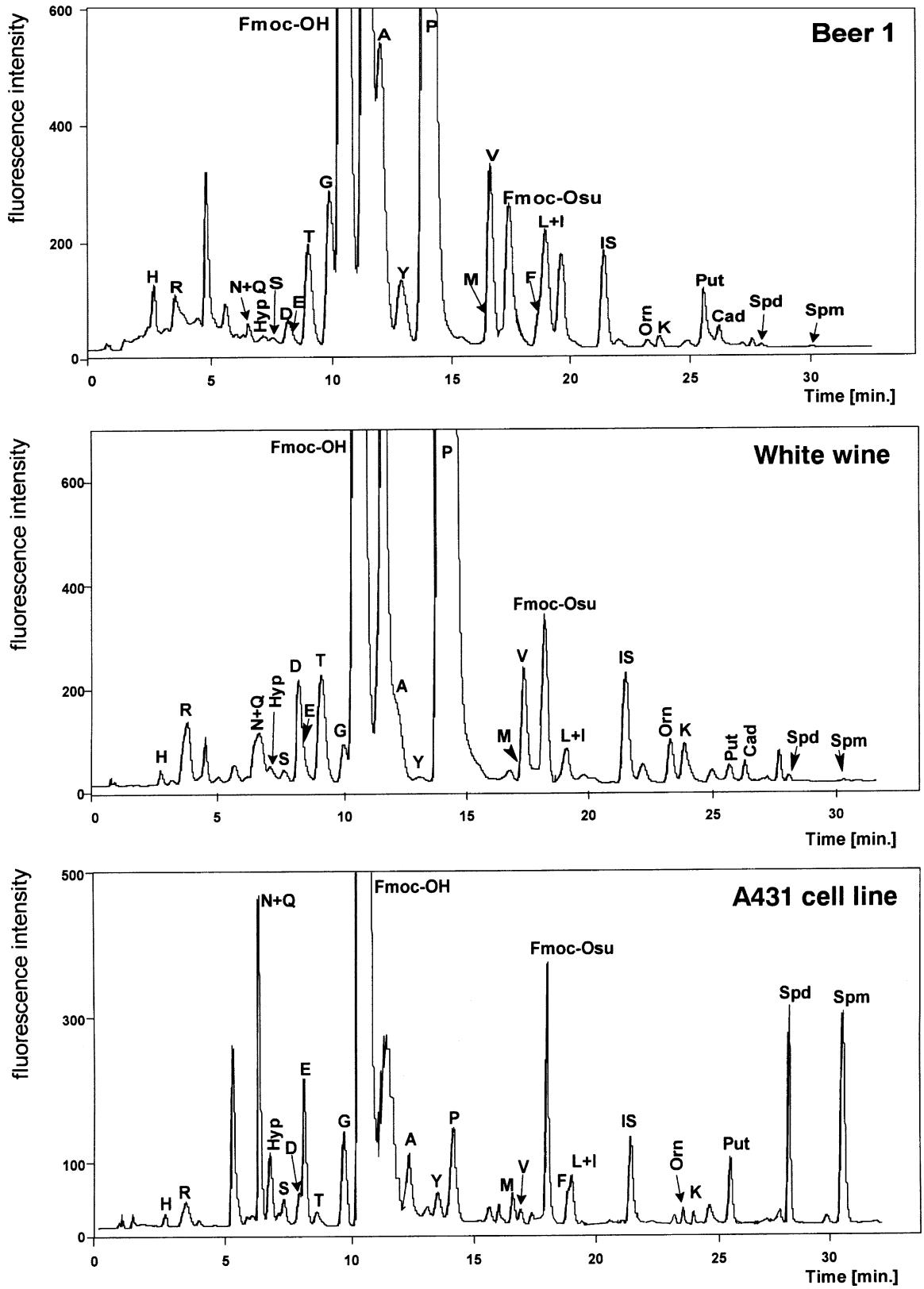

Fig. 3. Elution profiles of amino acids and polyamines derivatives obtained from beer, white wine and tumor cell line A431. Amino acid peaks are labeled with one letter—IS: internal standard (2-aminoheptanoic acid); Hyp: hydroxyproline; Orn: ornithine. For conditions see Section 2.

Table 4
Concentration of amino acids and polyamines in beers and wines (mg/l)

	Beers					Wines		
	1	2	3	4	5	Red 1	Red 2	White
His	7.89	5.98	2.41	6.80	9.92	2.35	2.25	2.18
Arg	9.11	5.62	2.23	6.88	10.95	3.22	4.59	10.19
Asn + Gln	3.49	3.94	1.20	3.15	1.98	1.35	0.97	7.78
Hyp	0.75	0.68	0.36	0.85	0.56	–	–	1.04
Ser	0.91	1.50	0.44	0.74	0.70	2.46	0.56	1.92
Asp	4.13	7.53	2.62	5.32	4.34	6.08	5.58	25.36
Glu	1.21	3.67	–	2.23	1.24	1.15	0.65	3.21
Thr	33.01	31.64	22.35	31.16	36.56	57.66	37.90	33.90
Gly	7.37	7.00	3.16	7.72	9.12	4.72	4.71	2.22
Ala	23.68	28.15	11.36	13.40	20.04	5.24	5.28	2.35
Pro	141.60	149.50	131.16	90.69	80.05	147.90	149.88	76.82
Tyr	4.06	2.24	2.63	3.63	8.27	1.26	1.56	0.79
Met	0.35	0.63	–	–	0.23	–	–	0.18
Val	9.12	8.55	10.01	6.36	14.74	1.19	1.28	4.56
Phe	0.61	0.34	0.53	0.75	0.85	1.05	1.62	–
Leu + Ile	5.90	5.34	3.21	4.82	3.78	5.06	5.62	0.30
Orn	0.79	0.63	0.25	0.54	0.43	0.69	0.12	2.14
Lys	1.28	0.84	0.18	0.41	0.46	0.77	0.29	1.90
Put	3.02	2.83	1.01	2.57	1.50	1.15	0.29	0.32
Cad	0.19	0.74	0.39	6.23	0.40	0.03	0.01	0.01
Spd	0.35	0.05	0.02	0.14	0.05	–	–	0.03
Spm	0.06	0.02	0.03	0.08	0.02	–	–	0.03
Total amino acids	255.26	256.73	195.10	192.45	204.22	241.91	222.86	174.62
Total polyamines	3.62	3.64	1.45	9.02	1.97	1.18	0.30	0.39

Reported values were averaged from three independent analyses of samples. 15 nM of 2-aminoheptanoic acid was used as internal standard.

The applicability of the reported procedure for simultaneously analysis of amino acids and polyamines has been demonstrated for the analysis of beer and wine samples (Fig. 3, Table 4). The reported method is routinely used in our laboratory for analysis of amino acids and polyamines contents of cell culture extracts.

4. Conclusion

A new method for simultaneous analysis of amino acids and biogenic polyamines after pre-column derivatization with *N*-(9-fluorenylmethoxycarbonyloxy)succinimide is proposed. The utilization of Fmoc-OSu eliminates some of the typical disadvantages of FMOC-Cl like: (i) reactivity towards imidazoles, phenolic hydroxyls and some alcohols; (ii) formation of unknown dipeptides during derivatization; (iii) presence of mono- and disubstituted derivatives of His and Tyr on chromatograms; and (iv) necessity of extraction steps. The described combination of columns and mobile phase significantly reduces the individual analysis costs, shortening the time of analysis, lowering the organic solvent consumption and extending the column life.

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