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Behavior and characteristics of the *o*-phthaldialdehyde derivatives of *n*-C_6–C_8 amines and phenylethylamines with four additive SH-containing reagents

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Abstract

The stability and characteristics of the C_6–C_8 *n*-aliphatic and phenylethylamines have been investigated as their *o*-phthaldialdehyde (OPA)/3-mercaptopropionic acid, OPA/*N*-acetyl-L-cysteine, OPA/2-mercaptoethanol and OPA/ethanethiol derivatives. Stoichiometric studies have been followed by photodiode array and fluorescence detection, simultaneously, while the composition of derivatives was confirmed by on line HPLC–electrospray ionization (ESI)-MS measurements. All four amines having in their original structure the NH_2–CH_2– moiety in accordance with the C_1–C_4 aliphatic, mono and diamines and amino acids of the same structure—furnished more than one OPA derivative: their initially formed isoindoles transform to further ones. Depending on the composition of the OPA reagents and on the pH of derivatizations different type of transformed species have been identified, in various proportions. Applying the OPA/SH additive reagent in the molar ratio of 1/3, favors the formation of one additional OPA molecule-containing isoindole, while using the OPA/SH additive (1/50) reagent resulted in the formation of one additional SH additive-containing species, identified and measured at the first time by HPLC. Transformation rate and stability of derivatives proved to be associated with the composition of the OPA reagent, with the type of the SH additive, with the pH of derivatizations, and, in selected cases also with the chain length of the amine. Results of stoichiometric and mechanism studies have been utilized to define optimum analytical conditions.

Keywords: Amines; Phenylethylamine; Phthaldialdehyde

1. Introduction

In the recent years several characteristics of the reaction between the primary NH_2 group-containing compounds and the OPA reagents have been clarified in our laboratory [1–10]: including several amines [7], such as C_1–C_4 aliphatic monoamines, diamines (ethylenediamine, 1,2-propylenediamine, 1,3-propylenediamine), ethanolamine and biogenic amines (agmatine, putrescine, cadaverine, histamine, tyramine, spermidine, spermine, etc.).

This paper, as a completion of our earlier studies on amines [7], describes the stability and transformation properties of the C_6–C_8 *n*-aliphatic amines (henceforth their collective designation: C_6–C_8 amines (abbreviated one by one: HexA, HepA, OctA) and phenylethylamine (abbreviated: PheEtA) as their *o*-phthaldialdehyde (OPA) derivatives: applying four different SH additive-containing reagents [3-mercaptopropionic acid (MPA), *N*-acetyl-*L*-cysteine (NAC), 2-mercaptoethanol (MCE), ethanethiol (ET), henceforth their collective designation: SH additives].

2. Experimental

2.1. Materials

OPA, MPA, NAC, MCE, ET and amines, such as C_6–C_8 *n*-aliphatic and phenylethylamines were obtained from Sigma (St. Louis, MO, USA) and from Serva (Heidelberg, Germany). HPLC-grade methanol and acetonitrile were purchased from Romil (Leics., UK). All other reagents were of the highest purity available.

2.2. Standard solutions

Standard solutions of free amines have been prepared with distilled water in the concentrations of $\sim$1–2 $\times$ 10^{-2} M and

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further diluted before use. Stock solution of OPA contained 0.25 g OPA (weighed with analytical precision) in 50 ml methanol (further on: methanolic OPA solution).

2.3. Buffer solution

Borate buffer was mixed in 50:50 (v/v) from 0.2 M boric acid (dissolved in 0.2 M potassium chloride) and 0.2 M sodium hydroxide (pH 9.9 ± 0.05).

2.4. Reagent solutions

OPA/SH additive (1/3) reagents were obtained by mixing, in order of listing, 5.0 ml methanolic OPA, 20.0 ml borate buffer and various amounts of MPA (49 μl) or MCE (40 μl) or ET (40 μl) solutions or with 0.091 g NAC dissolved previously in the borate buffer: finally, if necessary it was adjusted by 1 M sodium hydroxide, to various pH values (8.80, 9.30, 9.75, 10.25 ± 0.05); final volume 25.00 ml.

OPA/SH additive (1/50) reagents were obtained by mixing, in order of listing, 2.0 ml methanolic OPA, 4.0 ml methanol, 2 ml borate buffer and the corresponding amounts of MPA (325 μl) or MCE (262 μl) or ET (276 μl) solutions or 0.608 g NAC dissolved previously in the borate buffer): finally, if necessary, it was adjusted by 1 M sodium hydroxide, to various pH values (8.80, 9.30, 9.75, 10.25 ± 0.05); final volume 10.00 ml.

The molar ratios of OPA to the SH additive were given in the corresponding sections. [OPA]/[SH additive] = 1/3, and/or [OPA]/[SH additive] = 1/50.

2.5. Derivatization

2.5.1. Characterization of the reagent solutions

Blank elutions were performed with freshly prepared reagent solutions (reagent's age ≥ 90 min [2]), saved in the refrigerator (∼4 °C) and injected by the robotic autosampler, every day at least two times (Waters 717, thermostatted to ∼4 °C).

2.5.2. Studies with the OPA/SH additive reagents

Derivatizations were performed with reagents prepared at least 90 min earlier before use, and saved no longer than 9 days [2]. In the case of the OPA/ET reagent it should be freshly prepared, every second day. The calculated amounts of reagent solutions were mixed with the selected amounts of amines and react for 7 min before injection (if not otherwise stated).

2.6. Chromatography

2.6.1. Stability and stoichiometric studies: simultaneous photodiode array (DAD) and fluorescence (FL)

The system was a Waters HPLC instrument (Waters Pharmaceutical Division, Milford, MA, USA), with Waters 996 PDA and Waters 274 FL detectors, a Waters 600 controller quaternary pump with a thermostattable column area and a Waters 717 autosampler, operating with the Millennium Software (version 2010, 1992–95, validated by ISO 9002). The columns were Hypersil ODS bonded phase (5 μm), 200 mm × 4 mm + 20 mm × 4 mm guard column (column 1), or 150 mm × 4 mm + 20 mm × 4 mm guard column (column 2).

Detections have been performed simultaneously: DAD and FL detection systems were connected in order of listing. Blank tests, concentration dependence have been recorded between 190 and 400 nm (DAD) and evaluated at 334 nm, as well as at the optimum fluorescence wavelengths (excitation/emission = 337/454 nm).

On-line HPLC–electrospray ionization ESI-MS studies: simultaneous UV and MS detection (carried out at Central Service for Plant Protection and Soil Conservation Chemical Department, Budapest, Hungary).

The apparatus was a Spectra System (Thermo-Separation Products, San Jose, CA, USA), consisted of UV 2000 (for two wavelengths) and Finnigan Aqua (ThermoQuest, Manchester, UK) MS detectors, P 2000 quaternary pump, As 2000 Autosampler, operating with the Xcalibur software, RevisionB 1997 and column 2 was used.

Detections have been performed simultaneously, applying the UV 2000 and MSD Finnigan Aqua detectors, connected in order of listing. Blank tests, concentration dependence have been recorded between 190 and 900 nm (UV), evaluated at 334 nm (OPA/MPA-amines), MS detection was performed with ESI in the positive mode (mass range: 50–1600 mass units; gas temperature: 200 °C (flow rate 200 μl/min) or 380 °C (flow rate 1 ml/min); $V_{capillary}$: 3.5 kV.

2.7. Elution programs

According to the requirement of the various SH additive-containing derivatives different elution programs have been followed, applying eluents A and B, acetonitrile and methanol.

Eluent A was 0.05 M sodium acetate of pH 7.2. Eluent B was prepared from 0.1 M sodium acetate acetonitrile methanol (46:44:10, v/v) titrated with acetic acid or sodium hydroxide to pH 7.2.

Stability and fragmentation studies in the case of OPA/MPA derivatives of C_6–C_8 amines, one by one, or together, have been performed in the isocratic mode (flow rate 1 ml/min) applying eluent A (pH 7.2)–methanol (30:70, v/v), using column 2. The behavior of phenylethylamine was determined in the gradient mode: A–B (35:65) eluent was applied for 3 min, changed for A–B (20:80) for an additional 4 min, thereafter for A–B (35:65) eluent within 1 min, hold for 8 min (total elution time 16 min).

Stability studies of OPA/NAC derivatives of C_6–C_8 amines were carried out in the gradient mode: started with A eluent–methanol and changed to A eluent–methanol within 3 min, hold for 7 min. Thereafter changed to A–B (20:80) within 1 min, hold for 7 min (total elution time 18 min).

Table 1
Stability/characteristics of OPA/MPA, OPA/NAC, OPA/MCE and OPA/ET derivatives of hexyl (HexA), heptyl (HepA), octyl (OctA) and phenylethyl (PheEtA) amines as a function of reaction time obtained with the OPA/SH additive (1/3), at pH 9.3, based on simultaneous fluorescence (FL) and UV detection

Amine	UV max (nm)	[OPA]/[MPA] (1/3)						[OPA]/[NAC] (1/3)					[OPA]/[MCE] (1/3)						[OPA]/[ET] (1/3)					
		Retention time (min)	Response (%)[a]					Retention time (min)	Response (%)[a]				Retention time (min)	Response (%)[a]					Retention time (min)	Response (%)[a]				
			90 s	7 min	1 h	3 h	6 h		90 s	7 min	3 h	6 h		90 s	7 min	30 min	60 min	3 h		90 s	7 min	30 m	60 m	3 h
HexA1	333.9	6.18	99.4	84.2	64.4	51.6	39.4	3.66	100	98.2	84.5	78.1	4.70	99.5	99.3	99.1	98.7	98.1	3.55	100	100	100	100	100
HexA2	338.7	5.70	0.6	15.8	35.6	48.4	60.6	3.51	–	1.8	15.5	21.9	5.90	0.5	0.7	0.9	1.3	1.9	5.14					
Iu/pM: FL			***6.18***	***6.67***	***6.99***	***7.84***	***6.10***		***4.68***	***4.73***	***5.21***	***4.80***		***4.84***	***4.58***	***3.23***	***2.24***	***0.28***		***5.47***	***5.53***	***5.47***	***5.26***	***5.12***
UV			***0.47***	***0.55***	***0.59***	***0.59***	***0.59***		***0.52***	***0.51***	***0.51***	***0.47***		***0.34***	***0.31***	***0.22***	***0.15***	***0.02***		***0.35***	***0.36***	***0.36***	***0.33***	***0.32***
HepA1	333.9	8.84	97.7	89.5	66.3	40.4	25.6	4.67	99.3	97.6	76.7	67.2	6.01	99.5	99.4	99.4	99.4	99.0	6.43	100	100	100	100	100
HepA2	338.7	7.93	2.3	10.5	33.7	59.6	74.4	4.30	0.7	2.4	23.3	32.8	7.79	0.5	0.6	0.6	0.6	1.0						
Iu/pM: FL			***4.96***	***4.72***	***5.29***	***4.55***	***3.20***		***3.30***	***3.32***	***3.89***	***3.60***		***5.46***	***5.49***	***5.19***	***4.80***	***3.59***		***6.08***	***6.03***	***6.05***	***5.94***	***5.27***
UV			***0.38***	***0.39***	***0.39***	***0.32***	***0.22***		***0.36***	***0.39***	***0.38***	***0.35***		***0.38***	***0.37***	***0.35***	***0.33***	***0.25***		***0.39***	***0.39***	***0.39***	***0.38***	***0.34***
OctA1	333.9	13.13	98.0	89.1	69.2	44.0	30.0	6.37	99.6	97.8	70.6	57.2	7.61	99.0	99.1	98.9	98.9	98.7	7.86	99.6	99.5	99.5	99.5	99.8
OctA2	338.7	11.50	2.0	10.9	30.8	56.0	70.0	5.82	0.4	2.2	29.4	42.8	9.98	1.0	0.9	1.1	1.1	1.3	10.16	0.4	0.5	0.5	0.5	0.2
Iu/pM: FL			***4.37***	***4.37***	***4.91***	***3.67***	***2.58***		***3.27***	***3.32***	***3.81***	***3.50***		***5.46***	***5.49***	***5.19***	***4.80***	***3.59***		***5.90***	***5.80***	***6.07***	***5.85***	***5.47***
UV			***0.35***	***0.36***	***0.36***	***0.36***	***0.18***		***0.36***	***0.36***	***0.38***	***0.35***		***0.38***	***0.37***	***0.35***	***0.33***	***0.25***		***0.39***	***0.40***	***0.40***	***0.38***	***0.37***
PheEtA1	333.9	7.85	96.5	92.3	59.6	25.5	11.7	5.03	98.0	96.3	70.0	55.0	3.41	99.0	99.2	98.8	98.6	98.3	3.31	100	99.9	99.9	99.9	99.6
PheEtA2	338.7	8.43	3.5	7.7	40.4	74.5	88.3	6.27	2.0	3.7	30.0	45.0	4.00	1.0	0.8	1.2	1.4	1.7	5.85	–	0.1	0.1	0.1	0.4
Iu/pM: FL			***5.69***	***5.93***	***6.51***	***5.39***	***2.40***		***3.73***	***3.78***	***3.94***	***3.78***		***5.19***	***4.33***	***3.59***	***2.58***	***0.43***		***6.03***	***6.07***	***5.98***	***5.95***	***5.66***
UV			***0.35***	***0.35***	***0.36***	***0.29***	***0.13***		***0.38***	***0.40***	***0.38***	***0.38***		***0.37***	***0.34***	***0.26***	***0.18***	***0.04***		***0.41***	***0.41***	***0.40***	***0.40***	***0.38***

Indications: [OPA]/[SH additive])/[A] (20/60/1) ($1 = 1 \times 10^{-9}$ M) correspond to molar concentrations; – = no data available; Iu/pM integrator units/picomole.
[a] Based on the total of derivatives, obtained with FL detection.

In the cases of the OPA/MCE and OPA/ET derivatives isocratic elutions were performed as follows: OPA/MCE derivatives, B eluent–acetonitrile (50:50, v/v), flow rate 1.0 ml/min (30 °C, column 1); OPA/ET derivatives: B eluent–acetonitrile (30:70, v/v), flow rate 1.2 ml/min (30 °C, column 2).

(*Note*: Data in tables, without exception, were calculated to 1 ml/min elution rate.)

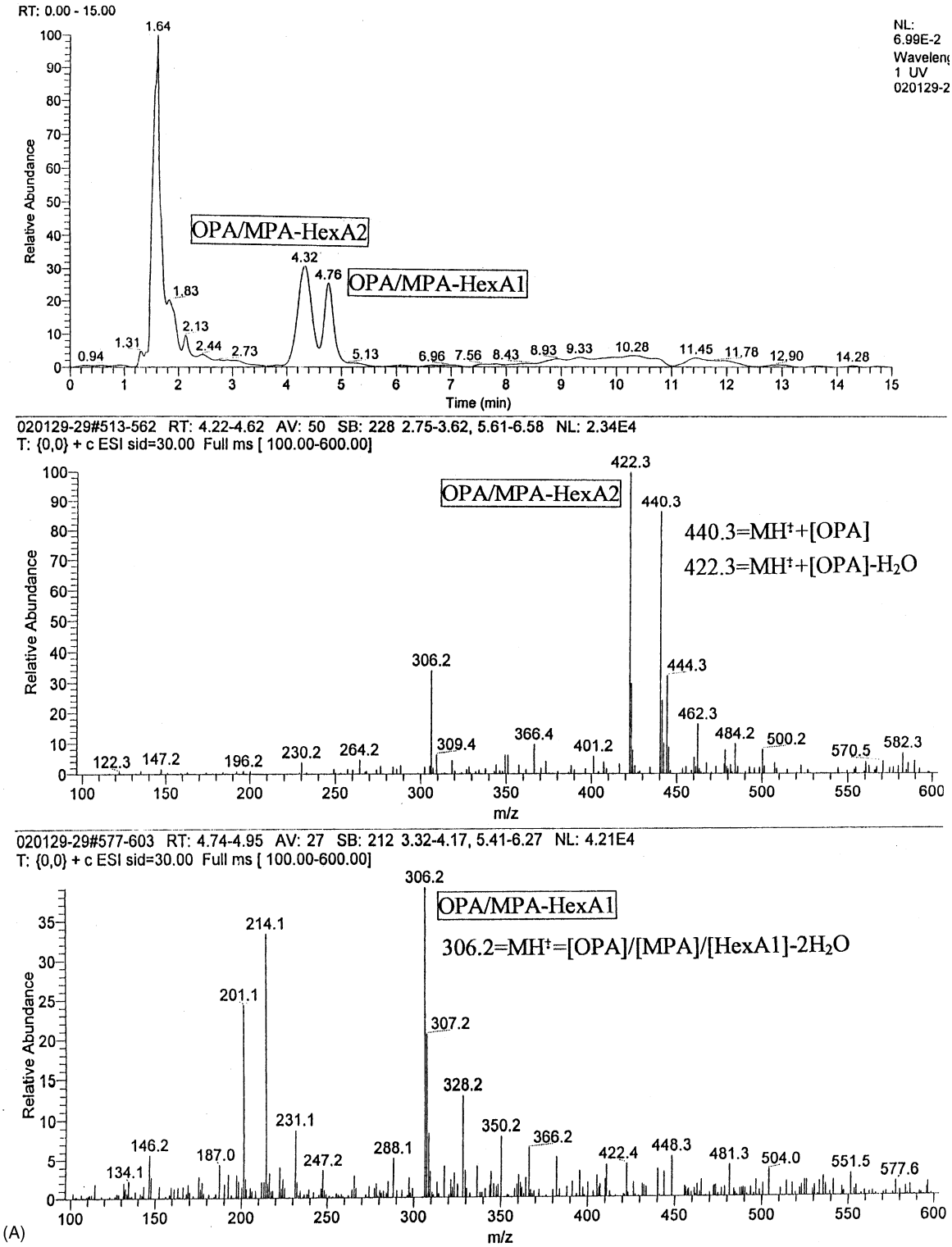

Fig. 1. (A–D) UV chromatograms (first lines) and MS spectra (second and third lines) of the initially obtained (indicated by number 1, i.e., hexylamine1: HexA1, heptylamine1 HepA1, octylamine1: OctA1, phenylethylamine1: PheEtA1) and their transformed OPA/MPA derivatives (indicated by number 2, i.e.,: hexylamine2: HexA2, heptylamine2: HepA2, octylamine2: OctA2, phenylethylamine2: PheEtA2) (detailed composition of fragments in the text).

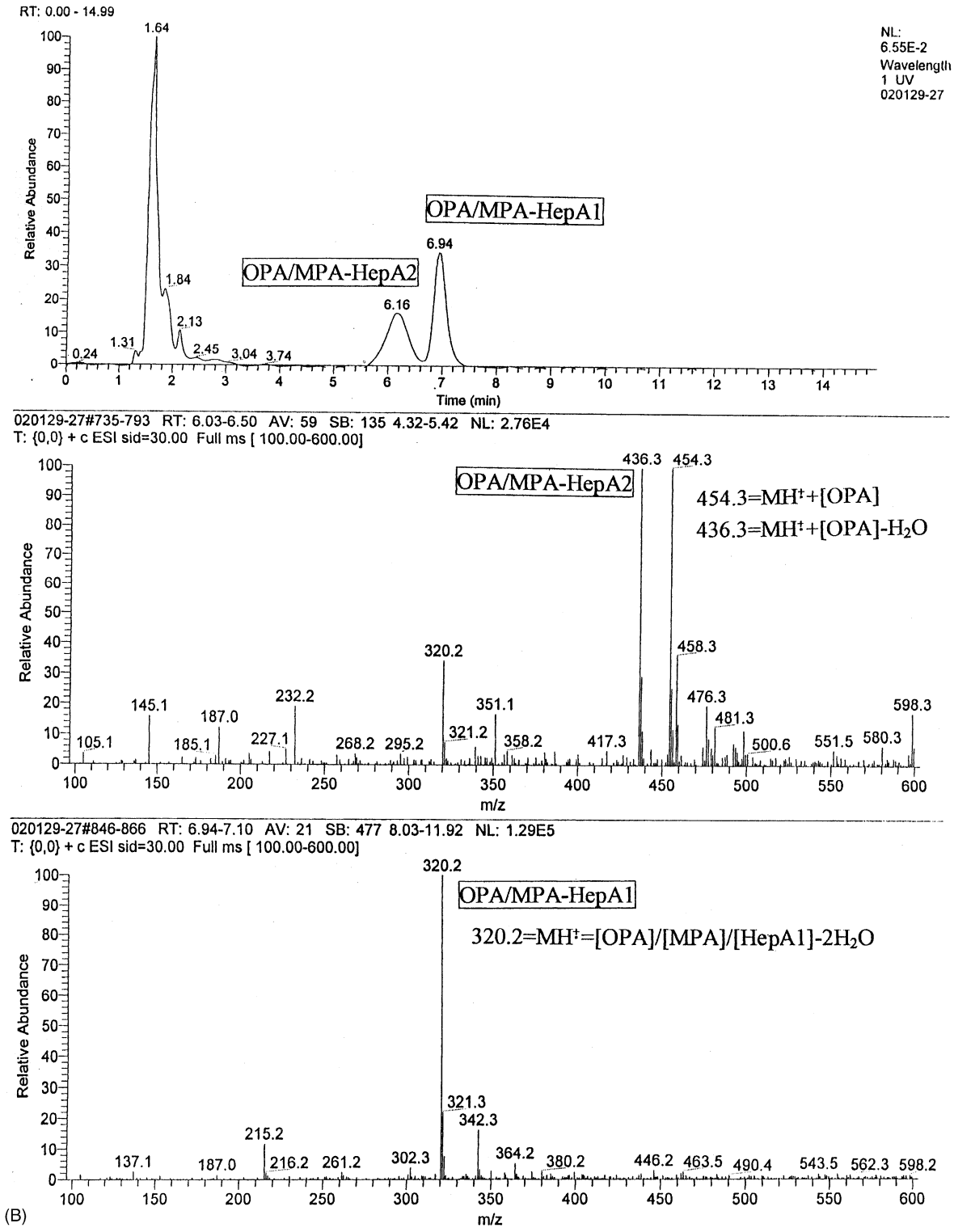

Fig. 1. (*Continued*)

3. Results and discussion

Introductory investigations, performed with the OPA/MPA and with the OPA/NAC reagents applying both of them in the 1/3 and in the 1/50 molar ratios, in solutions of pH 9.3, furnished two unexpected results:

(i) Derivatizations of the C_6–C_8 amines carried out with the OPA/MPA (NAC) (1/3) reagents resulted in the formation of the classical isoindoles and their expected,

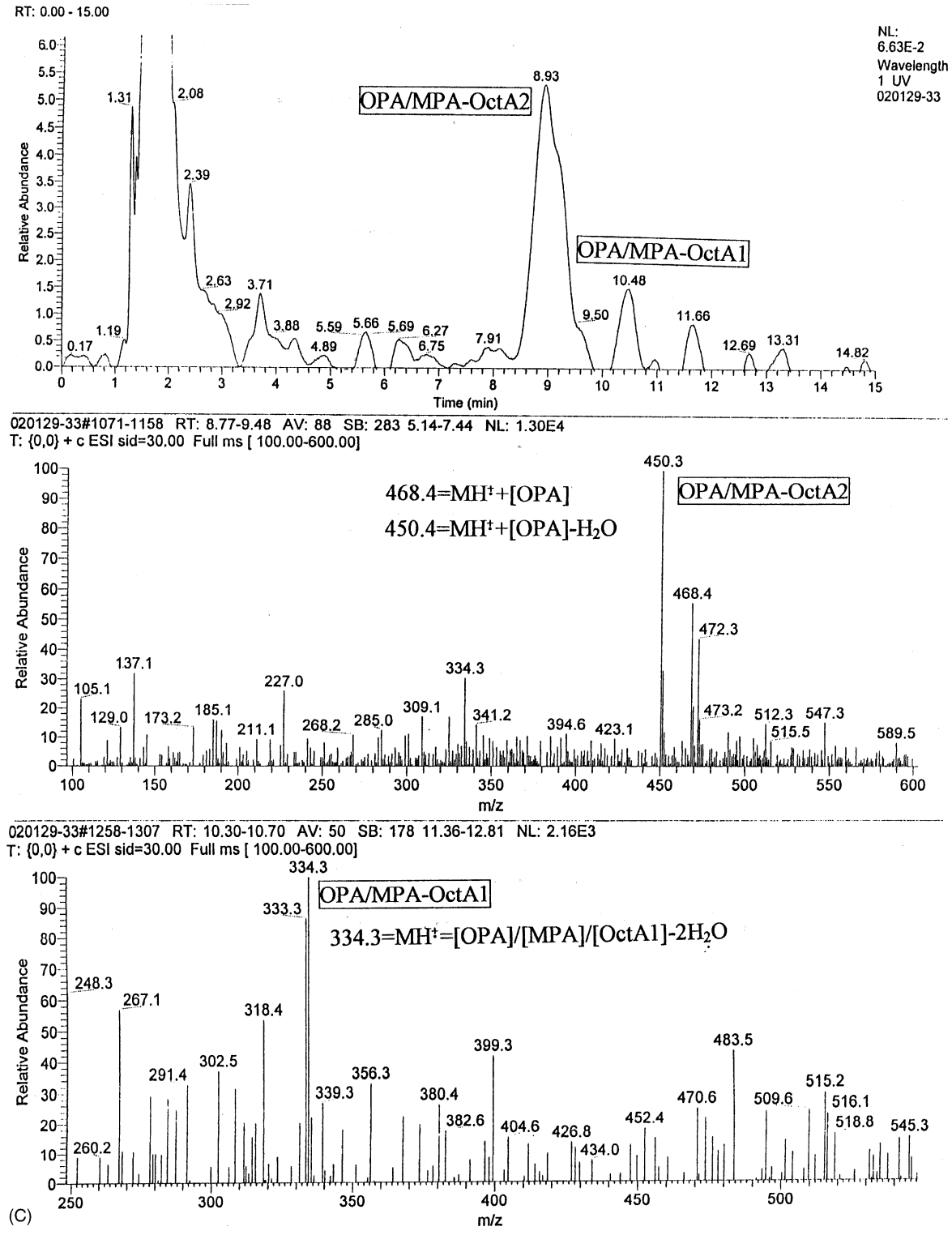

Fig. 1. (*Continued*)

transformed product, containing one additional OPA molecule [2–10]. However, these transformed species eluted before their classical isoindoles, in contrary to the transformed, corresponding species of amino acids [2–10] that eluted after their classical isoindoles [2,4,6,7,9].

(ii) Applying the OPA/MPA (NAC) (1/50) reagent (in order to eliminate/decrease the transformation of the initially formed isoindoles into the two OPA molecule-containing ones [7]) a new product was detected, with considerable smaller retention times, compared both to the initially formed classical isoindoles

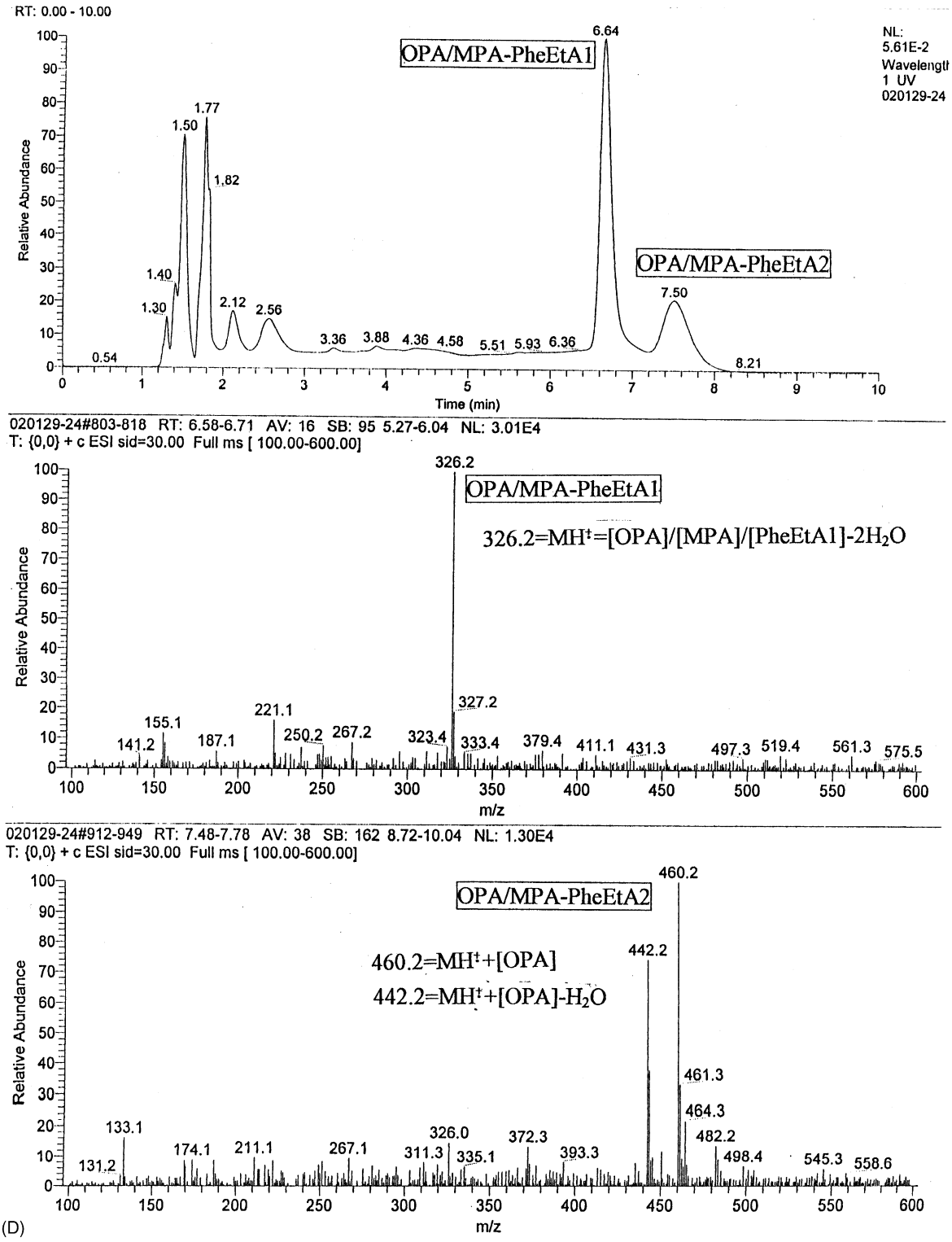

Fig. 1. (*Continued*).

and to the transformed derivatives, containing one additional OPA molecule.

Based on these introductory results it seemed to be inevitably necessary, in the frame of an exhaustive derivatization study, to compare the behavior of the OPA/MPA and OPA/NAC derivatives of C_6–C_8 and phenylethylamines also with the OPA/ET, and with the most commonly used [11,12], OPA/MCE derivatives.

3.1. Studies on the OPA/MPA, OPA/NAC, OPA/MCE and OPA/ET derivatives of the C_6–C_8 and phenylethylamines obtained with the OPA/SH additive (1/3) reagents

Results of stoichiometric studies, obtained as a function of the reaction time and reagent's composition, followed by DAD and FL detection, simultaneously were compiled in Table 1.

(i) Evaluating at first, the behavior of the OPA/MPA and OPA/NAC derivatives of the C_6–C_8 and phenylethylamines (Table 1: first two vertical columns, Fig. 1A–C, first line, UV detected chromatograms of the OPA/MPA derivatives), it is clear that in accord with the NH_2–CH_2− moiety-containing amino acids and amines [1–6], their initially formed derivatives (HexA1, HepA1, OctA1) became transformed (HexA2, HepA2, OctA2): their transformed species, elute with shorter retention times, probably due to their more polar properties (Figs. 1A–C, retention order: HexA2, HexA1, Hepta2, HeptA1, OctA2, OctA1). The transformed derivative of PheEtA, i.e., PheEtA2, in accord with earlier experiences [1–6], elutes after its initially formed isoindole (Fig. 1D, retention order: PheEtA1, PheEtA2).

The composition of the initially obtained and transformed species has been confirmed by their HPLC–ESI-MS spectra (Figs. 1A–D: spectra in second and third lines). As seen, without exception, the protonated (MH^+) molecular ions of the initially formed isoindoles (Figs. 1A-D, HexA, HepA, OctA, PheEtA, m/z values of spectra in the third lines, in order of listing: MH^+ = 306.2, 320.2, 334.3, 326.2) do transform into their two OPA molecule containing derivatives (MH^+ + OPA), including their corresponding dehydrated (MH^+ + OPA–H_2O) and/or cationized versions (MNa^+ + OPA) (Figs. 1A–D, spectra in the second lines, in order of listing, MH^+ + OPA = 440.3, 454.3, 468.4, 460.2) The mechanism of the transformation route of the classical isoindoles, resulting in the two OPA molecule containing species, was published recently [6].

(ii) As to the characteristics of the OPA/NAC derivatives of C_6–C_8 and phenylethylamines (Table 1: data in second vertical column) it has been repeatedly proven [2–4,6,7,9] that their responses are smaller and the stability of their initially formed isoindoles are more stable compared to their corresponding OPA/MPA ones.

(iii) In order to compare the characteristics of the OPA/MCE and OPA/ET derivatives of C_6–C_8 and phenylethylamines (Table 1, third and fourth vertical columns), they have been investigated, strictly, under the same conditions.

Results revealed that

(1) The OPA/MCE derivatives proved to be the less stable ones. Decomposition of the classical isoindoles, especially in the cases of HexA and PheEtA, takes place already after 7 min reaction time. Responses, expressed in integration units/picomole amine (Iu/pM), decrease in order of listing HexA, 90 s: 4.84, 7 min: 4.58; PheEtA, 90 s: 5.19, 7 min: 4.33.

(2) The stability of the OPA/ET derivatives is as excellent as of those of their OPA/MPA or OPA/NAC counterparts, with the accompanied, favored advantage of the almost side-reaction free derivatization. In the cases of the HexA and HepA transformation could not be detected, while transformation of OctA1 and PheEtA1 to OctA2 and PheEtA2 proved to be also quite negligible (less than 0.5% expressed in the total).

3.2. Studies on the OPA/MPA, OPA/NAC, OPA/MCE and OPA/ET derivatives of the C_6–C_8 and phenylethylamines obtained with the OPA/SH additive (1/50) reagents

3.2.1. Reactions with the OPA/MPA (1/50) reagents, as a function of the pH

Based on our earlier experiences, associated with the increased stability of the initially formed isoindoles of C_1–C_4 aliphatic amines [7], our first aim was to define the characteristics of the C_6–C_8 and phenylethylamines, strictly under the same conditions [OPA/MPA (1/50) reagent, pH 9.3; data in Table 2, second vertical column].

(i) As expected, the formation of the two OPA molecule containing species (derivatives designated by indices 2) could be decreased to negligible concentrations, but, simultaneously, a new species appeared, in considerable amounts: proportions of the new product (derivatives designated by indices 0) proved to be dependent on the pH of derivatizations.

(ii) Evaluating the impact of the pH of derivatizations (Table 2: proportions of transformed products at pH 8.8, 9.3, 9.75 and 10.25) it is clear that the higher the pH the smaller the extent of side reactions. After 7 min reaction, important from an analytical point of view, at pH 10.25, products of both side reactions could be unambiguously influenced: either quantitatively inhibited (HexA2, HepA2, OctA2) or considerably decreased (HexA: 1.1%, HepA0: 2.9%, OctA0: 0.8%).

From a theoretical point of view, it seemed to be of interest to examine the behavior of glycine and alanine too, upon derivatization with the OPA/MPA (1/50) reagents. Based on appearance of glycine0 and alanine0 the general tendency of formation of the dithio derivatives have been demonstrated: assuming the same behavior of all other, single (alanine) and more than one OPA derivative (glycine) providing amino acids. Thus,

Table 2
Stability/characteristics of OPA derivatives of hexyl (HexA), heptyl (HepA), octyl (OctA) and phenylethyl (PheEtA) glycine and alanine as a function of reaction time and the pH of reactions, obtained with the OPA/MPA (1/50) reagents, based on fluorescence (FL) and UV detections, applying isocratic elutions

Amine	UV max (nm)	Retention time (min)	pH 8.80				pH 9.30				pH 9.75				pH 10.25			
			Response (%)[a]				Response (%)[a]				Response (%)[a]				Response (%)[a]			
			7 min	1 h	3 h	6 h	7 min	1 h	3 h	6 h	7 min	1 h	3 h	6 h	7 min	1 h	3 h	6 h
HexA0	343.5	2.09	4.16	15.5	31.2	97.6	4.1	10.6	25.2	39.0	3.8	7.5	11.4	22.7	1.1	2.1	4.1	6.6
HexA1	333.9	4.56	95.8	83.5	66.9	2.0	95.9	88.6	73.4	59.7	96.1	91.5	87.5	76.2	98.9	97.9	95.9	93.4
HexA2	338.9	4.17	0.04	1.0	1.9	0.4	–	0.8	1.4	1.3	0.1	1.0	1.1	1.1	–	–	–	–
Iu/pM: FL			***2.05***	***4.38***	***4.46***	***4.95***	***3.98***	***5.03***	***5.19***	***5.36***	***5.02***	***5.00***	***4.92***	***4.86***	***4.77***	***4.89***	***4.86***	***4.79***
UV			***0.19***	***0.29***	***0.32***	***0.34***	***0.28***	***0.38***	***0.39***	***0.44***	***0.7***	***0.37***	***0.37***	***0.37***	***0.37***	***0.36***	***0.36***	***0.36***
HepA0	343.5	2.56	3.5	13.0	25.7	94.1	4.1	7.9	20.2	32.5	3.0	8.2	17.9	21.5	2.9	4.4	6.1	8.8
HepA1	333.9	6.71	96.4	85.6	72.0	5.1	95.8	91.1	78.1	65.9	96.8	90.3	80.4	76.9	97.1	94.8	92.7	90.2
HepA2	338.7	6.10	0.1	1.4	2.3	0.8	0.1	1.0	1.7	1.6	0.2	1.5	1.7	1.6	–	0.8	1.2	1.0
Iu/pM: FL			***2.63***	***4.61***	***4.79***	***4.92***	***4.48***	***5.20***	***5.16***	***5.15***	***5.40***	***5.08***	***4.72***	***4.62***	***5.29***	***5.32***	***5.16***	***4.26***
UV			***0.19***	***0.33***	***0.35***	***0.35***	***0.33***	***0.39***	***0.38***	***0.38***	***0.42***	***0.38***	***0.36***	***0.37***	***0.40***	***0.39***	***0.39***	***0.40***
OctA0	343.5	3.25	2.1	11.5	28.8	95.3	2.4	6.8	21.8	37.5	1.5	6.5	16.2	22.1	0.8	1.9	3.9	6.9
OctA1	333.9	9.95	97.8	87.0	68.9	4.1	97.5	92.2	76.3	60.8	98.4	92.0	82.0	76.2	99.2	97.5	95.2	92.1
OctA2	338.7	9.11	0.1	1.5	2.3	0.6	0.1	1.0	1.9	1.7	0.1	1.5	1.8	1.7	–	0.6	0.9	1.0
Iu/pM: FL			***2.54***	***4.51***	***4.67***	***5.65***	***4.27***	***4.96***	***4.58***	***4.33***	***5.16***	***4.28***	***4.14***	***3.93***	***5.31***	***5.09***	***4.88***	***3.98***
UV			***0.19***	***0.35***	***0.35***	***0.39***	***0.32***	***0.37***	***0.34***	***0.32***	***0.39***	***0.36***	***0.31***	***0.29***	***0.9***	***0.38***	***0.37***	***0.30***
Glycine0	343.5	2.12	1.2	10.2	23.2	43.9	–	7.4	16.0	26.8	–	–	–	–	0.2	0.7	1.5	2.8
Glycine1	333.9	2.90	98.8	89.8	76.8	56.1	–	92.6	84.0	73.2	–	–	–	–	99.8	99.3	98.5	97.2
Iu/pM: FL			***3.20***	***4.59***	***4.42***	***4.42***		***4.70***	***4.51***	***4.39***					***4.80***	***4.68***	***4.56***	***4.40***
UV			***0.26***	***0.39***	***0.39***	***0.39***		***0.41***	***0.41***	***0.39***					***0.9***	***0.38***	***0.38***	***0.36***
Alanine0	343.5	2.65	0.5	2.5	5.7	12.3	–	2.1	5.2	9.0	–	–	–	–		0.1	0.3	0.4
Alanine1	333.9	4.75	99.5	97.5	94.3	87.7	–	97.9	94.8	91.0	–	–	–	–	100	99.9	99.7	99.6
Iu/pM: FL			***0.89***	***2.96***	***3.83***	***4.06***		***4.23***	***4.23***	***4.23***					***3.83***	***4.24***	***4.23***	***4.23***
UV			***0.08***	***0.26***	***0.34***	***0.36***		***0.37***	***0.37***	***0.37***					***0.34***	***0.38***	***0.37***	***0.37***

Indications as in Table 1, as well as: [OPA]/[MPA]/[A] (20/1000/1) ($1 = 1 \times 10^{-9}$ M) correspond to molar concentrations.

similarly to amines, derivatizations at pH 10.25 proved to be the best choice also for glycine and alanine.

(iii) As to the spectral characteristics of derivatives (Fig. 2),

(a) In the cases of the initially formed products they proved to be the classical isoindoles manifesting an UV maximum at 333.9 nm (Fig. 2: HexA1, HepA1, OctA1, Tables 1–3 derivatives designated by indices 1).
(b) Their transformed versions, already identified [6,7] with the OPA/MPA and OPA/NAC (1/3) derivatives of amino acids and amines, providing UV maximum values shifted to 338.7 nm, indicating the different structure of species (Fig. 2: HexA2, HepA2, OctA2, Tables 1–3 derivatives designated by indices 2).
(c) The products identified at the first time, formed in considerable amounts, obtained with the OPA/SH additive (1/50) reagents manifested a maximum value at 343.5 nm; (Fig. 2: HexA0, HepA0, OctA0, Tables 1–3 derivatives designated by indices 0).
(d) The composition of these, at the first time identified compounds by HPLC, was assumed to correspond to the two SH additive containing "dithio" OPA derivatives.

This assumption is based on considerations as follows:

(a) The only literature data [13] relating to the identification of the OPA/di-*tert*-butylthiol derivative of *n*-propylamine, has been reported as a compound with an UV maximum of 344 nm.
(b) The formation of this type of compounds, in our practice, is unambiguously associated with the extremely high SH group-containing OPA reagents.
(c) The dithio OPA derivatives of C_3–C_8 amines obtained with the OPA/ET reagents have been identified recently in enormously high concentrations, by GC–MS [14].
(d) The above detailed assumptions have been confirmed by on-line HPLC–ESI-MS studies (Figs. 3A–C: UV chromatograms and spectra of the OPA/MPA derivatives of C_6–C_8 amines, obtained with the OPA/MPA (1/50) reagent, at pH 8.80).

First lines show the UV detected chromatograms of the OPA/MPA derivatives of amines at 343 nm; second lines furnish the spectra of the one additional SH additive-containing derivatives (HexA0, HepA0, OctA0); third lines represent the spectra of the protonated molecular ions (HexA1, HepA1, OctA1).

Results proved in all three cases unambiguously, that the one additional SH additive-containing OPA derivatives

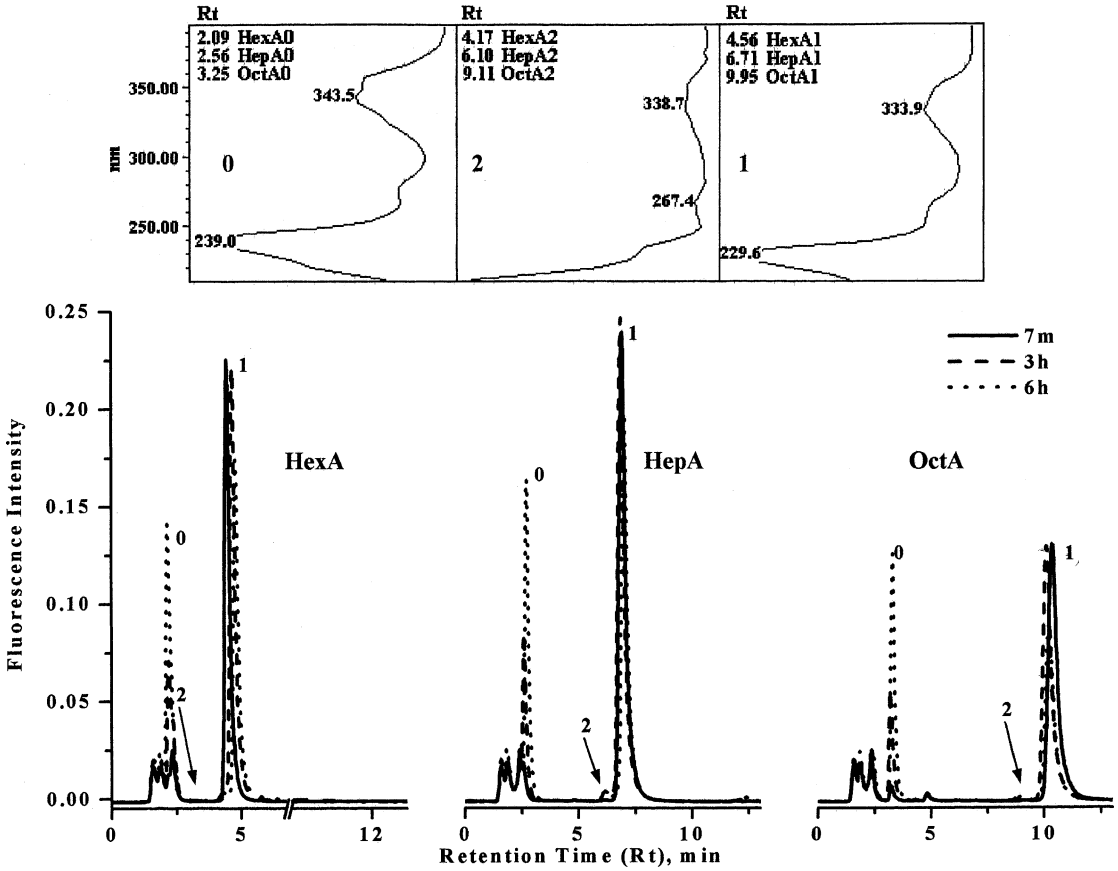

Fig. 2. (HexA, HepA, OctA) Fluorescence detected chromatograms shown with the corresponding DAD spectra at 343.5 nm: HexA0, HepA0 and OctA0 (0), at 338.9 nm HexA2, HeptA2, OctA2 (2) and at 333.9 nm Hexa1, HepA1 and OctA1 (1), respectively, obtained with the OPA/MPA (1/50) reagent.

Table 3
Stability/characteristics of OPA derivatives of hexyl (HexA), heptyl (HepA), octyl (OctA) and phenylethyl (PheEtA) amines as a function of reaction time obtained with the OPA/SH additive (1/50) reagents, based on fluorescence (FL) and UV detections, applying isocratic (OPA/MCE, OPA/ET) and gradient (OPA/NAC) Elutions (pH 8.80, pH 9.30)

Amine	UV max (nm)	[OPA]/[NAC] (1/50)					[OPA]/[MCE] (1/50)											[OPA]/[ET] (1/50)										
		Retention time (min)	Response (%)[a], pH 8.80				Retention time (min)	Response (%)[a], pH 8.80					Response (%)[a], pH 9.30					Retention time (min)	Response (%)[a], pH 8.80					Response (%)[a], pH 9.30				
			7 min	1 h	3 h	5 h		90 s	7 min	30 m	2 h	3 h	90 s	7 min	30 m	2 h	3 h		90 s	7 min	30 m	2 h	3 h	90 s	7 min	30 m	2 h	3 h
HexA0	343.5	7.05	0.2	1.4	3.1	6.8	3.87	–	0.1	0.5	3.5	11.5	–	–	–	–	–	3.67	–	–	–	0.1	0.4					
HexA1	333.9	9.68	99.8	97.9	95.5	91.5	4.71	99.5	99.4	98.8	95.8	88.0	99.4	99.3	98.1	97.6	99.7	5.34	100	100	100	99.9	99.6	100	100	100	100	100
HexA2	338.7	7.53	–	0.7	1.4	1.7	5.90	0.5	0.5	0.7	0.7	0.5	0.6	0.7	1.9	2.4	0.3		–	–	–	–	–					
Iu/pM: FL			***0.84***	***2.02***	***2.47***	***2.57***		***1.79***	***2.39***	***3.23***	***2.29***	***1.12***	***4.88***	***4.46***	***3.57***	***2.23***	***0.70***		***4.07***	***4.59***	***4.68***	***4.65***	***4.55***	***5.23***	***5.45***	***5.44***	***5.21***	***5.14***
UV			***0.12***	***0.28***	***0.35***	***0.36***		***0.12***	***1.16***	***0.23***	***0.16***	***0.08***	***0.33***	***0.32***	***0.25***	***0.15***	***0.05***		***0.30***	***0.33***	***0.33***	***0.32***	***0.32***	***0.32***	***0.34***	***0.34***	***0.33***	***0.32***
HepA0	343.5	7.30	0.1	0.8	1.4	1.3	4.56	0.3	0.4	0.8	2.1	6.0							–	–	–	–	–					
HepA1	333.9	10.35	99.9	98.1	93.8	91.7	5.83	99.3	99.0	97.6	95.7	92.6	99.5	99.6	99.2	98.6	98.3	6.68	100	100	100	100	100	100	100	100	100	100
HepA2	338.7	8.08	–	1.1	4.8	7.0	7.48	0.4	0.6	1.6	2.2	1.4	0.5	0.4	0.8	1.4	1.7	6.43	–	–	–	–	–	–	–	–	–	–
Iu/pM: FL			***0.83***	***1.96***	***2.27***	***2.50***		***1.89***	***2.75***	***3.75***	***3.11***	***1.85***	***5.61***	***5.29***	***5.09***	***4.76***	***4.03***		***4.40***	***4.61***	***4.78***	***4.88***	***4.70***	***5.79***	***5.98***	***5.99***	***5.64***	***5.53***
UV			***0.11***	***0.28***	***0.35***	***0.35***		***0.13***	***0.19***	***0.27***	***0.22***	***0.13***	***0.37***	***0.37***	***0.35***	***0.32***	***0.28***		***0.32***	***0.33***	***0.34***	***0.33***	***0.32***	***0.36***	***0.37***	***0.37***	***0.35***	***0.34***
OctA0	343.5	8.93	0.4	2.2	4.1	7.0	5.87	0.2	0.4	0.6	1.4	3.2	1.4	1.4	1.2	1.0	0.7	4.97	–	–	–	–	–	–	–	–	–	–
OctA1	333.9	11.00	99.6	96.8	94.8	92.0	7.46	98.9	98.4	97.5	95.6	93.3	97.6	97.6	97.8	97.7	97.7	7.86	100	100	100	100	100	100	100	100	100	100
OctA2	338.7	10.83	–	1.0	1.1	1.0	9.86	0.9	1.2	1.9	3.0	3.5	1.0	1.0	1.0	1.3	1.6		–	–	–	–	–	–	–	–	–	–
Iu/pM: FL			***0.89***	***2.36***	***2.55***	***2.60***		***1.79***	***2.94***	***3.96***	***3.89***	***2.98***	***5.65***	***5.45***	***5.28***	***5.19***	***4.86***		***4.49***	***4.76***	***4.94***	***4.92***	***4.61***	***4.82***	***5.97***	***5.87***	***5.47***	***5.29***
UV			***0.13***	***0.35***	***0.38***	***0.38***		***0.12***	***0.20***	***0.28***	***0.27***	***0.21***	***0.38***	***0.38***	***0.37***	***0.35***	***0.32***		***0.33***	***0.34***	***0.36***	***0.34***	***0.32***	***0.36***	***0.37***	***0.37***	***0.34***	***0.33***
PheEtA1	333.9		–	–	–	–	3.41	–	–	–	–	–	99.5	99.0	98.1	98.0	100	3.37	100	100	99.9	99.5	99.0	100	100	99.9	99.8	99.7
PheEtA2	338.7		–	–	–	–	4.00	–	–	–	–	–	0.5	1.0	1.9	2.0		6.33			0.1	0.5	1.0			0.1	0.2	0.3
Iu/pM: FL													***5.36***	***4.90***	***3.73***	***2.25***	***0.59***		***4.55***	***4.73***	***4.71***	***4.83***	***4.48***	***5.79***	***6.08***	***5.98***	***5.74***	***5.65***
UV													***0.37***	***0.35***	***0.20***	***0.16***	***0.04***		***0.35***	***0.35***	***0.35***	***0.35***	***0.33***	***0.38***	***0.39***	***0.39***	***0.38***	***0.37***

Indications as in Tables 1 and 2 as well as: [OPA]/[SH additive]/[A] (20/1000/1) (8×10^{-9} M) correspond to molar concentrations.

were identified (MH^+ + MPA, indicated as HexA0, HepA0, OctA0), formed according to Fig. 4.

These two SH additive-containing species (Figs. 3A–C, spectra in second lines, henceforth: dithio derivatives) were obtained together with their very informative crude molecular ions (M^+), originated from the corresponding dithio derivatives by the loss of one MPA molecule (Fig. 3A: HexA0 = MH^+ + MPA − 2H = m/z = 410.1

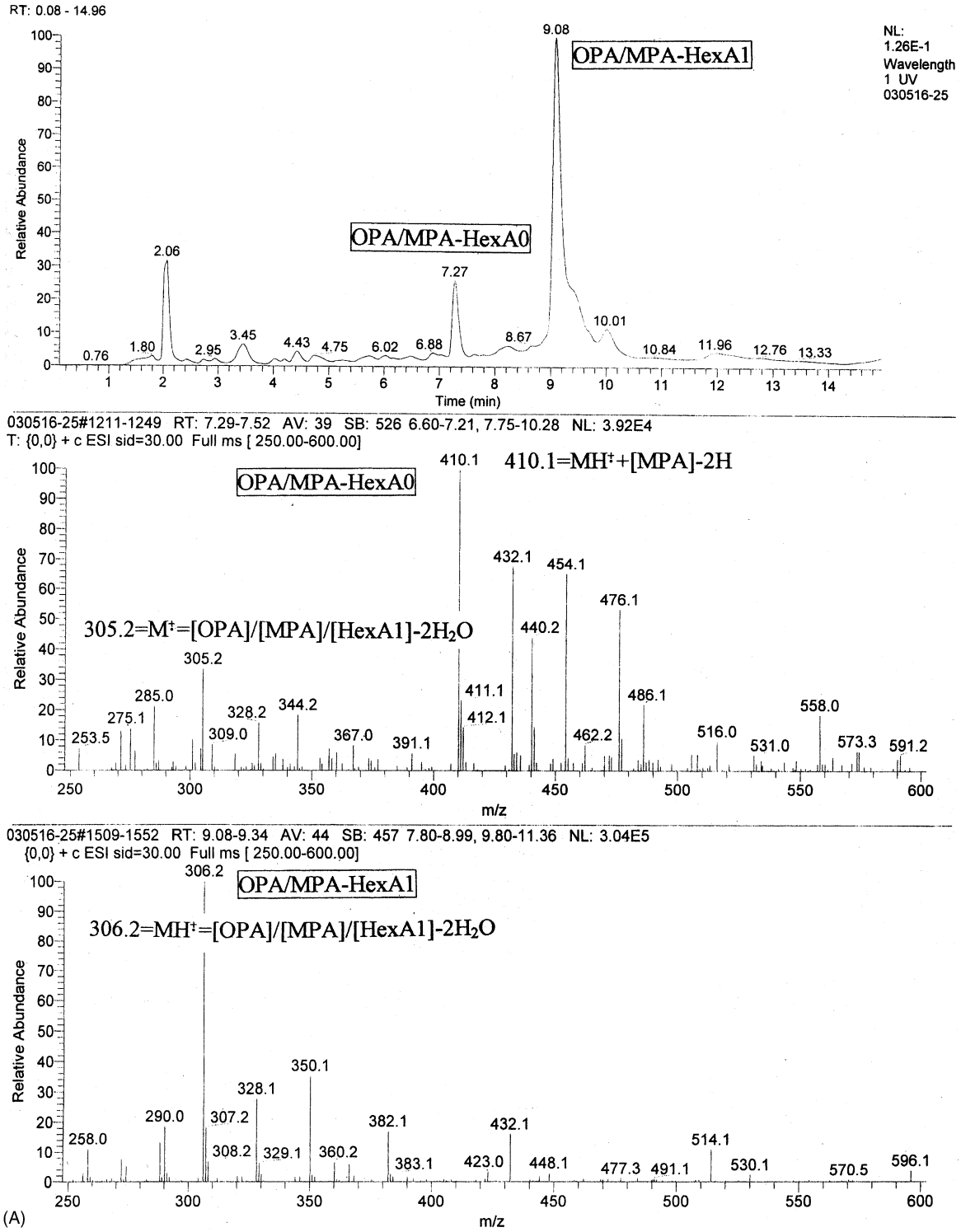

Fig. 3. (A–C) UV chromatograms (first lines) and MS spectra (second and third lines) of the initially obtained (indicated by number 1, i.e., hexylamine1: HexA1, heptylamine1: HepA1, octylamine1: OctA1) and their transformed OPA/MPA derivatives (indicated by number 0, i.e.,: hexylamine0: HexA0, heptylamine0: HepA0, octylamine0: OctA0 (detailed composition of fragments in the text).

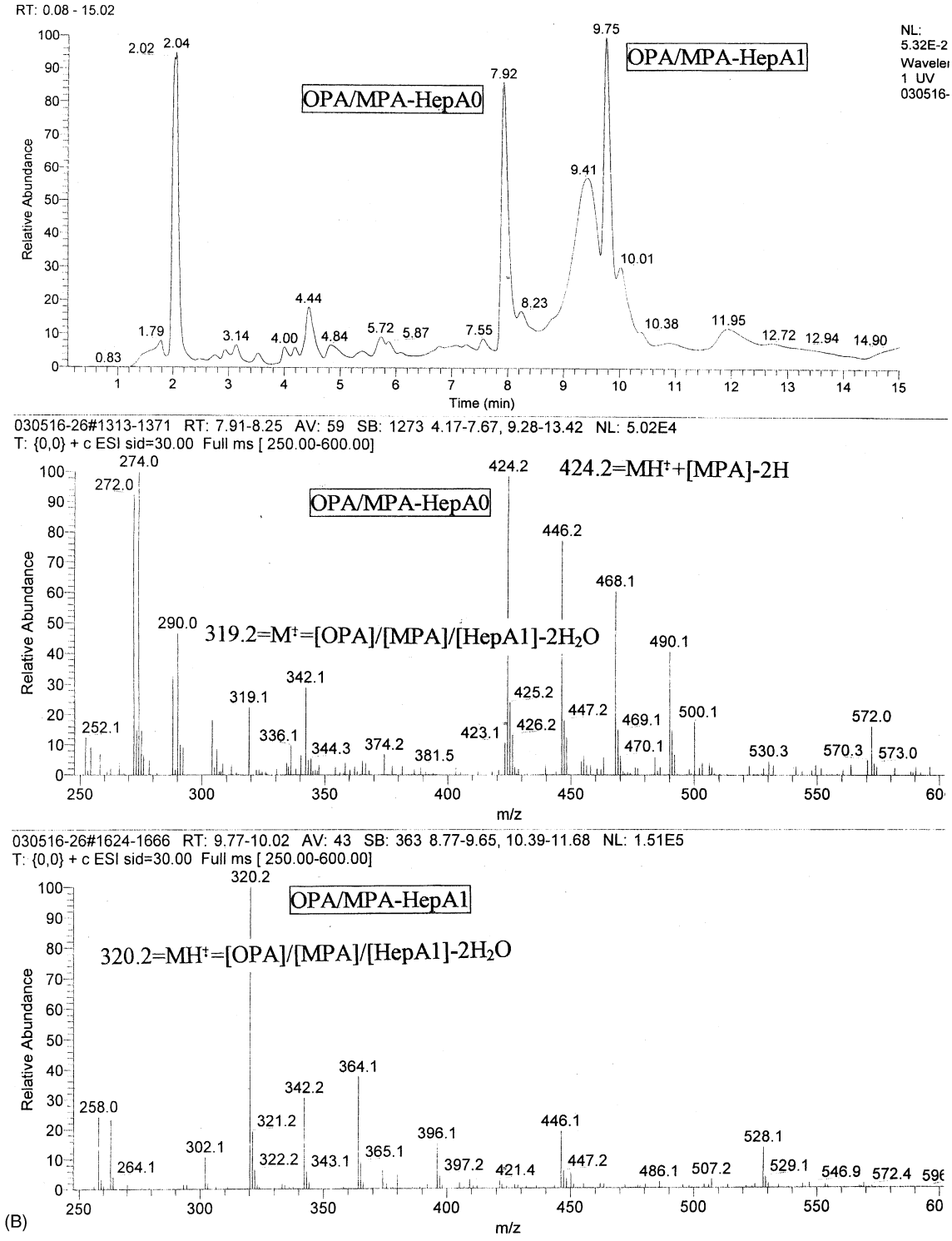

Fig. 3. (*Continued*)

and $M^{+} = 305.2$; Fig. 3B: HepA0 $= M^{+} + MPA - 2H = m/z = 424.2$ and $M^{+} = 319.2$; Fig. 3C: OctA0 $= M^{+} + MPA - 2H = m/z = 438.2$ and $M^{+} = 333.2$).

Certainly, beside the dithio derivatives also the initially formed classical, isoindoles have been repeatedly detected (Fig. 3A–C, spectra in third lines: HexA1 $= MH^{+} = m/z = 306.2$, HepA1 $= MH^{+} = m/z = 320.2$, OctA1 $= MH^{+} = m/z = 334.2$).

3.2.2. Reactions with the OPA/NAC, OPA/MCE and OPA/ET (1/50) reagents, at pH 8.80 and 9.30

Based on the behavior of C_6–C_8 amines, furnishing two different types of transformed derivatives in their reactions

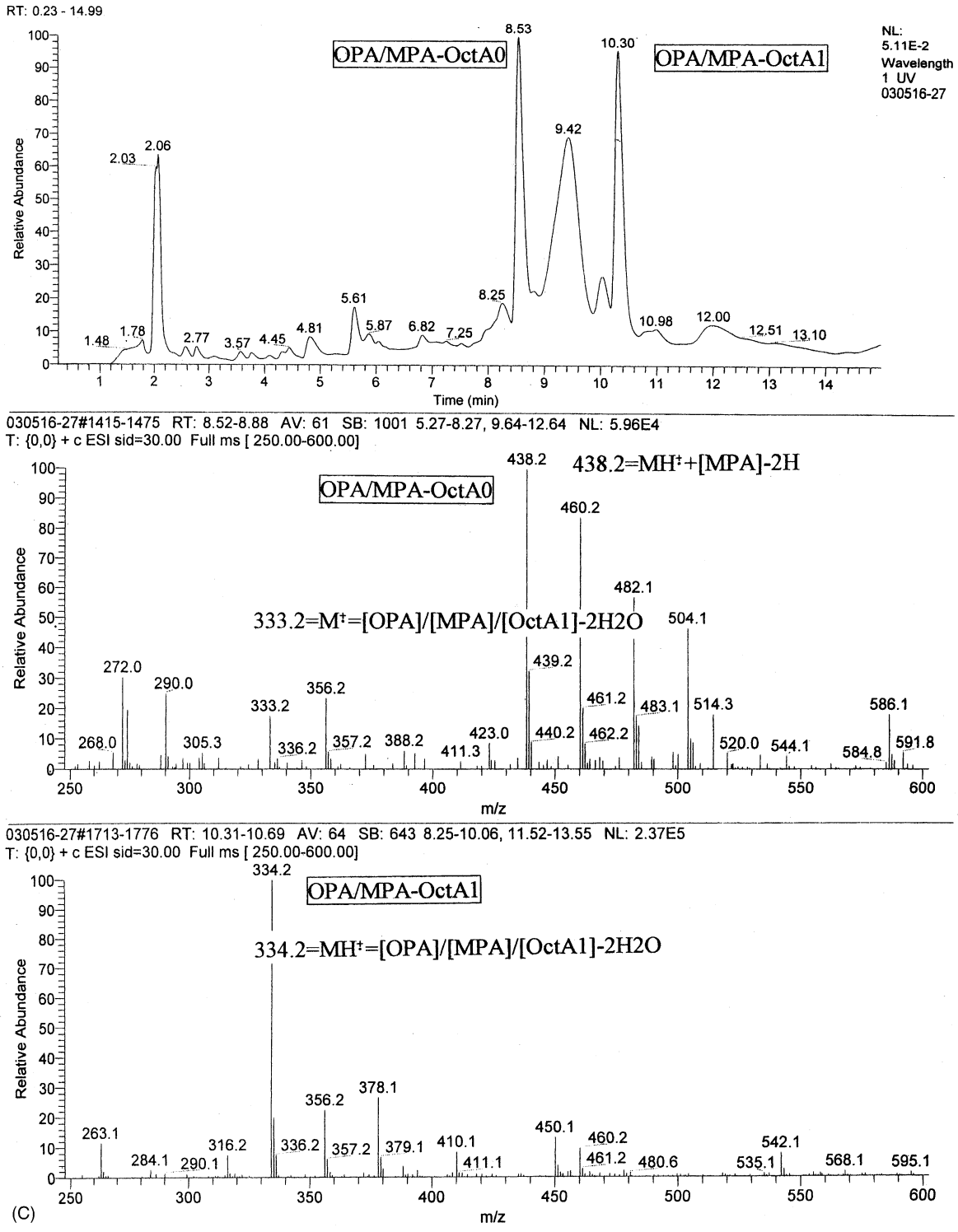

Fig. 3. (*Continued*).

with the OPA/MPA (1/50) reagent, it would be of interest to clarify also the characteristics of their OPA/NAC, OPA/MCE and OPA/ET derivatives, obtained under strictly the same conditions.

(i) Selection of pH values, based on data, shown in Table 2, was expected to be important in order to define the impact of SH additive on the proportions of transformed product(s), as well as, on the overall response values and stability of derivatives (Table 3).

(a) Comparing percentages of the dithio derivatives (HexA0, HepA0, OctA0 PheEtA0 in Tables 2 and 3) they decrease from the OPA/MPA through the

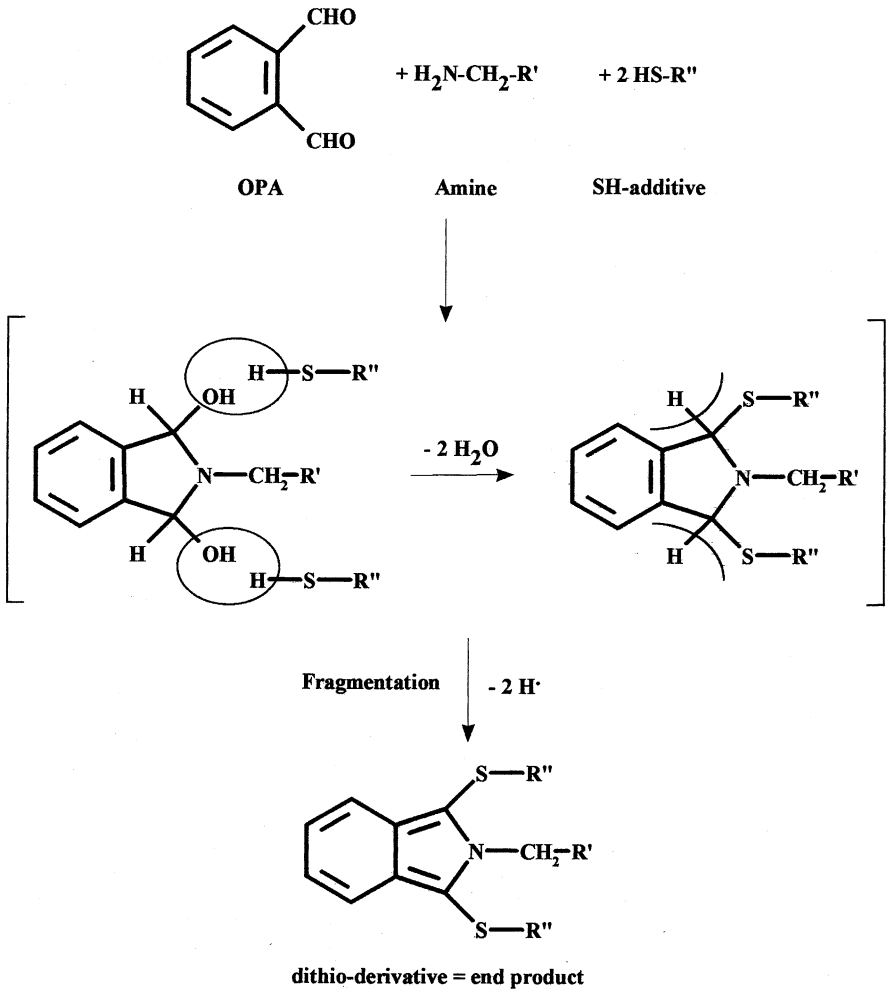

Fig. 4. Reaction of a NH_2 group-containing compound with the OPA/SH additive (1/50) reagent resulting in the dithio derivative.

OPA/NAC to the OPA/MCE derivatized ones, from pH 8.80 to 9.30. In case of the OPA/ET derivatizations dithio derivatives have not been found, at all.

Evaluating response values of C_6–C_8 and phenylethylamines, in general, obtained with the OPA/SH additive (1/50) reagents (Tables 2 and 3) it is clear that

(b) Beneficial effect of decreased, free OPA concentration [7], resulted in its limited reactivity could be enjoyed applying the OPA/MPA reagent, at pH 10.25, exclusively.

Table 4
Simultaneous quantitation of different amounts of hexyl (HexA), heptyl (HepA) and octyl (OctA) amines obtained with the OPA/ET (1/3) reagent, in model solutions, on the basis of their UV and fluorescence (FL) intensities

Amine	Retention time (min)	Integration units (Iu)/1 pM amino acid[a], injected (pM)										
		1800	900	450	225	112.5	62.25	31.125	15.625	7.81	Average[b]	R.S.D. (%)
HexA, Iu/pM: FL	5.58	–	7.52	7.53	7.49	7.53	7.53	7.42	7.70	7.54	7.53	0.97
UV		0.52	0.50	0.50	0.50	0.51	0.50	0.51	0.51	0.52	0.51	1.64
HepA, Iu/pM: FL	7.00	5.95	5.89	5.87	5.78	5.79	5.70	5.40	5.46	5.43	5.70	3.7
UV		0.40	0.39	0.39	0.38	0.38	0.36	0.36	0.36	0.38	0.38	3.9
OctA, Iu/pM: FL	8.95	6.94	6.82	6.72	6.58	6.61	6.43	6.14	6.16	6.14	6.50	4.7
UV		0.47	0.46	0.45	0.44	0.44	0.44	0.42	0.43	0.41	0.44	4.0

Indications: pM: picomole.

[a] Obtained from three separate derivatization tests.

[b] Averages, obtained with molar ratios of $[OPA]/[ET]:[A]^T = 7:1, 14:1, 28:1, 56:1, 112:1, 224:1, 448:1, 996:1, 1982:1$; $[OPA] = 1.86 \times 10^{-6}$ M.

(c) In the cases of the less stable OPA/MCE derivatives the decreased reactivity of the OPA reagents, at pH 8.80 resulted in decreased stability, i.e., in decreased response values.
(d) Derivatization of C_6–C_8 and phenylethylamines with the OPA/ET reagent(s), in particular at pH 9.30, can be regarded as a derivatization technique of choice. Practically, the amounts of transformed products are negligible, including also the products obtained with the OPA/ET (1/3) reagent (Table 1, last vertical column). Response values, obtained with the OPA/ET (1/3) and OPA/ET (1/50) reagents, equally reflect the reproducibility and stability of the process, predestinating these derivatives for analytical purposes.

3.3. Analytical consequences

Based on stoichiometric studies summarized in Tables 1–3 analytical reproducibility has been investigated with various concentrations of the C_6–C_8 amines, under the most promising conditions: applying the OPA/ET (1/3) reagent at pH (9.30) (Table 4).

As seen, derivatization of the C_6–C_8 amines is to be preferred by the OPA/ET reagent: furnishing acceptable R.S.D. values ($\leq$4.7%) and limit of quantitation (8 pM).

4. Conclusion

(1) The behavior and characteristics of the OPA/MPA, OPA/NAC, OPA/MCE and OPA/ET derivatives of C_6–C_8 aliphatic and phenylethylamines have been studied both from an analytical point of view and with respect to their composition.
(2) Performing derivatizations with the OPA/SH additive (1/3) reagents, the initially formed, classical isoindole transforms to the two OPA molecule-containing species.
(3) Applying the OPA/SH additive (1/50) reagents, in order to inhibit the formation of the two OPA derivative-containing product, resulted in an additional, transformed OPA derivative: detected and determined by HPLC at the first time (on the basis of on-line HPLC–ESI-MS measurements), proved to be the two SH additive-containing OPA derivatives.
(4) Proportion of the transformed derivatives can be unambiguously influenced by the quality of SH additive, by the composition of the OPA reagent, i.e., by the molar ratio of the OPA to the SH additive and by the pH of derivatizations.

As to the maximum yield of the dithio derivatives at pH 8.8, applying the OPA/MPA (1/50) reagent, we assume that their production in these extremely high concentrations is very likely associated with the characteristics of the SH additive, 3-mercaptopropionic acid, being a relatively strong acid ($pK_1 = 4.3$). Certainly the lower the pH, the higher the stability of the two SH additive-containing *o*-phthaldialehyde adduct molecule. However, high pH values (pH $\ll$ 8.8) favor the isoindole production. Thus, according to our results in this term pH 8.8 proved to be the optimum compromise, both to the adduct and to the isoindole formations.
(5) Regarding both the stability of OPA derivatives and the extent of side reactions, the impact of the SH additive component of the OPA reagent proved to be of primary importance. The OPA/MPA and OPA/ET derivatives of C_6–C_8 and phenylethylamines, under optimum derivatization conditions, provided the same stability. However in terms of side reaction free derivatization the OPA/ET reagents proved to be superb compared to the OPA/MPA one.
(6) Reproducibility studies carried out with the OPA/ET (1/3) (pH 9.30) reagent revealed that the OPA/ET derivatizations should be preferred: providing acceptable R.S.D. percentages ($\leq$4.7%) and limit of quantitation (8 pM).

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References

[1] I. Molnár-Perl, I. Bozor, J. Chromatogr. A 798 (1998) 37–46.
[2] I. Molnár-Perl, A. Vasanits, J. Chromatogr. A 835 (1999) 73–91.
[3] A. Vasanits, D. Kutlán, P. Sass, I. Molnár-Perl, J. Chromatogr. A 870 (2000) 271–287.
[4] D. Kutlán, I. Molnár-Perl, Chromatographia 53 (2001) 1–11.
[5] I. Molnár-Perl, J. Chromatogr. A 913 (2001) 284–304.
[6] Y. Mengerink, F. Tóth, D. Kutlán, I. Molnár-Perl, J. Chromatogr. A 949 (2002) 99–124.
[7] D. Kutlán, P. Presits, I. Molnár-Perl, J. Chromatogr. A 949 (2002) 235–248.
[8] I. Molnár-Perl, J. Chromatogr. A 987 (2003) 291–309.
[9] D. Kutlán, I. Molnár-Perl, J. Chromatogr. A 987 (2003) 311–322.
[10] R. Hanczkó, I. Molnár-Perl, Chromatographia 57 (2003) S103–S113.
[11] G. Lunn, L.C. Hellwig (Eds.), Handbook of Derivatization Reactions for HPLC, Reactive group: Amino Acids, Wiley, 1998, p. 625.
[12] I. Molnár-Perl, in: Z. Deyl, I. Mikšik, F. Tagliaro, E. Tesaøová (Eds.), Advanced Chromatographic and Electromigration Methods in BioSciences, Journal of Chromatography Library, vol. 60, Elsevier, Amsterdam, 1998, Chapter 11, pp. 415–467.
[13] S.S. Simons, D.F. Johnson, J. Org. Chem. 43 (1978) 2886–2891.
[14] T. Törő, Cs. Ágoston, I. Molnár-Perl, Chromatographia, in press.