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Journal of Chromatography A, 949 (2002) 235–248

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Review

Behavior and characteristics of amine derivatives obtained with *o*-phthaldialdehyde/3-mercaptopropionic acid and with *o*-phthaldialdehyde/*N*-acetyl-L-cysteine reagents

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Abstract

A comprehensive evaluation of papers dealing with the HPLC quantitation of amines as *o*-phthaldialdehyde (OPA) derivatives has been given and discussed in details. The stability and characteristics of selected representatives of mono [methyl-, ethyl-, *n*-/isopropyl, *n*-/isobutyl-, *tert*.-butyl-, *sec*.-butyl-, isoamyl amines and ethanolamine), di- and polyamines (ethylenediamine, 1,2-propylenediamine, 1,3-propylenediamine, agmatine, tyramine, putrescine, cadaverine, histamine, spermine, spermidine, and bis(hexamethylene)triamine] have been investigated as their OPA/3-mercaptopropionic acid (MPA) and OPA/*N*-acetyl-L-cysteine (NAC) derivatives, from an analytical point of view, performing photodiode array and fluorescence detection, simultaneously. All amines having in their original structure the NH_2-CH_2-R moiety, in accord with the amino acids of the same structure, furnished more than one OPA derivative: their initially formed species transformed to further ones. On the basis of on-line HPLC–MS the transformed derivatives were proved to be the corresponding isoindoles that contain an additional OPA molecule. In order to achieve optimum analytical conditions derivatization reagents have been applied in different composition, in parallel. The OPA and the SH-group additive contents of the reagents have been varied in the mole ratios of OPA/MPA(NAC)=1:3 and OPA/MPA(NAC)=1:50. Data obtained proved that performing derivatizations by means of the OPA/MPA(NAC)=1:50 reagents resulted in two benefits: both the stability of derivatives could have been increased and the number of the transformed derivatives decreased. In case of aliphatic amines and in ethanolamine, the transformation of the initially formed derivative can be either quantitatively avoided as in the case of ethanolamine, or considerably decreased, below 1%, as in the cases of the other aliphatic monoamines investigated. As to the behavior of di- and polyamines the stability of derivatives has been considerably improved, the number of species have been decreased from four to two with the exception of spermidine. Stability values characterized both by the UV and fluorescence responses, as a function of the reaction time (from 90 s up to 6 h) have been given in details.

Keywords: Reviews; Derivatization, LC; Amines; Biogenic amines; Amino acids

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0021-9673/02/$ – see front matter
PII: S0021-9673(01)01610-7

Contents

1. Introduction

In the last 3 years several characteristics of the *o*-phthaldialdehyde (OPA) derivatives of amino acids have been clarified in our laboratory [1–6]. Amino acids declared of low stability (glycine, β-alanine, γ-aminobutyric acid, histidine, ornithine and lysine), furnished more than one OPA derivative. Taking into account all of their products obtained, they proved to be as stable as all other amino acids providing a single OPA derivative. On the basis of our experiences with the OPA-amino acids [1–6] and on all those literature data [7–35] that deal with the determination of aliphatic mono- and diamines, including biogenic amines it seemed to be of interest to clarify the behavior of these OPA-derivatized amines.

In the early structure elucidation of the reaction product obtained from OPA and the primary amino group [7–9] (as well as in the pioneer stability studies of this product [10–13]), as model compound *n*-propylamine was investigated: the isoindole character of the product has been confirmed by NMR evidences performing various SH-group containing reagents [8] (2-mercaptoethanol, ethanethiol, *tert.*-butylthiol, thiophenol, etc.). At that time, in the early 1980s the product was not separated by a chromatographic technique. Consequently, its transformation into the successively formed product(s) was not realized, and, to the authors' knowledge, it has not been reported later on either. Unfortunately, when the chromatographic technique became common and the unambiguous phenomena of the considerably low stability of OPA-amines, in comparison even to the OPA amino acid derivatives, became known the intrinsic reason of this fact, for the time being, was not investigated. However, in order to enjoy the simple, selective and sensitive OPA-derivatization principle, also in the analytical practice of amines, according to a recent compilation, it has gained wide

acceptance [14–34]: the HPLC of amines proved to be performed in 23% as OPA derivatives [35] (Table 1).

As to the SH-group containing additive in the OPA derivatization of amines, 2-mercaptoethanol (MCE) [14–17,20–30], ethanethiol (ET) [17,21,31], *N*-acetyl-L-cysteine (NAC) [31–33], 3-mercaptopropionic acid (MPA) [34], or 1-thio-D-glucose (TG) [18] have been used, applying partly fluorescence (FL) [14–19,23,26–28,30–34], partly electrochemical (ED) [21,23,25,26,30] or UV detection [22].

Scientists, aiming to improve the stability of the OPA-amines, recommended their extraction into organic solvents [14–17,19], or, proposed the use of micellar surfactants [18], resulting in partly improved stability of the OPA-amine species [14–18]. The OPA/MCE derivatives [14–16] of histamine, norepinephrine, normetanephrine, dopamine, serotonin and tyramine obtained from plasma, urine and tissues [14], the norepinephrine, dopamine and serotonin content of plasma [15], as well as 11 amines of red must and wine [16] have been extracted, subsequently to their formation, into ethyl acetate: in these extracts derivatives proved to be stable for 20 h [14]. The recovery of added amines varied between 54.3 and 77.8%, including sample cleanup and extraction procedures [14].

Table 1
Distribution of derivatization methods used in the HPLC quantitation of amines

Derivative	No[a]	%[b]
Activated halide	70	11.4
Acyl halide	117	19.1
Alkene–anhydride	16	2.6
Naphthalene-2,3-dicarboxaldehyde/CN	23	3.8
Dialdehyde (not OPA)	8	1.3
OPA (SH-additive not defined)	10	1.6
OPA/2-mercaptoethanol	96	15.7
OPA/butanethiol–ethanethiol	13	2.1
OPA/3-mercapto(acetic)–propionic acid	15	2.4
OPA/NAC (chiral cysteines)	5	0.8
OPA/Na_2SO_3	2	0.3
OPA in total	141	23.0
Phenylthiocarbamyl	95	15.5
Substituted phenylthiocarbamyl	32	5.2
Succinimidyl ester	16	2.6
Dabsyl	36	5.9
Dansyl	31	5.0
Lactone–ketoaldehyde	6	0.9
Cobalt and hydroxylamine	7	1.1
Solid-phase reagent	3	0.5
Sulfonic acid/halide	5	0.8
Miscellaneous	9	1.4
In total	613	

[a] Number of papers referred.
[b] Distribution%, expressed in the total, taken from the period of 1992–1998 [35].

A basic research study [19] proved the increased stability of the OPA/ET derivatives of spermine, spermidine, putrescine, cadaverine and 1,6-hexanediamine in their ethyl acetate extracts: after an initial reaction time of 90 s followed by their quantitation from the ethyl acetate extracts after 2.5, 6.5 and 23 h, in order of listing, revealed the following recovery percentages: 80, 31 and 5% for spermine, 93, 83 and 67% for spermidine, 96, 92 and 90% for putrescine, 97, 93 and 85% for cadaverine and 97, 92 and 90% for 1,6-hexanediamine [19].

The histamine, tryptamine and tyramine contents of bacterial cultures have been derivatized in a 2-propanol–ethyl methyl ketone (10:90, v/v) mixture (1.3 ml) with the OPA/ET reagent (0.2 ml), in a capped tube and vortex mixed (15 s) [17]: after 30 min the organic phase, containing the OPA/ET derivatives, has been diluted with methanol and water, in the ratios of the organic phase–methanol–water (1:1:2, v/v) and injected into the HPLC system. The majority of amines may be estimated reliably with recoveries of added amounts ranging from 74 to 96%.

The addition of surfactant micelles (sodium dodecylsulfate) resulted in improved stability of the OPA/MCE derivatives of methylamine, tyramine, putrescine and cadaverine, but did not increase the stability of the corresponding OPA/MPA and OPA/NAC derivatives, respectively [18].

The histamine levels from various biological matrices [20], the histamine and serotonin contents [21,22,24] from human plasma and brain [21], from rat brain [22] and from cellular extracts [24], as well as the histamine and 1-methylhistamine contents from rat peritoneal mast cells [32] have been determined in the presence of ammonia, histidine, spermine and spermidine [20] performing post-column FL detection [20], ED [21,23,26] and pre-column FL detection [24,32].

The putrescine, cadaverine and 2-phenylethylamine content in various cheese samples have been

measured with isocratic elutions using UV detection [22].

The mono- and diamine contents of different wines [25–27,31] were quantitated partly by FL detection [27,31] and partly by ED [25,26]. A coulometric array of 16 electrodes increased the selectivity of the method [25].

The β-phenylethylamine content of human plasma [28], the agmatine concentrations in brain and plasma [29], as well as the norepinephrine and arginine [30] contents of plasma samples have been determined by FL detection [28–30].

The diamine content of urine and plasma samples have been measured separately from amino acids applying optimized eluent composition and the fully endcapped material containing Inertsil column [34].

On the basis of our experiences (data reported in this paper), after measuring the instability of the OPA-amines, in particular those of diamines, it seemed to be unbelievable that the transformation of the initially formed products to the forthcoming ones, with a single exception relating to ephedrine [33], was not realized [14–32]. The formation of double species of ephedrine obtained upon its pre- or post-column derivatization with the OPA/NAC reagent [33] was reported without any further explanation of the finding.

In this paper the characteristics and stability of the aliphatic mono- and diamines, including numerous, relevant biogenic amines have been studied, primarily from an analytical point of view, on the basic research level. Changes in responses of the OPA/NAC derivatized amines, in selected cases even those of the OPA/MPA derivatized ones, have been followed as a function of the reaction time and reagent's composition by UV photodiode array detection (DAD) and FL detection, simultaneously.

2. Experimental

2.1. Materials

OPA, MPA, NAC and amines, such as mono-[methyl-, ethyl-, *n*-/isopropyl, *n*-/isobutyl-, *tert*.-butyl-, *sec*.-butyl-, isoamyl amines, ethanolamine), di-[ethylenediamine, 1,2-propylenediamine, 1,3-propylenediamine, cadaverine: $NH_2(CH_2)_5NH_2$, putrescine: $NH_2(CH_2)_4NH_2$], and different polyamines [histamine: 4-(imidazolyl)ethylamine: $C_3H_3N_2CH_2$-CH_2NH_2, tyramine: 4-(2-aminoethyl)phenol: $HOC_6H_4(CH_2)_2NH_2$, agmatine: 4-(aminobutyl) guanidine: $NH_2(CH_2)_4NHC(=NH)NH_2$, spermine: $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, spermidine: $NH_2(CH_2)_4NH(CH_2)_3NH_2$, bis(hexamethylene)triamine: $NH_2(CH_2)_6NH(CH_2)_6NH_2$] were obtained from Sigma (St. Louis, MO, USA) and from Serva (Heidelberg, Germany). HPLC-grade methanol and acetonitrile were purchased from Romil Chemicals (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 concentrations of $\sim 1–2\times10^{-2}$ *M* and 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) ratios from 0.2 *M* boric acid (dissolved in 0.2 *M* potassium chloride)–0.2 *M* sodium hydroxide (pH 9.3±0.05).

2.4. Reagent solutions

OPA/MPA reagent was obtained by mixing, in order of listing, 5.0 ml methanolic OPA, 20.0 ml borate buffer and various amounts of MPA solutions: finally, if necessary it was adjusted by 1 *M* sodium hydroxide, to pH 9.3±0.05.

OPA/NAC reagent was prepared from 5 ml methanolic OPA solution, 20.0 ml borate buffer containing 0.1–0.4 g NAC: final pH 9.3±0.05.

The mole ratios of OPA to the MPA and NAC were, [OPA]/[MPA]([NAC])=1:3, and 1:50 as detailed in the corresponding sections.

2.5. Derivatization

2.5.1. Characterization of the reagent solutions

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

2.5.2. Studies with the o-phthaldialdehyde/3-mercaptopropionic acid(N-acetyl-L-cysteine)-amino acid solutions

Derivatizations were performed with reagents prepared at least 90 min earlier before use, and saved no longer than ≤9 days [2]. The calculated amounts of reagent solutions were mixed with the selected amounts of amines, and let react for 7 min before injection (if not otherwise stated).

2.6. Chromatography

The system was a Waters HPLC instrument (Milford, MA, USA), consisting of a Waters 996 photodiode array, and 274 fluorescence detectors, a Waters 600 controller quaternary pump with thermostattable column area, Waters 717 autosampler, operating with Millennium software (version 2.10, 1992–95, validated by ISO 9002). Columns were Hypersil ODS bonded phase: 150×4 mm, 5 μm, used with 20×4-mm guard columns (BST, Budapest, Hungary).

DAD and FL detections have been performed simultaneously, with the detectors connected in order of listing. Blank and test samples have been detected between 190 and 400 nm (DAD), evaluated at 334 nm (OPA/MPA(NAC)–amines), as well as at the optimum excitation (ex)/emission (em) wavelengths of 337/454 nm. The eluent system consisted of two components: eluent A was 0.05 *M* sodium acetate of pH 7.2, while eluent B was prepared from 0.1 *M* sodium acetate–acetonitrile–methanol (46:44:10) (mixed in volume ratios and titrated with glacial acetic acid or 1 *M* sodium hydroxide to pH 7.2).

Stability studies of amines one by one was performed in the gradient mode partly as described earlier {0–100 eluent B within 10 min [2], partly applying a faster gradient (50–75 eluent B within 5 min)}. (Note: data in the tables, without exception, were calculated to 1 ml/min elution rate.)

3. Results and discussion: derivatization study of amines as *o*-phthaldialdehyde/3-mercaptopropionic acid and *o*-phthaldialdehyde/*N*-acetyl-L-cysteine derivatives

3.1. Stability and characteristics of amines as their o-phthaldialdehyde/3-mercaptopropionic acid and o-phthaldialdehyde/N-acetyl-L-cysteine derivatives

3.1.1. Expectations based on experiences with amino acids: the role and impact of the mol ratios of the reagent's composition

In the knowledge of the reason and background of the special behavior [2–6] and characteristics of selected amino acids [6] (declared in the literature as the less stable ones), we wanted to clarify the intrinsic properties even of amines toward OPA derivatization. A promising and beaten path [3–6] was available to be followed: applying DAD and FL detection, simultaneously.

Recently [4], by varying the composition of the OPA reagent in its reaction with the more than one species providing amino acids, we demonstrated that the higher the concentration of the SH-group additive (MPA or NAC) the lower the amount of the transformed product. Changing the mole ratios of the OPA/NAC and OPA/MPA from 1:0.5 to 1:10 resulted on average in a 4-fold decrease of the transformed derivative ranging from 80% (at mole ratios of OPA/MPA and OPA/NAC=1:0.5) to 20% (at mole ratios of OPA/MPA and OPA/NAC=1:10), expressed in the total of derivatives formed [5]. This correlation implies that the free OPA concentration favors the transformation of the initially formed isoindole that is needed for the ''ready to react'' state, it means for those of free OPA molecules (OPA with the SH additive reacts as a preformed species with the amino group [7–9]).

In the light of the above detailed stoichiometric experiences, extended on the basis of on-line HPLC–MS data** [6], we altered the composition of the reagent in a considerable manner. Mole ratios of OPA to the SH-group containing additive have been

altered for OPA/MPA and OPA/NAC to 1:50. Thus, the behavior of amines has been compared in their reactions toward the OPA/SH additive=1:3 and 1:50 reagents, in parallel. We assumed that further increasing the concentration of the SH-group additive in the reagent might result in two benefits, simultaneously: (i) in a decrease of the transformation rate of the initially formed derivative, and (ii) in an increase of the overall stability of the total of derivatives (Figs. 1–3 and Tables 2–4).

(Note: HPLC–MS data** confirmed both the composition of the transformed derivatives that proved to be the corresponding isoindol plus an additional OPA molecule (Table 5), and the reaction pathway they are originating from [6].)

3.1.2. Introductory tests with the o-phthaldialdehyde/3-mercaptopropionic acid and=1:3 and o-phthaldialdehyde/N-acetyl-L-cysteine=1:3 reagents

Pilot investigations aiming to select the SH-group component (MPA or NAC) of the OPA reagent to be preferred, for sake of comparison, C_1–C_4 aliphatic amines and diamines (ethylenediamine, 1,2-propylenediamine, 1,3-propylenediamine, putrescine and cadaverine) were derivatized both with the OPA/MPA=1:3 and with the OPA/NAC=1:3 reagents, respectively.

In all cases investigated the transformation of the initially formed derivatives into the forthcoming ones proved to be slower with NAC as SH-group additive by the elution profiles of the OPA/MPA=1:3 derivatives of 1,3-propylenediamine, putrescine and cadaverine (Fig. 1) and the corresponding OPA/NAC=1:3 derivatives of putrescine and cadaverine (Fig. 3A,B). On the basis of these experiences the overwhelming part of further derivatizations, carried out as a function of the reaction time, have been performed as OPA/NAC derivatives.

3.2. Studies on the behavior and characteristics of the C_1–C_5 aliphatic amines and ethanolamine upon their reaction with the o-phthaldialdehyde/N-acetyl-L-cysteine=1:3 and the o-phthaldialdehyde/N-acetyl-L-cysteine (3-mercaptopropionic acid)=1:50 reagents

Exhaustive derivatization study with selected members of aliphatic monoamines (Fig. 2, Table 2: data for the OPA/NAC=1:3 derivatives) have been followed as a function of the reaction time. Responses, expressed in the total of derivatives were given in arbitrary units (integrator unit)/pM amines, from 90 s up to 6 h.

In the cases of C_1–C_5 aliphatic amines including ethanolamine both the decreased transformation rate of the initially formed OPA derivatives and the increased stability of the total of species proved to be equal to our expectations [6].

(i) The initially formed OPA derivatives, in all those cases where the neighboring group to the primary amino group was a CH_2 moiety, transformed to a second species of longer retention time containing additional OPA molecule, as expected: the composition of the transformed derivatives and the reaction pathway they are originating from have been confirmed on the basis of their molecular masses by on line HPLC–MS measurements [6]. The exceptions of isopropyl-, *sec.*-butyl- and *tert.*-butylamines that furnish a single OPA derivative, can be attributed to their initial molecular structure: their neighboring groups to their primary amino groups, in order of listing, are the –CH–, –CH– and –C= moieties, respectively.

(ii) Stability of derivatives prepared strictly under the same conditions, proved to be associated with the chain length of the aliphatic amines: the longer the chain length the slower the decomposition of the total of derivatives formed. Evaluating response values from an analytical point of view (shown in the horizontal lines of Table 2, expressed as integration unit/p*M* amines), they proved to be of importance in choosing optimum reaction time for the amine in question. In cases of ethanolamine, members of C_1–C_4 *n*-amines and isoamyl-amine, between 90 s and 7 min reaction times, maximum responses can be expected.

(iii) Unfortunately, the derivatization rate of the sterically hindered amino groups of the *sec.*- and *tert.*-aliphatic amines, applying the OPA/NAC-(MPA)=1:50 reagents, considerably decreased even in comparison to the OPA/NAC(MPA)=1:3 reagents. Thus, due to sterical hindrance, to apply optimum conditions for the C_1–C_4 *n*-monoamines and the corresponding isopropyl, isobutyl, *sec.*-butyl- and *tert.*-butylamines must be a matter of compromise.

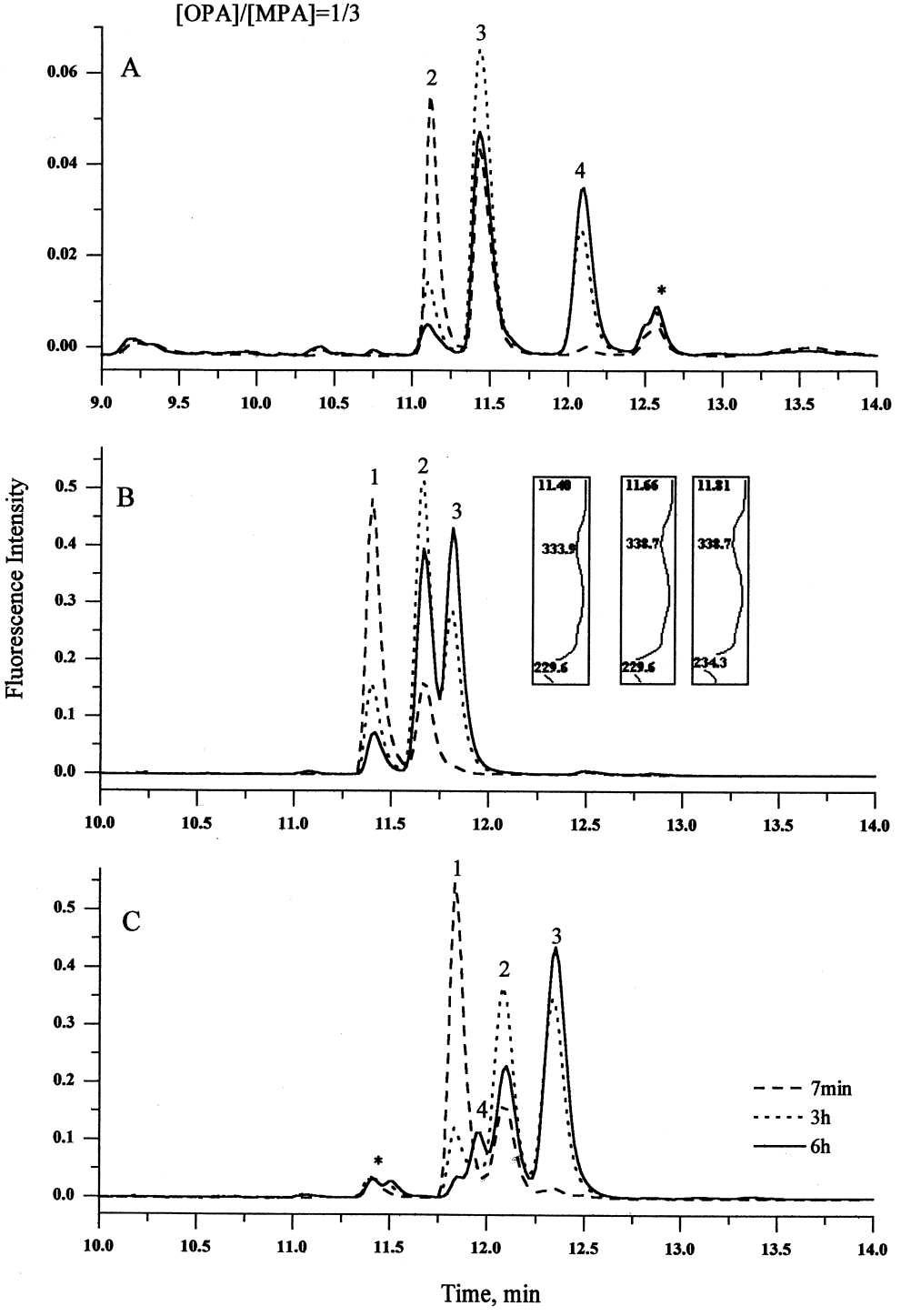

Fig. 1. Photodiode array–fluorescence detection chromatograms of 1,3-propylenediamine (A), putrescine (B) and cadaverine (C) obtained after various reaction times: (- - -) 7 min; (· · ·) 3 h; (———) 6 h, with reagent composition of [OPA]/[MPA]/[DA]=20:60:1 (1=1×10^{-9} *M*); *impurity peaks.

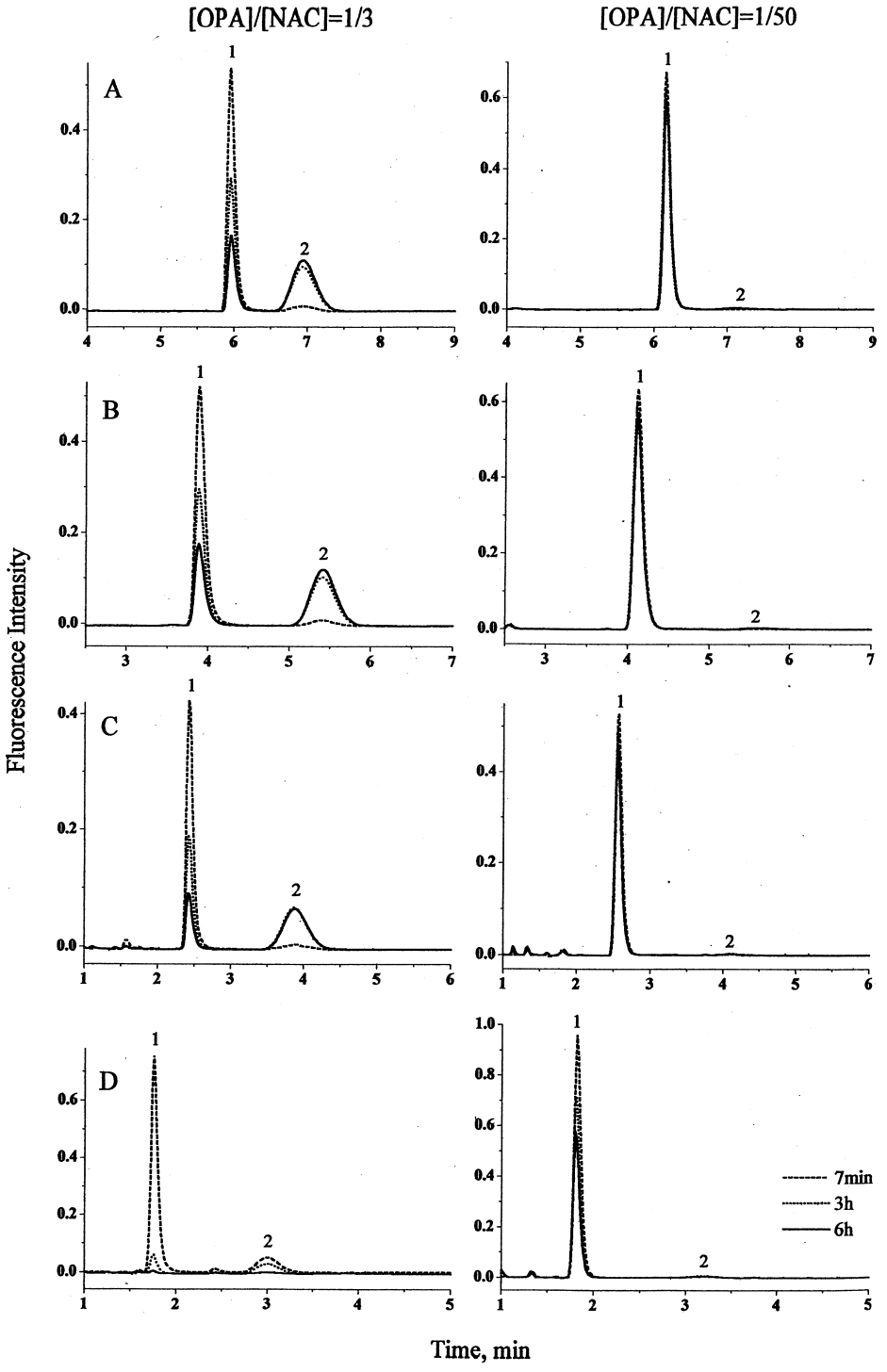

Fig. 2. FL detection chromatograms of *n*-butylamine (A), *n*-propylamine (B), ethylamine (C) and methylamine (D) obtained after various reaction times: (- - -) 7 min; (· · ·) 3 h; (———) 6 h, with reagents of various composition: [OPA]/[NAC]=1:3, i.e., [OPA]/[NAC]/[amine]=20:60:1, and [OPA]/[NAC]=1:50, i.e., [OPA]/[NAC]/[amine]=20:1000:1, ($1=1\times10^{-9}$ *M*). Detailed data in Table 2.

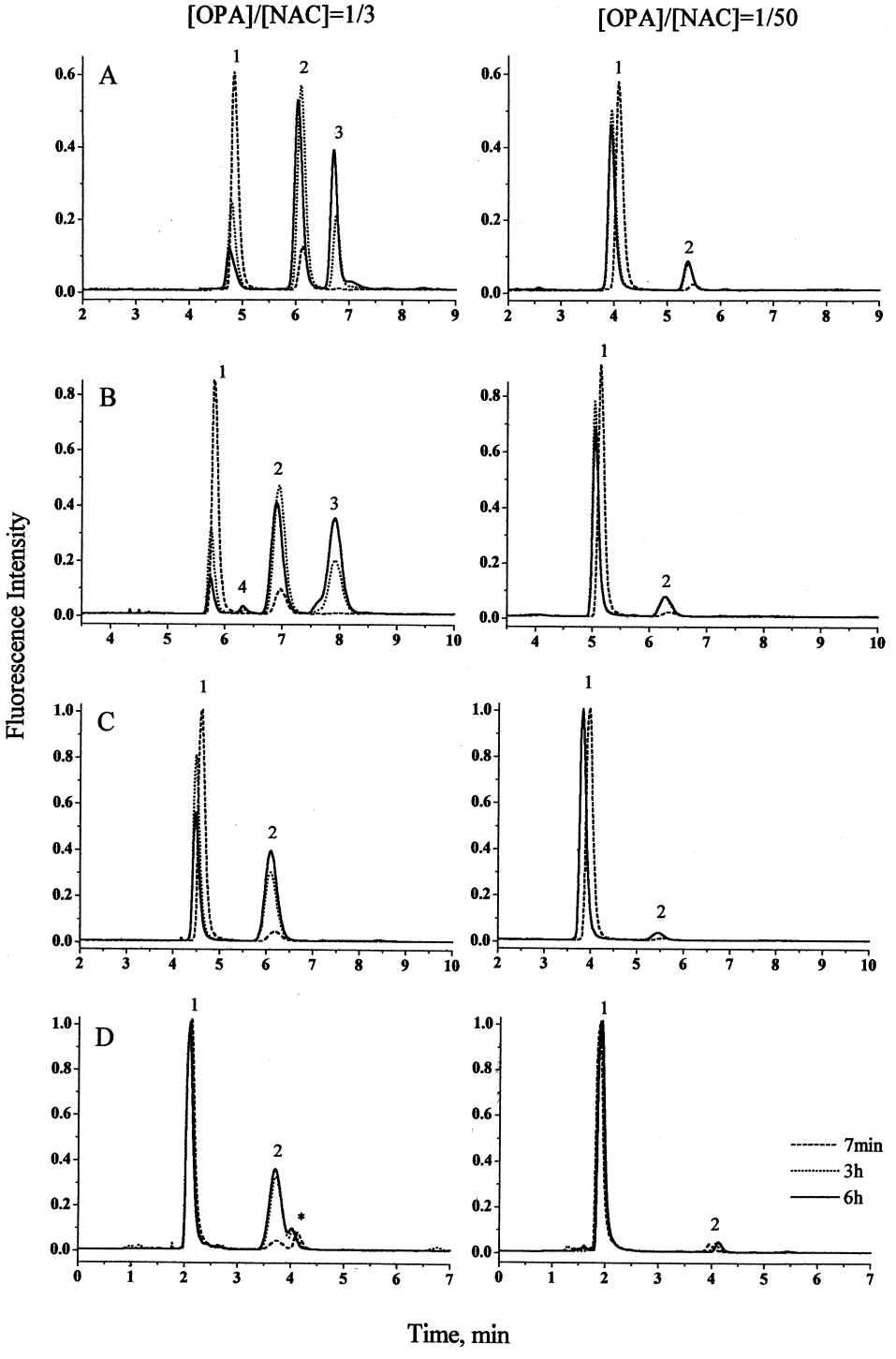

Fig. 3. FL detection chromatograms of putrescine (A), cadaverine (B), tyramine (C) and agmatine (D) obtained after (- - -) 7 min; (· · ·) 3 h; (———) 6 h, with reagents of various composition: [OPA]/[NAC]=1:50, i.e., [OPA]/[NAC]/[amine]=20:60:1, ($1=1\times10^{-9}$ *M*) and [OPA]/[NAC]=1:50, i.e., [OPA]/[NAC]/[amine]=20:1000:1, ($1=1\times10^{-9}$ *M*). Detailed data in Tables 3 and 4; *impurity peak.

Table 2
Stability and characteristics of the OPA/NAC and OPA/MPA derivatives of aliphatic amines and ethanolamine (EA) as a function of the reaction time and reagent composition, based on fluorescence (FL) and UV detection

Amines	Retention time (min)	Fluorescence detection										
		[OPA]/[NAC]=1:3, response (%)*				[OPA]/[NAC]=1:50, response (%)*				[OPA]/[MPA]=1:50, response (%)*		
		90 s	7 min	3 h	6 h	90 s	7 min	3 h	6 h	7 min	3 h	6 h
Ethanolamine1	1.35	98.9	98.6	97.7	97.9	100	100	100	100	100	100	100
Ethanolamine2	1.65	1.1	1.4	2.3	2.1	–	–	–	–	–	–	–
Int unit/pM:												
FL		4.07	3.79	2.99	2.20	3.96	3.71	3.62	3.54	3.90	3.36	2.98
UV		0.37	0.35	0.25	0.19	0.36	0.35	0.34	0.32	0.37	0.32	0.29
Methylamine1	1.85	93.8	82.0	38.5	42.4	99.1	97.9	96.4	96.4	98.2	96.5	97.2
Methylamine2	3.26	6.2	18.0	61.5	57.6	0.9	2.1	3.6	3.6	1.8	3.5	2.8
Int unit/pM:												
FL		4.68	4.50	0.86	0.11	4.55	4.35	3.39	2.76	4.51	2.84	1.89
UV		0.42	0.41	0.079	0.014	0.40	0.38	0.28	0.21	0.39	0.25	0.17
Ethylamine1	2.58	94.5	93.1	43.5	28.1	100	99.7	99.4	99.3	94.8	92.7	91.3
Ethylamine2	3.87	5.5	6.9	56.5	71.9	–	0.3	0.6	0.7	5.2	7.3	8.7
Int unit/pM:												
FL		4.42	4.44	4.33	3.37	4.41	4.40	4.04	3.76	4.48	3.68	3.09
UV		0.39	0.40	0.38	0.29	0.38	0.42	0.38	0.35	0.40	0.33	0.28
Isopropylamine	3.83	100	100	100	100	100	100	100	100	100	100	100
Int unit/pM:												
FL		4.25	4.82	4.74	4.67	2.73	4.81	4.69	4.89	3.90	4.83	4.77
UV		0.37	0.44	0.43	0.42	0.25	0.42	0.42	0.42	0.34	0.42	0.42
n-Propylamine1	4.17	98.2	95.0	53.3	37.6	100	99.5	98.2	98.1	98.5	97.7	97.7
n-Propylamine2	5.71	1.8	5.0	46.7	62.4	–	0.5	1.8	1.9	1.5	2.3	2.3
Int unit/pM:												
FL		4.69	4.71	4.82	4.18	4.57	4.56	4.34	4.19	5.04	4.32	3.72
UV		0.41	0.43	0.43	0.37	0.39	0.40	0.38	0.36	0.44	0.37	0.32
tert.-Butylamine	4.85	100	100	100	100	100	100	100	100	100	100	100
Int unit/pM:												
FL		0.58	0.71	1.33	1.29	0.029	0.050	0.45	0.68	0.055	0.46	0.69
UV		0.17	0.23	0.42	0.41	0.007	0.014	0.14	0.21	0.016	0.14	0.21
sec.-Butylamine	5.47	100	100	100	100	100	100	100	100	100	100	100
Int unit/pM:												
FL		4.34	4.23	4.19	4.15	1.74	2.63	4.18	4.22	2.27	3.34	3.18
UV		0.39	0.38	0.38	0.39	0.16	0.33	0.38	0.38	0.28	0.41	–
Isobutylamine1	5.88	98.9	98.5	83.9	74.1	100	99.7	97.8	97.6	98.8	96.1	96.0
Isobutylamine2	6.88	1.1	1.5	16.1	25.9	–	0.3	2.2	2.4	1.2	3.9	4.0
Int unit/pM:												
FL		4.01	4.06	4.31	4.48	4.37	4.30	4.21	4.13	4.31	3.85	3.38
UV		0.34	0.39	0.40	0.41	0.38	0.39	0.38	0.37	0.39	0.34	0.30
n-Butylamine1	6.23	98.3	94.2	50.3	33.9	100	99.4	97.7	97.6	98.7	97.1	97.1
n-Butylamine2	7.15	1.7	5.8	49.7	66.1	–	0.6	2.3	2.4	1.3	2.9	2.9
Int unit/pM:												
FL		4.07	4.04	4.18	3.62	4.02	4.06	3.88	3.68	3.96	3.24	2.71
UV		0.37	0.38	0.38	0.32	0.36	0.38	0.36	0.34	0.37	0.30	0.25
Isoamylamine1	7.71	99.8	96.3	72.1	58.5	100	99.2	97.5	97.6	nd	nd	nd
Isoamylamine2	8.06	0.5	3.7	27.9	41.5	–	0.8	2.5	2.4	nd	nd	nd
Int unit/pM:												
FL		4.46	4.28	4.41	4.30	4.30	4.24	4.07	3.84			
UV		0.40	0.42	0.43	0.42	0.41	0.42	0.40	0.38			

Indications: [OPA]/[MPA]([NAC])/[amine]=20:60:1 or 20:1000:1 ($1=1\times10^{-9}$ *M*) correspond to mole concentrations, indicated by OPA/NAC=1:3 or 1:50; response (%)*, based on the total of derivatives, obtained with FL detection; nd, not determined.

Table 3
Stability and characteristics of the OPA/NAC derivatives of 1,2-ethylenediamine (EDA), 1,2- and 1,3-propylenediamine (1,2-PDA and 1,3-PDA) depending on the reaction time and reagent composition based on fluorescence (FL) and UV detection

Diamines	Retention time (min)	UV max (nm)	Integrator units (pM)											
			OPA/NAC=1:3						OPA/NAC=1:50					
			FL detection			UV detection			FL detection			UV detection		
			7 min	3 h	6 h	7 min	3 h	6 h	7 min	3 h	6 h	7 min	3 h	6 h
1,2-EDA1	8.90	305	–	–	–	***0.25***	***0.26***	***0.26***	–	–	–	***0.09***	***0.09***	***0.09***
1,2-EDA2	12.02	234	–	–	–	***0.023***	***0.023***	***0.024***	–	–	–	***0.007***	***0.008***	***0.009***
1,2-EDA3	12.72	282	***0.018***	***0.075***	***0.123***	–	–	–	***0.67***	***1.11***	***1.27***	***0.019***	***0.030***	***0.033***
1,2-PDA1	9.02	305	–	–	–	***0.078***	***0.10***	***0.10***	–	–	–	***0.021***	***0.030***	***0.031***
1,2-PDA2	12.57	234	–	–	–	***0.13***	***0.12***	***0.12***	–	–	–	***0.029***	***0.02***	***0.030***
1,2-PDA3	13.60	282	***0.011***	***0.036***	***0.062***	–	–	–	***0.36***	***0.52***	***0.63***	***0.009***	***0.014***	***0.017***
1,3-PDA1	8.98	305	–	–	–	***0.032***	***0.082***	***0.087***	–	–	–	***0.005***	***0.042***	***0.059***
1,3-PDA2%*	10.06	334	85.1	25.8	16.6	95.0	55.3	38.0	99.5	94.6	94.4	100	100	100
1,3-PDA3%*	10.51	339	14.9	59.3	66.5	5.0	40.6	51.8	0.5	5.4	5.6	0.3	1.9	0.9
1,3-PDA4%*	11.28	339	–	7.4	16.9	–	4.1	10.2	–	–	–	–	–	–
Int.units/pM			***0.38***	***0.60***	***0.60***	***0.30***	***0.27***	***0.23***	***0.59***	***0.44***	***0.31***	***0.51***	***0.34***	***0.23***

Indications as in Table 2, as well as: (%)*, expressed in the total of derivatives obtained both by UV and FL detection.

(iv) As to the advantages and disadvantages of conditions to be selected, considerations depend on the compound(s) to be derivatized.

The OPA/NAC=1:50 reagent and 90 s reaction time proved to be the optimum (except if isopropylamine is also present): OPA/NAC derivatives of ethanolamine, ethyl, *n*-propyl, *n*- and isobutyl, as well as isoamylamines can be determined on the basis of a single derivative and that of methylamine on the basis of its two species (methylamine1 and methylamine2). In case of isopropylamine for its quantitative reaction 7 min in reaction time is needed. Except for methylamine (2.1% methylamine2), the amounts of transformed species were below 1%. Thus, depending on their absolute and relative concentrations they should be quantitated either on the basis of their single, or on both of their derivatives. Because of the very slow reaction rate of *tert*.-butyl- and *sec*.-butylamines their selected derivatization time with the OPA should be a matter of compromise: depending on the analyte's other components.

(v) Comparing the percentages of the transformed OPA/NAC derivatives to the corresponding OPA/MPA ones, the advantage of the OPA/NAC derivatization was repeatedly proved (higher responses, slower transformation of the initially formed derivatives).

3.3. Studies on the behavior of diamines upon reaction with the o-phthaldialdehyde/N-acetyl-L-cysteine=1:3 and 1:50 reagents

Introductory results obtained with the OPA/MPA=1:3 (Fig. 1) and OPA/NAC=1:3 reagents (Fig. 3, Tables 3 and 4) with selected diamines provided unexpected and in the same time horrifying results: taking into account that several protocols are based on the quantitation of their OPA derivatives. The only question remains, which of their derivatives were applied as the basis of their quantitation, described in the literature?

3.3.1. Characteristics of the 1,2-ethylenediamine and 1,2- and 1,3-propylenediamines

The first three members of the homologous series of aliphatic diamines provide more than one derivative of a different type: 1,2-EDA1, 1,2-EDA2, 1,2-PDA1,2 and 1,3-PDA1 furnish UV absorbancy, exclusively (Table 3) (EDA, ethylenediamine; PDA, propylenediamine).

(i) The fast forming and stable 1,2-EDA1, 1,2-EDA2 and 1,2-PDA2, as well as the continuously increased amounts of 1,2-PDA1 and 1,3-PDA1 derivatives obtained with the OPA/NAC=1:3 reagent manifested similar properties: they are not fluorescent, provided a UV maxima at 305 and 234 nm (i.e.,

Table 4
Stability and characteristics of the OPA/NAC/diamines depending on the reaction time and reagent composition based on fluorescence (FL) and UV detection

Diamines	Retention time (min)	UV max (nm)	OPA/NAC=1:3								OPA/NAC=1:50							
			FL detection, response (%)*				UV detection, response (%)*				FL detection, response (%)*				UV detection, response (%)*			
			90 s	7 min	3 h	6 h	90 s	7 min	3 h	6 h	90 s	7 min	3 h	6 h	90 s	7 min	3 h	6 h
Agmatine1	1.90	334	98.9	90.6	64.3	57.5	99.0	97.4	75.2	71.3	100	96.0	96.4	96.7	100	92.9	93.8	94.2
Agmatine2	3.97	339	1.1	9.4	35.7	42.6	1.0	2.6	24.8	28.7	0	4.0	3.6	3.3	0	7.1	6.2	5.8
Int.units/pM			***6.98***	***7.47***	***7.60***	***7.43***	***0.76***	***0.67***	***0.65***	***0.65***	***6.94***	***6.35***	***3.36***	***6.28***	***0.72***	***0.70***	***0.65***	***0.62***
Tyramine1	3.97	334	98.7	93.7	59.6	43.1	99.1	94.2	76.2	46.0	99.7	98.9	94.7	94.3	100	98.8	95.3	94.9
Tyramine2	5.60	339	1.3	6.3	40.4	56.9	0.9	5.8	23.8	54.0	0.5	1.4	5.3	5.7	0	1.2	4.7	5.1
Int.units/pM			***4.80***	***4.80***	***5.39***	***5.15***	***0.42***	***0.42***	***0.41***	***0.41***	***4.75***	***4.75***	***4.62***	***4.60***	***0.40***	***0.40***	***0.38***	***0.36***
Spermine1	3.81	334	98.1	98.8	99.5	99.7	96.1	98.2	99.7	99.7	97.2	98.8	99.4	99.3	93.4	97.8	99.4	99.1
Spermine2	6.46	334	1.9	1.2	0.5	0.3	3.9	1.8	0.3	0.3	2.8	1.2	0.6	0.7	6.6	2.2	0.6	0.9
Int.units/pM			***3.54***	***3.36***	***2.87***	***2.86***	***0.41***	***0.32***	***0.26***	***0.26***	***2.47***	***2.34***	***2.02***	***2.07***	***0.29***	***0.23***	***0.18***	***0.18***
Spermidine1	1.57	334	39.3	31.7	4.7	–	33.5	16.7	–	–	8.4	18.3	67.7	57.0	5.7	7.7	9.6	6.5
Spermidine2	3.59	334	59.7	63.3	84.0	100	64.2	55.7	5.4	2.7	90.8	81.7	32.3	43.0	84.3	60.1	5.5	6.6
Spermidine3	4.44	334	1.0	5.0	11.3	–	2.3	23.6	90.0	93.8	0.8	–	–	–	10.0	32.2	84.8	86.9
Spermidine4	6.20	339		–	–	–		4.0	4.6	3.6		–	–	–		–	–	–
Int.units/pM			***7.65***	***5.78***	***0.71***	***0.075***	***0.93***	***0.41***	***0.17***	***0.15***	***3.52***	***4.78***	***1.77***	***1.38***	***0.52***	***0.36***	***0.32***	***0.31***
Putrescine1	4.75	334	95.0	81.1	22.5	11.9	96.8	89.5	39.9	21.2	99.3	96.7	85.1	82.8	99.7	98.2	91.7	90.2
Putrescine2	6.03	339	5.0	18.4	58.6	57.9	3.2	10.5	50.7	83.4	0.7	3.3	14.9	17.2	0.3	1.8	8.3	9.8
Putrescine3	6.70	339		0.5	18.9	36.2		–	10.1	22.4		–	–	–		–	–	–
Int.units/pM			***2.03***	***2.07***	***3.06***	***3.22***	***0.73***	***0.77***	***0.76***	***0.67***	***2.05***	***2.09***	***1.99***	***1.87***	***0.76***	***0.69***	***0.63***	***0.57***
Cadaverine1	5.75	334	96.2	83.8	19.7	7.6	97.6	89.9	32.3	14.9	99.4	97.0	86.0	83.8	99.5	97.8	90.6	89.2
Cadaverine2	6.90	339	3.8	15.4	52.4	43.5	2.4	10.1	51.6	51.2	0.6	3.0	14.0	16.2	0.5	2.2	9.4	10.8
Cadaverine3	7.92	339		0.8	26.8	46.9		–	11.9	31.0		–	–	–		–	–	–
Cadaverine4	6.32	334		–	1.1	2.0		–	1.2	2.9		–	–	–		–	–	–
Int.units/pM			***2.43***	***2.52***	***3.76***	***4.00***	***0.72***	***0.76***	***0.76***	***0.67***	***2.50***	***2.50***	***2.37***	***2.15***	***0.74***	***0.74***	***0.67***	***0.58***
Histamine1	8.83	334	86.1	83.4	78.8	75.5	95.7	90.4	86.9	85.0	87.1	83.8	83.6	85.4	95.7	95.4	95.6	96.3
Histamine2	13.22	353	13.9	16.6	21.2	21.5	4.3	9.6	13.1	25.0	12.9	16.2	16.4	14.6	4.3	4.6	4.4	3.7
Int.units/pM			***3.65***	***3.51***	***2.29***	***1.62***	***0.29***	***0.31***	***0.19***	***0.14***	***3.58***	***3.81***	***3.42***	***3.25***	***0.28***	***0.25***	***0.27***	***0.26***
**BHMTA1	11.75	334	99.1	89.4	43.4	nd	99.3	91.8	46.0	31.2	99.7	99.3	98.3	98.4	99.8	99.4	98.7	98.8
**BHMTA2	12.08	339	0.9	9.5	44.7	nd	0.7	8.2	43.3	48.3	0.3	0.7	1.7	1.6	0.2	0.6	1.3	1.2
**BHMTA3	12.43	339	–	1.1	11.9	nd	–	–	9.7	20.5	–	–	–	–		–	–	–
Int.units/pM			***5.49***	***5.42***	***5.62***		***0.65***	***0.64***	***0.56***	***0.43***	***5.63***	***5.54***	***5.19***	***4.97***	***0.68***	***0.66***	***0.62***	***0.57***

Indications as in Tables 1 and 2 as well as: response (%)*, expressed in the total of responses; E, Ethylene; P, Propylene; **BHMTA, bis(hexamethylene)triamine; Int., Integrator. Note: number of derivatives like Cadaverine4 indicates the time order of their appearance, see also Fig. 3; nd, not determined.

Table 5
Composition of the OPA/NAC derivatives of putrescine, cadaverine, agmatine and tyramine confirmed by on-line HPLC–MS (detailed data in Ref. [6])

Biogenic amines	Dimer isoindoles				Transformed isoindoles				
	Molecular weight	MH^+ calc.	MH^+ obt.	MNa^+	MH^+ + OPA*	MNa^+ + OPA*	MH^+ + OPA–H_2O*	MH^+ + 2OPA**	MNa^+ + 2OPA**
Putrescine	88.2	611.5	**611.5**	633.5	745.6	**767.6**	727.6	879.5	**901.6**
Cadaverine	102.2	625.5	**625.5**	647.5	759.6	**781.6**	741.6	893.7	**915.6**
Agmatine	130.2	392.5	**392.4**	414.4	**526.5**				
Tyramine	137.2	399.5	**399.4**	421.3	533.5	555.4	**515.4**		

Indications: bold printed, abundant masses; *correspond to putrescine2, cadaverine2, agamatin2 and tyramine2; **correspond to putrescine3 and cadaverine3.

they are not of isoindole character, probably originated from the special reaction of the vicinal dioxo compounds with the vicinal diamines resulted in cyclic azine species, except in the case of 1,3-PDA1 which might result in a seven-membered ring). Their UV response values varied between 0.032 and 0.26 integrator units/p*M* in the range of 7 min and 6 h reaction times. Integrator units/p*M*, in order of increasing responses were: 0.032–0.087 for 1,3-PDA1, 0.078–0.10 for 1,2-PDA1 and 0.26 for 1,2-EDA. Derivatives of 1,2-EDA3 and 1,2-PDA3 manifest special behavior: (i) with the OPA/NAC=1:3 reagent they furnish fluorescence responses only, unfortunately with the time in increasing manner and with low intensities. (ii) With the OPA/NAC=1:50 reagent the fluorescence intensities of the OPA derivatives of 1,2-EDA3 and 1,2-PDA3 species considerably increased (0.67–1.27 and 0.36–0.63 integrator units/p*M*, in order of listing for the 1,2-EDA3 and for the 1,2-PDA3 derivatives, respectively).

It is worth mentioning that the formation of the OPA/MPA–1,2-EDA1 (data not shown) and the OPA/NAC–1,2-EDA1 derivatives proved to be proportional to the amines derivatized: in the concentration range of OPA/MPA(NAC)–1,2-EDA1= 20:60:1–200:600:1 the amine was measured with a RSD≤3.1%, consequently proper for analytical purposes. The amounts of these derivatives decreased by using the OPA/NAC=1:50 reagent: probably due to the decreased free OPA concentration needed for their formation: however, it is to be underlined that in the absence of NAC or MPA these derivatives are not formed.

(ii) As to the properties of derivatives of isoindole character (1,3-PDA2-4), the following lessons can be drawn:

The composition of the OPA derivatives of the vicinal diamines (1,2-EDA3 and 1,2-PDA3) is questionable, due to their fluorescence responses only with the OPA/NAC=1:3 reagent, and with the extremely different FL and UV responses obtained with the OPA/NAC=1:50 reagent.

The OPA derivatives of 1,3-PDA (1,3-PDA2-4), obtained both with the OPA/NAC=1:3 and with the OPA/NAC=1:50 reagents proved to be unambiguously of specific isoindole character. Namely, (i) the initially formed 1,3-PDA2 transformed to the 1,3-PDA3 and to the 1,3-PDA4 species, and (ii) both the number of the transformed derivatives and their percentages can be considerably decreased by using the OPA/NAC=1:50 reagent. (Note: since the specific fluorescence intensities of the transformed species are greater compared to the initially formed ones, in order to get reliable comparison of stability values, similarly as in our earlier papers [2–6], even in this study, the UV responses are to be taken into account.)

3.3.2. Characteristics of the biogenic amines and polyamines

(i) Investigations performed as a function of the reaction time confirmed (Table 4, Fig. 3) that the initially formed OPA derivatives of the biogenic amines, having in their initial structure the NH_2–CH_2– moiety, without exception, became transformed to further ones. As a consequence of our experiences obtained with two reagents of various composition, the use of a reagent containing the OPA/SH additive in the 1:50 mole ratio, and 90 s reaction time is to be preferred. In this case (1) agmatine can be determined on the basis of a single derivative, (2) tyramine, spermine, putrescine, cadaverine, histamine and bis(hexamethylene)triamine on the basis of two, while (3) spermidine with three derivatives.

(ii) As to the composition of the transformed derivatives (Table 5) in the case of *n*-propylamine and four diamines they have been determined as their OPA/NAC derivatives obtained with the OPA/NAC=1:3 reagent [6]. Data proved to be in thorough accord with the more than one OPA derivative providing amino acids (having in their initial structure the NH_2CH_2–R moiety). This means that the initially formed isoindoles (abundant masses of the protonated molecular ions (MH^+) became transformed to an additional OPA molecule-containing species (MH^++OPA); The reaction mechanism pathway of the transformation was also given in detail [6]). In case of putrescine and cadaverine, being diamines, both of their isoindoles do react with an additional molecule of OPA (Table 4: putrescine3, cadaverine3; Table 5: MH^++2OPA=putrescine3=m/z=879.5, MH^++2OPA=cadaverine3=m/z=893.7).

Results of our derivatization studies called atten-

tion to the fact that (iii) all those data found in the literature should be treated cautiously; in terms of reliability, and comparability. As to the, one by one, reported reproducibilities, no doubt they might be true, but not reliable and comparable: the peak that served as the basis for the quantitation of the amine in question, remains to be defined, because they could be different from procedure to procedure.

3.4. Conclusion

(1) A comprehensive summary of papers dealing with the HPLC quantitation of amines as OPA derivatives has been discussed in detail.

(2) The behavior and characteristics of the OPA/MPA and OPA/NAC derivatives of C_1–C_5 aliphatic amines and several diamines have been studied from analytical point of view.

(3) By using a reagent with a considerably increased molar excess of the SH-group additive corresponding to the OPA/SH additive=1:50 mole ratios resulted in two benefits: in an increased stability of the derivatives and in a decreased number of species formed.

Acknowledgements

This work was supported by the Hungarian Academy of Sciences and the Ministry of Education and Culture (project No.: OTKA T-033100 and FKFP-0191/1997).

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