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New aspects of the simultaneous analysis of amino acids and amines as their *o*-phthaldialdehyde derivatives by high-performance liquid chromatography
Analysis of wine, beer and vinegar

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Abstract

A new high-performance liquid chromatography method is described for the simultaneous quantitation of amino acids and amines for 37 compounds (20 amino acids+17 amines), as their *o*-phthaldialdehyde (OPA)−3-mercaptopropionic acid derivatives, within 53 min. Based on previously documented stoichiometric and reaction mechanism studies, derivatizations have been carried out with the OPA–SH-group=1:50 containing reagents. Reliability and reproducibility of analyses have been considerably improved. Average reproducibility data in a wide concentration range of derivatives had RSD≤3.4%.

Keywords: Wine; Beer; Food analysis; Derivatization, LC; Amino acids; Amines

1. Introduction

Conditions, characteristics and the mechanism of the *o*-phthaldialdehyde (OPA) derivatization of amino acids [1–6,8] and amines [6,7] have been discussed in detail [1–8]. Recent studies carried out outside [1] and inside the chromatographic system [2–8], demonstrated the reason and background for the ''instability'' of the overwhelming part of the primary amino-group containing compounds: all of those OPA derivatives that are providing the so-called ''less stable'' products contain in their initial structure the $-CH_2-NH_2-$, moiety. On the basis of these experiences, the mechanism of the reaction has been clarified [6], and, in order to quantitate amino acids [2–6,8] and amines [7], optimum derivatization conditions were developed. The practical utilization in the analysis of OPA derivatives was shown by the assay of the free amino acid contents of apples without any pretreatment [3].

On the basis of a detailed literature survey, it turned out that no attention had been paid to review the importance and possibilities of the simultaneous analysis of amino acids and amines, in spite of the fact that: (i) several matrices contain both groups, and (ii) the qualitative and quantitative knowledge of amino acids and amines, simultaneously, might be obligatory. Amino acids and amines are co-existing compounds in several biological and food matrices, taking place in various physiological and technical transformation processes. Therefore we summarized papers relating to the above topic [9] and also

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developed a new method using our many-sided experiences in OPA derivatization even in this term.

In the present paper, amino acid and amine contents of the same matrix are quantified without any preliminary extraction, in the presence of each other, as the same derivatives, from the same solution, by a single run. In the need to solve the task of the simultaneous analysis of amino acids and amines, the latter method is the method of choice. This means all other techniques, such as determination of amino acids and amines after their isolation from each other, in separate matrices, as different derivatives, lead to considerable losses of both groups, require more time and are more costly compared to the simultaneous ones. Extraction/separation, derivatization and chromatographic conditions, as well as criticism of proposals found in the literature, illustrated by more than 60 references, have been discussed in detail [9].

2. Experimental

2.1. Materials

OPA, 3-mercaptopropionic acid (MPA), *N*-acetyl-L-cysteine (NAC), amino acids and amines were obtained from Sigma (St Louis, MO, USA) and from Serva (Heidelberg, Germany). HPLC-grade methanol (MET) and acetonitrile (ACN) were purchased from Romil (Leics, UK). All other reagents were of the highest purity available.

Beer, wine and vinegar samples were purchased from a food store.

2.1.1. Beers

Dreher bak, Dreher classic and Dreher Lager were termed, in order of listing, as Dreher 1–3, Austrian hofpills, as hofpills.

2.1.2. Wines

''Badacsonyi Szürkebarát'', a Bulgarian Vermouth ''Vedmezsa Krov'' and a Hungarian red wine were termed as white, red 1 and red 2 wines, respectively. The vinegar was a Hungarian wine-vinegar, termed vinegar.

2.2. Standard solutions

Standard solutions of free amino acids and amines have been prepared with distilled water in the concentrations of $\sim 1\text{–}2\times10^{-2}$ *M* and further diluted before use. The wines, beers and vinegar were diluted twofold with distilled water before use. A stock solution of OPA contained 0.25 g OPA (weighed with analytical precision) in 50 ml methanol (termed methanolic OPA solution).

2.3. Buffer solution

Borate buffer was mixed in 50:50 volume 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

The OPA–MPA ≅ 1:50 reagent was obtained by mixing, in order of listing, 5.0 ml methanolic OPA, 20.0 ml borate buffer and 0.99 g MPA solution: finally, if necessary, it was adjusted with 1 *M* sodium hydroxide, to pH 9.3±0.05.

OPA–NAC reagent was prepared from 5 ml methanolic OPA solution and 20.0 ml borate buffer containing 1.52 g NAC: final pH 9.3±0.05.

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

2.5. Characterization of the reagent solutions

Blank elutions were carried out with freshly prepared (reagent age≥90 min [2]) reagent solutions, stored in the refrigerator (before use if necessary filtered on a funnel covered with glass fiber paper) (~4 °C) and injected by the robotic autosampler, every day at least two times (Waters 717, thermostatted for ~4 °C).

2.6. Derivatizations with the OPA–MPA(NAC)–amino acid solutions

Derivatizations were carried out with reagents prepared at least 90 min earlier and saved for no longer than 9 days [2]. The calculated amounts of reagent solutions were mixed with the selected

Table 1
Optimum gradient for the simultaneous elution of 17 amines

Step	Time (min)	Flow (ml)	A (%)	B (%)
1	0.00	1.00	60.0	40.0
2	24.00	1.00	25.0	75.0
3	27.00	1.00	0.0	100.0
4	27.10	1.40	0.0	100.0
5	30.00	1.40	0.0	100.0
6	31.00	1.40	60.0	40.0
7	38.00	1.40	60.0	40.0

amounts of amines or amino acids+amines and allowed to react for 15–30 min before injection (if not otherwise stated).

2.7. Chromatography

2.7.1. Reproducibility studies: simultaneous photodiode array (DAD) and fluorescence (FL) detection

The system was a Waters HPLC instrument (Waters Pharmaceutical Division, Milford, MA, USA) equipped with Waters 996 DAD and Waters 274 fluorescence detection systems, a Waters 600 controller quaternary pump with a thermostattable column area and a Waters 717 autosampler, operating with Millennium software (version 2010, 1992–95, validated by ISO 9002). The column was a Hypersil ODS bonded phase, 200 mm×4 mm, 5 μm, used with a 20 mm×4 mm guard column.

Detections have been carried out simultaneously: DAD (Waters 996) and FL (Waters 274) detection systems were connected in that order. Blank tests and concentration dependence have been recorded between 190 and 400 nm (DAD) and evaluated at 334 nm [OPA–MPA(NAC)-amino acids] as well as at the optimum fluorescence wavelengths (ex/em= 337/454 nm).

2.8. Elution programs

Reproducibility studies of 17 amines have been carried out in gradient mode consisting of two components: (A) eluent was 0.05 *M* sodium acetate, pH 7.2, while (B) eluent was prepared from 0.1 *M* sodium acetate–ACN–MET (40:45:15), mixed in volume ratios and titrated with glacial acetic acid or 1 *M* sodium hydroxide to pH 7.2 (Table 1).

Reproducibility studies of the simultaneous analysis of amino acids and amines have been carried out by means of a terner gradient system: (A) eluent was 0.05 *M* sodium acetate, pH 7.2, while (B) eluent was prepared from 0.1 *M* sodium acetate–ACN–MET (46:44:10), mixed in volume ratios and titrated with glacial acetic acid or 1 *M* sodium hydroxide to pH 7.2. Eluents C and D were MET and ACN, respectively (Table 2).

Table 2
Optimum gradient for the simultaneous elution of amino acids and amines

Step	Time (min)	Flow (ml)	A (%)	B (%)	C: MET (%)	D: ACN (%)
1	0.00	1.30	97.0	3.0	0.0	0.0
2	8.00	1.30	97.0	3.0	0.0	0.0
3	9.00	1.30	93.0	7.0	0.0	0.0
4	11.00	1.70	85.0	15.0	0.0	0.0
5	12.00	1.70	81.0	16.0	3.0	0.0
6	16.00	1.70	72.0	18.0	8.0	2.0
7	19.00	1.50	72.0	8.0	16.0	4.0
8	22.00	1.50	60.0	26.0	10.0	4.0
9	29.00	1.30	60.0	25.0	15.0	0.0
10	42.00	1.30	22.0	71.0	0.0	7.0
11	45.00	1.30	0.0	86.0	7.0	7.0
12	48.00	1.70	0.0	86.0	7.0	7.0
13	48.10	1.70	97.0	3.0	0.0	0.0
14	53.00	1.30	97.0	3.0	0.0	0.0

3. Results and discussion

3.1. Quantitation of 17 amines from a single run as their OPA–NAC and OPA–MPA derivatives

Our first aim was to determine as many amines as possible from a single run, in order: (i) to find a proper gradient program completing our stoichiometric and MS studies [6,7], and (ii) to define their reproducibility in a wide concentration range applying the OPA–SH-group containing additive in the mol ratios of OPA–SH-group=1:50 [7].

As to the reagent blank, i.e. the quantity and quality of impurities present in the OPA–MPA(NAC)=1:50 reagents (similar to those of the OPA–MPA(NAC)=1:3 reagents [2]), have been followed by simultaneous DAD and FL detection, from 1 day until 9 days (Table 3) applying a gradient program developed for amines (Table 1). It has been repeatedly proven that in order to obtain reproducible and reliable results, blank values should be extracted from the coeluting amino acid and/or amine derivatives. Certainly, depending both on the gradient program and on the amino acid or amine in question, which of the impurities is the coeluting, consequently the subtractable ones. As to the quantity of the impurities, accordingly our elution program (Table 1), in general, 20–25% of impurities proved to be the coeluting ones. It means that based on the total impurities detailed in Table 3, in the case of neglecting them, this can lead to the over-estimation of constituents as follows.

Taking into account an average of 500 integrator units (i.u.)/run for FL (2.5 i.u./p*M*) and of 300 i.e/run for UV responses (15 i.u./p*M*): (1) in the case of FL detection 250 p*M*/run (0.25×500×2.5=250), while (2) applying UV detection 1125 p*M*/run (0.25×300×15=1125) might be the total amount of

Table 3
Impurities in the OPA–MPA=1:50 reagent

Impurity peaks			Changes of integrator units with time (days)		
No.	Retention time (min)	Maxima (nm)	1	3	9
1FL	2.05		155	103	123
UV		225	32	80	31
2FL	2.24		93	85	111
UV		225	–	–	3
3FL	2.80		46	62	61
UV		252, 294	104	166	185
4UV	3.22	286, 215	27	41	21
5FL	4.22		5	5	10
6FL	5.50		152	42	64
UV		229	3	13	2
7FL	13.83		6	6	4
8FL	14.65		43	27	32
UV		229, 334	3	4	2
9UV	16.10	229, 334	3	3	11
10FL	16.55		13	19	11
11FL	31.32		–	40	25
12UV	32.53	248, 329	10	15	9
13FL	32.86		–	33	27
14FL	33.45		–	16	12
In total					
FL			513	438	480
UV			182	312	289

–, not detectable; ~2.5 integrator units (i.u.) correspond to 1 p*M* amino acid derivative with FL detection and ~15 p*M* derivative with UV detection.

impurities/run. Consequently, the amount of impurities cannot be neglected.

Our present consideration regarding the simultaneous elution of amino acids and amines was based on the fact that the gradient programs both for the OPA–MPA-amino acids and OPA–NAC-amino acids were already optimized [3]: we intended to complete our earlier optimized programs for amino acids with

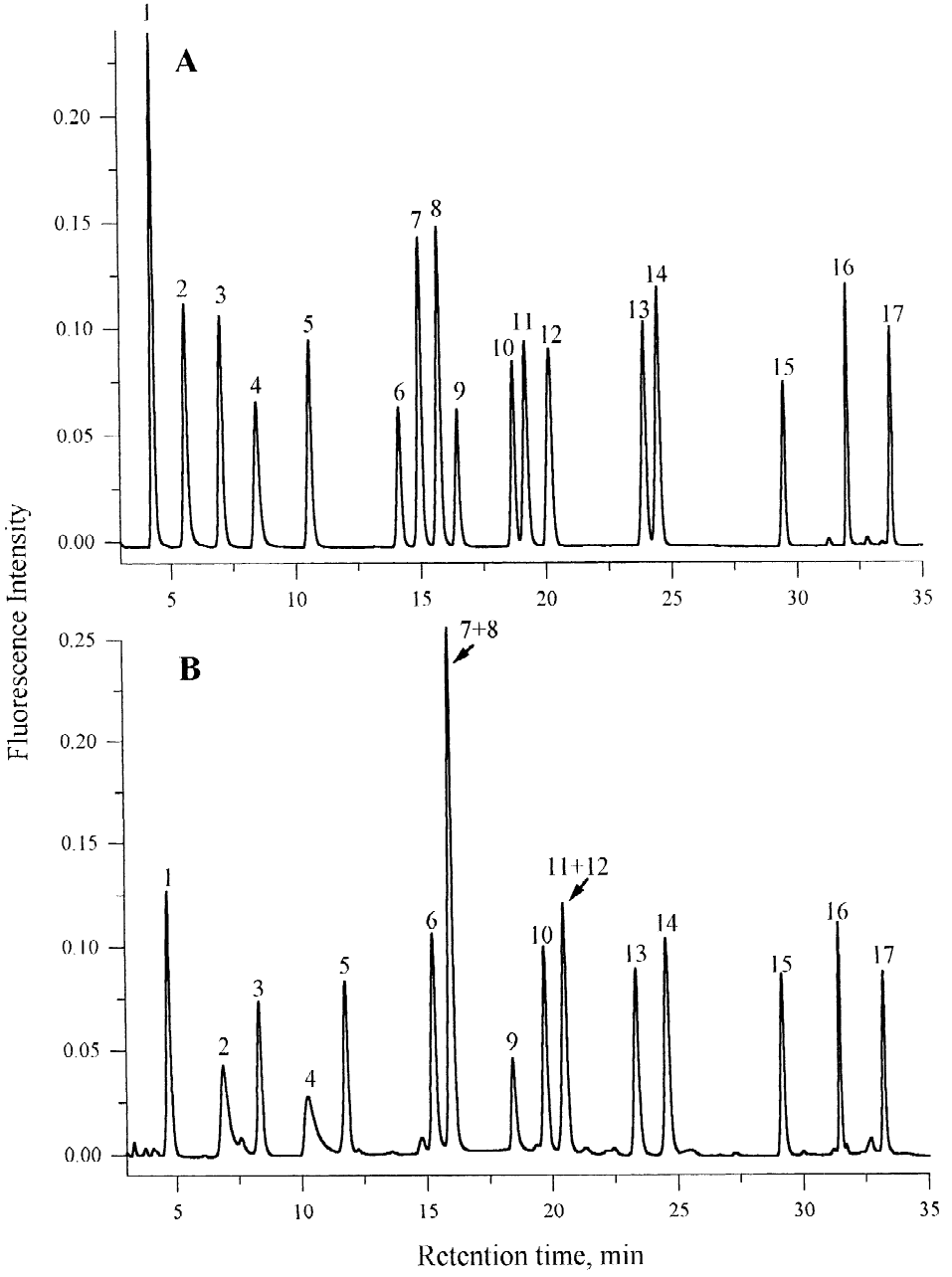

Fig. 1. Elution profile of the OPA–NAC-amines (A) and OPA–MPA-amines (B) applying optimum conditions (Table 1); peak numbers correspond to those indicated in Table 4.

the actually developed one for amines, combining the two programs, in a second step, with as few alterations as necessary (Fig. 1a,b).

As seen, the gradient program for the OPA–NAC-derivatized amines proved to be excellent (Fig. 1a) while the corresponding one for the OPA–MPA-amines (Fig. 1b) would have needed further improvement. Thus, the stoichiometric reproducibility studies of amines have been carried out with the OPA–NAC-amines.

However, we have been aware of the fact that for the simultaneous assay of amino acids and amines, from a practical point of view, the OPA–MPA derivatives are to be selected—in order to avoid the separate elution of the anomer pairs expected from natural matrices. Thus, the final chromatographic optimization of the OPA–MPA-amines was planned to finish simultaneously with the amino acids combining the two elution gradients with the necessarily needed alterations, step by step.

After an introductory study regarding optimum derivatization time both with the OPA–MPA and OPA–NAC=1:50 reagents, in accordance with our earlier experiences [6], the 15–30 min reaction time seemed to be the best compromise (Table 4). As seen, reproducibility values, in a wide concentration range (Table 4, vertical columns 4–7) proved to be acceptable (RSD≤3.1%). It is worth mentioning that remaining on the safe side in order to obtain quantitative derivatization of amines, the OPA–amine mol ratio should be equal to or higher than 8.97:1. The only exception was iso-propylamine: because of its slow reaction rate, its evaluation needs to be followed by a non-linear calibration curve.

3.2. Simultaneous determination of amino acids and amines in total 37 compound as their OPA–MPA derivatives

Chromatographic method development for the simultaneous separation of amino acids and amines was a gradient optimization study that had to be carried out, cautiously, step by step: the elution program, developed for the OPA–MPA-amino acids

Table 4
Reproducibility in the quantitation of different amounts of OPA–NAC-amines in model solutions on the basis of their fluorescence intensities

Amine	Retention time (min)	Arbitrary units (1 p*M* amino acid[a])						
		p*M* injected[c]	[OPA]–[NAC]:[amine]^T				Average[b]	RSD (%)
			8.97:1	17.93:1	35.87:1	71.74:1		
1 Ethanolamine	4.15	760	1.87	1.86	1.85	1.82	1.85	1.2
2 Histamine	5.30	700	1.75	1.78	1.65	1.73	1.73	3.2
3 Methylamine	6.65	610	1.90	1.88	1.76	1.80	1.84	3.6
4 Agmatine	8.00	666	1.43	1.47	1.48	1.56	1.49	3.7
5 Ethylamine	10.08	637	*1.55*	1.73	1.73	1.66	1.67	2.2
6 Isopropylamine	13.60	799	0.672	1.00	1.14	1.33	n.av.	
7 *n*-Propylamine	14.42	666	2.22	2.40	2.32	2.55	2.34	4.1
8 Tyramine	15.13	677	*2.34*	2.44	2.35	2.41	2.41	2.0
9 Putrescine	15.83	642	1.05	1.01	0.953	0.975	0.997	4.3
10 Cadaverine	18.60	664	1.24	1.28	1.25	1.28	1.26	1.7
11 Isobutylamine	19.53	587	*1.80*	1.92	1.94	2.05	1.96	3.1
12 *n*-Butylamine	20.35	674	1.76	1.89	1.86	1.90	1.85	1.9
13 Isoamylamine	23.85	728	1.58	1.62	1.56	1.61	1.59	1.7
14 Phenylethylamine	24.35	684	*1.92*	2.07	2.02	2.09	2.05	1.6
15 Hexylamine	29.03	607	1.50	1.60	1.56	1.60	1.57	3.0
16 Heptylamine	31.79	594	1.64	1.73	1.65	1.71	1.68	2.6
17 Octylamine	33.55	557	1.59	1.64	1.59	1.65	1.62	2.0

1–17=number of amines correspond to those in Fig. 1A; data in italics have been omitted from the mean; n.av.=not averagable.
[a] Obtained from at least three separate tests.
[b] Obtained with mol ratios of [OPA–(NAC)]:[amino acid]^T=8.97:1, 17.93:1, 35.87:1, 71.74:1, [OPA]=1.01×10^{-7} *M*.
[c] p*M*=the maximum amount of amines=[Amine]^T=11 263$\times10^{-12}$ *M*/injected (10 μl), [OPA]=1.01×10^{-7} *M*.

[3], was followed until 11 min with the only exception being the flow-rate which was increased from 11 min onwards to 1.7 ml/min. The ''hardest'' range to be optimized was the section between 11 and 42 min: this elution range proved to be common for selected amino acids and amines. The impact of ACN and MET was different towards the coeluting neighbors: consequently, their best achievable resolution had to be tested from neighbor to neighbor, one by one. The optimum chromatographic process is described in Table 2 and presented in Fig. 2. It is unnecessary to emphasis that in order to increase the stability of derivatives and to decrease their number formed, the OPA–MPA=1:50 reagent had to be applied [7] resulting in maximum and reproducible responses.

The concentration of constituents in this model solution was prepared according to the expected composition of beers, wines and the vinegar. This means selected amino acids are represented by high concentrations but amines by low concentrations: alanine was measured in the range of 1000–250 p*M*/injection, with ethylamine in the range of 44.9–5.6 p*M*/injection (Table 5: mol ratios from 8.47:1 to 67.78:1). Reproducibility values, in cases of amino acids and amines, in order of listing, on average, proved to be 3.6 and 3.3% (RSD%), respectively.

3.3. Simultaneous determination of amino acids and amines in beer, wine and in vinegar samples as their OPA–MPA derivatives

The quantitation of the amino acid and amine constituents in four beers, three wines and in a wine-vinegar, without any pretreatment was carried out to prove practical utility of our method (Table 6, Figs. 3 and 4). In comparison to all earlier simultaneous processes compiled in our review article [9], the advantages offered by the present developed technique can be summarized as follows:

(i) amino acids and amines, in a total of 37 compounds, were determined within 53 min.

(ii) Their derivatization was based on previously well documented stoichiometric studies [2–8]

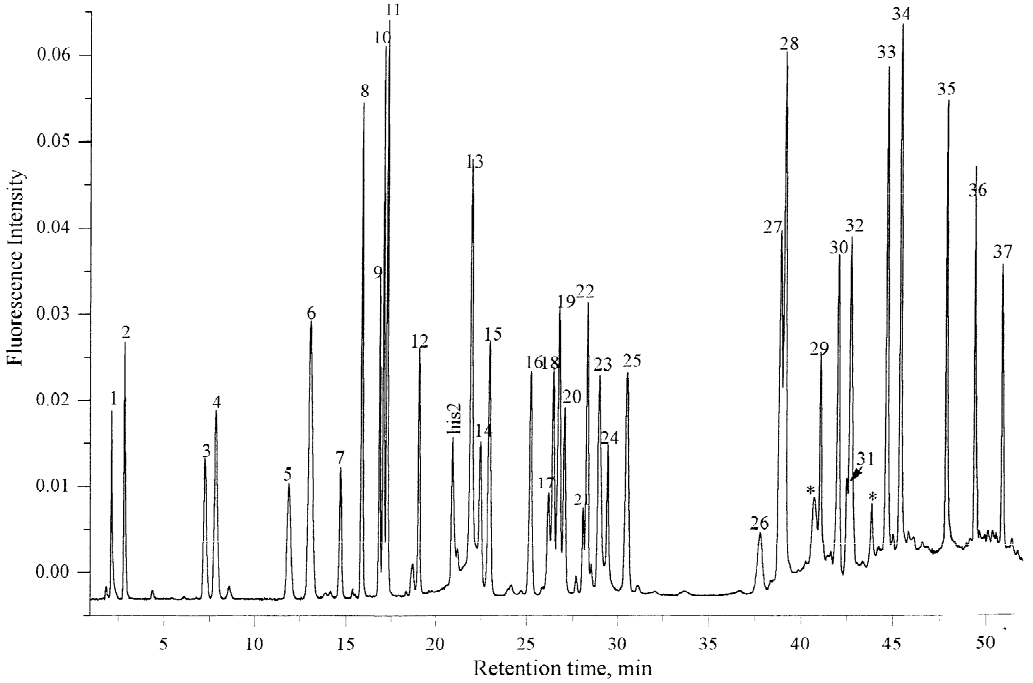

Fig. 2. Elution profile of amino acids and amines derivatized from model solutions with the OPA–MPA=1:50 reagent, applying optimum conditions (Table 2); peak numbers correspond to those indicated in Table 5 as well as: 17, histidine; 27, ornithine; 26, isopropylamine; 28, tyramine; 30, isobutylamine; 35, hexylamine; 36, heptylamine; 37, octylamine.

Table 5
Simultaneous quantitation of different amounts of OPA–MPA-amino acids and amines in model solutions on the basis of their fluorescence intensities

Amino acids or amines	Retention time (min)	Arbitrary units (1 p*M* amino acid[a])						
		p*M* injected[c]	[OPA]–[MPA]:[amino acid, amine][T]				Average[b]	RSD (%)
			8.47:1	16.9:1	33.8:1	67.7:1		
1 Aspartic acid	2.12	363.4	2.81	2.70	2.90	2.80	2.80	3.0
2 Glutamic acid	2.78	392.8	3.44	3.57	3.77	3.74	3.63	4.0
3 Asparagine	6.87	320.0	3.26	3.24	3.54	3.53	3.39	4.6
4 Serine	7.53	191.3	3.76	3.61	3.99	*4.15*	3.88	5.1
5 Glutamine	11.48	74.8	*3.73*	3.96	4.09	4.10	3.97	4.3
6 Glycine	12.72	225.7	4.09	4.10	4.31	4.42	4.23	3.9
7 Threonine	14.42	351.5	3.03	3.05	3.31	3.29	3.17	4.8
8 β-Alanine	15.80	397.6	2.10	2.09	2.21	2.29	2.17	4.4
9 Alanine	16.80	1003	3.40	3.39	3.58	3.47	3.46	2.5
10 Arginine	17.02	1795	4.76	4.91	5.05	4.89	4.90	2.6
11 γ-Aminobutyric acid	17.58	1439	3.73	3.80	3.90	3.99	3.85	3.0
12 Tyrosine	19.18	355.6	3.86	3.80	4.15	4.03	3.96	3.9
13 Ethanolamine	22.05	543.9	6.66	6.73	7.09	6.99	6.87	3.0
14 Valine	22.77	383.8	3.36	3.20	3.52	3.38	3.36	3.8
15 Methionine	23.05	400.0	2.19	2.11	2.21	2.17	2.17	2.7
16 Tryptophan	25.26	293.5	9.93	9.53	10.08	9.69	9.80	2.7
18 Phenylalanine	26.45	361.0	*5.06*	4.47	4.60	4.43	4.5	2.0
19 Methylamine	26.67	89.1	4.14	3.83	3.97	3.85	3.95	4.2
20 Isoleucine	27.03	364.0	3.33	3.55	3.66	3.67	3.55	4.2
22 Leucine	28.08	179.0	4.74	4.68	5.00	5.09	4.88	3.9
23 Agmatine	28.85	189.5	3.07	3.08	3.13	3.17	3.11	2.3
24 Lysine	30.12	182.0	1.27	1.27	1.24	1.29	1.26	2.7
25 Ethylamine	30.75	44.9	4.30	4.06	4.09	4.19	4.16	2.7
26 Spermine	39.00	491.1	10.26	10.16	9.82	10.05	10.07	2.6
29 Cadaverine	42.58	46.9	3.01	2.94	2.91	n.d.a.	2.95	1.9
31 Butylamine	43.50	47.6	3.27	3.13	3.16	n.d.a.	3.18	2.0
32 Putrescine	43.98	103.4	9.26	8.39	7.84	n.d.a.	8.49	8.7
33 Phenylethylamine	45.32	96.9	5.87	5.85	5.87	5.72	5.81	1.3
34 Isoamylamine	46.08	103.1	5.21	5.13	5.03	n.d.a.	5.12	2.0

1–34=numbers of amino acids and amines correspond to those in Fig. 2; The failing compounds (17, 21, 26, 28, 30, 35–37) were not included in the model solution; data in italics have been omitted from the mean; n.d.a.=no data available.

[a] Obtained from at least three separate tests.

[b] Averages, obtained with mol ratios of [OPA–(MPA)]:[amino acid, amine][T]=67.78:1, 33.89:1, 16.94:1, 8.47:1, [OPA]=9.17×10^{-8} *M*.

[c] p*M*=the maximum amount of amino acids+amines=1.0829×10^{-8} *M*.

ensuring stability, and consequently reliability and reproducibility to the maximum possible extent of the selected compounds to be derivatized.

4. Conclusions

A new, simultaneous analytical method was proposed, based on earlier stoichiometric and reaction mechanism studies of the OPA derivatization reaction. The separation and quantitation of 20 amino acids and 17 amines was carried out in a single run as their OPA–MPA derivatives within 53 min, ensuring the reproducible quantitation of amino acids and amines applying photodiode array and fluorescence detection, simultaneously. The practical utility of the proposed chromatographic technique was shown by the analysis of the amino acid and amine content of

Table 6
Quantitation of the free amino acid and amine content of beers, wines and vinegar, measured as their OPA–MPA derivatives, applying optimum conditions

Amino acid, amine	Amino acids and amines (mg/l)							
	Beers				Wines			Vinegar
	Dreher 1	Dreher 2	Dreher 3	Hofpills	White	Red 1	Red 2	
1 Aspartic acid	6.30	3.98	0.34	5.68	8.12	4.04	3.08	4.78
2 Glutamic acid	7.02	8.98	2.76	10.9	18.3	4.16	4.48	8.88
3 Asparagine	3.94	0.38	0.44	4.98	5.66	1.14	0.56	2.22
4 Serine	2.24	0.92	0.44	3.36	5.80	1.06	1.98	1.04
5 Glutamine	1.78	1.26	1.24	0.90	–	20.9	–	1.30
6 Glycine	16.6	14.4	7.20	3.28	7.50	1.94	2.68	9.04
7 Threonine	0.46	0.34	0.30	2.36	3.24	2.16	1.78	0.38
8 β-Alanine	1.52	1.36	1.00	0.46	1.36	0.20	0.64	0.46
9 Alanine	20.6	27.6	16.3	11.2	18.3	5.20	6.90	21.1
10 Arginine	7.42	20.9	17.5	21.5	50.0	2.56	1.62	21.3
11 γ-Aminobutyric acid	32.4	40.5	30.6	7.72	16.6	3.0	6.90	21.5
12 Tyrosine	16.9	12.5	6.16	3.28	3.70	0.76	0.88	14.1
13 Ethanolamine	3.58	4.66	4.04	4.26	5.56	2.30	2.06	3.44
14 Valine	14.2	12.3	2.26	2.76	3.08	1.40	2.44	12.0
15 Methionine	3.62	1.60	0.46	1.66	1.52	0.20	0.32	1.46
16 Tryptophan	3.84	4.28	5.14	2.78	5.44	–	0.40	4.64
18 Phenylalanine	15.9	10.7	1.66	4.84	4.24	1.04	1.80	15.1
19 Methylamine	0.58	0.20	0.16	0.18	0.20	0.060	0.12	0.26
20 Isoleucine	6.96	4.28	0.22	1.96	1.98	0.84	1.12	4.24
22 Leucine	12.4	9.18	–	6.64	7.68	2.30	1.88	9.00
23 Agmatine	13.0	12.1	9.76	–	–	–	–	18.2
24 Lysine	4.90	10.9	–	12.5	14.0	4.30	4.24	9.26
25 Ethylamine	0.18	0.14	–	0.34	0.64	0.20	0.40	0.10
26 Isopropylamine	–	–	–	0.88	0.14	0.080	0.060	–
27 Spermidine	0.44	0.14	0.74	5.18	1.34	0.56	0.42	0.32
28 Tyramine	1.16	0.52	–	–	–	–	1.34	1.04
29 Cadaverine	1.0	1.36	0.080	0.38	1.22	0.38	20.2	2.04
30 Isobutylamine	0.060	–	–	0.040	0.14	–	–	0.040
31 Butylamine	0.060	–	–	0.040	0.14	–	–	0.040
32 Putrescine	2.06	0.90	–	1.40	1.26	0.14	–	1.56
33 Phenylethylamine	0.24	0.14	0.036	0.040	0.16	–	0.22	0.12
34 Isoamylamine	0.14	0.040	0.034	0.24	0.18	0.020	0.52	0.14
Amino acids in total (mg/l)	201.5	206.5	108.9	121.7	187.6	60.9	69.0	189.1
10^{-9} M/10 μl[a]	50.3	51.6	27.2	30.4	46.9	15.2	17.3	47.3

Conditions as in Tables 4 and 5, as well as: quantitations were carried out at mol ratios of [OPA]–[MPA]–[amino acid, amine]^{T}=3×10^{-7} M: 150×10^{-7} M:15.2–51.6×10^{-9} M; the number of compounds corresponds to those in Figs. 3 and 4; sensitivity of the fluorescence detector (expressed as gain; g) has been changed throughout the elution procedure: 0–4 min=1 g, 4–16.6 min=10 g, 16.6–18.6 min=1 g, 18.6–53.0 min=100 g.

[a] The total amount of amino acids and amines was calculated uniformly on the basis of M_w=100.

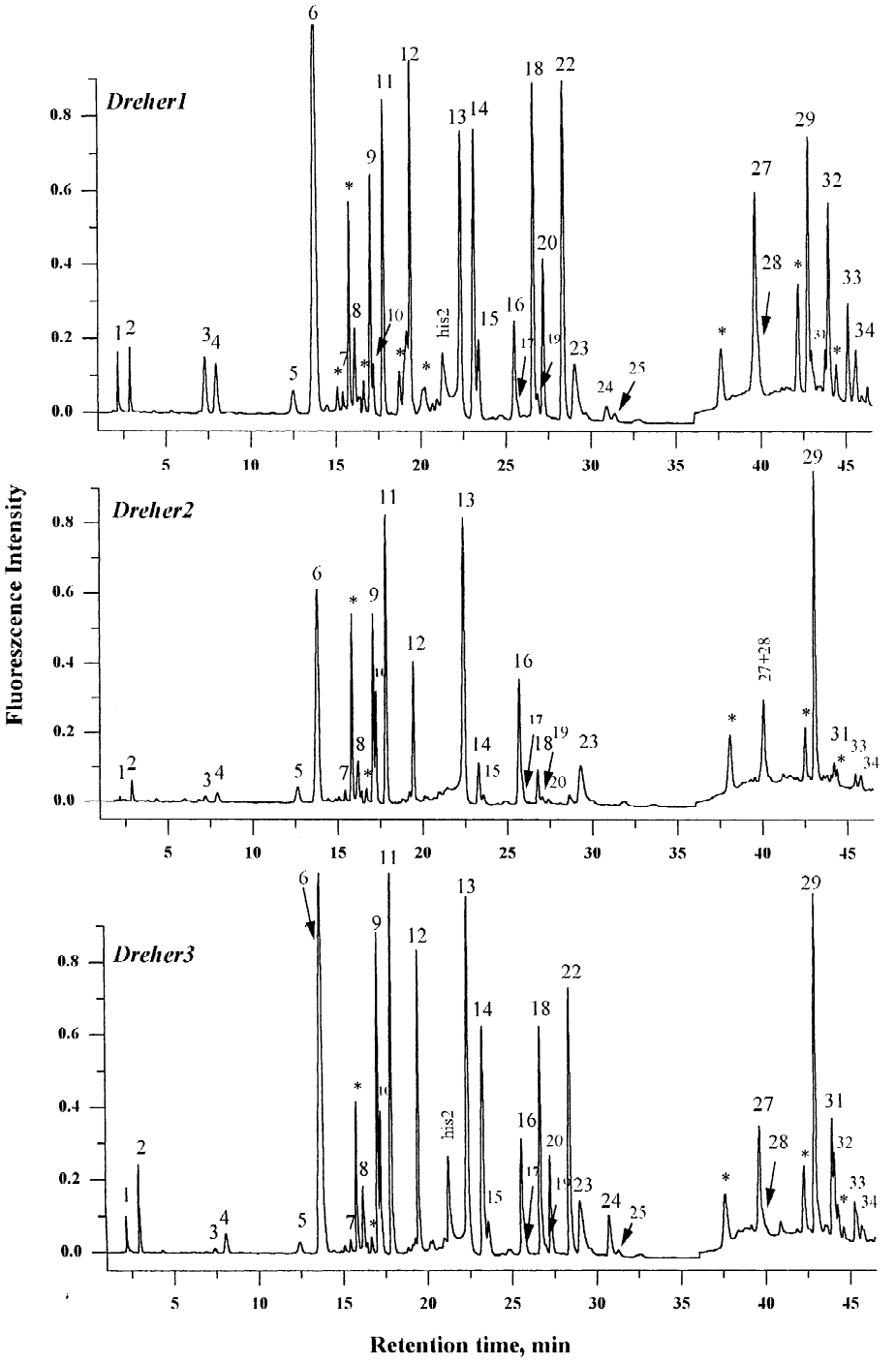

Fig. 3. Elution profile of the OPA–MPA derivatives obtained from beers (Dreher 1–3); peak numbers correspond to those indicated in Table 6).

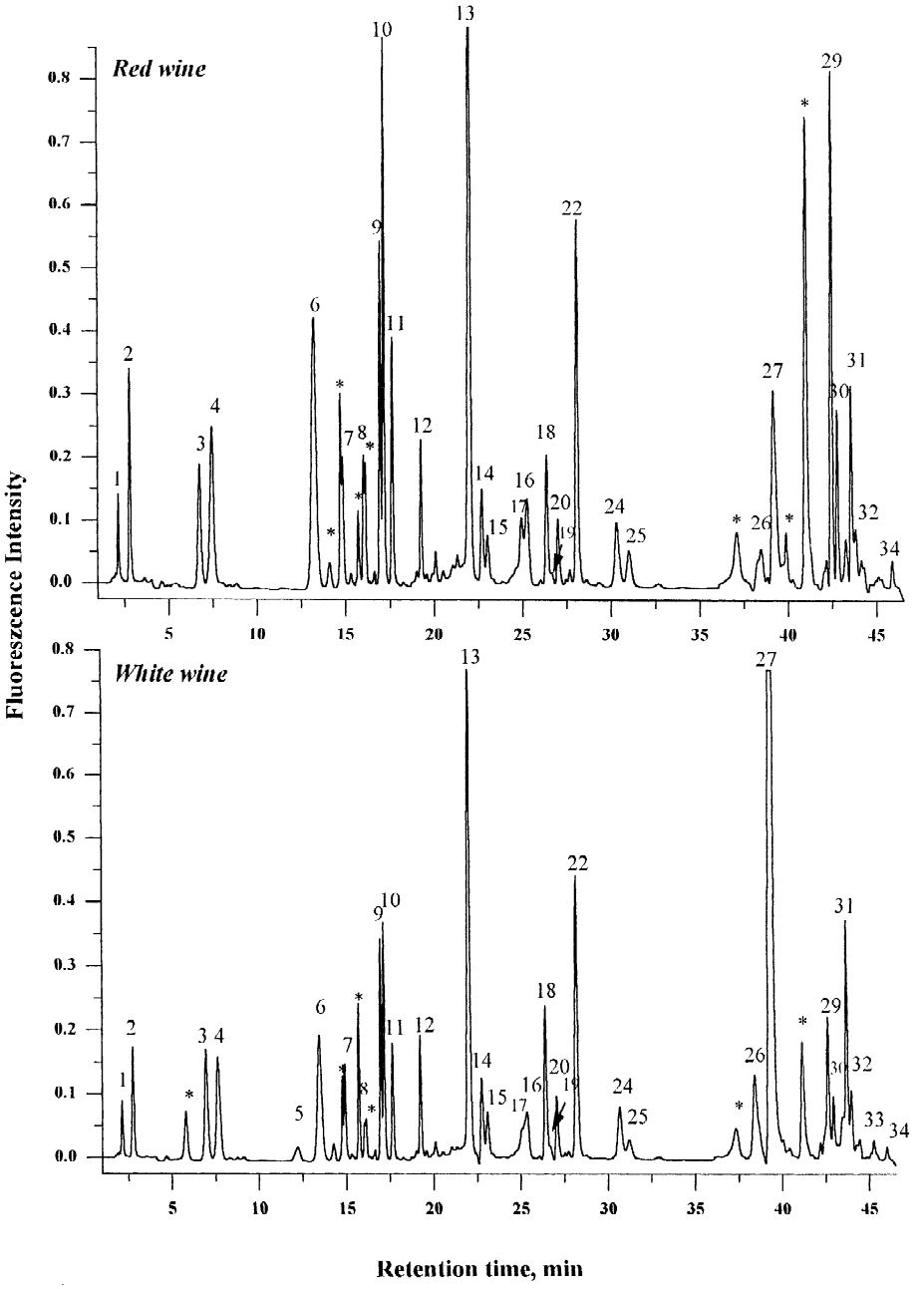

Fig. 4. Elution profile of the OPA–MPA derivatives obtained from red and white wine; peak numbers correspond to those indicated in Table 6.

beer, wine and vinegar samples without any preliminary separation or clean-up steps.

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