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Review

Quantitation of amino acids and amines in the same matrix by high-performance liquid chromatography, either simultaneously or separately

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Abstract

A literature overview is presented of chromatographic methods currently in use to determine amino acids and amines (i) simultaneously, (ii) in the presence of each other by separate methods, or (iii) amines alone subsequent to their isolation from amino acids. Separation, derivatization and chromatographic conditions are summarized. Advantages and drawbacks of all three possibilities are discussed and criticized in detail.

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

Contents

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1. Introduction

The derivatization mechanism and its conditions, characteristics of derivatives, as well as method developments for separation of various derivatives of amino acids [1–7] and amines [8] by chromatography have been discussed in detail [1–8]: including review articles [1,2], studies regarding optimization of different chromatographic techniques [1] and that of HPLC [2–8]. The author's literature [1,2] and research experiences [6–8] led to the recognition that there is a need for the simultaneous technique of reliable and reproducible quantitation of amino acids and amines, present in the same natural matrix, in considerably different concentrations. Based on the author's derivatization and chromatographic practice in GC [1] and HPLC [2–8] equally, it seemed to be most likely that in this case HPLC will be the method of choice.

After an exhaustive literature overview [9–68] it turned out that in the analyses of all those matrices (wine, cheese, fish, meat, other foods, plant tissues, human biological matrices) that consist of amino acids and amines—in different, changing concentrations, very often in a continuous transformation into each other—less attention has been paid to a proper simultaneous chromatographic technique. However, the qualitative and quantitative knowledge of this group of compounds, as well as their determination without loss and in a fast manner, are of primary importance from theoretical and practical points of view.

It was surprising to read [9] that the first paper dealing with a special emphasis on the quantitation of several amino acids and amines from a single run by HPLC, according to its authors was published 7 years ago [9] (i.e. ''. . .—a simultaneous determination of biogenic amines and amino acids, including the precursors of biogenic amines, has not yet been published''). Contrary to this remark [9] we found a number of additional papers [10–30], published already in 1978 [13].

In addition, instead of the simultaneous assay of amino acids and amines, even recently, in order to define the transformation process of amino acids into the corresponding amines [31–45], or determine amines only [46–65], prior to their chromatographic analyses, time-consuming and tedious separation techniques were selected, resulting in two main drawbacks: (i) in considerable loss, i.e. in poor and different recovery both of amino acids and amines and, (ii) in the additional cost and time consumption of separate methods carried out for the two different parts/extracts of samples.

2. Quantitation of amino acids and amines from a single run

Simultaneous determination of amino acids and amines was performed as dabsyl [9,10], as 9-fluorenylmethylchloroformyl [11,12], as *o*-phthaldialdehyde (OPA)–2-mercaptoethanol (ME) [13–18], as OPA–3-mercaptopropionic acid (MPA)–fluorenylmethyl chloroformate (FMOC) [19], as phenylthiocarbamyl (PTC) [21], as fluoresceinthiocarbamyl (FTC) [20–22], as well as, subsequent to their interaction with three recently introduced agents [24–26], i.e. with 5-furoylquinoline-3-carboxaldehyde [24], with carbazol-9-yl-acetyl [25] and carbazol-9-yl-propionyl chlorides [26]. Some proposals were associated with the analysis of the underivatized amino acids and amines [27–30]. Separation techniques were HPLC [9–12,15–19,25,26], CE [20–22,24,27], ion chromatography (IC) [28] and the classical ion-exchange chromatography (IEC) [13,14,30]. As to the detection methods, mainly fluorescence [9–16,8,19,25] as well as laser-induced fluorescence (LIF) [20–22], coulometric [17], UV [24,29], indirect photometric [27], integrated pulse amperometric (IPAD) [28] and condensation nucleation light scattering (CNLS) [30] detection systems were used. Sample preparation and derivatization procedures, chromatographic conditions, as well as reproducibility values and affectivity of the proposal, for selected papers, have been given in detail (Tables 1–3: [9–11,13–17,19–25,28–30]) and/or shown by original chromatograms (Figs. 1–8, taken from the following references: [9,11,12,18,19,21,25,26,29]).

2.1. Simultaneous analysis of amino acids and amines as dabsyl derivatives

The proteinogenic and physiological amino acids and biogenic amine contents of complex food ma-

Table 1
Simultaneous analysis of amino acids and amines from a single injection [9–20] as dabsyl [9,10], as FMOC [11–18] and as OPA derivatives [13–18,20], by HPLC [9–11,13,15,17,18,20] and by ion chromatography [16]

[Ref.] date	Sample preparation (P), derivatization (De)	Column (C), elution (E), flow-rate (F)	Detection	RSD %	Compounds/min
[9] 1995	P: 0.1–1 g cheese, meat, sausage, fish, plasma, tissue+10 ml 0.1 *M* HCl cont. 0.2% TDPA: homogenization, centrifugation, ultrafiltration (0.45-μm); De: 20 μl amino acids+amines (0.2–50 nM/component)+180 μl buffer+200 μl dabsyl-Cl reagent, 70 °C, 15 min	C: 150×4.6 mm (Spherisorb ODS 3 μm); E: A, 9 m*M* NaH_2PO_4 cont. 4% DMFA+1–2% TEA; B, 80% aqueous ACN; F: 1 ml/min, 50 °C	UV 436 nm	≤1.3–3.1	26/90: Asp, Glu, Asn, Gln, cit, Ser, Thr, Gly, Arg, Ala, GABA, Pro, Val, Met, Ile, Leu, Nleu, Phe, NH_3, cyst, Orn, Lys, His, Tyr, pheta, histn, tyrn
[10] 2001	P: 4 g cheese+15 ml 0.2 *M* $HClO_4$ homogenization, centrifugation, ultrafiltration (0.45-μm); De: dabsyl-Cl reagent, 70 °C, 15 min, ice bath 5 min, dilution with ACN+ET, centrifugation	C: 150×4.6 mm (Spherisorb ODS 3 μm); E: as in Ref. [9]	UV 436 nm	≤4	18/90: Gly, Ala, Pro, Val, Met, Arg, Ile, Leu, Lys, His, Tyr, put, cad, histn, sperm
[11] 1995	P/De: 20 μl wine+50 μl borate buffer (pH 8.5)+50 μl FMOC reagent (8 mg FMOC/1 ml ACN) after 3 min+300 μl quenching solution (ACN–acetic acid–water, 20:2:30, v/v)	C: 200×2.1 mm (Hypersil ODS, 5 μm); E: A, ACN cont. 2-octanol 1%, v/v; B, ACN 150 ml+H_3PO_4 8.8 ml+DCA 10 ml/1 l H_2O_2, pH 2.7; F: 0.3 ml/min	Fl, Ex/Em 263/313	≤1.6–13.6	13/110; His, Arg, agm, Phe, pheta, Orn, put, cad, Tyr, tyrn, histn, spermd, sperm
[13] 1985	P: tissue homogenized, urine diluted with 10 ml 0.2 *M* $HClO_4$ (1:10), centrifugation, supernatant analyzed. De: OPA–MCA post column	C: 250×4.6 mm, ODS+70×2.1 mm guard, 5 μm; E: A, 0.1 *M* NaAc (pH 4.5), B, 0.2 *M* NaAc–ACN 10:3 (A&B cont. 10 m*M* octanesulphonic acid; C, MET: 1 ml/min	UV and Fl	–	24/115: Tyr, anserine, Phe, carnosine, homocarnosine, acetylput, putreanine, 3-NH_2-propionic acid, S-adenosylmet, Trp, 5′-CH_3 thioadenosine, isoputreanine lact., isoputreanine, put, cad, decarboxylated S-adenosylmet, histn, spermd, sperm
[15] 1994	P: 2 μg/ml of standards and wines were stored +4 °C; De: OPA–ME then filtered (0.22 μm membrane)	C: 80×4.6 mm (ESA ODS 3 μm); F: 0.80 ml/min, progr., 37 °C	Coulometric	≤5.2	8/35: histn, try, try, tryn, pheta, put, cad, 1,6-diaminohexane
[16] 1996	P: urine/fruit juices cation-exchanged for basic and acidic fractions [15]; De: OPA–ME, post column, 70 °C	C: 150×4.6 mm (Inertsil ODS 5 μm); E: A, 5 m*M* SDS and 10 m*M* $HClO_4$ cont. water; B, ACN; F: 1–2 ml/min, progr., 30 °C	Fl Ex/Em 340/430	≤5.0	20/80: 18 amino acids+creatine and creatinine
[17] 1997	P: ~3 g muscle tissues of scallop or herring or mackerel+15 ml 0.2 *M* $HClO_4$ homogenization, filtration (1. Whatman 2V filter paper, 2. 0.2 μm filter); De: OPA–ME, post column	C: 150×4.6 mm (Hypersil ODS 5 μm); E: A, 0.1 M NaAc (pH 4.5), B, 0.2 *M* NaAc–ACN 10:3 (A&B cont. 10 m*M* octanesulphonic acid); C, MET: F: 1 ml/min	Fl Ex/Em 300/950	–	11/30: Arg, Orn, Lys, histn, Tyr, put, cad, His, agm, spermd, sperm
[19] 2001	P: musts after centrifugation, wines directly diluted 4× with nor-valine internal standard, filtered (0.45 μm); De: two injections, 1. OPA–MPA 2 min; 2. (112 min after the first) FMOC-Cl 2 min	C: 250×4.6 mm (Merck LichroCART, 100 RP-18 5 μm); E: A, 0.02 *M* NaAc cont. 0.018% TEA, 0.3% THF, EDTA~4×10^{-4}%; B, 20% NaAc 0.01 *M*, 40% ACN, 40% MET, 0.018% TEA; F: 0.7–1.2 ml/min	Fl Ex/Em 340/450 237/340	0.5–19.02	32/138: Asp, Glu, aspn, Ser, Gln, His, Gly, Thr, cit, Arg, Ala, GABA, Tyr, eta, Val, Met, Nval (I.S.), histn, Trp, meta, Phe, Ile, Leu, Orn, Lys, eta, tyrn, put, cad, tyra, isam, Pro
[20] in press	P: wine, beer, vinegar diluted twice; De: OPA–MPA 1:50, 15–20 min, +4 °C	C: 200×4.6 mm+guard 20×4.6 mm (Hypersil ODS 5 μm); E: A, 0.05 *M* NaAc (pH 7.2); B, A el–ACN–MET=46:44:10; C: MET; D: ACN; F: 1.3–1.7 ml/min, progr., 50 °C	UV and Fl	≤3.4	37/53: Asp, Glu, aspn, Ser, Gln, Thr, β-ala, Ala, Arg, GABA, Tyr, eta, Val, Met, Try, His, Phe, meta, Ile, Orn, Leu, agm, Lys, etam, sperm, ipa, tyrn, cad, iba, nba, put, pheta, ima, hexa, hepta, octa

Compounds/min, number of compounds/elution time, min (column equilibrium time included); OPA, *o*-phthaldialdehyde; ME, 2-mercaptoethanol; MPA, 3-mercaptopropionic acid; TDPA, 3,3′-thiodipropionic acid; DMFA, dimethylformamide; cont., containing; ACN, acetonitrile; ET, ethanal; FMOC, fluorenylmethyl chloroformate; DCA, dimethylcyclohexyl-amine; –, no data available; coulometric, array of 16 electrodes with increasing potential, 1–1200 mV; THF, tetrahydrofuran; MET, methanol.

trices [9] have been determined as dabsyl derivatives (Table 1, Fig. 1) with a good recovery (98–104%), reproducibility (1.3–3.1%), sensitivity (1–1250 p*M*) and low detection limit (0.12–0.52 p*M*). A practical application [10] of this method relates to the evaluation of the effect of temperature on evolution of the major free amino acids and amine contents of Azeitao cheese during storage. In both papers [9,10], derivatization was performed in deproteinized extracts at 70 °C, for 15 min, applying UV detection.

2.2. Simultaneous analysis of amino acids and amines as fluorenylmethyl chloroformate derivatives

Five amino acids and eight amines were measured simultaneously in 15 rose and 15 white wines [11]. The well-known disadvantage of FMOC derivatization, i.e. the disturbing effect of the wide reagent peaks (FMOC-Cl and/or FMOC-OH) can be seen in Fig. 2 [11]. The latter drawback in the analysis of amino acids and amines in wine, fruit, vegetable and cheese samples was intended to be eliminated on the basis of an exhaustive study [12] regarding: (i) derivatization conditions, such as the excess of the reagent and quenching compound, (ii) the optimum reaction time necessary for quantitative reactions, and (iii) the optimum amount of quenching compound. The authors proved [12] that in order to use FMOC reagent in ''high concentration''—5 m*M* was needed for quantitative derivatization, particularly in the case of amines—avoiding the reagent's precipitation in concentrated buffer solutions (0.2 *M*), instead of acetonitrile (ACN), acetone is the preferred solvent. To eliminate the high excess of FMOC-Cl requires the equivalent excess of the quenching compound, which also had to be held in solution. Thus, the amount of the reagent's and quenching compound's excesses proved to be also a matter of compromise. The optimum reaction time in the case of amino acids (3 min; more than 3 min led to peaks which had a strong pronounced shoulder, or double peaks), while in the case of amines with a

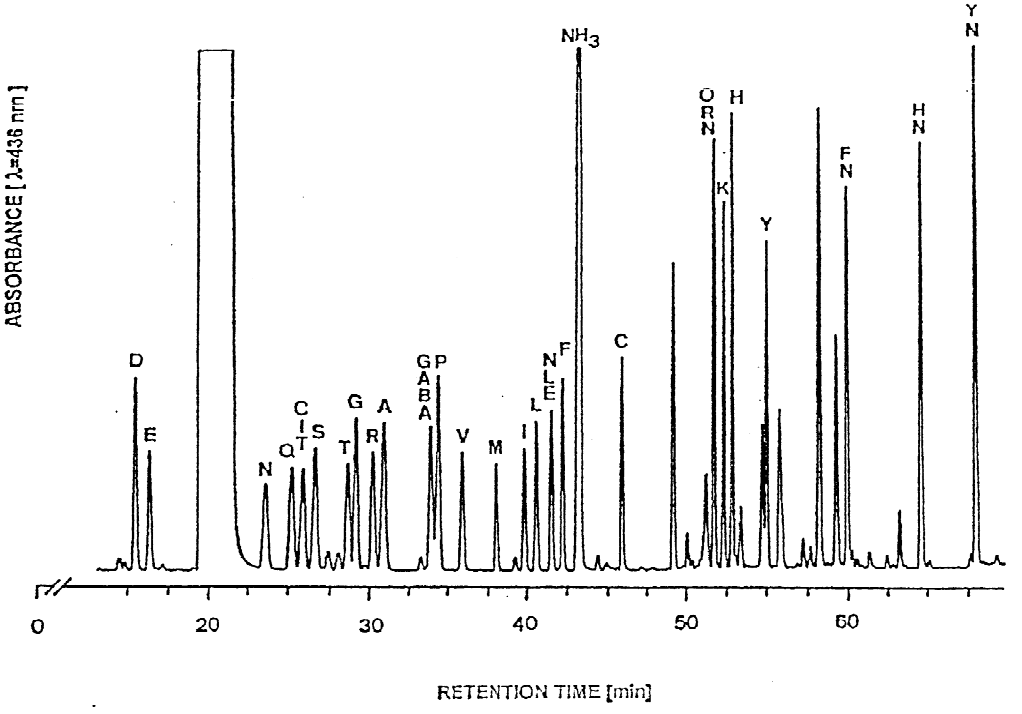

Fig. 1. RP-HPLC analysis of the dabsyl derivatives of proteinogenic and physiological amino acids and amines: D, Asp; E, Glu; N, Asn; Q, Gln; CIT, cit; S, Ser; T, Thr; G, Gly; R, Arg; A, Ala; GABA, GABA; P, Pro; V, Val; M, Met; I, Ile; L, Leu; NLE, Nle; F, Phe; C, cyst; ORN, Orn; K, Lys; H, His; Y, Tyr; FN, pheta; HN, histn; YN, tyrn; with permission from Ref. [9].

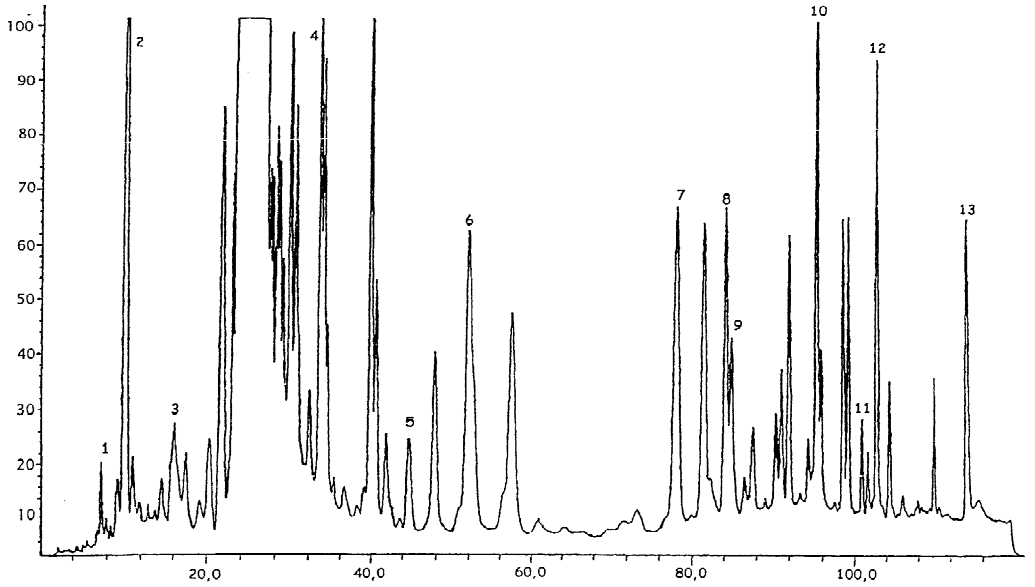

Fig. 2. Chromatogram of FMOC derivatives obtained from a wine sample overloaded with: 1, His 4.6 mg/l; 2, Arg 5.15 mg/l; 3, agm 4.1 mg/l; 4, Phe 4.7 mg/l; 5, pheta 5.2 mg/l; 6, Orn 3.6 mg/l; 7, put 4.0 mg/l; 8, cad 4.5 mg/l; 9, Tyr 4.7 mg/l; 10, tyrn 6.2 mg/l; 11, histn 4.6 mg/l; 12, spermd 3.9 mg/l; 13, sperm 3.9 mg/l; with permission from Ref. [11].

lower reaction rate (sperm, spermd, pheta) 5 min reaction time proved to be the optimum: consequently, as a compromise a 3-min reaction time was chosen. However, in spite of all of these precautions, reagent peaks could not be quantitatively eliminated (unsigned peaks in Fig. 3a and b taken from Ref. [12]).

2.3. Simultaneous analysis of amino acids and amines as o-phthaldialdehyde derivatives

For the simultaneous derivatization of amino acids and amines, mainly the OPA–ME reagent was selected [13–18], the only exception was the two-step derivatization carried out by the OPA–MPA–FMOC agents [19].

The first simultaneous analysis of amino acids and amines was performed in the late 1970s [13] as OPA–ME derivatives: separation and quantitation of 24 compounds, performed by the classical IEC required in total 115 min. Three different buffer eluents were applied with pH 5.55 for 37 min, pH 5.63 for 40 min and pH 5.73 for 93 min. Elution temperatures were 66 °C for 55 min and thereafter 78 °C until the end of the run. Washing (0.2 *M* NaOH containing 250 mg/l EDTA, for 15 min) and equilibration (first buffer for 30 min) processes needed an additional 45 min. The first HPLC of ternary gradient elution, in order to quantitate amino acids and amines simultaneously, present in urines and tissue extracts (a total of 24 compounds), was performed as their OPA–ME derivatives, applying fluorescence detection [14].

Tryptophan, together with seven biogenic amines (histn, trypn, tyrn, pheta, put, cad and 1,6-diaminohexane) were determined in wine samples as their OPA–ME derivatives by electrochemical (coulometric) detection, within 35 min total elution time (Table 1) [15].

A dual mode (UV and fluorescence detection), gradient HPLC method was developed in order to determine urinary bases (creatine, creatinine) and diagnostic amino acids in a single run as their OPA–ME derivatives, performing post-column derivatization and flow-rate gradient (1–2 ml/min) (Table 1) [16].

The amounts of four amino acids and seven amines—transforming under postmortem storage in the muscle of scallop, herring and mackerel samples—were followed as their OPA–ME derivatives

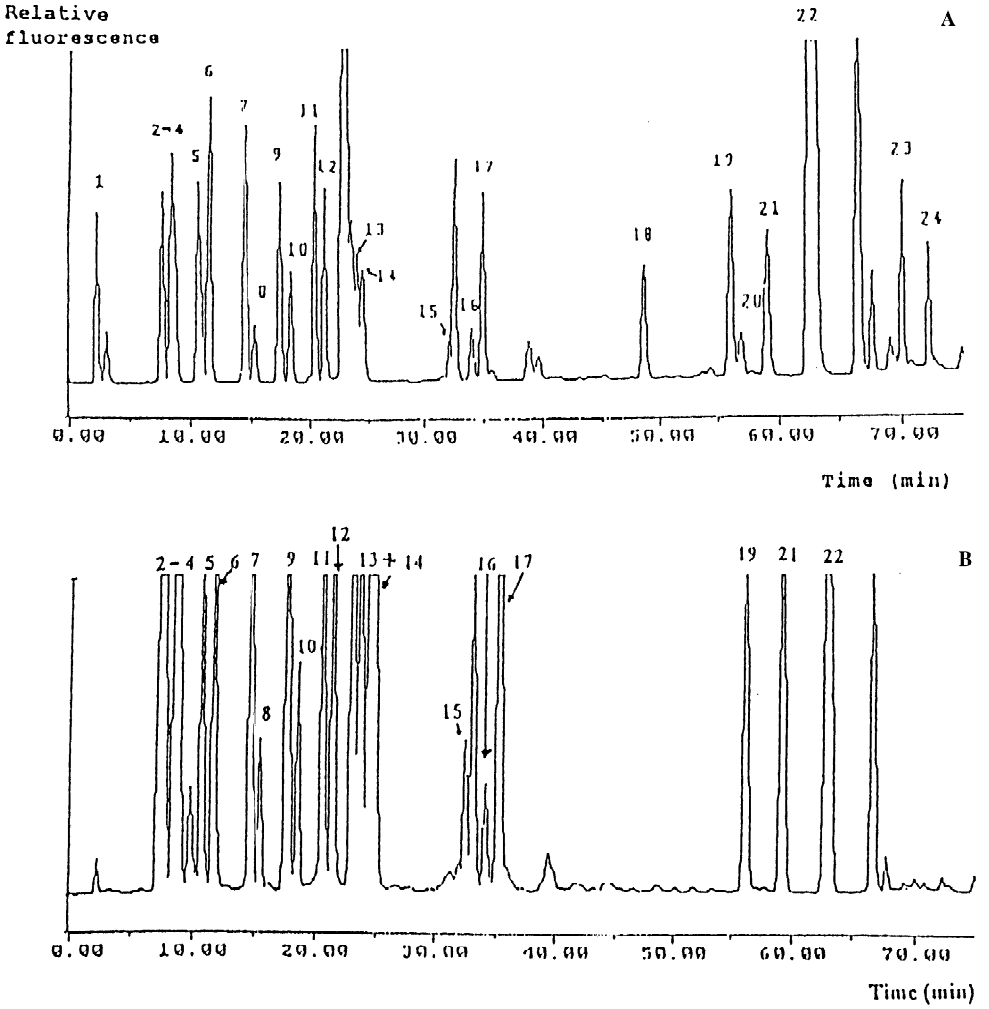

Fig. 3. HPLC chromatogram of biogenic amines and amino acids after derivatization with FMOC-Cl and removing the excess of FMOC reagent with heptylamine (hepta), from standard mixture (a, concentrations 5 μg/μl) and from cheese extract (b). Peaks: 1, cysteic acid; 2, Asp; 3, Ser; 4, Glu; 5, Thr; 6, Gly; 7, Ala; 8, Tyr; 9, Pro; 10, Met; 11, Val; 12, Phe; 13, Ile; 14, Leu; 15, His; 16, Tyr; 17, Lys; 18, pheta; 19, put; 20, histn; 21, cad; 22, hepta; 23, spermd; 24, sperm; with permission from Ref. [12].

applying post-column derivatization (Table 1, [17]). Twenty-four amino acids and amines have been determined with pre-column derivatization (OPA–ME) and fluorescence detection within 50 min (Fig. 4, taken from Ref. [18]).

The formation/transformation of free amino acid and amine constituents of 39 musts and 33 wines were followed during the fermentation process, as their OPA–MPA–FMOC derivatives, performing fluorescence detection (Table 1) [19]. The quantitative analysis of 32 amino acids and amines required 138 min (Fig. 5a and b taken from Ref. [19]). The simultaneous determination of amino acids and amines, in total 37 compounds, as their OPA–MPA derivatives has been developed recently (Table 1) [20]. A detailed description of the chromatographic method and its application to the analysis of beer, wine and vinegar samples can be found in this issue.

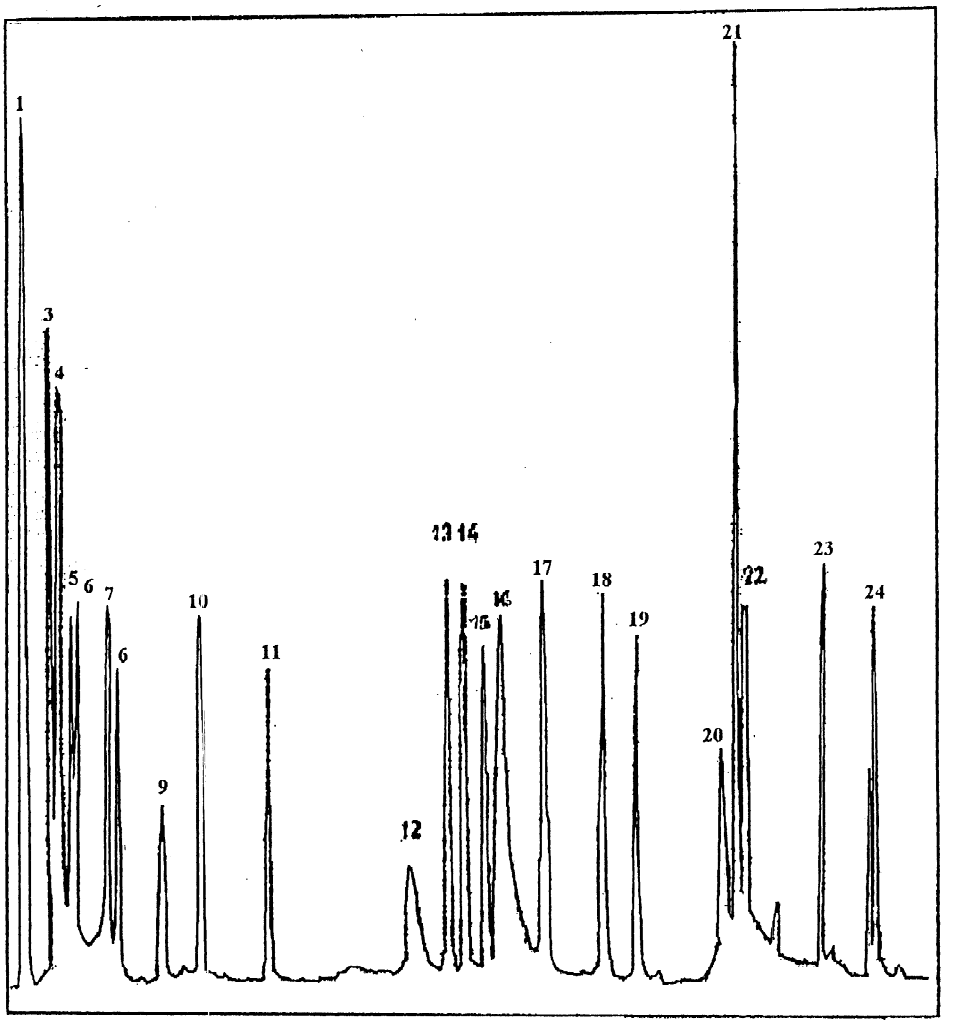

Fig. 4. HPLC profile of amines and amino acids, measured as OPA–ME derivatives. 1, Asp; 2, Glu; 3, Asn; 4, Ser; 5, Gln; 6, His; 7, Gly; 8, Thr; 9, Arg; 10, Ala; 11, Tyr; 12, agm; 13, Met; 14, Val; 15, Trp; 16, histn; 17, Phe; 18, Ile; 19, Leu; 20, Orn; 21, tyrn; 22, Lys; 23, put; 24, cad; with permission from Ref. [18].

2.4. Simultaneous analysis of amino acids and amines as fluorescein- and phenylthiocarbamyl derivatives

Histamine together with 24 amino acids in wine have been quantitated as their phenylthiocarbamyl derivatives by HPLC applying UV detection (Table 2) [21]. Free amino acids and primary amines (50 compounds/22 min: 21 quantitated, 19 identified) from child cerebrospinal fluid (Fig. 6a and b, [22]) and 28 amino acids and biogenic amines from red French wines over a 14-year period [23], were determined as their fluoresceinisothiocarbamyl derivatives by CE [22] and by micellar electrokinetic chromatography [23], applying in both cases [22,23] LIF detection (Table 2).

2.5. Simultaneous analysis of amino acids and amines as furoylcarboxyl and carbazol derivatives

Amino acids and biogenic amines were resolved and detected in a single run subsequent to their

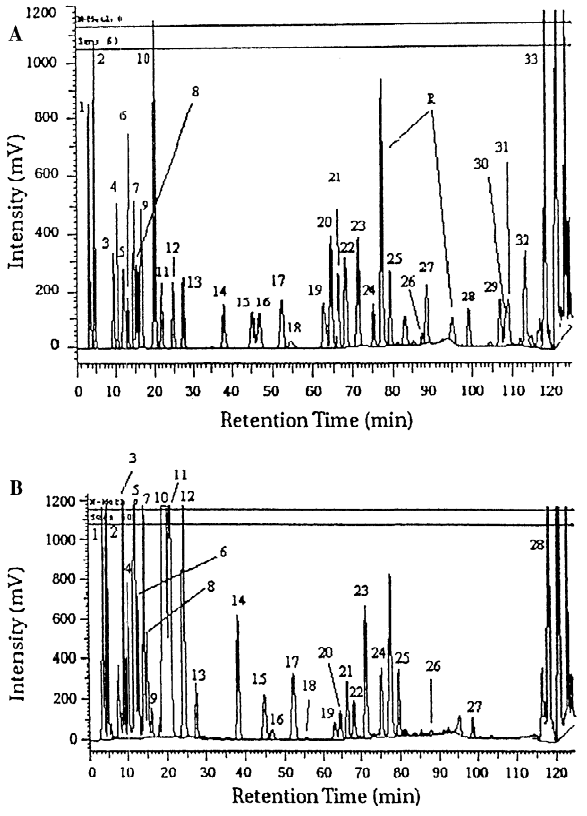

Fig. 5. Chromatogram of a standard solution (a) and a must sample at the beginning of alcoholic fermentation (b) obtained after derivatization with the OPA–MPA–FMOC reagent. Peaks: 1, Asp; 2, Glu; 3, Asn; 4, Ser; 5, Gln; 6, His; 7, Gly; 8, Thr; 9, cit; 10, Arg; 11, Ala; 12, GABA; 13, Tyr; 14, eta; 15, Val; 16, Met; 17, Nval; 18, histn; 19, Trp; 20, meta; 21, Phe; 22, Ile; 23, Leu; 24, Orn; 25, Lys; 26+27, tyrn; 28, put; 29, cad; 30, pheneta; 31, trypt; 32, iama; 33, Pro; R, reagent peak; with permission from Ref. [19].

derivatization with 5-furoylquinoline-3-carboxaldehyde (FQ) [24]. Sixteen FQ-tagged compounds in the brain sample were resolved in less than 16 min based on capillary electrophoresis (CE) and LIF detection (Table 2).

Rapid and sensitive pre-column derivatization methods [25,26] have been proposed using carbazol-9-yl-acetyl chloride (CRA-Cl) (Table 2) [25] and/or carbazol-9-yl-propionyl chloride (CRP-Cl) [26] as derivatizing reagents, for the simultaneous determination of amino acids and biogenic amines, by HPLC with fluorescence detection. Optimum derivatization and chromatographic conditions were given in detail [25,26]. The elution of the CRA derivatives needed 95 min (Table 2; Fig. 7a and b [25]) for 25 compounds, while those of the CRP derivatives were somewhat faster (Fig. 7c and d [26]), they needed 60 min.

2.6. Simultaneous analysis of amino acids and amines without derivatization

Six compounds (put, His, spermd, sperm, Lys and Arg) extracted from the tumor cells of brain samples were detected from nanolitre injection volumes by high-performance capillary electrophoresis (HPCE). As background electrolyte, quinine sulfate and indirect photometric detection was used [27].

Biogenic amines (put, cad, sperm, histn, spermd) and their precursor amino acids (His, Tyr) were determined simultaneously, in their underivatized form, in food samples (vegetables, cheese, fish) on a cation-exchange column performing integrated pulsed amperometric detection (IPAD) (Table 2) [28]. The analytes were extracted from the food samples with perchloric acid and the extracts were purified by liquid–liquid extraction using hexane. Good recoveries (85.5–97.4%) and RSDs (3.4–8.8%) were reported (Table 2) [28].

Ion-pair HPLC (heptanesulfonic acid) and UV detection (215 nm) were used for the quantitation of the most relevant amino acids (His, Tyr, Phe, Trp) and amines (histn, tyrn, pheta, trypn) in cheese samples, within 40 min, with perfect resolution (Fig. 8a and b) (Table 2) [29].

Four constituents of fish samples (His, histn, put, cad) were determined within 8 min applying IEC and CNLS detection [30]. The high acid concentration of the eluent (10 m*M* nitric acid containing 10% ACN) ensures the short retention times (Table 2).

3. Determination of amino acids and amines from the same matrix by separate chromatographic techniques

An earlier review of this field gave a short summary [31] of common methods with particular attention paid to the analysis of wines: all of these proposals follow the quantitation of amino acids and amines from a previously, differently isolated part of

Table 2
Simultaneous analysis of amino acids and amines from a single injection [21], as phenylthiocarbamyl (PTC) [21], as fluoresceinthiocarbamyl (FTC) [22,23], as carbazol-9-yl-acetyl [25] derivatives and without derivatization [28–30] by HPLC [21,25,28–30] and by CE [22–24]

[Ref] date	Sample preparation (P), derivatization (De)	Column (C), elution (E), flow-rate (F)	Detection	RSD %	Compounds/min
[21] 1991	P: wine filtered through micropartition system (Centifree MOS-1, Amicon); De: PITC–water–ET–TEA 1:1:7:1, prepared freshly	C: 250×4.6 mm+30×3.9 mm precolumn (Spherisorb ODS2, 5 μm); E: A 0.05 *M* NaAc, cont. 0.25% ACN+0.05% TEA+1 ppm EDTA (pH 6.8); B, ACN; F: 1 ml/min; 52 °C	PDA	2–7	25/40: Asp, Glu, OH-prol, Ser, Asn, Gly, Gln, His, GABA, Thr, Ala, Arg, Pro, ABA, Tyr, histn, Val, Met, cystine, Ile, Leu, Phe, Trp, Orn, Lys
[22,23] 1995–1997	P: 50 μl cerebrospinal fluid [22,23], 0.23 g cheese-paste extracted by 5 ml 0.1 *M* HCl, 3×, centrifuged [23] and 50 μl wine [19] diluted with 0.2 *M* carbonate buffer; De: 50 μl samples+50 μl 2.1×10^{-4} *M* FITC in acetone 2 h, in dark	C: 75 cm×50 μm fused-silica (effective length 42 cm); E: buffer 100 m*M* SDS+100 m*M* boric acid+NaOH until pH 9.0; Separation 20 kV, electrophoretic current 42 μA	LIF	–	28/30: put, Arg, Lys, histn, Orn, His, eta, tyrn, β-pheta, ammonia, spermd, sperm, Thr, Ile, Leu, Nleu, Pro, Phe+val, try, Asn, Tyr, Ser, Ala, cyst, taurine, Gly, Glu, Asp, cysteic acid
[24] 2001	P: 4 μl brain microdialysate from different tissues prepared in Ref. [22]; De: 4 μl sample+100 n*M* dry FQ, diluted with 0.2 *M* carbonate buffer+2 μl 25 m*M* KCN (pH 9.2), reaction in dark, at 65 °C, for 16 min	C: 40 cm×50 μm fused-silica; E: 60 m*M* SDS+20 m*M* borate (pH 9.0), or 45 m*M* SDS+15 m*M* borate+5 m*M* β-CD (pH 8.5); Separation 200 V/cm	LIF	CC= 0.96–1.0	16/18 (without flushing by NaOH, water, buffer): Ser, Thr, Gln, Glu, dopamine, His, Asn, Ala, taurine, Asp, GABA, Gly, Ser, norepi, Met, β-ala
[25] 1999	P: foods: 20 g cheese suspended with 40 ml 0.1 *M* HCl, stirred for 30 min (0–4 °C), centrifugation, filtration of supernatant. Residue extracted 2×, by 100 ml 0.1 *M* HCl; plant materials: 0.1–0.5 g barley seedling chopped, homogenized by 5 ml 5% $HClO_4$, extr. for 60 min. Centrifugation, filtration. Residue extracted 2×, with 2 ml 5% $HClO_4$. De: 40–400 l solution (pH 8.8)+50 μl CRA-Cl reagent 3–5 min	C: 200×4.6 mm (Spherisorb ODS 5 μm); E: A, 20 m*M* $NH_4H_2PO_4$ cont. 9 m*M* TEA (pH 6.5)–Met 9:1 (v/v); B, ACN; F: 1 ml/min, 35 °C	Fl Ex/Em 335/339	≤5	24/95: Asn, Gln, Ser, His, Gly, Thr, Ala, Pro, Tyr, Arg, Val, methi, Ile, Leu, Phen, cyst, Lys, pheta, put, histn, cad, hepta, sperm, spermd
[28] 1998	P: 10 g homogenized kiwi/meat/fish/cheese+30 ml $HClO_4$ (0.375 *M*), completed to 50–100 ml, extracted with hexane (2:1, v/v), centrifuged, filtrated 0.2 μm; De: –	C: cation-exchange anal. (IonPac CS10, Dionex); E: isocratic, 1 *M* $NaClO_4$–0.375 M $HClO_4$–water 81:5:14, v/v[a]; F: 1 ml/min	IPAD	3.4–8.8	7/30: put, His, Tyr, cad, histn, tyrn, spermd
[29] 1999	P: 1 g cheese homogenized for 2 min by 5 ml 5% TCA two times+ with 10 ml. Centrifuged supernatants combined, extracted 3× by 10 ml diethylether. Ether removed by N_2. Residue+25 ml water; injected 20 μl; De: –	C: 150×4 mm+20×4 mm pre-column (Spherisorb ODS2, 5 μm); E: A, 0.05 *M* KH_2PO_4 cont. 0.005 M heptanesulphonic acid, acidified by H_3PO_4 (pH 3.4); B, 60% A–40% ACN; F: 1 ml/min	UV 240	–	8/40: His, Tyr, Phe, histn, tyrn, try, β-phenylethylamine, trypn
[30] 1999	P: 6.5 g tuna fish/mackeral sardines washed, ground, homogenized (magnetic stirring 45 min)+20 ml water+10 ml 6 *M* HCl; 100 μl aliquot syringe filtered, injected	C: 100×4.6 mm (Alltech cation-exchange, filled with polybutadiene–maleic acid coated silica); E: 0.01 *M* HNO_3 cont. 10% ACN; F: 1 ml/min;	CNLSD	3.4–8.8	7/30: put, His, Tyr, cad, histn, tyrn, spermd

As in Table 1, as well as: ABA, α-aminobutyric acid; CRA-Cl reagent, 10 m*M* carbazol-9-yl-acetyl chloride (note: derivatization should be performed in 50% ACN and 0.2 *M* borate buffer, pH 8.8); SDS, sodium dodecylsulphate; IPAD, integrated pulsed amperometric detection; LIF, laser induced fluorescence; FITC, fluorescein isothiocyanate; FQ, 5-furoylquinoline-3-carboxaldehyde; CC, correlation coefficient; CNLSD, condensation nucleation light scattering detection.

[a] For detection the eluent was mixed by 0.25 *M* NaOH (0.8 ml/min).

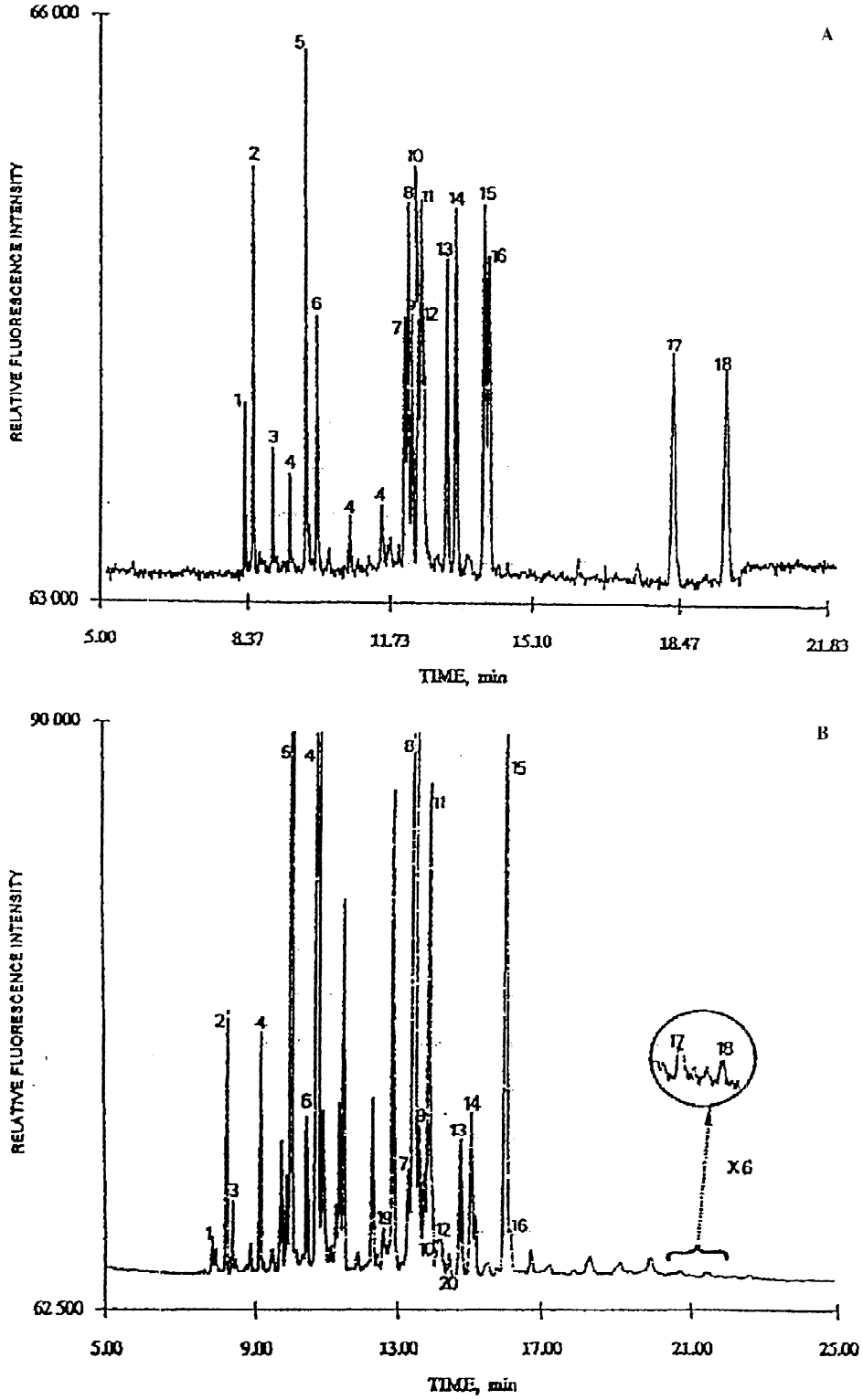

Fig. 6. Electropherogram of 17 FTC-standards (a, 10^{-9} *M*) and amino acids and amines from the cerebrospinal fluids of an acute lymphoblastic leukemic patient: 1, Lys; 2, Arg; 3, Orn; 4, blank; 5, ammonia; 6, tyrn; 7, Leu; 8, Gln; 9, Tyr; 10, Val; 11, Thr; 12, Phe; 13, Ser; 14, Ala; 15, taurine; 16, Gly; 17, Glu; 18, Asp; 19, cit; 20, Asn; with permission from Ref. [22].

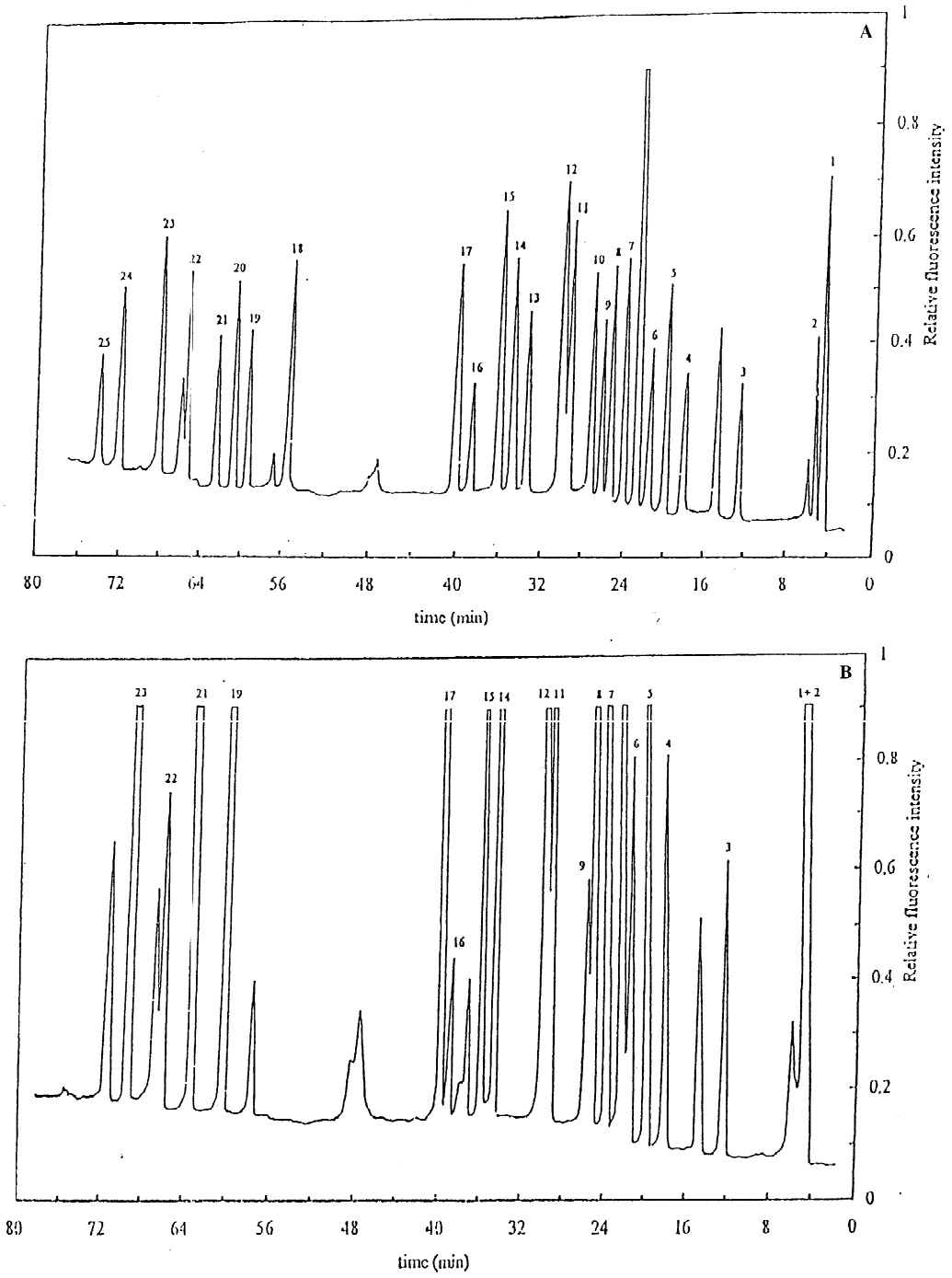

Fig. 7. LC chromatograms obtained with a standard mixture (a, c, 50 p*M*) of biogenic amines and amino acids, as well as with a cheese extract (b, d) after derivatization with carbazol-9-acetyl chloride (a, b [25]) and with carbazol-9-yl-propionyl chloride (c, d). Peaks 1–17 in (a)–(d) are the same: 1, Asp; 2, Glu; 3, Ser; 4, His; 5, Gly; 6, Thr; 7, Ala; 8, Pro; 9, Tyr; 10, Arg; 11, Val; 12, Met; 13, Ile; 14, Leu; 15, Phe; 16, Cys; 17, Lys, as well as in (a, b) in addition, 18, pheneta; 19, put; 20, histn; 21, cad; 22, unknown; 23, hepta; 24, spermd; 25, sperm; and in (c, d) in addition: 18, put; 19, cad; 20, hepta; 21, spermd; 22, sperm. With permission from Ref. [25] for (a, b) and from Ref. [26] for (c, d).

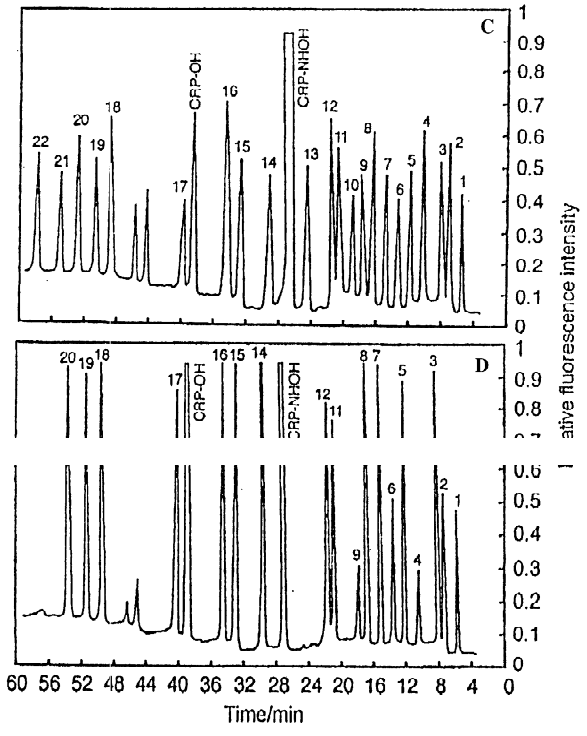

Fig. 7. (*continued*)

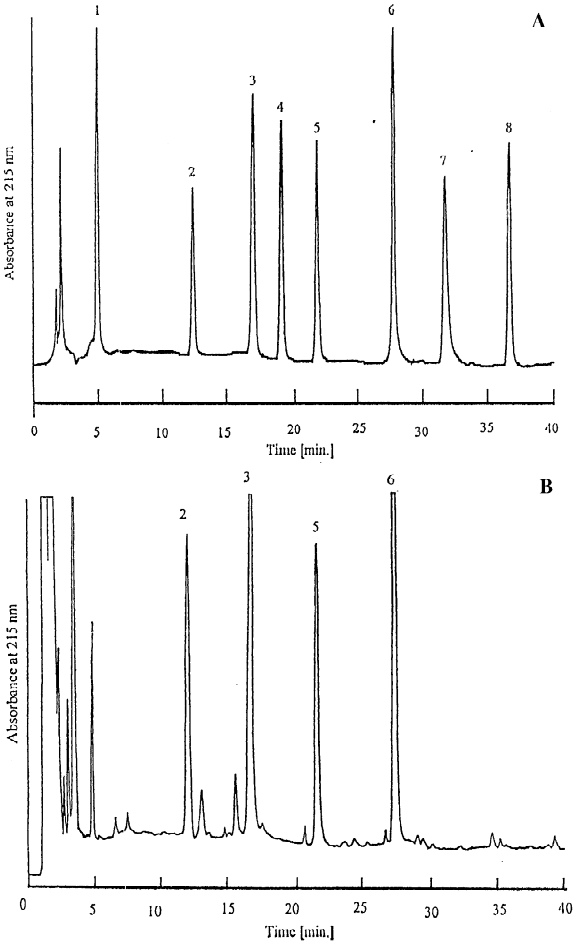

Fig. 8. HPLC chromatograms of standard amines and their precursor amino acids (a) as well as that of a sample of bacterial starters used for cheese production (b) measured with ion-pair chromatography and UV detection. Peaks: 1, His; 2, Tyr; 3, pheneta; 4, histn; 5, tyrn; 6, trp; 7, pheneta; 8, Trp. With permission from Ref. [29].

the matrix. Amino acids were quantitated mainly with classical IEC, while amines either by their OPA or via their dansyl derivatives.

According to recent proposals [32–45], amino acids and amines were derivatized with the common OPA–ME reagent in wine samples [32–34] and in microdialysates of biological species [35,36]. Derivatization of amino acids was performed directly in the diluted wine [32,33] (elution time: 85 min/21 amino acids), while amines subsequent to their isolation through a cartridge of polyvinylpyrrolidone (PYP) and Millipore filters (elution time 30 min/six amines) [34]. The chromatography of amino acids and amines obtained from the brain areas of rat using in vivo microdialysis [35] is detailed in Table 3.

Glutamic acid, glycine, taurine and γ-aminobutyric acid (GABA) content of microdialysis samples were measured in two runs as their OPA–ME derivatives [36]. Both elutions were really fast: glutamic acid, glycine and taurine were eluted together with an additional seven amino acids, within 5 min. The separation of GABA needed an extra 5 min. Quantitation in a single run would be easily achievable (author's remark).

Evolution of free amino acid and amino content of cured ham during ripening were determined as PTC-amino acid and dansyl amine derivatives [37,38]. Amino acids were derivatized in a deproteinized (by sulfosalicylic acid) homogenate after its centrifugation and filtration, while amines were derivatized in a chloroform–butanol (2:1, v/v) extract obtained from

Table 3
Determination of amino acids and amines from the same matrix by separate chromatographic techniques

[Ref.] date	Sample preparation (P), derivatization (De)		Column (C), elution (E), flow-rate (F)		Detection		Compounds/min	
	Amino acids	Amines	Amino acids	Amines	Amino acids	Amines	Amino acids	Amines
[35] 2001	P: brain microdialysate 5 μl; De: OPA–ME	P: brain microdialysate 25 μl; De: –	C: 250×4.6 mm (Spherisorb ODS2, 5 μm); E: A, 0.08 *M* NaH_2PO_4–MET–ACN–THF 90:24:2:1 (v/v); B, 0.04 *M* NaH_2PO_4–MET–THF 67:55.5:3 (v/v), pH 6.2; 30 °C	C: 150×2.1 mm, Waters Symmetry Shield RP8+Waters Symmetry Guard C_{18}; E: in 920 ml water 6.6 g NaH_2PO_4+197.2 g SOS+137.76 g EDTA+80 ml ACN (pH 2.9); 30 °C	Fl Ex/Em 260/450	Electr. 0.85 V	9/30: Asp, Glu, Asn, Ser, Gly, taur, Ala, GABA	5/30: NA, DA, DOPAC, HVA, 5-HIIA, 5-HT
[37] 1994	P: 10 g ham sample+5% SA homogenized (2 °C, 17 h) centrifuged, filtrated (Whatman no. 54 paper); De: PITC	P: 10 g ham extracted (2:1 chloroform–butanol); De: dansyl-Cl [38]	C: 250×4.6 mm (Supelcosil LC18, 5 μm); E: A, 0.03 *M* NaAc (pH 6.8); B, ACN–water 90:10; F: 1 ml/min; 35 °C	C: –; E: A, 0.01 *M* NaAc. cont. 5% THF (pH 4.2); B, ACN–THF 9:1; F: 1 ml/min	UV 254	UV 254	22/56.5: protein amino acids	7/47: put, trypn, cad, histn, tyrn, sperm, spermd
[42] 2002	P: wine dilution 2×filtr. (0.45 μm membrane); De: postcolumn ninhydrine, 121 °C	P: 15–20 ml wine evapor. to 1 ml+2 ml Na citr. (pH 2.2), filtr. (0.45 μm membrane); De: postcolumn ninhydrine, 121 °C	C: 36×0.37 cm; E: Na. citr. buffers, A, (pH 2.6); B, (pH 3.0); C, (pH 4.25); D, (pH 7.9); F: 0.3 ml/min; 60 °C	C: 6×0.37 cm; E: A, (pH 6.0); B, (pH 5.7); C, (pH 5.45); F; 0.3 ml/min; 60 °C	UV 570/440	UV 570	All stand. amino acids/92	7/101: histn, tyrn, put, cad, agm, spermd, sperm
[44] 1999	P: 50 mg leaf powder extraction by ET. Insoluble residue+1 ml water. De: 20 μl extract+AQC reagent (10 min, 50 °C)	P: detailed in the text; De: supernatant +Na_2CO_3+dansyl-Cl (60 °C, 1 h); Excess removed (proline), extracted by toluene	C: 150×3.9 mm (Waters Accq-Tag, 4 μm); E: A, 140 m*M* NaAc+17 m*M* TEA (pH 5.05); B, ACN–water 60:40 (v/v), F: 1 ml/min; 37 °C	C: 250×4.6 mm, Beckman C_{18} Ultrasphere 5 μm); E: water–MET gradient from 40:60 to 5:95 (v/v); F: 1 ml/min; 27 °C	UV 254	Fl Ex/Em 336/486	22/135: Asp, Asn, Ser, Glu, Gly, His+ +Gln, β-ala, Arg+Thr, and[a]	11/40: agm, tryp, dap, put, cad, octopamine and[b]

As in Tables 1 and 2, as well as: NA, noradrenaline; DOPAC, 3,4-dihydroxyphenylacetic acid; DA, dopamine; 5-HIIA, 5-hydroxyindole-3-acetic acid; HVA, homovanillic acid; 5-HT, 5-hydroxytryptamine (seretonine); SA, sulfosalicylic acid; EtAc, ethyl acetate; dap, 1,3-diaminopropane.

[a] Pro, GABA, Cys, Tyr, Val, Lys, Met, Orn, Ile, Leu, Phe.

[b] Methoxytyramine, spermd, sperm.

another deproteinized (by $HClO_4$) homogenate subsequent to its centrifugation and filtration (Table 3).

Derivatization of amino acids with phenylisothiocyanate (PITC) and amines with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) reagents, present in wines [39], resulted in the quantitation of six precursor amino acids and six amines; detailed conditions were not given.

The formation of biogenic amines and liberation of free amino acids during cheese ripening [40,41] were followed from the same sample by different techniques: amino acids were determined in both cases by the classical IEC method (in paper [40]: 37 compounds, in paper [41]: 41 constituents), amines in acidic extracts as their dansyl derivatives, while tryptophan alone from a basic extract.

Basic amino acids (histidine, arginine, ornitine, lysine) and polyamines (putrescine, spermidine and spermine) from complex biological samples, prior to their HPLC quantitation, were isolated by various ion-exchange clean-up processes [42]. The amino acid and amine contents of dried ion-exchanged residues were quantitated by HPLC as their FMOC amino acids and dansyl amine derivatives.

The amino acid and amine content of wine were determined recently [43] by classical IEC applying different columns and elution processes [42] (Table 3).

The amino acid, polyamine and betaine contents of freeze dried and ground plant tissues [44] (Table 3) were determined from two different extracts: (i) polyamines and betaine from the supernatant of a centrifuged homogenate obtained with 1 *M* ice-cold HCl, as their dansyl derivatives (derivatization: 60 min, 60 °C+ extraction by toluene; chromatography: 40 min), while amino acids from an ethanol extract obtained at 95 °C, prior to their precolumn derivatization with AQC (derivatization: 10 min, 50 °C; chromatography 135 min).

The amino acid, monoamines and their metabolites from biological tissue [45] were determined by two different HPLC–electrochemical detection (ED) processes using microbore columns. Amino acids were quantitated as their *tert*.-butylthio derivatives (at 700 mV versus Ag/AgCl reference electrode, derivatization time: 2 min, chromatography of 14 amino acids: 16 min), while amines were measured at 700 mV applying the Unijet microbore electrochemical detector (Bioanalytical System, chromatography of seven amines: 24 min).

4. Quantitation of amines only, following their separation from amino acids

The above methods relate mainly to the quantitation of amines [46–65] accompanied by amino acids, present in almost all matrices in overwhelming concentrations compared to amines. Chromatographic analyses of amines were carried out as their OPA [46–54] or their dansyl derivatives [55–65], as well as without derivatization by CE [66–68] and performed subsequent to their separation from amino acids by solvent extraction, IEC, solid-phase extraction, etc.

4.1. Determination of amines as their OPA derivatives by HPLC

Derivatization of amines subsequent to various separation processes were performed with the OPA–ME [46–53] and with the OPA–NAC [54] reagents. The histamine content of wines alone was measured after a Sep Pak C_{18} cell clean-up [46].

The quantitation of eight amines (agmatine (agm), cadaverine (cad), ethanolamine (etha), histamine (histn), phenylethylamine (pheta), tryptamine (try), tyramine (tyr), spermine (sperm) and spermidine (spermd)) present in red must and wines was performed without any preliminary isolation from amino acids [47]. The authors [47], after introductory experiences, rejected derivatization with dansyl chloride and fluorescamine (resulting in several by-products), and proposed the quantitation of these eight amines as their OPA–ME derivatives [47].

According to another proposal [48], in order to quantitate amines in wine and must after a 4-min reaction time, OPA–ME-amines were extracted into ethyl acetate [48] increasing their stability. Gradient elution, carried out with an acidic eluent (methanol–0.08 *M* acetic acid–acetonitrile, 52:45:3, v/v) at 35 °C, probably made it possible to avoid coelution with amino acids.

The most important wine amines (histn, tyr, put,

pheta, cad, isoamylamine (iama), heptylamine (hepta)) have been quantitated in the presence of amino acids of ~20 times excess [49]. According to the authors amino acids ''cause problems only when the histamine peak was very small''. However, the chromatogram presented is not convincing at all: containing unidentified compounds in enormously high concentrations beside the tiny amounts of amines.

Exhaustive extraction studies of 15 [50], later 17 [51] amines from wine were performed prior to their chromatographic analysis as OPA–ME derivatives [50,51]. Treatments with PVP, strong anion-exchange resins [50] and acidic cation-exchange cartridges [51], were tested and compared. Recoveries varied, in order of listing between 93 and 100% (PVP), 78 and 100% (C_{18} cartridge), 91 and 112% (strong anion-exchange resin), 31 and 104% (Amberlite CG-50, Sigma) and 10 and 90% (strong cation-exchange resin, Varian), respectively.

The reliability and sensitivity of the chromatographic analysis of biogenic amines in wine [52] was increased by cation-exchange clean-up completed by solid-phase extraction (SPE). A recovery study was not given.

An on-column derivatization technique [53] performed with the OPA–*N*-acetyl-L-cysteine (NAC) reagent was described for the quantitation of eight amines (derivatization+elution time 40 min) subsequent to their isolation from wines by two SPE cartridges (C_{18}, strong anion-exchange resin, both from Varian) reporting different recoveries (C_{18}, 65–105%; strong anion-exchange resin, 75–103%).

The post-column derivatized OPA–ME-biogenic amines of several Hungarian wines were measured after perchloric acid treatment, homogenization/centrifugation and filtration [54]: the poor separation of eight compounds needed 73 min.

The most advantageous separation idea [55] was the elution of the OPA–MPA-amino acids without the aim of their quantitation—prior to the assay of sperm, spermd, put and cad as their OPA–MPA derivatives. Applying a binary gradient, in the first 10 min of the gradient all amino acids elute, continued by the separation of the four amines (in the next 10 min) and the column equilibration time (11 min) (eluent A, 0.125 *M* sodium citrate containing 50 ml/l tetrahydrofuran; eluent B, citrate buffer–ACN–tetrahydrofuran (45:50:15, v/v) pH 6.4).

4.2. Determination of amines as their dansyl, dabsyl and benzoylated derivatives by high-performance liquid chromatography and high-performance thin-layer chromatography

Biogenic amine contents of different plant samples [56–58] have been determined in differently prepared acidic extracts.

The put, spermd and sperm content of lichens [56] were determined after the hydrolysis of samples. Free amines were extracted with 5% (w/v) trichloroacetic acid and then centrifuged. Dansylation was performed at room temperature for 16 h followed by a post-derivatization clean-up with toluene. RP-HPLC was performed from the toluene extract at 40 °C.

Five biogenic amines (1,3-diaminopropane, put, cad, sperm, sperm) from plant and animal tissues [57] were extracted with 10% (w/v) $HClO_4$ (15 mg dry tissue/ml), dansylated at room temperature overnight, or, at 70 °C for 10 min and separated within 15 min, including column equilibration time. Recoveries varied between 89.5 and 97.1%. Five polyamines from plant-seedlings [58] were determined after a clean-up of two steps: tissue homogenates were extracted with 0.7 *M* $HClO_4$ and the combined supernatants were further isolated by SPE (micropreparative Dowex 50-X8 column). Dansylation was performed in the dark, at 56 °C for 1 h. Dansyl amines were extracted into toluene and separated within 40 min by HPLC.

The content of amines in wine samples was quantitated as their dansyl [59,60] and dabsyl [61] derivatives. As a result of two different exhaustive isolation/enrichment procedures [59] ($method_1$: continuous liquid–liquid extraction for 3 days with Freon 11–dichloromethane (90:10, v/v); $method_2$: butanol–dichloromethane (70:60, v/v)) 25 amines were identified and measured in five red wines and in four white wines. Dansylation was followed at 65 °C for 20 min, elution time was 50 min. Recovery percentages for five amines, in order of listing, for $method_1$ and $method_2$ proved to be 9% and 72% (ethyl), 67% and 74% (butyl), 78% and 88% (di-

ethyl), 85% and 40% (phenylethyl), 93% and 25% (hexyl), as well as 96% and 10% (heptyl), respectively.

Nineteen amines from wines [60] were determined after a four-step isolation/enrichment process. Firstly, phenolic compounds were removed by PVP clean-up: the mixture of 1 g PVP+25 ml wine sample was stirred for 15 min and filtered (step 1). The phenolic-free wine samples were dansylated in the dark at 65 °C for 25 min (step 2). Dansyl amines were subjected to SPE (step 3). Finally, the acetonitrile eluates of SPE were concentrated by a rotary evaporator (step 4). The temperature of gradient elution was optimized (60 °C, 60 min). Recoveries varied in the range of 62–95%.

Eight biogenic amines [61] of red wines were derivatized by 4-(diamino)azobenzene-4′-sulfonyl (dabsyl) chloride (70 °C, 21 min) subsequent to their filtration and dilution (10-fold) by 0.1 *M* HCl containing 0.2% 3,3′-dithiopropionic acid. Gradient elution was performed at pH 5, for 50 min. Recoveries varied between 92.5 and 101.0%.

Five biogenic amines of fish meal prior to their HPTLC [62] were treated with 6% trichloroacetic acid and centrifuged. The supernatant was dansylated (37 °C, 2 h) and derivatives extracted with ethyl acetate. Separations needed 50 min; recoveries were not given.

Nine biogenic amines of various food samples (cheese, fish, meat) [63] were separated from amino acids in two steps: cheese with 0.1 *M* HCl, fish and meat with 5% trichloroacetic acid were extracted. The organic material containing extracts was saturated by NaCl and further extracted with butanol (cheese), or with butanol–chloroform (fish, meat). Dansylation of the organic extracts was followed at 40 °C for 1 h; elution on a 15×1.6 mm column (particle size 3 μm) was carried out within 6 min, with good resolution. Recoveries varied between 47 and 88% (salmon), 52 and 99% (tuna), 3 and 91% (salami), 3 and 80% (grana cheese) and 2 and 92% (gorgonzola cheese), respectively.

Changes in the polyamine contents of carbohydrates of various degree of polymerization was determined as their dansyl derivatives [64]. Prior to their derivatization was column chromatographic clean-up (Sephadex G-50), thereafter $HClO_4$ (5%, w/v) treatment was performed. An aliquot of the supernatant was also hydrolyzed. Free and acid-liberated amines were dansylated overnight in sealed vials. The chromatography of the toluene-extracted dansyl amines was performed within 30 min. Recoveries of 85% were reported.

Ten C_1–C_7 aliphatic amines, present in environmental waters at trace level, were quantitated as their dansyl derivatives applying peroxylate-based chemiluminescence detection [65]. A special set-up of instruments was developed for the continuous SPE, dansylation (65 °C, 10 min) and HPLC–peroxylate-based chemiluminescence detection of samples. The elution time was 45 min, recovery values were not given.

4.3. Quantitation of amines by capillary electrophoresis

Amine contents of beer [66] and wines [67,68] were derivatized by 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole [66], by AQC [67] and determined without derivatization [68], applying simultaneous UV and LIF [66], as well as UV [67] and indirect UV [68] detections.

Ethyl-, isoamyl- and dibutylamines of beer [66] were eluted without any pretreatment, within 17 min (including washing and equilibration time), together with several unknown compounds of overwhelming concentrations (present author's remark: unknown derivatives were very likely amino acids). Reproducibility values varied between 10 and 19% (RSD).

Separation of seven amines [67] from food samples was started by their extraction with 6% $HClO_4$ (shaking for 1 h) followed by centrifugation and filtration (0.45 μm membrane). Selective enrichment of amines present in food and wine samples was performed by column chromatography (Dowex 50W-X8). Derivatization needed 10 min (55 °C) and separation 30 min. Migration time and peak area values proved to be highly reproducible (<5%), recovery data were not given.

Direct determination of biogenic amines, developing continuous flow clean-up in wine, was optimized [68] regarding: (i) the type of SPE columns (out of the commonly used CBA, strong cation-exchange and C_{18} columns, the weak cation-exchanger CBA was selected), (ii) the pH of wine subjected to clean-up (increased pH by use of the NH_4OH–

NH_4Cl buffer ensuring the complete retention of amines), and (iii) the eluent (0.1 *M* HNO_3 in methanol). This process resulted in 96–98% recovery of amines from the SPE column. Indirect UV detection was carried out using 4 m*M* copper(II) sulfate, formic acid and 18-crown-6 as running buffer.

5. Conclusions

Chromatographic procedures related to the analysis of amino acid- and amine-containing matrices have been placed in three groups: (i) simultaneous analysis of amino acids and amines from a single run. (ii) Separate determination of amino acids and amines, mainly as different derivatives, applying various chromatographic methods, subsequent to their isolation into separate extracts. (iii) Quantitation of amines only, following the separation of amino acids from amines. The advantages and drawbacks of all three possibilities were characterized on the basis of recovery, reproducibility values, time and cost phenomena of methods; however, all of these characteristics were limited by data available in the paper in question.

1. From the point of view of the analytical chemist the simultaneous quantitation of amino acids and amines seems to be the best choice: however, to find the optimum conditions, with time not being considered, for amino acids and amines simultaneously occurring in a matrix, is an unambiguous challenge. In order to solve the problem quickly it helps to have practice in the derivatization and chromatographic conditions of the two groups (amino acids, amines) separately.
2. In cases when knowledge of the amine content of the sample only is needed, the best choice could be the fast chromatographic elution of amino acids without separation, prior to the slow, well-resolved separation of diamines, such as putrescine, cadaverine, spermine and spermidine, etc. Certainly, amines of low molecular mass, insert into the amino acid derivatives and are eluting together with them.
3. On the basis of the spectacularly low recoveries of amines—determined subsequent to their isolation/extraction from amino acids—this possibility can be regarded as the worst solution of the task.

6. Nomenclature

ACN	acetonitrile
agm	agmatine
Ala	alanine
β-ala	β-alanine
GABA	γ-aminobutyric acid
AQC	6-aminoquinolinyl-*N*-hydroxy-succinimidyl chloride
Asn	asparagine
Asp	aspartic acid
cad	cadaverine
CE	capillary electrophoresis
CRA-Cl	carbazol-9-yl-acetyl chloride
CRP-Cl	carbazol-9-yl-propionyl-chloride
cit	citrulline
CNLS	condensation nucleation light scattering
dabsyl	2,4-(dimethylamino)azobenzene-4′-sulfonyl chloride
dansyl	5-dimethylamino-1-naphtalenesulfonyl chloride
etam	ethanolamine
eta	ethylamine
FMOC	fluorenylmethyl chloroformate
FQ	5-furoylquinoline-3-carboxaldehyde
Glu	glutamic acid
Gln	glutamine
Gly	glycine
hepta	heptylamine
hexa	hexylamine
HPCE	high-performance capillary electrophoresis
His	histidine
IPAD	integrated pulse amperometric detection
IC	ion chromatography
IEC	ion-exchange chromatography
ima	isoamylamine
iba	isobutylamine
Ile	isoleucine
ipra	isopropylamine
LIF	laser-induced fluorescence
Leu	leucine
Lys	lysine
ME	2-mercaptoethanol

MPA	3-mercaptopropionic acid
MET	methanol
met	methionine
meta	methylamine
NAC	*N*-acetyl-L-cysteine
nba	*n*-butylamine
nle	norleucine
npra	*n*-propylamine
octa	octylamine
OPA	*o*-phthaldialdehyde
orn	ornithine
phe	phenylalanine
pheta	phenylethylamine
PITC	phenylisothiocyanate
PTC	phenylthiocarbamyl
DAD	photodiode array detection
PVP	polyvinylpyrrolidone
pro	proline
put	putrescine
ser	serine
spermd	spermidine
sperm	spermine
thr	Threonine
tryn	tryptamine
trp	tryptophan
tyrn	tyramine
tyr	tyrosine

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