ELSEVIER

Journal of Chromatography A, 707 (1995) 373–379

JOURNAL OF CHROMATOGRAPHY A

Short communication

Determination of biogenic amines and their precursor amino acids in wines of the Vallée du Rhône by high-performance liquid chromatography with precolumn derivatization and fluorimetric detection

T. Bauza[a,b,*], A. Blaise[a], F. Daumas[b], J.C. Cabanis[a]

[a]*Centre de formation et de Recherche en Oenologie, Institut Supérieur de la Vigne et du Vin, Faculté de Pharmacie, 15 Avenue Charles Flahault, 34018 Montpellier, France*

[b]*Comité Interprofessionnel des Vins d'A.O.C. de Côtes du Rhône et de la Vallée du Rhône, 6, Rue des trois faucons, 84000 Avignon, France*

First received 1 December 1994; revised manuscript received 2 March 1995; accepted 3 March 1995

Abstract

The presence of biogenic amines in foods and more particularly in wines is of current interest due to their pharmacological properties and the physiological disorders they may provoke in the human organism. Although at present compounds like histamine or cadaverine can be analyzed with precision in wine, less information is provided on the possible presence of spermine and spermidine. Using fluorenylmethylchloroformate (FMOC) as derivatization reagent we were able to determine these compounds in wines simultaneously with other biogenic amines such as histamine, tyramine, phenylethylamine, putrescine, agmatine, cadaverine, and precursor amino acids of these compounds, in their free state in wine: arginine, ornithine, histidine, phenylalanine, and tyrosine. Samples of 54 red wines, 15 rosé wines and 15 white wines from the Vallée du Rhône (France), all bottled and commercialized, have been analyzed by this method.

The polyamines cadaverine, spermine and spermidine are present in small quantities in these wines. Only agmatine and putrescine appear at levels significantly higher than 1 mg/l. The presence of putrescine is strongly correlated to that of its precursors arginine, ornithine and agmatine, as well as with the presence of tyramine and histamine.

On the other hand, no correlation (threshold of 5%) was found between the levels of phenylethylamine, tyramine and histamine, and those of their free precursor amino acids in the wine, phenylalanine, tyrosine and histidine.

Levels of putrescine, agmatine, histamine, tyramine, spermine, spermidine are higher in red wines than in the other types of wine.

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(95)00318-5

1. Introduction

Since their discovery in 1954 by Tarantola [1], the physiological influence on the human organism of the presence of biogenic amines in wines has been discussed [2,3]. More recently, high-performance liquid chromatography has enabled the development of good reliable analytical methods for the determination of the major biogenic amines in wines [4,5]. However, these methods are not able to detect some of the amines, such as spermidine and spermine. These amines, even though they appear less toxic than other compounds such as histamine or tyramine [6,7], possess nevertheless many functions at the cellular level. By their polycationic long chain structure, they interact with DNA, RNA, proteins and the membrane phospholipides [8,9]. They are also strongly implied in cellular growth phenomena. So, one finds them in great quantities in tumor cells, which possess high growth rates, and in smaller amounts in all living organisms, including viruses. The presence of these polyamines in grape berries, and the role that they may play have been studied for some years [10]. In the vine, potassium deficiency increases the putrescine content in leaves [11–13]. The presence of putrescine and cadaverine in wines is well documented [14,15]. On the other hand, few tests have been realized on spermine, spermidine and agmatine. The classical analytical method [4] for the determination of biogenic amines in wines by HPLC uses a precolumn derivatization with phthaldialdehyde (OPA). This method gives very good results for primary amines, but is not able to derivatize the secondary amines functions of spermine and spermidine. The utilization of fluorenylmethylchloroformate (FMOC) [16–19] allows the direct analysis in wines of the five polyamines putrescine (PU), cadaverine (CA), agmatine (AG), spermine (SM) and spermidine (SD), simultaneously with other biogenic amines, such as histamine (HA), phenylethylamine (PE), tyramine (TA), and their precursor amino acids arginine (AR), ornithine (OR), phenylalanine (PA), histidine (HD) and tyrosine (TS).

In order to measure the biogenic amine and amino acid precursor concentrations in wine, 84 samples of bottled and commercialized wines (54 red wines, 15 rosé wines and 15 white wines) have been taken from different sites in the region of the Vallée du Rhône, and analyzed with the method described below.

2. Experimental

2.1. Materials

Analyses were performed with a Hewlett-Packard 1050 series HPLC system consisting of a solvent degasser system, a quaternary pumping module at low pressure mixing, an autosampler, a fluorimetric detector series 1046A with excitation at 263 nm and detection at 313 nm. The system was managed by a Hewlett-Packard Vectra 286-S-20 data analysis station.

The separation column used was an ODS Hypersil column, 200 × 2.1 mm I.D., 5 μm (Hewlett-Packard). The injection volume was 2 μl for all analyses performed.

FMOC, all solvents, as well as the different biogenic amines and amino acids, were furnished by Fluka (Buchs, Switzerland).

2.2. Preparation of reagents

A standard solution containing 1 g/l of each compound is prepared in 0.1 *M* HCl. This solution can be stored for eight weeks in the refrigerator.

Solutions diluted 100- and 200-fold, are prepared daily for calibration. A buffer solution of pH 8.5 is prepared by adjusting the pH of a 0.2 *M* boric acid solution with 5 *M* NaOH.

A cleavage solution (0.5 *M* NH_3) and a quenching solution (acetonitrile–acetic acid–water, 20:2:3, v/v) are also used.

2.3. Derivatization reaction

In a 700-μl amber vial, 20 μl of sample are mixed with 50 μl of borate buffer. An 8-mg amount of FMOC is dissolved in 1 ml of acetonitrile, and 100 μl of this reagent are added to the

Table 1
Solvent gradient. Flow-rate 0.3 ml/min

Time (min)	A (%)	B (%)
0	15	85
18	15	85
18.1	38	62
25	40	60
30	40	60
67	42	58
103	85	15
110	85	15

vial. After 3 min, 50 μl of cleavage solution are added. After 3 min, 300 μl of quenching solution are added to the vial, which is then sealed and placed in the autosampler tray.

2.4. Chromatographic conditions

A solvent gradient with a flow-rate of 0.3 ml/min, is realized from two solutions according to Table 1: Eluent A, acetonitrile–2-octanol (1% v/v); Eluent B: acetonitrile 150 ml, phosphoric acid 8.8 ml, dimethylcyclohexylamine 10 ml, bidistilled water QSP 1 l. The pH must be near 2.7.

3. Results and discussion

FMOC reacts with primary and secondary amine functions. Optimization of the reaction conditions has been undertaken for the dosage of the polyamines putrescine, cadaverine, spermidine and spermine. The derivatization of spermine and spermidine requires larger quantities of reagent, because of the presence of the secondary functions. An excess of FMOC decreases the reaction efficiency despite the utilization of the cleavage solution. An amount of 6–10 mg/ml appears to be sufficient for derivatizing these compounds in wine. The cleavage solution is used at the end of the reaction to eliminate the excess of reagent, and to break complexes originating from multiple derivatizations of the same amine function. Its use increases the derivatization yield for spermine and spermidine analyzed directly in the wine, and harmonizes the results obtained with those of the standard solutions. An excess of cleavage solution (higher than 100 μl) reduces the efficiency of the derivatization of amines like spermine.

Figs. 1 and 2 show chromatograms obtained with aqueous standard solution and with a red wine.

The repeatability (relative error for six samples in the same analysing sequence) of the method ranges from 1.6% for agmatine to 13.6% for spermine.

When levels are not too high for the sensitivity of the detector, precursor the amino acids, arginine, ornithine, histidine, phenylalanine and tyrosine present in the free state in the wine and susceptible to be metabolized, have also been quantified.

The analysis of the 84 samples shows that wines from the Vallée du Rhône contain small quantities of biogenic amines (Table 2). Only 8% of the samples contain more than 20 mg/l of putrescine, and 1.2% more than 10 mg/l of histamine or tyramine. Phenylethylamine also is present in small quantities, 95% of samples containing less than 5 mg/l. The polyamines cadaverine, spermidine and spermine are rarely present in wine. Their levels were lower than 1 mg/l in 83.3% of the samples for spermidine, 98.9% for spermine and 98.8% for cadaverine. Agmatine, which is an intermediate in the synthesis of putrescine from arginine, appears at higher levels than the other polyamines, exceeding 20 mg/l in one third of the samples. Putrescine shows levels lower than this value in 91.7% of the samples (Table 2). There is a very strong correlation between the presence of putrescine and that of its precursors agmatine, arginine ($p < 0.001$), and ornithine ($p < 0.01$). High concentrations of these latter compounds favor the presence of putrescine in a wine. The presence of this amine is also correlated to the presence of tyramine and histamine ($p < 0.001$). On the other hand, no correlation exists ($p < 0.05$) between histamine and histidine (or the sum of the histidine and histamine contents), phenyl-

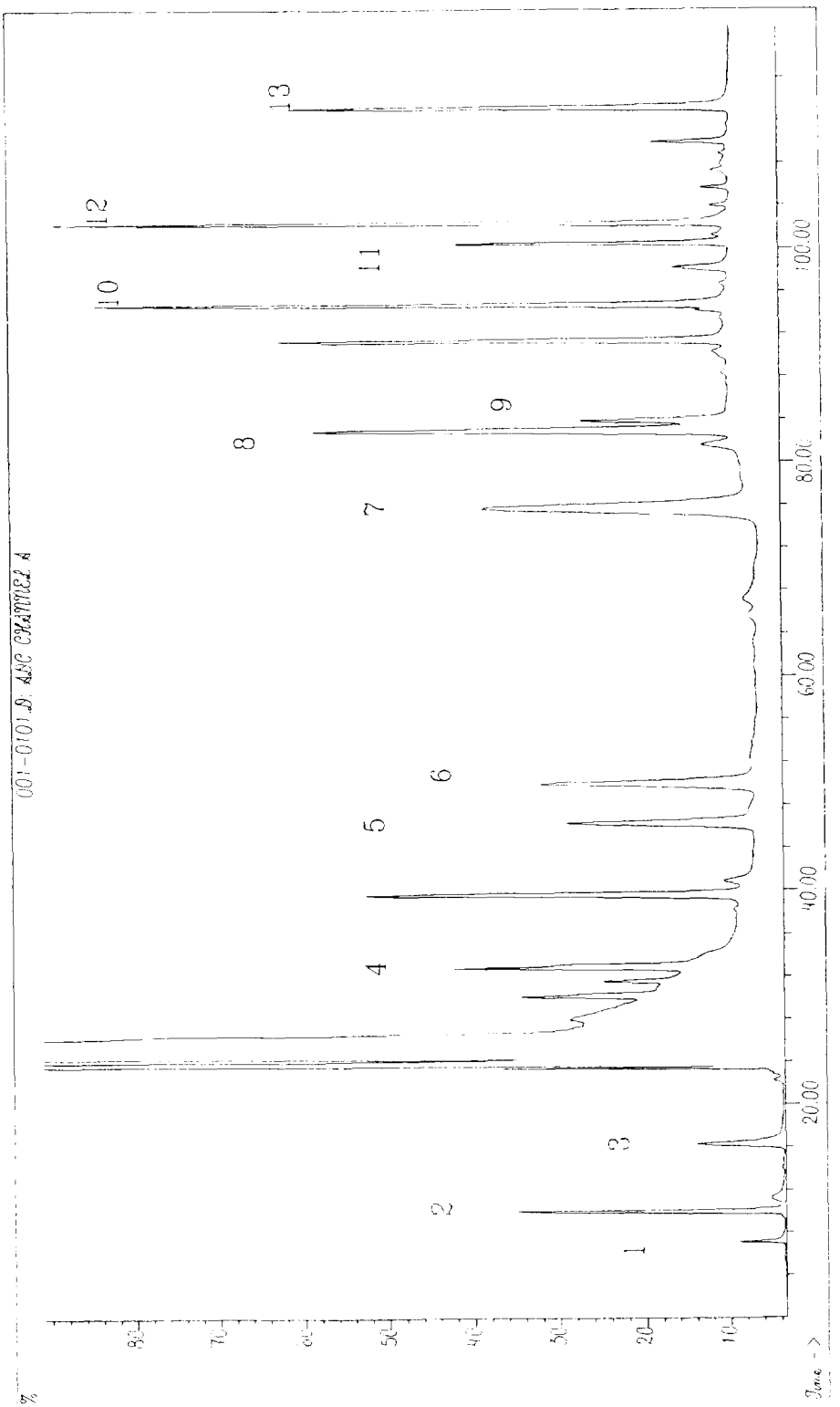

Fig. 1. Chromatogram of a standard solution. Peaks: 1 = histidine, 4.6 mg/l; 2 = arginine, 5.15 mg/l; 3 = agmatine, 4.1 mg/l; 4 = phenylalanine, 4.7 mg/l; 5 = phenylethylamine, 5.2 mg/l; 6 = ornithine, 3.6 mg/l; 7 = putrescine, 4 mg/l; 8 = cadaverine, 4.5 mg/l; 9 = tyrosine, 4.7 mg/l; 10 = tyramine, 6.2 mg/l; 11 = histamine, 4.6 mg/l; 12 = spermidine, 3.9 mg/l; 13 = spermine, 3.9 mg/l.

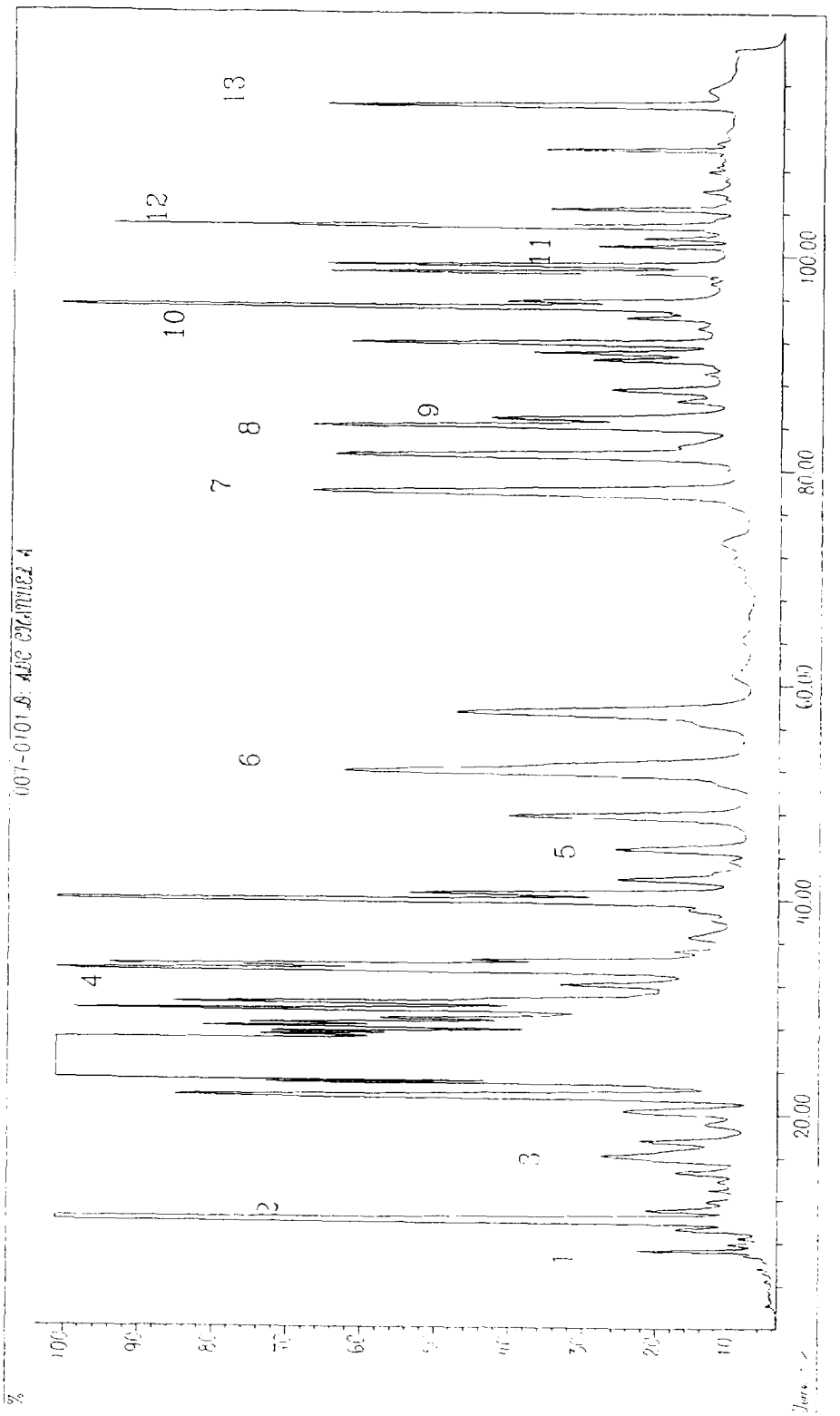

Fig. 2. Chromatogram of a wine sample overloaded with: 1 = histidine, 4.6 mg/l; 2 = arginine, 5.15 mg/l; 3 = agmatine, 4.1 mg/l; 4 = phenylalanine, 4.7 mg/l; 5 = phenylethylamine, 5.2 mg/l; 6 = ornithine, 3.6 mg/l; 7 = putrescine, 4 mg/l; 8 = cadaverine, 4.5 mg/l; 9 = tyrosine, 4.7 mg/l; 10 = tyramine, 6.2 mg/l; 11 = histamine, 4.6 mg/l; 12 = spermidine, 3.9 mg/l; 13 = spermine, 3.9 mg/l.

Table 2
Mean concentration and standard deviation for the different compounds found in wines of the Vallée du Rhône in mg/l

	All samples		Red wines		Rosé wines		White wines	
	Middle	Std. Dev.	Middle	Std. Dev.	Middle	Std. Dev.	Middle	Std. Dev.
AR	22.4	14.8	27.5	14.7	10.5	5.6	16.9	12.7
OR	3.7	4.4	5.0	4.8	1.0	1.2	1.9	2.5
AG	17.3	9.5	21.6	8.4	9.0	3.7	10.3	7.1
PU	7.7	6.8	10.8	6.7	2.5	0.9	1.9	0.7
CA	0.2	0.4	0.2	0.2	0.4	0.9	0.1	0.1
SD	0.5	0.6	0.6	0.6	0.4	0.5	0.3	0.3
SM	0.1	0.2	0.1	0.1	0.2	0.4	0.1	0.2
HI 8.9 10.9		8.2	6.7	13.5	22.0	7.3	3.7	
PA	13.0	5.8	13.7	5.9	11.3	4.8	12.6	6.3
TS	3.5	2.3	3.3	2.3	3.3	1.5	4.4	2.6
HA	2.5	2.6	3.7	2.5	0.4	0.6	0.1	0.2
PE	1.7	1.5	1.9	1.6	1.5	1.4	0.9	0.7
TA	3.1	2.1	3.7	2.3	2.3	1.7	2.2	1.4

$n = 84$ samples; red wines = 54, rosés wines = 15, white wines = 15. PU = putrescine, HI = histidine; HA = histamine; OR = ornithine; AR = arginine; AG = agmatine; PA = phenylalanine; PE = phenylethylamine; CA = cadaverine; TS = tyrosine; TA = tyramine; SD = spermidine; SM = spermine.

ethylamine and phenylalanine or between tyramine and tyrosine (or the sum of the tyramine and tyrosine contents).

The Kruskal–Wallis statistical test shows a significant difference between red wines and rosés and white wines with respect to the levels of arginine and ornithine, the precursor amino acids of putrescine, as well as histamine, putrescine, agmatine (1%), spermine, spermidine and tyramine (5%). Red wines are therefore richer in polyamines and their precursors than the other types of wine. Equally, they contain more tyramine and histamine.

4. Conclusions

The use of FMOC as derivatizing reagent seems to be an attractive choice for the determination of biogenic amine analysis in wines. This technique enables a direct analysis with satisfactory sensitivity of this family of compounds in the wine, particularly of spermine and spermidine, which contain secondary amines functions. It also allows the simultaneous determination of some of their precursor amino acids.

Wines of the Vallée du Rhône generally contain small quantities of biogenic amines. However, large differences may occur between different samples. Thus, red wines contain higher levels of histamine, tyramine, agmatine, putrescine, spermine and spermidine than the other types of wine. The levels of spermine, spermidine and cadaverine in wine are nevertheless insignificant from a physiological point of view. The amount of putrescine is linked to the presence of its precursors agmatine, arginine and ornithine, in the free state in the finished wine, as well as to that of the biogenic amines histamine and tyramine. On the other hand, the presence of tyramine and histamine is not linked to the presence of tyrosine and histidine. Similar results are found for phenylethylamine and its precursor phenylalanine.

References

[1] G. Tarantola, Atti Accad. Ital. Vite Vino, 6 (1954) 146.
[2] J.C. Cabanis, Bull O.I.V., 656–657 (1985) 1009.

[3] J. Aerny, Bull. O.I.V., 656–657 (1990) 1017.
[4] Ch. Tricard and J.M. Cazabeil, Office International de la Vigne et du Vin, Internal Communication, F.V. No. 850, 1990.
[5] P.K. Lehtonen, M. Saarinen, M. Vesanto and M.L. Riekkola, Z. Lebensm. Unters. Forsch., 194 (1992) 434.
[6] R.T. Coutts, G.B. Baker and F.M. Pasutto, Adv. Drug Res., 15 (1986) 169.
[7] H.M.L.J. Joosten, Neth. Milk Dairy J., 42 (1987) 25.
[8] C.W. Tabor and H. Tabor, Ann. Rev. Biochem., 53 (1984) 749.
[9] N. Seiler, Digestion, 46 (1990) 319.
[10] M. Brodequis, B. Dumery and Bouard, Conn. Vigne Vin, 23 (1989) 1.
[11] D.O. Adams, K.E. Franke and L.P. Christensen, Am. J. Enol. Vitic., 41 (1990) 121.
[12] A. Bertrand, M.C. Ingargiola and J. Delas, Rev. Fr. Oenol. (Cah. Sci.), 132 (1991) 7.
[13] D.O. Adams, D.J. Bates, D.F. Adams and K.E. Franke, Am. J. Enol. Vitic., 43 (1992) 239.
[14] J. Aerny, Office International de la Vigne et du Vin, Internal Communication, F.V. No. 881, 1990.
[15] M.C. Ingargiola and A. Bertrand, Office International de la Vigne et du Vin, Internal Communication, F.V. No. 896, 1992.
[16] S. Einarsson, B. Josefsson and S. Lagerkvist, J. Chromatogr., 282 (1983) 609.
[17] Z. Harduf, T. Nir and B.J. Juven, J. Chromatogr., 437 (1988) 378.
[18] P.A. Haynes, D. Sheumack, J. Kibby and J.W. Redmond, J. Chromatogr., 540 (1991) 177.
[19] H. Godel, P. Seitz and P. Verhoef, LC-GC Intl., 5 (1992) 44.