ELSEVIER

Journal of Chromatography A, 718 (1995) 309–317

JOURNAL OF CHROMATOGRAPHY A

Improvement of a solid-phase extraction method for determining biogenic amines in wines

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First received 22 February 1995; revised manuscript received 13 June 1995; accepted 14 June 1995

Abstract

Solid-phase extraction (SPE) was used simultaneously to clean up and concentrate samples prior to automatic derivatization to determine fifteen biogenic amines in wine. In the first step, SPE was used to remove polyphenolic compounds which interfere with further extraction and chromatographic analysis; in this study, treatments with polyvinylpyrrolidone, SAX and C_{18} cartridges were tested and compared. In the second step, C_{18} cartridges were used to concentrate the analytes after adding sodium octanesulfonate, sodium decanesulfonate and sodium dodecanesulfonate as ion-pair reagents. Reversed-phase chromatography with fluorimetric detection was performed on the extracted amines after automatic precolumn derivatization by treatment with *o*-phthalaldehyde. Biogenic amines can be separated and detected after the solid-phase extraction with an average sensitivity of the order of 20–90 $\mu g\ l^{-1}$. Recoveries were determined by the standard addition technique and the overall method was successfully applied to the determination of the above-mentioned amines in red wines from the Tarragona region.

1. Introduction

Biogenic amines are low-molecular-mass basic compounds that can be found in a variety of fermented foods and beverages, sometimes indicating product spoilage. In wines, amines occur as salts. They are odourless, but with the pH prevailing in the mouth, amines are released and their flavour can be tasted. Moreover, high amine contents, especially histamine, are related to several physiological effects, mainly when alcohol and acetaldehyde are present, which act as potentiators of toxicity. There is a positive correlation between the concentration of histamine and that of other undesirable biogenic amines, such as tyramine, tryptamine, β-phenethylamine, cadaverine and putrescine in wine.

More than 30 amines have been identified in wine [1–10]: butylamine, cadaverine, tryptamine, ethanolamine, 1,3-diaminopropane, dimethylamine, ethylamine, hexylamine, histamine, indole, iso- and *n*-propylamine methylamine, 2- and 3-methylbutylamine, morpholine, iso- and *n*-pentylamine, β-phenethylamine, piperidine, putrescine, pyrrolidine, 2-pyrrolidone, serotonin, tyramine, etc. Their concentration has been reported to range from a few mg l^{-1} to about 50 mg l^{-1} depending on the quality of the wine [11].

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0021-9673/95/$09.50
SSDI 0021-9673(95)00680-X

Numerous liquid chromatographic methods for the determination of biogenic amines in wine have been described, with ion-exchange [2,12] or reversed-phase columns; with pre- [7,16–18] or postcolumn [19] derivative formation and without derivatization [6,13–15] and with different detection means, mainly ultraviolet [10,18,20,21] or fluorescence [4,7,10,11,16,17]. Nevertheless, if biogenic amines are to be determined at low levels with no interference from other compounds, e.g., amino acids, previous clean-up and preconcentration steps are required.

Numerous methods for isolating amines from wine have been proposed and reported, including either liquid–liquid extraction (LLE) and solid-phase extraction (SPE) procedures. Several researchers, including Almy et al. [22], Lehtonen [10] and Bortolomeazzi [23], who extracted the amines with butanol after preconcentration in a rotary evaporator at pH 1.5, have used LLE before derivatization to isolate biogenic amines. Other workers, such as Walther et al. [24], who extracted dansylamides with ethyl acetate after acetone evaporation under a nitrogen stream, and Buteau et al. [6], who extracted five *o*-phthalaldehyde (OPA) derivatives with ethyl acetate, carried out the extraction after derivatization. Differences in polarity in the former LLE procedures result in low recoveries for some amines, while instability of the OPA derivatives in the latter results in poor reproducibility.

Nowadays, SPE is preferred to LLE because of its obvious advantages, and some studies have been carried out using this method before and after derivatization. However, the method commonly proposed for the determination of biogenic amines in wine (or aqueous samples in general) is based on cation-exchange extraction followed by derivatization of the fraction of interest with OPA [4,5,21,25–29]. In previous work, solid-phase extraction was successfully used to enrich the biogenic amines in wines after derivatization with dansyl chloride (DnsCl) using C_{18} sorbents [30] or with phenyl isothiocyanate (PITC) using SAX cartridges [21]. It has been demonstrated with phenolic substances in water [31] that the retention capacity of C_{18} cartridges for polar compounds increases when an ion pair reagent is used, so these ion pairs have been applied to wines.

For the procedure proposed here, several ion-pair reagents (octane-, decane- and dodecane sulfonate) were tested in order to improve the SPE recoveries for the determination of food-related biogenic amines. The method was applied to the determination of these compounds in red wines.

2. Experimental

2.1. Chemicals and reagents

The fifteen amines studied were ethanolamine, histamine, tyramine, ethylamine, isopropylamine, propylamine, methylamine, tryptamine, butylamine, phenethylamine, putrescine, 3-methylbutylamine, amylamine, cadaverine and hexylamine, all of which were supplied by Aldrich-Chemie (Beerse, Belgium). An individual standard solution of 2000 mg l^{-1} of each amine was prepared in HPLC-grade methanol (Scharlau, Barcelona, Spain) and stored in darkness at 4°C. A standard solution containing all the amines was prepared with an aliquot of each solution and subsequently diluted with methanol in a volumetric flask. More dilute solutions used in the different studies were prepared by diluting the standard solutions with water purified in a Milli-Q water apparatus (Millipore, Bedford, MA, USA).

Methanol, acetonitrile and tetrahydrofuran used in the chromatographic and extraction method were of HPLC grade (Scharlau). Sodium acetate (0.05 *M*) buffer solution was also supplied by Scharlau. For the automatic derivatization method, OPA (purity 99%) and mercaptoethanol (purity 98%) (Aldrich), HPLC-grade acetone (Scharlau) and sodium tetraborate and sodium hydroxide to adjust the pH were used.

Octanesulfonate (OSA), decanesulfonate (DeSA) and dodecanesulfonate (DoSA) sodium salts (Scharlau) were used to form amino–alkylsulfonate neutral pairs.

2.2. Equipment

Chromatographic experiments were performed using a Hewlett-Packard (Waldbronn, Germany) Model 1050 liquid chromatograph with an HP Model 1046A fluorescence detector. The samples were derivatized and injected with an HP Series 1050 automatic injector. Separation was performed using an ODS Basic cartridge (250 × 4.6 mm I.D., particle size 5 μm) preceded by an ODS Basic precolumn, both supplied by Teknokroma (Barcelona, Spain). Chromatographic data were collected and recorded on an HP ChemStation version A.01.01.

SPE experiments were performed using a Visiprep DL disposable liner solid-phase extraction vacuum manifold with individual flow control valves from Supelco (Bellefonte, PA, USA), which allowed twelve SPE tubes to be dried at a time.

2.3. High-performance liquid chromatographic method

Two solvent reservoirs containing (A) 1% tetrahydrofuran and 0.05 *M* sodium acetate in water and (B) methanol were used to separate all the amines with an HPLC elution programme which began with 55% of methanol in the mobile phase and finished 25 min later with 80% of the same solvent. Finally, the column was cleaned with a isocratic elution at this percentage of methanol for a further 3 min. The programme took a further 2 min to return to the initial conditions and stabilize the corresponding mobile phase. Determination was performed at 60°C with a flow-rate of 1 ml min^{-1} and the eluted POA derivatives were detected by monitoring their fluorescence at 330 and 445 nm as the wavelengths of excitation and emission, respectively. Under these conditions, all fifteen amines were eluted in less than 20 min.

2.4. Derivatization

The derivatization reagent was prepared with 45 mg of OPA, 200 μl of mercaptoethanol (ME) and 1 ml of methanol, diluted to a total volume of 10 ml with a buffer solution of sodium tetraborate (3.81 g dissolved in 100 ml of distilled water and adjusted to pH 10.5 with 10 *M* sodium hydroxide) so as to adjust the derivatization pH.

The derivatization was fully automated by means of an injector programme. The injection system mixed the reagents automatically. The OPA–ME derivatization reagent and the sample were drawn sequentially into the injection needle and the reactants were mixed by drawing them back and forth in the injection seat. Finally, the mixture was injected into the column and separated using gradient elution. The steps in the derivatization sequence are summarized in Table 1.

Table 1
Injection programme for the derivatization of primary amines with OPA

Step	Action	Amount	Details	Substance
10	Draw	2 μl	Air	
20	Draw	5 μl	From vial 2	OPA–ME (derivatizing agent)
30	Eject	5 μl	Into seat	
40	Draw	0 μl	From vial 1	MeOH for needle wash
50	Draw	2 μl	From sample	
60	Eject	2 μl	Into seat	
70	Draw	0 μl	Vial 1	MeOH for needle wash
80	Draw	5 μl	Vial 2	OPA–ME (derivatizing agent)
90	Eject	5 μl	Into seat	
100	Mix	12 μl	Ten cycles	
110	Wait	1 min		
120	Inject			

2.5. Solid-phase extraction

SPE consisted of two steps: the first removed the polyphenolic compounds and the second removed other polar compounds but retained the amines. Both C_{18} cartridges (1000 mg) (Varian, Harbor City, CA, USA) and SAX (500 mg) (Varian) were used in the first step, and in both cases they were activated with two fractions of 5 ml of methanol, and further conditioned with two fractions of 5 ml of Milli-Q-purified water.

When polyvinylpyrrolidone (PVP) was used to bleach wine, the conditions were the same as those outlined in a previous paper [30].

The second cartridge (C_{18}) was also activated with two fractions of 5 ml of methanol and then conditioned with another two fractions of 5 ml of the ion-pair solution (OSA, DeSA and DoSA) at a pH between 3.5 and 5.5, depending on the experiment. Then, the sample with ion-pair reagent at the established pH was passed through the cartridge. Amines were eluted with a determined volume of organic solvent and automatically derivatized prior to their chromatography.

3. Results and discussion

In a previous study [32], a binary mobile phase formed by a solution of triethanolamine in water and methanol as organic modifier was used to separate biogenic amines after their derivatization with OPA. The efficiency of the column clearly deteriorated after a few injections, revealed by the overlapping of the histamine derivative peak and other initial interfering peaks. It was therefore necessary to regenerate the column with an organic solvent to obtain the initial resolutions. The use of 0.05 *M* acetate buffer solution (pH 7) allows a different analysis to be carried out with no further column regeneration and with similar efficiency.

Fig. 1 shows the chromatogram which resulted from automatically injecting 5 mg l^{-1} of the fifteen OPA derivatives into the chromatograph under the conditions previously specified. Good resolution was obtained among all the peaks.

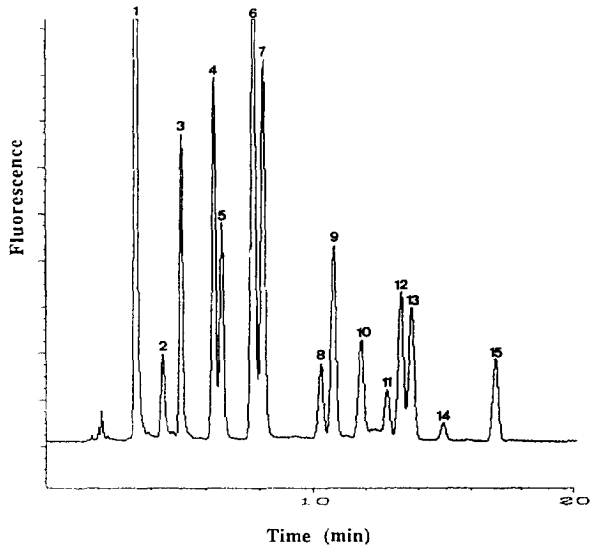

Fig. 1. Optimum chromatographic separation of an OPA-amine derivative standard solution of concentration 5 mg l^{-1}. Peaks: 1 = ethanolamine; 2 = histamine; 3 = methylamine; 4 = ethylamine; 5 = tyramine; 6 = isopropylamine; 7 = propylamine; 8 = tryptamine; 9 = butylamine; 10 = phenethylamine; 11 = putrescine; 12 = 3-methylbutylamine; 13 = amylamine; 14 = cadaverine; 15 = hexylamine. For experimental conditions, see text.

The precision and linearity of the precolumn derivatization method were examined. According to Buteau et al. [6], a comparison between results obtained by analysing samples using either an external or internal standard indicated that there was no significant difference between them. An external standard method was chosen here because it allows samples to be automatically derivatized using the injection programme explained above and which is more sensitive than the programmes which must involve an internal standard also.

In order to verify the linearity of the response of the different derivatives at the previously specified wavelengths for the working concentration, standard solutions of amines were prepared and injected. Calibration graphs of each amine were constructed by plotting the amine peak area against the amine concentration. Linear least-squares regression was used to calculate the slope, intercept and correlation coefficient, which was good in all cases (Table 2).

Table 2
Linearity of the fluorimetric detector response at 330 and 445 nm as excitation and emission wavelengths

Amine	Range of linearity (mg l^{-1})	Slope	Intercept	r^2
Ethanolamine	0.81–16.11	17381	−1336	1.000
Histamine	0.43–8.54	3326	−3024	0.999
Methylamine	0.22–4.46	12421	−731	1.000
Ethylamine	0.27–5.49	13363	−579	1.000
Tyramine	0.41–8.18	8147	−758	1.000
Isopropylamine	0.43–8.56	20162	226	1.000
Propylamine	0.47–9.41	12329	−584	1.000
Tryptamine	0.42–8.42	3095	−10	1.000
Butylamine	0.41–8.18	8562	−1132	1.000
Phenethylamine	0.40–8.10	4547	−219	1.000
Putrescine	0.94–18.72	1975	1211	0.999
3-Methylbutylamine	0.91–9.07	6825	−284	1.000
Amylamine	0.94–9.44	5369	−90	1.000
Cadaverine	0.74–7.44	871	1068	0.999
Hexylamine	0.86–8.58	4257	−522	1.000

The detection limit was calculated from the amount of amine required to give a signal-to-noise ratio of 3 by injecting 1 μl of sample without SPE, and ranged from 0.07 to 0.4 mg l^{-1}, whereas the quantification limits ranged from 0.2 to 0.9 mg l^{-1}, depending on the amine. The quantification limit was established as three times the detection limit and it was considered as the first point in calibration graphs.

Isolating biogenic amines from wine requires polyphenolic compounds to be previously removed because they block the C_{18} cartridge used in SPE, especially when dealing with red wines. In order to perform this bleaching treatment, PVP and two C_{18} and SAX cartridges were tested. In all cases a decolorized wine was obtained with good analyte recoveries. PVP treatment requires time for mixture stirring (1 g of PVP per 25 ml of wine, 15 min) and for filtration, which makes the analytical time much too long [30], whereas clean-up with C_{18} or SAX cartridges is easily semi-automated. A 15-ml volume of a red wine spiked with a standard solution of 3 mg l^{-1} of the biogenic amines was processed with either C_{18} or SAX cartridges. When SAX was used, the sample was necessarily adjusted to pH 8 in order to retain polyphenolic substances, which were converted into their respective anionic forms. Table 3 gives the results obtained when all the decolorizing agents were tried. No substantial amounts of amines were lost with any of them, but it was found that an increase in sample volume resulted in co-elution of polyphenols when C_{18} or SAX cartridges were used. As SAX showed the best recoveries, especially for phenethylamine and hexylamine, it was selected as the preliminary clean-up cartridge.

To perform the solid-phase extraction of amines with C_{18} cartridges, several variables were studied: the concentration of the ion-pair reagent, the volume of organic solvent to desorb analytes, the best ion-pair reagent and the volume of concentrated sample.

The volume of organic solvent to desorb amines from C_{18} cartridges was the first step in the optimization of these variables. A 15-ml volume of wine sample spiked with 0.4 mg l^{-1} of standard biogenic amine solution was adjusted to pH 8 and passed through the SAX cartridge to remove polyphenolic and other non-polar compounds. Then 100 μl of OSA solution [33] as the ion-pair reagent and a volume of 0.05 *M* sodium acetate solution to adjust the pH to 4.5 were added and passed through the activated C_{18} cartridge. Different volumes of methanol were

Table 3
Recoveries from triplicate bleaching of wine samples

Amine	PVP [30]		C_{18}		SAX	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
Ethanolamine	98	3	99	2	97	4
Histamine	93	2	100	2	96	5
Methylamine	96	4	90	5	103	6
Ethylamine	95	3	94	3	93	5
Tyramine	93	4	88	4	100	3
i-Propylamine	100	4	97	3	105	4
Propylamine	96	4	96	3	100	2
Tryptamine	94	8	92	7	98	5
Butylamine	98	4	90	6	108	4
Phenethylamine	100	2	86	6	106	5
Putrescine	97	4	100	2	91	6
3-Methylbutylamine	98	3	93	4	103	3
Amylamine	93	4	91	3	111	3
Cadaverine	95	2	100	3	101	3
Hexylamine	98	2	78	3	112	3

passed through to elute these compounds and the best results were obtained when 3 ml of methanol were used. Poor recoveries were obtained when smaller volumes were used and higher volumes only diluted the sample.

The pH and type of ion-pair reagent were tested in order to improve the recoveries. After the bleaching process, a new C_{18} cartridge was activated with methanol and the corresponding ion-pair reagent solution was adjusted to pH 3.5, 4.5 or 5.5, depending on the experiment. pH 4.5 gave the best recoveries, although no significant differences were observed.

Several ion-pair reagents were also tested to increase the retention of biogenic amines in C_{18} cartridges. OSA, DeSA and DoSA at different concentrations were added to the sample after clean-up and before the concentration step at pH 4.5. For this study 15 ml of red wine spiked with 0.4 mg l^{-1} of biogenic amines were adjusted to pH 8 and passed through the SAX cartridge. Then the sample was acidified (pH 4.5) and 100 μl of ion-pair reagent were added before it was passed through the C_{18} cartridge and eluted with 3 ml of methanol. Table 4 gives the results obtained when a concentration of 100 m*M* of each ion-pair reagent was added. As can be seen, the best results were obtained when DeSA was used. Similar results were obtained in three cases for less polar compounds, but an increase in recovery was observed for more polar analytes when DeSA was used. However, poor results were obtained in three cases for ethanolamine and histamine, whose recoveries were close to 30% and 50%, respectively, when DeSA was used.

The effect of the concentration of the ion-pair reagent was also studied. DeSA at concentrations of 50, 100 and 200 m*M* was added to 15 ml of the spiked sample at pH 4.5 prior to the concentration step. The results for 50 and 100 m*M* were very similar, but a clear increase in recovery for more polar compounds (mainly histamine and methylamine) was observed when 200 m*M* DeSA was used. Ethanolamine did not show an improved recovery in any of these experiments, whereas ethylamine, tyramine, propylamine and cadaverine showed better recoveries when increasing concentration of ion-pair reagent (see Table 5). For this reason, 200 m*M* DeSA was chosen as the best concentration of ion-pair reagent in the SPE. A further increase in the ion-pair reagent concentration was not used because it led to precipitation problems.

Under the optimized conditions, the volume of

Table 4
Comparison of OSA, DeSA and DoSA as ion-pair reagents

Amine	OSA		DeSA		DoSA	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
Ethanolamine	22	8	29	6	26	5
Histamine	33	6	49	4	31	7
Methylamine	31	6	73	5	30	7
Ethylamine	29	8	67	4	21	9
Tyramine	55	5	80	4	31	6
Isopropylamine	27	6	80	4	16	10
Propylamine	21	7	80	4	14	9
Tryptamine	67	5	63	5	53	6
Butylamine	60	5	76	5	48	6
Phenethylamine	79	4	73	6	70	5
Putrescine	56	5	83	5	63	5
3-Methylbutylamine	78	4	75	4	71	5
Amylamine	79	6	74	3	71	5
Cadaverine	66	6	65	6	51	4
Hexylamine	61	5	50	7	46	5

Concentration of ion-pair reagent = 100 m*M*. For other conditions, see text.

eluent was tested again. As no significant differences were obtained between OSA and DeSA when 1, 2, 3 or 4 ml of methanol were used to desorb amines from C_{18} cartridges, we did not consider an increase in the volume of eluent to obtain the best accuracy. Finally, under the optimum conditions (200 m*M* DeSA, pH 4.5), the volume of sample was increased, but there were considerable recovery losses, mainly for more polar compounds.

Table 5
Comparison of concentrations of DeSA which showed significant differences among them

Amine	Recovery (%)	
	100 m*M*	200 m*M*
Ethanolamine	29	31
Histamine	49	78
Methylamine	73	86
Ethylamine	67	75
Tyramine	80	93
Isopropylamine	80	81
Propylamine	80	85
Tryptamine	63	70
Butylamine	76	82
Phenethylamine	73	77
Putrescine	83	90
3-Methylbutylamine	75	79
Amylamine	74	80
Cadaverine	65	76
Hexylamine	50	54

pH 4.5. For other conditions, see text. The R.S.D. was between 3 and 7% in all the experiments.

The method developed allows biogenic amines to be determined at low levels with detection limits between 20 and 90 $\mu g\ l^{-1}$ and quantification limits between 40 and 200 $\mu g\ l^{-1}$. As can be seen, it improves the detection limits of the amines in wine samples. For this reason, the highest volume of samples must be treated to concentrate them to the maximum extent. Obviously, if the volume of sample had been decreased, the recoveries would have been better, but the detection limit would not have been improved with respect to other methods.

Fig. 2 shows the chromatograms resulting from the analysis of a red wine spiked with 0.1 mg l^{-1} of biogenic amines. The one shows that ethanolamine, histamine, tyramine and putrescine can be detected by direct injection of the spiked wine. The second shows the result of the optimized SPE treatment applied to the same wine. As can be seen, all of the amines can be detected and quantified, except for peaks 14 and 15,

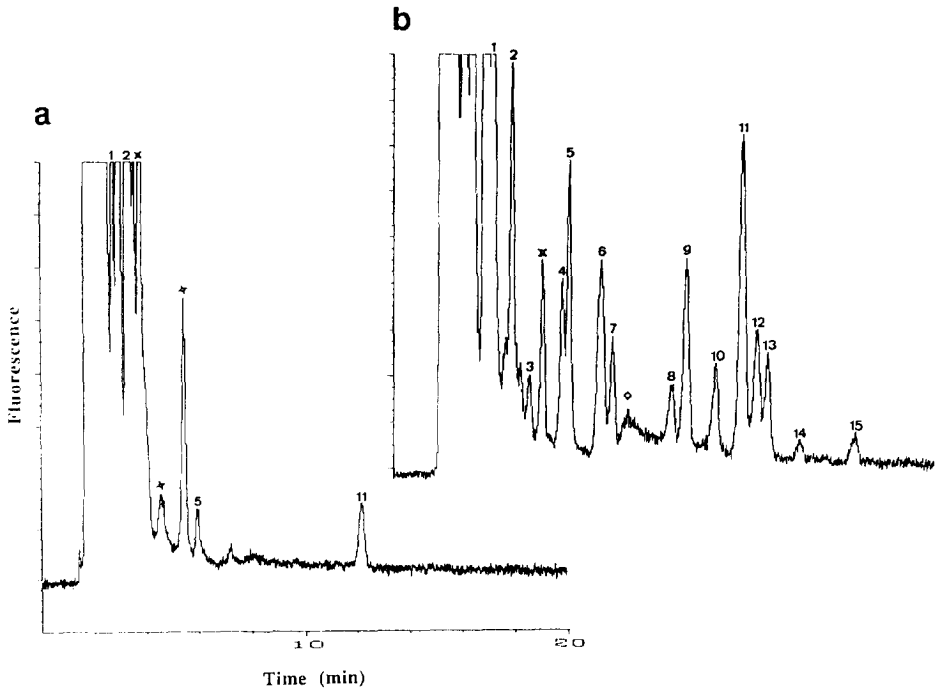

Fig. 2. Chromatograms showing the difference resulting from SPE. (a) Directly injected spiked wine; (b) the same spiked wine after SPE. Peaks: 1 = ethanolamine; 2 = histamine; 3 = methylamine; 4 = ethylamine; 5 = tyramine; 6 = isopropylamine; 7 = propylamine; 8 = tryptamine; 9 = butylamine; 10 = phenethylamine; 11 = putrescine; 12 = 3-methylbutylamine; 13 = amylamine; 14 = cadaverine; 15 = hexylamine. ◇ = Peak corresponding to the excess of OPA; * = unknown.

whose values are close to the detection limit of the method. At the same time, SPE simplifies the beginning of the chromatogram and histamine is eluted in a cleaner zone. In the middle of the chromatogram, a peak corresponding to the excess of OPA appeared, but it does not interfere with the other peaks.

Several red wines from the Tarragona region were analysed using the proposed procedure. In all of them, ethanolamine, tyramine and putrescine were identified and quantified either by direct injection (without SPE) or by applying the optimized SPE method. Histamine was identified in all of the chromatograms obtained, but only those corresponding to SPE treatment was it quantified because in the others it co-eluted with other compounds. The rest of the amines were identified and quantified in almost all of the wines analysed. Fig. 3 shows an example of the chromatograms produced by the SPE of one of these samples. In this case, peaks corresponding to tryptamine, phenethylamine and cadaverine can be identified at concentrations near their detection limits. Moreover, peaks corresponding to ethylamine, tyramine and isopropylamine can be quantified, whereas by direct injection they could only be identified.

4. Conclusions

The method described in this paper has an easy, fast clean-up and concentration step prior to analysis with precolumn automatic OPA derivatization and fluorescence detection. SAX cartridges improve the clean-up of the sample and retain more polyphenolic compounds than the other analytical procedures tested.

The use of an ion-pair reagent to increase recoveries in the concentration step was optimized by using C_{18} cartridges, which allowed

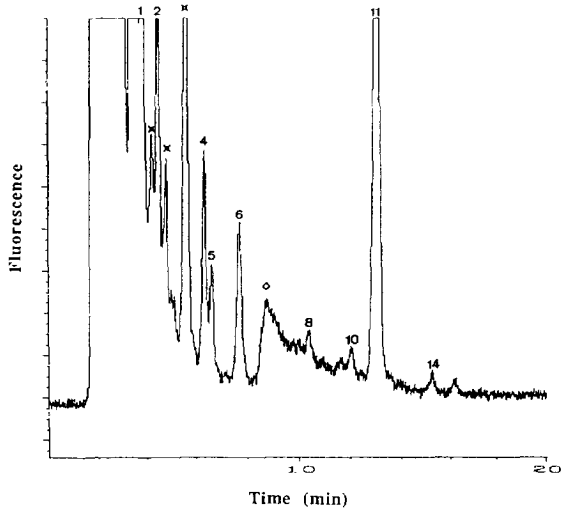

Fig. 3. Example of a red wine analysed using the proposed procedure. Peaks: 1 = ethanolamine; 2 = histamine; 4 = ethylamine; 5 = tyramine; 6 = isopropylamine; 8 = tryptamine; 10 = phenethylamine; 11 = putrescine; 14 = cadaverine. ◇ = Peak corresponding to the excess of OPA; * = unknown.

these compounds to be detected at $\mu g\ l^{-1}$ levels with no interferences.

The method was used to determine fifteen biogenic amines in red wines with satisfactory results.

Acknowledgement

The authors thank CICYT (project ALI 90-0890) for financial support.

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