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Journal of Chromatography A, 871 (2000) 75–83

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Optimization of chromatographic parameters for the determination of biogenic amines in wines by reversed-phase high-performance liquid chromatography

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Abstract

A method suitable for the determination of eight biogenic amines (histamine, tyramine, phenylethylamine, tryptamine, cadaverine, putrescine, spermidine and spermine) in wines has been developed. The method involves derivatization of the amines by treatment with dabsyl chloride, after which the derivates were analysed by reversed-phase liquid chromatography with gradient elution and spectrophotometric detection at 446 nm. Different variables affecting separation were optimized. Validation of the method included calibration experiments, the addition of standards amines for the determination of recovery and repeatability tests. Good linearity of the responses was obtained up to 500 $\mu g\ l^{-1}$, except for putrescine (up to 2100 $\mu g\ l^{-1}$). The detection limits ranged between 10 and 60 $\mu g\ l^{-1}$ for standard solutions. The method was successfully applied to the analysis of five Spanish wines.

Keywords: Wine; Food analysis; Optimization; Derivatization, LC; Biogenic amines

1. Introduction

Biogenic amines are organic bases occurring in a wide variety of foods, such as fish products, wine, beer, meat, cheese and other fermented foods. The consumption of foods containing high concentrations of these compounds may cause problems to some consumers, such as headaches, nausea, hypo- or hypertension, cardiac palpitations, etc. The interaction between ethanol (a monoamine oxidase inhibitor) and amines seems to be synergistic, which is important for those wine consumers who are sensitive to such compounds.

Biogenic amines in wines have been determined in a variety of ways, including fluorimetric, gas chromatographic, and liquid chromatographic methods [1–4]. The latter is the most commonly used, and within the last few years special attention has been paid to the determination of biogenic amines after precolumn derivatization with reagents such as dansyl chloride [5–7], *o*-phthalaldehyde [4,8–10] or dabsyl chloride [11], among others, normally with fluorimetric or spectrophotometric detection. Dabsyl chloride is an excellent reagent for the spectrophotometric detection of amines as it forms coloured compounds that can be detected in the visible zone, and the dabsyl derivates of primary and secondary amines are stable at room temperature [11]. In a previous work [12] we carried out the optimization of the main variables involved in this derivatization reaction.

In this paper, we describe the optimization of the variables involved in the chromatographic separation of eight biogenic amines as dabsyl derivates. The reliability of the proposed method in terms of

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0021-9673/00/$ – see front matter
PII: S0021-9673(99)00946-2

accuracy, repeatability and linearity has been studied. This method has been applied to the analysis of five Spanish wines.

2. Experimental

2.1. Apparatus and software

The liquid chromatograph consisted of a Hewlett-Packard 1050 series equipped with a UV–visible variable wavelength detector, a Rheodyne (Rheodyne, Cotati, CA, USA) 7125 loop injector with a 20-μl sample loop, and a 3396-A integrator. A LiChrospher 100 RP-18 (244×4.4 mm I.D., 5 μm) column, linked to a LiChrospher guard column (10× 4.6 mm I.D.) and thermostated at 40°C, was used for all separations. A Vortex Heidolph mixer, model Reax 2000, a thermostated Precisterm model s-137 bath and a BHG Fixette 2 centrifuge were used. All pH measurements were made with a Crison 2000 pH meter equipped with a combined AgCl–glass electrode assembly. The STATGRAPHICS [13] and SYSTAT [14] statistical software packages were used for data manipulation.

2.2. Chemical and reagents

2.2.1. Amine standard solutions

All amine standards were purchased as hydrochloride salts of the highest purity available. Tryptamine, phenylethylamine, spermine and spermidine were obtained from Fluka (Neu-Ulm, Germany), histamine, cadaverine, putrescine and tyramine from Sigma (St. Louis, MO, USA), and 1,7-diaminoheptane from Aldrich (Steinheim, Germany). Stock solutions of the biogenic amines containing 2.5 or 10 m*M* were prepared by dissolving in 0.1 *M* HCl containing 0.2% (w/v) 3,3′-thiodipropionic acid (TDPA) Fluka, as an antioxidant. They were kept refrigerated at −20°C. Composite amine standard was prepared from stock solutions to yield an overall concentration of 250 μ*M* per component.

2.2.2. Solutions for dabsylation reaction

Dabsyl chloride solution, 12.4 μ*M*, was prepared by dissolving 40 mg dabsyl chloride (Fluka) in 10 ml acetone, Merck (Darmstadt, Germany), following ultrasonic treatment for 15 min and filtering through a Merck Anotop filter into a brown glass vial. This solution was stored at −20°C. The reaction buffer medium consisted of 1.06 g Na_2CO_3 (Panreac, Barcelona, Spain) in 50 ml of water. The dilution solution was a mixture of 50 ml acetonitrile (Panreac HPLC grade), 25 ml ethanol (Panreac HPLC grade) and 25 ml of eluent A (see chromatographic solutions).

2.2.3. Chromatographic solutions

Eluent A, consisting of $4.0 \cdot 10^{-2}$ *M* sodium acetate (Panreac), 10% (v/v) dimethylformamide (DMF, Fluka) and 0.23% (v/v) triethylamine (TEA), Carlo Erba (Milan, Italy), was adjusted to pH 5.0 with diluted acetic acid (Panreac HPLC grade). Eluent B consisted of acetonitrile (Panreac)–*tert*.-butylmethyl ether (Fluka)–water (87.5:10:2.5, v/v/v).

All glassware was rinsed thoroughly with 70% ethanol and water and dried before use. Glass vials for standards and samples were heated at 500°C for 3 h to remove any organic contaminants. Highly purified water (Milli-Q, Millipore) was used throughout for the preparation of buffers and reagents. All the other reagents used were of analytical reagent grade.

2.3. Procedure

2.3.1. Sample preparation

Several commercial red wines from different Spanish regions were analysed. The samples were filtered through a 0.20-μm membrane Millipore filter, and 1 ml was diluted ten-fold with HCl–TDPA solution.

2.3.2. Dabsylation reaction

An aliquot of 1.5 ml of diluted wine or 2 ml of composite amine standards (if necessary diluted with 0.2% (w/v) TDPA in 0.1 *M* HCl) was transfered to a vial, adjusted to ≈pH 8.2 with reaction buffer and water added to 3.8 ml. After thorough mixing on a vortex-mixer, 1.6 ml of dabsyl chloride solution was added and it was mixed again. The mixture was heated in a water-bath for 21 min at 70°C, shaking at 1 and 15 min. Then, 4.6 ml of the dilution solution

Table 1
Gradient profile for amine analysis

Time (min)	% B
0	55
3	55
13	75
23	100
33	100
38	55
53	55

was added and allow to stand (≈20 min) in the water bath, shaking from time to time.

2.3.3. LC analysis

The C_{18} column was equilibrated at 40°C with a mobile phase consisting of eluent A–eluent B (45:55, v/v). An aliquot of 20 μl of the dabsyl derivates solution was injected, and eluted, at a flow-rate of 1.0 ml min^{-1}, using the gradient profile indicated in Table 1. The detection wavelength was 446 nm.

3. Results and discussion

In a previous work [12] we applied the experimental design to optimize the derivatization reaction of biogenic amines with dabsyl chloride, as a prior step to its HPLC determination. In order to improve the resolution among the amines to be separated, we decided to study the influence of the variables involved in the chromatographic process.

The initial chromatographic conditions were: mobile phase: Eluent A consisting of $9.0 \cdot 10^{-3}$ *M* dihydrogenphosphate (Merck), 4% (v/v) DMF and 0.18% (v/v) TEA and adjusted to pH 6.5 with diluted phosphoric acid (Merck). Eluent B consisted of acetonitrile–*tert.*-butylmethyl ether–water (80:10:10, v/v/v). For programmed elution, the conditions were as follows: 55% eluent B for 5 min, then 75% B in 15 min, then 100% B in 10 min, at 100% B for 10 min, then back to 55% B in 5 min and finally 55% B for 15 min; column temperature: 50°C; flow-rate: 1 ml min^{-1}.

The detection wavelength was checked between 416 and 496 nm, keeping the other chromatographic conditions constant. We selected 446 nm as the most appropriate wavelength because the peak areas and peak heights are maximum and constant between 436 and 456 nm.

The effect of varying the column temperature was evaluated by comparison of the chromatograms obtained between 35 and 62°C, using 446 nm as the detection wavelength and keeping the other chromatographic conditions constant. Resolution of bands is almost unaffected except for the cadaverine and histamine bands. The best chromatogram was obtained at 40°C.

The influence of the pH of the mobile phase was studied between 4 and 8, keeping the buffer concentration constant at 9 m*M* (DMF and TEA concentrations were also kept constant). pH 4–5 was adjusted with acetic acid–acetate buffer and pH 6–8 with phosphoric acid–dihydrogenphosphate buffer. The best resolution was obtained for a pH value of 5. Likewise, the concentration of the buffer was also studied among 11 and 130 m*M*, and a good resolution was obtained between 30 and 90 m*M*. We selected 40 m*M* as adequate.

The influence of TEA and DMF percentages in eluent A was studied jointly, using the response surface methodology (RSM) from sequential experimental Doehlert designs [15]. Following the multivariate methodology, a Doehlert design was used to obtain the maximum resolution region, but as the calculated maximum from the experimental data was close to the limits of the experimental domain, a second design was employed. The analysis of the results obtained in this design by means of ANOVA showed that neither TEA% nor DMF% were statistically significant. The quadratic effect of TEA% and DMF% and the interaction term between the two variables were not significant. The optimum values found were 10% (v/v) DMF and 0.23% (v/v) TEA.

The gradient profile was studied varying the time intervals and the percentage of water in solution B testing between 5 and 2% (v/v/v). We observed that resolution improved when the percentage of water decreased and the best chromatogram was obtained working with the elution profile shown in Table 1 and using the mobile phase described in the chromatographic solutions.

Fig. 1 shows a typical chromatogram obtained with the optimized chromatographic separation. All the analyte peaks are well resolved. The appearance

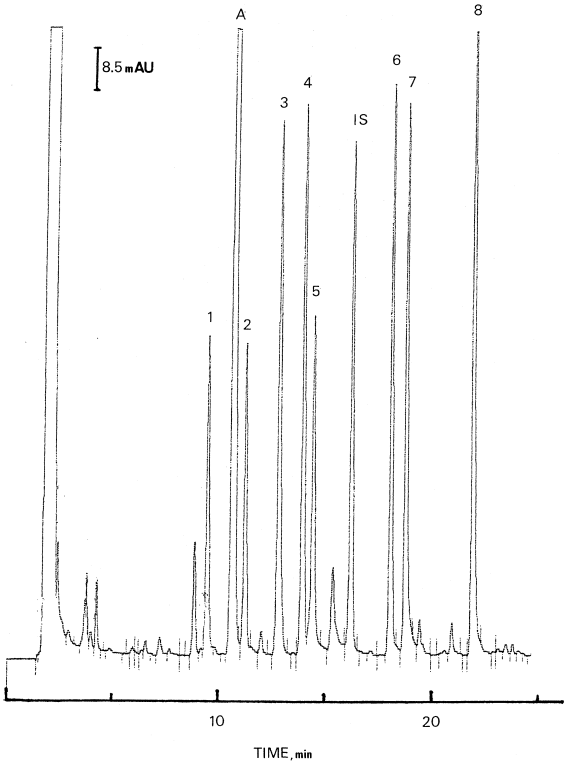

Fig. 1. Representative chromatogram of the dabsyl derivates of amines from a standard mixture in a Lichrospher 100 RP-18 (244×4.4 mm I.D., 5 μm). Mobile phase: eluent A, $4.0 \cdot 10^{-2}$ *M* sodium acetate, 10% (v/v) dimethylformamide (DMF), 0.23% (v/v) triethylamine (TEA), pH 5.0 with diluted acetic acid; eluent B, acetonitrile–*tert.*-butylmethyl ether–water. (87.5:10:2.5, v/v/v); gradient profile: see Table 1; flow-rate: 1 ml min^{-1}; column temperature: 40°C. Detection wavelength: 446 nm. Peaks: 1=tryptamine, 2=phenylethylamine, 3=putrescine, 4=cadaverine, 5=histamine, 6=tyramine, 7=spermidine, 8=spermine, A=methyl orange, I.S.=internal standard.

of other peaks can be observed, which can be assigned to secondary products of the dabsylation reaction. The peak labelled as A is due to methyl orange. These peaks do not affect the resolution of the peaks of interest.

3.1. Performance characteristics

Precision and linearity of the method were examined. The data were collected for five different concentrations (30–500 μg l^{-1} and up to 2 mg l^{-1} for putrescine) of biogenic amine standards, using triplicate responses at each concentration and a randomized arrangement. Tests for non-linearity (for a univariate linear calibration) were based on the analysis of the residual variance from a regression into parts owing to 'lack of fit' and 'pure error' in order to evaluate the absence of curvature of the concentration (linearity 'in-line' [16]). 1,7-Diaminoheptane, was used as a possible internal standard (I.S.) and added at a level of 2 mg l^{-1}.

The fit of the experimental data was carried out by using peak heights and peak areas and with and without I.S. The analysis of the data shows that, generally, quantification in areas is better, except for phenylethylamine and putrescine. Quantification by the external standard method was adopted since the use of I.S. does not improve the linearity. Table 2 shows the performance characteristics for the method. The linearity 'on-line', defined as LIN (%)=100 $(1 - s_b)$ [16], where s_b is the slope standard deviation, has been included as another figure of merit. This parameter indicates the greater or lesser dispersion of the data around the calibration line. On the

Table 2
Performance characteristics[a]

Amine	LR	Regression eq.	LIN (%)	RSD (c) (%)	DL (μg l^{-1})	AS (μg l^{-1})
Tryptamine	Up to 500	$A=29036+1132C$	97.94	3.45	32.5	14.2
Phenylethylamine	Up to 500	$A=-33419+1178C$	98.92	1.67	25.1	6.5
Putrescine	Up to 2100	$A=157466+3733C$	98.74	1.94	61.3	32.1
Cadaverine	Up to 500	$A=20413+3210C$	99.32	1.16	10.3	4.5
Histamine	Up to 500	$A=1184+2189C$	99.04	1.77	16.7	7.3
Tyramine	Up to 500	$A=33999+1886C$	99.31	1.13	10.3	4.4
Spermidine	Up to 500	$A=33705+2265C$	99.17	1.39	13.2	5.7
Spermine	Up to 500	$A=-12200+1681C$	98.34	3.67	26.9	11.6

[a] Abbreviations: LR, linear range (μg l^{-1}); *A*: peak area; *C*: concentration injected in μg l^{-1}; LIN (%): linearity on-line; RSD (c): relative standard deviation of the middle regression fitted line concentration; DL: detection limit; AS: analytical sensitivity.

Table 3
Statistics for the analysis of biogenic amines in red wine samples

Samples	Matrix effect	Youden blank	Significant difference between the slopes?		Accuracy test
			SC and AC	AC_1 and AC_2	
Wine A					
Putrescine	Yes[a]	Yes	Yes	No	$t_{cal}=1.450<t_{crit}=2.365$ ($\alpha=0.05$, df=7) No significant difference
Cadaverine	Yes	Yes	Yes	No	$t_{cal}=0.153<t_{crit}=2.365$ ($\alpha=0.05$, df=7) No significant difference
Histamine	No	Yes	No	–	$t_{cal}=1.803<t_{crit}=2.069$ ($\alpha=0.05$, df=23) No significant difference
Tyramine	No	Yes	No	–	$t_{cal}=1.392<t_{crit}=2.120$ ($\alpha=0.05$, df=16) No significant difference
Spermine	No	No	No	–	$t_{cal}=0.810<t_{crit}=2.101$ ($\alpha=0.05$, df=18) No significant difference
Tryptamine[b]	No	–	No	–	$t_{cal}=1.355<t_{crit}=2.365$ ($\alpha=0.05$, df=7) No significant difference
Phenylethylamine[b]	No	–	No	–	$t_{cal}=0.311<t_{crit}=2.145$ ($\alpha=0.05$, df=14) No significant difference
Spermidine[b]	Yes	–	Yes	No	$t_{cal}=0.556<t_{crit}=2.365$ ($\alpha=0.05$, df=7) No significant difference
Putrescine[b]	Yes	Yes	Yes	No	$t_{cal}=2.058<t_{crit}=2.365$ ($\alpha=0.05$, df=7) No significant difference
Cadaverine[b]	Yes	Yes	Yes	No	$t_{cal}=0.423<t_{crit}=2.365$ ($\alpha=0.05$, df=7) No significant difference
Histamine[b]	No	Yes	No	–	$t_{cal}=1.910<t_{crit}=2.069$ ($\alpha=0.05$, df=23) No significant difference
Tyramine[b]	No	Yes	No	–	$t_{cal}=2.066<t_{crit}=2.120$ ($\alpha=0.05$, df=16) No significant difference
Spermine[b]	No	No	No	–	$t_{cal}=1.681<t_{crit}=2.120$ ($\alpha=0.05$, df=16) No significant difference
Wine B					
Putrescine	No	No	No	–	$t_{cal}=2.015<t_{crit}=2.120$ ($\alpha=0.05$, df=16) No significant difference
Cadaverine	No	No	No	–	$t_{cal}=0.624<t_{crit}=2.120$ ($\alpha=0.05$, df=16) No significant difference
Histamine	No	No	No	–	$t_{cal}=1.143<t_{crit}=2.101$ ($\alpha=0.05$, df=18) No significant difference
Tyramine	No	No	No	–	$t_{cal}=0.003<t_{crit}=2.101$ ($\alpha=0.05$, df=18) No significant difference
Wine C					
Putrescine	No	No	No	–	$t_{cal}=0.203<t_{crit}=2.120$ ($\alpha=0.05$, df=16) No significant difference
Cadaverine	No	Yes	No	–	$t_{cal}=0.142<t_{crit}=2.120$ ($\alpha=0.05$, df=16) No significant difference
Histamine	No	No	No	–	$t_{cal}=0.227<t_{crit}=2.101$ ($\alpha=0.05$, df=18) No significant difference
Tyramine	No	No	No	–	$t_{cal}=0.112<t_{crit}=2.101$ ($\alpha=0.05$, df=18) No significant difference

other hand, the analytical sensitivity (AS), considered as the least variation of concentration that the analytical method is able to discern, was calculated following the expression: $AS = S_{A,C}/b$, where $S_{A,C}$ is the regression standard deviation of A to C, and b is the slope of the regression line.

The detection limits were calculated from the calibration data, following the criterion proposed by Sarabia et al. [17], which takes into account the statistics implied in the difference between the analytical signal and the blank signal, and also the variability produced for the presence of errors in the slope and in the intercept of the calibration graph. According to IUPAC recommendations, we assumed that there is a true and false positive of 0.05 ($\alpha = \beta = 0.05$).

3.2. Wine analysis

To check the applicability of the method, five Spanish wines were analysed. It was first necessary to determine whether there was a matrix effect in order to obtain bias-free analytical results. As the samples analysed were not standard reference materials, a validation study was carried out on the basis of different calibration procedures.

3.2.1. In the absence of a matrix effect

We applied a statistical protocol [18] based on three calibration procedures (i.e., standard calibration (SC), standard-additions calibration (AC), and Youden calibration (YC) with different sample sizes). The slope, the intercept, and the standard deviation of regression of each one of the calibration graphs were calculated.

First of all, using the *t*-test, the slopes of the lines obtained by the SC and AC calibrations are compared. If the difference between the two slopes is not significant, the standard-additions calibration can be used to validate the method. Then, we test whether the intercepts of the SC and YC regression lines differ significantly from each other, which would indicate that a systematic error due to the matrix components is present. In that case, the true blank of the sample, known as the Youden blank, is determined as the difference between the two intercepts.

Finally, the accuracy of the results is tested by

Table 3 (*continued*)

Samples	Matrix effect	Youden blank	Significant difference between the slopes?		Accuracy test
			SC and AC	AC_1 and AC_2	
Wine D					
Putrescine	No	No	No	–	$t_{cal} = 0.960 < t_{crit} = 2.228$ ($\alpha = 0.05$, df = 10) No significant difference
Cadaverine	Yes	Yes	Yes	No	$t_{cal} = 1.980 < t_{crit} = 2.365$ ($\alpha = 0.05$, df = 7) No significant difference
Histamine	Yes	No	Yes	No	$t_{cal} = 0.936 < t_{crit} = 2.228$ ($\alpha = 0.05$, df = 10) No significant difference
Tyramine	No	No	No	–	$t_{cal} = 1.390 < t_{crit} = 2.228$ ($\alpha = 0.05$, df = 10) No significant difference
Wine E					
Putrescine	No	No	No	–	$t_{cal} = 0.685 < t_{crit} = 2.120$ ($\alpha = 0.05$, df = 16) No significant difference
Histamine	No	Yes	No	–	$t_{cal} = 1.306 < t_{crit} = 2.101$ ($\alpha = 0.05$, df = 18) No significant difference
Tyramine	No	Yes	No	–	$t_{cal} = 1.870 < t_{crit} = 2.120$ ($\alpha = 0.05$, df = 16) No significant difference
Spermine	No	No	No	–	$t_{cal} = 0.313 < t_{crit} = 2.101$ ($\alpha = 0.05$, df = 18) No significant difference

[a] Saturated matrix–analyte interaction.
[b] Added to the sample before treatment.

comparing the analyte contents from the SC and AC graphs, using a *t*-test for the comparison of the two means. If the difference is not significant, it is concluded that the method is accurate, at least in relation with the matrix of the sample analysed. For samples with similar matrices (i.e. other red wine samples) the method is equally accurate. When the nature of the matrix changes drastically, it is necessary to apply the statistical protocol again in order to validate the method.

3.2.2. In the presence of a matrix effect

In this case, by applying the standards-addition methodology (AC), it is necessary that the proportional interactive effect not change with the matrix-to-analyte ratio [19,20]. This independence is evident in the AC plot [20], provided that the spike range is sufficiently large. An alternative way of showing the presence of a matrix effect consists of trying out two AC calibrations at different sample sizes [21]. This approach, based on four calibration procedures, SC, AC_1 (sample size 1), AC_2 (sample size 2), and YC, uses the statistical methodology to verify the accuracy of the analytical results in the presence of correctable systematic errors as it permits the detection of constant and proportional errors. The constant component of the error (Youden blank) is determined from the data set obtained in the YC with different sample sizes. To test the similarity between the slopes (SC, AC_1 and AC_2), a statistical technique known as the analysis of covariance (ANCOVA) is used [21]. This technique combines the aspects of

Table 4
Determination of biogenic amines in red wine samples

Sample	Analyte	Added (mg l^{-1})	Found (mg l^{-1})	Recovery (%)	RSD (%)
Wine A	Putrescine	–	13.67	–	3.4 ($n=7$)
	Cadaverine	–	1.88	–	4.3 ($n=6$)
	Histamine	–	3.21	–	7.2 ($n=6$)
	Tyramine	–	2.55	–	8.4 ($n=6$)
	Spermine	–	0.57	–	8.6 ($n=5$)
	Tryptamine	3.03	2.87	94.83	5.4 ($n=8$)
	Phenylethylamine	4.11	4.13	100.54	4.1 ($n=6$)
	Spermidine	2.88	2.90	101.00	4.8 ($n=8$)
	Putrescine	10.88	22.72	92.55	3.4 ($n=5$)
	Cadaverine	1.36	3.21	99.11	4.8 ($n=5$)
	Histamine	1.94	4.76	92.43	6.8 ($n=7$)
	Tyramine	2.45	4.77	95.33	5.8 ($n=6$)
	Spermine	0.53	1.02	92.73	5.5 ($n=5$)
Wine B	Putrescine	–	6.89	–	6.8 ($n=5$)
	Cadaverine	–	1.45	–	6.3 ($n=5$)
	Histamine	–	4.74	–	8.4 ($n=5$)
	Tyramine	–	5.91	–	4.3 ($n=5$)
Wine C	Putrescine	–	7.33	–	5.3 ($n=5$)
	Cadaverine	–	3.15	–	9.0 ($n=5$)
	Histamine	–	3.95	–	9.5 ($n=5$)
	Tyramine	–	3.58	–	4.1 ($n=5$)
Wine D	Putrescine	–	19.10	–	5.3 ($n=5$)
	Cadaverine	–	2.20	–	8.6 ($n=6$)
	Histamine	–	2.31	–	6.5 ($n=5$)
	Tyramine	–	3.17	–	8.0 ($n=6$)
Wine E	Putrescine	–	4.67	–	3.2 ($n=6$)
	Histamine	–	0.39	–	10.3 ($n=5$)
	Tyramine	–	2.24	–	8.3 ($n=6$)
	Spermine	–	0.38	–	7.0 ($n=5$)

variance and regression analysis, and among its many uses is the comparison of regression lines that permit the detection of the presence of any matrix–analyte interaction. Therefore, we conclude that at the sample size studied, there is a systematic error (a saturated matrix–analyte interaction) if the slopes of SC and AC_1 and SC and AC_2 are different and the slopes of AC_1 and AC_2 are statistically equal.

In order to obviate this systematic error, the analyte concentration in the measurement solutions, C_x (AC_i), is calculated using Eq. (1), where A'_{AC_i} is the corrected intercept of A_{ci} ($i=1$ and 2); a_Y is the intercept of the Youden calibration and b_p is the pooled slope of the calibrations AC_1 and AC_2

$$C_{x_i} = \frac{A'_{AC_i} - a_y}{b_p} \quad (1)$$

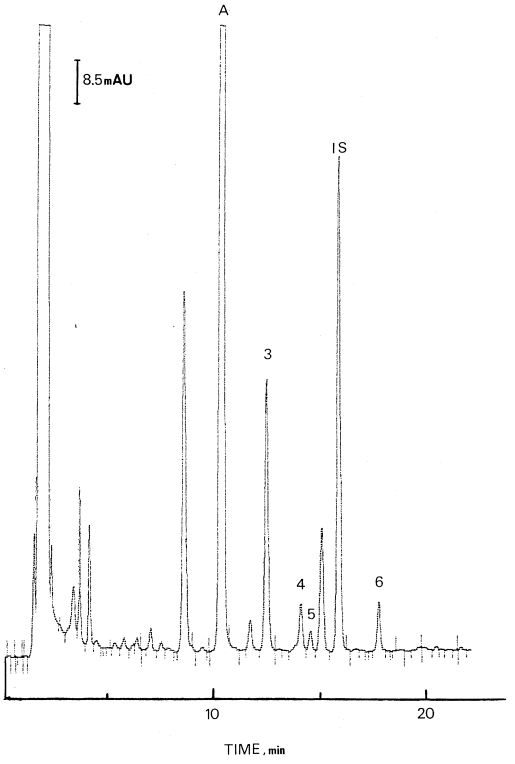

Fig. 2. Chromatogram of the dabsyl derivates of amines from wine sample D. Chromatographic conditions as in Fig. 1. Peaks: 3=putrescine, 4=cadaverine, 5=histamine, 6=tyramine, A=methyl orange, I.S.=internal standard.

Finally, the analyte concentration in the original sample, C_i, is calculated and the accuracy of the results obtained is evaluated with a *t*-test for $[n(AC_1)+n(AC_2)-3]$ degrees of freedom [21].

The statistical results obtained in the analysis of the wines are summarized in Table 3, where it can be observed that there is a saturated matrix–analyte interaction in the determination of putrescine and spermidine added in wine A, cadaverine in wines A and D, and histamine in wine D. In all cases, the accuracy test indicates that the method is reliable, at least for the samples analysed. Table 4 shows the analyte contents for the five red wines analysed. Putrescine, histamine and tyramine are always present, whereas tryptamine, phenylethylamine and spermidine are absent. In order to verify that these last amines can also be determined in this type of sample (matrix), a recovery study was carried out for wine A, with good results. We do not consider it necessary to perform a similar study for the other samples taking into account the similarity of the matrices. Likewise, the RSD values indicate a satisfactory precision. Fig. 2 shows the chromatogram obtained with one of the wines analysed, where it can be seen that the peaks of the amines present are well resolved.

4. Conclusions

The proposed method appears to be suitable for the easy determination of biogenic amines in wines. The optimization of the chromatographic conditions leads to a chromatogram in which the eight analysed amines are well resolved. Both good linearity and precision were obtained. The recovery results were satisfactory for all amines. We expect this method to be also suitable for the analysis of other beverages such as beers and liqueurs.

Acknowledgements

The authors are grateful to the Consejería de Educación y Ciencia of the Junta de Andalucía for financial assistance (group FQM 232 of II PAI). We are indebted to Christine Laurin for editing the English version of the text.

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