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Direct determination of biogenic amines in wine by integrating continuous flow clean-up and capillary electrophoresis with indirect UV detection

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Abstract

A flow-injection manifold for automating the determination of biogenic amines in wine using capillary electrophoresis (CE) with indirect UV detection was developed. The ensuing method involves clean-up and solid-phase extraction (SPE) of the target analytes in the sample. Various treatments involving different SPE minicolumns were tested and compared. The C_{18} minicolumn was chosen to concentrate the amines following addition of ammonium chloride and ammonium hydroxide as buffer to neutralize them. Additions of amine standards were used to determine recoveries. Biogenic amines can be separated and detected after SPE with limits of detection in the range 0.05–0.1 $\mu g\ ml^{-1}$ by using 4 m*M* copper(II) sulphate, formic acid and 18-crown-6 as running buffer. All the amines studied are eluted within 15 min under the optimum conditions established. The overall process was successfully used to identify biogenic amines in various types of wine from different Spanish regions. © 1998 Elsevier Science B.V.

Keywords: Wine; Food analysis; Sample preparation; Biogenic amines

1. Introduction

Amines may be found in virtually all foods because they are bacterial degradation products. Hence, investigating potential toxic effects of ingesting large amounts of these substances, is of special interest, as is the presumed relationship between high amine content and unsanitary conditions during winemaking. There are three possible origins for biogenic amines in wines. They can be present in the must, be formed by yeasts during alcoholic fermentation or result from the action of bacteria involved in malolactic fermentation. Histamine and tyramine in wines are products of microbial decarboxylation of their respective precursor amino acids: histidine and tyrosine [1]. Ethanolamine is one of several amines occurring in wine at low, but varying concentrations. Most frequently, it is formed from its precursor of 1,2-ethanediol, concentrations of which in grape must and wine can be used to estimate the amount of the diol that can be formed [2]. Cerutti and Remondi [3] suggested that wine produced under hygienically optimal conditions should be nearly free of amines. Some countries such as the US, Sweden, Austria and the Netherlands have established regulations and legal requirements for the maximum limits

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of biogenic amines (mainly histamine) in various types of foods. The lack of legislation on the tolerated contents of biogenic amines in wine, makes it difficult to correctly assess imports and exports of this product [4].

There are several methods for determining biogenic amines in foods, although almost all of which analyse for a single amine. Commonly, these methods are based on molecular spectroscopic techniques. When several amines in foods are to be determined a separation technique such as thin-layer chromatography, gas chromatography [5], mass spectrometry, liquid chromatography [6] or capillary electrophoresis (CE) [7] is generally employed. Current reference and official analytical methods use high-performance liquid chromatography (HPLC). All these methods involve manual treatment of the food sample (particularly those concerning with wine samples). Almost invariably, the main problem is sample preparation rather than the separation process itself. Occasionally, additional problems are encountered in detecting the previously separated compounds and a derivatization reaction must be used. Mahendradatta and Schwedt [8] developed a method for quantifying histamine in wine using CE, and Nouadje and coworkers [9,10] used micellar electrokinetic chromatography and laser-induced fluorescence for the determination of amines in wine. In this paper we report the first available method for the separation of biogenic amines in wines using CE and indirect UV detection coupled with a minicolumn for solid-phase extraction (SPE) in a flow system. The objective was to determine major amines such as histamine, putrescine, cadaverine, isoamylamine, phenethylamine, ethylamine, methylamine and tyramine, which are normally found at levels above 1 $\mu g\ ml^{-1}$ in wine.

Prior to separation by CE, the sample must be cleaned up to avoid interferences and, optionally, simultaneously concentrate the amines. The most usual clean-up techniques are liquid–liquid extraction and SPE. Liquid–liquid extraction is characterized by a long sample treatment which uses large volumes of organic solvents which could not be introduced in the CE and also it is difficult to automate. In this work, the flow system was coupled to the CE equipment via a programmable arm in order to automate the preliminary operations (viz., preconcentration, filtration and sampling) and sample introduction into the autosampler of the CE system. The method involves clean-up of the wine samples in the flow injection (FI) system by use of ion-exchange cartridges and a preconcentration step. SPE is used to simultaneously clean-up and concentrate sample prior to their analysis by CE [11]. Wine analyse are most often done with weak cation-exchange adsorbents formed by carboxylic groups (CBAs), strong cation exchangers (SCXs) made up of sulphonic groups and octadecylsilane (C_{18}). All were tested and compared in this paper. Sample handling is minimal because the pretreatment of the wine samples is fully automated. The proposed method allows the determination of a wide range of biogenic amines in less than 10 min.

2. Experimental

2.1. Apparatus

A Beckman P/ACE 5500 CE unit equipped with a diode array detector was used to separate and quantify of the analytes. Beckman capillary tubing of 57 cm×75 μm I.D.×375 μm O.D. was used. A Gilson Minipuls-3 peristaltic pump, a Rheodyne 5041 injection valve, three switching valves, PTFE tubing of 0.5 mm I.D. and a reactor 50 cm long were used to construct the manifold. The continuous filtration system was developed in our laboratory and tested with different types of microfilters from Millipore (pore sizes 0.8 μm, 0.45 μm and 0.22 μm). A laboratory-made programmable arm controlled by a microcomputer via an electronic interface was used [12] in order to automate introduction of the sample following pretreatment in the FI system.

2.2. Chemicals

Standard and buffer solutions were prepared in 18 MΩ deionized water from a Millipore Milli-Q water purification system. An individual standard solution of each of the following amines was prepared: ammonia, methylamine, 1,3-diaminopropane, putrescine, histamine, cadaverine, agmatine, ethylamine, spermidine, ethanolamine, propylamine, morpholine,

isopropylamine, diethylamine, butylamine, isobutylamine, spermine, amylamine, isoamylamine, 1-methylbutylamine, hexylamine, phenethylamine, heptylamine and tyramine. All were supplied by Aldrich. Working standard solutions were made by diluting the stock standard solutions with purified water or in a synthetic matrix of wine. C_{18} octadecyl, CBA carboxylic acid and SCX benzenesulphonic acid (Varian) were used as solid phases. The capillary was conditioned with a 0.1 *M* solution of sodium hydroxide. Methanol was used to condition the minicolumn and 0.1 *M* HNO_3 in MeOH as eluent. A synthetic wine matrix was prepared from ethanol, tartaric acid, citric acid, sucrose, glycerine, calcium chloride, sodium chloride and potassium hydrogentartrate, all supplied by Merck. A background electrolyte containing copper sulphate, formic acid and 18-crown-6 (supplied by Sigma) was used for electrophoretic separations.

2.3. Operating conditions

The running buffer used was a mixture of 4 m*M* copper sulphate, formic acid and 18-crown-6 ether (pH 4.5) [13]. The working voltage was +15 kV, the average current 14.5 μA, temperature 20°C and the wavelength 214 nm. Samples were hydrodynamically injected (by high pressure) for 10 s. Separation was effected from the positive to negative electrode. In order to maintain the capillary under good working conditions, its surface was regenerated once a day by consecutive washing with water (10 min), 0.1 *M* sodium hydroxide (2 min) and water (2 min) followed by the running buffer (15 min).

2.4. Wine samples

A group of commercially available wines were analysed. Samples spiked with analyte concentrations in the typical reported ranges were used [11,14]. Synthetic samples were prepared by mixing different amines compounds dissolved in a matrix medium containing ethanol, tartaric acid, citric acid, sucrose, glycerine, calcium chloride, sodium chloride and potassium hydrogentartrate in similar concentrations to those in real wine samples [14].

2.5. FI manifold

The on-line trace level enrichment process was carried out on an FI system coupled to CE equipment via a programmable arm (see Fig. 1). The manifold included two switching valves: one to select between four different types of wine and the other to switch between 0.1 *M* HNO_3 in MeOH and MeOH channels, respectively. The wine was passed through microfilters prior to insertion into the manifold. Two injection valves were needed: IV_1 was used to measure the volume of sample/buffer and IV_2 to hold the C_{18} minicolumn in its sample loop. Extracting the amines from the wine involved four steps. First, IV_1 was switched to its load position to

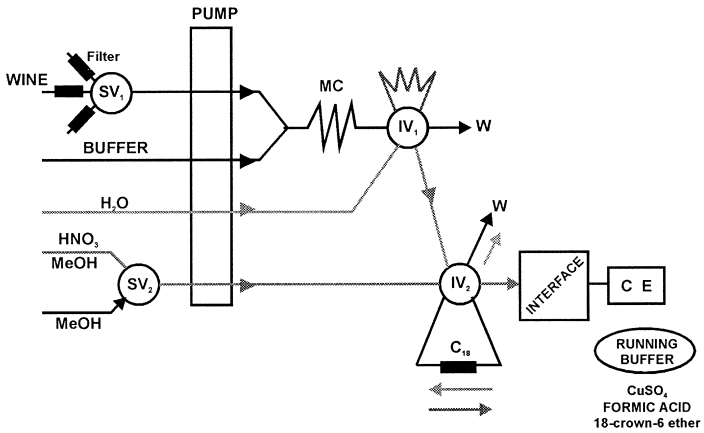

Fig. 1. FI manifold used for treating samples and introducing them into the CE system (IV=injection valve; SV=switching valve; MC=mixing coil; w=waste).

load a preset sample volume and IV_2 was switched to its empty position to condition the minicolumn with methanol (selected by valve SV_2). Second, IV_2 was changed to the load position to flush the C_{18} minicolumn with water (from IV_1). In the third step, IV_1 was switched to the empty position, to have the water carrier stream pass a controlled volume of wine plus buffer mixture through the C_{18} minicolumn, where neutral amines where retained. In the last step, IV_1 was switched to its load position and IV_2 was to its inject position to elute retained amines with 0.1 *M* HNO_3 in MeOH, the preconcentrated samples plug being driven to the CE equipment, the CE–FI system used in this work, was previously described by the authors [15]. Finally, valve SV_2 selected an MeOH stream to flush the minicolumn.

3. Results and discussion

Biogenic amines can be separated by CE using commonplace methods [7,13] for separating inorganic cations such as that based on copper sulphate, formic acid and 18-crown-6 ether (pH 4.5) buffer [13]. At this pH, the amines are positively charged, due to positive polarity used.

3.1. Sample clean-up in the FI system

Before the amines are separated, samples must be cleaned-up in order to ensure adequate selectivity. In fact, without this pretreatment of the wine sample (see Fig. 2), any peaks were identified due to matrix interferences (Fig. 2A). The SPE process eliminated matrix interferences and allowed the amines peaks to be identified (as Fig. 2B shows). The clean-up process was simultaneously used to preconcentrate the amines.

The most widely used adsorbents are weak cation exchangers with CBA, SCX and C_{18} exchangers. SCX was found to strongly retain the amines in the wine samples (pH 3.5). A 0.5 *M* HCl solution used for elution only extracted isoamylamine, phenethylamine and tyramine. HCl concentrations above 0.5 *M* could not be used because they gave rise to a broad negative peak that interfered with the amine peaks. A weak cation exchanger (CBA) was used to resolve this problem; however, the amines were only retained by about 30–40% when the wine (at pH 3.5) was passed through the exchanger. The problem was overcome by increasing the pH with NH_4OH–$ClNH_4$ buffer, which afforded complete retention of the amines by the CBA material. Elution with 0.1 *M* HNO_3 in MeOH provided recoveries of 96–98%. Cations contained in the wine matrix, which were

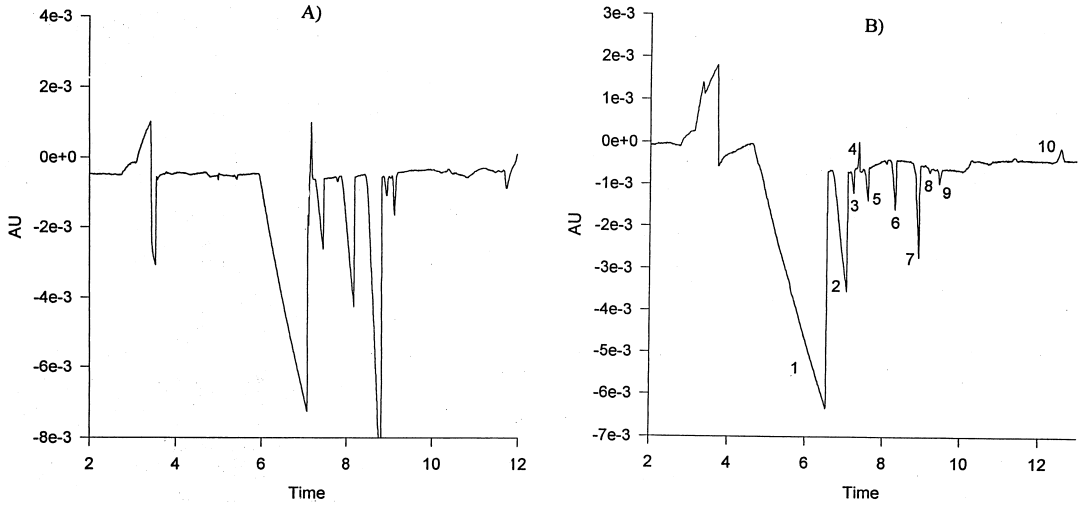

Fig. 2. Electropherogram for a red wine sample using the proposed CE method (A) without sample clean-up, (B) after sample clean-up (1, 2= unknown peaks; 3=putrescine; 4=histamine; 5=cadaverine; 6, 7=unknown peaks; 8=ethanolamine; 9=unknown peak; 10= phenethylamine).

also retained by CBA and eluted together with the amines, interfered with the CE method. The C_{18} adsorbent was found to avoid these problems. With this material, amines can be retained by using two alternative procedures. One involves an ion-pair formation with OSA (octanesulphonate sodium salt), the resulting ion-pairs being adsorbed in C_{18}; this, however, poses severe elution problems. The other (used in this work) involves the elimination of the positive charge of the amines by keeping the pH above 9. Under these experimental conditions, the amines were quantitatively retained on the C_{18} minicolumn and efficiently eluted from it by using 0.1 *M* HNO_3 in MeOH. Methanol was added to the eluent in order to decrease hydrophobic interactions between amines and the column material. The concentration of HNO_3 was optimised amines recoveries of 90% were achieved at concentrations below 0.05 *M*; on the other hand concentrations above 1 *M* produced an unstable baseline. The best results were achieved with 0.1 *M* HNO_3 with an average recovery of 97%. Other strong acids were tested but no advantages over HNO_3 were observed.

The optimum values for the hydrodynamic variables of the FI system were as follows: a sample volume of 2.5 ml, which was large enough to ensure adequate amounts of the amines from the wine samples; a buffer volume of 0.5 ml, which allows one to maintain a constant pH above 9 and, finally, elution with 1 ml of 0.1 *M* HNO_3 in MeOH, at 3 ml min^{-1}; a continuous filtration system including a microfilter of 0.45 μm pore size, which exhibited good performance; and an extraction column (5 cm× 2 mm I.D.) that was conditioned with 2 ml of methanol and 2 ml of water.

3.2. CE separation

While the 21 amines could be rapidly separated by CE only some of them are of interest to wine analysts (e.g., methylamine, histamine, tyramine, putrescine, cadaverine, ethanolamine, propylamine, isopropylamine, isoamylamine, tyramine and phenethylamine). The specific interferences of cations (potassium with methylamine, sodium with cadaverine, calcium with ethanolamine, magnesium with spermidine and manganese with propylamine) were avoided by using the C_{18} minicolumn at the optimised working pH (about 9). Different solutions were tested to keep these species at pH above 9, (NaOH, NH_4OH and NH_4Cl–NH_4OH). NaOH could not be used because the sodium peak interfered with the first amine peaks. A NH_4Cl–NH_4OH buffer solution was chosen because the ammonium peak (approx. 4.2 min) thus obtained interfered with none of the other peaks. Electrokinetic injection was found to be unsuitable because the sample contained nitric acid; hydrodynamic injection was thus used instead. A broad negative peak for HNO_3 in MeOH appeared at the electropherogram onset; however, no interference with other amine peaks was observed (see Fig. 2B).

3.3. Calibration curves

Calibration graphs were constructed by using liquid standards containing the amines of interest at concentrations in the range 0–10 μg ml^{-1} in a synthetic matrix of wine. Standard solutions were treated in the same way as real samples. External calibration method was used because no improvement was obtained with the internal standard method. The individual area–concentrations sets of value has been used to apply linear regression by minimum least square. The limit of detection (LOD) was calculated as the blank value plus 3-times its standard deviation, whereas the limit of quantitation (LOQ) was calculated as the blank value plus 10-times its standard deviation. The proposed method allows biogenic amines to be determined at low levels (with LODs between 0.05 and 0.1 μg ml^{-1}). Such low levels entail using a high sample volume, in order to retain large enough amounts of the amines.

The corresponding regression equations, and other characteristic parameters for the determination of these amines, are shown in Table 1. Standard deviation of residuals and the curve-fitting level (in percent) were obtained by analysis of variance (ANOVA) in validating of the calibration model.

3.4. Analytical applications

The method was initially used to determine biogenic amines in synthetic wine samples in order to evaluate its accuracy. Table 2 gives the recoveries

Table 1
Figures of merit of the proposed method for biogenic amine determination

Analyte	$y=a+bx$	r	R^2	$S_{y/x}$	LOD	LOQ
Methylamine	$a=7.3\cdot10^{-3}\pm7.3\cdot10^{-3}$ $b=0.22\pm2.7\cdot10^{-3}$	0.999	99.88	0.016	0.1	0.33
Putrescine	$a=0.018\pm0.017$ $b=0.55\pm6.5\cdot10^{-3}$	0.999	99.89	0.037	0.092	0.3
Histamine	$a=-2.8\cdot10^{-3}\pm0.011$ $b=0.35\pm4.5\cdot10^{-3}$	0.999	99.87	0.026	0.093	0.31
Cadaverine	$a=-0.01\pm8.2\cdot10^{-3}$ $b=0.13\pm3.1\cdot10^{-3}$	0.997	99.56	0.018	0.18	0.6
Ethanolamine	$a=4.0\cdot10^{-4}\pm2.1\cdot10^{-3}$ $b=0.07\pm7.9\cdot10^{-4}$	0.999	99.91	$4.6\cdot10^{-3}$	0.08	0.27
Propylamine	$a=2.5\cdot10^{-3}\pm3.4\cdot10^{-3}$ $b=0.08\pm1.2\cdot10^{-3}$	0.999	99.82	$7.4\cdot10^{-3}$	0.11	0.38
Isopropylamine	$a=-0.01\pm3.5\cdot10^{-3}$ $b=0.09\pm3.5\cdot10^{-3}$	0.994	98.87	0.02	0.11	0.37
Isoamylamine	$a=-6.3\cdot10^{-4}\pm8.6\cdot10^{-3}$ $b=0.28\pm3.2\cdot10^{-3}$	0.999	99.88	0.018	0.08	0.3
Tyramine	$a=2.0\cdot10^{-3}\pm1.6\cdot10^{-3}$ $b=0.08\pm6.0\cdot10^{-4}$	0.999	99.96	$3.5\cdot10^{-3}$	0.05	0.18
Phenethylamine	$a=2.8\cdot10^{-3}\pm2.0\cdot10^{-3}$ $b=0.09\pm1.3\cdot10^{-3}$	0.999	99.89	$4.1\cdot10^{-3}$	0.06	0.22

a=Intercept; b=slope; $S_{y/x}$=standard deviation of residuals; R=curve-fitting level (in percent) obtained by ANOVA for the validation of the model; LOD=limit of detection; LOQ=limit of quantitation; concentrations, LOD and LOQ in μg ml^{-1}.

obtained for typical amines (98–99% after SPE). Subsequently, it was used to determine the amines in three different types of wine (red, rose and white). Table 3 shows the results obtained in triplicate analyses of different wine samples. In order to validate the proposed method, the standard addition method was used to determine these biogenic amines. The *t*-test for the slopes of the calibration curves revealed no significant statistical differences in any case. As can be seen from the Table 3, red wines contain higher concentrations of biogenic amines than white wines for all the wines analysed in this work. This is usually ascribed to the greater significance of malolactic fermentation in red wines. Histamine was the most abundant amine, with concentrations higher than 2 μg ml^{-1}; the remainder, have different concentrations from 0.1 to 2.5 μg ml^{-1}. Not all the amines were found in each of the wines.

4. Conclusions

Technically, a new interface for coupling FI with CE was developed in order to automate the treatment of samples and their transfer to the CE equipment. This interface is an all-purpose device that makes discrete analytical equipment compatible with continuous flow systems. The assembly is highly suitable for automating multi-parameter determinations

Table 2
Analysis of synthetic samples by the proposed method (concentrations in μg ml^{-1})

Analyte		Wine 1	Wine 2	Wine 3	Wine 4
Methylamine	Added	0.2	0.5	1	3
	Found	0.19±0.03	0.52±0.05	1.03±0.08	2.93±0.15
	Recovery (%)	95	104	103	97.6
Putrescine	Added	0.4	0.8	2	5
	Found	0.42±0.04	0.81±0.05	2.2±0.1	4.87±0.4
	Recovery (%)	105	101	110	97.4
Histamine	Added	4.5	3.5	2.1	0.2
	Found	4.5±0.3	3.47±0.5	2.0±0.05	0.197±0.04
	Recovery (%)	100	99.1	95.2	98.5
Cadaverine	Added	2.5	3.5	0.3	0.5
	Found	2.3±0.1	3.7±0.2	0.3±0.06	0.49±0.08
	Recovery (%)	92	105	100	98
Ethanolamine	Added	5	4	3.5	1.5
	Found	5±0.2	3.8±0.4	3.6±0.6	1.47±0.2
	Recovery (%)	100	95	102	98
Propylamine	Added	0.2	0.4	1	2
	Found	0.21±0.05	0.39±0.1	1.1±0.08	2.07±0.1
	Recovery (%)	105	97.5	110	103
Isopropylamine	Added	0.3	0.8	1.5	3.5
	Found	0.29±0.05	0.82±0.04	1.5±0.09	3.47±0.2
	Recovery (%)	96.6	102.5	100	99.1
Isoamylamine	Added	1	2.5	0.2	0.4
	Found	1.1±0.09	2.4±0.1	0.2±0.05	0.42±0.02
	Recovery (%)	110	96	100	105
Tyramine	Added	0.8	0.5	2	3
	Found	0.8±0.02	0.5±0.07	2.1±0.05	2.98±0.3
	Recovery (%)	100	100	105	99.3
Phenethylamine	Added	4	2	1	0.5
	Found	3.9±0.09	2.16±0.1	1±0.08	0.48±0.02
	Recovery (%)	97.5	108	100	96

(especially in routine analyses) as it meets the typical laboratory quality requirements and affords a high throughput.

Analytically, the ensuing method allows the rapid determination of ten biogenic amines in wines. It involves simple, fast clean-up and preconcentration steps. The SPE material used avoids interferences from the matrix sample. The separation process is simpler than comparable chromatographic methods. The proposed CE–FI method for determining amines in wine is a good alternative to the conventional process where amines are manually extracted with a Vacuc/Elut system. It is also an alternative to other HPLC methods. Thus, biogenic amines are separated in less than 15 min by CE, in contrast to the 25 min taken by HPLC. Because aliphatic biogenic amines contain no chromophores significantly absorbing in the UV–Vis region, their HPLC determination entails

Table 3
Analysis of real samples by the proposal method (N.D.=not detected)

Amine determined	Type of sample	Concentration added ($\mu g\ ml^{-1}$)	Concentration found ($\mu g\ ml^{-1}$)	Recovery (%)	Concentration[a] ($\mu g\ ml^{-1}$)
Methylamine	Red wine 1	0.5	0.51	102	0.52
		1	1.23	123	
		2	2.19	109.5	
	Red wine 2	0.3	0.28	93.3	1.37
		0.5	0.52	104	
		0.7	0.71	101.1	
	White wine 1	0.2	0.19	95	0.52
		0.4	0.39	97.5	
		0.6	0.58	96.6	
	White wine 2	0.6	0.62	103.3	N.D.
		0.8	0.81	101.2	
		1	1.3	130	
	Rose wine 1	0.2	0.19	95	1.6
		0.4	0.37	92.5	
		0.8	0.83	103.7	
	Rose wine 2	0.3	0.32	106.6	N.D.
		0.6	0.61	101.6	
		2	2.2	110	
Putrescine	Red wine 1	1	1.1	110	2.1
		2	1.97	98.5	
		3	2.97	99	
	Red wine 2	0.5	0.51	102	3.4
		1	1.3	130	
		2	2	100	
	White wine 1	0.5	0.53	106	0.5
		2	2.4	120	
		4	3.87	96.75	
	White wine 2	0.2	0.21	105	2.6
		1	0.98	98	
		2	1.91	95.5	
	Rose wine 1	0.3	0.3	100	3.2
		1	1.2	120	
		2	2	100	
	Rose wine 2	0.4	0.41	102.5	2.1
		0.8	0.77	96.25	
		1.5	1.5	100	
Histamine	Red wine 1	1	0.93	93	4.87
		2	2.1	105	
		3	3.3	110	
	Red wine 2	2	1.9	95	7.2
		4	3.9	97.5	
		6	6.1	101	
	White wine 1	0.2	0.19	95	3.25
		0.4	0.38	95	
		0.6	0.61	101.6	

Table 3. Continued

Amine determined	Type of sample	Concentration added (μg ml^{-1})	Concentration found (μg ml^{-1})	Recovery (%)	Concentration[a] (μg ml^{-1})
	White wine 2	0.3	0.3	100	4.16
		1	1.2	120	
		2	2.1	105	
	Rose wine 1	0.5	0.51	102	2.13
		1.5	1.47	98	
		2.5	2.43	97.2	
	Rose wine 2	0.2	0.2	100	N.D.
		0.8	0.78	97.5	
		1.5	1.48	98.6	
Cadaverine	Red wine 1	0.2	0.18	90	0.34
		0.2	0.4	100	
		0.6	0.62	103.3	
	Red wine 2	0.6	0.61	101.6	N.D.
		0.8	0.82	102.5	
		1	0.98	98	
	White wine 1	0.3	0.31	103.3	0.57
		0.6	0.59	98.3	
		0.9	0.91	98	
	White wine 2	0.5	0.52	104	2.43
		0.7	0.7	100	
		1	1	100	
	Rose wine 1	0.4	0.41	102.5	N.D.
		0.6	0.6	100	
		0.8	0.78	97.5	
	Rose wine 2	0.8	0.83	103.75	0.62
		1	1.1	110	
		1.5	1.47	98	
Ethanolamine	Red wine 1	1	1.1	110	0.8
		2	1.9	95	
		3	3.1	103.3	
	Red wine 2	0.5	0.5	100	2.8
		1.5	1.57	104.6	
		2.5	2.4	96	
	White wine 1	1.5	1.51	100.6	N.D.
		2.5	2.48	99.2	
		3.5	3.43	98	
	White wine 2	0.2	0.2	100	N.D.
		0.4	0.42	105	
		1	1.23	123	
	Rose wine 1	0.6	0.62	103	1.3
		0.8	0.8	100	
		1.2	1.15	95.8	
	Rose wine 2	0.5	0.47	94	0.63
		1.5	1.48	98.6	
		2.5	2.5	100	

(Cont.)

Table 3. Continued

Amine determined	Type of sample	Concentration added ($\mu g\ ml^{-1}$)	Concentration found ($\mu g\ ml^{-1}$)	Recovery (%)	Concentration[a] ($\mu g\ ml^{-1}$)
Propylamine	Red wine 1	0.2	0.2	100	N.D.
		0.4	0.39	97.5	
		0.8	0.8	100	
	Red wine 2	0.3	0.31	103.3	0.17
		0.9	0.89	98.8	
		1	1.05	105	
	White wine 1	0.4	0.43	107.5	N.D.
		0.8	0.79	98.75	
		1	1	100	
	White wine 2	0.5	0.49	98	N.D.
		1	1.1	110	
		1.5	1.48	98.6	
	Rose wine 1	0.2	0.19	95	0.15
		0.6	0.6	100	
		0.8	0.8	100	
	Rose wine 2	1	1.05	105	0.23
		2	2.1	105	
		3	2.95	98.3	
Isopropylamine	Red wine 1	1	1	100	0.12
		2	2.15	107.5	
		3	2.96	98.6	
	Red wine 2	0.2	0.2	100	0.62
		0.6	0.59	98.3	
		1	1.3	130	
	White wine 1	0.5	0.5	100	0.18
		1	1.1	110	
		1.5	1.4	93.3	
	White wine 2	0.2	0.19	95	N.D.
		0.4	0.39	95	
		0.6	0.6	100	
	Rose wine 1	0.3	0.3	100	0.3
		0.8	0.79	98.75	
		1	1	100	
	Rose wine 2	0.4	0.4	100	0.38
		0.8	0.73	91.25	
		1.5	1.47	98	
Isoamylamine	Red wine 1	0.3	0.29	96.6	0.52
		0.7	0.71	101.4	
		0.9	0.92	102.2	
	Red wine 2	0.5	0.5	100	0.9
		1.5	1.49	99.3	
		2.5	2.47	98.8	
	White wine 1	0.3	0.31	103.3	0.48
		0.7	0.72	102.8	
		0.9	0.9	100	

Table 3. Continued

Amine determined	Type of sample	Concentration added (μg ml^{-1})	Concentration found (μg ml^{-1})	Recovery (%)	Concentration[a] (μg ml^{-1})
	White wine 2	0.5	0.49	98	0.53
		1.5	1.45	96.6	
		2.5	2.49	99.6	
	Rose wine 1	0.4	0.41	102.5	N.D.
		0.6	0.59	98.3	
		1.2	1.15	95.8	
	Rose wine 2	0.8	0.82	102.5	0.32
		1.6	1.57	98.1	
		2.5	2.49	99.6	
Tyramine	Red wine 1	0.1	0.095	96	0.93
		0.3	0.29	96.6	
		0.5	0.52	104	
	Red wine 2	1	1	100	N.D.
		2	2.05	102.5	
		3	2.9	96.6	
	White wine 1	0.5	0.48	96	N.D.
		1.5	1.45	96.6	
		2.5	2.42	96.8	
	White wine 2	0.4	0.38	95	0.3
		0.8	0.78	97.5	
		1.2	1.15	95.8	
	Rose wine 1	0.2	0.22	110	N.D.
		0.4	0.39	97.5	
		1.5	1.5	100	
	Rose wine 2	0.3	0.3	100	0.83
		0.9	0.88	97.7	
		1.3	1.42	109	
Phenylethylamine	Red wine 1	0.5	0.49	98	0.27
		1.5	1.6	106.6	
		2.5	2.45	98	
	Red wine 2	1.5	1.48	98.6	0.36
		2.5	2.55	102	
		3.5	3.46	98.8	
	White wine 1	0.2	0.23	115	0.2
		0.6	0.6	100	
		0.8	0.79	98.75	
	White wine 2	1	1	100	N.D.
		2	1.93	96.5	
		3	3.1	103.3	
	Rose wine 1	0.5	0.49	98	N.D.
		1.5	1.5	100	
		2.5	2.5	100	
	Rose wine 2	0.3	0.29	96.6	N.D.
		0.9	0.92	102.2	
		1.6	1.54	96.25	

[a] Concentration obtained by using the standard addition method.

derivatization, which is unnecessary in CE as it affords indirect detection.

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