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Determination of amines in wines by high-performance liquid chromatography with electrochemical coulometric detection after precolumn derivatization

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ABSTRACT

A sensitive and simple method for the simultaneous evaluation of histamine, tryptamine, tyramine, phenylethylamine, putrescine, cadaverine, 1,6-diaminohexane and tryptophan in wine was developed using HPLC and coulometric detection without clean-up pretreatment. After precolumn derivatization with *o*-phthaldialdehyde the compounds were separated on a reversed-phase column with gradient elution. To increase the selectivity of the method a coulometric array of sixteen electrodes at increasing potentials was used. The total analysis time was less than 35 min. The analytical performance of the methods is reported. The method was used to examine the levels of various amines in three different wines.

INTRODUCTION

Most amines found in wines come from the degradation of amino acids during malo-lactic fermentation [1]. Some of these amines generate distinct flavours which can affect the organoleptic quality of the wine. Other amines, such as histamine, tyramine, cadaverine and putrescine, can generate undesirable effects which include headache, vomiting and diarrhoea [2]. As a result, much attention has been given to the characterization and determination of amines in wine. However, the complexity of these natural products has made their identification and determination difficult. As amines are present in low concentrations in wines, they have been purified and preconcentrated by different methods [2,3], including *o*-phthaldialdehyde (OPA) derivatization with high-performance liquid chromatography (HPLC) followed by fluorescence detection [4] and derivatization of amines with dansyl chloride followed by electrochemical detection [4].

This paper reports the separation and the determination of eight amines (histamine, tryptamine, tyramine, phenylethylamine, putrescine, cadaverine, 1,6-diaminohexane and tryptophan) in a single run. It should be noted that tryptophan, an amino acid that is always present in wines, could present interference problems with the other amines. Separation and identification with the proposed method can be achieved without extraction or purification of the samples. This was done after their derivatization with OPA using gradient reversed-phase HPLC and electrochemical detection with an array of sixteen coulometric electrodes.

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EXPERIMENTAL

Chemicals

The two mobile phases used in the gradient runs were prepared by dissolving the appropriate chemicals in deionized water. Mobile phase A contained 0.1 *M* sodium acetate, 12.5% acetonitrile and 5% tetrahydrofuran (pH 6.5) and B contained 0.1 *M* sodium acetate, 25% acetonitrile and 30% tetrahydrofuran (pH 6.5). The mobile phases were filtered through a 0.22-μm PTFE lyophilic membrane (Millipore, Bedford, MA, USA) prior to use.

Acetonitrile (HPLC grade) and tetrahydrofuran, OPA, sodium acetate, mercaptoethanol and sodium tetraborate (all of analytical-reagent grade) were purchased from Sigma Chimica (Milan, Italy). The water used to prepare the mobile phases for the gradient runs was purified with a Milli-Q water-purification system (Millipore) and it was filtered through a 0.45-μm filter (Millipore). OPA solution was prepared by dissolving 27 mg of the compound in 1 ml of methanol. To this solution 5 μl of mercaptoethanol and 9 ml of 0.1 *M* sodium tetraborate (pH 9.3) were added. The final OPA solution was stored at room temperature and diluted 1:3 with sodium tetraborate (0.1 *M*, pH 9.3) just prior to use.

Apparatus

The chromatographic system used was a Coulochem Electrode Array System (CEAS) (ESA, Bedford, MA, USA). This instrument consisted of a refrigerated autosampler, two HPLC pumps capable of gradient operation and a detection system of four cell packs in series. These cell packs were contained with the column in a thermostatic compartment. Each pack contained four porous graphite working electrodes. Solutes were separated on a Model HR80 PTFE-lined column (80 × 4.6 mm I.D.) containing ODS, particle size 3 μm (ESA). The CEAS software monitored and controlled the autosampler, the two pumps, the detection system and the temperature of the column compartment.

Chromatographic method

A method capable of completely separating the eight compounds chosen was developed. It consisted of a gradient in which the organic modifier was altered during the run. The time line showing the gradient used in this separation is presented in Table I: the total flow-rate was 0.80 ml/min and the temperature of the column compartment was maintained at 37°C. The sixteen detector potentials were arranged in a symmetric array from 0 mV at electrode 1 to 1200 mV at electrode 16 with increments of 80 mV at each electrode. The indicated potentials refer to the solid-state palladium reference electrode built in the coulometric cells. Their absolute values were about 250 mV lower than the corresponding potential measured by using an Ag/AgCl reference electrode.

Standard and sample preparation

Pure standards of histamine, tryptophan, tryptamine, tyramine, phenylethylamine, putrescine, cadaverine and 1,6-diaminohexane were obtained from Sigma. Stock standard solutions were prepared by dissolving 10 mg of each component in methanol. These solutions were then subdivided into 1-ml portions and stored at −20°C. An eight-component working standard solution was prepared by combining and diluting with mobile phase A an aliquot of each of the stock standard solutions (final concentration 2 μg/ml). This method was used to measure the amines present in three wines: Gewürztraminer, Barolo and Port. The standards and the samples were kept refrigerated at 4°C up to the moment

TABLE I

GRADIENT PROFILE USED IN THE CHROMATOGRAPHIC SEPARATION

Time (min)	Phase A (%)	Phase B (%)
0	85	15
4.1	85	15
13	50	50
23	0	100
38	0	100
38.1	15	85
39	15	85

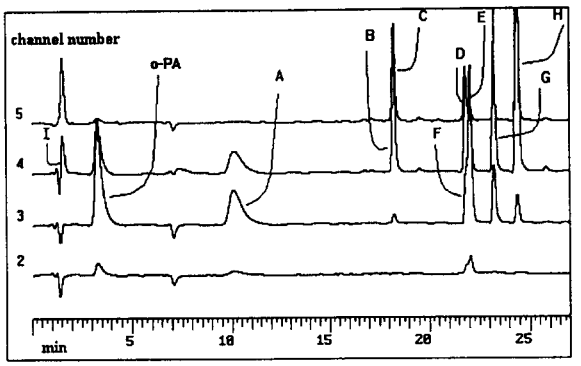

Fig. 1. Chromatogram of the OPA derivatives of (A) histamine, (B) tryptophan, (C) tryptamine, (D) tyramine, (E) phenylethylamine, (F) putrescine, (G) cadaverine and (H) 1,6-diaminohexane. Other peaks: injection void (I) and derivatizing reagent OPA. For clarity only four channels are presented. Electrode potentials: 2 = 80 mV; 3 = 160 mV; 4 = 240 mV; 5 = 320 mV. Full-scale sensitivity: 10 μA.

of derivatization. Prior to injection all samples were filtered through a 0.22-μm membrane (Millipore). The standards and samples, without any clean-up pretreatment, were then derivatized with OPA reagent and 20 μl were injected into the CEAS.

Precision

To investigate the within-run precision, twenty sets of the derivatized working standard mixture were injected and analysed under the conditions described above. Analysis of the same sample over a 10-day span (the sample was stored in aliquots at −20°C between assays) was used to measure the between-run precision.

RESULTS

Under the above conditions, a mixture containing the eight standards was separated and analysed. A typical separation of a 20-μl sample containing 25 ng of each of the eight components as external standard is shown in Fig. 1. Only four traces are shown, corresponding to electrodes 1–4. Although all sixteen channels were used, only a few signals are shown for the purpose of clarity. The total analysis time was less than 35 min. The detection characteristics of these eight standards are presented in Table II.

The retention time repeatability during the precision studies (carried out over a 10-day span) was found to be excellent (R.S.D. < 1.8%) for all standards. This was due to the strict control of both the gradient profile and the column temperature. The precision (within- and between-run), detection limits and dominant potentials are also reported (the dominant potential is that electrode potential where the maximum signal occurs for a given substance if eluted

TABLE II

CHROMATOGRAPHIC AND ELECTROCHEMICAL CHARACTERISTICS OF THE EIGHT EXTERNAL STANDARDS

25 ng of each component per injection of 10 μl.

Compound	Retention time (min)	Detection limit (mg/l)	Within-run R.S.D. (%)	Between-run R.S.D. (%)	Recovery[a] (%)	Dominant potential[b] (mV)
Histamine	10.05	0.016	2.2	3.2	93	80
Tryptophan	16.74	0.021	1.0	2.9	89	320
Tryptamine	17.14	0.018	1.8	3.5	96	320
Tyramine	18.23	0.016	0.7	3.2	92	320
Phenylethylamine	21.78	0.022	1.2	2.9	88	320
Putrescine	22.01	0.025	2.6	4.8	76	160
Cadaverine	23.19	0.021	2.1	5.2	89	160
1,6-Diaminohexane	24.34	0.012	3.1	3.9	103	160

[a] The recoveries are the means of three replicates from a sample of Gewürztraminer wine spiked with 10 ng of each of the eight amines.

[b] See text.

through a given coulometric array). Confirmation of peak identification was carried out in two steps. In the first, samples were spiked with the relevant standard. In the second, the ratio R between each standard was matched with the actual peaks of the samples (R is the ratio of the signal distribution between dominant and subdominant channels expressed as a percentage, where 1 corresponds to maximum match and 0 corresponds to no match) [5].

Several dilutions of the amine standards, having concentrations from 100 μg/ml to 10 ng/ml, were prepared and analysed to study the linearity of the method. The plots for the standards for all amines were linear throughout the range tested ($r = 0.997$, $p < 0.001$). The minimum detectable amounts (at a signal-to-noise ratio of 3:1) are reported in Table II. The recoveries for three replicates from a sample of Gewürztraminer wine are also reported in Table II. This sample was spiked with 10 ng of each of the eight amines and derivatized as described above.

The repeatability of the method was tested by repeated (twenty) injections of a standard solution containing 10 ng of each of the eight amines. The within-run variability (R.S.D.) ranged from 0.7 to 3.1%. The between-run variability was studied by analysing the same standard solution over a 10-day span. The R.S.D. ranged from 2.9 to 5.2%.

The standard mixture was used to examine the amine levels in three different wines: Gewürztraminer, a white, aromatic, rich-bodied wine, from Alto Adige, Italy; Barolo, a red, tannic, high-bodied wine aged 6 years in oak barrels, from Piemonte, Italy; and Port, an aged fortified wine, from Oporto, Portugal. These wines were selected since as a group they encompassed many different characteristics. The results are reported in Table III.

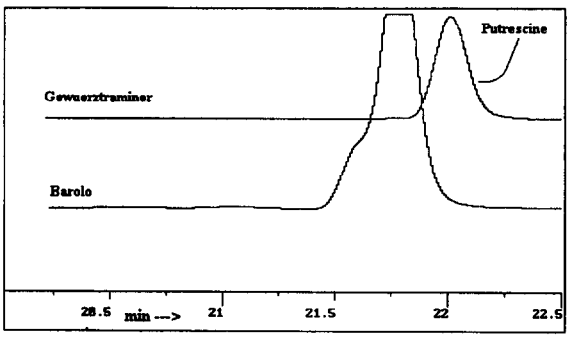

Fig. 2. Chromatograms of 20-μl samples of Barolo and Gewürztraminer wines after derivatization at 160 mV (channel 3). Full-scale sensitivity: 200 nA.

In Fig. 2, a comparison between a portion of the chromatogram of Barolo wine and one of Gewürztraminer wine is shown. The trace corresponds to the signal generated by electrode 3, which was set at 160 mV. The putrescine peak is visible in Gewürztraminer wine at a relatively high concentration whereas it is not present in Barolo wine. In addition, at this low potential a major unidentified peak is present in Barolo wine (not present in the external standard). This peak could interfere in the determination of

TABLE III

CONCENTRATIONS OF THE MEASURED COMPOUNDS IN THREE DIFFERENT WINES

Compound	Concentration (mg/l)		
	Gewürztraminer	Barolo	Port
Histamine	42.31	10.66	12.07
Tryptophan	9.52	0.74	–
Triptamine	0.40	2.94	1.09
Tyramine	25.49	5.65	1.95
Phenylethylamine	2.98	3.31	2.06
Putrescine	162.24	18.20	7.48
Cadaverine	0.28	0.76	0.31
1,6-Diaminohexane	0.25	0.12	1.59

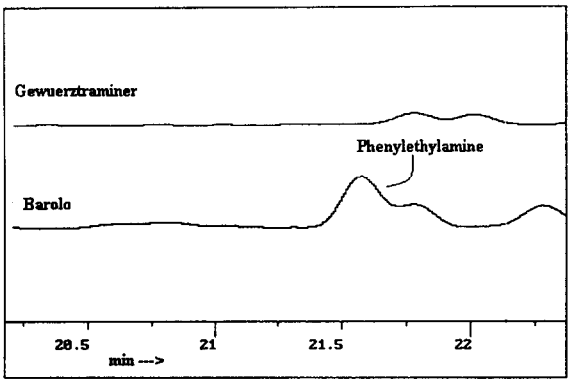

Fig. 3. Chromatograms of 20-μl samples of Barolo and Gewürztraminer wines after derivatization at 320 mV (channel 5). Full-scale sensitivity: 200 nA.

phenylethylamine. Fig. 3 shows the corresponding section of the chromatogram of electrode 5, which was set at 320 mV. At this electrode the signal of the unidentified substance shown in Fig. 2 disappeared. The substance was completely reacted by the coulometric electrodes placed before this electrode. As a result, it was possible to measure phenylethylamine without any interference at this channel.

DISCUSSION AND CONCLUSIONS

The use of CEAS for determining neurochemicals in tissues [6,7] and in cerebrospinal fluid [5] has been reported. More recently, a method for the evaluation of phenolic compounds in fermented beverages, fruit juices and plant extracts on the same analyser has been proposed [8].

In this work, we demonstrated that by combining reversed-phase chromatography with highly selective array electrochemical detection it is possible to determine a large number of amines in wines in a single run of 35 min. The derivatization procedure described allows for simple, rapid sample preparation at low cost and without any clean-up pretreatment. This method yields a high precision without preliminary separation of the different families of compounds which is necessary in other methods. The coulometric efficiency of each element of the array allows a complete voltammetric resolution of analytes as a function of their reaction potential. Some peaks may be resolved by the detector even if they are unresolved when they leave the chromatographic column. The detection limit is similar to that which was found with the measurement of dansyl derivatives followed by electrochemical detection [4]. However, it was better than the detection limit observed with OPA derivatization followed by fluorimetric detection [4]. In conclusion, the proposed method for the determination of amines in wines provides a simple, fast and reproducible sampling procedure with good selectivity and sensitivity over a wide concentration range.

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