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# Determination of biogenic amines as dansyl derivatives in alcoholic beverages by high-performance liquid chromatography with fluorimetric detection and characterization of the dansylated amines by liquid chromatography–atmospheric pressure chemical ionization mass spectrometry

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**Abstract**

A sensitive high-performance liquid chromatographic method for the simultaneous determination of 11 biogenic amines has been developed. The method involves addition of an internal standard (1,7-diaminoheptane), pre-column dansylation of the amines and subsequent solid-phase extraction of the derivatives through $C_{18}$ cartridges. The dansylamides were separated on an Inertsil ODS-3 column (250×4 mm I.D., 5 μm) using a 35-min gradient elution with a binary system of acetonitrile–water, a flow-rate of 1 ml $min^{-1}$ and fluorescence detection at excitation and emission wavelengths of 320 and 523 nm, respectively. The identity of the dansyl derivatives was confirmed by liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. Linearity of derivatization was obtained for concentrations ranging from 0.008 to 40.0 mg $l^{-1}$. The within- and between-day relative standard deviations ranged from 0.2 to 7.6% and 0.3 to 8.6%, respectively. The overall process was successfully applied to identify and quantify biogenic amines in white-, red- and Retsina Greek wines and Greek beers, after treatment with polyvinylpyrrolidone.


*Keywords:* Wine; Beer; Food analysis; Biogenic amines; Amines

## 1. Introduction

Biogenic amines are aliphatic, alicyclic or heterocyclic organic bases of low molecular mass that can occur in plants and in fermented foods. Biogenic amines are derived from microbial decarboxylation of the corresponding amino acids or by transamination of aldehydes by amino acid transaminases [1,2]. They are normal constituents of many foods and beverages and have been found to occur in cheese, wine, beer, sauerkraut, fishery products and aged meat, normally as a result of enzymatic degradation or fermentation processes [3–5]. Their presence in high amounts in foods is associated with food deterioration [6]. In non-fermented foods, the biogenic amines appear as a result of undesirable microbial activity. Buteau et al. [7]

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indicated the possibility that the biogenic amines are formed in wine by the action of contaminant microorganisms, while others [8] regarded histamine alone or together with the other amines to be an indicator of unsanitary conditions occurring during the wine production.

Biogenic amines in low concentrations are essential for many physiological functions, while at high concentrations may cause some deleterious effects. Several symptoms occur following excessive oral intake of biogenic amines, such as headache, hypo- or hypertension, nausea, cardiac palpitations, renal intoxication and in more severe cases intracerebral hemorrhage and death [1,4,9]. The interaction between ethanol and amines seems to be synergistic [10]. In view of the possible harmful effects of biogenic amines, their concentration levels in foods deserve careful investigation.

More than 20 amines have been identified in wine and their total 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]. The types and levels of biogenic amines in beers are affected mainly by raw materials, brewing techniques and hygienic conditions. Due to the high consumption of beer and the possible harmful effects of biogenic amines, it is important to determine their levels. The levels of biogenic amines in American, European and Brazilian beers have been reported [1,12,13]. However, no information was found on levels of biogenic amines in Greek beers.

The determination of biogenic amines has been carried out with different chromatographic methods. High-performance liquid chromatography (HPLC) is the preferred method [1,2,4–6,11,14–16] but, even so, it is necessary to increase the sensitivity of the analytical method, by using pre- or post-column derivatization, because amines lack an easily detectable common chromophore. The determination of biogenic amines in wines and beers has been carried out mostly by HPLC [1,5,11–14,17–23] after derivatization with reagents such as *o*-phthalaldehyde (OPA) [5,13,14,17,18,24,25] dansyl chloride (Dns-Cl) [11,12] or dabsyl chloride [22]. OPA has the disadvantages that it reacts only with primary amines and that their fluorescent derivatives are unstable, while dabsyl-chloride and Dns-Cl react with both primary and secondary amino groups providing very stable derivatives. Dansyl derivatives combine the unique feature of being both fluorescent and detectable in the UV region. To our knowledge, there are only three published works reporting HPLC methods for the determination of dansylated amines with fluorimetric detection [26–28], but none of them is applied to alcoholic beverages and only one is reported for the determination of more than five amines.

Regardless of the derivatizing reagent used, if biogenic amines are to be determined at low levels in complex matrices such as wine or beer, with no interference from other compounds (e.g. amino acids, phenolic compounds) previous clean-up and preconcentration steps are required. Numerous methods for isolating amines from wine have been reported, including liquid–liquid extraction (LLE) either before [4,23] or after [15] derivatization and solid-phase extraction (SPE) procedures. Nowadays, SPE is preferred to LLE because of its obvious advantages and several studies have been carried out using SPE, based on cation-exchange extraction before derivatization [24,25] or as a means to enrich biogenic amines in wines after derivatization using $C_{18}$ sorbents [11] or strong anion-exchange (SAX) cartridges [25].

For quality assurance and quality control purposes, the verification of analyte peak purity and identity of peaks is of primary importance, especially in complex matrices. In this work, a HPLC–diode array detection–atmospheric pressure chemical ionization mass spectrometry (HPLC–DAD–APCI-MS) system was used for the determination of analyte peak purity and characterization of the peaks. To our knowledge, only three publications on the characterization of dansylamides, in various matrices, have been reported so far [29–31].

In this paper, 11 of the most oenologically important biogenic amines are determined in wines and beers after pretreatment with polyvinylpyrrolidone (PVP), which removes the substances that interfere in the derivatization and subsequent quantification of the derivatives. The applied post-derivatization SPE procedure increases the selectivity and sensitivity of the method. The proposed method offers several advantages over previously reported ones, including: the use of a simple gradient which effectively separates all the amines in a relatively short time; a

simple, time- and cost-effective pretreatment of the samples which is applied for the first time to beers; a sensitive and selective detection method offering at least one order of magnitude higher sensitivity for all amines (except histamine); wider linear ranges covering two orders of magnitude and improved precision and recovery over other methods using derivatization with OPA and fluorimetric detection. Finally, the use of a HPLC–DAD–APCI-MS system, for peak purity control and for characterization of the peaks, which is applied for the first time in the literature to dansylated wine and beer samples.

## 2. Experimental

### 2.1. Reagents and materials

Methylamine and histamine were obtained from Lancaster (Morecambe, UK). Putrescine and cadaverine were obtained from Sigma (St. Louis, MO, USA). Isoamylamine was obtained from Aldrich (Steinheim, Germany). Ethylamine, tyramine, spermidine, spermine, tryptamine and 2-phenylethylamine were obtained from Fluka (Buchs, Switzerland). All amines, except for isoamylamine were purchased as hydrochloride salts and the concentrations of standard solutions were corrected on the basis of their purity and referred to as free base. 1,7-Diaminoheptane obtained from Lancaster was used as the internal standard (I.S.). Dns-Cl, obtained from Fluka, was used as the derivatization reagent at a concentration of 1% (w/v) in acetone. PVP, obtained from Aldrich, was used as the wine bleaching agent. Acetonitrile was purchased from Merck (Darmstadt, Germany). Methanol and acetone were obtained from Riedel-de Haën (Hannover, Germany). Sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) and sodium hydroxide Titrisol 10 *M*, used for the preparation of buffer, were obtained from Riedel-de Haën and Merck, respectively. The borate buffer solution was prepared by dissolving 3.81 g of sodium tetraborate in 100 ml of Milli-Q water (Millipore, Bedford, MA, USA) and the pH was adjusted to 10.5 with 10 *M* sodium hydroxide. This buffer was used to adjust the sample pH before derivatization. All the reagents used for the assay were of analytical-reagent grade, except for methanol and acetonitrile, which were of HPLC grade. Milli-Q water was used throughout the work. Wine and beer samples were obtained from commercial sources. Types and brands of massive levels of consumption and production were sampled.

Stock standard solutions (1000 mg $l^{-1}$) of each amine and of the internal standard were prepared in methanol–water (1:1). Dilute solutions (100 mg $l^{-1}$ and 10 mg $l^{-1}$) of the amines were prepared weekly, in water. All solutions were kept in the dark under refrigeration, at 4 °C. The standard mixtures used for calibration were prepared just prior to derivatization.

SPE cartridges Discovery $C_{18}$ (500 mg 3 $ml^{-1}$) obtained from Supelco (Bellefonte, PA, USA) were used for the solid-phase clean-up of the derivatives.

Schleicher & Schuell (Dassel, Germany) 0.2 μm filters were used for the filtration of standard solutions and real samples.

### 2.2. Equipment

The HPLC–fluorescence system, initially used for the quantification of amines, consisted of a model LC-9A Shimadzu binary gradient pump (Kyoto, Japan), equipped with a Rheodyne 7725i injection valve (Cotati, CA, USA), having a 20-μl loop. The detector was a model RF-551 Shimadzu spectrofluorometer, connected to a model 4290 Spectra-Physics integrator (San Jose, CA, USA), for the recording of the results and evaluation of peak areas and retention times.

Peak purity control and identification of the peaks was performed serially, on a separate HPLC–DAD–APCI-MS system, Hewlett-Packard HP series 1100, (Waldbron, Germany and Palo Alto, CA, USA) consisting of a HP G1311A quartenary gradient pump, a HP G1316A column oven, a HP G1322A degasser, a HP G1313A autosampler and a HP G1947A APCI-MS system.

In both HPLC systems, the analytical column used was a reversed-phase Inertsil ODS-3 (250×4 mm I.D., 5 μm) obtained from MZ Analysentechnik (Mainz, Germany).

The SPE study was performed on a Vac-Elut manifold system, having a 10-position capacity, obtained from Varian (Harbor City, USA) and the evaporation of solvents was carried out in a water-

bath at 45 °C under a stream of nitrogen, by means of a Pierce model 18780 Reacti-Vap device (Rockford, IL, USA).

A Glass-col Terre Haute In 47802 vortexer and a model Z230 centrifuge (B. Hermle, Gosheim, Germany) were employed for the treatment of real samples.

A glass vacuum solvent-filtration apparatus obtained from Alltech Associates (Deerfield, IL, USA) was employed for the filtration of water prior to its use in the mobile phase.

### 2.3. Chromatographic conditions

Two solvent reservoirs, containing (A) water and (B) acetonitrile, were used to separate the amines at ambient temperatures. An initial linear gradient elution from 40% of solvent B to 80% in 25 min was used, followed by another linear elution from 80% to 100% of B in 5 min. A 5-min additional step was included finally to reach the initial conditions and achieve mobile phase stabilization. Separation was performed with a flow-rate of 1 ml $min^{-1}$ and all amines were eluted in ~35 min. Quantification of the dansylamides was carried out by fluorescence detection, at 320 nm (excitation wavelength, $\lambda_{ex}$) and 523 nm (emission wavelength, $\lambda_{em}$), with a gain setting of ×1 and sensitivity range 1.

Peak purity control and identification of the peaks in a standard solution and a pooled sample (derived from five red and five white wines) was performed with a HPLC–DAD–APCI-MS system under the same chromatographic conditions as those used for the fluorimetric determination of the amines, except for column thermostating at 25 °C. The peaks were detected at 230, 254 and 280 nm and the effluent from DAD was injected into the APCI-MS system. The APCI interface conditions were: vaporizer temperature at 400 °C, nebulizing gas temperature at 350 °C, nebulizer pressure 50 p.s.i., drying gas ($N_2$) flow-rate 5 l $min^{-1}$ and corona current 4 μA (1 p.s.i.=6894.76 Pa). The fragmentor was set at 110 V. Scan spectra were recorded in positive ion mode over the range $m/z$ 100–1300 amu. The injection volume was 10 μl solution of dansylamides in acetonitrile.

### 2.4. Procedures

#### 2.4.1. Standard solutions

Dilute standard solutions (100 mg $l^{-1}$ and 10 mg $l^{-1}$) of each amine were prepared by serially diluting the corresponding 1000 mg $l^{-1}$ stock solution in 10-ml volumetric flasks with water. Six standard mixtures of all amines, at different concentrations (ranging from 0.008–0.8 mg $l^{-1}$ for methylamine and ethylamine, 0.014–1.4 mg $l^{-1}$ for putrescine and cadaverine, 0.015–1.5 mg $l^{-1}$ for isoamylamine, 0.02–2.0 mg $l^{-1}$ for 2-phenylethylamine, 0.03–3.0 mg $l^{-1}$ for spermidine, 0.04–4.0 mg $l^{-1}$ for tryptamine and spermine, 0.08–8.0 mg $l^{-1}$ for tyramine and 0.4–40 mg $l^{-1}$ for histamine) were prepared by adding the appropriate amount of the 100 or 10 mg $l^{-1}$ amine solutions, in 10-ml volumetric flasks and bringing to volume with water. These standard mixtures were used for the construction of calibration curves, after derivatization and subsequent SPE.

The dilute standard amines were prepared weekly and kept in dark under refrigeration, at 4 °C. The standard mixtures, used for calibration, were prepared just prior to derivatization, because some amines, especially histamine, are subject to decomposition, resulting in low yields of derivatives, which in turn give rise to LC measurements of decreased sensitivity.

#### 2.4.2. Sample treatment

A 0.5-g quantity of PVP was added to 10 ml of standard solutions (mixture of the amines) or real samples in a beaker. The resulting mixtures were stirred for 15 min on a magnetic stirrer and were then centrifuged for 15 min at 3500 *g*. The supernatants were finally filtered through 0.2 μm filters. The beer samples were degassed by ultrasonication for 15 min prior to the PVP treatment.

#### 2.4.3. Derivatization process

Aliquots of 5 ml of the filtered standard solutions or real samples were transferred to appropriate vials and the pH was adjusted to 9.5 with the borate buffer (prepared as described in Section 2.1). A 2-ml volume of the resulting solutions was transferred to 5-ml reaction vials and 400 μl of an 8.43 mg $l^{-1}$

internal standard solution and 800 μl of a 1% (w/v) Dns-Cl solution were then added and the mixtures were brought to a total volume of 4 ml with acetone–water (3:1). After vigorous agitation the reaction mixtures were left in darkness for 30 min at 65 °C.

*2.4.4. Solid-phase extraction clean-up*

After derivatization, the reaction vials were left to cool at room temperature and acetone was removed under a stream of nitrogen. The remaining aqueous phase of the derivatized standard solutions or real samples was then applied to Supelco Discovery $C_{18}$ cartridges (500 mg 3 $ml^{-1}$), which had been previously activated with two cartridge volumes of methanol followed by two volumes of water. After the samples had passed through, the cartridges were washed with two cartridge volumes of water–acetone (80:20, v/v) and taken to dryness under vacuum. The samples were finally eluted with 3 ml of acetonitrile and the eluates were evaporated to dryness under nitrogen. The residues were reconstituted to 2 ml with acetonitrile and the resulting solutions were repeatedly injected into the chromatograph.

*2.4.5. Identification and purity check of dansylated amines*

A derivatized standard solution and a derivatized pooled sample (derived from five red and five white wines) were analyzed by HPLC–DAD connected in series with APCI-MS. The samples (10-μl injection) were monitored at 230, 254 and 280 nm and under the HPLC conditions used, all peaks showed a high purity factor, both in the standard and the sample, except for dansyl tryptamine, which showed a low purity in the sample. To confirm identification of the peaks, HPLC–APCI-MS in positive ion mode was applied.

## 3. Results and discussion

In the analytical method developed, an optimization of the derivatization procedure was carried out by investigating the influence of Dns-Cl concentration, pH, derivatization time and derivatization temperature, on the output of the dansylation reaction. The post-derivatization SPE clean-up was also optimized, by examining the extraction efficiency of different sorbent types ($C_{18}$ and $C_8$) from different brand names, with several extraction protocols, applied to a standard mixture of dansylated amines. The extraction efficiency was evaluated in terms of highest peak areas obtained for all dansylamides and of highest selectivity (fewest interferences from matrix). Finally, the matrix effect was evaluated and expressed as percent extraction recovery. The percent extraction recovery was calculated by comparing the peak area of the extracted amines from a spiked amine-free wine sample with the peak area of the extracted amines from a standard solution, at the same concentration level, measured the same day. Overall, the best results were obtained with Supelco Discovery $C_{18}$ cartridges, which yielded the highest extraction efficiency, among other cartridges tested, and showed a similar extraction behavior of the amines in spiked samples as in standards. The average percent extraction recovery, in the latter case, was 95.0%.

Fig. 1 shows the chromatogram of a standard solution of dansylamides, obtained with the gradient profile described in Section 2.3, and as can be seen, all the analyte peaks are well resolved. The presence of other peaks (as those at ~3, 18 and 19 min) can be assigned to side-products of the dansylation reaction.

The fluorescence parameters were optimized and spectra of all peaks of interest were acquired by using the stop flow technique during the chromatographic run. The spectra are given in Fig. 2. The choice of excitation and emission wavelengths was made on the basis of highest sensitivity for all dansylamides and low background noise.

*3.1. Mass spectrometric characterization of the peaks*

Identification of peaks in a standard and a pooled dansylated sample was performed by HPLC–APCI-MS, in positive ion mode, and it was based on their mass spectra. The results are presented in Table 1. The dansyl derivative of each amine produced a very stable and intense $[M+H]^+$ ion (of relative intensity of ~100%), which showed no fragmentation under the conditions used. The results show that the dansylation reaction produces the expected deriva-

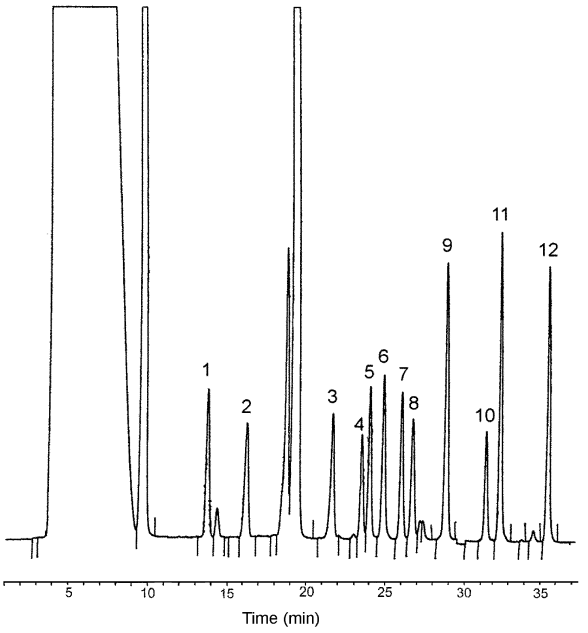


Fig. 1. Representative (RP-HPLC) chromatogram of the derivatives of amines from a standard mixture. Peaks: 1, methylamine (0.08 mg $l^{-1}$); 2, ethylamine (0.08 mg $l^{-1}$); 3, tryptamine (0.40 mg $l^{-1}$); 4, 2-phenylethylamine (0.18 mg $l^{-1}$); 5, isoamylamine (0.15 mg $l^{-1}$); 6, putrescine (0.14 mg $l^{-1}$); 7, cadaverine (0.14 mg $l^{-1}$); 8, histamine (4.0 mg $l^{-1}$); 9, internal standard (0.843 mg $l^{-1}$); 10, tyramine (0.80 mg $l^{-1}$); 11, spermidine (0.30 mg $l^{-1}$); 12, spermine (0.40 mg $l^{-1}$); attenuation = 512; chart speed = 0.5 cm $min^{-1}$; $\lambda_{ex}$ = 320 nm, $\lambda_{em}$ = 523 nm; column, Inertsil ODS-3 (250×4 mm I.D., 5 μm); mobile phase, acetonitrile (40–80% from 0–25 min, 80–100% from 25–30 min, 100–40% from 30–35 min)–water; injection solvent, acetonitrile; injection volume, 20 μl.

tives, and that in the case of di- or polyamines, all amino-groups were substituted. It should also be mentioned that in the case of tyramine, Dns-Cl reacts also with the phenolic hydroxyl group.

The unknown peaks at about 3 and 18 min, present both in standard and pooled dansylated samples, were identified as derivatization by-products: dansylamide (Dns-$NH_2$, $M_r = 250$) and dansyl sulphonic acid (Dns-OH, $M_r = 251$), respectively [26]. The unknown peak (at 19 min), which co-elutes with the dansyl derivative of tryptamine, only in real samples, was identified as dansyl hydrazine (Dns-$N_2H_3$, $M_r = 265$).

### 3.2. Method validation

#### 3.2.1. Linearity

The linearity of the method for each amine assayed was examined. The data were collected for six different concentrations of biogenic amines in mixtures (ranging from 0.008–0.8 mg $l^{-1}$ for methylamine and ethylamine, 0.014–1.4 mg $l^{-1}$ for putrescine and cadaverine, 0.015–1.5 mg $l^{-1}$ for isoamylamine, 0.02–2.0 mg $l^{-1}$ for 2-phenylethylamine, 0.03–3.0 mg $l^{-1}$ for spermidine, 0.04–4.0 mg $l^{-1}$ for tryptamine and spermine, 0.08–8.0 mg $l^{-1}$ for tyramine and 0.4–40 mg $l^{-1}$ for histamine) with a concentration of 0.843 mg $l^{-1}$ of I.S., using triplicate analysis for each mixture. Calibration graphs were constructed by plotting the amine to I.S. peak-area ratios against the amine concentrations. Linear least squares regression was used to calculate the slope, intercept and correlation coefficient and the results are given in Table 2. In all cases a good correlation coefficient was obtained, ranging from 0.9994 to 0.9999.

#### 3.2.2. Precision

The repeatability and reproducibility of the method was assessed by injecting eight times each of three standard dansylamide mixtures at low, medium and high concentration levels, during the same day and over a period of 10 days, respectively. In the latter case, the 10 measured values, representing the means of three determinations per day and per standard, were used for the evaluation of the overall between-day precision of the method. The within-day relative standard deviations (RSDs) ranged from 0.2 to 7.6% and the between-day RSDs from 0.3 to 8.6%, indicating a high degree of precision.

#### 3.2.3. Recovery

The overall recovery of the method was determined by spiking 10-ml aliquots of a pooled wine sample (derived from five red and five white wines) and of an amine-free red wine, with known amounts of four standard amine mixtures. The initial amine content of the pooled wine sample had been determined six times. After addition, the samples were subject to the whole analytical procedure and the concentration of each sample was measured three

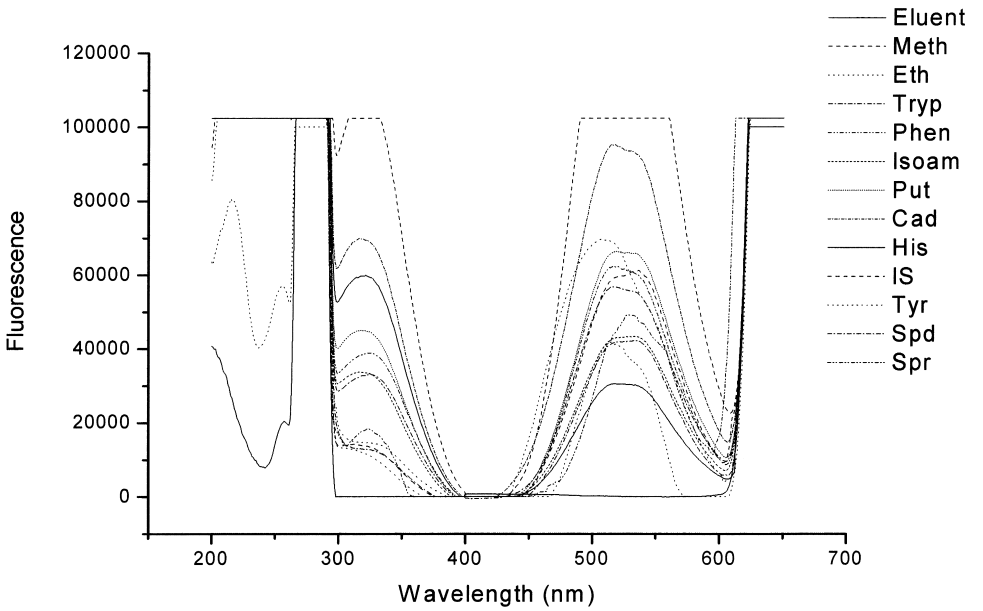


Fig. 2. Fluorescence spectra of the dansylamides in acetonitrile. Concentrations: methylamine 0.08 mg $l^{-1}$; ethylamine 0.08 mg $l^{-1}$; tryptamine 0.40 mg $l^{-1}$; 2-phenylethylamine 0.18 mg $l^{-1}$; isoamylamine 0.15 mg $l^{-1}$; putrescine 0.14 mg $l^{-1}$; cadaverine 0.14 mg $l^{-1}$; histamine 4.0 mg $l^{-1}$; internal standard 0.843 mg $l^{-1}$; tyramine 0.80 mg $l^{-1}$; spermidine 0.30 mg $l^{-1}$ and spermine 0.40 mg $l^{-1}$.

Table 1
Values of characteristic mass fragments of dansylamides obtained by HPLC–APCI-MS

| Peak | Dansylamide | M ($m/z$) | $[M+H]^+$ ($m/z$) |
|---|---|---|---|
| Methylamine | Dns-methylamine | 264 | 265 |
| Ethylamine | Dns-ethylamine | 278 | 279 |
| Tryptamine | Dns-tryptamine | 393 | 394 |
| 2-Phenylethylamine | Dns-2-phenylethylamine | 354 | 355 |
| Isoamylamine | Dns-isoamylamine | 320 | 321 |
| Putrescine | $(Dns)_2$-putrescine | 554 | 555 |
| Cadaverine | $(Dns)_2$-cadaverine | 568 | 569 |
| Histamine | $(Dns)_2$-histamine | 577 | 578 |
| I.S. | $(Dns)_2$-I.S. | 596 | 597 |
| Tyramine | $(Dns)_2$-tyramine | 603 | 604 |
| Spermidine | $(Dns)_3$-spermidine | 844 | 845 |
| Spermine | $(Dns)_4$-spermine | 1134 | 1135 |
| U.P.1[a] | Dns-$NH_2$ | 250 | 251 |
| U.P.2[b] | Dns-OH | 251 | 252 |
| U.P.3[c] | Dns-$N_2H_3$ | 265 | 266 |

[a] Unknown peak at about 3 min.
[b] Unknown peak at about 18 min.
[c] Unknown peak at 19 min, present only in real samples.

Table 2
Regression analysis equations and linear ranges for dansylamides using HPLC–fluorescence

| Amine | Regression equation $y = (a \pm SD_a) + (b \pm SD_b)x$ | Correlation coefficient | Linear range (mg $l^{-1}$) |
|---|---|---|---|
| Methylamine | $y = (0.0336 \pm 0.0276) + (6.572 \pm 0.0742)x$ | 0.9997 | 0.008–0.8 |
| Ethylamine | $y = (0.0716 \pm 0.0265) + (4.794 \pm 0.0712)x$ | 0.9995 | 0.008–0.8 |
| Tryptamine | $y = (0.0263 \pm 0.0458) + (1.385 \pm 0.0246)x$ | 0.9994 | 0.04–4.0 |
| 2-Phenylethylamine | $y = (-0.0057 \pm 0.0213) + (2.475 \pm 0.0254)x$ | 0.9998 | 0.02–2.0 |
| Isoamylamine | $y = (0.0613 \pm 0.0303) + (3.544 \pm 0.0439)x$ | 0.9997 | 0.015–1.5 |
| Putrescine | $y = (0.0243 \pm 0.0185) + (4.269 \pm 0.0284)x$ | 0.9999 | 0.014–1.4 |
| Cadaverine | $y = (0.0657 \pm 0.0340) + (3.445 \pm 0.0522)x$ | 0.9995 | 0.014–1.4 |
| Histamine | $y = (0.0627 \pm 0.0332) + (0.1102 \pm 0.0018)x$ | 0.9995 | 0.4–40 |
| Tyramine | $y = (0.0395 \pm 0.0332) + (0.5530 \pm 0.0089)x$ | 0.9995 | 0.08–8.0 |
| Spermidine | $y = (0.1040 \pm 0.0682) + (3.038 \pm 0.0488)x$ | 0.9995 | 0.03–3.0 |
| Spermine | $y = (0.0394 \pm 0.0453) + (2.069 \pm 0.0243)x$ | 0.9997 | 0.04–4.0 |

$y$, peak area ratio of each amine to internal standard (mean of three determinations); $x$, concentration in mg $l^{-1}$; $a$, intercept; $b$, slope; $SD_a$, $SD_b$, standard deviations of intercept and slope, respectively.

times. Satisfactory recovery values for all dansylamides were obtained (except for dansyl tryptamide), ranging from 73.8 to 114.0% for the spiked sample and from 85.0 to 107.0% for the amine-free sample. Dansyl tryptamide could not be determined due to the interfering peak of dansyl-hydrazine present only in real samples. When the concentration found was plotted against the concentration added for the spiked pooled sample, linear plots were obtained with correlation coefficients ranging from 0.9950 to 0.9998.

#### *3.2.4. Limits of detection and quantification*

The limits of detection were calculated from the amount of amines required to give a signal-to-noise ratio of 3 and were found to be 0.08 ng for methylamine and ethylamine, 0.1 ng for putrescine and cadaverine, 0.2 ng for tryptamine, 2-phenylethylamine, spermidine and spermine, 0.4 ng for tyramine, 0.15 ng for isoamylamine and 2.0 ng for histamine injected on-column. The limits of quantification were determined with a signal-to-noise ratio of 10 and were found to be 0.16 ng for methylamine and ethylamine, 0.28 ng for putrescine and cadaverine, 0.3 ng for isoamylamine, 0.4 ng for 2-phenylethylamine, 1.6 ng for tyramine, 0.6 ng for spermidine, 0.8 ng for tryptamine and spermine and 8.0 ng for histamine injected on-column.

### *3.3. Method application*

The method developed herein was applied to determine the content of amines in white-, red- and Retsina wines and Greek beers. All samples were of different origin and varieties. Retsina wines and beers were diluted once before the analysis. Five samples from each beverage category were analyzed six replicate times each. Ranges of amine-concentrations found in the samples are reported in Table 3. Fig. 3 shows the chromatograms of a white (A) and a red wine (B) and Fig. 4 the chromatogram of a Retsina wine (A) and a beer sample (B). These samples were treated and analyzed under the same conditions as the standard solutions. Due to the dansyl-hydrazine interfering peak, present in real samples, the determination of tryptamine was not possible.

## 4. Conclusions

Until recently, HPLC–UV has been the most widely used analytical approach to assay dansylated amines in wines. Although fluorescence detection has been used in a few cases in the past, to measure some biogenic amines in biological fluids or tissues, it had never been applied to alcoholic beverages

Table 3
Precision of determination in six replicate analyses of four different categories of Greek alcoholic beverages

| Amine | | White wines | Red wines | Retsina wines | Beers |
|---|---|---|---|---|---|
| Methylamine | Mean (mg $l^{-1}$)[a] | 0.513–0.903 | 0.588–1.503 | 0.022–0.220 | 0.030–0.216 |
| | SD[a] | 0.003–0.022 | 0.005–0.073 | 0.001–0.002 | 0.002–0.008 |
| | RSD (%)[a] | 0.3–4.3 | 0.6–7.7 | 0.4–3.6 | 0.9–6.7 |
| Ethylamine | Mean (mg $l^{-1}$)[a] | 0.537–2.162 | 0.544–2.639 | 0.152–0.394 | 0.189–0.893 |
| | SD[a] | 0.008–0.148 | 0.012–0.064 | 0.002–0.009 | 0.001–0.076 |
| | RSD (%)[a] | 1.1–6.8 | 0.5–5.9 | 0.6–3.9 | 0.5–8.5 |
| 2-Phenylethylamine | Mean (mg $l^{-1}$)[a] | 0.127–0.154 | 0.367–0.720 | NQ | – |
| | SD[a] | 0.002–0.006 | 0.008–0.011 | – | – |
| | RSD (%)[a] | 1.3–4.7 | 1.1–3.0 | – | – |
| Isoamylamine | Mean (mg $l^{-1}$)[a] | 0.480–0.974 | 0.430–1.244 | 0.112–0.331 | 0.048–3.179 |
| | SD[a] | 0.008–0.023 | 0.005–0.019 | 0.001–0.005 | 0.002–0.055 |
| | RSD (%)[a] | 1.4–4.5 | 0.8–2.2 | 0.4–2.7 | 1.3–4.2 |
| Putrescine | Mean (mg $l^{-1}$)[a] | 0.528–2.539 | 0.900–3.148 | 0.294–0.986 | 3.210–5.228 |
| | SD[a] | 0.006–0.055 | 0.011–0.110 | 0.005–0.013 | 0.026–0.124 |
| | RSD (%)[a] | 0.7–2.3 | 0.5–3.5 | 1.1–1.9 | 0.5–3.5 |
| Cadaverine | Mean (mg $l^{-1}$)[a] | 0.118–0.208 | 0.037–0.528 | 0.022–0.136 | 0.262–2.099 |
| | SD[a] | 0.004–0.017 | 0.001–0.013 | 0.001–0.008 | 0.006–0.061 |
| | RSD (%)[a] | 3.4–13.2 | 0.8–5.4 | 1.6–9.1 | 1.1–7.3 |
| Histamine | Mean (mg $l^{-1}$)[a] | 0.250–0.989 | 0.276–2.626 | 0.118–0.491 | 0.572–2.304 |
| | SD[a] | 0.006–0.015 | 0.007–0.044 | 0.007–0.015 | 0.009–0.062 |
| | RSD (%)[a] | 0.7–2.4 | 0.7–4.0 | 3.0–5.9 | 1.0–2.9 |
| Tyramine | Mean (mg $l^{-1}$)[a] | 0–1.294 | 0.524–1.583 | 0.255–0.383 | 1.511–6.056 |
| | SD[a] | 0.010 | 0.014–0.016 | 0.001–0.008 | 0.007–0.335 |
| | RSD (%)[a] | 0.8 | 0.9–3.1 | 0.3–3.1 | 0.2–5.5 |
| Spermidine | Mean (mg $l^{-1}$)[a] | 0–0.136 | 0.137–0.344 | 0.126–0.231 | 1.196–4.454 |
| | SD[a] | 0.003–0.008 | 0.003–0.014 | 0.0005–0.006 | 0.008–0.045 |
| | RSD (%)[a] | 5.9–6.1 | 2.2–4.1 | 0.4–2.8 | 0.3–3.0 |
| Spermine | Mean (mg $l^{-1}$)[a] | NQ | 0.014–0.209 | – | 0.202–1.409 |
| | SD[a] | – | 0.001–0.006 | – | 0.006–0.034 |
| | RSD (%)[a] | – | 2.9–4.3 | – | 0.7–3.0 |

[a] Range of values for five samples in each sample category.

before. The method presented herein is the first HPLC–fluorescence method developed to permit simultaneous quantification of some of the most oenologically important biogenic amines in wines and beers.

Likewise, although a couple of studies have been reported on the identification of dansylated amines in biological fluids by LC–MS, analytical data from a HPLC–DAD–APCI-MS study of dansylated amines, in alcoholic beverages, is presented here for the first time in the literature.

The method combines an efficient sample pretreatment using PVP and a post-derivatization clean-up step by means of SPE using $C_{18}$ cartridges. It is suitable for the simultaneous quantification of 10 biogenic amines (methylamine, ethylamine, 2-phenylethylamine, isoamylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine), in complex matrices such as wines and beers. The good linearity, the satisfactory recovery results for all amines and the high levels of sensitivity and precision recommend the use of the proposed method

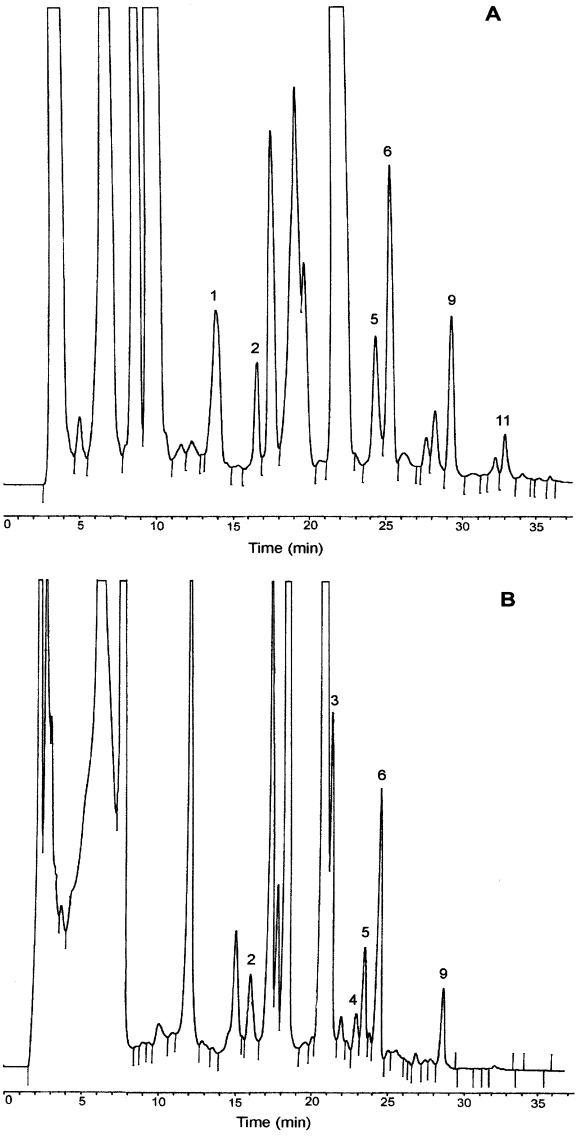


Fig. 3. RP-HPLC chromatograms of (A) white wine; peaks: 1, methylamine (0.119 mg $l^{-1}$); 2, ethylamine (0.082 mg $l^{-1}$); 5, isoamylamine (0.145 mg $l^{-1}$); 6, putrescine (0.343 mg $l^{-1}$); 9, internal standard (0.843 mg $l^{-1}$); 11, spermidine (0.059 mg $l^{-1}$); attenuation = 512 and (B) red wine; peaks: 2, ethylamine (0.098 mg $l^{-1}$); 4, 2-phenylethylamine (0.178 mg $l^{-1}$); 5, isoamylamine (0.174 mg $l^{-1}$); 6, putrescine (0.432 mg $l^{-1}$); 9, internal standard (0.843 mg $l^{-1}$); attenuation = 1024. Chart speed = 0.5 cm $min^{-1}$; $\lambda_{ex}$ = 320 nm, $\lambda_{em}$ = 523 nm; column, Inertsil ODS-3 (250 × 4 mm I.D., 5 μm); mobile phase, acetonitrile (40–80% from 0–25 min, 80–100% from 25–30 min, 100–40% from 30–35 min)–water; injection solvent, acetonitrile; injection volume, 20 μl.

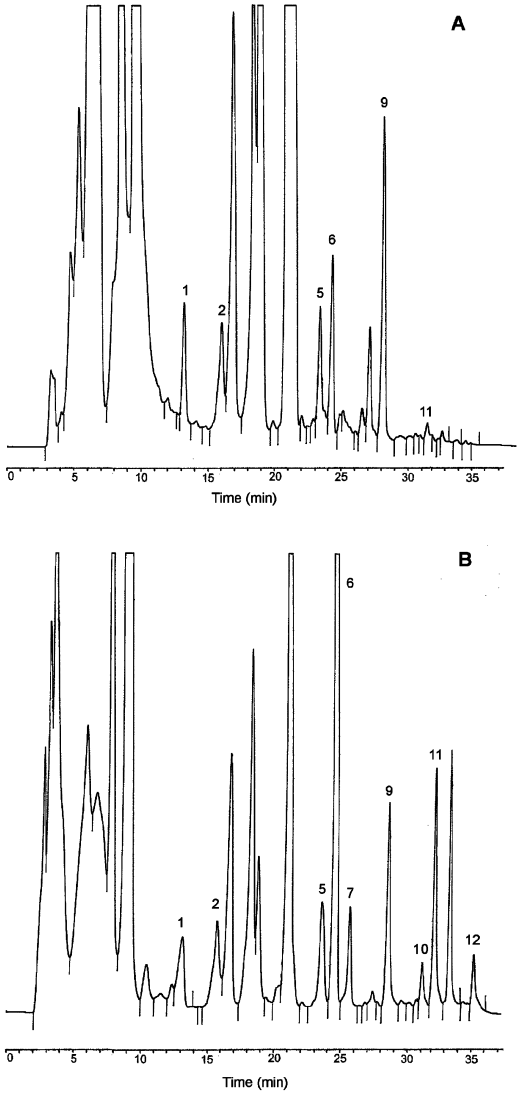


Fig. 4. RP-HPLC chromatograms of (A) Retsina wine; peaks: 1, methylamine (0.028 mg $l^{-1}$); 2, ethylamine (0.022 mg $l^{-1}$); 5, isoamylamine (0.043 mg $l^{-1}$); 6, putrescine (0.055 mg $l^{-1}$); 9, internal standard (0.843 mg $l^{-1}$); 11, spermidine (not quantified); attenuation = 128 and (B) beer; peaks: 1, methylamine (0.085 mg $l^{-1}$); 2, ethylamine (0.098 mg $l^{-1}$); 5, isoamylamine (0.127 mg $l^{-1}$); 6, putrescine (0.470 mg $l^{-1}$); 7, cadaverine (0.110 mg $l^{-1}$); 9, internal standard (0.843 mg $l^{-1}$); 10, tyramine (0.390 mg $l^{-1}$); 11, spermidine (0.269 mg $l^{-1}$); 12, spermine (0.098 mg $l^{-1}$); attenuation = 512. Chart speed = 0.5 cm $min^{-1}$; $\lambda_{ex}$ = 320 nm, $\lambda_{em}$ = 523 nm; column, Inertsil ODS-3 (250 × 4 mm I.D., 5 μm); mobile phase: acetonitrile (40–80% from 0–25 min, 80–100% from 25–30 min, 100–40% from 30–35 min)–water; injection solvent, acetonitrile; injection volume, 20 μl.

whenever the need for results of high analytical quality is warranted.

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