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Reverse phase HPLC/DAD determination of biogenic amines as dansyl derivatives in experimental red wines

G. mo Dugo *, F. Vilasi, G.L. La Torre, T.M. Pellicanò

Department of Organic and Biological Chemistry, University of Messina, Salita Sperone, 31-98166 Messina, Italy

Abstract

An accurate, rapid and very sensitive high-performance liquid chromatographic (HPLC) method for the determination of dansyl derivative of 11 biogenic amines, in experimental red wine samples from the crop year 2000 produced by Sicilian and French viticultures, was developed. The method involved the derivatisation with dansyl chloride followed by liquid chromatography gradient elution analysis without any other sample pre-treatment. The chromatographic system was equipped with a reversed-phase C-18 column and a DAD detector. A mobile phase of acetonitrile/water was used. The levels of biogenic amines in the samples ranged from 0.10 to 0.80 μg/ml. The HPLC method developed, showed a good linearity, sensitivity and repeatability. Recovery test ranged from 85.0% to 98.6% while detection limit was 0.01 ng/ml for all the studied compounds.

Keywords: Biogenic amines; Dansyl chloride; HPLC; Wine

1. Introduction

Biogenic amines are nitrium organic compounds of different nature: aliphatic, aromatic and heterocyclic. They are produced in food and beverages by *lactic acid bacteria* during the process of fermentation for example in cheese, sausages, fish, beer and wine (Lonvaud-Funel, 2001), from amino acid decarboxylation. They are undesirable in all foods and beverages because of their toxicity.

At high a concentration, they may induce headaches, respiratory distress, heart palpitation, hyper- or hypotension (Romero, Gazquez, Bagur, & Sauchez-Vines, 2000). Biogenic amines are synthesised in several parts of *Vitis Vinifera*, including berries and leaves (Adams, Frankel, & Christensen, 1990).

In wine, some amino acids can be decarboxylated; as a result histamine, tyramine, putrescine, cadaverin are usually found. Their presence in wine, particularly red wines, can also be a consequence of malolactic fermentation or the action of yeasts in primary fermentation (Maga, 1978).

Histamine, tyramine and putrescine are the principal biogenic amines in wines. In most wines, biogenic amines levels are low after the alcoholic fermentation, and increase during malolactic fermentation. Recent studies demonstrated that the interaction between ethanol (a monoamine oxidase inhibitor) and amines, seems to be synergistic. This is important for wine consumers that are sensitive to such compounds (Romero et al., 2000). Even though biogenic amines are undesirable components, their presence in wine may be correlated to cultivar, region of provenience and enological techniques.

This class of compounds are difficult to analyse because of their structural diversity and lack of an easily detectable common chromophore. The usual approach, therefore, has been to derivatise free amines with an easily detectable label group.

Different chromatographic methods has been developed for the determination of biogenic amines in wines

* Corresponding author. Tel./fax: +39 90 676 5180.
E-mail address: dugogia@isengard.unime.it (G. mo Dugo).

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sample: gas-chromatographic (GC; GC–MS) and liquid-chromatographic (HPLC; LC–MS) (Busto, Guasch, & Borrull, 1995; Soleas, Carey, & Goldberg, 1999) with pre- and post-column derivatisation (Busto, Guasch, & Borrull, 1996, 1997; Moret & Conte, 1996).

The aim of this work is to optimise the extraction of biogenic amines, the derivatisation conditions and their determination in experimental red wine samples produced by Sicilian and French viticultures.

2. Experimental

2.1. Chemical reagents

Standard solution including: 1,5-diamine pentane (CAD), 1,4-diamine butane (PUT), histamine (HIST), tyramine (TYR), tryptamina (TRYP), phenylethylamine (PHE), 1,7-diamine pentane (DAP), dansyl chloride and Na_2CO_3 were obtained from Sigma–Aldrich; spermidine (SPD), spermine (SPM), *o*-metilhydrossilamine (MIA), ethanolamina (ETA) were supplied by Fluka. Acetonitrile and water of HPLC grade was Carlo Erba reagents.

2.2. Preparation of standard amines solution

A stock of standard solution was prepared by adding an accurately weighed amount of each amine (ca. 10 mg) to a 10 ml volumetric flask and brought to the mark with 0.1 M HCl. The standard solutions were stored at 4 °C until the use. A calibration straight for each dansylated amine was obtained by analyzing the standard solutions diluited at different concentrations. A standard mixture of biogenic amines was prepared by adding 0.1 ml of each amine stock solution in a 10 ml volumetric flask and making up to the mark with 0.1 M HCl. These solutions were stored at 4 °C until the use.

Dansyl chloride solution (10 mg/ml) was prepared dissolving 100 mg in 10 ml of acetone, it was stored at −4 °C until the use. A solution of $Na_2CO_3$was prepared for the derivatisation reaction.

2.3. Derivatisation reaction

The derivatisation reaction was carried out by adding 1.6 ml of dansyl chloride solution to 1.5 ml of amine solution and adjusted to pH 8.2, with Na_2CO_3solution. The mixture was heated in a water-bath for an hour at 40 °C. After the reaction, the acetone was removed in a slight stream of N_2. Then, the volume was made up to 1 ml with acetonitrile, and before HPLC analysis, a filtration through a 0.45 μm membrane millipore filter was made. The resulting sample was injected three times.

For wine samples dansylation reaction was performed as described earlier for standard solutions, using 1.5 ml of wine. The samples were filtered through a 0.45 μm membrane millipore filter, and 20 μL were inject into the HPLC system. To ensure that the dansylation reaction was completed, the peaks area of the diluted pure dansyl derivatives were compared with the theoretical areas taken from the calibration curves. Yields of above 97% were obtained.

2.4. HPLC/DAD analysis

The HPLC determination of dansyl derivatives of the biogenic amines, were performed with a Shimadzu liquid chromatography system coupled with a system controller SCL-10A-Vp; two pumps LC-10A-Vp; DAD detector SPD-M 10A Vp.; a degasser GT-154, Rheodyne injector with a 20 μL loop, and a column C-18 Supelco Discovery 150 × 2.1 mm, 5 μm, with a precolumn of the same material. The mobile phase was water (W) and acetonitrile (A). For a better separation a 0.4 ml/min flow rate and a gradient elution mode (Table 1) were used. The quantitative analysis was carried out by the external standard method. Using a wine sample spiked with the biogenic amines standard solutions, the retention time of each amine was confirmed. A wavelength of 254 nm was used. The HPLC chromatogram of the biogenic amines standard solution is given in Fig. 1.

2.5. Samples

Fifteen red wine samples produced in Sicily in the crop year 2000, were analysed. They were from experimental viticulture of ''Istituto Regionale della vite e del vino'' (Marsala, Italy) and grown in a level ground (200 m elevation). The samples were: Frappato, Nero d'Avola, Merlot, Cabernet Sauvignon, Syrah, Petit Verdot, Tannat, Aglianico, Cabernet-French (Monreale), Pinot Noir (Valle d'Olmo-Regaleali), Pinot Noir (Colline Siciliane-Rapitalà), Pinot Noir (S. Venerina–M. Etna), Nero d'Avola (Biesina–Marsala), Nerello Mascalese, Tempranillo.

The grapes were manually harvested at maturity and transported to the experimental wine-production centre (Cantina G. Dalmasso of Marsala – Istituto Regionale della Vite e del Vino). The procedure of experimental vinification was the same for all the variety grapes, but

Table 1
Gradient elution for biogenic amines analysis

Time (min)	% W	% A
0	65	35
6	35	65
10	20	80
15	10	90
20	65	35

W, H_2O; A, CH_3CN.

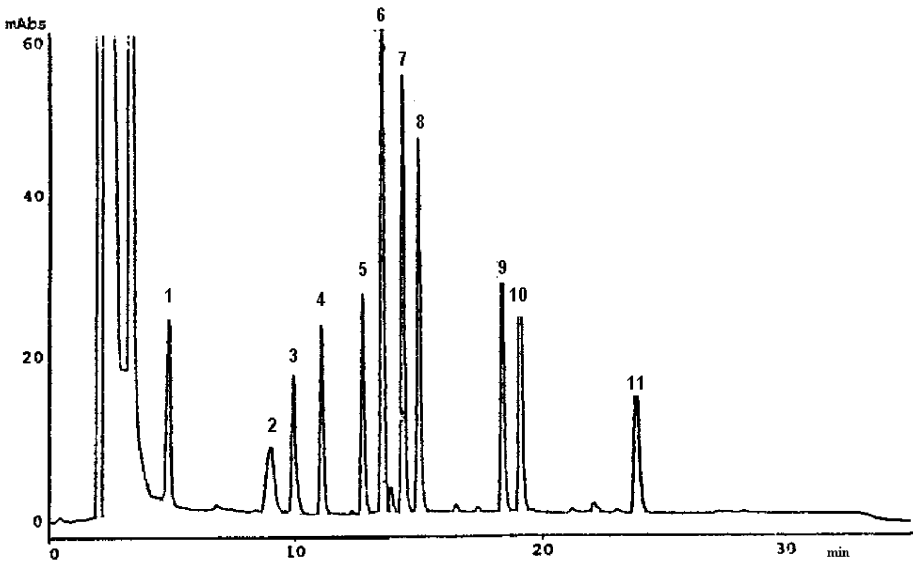

Fig. 1. HPLC chromatogram of the biogenic amines standard solution: (1) Tryptamine (**TRY**); (2) 2-phenylethylamine (**PHE**); (3) Putrescin (**PUT**); (4) Cadaverin (**CAD**); (5) 1,7-diamine heptane (**DAP**); (6) Histamine (**HIST**); (7) Tyramine (**TYR**); (8) Spermidine (**SPD**); (9) Spermine (**SPM**); (10) *o*-methyl hydroxyl-amine (**MIA**); (11) Ethanolamina (**ETA**).

the harvest-time varied according to the different variety grapes.

2.6. Red vinification

The grape skins and all solid materials were removed by filtration using a system of serial filters; afterwards, the must was spiked with SO_2 (5 g/hl), and selected yeasts (30 g/hl) and let to fermentate at 26–28 °C for 6–8 days, effecting three fullings a day. The wine was drawn from the vat and the grapes marc were crushed by hydraulic press. The malolactic fermentation, inducted by Leoconostoc oenos, was made with good outcome for all the samples except for Merlot and Syrah samples because of the higher alcoholic concentration. The lees were removed and the wine was spiked with SO_2 (5 g/hl). Subsequently, after 30 days, the wine was spiked again with SO_2 (5 g/hl) and bottled in dark bottles. All the samples were separately vinified following the protocol mentioned above and stored in the dark at 16 °C. Each sample was opened immediately prior to the analysis.

3. Results and discussion

3.1. Performances

The linearity was tested by analysing standard solutions at different concentrations. The calibration lines were obtained with good correlation coefficent for all the compounds (0.99). The sensibility test (detection limit) of the analytical method was calculated with a signal to noise ratio of four and corresponded to a concentration of 0.01 ng/ml.

Recoveries of the biogenic amines were executed by spiking the wine samples at different levels with known amounts of each amines; spiked and unspiked samples were analysed in triplicate.

Obtained results are shown in Table 2. The recoveries ranged from 85.0% to 98.6%.

Figs. 1 and 2 show a typical chromatogram of dansylated amines standards and a wine sample, obtained by an optimised chromatographic separation. All the analyte peaks were separated and could be identified by their retention time. Table 3 reports the biogenic amines content in the studied samples of wine.

The levels of biogenic amines ranged from 0.10 to 0.80 μg/ml.

Ethanolamina was found in all samples. Among Sicilian viticultures, the highest ethanolamina content was found in Nero d'Avola Biesina sample (0.30 μg/ml) followed by Nero d'Avola, Tempranillo and Nerello

Table 2
Performances of analytical method

	Recovery (%)[a]	Detection limit (μg/ml)[b]
1,5-Diaminepenthane (cadaverin)	95.2 ± 2.6	0.01
1,4-Diamine butane (putrescin)	86.3 ± 1.8	0.02
Histamine	96.7 ± 2.4	0.01
Tyramine	85.1 ± 1.4	0.01
Tryptamina	85.8 ± 1.7	0.02
2-Phenylethylamine	98.6 ± 2.6	0.01
1,7-diamine epthane	91.2 ± 2.3	0.01
Spermidine	87.5 ± 1.8	0.01
Spermine	88.3 ± 1.5	0.02
o-Metilhydrossilamine	87.4 ± 1.9	0.01
Ethanolamina	86.8 ± 2.2	0.01

[a] Average of three replicates.
[b] Four times the noise signal.

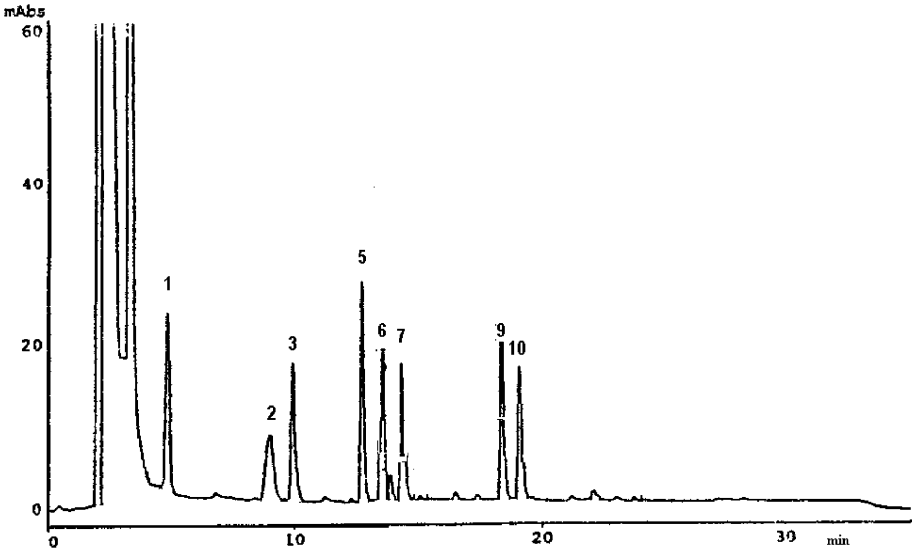

Fig. 2. HPLC chromatogram of wine sample.

Table 3
Biogenic amines content (μg/ml) in Sicilian wine samples

Sicilian wine samples	CAD	PUT	HIST	TYR	TRYP	PHE	DAP	SPD	SPM	MIA	ETA
Frappato	n.d.	0.2	0.1	0.1	0.3	n.d.	0.1	n.d.	n.d.	0.1	0.1
Nero d'Avola	0.2	0.6	0.1	0.2	0.2	0.3	0.1	n.d.	n.d.	0.1	0.2
Aglianico	0.1	0.5	0.3	0.2	0.3	0.1	0.2	n.d.	n.d.	0.1	0.1
Nero d'Avola (Biesina)	0.1	0.5	0.2	0.2	0.2	0.2	0.1	n.d.	n.d.	0.1	0.3
Nerello Mascalese	n.d.	0.4	0.1	0.1	0.1	0.3	0.1	n.d.	n.d.	0.1	0.2
Tempranillo	0.2	0.1	n.d.	n.d.	n.d.	0.1	n.d.	n.d.	n.d.	0.1	0.2

Mascalese wine samples (0.20 μg/ml). The lowest *ethanolamina* concentration was obtained in Frappato and Aglianico wine samples (0.10 μg/ml).

Among French viticultures, the highest *ethanolamina* level was found in Pinot Noir Valle d'Olmo Regaleali and Pinot Noir S. Venerina samples (0.80 μg/ml) followed by Pinot Noir Colline Siciliane (0.70 μg/ml); Syrah and Tannat samples had the lowest *ethanolamina* content (0.20 μg/ml).

All Sicilian wine samples showed a similar level of *o*-metilhydrossilamine. In French wine samples the highest *o*-metilhydrossilamine content was found in Cabernet Sauvignon sample (0.30 μg/ml); the lowest was found in Merlot sample (0.10 μg/ml) (see Table 4).

Spermine was not found in Sicilian, Syrah and Petit Verdot wine samples. Among French viticultures the highest *spermine* content was found in Merlot, French Cabernet, Pinot Noir Colline Siciliane and Pinot Noir S. Venerina samples (0.20 μg/ml).

S*permidine* was not found in Sicilian, Syrah and Petit Verdot wine samples. The highest *spermidine* level found was 0.20 μg/ml.

Among wine samples produced from sicilian viticultures the highest *1,7-diamine epthane* content was found

Table 4
Biogenic amines content (μg/ml) in French wine samples

French wine samples	CAD	PUT	HIST	TYR	TRYP	PHE	DAP	SPD	SPM	MIA	ETA
Merlot	0.2	0.3	n.d.	0.2	0.1	0.2	0.1	0.1	0.2	0.1	0.5
Cabernet Sauvignon	0.4	0.2	n.d.	0.4	0.3	0.1	n.d.	0.1	0.1	0.3	0.4
Syrah	0.3	0.4	0.2	0.1	0.1	0.1	n.d.	n.d.	n.d.	0.1	0.2
Petit Verdot	0.4	0.7	0.1	0.1	0.2	0.1	0.1	n.d.	n.d.	0.2	0.3
Tannat	0.2	0.2	0.1	0.3	0.1	0.2	0.1	0.1	0.1	0.2	0.2
French Cabernet Monreale	0.5	0.4	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.2	0.6
Pinot Noir Valle d'Olmo	0.5	0.5	0.6	0.2	0.3	0.6	0.1	0.2	0.1	0.1	0.8
Pinot Noir Colline Siciliane	0.4	0.3	0.4	0.2	0.1	0.5	0.2	0.2	0.2	0.2	0.7
Pinot Noir S. Venerina	0.4	0.5	0.8	0.3	0.3	0.5	n.d.	0.1	0.2	0.2	0.8

in Aglianico sample (0.20 μg/ml). Among French viticultures, the highest 1,7-diamine epthane level was found in Pinot Noir Colline Siciliane(0.20 μg/ml).

The highest *phenylethylamine* content, among Sicilian viticultures wine samples, was found in Nero d'Avola Biesina and Nerello Mascalese samples (0.30 μg/ml). *Phenylethylamine* was not found in Frappato wine. For French viticultures samples, the highest *phenylethylamine* level was found in Pinot Noir Valle d'Olmo Regaleali (0.60 μg/ml) followed by Pinot Noir Colline Siciliane and Pinot Noir S. Venerina (0.70 μg/ml).

Tryptamina was found in all French wine samples. The highest content was obtained in Cabernet Sauvignon, Pinot Noir Valle d'Olmo Regaleali and Pinot Noir S. Venerina wines (0.30 μg/ml).

Among Italian viticultures, the highest tryptamina content was obtained in Frappato and Aglianico samples; it was not found in sample of Tempranillo.

The highest *tyramine* level was found in Cabernet Sauvignon wine sample (0.40 μg/ml).

Among wine samples produced from sicilian viticultures the highest tyramine content was found in Nero d'Avola and Aglianico samples (0.20 μg/ml); it was not found in Tempranillo wine.

Histamine was not found in Merlot, Cabernet Sauvignon and Tempranillo wine samples. The highest *histamine* content was found in Pinot Noir S. Venerina sample (0.80 μg/ml).

Among Italian viticultures, the histamine highest content was found in Aglianico wine (0.30 μg/ml) followed by Frappato, Nero d'Avola and Nerello Mascalese.

Putrescin was found in all samples; its highest content was 0.70 μg/ml in Petit Verdot, for French wines, and 0.60 μg/ml in Nero d'Avola, for Sicilian wines.

Cadaverin was not found in Frappato and Nerello Mascalese wine samples. French wine samples show a cadaverin content higher than Sicilian ones; its ranged from 0.20 (in Merlot sample) to 0.50 μg/ml (in French-Cabernet and Pinot Noir Valle d'Olmo Regaleali).

Among Italian viticultures the highest cadaverin value was found in Tempranillo and Nero d'Avola samples (0.20 μg/ml).

Among French viticultures, analysed in this work, all biogenic amines were found in French-Cabernet, Pinot Noir Valle d'Olmo Regaleali, Pinot Noir Colline Siciliane and Tannat wine samples.

In Pinot Noir S. Venerina wine, of all the studied biogenic amines, only 1,7-diamine epthane was not found.

Among Sicilian grapes samples all biogenic amines were found in Nero d'Avola and Aglianico samples, while cadaverin was not found in Nerello Mascalese wine sample.

4. Conclusions

The method described in this paper allows the simultaneous determination of 11 biogenic amines present in wine samples. The HPLC method described besides being sensitive and reproducible does not require any sample pre-treatment but the derivatisation reaction with dansyl chloride.

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References

Adams, D. O., Frankel, K. E., & Christensen, L. P. (1990). Elevated putrescine levels in grapevine leaves that display symptoms of potassium deficiency. *American Journal of Enology and Viticulture, 41*, 121–125.

Busto, O., Guasch, J., & Borrull, F. (1995). Improvement of a solid-phase extraction method for determining biogenic amines in wines. *Journal of Chromatography A, 718*, 309–317.

Busto, O., Guasch, J., & Borrull, F. (1996). Determination of biogenic amines in wine after precolumn derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidy carbamate. *Journal of Chromatography A, 737*, 205–213.

Busto, O. M., Guasch, J., & Borrull, F. (1997). Determination of biogenic amines in wines by high-performance liquid chromatography with on column fluorescence derivatization. *Journal of Chromatography A, 757*, 311–318.

Lonvaud-Funel, A. (2001). Biogenic amines in wines: role of lactic acid bacteria. *FEMS Microbiology Letters, 199*, 9–13.

Maga, J. A. (1978). Amines in food. *CRC Critical Reviews in Food Science and Nutrition, 10*, 373–403.

Moret, S., & Conte, L. S. (1996). High performance liquid chromatographic evaluation of biogenic amines in foods an analysis of different methods of sample preparation in relation to food characteristics. *Journal of Chromatography A, 729*, 363–369.

Romero, R., Gazquez, D., Bagur, M. G., & Sauchez-Vines, M. (2000). Optimization of chromatographic parameters for the determination of biogenic amines in wines by reversed-phase high-performance liquid chromatography. *Journal of Chromatography A, 871*, 75–83.

Soleas, G. J., Carey, M., & Goldberg, D. M. (1999). Method development and cultivar-related differences of nine biogenic amines in Ontario wines. *Food Chemistry, 64*, 49–58.