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Analysis of biogenic amines in wines by salting-out assisted liquid–liquid extraction and high-performance liquid chromatography with fluorimetric detection

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ABSTRACT

Biogenic amines are nitrogenous organic compounds of low molecular weight that are either formed or metabolized in cells of living organisms and can be found in several food products, being produced mainly by amino acid decarboxylation. When ingested in high concentrations they can induce several health problems in humans. In alcoholic beverages, and especially in wine, they are formed during the vinification process as a result of the action of microorganisms.

In this work it is proposed a new methodology for the determination of biogenic amines in wines, which includes a sample preparation approach based on salting-out assisted liquid–liquid extraction, the use of dansyl chloride for the derivatization and chromatographic separation by high-performance liquid chromatography with fluorimetric detection. The salting-out effect is used to promote phase separation between water and a water-miscible organic solvent, while improving the extraction of organic or inorganic species. Several extraction parameters were optimized, such as the dansyl chloride concentration, pH and the effects caused by the order in which the extraction and derivatization were performed. Extraction of amines, and consequent detection, depends on the presence of dansyl chloride in solution prior to extraction. The results showed the possibility to simultaneously perform the extraction and the derivatization, making sample preparation easier and less time-consuming. The methodology was successfully applied to the determination of biogenic amines in five wines (white, red and *rosé*). This method has the potential to be a good alternative to existing methods since it is cheaper, easier and simplifies the sample preparation step.

1. Introduction

Biogenic amines (BA) are a very important group of organic compounds that are commonly found in a wide variety of foods and beverages such as cheese, wine, beer, fishery products and meat [1–6]. They are generally formed by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones [7,8]. BA are commonly divided in three groups, according to their chemical structure: aliphatic (methylamine (MA), dimethylamine (DMA), ethylamine (EA), putrescine (PUT), cadaverine (CAD), isopentylamine (ISO), spermidine (SPD), spermine); aromatic (tyramine, phenylethylamine (PHE)) and heterocyclic (histamine (HIS), tryptamine). BA are food quality markers associated to the degree of degradation and fermentation in foods. These compounds can either be beneficial and harmful to human health, as in low concentrations they can be easily tolerated by the human body and actually help regulate several physiological functions; on the other hand at high concentrations they can induce neurological disorders, headaches, hypo- or hypertension, nausea, cardiac palpitations, renal intoxication among others [7,9,10].

In wine, BA are formed throughout the vinification process due to the action of various microorganisms during alcoholic and malolactic fermentations. The malolactic fermentation, which is catalyzed by the lactic acid bacteria (LAB), is considered one of the most important steps during winemaking and it is also when most BA are produced. This production is dependent on the presence of LAB, the availability of the precursor amino acids, the duration of the initial fermentation phase, the levels of sulfur dioxide, the pH and the period of contact between must and grape skin [11,12]. For those reasons, red wines have generally higher contents of BA than white wines. As for beer, the formation and presence of BA is related to the action of several yeast strains, LAB and the quality of the precursor raw materials [13]. The presence of BA in alcoholic beverages has received continuous attention since it was found that the interaction between ethanol and BA might be synergistic, as ethanol can directly or indirectly inhibit amine oxidases (monoamine oxidase) [14,15].

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The simultaneous and rapid determination of BA in wines requires inexpensive, reliable and simple methodologies. This is not an easy task, due to their low levels in complex matrices such as wine. In the literature we found several methodologies used for the determination of biogenic amines that are based on capillary electrophoresis (CE), gas chromatography (GC) and most commonly high-performance liquid chromatography (HPLC), in combination with several detectors [16–18]. For HPLC analysis using spectrophotometric detectors the determination of BA needs a derivatization because most BA lack of a chromophore. This derivatization, that occurs via amino groups with different tagging reagents, helps to improve selectivity and sensitivity of the methodology. The most common derivatizing reagents used are *o*-phthalaldehyde (OPA) [19,20], dansyl chloride (DNS-Cl) [21–23], phenyl isothiocyanate (PITC) [3], 4-chloro-3,5-dinitrobenzotrifluoride (CNBF) [24] and 1,2-naphthoquinone-4-sulfonate (NQS) [25]. DNS-Cl is probably the most used because derivatives are more stable than those obtained with other reagents and can react with primary and secondary amines [21] (Fig. 1).

Regardless of the derivatization reagent used, to obtain optimal analysis conditions, a clean-up or concentration step is required for the determination of biogenic amines in complex matrices, such as wine and beer. Many analytical methods reported in the literature, such as liquid–liquid extraction (LLE), include these steps, but usually involve the use of large quantities of hazardous solvents [26,27] while being a time-consuming step. To overcome this, other techniques like solid-phase extraction (SPE) [28], solid-phase microextraction (SPME) [29] or dispersive liquid–liquid microextraction (DLLME) [30] have been used.

In this work we propose a simple and quick methodology for the determination of nine BA found in wines, using a straightforward sample preparation procedure based on a salting-out assisted liquid–liquid extraction (SALLE) and derivatization with DNS-Cl for the analysis by high-performance liquid chromatography with fluorimetric detection (HPLC-FLD). This methodology uses the salting-out effect as the basis for a homogeneous liquid–liquid extraction, and besides promoting phase separation the addition of salt improves the extraction of molecular species to the organic phase. This methodology, that already proved to be valuable in the determination of α-dicarbonyl compounds [31], is a simple, quick and reliable way to determine BA in wine samples.

2. Materials and methods

2.1. Chemicals and samples

All the reagents used in this work, except when mentioned otherwise, were of analytical grade and were used without further purification. Ultrapure water with a resistivity not lower than 18.2 MΩ cm (Direct-Q® 3UV water purification system) was used for all chemical analyses and glassware washing. HPLC grade acetonitrile was from Fisher (USA). All chromatographic eluents were filtered through a Nylon filter of 0.45 μm pore size (Whatman, USA) prior to use. DNS-Cl and all BA were from Sigma-Aldrich (Steinheim, Germany).

Individual stock standard solutions (1 g L^{-1}) of each biogenic amine were prepared by diluting or dissolving the appropriate amount of the commercial reagent in high-purity water and stored at 4 °C. The working solutions were daily prepared by dilution of the stock solutions (1 g L^{-1}). Phosphate buffer 0.2 mol L^{-1} (pH 12) was prepared by dissolving the appropriate amount of disodium hydrogen phosphate in water, and the pH was adjusted with sodium hydroxide (4 mol L^{-1}); acetate buffer 0.01 mol L^{-1} (pH 4.0) was prepared with sodium acetate and acetic acid. These reagents were from Merck.

The DNS-Cl solution (3.5 mg mL^{-1}) was prepared by dissolving 80 mg of the reagent in 25 mL of acetonitrile; it was stored at 4 °C until use.

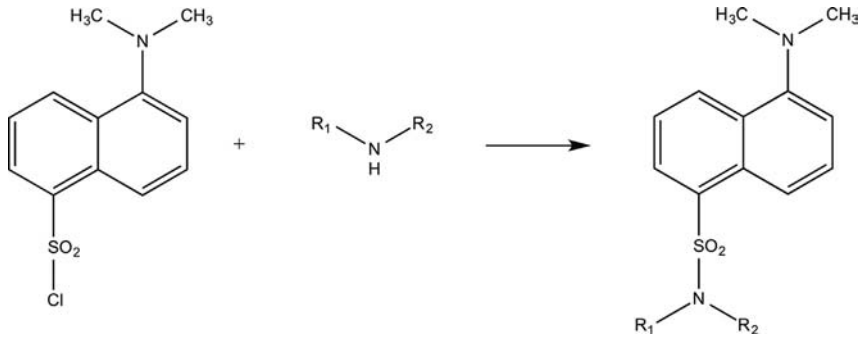

Fig. 1. Representation of the reaction between biogenic amines and dansyl chloride.

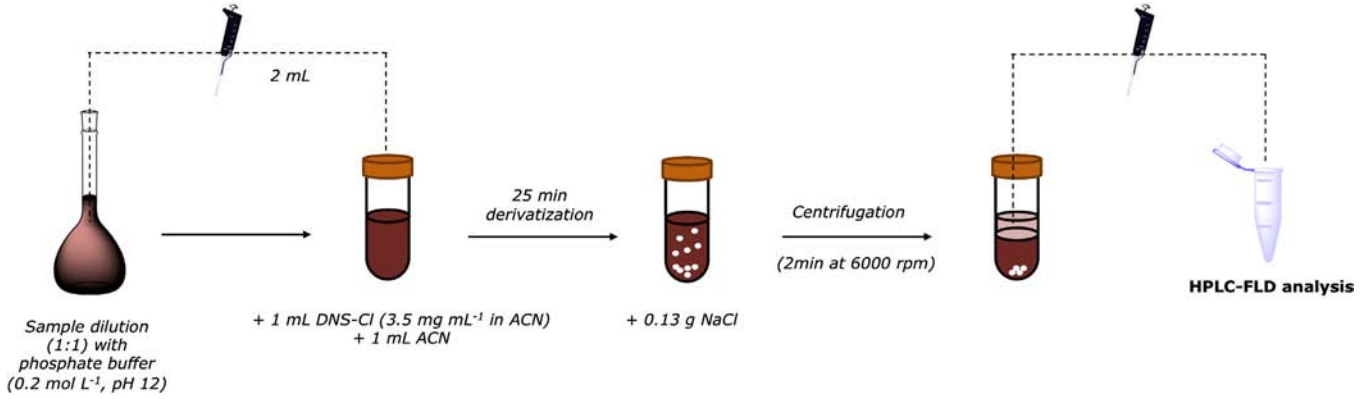

Fig. 2. Scheme of the experimental procedure proposed for the extraction of BA.

The wine samples used in this work were purchased in local supermarkets.

2.2. Adopted extraction procedure

The experimental SALLE procedure followed was based on the one described previously by Valente et al. [31] with appropriate modifications. 2 mL of a wine sample were mixed with 1 mL of acetonitrile and 1 mL of DNS-Cl obtaining a ratio of 1:1 (v/v) between the aqueous and the organic phase. To ensure a correct derivatization step, wine samples were previously mixed with phosphate buffer 0.2 mol L^{-1} (pH 12.0) in a ratio of 1:1 (v/v), to increase the pH of the medium. After 25 min of derivatization, 0.13 g of NaCl (1 mol L^{-1} in the solution) were weighed and added to the mixture; the tubes were centrifuged at 6000 rpm for 2 min for phase separation. The upper phase was collected for HPLC-FLD analysis. The procedure is summarized in Fig. 2.

2.3. HLPC-FLD analysis

The HPLC system (Jasco Corporation, Japan) was composed by a low-pressure quaternary gradient unit with an in-line degasser (model PU-2089 Plus), a fluorimetric detector (model FP-2020 Plus) and a manual injector (Rheodyne, model 7725i). The system control and data analysis was made with a Jasco ChromPass Chromatography Data software (version 1.7.403.1). The separation of the DNS-Cl derivatives was achieved on a *Phenomenex* Gemini C_{18} column (250 mm × 4.60 mm, 5 μm particle size) and a guard column in gradient mode. Mobile phase consisted of acetonitrile and acetate buffer (0.01 mol L^{-1}, pH 4.0). In the initial 8 min acetonitrile increased linearly from 35% to 65%; in the next 17 min acetonitrile increased to 80% and in the following 10 min to 100%; from 35 to 40 min the mobile phase composition returned to 35% acetonitrile; an additional 5 min step was used for conditioning before the next injection. The flow rate was 1.0 mL min^{-1}; the injection volume was 20 μL; the excitation wavelength (λ_{ex}) used for DNS-Cl derivatives detection was 320 nm and the emission wavelength (λ_{em}) was set to 523 nm.

3. Results and discussion

BA lacks of chromophore properties so for a suitable detection a derivatization reaction had to be performed. DNS-Cl reacts with amines to form stable covalently bound sulfonamides (DNS-Cl derivatives), which have fluorescent properties (Fig. 1).

Some parameters of the SALLE extraction, such as the effect of the organic solvent, type and salt concentration and the volume of the organic solvent were previously evaluated by Valente et al. [31] and were considered for the present work.

Besides the optimization of the chromatographic conditions, several extraction parameters had to be optimized, such as the influence of extraction time, the influence of pH and DNS-Cl concentration.

The extraction and the derivatization steps are generally considered independently since, in most cases, the extraction is performed first, followed by derivatization of the analytes. Since DNS-Cl was prepared in acetonitrile, which is also the organic solvent used for the extraction, it was studied the incorporation of the reagent on the extraction procedure. With that in mind three different extraction/derivatization procedures were tested: (A) derivatization followed by extraction; (B) simultaneous derivatization and extraction and (C) extraction followed by derivatization.

In the first studied procedure A, 2 mL of standard solution were mixed with 1 mL of DNS-Cl and 1 mL of acetonitrile and let to react during a defined period of time (0, 20, 40, 60 and 80 min). At the end of the derivatization period the appropriate amount of NaCl was added to promote phase separation and extraction of the analytes. As can be seen in Fig. 3A, for the three BA used in this study (PUT, CAD and SPD), there is an increase of the chromatographic signal from 0 to 20 min followed by a small increase and stabilization until 80 min of derivatization time. At this point a derivatization time between 20 and 30 min would be adequate and that the extraction achieved good recoveries.

In procedure B the derivatization and the extraction were performed simultaneously by mixing in the same tube the standard solution, DNS-Cl, acetonitrile and NaCl. After shaking, phase separation occurred and the derivatization was accomplished in the same time intervals of the previous situation. The results (Fig. 3B) showed that it is possible to perform simultaneously the derivatization and phase separation, despite the slightly smaller recoveries obtained when compared to procedure A. The possibility to perform both extraction and derivatization in one single step makes sample preparation for BA analyses simpler and less time-consuming. It should be noted that similar to the observed for procedure A, an increase on derivatization time above 20 min does not cause significant changes on BA signals.

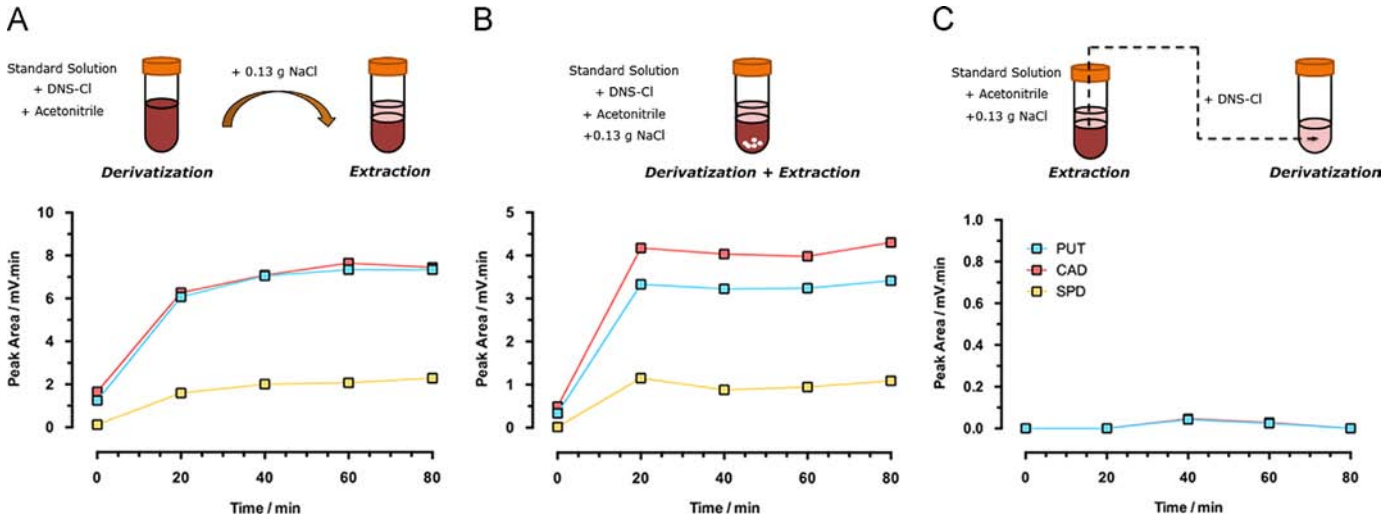

Fig. 3. Peak area as a function of the derivatization time (0, 20, 40, 60 and 80 min) for the extraction of BA from a standard solution (0.5 mg L^{-1}) using different extraction protocols: (A) derivatization followed by extraction; (B) simultaneous derivatization and extraction; and (C) extraction of the amines followed by derivatization. Model solutions of three BA, Putrescine (PUT), Cadaverine (CAD) and Spermidine (SPD), were used in this study.

In the third tested procedure (C) extraction of analytes was firstly carried out with acetonitrile and after phase separation the extract was collected and the derivatization was performed (Fig. 3C). Contrary to what happened in the previous situations (A and B) in this case the recoveries of BA are residual. This behavior is common to all three BA analyzed and is independent of the derivatization time. This result suggests that the extraction of BA to the organic phase is greatly influenced by the presence of the derivatization agent in solution in order to convert the amines into less polar species, the DNS-Cl derivatives.

Since procedure A had the best recoveries between the three and since, compared to procedure B, only requires an additional step of salt addition, it was the adopted procedure.

3.1. Effect of derivatization reagent concentration

There is no general agreement in literature about the DNS-Cl concentration that should be used for BA determination. This concentration varies from work to work and is dependent on the solvent in which it is prepared and the sample being analyzed. For DNS-Cl, one can find in literature concentrations typically higher than 7 mg mL^{-1} [22,28]. The concentration used has to be in excess in order to derivatize BA even if large amounts of other compounds that could potentially interact with the derivatization reagent are present in samples. It should also be noted that in most cases DNS-Cl is prepared in acetone. However, in our procedure acetonitrile is used instead with success. Due to this adaptation in the derivatizing process we found necessary to study this experimental parameter in order to find the optimal DNS-Cl concentration.

Following the procedure shown in Fig. 2, and with a derivatization time of 25 min, extractions were carried with standard solutions containing 0.5 mg L^{-1} of three BA (PUT, CAD, and SPD) using increasing DNS-Cl concentrations (from 0 to 10.0 mg mL^{-1}). The effect of DNS-Cl concentration on the analytes recoveries is shown in Fig. 4. It can be seen that the extraction recovery is strongly influenced by DNS-Cl concentration in the mixture. Using a DNS-Cl concentration between 1.0 and 2.5 mg mL^{-1} in the organic phase the maximum in chromatographic peaks is attained for the three BA. Therefore, a concentration of 1.8 mg mL^{-1} was chosen, corresponding to the addition of 1 mL DNS-Cl (3.5 mg mL^{-1}) to the mixture. For concentrations higher than 2.5 mg mL^{-1} the chromatographic peaks of the three BA decrease abruptly and for concentrations above 5.0 mg mL^{-1} the detection of BA is inhibited. According to these results the DNS-Cl concentration is a very important experimental parameter and should be strictly controlled.

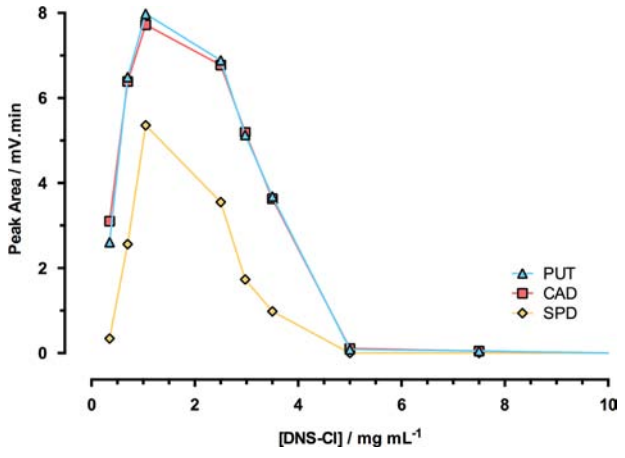

Fig. 4. Peak area as a function of DNS-Cl concentration for the extraction of model solutions of three BA (PUT, CAD and SPD) from a standard solution (0.5 mg L^{-1}, each) with increasing DNS-Cl concentration. Derivatization time: 25 min.

3.2. Influence of pH

pH control of sample solution, during the derivatization step, is of utmost importance since it greatly influences the partition equilibrium of amines derivatives between the two phases. This can also affect the extension of the derivatization reaction, and, as a rule of thumb, the sample pH has to be above the pKa of amines so that they could be deprotonated. This is usually accomplished by adding an alkaline solution in order to raise pH to values above 8. Since pH can easily influence the extraction and determination of BA, we decided to take a further study on this parameter. Several pH

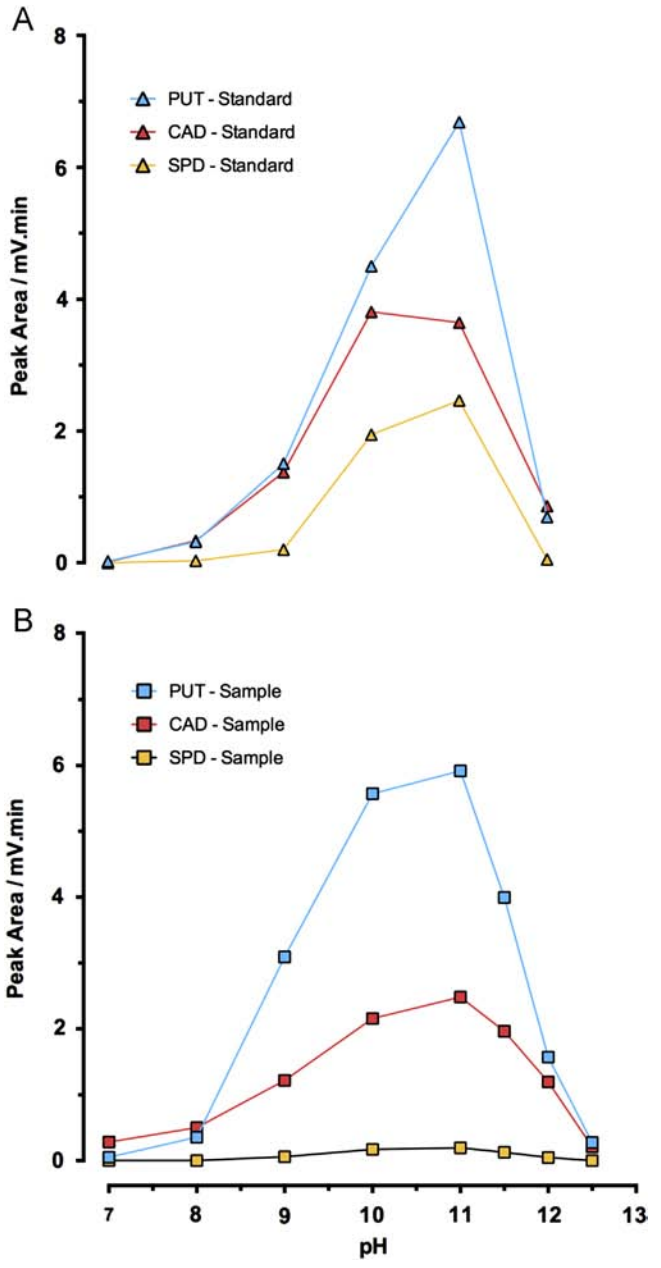

Fig. 5. Peak area for the extraction of model solutions of three BA from a (A) standard solution (0.5 mg L^{-1}) and (B) from a spiked sample with different pH values. Standards and samples were prepared and diluted (1:1, v/v), respectively, with buffer solution (0.2 mol L^{-1}) prior to the extraction. Procedure A was used in this study.

values (between 7 and 12.5) were tested both in standard and sample solutions, as represented in Fig. 5. For pH below 8, there is hardly any detection of BA, which is in agreement with the previous assumption; for pH 9–11 there is an increase in BA signal, reaching its maximum at pH 11. From this point forward higher pH values would cause smaller recoveries of BA and poor detection at pH above 12. This study reveals the importance of accurately control and adjust sample's pH before the extraction procedure because it greatly influences the recovery of amines. Although the optimum pH is dependent on each amine, due to their different chemical structure, we believe that pH 11 is the most adequate to run the procedure since it gives the best compromise between sensitivity and recovery for the amines used in the optimization process.

3.3. Validation of the method

In order to verify the linearity of the response of the BA derivatives under study, standard solutions containing all amines (concentrations ranging from 0.03 to 1.7 mg L^{-1}) were prepared and submitted to the adopted procedure. Linear least-squares regression was used to calculate the slope, intercept and determination coefficient (r^2). Determination coefficients between 0.977 and 0.999 were obtained; this evidences a good linearity for all BA (Table 1).

Detection (LD) and quantification (LQ) limits of the proposed methodology were calculated as three and ten times the standard deviation of the intercept/slope of the calibration curves. The LD and LQ ranged from 0.003 to 0.22 μg L^{-1} and 0.09 to 0.74 μg L^{-1}, respectively.

The repeatability of the methodology was obtained by analyzing in the same day five replicates of a spiked sample ($n=5$).

The influence of the sample matrix on the extraction was determined by analyzing spiked samples at four concentration levels, which were chosen according to the concentration of the analytes in the sample, in order to give BA signal increase between 50% and 200% of the area of the non-spiked sample. Since the slopes of the standard addition curves were statistically different (Student's *t*-test, 99% confidence interval) from those of the calibration curves, the standard additions method was used for the quantification of BA in the wine samples.

3.4. Analysis of wine samples

The proposed methodology was applied to the determination of BA content in five wines. All wines were of different origins and varieties and were constituted by three white wines, one red wine and one *rosé* wine (Table 2). The extraction and derivatization were performed according to the procedure previously described. Wine samples were diluted 1:1 (v/v) with phosphate buffer (0.2 mol L^{-1}, pH 12.0) to control pH and decrease their ethanol content. For HIS, we performed a higher dilution in order to account for higher concentrations found in samples. Some variability within samples existed; red wine had the highest amounts of BA, as expected considering its vinification process. Regarding the nine BA found in the wine samples, HIS and PUT showed the highest concentrations, which is in agreement with what is generally found in the literature for wines.

Regarding the stability of the amines derivatives, after a 12 h time-span we have not noticed a significant loss of the signal intensity.

It should be highlighted that difficulties were experienced while analyzing this set of BA in wines. In fact, in the case of spermidine, which is a non-volatile amine generally found in low concentrations or not even detected in wines [23,32], we were able to determine this amine in the aqueous model solutions following the optimized protocol; however, in wine samples we were not able to detect it, even when standard additions were done to the samples. This may be connected with the extraction

Table 1
Figures of merit of the proposed methodology.

Amine	Calibration curve equation $y=(a\pm SD)x+(b\pm SD)$[a]	(r^2)[b]	LD (mg L^{-1})	LQ (mg L^{-1})	RSD[c] (%)
HIS	$y=(0.26\pm0.023)x+(-0.12\pm0.025)$	0.977	0.22	0.74	16.8
MET	$y=(7.21\pm0.22)x+(0.059\pm0.029)$	0.997	0.012	0.039	8.4
ETA	$y=(5.91\pm0.12)x+(0.0081\pm0.015)$	0.999	0.010	0.030	6.1
DMA	$y=(4.74\pm0.26)x+(0.48\pm0.032)$	0.991	0.023	0.077	25.8
2-PHE	$y=(2.42\pm0.093)x+(-0.011\pm0.013)$	0.996	0.017	0.058	10.1
ISO	$y=(4.26\pm0.053)x+(-0.020\pm0.007)$	0.999	0.005	0.018	3.4
PUT	$y=(8.29\pm0.40)x+(0.0050\pm0.068)$	0.993	0.028	0.094	18.5
CAD	$y=(6.52\pm0.11)x+(0.020\pm0.013)$	0.999	0.007	0.023	18.0
SPD	$y=(13.0\pm0.09)x+(0.030\pm0.014)$	0.999	0.003	0.009	nd[d]

[a] Calibration curve equation; $y=ax+b$; where *a*: slope ± standard deviation, expressed in mg^{-1} L; *b*: intercept ± standard deviation, expressed in mV min.
[b] Determination coefficient.
[c] RSD: relative standard deviation expressed as percentage of the mean value; spiked sample ($n=5$).
[d] Not detected.

Table 2
Quantification of biogenic amines in wine samples.

Sample	**Concentration** (mg L^{-1})								
	HIS	MET	ETA	DMA	2-PEA	ISO	PUT	CAD	SPD
White wine 1	8.94 ± 0.62	0.17 ± 0.03	0.48 ÷ 0.07	0.12 ± 0.01	< LQ	< LQ	0.17 ± 0.01	0.016 ± 0.002	< LQ
White wine 2	4.44 ± 0.24	0.15 ± 0.02	0.50 ÷ 0.08	0.11 ± 0.01	0.089 ± 0.006	< LQ	0.21 ± 0.004	0.080 ± 0.004	< LQ
White wine 3	2.83 ± 0.13	0.37 ± 0.08	0.48 ± 0.14	0.14 ± 0.02	0.19 ± 0.03	0.13 ± 0.02	0.27 ± 0.02	< LQ	< LQ
Red wine 1	23.1 ± 2.2	0.066 ± 0.014	1.14 ± 0.11	0.32 ± 0.013	0.15 ± 0.014	0.051 ± 0.023	1.50 ± 0.014	0.068 ± 0.038	< LQ
Rosé wine 1	15.1 ± 2.4	0.26 ± 0.05	0.76 ± 0.15	0.16 ± 0.01	< LQ	1.0 ± 0.2	2.16 ± 1.02	0.027 ± 0.036	< LQ

protocol itself or with interferences from the wine matrix. Additional work is underway to address this topic.

4. Conclusions

In this work we propose a simple and fast methodology for the determination of BA in wines, based on SALLE. LLE methodologies were viewed in the past as worst procedures when compared to SPE or SPME, mainly due to the use of large quantities of hazardous organic solvents and to be labor intensive. The proposed methodology uses a less hazardous organic solvent for the extraction, acetonitrile, and the extraction procedure is very straightforward. This is the main advantage of the proposed method since extraction can be simultaneous executed with derivatization, thus reducing the number of stages before the chromatographic analysis. Additionally, this can be accomplished in 25 min time-span. In comparison with a common extraction procedure, solid-phase extraction (SPE), SALLE has lower solvent consumption, less stages; therefore, there is a lower probability of analytes loss and is simpler to execute. Several extraction parameters were optimized, such as derivatizing reagent concentration, pH and extraction time. It was shown the influence of pH and the importance to accurately control it prior to the extraction.

This methodology was successfully applied to the analysis of nine BA normally present in foods and beverages. It presents itself as an easier, faster and cheaper methodology for the analysis of this class of compounds and one that could easily be implemented in quality control experiments.

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References

[1] S. Moret, D. Smela, T. Populin, L.S. Conte, Food Chem. 89 (2005) 355–361.
[2] S. Casal, M. Oliveira, M.A. Ferreira, J. Liq., Chromatogr. Rel. Technol 25 (2002) 2535–2549.
[3] I.M. Valente, C.M. Santos, L.M. Goncalves, J.A. Rodrigues, A.A. Barros, Anal. Methods 4 (2012) 2569–2573.
[4] G. Suzzi, F. Gardini, Int. J. Food Microbiol. 88 (2003) 41–54.
[5] R. Romero, M.G. Bagur, M. Sanchez-Vinas, D. Gazquez, Anal. Bioanal. Chem. 376 (2003) 162–167.
[6] J. Lange, C. Wittmann, Anal. Bioanal. Chem. 372 (2002) 276–283.
[7] M.H.Silla Santos, Int. J. Food Microbiol. 29 (1996) 213–231.
[8] B. ten Brink, C. Damink, H.M.L.J. Joosten, J.H.J. Huis in 't Veld, Int. J. Food Microbiol. 11 (1990) 73–84.
[9] R.E. Anli, M. Bayram, Food Rev. Int. 25 (2009) 86–102.
[10] A.R. Shalaby, Food Res. Int. 29 (1996) 675–690.
[11] C. Ancin-Azpilicueta, A. Gonzalez-Marco, N. Jimenez-Moreno, Crit. Rev. Food Sci. Nutr. 48 (2008) 257–275.
[12] A.P. Marques, M.C. Leitao, M.V.S. Romao, Food Chem. 107 (2008) 853–860.
[13] P. Kalac, M. Krizek, J. Inst. Brew. 109 (2003) 123–128.
[14] L.S. Maynard, V.J. Schenker, Nature 196 (1962) 575–576.
[15] R. Romero, D. Gazquez, M.G. Bagur, M. Sanchez-Vinas, J. Chromatogr. A 871 (2000) 75–83.
[16] A. Onal, S.E.K. Tekkeli, C. Onal, Food Chem. 138 (2013) 509–515.
[17] C. Simo, M.V. Moreno-Arribas, A. Cifuentes, J. Chromatogr. A 1195 (2008) 150–156.
[18] S. Cortacero-Ramirez, D. Arraez-Roman, A. Segura-Carretero, A. Fernandez-Gutierrez, Food Chem. 100 (2007) 383–389.
[19] O. Busto, M. Miracle, J. Guasch, F. Borrull, J. Chromatogr. A 757 (1997) 311–318.
[20] V. Pereira, M. Pontes, J.S. Câmara, J.C. Marques, J. Chromatogr. A 1189 (2008) 435–443.
[21] V. Konakovsky, M. Focke, K. Hoffmann-Sommergruber, R. Schmid, O. Scheiner, P. Moser, R. Jarisch, W. Hemmer, Food Addit. Contam. A 28 (2011) 408–416.
[22] C. Proestos, P. Loukatos, M. Komaitis, Food Chem. 106 (2008) 1218–1224.
[23] E.H. Soufleros, E. Bouloumpasi, A. Zotou, Z. Loukou, Food Chem. 101 (2007) 704–716.
[24] T. Tang, T.Y. Shi, K. Qian, P.L. Li, J.Q. Li, Y.S. Cao, J. Chromatogr. B 877 (2009) 507–512.
[25] N. Garcia-Villar, S. Hernandez-Cassou, J. Saurina, J. Chromatogr. A 1216 (2009) 6387–6393.
[26] N. García-Villar, J. Saurina, S. Hernández-Cassou, Anal. Chim. Acta 575 (2006) 97–105.
[27] S. Moret, L.S. Conte, J. Chromatogr. A 729 (1996) 363–369.
[28] Z. Loukou, A. Zotou, J. Chromatogr. A 996 (2003) 103–113.
[29] M.A. Awan, I. Fleet, C.L.P. Thomas, Food Chem. 111 (2008) 462–468.
[30] K.J. Huang, C.X. Jin, S.L. Song, C.Y. Wei, Y.M. Liu, J. Li, J. Chromatogr. B 879 (2011) 579–584.
[31] I.M. Valente, L.M. Gonçalves, J.A. Rodrigues, J. Chromatogr. A 1308 (2013) 58–62.
[32] G. Dugo, F. Vilasi, G.L. La Torre, T.M. Pellicano, Food Chem. 95 (2006) 672–676.