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Comparison between phase-transfer and cloud-point methodologies for the micellar extraction of biogenic amines

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ABSTRACT

We compared the recently developed surfactant-based extraction with sodium *bis*-[2-ethylhexyl]-sulphosuccinate (AOT) in heptane (the phase-transfer method) and the well established methodology with poly(oxyethylene)-7,5-(p-*tert*-octylphenyl) ether (Triton X-114; the cloud-point technique) to extract and preconcentrate biogenic amines. Both procedures were optimized and applied to the extraction of biogenic amines from a beer sample. In order to improve the degree and monitoring of the extraction otherwise achieved with underivatized amines, the fluorescent reagent dansyl chloride was used. Since the different procedures described in the literature are not in agreement, we optimized the derivatization reaction. Accordingly a systematic investigation of the experimental variables, one by one, yielded the following optimum conditions: dansyl chloride, 5 mg/mL; 27 °C; reaction time, 25 min; pH, 10.35 at ionic strength, 0.7 mol/L. Both surfactant-based extraction procedures are simple and sensitive, but the use of AOT instead of Triton X-114 offers certain advantages. Detection limits of between 0.03 and 0.8 pmol injected were obtained when AOT was used, whose range is similar to or better than the other published techniques; while the corresponding values for Triton X-114 were between 0.2 and 1.2 pmol injected. The phase-transfer extraction is faster than the cloud-point method, and no heating is required. For both approaches, the recovery was very high for all the amines studied and the reproducibility quite good for almost all. Upon comparison of calibration curves in pure water with those in the presence of samples, matrix effects were detected.

1. Introduction

Biogenic amines (BAs) are generated in foods and beverages by an enzymatic decarboxylation of free amino acids [1]. The toxic and carcinogenic effects of these amines are well known, and their concentration is employed as an indicator of preservation quality in consumable products [2,3]. Because of their low concentration levels and matrix effects, the determination/quantification of biogenic amines constitutes a continuing problem in food safety.

HPLC is the most popular analytical method employed to screen for and quantitate BAs [4a-c]. Since these compounds lack chromophores or fluorophores, they must first be derivatized with absorbent or fluorescent compounds for their detection at trace levels [4–7].

Surfactant-based extraction methodologies such as the cloud-point technique are becoming widespread for the preconcentration of analytes present at only trace levels [8,9]. In this technique, when an aqueous solution containing the analyte and some nonionic surfactant in amounts above their critical micellar concentration is heated above a critical temperature (the cloud-point temperature, CPT), the solution becomes cloudy and the surfactant separates into a second phase [10,11]. This surfactant phase, constituting a very small portion of the original volume of the mixture, usually contains all the analytes. After that step, the micellar phase must be separated from the aqueous phase and diluted with a nonviscous solvent such as acetonitrile or methanol to allow injection into an HPLC system. The main drawback is that the method is not suitable for the extraction of heat-labile analytes if the surfactant used has a high CPT or if much higher temperatures than the CPT must be maintained for too long a period of time in order to allow maximum extraction [12,13].

A different surfactant-based methodology, not requiring the application of heat for extraction, is the use of the phase-transfer method [13,14]. After the vigorous shaking of an aqueous salt solution with an added immiscible organic solvent (*e.g.*, heptane) and the appropriate time interval for enabling a separation of the resulting two clear phases, reverse micelles are formed in the organic phase. In this method, the surfactant sodium *bis*-[2-ethylhexyl] sulphosuccinate (AOT) has been widely employed for micellar extraction of metals, amino acids, and proteins from aqueous salt

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solutions [13–15]. In a previous study, we used this same phase-transfer methodology to extract and preconcentrate BAs present in fruit juices and compared this methodology with the cloud-point technique by using Triton X-114 at the same percent concentration as AOT [16]. We did not, however, optimize the experimental conditions for the derivatization and cloud-point extraction steps and we further merely employed standard procedures obtained from the literature.

In these present experiments we compared the results from the phase-transfer methodology with AOT in heptane as the extracting phase with those obtained by an optimized cloud-point procedure with Triton X-114 for the measurement of tryptamine (Trp), cadaverine (Cad), tyramine (Tyr), putrescine (Put), spermine (Spm), spermidine (Spd), histamine (His), and phenylethylamine (Phen). The techniques were then employed for the extraction of the BAs from a beer sample. There are only a few extant investigations on the measurement of BAs in beers, especially when compared to those conducted in wines [17–19]. Considering the high consumption of beer along with the harmful effects of the BAs, more information about their contents in beers would certainly appear to be necessary. In this study we performed the cloud-point extractions at different amine concentrations, temperatures, sodium-chloride concentrations, and extraction times and compared the advantages and disadvantages of both extraction procedures with respect to the total analysis time, the detection limits, preconcentration factors, and the recoveries-among other variables. Since in the literature several derivatization procedures for BAs have been employed and no complete agreement currently exists among those approaches, we optimized the derivatization reaction using 5-dimethylamino-1-naphthalenesulphonyl chloride (dansyl chloride; DnsCl) as the fluorophore. Since we detected matrix effects by comparing the slopes of the calibration curves in pure water with those in the presence of the sample, we used the standard-addition method for quantification.

2. Experimental

2.1. Chemicals

AOT (Sigma) was dried in a vacuum oven at 40 °C for 2 days and used without further purification. Triton X-114 (Sigma) and reagent-grade *n*-heptane (Baker) were used as received. Water was purified with a Milli-Q system (Millipore Co.). Methanol was obtained from Mallinckrodt. All salts and inorganic acids were reagent grade or better. Put; the hydrochlorides of Trp, Phen, Cad, His, Tyr, Spm, and Spd; polyvinylpyrrolidone (PVP); and 3,3′-thiodipropionic acid were obtained from Aldrich. The internal standard, 3-amino-1-phenylbutane, and the DnsCl were purchased from Fluka and the trifluoracetic acid (TFA), sodium carbonate, and NaCl from Merck. All solutions were filtered through 0.22-μm membranes (Micron Separations, Westborough, MA, USA) before injection into the HPLC column.

2.2. Chromatographic methods and equipment

Chromatographic analysis of the dansylated biogenic amines (Dns-BAs) was performed on a Zorbax SB-C18 column (150 mm × 3.0 mm, i.d.) packed with 5-μm particles and connected to a guard column in an HP 1100 liquid chromatograph, equipped with a binary pump, thermostatized column compartment, autoinjector, degasser, and fluorescence detectors and linked to an HP workstation. The flow-rate protocol was at 0.6 mL/min in a mobile phase of composition MeOH–water (70:30) between times 0 and 16 min followed by a mobile-phase gradient of 70–100% methanol between times 16 and 25 min. Then the initial composition was reestablished for 5 min. For fluorescence detection of Dns-BAs, the optimum wavelengths were 340 nm for excitation and 520 nm for emission.

A thermostatically controlled bath (Instrumentos Alycar, Ind. Arg.) maintained at the appropriate temperatures was used for the derivatization and cloud-point experiments. A Vortex Genie 2 (Scientific Industries, USA) mixer employed for thorough vortexing of the aqueous and the organic phases containing the added AOT. Phase separation was effected by an Eppendorf 5417C/R centrifuge (5000 × *g*, 5 min).

2.3. Preparation of standard solutions and samples

Biogenic-amine stock solutions (1 mg/mL) were prepared in 0.1 M HCl containing 0.2% (w/v) of 3,3′-thiodipropionic acid as antioxidant and stored at 4 °C in the dark. Working standard solutions for calibration were prepared by the appropriate dilution of the stock solutions and used within the same week. DnsCl was dissolved in acetone (5 mg/mL).

Beer samples were treated with PVP (0.5 g in 10 mL of the sample) to remove polyphenols, which compounds interfere in the derivatization reaction; then stirred for 15 min, centrifuged for another 5 min at 5000 × *g*, and filtered through 0.22-μm membranes. The samples were finally degassed by sonication for 5 min before analysis.

2.4. Derivatization procedure

Optimization of the derivatization conditions was performed by selecting different temperatures (27, 40, and 60 °C), reaction times (5, 25, 60, and 90 min), and pHs (8.23, 9.83, and 10.35). These pH values result from mixing the BA solutions in hydrochloric acid with different proportions of sodium carbonate and/or bicarbonate. All these experiments were done at an ionic strength of 0.7 mol/L. After the optimum temperature, reaction time, and pH were ascertained; two different ionic strengths (0.6 and 1.2 mol/L) and two different DnsCl concentrations (3 and 7 mg/mL) were assayed. In all instances the same amount of amine was employed.

2.5. Surfactant-based extractions of the Dns-BAs

The phase-transfer method with AOT in heptane as the extracting solvent and the cloud-point technique with Triton X-114 in water were employed for the extraction of Dns-BAs from the standard solutions and samples.

2.5.1. Phase-transfer experiments

The following experimental conditions for extraction were optimized in our previous publication [16]. After evaporation of the acetone, dansyl derivatives obtained from standard solutions or from sample solutions are mixed with 350 μl NaCl (final concentration, 0.8 mol/L) and 300 μl AOT at 0.3 mol/L in heptane. The mixture is shaken and centrifuged as described above and 5 μl of the resulting organic phase injected into the chromatographic column.

2.5.2. Cloud-point experiments

Three milliliters of the samples and 30 μl of the internal standard (diluted 1:10 from the stock) were mixed with 2 mL of DnsCl in acetone (5 mg/mL) and 2 mL of sodium carbonate (0.23 mol/L). After derivatization, the acetone was removed and the NaCl and Triton X-114 solutions added. The mixture was placed in a water bath and the temperature raised at 2 °C per min up to 40 °C or to 60 °C and maintained for half an hour at those temperatures. The tubes were finally centrifuged for 5 min at 5000 × *g* for phase separation and placed in an ice bath for 2 min to increase the viscosity of the micellar phase. The aqueous phase was separated by inverting

the tube, and the sticky micellar phase at the bottom of the tube was finally mixed with 300 μl MeOH. The admixture was not fast (the rapidity can be observed in colored samples but would not be obvious in clear solutions). The tubes were therefore placed in an ultrasonic bath for 1 min in order to detach the surfactant from the tube walls. After this step, the samples were filtered and injected into the chromatographic column.

2.6. Statistical analysis

Data treatment and calibration curves were performed by using the Excel data-analysis tools (Microsoft Excel, Microsoft Corp.).

3. Results and discussion

3.1. Optimization of the derivatization conditions

In our previous paper [16] the experimental conditions most frequently employed in the literature were selected (reaction time, 2 h; pH 8.45 with saturated $NaHCO_3$; temperature, 40 °C; DnsCl concentration, 5 mg/mL). The diversity of protocols for derivatization of biogenic amines [4,20–25] (with some of the conditions indicated being contradictory) would point to the need for a thoroughly optimized derivatization procedure. Zoutou et al. [25] have previously optimized the derivatization reaction with DnsCl at a fixed ionic strength, but those authors used a reaction-temperature range of between 25 and 40 °C, higher than that used in this work, which excessive temperatures can favor the decomposition of the dansyl derivatives. The optimized variables obtained in this paper are otherwise in essential agreement with those obtained by Zoutou et al.

Table 1 (A, B, and C) shows the results from normalizations of the peak areas (*i.e.*, the peak area divided by the largest area obtained for a given amine under the different reaction conditions) obtained at an ionic strength of 0.7 mol/L. The maximum area for each amine is not obtained under the same experimental conditions. The best reaction efficiencies were realized at pH 9.83 and 10.35, with six of the nine amines showing optimum efficiencies at the lower of these two values (Table 1B). This pH range agrees quite well with the pH optimum of 9.5 reported in Ref. [25], but at different reaction times and temperatures depending upon the amine. Those authors do not, however, specify whether that optimum occurs for all the amines or only for some. It is thus difficult to obtain a consensus field among the different experimental variables wherein the derivatization of all compounds is maximal. At pH 10.35 efficiencies are very good for all the amines, principally at 27 °C and a 25-min reaction time (Table 1C). This pH value is also very close to the one employed by Zotou et al. [25], though other authors do not mention the pH *per se*, but simply state conditions such as "saturated carbonate" or "saturated bicarbonate" [21]. Finally, the reaction time furthermore agrees with the one used in Ref. [25] (30 min), but the reaction temperature they examined was 65 °C. They did, however, study a temperature range between 60 and 80 °C, which values could be high enough to cause a decomposition of the dansyl derivatives.

Despite all these previous investigations, we found no optimization studies in which the ionic strength had been varied. For this reason we decided to investigate other ionic strengths (0.6 and 1.2 mol/L) while employing the optimized conditions for temperature, reaction time, and pH (Table 2). The reaction efficiencies at all ionic strengths tested were lower than that obtained at 0.7 mol/L. Therefore, on the basis of these observations and considerations, we selected this ionic strength for the experiments presented here.

Finally, the DnsCl concentration was changed to 3 and 7 mg/mL. A DnsCl concentration of 3 mg/mL, while giving a larger chromatographic-peak area for Trp, produced no significant change in the areas corresponding to Phen, Cad, and Put and moreover decreased the size of the peaks representing Tyr, His, Spm, and Spd. At a DnsCl concentration of 7 mg/mL, all the chromatographic peaks were reduced in size. Thus, a DnsCl concentration of 5 mg/mL would represent a reasonable compromise. At this concentration, it being

Table 1
Normalized chromatographic-peak areas obtained for different BAs at ionic strength, I = 0.7 mol/L.

t (min)	Trp	Phen	Put	Cad	Std	His	Tyr	Spd	Spm
(A) pH = 8.23									
27 °C									
5	16	14	3	5	16	15	22	1	1
25	24	26	20	11	36	43	53	4	4
60	22	23	21	14	40	48	57	11	8
90	19	20	21	12	32	47	56	8	11
40 °C									
5	46	42	7	13	28	21	27	3	2
25	67	73	36	43	63	62	63	23	15
60	55	61	33	40	56	55	57	26	18
90	46	51	27	35	49	48	49	25	15
60 °C									
5	71	76	42	65	66	53	63	44	28
25	73	77	46	69	68	53	62	48	31
60	76	79	48	67	67	50	57	45	30
90	73	74	45	61	62	44	50	39	26
(B) pH = 9.83									
27 °C									
5	**100**	**100**	55	60	96	27	92	42	19
25	82	95	78	91	96	81	83	77	59
60	81	91	83	97	96	**100**	86	90	82
90	69	78	70	83	84	88	73	86	73
40 °C									
5	74	77	54	78	68	31	72	69	48
25	75	83	65	91	80	52	73	87	72
60	71	79	61	85	77	50	66	85	69
90	62	69	61	70	67	43	54	76	59
60 °C									
5	78	82	69	91	74	36	93	93	79
25	86	87	77	**100**	94	45	**100**	**100**	88
60	79	80	68	88	94	36	83	95	75
90	68	69	57	63	79	20	56	65	52
(C) pH = 10.35									
27 °C									
5	71	77	77	79	86	49	86	72	60
25	76	80	**100**	96	97	94	89	90	**100**
60	70	77	86	86	**100**	91	83	85	96
90	68	74	83	81	91	87	81	82	92
40 °C									
5	74	80	78	91	81	46	84	86	76
25	72	79	76	86	80	48	81	84	83
60	76	83	80	91	88	55	84	86	89
90	71	77	74	83	80	49	79	84	81
60 °C									
5	58	61	57	64	52	25	75	66	63
25	62	61	59	64	53	22	71	64	63
60	63	62	58	61	52	14	64	58	55
90	61	60	55	57	49	11	59	54	52

Table 2
Normalized chromatographic-peak areas obtained for different BAs at two ionic strengths by using the optimal conditions from Table 1 ($HCO_3^-/CO_3^=$ at pH = 10.35, 27 °C and 25 min).

t (min)	Trp	Phen	Put	Cad	Std	His	Tyr	Spd	Spm
I = 0.6 (mol/L)									
25	66	76	62	89	74	74	89	98	74
I = 1.2 (mol/L)									
25	63	48	18	42	37	16	87	36	25

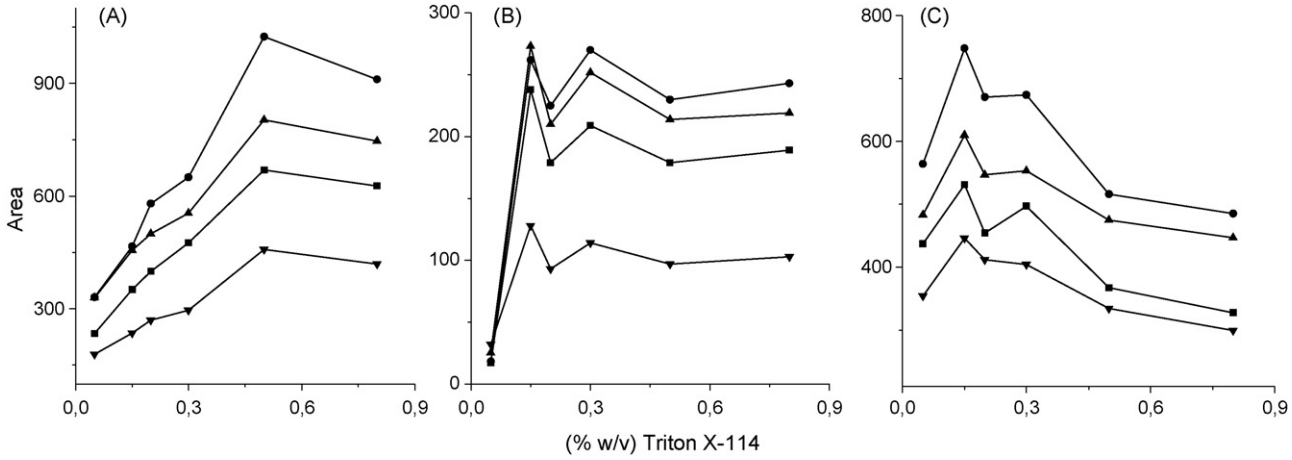

Fig. 1. Peak areas obtained for Trp (■), Phen (●), Put (▲), and Std (▼) after cloud-point extraction with different amounts (% w/v) of Triton X-114. Initial concentration of each amine: (A) 5 mg/L; (B) 10 mg/L; (C) 20 mg/L.

commonly used in the literature [21], the molar ratios between the DnsCl and the amines were higher than 100, which molar excess insures that the reaction proceeds to completion. In Ref. [25] the authors have employed 0.5, 0.75, 1.0, and 1.5% (w/v) DnsCl (*i.e.*, from 1.5 to 5 mg/mL) and observed the optimum concentration to be 1% (w/v). Fu et al. [26] reported that at high DnsCl concentrations side reactions occurred with resulting decreases in the yields of the dansyl derivatives.

Therefore, the optimum conditions for obtaining acceptable reaction efficiencies for all the BAs would be a combination of the following experimental variables: a concentration of 5 mg/mL of DnsCl, 27 °C, a 25-min reaction time, and a pH of 10.35 at an ionic strength of 0.7 mol/L.

3.2. Optimization of the cloud-point extractions

The characterization of the optimum extraction conditions with Triton X-114 was made on the basis of four representative amines: Trp, Phen, Put, and the internal standard (IS) 3-amino-1-phenylbutane. The first step was the selection of the optimum surfactant concentration. Fig. 1 shows the chromatographic-peak areas at different percentages of surfactant and initial amine concentrations (5, 10, and 20 mg/L). At an amine concentration of 20 mg/L, the optimum amount of Triton X-114 is about 0.15% (w/v) for all four selected amines. We observed that the drop of micellar phase obtained at the bottom of the tube becomes greater with increasing percentages of surfactant. This typical feature of the cloud-point process with all nonionic surfactants [9] is inconvenient since the purpose of the extraction is to increase concentration in that phase for the greatest sensitivity of detection. In the optimization experiments not concerned with maximum detection, the surfactant drop was diluted to a final volume of 0.5 mL before injection into the HPLC system in order to enable an informative comparison of the chromatographic-peak areas. Thus, the maxima present in Fig. 1 are a consequence of an increased initial extraction with higher amounts of surfactant. The decreased extraction occurring after the maximum could be attributed to increased amounts of surfactant monomers or aggregates remaining in the aqueous phase, which lack of partitioning would increase the solubility of the analyte in that phase. When, however, the initial concentration of amine was 40 mg/L, the maximum previously observed at the lower concentration was shifted to 0.5% (w/v). This difference was not surprising since more micelles would be required to extract higher amounts of analytes. Most of the BAs in beers are present in the range of 0–20 mg/L [25,33]. Thus, depending upon the type and amount of sample to be extracted, different percentages of surfactant can be necessary. On the basis of these results, we selected 0.15% (w/v) of Triton X-114 for the remainder of the experiments.

At 0.15% (w/v) surfactant concentration, the extraction time, NaCl concentration in the aqueous phase, and the extraction temperature were assayed at two different amine concentrations (5 and 10 mg/L). Fig. 2 shows the chromatographic-peak areas corresponding to the micellar phases obtained.

Carabias-Martínez et al. [9] recommended raising the system temperature above the CPT and maintaining this condition for a given time to enhance preconcentration factors. Temperatures of 40 and 60 °C have been normally found to be optimum for most extractions [8,9,27]. We thus compared extraction efficiencies at 60 °C with those at 40 °C. The extraction efficiencies were indeed higher at 60 °C, in agreement with the literature, and were better when the contact time between the micellar and aqueous phases was longer, as the extractions proved to be more complete for 45 min than for 15 min.

The NaCl concentration in the aqueous phase exerts only a small effect on the extraction yield compared to the other variables. Depending on the analyte concentration, extraction temperature, and extraction time, an either negligible or quite significant effect was observed. Therefore, the presence of some salt in the aqueous phase seems to be necessary for the extraction of certain analytes, probably because of the so-called salting-out process.

Upon consideration of all these results, we conclude that the optimal extraction conditions for diluted samples of Dns-BAs are: a Triton X-114 concentration of 0.15% (w/v), heating up to 60 °C from room temperature, 45 min of incubation or contact time, and 3% (w/v) NaCl in the aqueous phase. These optimum conditions are in good accord with those found in the literature [29,30].

3.3. Reproducibility of both extraction methods

The reproducibility of the entire method was assessed in octuplicate by dansylating a beer sample along with added standards and the IS, extracting the dansylated amines with AOT and Triton X-114 under the optimized conditions for each, and then injecting the extracted products into the HPLC system. The values for standard deviation expressed as a percent of the mean were between 2% and 5% for almost all the amines in the extractions with AOT-with the sole exception of Spd, where the figure was 18%. By contrast, the relative standard deviation, expressed as a percent (RSD%) for the extractions with Triton X-114 were lower than 5% for Put, Cad, Phen, and Trp; but were between 11% and 23% for His, Tyr, Spd, and Spm. For this reason, the calibration curves

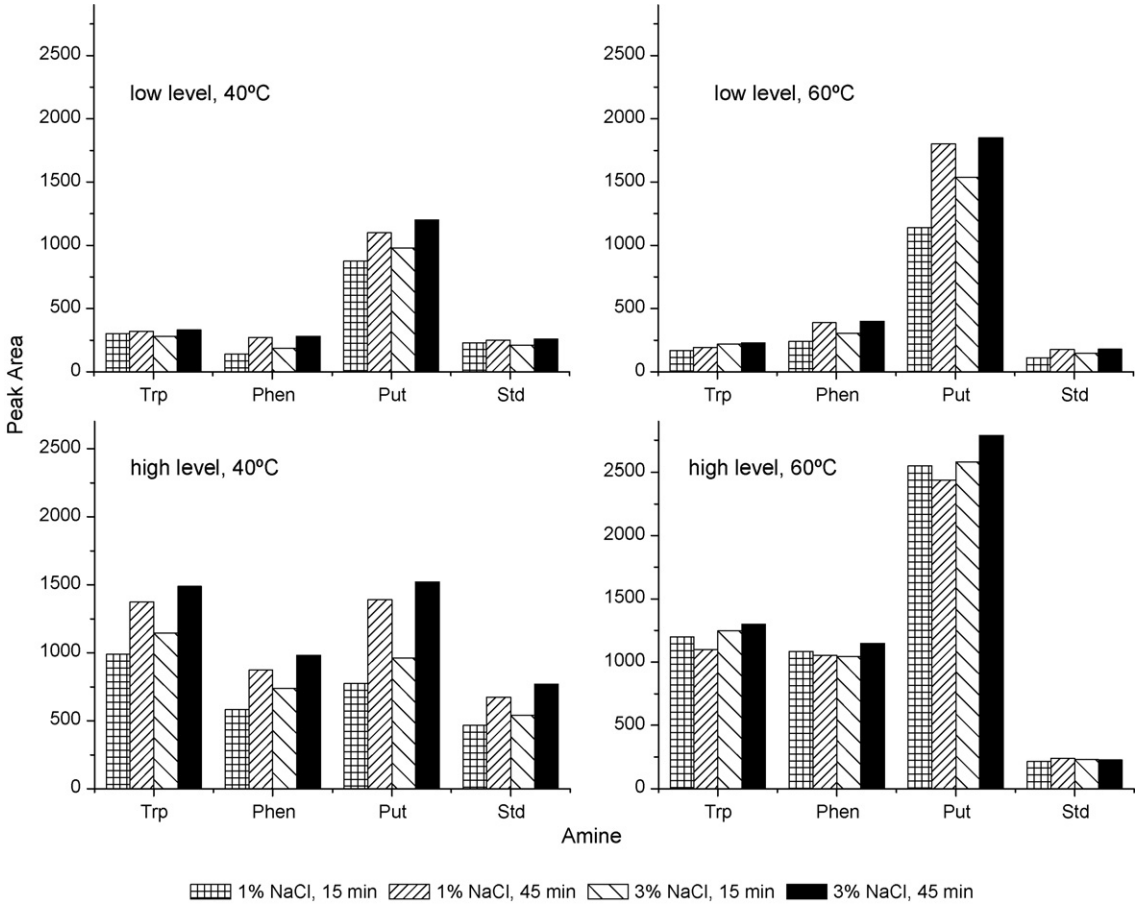

Fig. 2. Optimization of cloud-point extraction for four biogenic amines at two different concentration levels.

for these last four amines (see Section 3.4) were not included in Table 3.

The low reproducibility for the extraction of His, Tyr, Spd, and Spm when Triton X-114 was used could be attributed to the temperature at which the analyte solution was mixed with the surfactant solution (*i.e.*, the mixing temperature). The CPTs of the Triton X-114 solutions are dependent on both the surfactant concentration and the presence of water-soluble additives. As the surfactant concentration decreases and the additive concentration increases, the CPT decreases [8,28]. The CPT obtained in this work was 21 °C for a 0.15% (w/v) Triton X-114 solution in water. This figure is in agreement with the values reported in the literature [28]. The CPT observed for the samples containing the derivatized amines, however, was 9 °C. This lower value results from the presence of the NaCl added to increase the extraction efficiency, sodium carbonate coming from the derivatization step, the analytes, and the other components of the matrix. Therefore, it might be necessary for the temperature at which all the species (analytes and additives) are mixed with the surfactant solution to be below the CPT in order to insure the homogeneity of the solution and thus obtain more efficient and reproducible extractions. In this regard, mixing at 0 °C or at 10 °C, depending on the surfactant concentration and the additives involved, was examined by some authors [31,32]; while the samples and the surfactant solution were combined at close to room temperature or even higher by others [10,33,34]. Even when no appropriate mixing can be achieved at room temperature (when this temperature is above the CPT), micelles are nevertheless dynamic systems that will allow the analytes to be exchanged between the surfactant aggregates and the bulk solution. Thus, the analyte-trapping process in the organic phase should continue in the extraction step used here (*i.e.*, at 60 °C for 45 min, as described in the previous section). For this reason, we have compared the reproducibility of the extraction process for His, Tyr, Spm, and Spd at initial mixing temperatures of both 0 and 20 °C. Although the extraction yields obtained were very similar at the two temperatures, the RSD%s were still not acceptable; thus indicating that the low reproducibility observed for those amines when Triton X-114 was used was not a consequence of the initial mixing temperature (*i.e.*, room temperature). In light of these results, the admixture at room temperature between the sample and the surfactant solution is completely justified as prescribed in the previous

Table 3
Calibration equations for extractions with AOT and Triton X-114 from standards in water.

Amine	Lineal regression	*R*	SD	*N*
Extractions with AOT				
Trp	$y=(0.55\pm0.02)x-(0.02\pm0.02)$	0.9943	0.03	12
Cad	$y=(0.81\pm0.05)x+(0.08\pm0.05)$	0.9811	0.05	15
Phen	$y=(0.76\pm0.02)x-(0.004\pm0.01)$	0.9980	0.02	12
Put	$y=(0.118\pm0.003)x-(0.03\pm0.02)$	0.9950	0.03	15
His	$y=(0.036\pm0.002)x-(0.02\pm0.06)$	0.9881	0.09	11
Tyr	$y=(0.20\pm0.02)x+(0.07\pm0.04)$	0.9836	0.07	10
Spm	$y=(0.64\pm0.07)x-(0.09\pm0.09)$	0.9898	0.08	9
Extractions with Triton X-114				
Trp	$y=(1.15\pm0.03)x-(0.07\pm0.06)$	0.9984	0.02	9
Cad	$y=(1.37\pm0.04)x+(0.08\pm0.05)$	0.9811	0.05	11
Phen	$y=(1.53\pm0.05)x-(0.03\pm0.03)$	0.9925	0.04	11
Put	$y=(2.06\pm0.08)x-(0.02\pm0.03)$	0.9979	0.04	8
Tyr	$y=(0.49\pm0.02)x+(0.07\pm0.04)$	0.9839	0.01	10

Table 4
Standard-addition method for extractions with AOT and Triton X-114 in a beer sample.

	Regression equations	*R*	SD	*N*	[Amine] (mg/L)	*R*%
Extractions with AOT						
Trp	$y=(1.04\pm0.05)x+(0.15\pm0.06)$	0.9858	0.1	13	0.949 ± 0.004	103
Cad	$y=(0.63\pm0.09)x+(0.05\pm0.07)$	0.9880	0.07	13	0.918 ± 0.008	113
Phen	$y=(1.13\pm0.06)x+(0.004\pm0.05)$	0.9902	0.08	10	0.29 ± 0.01	111
Put	$y=(0.516\pm0.04)x+(0.28\pm0.02)$	0.9933	0.03	9	0.203 ± 0.004	96
His	$y=(0.018\pm0.002)x+(0.12\pm0.06)$	0.9796	0.1	11	0.48 ± 0.01	98
Tyr	$y=(0.0274\pm0.0002)x+(0.0699\pm0.0008)$	0.9999	0.0008	9	0.18 ± 0.01	100
Spm	$y=(0.51\pm0.06)x+(0.031\pm0.003)$	0.9818	0.08	8	2.17 ± 0.01	89
Extraction with Triton X-114						
Trp	$y=(0.37\pm0.02)x+(0.063\pm0.009)$	0.9901	0.02	10	0.816 ± 0.004	98
Cad	$y=(0.60\pm0.04)x+(0.2\pm0.04)$	0.9859	0.08	11	1.052 ± 0.005	101
Phen	$y=(0.81\pm0.05)x+(0.10\pm0.06)$	0.9819	0.12	13	0.36 ± 0.01	88
Put	$y=(0.23\pm0.01)x+(0.30\pm0.01)$	0.9930	0.02	8	0.185 ± 0.007	102

section on the basis of both the reproducibility obtained and the analysis time.

3.4. Calibration curves including extractions with Triton X-114 and AOT from aqueous samples and from beer samples: matrix-effect studies

In order to investigate if matrix effects were present in the quantitative determinations, we compared the slopes obtained in the least-squares-regression equations for both extraction procedures: from aqueous solutions (the external-standard method) and from a beer sample (the standard-addition method). The sample was spiked with the standards before the derivatization and extraction procedures. For the purpose of these comparisons, we choose the following *t*-test according to Eq. (1) [35]:

$$t = \frac{b_2 - b_1}{\sqrt{s_1^2 - s_2^2}} \quad (1)$$

where b_1 and b_2 are the slopes of the regression equations to be compared and s_1 and s_2 are the respective standard deviations. Table 3 shows the regression equations for extractions with AOT and Triton X-114 from aqueous samples (no matrix present). As expected, satisfactory regression coefficients and standard deviations were obtained with the intercept being virtually zero in all instances. Table 4 depicts the regression results for extractions with AOT and Triton X-114 from a beer sample. Here acceptable standard deviations and regression coefficients were obtained, and moreover the intercepts reflect the biogenic-amine concentration in the sample. Since the extraction of Spd from the beer sample was not reproducible for both extraction procedures, nor was the extraction of His, Tyr, and Spm with Triton X-114, the standard-addition least-square regressions for these amines are not included. Through the use of Eq. (1), we compared, at a 5% probability, the slopes obtained for Cad and Phen by the standard-addition method with both extraction methodologies for the beer sample. The first row of Table 5 shows that the calculated *t* value for Cad is almost the same as the observed value. Thus, both extraction procedures give the same calibration curve for this amine. By contrast, the theoretical and empirical slopes of the calibration curves for Phen are significantly different. In the second and third row of Table 5, the slopes obtained with the external-standard method were compared with those observed with both surfactant-based extractions from the samples with added standards. The slopes obtained in the presence of matrix are significantly different from those recorded when no matrix is present since the calculated *t* values are higher than the observed ones regardless of the surfactant employed. These results show that matrix effects occur in the extractions with both surfactants, at least for these two amines. The standard-addition method including the extraction step must, however, be applied independently whether or not matrix effects are present for the other BAs since the latter are quantified simultaneously.

Table 5
t-Test at 5% probability for comparison of the calibration curves slopes obtained for extractions with AOT and Triton X-114 from beer samples (standard-addition method) and from standard solutions.

Method	Extraction system	*t* (calculated)	*t* (tabulated)
Standard addition	*Triton X-114*	Cad: 2.16	2.10
Standard addition	*AOT*	Phen: 6.99	2.10
Standard addition	*Triton X-114*	Cad: 34.41	2.06
External standard	*Triton X-114*	Phen: 39.65	1.96
Standard addition	*AOT*	Cad: 4.77	2.06
External standard	*AOT*	Phen: 80.30	2.10

3.5. Biogenic amines in the beer sample: comparison of the recoveries and detection limits before and after extraction

Fig. 3 compares the chromatograms before and after both surfactant-based extractions, while Table 4 shows the biogenic-amine contents of the beer sample assayed by both extraction procedures along with the recoveries. The amine contents found in the beer sample are in satisfactory agreement between both extraction procedures, mainly if the correction for the recoveries is taken into account. We need to emphasize that the errors obtained are the summation of those occurring in both the derivatization and the extraction procedures, which steps are the most significant contributors. The quantities measured are either of the same

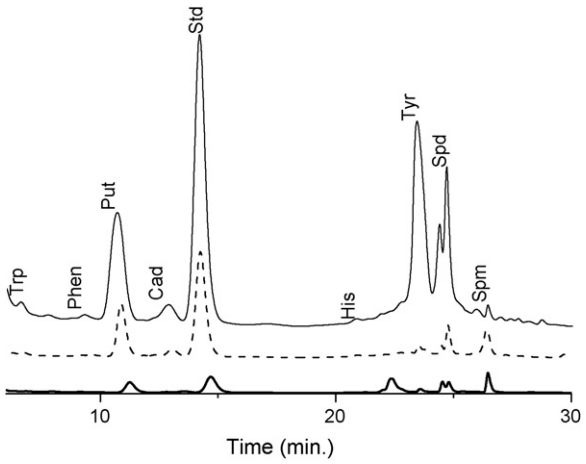

Fig. 3. Chromatograms for a beer sample without extraction (—) and after extraction with Triton X-114 (- - -) and AOT (—).

Table 6
Comparison of the detection limits and preconcentration factors for both surfactant-based extractions.

Amine	LOD_{der} [a] (pmol)	LOD_{AOT} [b] (pmol)	f_c^{AOT}	LOD_{Triton} [b] (pmol)	f_c^{Triton}
Put	2.30	0.61	31	0.69	20
His	70.96	0.84	33	–[c]	–[c]
Tyr	2.98	0.08	32	0.72	22
Phen	3.46	0.10	30	0.32	18
Cad	2.46	0.08	32	1.03	21
Spm	8.51	0.02	27	–[c]	–[c]
Spd	13.40	0.03	30	–[c]	–[c]
Trp	3.88	0.07	31	0.34	19

[a] LOD_{der} is the detection limit (S/N = 3), after derivatization without extraction.
[b] LOD are the corresponding values including the extraction step with AOT and Triton X-114.
[c] Not included due to the poor reproducibility for these amines.

order of magnitude or below the biogenic-amine concentrations found by other authors [24,33]. The recoveries were calculated from one level in the standard-addition curve and were expressed as: 100 (amount detected/amount with standards present). High values were obtained for all BAs, excepting Phen extracted with Triton X-114.

Table 6 compares the detection limits, LODs, and preconcentration factors, f_c, for both extraction methodologies together with the LODs obtained without extraction. The LODs after extraction are lower for the AOT system (0.08 pmol for Cad and 0.84 pmol for His) than for Triton X-114 (1.03 pmol for Cad and 1.24 pmol for His) and much lower than the LODs without extraction. The LODs for both surfactant-based extractions are furthermore considerably lower than those obtained in Ref. [20], where a solid-phase-extraction preconcentration step was included. For example, we obtained respective LODs for Phen and Put at 0.1 and 0.61 pmol, as opposed to 2.54 and 1.13 pmol in Ref. [20] (after conversion of their ng values to pmol). The f_c values are higher for AOT than for Triton X-114; since the final volumes of the surfactant phase, before injection into the HPLC column, were the same for both extraction procedures, a direct comparison of the chromatograms obtained can be made. These results are consistent with the previous observation (Ref. [16]) that AOT is a more efficient surfactant than Triton X-114 (probably because the latter is nonionic and the protonated amines interact more weekly than with the anionic surfactant AOT). In fact, as was observed in Ref. [16], some amines still remain in the aqueous solution for the extractions with Triton X-114, while no such peaks are observed for that fraction in the extractions with AOT.

4. Conclusions

We have fully optimized the derivatization of BAs with DnsCl with respect to several experimental variables such as the reaction temperature, the reaction time, the pH, the buffer employed (carbonate *vs*. mixtures of carbonate/bicarbonate), the DnsCl concentration, and the ionic strength. We have also optimized the cloud-point extractions with Triton X-114 with respect to the surfactant concentration, the extraction time, the extraction temperature, and the salt concentration in the aqueous phase.

After comparing both fully optimized extraction procedures we conclude that the reverse micelles of AOT in heptane represent a better extraction system than the cloud-point extraction with Triton X-114 since lower detection limits and better preconcentration factors are obtained, and the reproducibility is better. These findings confirm the initial results reported in our previous paper, where we observed that AOT was an excellent extracting surfactant for Dns-BAs. This preliminary conclusion had been based on the observation that in the cloud-point extraction small amounts of the analytes had remained in the aqueous phase, while the extraction with AOT had been almost complete (with no chromatographic peaks being detected in the aqueous phase). The optimized extraction procedure with AOT is at once faster, more sensitive, and more reproducible than extractions with Triton X-114. Additionally, the proposed procedure is extremely simple and requires no heating of the sample.

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References

[1] D.M.L. Morgen, Biochem. Soc. Trans. 18 (1990) 1080.
[2] M.J.R. Nout, Food Res. Int. 27 (1994) 291.
[3] D.M. Kuhn, W. Lovenberg, in: J.N. Hathcock (Ed.), Nutritional Toxicology, vol. I, Acad. Press, NY, 1982.
[4] (a) S. Moret, R. Bortolomeazzi, G. Lercker, J. Chromatogr. 591 (1992) 175; (b) V. Pereira, M. Pontes, J.S. Câmara, J.C. Marques, J. Chromatogr. A 1189 (2008) 435; (c) C. Proestos, P. Loukatos, M. Komaitis, Food Chem. 106 (2008) 1218.
[5] M.R. Alberto, M.E. Arena, M.C. Manca de Nadra, Food Control 13 (2002) 125.
[6] J.K. Lin, J.K. Chang, Anal. Chem. 47 (1985) 1634.
[7] S. Oguri, Y. Yoneya, M. Mizunuma, Y. Fujiki, K. Otsuka, S. Terabe, Anal. Chem. 74 (2002) 3463.
[8] J. Szymanowski, J. Radioanal. Nucl. Chem. 246 (2000) 635.
[9] R. Carabias-Martínez, E. Rodríguez-Gonzalo, B. Moreno-Cordero, J.L. Pérez-Pavón, C. García Pinto, E. Fernández Laespada, J. Chromatogr. A 902 (2000) 251.
[10] E.K. Paleologos, S.D. Chytiri, I.N. Savvaidis, M.G. Kontominas, J. Chromatogr. A 1010 (2003) 217.
[11] S.R. Sirimanne, D.G. Patterson Jr., L. Ma, J.B. Justice Jr., J. Chromatogr. B 716 (1998) 129.
[12] E.W. Gunter, W.J. Driskel, P.R. Yeager, Clin. Chim. Acta 175 (1988) 329.
[13] (a) E.B. Leodidis, T.A. Hatton, J. Phys. Chem. 94 (1990) 6400, 94 (1990) 6411; (b) E.B. Leodidis, A.S. Bommarius, T.A. Hatton, J. Phys. Chem. 95 (1991) 5943; (c) E.B. Leodidis, T.A. Hatton, J. Phys. Chem. 95 (1991) 5957.
[14] T.K. De, A. Maitra, Adv. Colloid Interface Sci. 59 (1995) 95.
[15] H.R. Rabie, J.H. Vera, Langmuir 11 (1995) 1162.
[16] L. Romero, S. Keunchkarian, M. Reta, Anal. Chim. Acta 565 (2006) 136.
[17] S. Gómez-Alonso, I. Hermosín-Gutiérrez, E. García-Romero, J. Agric. Food Chem. 55 (2007) 608.
[18] S. Cortacero-Ramírez, D. Arráez-Román, A. Segura-Carretero, A. Fernández-Gutiérrez, Food Chem. 100 (2007) 383.
[19] P. Kalač, M. Křižek, J. Inst. Brew. 109 (2003) 123.
[20] Z. Loukou, A. Zotou, J. Chromatogr. A 996 (2003) 103.
[21] N.P.J. Price, J.L. Firmin, D.O. Gray, J. Chromatogr. 598 (1992) 51.
[22] S. Moret, D. Smela, T. Populin, L.S. Conte, Food Chem. 89 (2005) 355.
[23] N.M. Tamin, L.W. Bennett, T.A. Shellem, J.A. Doerr, J. Agric. Food Chem. 50 (2002) 5012.
[24] G. Lunn, L.C. Hellwig, Handbook of Derivatizations Reactions for HPLC, Wiley Interscience, 1998.
[25] A. Zoutou, Z. Loukou, E. Soufleros, I. Stratis, Chromatographia 57 (2003) 429.
[26] S. Fu, X. Zou, X. Wang, X. Liu, J. Chromatogr. B 709 (1998) 297.
[27] M. Izquierdo-Pulido, T. Hernández-Jover, A. Mariné-Font, M.C. Vidal-Carou, J. Agric. Food Chem. 44 (1996) 3159.
[28] R. Ferrer, J.L. Beltrán, J. Guiteras, Anal. Chim. Acta 330 (1996) 199.
[29] A. Favre-Réguillon, M. Draye, G. Lebuzit, S. Thomas, J. Foos, G. Cote, A. Guy, Talanta 63 (2004) 803.
[30] T.I. Sikalos, E.K. Paleologos, Anal. Chem. 77 (2005) 2544.
[31] C. Bordier, J. Biol. Chem. 256 (1981) 1604.
[32] B.R. Ganong, J.P. Delmore, Anal. Biochem. 193 (1991) 35.
[33] J.L. Manzoori, G. Karim-Nezhad, Anal. Chim. Acta 521 (2004) 173.
[34] D.L. Giocas, V.A. Sakkas, T.A. Albinis, D.A. Lampropoulou, J. Chromatogr. A 1077 (2005) 19.
[35] B.G.M. Vandeginste, D.L. Massart, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics, Elsevier, 1998.