Journal of Chromatography A, 1218 (2011) 4332–4339

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Hydrazone-based ligands for micro-solid phase extraction-high performance liquid chromatographic determination of biogenic amines in orange juice

Chanbasha Basheer[a], Weishan Wong[b], Ahmad Makahleh[c], Abdassalam Abdelhafiz Tameem[c,d], Abdussalam Salhin[c], Bahruddin Saad[c,*], Hian Kee Lee[b,*]

[a] Department of Chemistry, King Fahd University of Petroleum and Minerals, KFUPM Box 1059, Dhahran 31261, Saudi Arabia
[b] Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore
[c] School of Chemical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia
[d] Faculty of Sciences, Sebha University, 71 Sebha, Libya, Penang, Malaysia

ARTICLE INFO

Article history:
Received 18 February 2011
Received in revised form 15 April 2011
Accepted 26 April 2011
Available online 6 May 2011

Keywords:
Micro-solid phase extraction
Sol–gel
Biogenic amines
High performance liquid chromatography
Hydrazone

ABSTRACT

Eight hydrazone-based ligands were synthesized, trapped in a silica sol–gel matrix, and were subsequently used in the micro-solid phase extraction (μ-SPE) of biogenic amines (BAs). The BAs investigated were tryptamine, phenylethylamine, putrescine, histamine, tyramine and spermidine. Prior to the extraction, dansyl chloride was added to the samples which were heated to 70 °C for 10 min. The samples were extracted with μ-SPE, after which analytes were desorbed using acetonitrile via ultrasonication. The extracts were analysed by high performance liquid chromatography (HPLC) with ultraviolet detection. Of the eight ligands investigated as sorbents, benzophenone 2,4-dinitrophenylhydrazone was found to be the most promising. The enhanced π–π interaction between the analytes and the ligand facilitated the adsorption process. Under the most suitable extraction conditions, the method demonstrated good linearity with correlation coefficient of more than 0.985 over a concentration range of 1–50 μg L^{-1}. Satisfactory repeatability with relative standard deviations of 7.43–11.30% ($n = 3$) were obtained. Detection limits ranged from 3.8 to 31.3 ng L^{-1}. The μ-SPE method exhibited lower recoveries (71.5–87.4%) when compared to the solid phase extraction technique (79.7–95.0%), but enrichment factors of 94–460 were obtained. The proposed μ-SPE-HPLC method was applied to the determination of BAs in orange juice purchased from local supermarkets, with satisfactory results. The orange juices were characterized by the presence of relatively high levels of putrescine (range, 550–2210 μg L^{-1}) but tryptamine and phenylethylamine were not detected in any of the tested samples.

1. Introduction

Biogenic amines (BAs) are organic bases with either aromatic (e.g., tyramine, phenylethylamine, histamine, and tryptamine) or aliphatic (e.g., putrescine and spermidine) structures (Fig. 1). They occur as natural constituents in living organisms that are formed and broken down by metabolic processes in plants, animals and microorganisms [1]. They are needed in low concentrations for physiological functions such as cell proliferation, fruit growth and development, and stabilization of cell membranes [2,3].

BAs can also be found in a variety of foods such as fish, cheese, wine, beer, and fruit juices. They can be produced during storage or processing of products by thermal or bacterial enzymatic decarboxylation of amino acids [4]. Intake of high concentration of BAs in food poses some health concerns to the public due to their toxicological and physiological effects. These include migraine, nausea, hypertension and in severe cases, death and intracerebral haemorrhage [5,6]. BAs can also be potential cancer markers as rapid tumor growth has been associated with changes in polyamine concentration (e.g., putrescine and spermidine) [7]. Therefore, it is imperative to develop a suitable analytical technique to quantify BAs in view of their widespread presence.

Several analytical techniques have been reported for the determination of BAs in food, e.g., high performance liquid chromatography (HPLC) [8–11], thin layer chromatography [12] and capillary electrophoresis [13]. Of these, HPLC with ultraviolet detection (UV) is commonly used [14]. However, due to the lack of chromophores, derivatization of the BAs is often needed to increase the sensitivity of UV detection. Common derivatization agents such as dansyl chloride (Dn-Cl) [15], opthaldialdehyde [16] and dabsyl chloride [17] have been used. Dn-Cl is preferred as it can react with both primary and secondary amino groups, forming stable fluorescent and UV derivatives [2].

* Corresponding authors. Tel.: +65 6516 2995; fax: +65 6779 1691.
E-mail addresses: bahrud@usm.my (B. Saad), chmleehk@nus.edu.sg (H.K. Lee).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.04.073

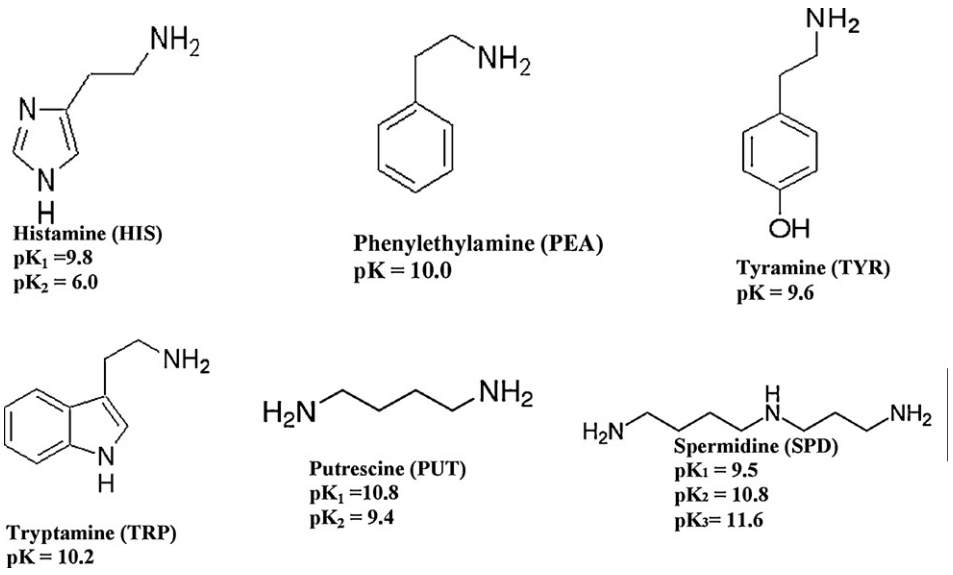

Fig. 1. Structure and pK_a of BAs studied.

Common sample preparation techniques prior to chromatographic or other analysis include liquid–liquid extraction (LLE) [8] and solid-phase extraction (SPE) [18]. The main drawbacks of LLE are the utilization of large amounts of toxic organic solvent and multiple extraction steps, which are not only tedious but also time consuming [19]. Although, SPE uses less organic solvent than LLE, the extraction procedures are nonetheless time consuming. Moreover, an SPE cartridge is generally non-reusable and the technique can therefore be relatively expensive.

Recently, a miniaturized, solvent-minimized extraction technique known as micro-solid phase extraction (μ-SPE) has been developed, in which the sorbent material is secured within a porous membrane envelope to afford an extraction device. The advantages of μ-SPE over SPE and LLE are the minimization of solvent, and ease and convenience of the extraction process. μ-SPE is also suitable for processing complex matrices as sample clean-up and extraction are carried out simultaneously. Sorbent materials such as C_{18}, C_2, C_8, HayeSep A, HayeSep B have been previously used in μ-SPE [20,21]. We have reported the use of sorbent materials for the extraction of BAs based on crown ethers [22] and the hydrazone (4-hydroxy-*N*′-[(*E*)-(2-hydroxyphenyl)methylidene]benzohydrazide) [23]. Both sorbents were studied in the SPE format where the BAs were extracted before the derivatization. The mechanisms of the retention were mainly based on hydrogen and charge–charge interactions, respectively. The crown ether-based sorbents exhibited good selectivity towards spermidine only, while the hydrazone-based sorbent showed high extraction efficiency towards all the aliphatic BAs, but not the aromatic BAs. A three phase liquid microextraction for the determination of BAs in food items was also performed, with the hope of enhancing the selectivity [24]. This approach resulted in good enrichment factors for all the tested BA standards. However, low recoveries and lack of sensitivity were found when the method was applied to real samples. Based on the experience these previous studies, it was anticipated that the derivatized BAs can be better extracted using sorbents that contained aromatic-based ligands. In this study, a few synthesized hydrazone ligands were physically trapped in the sol–gel network and used in the μ-SPE extraction of the derivatized BAs. The most suitable extraction conditions were assessed, and the technique was subsequently applied to the preconcentration of these compounds in orange juice, with HPLC determination.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade methanol, acetone, dichloromethane and acetonitrile were purchased from Tedia (Fairfield, OH, USA). Derivatization reagent dansyl chloride (Dn-Cl), 3-hydroxy-4-nitrobenzaldehyde, 2-hydroxy-5-nitrobenzaldehyde and 4-hydroxybenzhydrazide were purchased from Fluka (Buch, Switzerland). Sodium hydroxide of analytical grade was obtained from J.T. Baker (Philipsburg, NJ, USA). Concentrated sulfuric acid (98%) and hydrochloric acid (36%) were obtained from Acros Organics (Morris Plains, NJ, USA), benzophenone from BDH Chemicals (Poole, England), formaldehyde from Ajax Chemicals (Melbourne, Australia), salicylaldehyde from Riedel-De Haen (Steinheim, Germany), tetrahydrofuran (THF) from Lab-Scan Asia (Bangkok, Thailand). Spermidine trihydrochloride (SPD), tyramine hydrochloride (TYR), histamine dihydrochloride (HIS), putrescine dihydrochloride (PUT), phenylethylamine hydrochloride (PEA), tryptamine hydrochloride (TRP), ethanol 95% and tetraethoxysilane (TEOS) were obtained from Sigma–Aldrich (Milwaukee, WI, USA). Ultrapure water was produced from a Nanopure water purification system (Barnstead, Dubuque, IA, USA). Stock solutions containing 1000 mg L^{-1} TRP, PEA, PUT, HIS, SPD and TYR were prepared individually in water. A standard working solution mixture containing 10 mg L^{-1} of each BAs was then prepared in water by dilution. All standard solutions were stored at 4 °C.

2.2. Preparation of ligands

Benzophenone 2,4-dinitrophenylhydrazone (**I**), formaldehyde 2,4-dinitrophenylhydrazone (**II**), salicylaldehyde 2,4-dinitrophenylhydrazone (**III**), 3-hydroxy-4-nitrobenzaldehyde 2,4-dinitrophenylhydrazone (**IV**) and 2-hydroxy-5-nitrobenzaldehyde 2,4-dinitrophenylhydrazone (**V**) were synthesized as described previously [25–27]. In the preparation of ligands **I**–**V**, 2,4-dinitrophenylhydrazine was dissolved in ethanol (50 mL), followed by the addition of 5 mL of concentrated sulfuric acid in order to complete the dissolution of hydrazine. The mixture was added drop wise to equivalent amounts of benzophenone, formaldehyde, salicylaldehyde, 3-hydroxy-4-nitrobenzaldehyde, 2-hydroxy-5-nitrobenzaldehyde that had been separately dissolved in ethanol.

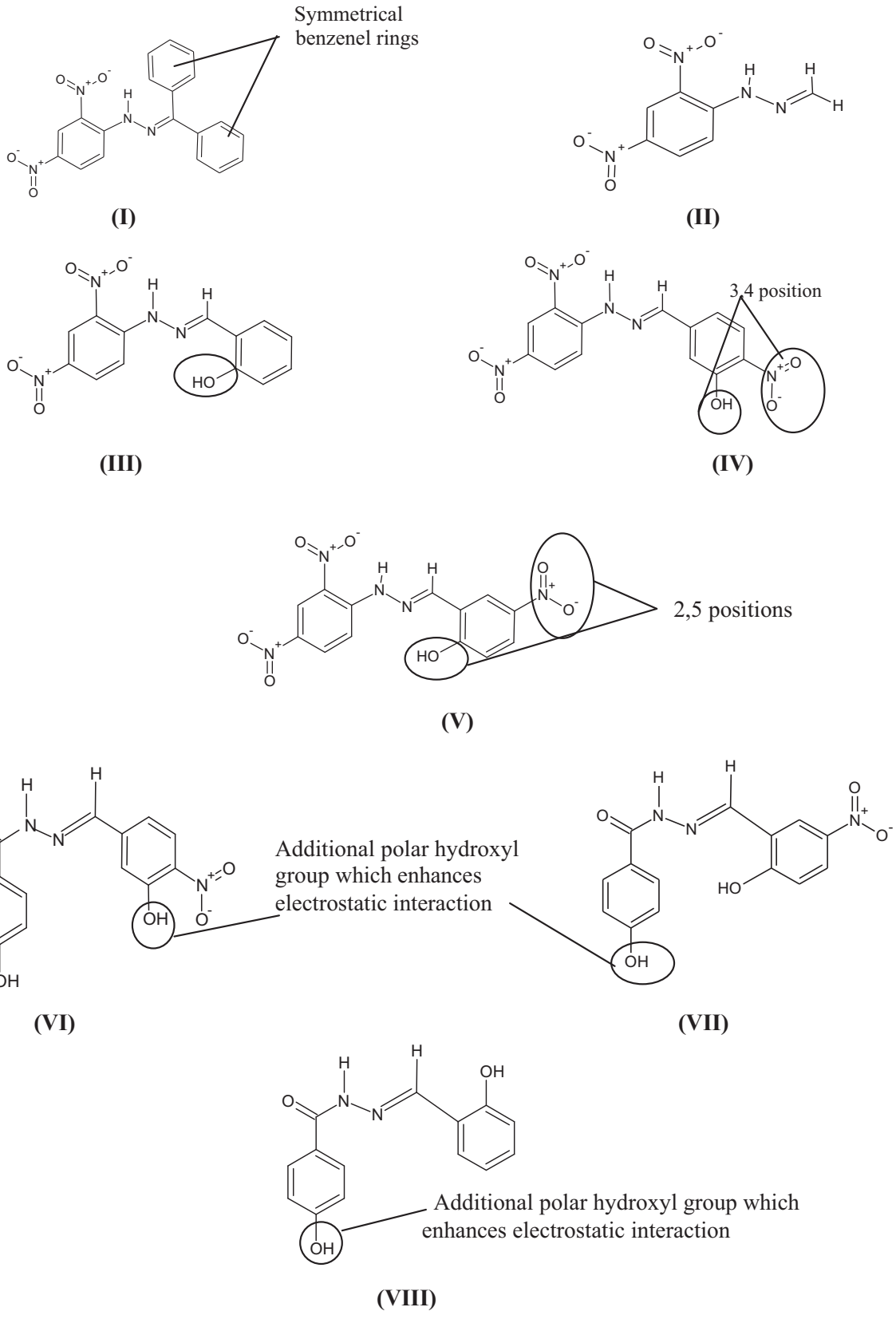

Fig. 2. Structures of hydrazone ligands studied.

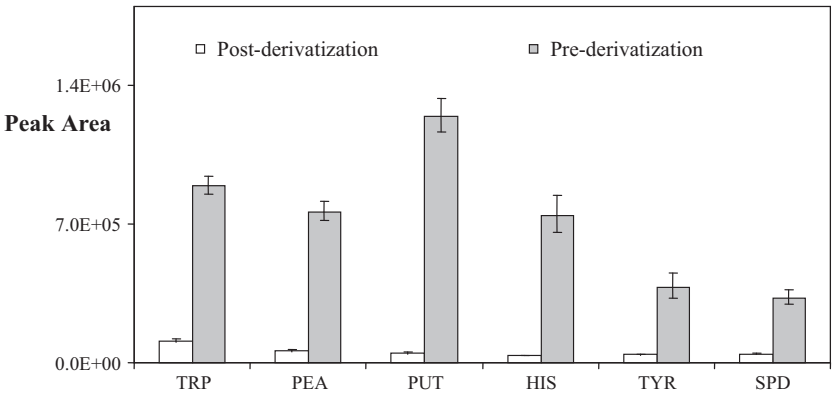

Fig. 3. Effects of pre- versus post derivatization on the extraction. Extraction conditions for (a) pre-derivatization: extraction time, 20 min; sorbent **VIII**; 50 μL of Dn-Cl was added to the sample solution and then heated to 70 °C for 10 min; stirring speed, 105 rad s^{-1}; desorption time, 10 min; and desorption solvent, acetonitrile; (b) post-derivatization: samples were extracted as earlier (without adding Dn-Cl and heating), the Dn-Cl was added to the desorption solvent, after the desorption (ultrasonication), the extract was then heated to 70 °C for 10 min.

The respective mixtures were stirred for 15 min, and left to stand further at room temperature for 30 min. The ligands 3-hydroxy-4-nitrobenzaldehyde-4-hydroxybenzoylhydrazone (**VI**), 2-hydroxy-5-nitrobenzaldehyde-4-hydroxybenzoylhydrazone (**VII**) and 4-hydroxy-*N*′-[(*E*)-(2-hydroxyphenyl)methylidene]benzohydrazide (**VIII**) were also synthesized (Fig. 2) according to our previous report [28]. A solution of 3-hydroxy-4-nitrobenzaldehyde, 2-hydroxyl-5-nitrobenzaldehyde or salicyaldehyde in methanol (10 mL) was separately added drop wise to a methanol solution that contained an equivalent amount of 4-hydroxybenzhydrazide and each mixture was refluxed for 2 h. The targeted ligands were collected after steam evaporation of the reaction solvent. All ligands were purified by re-crystallization from ethanol and methanol, and dried in air, except that ligand (**VIII**) was obtained in a crystalline form after evaporation of the reaction solvent.

2.3. Preparation of ligand immobilized sorbent

A mixture of TEOS (3.28 mL), ethanol (4.56 mL), and hydrochloric acid (0.36 mL, 4 M) was prepared and stirred for 15 min to yield a sol dispersion. Next, the hydrazone ligand (dissolved in 10 mL THF) was added to the sol dispersion and was stirred vigorously for 45 min. The solution mixture was clear and homogeneous, which was later aged in an oven (60 °C) for 2 days. The sol–gel that was formed was soaked in water for 1 day for conditioning. The sorbent was dried (at 60 °C) for 1 day and ground into small pieces using a mortar and pestle.

2.4. HPLC conditions

A Shimadzu (Kyoto, Japan) HPLC system, equipped with Shimadzu quaternary pump, SPD-20A UV detector and SIL-20A autosampler was used. Separation was carried out using COSMOSIL 5C18-MS-I column (250 mm × 4.6 mm) purchased from Nacalai (San Diego, USA), with detection wavelength set at 254 nm. A mobile phase comprising acetonitrile:water (70:30) was used. The flow rate was as follows: 1 mL min^{-1} (0–11.0 min), 1.5 mL min^{-1} (11.1–19.0 min), 1.0 mL min^{-1} (19.1–20.0 min).

2.5. μ-SPE

Q3/2 Accurel 2E HF (R/P) polypropylene sheet membrane (157 μm thickness, 0.2 μm pore size) was purchased from Membrana (Wuppertal, Germany). 600 μL microcentrifuge tubes, used for desorption were obtained from Axygen Scientific (Union City, CA, USA).

The μ-SPE device consisted of sol–gel sorbent materials (20 mg) that were enclosed within the polypropylene membrane sheet envelope. The device was prepared by folding over the longer edge of the polypropylene sheet (~0.5 cm width), the edge of the flap was heat-sealed to the main sheet. The fold-over section was trimmed

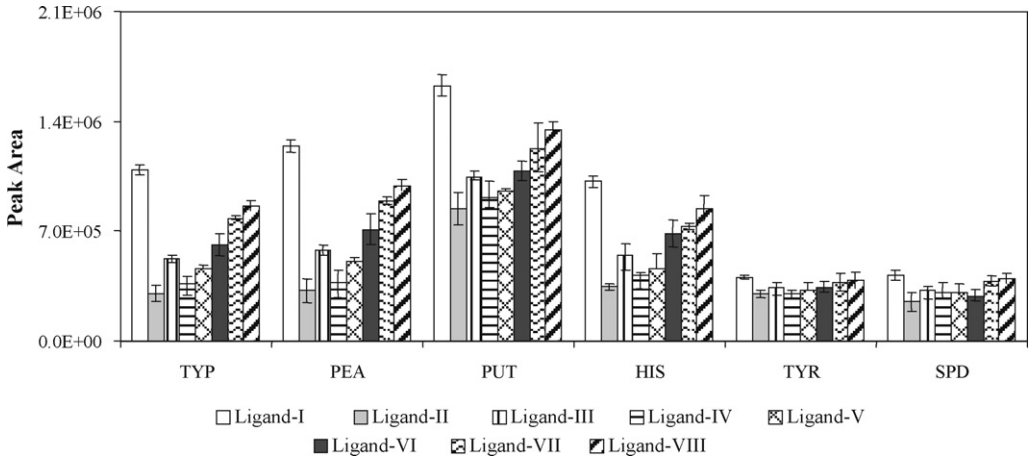

Fig. 4. Selection of suitable sorbent for the extraction of BAs. Extraction conditions for extraction time, 20 min; 50 μL of Dn-Cl was added to the 50 μg L^{-1} spiked sample solution and then heated to 70 °C for 10 min; samples were extracted for 20 min at stirring speed of 105 rad s^{-1}; desorption time, 10 min; and desorption solvent, acetonitrile.

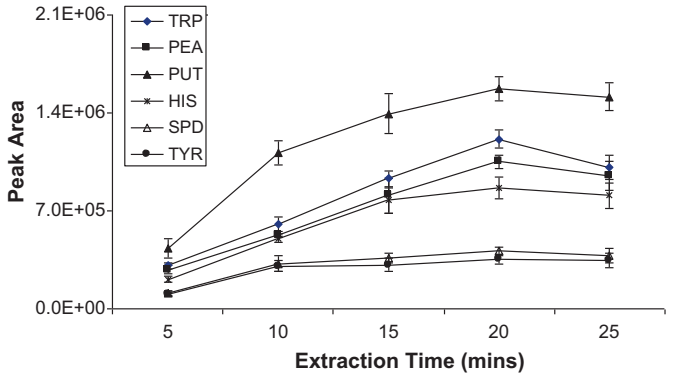

Fig. 5. Effects of extraction time on μ-SPE. Extraction conditions: sorbent, ligand **I**; 50 μL of Dn-Cl was added to the 50 μg L^{-1} spiked sample solution and then heated to 70 °C for 10 min; samples were extracted for 20 min at stirring speed of 105 rad s^{-1}; sample pH, 10; desorption time, 10 min; and desorption solvent, acetonitrile.

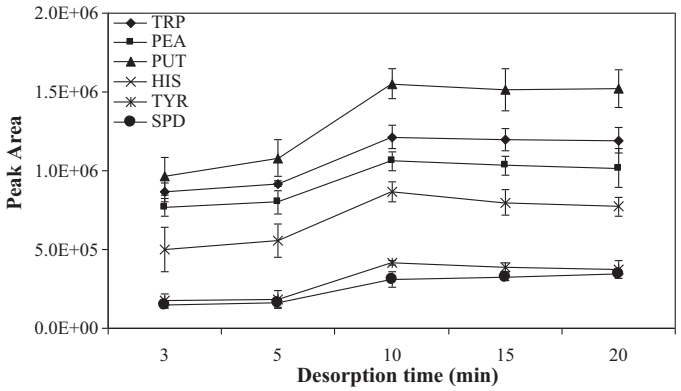

Fig. 6. Effects of desorption time on μ-SPE. Extraction conditions: sorbent, ligand **I**; 50 μL of Dn-Cl was added to the 50 μg L^{-1} spiked sample solution and then heated to 70 °C for 10 min; samples were extracted for 20 min at stirring speed of 105 rad s^{-1}; sample pH, 10; and desorption solvent was acetonitrile.

off from the main membrane sheet and cut into individual rectangular (0.5 cm × 1.5 cm) pieces. One of the two remaining open ends was heat-sealed. After the sorbent was introduced through the last open end, this end was heat-sealed to secure the contents.

Each μ-SPE device was conditioned by ultrasonication in water (5 min) followed by methanol (10 min). The device was then stored in methanol until use. Sample solutions (10 mL sample adjusted to pH 10 and spiked at various concentrations of BAs) were prepared, followed by the addition of 50 μL dansyl chloride (1 M in acetone). The solution was then heated at 70 °C for 10 min. The excess dansyl chloride was removed by adding 50 μL of 1 M glutamic acid. Subsequently, two μ-SPE devices were placed into the sample solution which was stirred at 105 rad s^{-1}. After extraction, the devices were removed with a pair of tweezers, dried with lint-free tissue and placed in a 600 μL micro-centrifuge tube. The analytes were desorbed in acetonitrile (100 μL) via ultrasonication (10 min). No further clean-up step was required. Lastly, the extracts (20 μL) were analysed by HPLC. The device could be reused after being ultrasonically cleaned in methanol.

Several parameters that affect the extraction efficiency were investigated, i.e. pre- or post-extraction derivatization, type of ligand, extraction time, desorption solvent, desorption time and number of μ-SPE devices used for each extraction. Each experiment was performed in triplicate and extraction efficiencies were indicated based on chromatographic peak areas.

2.6. Solid phase extraction (SPE)

The modified SPE procedure was adapted from previous work [29]. SPE was performed using commercially available Waters Oasis-HLB-SPE cartridges (200 mg, 6 mL) (Milford, MA, USA). The cartridge was first conditioned with methanol (5 mL), water (5 mL) at pH 4, 10 mM sodium hydroxide:methanol (70:30) solution (5 mL) followed by 5 mL of 10 mM calcium chloride:methanol (70:30) solution. Prior to SPE, analytes were derivatized with dansyl chloride as above (for the 10 mL sample, 50 μL dansyl chloride (1 M in acetone) was added and then heated at 70 °C for 10 min). The excess dansyl chloride was removed by adding 50 μL of 1 M glutamic acid. The derivatized sample solution was passed through the SPE cartridge under vacuum at a flow rate of 2 mL min^{-1}. Analytes were then eluted with methanol (5 mL). The eluates were reconstituted with acetonitrile (100 μL), after evaporation to dryness under a gentle stream of nitrogen gas. Twenty μL of extract was used for the HPLC analysis.

2.7. Orange juice samples

Five different brands of orange juice were bought from local supermarkets, the sample was used directly for the analysis of TRP, PEA, and HIS, while a series of dilutions were done for the analysis of the other BAs (PUT, TYR, and SPD) due to its high content in the selected samples.

3. Results and discussion

BAs ranging from 0 to 10% were extracted when blank sorbents were used The extraction was significantly enhanced when ligands were immobilized in the sol–gel matrix.

Initially, sorbent containing ligand **VIII** was used for the studies. Larger peak area was obtained from pre-derivatization, compared to the post-derivatization, extraction (Fig. 3). This is because ligand **VIII** (4-hydroxy-*N*′-[(*E*)-(2-hydroxyphenyl)methylidene]benzohydrazide), which is relatively non-polar, interacts better with the derivatized amines (relatively non-polar). Prior to derivatization, ligand **VIII** cannot compete with water effectively to extract the underivatized polar biogenic amines efficiently. Therefore, pre-derivatization extraction approach was selected.

3.1. Type of ligand

Selection of a suitable ligand is important as each ligand contains different functional groups, and hence has different affinity for the target analytes. Therefore, eight sol–gel sorbents containing immobilized hydrazone ligands **I**–**VIII** were investigated (Fig. 2).

The most hydrophobic ligand (**I**) achieved the highest extraction capability (Fig. 4). The additional benzene rings of **I** resulted in an enhancement of π–π interaction with the analytes. In contrast, the least non-polar ligand (**II**) gave the lowest peak areas, due to the weakest interaction with the analytes.

Ligands **III**–**V** have almost similar chemical structures except that ligand **III** contains a hydroxyl group as substituent, while ligands **IV** and **V** contain a hydroxyl and nitro group substituted at positions 3 and 4, and, 2 and 5, respectively. Between them, ligand **IV** experienced the least extraction efficiency, due to its bulky structure which conceivably hinders the bonding interactions between the analytes and the aromatic rings.

Generally sorbents based on ligands **VI**–**VIII** exhibited higher peak areas than those based on ligands **III**–**V** as they are less sterically hindered. In addition, ligands **VI**–**VIII** possess an additional polar hydroxyl group each, which may interact with the under-

Table 1
μ-SPE validation data.

Analyte	Linearity ($\mu g\,L^{-1}$)	RSD (%) ($n=3$)	LOD ($ng\,L^{-1}$)	LOQ ($ng\,L^{-1}$)	Correlation coefficient (r^2)	Enrichment factor
TRP	1–50	≤7.79	7.12	23.70	0.990	460
PEA	1–50	≤8.47	6.65	22.10	0.988	130
PUT	1–50	≤9.13	3.82	12.70	0.992	278
HIS	1–50	≤7.43	8.22	27.39	0.986	314
TYR	1–50	≤10.90	12.50	41.80	0.989	168
SPD	1–50	≤11.30	31.30	104.00	0.995	94

ivatized secondary amines. However, the electrostatic interaction between the target analytes and the sorbent was apparently not sufficient to achieve higher extraction efficiency, the hydrophobicity and π–π interaction were dominant, as observed with ligand **I**. Based on these results, sorbents containing ligand **I** appear to be most promising and was used for further studies (Fig. 2).

3.2. Extraction time

μ-SPE is a non-exhaustive extraction technique which is based on the partitioning of analytes between the sorbent materials and sample solution. Extraction time was evaluated between 5 and 25 min, at 5 min intervals. Fig. 5 shows that there was a rapid increase in extraction from 5 to 15 min, followed by a gradual increase to a maximum at 20 min. Extraction time beyond 20 min led to a slight decrease in peak area, which is often observed in non-exhaustive extraction techniques. This is due to the desorption of analyte beyond the optimum time.

3.3. Desorption conditions

The selection of a desorption solvent was based on several considerations such as the solubility of analytes and membranes, and polarity of the solvent. Taking into consideration that the analytes are relatively polar and both Dn-Cl and polypropylene membrane are unreactive with common organic solvents (e.g., acetonitrile, acetone, methanol and dichloromethane), these solvents were therefore tested. Acetonitrile was found to be the best solvent as it yielded the largest peak areas. Desorption of the target analytes were carried out by ultrasonicating the μ-SPE devices in acetonitrile (100 μL). Desorption time over 3–20 min was investigated. It was found that desorption was completed within 10 min (Fig. 6). Relative low peak areas were observed at shorter time due to incomplete desorption while there is no considerable increase in the peak area when desorption time exceeded 10 min. Thus, a desorption time of 10 min was selected.

Carryover effects were also examined by desorbing the μ-SPE devices in acetonitrile for the second time. No analyte was detected and the device could be reused for about 30 extractions. In addition, the extraction efficiency was not significantly affected when it was used repeatedly.

The influence of the number of μ-SPE device on the extraction efficiency was evaluated using one to three devices. There was no significant additional enhancement in the peak areas when more than two devices were used. Therefore, two μ-SPE devices were sufficient to achieve the maximum extraction efficiency.

3.4. Method validation

The adopted parameters that were used for the quantitative method evaluation studies were as follows: pre-extraction derivatization; sample pH, 10; ligand **I**; extraction time, 20 min; desorption solvent, acetonitrile; desorption time, 10 min; number of μ-SPE devices, 2.

The μ-SPE technique was evaluated by determining the linearity, repeatability, limits of detection (LOD), limits of quantification (LOQ), enrichment factor and recovery. The linearity of the method was investigated for five different concentrations over the range 1–50 $\mu g\,L^{-1}$. Calibration plots were obtained by plotting the peak areas of the analytes versus their corresponding spiked concentrations in water. Generally, BAs exhibited good linearity with correlation coefficient, $r^2 > 0.985$. Repeatability was determined at various analyte concentrations (1–50 $\mu g\,L^{-1}$), with triplicate analysis. Relative standard deviations (RSD) from 7.43 to 11.30% were achieved. The LOD was evaluated by at a signal-to-noise ratio (S/N) of 3, while the LOQ was determined at S/N = 10. The LODs and LOQs for BAs were found to be in the range of 3.8–31.3 and 12.7–104 $ng\,L^{-1}$, respectively (Table 1). These values were comparatively lower than those obtained in previously reported procedures [30–32].

Enrichment factors, determined by comparing the peak areas of analytes after and before extraction, were between 94- and 460-fold. TRP achieved the highest enrichment factor, the probable reason being that it has two aromatic rings which allow optimum interaction with the sorbent (Table 1). The π–π interaction with the sorbent is also enhanced by the TRP conjugated system. The presence of nitrogen atoms in TRP may permit hydrogen bonding interaction with the NO_2 groups of ligand **I**. HIS has the second highest enrichment factor due to the presence of the aromatic system to enhance π–π interaction. On the other hand, SPD achieved the lowest enrichment factor due to the presence of bulky aromatic rings (in the derivatized form), which conceivably hindered interaction between the ligand and the analytes.

Table 2
Comparison of μ-SPE and SPE recoveries with spiked samples.

Analyte	Recovery, % (samples spiked at 5 $\mu g\,L^{-1}$) ($n=3$)		Recovery, % (samples spiked at 25 $\mu g\,L^{-1}$) ($n=3$)	
	μ-SPE	SPE	μ-SPE	SPE
TRP	83.0	84.0	87.4	91.3
PEA	81.0	85.7	85.2	93.1
PUT	71.5	79.7	75.3	86.3
HIS	86.3	90.3	81.4	92.4
TYR	77.6	87.4	76.9	95.0
SPD	83.1	89.7	77.1	94.2
Mean ± SD	80.4 ± 5.2	86.1 ± 3.9	80.6 ± 4.9	92.1 ± 3.1

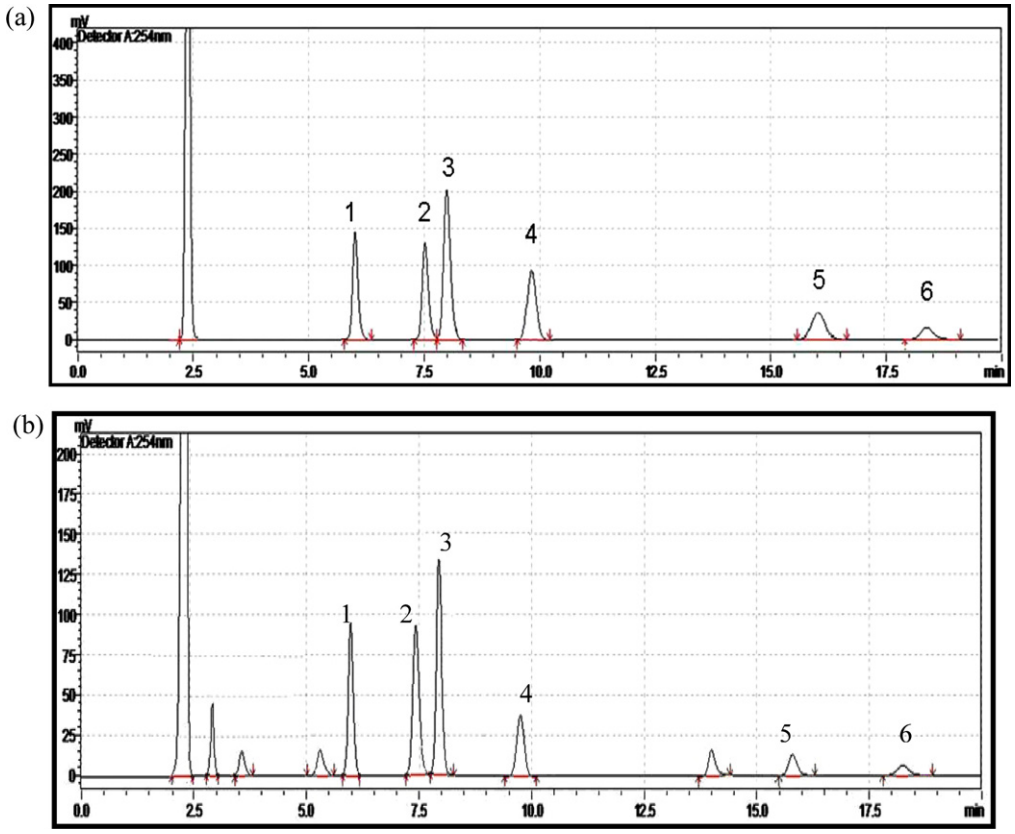

Fig. 7. Chromatogram obtained from the μ-SPE (a) ultrapure water that had been spiked with 50 μg L^{-1} of analytes conducted under the optimized extraction conditions and (b) analytes extracted from diluted orange juice spiked with 25 μg L^{-1}, optimized extraction conditions used. Peak identifications: (1) TRP, (2) PEA, (3) PUT, (4) HIS, (5), and (6) SPD.

3.5. Comparison of recoveries and enrichment factors of μ-SPE with SPE

The extraction principles of μ-SPE are similar to conventional SPE. The μ-SPE extraction recoveries were compared to SPE (conditions are given in the experimental section) by considering orange juice samples spiked at 5 and 25 μg L^{-1} concentrations. The comparison was carried out by evaluating the percentages of recoveries by standard addition method.

Generally, μ-SPE (Table 2) exhibited lower percentage recovery than the SPE technique as it is a non-exhaustive extraction technique. Nevertheless, the μ-SPE technique was able to achieve reasonable recoveries. The enrichment factor was evaluated by comparing the peak areas of the analytes after the extraction of analytes that were spiked to nanopure water, and the peak areas of the analytes without extraction. Fig. 7 shows the comparison chromatograms of ultrapure water spiked extract and orange juice spiked extract. The chromatograms demonstrate that no matrices effect was observed in μ-SPE procedure.

3.6. Analysis of genuine samples

The optimized μ-SPE method was applied to the determination of BAs in orange juice. Results are shown in Table 3. TRP and PEA were not detected in all the samples studied. Relatively low levels of HIS, TYR and SPD were noted, PUT ranging from 550 to 2210 μg L^{-1} was found. Previous studies with orange juice had also indicated the presence of PUT [33–36], HIS [36], TYR [36,37], SPD [34–36]. TYR is expected to be present in citrus fruit as it is a precursor of synephrine which is a bioactive amine in orange juice [38]. The presence of HIS in orange juice could be due to its protective role against predators in the plants [39]. Based on our findings, PUT was the predominant BA in all the orange juices tested. The presence of high amounts of PUT is characteristic of citrus fruits [34,35], in agreement with previous studies [11,33,40]. The presence of SPD was expected, as PUT, being the most prevalent amine in orange juice, is an intermediate in the synthesis of SPD [34,39,41].

Table 3
Concentration of biogenic amines found in different brands of orange juice.

Biogenic amines	Biogenic amines (μg L^{-1}) ($n=6$)				
	A	B	C	D	E
TRP	N.D.	N.D.	N.D.	N.D.	N.D.
PEA	N.D.	N.D.	N.D.	N.D.	N.D.
PUT	550 ± 60.5	1960 ± 217	2050 ± 238	2210 ± 250	700 ± 88.1
HIS	1.12 ± 0.01	2.6 ± 0.7	10.8 ± 1.7	40 ± 5.5	1.9 ± 0.2
TYR	15.3 ± 1.9	3.9 ± 0.4	24.7 ± 6.3	12.8 ± 1.1	64.2 ± 6.5
SPD	80.3 ± 13.6	139 ± 15.1	155 ± 24.6	160 ± 15.7	89 ± 10.0

N.D.: not detected.

4. Conclusion

The proposed μ-SPE, used in tandem with HPLC-UV, offers an interesting and effective option for the determination of BAs in orange juice. In addition, high enrichment factors of 94–460 were achieved due to the high affinity between the sol–gel sorbent that contained benzophenone 2,4-dinitrophenylhydrazone ligand and the target analyte. The BAs were first derivatized and then extracted, while in the previous work (using ligand **VIII**) [23] the BAs were extracted, desorbed and derivatized. The mechanism of extraction is based on π–π interactions, unlike in the earlier work

where the extraction is predominantly due to charge–charge interactions [23]. In contrast to the previous work [23], the proposed method is able to extract not only the aliphatic but also the aromatic BAs efficiently. The ligand can be easily synthesized at room temperature using a single step. The major advantages of μ-SPE over SPE include reduced solvent consumption and time, and high pre-concentration. Furthermore, the μ-SPE device can be easily fabricated, is inexpensive and reusable. Comparable recoveries (>71%) were obtained by both μ-SPE and SPE techniques. The porous polypropylene membrane of the μ-SPE device served as a barrier to exclude interferences; hence no further clean-up procedure was needed during the extraction.

Acknowledgements

Financial support by the National University of Singapore and Universiti Sains Malaysia Research University Grant scheme towards this research is gratefully acknowledged.

References

[1] M.H. Silla Santos, Int. J. Food Microbiol. 29 (1996) 213.
[2] A. Zotou, Z. Loukou, E. Soufleros, I. Stratis, Chromatographia 57 (2003) 429.
[3] F. Kvasnička, M. Voldřich, J. Chromatogr. A 1103 (2006) 145.
[4] A. Önal, Food Chem. 103 (2007) 1475.
[5] P. Kalač, V. Hlavatá, M. Křížek, Food Chem. 58 (1997) 209.
[6] S. Moret, R. Bortolomeazzi, G. Lercker, J. Chromatogr. A 591 (1992) 175.
[7] N. Seiler, C.L. Atanassov, F. Raul, Int. J. Oncol. 13 (1998) 993.
[8] J. Kirschbaum, K. Rebscher, H. Brückner, J. Chromatogr. A 881 (2000) 517.
[9] P. Kalač, S. Švecová, T. Pelikánová, Food Chem. 77 (2002) 349.
[10] J. Lange, K. Thomas, C. Wittmann, J. Chromatogr. B 779 (2002) 229.
[11] S. Moret, D. Smela, T. Populin, L.S. Conte, Food Chem. 89 (2005) 355.
[12] J. Lapa-Guimarães, J. Pickova, J. Chromatogr. A 1045 (2004) 223.
[13] R.E. Paproski, K.I. Roy, C.A. Lucy, J. Chromatogr. A 946 (2002) 265.
[14] M. Saaid, B. Saad, N.H. Hashim, A.S. Mohamed Ali, M.I. Saleh, Food Chem. 113 (2009) 1356.
[15] O. Busto, Y. Valero, J. Guasch, F. Borrull, Chromatographia 38 (2000) 571.
[16] M.R. Alberto, M.E. Arena, M.C. Manca de Nadra, Food Control 13 (2002) 125.
[17] R. Romero, D. Gázquez, M.G. Bagur, M. Sánchez-Viñas, J. Chromatogr. A 871 (2000) 75.
[18] F. Calbiani, M. Careri, L. Elviri, A. Mangia, L. Pıstarà, I. Zagnoni, J. Agric. Food Chem. 53 (2005) 3779.
[19] H. Lord, J. Pawliszyn, J. Chromatogr. A 902 (2000) 17.
[20] C. Basheer, H.G. Chong, T.M. Hii, H.K. Lee, Anal. Chem. 79 (2007) 6845.
[21] C. Basheer, K. Narasimhan, M. Yin, C. Zhao, M. Choolani, H.K. Lee, J. Chromatogr. A 1186 (2008) 358.
[22] M. Saaid, B. Saad, I.A. Rahman, A.S.M. Ali, M.I. Saleh, Talanta 80 (2010) 1183.
[23] A.A. Tameem, B. Saad, A. Makahleh, A. Salhin, M.I. Saleh, Talanta 82 (2010) 1385.
[24] M. Saaid, B. Saad, A.S.M. Ali, M.I. Saleh, C. Basheer, H.K. Lee, J. Chromatogr. A 1216 (2009) 5165.
[25] A.A. Tameem, A. Salhin, B. Saad, I.A. Rahman, M.I. Saleh, S.-L. Ng, H.-K. Fun, Acta Crystallogr. E 62 (2006) o5686.
[26] A.A. Tameem, A. Salhin, B. Saad, I.A. Rahman, M.I. Saleh, S.-L. Ng, H.-K. Fun, Acta Crystallogr. E 63 (2007) o57.
[27] A.A. Tameem, B. Saad, A.M. Salhin, S.R. Jobas, H.-K. Fun, Acta Crystallogr. E 64 (2008) o679.
[28] A. Salhin, A.A. Tameem, B. Saad, S.-L. Ng, H.-K. Fun, Acta Crystallogr. E 63 (2007) o2880.
[29] A. Peña-Gallego, P. Hernández-Orte, J. Cacho, V. Ferreira, J. Chromatogr. A 1216 (2009) 3398.
[30] A. Okamoto, E. Sugi, Y. Koizumi, F. Yanagida, S. Udaka, Biosci. Biotechnol. Biochem. 61 (1997) 1582.
[31] Z. Loukou, A. Zotou, J. Chromatogr. A 996 (2003) 103.
[32] A.L. Cinquina, A. Calì, F. Longo, L. De Santis, A. Severoni, F. Abballe, J. Chromatogr. A 1032 (2004) 73.
[33] A. Tassoni, M.A. Germanà, N. Bagni, Food Chem. 87 (2004) 537.
[34] S. Bardócz, G. Grant, D.S. Brown, A. Ralph, A. Pusztai, J. Nutr. Biochem. 4 (1993) 66.
[35] K.A. Eliassen, R. Reistad, U. Risøen, H.F. Rønning, Food Chem. 78 (2002) 273.
[36] S.M. Vieira, K.H. Theodoro, M.B.A. Glória, Food Chem. 100 (2007) 895.
[37] R.T. Coutts, B.B. Glen, F.M. Pasutto, Adv. Drug Res. 15 (1996) 169.
[38] T.A. Wheaton, I. Stewart, Anal. Biochem. 12 (1965) 585.
[39] H.E. Flores, C.M. Protacio, M.W. Sign, Recent Adv. Phytochem. 23 (1989) 329.
[40] A.R. Shalaby, Food Res. Int. 29 (1996) 675.
[41] D.R. Walters, Phytochemistry 64 (2003) 97.