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Solid Phase Extraction Preconcentration and HPLC Determination of Biogenic Amines Using A Hydrazone Derivative Immobilized on Sol-Gel Matrix

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Abstract

A sorbent material based on a hydrazone ligand, formaldehyde-2,4–dinitrophenylhydrazone was prepared by immobilizing the ligand into a silica sol-gel matrix. The capability of the sorbent material for the extraction of seven biogenic amines was investigated. Under the optimum condition, the sorbent material exhibited preferential selectivity towards the aliphatic (putrescine, PUT; cadaverine, CAD; spermidine, SPD) and the heterocyclic (histamine, HIS) over the aromatic (tryptamine, TRY; 2-phenylethylamine, PEA, tyramine, TYR) biogenic amines.

Keywords: *Hydrazone, biogenic amine. Sol-gel, extraction*

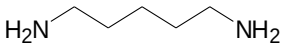
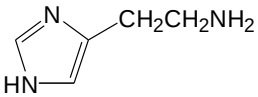
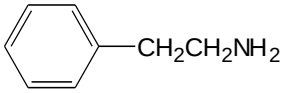
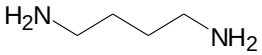
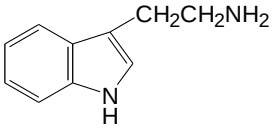
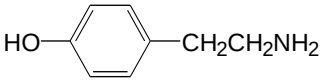
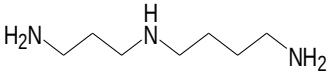
1. Introduction

Biogenic amines (BAs) are produced in plants and animals by microbial decarboxylation of amino acids resulted from removing α-carboxyl group to produce the corresponding amine. BAs can have an aliphatic, aromatic or heterocyclic structure, and their study is of interest for their role as biomarkers in toxicological risk studies and as indicators of food quality [1]. They have also toxicological effects but the exact toxicity threshold values for compounds are different, since the toxic dose is strongly dependent on the efficiency of the detoxification mechanisms of each compound. A considerable number of researchers had elaborately studied and reported BAs and their occurrences in human tissues [2], in plants [3], in foods and food products such as in fish and seafood [4,5], in meat products [6,7], in bean products [8], in fruits and vegetables [9,10], and in wine and alcoholic beverages [11,12]. Specifically, the most frequent BAs important in food analysis are the diamines, e.g. putrescine (PUT) and cadaverine (CAD); polyamines, e.g.

spermidine (SPD) and spermine (SPM); aromatic amines, e.g. tyramine (TYR); and heterocyclic amines, e.g. tryptamine (TRY) and histamine (HIS) [13]. Table 1 shows an example of these compounds. Generally, food products that depend on natural fermentation are more susceptible to biogenic amines as a result of a contaminating microflora exhibiting amino acid decarboxylase activity, such as cheese, soy bean products and alcoholic beverages[14].

There are many different analytical approaches advocated to extract BAs compounds from food products. Common methods applied for this application were: Liquid-liquid extraction method (LLE), Liquid-phase microextraction method (LPME), Solid-phase extraction method (SPE) [15] and Solid-phase microextraction method (SPME) [16].

Table1. Structures of biogenic amines [8]

Name	**Abbreviation**	**Structure formula**	**Molecular weight**
Cadaverine	CAD		175.1
Histamine	HIS		111.1
Phenylethylamine	PEA		121.2
Putrescine	PUT		88.2
Tryptamine	TYP		160.2
Tyramine	TYR		137.2
Spermidine	SPD		145.3

The abovementioned separation methods are usually used in conjunction with BA detection methods. The common separation and determination techniques used intensively for this purpose were known to be: Fluorescence-based methods [17], Capillary electrophoresis [18], Molecular/enzyme-based methods such as Polymerase Chain Reaction (PCR) method [19] and Enzyme-Linked Immunosorbent Assay (ELISA) method [20]. Chromatographic-based methods

such as the techniques of Thin Layer Chromatography (TLC) [21], High Performance Liquid Chromatography (HPLC) [22,23], Gas Chromatography (GC) [24]. Liquid Chromatography (LC) [25] and Ion-Exchange Chromatography (IC) [26].

Many methods have shown considerable variation in response to BA in terms of derivatization, suitability to BA compounds, detectability, recovery, selectivity, linearity, repeatability, reproducibility, sensitivity, accuracy, cost- and time-effectiveness, technical skills required, and instrumentation and preparation procedures. HPLC with pre- or post-column derivatisation is considered the most extensively reported method used in separation and quantification of BA due to its higher resolution, sensitivity, great versatility and simple treatment [27]. The present study comes profoundly to meet the aforementioned aspirations, by introducing hydrazone as a novel sorbent synthesized ligand. Since there is no work have been reported for the extraction of BAs using hydrazone compounds.

2. Experimental

Analytical grade chemicals, reagents and solvents were used as received without additional purification. 18 mΩ ultrapure water was used throughout. A Perkin Elmer 200-series HPLC unit and an Ultraviolet-visible lambda 35 UV -vis spectrometer were used for separation and detection, respectively. Stuart Scientific flask shaker SFI (500 osc min^{-1}) was used for the extraction. The chromatographic separation in the isocratic mode was carried out using a C_{18} ODS Hypersil column (250 × 4.5 mm, 5 µm) at ambient temperature. The mobile phase was 60:25:15 (v/v/v) acetonitrile:water:methanol at a flow rate of 1.0 mL min^{-1}; wavelength, 254 nm; and injection volume, 50 µL. The preparation of the ligand, the sorbent material and stock solution (1000 mg L^{-1}) of a mixture of seven BAs (TRY, PEA, PUT, CAD, HIS, TYR and SPD) were carried out as previously reported [28,29].

2.1 Characterization of the ligand and sorbent

Because of the failure of the infrared technique to provide a conclusive evidence on the presence of the hydrazone compound in the gel matrix, which could be attributed to the too small amount of the ligand in the network, solid state UV-Visible analysis was carried out. A comparison between the free ligand and its corresponding sol-gel sorbent was carried out using solid state UV-Vis technique in the region from 200-800 nm. The free ligand showed red shift with maximum absorption (λ_{max}) around 420 nm. The UV–Vis spectrum of the free ligand, blank and the hydrazone immobilized sorbent is shown in Fig. 1. The difference in the spectrum of the blank and sorbent is a strong evidence that indicates the successful incorporation of the hydrazone ligand into

the sol–gel network. However, incorporating the ligand into the silica-gel causes a shift in thewavelength (λ_{max}) of the free ligand ($\Delta\lambda_{max}$= -29). This could be due to the intermolecular interactions between the ligand and the silica which can change the characteristics of the absorption spectra.

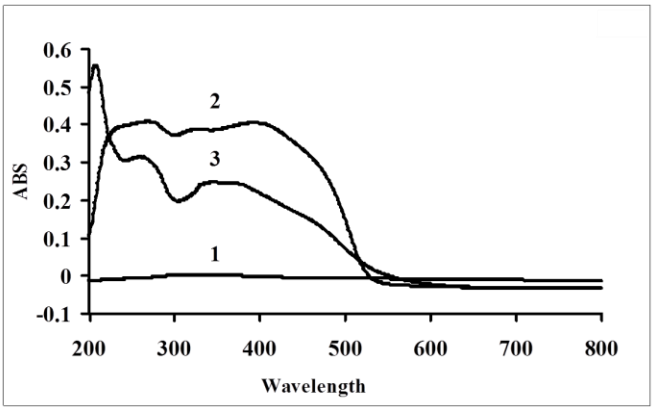


Figure 1. UV-Vis spectra of (1) blank sol-gel, (2) free ligand, and (3) sol-gel sorbent

2.2 Extraction method

Batch method of extraction was conducted. The sorbent (25 mg) was placed in a glass vial along with 1 mL BAs standard mixture and 4 mL of 0.1 M Tris buffer (pH 9). The mixture was shaken mechanically at room temperature for 15 min. After the equilibrium time, the mixture was filtered and the filtrate (1 mL) was derivatizedas previously reported [28]. The amount of the un-extracted BAs left in the solution after the extraction was determined by reversed phase HPLC (Fig. 2). The extracted amount was calculated by the difference.

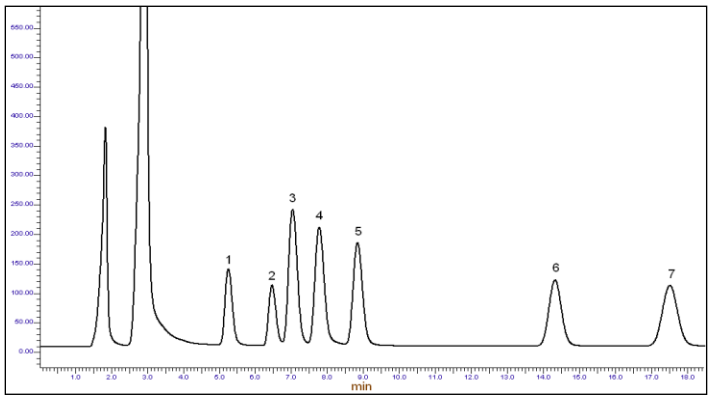


Figure 2. Chromatogram of BA (20 mg L^{-1} each) standard solution.

TRY (1); PEA (2); PUT (3); CAD (4); HIS (5); TYR (6); SPD (7).

3. Results and Discussion

The extraction was optimized by using 20 mg L^{-1} BAs standard solution. Important parameters that affects the extraction efficiency were investigated, i.e., sample pH, ligand concentration within the sorbent material, extraction time, and sorbent capacity.The extraction was also carried out using blank sorbent (i.e. does not contain the studied ligand) as control. The enhancement on the extraction behaviour between the blank and the ligand immobilized sorbents demonstrated the capability of hydrazone ligand in the extraction process (Fig. 3).

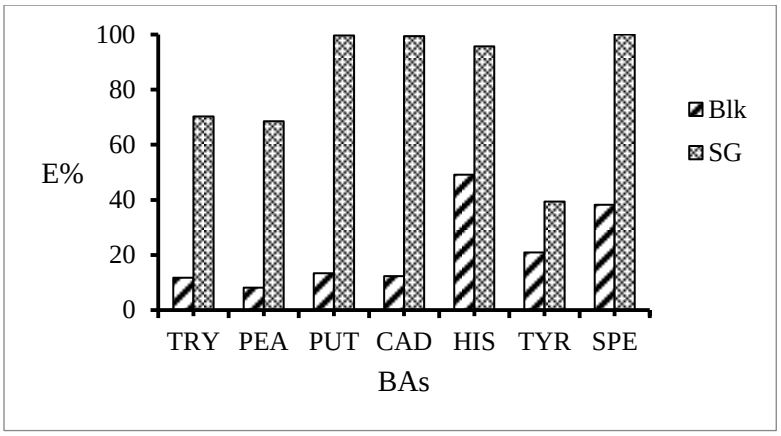


Figure 3: Extraction of BAs by the sol-gel matrix

3.1 Effect of contact time

The effect of contact time on the extraction efficiency was studied by shaking the BAs mixture with the sorbent material ranging from 5 to 60 min (Fig.4). Good extraction was observed after 5 min of contact time (%E >70 %) for all the studied BAs. However, maximum extraction was achieved after 15 min. Increasing the contact time further has no significant improvement of the %E. Therefore, 15 contact time was chosen for subsequent studies.

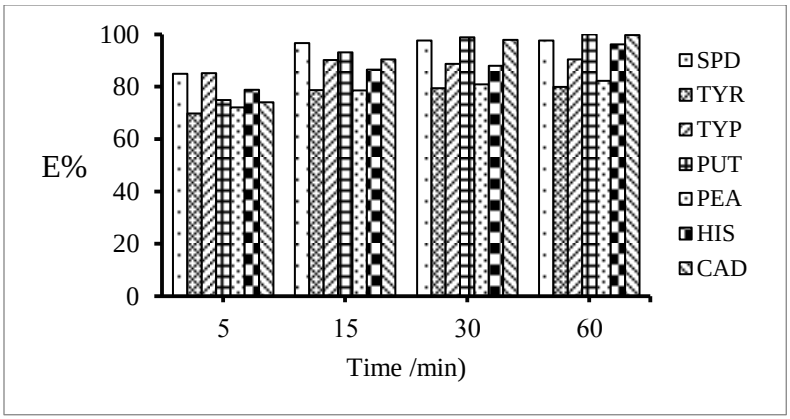


Figure 4: Effect of contact time on the extraction of BAs

3.2 Effect of ligand concentration in the sol-gel network

Sorbent materials containing different concentrations of ligand (0.85% - 6.8%)were prepared and their extraction efficiency was examined at the optimum pH 9 and extraction time of 15 min. It was observed that the highest extraction (%E >80) for all BAs was obtained when 0.85% ligand in the sorbent was used (Fig.5). Further increase of the ligand concentration does not seem to affect the extraction efficiency.

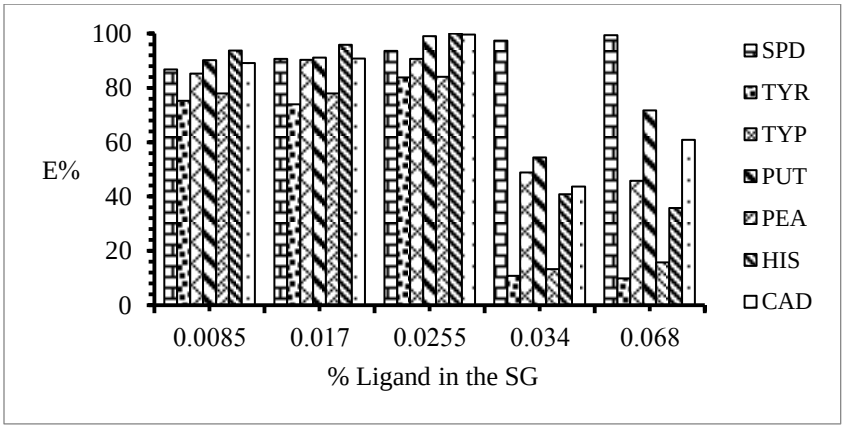


Figure 5 : Effect of ligand concentration on the extraction of BAs

3.3 Effect of BAs concentration

Five different concentrations (50 - 400 mg L^{-1}) of BAs were used to study the extraction efficiency and the capacity of the sorbent (Table2). It was found that the aliphatic BAs (PUT, CAD, and SPD) were quantitatively extracted (%E 100 %) up to 200 mg L^{-1}. At concentrations higher than 200 mg L^{-1} a decrease in the extraction of PUT and CAD was observed, while no decrease was found for SPD. The aromatic BAs were also poorly extracted than the aliphatic. The presence of the aromatic ring resulted in a decrease in the extraction efficiency due to steric effects. HIS was extracted more than the other aromatic BAs due to its smaller size.

Table 2 Effect of BAs concentration

BAs	Concentration (ppm)											
	5		50		100		200		400		500	
	BLK	SG	BLK	SG	BLK	SG	BLK	SG	BLK	SG	BLK	SG
TYR	0	100	64.10	77.68	47.18	67.36	38.23	56.13	34.42	46.66	32.46	45.67
PEA	0	0	0	24.01	4.94	31.02	9.19	29.37	13.43	29.92	14.92	31.19
PUT	54.15	100	29.47	100	30.18	100	21.08	100	21.67	61.61	24.05	51.51
CAD	50.19	100	35.76	100	34.29	100	24.31	97.42	24.32	52.97	27.15	44.54
HIS	100	100	74.75	99.39	48.36	99.85	36.02	68.89	30.17	44.29	31.43	42.06
TRY	0	51.30	13.37	61.41	14.07	32.43	11.51	30.61	18.29	28.10	33.06	29.77
SPD	57.19	100	48.46	100	44.99	100	34.03	100	42.94	98.95	53.17	95.92

BLK= blank sol-gel, SG= immobilized sol-gel (sorbent)

4. Conclusion

The studied hydrazone ligand shows preferential selectivity towards the aliphatic (PUT, CAD, SPD) and the heterocyclic (HIS) over the aromatic (TRY, PEA, TYR) BA. The high extraction efficiency of the sorbent was achieved due to the high affinity between the sol-gel sorbent that contained the hydrazone ligand and the target analyte. A significant advantage of

hydrazone compounds when compared with sorbents based on other ligands such as crown ethers is that the hydrazones can be easily synthesized using a one simple step at room temperature.

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