PÉTER FORGÓ*, ATTILA KISS

DEVELOPMENT AND ADAPTATION OF NOVEL HPLC PROCEDURE TO CHARACTERIZE BIOGENIC AMINES IN FOOD SAMPLES

Abstract: A high performance chromatographic (HPLC) method for the simultaneous determination of seven biogenic amines (histamine (HI), tryptamine (TR), tyramine (TY), putrescine (PUT), cadaverine (CAD), spermidine (SPD) and spermine (SPM)) in food products has been developed. The technique was applied to determine the biogenic amine content of wine and bear samples, which were treated with dansyl-chloride to form dansyl derivatives of biogenic amines. A gradient elution program was applied in the separation process involving water (A) and acetonitrile (B) solvents. Quantitative detection of the compounds was carried out with light absorbance at 336 nm, the components were identified with a mass-spectrometer detector by their molecular mass. The sensitivity of the applied method was 3-5μM for the studied derivatives. The method was tested on commercially available beer and wine samples, where histamine was proven to have the highest level (0.25-0.32 mM in beers, while wines showed higher histamine level 0.33-0.41mM). Beers contained detectable amount of tryptamine (around 0.01 mM), while wines had notable tyramine and tryptamine content.

Keywords: food products, biogenic amine, HPLC-MS, derivatization, diode-array detection.

Introduction

Biogenic amines (BAs) represent a unique class of natural compounds and can be characterized as small or medium sized organic amine based derivatives with aliphatic, aromatic and heterocyclic moieties.[1] Biogenic amines are produced in biochemical metabolism during fermentation or brewing food production processes[2] by microbial degradation[2, 3] and decarboxylation process from amino acids associated with food spoilage. Moreover, biogenic amines can be formed "in vivo" from the corresponding aldehydes[4]. The members of this molecular class can be found in a wide range of food products including fish, meat, vegetables, fruits, nuts, dairy products, brewed drinks and fermented food products. However, the total biogenic amine content is strongly correlated to the

* Eszterházy Károly College, EGERFOOD Regional Knowledge Centre, Leányka str. 6, H-3300, Eger, Hungary. Corresponding author: Dr. Peter Forgo, forgop@ektf.hu

microorganisms present in the sample. Several studies have pointed out their high biological activity that can increase hypertension as tryptamine[5], can affect cardiovascular, muscle, neural, gastric functions[6, 7] and can cause food poisoning as histamine. Furthermore, the members of the family have a potential to form carcinogen nitrosamines with nitrite.[8] Nevertheless, the putrescine based polyamines play an important role in cellular growth and metabolism.[9]

As a result of the high biological relevance, several analytical methods have been developed for the qualitative and quantitative detection of biogenic amines. Fluorescence based protocols have been developed for the detection of histamine in fish[10, 11], cheese and other fermented food products[12]. Thin layer chromatographic methods have been applied to determine natural[13] and derivatized compounds[14] involving densitometric detection[13]. Gas-chromatographic and HPLC[15] methods coupled with diode array and mass spectroscopic detection[16] have been utilized to study the biogenic amine content in brewed[17] and other food products[18, 19, 20]. Capillary electrophoretic[21, 15] separation and pulsed amperometric[22] detection methods have also been used to determine biogenic amine contents in several food products. Electrochemical biosensors[23] have also been widely used in the detection of biogenic amines.[24, 25]

Our purpose was to develop an efficient HPLC-MS method in order to detect biogenic amines in food products and brewed drinks. The method was optimized for the detection and quantitative analysis of seven biogenic amines: histamine (HI)(**1**), tryptamine (TR)(**2**), tyramine (TY)(**3**), putrescine (PUT)(**4**), cadaverine (CAD)(**5**), spermidine (SPD)(**6**) and spermine (SPM)(**7**), the experiments were run with the presence of diamino-heptane (DAH)(**8**) as internal standard.

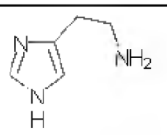
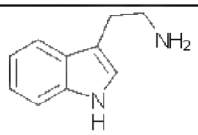
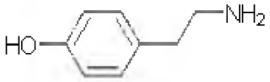
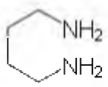
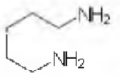
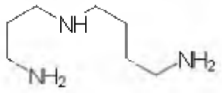
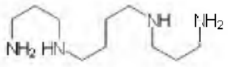
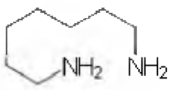
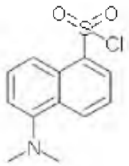
		
Histamine (HIS) (1)	Tryptamine (TR) (2)	Tyramine (TY) (3)
		
Putrescine (PUT) (4)	Cadaverine (CAD) (5)	Spermidine (SPD) (6)
		
Spermine (SPM) (7)	Diamino-heptane (DAH) (8)	Dansyl-chloride (9)

Figure 1. The structure of the investigated compounds.

Experimental procedure

Approximately 9 mg of the standard seven biogenic amines and diamino-heptane (DAH) were dissolved in a 25 ml volumetric flask. A standard dansyl-chloride(9) solution was also prepared in acetone solution (23.6 mg dansyl-chloride in 1.18 ml acetone). Standard solutions (250, 200, 150, 100 and 50 µl from the stock solution were used to dilute the solution to 0.85 ml) were prepared by the dilution of the stock solution and adding sodium-hydroxide and dansyl-chloride. The standard solutions were kept in 60°C for one hour, then the excess dasyl-chloride was decomposed by the addition of ammonia (50 µl). After filtration and addition of acetonitrile (0.5 ml) 10µl of the standard solution was injected for HPLC analysis. Real samples were treated the same way as standard solutions, 250 µl of wine and beer samples were used for the HPLC analysis with the addition of sodium-hydroxide and dansyl-chloride stock solutions. The derivative producing reaction took place during one hour at 60°C and the excess dasyl-chloride was decomposed by the addition of ammonia (50 µl). After filtration and addition of acetonitrile (0.5 ml) 10µl of the sample solution was injected for HPLC analysis. A Shimadzu LCMS-2010 HPLC instrument was used for the separation, which was coupled to a mass detector with atmospheric pressure chemical ionization (APCI) in the m/z range of 100-1200 amu with positive ion detection mode and 1.5 kV detector voltage. The diode-array detector was used for quantitative analysis and it covered a 190-800 nm wavelength range, the biogenic amine content was analyzed at 336 nm. The chromatographic column was a YMC-Pack ODS-AQ 250x4.6 mm, 5µm. A gradient elution was carried out with acetonitrile (B) and water(A) solvents at a flow rate of 0.4 ml/min and with the following gradient profile: 0 min (65% B), 2 min (65% B), 20 min (80 % B), 24 min (90 % B), 32 min (100 % B), 40 min (100 % B), 42 min (90 % B), 44 min (80 % B), 47 min (65 % B).

Results and discussion

Specific chromatographic conditions have been elaborated and adjusted throughout our experiments. Variable parameters have been tested such as eluents and flow rates. Acetonitrile-water solvent system with gradient elution proved to be the most efficient, while 0.4 ml/min was found to be most appropriate flow rate.

The seven biogenic amine derivatives and the reference diamino-heptane appeared separately in the chromatogram with the following retention times: HIS: 11.19 min, TR: 18.83 min, PUT: 22.71 min, CAD: 24.30 min, DAH: 28.61 min, TY: 32.55 min, SPD: 33.58 min, SPM: 38.96 min. (Figure 2). The signals in the chromatograms were assigned by their mass spectrum extracted from the total ion chromatogram (Figure 3). The calibration curve of the signal intensities as a

function of the injected amount of eight derivatives showed linear characteristics in the $5X10^{-10}$-$5X10^{-9}$ mole concentration region (figure 4). The calculated sensitivity using the calibration curves fall to the 3-5μM concentration region. These experimental conditions were used to determine the biogenic amine content of commercially available ten beer and five wine samples The main biogenic amine component was histamine (HIS), the detected level varied between 0.25 mM and 0.31 mM. The tryptamine content was around 0.01 mM in the ten beer samples, tyramine, putrescine, cadaverine, spermine and spermidine could not be determined quantitatively in beer samples. The histamine content of the five wine samples were a little higher than in beers, the values varied between 0.33 mM and 0,41 mM. Tryptamine content was proven to be between 0.05mM and 0.06mM, while the thyramine concentration was even more lower (0.006-0.008 mM). Putrescine contrentrations in wine samples were between 0.03 mM and 0.04 mM. Cadaverine, spermine and spermidine could not be determined quantitatively in wine samples.

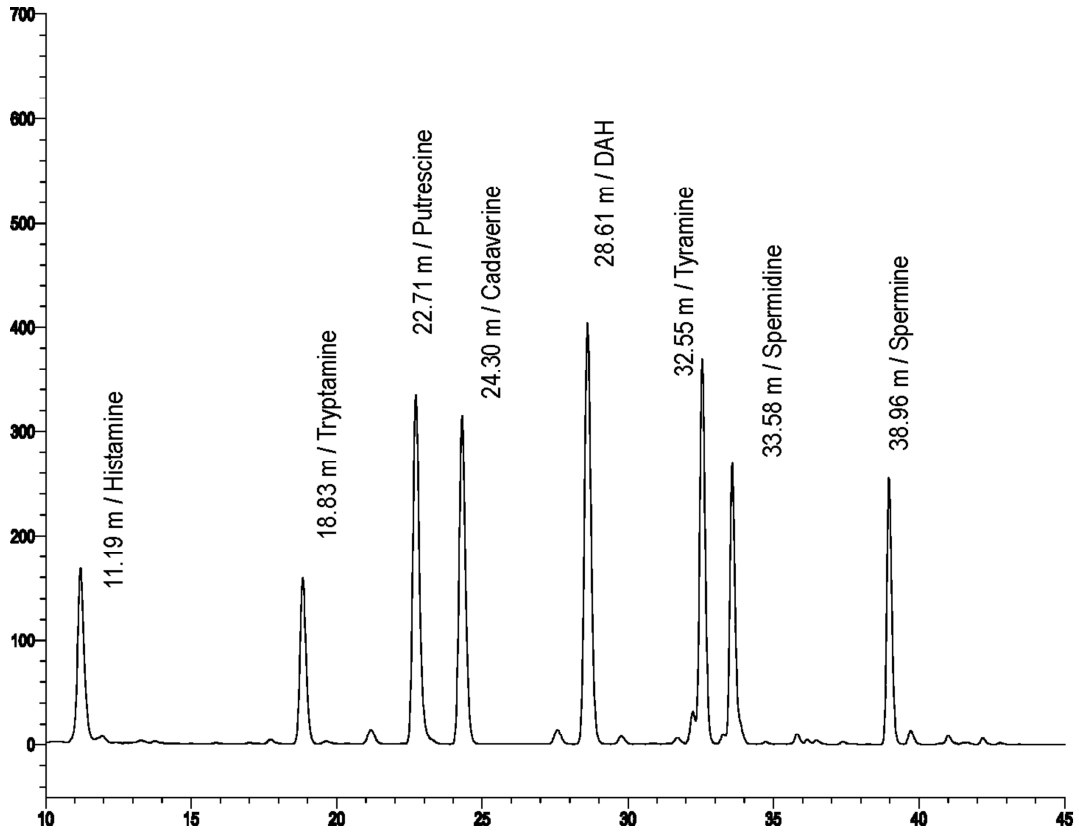


Figure 2. Chromatogram of the biogenic-amine reference solution with the highest concentration detected with a diode-array detector at 336 nm.

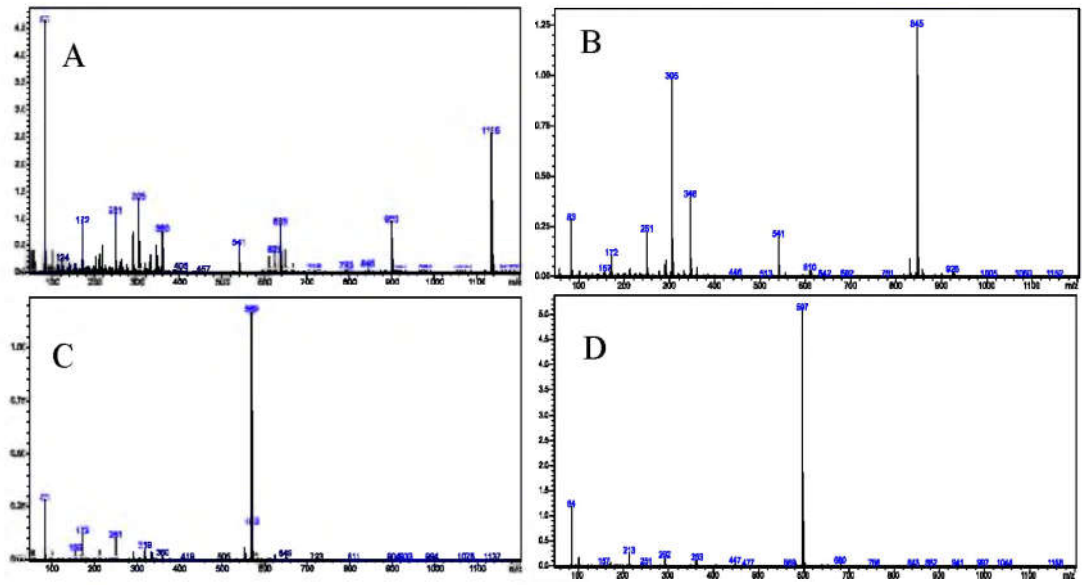


Figure 3. Mass-spectra of four selected biogenic amine derivatives, A- SPM, B- SPD, C- CAD, D- DAH.

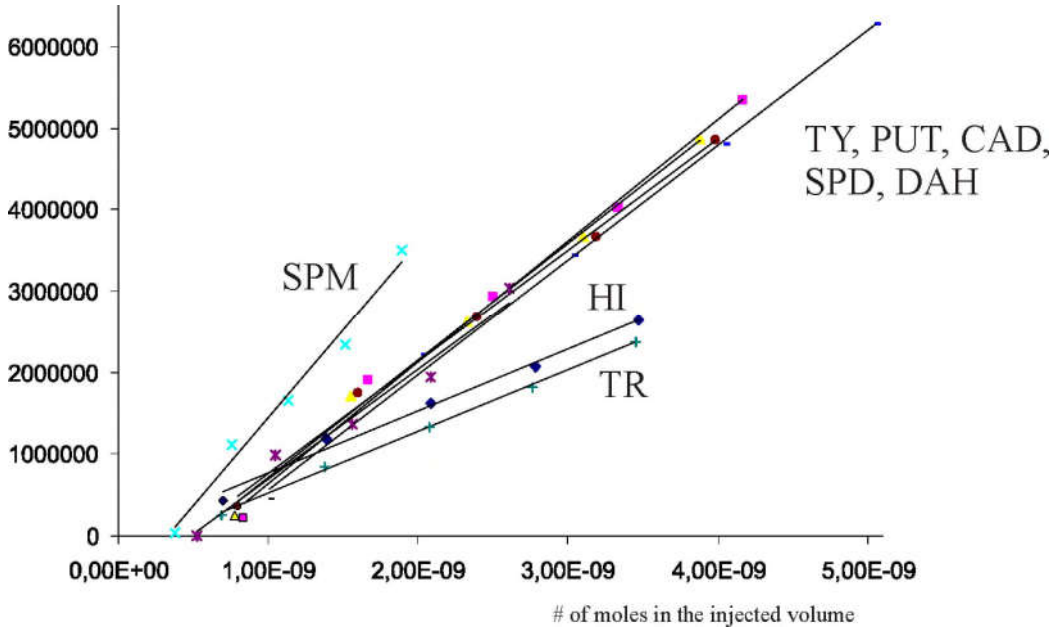


Figure 4. The calibration curves for the biogenic amine derivatives, signal intensities at 336 nm as a function of the injected number of moles.

Table 1. The obtained values of biogenic amine content for commercially available light beers and wines.

	Histamine	Tryptamine	Tyramine	Putrescine
Beer #1	0,28	0,009	nd	nd
Beer #2	0,31	0,010	nd	nd
Beer #3	0,25	0,008	nd	nd
Beer #4	0,27	0,009	nd	nd
Beer #5	0,32	0,010	nd	nd
Beer #6	0,27	0,009	nd	nd
Beer #7	0,26	0,008	nd	nd
Beer #8	0,27	0,009	nd	nd
Beer #9	0,29	0,009	nd	nd
Beer #10	0,31	0,010	nd	nd
Wine #1	0,38	0,061	0,007	0,042
Wine #2	0,41	0,066	0,008	0,046
Wine #3	0,33	0,053	0,006	0,037
Wine #4	0,37	0,060	0,007	0,041
Wine #5	0,39	0,063	0,007	0,043

Conclusions

A reliable chromatographic method was developed to determine the biogenic amine content in food products, the seven biogenic amine derivative can be reliably determined in food matrices. The method uses a dansyl derivative making step which allows the uv/vis detection of the amine derivatives. The studied ten beer products had histamine levels between 0.25 mM and 0.32 mM, the second detectable biogenic amine was tryptamine, but with much lower levels. In case of wine samples the histamine level was found to be higher (0.33-0.41 mM) and tryptamine, tyramine and putrescine were also in detectable amount. The method can be generally used to detect biogenic amines in food product with the use of appropriate sample preparation protocol.

Acknowledgements

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