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VISUAL EXPERIMENT

Simultaneous detection and quantification of different biogenic amines

Vishal Kumar, Ashutosh Bahuguna, and Myunghee Kim

Department of Food Science and Technology, Yeungnam University, Gyeongsan 38541, Gyeongsangbuk-do, Korea.

Correspondence: foodtech@ynu.ac.kr

First two authors contributed equally

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ABSTRACT

Biogenic amines are a group of low molecular weight nitrogenous organic compounds that have an important physiological role in cell proliferation, differentiation, and signal transduction. Also, certain biogenic amines act as an important biomarker for the detection of neuroendocrine and cardiovascular disorders in humans. Besides this, the abundance of biogenic amines in food is recognized as a toxin and anti-nutritional element that have several health implications. This visual experiment demonstrates all the critical steps required for the extraction of biogenic amines followed by derivatization and high performance liquid chromatography analysis for the successive detection and quantification of biogenic amines such as histamine, cadaverine, tryptamine, agmatine, putrescine, 2-phenylethylamine, spermine, tyramine, and spermidine.

INTRODUCTION

Biogenic amines are the physiological important group of low molecular weight nitrogenous compounds that are mainly formed by the decarboxylation of the amino acids catalyzed by endogenous enzymes or by microorganisms. Based on the presence of the amine group biogenic amine can be classified as monoamines (tyramine, 2-phenylethylamine, histamine, tryptamine, dopamine), diamines (putrescine, cadaverine), and polyamines (spermine and spermidine) (Linares et al., 2011). Moreover, biogenic amines can be classified further based on their biological role e.g. vasoactive biogenic amines (tryptamine and tyramine), psychoactive biogenic amines (histamine, cadaverine, and putrescine), and neurotransmitter biogenic amines (dopamine, norepinephrine, epinephrine, serotonin, and histamine) (Linares et al., 2011). Biogenic amines play an important role in cell propagation and differentiation, signal transduction, and membrane stability. Besides many biogenic amines found vital to regulate body temperature, blood pressure, nutrient intake, neural transmission, allergic response, and the synthesis of alkaloids and hormones (Plenis et al., 2019). Additionally, biogenic amines are also characterized as important biomarkers in the diagnosis, prognosis, and therapy of cardiovascular and neuroendocrine disorders such as Parkinson's, schizophrenia, and neuroendocrine tumors. Active involvement of biogenic amines such as histamine, dopamine, and serotonin were observed in many neurological and neuroinflammatory diseases (Sánchez-Jiménez et al., 2013). Besides the endogenous synthesis of biogenic amines, food is the major exogenous source of biogenic amines for humans and animals. In food, the excessive-high concentration of biogenic amines reflects poor food quality and is a major cause of food poisoning (Naila et al., 2010). Notably, intake of food with an abundance of biogenic amines, like putrescine, tyramine, and 2-phenylethylamine results in high blood pressure and is demonstrated to cause heart disease and brain hemorrhage (Naila et al., 2010). The most concerning biogenic amine in food is histamine responsible for histamine poisoning (scombroid poisoning) (Naila et al., 2010). Owing to the high toxicity of histamine, many food regulatory authorities recommended a legal limit of histamine in food. United states food and drug administration (US FDA) suggested 50 ppb as the maximum limit of histamine for fishes (US FDA, 2011). Likewise, Korea and China suggested 200-400 μg/kg as the maximum allowable limit of histamine in fermented foods (MFDS, 2017; CNS 2016). Knowing the various physiological and toxic roles of biogenic amines, their estimation in animals and food is of great interest, however, identification and quantification of the biogenic amines is a complex process. Keeping this in mind the visual experiment

was designed to demonstrate the sequential methodology for the extraction of biogenic amines, followed by derivatization to improve signals for rapid, accurate, and simultaneous detection of various biogenic amines employing high performance liquid chromatography (HPLC).

MATERIALS AND EQUIPMENT

1. Standard biogenic amines (Sigma-Aldrich, USA)
    a) Agmatine
    b) Tryptamine
    c) 2-Phenylethylamine
    d) Putrescine
    e) Cadaverine
    f) Histamine
    g) Tyramine
    h) Spermine
    i) Spermidine
2. Perchloric acid
3. Sodium hydroxide (NaOH)
4. Sodium hydrogen carbonate ($NaHCO_3$)
5. Dansyl chloride
6. Ammonium acetate
7. Acetonitrile
8. Ammonium hydroxide (NH_4OH)
9. Falcon tubes (50 mL)
10. Eppendorf tube (5 mL)
11. Oak Ridge centrifugation tube (50 mL)
12. Glass funnel
13. Micropipettes
14. Whatman filter paper 1
15. Syringe filters (0.2 µm)
16. Nylon membrane filters (0.2 µm)
17. Syringe (1 mL)
18. Vacuum filtration assembly
19. Glass vials (HPLC) with pre-slitted lids
20. Water bath
21. Centrifuge
22. C_{18} column
23. HPLC machine equipped with UV detector
24. Biological sample (*doenjang:* a traditional Korean fermented food, and tuna fish)

PREPARATION OF REAGENTS

1. 0.4 M perchloric acid (measure 34.5 mL perchloric acid and mix in the distilled water to make up the volume of 1 L).

2. 2 M NaOH (In 50 mL of distilled water dissolve 4 g of NaOH).
3. Saturated solution of sodium hydrogen carbonate (dissolve 12 g of $NaHCO_3$ in 100 mL of distilled water, mix well and subsequently filter through Whatman filter paper 1).
4. Dansyl chloride solution (10 mg/mL) [dissolve 0.25 g of dansyl chloride in 25 mL of acetone].
5. 25% ammonium hydroxide (Mix 2.5 mL of NH_4OH in distilled water to make the volume 10 mL)
6. 0.1 M ammonium acetate (In 500 mL of distilled water dissolves 3.85 g ammonium acetate). The prepared solution was vacuum filtered through 0.2 µm nylon membrane filters followed by 10 min of sonication for degassing.
7. Standard biogenic amine (dissolve 1 mg of different biogenic amines in 1 mL of distilled water).

PROTOCOL

EXTRACTION OF BIOGENIC AMINES

1. 10 g of sample was precisely weighed in a falcon tube (50 mL). Subsequently, 25 mL of 0.4 M perchloric acid was added and the sample was homogenized at ~10,000 rpm. After 3 min of homogenization, the content was transferred into Oak Ridge centrifugation tube (50 mL) and centrifuged at ~3,500 × *g*.
2. After 5 min of centrifugation, the supernatant was filtered through Whatman filter paper 1. The pellet was re-extracted with 25 mL of 0.4 M perchloric acid and subsequently centrifuged at ~3,500 × *g* for 5 min.
3. The supernatant was passed through Whatman filter paper 1. The filtered content was pooled with the earlier collected fraction and processed for derivatization.

DERIVATIZATION OF BIOGENIC AMINES

1. 1 mL of the extracted samples was transferred into a 5 mL centrifuge tube. Similarly, 100 µL of standard biogenic amine (1 mg/mL) was mixed with 900 µL of water in a 5 mL tube to achieve 1 mL volume of 100 µg/mL concentration.
2. The content in the tubes was mixed sequentially with 2 M NaOH (200 µL), saturated $NaHCO_3$ (300 µL), 10 mg/mL of dansyl chloride (2 mL).
3. The content in the tubes was mixed properly by vigorous shaking and the tubes were incubated at 40°C in a water bath.
4. After 45 min of incubation, 100 µL of ammonium hydroxide (25%) was added and tubes were incubated at room temperature (30 min).
5. After incubation, the volume of each tube was adjusted up to 5 mL by adding acetonitrile followed by shaking.
6. Finally, 1 mL fraction was filtered through a syringe filter (0.2 µm) and processed for the HPLC analysis.

CONDITIONS FOR HPLC ANALYSIS

1. For the detection and quantification of biogenic amines 20 µL of derivatized samples and standard biogenic amines were injected into the C_{18} column (column length 250 mm, and internal diameter 4.6 mm). The column was maintained at a constant temperature of 40°C.
2. A constant flow (1.0 mL/min) of mobile phase (ammonium acetate (0.1 M) and acetonitrile) in a gradient manner was used to separate the biogenic amines. The gradient elution was carried out for 35 min as the sequence mentioned below:

Time (min)	0.1 M ammonium acetate (%)	Acetonitrile (%)
0.00	65	35
5.00	55	45
10.05	35	65
17.05	20	80
26.05	10	90
35.00	65	35

3. The segregated biogenic amines were detected at 254 nm by UV-detector. The detection and quantification of biogenic amines in the samples were determined by equating them with standard biogenic amines of known strength.

VIDEO CLIPS

Duration 12 min 59 sec

DISCUSSION

Biogenic amine quantification is quite tedious owing to the light sensitivity, chemical instability, and spontaneous oxidation of biogenic amines. Moreover, the low concentration of biogenic amines in the biological samples needs a sensitive method for extraction and accurate quantification. The most reliable extraction methods include solid-phase extraction, solid-liquid extraction, and liquid-liquid extraction (Plonka 2012). The choice of the extraction method depends on the sample type; however, the most acceptable process for biogenic amines extraction in solid samples is the solid-liquid extraction method. A sophisticated and sensitive technique based on capillary electrophoresis, gas chromatography (GC), and high-performance liquid chromatography (HPLC) is used for the accurate detection and quantification of the biogenic amines (Önal et al., 2007). Due to high sensitivity, HPLC coupled with a UV detector is the most reliable method for the detection and quantification of biogenic amines. Despite the high sensitivity of the HPLC method, biogenic amines exhibited considerable interaction with free silanol groups in the silica-based column such as C_{18}, resulting in poor separation and peak trailing (Jubele, 2018). Furthermore, biogenic amines do not have chromophores that make poor signals in the UV detector, consequently inappropriate detection and quantification (Önal et al., 2007). To overcome these hurdles sample derivatization is an essential step for rapid, accurate, and simultaneous detection of different biogenic amines. Several derivatization agents such as dansyl chloride, benzoyl chloride, babsyl chloride, fluorescein, and o-phthaldialdehyde make a complex with biogenic amines resulting in steric hindrance to prevent the interaction of biogenic amines with silanol groups of stationary phase (C_{18}) (Jubele, 2018; Önal et al., 2007). In addition, the derivatizing agents impart the chromophore properties for efficient and simultaneous detection of different biogenic amines using a single wavelength in UV-detector. Among the different derivatization agents, dansyl chloride is most frequently used owing to its water-soluble nature, high selectivity, and sensitivity (Munir and Badri, 2020). Dansyl chloride reacts with the amino group of biogenic amines and makes a complex that is easily detected at a wavelength of 254 nm (figure 1).

The outcome of the present study showed the presence of 2-phenylethylamine, cadaverine, histamine, tyramine, spermine, and spermidine with characteristic retention times (RT) of 17.84, 19.15, 19.80, 23.24, 29.44 and 24.10 min, respectively, in sample-1 (*doenjang*: a traditional soybean-based Korean fermented food). While the presence of putrescine (RT 18.41 min), cadaverine (RT 19.51 min), histamine (RT 19.80 min), and tyramine (RT 23.24 min) in the sample-2 (Tuna fish). The eluted biogenic amines have different chemical structures but all of them were simultaneously detected in a single chromatographic run at a wavelength of 254 nm, due to the derivatization with dansyl chloride.

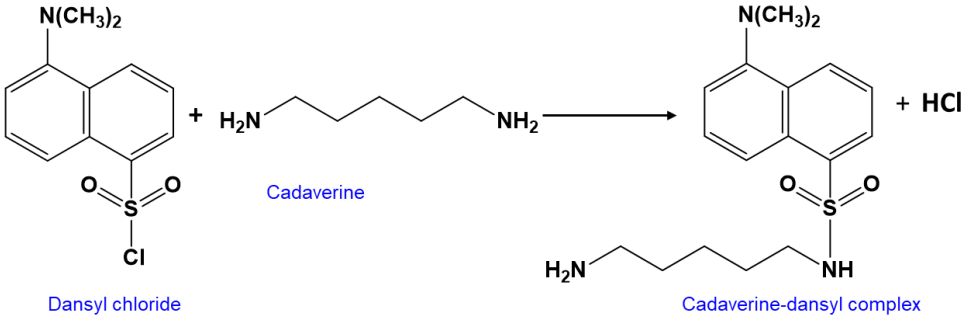

Figure 1: Dansyl chloride mediated derivatization of biogenic amine. Cadaverine was used as representative biogenic amine

PRECAUTIONS

Homogenize the samples properly to achieve proper recovery of biogenic amines.

Work carefully with dansyl chloride, as it is a strong irritant for skin and eyes.

Biogenic amines are the potential toxins, therefore, need to be handle carefully.

All the solvents used for HPLC analysis must be filtered and degassed.

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