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High-sensitivity detection of biogenic amines with multiple reaction monitoring in fish based on benzoyl chloride derivatization

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ABSTRACT

In this study an efficient and sensitive method for biogenic amines detection was established by combining rapid extraction and derivatization with multiple reaction monitoring (MRM) based on ultra high performance liquid chromatography-triple quadrupole mass spectrometry. Bead-beating disruption and extraction using 5-sulfosalicylic acid not only improved the extraction efficiency but also was environmentally friendly. Benzoyl chloride derivatization could obtain stable biogenic amine derivatives with a shorter reaction time. By combining with MRM detection mode, higher detection sensitivity for biogenic amines could be achieved. The method showed a good linearity with linear range of 3–4 orders of magnitude and regression coefficients ranging from 0.9966 to 0.9999. The limit of detection and limit of quantitation could even reach lower pg/mL level. Satisfactory recovery was obtained from 74.9% to 119.3%. And the derivatives were stable within 48 h at 4 °C. The method established was used to determine content of biogenic amines in different fishes at different storage conditions. The results indicated that this method was suitable for analysis of biogenic amines.

1. Introduction

Biogenic amines (BAs) including histamine, cadaverine, putrescine, tyramine, spermine, spermidine, 2-phenethylamine, tryptamine and agmatine are nitrogen-contained low molecular weight organic compounds. Their structures are shown in Fig. 1. BAs in food are usually generated from decarboxylation of corresponding amino acids caused by decarboxylase produced from microorganisms [1]. They exist in animal and plant body, fermented foods, meat products and aquatic products [2]. BAs are the indispensable parts of the biological active cells and have important biological function [3]. But if the intake of BAs was excessive in human body, it will cause some adverse reactions including headache, nausea and anaphylaxis, and so on [4]. So the content of BAs in foods can be used as indicator of food quality [5]. Based on this, it is of great significance to establish fast and efficient analysis method to detect BAs in foods.

Nowadays various analytical methods have been applied in detection of BAs, such as thin-layer chromatography (TLC) [6,7], capillary electrophoresis (CE) [8,9], gas chromatography (GC) [10,11] and liquid chromatography (LC) [12,13]. But TLC, CE and GC are not the common techniques [14]. Because of the high sensitivity and resolution, LC especially high performance liquid chromatography (HPLC) has become the important means for determination of BAs. And the most commonly used detectors involve ultraviolet detector (UV) [15,16] and fluorescence detector (FL) [17,18]. In recent years, HPLC coupled with mass spectrometry (MS) has also become a kind of important analysis method [19–21]. What's more, MS detection can provide higher sensitivity than UV and FL detection.

In LC-UV or FL or MS analysis, derivatization is often used to improve the response of BAs. For UV and FL detection, derivatization can help BAs without chromophores to be detected more easily [14]. For MS analysis, derivatization can help to strengthen retention, improve ionization efficiency and increase sensitivity [22]. The biogenic amines (Fig. 1) usually contain the amino group. Then the most commonly used derivatization reagents include dansyl chloride (Dns-Cl) [23,24], *o*-phthalaldehyde (OPA) [25] and benzoyl chloride [26,27]. Dns-Cl derivatization usually needs heating condition and reaction time is longer, while OPA only reacts with primary amines and the derivatives are not stable. By contrast, benzoyl chloride derivatization is simpler which only needs a short reaction time under mild reaction conditions. What's more, benzoyl chloride derivatization method has been used in analysis of

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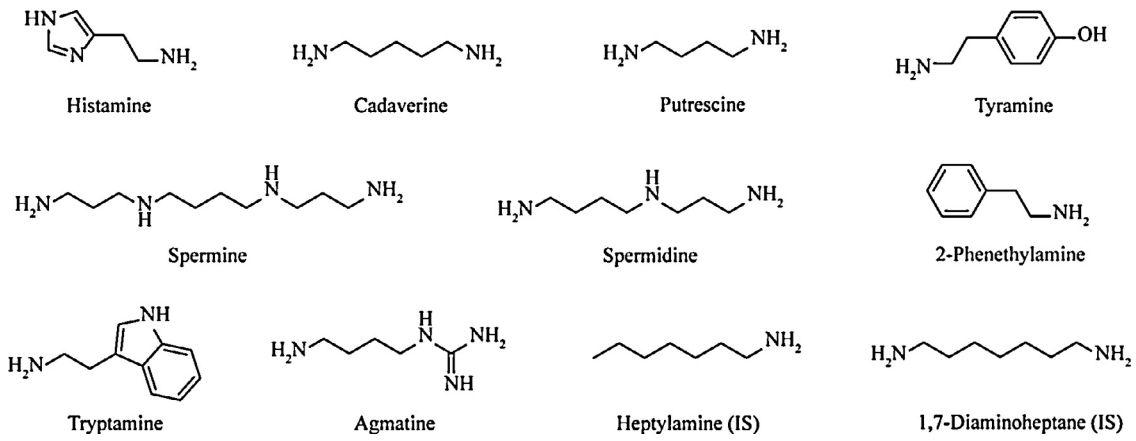

Fig. 1. Structures of nine biogenic amines and two internal standards (IS).

neurotransmitters to improve chromatographic retention and mass sensitivity [28–30].

Sample pretreatment is also important in the analysis of BAs. There are some commonly used techniques including solid-phase extraction (SPE) and liquid–liquid extraction (LLE) [14]. Meanwhile some other techniques, including matrix solid-phase dispersion (MSPD) [31], liquid–phase microextraction (LPME) [32], and so on, are also developed, but they are not commonly used. LLE needs more solvents and the recovery obtained in LLE is basically lower than that obtained in SPE, while the problem of SPE is that the operation is complex and time consuming [14]. According to existing literatures, direct solvent extraction with trichloroacetic acid (TCA) [33,34], perchloric acid (HClO) [16,35,36] and hydrochloric acid (HCl) [20,37] is also a kind of popular extraction method in BAs analysis based on the LC-UV or FL. While in LC–MS analysis, these chlorinated extraction reagents are not the optimal choice in theory, because they are easy to retain in ion channel and produce ion suppression in negative detection mode. In addition there are also other solvents to be used in extraction of BAs. For example, sulfosalicylic acid had also been used in extraction of polyamines in milk and the results showed it could provide better satisfactory recovery [38]. The use amount of tissue for analysis usually ranges from 10 mg to 50 mg, which is considered to obtain satisfactory results [39–43].

In our study, a comprehensive and effective analysis method was developed to determine the BAs content in fishes. This method integrated ultra high performance liquid chromatography–triple quadrupole mass spectrometry (UHPLC–TQMS) with bead-beating disruption extraction (5-sulfosalicylic acid as extraction reagent) and benzoyl chloride derivatization to analyze BAs in different fishes. This method not only simplified the sample pretreatment process greatly but also improved the detection sensitivity. Based on the established method four types of fishes from different markets were analyzed.

2. Experimental

2.1. Materials and chemicals

Histamine, cadaverine, putrescine dihydrochloride, tyramine, spermine, spermidine, 2-phenethylamine were purchased from Sigma-Aldrich (St. Louis, Mo, USA). Tryptamine (98%), agmatine sulfate (98%), 1, 7-diaminoheptane and 5-sulfosalicylic acid dihydrate (99%) were obtained from J&K scientific LTD (Beijing, China). Heptylamine (99%) was purchased from Alfa Aesar (Tianjin, China). Acetonitrile (ACN, HPLC grade) was purchased from Merck (Darmstadt, Germany). Ethanol (EtOH, AR) was obtained from Damao Chemical Reagent Factory (Tianjin, China). Ultrapure water (H_2O) was prepared by Milli-Q system (Millipore, Billerica, MA, USA). Sodium tetraborate decahydrate (ACS reagent, ≥99.5%), benzoyl chloride (≥99%) and formic acid (FA) were bought from Sigma-Aldrich (St. Louis, Mo, USA).

2.2. Preparation of stock solution

Individual stock solution of histamine, cadaverine, putrescine, spermine, spermidine, agmatine, heptylamine and 1, 7-diaminoheptane was prepared in 20% ACN. The structures of heptylamine and 1, 7-diaminoheptane are displayed in Fig. 1. Individual stock solution of tyramine, 2-phenethylamine and tryptamine was prepared in EtOH. The concentration of each individual stock solution was 10 mg/mL. 1 mg/mL mixed stock standard solution was prepared by mixing individual stock solution of nine BAs and diluting with 20% ACN. 1 mg/mL mixed internal standard (IS) stock solution was prepared by mixing individual stock solution of heptylamine and 1, 7-diaminoheptane. In this experiment, the mixed work solutions were obtained by diluting the mixed stock standard solution. All these solutions were stored at −20 °C.

2.3. Sample information and sample pretreatment

Four kinds of fishes including mackerel, yellow croaker, hairtail and pomfret were purchased from morning market and supermarket in Dalian, respectively. The bones, skins and internal organs of these fishes were removed immediately, and then the fish tissue was homogenized roughly in a blender (MJ-BL25B3, Midea, Guangdong, China). One part of each fish tissue was analyzed immediately, and remaining tissues were divided into several parts, and stored at 22–24 °C (room temperature, RT) and −20 °C, respectively, for further analysis.

Direct solvent extraction with bead-beating disruption was used for further preparation of fish samples. To be specific, 20.0 mg (±0.5 mg) fish tissue after rough homogenization was weighed into a 2 mL centrifuge tube. Then 300 μL 5-sulfosalicylic acid solution (5 mg/mL, in H_2O), 100 μL IS solution (4.5 μg/mL, in 20% ACN) and 100 μL blank solvent (20% ACN) or mixed work solution (concentration was determined according to the corresponding experiment) were added into the 2 mL centrifuge tube in order. In the end, zirconia bead was added into the centrifuge tube for the simultaneous homogenization and extraction of fish tissue in a mixed grinding apparatus (MM400, Retsch, Germany) at 20 Hz for 1 min for twice. Later sample was put into a Sorvall Biofuge Stratos Centrifuge System (Thermo Fisher Scientific) for centrifugation under