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Direct separation and detection of biogenic amines by ion-pair liquid chromatography with chemiluminescent nitrogen detector

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ABSTRACT

Analysis of biogenic amines is critical to pharmaceutical and food industry due to their biological importance. For many years, the determination of biogenic amines has relied on high performance liquid chromatography (HPLC) coupling with pre-, on-, or post-column derivatization procedures to enable UV or fluorescent detections. In this study, 14 biogenic amines were separated on a Phenomenex Luna® Phenyl-Hexyl column by an ion-pair liquid chromatography method using perfluorocarboxylic acids as ion-pair reagents and detected by a chemiluminescent nitrogen detector (CLND). This direct separation and detection HPLC method eliminated the time consuming and cumbersome derivatization procedures. Compared with HPLC–UV (post-column derivatization with ninhydrin) and HPLC-charged aerosol detector (CAD) methods, this HPLC–CLND technique provided narrower peaks, better baselines, and improved separations and detections. Excellent linearity was acquired by CLND for each of the 14 biogenic amines ranging from less than 1 ng to about 1000 ng (on-column weights). The relative response factors determined by this LC–CLND method were proportional to the numbers of nitrogen atoms in each compound, which has been the characteristic of the equimolar determinations by CLND. In addition, a number of samples including beer, dairy beverage, herb tea, and vinegar were analyzed by the LC–CLND method with satisfactory precision and accuracy.

1. Introduction

As products of decarboxylation of amino acids, biogenic amines play diverse roles in cellular growth and metabolism. Due to their biological importance, biogenic amines had been determined by a variety of analytical techniques in plants [1]; foods–fruits [2], vegetable products [3], meat [4], fish [5] and milk products [6]; beverages–beer [7,8] and wine [9,10]; biological samples – fluids [11], tissues [12,13], and urines [14]. As depicted in Fig. 1, putrescine (1,4-diaminobutane/butanediamine), cadaverine (1,5-diaminopentane), spermidine, and spermine have no chromophores or fluorophores; and β-alanine (3-aminopropionic acid), γ-aminobutyric acid (GABA, 4-aminobutanoic acid), and agmatine have very weak UV absorbance – not suitable for low level detections. In addition, these small, polar amines hardly retain on a reversed phase HPLC column. Therefore, liquid chromatography coupling with pre or post-column derivatization had been the most common practices in analyzing variety of samples containing biogenic amines.

Dansyl chloride [15] and 9-fluorenylmethyl chloroformate (FMOC-Cl) [10,11,16] are often used in pre-column derivatization to convert biogenic amines into adducts to improve the on-column separation and spectroscopic responses. Depending on the complexity of derivatization and sample matrix, multiple sample preparation steps may have to be carried out prior to HPLC analysis. Thus, recovery studies are warranted to demonstrate the accuracy and precision of sample preparation processes. Pre-column derivatization is nevertheless preferred in a number of cases as it is less demanding on instrumentation and system setups.

Post-column derivatization, on the contrary does not require the extra sample preparations as biogenic amines can be derivatized online after HPLC separation prior to detection. O-phthaldialdehyde (OPA) and ninhydrin [17] had been widely used in post-column derivatization. The post-column approach did provide the benefits of automatic online derivatization – eliminating the extra sample manipulations which could have brought in imprecision and prolonged sample preparations. However, post-column derivatization requires the addition of extra chromatographic components, stable supply of derivatization reagents with acceptable purities, and delicate setups. The turbulence generated during the online mixing must be controlled to maintain a satisfactory chromatographic baseline. It is inevitable that the increased system dwell volume and instrument bandwidth will

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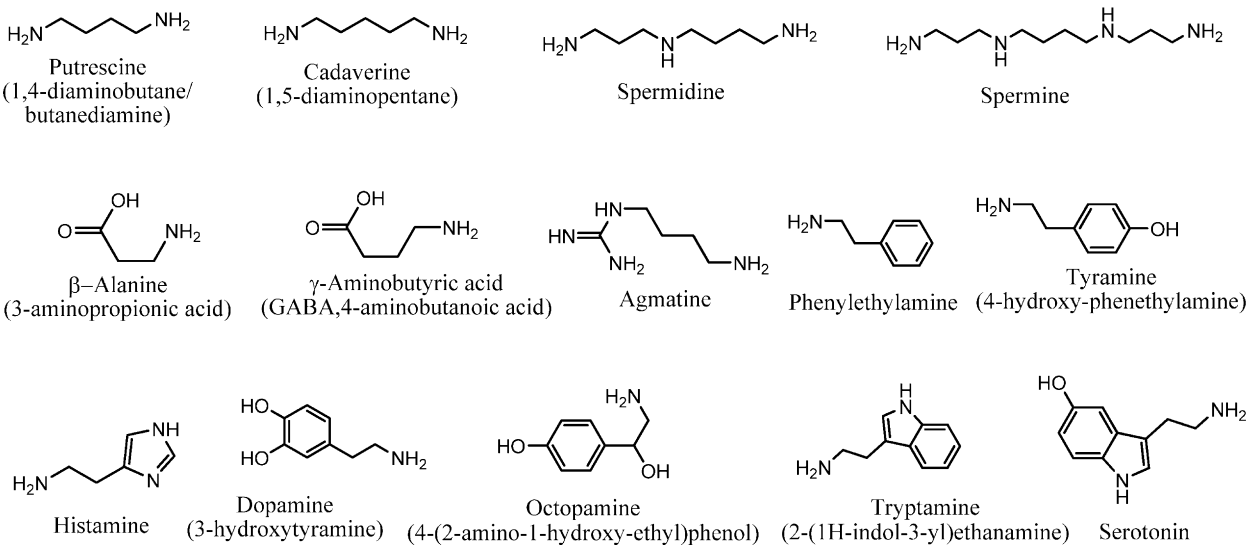

Fig. 1. Structures of 14 biogenic amines.

bring about peak broadening and sample dilution. This has been the inherent drawback of post-column derivatization method and creates formidable challenges for a HPLC practitioner improving resolution, signal-to-noise ratio, and peak capacity.

A variety of new techniques had been developed over time to overcome the inconveniences and imperfections of pre and post-column derivatizations such as on-column fluorescent derivatization method [18], capillary electrophoresis with pulsed amperometric detection [19], ion-exchange chromatography with conductivity detector [20] and integrated square-wave electrochemical detection [21]. Based on our extensive literature searches so far, no work was published demonstrating direct separation and detection of biogenic amines by a LC–CLND method.

Rather than develop and validate another LC-derivatization method for a new sample matrix with the familiar approaches, the aim of our work is to develop a simple chromatographic separation method with a direct detection of biogenic amines to eliminate the derivatization and other drawbacks. Petritis et al. [22] had extensively studied the compatibilities of a number of long perfluorinated carboxylic acids as ion-pair reagents with various detectors in the determinations of amino acids by HPLC and concluded that electrospray mass spectrometry in tandem mode and CLND are the most promising ones due to their higher sensitivity and specificity. Given that CAD was not included in their detector comparison and no derivatization was required for the amino acids LC–UV method, we decide to focus our efforts on identifying the most suitable chromatographic conditions that enables the detections of the 14 biogenic amines by UV derivatization, CAD, and CLND. In our study the impacts of perfluorocarboxylic acids as ion-pair reagents, their concentrations, and the column temperatures were closely examined. As there are no adjacent nitrogen atoms present in any of biogenic amines, equimolar determinations by CLND should be expected and the performances of the three detection techniques can be compared. Finally LC–CLND method was applied in the determination of biogenic amines in a variety of samples to demonstrate its precision and accuracy.

2. Materials and methods

2.1. Samples

Beer, herb tea, dairy beverage, and vinegar were purchased from retail stores in California, USA. All samples were filtered through a 0.2 μm cellulose acetate membrane filter prior to HPLC analysis. The contents in the herb teabag were extracted by hot drinking water. The dairy beverage was first centrifuged at 13,000 rpm for 5 min to facilitate the filtration.

2.2. Chemicals

The water used in all experiments was obtained from Milli-Q system (Millipore, Billerica, MA, USA). The other solvents/reagents used were HPLC grade methanol, isopropyl alcohol (IPA) and trifluoroacetic acid (TFA) from J.T. Baker (Phillipsburg, NJ, USA); long-chain perfluorocarboxylic acids including pentafluoropropionic acid (PFPA, 97%), heptafluorobutyric acid (HFBA, 99%), nonafluoropentanoic acid (NFPA, 97%, synonyms: perfluoropentanoic acid or nonafluorovaleric acid), and undecafluorohexanoic acid (UFHA, 97%, synonyms: perfluorocaptroic acid or perfluorohexanoic acid) from Sigma–Aldrich (St. Louis, MO, USA); Trione ninhydrin reagent T100 from Pickering Laboratories (Mountain View, CA, USA). All 14 biogenic amines: spermidine (SD), spermine (SM), phenethylamine (PHE) hydrochloride, dopamine (DO, synonym: 3-hydroxytyramine) hydrochloride, serotonin (SE) hydrochloride, octopamine (OC, synonym: 4-(2-amino-1-hydroxy-ethyl)phenol) hydrochloride, histamine (HI) dihydrochloride, agmatine (AG) sulfate, γ-aminobutyric acid (GABA, synonym: 4-aminobutanoic acid), putrescine (PU, synonym: 1,4-diaminobutane/butanediamine), cadaverine (CA, synonym: 1,5-diaminopentane), β-alanine (BAL, synonym: 3-aminopropionic acid), tyramine (TY, synonym: 4-hydroxy-phenethylamine), and tryptamine (TR, synonym: 2-(1H-indol-3-yl)ethanamine) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.3. Stock standard solution preparation

0.2% TFA in water was prepared as sample diluent by adding 2 mL TFA in 1 L water. All biogenic amines except TR have good solubility in the 0.2%TFA. To make an approximately 800 μg/mL stock standard solution of it, about 20 mg TR was weighed and transferred into a 25 mL volumetric flask, added 1 mL methanol to dissolve and diluted to volume with sample diluent. As for the other 13 biogenic amines, about 20 mg equivalent free base of each amine was weighed and dissolved separately in 25 mL sample diluent. The free base concentration of each amine in each stock standard solution was ~800 μg/mL. To make a mixing stock standard solution, 1 mL