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Selective and sensitive liquid chromatographic determination method of 5-hydroxyindoles with fluorous and fluorogenic derivatization

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ABSTRACT

A liquid chromatographic (LC) method with improved selectivity for the simultaneous determination of 5-hydroxyindoles (5-HIs; 5-hydroxytryptophan, 5-hydroxytryptamine, *N*-acetyl-5-hydroxytryptamine, 5-hydroxyindole-3-acetic acid, and 5-hydroxytryptophol) is described. This method involves precolumn derivatization with 4-(3′,3′,4′,4′,5′,5′,6′,6′,7′,7′,8′,8′,9′,9′,10′,10′,10′-heptadecafluorodecyl)benzylamine (HFBA) and separation of the derivatives using a fluorous LC column. In this study, stable benzoxazole derivatives of 5-HIs with HFBA have been obtained by a simple derivatization procedure; their fluorescent properties enabled highly sensitive detection. In addition, only the HFBA derivatives of 5-HIs has been selectively retained on the fluorous LC column via fluorous interaction whereby perfluoroalkyl compounds show affinities with each other, while the non-fluorous compounds did not. The HFBA derivatives were separated within 30 min and the detection limits for 5-HIs in a 20-μL injection volume were 1.2–14 fmol (*S*/*N* = 3). Furthermore, this method was applied to the analysis of 5-HIs in the human plasma from healthy subjects.

1. Introduction

5-Hydroxyindoles (5-HIs), originating from tryptophan, are produced via the corresponding biosynthetic pathways in the human body [1] and play important roles in the control and regulation of the functions of the central and peripheral nervous systems; they also exhibit associations with various diseases [2–5]. In particular, 5-hydroxytryptamine (5-HT) occurs mainly in the central nervous system, blood platelets, and enterochromaffin cells of the gastrointestinal tract. 5-HT is sequentially metabolized, first by monoamine oxidase and then by aldehyde dehydrogenase, to form 5-hydroxyindole-3-acetic acid (5-HIAA). 5-HIAA is the most abundant end product of both central and peripheral 5-HT metabolism and is excreted into urine. The correlations of 5-HIAA levels in plasma and urine to some pathological conditions have been reported [4,5]. Thus, a simple, selective, sensitive, and simultaneous determination method of 5-HIs in the human body is of great importance in the research involving 5-HIs or the diagnosis of diseases related to 5-HIs. A number of methods have been described for the determination of 5-HIs; of these, liquid chromatography (LC) with native fluorescent detection [6–8], electrochemical detection [9–11], and mass spectrometry (MS) [12–16] have been most widely used. Although these methods are reliable, a number of peaks attributed to coinciding compounds in biological samples may interfere with the corresponding chromatogram expect for LC-MS. Therefore, highly sophisticated LC separation conditions and troublesome clean-up procedures are required to eliminate the interferences caused by other coexisting compounds. In contrast, MS and MS/MS detection methods are selective, sensitive, and highly reliable; however, these techniques are sometimes interfered with by matrix components present in biological samples and accurate determination may not be obtained.[17,18].

We have previously reported selective and sensitive fluorogenic derivatization methods for 5-HIs and catecholamines in biological samples [19–24]. These methods were based on the reactions of these analytes (weakly fluorescent) with non-fluorescent benzylamine (BA) in a weakly alkaline medium in the presence of an oxidizing agent, yielding highly fluorescent benzoxazole derivatives. Furthermore, we have also applied some BA derivatives as

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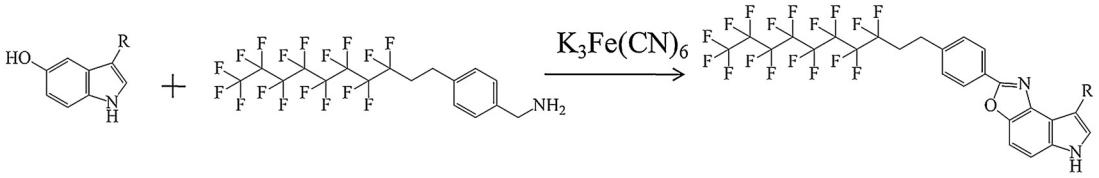

5-HIs	Abbreviation	R
5-Hydroxytryptamine	5-HT	$-CH_2CH_2NH_2$
5-Hydroxyindole-3-acetic acid	5-HIAA	$-CH_2COOH$
N-Acetyl-5-hydroxytryptamine	NAS	$-CH_2CH_2NHCOCH_3$
5-Hydroxytryptophol	5-HTOL	$-CH_2CH_2OH$
5-Hydroxytryptophan	5-HTRP	$-CH_2\underset{\displaystyle COOH}{\overset{\displaystyle |}{C}}HNH_2$

Fig. 1. Fluorous–fluorogenic derivatization reaction for 5-HIs with HFBA.

fluorogenic derivatization reagents for 5-HIs and catecholamines, resulting in improved analytical sensitivities for their detection [25–28].

Recently, we have also developed LC analytical methods for native fluorescent compounds based on "fluorous derivatization," in which "fluorous" refers to the unique affinity between highly fluorinated compounds [29–31]. The principle of this method is that native fluorescent analytes are derivatized precolumn by attachment of a non-fluorescent fluorous compound to a particular functional group; thereafter, the fluorous-derivatized analytes are extremely retained on a fluorous phase LC column, thus achieving selective separation of analytes from biological sample matrices. Neither non-fluorescent compounds nor underivatized native fluorescent compounds interfere with the detection. This method was successfully applied to the determination of pharmaceutical agents possessing carboxylic groups in the human plasma and urine after administration [30], as well as native fluorescent biogenic amines in the human urine [31].

In this study, we report a novel derivatization method for the highly selective and sensitive determination of 5-HIs by combining both of the above-mentioned fluorous and fluorogenic derivatization methods. To the best of our knowledge, this is the first report on analyses with both fluorogenic derivatization using a fluorous labeling reaction. 5-Hydroxytryptophan (5-HTRP), 5-HT, *N*-acetyl-5-hydroxytryptamine (NAS), 5-HIAA, and 5-hydroxytryptophol (5-HTOL) were selected as model 5-HI compounds (Fig. 1). 5-HTRP, NAS, and 5-HTOL as well as 5-HT and 5-HIAA are produced from tryptophan in human body and compose the biosynthetic and metabolic pathways of serotonin [1]. 4-(3′,3′,4′,4′,5′,5′,6′,6′,7′,7′,8′,8′,9′,9′,10′,10′,10′-Heptadecafluorodecyl)benzylamine (HFBA), possessing a fluorous moiety and benzylamine structure, was used as a derivatization reagent (Fig. 1). This determination method has both high selectivity based on fluorous interactions and high sensitivity based on fluorogenic derivatization. The conditions for derivatization, LC separation, and fluorescent detection were optimized using standard solutions of 5-HIs. After optimization studies, this method was applied to the analysis of 5-HT and 5-HIAA in the human plasma from healthy subjects without the need for any other enrichment or clean-up procedure.

2. Experimental

2.1. Reagents and materials

Unless stated otherwise, all chemicals mentioned below were of the highest purity available and were used as received. 5-HTRP (5-hydroxytryptophan dihydrate), 5-HTOL, and HFBA were purchased from Sigma–Aldrich (St. Louis, MO, USA). 5-HT (5-hydroxytryptamine hydrochloride), 5-HIAA, and benzylamine hydrochloride (BA) were purchased from Wako Pure Chemicals (Osaka, Japan). NAS, *N*-cyclohexyl-3-aminopropanesulfonic acid (CAPS), and potassium hexacyanoferrate(III) were purchased from Tokyo Chemical Industry (Tokyo, Japan), Dojindo Laboratories (Kumamoto, Japan), and Kishida Chemical (Osaka, Japan), respectively. All organic solvents were of LC grade. It should be noted that these reagents and solvents are toxic to the eyes, lungs, and skin, and should be used carefully according to guidelines specified in the latest material safety data sheets. Ultrapure water, purified using a Milli-Q gradient system (Merck Millipore, Billerica, MA, USA), was used to produce all aqueous solutions.

The respective stock solutions (1.0 mM) of analytes were prepared in water and stored at −30 °C. The solutions were stable for at least 1 week and diluted further with ultrapure water to the required concentrations before use. A solution of 10-mM HFBA in tetrahydrofuran (THF) was usable for at least 1 week when stored at room temperature. A CAPS buffer (30 mM) was prepared in water and adjusted to pH 12 with aqueous sodium hydroxide. Potassium hexacyanoferrate(III) solution (20 mM) was prepared using the above-prepared 30-mM CAPS buffer (pH 12) and used within 1 day.

2.2. Derivatization procedure

To the sample solution (200 μL) placed in a polypropylene 1.5-mL vial were added 125 μL of 10-mM HFBA and 25 μL of 20-mM