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Rapid, simple and simultaneous measurement of kynurenine and tryptophan in plasma by column switching-HPLC method

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Abstract. We established a highly reproducible, simple and rapid simultaneous measurement method in which blood plasma can be directly injected into high-performance liquid chromatography (HPLC) without conducting conventional extraction of kynurenine (Kyn) and tryptophan (Trp) from blood plasma. In this method, Kyn and Trp were first separated from proteins present in blood plasma using a pre-column. Then, by column-switching, the separated Kyn and Trp were automatically injected into a separation column. The recoveries of Kyn and Trp determined in blood plasma by this method were 100.0±0.9 and 100.0±1.4%, respectively.

Keywords: Tryptophan; Kynurenine; HPLC; Proteins

1. Introduction

Tryptophan (Trp) is one of the essential amino acids and is metabolized to various biologically active substances in vivo such as serotonin, melatonin, nicotinic acid, and nicotinamide adenine dinucleotide (NAD). On the other hand, Kynurenine (Kyn) is an important intermediate of Trp metabolism, because Kyn is generated by cleavage of the indole ring of Trp and metabolized in at least three pathways. Therefore, the development of

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a convenient quantitative analysis of Kyn and Trp present in blood and tissues should be very useful for the study of Trp metabolism.

In the past, many methods using high-performance liquid chromatography (HPLC) have been reported for the measurement of Trp and its metabolites in blood and tissues. Recently, Morita et al. [1] reported the HPLC-fluorescence method for Trp and its metabolites and the HPLC-UV method for Kyn and Kyn-derived metabolites using blood serum and plasma as samples. In addition, Widner et al. [2] and Vignan et al. [3] measured serum Trp by the HPLC-fluorescence method and serum Kyn by the HPCL-UV method. Herve et al. [4] reported that Trp, 3-hydroxyanthranilic acid (Kyn metabolite) and kynurenic acid in serum were measured by the HPLC-UV or fluorescence method and Kyn and 3-hydroxykynurenine in serum were measured by the HPLC-UV method. Vaarmann et al. [5] measured 13 substances present in plasma such as catecholamines, Trp and its metabolites and Kyn and its metabolites by the HPLC method with electrochemical detection (HPLC-ECD method) under the following conditions: +900 mV was used for Trp and +1000 mV for Kyn. Maneglier et al. [6] measured Trp and Kyn in human plasma and cultured human cells by the HPLC-ECD method using +600 mV. In all these methods, however, the removal of proteins in samples was conducted using perchloric acid and trichloroacetic acid. Thus, in the above-described HPLC methods, the measurements of Trp and Kyn in samples by HPLC were conducted by changing the UV wavelength and voltage according to the substances measured or by use of two of the same type detectors at a time. The same sample was measured twice by the same HPLC method by changing the wavelength and voltage for detection. Marklova et al. [7] analyzed simultaneously 10 Trp metabolites in urine and plasma, including Kyn and Trp, by the HPLC-UV method at a single wavelength (254 nm) after preparation of HPLC samples by the solid-phase extraction method. However, it takes a long time to prepare samples by the solid-phase extraction method before HPLC measurement.

Therefore, we attempted to develop a simple and rapid simultaneous analysis of plasma Kyn and Trp by HPLC.

2. Materials and methods

2.1. Materials

Special grade reagents of kynurenine, tryptophan and acetonitrile (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were used. For other reagents, commercial special grade reagents were used. For HPLC equipment, the PU-980 Interijent HPLC Pump was used as the pump and the HV-992-01 System Controller 802-SC (JASCO Corporation, Tokyo, Japan) as the high-pressure switching hexagonal valve. The NANO SPASE S-1 (Shiseido Co., Ltd., Tokyo, Japan) was used as the UV detector; the measuring wavelength was set at 256 nm. The Model 7125 (Rheodyne, Cotati, CA, USA), equipped with a 20 μl loop, was used as the injector. The analytical column used was the CAPCELL MF SCX S-5 (4.0 mm I.D. × 20 mm; 5 μm particle size; Shiseido Co., Ltd., Tokyo, Japan) and the pre-column used was the CAPCELL PAK C18 AQ (4.6 mm I.D. × 250 mm; 5 μm particle size; Shiseido Co., Ltd., Tokyo, Japan). The column temperature was set at room temperature. The C-R5A CHROMATOPAC (SHIMAZU Corporation, Kyoto, Japan) was used as the recorder.