Determination of homocysteine and its related compounds in hair by HPLC-fluorescence method (1) -Estimation of extraction conditions-

[1]Mitsuhiro WADA, [1]Shinichi NAKAMURA, [2]Kenichiro NAKASHIMA

[1]School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, 1714-1 Yoshino-machi, Nobeoka, Miyazaki 882-8508, Japan.

[2]Faculty of Pharmaceutical Sciences, Nagasaki International University, 2825-7 Huis Ten Bosch, Sasebo, Nagasaki 859-3298, Japan.

Introduction

Homocysteine (Hcy), one of the sulfur-containing amino acids, is an intermediate metabolite of methionine (Met) to metabolite cysteine (Cys). The elevated level of Hcy and its related compounds in organisms play an important role in a variety of diseases. Therefore, the simultaneous determination of Hcy and its related compounds such as Met and Cys is required to appropriate clinical management. The purpose of the study is to develop the HPLC-fluorescence (-FL) method combined with 4-(*N*,*N*-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) for determination of Hcy and its related compounds in hair as a non-invasive sample. In this presentation, the extraction condition of Hcy from hair matrix was examined.

Materials and Methods

Extraction: One milligram of grinded hair was treated as follows: 1) 6M NaOH (80℃, 1 h), 2) 6 M HCl (110 ℃, 24 h), 3) 5% HCl in MeOH (1 h sonication) and 4) 5% trifluoroacetic acid in MeOH (1 h sonication). After reduction with 4 mM dithioerythritol in 400 mM borate buffer (pH 8.5) at 80 ℃ for 15 min, the solution was applied to the derivatization reaction. Derivatization: The extract was added to 60 μL of 400 mM borate buffer (pH 8.5) and 60 μL of DBD-F in CH_3CN. After heating at 80℃ for 15 min the mixture was centrifuged at 2670 *g* for 10 min. Then, 150 μL of the mixture were cleaned up by liquid-liquid extraction with 300 μL of ethyl acetate. The organic layer was dried up and reconstituted with 150 μL of mobile phase. HPLC: The DBD derivatives were separated on a Daisopak SP-120-5-ODS-BP (250 × 2.0 mm, i.d.) with 25 mM phosphate buffer (pH 2.0)/CH_3CN/MeOH (=57:37:6, v/v/v%) as a mobile phase. The detection wavelength at 400 (λ_{ex}) and 570 nm (λ_{em}) was used.

Results and Discussion

The DBD-Hcy could be observed in the hair samples extracted with acid-MeOH, however, no peak from the samples with acid or alkaline treatment. On the other hand, Met and Cys in sample treated with 6 M NaOH were detected effectively.

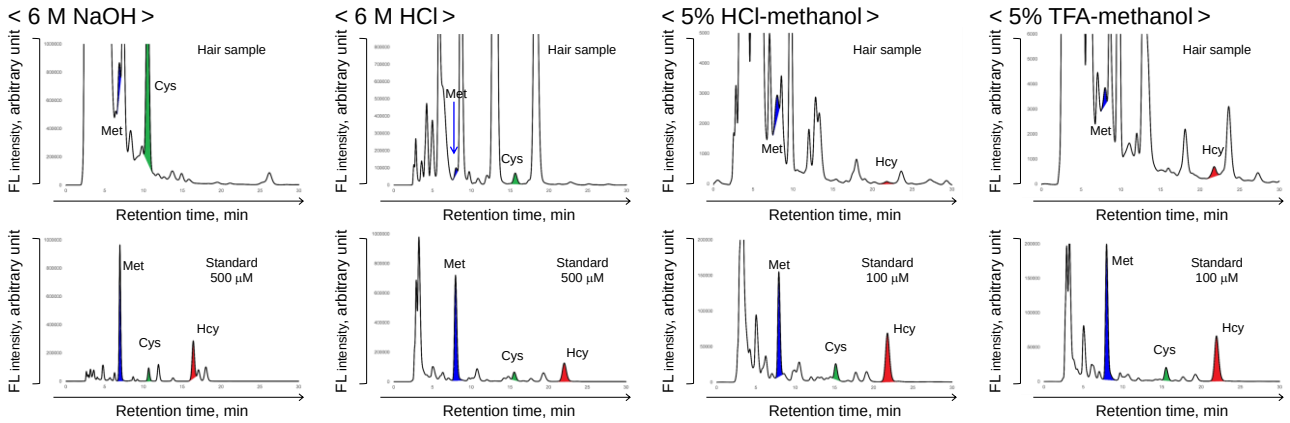

Figure 1. Chromatograms of hair sample and standards.

To improve the extraction yield of Hcy and related compounds the conditions in detail should be examined.