Construction of a Gradient μLC/MS System and Its Application

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Abstract

A gradient LC/MS system was constructed and applied for separation of biological samples. For example, a rapid and simple analytical method without pretreatment based on gradient μLC/MS with a disposable microcolumn has been developed to determine B group vitamins in urine. Urine samples were directly injected to the disposable home-made microcolumn. The microcolumn can be emptied after being used for a series of urine samples, and repacked with fresh stationary phase. An overdose of vitamin pills were swallowed by healthy volunteers and the urine samples were taken 1,2,3,5, and 8 hours after swallowing. Vitamins immediately showed up in urine, hit the maximum, and disappeared swiftly. This technique is expected to have some application for clinical purposes.

INTRODUCTION

Vitamins are essential food constituents that are required in small amounts for maintaining life. Vitamins are generally divided into two main groups; fat- and water-soluble vitamins. While fat-soluble vitamins have been analyzed by both GC and LC techniques, water-soluble vitamins have been determined exclusively by HPLC techniques[1-6]. In most HPLC analyses of water-soluble vitamins, pretreatment procedures were required to concentrate vitamin components and/or to eliminate interfering materials[4-8]. In addition, vitamins are often unstable in such biological matrices[9].

In this study, we have developed a rapid and sensitive method for determining B group vitamins in urine without pretreatment by the gradient μLC/MS system. Various studies on the determination of vitamins by LC/MS were reported[10-14]. Determination of water soluble vitamins in urine by LC/MS has not been reported yet. This pseudo-on-time determination method was enabled by incorporation of a cheap disposable home-made microcolumn.

EXPERIMENTAL

Apparatus

Two Shimadzu(Tokyo, Japan) 10AD pumps, a Shimadzu DGU-14A membrane degasser, a Tee union with a 1/16 inch I.D. stainless steel frit(as a micromixer), a Rheodyne (Cotati, CA,

USA) 7520 injector with a 0.5μL injection loop, and a home-made 0.5mm I.D. microcolumn were combined to construct the μLC part of the system[15-18].

The mass spectrometer was a VG Biotech(Manchester, UK) Quattro triple quadrupole system with a nitrogen-flow assisted electrospray interface. The electrospray voltage was set at 3.5 kV, and the cone voltage, at 20V. The source temperature was controlled at 90 oC. The nitrogen gas flow rate was adjusted to 0.3 L/min. The mass spectrometer was operated in the selective ion recording(SIR) mode.

Preparation of standard solutions in blank urine

The blank urine was collected in a week advance and filtered through a 0.45μm membrane filter. The blank urine was found to have no vitamins at any recognizable level. Each vitamin was exactly measured(10mg) and dissolved in 50ml blank urine on the same day of excretion experiment. The mother solutions of individual vitamins were mixed in appropriate rates and diluted with blank urine to give standard solutions over a proper range of concentration. The mother solutions and standard solutions were stored in a refrigerator at 4 °C. We used the blank urine to prepare standard solutions since we had found that standard solutions made in pure water gave faulty results.

The internal standard was measured(cytosine 50mg) and dissolved in 50mL blank urine.

Urine sample preparation

Three commercial vitamin pills were taken with 200mL water by a healthy man in the morning without eating breakfast. He ate lunch 5 hours later. Each pill contained 50mg vitamin B1, 2.5mg vitamin B2, 2.5mg vitamin B6, and 5.22 μg vitamin B12. The man took water continuously little by little at a rate of about 100 mL/hr. His urine samples(about 30mL each time) were taken 1,2,3,5, and 8 hours after eating the pills. Each urine sample was filtered immediately through a 0.45μm membrane filter. A 19mL aliquot of filtered urine was taken and 1 mL of internal standard stock solution was added and immediately injected for analysis by Gradient μLC/MS.

Chromatographic procedures

The home-made Alltima C18 column(300 0.5mm) was used. The gradient elution flow rate was fixed at 10 μL/min. The solvent A was 0.1% TFA(trifluoroacetic acid) in methanol, and B, 0.1% TFA in water. The eluent composition was initially 30% A + 70% B, held for 2min, and was linearly changed to 90% A + 10% B in 4 min, held for 4 min, then swiftly returned to the initial composition.

RESULTS AND DISCUSSION

We have analyzed vitamins in urine using a μLC/MS system with a home-made disposable microcolumn. The merit of our method is elimination of pretreatment of urine samples. If a conventional HPLC method with a UV detector is used, tedious pretreatment is necessary to concentrate the components and to eliminate matrix interferences.

The SIR chromatograms for individual components obtained by the gradient μLC/MS

system for a standard solution and the urine sample taken 3 hours after eating vitamin pills are shown in Figure 1.

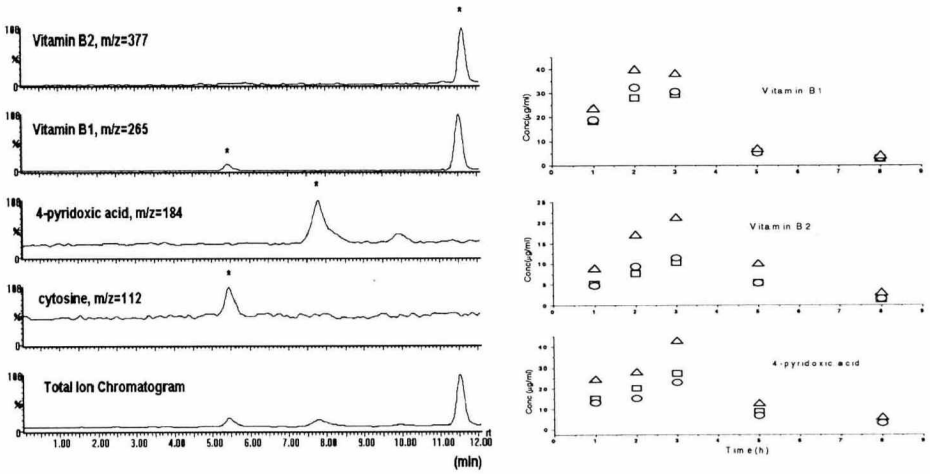

Figure 1. The SIR chromatograms of individual components obtained by gradient μLC/MS for the urine sample taken 3 hours after eating vitamin pills. The component of interest in each SIR chromatogram is marked with an asterisk.

Figure 2. The trends of concentrations(with their reliability ranges) of vitamin B1, B2 and the metabolite of vitamin B6(4-pyridoxic acid) in urine of three volunteers with respect to elapsed time after swallowing 3 vitamin pills. □:person A, ○:person B, △:person C.

The calibration curves of individual components by standard solutions show good linearity for each component over the concentration range examined.

The excretion patterns of vitamin B1, B2, and 4-pyridoxic acid with regard to elapsed time for the three volunteers are compared in Figure 2. The concentrations of vitamin B1, B2, and 4-pyridoxic acid in urine increased rapidly after eating vitamin pills and reached the peak when 3 hours elapsed, and decreased rapidly. The general trends were similar although some variances in concentrations of vitamins in urine among the three volunteers were observed. Vitamin B6 and B12 were not found in the urine samples. Vitamin B6 is quickly metabolized to 4-pyridoxic acid, and a very small amount of vitamin B12 (5.22μg per pill) was contained in the vitamin pill, thus the concentrations of vitamin B6 and B12 in urine would be negligible.

Direct excretion of a vitamin component was found insignificant compared to decomposition by metabolism. For example, the total amount of vitamin B1 excreted in urine of person A was estimated to be ca 2mg while the total amount of vitamin B1 swallowed was 150mg. The swallowed amount of vitamin B2 was 7.5mg but only 0.6mg was

excreted in its original form in urine. Nevertheless, the concentration of a vitamin component in urine should be a measure of level of the component in the body.

Rapid and easy monitoring of components of biological interest in urine will be often very important for clinical purposes. This study shows that μLC/MS techniques can serve nicely for such purposes when a cheap disposable home-made microcolumn is incorporated.

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