Simultaneous Determination of Water-Soluble Vitamins (Vitamin B₁, B₂, B₃, B₆ and C) in Dietary Supplements by High-Performance Liquid Chromatography

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영양보충용 식품 중 수용성비타민(Vitamin B₁, B₂, B₃, B₆ and C)의 HPLC를 이용한 동시분석법
서 희 재 · 김 소 희
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Abstract

시중에 유통 중인 영양보충용 제품의 수용성 비타민 B₁ (thiamin), B₂ (riboflavin), B₃ (nicotinic acid and nicotine amide), B₆ (pyridoxine), C (ascorbic acid)의 신속한 동시분석 방법을 확립하기 위하여 본 연구를 실시하였다. 영양보충용 제품은 정제, 연질캡슐, 분말, 액상의 4가지 제형에 대해 27종의 제품을 구입한 후, Ion-pair 분리기법을 사용하여 HPLC-UVD를 이용한 동시분석 방법을 검토하였다. 비타민 B₁, B₂, B₃, B₆, C의 HPLC에 의한 동시분석 조건을 검토한 결과, 이동상은 0.02% triethylamine, 17.5% 메탄올, 5 μM sodium hexanesulfonic acid가 함유된 pH 3.5(acetic acid로 조절)의 수용액을 사용하였고, 용출시간은 다른 피크의 영향을 받지 않도록 30분으로 하였다. 수용성 비타민의 회수율은 96% 이상이었다. 본 연구에 의해 확립된 수용성 비타민의 동시분석 조건은 검량선의 직선성, 정밀성, 정확성 등이 USP 및 ICH 기준에 적합하며 HPLC의 동시분석 방법으로 향상하였다. 수용성 비타민의 수출 용배는 제형에 따라 약간의 차이를 보이긴 했으나, 물이나 산성조건을 갖춘 HPLC의 이동상이 메탄올이나 메탄올보다 높은 수출 효율을 보였다. 초음파 추출기에 의한 추출 시간은 20분이 가장 적당하였다. 본 연구의 결과는 수용성 비타민의 신속한 추출 및 분석에 매우 효율적으로 이용될 것으로 기대된다.

Key words: dietary supplement, water-soluble vitamin, simultaneous determination, HPLC

INTRODUCTION

Vitamin performs a wide variety of function in human body and is essential for good health (NIH 2001). For example, vitamin B₁ (thiamin) works as a cofactor for the metabolism of carbohydrates and needs for nerve transmission. Vitamin B₂ (riboflavin) works as a coenzyme for a wide variety of enzymes in the intermediate metabolism (Lynch & Young 2000). Vitamin B₃ (niacin and its amide) is very helpful for lowering high cholesterol level and elevating high-density lipoprotein cholesterol level (HDL) (NIH 2001). Vitamin B₆ (pyridoxine) is need for more than 100 enzymes involved in protein metabolism and essential for red blood cell metabolism, nervous and immune systems (NIH 2001). Vitamin C (ascorbic acid) can protect tissues and cells against oxidative damages by free radicals (Morisaki & Ozaki 1996). Individuals with a poor quality diet or an inadequate vitamin intake for an extended period may benefit from taking a vitamin supplement if they are...
unable to increase their dietary intake of vitamin (Leklem 1999; NIH 2001). Nowadays, various dietary supplements in the form of tablet, powder, capsule and liquid are available in the market. In order to determine vitamin concentration in foodstuff, fluorometric, spectrometric, titrimetric, gas chromatographic and high-performance liquid chromatographic methods have been described, but some of these methods are currently rarely used (Albalá-Hurtado et al. 1997). High-performance liquid chromatographic methods (HPLC) to be developed for the determination of water-soluble vitamins in various foods have enabled rapid, specific and sensitive analysis. Recently, several ion-pairing chromatographic methods using reversed phase column have been reported to determine of water-soluble vitamins, such as infant milk (Viñas et al. 2003), baby foods (De Beer et al. 2003), multivitamin preparations (Lam et al. 1984) and liquid tonics (Maeda et al. 1989).

However, no references are available for the simultaneous determination of water-soluble vitamins in dietary supplements. In this study, we have developed a rapid and precise method for simultaneous determination of water-soluble vitamins in tablet, powder, capsule and liquid dietary supplements. The separation of water-soluble vitamins such as vitamin B\textsubscript{1} (thiamin), vitamin B\textsubscript{2} (riboflavin), vitamin B\textsubscript{3} (nicotinic acid and nicotine amide), vitamin B\textsubscript{6} (pyridoxine) and vitamin C was optimized using ion-pair HPLC method with a reversed phase column and an UV detection.

MATERIALS AND METHOD

1. Reagents and Solvents

Methanol, ethanol and water were of HPLC grade (Merck, Darmstadt, Germany). Acetic acid was of reagent grade. Sodium hexane-sulfonic acid and triethylamine were supplied by Sigma-Aldrich (St. Louis, MO, USA). Standards of thiamin hydrochloride, riboflavin, pyridoxine hydrochloride, nicotine amide, nicotinic acid and ascorbic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Standards solutions were prepared as follows; 1, 5, 10, 50 and 100 \(\mu\)g/ml of thiamin hydrochloride, riboflavin, pyridoxine hydrochloride, nicotine amide, nicotinic acid and ascorbic acid in mobile phase. All standard solutions were filtered through a 0.45 \(\mu\)m membrane (Millipore, Bedford, MA, USA), stored in a dark and cool space (at 4°C in a refrigerator).

The mobile phase containing 0.02% triethyamine was prepared as described below;

1. Reference solution: (a) Adjust pH of the aqueous solution to 3.5, 5.0 and 6.5. (b) Add 1, 5 and 10 \(\mu\)M of ion-pairing reagent (sodium hexane-sulfonic acid) to the aqueous solution. (c) Add 12.5, 17.5 and 22.5 ml of LC grade methanol in 100 ml aqueous solution containing 0.02% triethyamine.

2. Working mobile phase: The mobile phase was prepared to mix 0.02% triethyamine, 5 \(\mu\)M sodium hexane-sulfonic acid and 17.5% methanol in LC grade water adjusted to pH 3.5 with acetic acid. Filter the aqueous solution through a 0.45 \(\mu\)m membrane (Millipore, Bedford, MA, USA) before HPLC injection.

2. Apparatus

The pH of the mobile phases was measured with a pH 210 (Hanna, Italy) pH meter, equipped with a HI 1131B pH glass electrode. The ultrasonic processor power sonic 420 (Whasin, Korea) was used for extraction of vitamins from the samples.

The HPLC system was operated on a Shiseido Nanospace SI-2 separation module (Tokyo, Japan) equipped with Shiseido Nanospace SI-2 UV/VIS detector controlled by Millennium 32 chromatography manager data acquisition system. The column used for the analysis was a Capcell-Pak C\textsubscript{18} MG Column (150 \(\times\) 3.0 mm, 5 \(\mu\), Shiseido, Tokyo, Japan), used for separation at 40°C. The flow rate was 500 \(\mu\)l/min. The detector wave-length set at 254 nm.

3. Sample Preparation

The dietary supplements were presented in the form of powder, liquid, tablet and capsule. In order to improve the extraction efficiency of vitamins, different types of dietary supplements has been prepared as follows method. (a) Powder and liquid form samples: Powder and liquid samples were weighed as amount 10 to 50 \(\mu\)g/ml as water-soluble vitamins (based on the Nutrition Facts of the product) into four 50 ml volumetric flask. Then, 10 ml of four types of solution (mobile phase, water, ethanol and methanol) were added into the flask, respectively. The four flasks were protected from light by covered with aluminum foil and the mixtures were thoroughly extracted for 1, 10, 20, and 30 min at ultra sonic bath. The extracts were filtered through a 0.45 \(\mu\)m membrane filter (Millipore, Bedford, MA, USA) before HPLC analysis. A sample blank and control sample were included in each analytical schedule. (b) Other types of samples: The Korean Food Code stipulates that the experimental method for net contents must be performed with collected twenty tablets.