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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

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**Determination of thiamine hydrochloride, pyridoxine
hydrochloride niacin, niacinamide and caffeine in health foods
(English Translation)**

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Foreword

This Standard was proposed by and shall be under the jurisdiction of the Ministry of Health of the People's Republic of China.

Chief drafting organization of this Standard. Institute of Food Safety Control and Inspection Ministry of Public Health.

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Introduction

Thiamine hydrochloride, pyridoxine hydrochloride, niacin, niacinamide and other vitamins-B family are the indispensable nutrients for human beings; they play an important role in prevention and treatment of various diseases. Currently vitamins-B family and caffeine are added into health food as functional component. On basis of the reference to the vitamin detection method of Japanese Taisho pharmaceutical Co., Ltd, and combined with the specific conditions of instrument analysis in our country, this method is formulated as a high performance liquid chromatographic method that can determine the thiamine hydrochloride, pyridoxine hydrochloride, niacin, niacinamide and other vitamins-B family and caffeine that are added in tablet, capsule, oral liquid, beverages as well as other health foods.

Determination of thiamine hydrochloride, pyridoxine hydrochloride, niacin, niacinamide and caffeine in health foods

1 Scope

This Standard specifies the high performance liquid chromatography determination method of thiamine hydrochloride, pyridoxine hydrochloride, niacin, niacinamide and caffeine in health foods.

This Standard is applicable to the high performance liquid chromatography determination method for ADDING thiamine hydrochloride, pyridoxine hydrochloride, niacin, niacinamide and caffeine that are served as dietary supplements INTO test sample types such as tablet, capsule, oral liquid and drink.

Detection limit of this method is. 0.02 μ g for thiamine hydrochloride, 0.02 μ g for pyridoxine hydrochloride, 0.10 μ g for niacin, 0.06 μ g for niacinamide, and 0.05 μ g for caffeine. The linear range is. 0.500 μ g/mL ~ 50.0 μ g/mL for thiamine hydrochloride; 0.500 μ g/mL ~ 50.0 μ g/mL for pyridoxine hydrochloride; 1.00 μ g/mL ~ 100 μ g/mL for niacin; 1.00 μ g/mL ~ 100 μ g/mL for niacinamide; and 5.00 μ g/mL ~ 500 μ g/mL for caffeine.

2 Principle

Use methanol + water + phosphoric acid=100+400+0.5 to extract and dilute the uniformly crushed tablet, capsule or liquid test samples; then conduct qualitative and quantitative determination according to the external standard method of high performance liquid chromatography UV detector.

3 Reagents

Unless otherwise specified, the water used in the analysis is double-distilled water.

3.5 Sodium 1-decanesulfonate. High performance liquid chromatography (HPLC) reagents.

3.7 Standard stock solution B. Accurately and respectively weigh 0.0400g of standard

nicotinamide and 0.0400g of standard niacin of which the moisture has been deducted; dissolve into water and fix the volume to 20mL.

5.1.1 Take more than 20 tablets or capsule samples; grind it or mix it evenly; weigh a

certain amount of sample (accurate to 0.001g) and place into a test tube; add methanol + water + phosphate = 100 + 400 + 0.5 mixed solution; make the concentration to be - in each 1mL, it contains thiamine hydrochloride, pyridoxine hydrochloride 0.25 mg; and it contains niacin, niacinamide 1 mg. After the supersonic extraction for 5 min, centrifuge it at 3000r/min for 5 minutes; filter the supernatant through 0.45μm membrane, it is ready for injection.

5.2.3 Detection wavelength. For thiamine hydrochloride, 260 nm. For caffeine, niacin,

nicotinamide, pyridoxine hydrochloride, 280nm.

6 Result calculation

Reserve two significant digits for the calculation results.

7 Precision

The absolute difference of two independent determination results, under the repeatability condition, shall not be over 10% of the arithmetic mean.

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