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

保健食品中盐酸硫胺素、盐酸吡哆醇、烟酸、烟酰胺和咖啡因的测定

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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: $0.02\mu g$ for thiamine hydrochloride, $0.02\mu g$ for pyridoxine hydrochloride, $0.10\mu g$ for niacin, $0.06\mu g$ for niacinamide, and $0.05\mu g$ for caffeine. The linear range is: $0.500\mu g/mL \sim 50.0\mu g/mL$ for thiamine hydrochloride; $0.500\mu g/mL \sim 50.0\mu g/mL$ for pyridoxine hydrochloride; $1.00\mu g/mL \sim 100\mu g/mL$ for niacin; $1.00\mu g/mL \sim 100\mu g/mL$ for niacinamide; and $5.00\mu g/mL \sim 500\mu g/mL$ 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.

- **3.1** Methanol: Guaranteed reagent.
- 3.2 Acetonitrile: Chromatographically pure.

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- 3.3 Phosphoric acid: Analytically pure.
- **3.4** Sodium dodecylsulhte: High performance liquid chromatography (HPLC) reagents.
- **3.5** Sodium 1-decanesulfonate: High performance liquid chromatography (HPLC) reagents.
- **3.6** Standard stock solution A: Accurately and respectively weigh 0.0500g of standard thiamine hydrochloride and 0.0500g of standard pyridoxine hydrochloride of which the moisture has been deducted; dissolve into water and fix the volume to 50mL.
- **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.
- **3.8** Mixed standard working solution: Accurately weight 0.0500 g of caffeine that has been dried at 80° C for 4h; add 5mL of standard stock solution A and 10mL of standard stock solution B into the caffeine; then add mixed solution of methanol + water + phosphoric acid = 100 + 400 + 0.5 TO 20 mL.

4 Instruments

- **4.1** High performance liquid chromatograph: attached with ultraviolet detector.
- 4.2 Acoustic cleaner.
- 4.3 Centrifuge.

5 Analysis procedures

5.1 Sample treatment

- **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 0.45μ m membrane, it is ready for injection.
- **5.1.2** Liquid sample is directly diluted with methanol + water + phosphate = 100 + 400 + 0.5 mixed solution; mixed evenly; filter through 0.45 µm membrane, it is ready for

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