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Vitamins B and C Injection

General Notices

DEFINITION

Vitamins B and C Injection is a sterile solution of Thiamine Hydrochloride, Pyridoxine Hydrochloride, Riboflavin Sodium Phosphate, Nicotinamide and Ascorbic Acid (as the sodium salt) in Water for Injections, containing Glucose for the intravenous injection or Benzyl Alcohol for the intramuscular injection.

Vitamins B and C Injection is prepared immediately before use by mixing the contents of two ampoules, (1) and (2). Ampoule (1) contains Thiamine Hydrochloride, Pyridoxine Hydrochloride, Riboflavin Sodium Phosphate and, where appropriate, Benzyl Alcohol. Ampoule (2) contains Nicotinamide and Ascorbic Acid and, where appropriate, Glucose. The air in ampoule (2) is replaced by nitrogen or other suitable inert gas.

The injection complies with the requirements stated under Parenteral Preparations and the contents of ampoules (1) and (2) comply with the following requirements, as appropriate.

Content of thiamine hydrochloride, $C_{12}H_{17}ClN_4OS,HCl$

92.0 to 106.0% of the stated amount.

Content of pyridoxine hydrochloride, $C_8H_{11}NO_3,HCl$

90.0 to 110.0% of the stated amount.

Content of riboflavin, $C_{17}H_{20}N_4O_6$

90.0 to 110.0% of the stated amount.

Content of nicotinamide, $C_6H_6N_2O$

95.0 to 105.0% of the stated amount.

Content of ascorbic acid, $C_6H_8O_6$

95.0 to 105.0% of the stated amount.

Content of glucose, $C_6H_{12}O_6$

Where appropriate, 90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 60 volumes of *methanol* and 40 volumes of a solution containing 0.14% w/v of *potassium dihydrogen orthophosphate* and 0.5% w/v of *disodium edetate* as the mobile phase. Apply separately to the plate 2 µL of each of the following solutions in *water*. For solution (1) use a volume of the contents of ampoule (1) diluted, if necessary, with *water* to contain 0.2% w/v of Pyridoxine Hydrochloride. For solution (2) use a volume of the injection in ampoule (2) diluted, if necessary, with *water* to contain 2% w/v of Ascorbic Acid. Solutions (3) and (4) contain 1% w/v and 0.4% w/v respectively of *thiamine mononitrate BPCRS*. Solution (5) contains 0.2% w/v of *pyridoxine hydrochloride BPCRS*. Solution (6) contains 0.022% w/v of *riboflavin sodium phosphate BPCRS*. Solution (7) contains 0.64% w/v of *nicotinamide BPCRS*. Solution (8) contains 2% w/v of *ascorbic acid BPCRS*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm and 365 nm*). The principal spots in the chromatogram obtained with solution (1) correspond to those in the chromatograms obtained with either solutions (3), (5) and (6) or solutions (4), (5) and (6). The principal spots in the chromatogram obtained with solution (2) correspond to those in the chromatograms obtained with solutions (7) and (8).

B. *For injections containing Glucose*, heat 1 mL of the contents of ampoule (2) with *cupri-tartaric solution R1*. A copious precipitate of copper(I) oxide is produced.

ASSAY

For thiamine hydrochloride, pyridoxine hydrochloride and nicotinamide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*. Solution (1) contains 0.01% w/v of *thiamine mononitrate BPCRS* and 0.002% w/v of *pyridoxine hydrochloride BPCRS*. Solution (2) contains 0.01% w/v of *thiamine mononitrate BPCRS* and 0.005% w/v of *pyridoxine hydrochloride BPCRS*. Solution (3) contains 0.016% w/v of *nicotinamide BPCRS*. For solution (4) dilute a suitable volume of the contents of ampoule (1) with *water* to contain 0.01% w/v of Thiamine Hydrochloride. For solution (5) dilute a suitable volume of the contents of ampoule (2) to contain 0.016% w/v of Nicotinamide.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) as the mobile phase with a flow rate of 1 mL per minute, 0.22% w/v of *sodium heptanesulfonate* in a mixture of 75 volumes of a 1.36% w/v solution of *potassium dihydrogen orthophosphate* and 25 volumes of *methanol*, the pH of the final mixture being adjusted to 3.0 with *orthophosphoric acid*, and (c) a detection wavelength of 280 nm.

Calculate the content of $C_{12}H_{17}ClN_4OS,HCl$ using the declared content of $C_{12}H_{17}N_5O_4S$ in *thiamine mononitrate BPCRS*. Each mg of $C_{12}H_{17}N_5O_4S$ is equivalent to 1.030 mg of $C_{12}H_{17}ClN_4OS,HCl$. Calculate the content of $C_8H_{11}NO_3,HCl$ using the declared content of $C_8H_{11}NO_3,HCl$ in *pyridoxine hydrochloride BPCRS*. Calculate the content of $C_6H_6N_2O$ using the declared content of $C_6H_6N_2O$ in *nicotinamide BPCRS*.

For riboflavin

Dilute a volume of the contents of ampoule (1) containing the equivalent of 4 mg of riboflavine with sufficient *phthalate buffer pH 4.0* to produce 200 mL and measure the *absorbance* of the resulting solution at the maximum at 446 nm, Appendix II B. Calculate the content of $C_{17}H_{20}N_4O_6$ taking 323 as the value of A(1%, 1 cm) at the maximum at 446 nm.

For ascorbic acid

Dilute a volume of the contents of ampoule (2) with *water* to produce a solution containing 1% w/v of Ascorbic Acid. To 20 mL of the resulting solution add 5 mL of 1M *sulfuric acid* and 50 mL of *0.05M iodine VS* and titrate the excess iodine with 0.1M *sodium thiosulfate VS* using *starch mucilage* as indicator. Each mL of *0.05M iodine VS* is equivalent to 8.806 mg of $C_6H_8O_6$.

For glucose

For injections containing Glucose, dilute a volume of the contents of ampoule (2) containing 0.8 g of Glucose to 500 mL with *water* and dilute 2 mL of the resulting solution to 100 mL with *water*. Transfer 3 mL of this solution to a boiling tube previously cleaned with *chromic-sulfuric acid mixture* and rinsed with *water*. In two similar tubes place separately 3 mL of *glucose standard solution* and 3 mL of *water*. Add 6 mL of *anthrone reagent* in such a manner as to ensure rapid mixing, allow to stand for 10 minutes, cool quickly and measure the *absorbance*, Appendix II B, of the test solution and of the

standard solution at the maximum at 625 nm using the solution prepared with *water* in the reference cell. Calculate the concentration of $C_6H_{12}O_6$ in the test solution.

STORAGE

Ampoules (1) and (2) for Vitamins B and C Injection should be protected from light.

LABELLING

For each ampoule the label states (1) the directions for the preparation of the injection; (2) whether the final injection is for intravenous or intramuscular use; (3) that the final injection is a high potency injection.

For ampoule (1) the label states the quantity of Riboflavin Sodium Phosphate in terms of the equivalent amount of riboflavin.