



Shake 0.7 g in 10 mL of [water](#) for 5 minutes and centrifuge. To 5 mL of the supernatant add 45 mL of [water](#) and 3 drops of [phenolphthalein solution R1](#) and 0.1 mL of [0.1M hydrochloric acid VS](#). A red colour is not produced.

### Related substances

Carry out the method for [liquid chromatography, Appendix III D](#), using the following solutions.

- (1) Dissolve 50 mg of the substance being examined in 20 mL of [tetrahydrofuran](#) and 2 mL of [glacial acetic acid](#) and dilute with sufficient [absolute ethanol](#) to produce 100 mL.
- (2) Dilute 1 mL of solution (1) to 100 mL with [absolute ethanol](#).
- (3) Dissolve 5 mg of [dantrolene sodium BPCRS](#) and 0.1 g of [theophylline BPCRS](#) in 20 mL of [tetrahydrofuran](#) and 2 mL of [glacial acetic acid](#) and dilute with sufficient [absolute ethanol](#) to produce 100 mL. Further dilute 10 mL of this solution to 100 mL with [absolute ethanol](#).

### CHROMATOGRAPHIC CONDITIONS

- (a) Use a stainless steel column (15 cm × 4.6 mm) packed with [silica gel for chromatography](#) (5 µm) (Zorbax Sil is suitable).
- (b) Use isocratic elution and the mobile phase described below.
- (c) Adjust the flow rate of the mobile phase so that the retention time of the peak corresponding to Dantrolene Sodium is about 8 minutes.
- (d) Use a column temperature of 30°.
- (e) Use a detection wavelength of 300 nm.
- (f) Inject 10 µL of each solution.
- (g) For solution (1) allow the chromatography to proceed for at least twice the retention time of the principal peak.

### MOBILE PHASE

9 volumes of [absolute ethanol](#), 10 volumes of [glacial acetic acid](#) and 90 volumes of [hexane](#).

### SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the [resolution](#) between the peaks corresponding to theophylline and dantrolene is at least 6.

### LIMITS

In the chromatogram obtained with solution (1):

the total area of all the [secondary peaks](#) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

### Water

14.5 to 17.0% w/w, [Appendix IX C](#). Use 0.2 g

## ASSAY

Carry out the method for [liquid chromatography, Appendix III D](#), using the following solutions.

- (1) Dissolve 60 mg of the substance being examined in 50 mL of [dimethylformamide](#) and dilute 1 volume of the resulting solution to 100 volumes with the mobile phase.
- (2) Dilute 1 volume of a 0.12% w/v solution of [dantrolene sodium BPCRS](#) in [dimethylformamide](#) to 100 volumes with the mobile phase.

### CHROMATOGRAPHIC CONDITIONS

- (a) Use a stainless steel column (15 cm × 4.6 mm) packed with spherical particles of silica, 5 µm in diameter, the surface of which has been modified with chemically-bonded nitrile groups (Spherisorb CN is suitable).
- (b) Use isocratic elution and the mobile phase described below.
- (c) Use a flow rate of 1 mL per minute.
- (d) Use an ambient column temperature.
- (e) Use a detection wavelength of 262 nm.

(f) Inject 20 µL of each solution.

#### MOBILE PHASE

15 volumes of [acetonitrile](#) and 85 volumes of a phosphate buffer pH 6.8 prepared by dissolving 11.88 g of [disodium hydrogen orthophosphate](#) and 9.08 g of [potassium dihydrogen orthophosphate](#) in 1000 mL of [water](#).

#### DETERMINATION OF CONTENT

Calculate the content of  $C_{14}H_9N_4NaO_5$  in the substance being examined using the declared content of  $C_{14}H_9N_4NaO_5$  in [dantrolene sodium BPCRS](#).