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Notes: Revised Related Substances and Assay	

Spironolactone Tablets

Spironolactone Preparations

Action and use
Aldosterone receptor antagonist; potassium-sparing diuretic.

DEFINITION

Spironolactone Tablets contain Spironolactone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of spironolactone, $C_{24}H_{32}O_4S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Spironolactone with 10 mL of *dichloromethane* and filter (Whatman GF/C is suitable).

(2) 0.2% w/v of *spironolactone BPCRS* in *dichloromethane*.

(3) 0.01% w/v of each of *spironolactone BPCRS* and *canrenone EPCRS* in *dicloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254} (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light* (*254 nm*).

MOBILE PHASE

1 volume of *water*, 24 volumes of *cyclohexane* and 75 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 1000 mL of 0.1M *hydrochloric acid* containing 0.1% w/v *sodium dodecyl sulfate* and rotate the paddle at 75 revolutions per minute. Withdraw a sample of 10 mL of the medium, filter and measure the *absorbance* of the filtrate, suitably diluted if necessary, at the maximum at 242 nm, Appendix II B. Calculate the total content of $C_{24}H_{32}O_4S$ in the medium taking 445 as the value of A(1%, 1 cm) at the maximum at 242 nm.

Related substances

Carry out the following procedure protected from light and prepare samples immediately before use. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A.

Solution A

10 volumes of *methanol*, 20 volumes of a 0.0029% w/v solution of *orthophosphoric acid*, 20 volumes of *water* and 50 volumes of *acetonitrile*.

(1) Shake a quantity of powered tablets containing 50 mg of Spironolactone with 400 mL of solution A. Dilute this solution to 500 mL with solution A and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A.

(3) Dilute 1 volume of solution (2) to 10 volumes with solution A.

(4) 0.0001% w/v of each of *spironolactone BPCRS* and *canrenone EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable) fitted with a guard column (4 mm × 3 mm) packed with the same material.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A

5 volumes of *acetonitrile*, 35 volumes of *methanol* and 60 volumes of *water*.

Mobile phase B

25 volumes of *acetonitrile*, 35 volumes of *methanol* and 40 volumes of *water*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	100	0	isocratic
2-35	100→0	0→100	linear gradient
35-43	0	100	isocratic
43-44	0→100	100→0	linear gradient
44-49	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to spironolactone and canrenone is at least 2.0

LIMITS

In the chromatogram obtained with solution (1):

identify any peak corresponding to canrenone using solution (4) and multiply the area of this peak by the correction factor of 1.7;

the area of any peak corresponding to canrenone is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the following procedure protected from light and prepare samples immediately before use. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A.

Solution A

10 volumes of *methanol*, 20 volumes of a 0.0029% w/v solution of *orthophosphoric acid*, 20 volumes of *water* and 50 volumes of *acetonitrile*.

(1) Weigh and powder 20 tablets. Shake a quantity of powered tablets containing 50 mg of Spironolactone with 400 mL of solution A. Dilute this solution to 500 mL with solution A and filter.

(2) 0.01% w/v of *spironolactone BPCRS* in solution A.

(3) Dilute 1 volume of solution (2) to 10 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to spironolactone and canrenone is at least 2.0

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{32}O_4S$ in the tablets using the declared content of $C_{24}H_{32}O_4S$ in *spironolactone BPCRS.*

STORAGE

Spironolactone Tablets should be protected from light.