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Abacavir and Lamivudine Tablets

General Notices

Action and use

Nucleoside reverse transcriptase inhibitor; antiviral (HIV).

DEFINITION

Abacavir and Lamivudine Tablets contain Abacavir Sulfate and Lamivudine.

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

Content of abacavir, $C_{14}H_{18}N_6O$

95.0 to 105.0% of the stated amount.

Content of lamivudine, $C_8H_{11}N_3O_3S$

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 powdered tablets containing the equivalent of 0.2 g of abacavir with 50 mL of *water*, filter and use the filtrate.
(2) 0.23% w/v of *abacavir sulfate BPCRS* in *water*.
(3) 0.1% w/v of *lamivudine BPCRS* in *water*.

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

MOBILE PHASE

3 volumes of *glacial acetic acid*, 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (1) shows two clearly separated spots.

CONFIRMATION

The chromatogram obtained with solution (1) shows two principal spots corresponding in position and size to the principal spots in the chromatograms obtained with solutions (2) and (3).

B. In the Assay, the chromatogram obtained with solution (1) shows principal peaks with the same retention time as the principal peaks due to abacavir and lamivudine in the chromatograms obtained with solutions (2) and (3) respectively.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 and rotate the paddle at 75 revolutions per minute.
(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A: Dissolve 1.9 g of *ammonium acetate* in 900 mL of *water*, adjust the pH to 3.9 with *glacial acetic acid* and dilute to 1000 mL.

(1) After 45 minutes withdraw a sample of the medium and filter. Dilute with the dissolution medium, if necessary, to produce a solution containing the equivalent of 0.067% w/v of abacavir.
(2) 0.078% w/v of *abacavir sulfate BPCRS* and 0.033% w/v of *lamivudine BPCRS* in solution A.
(3) 0.01% w/v of *lamivudine impurity standard BPCRS* and 0.016% w/v of *abacavir sulfate BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the total content of abacavir, $C_{14}H_{18}N_6O$, and lamivudine, $C_8H_{11}N_3O_3S$, in the medium using the declared contents of $C_{14}H_{18}N_6O$, in *abacavir sulfate BPCRS* and of $C_8H_{11}N_3O_3S$ in *lamivudine BPCRS*.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *lamivudine impurity standard BPCRS* and the retention of abacavir relative to lamivudine is about 2.6;

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to lamivudine impurity B and lamivudine is at least 2.0.

LIMITS

The amounts of abacavir and lamivudine released are not less than 75% (Q) of the stated amounts.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A.

Solution A: Dissolve 1.9 g of *ammonium acetate* in 900 mL of *water*, adjust the pH to 3.9 with *glacial acetic acid* and dilute to 1000 mL.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.1 g of abacavir in 60 mL with the aid of ultrasound for 30 minutes, dilute to 100 mL and filter. Dilute 1 volume of the filtrate to 5 volumes.
(2) Dilute 1 volume of solution (1) to 50 volumes. Further dilute 1 volume to 10 volumes.
(3) 0.01% w/v of *lamivudine impurity standard BPCRS* and 0.016% w/v of *abacavir sulfate BPCRS*.
(4) 0.0001% w/v each of *salicylic acid BPCRS* (lamivudine impurity C) and *cytosine* (lamivudine impurity E).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (YMC ODS-A is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use a column temperature of 30°.
(e) Use a detection wavelength of 270 nm.
(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.025M *ammonium acetate*, adjusted to pH 3.9 with *glacial acetic acid*.

Mobile phase B *methanol*.

Mobile phase C *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Mobile phase C (% v/v)	Comment
0-15	95	5	0	isocratic
15-30	95→70	5→30	0	linear gradient
30-38	70	30	0	isocratic
38-60	70→0	30→0	0→100	linear gradient
60-65	0	0	100	column wash
65-66	0→95	0→5	100→0	linear gradient
66-75	95	5	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the relative retentions with reference to lamivudine (retention time about 15 minutes) are lamivudine impurity E, about 0.2; lamivudine impurity F, about 0.3; lamivudine impurity A, about 0.35; lamivudine impurity H, about 0.37; lamivudine impurity C, about 0.39; lamivudine impurity G, about 0.4; lamivudine impurity B, about 0.9; lamivudine impurity J, about 1.5 and abacavir, about 2.6.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *lamivudine impurity standard BPCRS* and the retention of abacavir relative to lamivudine is about 2.6;

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to lamivudine impurity B and lamivudine is at least 2.0.

LIMITS

Using the chromatogram obtained with solutions (3) and (4), the reference chromatogram supplied with *lamivudine impurity standard BPCRS* and the relative retentions identify any peaks in solution (1) corresponding to the named lamivudine impurities. Multiply any peaks areas corresponding to lamivudine impurity C and lamivudine impurity E by the following correction factors; impurity C, 1.3; impurity E, 0.6.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to lamivudine impurity J is not greater than 2.5 times the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to lamivudine impurity A is not greater than 1.5 times the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to lamivudine impurities B, C, E, F, G and H is not greater than the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than the area of the peak due to abacavir in the chromatogram obtained with solution (2) (0.2%).

The total impurity content is not greater than 2.2%.

Disregard:

any peak corresponding to a lamivudine impurity with an area less than half the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.1%);

any other peak with an area less than half the area of the peak due to abacavir in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in solution A.

Solution A: Dissolve 1.9 g of *ammonium acetate* in 900 mL of *water*, adjust the pH to 3.9 with *glacial acetic acid* and dilute to 1000 mL.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.2 g of abacavir with 60 mL of solution A in a 100-mL amber volumetric flask for 30 minutes, dilute to 100 mL and filter. Dilute 1 volume to 5 volumes.
(2) 0.046% w/v of *abacavir sulfate BPCRS*
(3) 0.02% w/v of *lamivudine BPCRS*.
(4) 0.01% w/v of *lamivudine impurity standard BPCRS* and 0.016% w/v of *abacavir sulfate BPCRS*.

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 (4):

the chromatogram obtained with solution (4) closely resembles the reference chromatogram supplied with *lamivudine impurity standard BPCRS* and the retention of abacavir relative to lamivudine is about 2.6;

the *resolution* between the peaks due to lamivudine impurity B and lamivudine is at least 2.0.

DETERMINATION OF CONTENT

Using solutions (1) and (2), calculate the content of $C_{14}H_{18}N_6O$ in the tablets from the chromatograms obtained using the declared content of $C_{14}H_{18}N_6O$ in *abacavir sulfate BPCRS*.

Using solutions (1) and (3) calculate the content $C_8H_{11}N_3O_3S$ in the tablets from the chromatograms obtained using the declared content of $C_8H_{11}N_3O_3S$ in *lamivudine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Abacavir Sulfate, excluding impurity A, and Lamivudine.