Azithromycin Eye Drops

Azithromycin Eye Drops is a sterile solution of Azithromycin in a suitable oily vehicle.

Azithromycin Eye Drops contain not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of azithromycin, $C_{38}H_{72}N_2O_{12}$.

Usual strength. 1.0 per cent w/v.

Identification

A. Mix a quantity of the eye drops containing 100 mg of Azithromycin with 10 ml of *ethanol.* Allow to stand, retain the upper ethanolic layer and evaporate it to dryness under a stream of nitrogen. Wash the residue with 10 ml of *hexane* followed by a further 50 ml of *hexane* and allow to dry in air. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *azithromycin IPRS* treated in the same manner or with the reference spectrum of azithromycin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE - Prepare the solutions immediately before use.

Solvent mixture. 20 volumes of *dichloromethane* and 80 volumes of *methanol*.

Test solution. Dilute a suitable volume of the eye drops with the solvent mixture to obtain a solution containing 0.8 per cent w/v of Azithromycin.

Reference solution (a). A 0.008 per cent w/v solution of *azithromycin IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v of *azithromycin IPRS* and *6-demethyl-azithromycin IPRS (azithromycin impurity A)* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end-capped octadecylsilane amorphous organosilica polymer (5 µm) (Such as X-terra MS),
- column temperature: 60°,
- mobile phase: A. a 0.18 per cent w/v solution of *anhydrous disodium hydrogen phosphate*, adjusted to pH 8.9 with *dilute orthophosphoric acid* or with *dilute sodium hydroxide solution*,
  B. a mixture of 25 volumes of *methanol* and 75 volumes of *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile Phase B (per cent v/v)
0	50	50
25	45	55
30	40	60
80	25	75
81	50	50
93	50	50

Name	Relative retention time	Correction factor
Azithromycin impurity L	0.29	2.3
Azithromycin impurity M	0.37	0.6
Azithromycin impurity E	0.43	---
Azithromycin impurity F	0.51	0.3
Azithromycin impurity D	0.54	---
Azithromycin impurity J	0.54	---
Azithromycin impurity I	0.61	---
Azithromycin impurity C	0.73	---
Azithromycin impurity N	0.76	0.7

Azithromycin impurity H	0.79	0.1
Azithromycin impurity A	0.83	---
Azithromycin impurity P	0.92	---
Azithromycin (Retention time: about 45-50 minutes)	1.0	---
Azithromycin impurity O	1.23	---
Azithromycin impurity G	1.26	0.2
Azithromycin impurity B	1.31	---

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromycin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak eluting with relative retention time of about 1.3 due to 3-deoxyazithromycin (azithromycin impurity B) is not more than twice the area of principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent) and the sum of the areas of all the other secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). Ignore the peaks eluting before azithromycin impurity L and after azithromycin impurity B.

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 20 volumes of *dichloromethane* and 80 volumes of *methanol*.

Test solution. Dilute a volume of eye drops containing 0.05 g of Azithromycin to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.05 per cent w/v solution of *azithromycin IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.05 per cent w/v, each of, *azithromycin IPRS* and *azithromycin impurity A IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane vinyl polymer (5µm) (Such as Asahipak ODP-50),
- column temperature: 40º
- mobile phase: a mixture of 40 volumes of 0.67 per cent w/v solution of *dipotassium hydrogen orthophosphate*, adjusted to pH 11 with a 56 per cent w/v solution of *potassium hydroxide* and 60 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to impurity A and azithromycin not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{38}H_{72}N_2O_{12}$ in the eye drops.