EAG/Panel/Working Party	Antibiotics
Contact Details	peter.crowley@mhra.gov.uk stephen.maddocks@mhra.gov.uk
Deadline for Comment	31st December 2018
Target Publication Date (subject to change)	BP 2020
Notes: New Monograph. Would appreciate feedback on Extraction procedure for Azithromycin.	

Azithromycin Eye Drops

Azithromycin Preparations

Action and use
Macrolide antibacterial.

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

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of Azithromycin, $C_{38}H_{72}N_2O_{12}$
90.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Add 10 mL of *absolute ethanol* to a quantity of the eye drops containing 0.1 g of Azithromycin and mix. Allow to stand, remove the upper ethanolic layer and evaporate to dryness under a stream of nitrogen. Add 10 mL of *hexane* to the residue, mix (a precipitate is formed) and filter the resulting mixture. Wash the residue with 50 mL of *hexane* and allow to dry in air. The infrared absorption spectrum of the dried residue, Appendix II A, is concordant with the reference spectrum of azithromycin (RS xxx)

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

TESTS

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of *dichloromethane* and 80 volumes of *methanol*.

(1) Dilute a quantity of the eye drops to obtain a solution containing 0.8% w/v azithromycin.

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

(3) Dissolve the contents of a vial of *azithromycin for system suitability EPCRS* in 1.0 mL.

(4) 0.8% w/v of *azithromycin for peak identification EPCRS*.

(5) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with end-capped octadecylsilyl amorphous organosilica polymer for mass spectrometry (5 µm) (Xterra MS C18 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 60°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 0.180% w/v solution of *anhydrous disodium hydrogen orthophosphate* adjusted to pH 8.9 with dilute *orthophosphoric acid* or with *dilute sodium hydroxide solution*.

Mobile phase B *25 volumes of methanol R1* and 75 volumes of *acetonitrile R1*.

Azithromycineyedrops_2020_1_tb.tif

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	50→45	50→55	linear gradient
25-30	45→40	55→60	linear gradient
30-80	40→25	60→75	linear gradient
80-81	25→50	75→50	linear gradient
81-93	50	50	isocratic

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to azithromycin (retention time about 45 minutes) are: impurity L, about 0.29; impurity M, about 0.37; impurity E, about 0.43; impurity F, about 0.51; impurity D, about 0.54; impurity J, about 0.54; impurity I, about 0.61; impurity C, about 0.73; impurity N, about 0.76; impurity H, about 0.79; impurity A, about 0.83; impurity P, about 0.92; impurity O, about 1.23; impurity G, about 1.26; impurity B, about 1.31.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the peak-to-valley ratio is at least 1.4, where Hp is the height above the baseline of the peak due to impurity J and Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to Impurity F.

LIMITS

In the chromatogram obtained with solution (1):

identify any peaks corresponding to impurities F and H using solution (3) and any peaks corresponding to impurities G, L, M, and N using solution (4). Multiply the

area of these peaks by corresponding correction factors: impurity F, 0.3, impurity H, 0.1; impurity G, 0.2; impurity L, 2.3, impurity M, 0.6; impurity N, 0.7;

the area of any peak due to impurity B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the areas of any peaks due to azithromycin impurities A, C, E, F, H, I, L, M, N, O, P is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any peaks due to impurities D and J is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other secondary peak is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of the areas of all secondary peaks is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peaks eluting before impurity L and after impurity B and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of *dichloromethane* and 80 volumes of *methanol*.

(1) Dilute a quantity of the eye drops to obtain a solution containing 0.05% w/v azithromycin.

(2) 0.05% w/v of *azithromycin EPCRS*.

(3) 0.05% w/v each of *azithromycin EPCRS* and *azithromycin impurity A EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl vinyl polymer for chromatography* (5 µm) (Asahipak ODP-50 is suitable).

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

(c) Use a flow rate of 1 ml per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

40 volumes of a 0.67% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 11.0 with a 56% w/v solution of *potassium hydroxide*, and 60 volumes of *acetonitrile R1*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity A and azithromycin is at least 1.5.

DETERMINATION OF CONTENT

Calculate the total content of azithromycin, $C_{38}H_{72}N_2O_{12}$ in the eye drops using the declared content of $C_{38}H_{72}N_2O_{12}$ in *azithromycin EPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Azithromycin.