A-224-02-0199

ANALYTICAL METHOD PROCEDURES

TOTAL PAGES: 8

AZITHROMYCIN POWDER FOR ORAL SUSPENSION - 200mg Azithromycin Dihydrate per 5mL suspension after reconstitution as Anhydrous Azithromycin
IMPURITIES BY TLC FOR STABILITY STUDIES

IMPURITIES DETERMINATION

General Index:

1. PRODUCT SPECIFICATIONS
2. IMPURITIES BY TLC
3. TLC SETUP
4. STANDARD PREPARATION
5. SAMPLE PREPARATION
6. PROCEDURE
7. CALCULATION
8. TYPICAL PLATES (SCHEMATIC)

Method is suitable for:
☒ In-process control
☒ Product Release
☑ Stability indicating analysis

ED. N0: NEW	Effective Date :	APPROVED:			
Ed. Status : ORIGINAL	DD / MM / 199Y	*C. Latham*	*Edanna Holman*	*C Frost*	*Pat West*
		ANALYST	SUPERVISOR	QC	HEAD

A-224-02-0199

ANALYTICAL METHOD PROCEDURES

TOTAL PAGES: 8

AZITHROMYCIN POWDER FOR ORAL SUSPENSION - 200mg Azithromycin Dihydrate per 5mL suspension after reconstitution as Anhydrous Azithromycin
IMPURITIES BY TLC FOR STABILITY STUDIES

ASSAY SPECIFICATIONS

PRODUCT:	**AZITHROMYCIN SUSPENSION** 200 mg/ 5mL
Description Color Odor & Clarity	[White to light gray] uniform homogeneous **Suspension** with banana / vanilla flavor and odor
Identification A:	The Chromatogram of the sample solution exhibits a peak with the same retention time as the standard solution
Fill Volume [15.0 mL] (±5.0%) mL	**Target 15.0**
Fill Volume [22.5 mL] (±5.0%) mL	**Target 22.5**
pH (± 1.0 unit)	**Target 10.0** **Limit: 9.0 - 11.0**
Viscosity **Brookfield, Spindle #[LV2] After [6]rpm**	Target 1200 Limit: 800 - 1800 cp
Assay [for Stability]	Limit: 90**.0** - 110**.0**% of labeled amount **[180**.0**] - [220**.0**] mg / [**5**] mL**
Impurity determination - Any Individual - IMP.1 - IMP.2 - Total:	 **NMT 0.5% of the labeled amount** **NMT 0.8% of the labeled amount** **NMT 0.5% of the labeled amount** **NMT 2.0% of the labeled amount**
Container Closure system	50 mL HDPE Bottle with CRC HDPP Cap Security Cap has twist off-tamper evident ring
IMPURITIES KEY:- IMP1. N-Demethylazithromycin IMP2. Desosaminylazithromycin	

ED. N0: NEW	Effective Date :	APPROVED:			
Ed. Status : ORIGINAL	DD / MM / 199Y	*C. Latham*	*Edanna Holman*	*C Frost*	*Pat West*
		ANALYST	SUPERVISOR	QC	HEAD

A-224-02-0199

ANALYTICAL METHOD PROCEDURES

TOTAL PAGES: 8

AZITHROMYCIN POWDER FOR ORAL SUSPENSION - 200mg Azithromycin Dihydrate per 5mL suspension after reconstitution as Anhydrous Azithromycin
IMPURITIES BY TLC FOR STABILITY STUDIES

TLC / SETUP		
REFERENCE		**A-124-02-0199**
Plate	**:**	Silica gel 60 F254, 20x20cm plate with 250µ precoated layer
Drying Temperature	**:**	100 ± 3°C / 10 min
Spray [1]	**:**	**Dragendorff's** reagent
Spray Composition	**:**	**Dragendorff's** reagent
Detector	**:**	N/A
Sample volume	**:**	20µL
Standard volume	**:**	20µL
Mobile Phase	**:**	Chloroform : Methanol (1:1, v/v)
Loading	**:**	Apply 50µL of the Sample and Standard solutions as a 1.5cm long streak from the base line
Front Length	**:**	**2.5** cm from top of plate
Spot Color	**:**	Dark band in off-white background
Solvent System		Hexane : Ethyl acetate : Diethylamine (75 : 25 : 10 V/V) in a pre-saturated tank suitable for TLC chromatography for at least 1 hour with the vapors of the solvent system

SPECIAL NOTE AND CAUTION:

1. All solvents used must be of TLC grade.
2. All samples and standard solution must be protected from Light.

It is necessary to ensure that the chamber is kept covered throughout the spot resolution to prevent solvent loss.

A-224-02-0199

ANALYTICAL METHOD PROCEDURES

TOTAL PAGES: 8

AZITHROMYCIN POWDER FOR ORAL SUSPENSION - 200mg Azithromycin Dihydrate per 5mL suspension after reconstitution as Anhydrous Azithromycin
IMPURITIES BY TLC FOR STABILITY STUDIES

STANDARD PREPARATION

The Analytical Standard refers to the following: ☑Primary Reference Standard (A.S.);

STANDARD SOLUTIONS [A] / [B] / [C] / [D]

[A]. Accurately weigh 8.7mg of N-Demethylazithromycin A.S. into a 50mL volumetric flask. Dissolve in and make up to volume with Diluent (concentration equivalent to 0.7% of the concentration of Azithromycin in the Sample solution).

[B]. Accurately weigh 7.2mg of Desosaminylazithromycin A.S. into a 100mL volumetric flask. Dissolve in and make up to volume with Diluent (concentration equivalent to 0.3% of the concentration of Azithromycin in the Sample solution).

[C]. Azithromycin Powder held at 5°C as a control: Prepare the solution as directed in the sample preparation and use the clear supernatant as the control sample.

[D]. Accurately weigh about 10.5 mg Standard (AZITHROMYCIN AS) into a 200mL volumetric flask. Make up to volume with diluent and mix. Sonicate for 10 minutes to dissolve. (Concentration of solution equivalent to 0.2% of the concentration of Azithromycin in the Sample solution.)

TEST SOLUTION

Accurately weigh sufficient powder equivalent to 250mg of Azithromycin into a 10mL volumetric flask. Add about 5mL of Diluent and sonicate for 10 minutes. Make up to volume with Diluent and mix. Centrifuge this suspension for 10 minutes. Use the clarified extract for analysis.

DILUENT - Mixture of Chloroform : Methanol (1:1; V/V)

Solvent System: - Hexane : Ethyl acetate : Diethylamine (75 : 25 : 10 V/V) in a pre-saturated tank for at least 1 hour with the vapors of the solvent system.

This is the Mobile Phase for the TLC IMPURITY Test.

A-224-02-0199

ANALYTICAL METHOD PROCEDURES

TOTAL PAGES: 8

AZITHROMYCIN POWDER FOR ORAL SUSPENSION - 200mg Azithromycin Dihydrate per 5mL suspension after reconstitution as Anhydrous Azithromycin
IMPURITIES BY TLC FOR STABILITY STUDIES

PROCEDURE:

CHROMATOGRAPHIC CONDITIONS

■ **Plate** : Silica gel 60 F254 plate, Layer thickness = 0.25mm Pre-coated (E. Merck).

■ **Solvent system**: Hexane : Ethyl acetate : Diethylamine (75 : 25 : 10 V/V) in a suitable tank for TLC chromatography. Pre-saturate the tank with the solvent vapor system for not less than 30 minutes.

■ **Loading** : Separately apply equal volumes (20µL) of the Standard solutions A, B, C, D and the Sample solution as solutions as a 1.5cm long streak from the base line of the thin-layer plate.

■ Air-dry the plate for about 10 minutes before inserting into the tank.

■ **Development**: Note - All streaks on the plate must be completely dry before development.

■ Develop the plate in the tank up to 15cm (one pass).

Dragendorff's reagent Air-dry the plate for 5 minutes and then re-insert it into the developing chamber for a second pass. Remove the plate from the tank and allow to dry at room temperature.

Detection Reagents

Dragendorff's Reagent:

Solution 1: Weigh about 425mg of Bismuth subnitrate and dissolve in 25mL mixture of glacial acetic acid : water (20:80, V/V).

Solution 2: Weigh about 4g of Potassium iodide and dissolve in 10mL of water.

Solution 3: Weigh about 2.5g of Sodium nitrite and dissolve in 50mL of water.

Immediately prior to use, combine 5mL of Solution 1, 5mL of Solution 2 and 20mL of Glacial acetic acid. Dilute this solution to 100mL with water.

Spray the plate with the above mixture and air-dry the plate for 5-10 minutes. Finally spray the plate with Solution 3.

A-224-02-0199

ANALYTICAL METHOD PROCEDURES

TOTAL PAGES: 8

AZITHROMYCIN POWDER FOR ORAL SUSPENSION - 200mg Azithromycin Dihydrate per 5mL suspension after reconstitution as Anhydrous Azithromycin
IMPURITIES BY TLC FOR STABILITY STUDIES

CALCULATION.

Measure the length of the Test and Reference Standard spots (impurities) from the base line and divide by the length of the solvent front. This is the Rf value.
Calculate the Rf of the Sample $[C_{38}H_{72}N_2O_{12}]$
Calculate the Rf of the Standard $[C_{38}H_{72}N_2O_{12}]$

ACCEPTANCE CRITERIA

The relative mobility (Rf) of the main streak produced by the Sample solution (about 0.55) should correspond to that produced by the Standard solution C.

The streak at Rf of about 0.18 in the Sample solution exhibits an intensity not greater than the corresponding streak in the Standard solution A.

The streak at Rf of about 0.42 in the Sample solution exhibits an intensity not greater than the corresponding streak in the Standard solution B.

For evaluating other additional or existing streaks compare the chromatogram of the Sample solution with the chromatogram of the Standard solution D (5°C control powder for suspension).

The intensity of any additional streaks (not existing in Standard solution D) as well as any increase in intensity of existing bands, is less than that of the band, produced by the Standard Solution C.

A-224-02-0199

ANALYTICAL METHOD PROCEDURES

TOTAL PAGES: 8

AZITHROMYCIN POWDER FOR ORAL SUSPENSION - 200mg Azithromycin Dihydrate per 5mL suspension after reconstitution as Anhydrous Azithromycin
IMPURITIES BY TLC FOR STABILITY STUDIES

CALCULATION.

Measure the length of the Test and Reference spots from the base line and divide by the length of the solvent front. These are the Rf values.
Calculate the Rf of the Test [$C_{38}H_{72}N_2O_{12}$]
Calculate the Rf of the Standard [$C_{38}H_{72}N_2O_{12}$]

Spot length**/**Solvent front = Rf. T^1 = Rf. S^1

$$\frac{\text{Rf Test}}{\text{Rf Std}} = \text{Relative Rf Value} \approx 1.0$$

CONCLUSION.

Method **A-224-02-0199** is suitable stability indicating TLC analysis for the identification of the potential impurity profile of the active material in the dosage form.

ED. N0: NEW	Effective Date :	APPROVED:			
Ed. Status : ORIGINAL	DD / MM / 199Y	*C. Latham*	*Edanna Holman*	*C Frost*	*Pat West*
		ANALYST	SUPERVISOR	QC	HEAD

A-224-02-0199

ANALYTICAL METHOD PROCEDURES

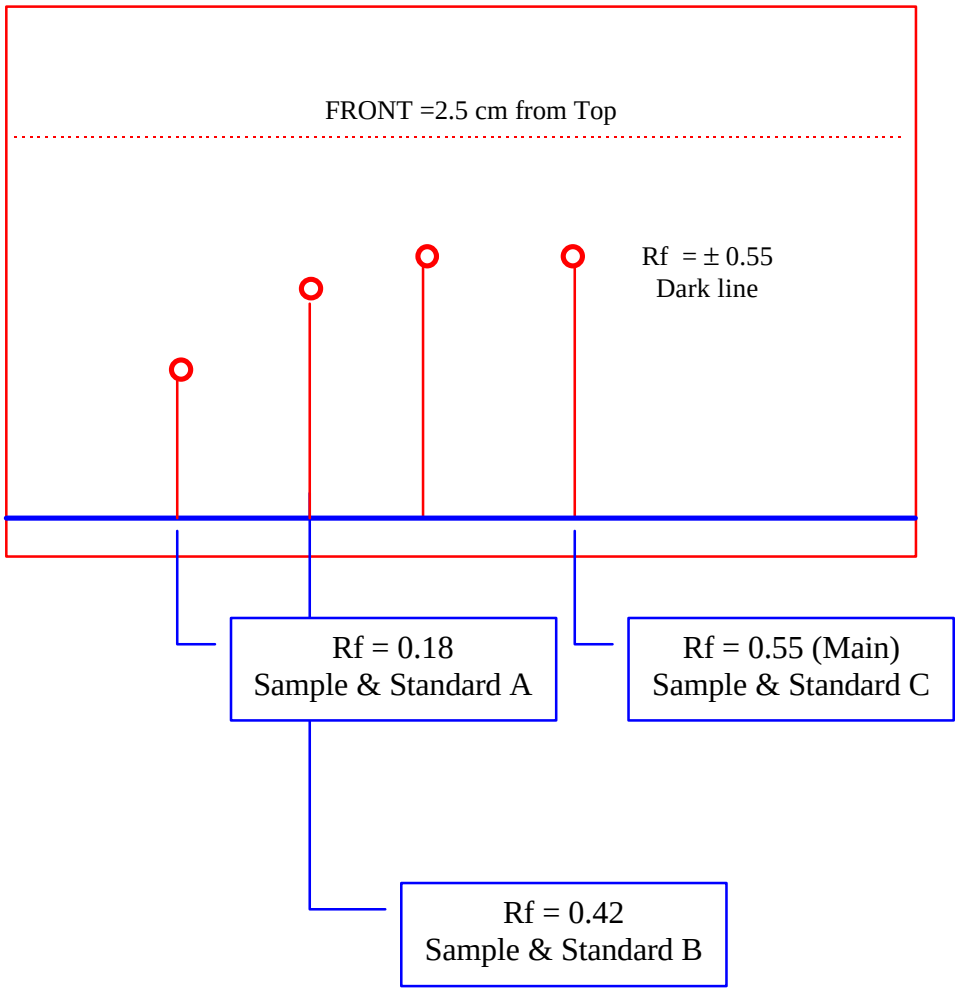
TOTAL PAGES: 8

AZITHROMYCIN POWDER FOR ORAL SUSPENSION - 200mg Azithromycin Dihydrate per 5mL suspension after reconstitution as Anhydrous Azithromycin
IMPURITIES BY TLC FOR STABILITY STUDIES

TYPICAL CHROMATOGRAM

(Representative TLC Chromatogram)

IMP1. N-Demethylazithromycin A.S.
IMP2.Desosaminylazithromycin A.S



NB: Chromatogram has been resolved horizontally for clarity

ED. N0: NEW	Effective Date :	APPROVED:			
Ed. Status : ORIGINAL	DD / MM / 199Y	*C. Latham* ANALYST	*Edanna Holman* SUPERVISOR	*C Frost* QC	*Pat West* HEAD