A-126-01-129Y

ANALYTICAL METHOD PROCEDURES

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THIS SOP IS 'SWITCHED: ☑ON'

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

ASSAY DETERMINATION

General Index:

Method is suitable for:

☒ In-process control
☑ Product Release
☑ Stability indicating analysis

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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	

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HPLC / SETUP

REFERENCE	USP 23 Page 141 - 142
Pre-Column : (ES Industries cat.no. 115271-γRP1/P)	Alumina based hydrocarboneous spherical particles, 5µ, 80°A pore size, C-18, 500 x 4.6mm
Column (Waters) : (ES Industries cat.no. 115271-γRP1/P)	Alumina based hydrocarboneous spherical particles, 5µ, 80°A pore size, C-18, 500 x 4.6mm
Column Temperature :	(00 ± 2°C)
Pressure :	00.0 atm
Flow rate :	0.8 mL/min. (1.2mL/min with pre-column)
Detector :	Coulometric electrochemical detector
Sample volume/Loop :	50 µL
Mobile Phase (pH = 11.0) :	Mixture of 0.02M Potassium dihydrogen phosphate (KH2PO4):Acetonitrile (71 : 29, V/V) with final pH=11.0[1]

SPECIAL NOTE AND CAUTION:

■ **An in-line graphite filter element must be placed before every coulometric type electrochemical cell.**

■ Flow rate may be varied in order to achieve the required System Suitability

■ **Due to slight batch to batch variation in column packing, retention times may change. In that case, the other system suitability parameters should be met.**

■ All solvents used must be of HPLC grade.

■ All samples and standard solution should be protected from Light.

■ Working Standard Solution can be used for **48 hrs** when stored in a refrigerator.

NOTE: Degas the mobile phase by sonication for 30 minutes.

■ **If a Guard cell (Model 5020) is used, it is placed between the pump and the injector, and operated at the potential of 0.05V higher than E2.**

■ **If a conditioning cell (Model 5021) is used, it is placed immediately before the analytical cell and operated at the same potential as E1.**

■ **It is necessary to ensure that the column is kept at constant temperature through the analysis time to prevent changes in the retention time**

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HPLC Assay of Azithromycin

SCOPE:-

Azithromycin assay is determination by HPLC with electrochemical detection after extraction of active Azithromycin from powder for oral suspension with Diluent.

Detection

Azithromycin is detected by dual porous graphite coulometric electrodes operated in the oxidative screen mode.

Detector:

Coulometric electrochemical detector with dual series capability using a ESA Coulochem II Multi-Electrode Detector with dual series porous graphite coulometric electrodes.

Analytical Cell:

Model 5010 standard analytical cell.

Or/alternative - high sensitivity cell

Model 5011 analytical cell [high sensitivity] operated in the oxidative screen mode at the following parameters:

Cell potential:

E1 (first electrode):	0.600-0.730V
E2 (working electrode):	0.800-0.870V
Full scale range:	1,2 or 5μA
Filter:	2,5 or 10 sec
Signal output voltage:	100mV

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Diluent for internal standard:
Mixture of 0.02M Potassium dihydrogen phosphate (KH2PO4):Acetonitrile (71:29, v/v) with final pH = 8.0[1].

Diluent:-
Mixture of 0.01M Potassium dihydrogen phosphate (KH2PO4):
2-propanol:Ethanol:Acetonitrile (53 **:** 20 **:** 16 **:** 11, V/V) with final pH = 8.4[2]

[1]**Mobile phase and Diluent for internal standard preparation**

- Weigh 11.6gr of Potassium dihydrogen phosphate into a large glass container (6 liter capacity),
- Add 4260mL of *HPLC grade Water* and mix to dissolve.
- Add 1740mL *Acetonitrile* and mix well.
- Divide the solution into two portions (2:1) and adjust the pH of the larger portion (4L) to pH=11.0±0.1 with 10M Potassium hydroxide.
- Filter through 0.2µ membrane filter.
- **This is the Mobile phase.**

- Adjust the pH of the smaller portion (2L) to pH=8.0 ±0.1 with 10M Potassium hydroxide.
- **This is the Diluent for internal standard.**

[2]Diluent preparation

- Weigh 4.3gr of Potassium dihydrogen phosphate into a 6 liters glass container
- Add 3180mL of *HPLC grade water* and mix to dissolve.
- Add 1200mL of 2-propanol, 960mL of Ethanol and 660mL of Acetonitrile and mix well.
- Adjust the final pH of Diluent to 8.4 with 10M Potassium hydroxide.
- **This is the diluent.**

NOTE: Flow rate and mobile phase proportions may be varied in order to achieve the required system suitability.

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STANDARD PREPARATION

The Analytical Standard refers to one of the following:

☑ Primary Reference Standard (A.S.);

☑ Active Drug Substance analyzed by the Analytical laboratory according to a specific monograph in comparison with current Reference Standard. After release this material becomes an in-house *working standard*.

SOLUTION PREPARATION

Solution A: - Internal Standard Solution
Accurately weigh and dissolve **3** mg of Diphenhydramine hydrochloride A.S. into 2L of diluent for internal standard.

Standard Preparation

■ Accurately weigh about 23.5mg of Azithromycin dihydrate A.S. into a **50**mL volumetric flask.

■ Dissolve in and dilute to volume with **Diluent**. Sonicate for 5 minutes.

■ Pipet 5mL of the above solution into a **50**mL volumetric flask and bring up to volume with the internal standard solution.

■ Dilute **5**mL of the resulting solution to a **50** mL with the internal standard solution.

This is the Standard Solution.

SYSTEM SUITABILITY TEST

SYSTEM SUITABILITY TEST

■ Inject the Standard Solution.

■ The number of theoretical plates (calculated according to USP) for the Azithromycin peak should be **greater** or equal to 1500.

■ The tailing factor for both analyte peaks, Diphenhydramine and Azithromycin (when calculated according to USP) should be not more than **2.0**

■ The relative standard deviation for 5 replicate injections is not more than 2.0%.

■ The resolution factor between Azithromycin and Diphenhydramine peaks(when calculated according to USP) should be not less than **1.5**

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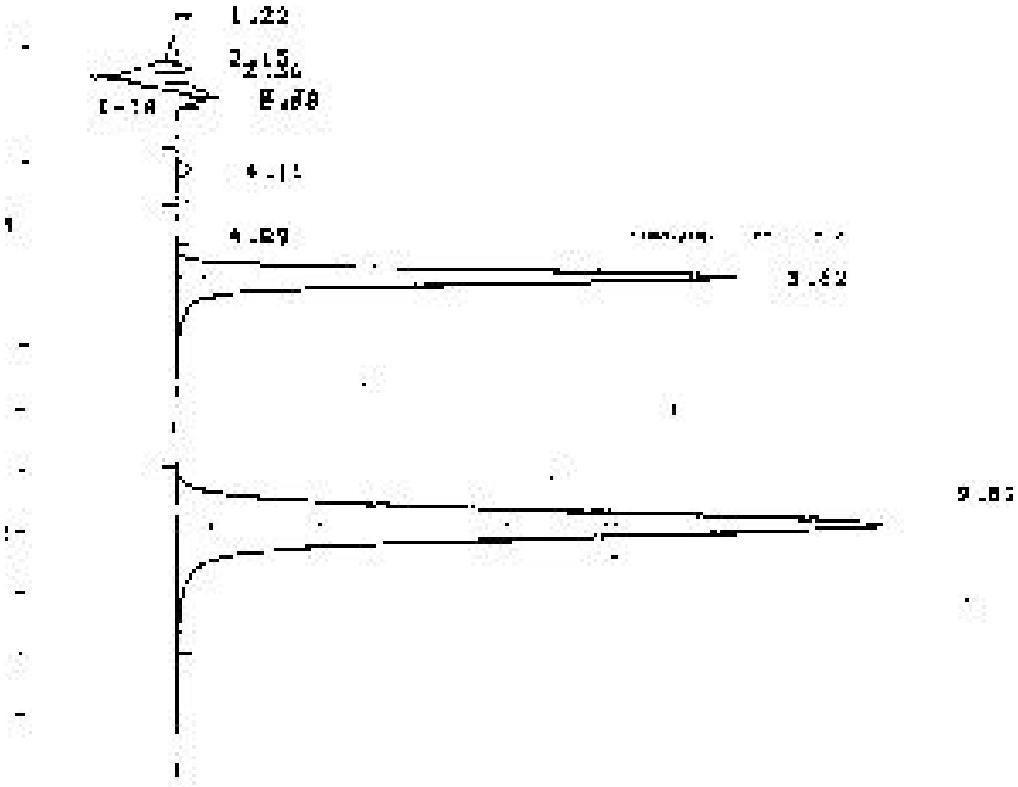
SYSTEM SUITABILITY CHROMATOGRAM

(Representative Chromatogram)

RT [1] = 5.62 min RT [2] = 9.83 min



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SAMPLE PREPARATION:

■ Test THREE bottles individually for Azithromycin content as BELOW:

■ Tap the Azithromycin container to initially loosen the powder.

■ Reconstitute by weighing the correct volume of water into the bottle that is **9**mL for **15**mL final volume (after reconstitution) or **12**mL for **22.5** mL final volume (after reconstitution.)

■ Shake the reconstituted container for approximately 15 minutes.

■ Allow the contents of the container to stand for 30 minutes.

■ Repeat Shaking procedure of the container for an additional 15 minutes.

■ Shake the bottle for 20 seconds immediately prior to sampling in order to accomplish homogeneity of suspension.

■ Via a plastic syringe calibrated to 0.2mL, transfer **5**mL suspension into a 500mL volumetric flask (for the 200mg/5mL dosage constituted oral suspension)

Use a 250mL volumetric flask (for the 100mg/5mL dosage constituted oral suspension).

■ When sampling with syringe, avoid inclusion of large air bubbles.

■ Record the weight of the suspension aliquot delivered into the volumetric flask.

■ Add about 70% volume of Diluent to the flask and shake for 30 minutes.

■ Make up to volume with Diluent.

■ Pipet **5**mL of above solution into a **50**mL volumetric flask and make up to volume with the internal standard solution.

■ Pipet **5**mL of the resulting solution into a **50**mL volumetric flask and make up to volume with internal standard solution. **This is the Sample solution**.

■ Determine the density of the freshly prepared suspension using a calibrated pycnometer.

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PROCEDURE:

■ Separately - inject equal volumes of Standard and Sample solutions into the chromatograph.

■ Record the chromatograms

■ Determine the resulting heights for both the Diphenhydramine peak and the Azithromycin peak using a suitable integrator Measure the responses for the major peaks

■ Retain chromatograms showing the calculate values in mg/mL

■ NOTE: Inject the Standard solution after each third injection of the Sample solution

CALCULATION.

$$\frac{\text{Peak height of Azithromycin smp}}{\text{Peak height of Diphenhydramine smp}} = \text{Rsmp}$$

$$\frac{\text{Peak height of Azithromycin Std}}{\text{Peak height of Diphenhydramine Std}} = \text{Rstd}$$

$$\frac{\text{Rsmp x Std wt}^{1}\text{(mg) x F}^{3}\text{ x 5 x 100}}{\text{Rstd x Vsmp}^{2}\text{ (mL) x Dosage (mg / 5mL)}} = \text{\% Azithromycin of labeled claim}$$

[1]Std wt is corrected according to % Assay[1]and % Water in the standard.

[2]$\text{Vsmp} = \frac{\text{Smp wt(g)}}{\text{Density (g / mL)}}$

[3] F - factor = 5 for dosage 100mg per 5mL
= 10 for dosage 200mg per 5mL

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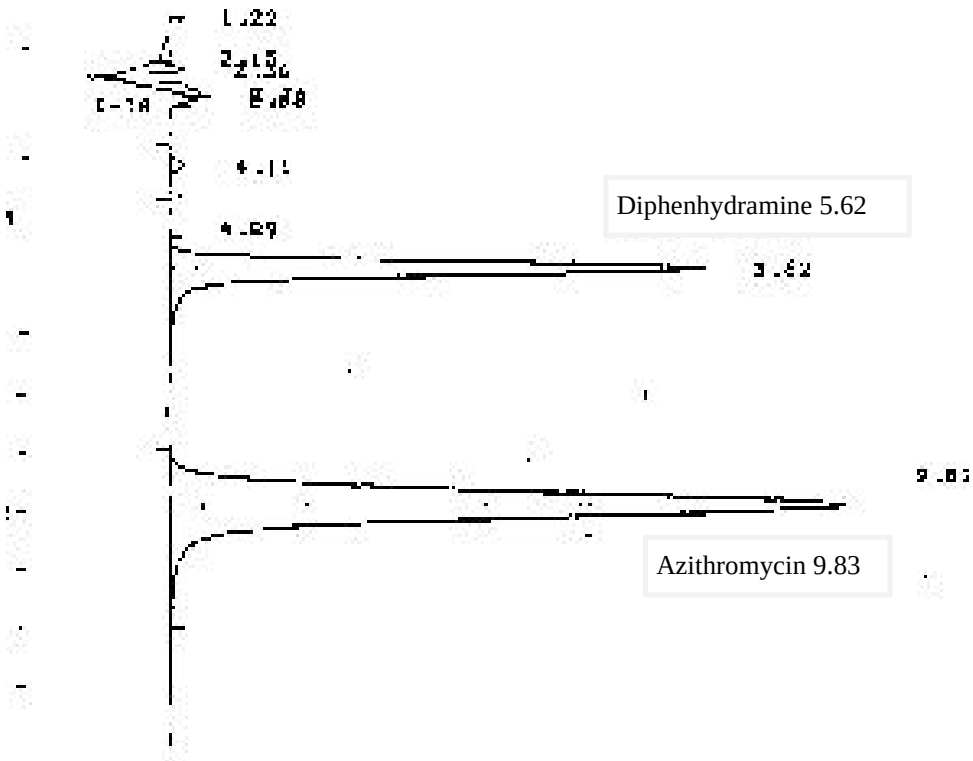
CONCLUSION.

Method **AMP-126-01-1298** is suitable for the assay of the active ingredient in the dosage form.

TYPICAL CHROMATOGRAMS

(Representative Chromatogram)

RT [1] = 5.62 min RT [2] = 9.83 min



DEGRADATION PROFILE:-

Decomposition Products Found	
IMP1. N-Demethylazithromycin	NMT 0.8 %
IMP2. Desosaminylazithromycin	NMT 0.5 %

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