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Determination Of Amphotericin B In Bulk And Pharmaceutical Formulation By Spectrophotometer.

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ABSTRACT

Nowadays the methods available for determination of Amphotericin B are costly. Till date there is no economic and reproducible spectrometric method for quality control of Amphotericin B in bulk and pharmaceutical dosage has been reported. To develop economic, accurate, reproducible and validated spectrometric method for quality control of pharmaceutical formulations containing Amphotericin B. Spectrometric method was used for estimation of Amphotericin B using Dimethyl sulfoxide [DMSO] and Water 1:1 as a solvent. On basis of absorption of visible light, Amphotercin B spectra in DMSO and Water 1:1 ratio showed maximum absorption wavelength (Lambda max) at 415 nanometer [nm]. The calibration curve was plotted and linear over the concentration range from 2-20µg/ml of Amphotericin B with correlation coefficient 0.9975. Validation was performed as per International Council of Harmonization [ICH] guidelines for linearity, accuracy, precision; recovery and results were within required limits. LOD and LOQ found to be 0.0570 and 0.1574 respectively by simple UV spectroscopy. This method has Percent Relative standard deviation [% RSD] less than one and reproducible. From the results the developed method proves to be valid, sensitive and linear over the concentration range studied and applicable for quality control, routine analysis and determination of Amphotericin B in Bulk and pharmaceutical dosage form.

Keywords: Amphotericin B, spectrophotometric method, ICH guidelines.

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INTRODUCTION

The first antifungal drug used in treatment of fungal, yeasts, candidiasis, and invasive aspergillosis is amphotericin B. It mainly binds to ergosterol following increase in cell membrane permeability which causes death.[1]

Amphotericin B is an antifungal antibiotic derived from a strain of Streptomyces nodosus. The activity of Amphotericin B which is shared in common with other polyenes, is based on the binding of the hydrophobic moiety of the Amphotericin B molecule to the fungal cell membrane ergosterol moiety, producing an aggregate that forms transmembrane channel.[2]

These defects cause depolarization of the membrane and an increase in membrane permeability to protons and monovalent cations. Intermolecular hydrogen bonding inter- actions among hydroxyl, carboxyl, and amino groups stabilize the channel in its open form, destroying activity and allowing the cytoplasmic contents to leak out, leading to cell death.[3]

Histoplasma meningitis has been treated successfully with intravenous amphotericin B, and most investi- gators think that intrathecal therapy is not usually needed. Intravenous amphotericin B is the only potentially effective treatment for cerebral zygomy- cosis.[4]

Mechanism of Action: The mechanism of action of amphotericin B which is shared in common with other polyenes, is based on the binding of the hydrophobic moiety of the amphotericin B molecule to the fungal cell membrane ergosterol moiety, producing an increase in membrane permeability to protons and monovalent channels. An increase in membrane permeability to protons and monovalent cations and also these defect cause depolarization of the membrane. In its open form intermolecular hydrogen bonding interactions among hydroxyl, carboxyl, and amino groups stabilize the channel. Destroying activity and allowing the cytoplasmic contents to leak out, leading to cell death. Amphotericin B also has the capability of binding to the cholesterol of mammalian cell membranes, which is responsible for a major fraction of its toxic potential. Amphotercin B has less affinity for cholesterol containing membranes than for ergosterol-containing membranes.[5]

Amphotericin B has large affinity towards ergosterol which is major part of fungal membrane. The drug and membrane interaction creates channels through which ion and molecules species and produce ionic imbalance. In MOA the intake of Na+ and K+ Mg+ goes out through pores due to activation of Na+/K+ ATPase pump. Oxygen consumption is increased to maintain intracellular ATP level. When Demand of ATP exceeds free radicals formed and intracellular accumulation of calcium. All these processes combine together lead to damage to cells and death occurs.[10]

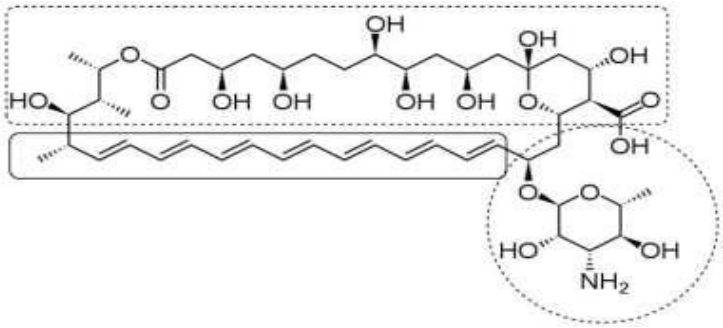

Figure 1: Structure of Amphotericin B[11]

Analysis is most critical parameter in development and formulation of new drug moieties. So it is necessary to produce accurate and reproducible and precise method determination of drug in formulation. Primary objective to develop validate visible UV spectrometric method as per ICH guidelines. [6-7]

Amphotericin B has absorption limitation due to poor water solubility. Most prominent adverse effect of amphoterecin B is nephrotoxicity. Beside these problems Amphotericin B is most effective drug for the treatment of fungal infections.[10]

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MATERIALS AND METHODS

Instrument

Double beam UV visible spectrophotometer: Make-Shimadzu, Model-UV1800.

Cleaning

Mixture of chromic acid and sulphuric acid used to soak the glass overnight and rinsed with distilled water prior use and dried in hot air oven. [8]

Material

Pure sample

Amphotericin B obtained as gift sample from Lifecare Innovations Pvt. Ltd. Vadodara, India.

Reagents and Chemicals

Dimethylsulfoxide (DMSO) (OZONE International. Pvt. Ltd. Mumbai, Maharashtra), Distilled water,Other chemicals& reagents used were of analytical grade.

Preparation of standard stock solution:

Stock solution I:Concentration of 1mg/ml ie 1000 µg/ml was prepared by dissolving 100 mg of Amphotericin B in little amount of 1:1 ratio of distilled water and DMSO in a volumetric flask of 100 ml and final volume was adjusted with DMSO and distilled water 1:1 ratio.

Stock Solution II: 100µg/ml concentration was prepared by withdrawing 10 ml of solution from stock solution: I and make up the final volume in 100ml volumetric flask with same solvent.

Determination of Lambda max[λmax]

The stock solution:II diluted further and scanned in the range of 400 to 800 nm against blank. The wavelength of maximum absorbance was found at 415 nm further used for development of standard calibration curve.[8] **(Figure no. 2)**

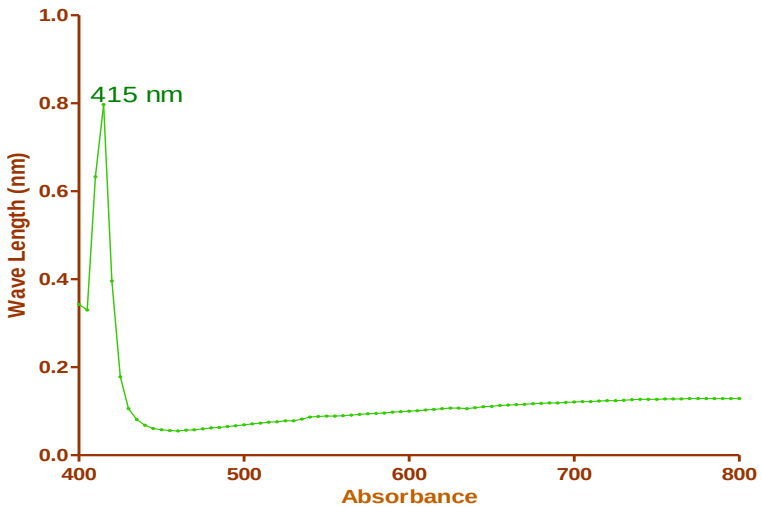

Figure 2: Determination of λmax of Amphotericin B

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Preparation of Calibration Curve

Stock solution I:Concentration of 1mg/ml ie 1000 µg/ml was prepared by dissolving 100 mg of Amphotericin B in little amount of 1:1 ratio of distilled water and DMSO in a volumetric flask of 100 ml and final volume was adjusted with DMSO and distilled water 1:1 ratio.

Stock Solution II: 100µg/ml concentration was prepared by withdrawing 10 ml of solution from stock solution: I and make up the final volume in 100ml volumetric flask with same solvent.

Concentrations of 2 4,6,8,10,12,14,16,18,and 20 µg/ml were prepared by withdrawing 2,4,6,8,10,12,14,16,18,and 20 ml solutions from stock solution:II respectively and make up the final volume up to 100ml by using DMSO and distilled water 1:1 as solvent. These solutions scanned at 415 nm to get the absorbance values.[9]

From the obtained readings a graph of concentration v/s absorbance was plotted to get the standard calibration curve as shown in **Table no.1 and Figure no.3.**

Table 1: Calibration data for the method development.

Sr .No.	Concentration (µg/ml)	Absorbance at 415 nm ± standard deviation[S.D]
1	2	0.176±0.002
2	4	0.321±0.006
3	6	0.430±0.0026
4	8	0.594±0.0021
5	10	0.716±0.0036
6	12	0.855±0.0029
7	14	1.011±0.0022
8	16	1.141±0.0024
9	18	1.289±0.0025
10	20	1.502±0.0023

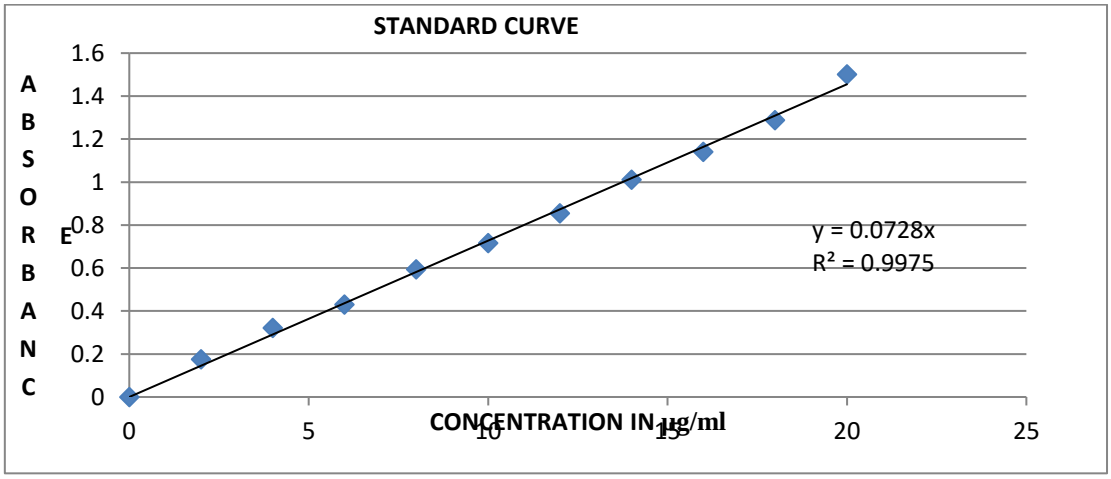

Figure 3: Calibration Curve for Amphotericin B

METHOD VALIDATION

Linearity and Range

The standard calibration curve found to be linear over the range of 2 to 20 µg/ml concentration and linear regression analysis used to treat the data as shown in Table no.03.The calibration curve equation for

Amphotericin B was y = 0.0728x + 0.00 and calibration curve was found to be linear in the above mentioned concentrations (The correlation coefficient (R^2) of determination was (0.9975)

Precision

Precision is a unique tool used for determination of repeatability.10 µg/ml solution of amphotericin B analyzed for six times to determine repeatability at 415nm. Results are reported in (Tableno. 2)

Table 2: Data showing Repeatability of Absorbance's

Sr. No.	Conc. (µg/ml)	Absorbance	Mean± S.D.	%R.S.D
1	10	0.711	0.716 ±0.00368	0.513
2		0.714		
3		0.718		
4		0.721		
5		0.715		
6		0.719		

S.D. = Standard Deviation, R.S.D.= Relative Standard Deviation

Intra-day and inter-day variation play a key role in study of precision of developed method.

By analyzing 4,8 and 12 µg/ml solution amphotericin-B thrice a day intraday precision was determined also by analyzing same concentration of solution thrice a day inter-day precision was measured, results were shown in (Table no.03)

Table3: Results for Intra-day and Inter-day precision of Amphotericin-B

Drug	Conc. (µg/ml)	Intra-day Mean Abs.	Absorbance ± S.D.	%RSD	Inter-day Mean Abs.	Absorbance ± S.D.	%RSD
Amphotericin B	04	0.315	±0.00158	0.503	0.330	±0.0010	0.330
	08	0.590	±0.00170	0.288	0.598	±0.0020	0.334
	12	0.848	±0.00235	0.277	0.864	±0.0026	0.306
Mean %RSD				0.356			0.323

Recovery Study

Accuracy of developed method was analyzed by using commercially available Amphotericin B Injection Indian Pharmacopeia [I.P] (Fungitericin Intravenous 50 mg)Mfg.By: Lifecare Innovations Ptv.Ltd.Gujrat. Amphotericin B Injection Indian Pharmacopeia [I.P] (Fungitericin Intravenous 50 mg) equivalent to 50 mg of amphotericin B was calculated and measured and transferred to 100 ml of volumetric flask containing 10 ml of DMSO and distilled water 1:1 ration as a solvent. The final volume was make up to 100 ml with same solvent with frequent shaking for 10-15 minutes. By using whattamn filter paper the solution was filterd.The filtrate was suitably diluted to 05µg/ml concentration by using same solvent. Absorbance was measured against the blank solution. The recovery study was done by using 03 different levels of addition that are 80%, 100% and 120% addition. At three different levels a known amount of standard drug solution was added to pre-analyzed sample solution and absorbance was measured.[9]

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By using standard calibration curve drug content was calculated. Results of estimated amount of drug by using this method shown in Table no.04

Table 4: Determination of Accuracy by Percentage Recovery Method.

Drug	Amphotericin-B Injection amount (µg/ml)	Level of addition (%)	Equivalent Amount of formulation added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Average% Recovery
Amphotericin-B	05	80	8	12.96	99.73	99.69
	05	100	10	14.96	99.77	
	05	120	12	16.93	99.59	

Table 5: Validation parameters for Amphoterecine-B

Sr. No.	Parameters	Results
1	Absorption maxima (nm)	415 nm
2	Linearity range (µg/ml)	2-20 µg/ml
3	Standard Regression Equation	y = 0.00728x + 0.00
4	Correlation coefficient (R^2)	R^2 = 0.9975
5	Specificity	A 10 µg /ml solution of candidate drug in solvent DMSO and distilled Water 1:1 at UV detection of 415 nm will show an absorbance value of 0.716 ±0.00368
6	Accuracy (% Recovery)	99.69
7	Precision RSD Repeatability (n=6) Intra-day(n=3) Inter-day(n=3)	0.513 0.356 0.323
8	Molar Absorptivity	$6.9052*10^4$ L/mol.cm
9	LOD	0.0570 µg /ml
10	LOQ	0.1574 µg /ml

The absorption maxima i.elabda max found at 415nm and calibration curve linear over the range of 2-20 microgram/ml.The standard regression equation and correlation coefficient fond to be y = 0.00728x + 0.00, R^2 = 0.9975 respectively.Molar absorptivity found to be $6.9052*10^4$ L/mol.cm .The accuracy of the method found to be 99.69% conformed by accuracy study.4

RESULTS AND DISCUSSION

Molar absorptivity of the developed method was$6.9052*10^4$ L/mol.cm.Lambda max of amphotericin B found to be at 415 nm and standard calibration curve found to be linear over the range of 02-20µg/ml.By using mean % recovery, accuracy of the developed method was calculated and it was found to be 99.69%.Percentage RSD found to be less than one and .LOD and LOQ found to be 0.0570 and 0.1574 respectively by simple UV spectroscopy.Results of precision,repeatability,inter-day,intra-day variation shown in Table no.03 and 05.

CONCLUSION

From the results of accuracy, precision, and recovery it is concluded that the developed method of amphotericinB was coast effective, accurateand reproducible and can be used for quality control analysis in bulk and pharmaceutical dosage forms.

List of Abbreviations

1. UV-ULTRAVIOLET
2. DMSO-DIMETHYL SULFOXIDE
3. nm-NANOMETER
4. µg-MICROGEAM
5. ICH-INTERNATIONAL COUNSIL OF HARMONISATION
6. %RSD-PERCENT RELATIVE STANDARD DEVIATION
7. λmax-LAMBDA MAXIMUM
8. SD-STANDARD DEVIATION
9. I.P-INDIAN PHARMACOPOEIA

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REFERENCES

[1] A.Flok, C. Cotoraci and C.belta. Evaluation and Hepatotoxicity with treatment dose of flucytosine and amphoterecin B for invasive fingalinfections.BiomedReseach international .,1,1-9 (2016)

[2] J.Brajtburg ,J.Bolard. Carrier effects on biological activity of Amphotericin B. ClinMicrobiol .,9,512–31 (1996)

[3] R.H.Johnson ,H.E.Einstein. Amphotericin B and coccidioidomicosis. Ann NY Acad Sciences., 1111,434–41(2007);

[4] W.E.Joseph ,T.Myint.Central nervous system histoplasmosis observational study.Medicine Baltimore.,97,13 1-8 (2018);

[5] L.L.RafaelN.Maria Amphotericin B: side effects and toxicity.RevistaIberoamericana de Micologia., 26(4), 223-227(2009);

[6] D.A.Skoog,F.J.Holler, and D.A.Nieman. Principle of Instrumental Analysis,6 Edition Reprint, Thomson Brooks Colepublication.,300-351 (2004);

[7] J.H,ElizabethM.Johnson. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts.Cliniacal and Laboratory standards institute (CLSI).,29(17),1-23 (2009);

[8] S.M.Patil .Development of UV spectrophotometric method for estimation of letrozolein pure and pharmaceutical dosage form. Indo American Journal of Pharm Research.,2(6),2345-2353(2013);

[9] S.M.Patil.Development of validated UV spectrophotometric method for estimation of fosfestrol in bulk and pharmaceutical dosage formWJPR.,3(7).5541-5548(2013);

[10] G.Singh ,T.Kaur , A.Kaur , R. Kaur.Analytical methods for determination of Amphotericin B in biological samples: a short review. Annals of Applied Bio-sciences., 1,26-32 (2014);

[11] S.Ketllyn,V.Bárbara,L. Fermino. Difficulties in antifungal therapy with amphotericin B and the continuous earch for new formulations: A literature review.African journal of pharmacy and pharmacology.,10(24),512-520 (2016);