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VALIDATED RP-HPLC METHOD FOR THE QUANTIFICATION OF ANIDULAFUNGIN IN BULK SAMPLE AND PARENTERAL DOSAGE FORM AND ITS APPLICATION TO FORCED DEGRADATION STUDIES IN BULK SAMPLE

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ABSTRACT

A simple, selective, precise and stability-indicating high-performance liquid chromatographic method of analysis of Anidulafungin in pharmaceutical dosage form was developed and validated. The solvent system consisted of acetonitrile: water: 0.1% v/v trifluoroacetic acid (48:52:1) with pH 4.7. Retention time of Anidulafungin in C-18 column was 8.86 ± 0.5 min at the flow rate 1.5ml/min. Anidulafungin was detected at 300 nm at room temperature. The linear regression analysis data for the linearity plot showed good linear relationship with correlation coefficient value, R^2 = 0.999 in the concentration range 250 – 1500 µg/ml with slope 12057.1248, intercept -119267.88. The method was validated according to the International Conference on Harmonization (ICH) guidelines for linearity, range, accuracy, precision and specificity and applied on bulk powder and pharmaceutical formulations. Anidulafungin was determined in sterile dosage form in range of 99.73% with 0.327 standard deviation. The accuracy of the method was validated by recovery studies and was found to be significant and under specification limits, with % Recovery 99.35 – 100.96 (within acceptable range (98 – 102%). The method was also found to be robust. The drug was subjected to stress conditions of acidic, basic, oxidation, photolysis and thermal degradation. Considerable degradation was found in all stress conditions.

Keywords: - RP-HPLC, Anidulafungin, Validation, Stability.

1.1 INTRODUCTION

Anidulafungin is chemically N-[(3S,6S,9S,11R,15S,18S,20R,21R,24S,25S,26S)-6-[(1S,2S)-1,2-dihydroxy-2-(4-hydroxyphenyl)ethyl]-11,20,21,25-tetrahydroxy-3,15-bis[(1R)-1-hydroxyethyl]-26-methyl-2,5,8,14,17,23-hexaoxo-1,4,7,13,16,22-hexaazatricyclo[22.3.0.0 ^{9, 13}]heptacosan-18-yl]-4-{4-[4-(pentyloxy)phenyl]phenyl}benzamide with pale yellow coloured amorphous powder and is used in the treatment of invasive candidiasis. The molecular formula of Anidulafungin is $C_{58}H_{73}N_7O_{17}$ and molecular weight is 1140.2369. Anidulafungin undergoes slow chemical degradation at physiologic temperature and ph to a ring-opened peptide that lacks antifungal activity. It is a semi-synthetic echinocandin with antifungal activity and inhibits glucan synthase, an enzyme present in fungal, but not mammalian cells and formation of 1,3-β-D-glucan, an essential component of the fungal cell wall, which leads to osmotic instability and cell death.[1]

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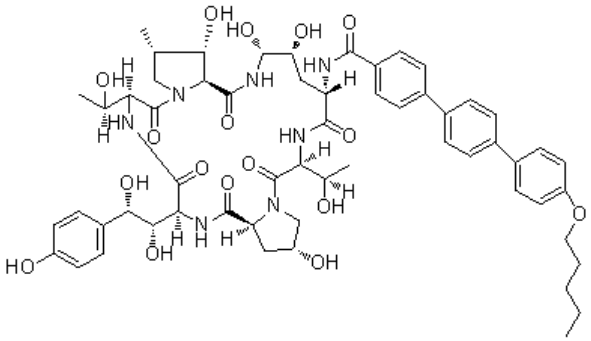


Fig.1. Structure of Anidulafungin

Anidulafungin is available in sterile dosage form with the label claim of 50mg, 100mg, and 200mg commercially by different manufacturers. As per literature survey a few analytical method have been reported for the determination of Anidulafungin in pure drug, pharmaceutical dosage form and in biological samples using Liquid Chromatography. No reports were found for the method for forced degradation studies in dosage form.[2, 3]

Stress testing carried out to elucidate the inherent stability characteristic of the active substances and forms an important part of the API. It suggests that degradation products that are formed under a variety of conditions should be identified and degradation pathways

be established. The purpose of stress testing is to provide evidence on how the quality of drug substance varies with time under the effect of varieties of factors such as acidic, alkaline, temperature, light and presence of oxygen. An ideal stability-indicating method is one that quantifies the drug and also resolves its degradation products.

The aim of present work is to develop a simple, specific, sensitive, accurate and stability indicating HPLC analytical procedure for the analysis of Anidulafungin and validated as per ICH guidelines.

1.2 EXPERIMENTAL

1.2.1. Materials and Reagents

Working standard of pharmaceutical grade Anidulafungin was obtained as generous gift from Therdose Pharma Pvt. Ltd., Kukatpally (Hyderabad, India). Formulation available in the market is parenteral form, containing 50 mg Anidulafungin and was procured from Pfizer. Chemicals and reagents of analytical-grade were purchased from Rankem grade and were purchased from Rankem, Mumbai, India.

1.2.2. Apparatus

The HPLC system employed in the method development, forced degradation studies and assay method validation was Schimadzu Seperations Module (LC-20AD) system with PDA (SPD-M20A) detector. The output signal was monitored and processed using LC Solutions software. The column used was YMC ODS Pack AQ C_{18} (150x4.6 i.d., 3 µm) with an injection system of auto sampler (SIL-20AC ^{HT}) and injection volume is 10 µL. The mode of separation was isocratic.

1.2.3. HPLC Method

1.2.3.1 Stock Solution

Accurately 100mg of the Anidulafungin pure drug was weighed and transferred into 50ml clean, dry standard volumetric flask. The volume was made up to the mark with methanol (2000 µg/ml).

1.2.3.2 Standard Solution

5 ml of Anidulafungin stock solution was transferred to a 10 ml clean volumetric flask and the volume was made up with methanol and mixes well (500 µg/ml) and then filtered through 0.45 µm Ultipor N66 nylon filter. The final solution (500µg/ml) was injected into the HPLC system.

1.2.3.3 Chromatographic condition

The mobile phase consisted of acetonitrile, water, 0.1% v/v trifluoroacetic acid in the ratio of 48:52:1. Contents of the mobile phase were filtered before use through a 0.45µ membrane and degassed for 15min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.5ml/min and the injection volume was 10µl. The column temperature was maintained at 25±1^{0}C. The eluents were monitored at 300nm.

1.2.3.4 Calibration of HPLC method

Different volumes of stock solutions were accurately transferred in to 10 ml volumetric flask and diluted to mark to yield concentration range 250-1500 µg/ml for Anidulafungin. 11 solutions were prepared and the final volume was made up to the mark with diluent. The calibration line was obtained by plotting the peak area against concentration of drug.

1.2.3.5 Assay of Anidulafungin

From 50mg per vial 7.5ml was pipette out and taken in 25ml volumetric flask and was made up to the mark with methanol to obtain 1000µg/ml. 5ml was transferred into a 10ml volumetric flask and the volume was made up to the mark with mobile phase to obtain 500µg/ml of Anidulafungin. The solution was sonicated for 10min and injected under above chromatographic conditions and peak area was measured.

The assay procedure was made triplicate and weight of sample taken for assay was calculated. The percentage of drug found in formulation, mean and standard deviation in formulation were calculated.

1.2.4 Validation of the Developed Method

1.2.4.1 Specificity and selectivity

The specificity of the method was evaluated with regard to interference due to presence of any other excipient. The figure shows that drug was clearly separated from its excipient. Thus, the HPLC method presented in this study is selective.

1.2.4.2 Accuracy

Accuracy of the method was determined by recovery experiments. To the formulation, the reference standards of the drug were added at the level of 75%, 100%, 125%. The recovery studies were carried out three times and the percentage recovery and %RSD of the recovery were calculated and shown in *table: 4.*

1.2.4.3 Precision

The precision of the method was demonstrated by method precision, system precision, inter-day and intra-day variation studies. In method precision studies six different weights of standard solution (500µg/ml) were weighed and two repeated injections for single preparation was made and response factor of drug peak and % RSD were calculated and presented in *table: 6.* In system precision studies, six repeated injections of standard solution (500µg/ml) were made and the response factor of drug peak and %RSD were calculated and presented in *table: 5.*. In the intra-day studies, three repeated injections of standard solution (250, 500, 750µg/ml) were made and the response factor of drug peak and % RSD were calculated and presented in *table: 7.* In the inter-day studies, three repeated injections of standard solution (250, 500, 750µg/ml) were made for three consecutive days and response factor of drugs peak and % RSD were calculated presented in *table: 8.*

1.2.4.4 Linearity

The linearity of the method was demonstrated over the concentration range of 250-1500 µg/ml of the target concentration. By assaying the samples in triplicate on three separate occasions, by analyzing different concentrations of the pure drug from the chromatogram calibration curve is produced and was shown in *Fig: 3.* The correlation coefficients for the average area at each level versus concentration of analyte were calculated and are presented in *table: 2.*

1.2.4.5 Limit of Detection (LOD)

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

From 1000µg/ml stock solution, 1ml was taken into 100ml volumetric flask and the volume was made up to the mark with methanol. 2.5ml was taken into 100ml volumetric flask and the volume was made up to the mark with methanol (250ng/ml). The Limit of detection was shown in *table: 9.*

1.2.4.6 Limit of Quantitation (LOQ)

Based on the LOD strength (0.1 mcg/ml, standard solution) the LOQ values were calculated by multiplication with three times.

From 1000µg/ml stock solution, 1ml was taken into 100ml volumetric flask and the volume was made up to the mark with methanol. 2.5ml was taken into 100ml volumetric flask and the volume was made up to the mark with methanol. 3.3ml was taken into 100ml volumetric flask and the volume was mad up to the mark with methanol (82.5ng/ml). The Limit of quantitation was shown in *table: 9.*

1.2.4.7 Robustness

The optimum HPLC conditions set for this method have been slightly modified for samples of Anidulafungin dissolved in the drug matrix as a means to evaluate the method robustness. The small changes include - the flow rate, change in the composition of aqueous phase, λmax. The results were shown in *table: 10.*

1.2.4.8 System suitability

It is defined as tests to measure the method that can generate result of acceptable accuracy and precision. The system suitability was carried out after the method has been developed. Accurately weighed and transferred 25.56mg of Anidulafungin drug substance into a 50ml volumetric flask and made up to the mark with methanol (concentration 500µg/ml). For this, parameters like plate number (N), resolution (R), relative retention time (α), HETP, peak symmetry of samples were measured, and shown in *table: 12.*

1.2.4.9 Solution stability

25mg of drug substance was weighed and taken in 25ml volumetric flask and made up to the mark with diluent (1000µg/ml). From it, at different time intervals like initial, 4 Hrs, 8 Hrs and 24 Hrs, 5ml was taken in to 10ml volumetric flask and diluted up to the mark with the diluent (methanol) and obtained the final concentration of 500µg/ml and injected in to the chromatographic conditions. For this, parameters like retention time, peak area, theoretical plates and tailing factor were measured and shown in *table: 11.*

1.2.5 Degradation studies of Anidulafungin

Degradation studies were carried out as per ICH guidelines. The objective of the study was to find out the degradation products, which in turn help in the establishment of degradation pathways and the intrinsic stability of drug molecule. In order to check the selectivity of the proposed method, degradation studies were carried out by using acidic, basic, oxidative, photolytic, thermal and hydrolytic conditions.

1.2.5.1 Procedure for forced degradation studies

To determine, whether the analytical method and assay were stability indicating, Anidulafungin active pharmaceutical ingredient (API) powder was stressed under various conditions to conduct forced degradation studies. Intentional degradation was attempted to stress conditions of acidic (0.1N HCl), basic (0.1N NaOH), oxidative degradation (3%H_2O_2), thermal treatment (heated at 80^0c) and photolytic to evaluate the ability of the proposed method to separate Anidulafungin from its degradation products.

1.2.5.2 Acidic degradation

25mg of Anidulafungin drug substance was weighed accurately and transferred into a 25ml volumetric flask, added 2.5ml of 0.1N HCl and made up to mark with diluent (concentration 1000µg/ml). This solution was analyzed initially and the remaining solution was stored at 60°C. 2.5ml acid degradation stock solution was transferred into a 5ml volumetric flask and made up to the mark with diluent (500µg/ml).The results were shown in *table: 13.*

1.2.5.3 Basic degradation

25mg of Anidulafungin drug substance was weighed accurately and transferred into a 25ml volumetric flask, added 2.5ml of 0.1N NaOH and made up to mark with diluent (concentration 1000µg/ml). This solution was analyzed initially and the remaining solution was stored at 60°C. 2.5ml base degradation stock solution was transferred into a 5ml volumetric flask and made up to the mark with diluent (500µg/ml). The results were shown in *table: 13.*

1.2.5.4 Oxidative degradation

25mg of Anidulafungin drug substance was weighed accurately and transferred into a 25ml volumetric flask, added 2.5ml of 3% H_2O_2 and made up to mark with diluent (concentration 1000µg/ml). This solution was analyzed initially and the remaining solution was stored at 60°C. 2.5ml oxidative degradation stock solution was transferred into a 5ml volumetric flask and made up to the mark with diluent (500µg/ml). The results were shown in *table: 13.*

1.2.5.5 Thermal Degradation

1.2.5.5.1 Thermal degradation of liquid sample at NMT 80^0c

Forced degradation in thermal degradation was performed by taking 25mg of drug into 25ml volumetric flask and diluted up to the mark with methanol to obtain concentration of 1000µg/ml. The flask was exposed to a controlled temperature in oven NMT 80^0c. From that solution 5 ml was taken into 10ml volumetric flask and diluted with methanol to obtain a final concentration of 500µg/ml and injected. The results were shown in *table: 13.*

1.2.5.5.2 Thermal degradation of solid sample At NMT 80^{0}c

Anidulafungin pure drug was exposed to a controlled temperature in oven NMT 80^{0}c. At sampling time, accurately about 25mg of the pure drug was weighed and transferred into 25ml clean, dry standard volumetric flask. The volume was made up to the mark with methanol. 5ml of the above solution was transferred to a 10ml volumetric flask and the volume was made up to the mark with methanol to obtain 500µg/ml concentration of Anidulafungin and injected. The results were shown in *table: 13.*

1.2.5.6 Photolytic Degradation

1.2.5.6.1 Photo degradation of liquid sample

Forced degradation in photo degradation was performed by taking 25mg in 25ml volumetric flask and made up to the mark with methanol (1000µg/ml). The flask was exposed to sunlight directly. From that solution 5 ml was taken in to 10ml volumetric flask and diluted with methanol to obtain a final concentration of 500µg/ml. The results were shown in *table: 13.*

1.2.5.6.2 Photo degradation of solid sample

Anidulafungin pure drug was exposed to sunlight directly. At sampling time, accurately about 25mg of the pure drug was weighed and transferred into 25ml clean, dry standard volumetric flask. The volume was made up to the mark with methanol. 5ml of the above solution was transferred to a 10ml volumetric flask and the volume was made up to the mark with methanol to obtain 500µg/ml concentration of drug. The results were shown in *table: 13.*

1.2.5.7 Hydrolytic Degradation

25mg of Anidulafungin drug substance was weighed accurately and transferred into a 25ml volumetric flask, added 5ml of methanol and made up to mark with water (concentration 1000µg/ml). This solution was analyzed initially and the remaining solution was kept aside. 2.5ml hydrolytic degradation stock solution was transferred into a 5ml volumetric flask and made up to the mark with water (500µg/ml). The results were shown in *table: 13.*

1.3 RESULTS AND DISCUSSION

The selected drug Anidulafungin was estimated by external standard method and stability studies was carried out as per ICH Guidelines. The external standard method was optimized in the mobile phase ratio acetonitrile: water: 0.1% v/v trifluoroacetic acid (48:52:1) with pH – 4.7. The detection was carried out at wavelength 300 nm with a retention time of 8.86 mins and peak asymmetry of 0.8.

The method was validated for all validation parameters as per ICH guidelines. The linearity range for Anidulafungin was 250 – 1500 µg/ml. with R^2 value of 0.999. The % RSD for method and system precision was < 2%. The method has been validated in assay of sterile dosage forms. The accuracy of the method was validated by recovery studies and was found to significant and under specification limits, with % Recovery 99.35 – 100.96 (within acceptable range (98 – 102%). The assay results were found to be 99.73% (i.e. within 98 – 102%). The method passedthe robustness parameters for change in mobile phase ratio, flow rate and λ_{max} .

The sample solutions were subjected to acidic, basic, and oxidative degradations at room temperature. The % degradation at room temperature was more in case of acidic (11.8 % in 1 Hr 30min), basic (15.13 % in 1Hr), oxidative (8.49% in 8hrs) degradation, hydrolytic (3.16% in 48 Hrs), thermal solid (10.42% in 12 Hrs), thermal liquid (12.3% in 10 Hrs), photolytic solid (9.17% in 12 Hrs), photolytic liquid (10.14% in 10 Hrs).

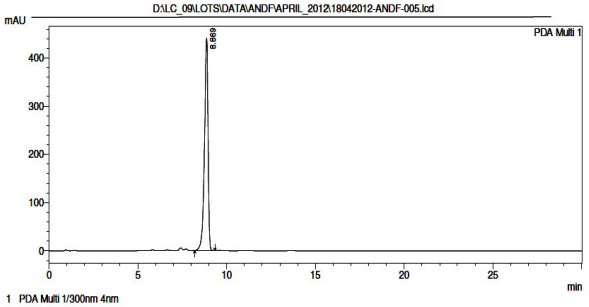
1.4 CONCLUSION

A validated HPLC method was developed for Anidulafungin, using the stress-testing route suggested by ICH. The developed method is simple, accurate, precise, specific, and could separate drug from degradation products. It is suggested for use in analysis of samples generated during stability studies on Anidulafungin and its formulations.

A forced degradation study on Anidulafungin was carried out and an efficient HPLC method for the quantification of Anidulafungin and its degradation products in bulk drug was developed and validated. The results of stress testing of the drug, undertaken according to the ICH guidelines, revealed that degradation products were formed under acidic, alkaline, thermal, photolytic and oxidizing conditions.

Table 1: Optimized Chromatographic conditions

S. No	Parameters	Conditions
1	Mobile Phase Optimized	ACN : Water :0.1% v/v TFA (48:52:1)
2	Stationary Phase	C_{18} (150 × 4.6 mm i.d.,3 µm)
3	Flow Rate	1.5 ml / min
4	Run Time	30 min
5	Column Temperature	25^{o}C
6	Volume of Injection	10 µL
7	Detection Wavelength	300 nm
8	Retention time of Drug (min)	8.86 min



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PDA Ch1 300nm 4nm

Peak#	Name	Ret. Time	Area	Area %	Theoretical Plate#	Tailing Factor
1	ANDF	8.87	6173880	100.00	9056.65	0.80
Total			6173880	100.00		

Fig. 2: Optimized chromatogram of Anidulafungin sample

Table 2: Linearity of Anidulafungin

S. No	Level	Concentration (mcg/ml)	Peak area Mean ± SD (n=3)	%RSD
1.	50%	250	2897483 ± 17185.79	0.59
2.	75%	375	4386414 ± 2429.28	0.06
3.	100%	500	5960736 ± 2303.99	0.04
4.	125%	625	7360635 ± 1290.23	0.02
5.	150%	750	9017954 ± 7625.20	0.08
6.	175%	875	10234965 ± 5668.14	0.06
7.	200%	1000	11984902 ± 7370.28	0.06
8.	225%	1125	13511751 ± 29328.15	0.22
9.	250%	1250	14982995 ± 65865.68	0.44
10.	275%	1375	16517655 ± 18006.26	0.11
11.	300%	1500	17882391 ± 7516.20	0.04
Y=12057x-11926			**R^2 = 0.999**	

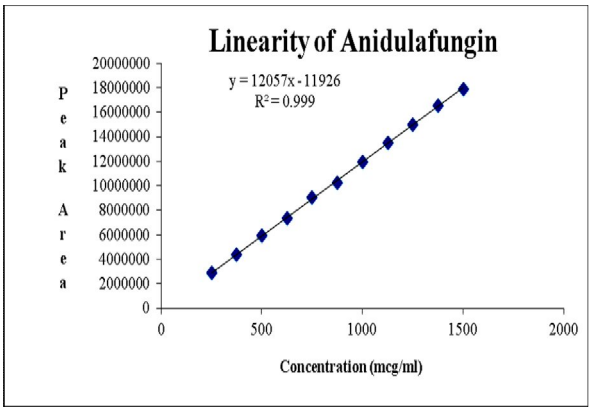

Fig 3: Linearity plot of Anidulafungin

Table 3: Assay of formulation

S. No	Formulation	Label claim	Amount Found Mean ± SD (n=3)	Assay	%RSD
1.	Eraxis	50mg	49.86 ± 0.327mg	99.73%	0.82

Table 4: Recovery studies

S. No	Pre analyzed sample conc. (µg/ml)	Recovery level	Amount Spiked (µg/ml)	Amount Recovered (µg/ml) Mean ± SD (n=3)	%Recovery	%RSD
1	375 (LQC)	75%	281.61	279.85 ± 0.2843	99.37	0.07
		100%	375.36	378.92 ± 1.1810	100.94	0.36
		125%	467.51	465.72 ± 0.3709	99.61	0.11
2	500 (MQC)	75%	375.23	372.82 ± 0.2660	99.35	0.13
		100%	500.21	505.03 ± 1.5831	100.96	0.30
		125%	625.14	622.73 ± 0.5008	99.61	0.09
3	625 (HQC)	75%	468.34	465.47 ± 0.3322	99.38	0.08
		100%	625.51	631.28 ± 1.9804	100.92	0.28
		125%	781.22	778.32 ± 0.6896	99.62	0.10

Table 5: System Precision Studies

S. No.	Retention Time	Peak Area	Theoretical Plates	Tailing Factor
1	8.83	6153193	8872.0	0.80
2	8.84	6180109	9129.7	0.79
3	8.84	6184140	9410.9	0.78
4	8.86	6174198	9074.9	0.79
5	8.86	6148703	9092.0	0.80
6	8.87	6173880	9056.7	0.80
MEAN	8.85	6169037	9106.0	0.79
STDV	0.02	14596	174.28	0.01
%RSD	0.18	0.24	1.91	1.02
Acceptance Criteria	NMT - 1.0%	NMT - 2.0%	NLT or Equal to 2500	NMT - 2.0%
Result	Pass RSD - 0.18	Pass RSD - 0.24	Pass	Pass RSD - 1.02

Table 6: Method Precision studies

S. No	Concentration (µg / ml)	Peak area (n = 6)	% Assay	% RSD
1	500	6123006	98.9	0.71
		6184961	99.9	
		6143002	99.2	
		6064085	98.9	
		6060038	98.5	
		6125942	99.3	

Table 7: Intraday Precision studies

S. No	Concentration µg/ml	Peak area mean ± SD (n=3)	%RSD
1	250 (LQC)	2910446 ± 282.8427	0.01
2	500 (MQC)	5968948 ± 7109.252	0.12
3	750 (HQC)	7412552 ± 3750.494	0.05

Table 8: Interday Precision studies

S. No	Concentration (µg/ml)	Peak area mean ± SD(n=3)	%RSD
1	250 (LQC)	2902816 ± 20494.78	0.05
2	500 (MQC)	5960662 ± 3253.398	0.02
3	750 (HQC)	7360228 ± 1528.765	0.71

Table 9: LOD and LOQ values of Anidulafungin

S. No	Concentration (mcg/ml)	Peak area mean ± SD (n=3)	%RSD	S/N ratio
1	82.5ngm (LOD)	1025 ± 62.067	0.12	5.161575
2	250ngm (LOQ)	2323 ± 1163.372	0.316	10.041811

Table 10: Robustness studies

S. No	Parameter	Modification	Retention time (in mins)	Tailing Factor
1	Flow rate (ml/min)	1.3	8.829	0.865
		1.4	8.843	0.835
		1.5	**8.84**	**0.80**
		1.6	8.824	0.782
		1.7	8.971	0.761
2	λ_{max} (nm)	297	8.829	0.865
		300	**8.84**	**0.80**
		303	8.828	0.835
3	Mobile phase ratio	45:55:1	8.855	0.79
		48:52:1	**8.84**	**0.82**
		52:48:1	8.841	0.778
		55:45:1	8.829	0.865

Table 11: Solution stability studies

S. No.	Solution(hrs)	Retention Time	Peak Area	Tailing Factor	Theoretical Plates
1	4	8.91	6000132	0.8	9939
		8.92	5997061	0.8	9982
2	8	8.87	5990008	0.8	9882
		8.86	5988164	0.8	9860
3	24	8.97	5996580	0.8	9842
		8.96	5996545	0.8	9849
MEAN		8.915	5994748	0.8	9892
STDV		0.045	4620.38	0.01	56.18
%RSD		0.51	0.08	0.71	0.57
Acceptance Criteria		**NMT - 1.0%**	**NMT - 2.0%**	**NMT 2.0%**	**NLT or Equal to 2500**
Result		**Pass RSD - 0.51**	**Pass RSD - 0.08**	**Pass RSD 0.71**	**Pass**

Table 12: System Suitability parameters

S. No	Parameter	ANIDULAFUNGIN
1	Retention time (R_t)	8.99
2	Theoretical plates	10046
3	Peak Asymmetry	0.8
4	Peak Area	5993115
5	% RSD	0.36

Table 13: Results of Stress degradation of Anidulafungin

Stress Degradation	Stress Condition	Time	% Degradation
Acidic	0.1 N HCl	1Hr 30min	11.8
Alkaline	0.1 N NaOH	1Hr	15.13
Hydrolytic	H_2O	48 Hrs	3.16
Oxidative	3% H_2O_2	8 Hrs	8.49
Thermal (Solid)	80ºC	12 Hrs	10.42
Thermal (liquid)	80ºC	10 Hrs	12.3
Photolytic (solid)	Sun light	12 Hrs	9.17
Photolytic (Liquid)	Sun light	10 Hrs	10.14

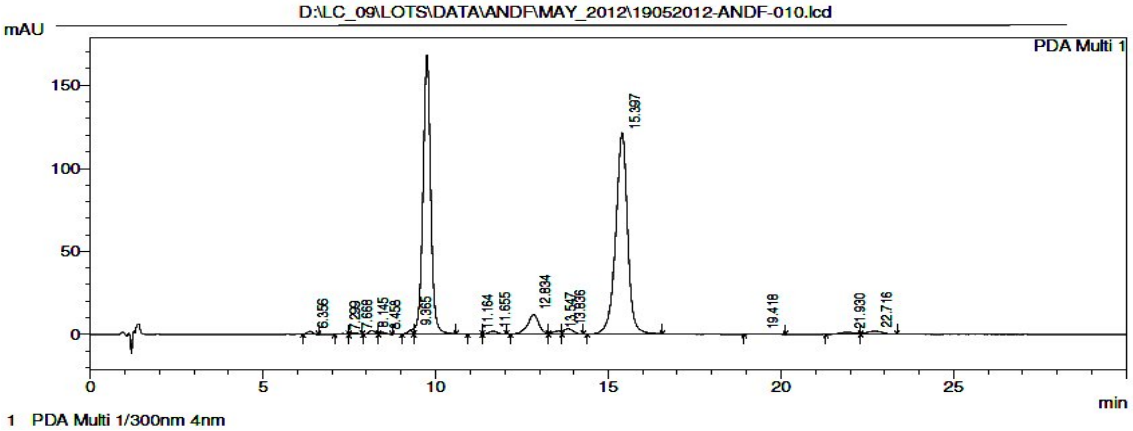

Fig. 4: Degradation in 0.1 N HCL

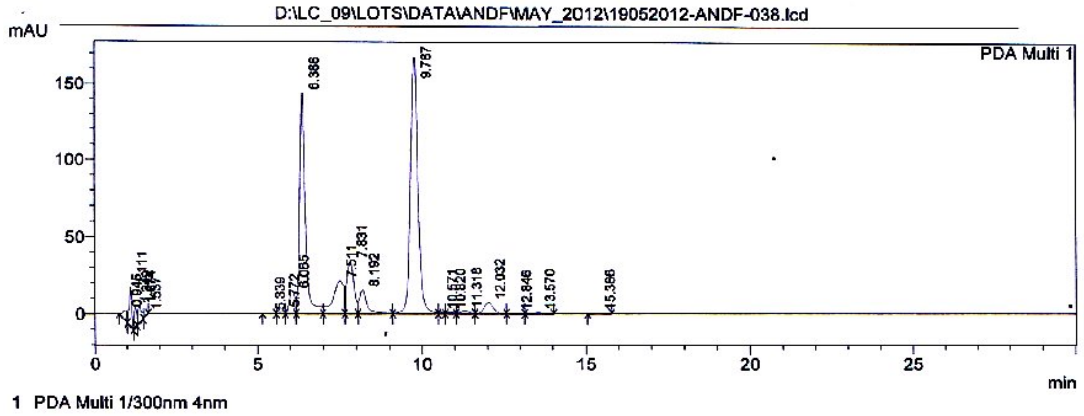

Fig. 5: Degradation in 0.1 N NaOH

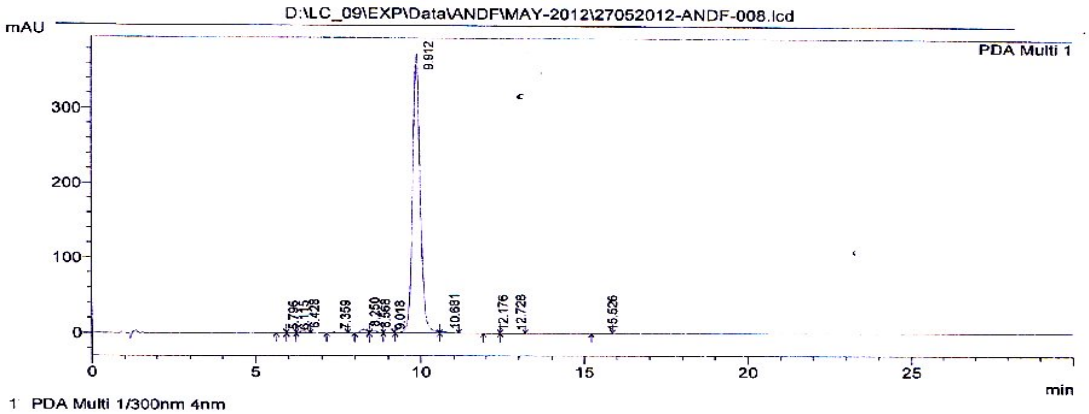

Fig. 6: Hydrolytic degradation

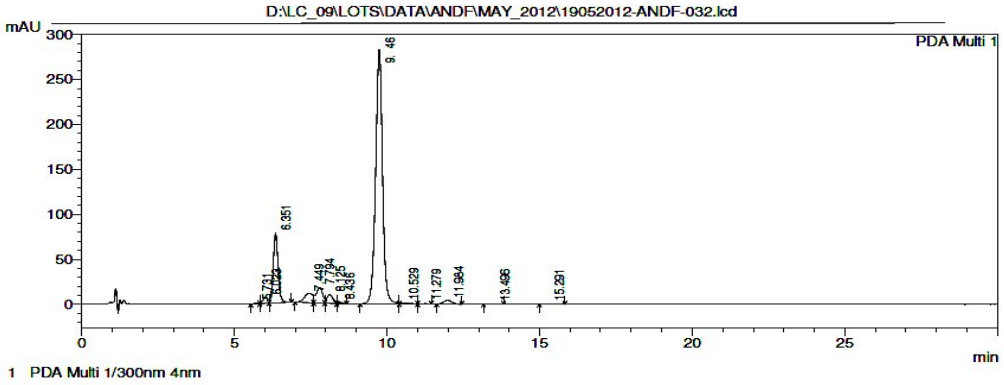

Fig. 7: Degradation in 3% H_2O_2

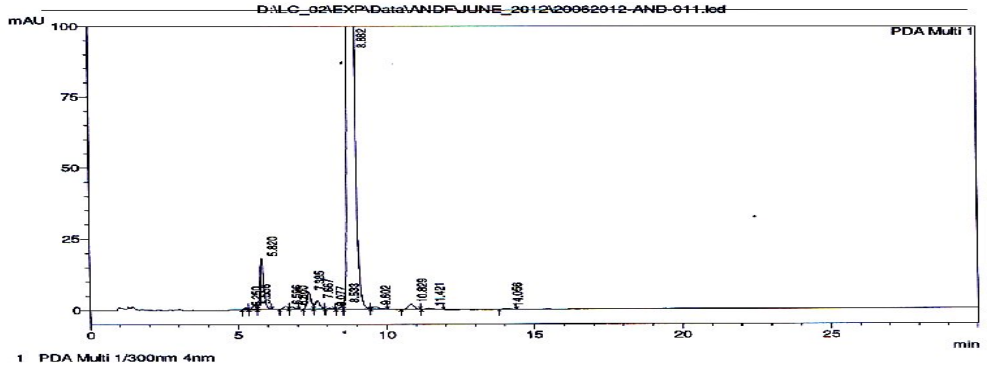

Fig. 8: Thermal Degradation solid

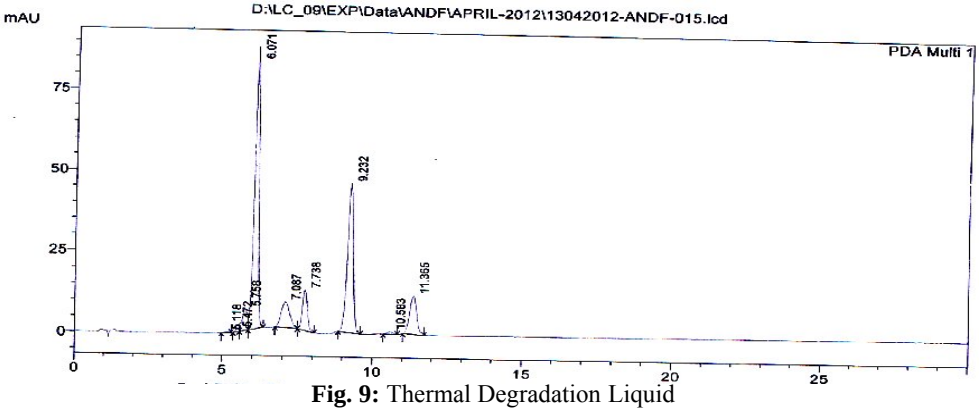

Fig. 9: Thermal Degradation Liquid

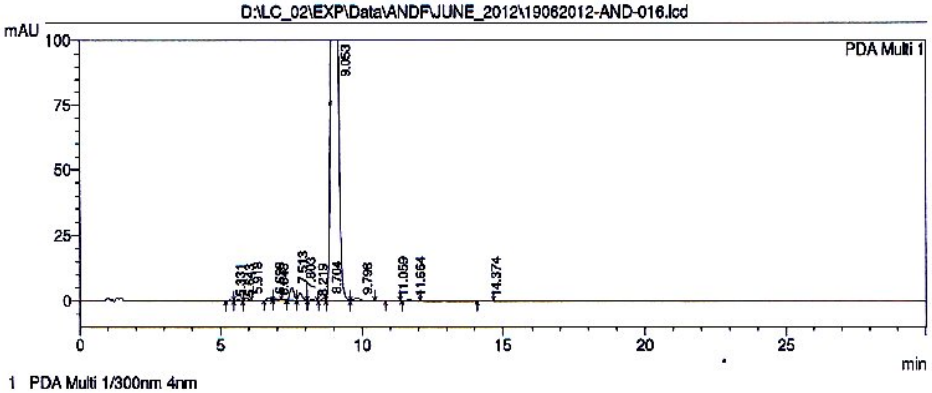

Fig. 10: Photolytic Degradation solid

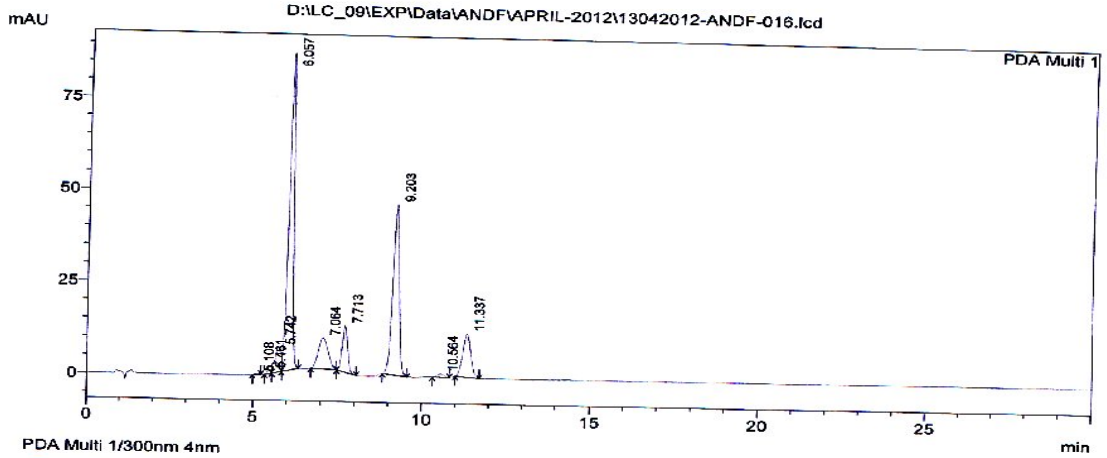

Fig. 11: Photolytic Degradation liquid

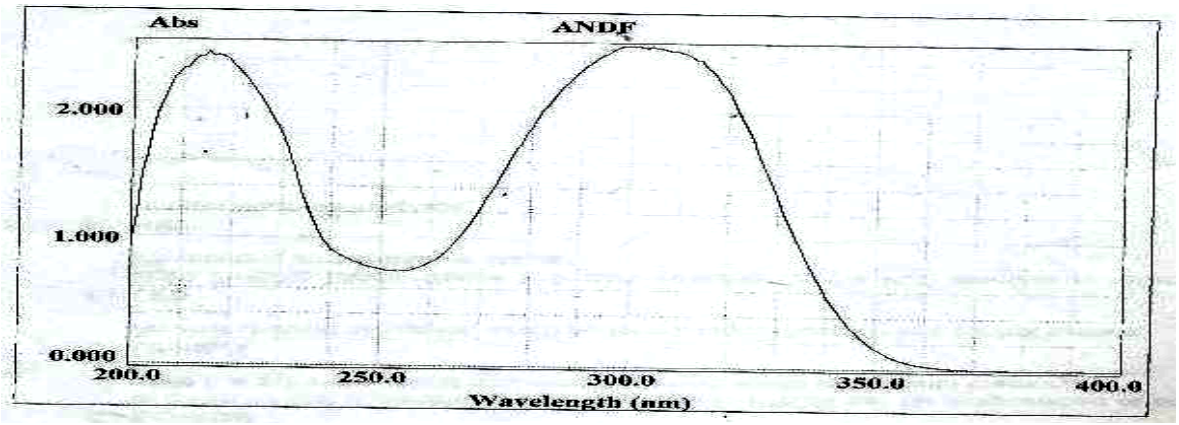

Fig. 12: UV Spectrum of Anidulafungin

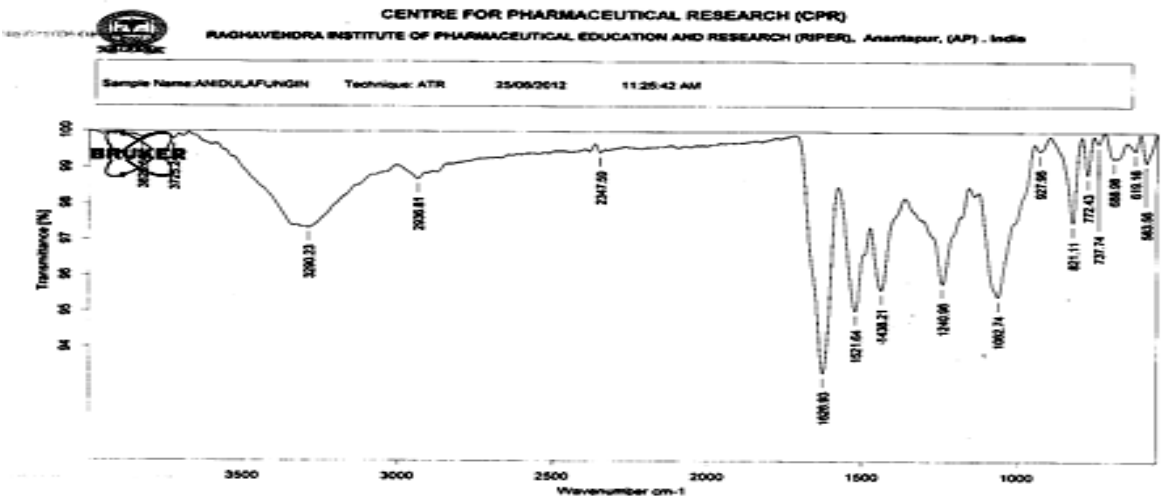

Fig. 20: IR Spectrum of Anidulafungin

1.5 ACKNOWLEDGEMENT

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1.6 REFERENCES

1. www.drugbank.ca/drugs/DB00362
2. Christina A Sutherland, David P. Nicolau, Joseph L Kuti, 2011. "Development of an HPLC method for the determination of Anidulafungin in Human Plasma and Saline". Journal of Chromatographic Science. Volume 49, 397 – 400.
3. Jens Martenns – Loben Hoffer, Victoria Rupprecht, Stefanie M, Bode- Boger, 2011.

"Determination of Micafungin and Anidulafungin in Human Plasma: UV- or Mass Spectrometric quantification?" Journal of Chromatography B. Volume 879, Issue 22, 2051-2056.
4. Kamboj PC. Pharmaceutical Analysis. Vol. I. 2nd Ed. New Delhi: Vallabh Publications; 2007. 1-2.
5. Michael W. Dong. Modern HPLC for practicing scientists, John Wiley and Sons, New Jersey; 2006, 193-196.
6. http:/en.wikipedia. org/wiki/stabilitydata
7. www.rxlist.com/eraxis-drug.htm
8. Clarke's analysis of drugs and poisons, 3rd edition, 2005ICH: Q2B, Analytical validation-methodology; 1996.
9. Fong GW, Lam SK. HPLC in the Pharmaceutical industry. New York: Marcel Dekker Inc; 1991, Vol47, 16-56.
10. Kazakevich Y, Lobrutto R. HPLC for Pharmaceutical Scientists. London: John wiley and sons; 2007, 10-22.
11. Kromidas S. HPLC made to measure. Weinheim: Willey VCH verlag Gambh and Co. KGaA; 2006. 151-347.
12. P.D.Sethi High Performance Liquid Chromatography CBS publishers volume 1, 59-63.

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