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Formulation and Evaluation of Nystatin Vaginal Tablet

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ABSTRACT

The objective is to formulate vaginal tablet of Nystatin and evaluate the in vitro antimycotic effectiveness against the marketed product in Candida albicans. Nystatin is an antimycotic polyene antibiotic obtained from Streptomyces noursei. Vaginal tablet of Nystatin were formulated with 100,000 Units of Nystatin. The tablets were made with Ethyl Cellulose, 4 cps as polymer, Pregelatinized Starch (Starch 1500) as binder, PEG 8000 as wetting agent, Co-processed mixture of Lactose–Microcrystalline Cellulose (Cellactose) as diluent and Stearic Acid (Stellipress 1200 Poudre) as lubricant. The optimized formulation was subjected to stability studies at accelerated condition. Test and marketed formulations were evaluated for appearance, weight variation, thickness, hardness, friability, drug content and in vitro drug release. In vitro studies on anti mycotic evaluation were carried out for the optimized formulation in three sets of medium cultured with Candida albicans and the zone of inhibition were compared with the marketed one.

Keywords: Nystatin, USP, Vaginal tablet, Direct blending, Compression process

INTRODUCTION

Nystatin was discovered in the New York State Health Laboratory is a antimycotic polyene antibiotic obtained from Streptomyces noursei. It is structurally similar to amphotericin B and has the same mechanism of action [1-3].

Nystatin is 20-(4-amino-3,5-dihydroxy-6-methyl-oxan-2-yl)oxy-4,22,24,28,29,32,34,36-octahydroxy-2,3,5-trimethyl-26,38-dioxo-1-oxacyclooctatriaconta-6,8,12,14,16,18-hexaene-23-carboxylicacid. Its molecular formula is $C_{47}H_{75}NO_{17}$. Nystatin is a yellow to light tan powder. It is freely soluble in dimethylformamide, slightly sparingly soluble in methyl alcohol, n-propyl alcohol and n-butyl alcohol, practically insoluble in alcohol, chloroform, and ether and insoluble in water. Nystatin vaginal tablet, 100,000 Units is available in the market manufactured by Duramed pharmaceutical, Inc., NY, USA.

Microbiology

Nystatin is both fungi static and fungicidal *in vitro* against a wide variety of yeasts and yeast like fungi. Nystatin acts by binding to sterols in the cell membrane of sensitive fungi with a resultant change in membrane permeability allowing leakage of intracellular components. Nystatin exhibits no appreciable activity against bacteria, protozoa, trichomonas or viruses. Nystatin is not absorbed from intact skin or mucous membranes [1,2,4].

Chemistry and stability

Nystatin is an antifungal antibiotic produced by Streptomyces noursei. The drug is an amphoteric polyene macrolide that occurs as a hygroscopic, yellow to light tan powder with a cereal-like odour and is very slightly soluble in water and slightly to sparingly soluble in alcohol. Each mg of Nystatin contains not less than 4400 units of activity [5,6]. Nystatin deteriorates on exposure to heat, light, moisture, or air. Nystatin oral suspension and tablets should be stored in tight, light-resistant containers at room temperature (e.g. 15-30°C); exposure of the tablets to temperatures exceeding 40°C and freezing of the oral suspension should be avoided. Nystatin powder should be stored in tight, light-resistant containers and refrigerated at 2-8°C. Since extemporaneously prepared oral suspensions of Nystatin do not contain a preservative, such suspensions should be used immediately after preparation and should not be stored [7-9].

Indications and usage

The Nystatin vaginal tablet is effective for the local treatment of vulvovaginal candidiasis (moniliasis). The diagnosis should be confirmed, prior to therapy, by KOH smears and/or cultures. Other pathogens commonly associated with vulvovaginitis (*Trichomonas* and *Haemophilus vaginalis*) do not respond to Nystatin and should be ruled out by appropriate laboratory methods [4,8].

Dosage and administration

The usual dosage is one tablet (100,000 units Nystatin) daily for two weeks. The tablet should be deposited high in the vagina by means of the applicator [1,8].

MATERIALS AND METHODS

Materials

Nystatin was obtained from Shreeji Pharma International. Ethyl Cellulose (Ethocel 4 cps) and Pregelatinized Starch (Starch 1500 LM) was obtained from Colorcon. Polyethylene Glycol 8000 (PEG 8000) was obtained from Dow. Co-processed mixture of Lactose & Microcrystalline Cellulose (Cellactose 80) was obtained from Meggle. Stearic Acid (Stellipress 1200 Poudre) was obtained from Stearinerie Dubois.

Drug-excipient compatibility study

The active ingredients and excipients were mixed in appropriate ratio and mixed well in a polybag and it is passed through 40 ASTM Sieve and then taken in 2 ml glass vials [10]. Then these vials are kept at room temperature (control), 40°C ± 2°C/75 ± 5% RH and at 55°C. The samples were withdrawn at 2nd week for 55°C and at 4th week for 40°C ± 2°C/75 ± 5% RH and analysed for Appearance (Table 1), Fourier Transform Infrared Spectroscopy (Table 2), Assay (Table 3), and Moisture Content (Table 4).

Prerequisite design attributes

Nystatin Vaginal Tablet to be prepared will be designed to be similar to that of reference product; Excipients shall be similar to that of brand listed excipients. Tablet dimension is 25.53 mm × 12.75 mm oval shaped tablets. Tablet weight is 1100 mg. Disintegration Time about 5 min. Hardness is about 13 kP. % Friability NMT 1.0%. Dissolution profile should be comparable with the profile of reference product when tested in pH 4.5 acetate buffer (pH of Vaginal Fluid) Stability profile should have a comparable stability with that of reference product at accelerated stability condition (40°C/75%RH) for 12 weeks (Figure 1).

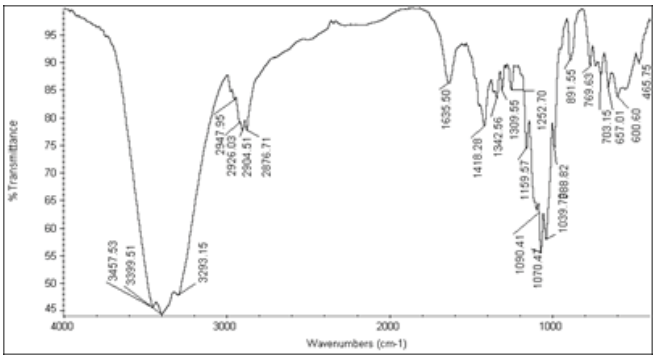
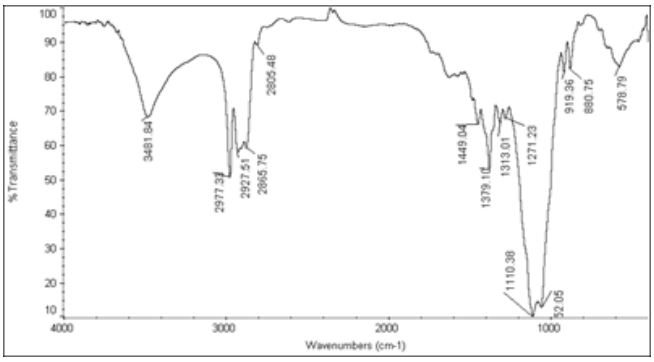
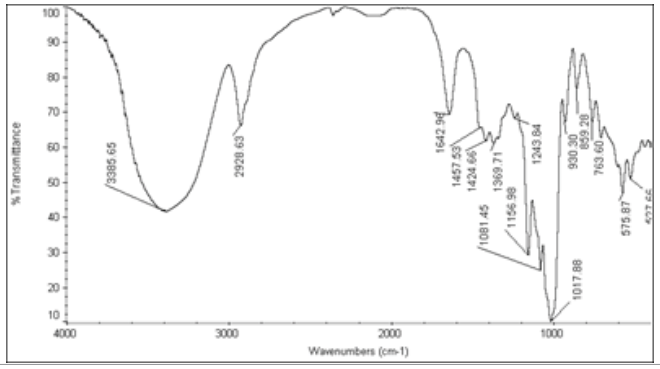
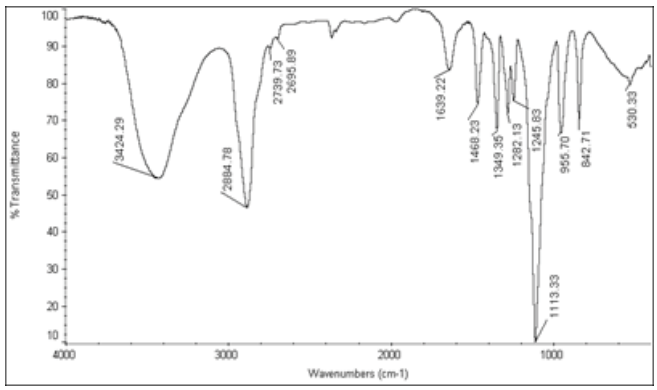
Formulations

Tablets were made by direct blending and compression process [4]. Formulation details are shown in (Table 5). All the lists of ingredients were weighed accurately (Except Lubricant) and passed through # 20 ASTM sieve. Sifted materials were blended for 10 min in a Double Cone Blender at 15 RPM. The blended materials were again sifted through #30 ASTM sieve. The sifted materials were again blended for 15 min in a Double Cone Blender at 15 RPM. The lubricant is sifted through # 60 ASTM sieve and added into the above blended materials, and blended for 5 min at 15 RPM. Compression was done in a Cadmach 16 station tablet press using 'D' type, 25.53 mm × 12.74 mm oval shaped flat-faced, bevel-edged punch tooling.

Table 1: Physical observation of drug-excipient compatibility study

Physical Observation					
Pack: Glass Vials					
Ingredient	Appearance				
	Title	D-E Ratio	Initial	55°C 2nd Week	40°C/75%RH 4th Week
Nystatin	A	1	Yellow to light tan powder	Yellow to light tan powder	Yellow to light tan powder
Nystatin+Ethocel 4 cps	B	1:25	Yellow to light tan powder	Yellow to light tan powder	Yellow to light tan powder
Nystatin+Starch 1500 LM	C	1:50	Yellow to light tan powder	Yellow to light tan powder	Yellow to light tan powder
Nystatin+Polyethylene Glycol 8000	D	1:25	Yellow to light tan powder	Yellow to light tan Semi solid mass	Yellow to light tan Semi solid mass
Nystatin+Cellactose 80	E	1:25	Yellow to light tan powder	Yellow to light tan powder	Yellow to light tan powder
Nystatin+Stellipress 1200 Poudre	F	1:1	Yellow to light tan powder	Yellow to light tan Semi solid mass	Yellow to light tan Semi solid mass

Table 2: FTIR interpretation graphs of 55°C-2nd week

S.No	Particulars	55°C - 2nd Week
1	Nystatin	
2	Nystatin+ Ethocel 4 cps	
3	Nystatin Starch 1500 LM	
4	Nystatin+Polyethylene glycol 8000	

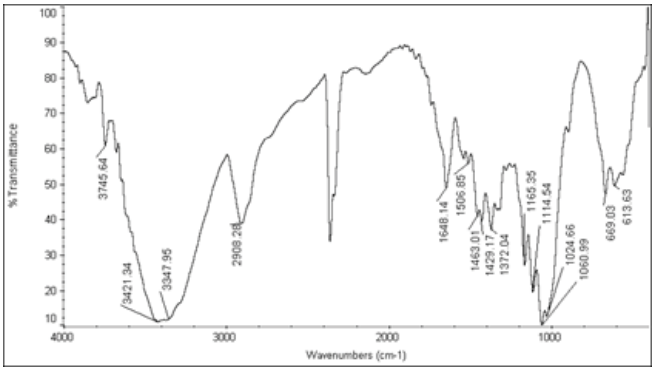
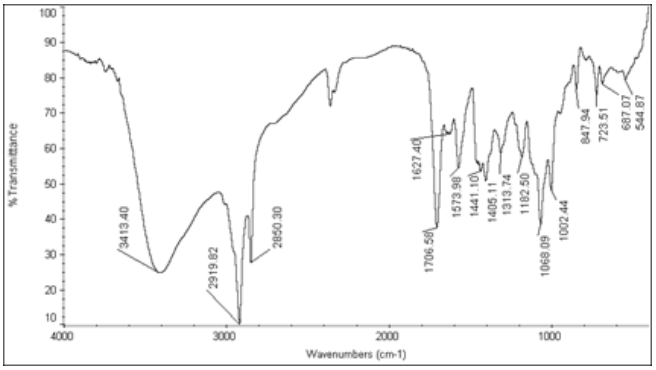
5	Nystatin+Cellactose 80	
6	Nystatin+Stellipress 1200 Poudre	

Table 3: Assay (% drug content) of drug-excipient compatibility study

Assay (% Drug Content)					
Pack: Glass Vials					
Ingredient	Appearance				
	Title	D-E Ratio	Initial	55°C 2nd Week	40°C/75%RH 4th Week
Nystatin	A	1	98.5	96.8	97.2
Nystatin+Ethocel 4 cps	B	1:25	99.2	95.1	96.3
Nystatin+Starch 1500 LM	C	1:50	98.5	97.9	98.1
Nystatin+Polyethylene Glycol 8000	D	1:25	99.5	93.1	94.3
Nystatin+Cellactose 80	E	1:25	99.5	96.2	97.4
Nystatin+Stellipress 1200 Poudre	F	1:1	98.7	92.8	96.8

Table 4: Moisture content (% w/w) of drug-excipient compatibility study

Moisture Content (% w/w)					
Pack: Glass Vials					
Ingredient	Appearance				
	Title	D-E Ratio	Initial	55°C 2nd Week	40°C/75%RH 4th Week
Nystatin	A	1	4.97	4.63	4.92
Nystatin+Ethocel 4 cps	B	1:25	3.95	2.97	3.24
Nystatin+Starch 1500 LM	C	1:50	8.57	6.98	8.27
Nystatin+Polyethylene Glycol 8000	D	1:25	3.12	2.57	3.07
Nystatin+Cellactose 80	E	1:25	4.26	3.56	4.09
Nystatin+Stellipress 1200 Poudre	F	1:1	3.12	2.25	2.98
Note: Moisture content by loss on drying at 105°C–15 min auto mode in IR moisture balance					

Evaluation

The tablets were evaluated for different physicochemical parameters such as appearance, weight variation, thickness, hardness, friability, drug content and *in vitro* release [10,11]. *In vitro* release was studied using Apparatus-II (Paddle)

Table 5: Formulation development and evaluation of nystatin vaginal tablet

Ingredients		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
		mg/tab				
Nystatin		15.58	15.58	15.58	15.58	15.580
Ethocel 4 cps		24.00	24.00	12.00	6.00	3.00
Starch 1500 LM		82.50	82.50	82.50	123.75	165.000
Polyethylene Glycol 8000		15.50	15.50	15.50	15.50	15.500
Cellactose 80		956.92	951.42	963.43	928.17	889.92
Stellipress 1200 Poudre		5.50	11.00	11.00	11.00	11.000
Total Tablet Weight		1100.00	1100.00	1100.00	1100.00	1100.000
Final Blend Characteristics						
Bulk Density (g/ml)		0.69	0.66	0.74	0.75	0.71
Tapped Density (g/ml)		1.01	0.99	0.98	1.02	1.03
Compressibility Index (%)		31.7	33.3	24.5	26.5	31.1
Hausner Ratio		1.46	1.50	1.32	1.36	1.45
Angle of Repose (°)		30	30	31	33	29
Loss On Drying (% w/w)		1.66	1.72	2.12	2.57	2.55
Blend Uniformity (% Assay) in Final blend (10 no's) (2.0 cc die used twice)		Avg: 96.20 Min: 91.20 Max: 101.20 %RSD: 5.20	Avg: 94.30 Min: 90.90 Max: 97.60 %RSD: 3.55	Avg: 99.20 Min: 97.20 Max: 101.20 %RSD: 2.02	Avg: 97.95 Min: 95.20 Max: 100.70 %RSD: 2.81	Avg: 98.05 Min: 95.80 Max: 100.30 %RSD: 2.29
Tablet Characteristics						
Weight (mg)		1089-1103	1102-1118	1095-1111	1093-1115	1098-1108
Thickness (mm)		3.72-3.83	3.71-3.80	3.68-3.76	3.77-3.83	3.74-3.80
Hardness (kP)		13.2-17.1	12.8-15.7	12.5-17.4	11.7-14.8	13.2-16.8
Friability (%)		0.23	0.35	0.29	0.42	0.19
Disintegration Time (mins)		15.10-18.40	16.20-17.45	11.32-13.11	8.12-9.41	3.45-5.12
pH of 1% Tablet Slurry		6.25	6.13	6.47	6.32	6.38
Loss On Drying (% w/w)		1.88	1.83	2.30	2.71	2.69
Remarks		Picking, Sticking and Striation Observed. DT to be reduced.	No Tablet defects observed. DT to be improved.	No Tablet defects observed. DT to be improved.	No Tablet defects observed. DT to be improved.	No Tablet defects observed. DT is satisfactory.
Uniformity of Dosage Units (% Assay) Composite Sample (10 no's)		Avg: 96.00 Min: 91.60 Max: 100.40 %RSD: 4.58	Avg: 95.35 Min: 93.80 Max: 96.90 %RSD: 1.63	Avg: 97.90 Min: 96.10 Max: 99.70 %RSD: 1.84	Avg: 98.10 Min: 95.90 Max: 100.30 %RSD: 2.24	Avg: 98.20 Min: 96.50 Max: 99.90 %RSD: 1.73
Dissolution Apparatus-II (Paddle), 50 RPM, 900 ml, pH 4.5 Acetate Buffer	**Time (min)**	**% Drug Dissolved (Mean of n=6 Units)**				
	10	4.8	5.0	13.0	18.3	22.3
	15	7.2	6.9	18.2	21.4	31.6
	30	9.1	9.2	20.7	26.3	36.3
	45	9.8	10.3	22.2	29.7	37.6
	60	12.0	11.3	24.0	33.1	43.5

dissolution test apparatus in pH 4.5 Acetate buffer for a period of 60 min. HPLC method was used as the method of analysis [11,12].

Stability studies

The finalized formulation (Trial 5) was loaded for stability in Alu-Alu blister packs at accelerated condition (40°C/75%RH for 12 weeks). The results are shown in Table 6; the stability results show the stability of Nystatin in Nystatin Vaginal Tablet. The results obtained for each tests is well within the prescribed and predefined in-house limits [7,9].

Anti-mycotic effectiveness study

Procedure

Test organism was spread on the test plates- Muller Hinton Agar (MHA). Sterile wells were made with the help of a borer with 30 mm diameter-with the test and reference samples. Samples were prepared in Stock with DMSO. The test plates were incubated for 48 h. The zone of inhibition (in mm diameter) were read and taken as the activity of the sample against the test organisms (Table 7). Strain-*Candida albicans* (MTCC 227) (Figures 2 and 3).

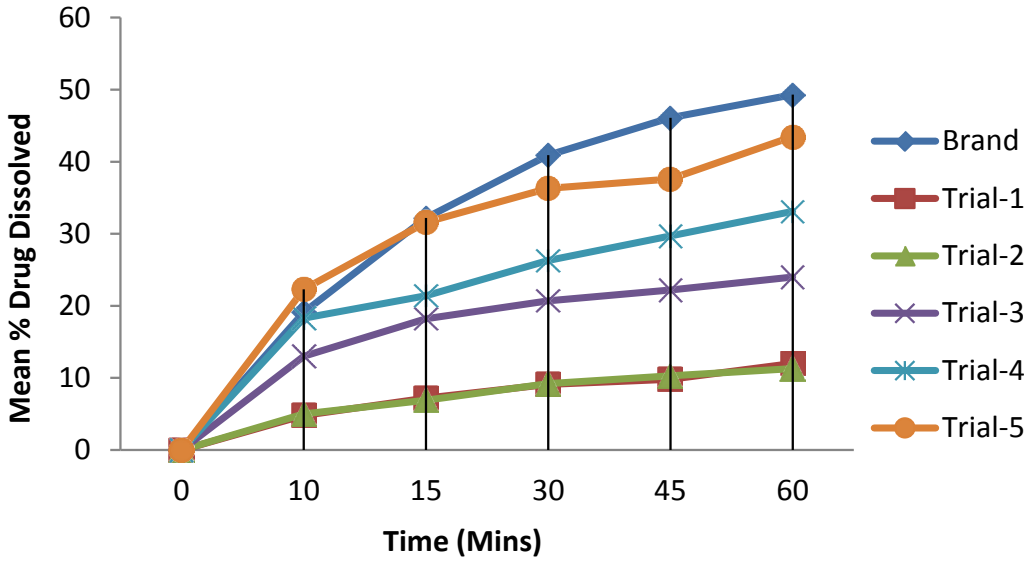


Figure 1: *In-vitro* dissolution profile of nystatin vaginal tablet

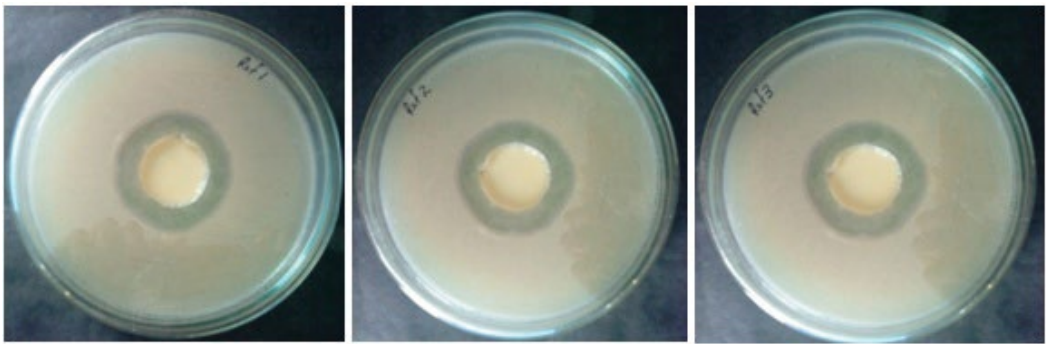


Figure 2: Reference sample treated *Candida albicans*

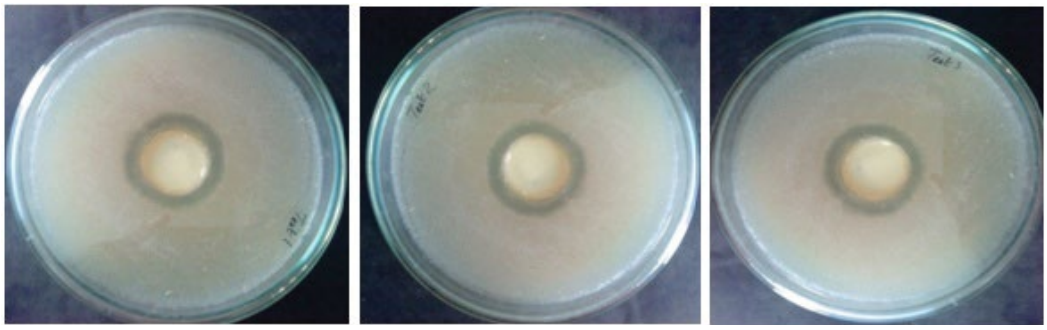
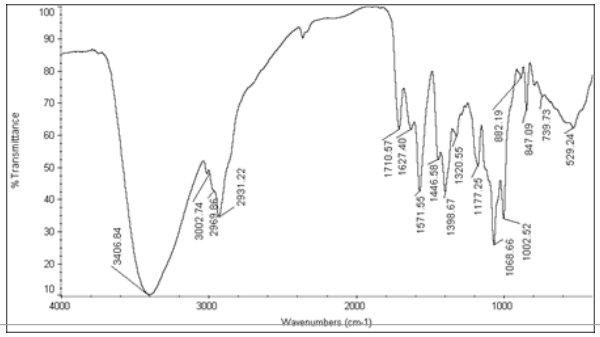
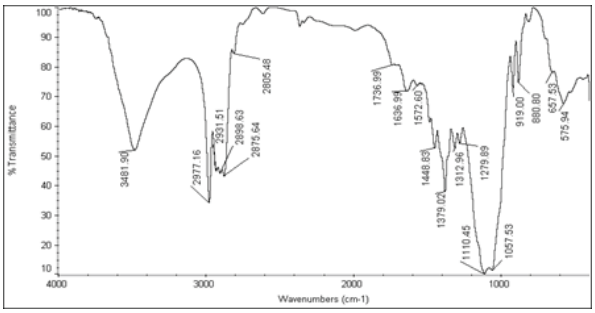
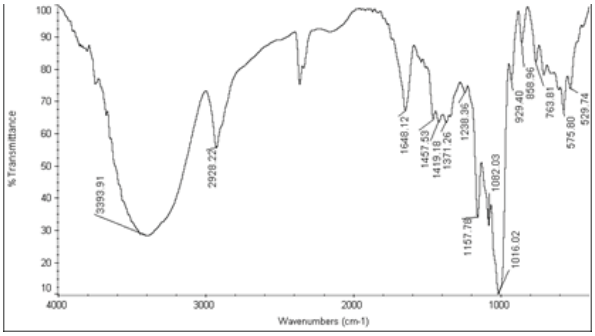
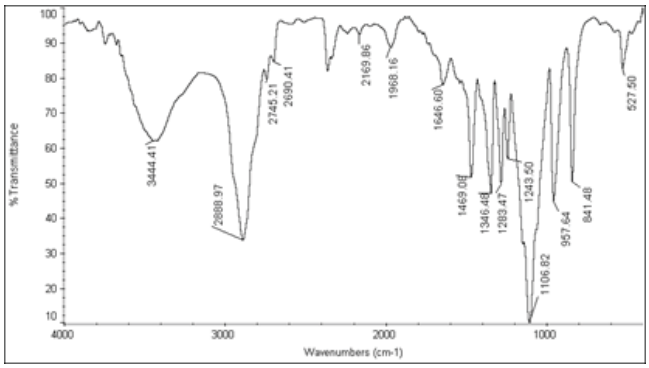


Figure 3: Test sample treated *Candida albicans*

RESULTS AND DISCUSSION

Drug Excipient compatibility data shown in Tables 3 and 4 suggests that both the temperature and moisture doesn't affect the stability of mixture indicating compatibility of drug with Excipients studied. All the five Trial blend flow is good. Drug distribution in the blend and in tablets is satisfactory [13,14]. Able to achieve hardness up to 16 kP to 17 kP. Tablet defects of trial-1 (Picking, Sticking and Striation) observed during compression and trial 2, 3, 4, 5 (Picking, Sticking and Striation) was not observed during compression. Disintegration time needs to be reduced and dissolution profile needs to be improved in Trial 1, 2, 3, 4. The Trial-5 Disintegration Time is similar to that of marketed product. Dissolution profile is comparable with that of marketed product (Table 8).

Table 6: FTIR interpretation graphs of 40°C/75% RH 4th week

S. No.	Particulars	40°C/75% RH 4th Week
1	Nystatin	
2	Nystatin+Ethocel 4 cps	
3	Nystatin+Starch 1500 LM	
4	Nystatin+Polyethylene glycol 8000	

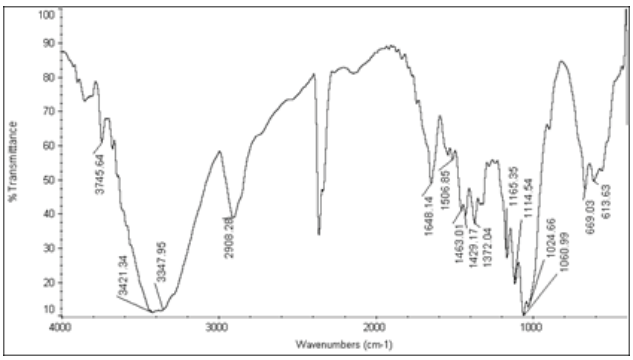
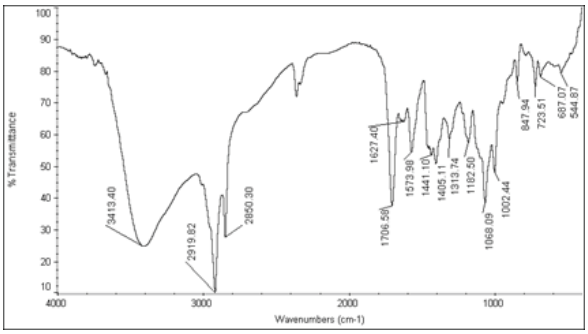
5	Nystatin+Cellactose 80	
6	Nystatin+Stellipress 1200 Poudre	

Table 7: Zone of inhibition for reference and test sample treated with *Candida albicans*

S. No.	Culture	Well (diameter–mm)	Sample Content (ml)	Test (zone–mm)	Reference (zone–mm)
1	*Candida albicans*	30	10	49	52
2	*Candida albicans*	30	10	49	52
3	*Candida albicans*	30	10	48	53
Average				49	52
% Activity of Test with respect to Reference (Avg T/ Avg R) × 100				**94%**	

Table 8: Stability studies of finalized formulation (Trial-5)

S. No.	Tests	Specification	Initial	40°C/75%RH	
				4th Week	12th Week
1	Description	Pale yellow mottled oval shaped, flat face bevel edged tablet	Complies	Complies	Complies
2	Assay (%)	NLT 90.0% to NMT 110.0% of labeled amount of Nystatin	98.2	96.4	94.9
3	Related Substances (%)	NLT 85.0% of Nystatin A1 is found. NMT 4.0% of any other individual compound.	Nys A1–92.8 UI–0.78	Nys A1–91.5 UI–1.12	Nys A1–90.9 UI–1.54
4	Loss On Drying (% w/w)	NMT 5.0%	2.69	2.51	2.57
5	Disintegration Time (min)	NMT 15 min	4 min 30 s	5 min 41 s	6 min 18 s
6	*Dissolution (% Drug Dissolved)	NLT 35% (Q) at the end of 60 min.	43.5 (S1)	44.1 (S1)	43.8 (S1)
*Apparatus-II (Paddle) 50 RPM 900 ml pH 4.5 Acetate Buffer Nys A1–Nystatin A1,UI–Unknown Impurity					

CONCLUSION

The marketed product of Nystatin Vaginal tablet (Manufactured by Duramed Inc., USA) was characterized for

Table 9: FT-IR interpretation drug-excipient compatibility study

FTIR Interpretation					
Pack: Glass Vials					
Ingredient	**Finger Print Comparisons**				
	Title	**D-E Ratio**	**Initial**	**55°C 2nd Week**	**40°C/75%RH 4th Week**
Nystatin	A	1	Typical Wave Numbers (cm^{-1}) of Nystatin includes, 3400, 2930, 1710, 1627, 1570, 1446, 1398, 1320, 1177, 1068, 1002, 847, 739 and 529		
Nystatin+Ethocel 4 cps	B	1:25	Complies	Complies	Complies
Nystatin+Starch 1500 LM	C	1:50	Complies	Complies	Complies
Nystatin+Polyethylene Glycol 8000	D	1:25	Complies	Complies	Complies
Nystatin+Cellactose 80	E	1:25	Complies	Complies	Complies
Nystatin+Stellipress 1200 Poudre	F	1:1	Complies	Complies	Complies

Dimension, Weight, Thickness, Hardness, Disintegration Time, Dissolution, pH of Tablet Slurry and Loss on Drying. A systematic Drug-Excipient compatibility study was done by packing the samples in Glass vials and exposed at 55°C–2 weeks and 40°C/75% RH–4 weeks and the exposed samples were characterized for Morphological Change (Physical Observation), Assay, Related Substances, Moisture Content and FTIR Finger Printing with respect to Initial samples (Table 9). This study was done foremost before taking up the prototype formulation activity [15,16]. Intra-vaginal tablet of Nystatin, 100,000 units was prepared by a simple direct blending manufacturing process which provided the necessary uniform distribution of drug in the blend for tableting and comparable disintegration time and dissolution profile to that of marketed product was achieved. Physical characteristics of the prepared vaginal tablet like Weight, Thickness, Hardness, Friability, Disintegration Time, Loss On Drying and pH of 1% Tablet Slurry were determined and were comparable to that of marketed product. Dissolution test was conducted for both test and reference products in Apparatus-II (Paddle), 50 RPM, 900 ml, pH 4.5 Acetate Buffer, Time Points: 10, 15, 30, 45 and 60 min; and the results were comparable, based on which an In-house single point dissolution limit was fixed as NLT 35% (Q) at the end of 60 min. The finalized Nystatin Vaginal Tablet were packed in Alu-Alu blister packs and charged for stability at accelerated condition (40°C/75%RH for 12 Weeks) and was characterized for Description, Assay, Related Substances, Loss On Drying, Disintegration Time and Dissolution. The results were comparable with the initial values showing the stability of Nystatin in prepared vaginal tablet. Anti-mycotic effectiveness of the prepared Nystatin Vaginal tablet against the marketed product (in triplicate) was done in the cultures of Candida albicans (Outsourced and monitored the activity in Biozone Research Technologies based at Chennai) The prepared Nystatin Vaginal Tablet showed 94% anti-mycotic activity with respect to the marked product.

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