

Simultaneous Spectrophotometric Estimation of Nystatin and Triamcinolone Acetonide in Topical Dosage Form.***¹Sanjeevani R. Desai, ²John I. D'souza.**¹JJT University, Jhunjhunu, Rajasthan, India, ²Dept. of Pharmaceutics, Tatyasaheb Kore College of Pharmacy, Warananagar, Kolhapur, MS. India.**Abstract**

The simple, accurate, precise and economic spectrophotometric method in UV/VIS region has been developed for the determination of Nystatin and Triamcinolone Acetonide in Topical formulation. Nystatin and Triamcinolone Acetonide are widely aimed for treatment of fungal infection. The wavelengths selected for Simultaneous equation method were 304 nm and 240 nm i.e. the respective λ_{\max} of both the drugs. This method was applied to the assay of the drug in marketed formulation, which were found in the range of 97 - 98 % of the labeled value for both Nystatin and Triamcinolone acetonide. The method was validated for linearity, accuracy and precision. Hence, the method herein described can be successfully applied in quality control of combined pharmaceutical dosage form.

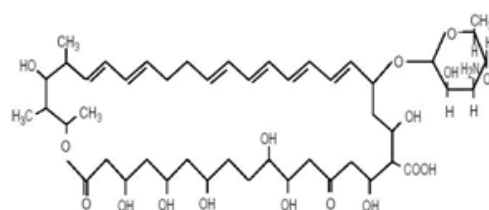
Key Words

Simultaneous equation method, Wavelength, Nystatin, Triamcinolone Acetonide.

Introduction

Analytical branch gives the selectivity of the drug. It involves the more sensitive, simple and specific data for the bulk drug powders and its dosage form. It is easy for the detection of sample purity and standardization with accurate result for pharmaceutical uses¹. We have selected Nystatin and Triamcinolone Acetonide for present study. Nystatin is Official in BP. Nystatin is a polyene antimycotic obtained from *Streptomyces noursei*². Due to its toxicity profile, there are currently no injectable formulations of this drug on the US market³. It is a yellow to light tan powder with a cereal-like odor,

very slightly soluble in water, and slightly too sparingly soluble in alcohol. It has a molecular Formula of $C_{47}H_{75}NO_{17}$ and a molecular weight of 926.13.

**Fig 1:** Structure of Nystatin.

Triamcinolone acetonide is designated chemically as Pregna-1, 4-diene-3, 20-dione, 9-fluoro-11, 21-dihydroxy-16, 17-[(1-methylethylidene) bis (oxy)]-, (11 β , 16 α -). It is official in USP. The white to cream crystalline powder has a slight odor, is practically insoluble in water, and very soluble in alcohol.

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It has a molecular formula of $C_{24}H_{31}FO_6$ and a molecular weight of 434.50⁴.

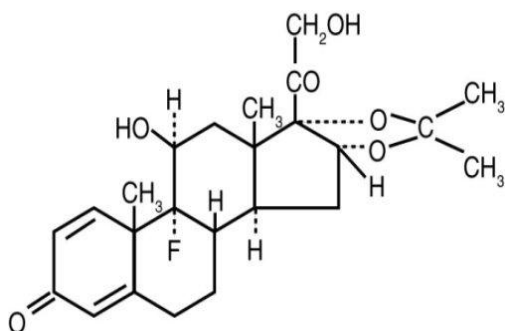


Fig 2: Structure of Triamcinolone Acetonide

A detailed survey literature was carried out and revealed that several methods based on varied techniques, viz. High Performance Liquid Chromatography, High Performance Thin Layer Chromatography and Spectrophotometry were available individually for both Nystatin and Triamcinolone Acetonide⁵⁻⁷. No method is available for the simultaneous estimation of these drugs by UV spectrophotometric method. So, a successful attempt has been made to estimate the two drugs simultaneously by UV spectrophotometric analysis. Paper describes simple, rapid, accurate, reproducible and economical methods for the simultaneous determination of Nystatin and Triamcinolone Acetonide in topical formulations using simultaneous equation method.

Materials and Methods

A Shimadzu UV/ Visible spectrophotometer, model 1700 was employed with 1cm matched quartz cells were used for all the special measurements. A Shimadzu electronic

analytical balance was used for weighing the sample.

Reagent and Chemicals

Analytical pure samples of Nystatin and Triamcinolone Acetonide (Cipla Pvt. Ltd., Mumbai) were used in the study. All the chemicals used were of A.R. Grade.

Preparation of Standard Stock Solution

The Stock solution 1000 $\mu\text{g/mL}$ of Nystatin and Triamcinolone Acetonide were prepared separately by dissolving 10 mg drug in 10 mL Ethanol.

Preparation of Calibration Curves

Solutions of 10 $\mu\text{g/mL}$ of Nystatin and Triamcinolone Acetonide were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of Nystatin and Triamcinolone Acetonide was observed at 304 nm and 240 nm respectively. Nystatin and Triamcinolone Acetonide showed linearity in the concentration range of 2- 10 $\mu\text{g/mL}$ and 5 – 30 $\mu\text{g/mL}$ at their respective maxima as shown in fig 4 & 5. The coefficient of correlation was found to be 0.998 for Nystatin and 0.976 for Triamcinolone Acetonide. The results are shown in Table 1.

Simultaneous Equation Method

Standard solution of 10 $\mu\text{g/mL}$ of Nystatin and Triamcinolone Acetonide were scanned in the wavelength range of 200-400 nm as shown in fig. 3. Wavelengths of Nystatin 304nm, wavelength of Triamcinolone Acetonide 240 nm were selected for formulation of the

simultaneous equation method. The absorptivities ($A_1\%$, 1 cm) of both the drugs at the wavelengths were determined. The absorbance and absorptivities values at the particular wavelength were substituted in the following to obtain the concentration^{8, 9}. Concentrations in the sample were obtained by using following equations.

$$C_x = \frac{(A_1 a_{y2} - A_2 a_{y1})}{(A_{x1} a_{y2} - a_{x2} a_{y1})} \text{-----Eq. (i)}$$

$$C_y = \frac{(A_1 a_{x2} - A_2 a_{x1})}{(a_{y1} a_{x2} - a_{y2} a_{x1})} \text{-----Eq. (ii)}$$

Where, A_1 and A_2 are absorbances of mixture at 304 nm and 240 nm respectively, a_{x1} and a_{x2} are absorptivities of Nystatin at λ_1 and λ_2 respectively and a_{y1} and a_{y2} are absorptivities of Triamcinolone Acetonide at λ_1 and λ_2 respectively. C_x and C_y are concentrations of Nystatin and Triamcinolone Acetonide respectively.

Validation

The method was validated according to ICH Q2 B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte^{10, 11}.

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (50%, 80% and 100%). Percent recovery for Nystatin and Triamcinolone Acetonide, by both the methods, was found in the range of 97% to 99 %.

Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of Nystatin and Triamcinolone Acetonide. For simultaneous equation method the Beer- Lambert's concentration range was found to be 2 – 10 $\mu\text{g/mL}$ for Nystatin and 5 – 30 $\mu\text{g/mL}$ for Triamcinolone Acetonide.

Precision

Precision was studied to find out intra and inter-day variations in the test method of Nystatin and Triamcinolone Acetonide. Calibration curves prepared in medium were run in triplicate in same day and for three days. The results are tabulated in Table 2.

Results and Discussion

The method was developed under the experimental conditions described. The assay of Topical formulation and recovery studies was performed. The developed method was validated as per ICH guidelines for linearity, repeatability, intermediate precision (interday and intra-day precision studies) as shown in Table 2. The mean % drug content of formulation by the developed methods was 97.5 % and 98 % respectively. The mean % recoveries of Nystatin and Triamcinolone Acetonide were found to be 98.99% and 97.21% respectively.

Conclusion

Nystatin and Triamcinolone Acetonide are available in combined pharmaceutical dosage form for the treatment of fungal infection. Here, simple UV spectrophotometric Simultaneous equation method was

developed for their simultaneous analysis. The standard deviation calculated for the methods is low, indicating high degree of precision of the methods. The RSD is also less than 2% as required by ICH guidelines. The developed methods are simple, rapid, precise, accurate and can be employed for the routine estimation of Nystatin and Triamcinolone Acetonide in Formulation

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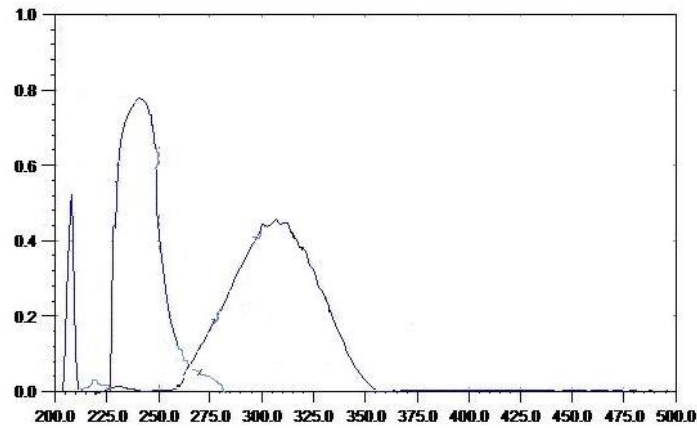


Fig. 3: Overlaid spectra of Triamcinolone Acetonide and Nystatin.

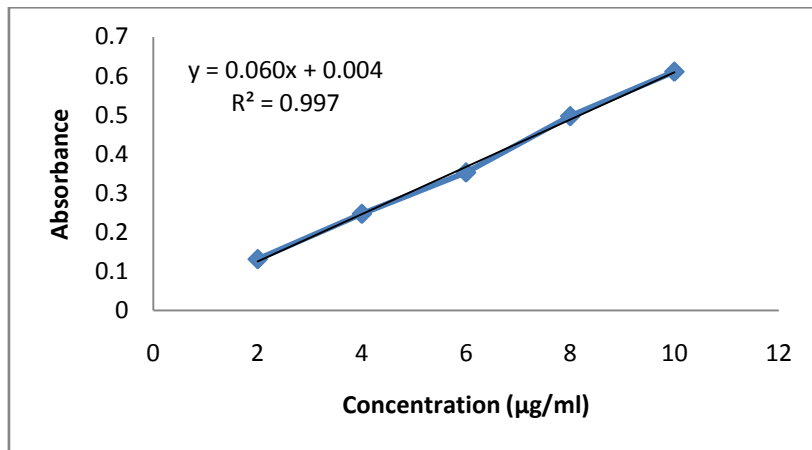


Fig. 4: Regressed standard curve of Nystatin at λ_{max} 304 nm.

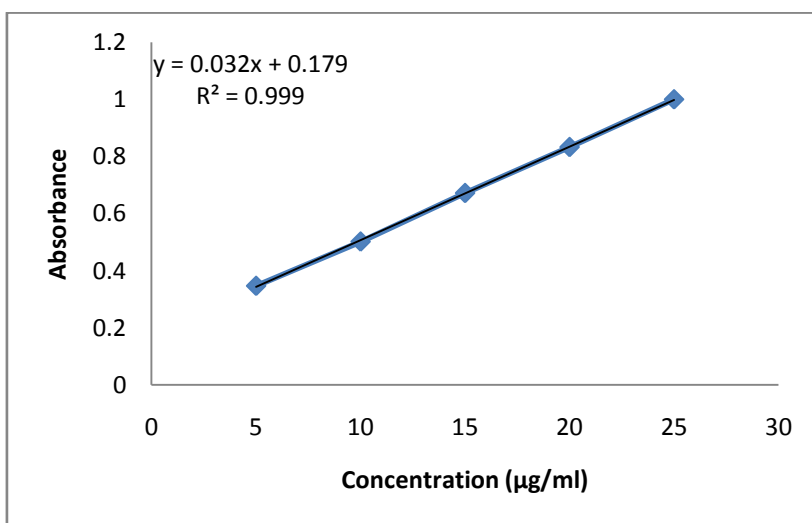


Fig. 5: Regressed standard curve of Triamcinolone Acetonide at λ_{max} 240nm.

Table 1: Simultaneous Equation data of Nystatin and Triamcinolone acetoneide.

Nystatin									
Sr. No.	Conc. (µg/ml)	Absorb.	304 nm			Absorb.	240 nm		
1.	0	0	Slope 0.060	Intercept 0.004	R ² 0.997	0.00	Slope 0.014	Intercept 0.054	R ² 0.959
2.	2	0.131				0.172			
3.	4	0.247				0.225			
4.	6	0.353				0.279			
5.	8	0.497				0.362			
6.	10	0.611				0.396			
Triamcinolone Acetoneide									
Sr. No.	Conc (µg/ml)	Absorb.	304nm			Absorb.	240nm		
1.	0	0.00	Slope 0.001	Intercept 0.028	R ² 0.973	0.00	Slope 0.032	Intercept 0.179	R ² 0.999
2.	5	0.04				0.347			
3.	10	0.043				0.502			
4.	15	0.051				0.672			
5.	20	0.059				0.833			
6.	25	0.068				1.000			

Table 2: Validation of the proposed method.

Parameters	Nystatin	Triamcinolone Acetoneide
Linearity Range (µg/ml)	2 – 10 µg/ml	5 – 25 µg/ml
Accuracy (% Recovery)	98.99	97.21
Precision (% Obtained ±S.D.)*		
Interday	99.08± 0.389	99.08 ± 0.438
Intraday	98.91± 0.529	99.97± 0.699

*Indicates the ±Standard deviation (S.D.) value of the % estimation at different conditions where n=3.

Table 3: Results of Commercial Sample Analysis.

Pharmaceutical Formulation	labeled amount tablet (mg)	Amount found (mg)	% Drug Obtained	%RSD*
Nystatin	1,00,000 unit	24.37 mg	97.5%	0.458
TA	1mg	0.98 mg	98%	1.024

*Indicates RSD = Relative Standard deviation where n = 3

Conflict of Interest: Not declared
