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Research Article

VALIDATED HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF BETAMETHASONE VALERATE AND NEOMYCIN SULPHATE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise, accurate, and reliable HPTLC method has been developed and validated for the analysis of BETA- Betamethasone Valerate and NEO- Neomycin Sulphate in their combined dosage form. Separation and analysis were performed on precoated silica gel 60 F_{254} plate having thickness of layer 0.2 mm , which were then eluted with n- propanol:ammonia (7:3) (v/v) .Calibration plots were established showing the dependence of response (peak area) on the amount chromatographed. The validated calibration ranges were 200–600 ng/spot and 1000-30000 ng/spot for BETA and NEO respectively with correlation coefficient (R^2) 0.994 and 0.991, respectively. Average % recovery was between 98.65–100.3% and 97.19–100% for BETA and NEO, respectively and give Rf 0.82 and 0.05 For BETA and NEO respectively. The spots were scanned at 245 nm for BETA and the Ninhydrin(post derivatizing reagent) reagent sprayed on plate for visualization of NEO and plate scan at 540nm. The proposed method was validated as per ICH guidelines and successfully applied to the estimation of BETA and NEO in their combined semisolid dosage form.

KEY WORDS

Betamethasone Valerate, Neomycin Sulphate, HPTLC, post derivatization, Pharmaceutical Dosage form, simultaneous Estimation

INTRODUCTION

BETA is chemically 9α–fluoro-11β,17α,21-trihydroxy- 16β-methyl-pregna-1,4-diene-3,20-dione-17-valerate figure 1. [1] BETA is a type of medicine called a topical corticosteroid. It is used for relief of the inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses also having Immunosuppresive activity.[2,3] BETA is official in Indian pharmacopoeia , British pharmacopoeia and United state pharmacopoeia. [1,4,5]

Chemically NEO is (2R,3S,4R,5R,6R)-5-amino-2-(aminomethyl)-6-{[(1R,2R,3S,4R,6S)-4,6-diamino-2-{[(2S,3R,4S,5R)-4-{[(2R,3R,4R,5S,6S)-3-amino-6-(aminomethyl)-4,dihydroxyoxan-2-yl]oxy}-3-hydroxy-5-(hydroxymethyl)oxolan-2-yl]oxy}-3-hydroxycyclohexyl]oxy}oxane-3,4-diol Figure 2. [6] NEO is an Amino glycoside antibiotic and used to treat infections with bacteria. Its having topical application to the skin and eye.[2,3] NEO is official in Indian pharmacopoeia ,

British pharmacopoeia. [1,4,] NEO having Chromophore group so, it require derivatizing reagent. So, $CuCl_2.2H2O$ used to Derivatized NEO.

Literature survey for BETA reveals UV-Visible spectrophotometry[7] , High Performance Liquid Chromatographic (HPLC) [8,9] , RP-HPLC [10] , Stability indicating HPLC [11] , Stability indicating RP-LC[12] and HPTLC[13,14,15] have been reported for single form and in combination with other drugs.

Literature survey for NEO reveals Derivative spectrophotometry[16] , HPLC[17] , RP-LC [18] ,RP-HPLC[19] , Normal Phase High Performance Liquid Chromatographic (NP-HPLC) [20] ,Capillary electrophoresis[21] ,Flow Injection Chemiluminescence [22] and TLC [23,24] have been reported for single form and in combination with other drugs.

Yet from literature review we can reveled that , No, method has been reported for simultaneous estimation of both drug by HPTLC method. This paper describes a simple, accurate, sensitive and validated HPTLC method for simultaneous quantification of these compounds in cream combined dosage forms. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines

MATERIALS AND METHODS

Reagents and Chemicals:

Pure Standard gift sample of Betamethasone Valerate(BETA) and Neomycin Sulphate (NEO) provided by Zymethwelness,Ahemdabad and Iniana Opthalmics, Wadhvan. cream of BETNOVATE-n (BETA- 0.1% W/W,NEO- 0.5% W/W) were purchased from local market. Analytical grade methanol and HPLC water were purchased from Merck chemicals, Mumbai, India. Ninhydrin post derivatizing reagent.

Preparation of solutions

Stock -1 Preparation of Standard Stock BETA:

Accurately weighed quantity of BETA (10 mg) was transferred into 10 ml volumetric flask, dissolved and diluted up to mark with Methanol:Water(50:50). (1000 µg/ml)

Stock-2 Preparation of Standard Stock Solution of NEO :

Accurately weighed quantity of NEO (50 mg) was transferred into 10 ml volumetric flask, dissolved and diluted up to mark with Methanol:Water(50:50). (5000 µg/ml)

Stock-3 Preparation of Combined Working Standard Solution Containing BETA and NEO in Ratio of 1:5

Accurately weighed 10 mg BETA and 50 mg NEO were transferred into 10 ml volumetric flask, dissolved in sufficient amount of Methanol: water and diluted up to mark with Methanol: Water to give concentration of 1000 µg/ml of BETA and 5000 µg/ml of NEO. From this solution 1ml was taken and further diluted to 10 ml with Methanol: Water(50:50) to get 100 µg/ml of BETA and 500 µg/ml of NEO.

Stock-4 Preparation of Sample solution:

1gm of drug dissolved in 10 ml of mixture of methanol:water in 10ml volumetric flask , filtered the solution . from this solution withdrawn 1ml of solution and diluted with mixture of methanol:water(50:50) in 10ml volumetric flask which give 10 and 50 µg/ml of concentration. Then above solution applied 5 µl (500 and 2500 ng/spot for ,BETA and NEO, respectively) was applied on HPTLC plate that was developed in Optimized mobile phase.

Instrumentation and Conditions:

Chromatography was performed on 20.0 x 10.0 cm on precoated silica gel 60 F_{254} aluminium sheet (E. Merck, Mumbai, India). Samples were applied as 8 mm bands by means of a Camag Linomat 5 semi-automatic(Muttenz, Switzerland) sample applicator equipped with 100 µL syringe; operated with settings of band length, 8 mm; distance between bands, 13.3 mm. The constant application rate was 15 s µL, and a nitrogen aspirator was used. Ascending development of plate, migration distance

80 mm was performed at ambient temperature with n-propanol :ammonia(7:3)(v/v) mobile phase in a 20 cm × 20 cm Camag twin-trough chamber previously saturated for 15 min. After development the plates were dried with hot-hair dryer and viewed in a CAMAG UV cabinet. Densitometric scanning at 245 nm for BETA and Neo was scan at 540nm after spraying plate with ninhydrin reagent,then performed with a Camag TLC Scanner 4 equipped with winCATs software. The scanning rate was 20 mm s. The source of radiation used was the deuterium lamp.

HPTLC method and chromatographic Conditions

The HPTLC procedure was optimized to develop a simultaneous assay method for BETA and NEO. The mixed standard stock solution (0.1 mg/ml BETA, and 0.5 mg/ml NEO) was spotted onto HPTLC plate and developed in different mobile phase, Chloroform:Methanol:Water(18:5:0.5), Ethanol:Toluene:Chloroform:Glacial acetic acid(3:10:7:0.2), Chloroform:acetone:Glacial acetic acid(17:2:15), Methanol:ammonia(4:6), Methanol:ammonia:acetone:Chloroform(8.75: 5:5:1.25), N-Propanol:Ammonia(7:3). Development of a Simultaneous assay method for BETA and NEO was very critical because the sensitivity of NEO is very less to UV detection while Initially, different mobile phase were tried such as a toluene-Chloroform: Methanol:Water (18:5:0.5)was tried, but in this system BETA moved and but NEO did not moved. scanning, it require derivatization by using derivatizing reagent. Hence, in order to move NEO , Methanol:ammonia(4:6), But effective separation was not observed. Finally, a mobile phase consisting of n-Propanol:ammonia(7:3) was found to be optimum. After chamber saturation 15 min, the plates were developed to a distance of 80 mm and then dried in hot air. Densitometric analysis was carried out using a camag TLC Scanner 4 (camag) in the absorbance mode at 245 nm for BETA and 540nm for NEO. The slit dimension was kept at 6.00 x 0.45 mm, and a scanning speed of 20 mm/s was employed. The chromatograms were integrated using wincats evaluation software .

Analysis of a Marketed Formulation

To determine the content of BETA and NEO in a pharmaceutical Cream formulation (brand name: BETNOVATE-n); label claim: 0.1g w/w BETA, and 0.5gm w/w NEO). 1gm of drug dissolved in 10 ml of mixture of methanol:water(50:50) in 10ml volumetric flask , filtered the solution . from this solution withdrawn 1ml of solution and diluted with mixture of methanol:water in 10ml volumetric flask which give 10 and 50 µg/ml of concentration. Then above solution applied 5 µl (500 and 2500 ng/spot for ,BETA and NEO, respectively) was applied on HPTLC plate that was developed in Optimized mobile phase. The analysis was repeated in triplicate.figure no.3,4,5,6

Validation of the Method

Validation of the optimized HPTLC method carried out with respect to the following parameters:

Linearity

Calibration graphs were constructed by plotting peak areas versus concentrations of BETA and NEO, and the regression equations were calculated. The calibration graphs were plotted over 5 different concentrations in the range of 200–600 ng/spot for BETA and 1000–3000 ng/spot for NEO by applying different volumes stock solution containing BETA and NEO (100 µg/ml of BETA and 500 µg/ml of NEO). The calibration graphs were developed by plotting peak area versus concentrations with the help of the winCATS software. Figure 7,8.

Accuracy

Accuracy was measured by applying the method to preanalyzed drug sample (BETA,

and NEO combination cream) to which known amounts of BETA and NEO standard powder corresponding to 80, 100, and120% of the label claim had been added (standard addition method). The drug sample and spike were mixed, and the powder was extracted and analyzed by the optimized method.

Precision

Precisions of the proposed HPTLC methods were determined by analyzing mixed standard solution of BETA and NEO at concentrations (500 ng/spot for BETA and 3000ng/spot for NEO) 3 times in the same day and in 2 different days. The results are reported in terms of %RSD.

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for BETA and NEO in sample was confirmed by comparing the Rf and spectra of the spots with that of standards. The peak purity of BETA and NEO were assessed by comparing the spectra at three different levels, i.e. peak start, peak apex and peak end positions of the spot.

Limit of Detection and Quantitation

The limits of detection (LOD) and quantification (LOQ) were calculated from the slope (s) of the calibration plot and the standard deviation of the response (SD).

RESULTS AND DISCUSSION

The results of validation studies on the simultaneous HPTLC determination method developed for BETA and NEO with n-Propanol:Ammonia (7:3, v/v) as the mobile phase are given below.

Linearity

The drug response was linear over the concentration range between 200-600 ng/spot for BETA and 1000-3000 ng/spot for NEO in Table 1.

Recovery studies

As shown from the data in table good recoveries of the BETA and NEO in the range from 97 to 100%were obtained for the various added concentrations. Table 2

Precision

The results of the Interday and Intraday precision experiments are shown in table. The developed method was found to be precise, with RSD values for Interday and Intraday precision studies below 2% as recommended by ICH guidelines. Table 3

Specificity

Excipients and Diluents used in the specificity studies did not interfere with the estimation of either of the drugs by the proposed methods. Hence, the methods were found to be specific for estimation of BETA and NEO.

Assay of the Dosage Form

(BETA 100 mg and NEO 500 mg) The proposed validated method was successfully applied to determine BETA and NEO in their dosage form (BETNOVATE-n). The results obtained for BETA and NEO was comparable with the corresponding labelled amounts (Table)

Parameter	Betamethasone valerate	Neomycin Sulphate
Linearity range ng/spot	200-600	1000-3000
r2	0.994	0.991
Slope	5.883	0.419
Intercept	1855	243.3

n = 5, r^2 -coefficient of correlation

Table 1. Linear regression data for calibration plots

Drug	Amount added %	Amount of sample drug taken (ng/spot)	Amount of standard drug added (ng/spot)	Amount recovered(ng/spot)±%	Recovery %
BETA	80	200	240	218.8± 0.0009	97.19
	100	200	300	286.1 ± 0.00189	99.12
	120	200	360	307.4 ± 0.00160	100
NEO	80	1000	1200	873.1 ± 0.0030	99.89
	100	1000	1500	1010.3 ± 0.00217	100
	120	1000	1800	1038 ± 0.0006	99.2

Table 2.Recovery studies

Drug(ng/spot)	Interday (Peak area)	%RSD	Intraday (Peak area)	%RSD
	4765.2	0.020	4765.5	0.100
BETA(500)	4765.1		4770.1	
	4763.5		4770	
	1211.7	1.422	1246.1	1.284
NEO(2500)	1181		1215.6	
	1209.3		1238.3	

Table 3. Precision studies

Sample	Label Claim(mg)	% Assay
BETA	100mg	98.93
NEO	500mg	102.2

Table 4. Applicability of the HPTLC method for the analysis of the pharmaceutical formulations

Parameter		BETA	NEO
Linearity Range(ng/spot)		200-600	1000-3000
Correlation Coefficient		0.994	0.991
Limit Of Detection (ng/spot)		100	1000
Limit Of quantification (ng/spot)		300	3000
%Recovery(n=3)	**80%**	98.65	99.75
	100%	100	99.02
	120%	100.3	98.68
Precision (n=3),(%RSD)	**Interday**	0.020	0.100
	Intraday	1.422	1.284

Table 5. The data of summary of validation parameters are listed

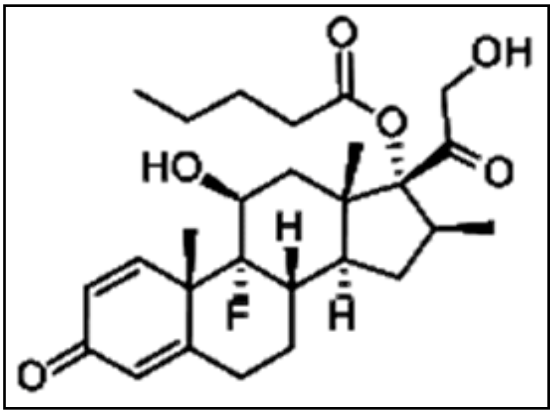

Figure 1. Structure of BETA

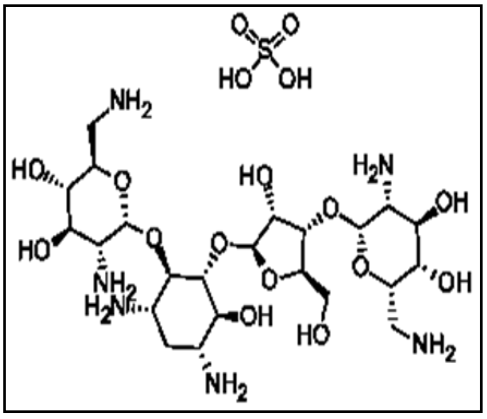

Figure 2: Structure of NEO

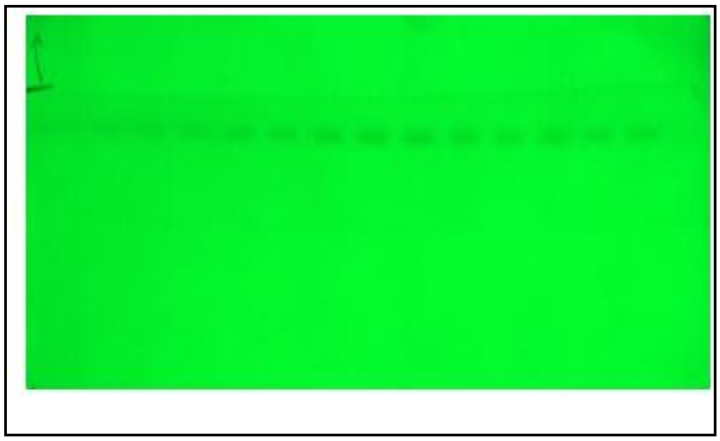

Figure 3.UV image of developed Chromatogram BETA

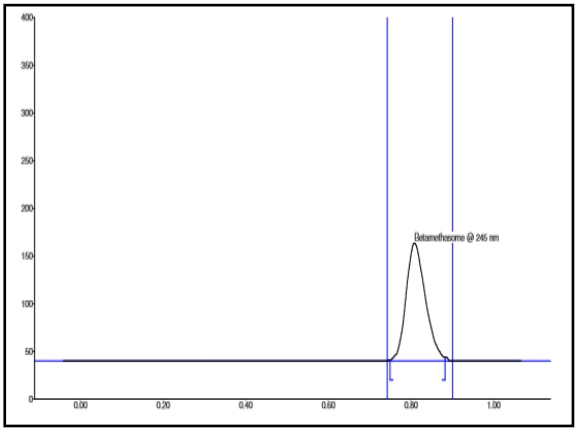

Figure 4:: HPTLC Chromatogram of Betamethasone Valerate Scan at 245nm

Figure 5:UV image of developed Chromatogram NEO

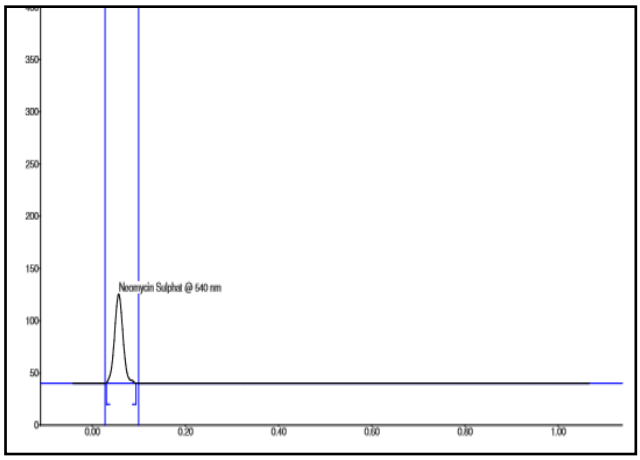

Figure 6: HPTLC Chromatogram of Neomycin Sulphate scan at 540nm after spreading of dipping agent show dark pink against pink background

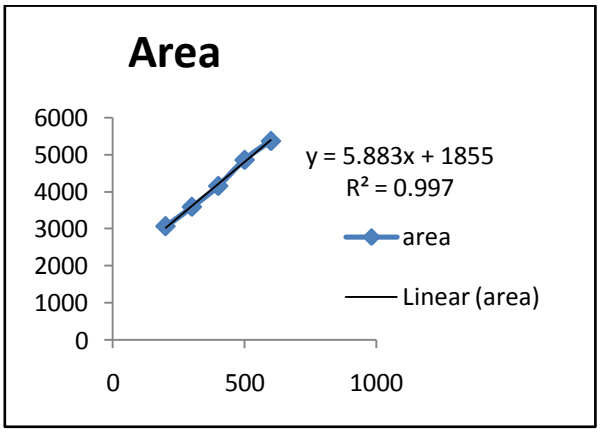

Figure 7:Calibration curve for BETA

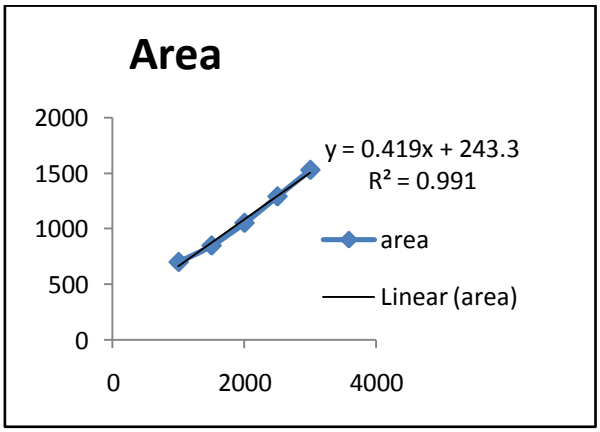

figure 8:Calibration curve for NEO

CONCLUSION

Thus, the objective of project work was development and comparison of analytical method of BETA and NEO in their combined dosage form. The developed and validated HPTLC method for BETA and NEO was found to be simple, specific, and cost effective and can be routinely applied for analysis of BETA and NEO in their combined dosage form. We can say that HPTLC method is more sensitive giving precise results (interday, intraday) for both drugs, and also HPTLC method is more sensitive in terms of LOD and LOQ. It also requires least solvents for analysis. The proposed method has the advantages of simplicity and convenience for the separation and quantitation of BETA and NEO in combination and can be used for the assay of their dosage form. Also, the low solvent consumption and short analytical run time lead to environmentally friendly chromatographic procedures. The additives usually present in the pharmaceutical formulations of the assayed analytes did not interfere with determination of BETA and NEO. The method can be used for the routine simultaneous analysis of BETA and NEO in pharmaceutical preparation.

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