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First derivative UV–Spectrophotometric Method for the simultaneous estimation of Neomycine and Betamethasone in their combined dosage form

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Abstract:

A simple, rapid, accurate, economical UV–Visible First order derivative Spectrophotometric method was developed and validated for simultaneous estimation of Neomycin and Betamethasone in bulk and dosage form. The method involved determination of Neomycin and Betamethasone using first derivative spectrophotometric method at 224nm and 234nm respectively over the concentration range of 2.5 – 15µg/ml and 0.6 – 3.6µg/ml with the Correlation Coefficien(r^2) 0.9997 and 0.9991. The recovery studies confirmed accurace of proposed method and low value standardeviation confirmed precession of method. The method is validated as per ICH guidelines.

Key words: Neomycin, Betamethasone, Derivative Spectrophotomeric.

INTRODUCTION

Neomycin(NMC) is an aminoglycosideantibiotic found in many topical medications such as creams, ointments, and eyedrops. It is drug used to works by stopping the growth of bacteria in the intestines. chemically it is known as (2*RS*,3*S*,4*S*,5*R*)-5-amino-2-(aminomethyl)-6-((2*R*,3*S*,4*R*,5*S*)-5-((1*R*,2*R*,5*R*,6*R*)-3,5-diamino-2-((2*R*,3*S*,4*R*,5*S*)-3-amino-6-(aminomethyl)-4,5-dihydroxytetrahydro-2*H*-pyran-2-yloxy)-6-hydroxycyclo hexyloxy)-4-hydroxy-2-(hydroxymethyl) tetrahydrofuran-3-yloxy)tetrahydro-2*H*-pyran-3,4-diol (fig.1).

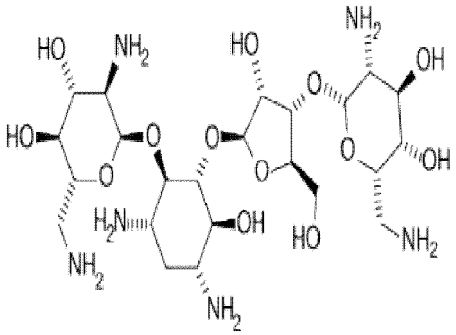

Fig. 1 Chemical structure of Neomycin

Betamethasone (BMS) is a steroid medication, It is used for a number of diseases including rheumatic disorders such as rheumatoid arthritis and systemic lupus erythematosus, skin diseases such as dermatitis and psoriasis, allergic conditions such as asthma. Chemically its is known as (8S,9R,10S,11S,13S,14S,16S, 17R)-9-fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl- 6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro- 3H-cyclopenta[a] phenanthren-3-one (fig. 2).

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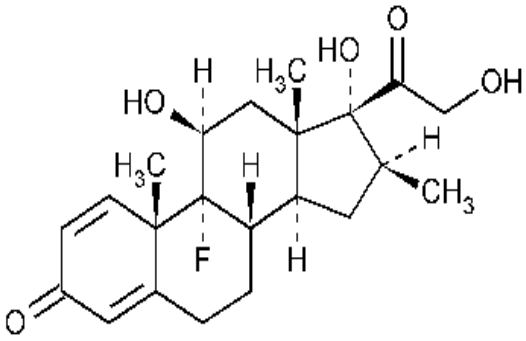

Fig. 1 Chemical structure of Betamethasone

The literature survey reveled that many analytical methods like Electrophoresis [1], Colorimetric [2], kinetic spectro-photometric[3], derivative Specro- photmetric [4], TLC–Densitometric [5] and HPLC [6–9] were reported for estimation of Neomycin and Densitometric method [10], spectrophotometric methods [11-16], HPLC methods [17-25] and LC-MS methods [26-30] were reported for Betamethasone.

Materials and Methods:

Instrumentation:

Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Standard cuvettes of 10mm path length are used for analysis. Sonicator (1.3L) Ultrasonicator was used to sonicating the standard and formulation sample. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234).

Diluent Used: Methanol and Water in the ratio of 6:4 (v/v).

Preparation of standard drug solution:

10mg of standard drug Neomycin and Betamethasonewas accurately weighed separately and dissolved in 5ml diluent then transferred to a 10ml volumetric flask sonicate it for 5min, finally volume was made up to the mark with same solvent to make 1000µg/ml stock solution. From this 1ml was again diluted to 10ml to get a concentration of 100µg/ml solution of Neomycin and Betamethasonewere obtained separately. From the solution, required concentration were prepared separately, then 1ml from each of the solution was mixed to obtained a combined solution for the simultaneous estimation of Neomycin and Betamethasone.

Preparation of working sample solution:

3 formulation ointments from different batch numbers of Betamethasone and Neomycin [Bonivit-N; Betamethasone – 0.12% (w/w) and Neomycin – 0.5% (w/w)] were mixed. From the ointment, an amount equivalent to 1mg of Neomycin standard was weighed accurately and was dissolved in 100ml solvent. Sonicate the content for 10-15min to dissolve the drug completely in mobile phase. Then it was filtered and makes up to 10ml with same diluents to make 10µg/ml Neomycin stock solution. As per the label claim of the two drugs a Betamethasone concentration of 2.4µg/ml was obtained. The resultant solution was used for the simultaneous estimation of Betamethasone and Neomycin in combined dosage forms.

First order spectrophotometric method:

The standard solution of Neomycin and Betamethasone were scanned separately in the UV range and First-order spectra for NMC and BMS were recorded shown in fig. 3. The first order derivative absorption at 224nm (zero cross point for BMS) was used for NMC and 234nm(zero cross point for NMC) for BMS. These two wavelengths can be applied for the

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determination of NMC and BMS with out any interference from the other drug in their pure and combined dosage forms.

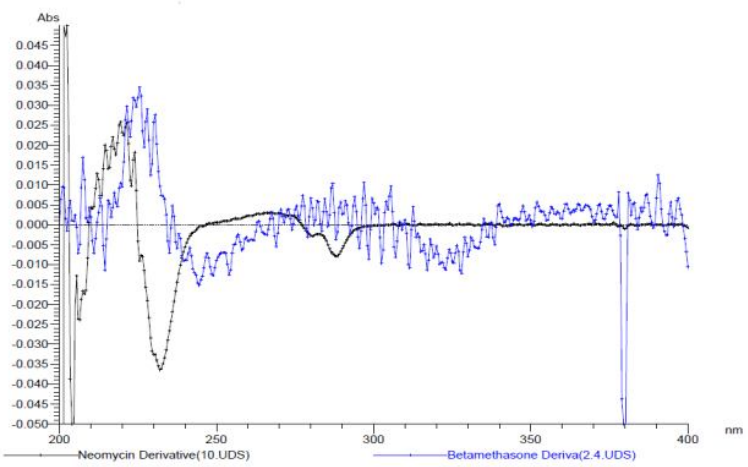

Fig. 3 Overlain first order derivative absorption spectra of NMC and BMS

The proposed method was validated as per ICH guideline [31].

Calibration Curve:

Calibration curves were constructed by plotting drug concentration versus the absorbance values of the first derivative spectrum at 224nm for NMC and 234nm for BMS. Statistical data for calibration curvesare depicted in table–1

Accuracy:

Recovery studies were performed by standarded addition method at three intervals i.e. 50%, 100% and 150% known amounts of pure NMC and BMS were added to pre–analysed sample of marked formulation and there were subjected to analysis by the proposed method. Results of recovery studies are shown in table.

Method Precession:

The precision of the method was performed by find out intra–day and inter–day variation. The results of the precision studies are shown in table. The values of standard deviation less than 2% indicated high degree of precision.

LOD and LOQ: The LOD and LOQ of the drugs were derived by Calculating the signal to noise ratio(S/N, i.e. 3.3 for LOD and 10 for LOQ) using the following equation designated by ICH guidelines

$$LOD = 3.3 * \sigma / S$$
$$LOD = 10 * \sigma / S$$

Where

σ = the standard deviation of the response
S= slope of the calibration curve.

Results and Discussion:

The high correlation coefficient(r2) values i.e. 0.9997 for NMC and 0.9991 for BMS indicated excellent linearity between their peak areas and standard drug concentrations in the range 2.5–15.0µg/ml for NMC at 224nm and 0.6–3.6µg/ml for BMS at 234nm respectively. Intraday and inter day precision results in terms of % RSD were found to be 0.2111 and 0.283

for NMC and 0.123 and 0.162 for BMS (< 2%) which indicated that the proposed method is highly precise and reproducible.

Accuracy results were found in the range of 99.24 to 99.92% for NMC and 99.04 to 99.75% for BMS respectively which indicates high recovery of the method. LOD and LOQ values were found to be 0.03µg/ml and 0.10µg/ml for NMC at 224nm, for BMS they were found to be 0.015µg/ml and 0.05µg/ml at 234nm respectively. These values showed that the method is more sensitive for simultaneous determination of NMC and BMS. The proposed method was applied for the analysis of NMC and BMS in pharmaceutical dosage form. % assay of NMC and BMS were found to be 99.750% for NMC and 98.917% for BMS respectively in the marketed formulations. The summary of the results were given in table–1.

Conclusion:

The developed first order derivative spectrophotometric method for the simultaneous estimation of NMC and BMS in pharmaceutical dosage form is simple, rapid, accurate and economical for routine quality control analysis (Further more it requires simple and rapid sample preparation when applied to the analysis of Ointment dosage form) Hence it is concluded that derivative spectro- photometry can be successfully applied for the simultaneous determination of NMC and BMS.

Table. 1 Regression analysis data and summary of validation parameters for NMC and BMS

Parameters	Neomycin	Betamethasone
Absorption maxima(nm)	224	234
Linearity range(µg/ml)	2.5–15.0	0.6–3.6
Correlation Coefficient (r2)	0.9997	0.9991
LOD(µg/ml)	0.03	0.015
LOQ(µg/ml)	0.10	0.05
Regression equation Y=mX+c	Y=0.0014X+0.0016	Y=0.0167X+0.0085
Precision (% RSD, n=6)		
Intra day	0.211	0.123
Inter day	0.283	0.162
Accuracy±SD (%Recovery, n=3)	99.632±0.351	99.308±0.385

Table. 2 Results of assay of marketed formulation – BONIVIT - N

Sl. No	Drug	Brand Name	Available form	Label Claim (w/w)	Amount Prepared µg/ml	Amount Found µg/ml	% Assay
1	Neomycin	BONIVIT - N	Ointment	0.5%	10	9.975	99.750
2	Betamethasone			0.12%	2.4	2.374	98.917

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