



# Development and Validation of New Analytical Method for the Estimation of Beclomethasone Dipropionate, Clotrimazole and Neomycin Sulphate in Bulk and Pharmaceutical Dosage Forms

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**ABSTRACT:** A new simple, precise, accurate, economic and selective HPLC method has been developed and validated for the estimation of Beclomethasone dipropionate, Clotrimazole, and Neomycin sulphate in bulk and Pharmaceutical dosage form. Column is Zorbax C18 150×4.6 mm, 5 µm, wave length 239 nm, injection volume 20 µL, column temperature is ambient and flow rate 1.0 mL/min. Retention time of clotrimazole, neomycin sulphate, beclomethasone dipropionate is about 2.209, 4.7 and 8.4 min respectively and also estimated by uv-visible spectrophotometry the solvent is 0.1 N NaoH, wave length is 421 nm, linearity is 2-10 µg/mL. The developed methods have been validated statistically as per ICH guidelines. The method showed good reproducibility and recovery with %RSD less than 2. So, the proposed methods were found to be simple, specific, precise, accurate and linear. Hence it can be applied for routine analysis of Beclomethasone dipropionate, Clotrimazole, Neomycin sulphate in bulk drug and Pharmaceutical preparations. © 2018 iGlobal Research and Publishing Foundation. All rights reserved.

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**Keywords** Beclomethasone dipropionate; Clotrimazole; Neomycinsulphate; RP-HPLC; Validation; C-18 column.

## INTRODUCTION

HPLC is an analytical technique used to separate, identify and quantify the component. It finds its use for research, manufacturing, medical, legal purposes. The development of an analytical method for the identification and quantification of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. In the present study attempt is made to estimate the following three drugs simultaneously.

Beclomethasone dipropionate IUPAC name 2-[(1R, 2S, 10S, 11S, 13S, 14R, 15S, 17S)-1-chloro-17-hydroxy-2, 13, 15-trimethyl-5-oxo-14-(propanoyloxy)tetracyclo[8.7.0.0{2, 7}.0{11, 15}] heptadeca-3, 6-dien-14-yl]-2-

oxoethylpropionate. It is soluble in methanol; sparingly soluble in ethanol; slightly soluble in ethyl acetate, dichloromethane and acetonitrile; It is used for the prophylaxis of asthma It is used for the treatment of rhinitis(e.g. hay fever). It acts as a anti-inflammatory, and it is a synthetic corticosteroid.

Clotrimazole IUPAC name 1-[(2-chlorophenyl)diphenylmethyl]-1H-imidazole It is soluble in DMSO (25 mM), chloroform (50 mg/mL), DMF, ethyl acetate, ethanol, It is an anti-fungal agent, 14-alpha demethylase inhibitor and anti-infective agent.

Neomycin sulphate IUPAC name (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, 5- dihydroxyoxan-2-yl]oxy}-3-hydroxy 5 (hydroxymethyl)oxolan-2-yl]oxy}-3-hydroxycyclohexyl]oxy}oxane-3, 4-diol. It is a yellow colour powdered form and soluble in NaOH, ethanol (0.10mg/ml), methanol (0.23mg/mL), isopropanol (0.08mg/mL), isoamyl alcohol(0.25mg/mL) and benzene(0.05mg/mL). It is insoluble in acetone, chloroform and ether. It belongs to bactericidal aminoglycoside antibiotic and it is used as aminoglycoside antibiotic found in many topical medications such as creams, ointments and eyedrops.

**MATERIALS AND METHODS**

**Chemicals**

Beclomethasone dipropionate, Clotrimazole and Neomycin sulphate pure drugs procured from Nirmala college of Pharmacy, Atmakuru, Guntur district. HPLC grade Acetonitrile, HPLC grade Water were purchased from Merck (India) Ltd., Mumbai, India. All other chemicals are of analytical grade from S.D.Fine Chemical Ltd., Worli, India. Ointment (QUADRI DERM) was purchased from Indian market, containing beclomethasone dipropionate (0.025 mg), clotrimazole (1 mg) and neomycin sulphate (0.5 mg) manufactured by fulford india ltd.

**Instrumentation**

Analysis was performed on HPLC instrument equipped with UV detector, Rheodyne injector and C-18 column compartment with EX1600SM soft ware (Cyber Lab corporation, USA.) Other equipments used in the study were, UV-Visible Spectrophotometer (Evolution-201;Thermo Scientific, India) and ultrasonic bath (Amkette Industries India).

**Method development**

**Preparation of Standard solution for Beclomethasone Dipropionate clotrimazole, neomycin sulphate**

Accurately weighed equal amounts 50 mg of beclomethasone, clotrimazole, neomycin sulphate working standards were transferred into three 100 mL volumetric flasks. 70 mL of diluents is added to each flask, sonicated and made up the volume to 100 mL with diluent. Further dilutions of each drug was 0.1 mL to 100 mL with the diluents.

**Chromatographic conditions**

Zorbax C18 Column (250 mm x 4.6 mm, 5 µm) was used for chromatographic separation. The mobile phase composed of acetonitrile and buffer (30:70) v/v; at a flow rate of 1.0 ml/min with run time of 10 min. Mobile phase and sample solutions were filtered through a 0.45µ nylon syringe filter (Millipore, USA) and degassed. The detection of three drugs was carried out at 239 nm (Isoabsorptive point).

**Analysis of marketed ointment (QUADRI DERM)**

A 100 mg cream equivalent to the label claim of the drugs of sample is weighed into a 500 mL volumetric flask. 100 mL of diluent is added, sonicated to dissolve and made up to volume with diluent. Further diluted 0.5 to 100 mL with the diluent. Filtered through 0.45µ Nylon syringe filter. Injected 20µL of Standard preparation five times and Sample preparation into

the Chromatographic column. The chromatograms are recorded and the peak responses for Clotrimazole, Beclomethasone dipropionate and Neomycin sulphate are measured. The System suitability parameters are met. From the peak responses, the content of Clotrimazole, Beclomethasone and Neomycin in the sample are calculated.

**Assay calculations**

Drug	Labeled amount(mg)	Amount present(mg)	% Assay
Beclomethasone Dipropionate	0.025	1.07	100.7
Clotrimazole	1	1.02	100.2
Neomycin Sulphate	0.5	0.504	100.4

**Validation of method**

The HPLC method was validated in accordance with ICH guidelines. The linearity of detector response for beclomethasone, clotrimazole, neomycin sulphate was established for five concentrations. The final concentrations of each solution in µg/mL was calculated and plotted against area response. The slope, y-intercept, correlation coefficient were calculated. System precision of the method was verified by six injections from the same standard preparations were made and the relative standard deviation for the replicate injections was calculated. The method precision was carried out the six times using the proposed method. Repeatability was measured by multiple injections of a homogenous sample of beclomethasone, dipropionate, clotrimazole, neomycin sulphate. Accuracy was conducted for Beclomethasone dipropionate, Clotrimazole, Neomycin sulphate. Assay in triplicate (50%, 100% and 150%) as per test equivalent of drugs containing beclomethasone, dipropionate clotrimazole, neomycin sulphate into each volumetric flasks for each spike level to get concentration of these three drug solutions equivalent to 50%, 100%, and 150% of the labeled amount as per test method. The average % recovery was calculated. Solution stability of beclomethasone dipropionate, Clotrimazole, neomycin sulphate in diluents was determined by storing sample solution (30ppm) in a tightly closed in volumetric flask at room temperature for 24 h. later these drug solutions measured at different time interval like 4, 12, and 24 hrs and finally result is obtained were compared with freshly prepared solutions. Robustness was evaluated by analysis of aliquots from homogenous lots by different physical parameters like flow rate, mobile phase composition, wave length which may differ but the response still within the specified limits of assay.

**RESULTS AND DISCUSSION**

HPLC is an analytical technique used to separate, identify and quantify the component. It finds its use for research, manufacturing, medical, legal purposes. The development of an analytical method for the identification and quantification of drugs by HPLC has received considerable attention in

recent years because of their importance in quality control of drugs and drug products. The objective of the present study was to develop a simple, rapid, precise, accurate and sensitive HPLC method for the analysis of Beclomethasone Dipropionate, Clotrimazole, Neomycin Sulphate in bulk and its pharmaceutical dosage form by using solvent system of ACN: OPA in the ratio 70:30 and Kromasil C18, 150 mm x 4.6mm, 5µm stationary phase. The chromatographic condition is optimized at flow rate of 1ml/min with UV detection at 239 nm. Validation studies are carried out by using freshly prepared solutions as per ICH requirements.

Parameter	Beclomethasone dipropionate	Clotrimazole	Neomycin sulphate
Linearity Range correlation coefficient	0.05-0.038 ppm R <sup>2</sup> =0.999	0.200-1.506ppm R <sup>2</sup> =0.998	0.100-0.750ppm R <sup>2</sup> = 0.997
System Precision	% RSD =0.157	%RSD = 0.157	% RSD = 1.182
Intermediate Precision	%RSD =0.219	%RSD = 0.189	% RSD = 0.243
Method precision	%RSD 0.354	% RSD = 0.145	% RSD =0.340
Accuracy	% recovery=100.4	% recovery = 100.4	% recovery = 100.6
Solution stability	Stable up to 24 h %RSD =1.075	Stable up to 24 h % RSD = 0.414	Stable up to 24 h % RSD = 0.188

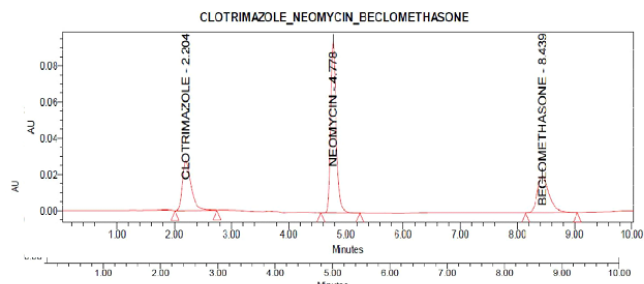


Fig 1: A representative chromatogram of standard

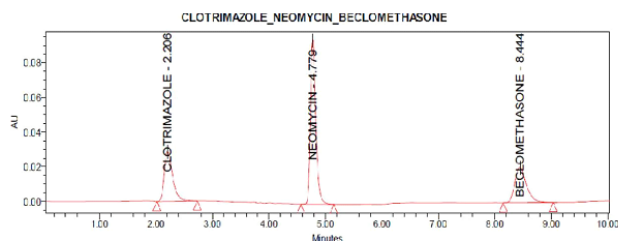


Fig. 2: A representative chromatogram of sample

**CONCLUSION**

In the present investigation a new analytical method was developed for potent drugs beclomethasone dipropionate, clotrimazole and neomycin sulphate. Since there is no analytical method available to estimate the combination of beclomethasone dipropionate, clotrimazole and neomycin sulphate using RP-HPLC this method will provide a choice of routine determination of beclomethasone dipropionate, clotrimazole and neomycin sulphate in bulk and pharmaceutical dosage forms.

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