ejpmr, 2017,4(1), 338-342

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF MUPIROCIN AND BECLOMETHASONE DIPROPIONATE IN PHARMACEUTICAL DOSAGE FORM

Prasanna Pradhan*, Dhanga Nehal, Chaudhari Sandhya, Shreya Shah and Umesh Upadhyay

Sigma Institute of Pharmacy, Bakrol, Vadodara.

***Corresponding Author: Prasanna Pradhan**

Sigma Institute of Pharmacy, Bakrol, Vadodara.

Article Received on 28/10/2016 **Article Revised on 18/11/2016** **Article Accepted on 08/12/2016**

ABSTRACT

A simple, rapid, economical, precise and accurate Stability indicating RP-HPLC method for simultaneous estimation of Mupirocin and Beclomethasone dipropionate in Their Combined Dosage Form has been developed. The separation was achieved by LC- 20 AT C18 (250mm x 4.6 mm x 2.6 µm) column and Buffer (pH 4.5): Acetonitrile (60:40 v/v) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 236 nm. Retention time of Mupirocin and Beclomethasone dipropionate were found to be 3.55 min and 5.18 min, respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Mupirocin 20-60 µg/ml and for Beclomethasone dipropionate 0.5-1.5 µg/ml. The percentage recoveries obtained for Mupirocin and Beclomethasone dipropionate were found to be in range of 100.22 ± 0.60 and 100.59± 0.20 respectively. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial combined dosage form.

KEYWORDS: Mupirocin, Beclomethasone dipropionate, Stability indicating RP-HPLC Method, Validation.

INTRODUCTION

Mupirocin (MUP) chemically designated as 9-{[(2E)-4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-{[(2S,3S)-3-[(2S,3S)-3-hydroxybutan-2-yl]oxiran-2-yl]methyl}oxan-2-yl]-3-methylbut-2-enoyl]oxy}nonanoic acid[1] [Figure 1a], an antibiotic isolated from Pseudomonas fluorescens. It is a topically acting antibiotics, primarily effective against Gram-positive bacteria. It acts by selectively binding bacterial isoleucyl- tRNA synthetase, which halts the incorporation of isoleucine into bacterial proteins.

Beclomethasone dipropionate (BEC) chemically designated as 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-oxoethyl propanoate is an glucocorticoid[2] [Figure 1b], It is a prodrug of the free form, Beclomethasone (beclomethasone-17-monopropionate). A synthetic corticosteroid, used topically as an anti-inflammatory agent.

The literature survey suggests UV method[3], HPLC[4,5] method and RP-HPLC[6,7–12] method method for MUP and BEC. The aim of the present study was to develop simple, rapid, accurate, specific and precise RP-HPLC method for the estimation of MUP and BEC in the bulk and pharmaceutical formulation.

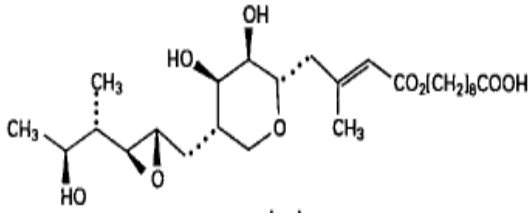

Fig. 1a: Structure of Mupirocin

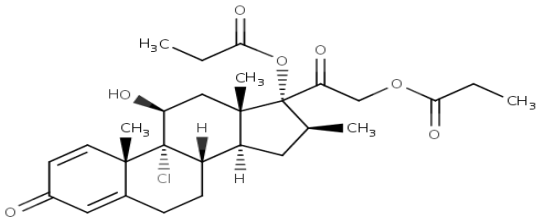

Fig 1b: Structure of Beclomethasone dipropionate

MATERIALS AND METHOD

Materials and Reagents: Mupirocin was donated by **Dermocare Pvt. Ltd**, Changodar, Ahmedabad and Beclomethasone Dipropionate was generously gifted by **Halewood Lab**, Vatwa, Ahmedabad. Acetonitrile (HPLC grade) was purchased from Qualigens fine chemicals, Mumbai, India. Double distilled water used for HPLC analysis and preparation of buffer. All the other chemicals used were of analytical grade. For the

estimation of commercial formulation, Supirocin®-B plus having (2% Mupirocin and 0.05% Beclomethasone Dipropionate in 5g ointment) manufactured by Glenmark Pharmaceuticals Ltd. were procured from the local vendor.

Instrumentation and Chromatographic conditions
The chromatographic system consisted of LC-20 AT prominence solvent delivery module, manual Rheodyne injector with a 20-µl fixed loop with SPD-20A Prominence detector working in UV–visible range. The chromatogram was recorded by means of Spinchrom (CFR Version 2.4.0.195) Software and a C18 Column [Hypercil BDS, 150mm x4.6mm 5µ].

Preparation of mobile phase: The mobile phase finalized for this method is composed of Buffer (pH 5): Acetonitrile (60: 40 v/v). It was filtered through a 0.45 µ membrane filter and degassed for 15 minutes. The flow rate of the mobile phase was maintained at 1 ml/min. Detection was carried out at 236 nm at ambient temperature.

Standard Solutions Preparation: Stock solution was prepared by dissolving accurately weighed 40 mg of MUP and 1 mg of BEC in a 100 ml volumetric flask separately to obtain 400 µg/mL and 10 µg/mL of MUP and BEC respectively using HPLC grade methanol as solvent. Standard solution of binary mixtures containing MUP (40 µg/mL) and BEC (1 µ g/mL) prepared from above standard stock solution and used during method development. The stock solution was further diluted suitably with methanol to obtain concentrations for calibration curve.

Sample solution preparation: Accurately weighed ointment of 5g containing equivalent to 2% of MUP and 0.05% of BEC as per the label claim, was transferred to flask containing 60mL of Methanol. The flask allowed to shake for 5-10 min and sonicated for 15min. Resulting solution transferred to 100mL volumetric flask and made up the volume with Methanol. The resulting solution was filtered through 0.45 µm membrane filter. From the above solution 0.4mL of aliquots transferred to 10mL volumetric flask and make up the volume with Phosphate buffer pH 5: acetonitrile 60:40% (v/v).

RESULTS AND DISCUSSION

Development and Optimization of the Stability-Indicating HPLC Method: Both MUP and BEC have limited aqueous solubility compelled to use methanol for the extraction of drugs from the formulations and for preparation of stock solutions. MUP shows UV maxima at 218 and 271 nm, whereas BEC shows maxima at 236 nm. In formulation the concentration of MUP is 2% and BEC concentration is much i.e. 0.05%. So in order to gain maximum and reliable sensitivity 236 nm was selected for the study.

Mobile phase optimization was initiated using water and methanol (50:50, %v/v) at the flow rate of 1.0 ml/min. Only one peak of BEC observed. Despite of changing the % of organic modifier the observation remains same. Same was tries with water and acetonitrile (50:50, %v/v), the retention time decreases but again only single peak observed.

Keeping in mind pKa of MUP and BEC, Phosphate buffer with pH 5.0 was tried, as higher pH in comparison to pKa will keep acidic drug substance in ionized form.

Mobile phase with composition Buffer (pH 5): Acetonitrile (50: 50, % v/v), both drugs shows peak. With this mobile phase both the drug substances became polar and have less affinity for the stationary phase and eluting quickly, close to void volume. In order to allow them to spend more time in column, % of organic phase in composition of mobile phase has to be reduced.

Reducing acetonitrile from 50% to 40% leads to increased retention time of both. But as BEC is less polar it is more retained, retention time changes from 3.2 to 5.21min. In comparison to that MUP is more polar and elute out relatively more quickly, retention time changes from 2.65 to 3.57min. Hence smaller volume of acetonitrile results in increase in resolution between two peaks to 7.43, sufficient to accommodate degradation peak.

Finally, the mobile phase composition Buffer (pH 5): Acetonitrile (60: 40 v/v), at a flow rate of 1 mL/min was found to give good separation of peaks.

The method developed above is simple, uses isocratic flow system and UV detector for detection of drug. The chromatograms are given in the [Figure 2].

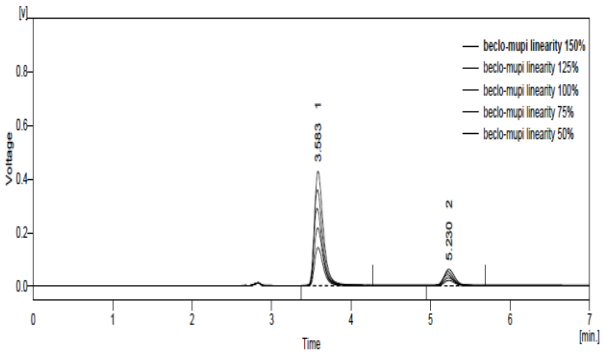

Fig 2: Chromatogram of MUP and BEC in Buffer (pH 4.5): Acetonitrile (40:60)

Method Validation

System suitability: The system suitability was determined by injecting six replicates of the standard solutions containing MUP (40 µg/ml) and BEC (1 µg/ml) and analyzing each active ingredient for its peak area, peak tailing factor, resolution, number of theoretical plates and capacity factor. The resolution was

found to be 7.43 with retention time 3.43 min. and 5.21min. for MUP and BEC respectively. The percent RSD of retention time for both the drugs was less than 0.3% indicating high stability of the system. The percent RSD of peak area was well within the range of 2% limit signifying suitability of the system [Table 1]. The tailing factor was 1.8 (mean) for MUP and 1.3 (mean) for BEC.

Table 1: System suitability test parameter

Statistical parameter	MUP (40 µg/ml)		BEC (1 µg/ml)	
	Retention time (min)	Peak area	Retention time (min)	Peak area
Mean ± SD	3.75 ± 0.01	2224.0 ± 35.67	5.21 ± 0.04	367.7 ± 2.72
% RSD	0.17	1.25	0.26	0.74
Theoretical plate	5197		7338	
Tailing Factor	1.8		1.3	
Resolution	7.43			

Linearity and Range: As the test concentration for MUP (40 µg/ml) and BEC (1 µg/ml), linearity was studied simultaneously for the range of 20-60 µg/ml for MUP and 0.5-1.5 µg/ml for BEC which covers 50% to 100% of test concentration. The concentration and peak area were used to plot calibration curve evaluated by its correlation coefficient. The calibration curve was evaluated by its correlation coefficient. The correlation coefficients for both the calibration plots of drugs were more than 0.999 [Table 2].

Table 2: Regression analysis of linearity data of MUP and BEC

Parameter	MUP	BEC
Concentrate Range(µg/ml)	20-60	0.5–1.5
Regression equation	y =55.234x + 12.78	y = 366.6x + 0.72
Correlation coefficient (r2)	0.999 ± 0.0004	0.999 ± 0.0003
LOD (µg/ml)	0.65	0.032
LOQ (µg/ml)	1.98	0.098

Precision: System precision (injection repeatability) was determined by performing six repeated analysis of working standard solution. Intermediate precision was assessed by analyzing three replicate injections of reference solutions at three levels on the same day and on three different days. Precision calculated for samples during intraday and inter day run are given in [Table 3]. The % RSD for intraday and interday below 2% inferred the precision of the method.

Table 3: Intra- and Inter-day accuracy and precision of HPLC assay for MUP and BEC

Parameters	MUP			BEC		
Nominal concentration	20 µg/ml	40 µg/ml	60 µg/ml	0.5 µg/ml	1.0 µg/ml	1.5µg/ml
Intra day						
Avg.	1103.17	2238.69	3326.71	183.39	366.90	555.29
SD	14.66	13.63	38.92	0.78	4.19	3.84
%RSD	1.33	0.61	1.17	0.43	1.14	0.69
Inter day						
Avg.	1094.41	2226.73	3283.85	180.88	367.08	546.26
SD	15.38	21.38	46.61	2.37	5.25	4.00
%RSD	1.41	0.96	1.42	1.31	1.43	0.73

Accuracy: The accuracy of the proposed method was evaluated by calculating the recovery studies of the test drug at three different concentration levels (50%, 100% and 150%) by standard addition method. A known amount of MUP and BEC was added to pre-quantified sample solution and three replicates of each concentration were injected in developed chromatographic conditions. The mean percentage recovery of MUP and BEC was varied between 99.81 - 100.74% and 100.32 - 101.39% respectively indicating that the developed method was found to be accurate. The % recovery results were shown in [Table 4].

Table 4: Recovery Studies Data for MUP and BEC

Drug	Level (%)	Spiked Conc. (µg/mL)	Mean Conc. recovered (µg/mL)	Mean recovery ± SD (%, n=3)
MUP	80	16	16.05	100.11 ± 1.02
	100	20	20.00	100.74 ± 0.63
	120	24	24.20	99.81 ± 1.06
BEC	80	0.8	0.79	100.32 ± 1.19

	100	1.0	1.0	100.70 ± 1.11
	120	1.2	1.21	101.39 ± 0.90

Robustness: Robustness of the proposed method has been evaluated by small deliberate changes in the system parameters such as flow rate, mobile phase composition and pH of the mobile phase. It was found that none of the above parameters caused alteration in the peak area, retention time and USP tailing by small changes like ±0.1mL change in flow rate, ±5% change in mobile phase and ±5∘C change in temperature. The %RSD was found to be within the limits and the method was found to be robust. The robustness results were shown in [Table 5].

Table 5: Robustness Data for MUP and BEC

Parameter	Variation	Average peak area ± SD (n=3)		% RSD	
		MUP	BEC	MUP	BEC
	1mL/min, 60:40 pH 5	2238.68 ±13.63	366.90 ± 4.19	0.60	1.14
Flow rate (mL/min)	**1.2**	2235.29 ± 35.17	371.52 ± 2.62	1.57	0.70
	0.8	2201.40 ± 6.92	363.25 ± 2.08	0.31	0.57
Mobile phase (Phosphate Buffer pH 5: Acetonitrile)	**58:42**	2263.90 ±13.80	372.96 ± 4.32	0.60	1.15
	62:38	2155.74 ±17.28	352.57 ±5.30	0.80	1.50
Mobile phase pH	**7**	2201.68 ±7.77	362.21 ± 4.41	0.35	1.21
	3	2143.28 ±33.01	356.79 ±1.43	1.54	0.40

Forced Degradation Study: Forced degradation study was done on drug combination by Acid, Base, Photo Oxidative and thermal methods. Labeling of all degradation products was done by a degrading individual drug with a similar condition as used for the combination. Retention time and wavelength of degradation product were useful parameters to label degradation products. Such labeling was very useful to identify common degradation products among different degradation conditions was found to be a common degradation product under thermal degradation condition. Preliminary trials on individual drugs and those in combination were conducted to optimize various stress conditions. Samples were withdrawn at 4 hours intervals, to monitor the rate of degradation and optimize the stress conditions. All drugs showed degradation in Acid, Base, Photo, Oxidative and thermal methods at room temperature for 24 hours. [Figure 3] MUP was easily susceptible to degradation in comparison of BEC in drastic condition. BEC was comparatively more prone to degradation under acid, basic, photo, oxidative and thermal conditions. [Table 6].

Table 6: Degradation sample data of MUP and BEC

Parameter	MUP				BEC			
	Standard		Sample		Standard		Sample	
	Area	% Deg.	Area	% Deg.	Area	% Deg.	Area	% Deg.
Acid	1886.6	13.91	1826.6	16.65	327.30	14.05	327.87	13.9
Base	1749.3	20.17	1764.9	19.46	323.50	15.04	323.98	14.9
Thermal	1893.4	13.60	1935.8	11.66	328.77	13.66	336.78	11.5
Oxidation	1894.4	13.55	1882.8	14.08	333.17	12.52	331.80	12.8
Photolytic	1808.9	17.45	1852.7	15.46	322.47	15.32	310.18	18.5

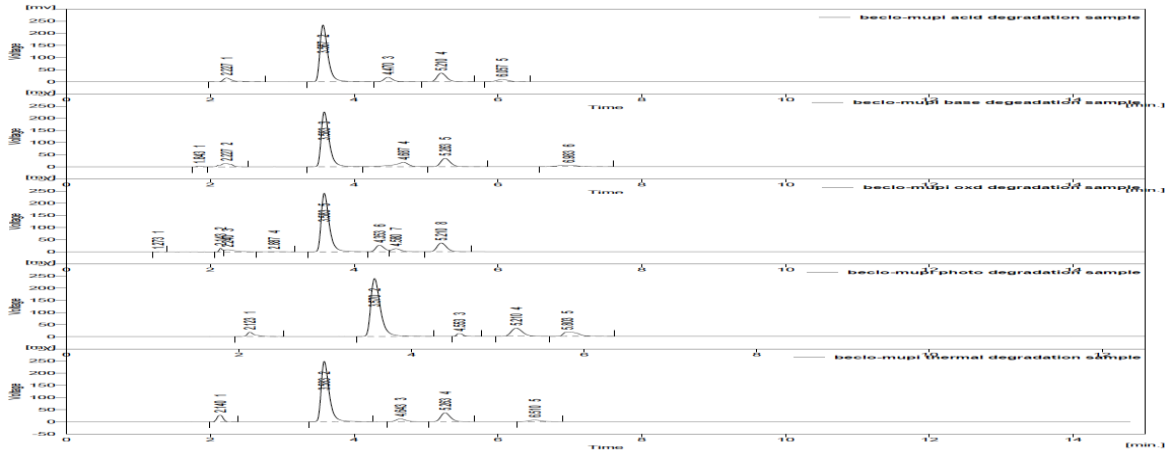

Figure 3: MUP and BEC Degradation sample

Assay of Marketed formulation: 5g of ointment weighed accurately which is equivalent to 2% of Mupirocin and 0.05% of Beclomethasone dipropionate as per the label claim, was transferred to flask containing 60mL of Methanol. The flask allowed shaking for 5-10 min and Sonicator for 15min. Resulting solution transferred to 100mL volumetric flask and make up the volume with Methanol. The resulting solution was filtered through whatman filter paper and the 0.4mL of aliquot transferred to 10mL volumetric flask and make up the volume with Phosphate buffer pH 5: Acetonitrile 60:40% (v/v). (MUP 40 µg/mL and BEC 1 µg/mL) [Table 7].

Table 7: Assay results of marketed formulation

Ointment Supirocin®- B Plus		% Label Claim	Amount of Drug taken (mg)	Amount of Drug found (mg)	% Assay ± SD
	MUP	2.00	100.0	99.21	99.21 ± 0.4
	BEC	0.05	2.5	2.51	100.4 ± 0.9

SD: standard deviation; n= 3.

CONCLUSION

The developed method has proven to be rapid, accurate, and stability- indicating for the simultaneous determination of the combined Mupirocin and Beclomethasone in its formulation in the presence of excipients and the degradation products. There was always a complete separation of both ingredients from their degradation products and from the placebo. Method was validated in terms of linearity and range, recovery, precision and ruggedness. This method offers advantages of using simple mobile phase; rapid sample preparation and comparatively short run time hence the proposed RP-HPLC method could be adopted for the quantitative quality control and routine analysis of this combination in its formulation.

ACKNOWLEDGEMENT

We acknowledge the Sigma Institute of Pharmacy, Vadodara for providing the necessary facilities to carry out the research work.

REFERENCES

1. PubChem Compound Database. Mupirocin [Internet]. National Center for Biotechnology Information. 2016 [cited 2016 Sep 4]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/446596.
2. PubChem Compound Database. Beclomethasone dipropionate [Internet]. National Center for Biotechnology Information. 2016 [cited 2016 Sep 4]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/446596.
3. Shah PD, Koradia S. Simultaneous Determination of Beclomethasone Dipropionate and Formoterol Fumarate in Rotacap Dosage Form Using Two Different Spectrophotometric methods. World J Pharm Pharm Sci. 2014; 3(5): 611–23.
4. Ribeiro de SAL, Cintia A, Kelly M, Moreira S, Chorilli M. Validation of a stability-indicating HPLC method with diode array detection for the determination of beclomethasone dipropionate in aqueous suspension for nebulizer. Asian J Biomed Pharm Sci. 2013; 3(19): 4–9.
5. Orsi D De, Gagliardi L, Chimenti F. HPLC Determination of Beclomethasone Dipropionate and Its Degradation Products in Bulk Drug and Pharmaceutical Formulations. 2006; (September 2014): 37–41.
6. Amrutiya N, Madan M, Bajaj A. Development and validation of RP-HPLC method for simultaneous estimation of prednicarbate, mupirocin and ketoconazole in topical dosage forms. J Anal Chem [Internet]. 2010; 65(11): 1148–54.
7. Chassot JM, Ferreira LM, Gomes FP, Cruz L, Tasso L. Stability-indicating RP-HPLC method for determination of beclomethasone dipropionate in nanocapsule suspensions. Brazilian J Pharm Sci. 2015; 51(4): 803–10.
8. Dhudashia K, Patel A, Patel C. Development and validation of a reversed-phase HPLC method for simultaneous estimation of clotrimazole and beclomethasone dipropionate in lotion and cream dosage form. Chronicles Young Sci [Internet]. 2013; 4(2): 102.
9. Martis E a, Gangrade DM. Reverse phase isocratic HPLC method for simultaneous estimation of salbutamol sulphate and beclamethasone diproprionate in rotacaps formulation dosage forms. Int J Pharm Pharm Sci. 2011; 3(1): 64–7.
10. Stephen E. Johnston, Nicole L. Gill, Yu-Chien Wei, Robert Markovich, Abu M. Rustum. Development and validation of a stability-indicating RP-HPLC method for simultaneous assay of betamethasone dipropionate, chlorocresol, and for the estimation of betamethasone dipropionate related compounds in a pharmaceutical cream and ointment. J Chromatogr Sci. 2010; 48(9): 733–41.
11. Saha CN, Bhattacharya S. A validated simultaneous RP-HPLC method for determination of betamethasone dipropionate and tolnaftate in combined semisolid formulation. Int J ChemTech Res. 2009; 1(3): 671–4.
12. Kumar N, Pradhan PK, Rajput P, Joshi B. Rp-Hplc Method Development and Validation for Simultaneous Estimation of Cefoperazone and Tazobactam in Their Combined Dosage Form. World Journal Of Pharmacy and Pharmaceutical Sciences, 2014; 3(8): 1083-1093.