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NOVEL, RAPID, ISOCRATIC RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF BETAMETHASONE DIPROPIONATE, MICONAZOLE NITRATE AND CHLOROCRESOL IN PHARMACEUTICAL DOSAGE FORM

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Keywords:

RP-HPLC, Betamethasone dipropionate, Miconazole nitrate, Chlorocresol

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ABSTRACT: A novel, rapid, isocratic, reversed-phase HPLC method was developed to simultaneously determine betamethasone dipropionate, miconazole nitrate and chlorocresol in a topical dosage form. The separation was performed on the C18 prontosil (250 x 4.6mm x 5µ) column. The mobile phase used for this study consists of acetonitrile and phosphate buffer of pH 2 in the ratio of 70:30 v/v, respectively. The flow rate was set as 1 ml per minute. This method used a UV detector with a wavelength of 235 nm. The retention time of chlorocresol, miconazole nitrate, and betamethasone dipropionate was 4.30 min, 5.13min, and 6.49 min, respectively. Linearity was observed for betamethasone dipropionate, miconazole nitrate, and chlorocresol in the concentration range of 1 to 3 µg/ml, 40 to 120 µg/ml and 2 to 6 µg/ml, respectively. The novel, rapid, reversed-phase HPLC method can be used in the regular analysis of any drug or combination in the pharmaceutical dosage form.

INTRODUCTION: High-performance liquid chromatography (HPLC) was derived from traditional column chromatography and is one of the most significant analytical chemistry tools today [1]. HPLC mainly uses a column with packing material, the stationary phase, a pump that helps to move the mobile phase through the column and a detector that helps to show the retention time of the molecules. Retention time changes depending on the interactions between the stationary phase sample and solvent(s) used [2].

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Pharmaceutical creams and ointments containing betamethasone dipropionate are widely used as an anti-inflammatory agent to relieve a wide variety of skin conditions *eg.* dermatitis and psoriasis [3]. Miconazole is from the imidazole category with antifungal properties and is used in treating skin infections. Miconazole can be taken by oral route for the therapy of oropharyngeal and intestine candidiasis.

Miconazole nitrate is usually applied in the treatment of fungal infections of the skin, including candidiasis, dermatophytosis and pityriasis versicolor. To cure skin infections like candidiasis and dermatophytosis and pityriasis vesicolor, miconazole nitrate is used. It is also used for the healing of vaginal candidiasis [4]. Chlorocresol is a bactericide closely related to carbolic acid.

Chlorocresol is also used as a preservative in pharmaceutical formulations. Preservative systems perform an important part of any cream or ointment formulation. In cream or ointment formulation, preservatives perform an important role. Knowing the actual concentration of preservative(s) in different formulations is vital in establishing the shelf-life of a product it is crucial to know the concentration of preservative(s) for the product [5]. The developed method was validated as per International Council for Harmonization (ICH) guidelines.

MATERIALS AND METHODS:

Material and Reagent: The reference standard of Miconazole Nitrate was purchased from Sisco Research Lab Pvt. Ltd with purity 98 %. Reference standard of betamethasone dipropionate was procured from Zydus Cadilia, Mumbai, India with 99 % purity. Reference standard of chlorocresol was purchased from Sigma Aldrich with a purity 99%.

HPLC grade acetonitrile was purchased from Thermo Fisher Scientific India Pvt. Ltd. The formulation Betamil GM (Pocter& Gamble Health Ltd.) was procured from the local market.

Apparatus and Chromatographic Conditions: This method employed the RP HPLC Shimadzu (LC2030) model with "Lab solution" software and a double wavelength UV detector. The analytical column used to separate the analyte was the prontosil (250 x 4.6 mm x 5 µ) column.

Preparation of Standard Solutions: 0.1 g each of betamethasone dipropionate, miconazole nitrate, chlorocresol were accurately weighed and transferred into a 100 ml volumetric flask separately. About 70 ml of solvent acetonitrile was added, sonicated to dissolve and volume was made up with solvent acetonitrile (1000 µg/ml). The final concentration of betamethasone dipropionate, miconazole nitrate, chlorocresol was made to 2 µg/ml, 80 µg/ml, 4 µg/ml, respectively by solvent acetonitrile.

Preparation of Mixed Standard Solution: Accurately weighed betamethasone dipropionate, miconazole nitrate, chlorocresol reference standard was dissolved in acetonitrile and diluted stepwise to obtain a mixed standard solution of 2 µg/ml, 80 µg/ml and 4 µg/ml of betamethasone dipropionate, miconazole and chlorocresol respectively.

Preparation of Sample Solution: Accurately weigh the 1 g of Betamil GM cream containing 0.05 g of betamethasone dipropionate, 2 g of miconazole nitrate,0.1 g of chlorocresol in 50 ml volumetric flask, add 30 ml of acetonitrile and sonicate it. Sonication was done to dissolve the APIs completely, then make up the volume to the mark with the same diluent. Mixed APIs were completely dissolved by sonication and volume was made up by the same solvent (acetonitrile). The final solution was filtered through 0.45 µm of pore size.0.45 µm filter was used for the filtration of the final solution.

Preparation of 0.05M Phosphate Buffer (pH 2): About 6.8 gm of potassium dihydrogen phosphate was accurately weighed and dissolved in 950 ml water. The Orthophosphoric acid was used to adjust the pH 2 and the volume was made up to 1000 ml in a volumetric flask. The solution was then filtered using 0.45 µ membrane filter.

RESULTS AND DISCUSSION:

Selection of Wavelength: The UV scanning spectrum of betamethasone dipropionate, miconazole nitrate, and chlorocresol ranging from 200 to 400 nm revealed that the maximum absorption wavelength was 235 nm for betamethasone dipropionate 225 nm for miconazole nitrate and 228 nm for chlorocresol. Considering the content of betamethasone dipropionate in the formulation, which was much less than miconazole nitrate and chlorocresol, UV maxima of beclomethasone dipropionate (235 nm) were selected as detection wavelength **Fig. 1.**

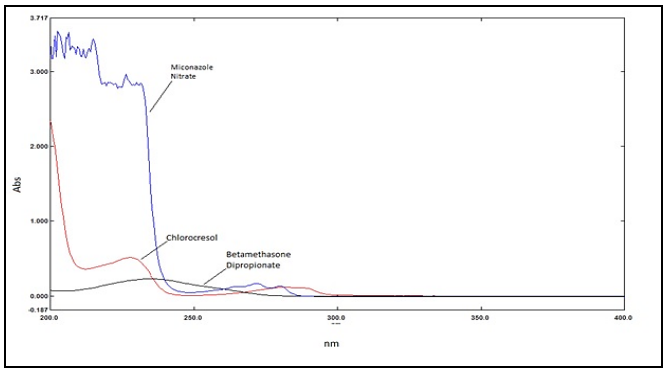

FIG. 1: UV OVERLAIN SPECTRA OF BETAMETHASONE DIPROPIONATE, MICONAZOLE NITRATE, CHLOROCRESOL

Method development: A preliminary study with several mobile phases was performed to optimize the HPLC methods for drug analysis. Parameters such as the optimum pH and ideal mobile phase proportion were studied to achieve the separation of analytes. When separation was tried with acetonitrile: phosphate buffer (70:30 v/v) with pH 2.9 the peaks of miconazole nitrate and betamethasone dipropionate were overlapping. With the same mobile phase at pH 2.5 the peaks of miconazole nitrate and betamethasone dipropionate did not separate properly. After several other trials, a satisfactory result was achieved at acetonitrile: phosphate buffer (70:30 v/v) with pH 2 adjusted with ortho-phosphoric acid. The optimized method used the Prontosil C18 (250 x 4.6 mm x 5 µ) column. The retention time of chlorocresol, miconazole nitrate and betamethasone dipropionate was 4.30 min, 5.13 min, and 6.49 min, respectively, at detection wavelength 235 nm with a run time of 8 min. The injection volume was 10 µL with a 1.0 ml/min flow rate.

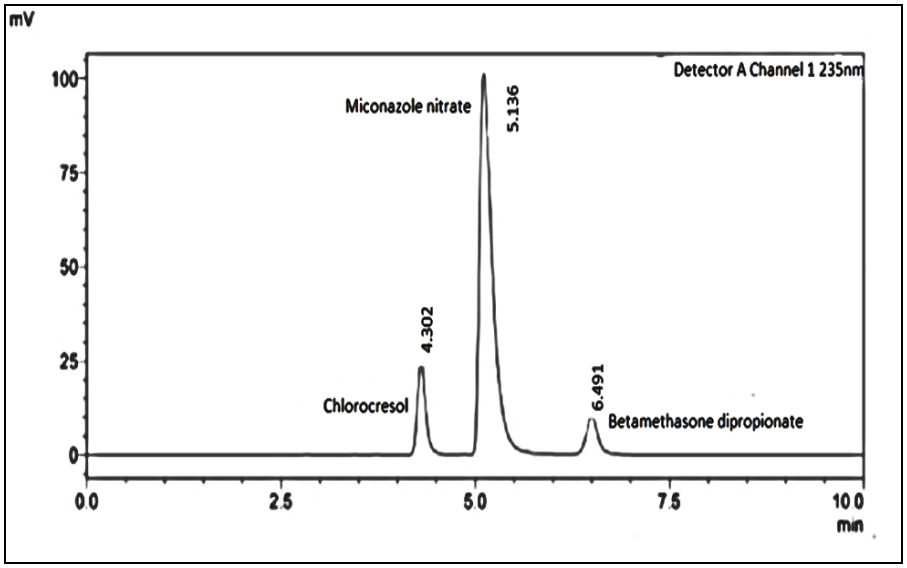

FIG. 2: HPLC CHROMATOGRAM FOR THE STANDARD SOLUTION

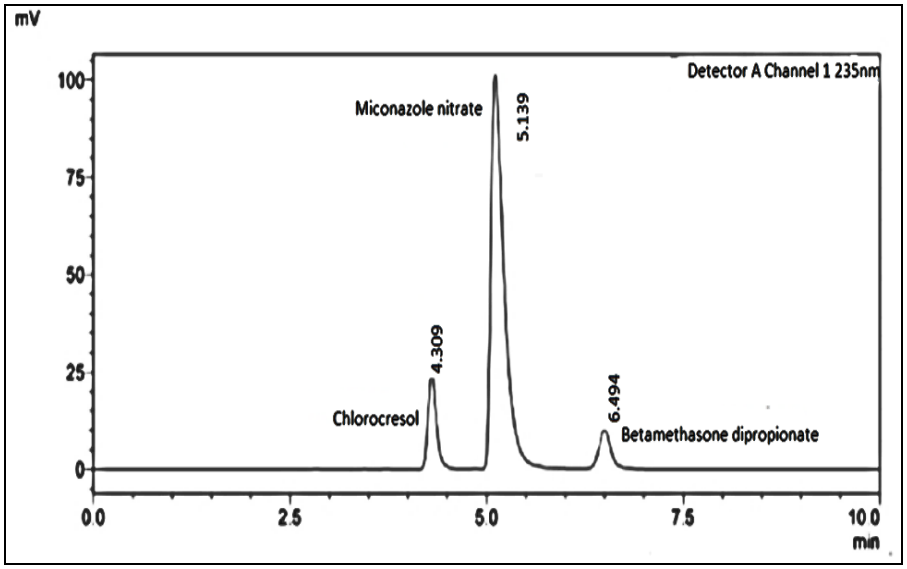

FIG. 3: HPLC CHROMATOGRAM OF SAMPLE SOLUTION

Linearity: Calibration curves were constructed by plotting the peak areas (Y) versus concentration with the resulting level shown in the table. Peak areas (Y) versus concentration were plotted for the construction of the calibration curve. The correlation coefficient Found was greater than 0.99 for each drug marker, which meets the method validation acceptance critical; hence, the method is said to be linear **Table 1.**

TABLE 1: LINEARITY OF MIXED STANDARD SOLUTION

Compound	Regression equation	R2	Range of conc µg/ml
Betamethasone dipropionate	y = 1028.6x + 6428.1	0.9958	1 to 3
Miconazole nitrate	y = 11212x+37784	0.9916	40 to 120
Chlorocresol	y = 1894.4x-5706.4	0.9921	2 to 6

Specificity: The chromatographic behaviors of betamethasone dipropionate, miconazole nitrate, and chlorocresol were out of interference by another compound.

The complete separation of the chromatogram of betamethasone dipropionate, miconazole nitrate and chlorocresol was achieved. As the retention time of standard drugs and the retention time of the drugs in the sample solution were the same, so the method was found to be specific. This method was found to be specific as the retention time of drugs in the sample solution and standard drugs were identical.

Precision:

System Precision: Six replicate injections of standard solutions at working concentration showed a percent relative standard deviation (%RSD) less than 2 concern the area for each marker drug, which shows the acceptable reproducibility and thereby system precision **Table 2.**

TABLE 2: RESULT FOR SYSTEM PRECISION

S. no.	Peak area		
	Betamethasone dipropionate (2 µg/ml)	Miconazole Nitrate (80 µg/ml)	Chlorocresol (4 µg/ml)
1	110536	1185365	172399
2	112169	1211697	173399
3	111809	1189885	175399
4	110592	1205923	172399
5	111499	1194998	174099
6	110268	1202680	173399
Average	111146	1198425	173516
RSD %	0.70	0.84	0.65

Method Precision: Method precision was determined by analyzing six sample replicas under the test of repeatability at working concentration. Analysis of six replicas of the sample at working concentration was performed to determine the method's precision. The % RSD of peak areas was less than 2, indicating acceptable reproducibility and method precision **Table 3.**

TABLE 3: RESULT FOR METHOD PRECISION

	% Assay		
S. no.	Betamethasone dipropionate	Miconazole nitrate	Chlorocresol
1	99.5	98.92	99.5
2	99.8	99.87	99.8
3	100.6	100.85	100.6
4	98.3	99.89	98.89
5	100.2	100.98	100.2
6	98.5	100.02	98.5
Average	99.48	100	99.58
SD	0.91	0.75	0.79
%RSD	0.92	0.75	0.79

Robustness: To discover developed method robustness, experimental conditions were purposefully altered and the system suitability parameters such as tailing factor and peak area were evaluated. The solution was prepared as per the sample preparation test method described earlier and injected at different variable conditions like column temperature (26 ℃ and 30 ℃), flow rate (0.9 ml and 1.01 ml/min) and detection wavelength (233 nm and 237 nm). The result for robustness is shown in **Table 4.**

TABLE 4: RESULT FOR ROBUSTNESS

Parameters	% Assay		
	Betamethasone dipropionate	Miconazole nitrate	Chlorocresol
Minus Temp (26 ℃)	100.24	99.29	100.68
Plus Temp (30 ℃)	100.61	99.06	99.87
Minus flow rate 0.9 ml	100.88	100.79	100.01
Plus flow rate 1.1 ml	99.46	100.14	100.08
Minus wavelength 233 nm	100.74	99.7	100.66
Plus wavelength 237 nm	100.46	100.56	100.52

Accuracy: Accuracy was done by means of recovery experiments using the standard addition method at 3 different levels (at three different levels by using the standard addition method) (80%, 100% and 120%). The known amount of standard solution containing betamethasone dipropionate (0.6 µg/ml, 1 µg/ml, 1.4 µg/ml) miconazole nitrate (24 µg/ml, 40 µg/ml, 56 µg/ml) chlorocresol (1.2 µg/ml, 2 µg/ml, 2.8 µg/ml) was added to pre quantified sample solution to achieve the 80%, 100%, 120% levels These samples were examined by injecting the sample solution and recovery was calculated **Table 5.**

TABLE 5: RESULT OF ACCURACY AND RECOVERY

Component	Level	Sample added (µg/ml)	Standard added (µg/ml)	Total amount (µg/ml)	Peak area	Recovery (µg/ml)	% Recovery
Betamethasone dipropionate	80%	1	0.6	1.6	88987	1.59	99.7
	100%	1	1	2	110131	1.97	98.8
	120%	1	1.4	2.4	133611	2.39	99.87
Miconazole nitrate	80%	40	24	64	951461	63.97	99.95
	100%	40	40	80	1186315	79.76	99.7
	120%	40	56	96	1422656	95.65	99.64
Chlorocresol	80%	2	1.2	3.2	137488	3.19	99.69
	100%	2	2	4	170244	3.95	98.97
	120%	2	2.8	4.8	206447	4.79	99.87

CONCLUSION: In the present work, a novel, rapid, isocratic RP HPLC method is developed and validated according to the ICH guidelines for simultaneous estimation of betamethasone dipropionate, miconazole nitrate and chlorocresol for the first time. Using the optimized chromatographic conditions, proper resolution of peaks with acceptable tailing factor and short retention time is observed. In the validation studies, the method is linear with a regression coefficient of more than 0.999 for all three drugs within the tested range.

The % recovery at each spike was found NLT 98% and NMT 102% of added amount indicating accuracy of the method. The method is found to be precise, with % RSD less than 2. The method is found to be robust for the minor deliberate changes done. Thus, the developed method is linear, precise, accurate, robust, simple and can be used for routine quality control analysis of formulations containing the selected ingredients. The RP HPLC method for simultaneous determination of betamethasone dipropionate, miconazole nitrate, and chlorocresol has not yet been reported [6-9]. It can be said that the proposed HPLC method for simultaneous analysis of betamethasone dipropionate, miconazole nitrate, chlorocresol in pharmaceutical dosage forms a good resolution and a short analysis time of 8 min.

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CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

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