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Reversed Phase HPLC Method Development and Simultaneous Determination of Topical Dosage Form

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Abstract: ***A novel, rapid, isocratic, reversed phase HPLC method was developed for simultaneous determination of betamethasone dipropionate, miconazole nitrate, chlorocresol in topical dosage form. The separation was carried out on 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 kept as 1 mL per minute. In this method, UV detector was used with wavelength 235 nm. The retention time of chlorocresol, miconazole nitrate, betamethasone dipropionate was 4.30 min, 5.13 min, 6.49 min, respectively. Linearity was observed for betamethasone dipropionate, miconazole nitrate, chlorocresol in concentration range of 1 to 3 µg/mL, 40 to 120 µg/mL, 2 to 6 µg/mL respectively.***

Keywords: High performance liquid chromatography, Simultaneous determination, Betamethasone dipropionate, Miconazole nitrate, Chlorocresol.

1. Introduction

High performance liquid chromatography (HPLC) was derived from the traditional column chromatography and is one of the most significant tools of analytical chemistry today [1]. HPLC mainly use a column with the packing material (stationary phase), a pump that moves the mobile phase (s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent (s) used. [2] 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, psoriasis [3]. Miconazole is an imidazole antifungal agent used as miconazole base or miconazole nitrate for the treatment of superficial candidiasis and of skin infections dermatophytosis and pityriasis versicolor. Miconazole can be given by mouth in for the treatment of oropharyngeal and intestine candidiasis. Miconazole nitrate is usually applied in the treatment of fungal infections of skin including candidiasis, dermatophytosis, and pityriasis versicolor. It is used for treatment of vaginal candidiasis [4]. Chlorocresol is a bactericide, closely related to carbolic acid. It is used as preservative in pharmaceutical formulations. Preservative systems are important parts of any cream or ointment formulations. Knowing the actual concentration of preservative (s) in different formulations is vital in establishing the shelf-life of a product [5]. The developed method was validated as per International Council for Harmonization (ICH) guidelines [6].

2. Experimental

2.1 Material and Reagent

Reference standard of Miconazole Nitrate was purchase from Sisco Research Lab Pvt. Ltd with purity 98 %. Reference standard of betamethasone dipropionate was procured as gift sample from Zydus Cadilia, Mumbai, India with 99 % purity. Reference standard of chlorocresol was purchase from Sigma Aldrich with 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 local market.

2.2 Apparatus and Chromatographic Conditions

RP HPLC Shimadzu (LC2030) model with “Lab solution” software and double wavelength UV detector was employed in this method. Analytical column used for separation of analyte was prontosil (250 x 4.6 mm x 5 µ) column.

2.3 Preparation of standard solutions

2.3.1 Preparation of standard solution

0.1 g each of betamethasone dipropionate, miconazole nitrate, chlorocresol were accurately weighed and transfer into 100 mLvolumetric flask separately. About 70 mL of solvent acetonitrile was added, sonicated to dissolve and make up the volume with solvent acetonitrile (1000 µg/mL). Final concentration of betamethasone dipropionate, miconazole nitrate, chlorocresol were made to 2 µg/mL, 80 µg/mL, 4 µg/mL respectively by solvent acetonitrile.

2.3.2 Preparation of mixed standard solution

Accurately weighed betamethasone dipropionate, miconazole nitrate, chlorocresol reference standard were dissolved in acetonitrile and diluted stepwise to obtain mixed standard solution of 2 µg/mL, 80 µg/mL, 4 µg/mL of betamethasone dipropionate, miconazole and chlorocresol respectively.

2.3.3 Preparation of sample solution

Accurately weigh the quantity of 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 and mix. Final solution was filtered through 0.45 µm of pore size.

2.3.4 Preparation of 0.05M phosphate buffer (pH 2)

About 6.8 gm of potassium dihydrogen phosphate was accurately weighed and dissolve in 950 ml water. The pH was adjusted to 2 with *ortho* phosphoric acid and the volume was made up to 1000 ml in volumetric flask. The solution was then filtered using 0.45 µ membrane filter.

3. Result and Discussions

3.1 Selection of wavelength

The UV scanning spectrum of betamethasone dipropionate, miconazole nitrate, chlorocresol ranging from 200 to 400 nm reveled that 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 formulation which was much less than miconazole nitrate and chlorocresol, so UV maxima of beclomethasone dipropionate (235 nm) was selected as detection wavelength. (Figure 1).

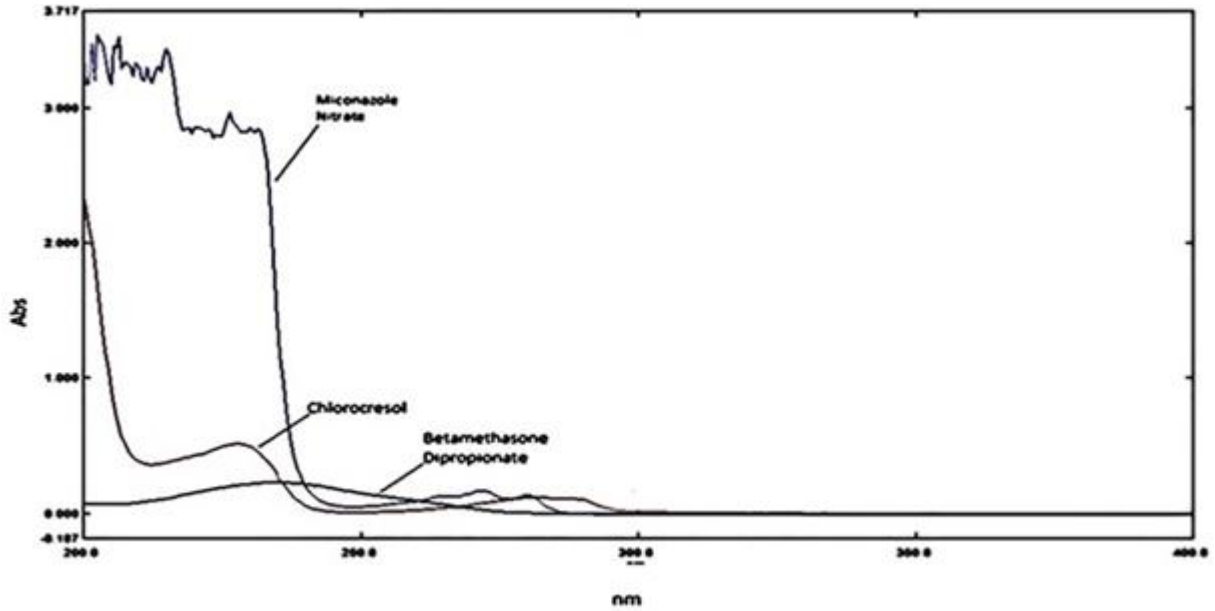


Figure 1: UV overlain spectra of betamethasone dipropionate, miconazole nitrate, chlorocresol

3.2 Method development

To optimize the HPLC methods for drug analysis, a preliminary study with several mobile phase was performed. Parameters such as the ideal mobile phase and their proportion at optimum pH 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, satisfactory result was achieved at acetonitrile: phosphate buffer (70: 30 v/v) with pH 2 adjusted with ortho-phosphoric acid. The optimized chromatographic conditions are shown in Table 1. The chromatogram of standard solution and sample solution are shown in Figure 2 and Figure 3.

Table 1: Optimized chromatographic conditions

Parameters	Optimized condition
Column	Prontosil C18 (250 x 4.6 mm x 5 µ)
Mobile phase	Acetonitrile: phosphate buffer 70: 30 v/v at pH 2 with *ortho*-phosphoric acid.
Flow rate	1.0 mL/min
Run time	8 min
Column temperature	28 ºC
Injection volume	10 µL
Detection wavelength	235 nm
Retention time	4.30 min, 5.13 min, 6.49 min

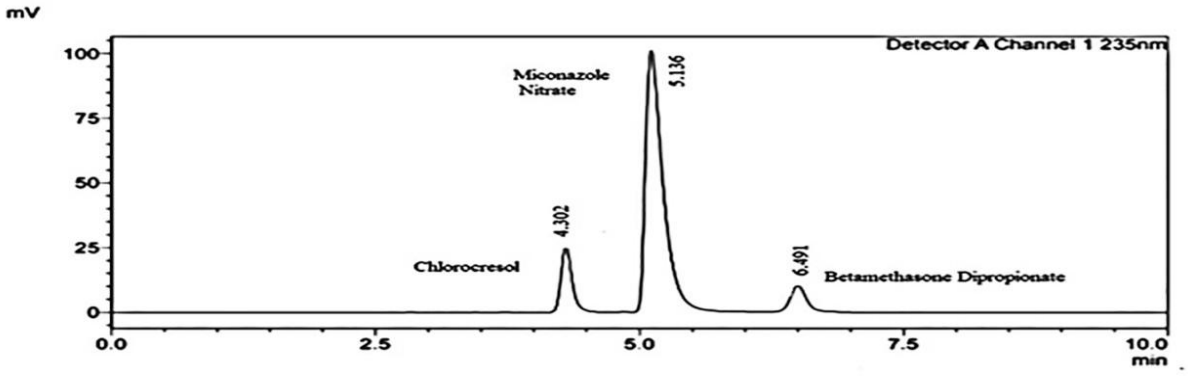


Figure 2: HPLC chromatogram for standard solution

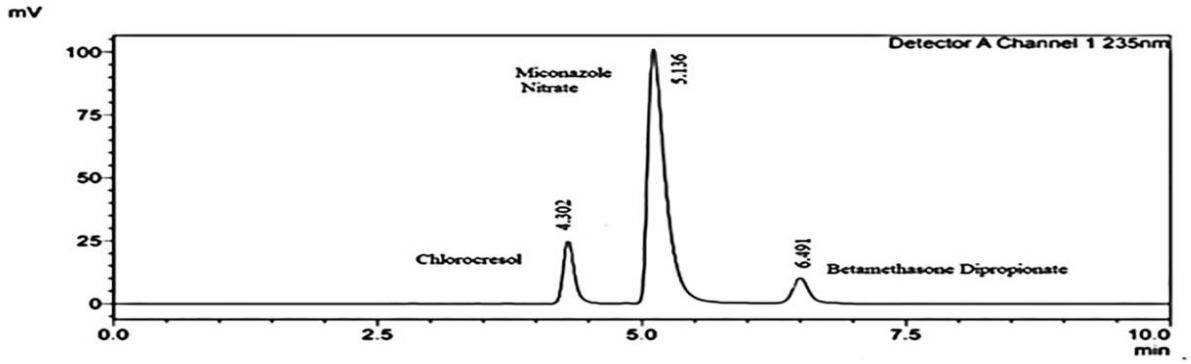


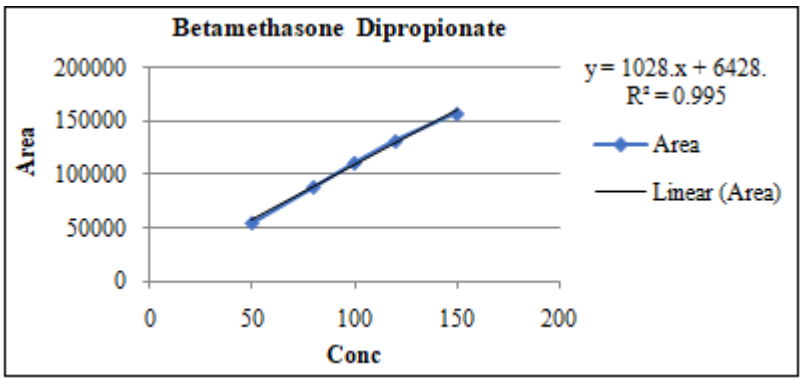
Figure 3: HPLC chromatogram of sample solution

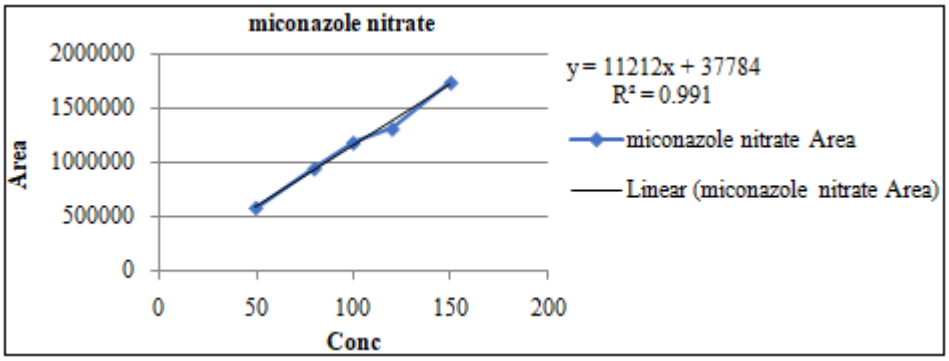
3.3 Linearity

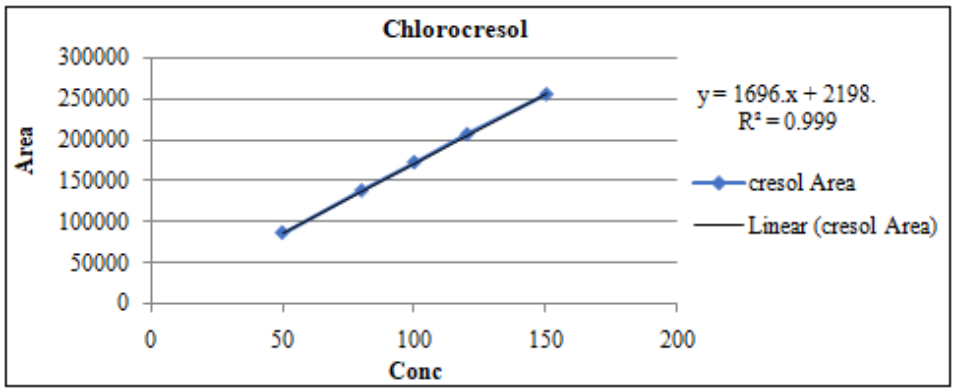
Calibration curves were constructed by plotting the peak areas (Y) versus concentration with the result level shown in the table. The correlation coefficient were greater than 0.99 for each marker, which meet the method validation acceptance critical and hence method is said to be linear (Table **2**).

Table 2: Linearity of mixed standard solution

Compound	Regression equation	R^2	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







3.4 Specificity

The chromatographic behaviors of betamethasone dipropionate, miconazole nitrate and chlorocresol were out of interference by another compound. The complete separation of 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 sample solution were same, so the method was found to be specific.

3.5 Precision

3.5.1 System precision

Six replicate injection of standard solutions at working concentration showed percent relative standard deviation (%RSD) less than 2 concern the area for each marker, which indicate the acceptable reproducibility and thereby system precision (

Table ***3***).

Table 3: Result for system precision

	Peak area		
Sr No.	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

3.5.2 Method precision

Table 4: 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

Method precision was determined by performing the analysis of six replicas of the sample under the test of repeatability at working concentration. The % RSD of peak areas was less than 2 which indicate acceptable reproducibility and there by method precision

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3.6 Robustness

Table 5: 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 1.1mL	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

To determine the robustness of developed method, experimental conditions were deliberately altered, and the system suitability parameter tailing factor and peak area were evaluated. The solution was prepared as per the sample preparation test method describe 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 % RSD was found to be within limit and therefore method was robust (Table 5).

3.7 Accuracy

Accuracy was determined by means of recovery experiments, using standard addition method at three different levels (80 %, 100 %, 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) were added to pre quantified sample solution to reach the 80 %, 100 %, 120 % levels These samples were analyzed by injecting the sample solution and recovery was calculated (

Table *6*).

Table 6: 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

4. Conclusion

The RP HPLC method for simultaneous determination of betamethasone dipropionate, miconazole nitrate, chlorocresol has not yet been reported [6] [7] [8] [9]. Its can be concluded that the proposed HPLC method is sensitive, accurate, precise, robust and reproducible in simultaneous analysis of betamethasone dipropionate, miconazole nitrate, chlorocresol in pharmaceutical dosage forms with the good resolution and a short analysis time of 8 min.

List of Abbreviation

APIs: Active Pharmaceutical ingredients; ICH: International Council for Harmonization; RP-HPLC: Reversed-phase high-performance liquid chromatography; RSD: Relative standard deviation; UV: Ultraviolet.

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