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Research Article

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Development and validation of analytical method for simultaneous estimation of Betamethasone dipropionate, Tolnaftate, Iodochlorhydroxyquin in pharmaceutical dosage form.

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Abstract A simple, accurate and precise RP-HPLC method was developed for simultaneous estimation of Betamethasone dipropionate, Tolnaftate and Iodochlorhydroxyquin. EclipseC_{18} (250mm×4.6mm) 5µ (particle size) was used as stationary phase. The mobile phase used was KH_2PO_4 buffer of pH 6.3: Acetonitrile 30:70 V/V. The mobile phase was delivered at flow rate 1.5 ml/min. UV detection was set at 254nm. The retention time of Betamethasone dipropionate, tolnaftate and Iodochlorhydroxyquin was found to be 5.2 minutes, 9.8 and 7.2 minutes respectively. Linearity was observed over the concentration range of 3.9-9.1µg/ml, 60-140 µg/ml and 60-140 µg/ml for Betamethasone dipropionate, Tolnaftate and Iodochlorhydroxyquin respectively. The LOD was found to be 0.44 µg/ml, 6.49 µg/ml and 4.64µg/ml for Betamethasone dipropionate, Tolnaftate and Iodochlorhydroxyquin respectively. Whereas LOQ was found to be 1.34 µg/ml,19.67 µg/ml and 14.08 µg/ml for Betamethasone dipropionate ,Tolnaftate and Iodochlorhydroxyquin respectively Moreover, the % RSD for repeatability, inter and intraday precision was found to be less than 2%, which reveals that the method is precise. The % recovery was found to be 100.84% for betamethasone dipropionate, 101.11% for tolnaftate and for 100.53% Iodochlorhydroxyquin. However, the change in flow rate and mobile phase ratio also did not show any significant variance. Assay of the combined dosage form finalized the applicability of this method for simultaneous estimation of Betamethasone dipropionate, Tolnaftate and Iodochlorhydroxyquin in combined dosage form.

Keywords RP-HPLC, betamethasone dipropionate, tolnaftate, iodochlorhydroxyquin

Introduction

Betamethasone dipropionate is glucocorticoids used for its anti-inflammatory or immunosuppressive properties. It is glucocorticoid agonist. Tolnaftate is synthetic over the counter anti-fungal agent used as topical fungicide. Iodochlorhydroxyquin is a broad-spectrum antibacterial with antifungal properties. From the literature survey, it was found that there are methods available for combination of Betamethasone dipropionate and dexamethasone, betamethasone and tolnaftate, salicylic acid and tolnaftate, betamethasone valarate and clioquinol [1-3].

The aim of this project work is to development and validation of analytical method for estimation of Betamethasone dipropionate, Tolnaftate and Iodochlorhydroxyquin in Pharmacetical dosage form [4-6].

Materials and Methods

Equipment

Chromatographic separation was performed on HPLC System – LC-2010 Shimadzu, Japan PDA detector equipped with a solvent delivery pump, sample injector and column thermostat. Empower system software was applied for data collecting and processing.

Chemicals and Reagent

1. Acetonitrile (HPLC Grade)
2. Water (HPLC Grade)
3. Potassium dihydrogen phosphate
4. Methanol (HPLC Grade)

Amiderm cream was procured from local market. Reference standard of Betamethasone dipropionate, Tolnaftate and Iodochlorhydroxyquin were obtained from Accuprec Laboratory.

Preparation of Solutions

Preparation of Mobile phase solution (pH 6.3)

Potassium dihydrogen Phosphate, 0.680 gm, was dissolved in 250 ml of water (20mM) and the pH was adjusted to 6.3 with 1M Potassium hydroxide. The mixture of Potassium dihydrogen Phosphate and Acetonitrile was then taken in 30:70, v/v ratio.

Mobile phase preparation

Selected solvents were mixed in required proportions either with water, other solvents or buffer and sonicated for specified time and then filtered through 0.45µ Whatmann filter paper for further use.

Preparation of standard stock solution of API

Accurately weighed quantity of Betamethasone dipropionate,tolnaftate and Iodochlorhydroxy10 mg was transferred into 10 ml volumetric flask containing methanol (1000 µg/ml), take 1 ml from 1000µg/ml dissolved and diluted in 10 ml of methanol up to mark with methanol (100 µg/ml).

Preparation of standard of binary mixture

Standard stock solution of Betamethasone Dipropionate, Tolnafate and iodochlorhydroxyquin 100µg/ml were prerpared. After the preparation of stock solution take 0.65ml betamethasone, 1ml tolnaftate and 1ml iodochlorhydroxyquin were taken and diluted up to 10 ml of methanol.

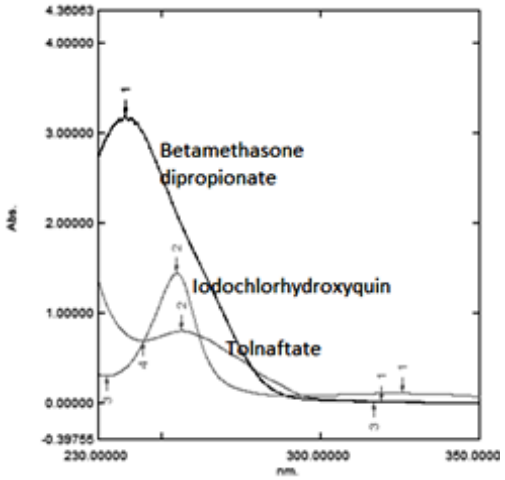

Figure 1: *Selection of Wavelength*

Results and discussion

Selection of wavelength

Standard solution of Betamethasone dipropionate (10 µg/ml), Tolnaftate (10 µg/ml), and iodochlorhydroxyquin (10 µg/ml) were scanned between 200-400 nm using UV-visible spectrophotometer. All solutions were scanned between 200 - 400 nm. Wavelength (254 nm) was selected for further method development.

Linearity

The linearity for Betamethasone dipropionate, Tolnafate and Iodochlorhydroxyquin were assessed by analysis of combined standard solution in range of 3.9-9.1 µg/ml,60-140 and 60-140 µg/ml, respectively.

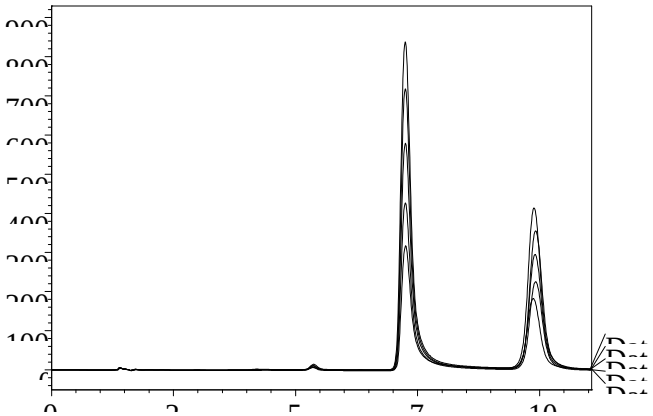

Figure 2*: Chromatogram for calibration curve of Betamethasone dipropionate, Tolnaftate and Iodochlorhydroxyquin*

Table 1: Linearity data for Betamethasone dipropionate, Tolnaftate and Iodchlorhydroxyquin

S No	Concentration (µg/mL)			Area (n=3)		
	BMD	**TLF**	**IOD**	**BMD**	**TLF**	**IOD**
1.	3.9	60	60	55943.33	3449913	4428085
2.	5.2	80	80	75480.67	4378600	6087046
3.	6.5	100	100	95431.67	5671479	7649999
4.	7.8	120	120	113130.7	6879485	9644577
5.	9.1	140	140	138343.3	7852930	11196627

Table 2: Statistical data* for Betamethasone Dipropionate, Tolnaftate and Iodochlorhydroxyquin by HPLC method

Parameters	Results		
	BMD	TLF	IHQ
Linear Range(µg/ml)	3.9-9.1	60-140	60-140
Slope	15573.07	56534.59	85473.06
Intercept	-5559.06	-6978.46	-746040.16
Limit of Detection (µg/ml)	0.44	6.49	4.64
Limit of Quantitation (µg/ml)	1.34	19.67	14.08

Precision: The precision of this method is determined by Intraday and Interday precision. The % RSD was found less than 2, this indicate that the method is precise. The results of precision study are shown in Table 3.

Table 3: precision of RP-HPLC

Precision	BMD	TLF	IHQ
Intraday	0.77-1.79	0.28-1.15	0.51-1.10
Interday	1.23-1.80	0.32-0.97	0.37-1.48

Limit of Detection and Limit of Quantification (LOD and LOQ)

The sensitivity of method is described in terms of LOD and LOQ. LOD and LOQ values for Betamethasone dipropionate were found to be 0.44µg/ml and1.34 µg/ml, Tolnaftate LOD and LOQ were found to be 6.49 µg/ml and 19.57µg/ml and Iodochlorhydroxyquin 4.64µg/ml and 14.08 µg/ml.

Accuracy

The accuracy was evaluated by recovery of Betamethasone dipropionate, Tolnaftate and Iodochlorhydroxyquin at three different levels (80, 100 and 120). %RSD was found to be less than 2, ensuring that the method is accurate. The result of accuracy are shown in table 4.

Table 4: Accuracy of Betamethasone dipropionate, Tolnaftate and Iodochlorhydroxyquin

S. No.	Formulation	Level of spiking	Average area	% recovery	%RSD
1.	**BMD**	80%	94124	102.01	1.05
2		100%	114072	101.83	1.41
3.		120%	136752	98.68	0.51
1.	**TLF**	80%	4602301	100.11	1.22
2.		100%	5813477	101.16	0.92
3.		120%	7004765	102.06	1.34
1.	**IHQ**	80%	6598832	100.29	1.11
2.		100%	8089732	98.36	0.50
3.		120%	10160647	102.95	1.01

Repeatability

The experimental value obtained for the repeatability of BMD, TLF and IHQ in sample is present in Table 5. The result obtained shows %RSD less than 2, indicating good repeatability of method.

Table 5: Repeatability of Betamethasone dipropionate, Tolnaftate and Iodochlorhydroxyquin

Parameter	Area		
	BMD	**TLF**	**IHQ**
AVERAGE	104490.2	5911624	8135525
SD	1916.25	32579.29	40734.44
% RSD	1.83	0.55	0.50

Robustness

Robustness of the method was carried out by deliberately made small change in flow rate of Mobile Phase and Mobile phase composition. The results are shown in table 6.

Specificity

Specificity was observed that diluents did not interfere with detection of Betamethasone dipropionate, Tolnaftate and Iodochlorhydroxyquin.

Table 6: Result of Robustness Study

Parameter	Area		
	BMD	**TLF**	**IHQ**
AVERAGE	104490.2	5911624	8135525
SD	1916.25	32579.29	40734.44
% RSD	1.83	0.55	0.50

Label claim recoveries from tablets

The proposed method was evaluated in the assay of commercially available tablet containing BMD (0.643), TLF (10 mg) and IHQ (10 mg). Three replicated determination were carried out on and accurately weighted amount of tablet equivalent to BMD (0.643), TLF (10 mg) and IHQ (10 mg). The result of label claim studies is shown in table 7.

Table 7: Result of essay of Tablet formulation

	Label Claim	**Avg %Assay**	**SD**	**%RSD**
BMD	0.64mg	99.31	1329.60	1.25
TLF	10 mg	100.93	21379.6	0.36
IHQ	10 mg	98.06	53196.83	0.65

Conclusion

The developed RP-HPLC Method was accurate, precise, reproducible and robust. It can be used as simultaneous determination of BMD, TLF and IHQ in pharmaceutical dosage form. The method was validated as per ICH Guideline [7].

References

1. Skoog D.A, West D.M and Holler D.F; Fundamentals of Analytical Chemistry; 8th Edn, Saunders College Publishing, 2004, pp 973-992.
2. Sharma B.K, Instrumental Methods of Chemical Analysis; 25th Ed; GOEL Publication House, Meerut, pp 286-385.
3. Betamethasone dipropionate "Drug profile",Oct2014 ,http://www.drugbank.ca/drugs
4. Tolnaftate"Drugprofile",Oct2014, http://www.drugbank.ca/drugs
5. Clioquinol"Drugprofile",Oct2014, http://www.drugbank.ca/drugs
6. Indian Pharmacopoeia–2007, Government of India, Ministry of Health and Family Welfare, Published by: IPC Ghaziabad, Volume 3, page no. 1641
7. ICH guideline Q2B (R1), Validation of analytical procedure methodology, International conference on Harmonization, IFPMA, Geneva.