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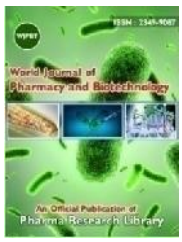

Research Article **Open Access**

Analytical Method Development and Validation for the Simultaneous Estimation of Beclomethasone Dipropionate and Clotrimazole by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Beclomethasone dipropionate and Clotrimazole by using column C18 [Macherey-Nagel Nucleosil], 4.6x250mm, 5 microns flow rate was 1.2 ml/min, mobile phase ratio was Phosphate buffer: Methanol (30:70 v/v), detection wavelength was 254 nm. The Spectroscopic method was done in solvent using methanol and the instrument used was DIONEX HPLC system. UV D340U detector. The retention times for Beclomethasone dipropionate and Clotrimazole were found to be 9.5 min and 14.5 min. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Bclomethasone dipropionate and Clotrimazole was found in the concentration range 6.25-18.75 mcg/ml and 250-750 mcg/ml and correlation coefficient (r^2) was found to be 0.999 and 0.999 respectively, % recovery was found to be 99.44 to 100.20 and 99.75 to 100.05% respectively. %RSD for repeatability and precision was found to be <2. LOD values for Beclomethasone dipropionate and Clotrimazole were found to be within the limits and LOQ values for Beclomethasone dipropionate, Clotrimazole were found to be within the limits respectively. .

Keywords: Beclomethasone dipropionate, Clotrimazole, HPLC

A R T I C L E I N F O

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1. Introduction

Analytical methods: Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available

Description of the Various Analytical Methods

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [2, 3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements. Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [5, 6].

Chromatography:

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate [7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC [9].

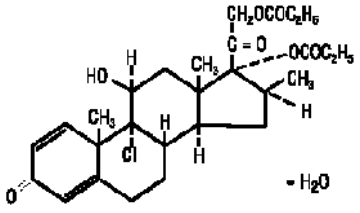

Figure 1: Beclomethasone dipropionate

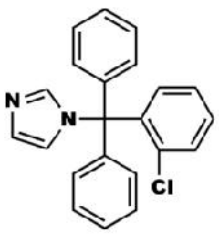

Figure 2: Clotrimazole

2. Materials and Methods

Apparatus

The instrument used for the study was DIONEX HPLC system Chromatographic system. UV D340 U photo diode array detector.

Reagents and Materials

Methanol of HPLC grade, and Di-sodium hydrogen orthophosphate by Merck Chemicals and S.D. Fine chemicals, Water HPLC grade from Milli-Q RO system was used [10].

Selection of detection wavelength:

The sensitivity of method that uses UV- Vis detector depends upon the proper selection of wavelength. An ideal wavelength is that gives maximum absorbance and good response for both the drugs to be detected [11]. Standard solutions of Beclomethasone dipropionate and clotrimazole were scanned in the UV range (200-400nm) and the spectrums obtained were overlaid and the overlain spectrum was recorded. From the overlain spectrum, 254 nm was selected as the detection wavelength for the present study [12].

Selection of mobile phase

Initially the mobile phase tried was methanol and water, methanol and Methanol, buffer and water in various proportions. Finally, the mobile phase was optimized to Buffer: Methanol in proportion 30:70 v/v respectively [13].

Optimization Chromatographic trials for Simultaneous Estimation of Beclomethasone dipropionate and Clotrimazole by RP- HPLC.

Optimization Chromatographic conditions

Column : C18 [Macherey-Nagel Nucleosil], 4.6x250mm, 5 microns.
Mobile phase ratio: Phosphate buffer: Methanol (30:70 v/v)
Detection wavelength: 254 nm
Flow rate : 1.2ml/min
Injection volume : 20µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 15 min
Retention time : 9.5 and 14.5 mins

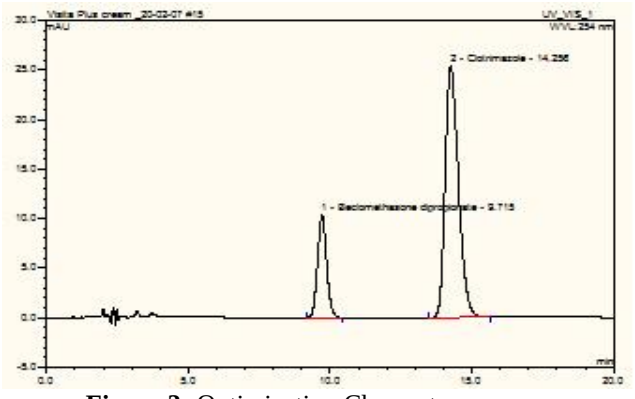

Figure 3: Optimization Chromatogram

Observation: The separation of two analytical peaks was good. The plate count also above 2000, tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method.

Procedure

Preparation of phosphate buffer

Dissolve 6.6g of Di-sodium hydrogen orthophosphate into a 1000ml of water and mix it. It was filtered through 0.45µm nylon membrane filter and degassed with sonicator. It was used as a diluent for the preparation of sample and standard solution [14].

Preparation of mobile phase

Mobile phase consist of buffer: Methanol (30:70) was taken sonicated and degassed for 10min and filtered through 0.45 µm nylon membrane filter [15].

Beclomethasone dipropionate & Clotrimazole standard preparations

Solution 1:

Weigh accurately about 50mg of Beclomethasone Dipropionate WS into a 200ml volumetric flask. Add 100ml of methanol. Shake to dissolve [16]. Make upto volume with methanol.

Solution 2:

Weigh accurately about 50mg of clotrimazole WS into a 100ml volumetric flask. Add 40ml of methanol [17]. Shake to dissolve. Add 5ml of solution 1.Make upto volume with methanol and mix it [18].

Sample solutions preparation

Weigh accurately about 5 g of sample into a 100ml volumetric flask. Add 50ml of methanol. Warm on a boiling water bath for 5 minutes and sonicate for 15 minutes [19, 20]. Cool the content, make up to volume with methanol. Filter the solution through Whatman No.42 filter paper [21]]. Discard first 5ml of filtarte. Use this solution as sample. Inject Standard solution 2 five times and calculate the % rsd [22]. It should not be more than 2 %.Inject standard and sample solution and record the chromatograms

3. Results and Discussion

Method Validation Parameters

1. Specificity

Weigh accurately about 5 g of sample into a 100ml volumetric flask. Add 50ml of methanol. Warm on a boiling water bath for 5 minutes and sonicate for 15 minutes. Cool the content, make up to volume with methanol. Filter the solution through Whatman No.42 filter paper. Discard first 5ml of filtarte. Use this solution as sample. Inject Standard solution 2 five times and calculate the % rsd. It should not be more than 2 %.Inject standard and sample solution and record the chromatogram [24, 25].

2. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Serial dilutions of Amlodipine and Perindopril (6.25-18.75µg/ml and 250-750 µg/ml) were injected into the column and detected at a wavelength set at 254 nm. The calibration curve was obtained by plotting the concentration vs. peak area [23]

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range: Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 6.25µg/ml-18.75µg/ml and 250µg/ml to 750µg/ml of Beclomethasone dipropionate and Clotrimazole respectively.

4. Accuracy

Spiked Placebo Method

The accuracy of the Method is determined by % recovery data obtained by spiking the placebo with 80, 90,100,110 and 120 % of the declared content of the active ingredients and analyzing according to the test procedure. At each of the concentration level three replicate samples has been analyzed. Hence the total number of samples used was fifteen.

5. Precision

System Precision

The system precision is determined by analyzing replicates [ten replicates] of the Standard Solution of Median Concentration of the Range [50-150%] of the theoretical quantity of analytes] under similar conditions of operation and composition and analyzing as per the test procedure.

Method precision:

The Method Precision is determined by using six standard solutions [not replicates from same solution] at the median concentration used for checking the Linearity of the Method and analyzing as per the test procedure.

Validation of the method

Linearity

The linearity study was performed for the concentration of 6. 25ppm to 18.75 for Beclomethasone dipropionate and 250ppm to 750ppm for Clotrimazole and chromatograms are shown below.

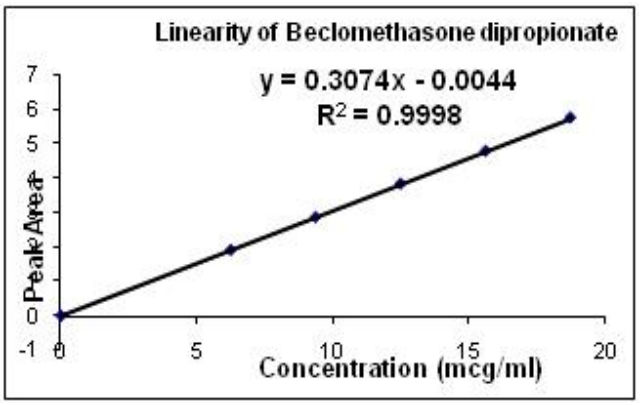

Figure 4: Linearity graph of Beclomethasone dipropionate

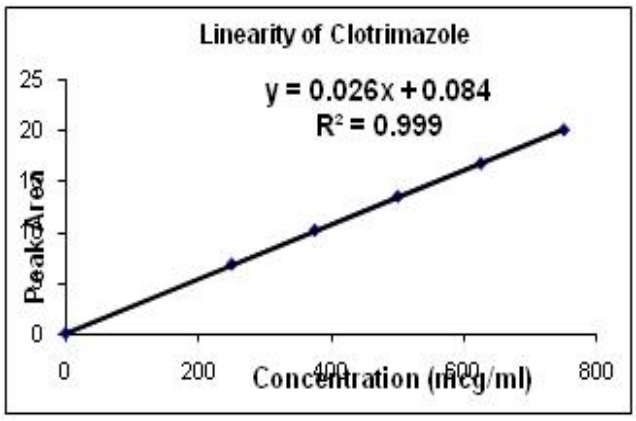

Figure 5: Linearity graph of Clotrimazole

Table 1: Data for Determination of Specificity of Method for Beclomethasone

Conc. of Placebo	Conc.* of Beclometha-sone [Added] [mcg/ml]	Conc.* of Beclometha-sone [Recovered] [mcg/ml]	% Recovery	Mean	Standard Deviation	% Relative Standard Deviation
80%	12.50	12.49 12.48 12.51	99.92 99.84 100.08	99.95	0.122	0.122
90%	12.47	12.48 12.46 12.45	100.08 99.92 99.84	99.95	0.122	0.123
100%	12.48	12.48 12.50 12.47	100.00 100.16 99.92	100.03	0.122	0.122
110%	12.49	12.51 12.50 12.48	100.16 100.16 99.92	100.08	0.139	0.139
120%	12.51	12.50 12.49 12.52	99.76 99.92 99.84	99.84	0.080	0.080

Table 2: Data for Determination of Specificity of Method for Clotrimazole

Conc. of Placebo	Conc.* of Clotrimazole [Added] [mcg/ml]	Conc.* of Clotrimazole [Recovered] [mcg/ml]	% Recovery	Mean	Standard Deviation	% Relative Standard Deviation
80%	500	498.25 499.32 499.21	99.65 99.86 99.84	99.78	0.12	0.12
90%	501	500.14 501.45 500.87	99.82 100.08 99.97	99.96	0.13	0.13
100%	500	501.20 496.85 505.12	100.24 99.37 101.02	100.21	0.83	0.82

110%	501	500.25 501.32 501.10	99.85 100.06 100.01	99.97	0.11	0.11
120%	502	502.21 501.99 502.33	100.04 99.98 100.06	100.03	0.04	0.04

Table 3: Calibration data of Beclomethasone dipropionate and Clotrimazole

Analyte	Day	Regression Equation	Slope	Intercept	Correlation Coefficient
Beclomethasone Dipropionate	I	y = 0.3074x - 0.0044	**0.3074**	**0.0044**	**0.9998**
	II	y = 0.3073x - 0.0055	**0.3073**	**0.0055**	**0.9998**
	III	y = 0.3073x - 0.0029	**0.3073**	**0.0029**	**0.9999**
Clotrimazole	I	y = 0.0268x + 0.843	**0.0268**	**0.0843**	**0.9999**
	II	y = 0.0268x +0.0844	**0.0268**	**0.0844**	**0.9999**
	III	y = 0.0268x +0.0843	**0.0268**	**0.0843**	**0.9999**

Recovery studies: Sample solutions at different concentrations were prepared and the % recovery was calculated.

Table 4: Data for Determination of Accuracy of Method by Spiked Placebo Method for Beclomethasone Dipropionate Content:

Beclomethasone Conc.	Conc.* of Beclometh-asone [Added] [mcg/ml]	Conc.* of Beclometha-sone [Recovered] [mcg/ml]	% Recovery	Mean	Standard Deviation	% Relative Standard Deviation
80%	10.00	9.99 10.01 10.02	99.90 100.10 100.20	**100.07**	**0.153**	**0.153**
90%	11.25	11.23 11.21 11.26	99.82 99.64 100.09	**99.85**	**0.224**	**0.224**
100%	12.50	12.43 12.45 12.49	99.44 99.60 99.92	**99.65**	**0.244**	**0.245**
110%	13.75	13.70 13.71 13.76	99.64 99.71 100.07	**99.81**	**0.234**	**0.234**
120%	15.00	14.99 15.01 15.02	99.93 100.07 100.13	100.04	0.102	0.102

Theoretical Concentration 100% is taken to be 12.50 mcg per ml.

Table 5: Data for Determination of Accuracy of Method by Spiked Placebo Method for Clotrimazole Content

Conc.* of Clotrimazole [Added] [mcg/ml]	Clotrimazole Conc.	Conc.* of Clotrimazole [Recovered] [mcg/ml]	% Recovery	Mean	Standard Deviation	% Relative Standard Deviation
400	80%	399.01 400.10 400.20	99.75 100.03 100.05	**99.94**	**0.165**	**0.165**

450	90%	450.01 450.21 449.24	100.00 100.05 99.83	**99.96**	**0.114**	**0.114**
500	100%	500.12 500.24 499.12	100.02 100.05 99.82	**99.97**	**0.123**	**0.123**
550	110%	550.25 550.10 550.05	100.05 100.02 100.01	**100.02**	**0.019**	**0.019**
600	120%	600.20 600.14 600.07	100.03 100.02 100.01	**100.02**	**0.011**	**0.011**

***** Theoretical Concentration 100% is taken to be 500 mcg per ml

Table 6: Data for Repeatability of the Method with mobile phase composition variation: Beclomethasone dipropionate content

Volume of Methanol	Conc. of Beclomethasone [Added] [mcg/ml]	Conc. of Beclomethasone [Recovered] [mcg/ml]	% Recovery	Mean	Relative Standard Deviation	% Relative Standard Deviation
68 %	12.50	12.54	100.32	**100.08**	**0.438**	**0.438**
		12.46	99.68			
		12.45	99.60			
		12.58	100.64			
		12.52	100.16			
70%	12.50	12.59	100.72	**100.336**	**0.613**	**0.611**
		12.48	99.84			
		12.52	100.16			
		12.47	99.76			
		12.65	101.20			
72%	12.50	12.61	100.88	**100.16**	**0.557**	**0.556**
		12.58	100.64			
		12.48	99.84			
		12.47	99.76			
		12.46	99.68			

Table 7: Data for Repeatability of the Method with mobile phase composition variation Clotrimazole Content

Volume of Methanol	Conc. of Clotrimazole [Added] [mcg/ml]	Conc. of Clotrimazole [Recovered] [mcg/ml]	% Recovery	Mean	Relative Standard Deviation	% Relative Standard Deviation
68 %	500	505.31	101.06	**99.35**	**1.136**	**1.144**
		499.26	99.85			
		495.32	99.06			
		492.60	98.52			
		491.25	98.25			
70%	500	492.35	98.47	**100.28**	**1.164**	**1.161**
		506.15	101.23			
		506.23	101.25			
		503.14	100.63			
		499.23	99.85			

72%	500	497.96	99.59	**99.96**	**0.525**	**0.526**
		496.46	99.29			
		500.45	100.09			
		503.11	100.62			
		501.10	100.22			

Table 8: Data for System Precision of the Method

Analytes Name	Peak Area*	Mean	Standard Deviation**	% Relative Standard Deviation
Beclomethasone dipropionate	3.8613 3.8951 3.9332 3.8601 3.9057 3.9776 3.8873 3.9112 3.8765 3.8783	**3.8986**	**0.036**	**0.919**
Clotrimazole	13.4992 13.8313 13.7213 13.7668 13.7568 13.8212 13.7654 13.7687 13.7698 13.8765	**13.7577**	**0.101**	**0.735**

4. Conclusion

The multi-drug therapy is an ancient phenomenon to combat interrelated symptoms of diseased status of human beings. Since it ensure timely and complete medication for disorder and it has patient compliance, as it reduces the number of formulations to be taken at a time. Therefore, the pharmaceutical formulations with combinations of drugs have shown an increasing trend to counteract other symptoms specific to one drug n formulation, and hence analytical chemist will have to accept the challenge of developing reliable methods for analysis of drugs in such formulation. Simultaneous analysis procedures are now being used more frequently for estimation of drugs in multi-component pharmaceutical formulations due to their inherent advantages viz. avoid time consuming extraction and separation, economical in the sense that use of expensive regents is minimized are equally accurate and precise. For the estimation of multi-component formulation, the instrumental techniques, which are commonly employed, are spectrophotometery, GLC, high performance thin layer chromatography (HPTLC), HPLC etc. The validation of methods has to validate by using same parameters as per ICH guidelines. A HPLC method is developed for the simultaneous estimation of Beclomethasone dipropionate and Clotrimazole in combination using instrument High performance chromatograph. Dionex HPLC system is being used, having UV D340U detector fitted with injector and column C18 [Macherey-Nagel Nucleosil], 4.6x250mm, 5 microns. Injection volume of 20 µl is injected and eluted with the mobile phase of 30-volume of0.66% di soddium ortho phosphate and 70 volume of methanol at the flow rate of 1.2 ml/min and detected by UV detector at 254nm. The peaks of Beclomethasone dipropionate and Clotrimazole are found well separated at 9.5 min and 14.5 min respectively. The developed method is validated for various parameters as per ICH guidelines like accuracy, precision, linearity, specificity, ruggedness and robustness. The results obtained are within the acceptance criteria. The proposed method is applied for determination of Beclomethasone dipropionate and Clotrimazole in marketed formulations. The assay results confirmed with the label claim of the formulation. Hence the proposed method is found to be satisfactory and could be used for the routine analysis of Beclomethasone dipropionate and Clotrimazole in their formulations.

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