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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR SIMULTANEOU ESTIMATION OF BEMOTRIZINOL AND BISOCTRIZOLE IN CREAM DOSAGE FORM

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ABSTRACT

Bemotrizinol and Bisoctrizole is used as Sunscreen active ingredient. Sunscreen has been proven to decrease the development of skin cancer. It helps to prevent facial brown spots and skin discolorations. The present work was aim to develop and validate RP-HPLC method for the estimation of Bemotrizinol and Bisoctrizole in cream dosage form using Syncronis C18 (250mm × 4.6 mm) (5µm) as column and flow rate 1.2 ml/min. The whole mobile phase consist of 1,4 Dioxane: Ammonium Acetate buffer (80:20 v/v) and detection was carried out at 340 nm. The retention time for Bemotrizinol was 14.23 min and Bisoctrizole 11.00 min. The linearity range was found to be 10-30 µg/ml (r2=0.9995) for Bisoctrizole and 20-100 µg/ml (r2 =0.9984) for Bemotriznol. The limits of detection for Bemotrizinol and Bisoctrizole were 0.18 and 0.64 µg/mL, respectively. The limits of quantification for Bemotrizinol and Bisoctrizole were 0.55 and 1.93 µg/mL, respectively. The assay was found within the range of 98-102%. A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for the simultaneous estimation of Bemotrizinol and Bisoctrizole. The method was successfully applied for the estimation of Bemotrizinol and Bisoctrizole in cream dosage form.

Keywords: Bemotrizinol, Bisoctrizole, Validation, RP-HPLC

INTRODUCTION:

Bemotrizinol is an oil-soluble organic compound that is added to sunscreens to absorb UV rays. Bemotrizinol is a broad-spectrum UV absorber, absorbing UVB as well as UVA rays. It has two absorption peaks, 310 and 340 nm **[1, 2]**. It is highly photostable. Even after 50 MEDs (minimal erythemal doses), 98.4% remains intact. It helps prevent the photodegradation of other sunscreen actives like avobenzone. Bemotrizinol has strong synergistic effects on the SPF when formulated with bisoctrizole, ethylhexyl triazone or iscotrizinol **[3]**. It is the most effective UV absorber available measured by SPF, based on the maximum concentration permitted by European legislation. Bemotrizinol is not approved by the United States Food and Drug Administration, but is approved in the European Union since the year 2000 and other parts of the world, including Australia. Unlike some other organic sunscreen actives, it shows no estrogenic effects in vitro **[4]**.

Bisoctrizole is a benzotriazole based organic compound that is added to sunscreens to absorb UV rays. Bisoctrizole is a broad-spectrum ultraviolet radiation absorber, absorbing UVB as well as UVA rays. It also reflects and scatters UV **[5]**. Bisoctrizole is a hybrid UV absorber, the only organic UV filter produced in microfine organic particles (< 200 nm), like microfine zinc oxide and titanium dioxide. Where other organic UV absorbers need to be dissolved in either the oil or water phase, bisoctrizole dissolves poorly in both. Bisoctrizole is added to the water phase of a sunscreen as a 50% suspension, whereas mineral micro pigments are usually added to the oil phase **[6, 7]**. The bisoctrizole particles are stabilized by the surfactant decylglucoside. Bisoctrizole shows very little photodegradation and has a stabilizing effect on other UV absorbers, octyl methoxycinnamate (octinoxate) in particular. When formulated into a sunscreen, bisoctrizole has minimal skin penetration **[8]**. Unlike some other organic sunscreen actives, it shows no estrogenic effects in vitro **[9]**. Bisoctrizole is not approved by the U.S. Food and Drug Administration (FDA), but is approved in the EU and other parts of the world **[10]**.

Literature reviews revels that several individual analytical methods are available for Bemotrizinol and Bisoctrizole individually or in combination with other drugs but no method has been reported for simultaneous estimation of Bemotrizinol and Bisoctrizole by RP-HPLC and UV. So, purpose of described method is to develop and validate a simple, accurate and precise analytical

method for simultaneous estimation of Bemotrizinol and Bisoctrizole by RP-HPLC.

MATERIALS AND METHODS

Instrumentation:

Integrated high performance liquid chromatographic systems LC- 2010AHT from Shimadzu Corporation (Chromatographic and Spectrophotometric consisted of a binary gradient system, a high speed UV–vis detector. BioSepSECS2000, 300mm7.8 mm analytical column. Chromato- grams were recorded and integrated on PC installed with LC solution chromatographic software,

Reagent and chemicals:

Bemotrizinol and Bisoctrizole was obtained from ARK Chemicals, Mumbai. Solvent 1,4-dioxane, methanol was obtained from sigma Aldrich.

Chromatographic condition: Gradient mobile phase consisted of a mixture of 1,4-dioxane:Ammonium Acetate Buffer pH-3 (80:20% v/v) at 340 nm. The mobile phase was filtered and degassed through membrane filter of 0.45 µm porosity under vacuum. A constant flow rate of l.0 mL/min was employed throughout the analysis. Variable UV–vis detector wavelength was set at 340 nm. All pertinent analyses were made at 25 1C and volume of solution injected on to the column was 10 µL.

Solution Preparation:

Preparation of standard stock solution:

Take 10mg of drug and dilute up to the 100ml with 1, 4-Dioxane in 100ml volumetric flask (100µg/ml).

Preparation of working solution:

From the Stock solution (100µg/ml) takes 1, 2, 3, 4, 5ml of aliquot of stock solution in 10ml volumetric flask and adjusted up to mark with mobile phase give 10, 20,30,40,50 µg/ml solutions. The solution was filtered through0.45 µm membrane filter and 10 µL was injected.

Validation

The developed HPLC method was validated in accordance with ICH (International Council on Harmonization) recommendations Q2 (R2) in terms of accuracy, precision, linearity, limit of detection (LOD), limit of quantitation (LOQ), and solution stability.

Specificity

The ability of analytical method to accurately evaluate the analyte in the presence of excipients, degradants, and contaminants that may be expected to be present is known as specificity **[11]**. By analyzing Bemotrizinol and Bisoctrizole in the presence of excipients including starch, talc, and magnesium stearate, the specificity of the approach was confirmed and excipient interference was observed **[12]**.

Linearity and range

By using the suitable aliquot of the working standard solution in various 10 mL volumetric

flasks, calibration curves were created. To obtain a final concentration of 10-50 µg/ml for Bemotrizinol and in the range of 20-100µg/ml for Bisoctrizole, the volume was adjusted to 10 mL utilizing mobile phase as a solvent. Mean peak area versus concentration were used to plot the calibration curve **[13]**. For each of the components, the regression equation was computed and the correlation coefficients were found.

Precision

The intra-day and inter-day precisions were employed to evaluate precision. By analyzing sample solutions of mixture containing both components at three distinct concentration levels (20, 30 and 40 µg/mL) for Bemotrizinol and (30, 40 and 50 µg/mL) for Bisoctrizole on the same day (n = 3), covering the whole range of the calibration curve (low, medium, and high concentrations), the intra-day precision was established **[14]**. Inter-day accuracy was assessed by examining sample combination solutions at three distinct concentration levels, covering the complete range (low, medium, and high concentrations), over the course of three consecutive days (n = 3) **[15]**. The mean, SD, and relative standard deviation (% RSD) values were calculated using the peak areas that were obtained. The middle concentration of both components was examined six times to assess the repeatability of peak area measurement.

LOD and LOQ

The limits of detection (LOD) and limits of quantitation (LOQ) were used to evaluate the sensitivity of the developed method. For the determination of LOD and LOQ, linearity of the standards was performed three times to obtained standard deviation of the intercept (SD) and

slope of the regression equation (S) value. LOD and LOQ were determined by the standard deviation method and calculated as follows:

Limits of detection = 3.3 × SD/S and Limits of quantitation = 10 × SD/S

Robustness

To observe the impact of such alterations, small intentional changes were made to the chromatographic conditions **[16]**. The effects of changing the flow rate, wavelength, and mobile phase composition by up to ±2% were studied. The method's robustness was assessed at concentration levels (20, 30 and 40 µg/mL) for Bemotrizinol and (30, 40 and 50 µg/mL) for Bisoctrizole. The mean peak area and % RSD values were calculated.

Accuracy

The accuracy of the method was evaluated by using the standard addition method to calculate the % recovery of Propyphenazone, Flurbiprofen, and prodrug **[17, 18]**. Standard stock solutions with known concentrations were added to the sample. The proposed

approach was used to examine the solution after it had been injected into the HPLC apparatus. By measuring the areas and then fitting these values to the straight-line equations of the calibration curves, the amounts of Bemotrizinol, and Bisoctrizole were calculated.

Assay of synthetic mixture

The synthetic mixture was prepared by mixing Bemotrizinol, and Bisoctrizole in a (1:2) ratio with common excipients used for the tablet formulation. To determine drug content 10 mg equivalent powder content taken and dissolve in methanol. Final test solution (10 µg/mL) was prepared using same solvent **[19]**.

Solution stability

Solution stability was determined by storing the stock solution (100 µg/mL) of Bemotrizinol, and Bisoctrizole at room temperature for 24 h and analyzing them at different intervals *viz.* 0, 4, 8 and 24 h **[20]**.

RESULT AND DISCUSSION

Chromatography:

Chromatographic system comprising a mixture of 1,4-Dioxane and ammonium acetate buffer (80:20) as mobile phase and flow rate 1.2 ml/min at 340nm **(Figure 1)**.

System Suitability Parameter:

The suitability of the developed method was confirmed by analyzing the chromatogram of standard solutions containing bemotrizinol and Bisoctrizole. Various chromatographic characteristics, including retention time, theoretical plates, resolution, and tailing factor, were derived from the chromatogram. The results of these analyses are presented in **Figure 2 and Table 1**. The obtained values of the system suitability parameters indicated that the procedure was appropriate and suitable for the specified chromatographic conditions.

Validation of RP-HPLC method

Specificity:

For the determination of specificity a solution of blank and drugs were injected. The chromatogram were injected and observed for interference of diluents with the drugs peak **(Figure 3)**.

Linearity and Range:

Linear relation was obtained between mean peak area and concentration of the drug in the range of 10-50 µg/ml for Bemo and in the range of 20-100µg/ml for Biso. Calibration curve of Bemo and Biso were obtained by plotting the mean

Linear relation was obtained between mean peak area and concentration of the drug in the range of 10-50 µg/ml for Bemo and in the range of 20-100µg/ml for Biso. Calibration curve of Bemo and Biso were obtained by plotting the mean peak area against concentration (µg/ml). The data of the peak areas obtained with the respective

concentrations in µg/ml are shown in **Table 2** for Bemo and Biso. The linearity curves for Bemo and Biso are shown in **Figure 4**.

Precision

Repeatability:

The % R.S.D. for repeatability was found to be 0.6181 (Bemo) and 0.5537 (Biso) was within the acceptable criteria. Hence it could be said that the method provides satisfactory results on repeated sampling.

Intraday Precision:

Three replicates of three concentrations of standard solution of both the drug. Total nine determinations were analyzed at same day and Peak areas were measured and %RSD was calculated.

Inter Day Precision:

Three replicates of three concentrations of standard solution of both the drug. Total nine determinations were analyzed at three consecutive day and Peak area were measured and %RSD was calculated.

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. % recovery was found to be 99.67-99.83 for Bemo and 99.10-101.6 for Biso.

Robustness

Three concentrations of standard solution of both the drug were analyzed at three different wavelength and three different flow rate and %RSD was calculated and found out to be less than 2%, which demonstrated that the proposed method was robust.

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

The LOD & LOQ of the Bemo and Biso by HPLC Method were obtained by repeating the calibration curve 3 times. The results were shown in the **Table 2**.

Assay

The applicability of the method was confirmed by determining % Assay of Synthetic mixture by HPLC Method. The % Recovery of bemotrizinol and bisoctrizole was found to be 99.57% and 98.325 respectively.

Solution stability

The results of the analysis showed that the absorbance of the analytes (100 µg/mL stock solutions of bemotrizinol and bisoctrizole) remained almost unchanged and no significant degradation was observed during this period, with less than 2% RSD. Thus, these solutions were stable for 24 h when stored at room temperature.

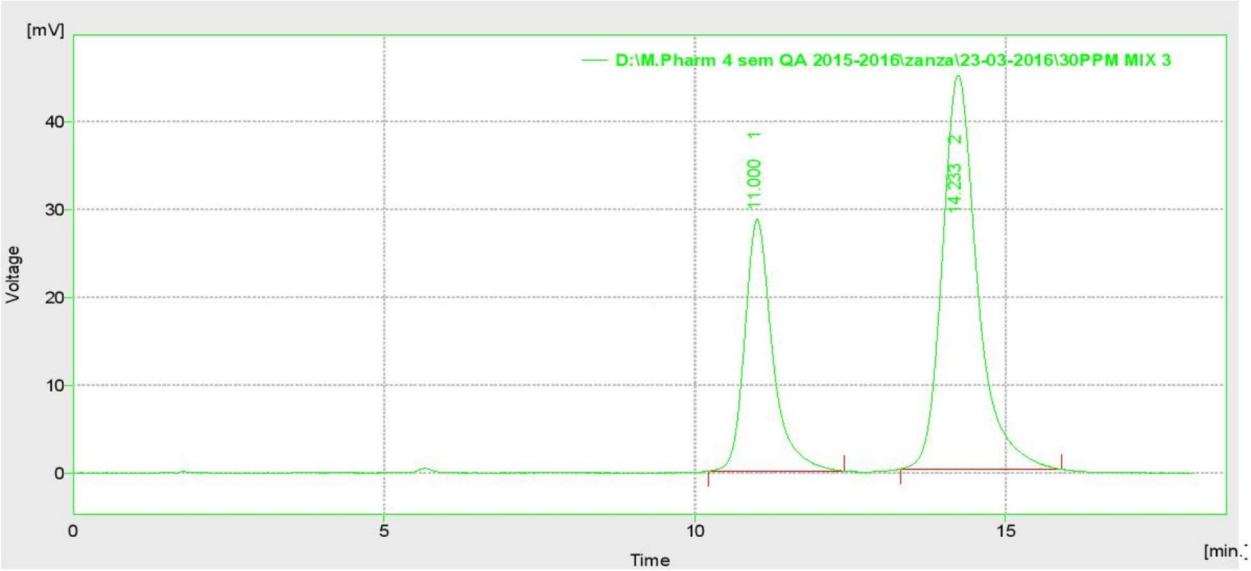

Figure 1: Chromatogram of mixture in 1, 4-dioxane: Ammonium Acetate Buffer pH-3 (80:20% v/v) at 340 nm

Table 1: Data of System Suitability

Sr. No.	Retention Time		Tailing Factor		Theoretical Plates		Resolution
	Bemo	Biso	Bemo	Biso	Bemo	Biso	
1	14.23	11.0	1.44	1.49	3454	3310	3.75
2	14.22	11.01	1.43	1.47	3502	3315	3.79
3	14.209	11.02	1.44	1.49	3605	3350	3.73
4	14.191	11.01	1.45	1.47	3430	3357	3.75
5	14.231	11.03	1.46	1.44	3477	3377	3.65
6	14.220	11.08	1.45	1.47	3455	3380	3.73
MEAN	14.2168	11.015	1.445	1.4816	3487.16	3348.167	3.733
SD	0.01498	0.0104	0.0104	0.012	62.627	29.94	0.04633
%RSD	0.1050	0.0952	0.7258	0.897	1.79	0.894	1.2409

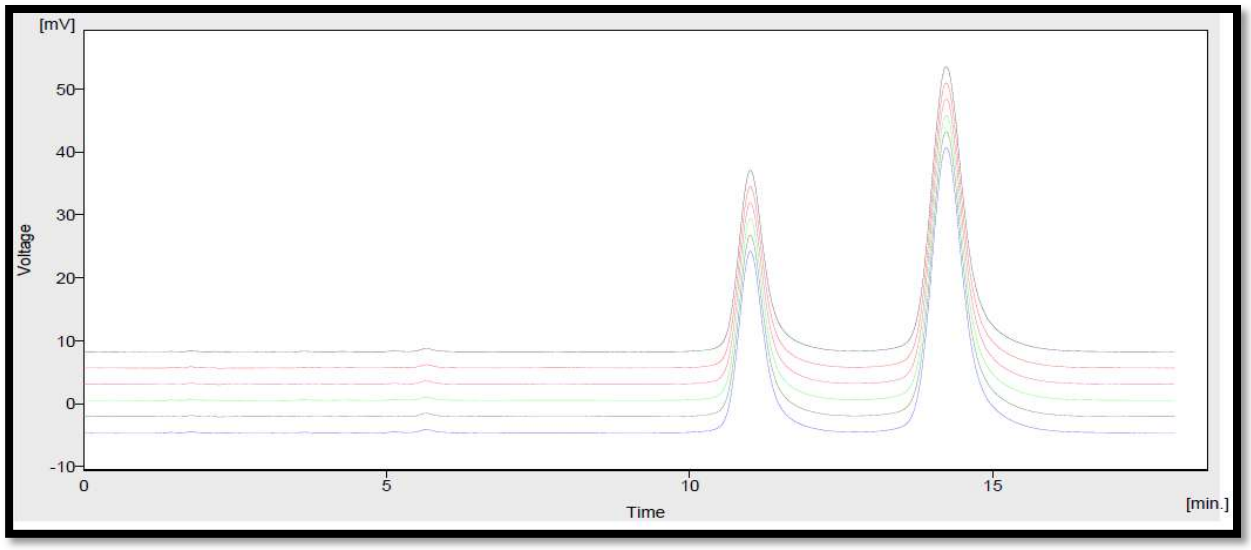

Figure 2: Overlay Chromatogram of system suitability

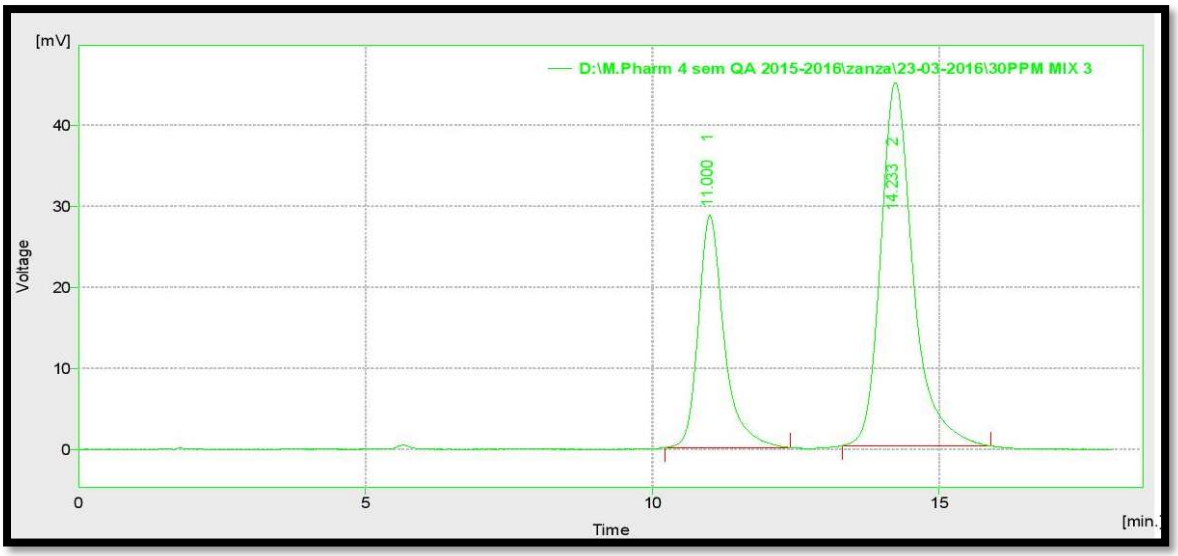

Figure 3: Chromatogram of mixture

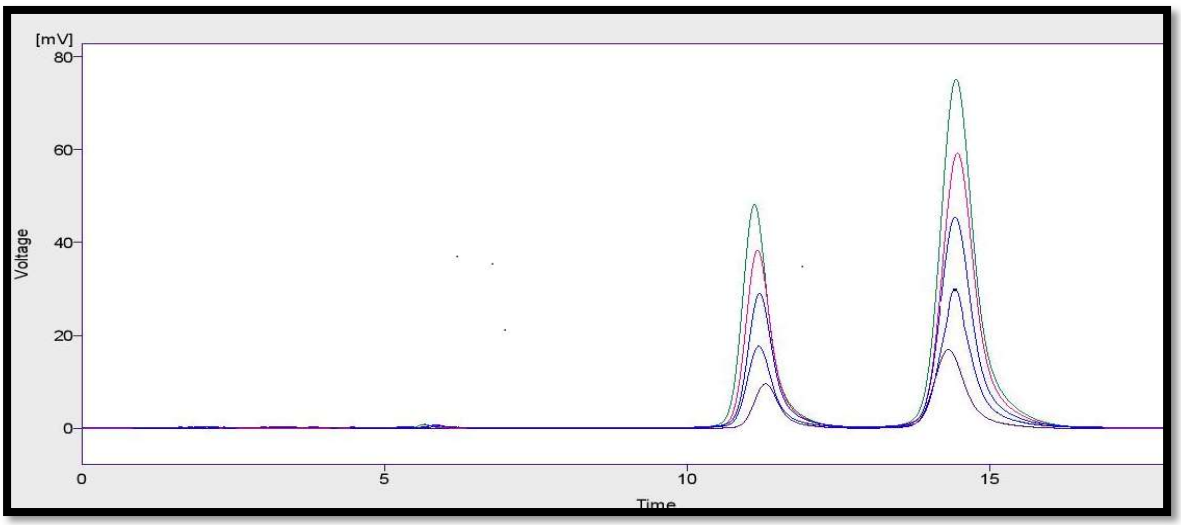

Figure 4: Chromatogram of Linearity

Table 2: Data of Linearity, LOD and LOQ

Drug	Concentration range (µg/ml)	Equation	Regression coefficient	LOD	LOQ
Bemotrizinol	10 – 50	y = 61.395x + 25.16	R^2 = 0.9991	0.1815	0.6401
Bisoctrizole	20 – 100	y = 16.099x – 53.977	R^2 = 0.9985	0.55	1.9399

Table 3: Data of Precision

Parameters		Bemotrizinol	Bisoctrizole
Precision	%RSD (Repeatability)	0.5486	0.9614
	%RSD (Inter-day)	0.602933	1.006867
	%RSD (Intra-day)	0.554933	0.4395
Robustness	Change in wavelength	0.2299	0.441867
	Change in flow rate	0.1309	0.228833

Table 4: Data of Accuracy

Drug	Level	Amt of sample (ppm)	Amt of std. spiked(ppm)	Total Amount (ppm)	Amt. found	% Recovery
BEMO	**50**	**20**	**10**	**30**	**29.90**	**99.67**
	100		**20**	**40**	**40.13**	**99.67**
	150		**30**	**50**	**49.91**	**99.83**
BISO	**50**	**40**	**20**	**60**	**59.47**	**99.10**
	100		**40**	**80**	**81.29**	**101.6**
	150		**60**	**100**	**103.49**	**100.19**

CONCLUSION

From the results, it was concluded that the developed method is precise, accurate, sensitive and simple. All the methods are suitable for QC Laboratories, where economy and time considerations are essential.

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