RESEARCH ARTICLE

Challenges of HPLC Method Development and Validation for the Assay of Bemotrizinol from Complex Matrix in Cosmeceutical Preparation

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ABSTRACT

A simple high performance liquid chromatography (HPLC) method was developed for the assay of bemotrizinol (Tinosorb-S) from the complex pharmaceutical cosmetics matrix. Unlike the existing methods, the proposed mobile phase used in this method is very simple and excluding buffer. The use of buffer reducing column longevity and also a time-consuming process which increases the cost of analysis. To overcome all the referred problems, the present article was developed and validated as per International Council for Harmonization (ICH) guidelines. The reverse-phase chromatography was performed on Shimadzu model no. SPD-M10A VP with LC solution software, µBondapack (3.9 × 300 mm, 10-micron particle size) column with methanol (100%) as mobile phase at a flow rate 2.5 mL per minutes and UV detection at 254 nm. The retention time of bemotrizinol was found in 17.599 minutes, and the linear regression analysis data for the calibration plots showed a good linear relationship in the concentration range 70 to 130 µg/mL. The value of the correlation coefficient, slope, and intercept were 0.996, 7,715, and 15,320, respectively. The limit of quantification (LoQ) and limit of detection (LoD) were found to be 1.32 and 0.44, respectively. The relative standard deviation (RSD) for intra-day sample A 1.0858, sample B 0.8859, and inter-day sample A 0.9921, sample B 0.967 which were found to be lesser than 2%. The developed method was validated with regard to linearity, accuracy, precision, selectivity, and robustness, and the method was found to be simple, cost-effective, precise, accurate, linear, and specific for the successful identification and determination of bemotrizinol in pharmaceutical cosmetic preparation.

Keywords: Bemotrizinol **(**Tinosorb-S), HPLC, Validation.

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INTRODUCTION

The use of sunscreen products is widely accepted for the prevention of skin cancer due to the ultraviolet (UV) sunlight and it is also used for primary prevention of long-term exposure to strong sunlight which may result in skin burns (i.e., "sunburn"). Bemotrizinol is (Figure 1) chemically known as bis-ethylhexyloxyphenol methoxyphenyl triazine, molecular formula $C_{38}H_{49}N_3O_5$, molecular weight 627.81 g/mol. Bemotrizinol is an oil-soluble broad-spectrum organic compound that is added to sunscreen to absorb UV rays, UVB (290–320), as well as, UVA (320–400) rays. It is highly efficient at low concentration and perfect for long-lasting sun care and face care products that offer a distinctive sensory experience. It has two absorption peaks, 310 and 340 nm.[1] It is highly photostable. Even after 50 minimal erythemal doses (MEDs), 98.4% remains intact. It helps prevent the photodegradation of other sunscreen actives, like avobenzone.[2] Bemotrizinol has strong synergistic effects on the sun protection factor (SPF) when formulated with bisoctrizole, ethylhexyl triazone, or iscotrizinol. However, excessive addition of sunscreen agents for the long term may increase the risk of developing a skin allergy to light, as well as, birth defects.[3] So the types and amounts of sunscreen agents in cosmetics are strictly regulated. The permitted active ingredients of sunscreen agents in sunscreen cosmetics in the

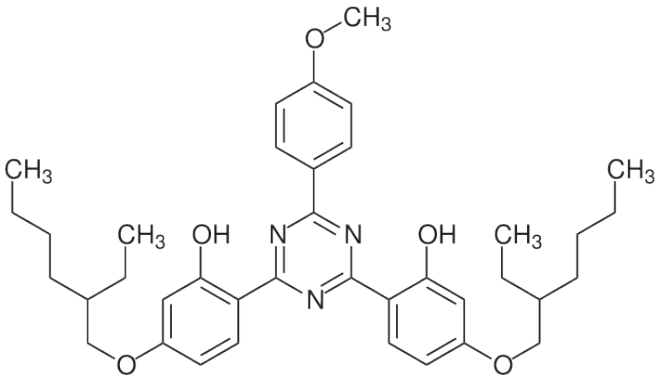

Figure 1: Bemotrizinol

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European Union (EU), the United States (USA), China (CHN), and Japan (JP) are listed in Table 1.[4-7]

Some sunscreen cosmetics are still adding phenyl salicylate which may cause potential human health problems that are not listed in these regulations.[3] Therefore, it is necessary to detect multiple sunscreen agents in different sunscreen formulations. The application of HPLC has been applied extensively for quantitative determination.[8-12] Very few methods are reported in the literature and for the quantitative determination of bemotrizinol; buffer is used for mobile phase preparation. The reported methods in the literature suffer from one or the other disadvantage, such as, long run time, poor sensitivity, column longevity, very narrow linearity range, etc. Since pharmacopeias do not describe a suitable method for the determination of bemotrizinol in pharmaceutical cosmetic formulations, in the present work we developed a simple, cost-effective, precise, accurate, linear, and specific reverse phase liquid chromatographic method for the determination of bemotrizinol cream as an alternative method. This method can be used for the analysis of a large number of samples. The HPLC method was validated in terms of linearity, precision, accuracy, robustness, LoD, and LoQ according to ICH guideline.[13-16]

AIMS AND OBJECTIVES

To develop a simple, cost-effective, time-effective, and efficient HPLC method for the determination of bemotrizinol, a sunscreen agent from the complex pharmaceutical cosmetics matrix in different sunscreen products due to the potential developing a skin allergy to light and birth defects in humans.

MATERIALS AND METHODS

The sunscreen cream Sunmate SPF 30+ gel cream from Palsons Derma Pvt. Ltd. and Photoderm MAX SPF50+ from Bioderma, respectively, used for analysis. Pure drug sample bemotrizinol was kindly supplied as a gift sample by Palsons Derma

Table 1: Active ingredients of sunscreen agents in sunscreen cosmetic and their permitted concentrations in different parts of the world

	Permitted concentration			
Active ingredients	*USA*	*USA EU P*	*CHN*	*H JP N JP*
p-aminobenzoic acid	15%	Forbidden	5%	Forbidden
2-hydroxy-4-methoxybenzophenone	6%	10%	10%	5%
3,3,5-trimethylcyclohexyl salicylate	15%	10%	10%	10%
2-ethylhexyl-4-methoxycinnamate	7.5%	10%	10%	20%
2-ethylhexyl salicylate	5%	5%	5%	10%
2-hydroxy-4-methoxy-benzophenone-5-sulfonic acid	10%	5%	5%	10%
Avobenzone butylmethoxydibenzoylmethane	3%	5%	5%	10%
3-(4-methylbenzylidene) camphor	Forbidden	4%	4%	Forbidden
Octocrylene	10%	10%	10%	10%
Menthyl anthranilate	5%	Forbidden	Forbidden	Forbidden
Trolamine salicylate	12%	Forbidden	Forbidden	Forbidden
Ecamsule	3%	10%	10%	Forbidden
Titanium dioxide	25%	25%	25%	No limit
Zinc oxide	25%	25%	25%	No limit
Tinosorb M	Forbidden	10%	10%	10%
Tinosorb S	Forbidden	10%	10%	3%
Neo heliopan AP	Forbidden	10%	Forbidden	Forbidden
Mexoryl XL	Forbidden	15%	15%	Forbidden
Benzophenone-9	Forbidden	Forbidden	Forbidden	10%
Uvinul T 150	Forbidden	5%	Forbidden	3%
Uvinul A plus	Forbidden	10%	Forbidden	10%
Uvasorb HEB	Forbidden	Forbidden	10%	5%
Parsol SLX	Forbidden	10%	10%	10%
Isopentenyl-4-methoxycinnamate	10%	10%	10%	Forbidden
Padimate-O	8%	Forbidden	8%	10%
Phenylbenzimidazole sulfonic acid	4%	8%	8%	3%
Cinoxate	3%	Forbidden	Forbidden	Forbidden
Dioxybenzone	3%	Forbidden	Forbidden	Forbidden

Note: USA *(*United States of America); EU *(*European Union); CHN *(*China); JP *(*Japan)

Pvt. Ltd. The sample is used without further purification. HPLC grade methanol, chloroform was procured from Merck, Qualigen, and Spectrochem, Rankem, respectively. 0.2 µm PTFE syringe filter, and also 0.2 µm nylon syringe filter were procured from Millipore.

Instrumentation and Chromatographic Condition

The HPLC system Shimadzu model no. SPD-M10A VP with LC solution software consisted of a binary pump (model no. LC-10AT VP and LC20AD), autosampler (model no. SIL-20AHT), column oven (model no. CTO-10AS VP), and PDA detector. Isocratic elution was achieved on µBond pack column, (3.9 × 300 mm, 10 microns) and column temperature maintained at 27ºC by using a column oven. Methanol 100% was used as a mobile phase. Chloroform and methanol were used as diluents. Other parameters, such as, run time 30 minutes, 2.5 mL/minutes as flow rate, and the injection volume was 20 µL. Detection was done by spectrophotometer set at 254 nm. The chromatogram of standard and sample solution is shown in Figures 2 and 3.

Procedure

Standard Solution Preparation

Weigh accurately about 0.013-gram of bemotrizinol working standard in a 100 mL volumetric flask; add about 10 mL of chloroform, sonicated for five minutes in an ultrasonic bath to dissolve and volume makeup with the methanol. Mix well and filter through 0.2 µm syringe filter before injection.

Sample Solution Preparation

Weigh accurately sample equivalent to 0.025-gram of bemotrizinol in a 50 mL volumetric flask, add about 25 mL of chloroform, sonicated for 10 minutes in an ultrasonic bath to dissolve and volume makeup with the same solvent. Take 5 mL into a 20 mL volumetric flask and volume makeup with methanol. Mix well and filter through 0.2 µm syringe filter before injection.

Analysis of Commercial Formulations

The proposed method was developed to the determination of bemotrizinol in the Photoderm MAX ^{SPF}50+ (sample-A) and Sunmate SPF 30+ gel cream (sample-B) sunscreen formulation.

RESULTS

For the assay, each brand was calculated by comparing the peak area of the standard solution and sample solution. The result of these assays found 99.36% (sample-A) and 99.54% (sample-B), respectively. The results were shown in Table 2. The assay result indicates that the method is very selective to determine the bemotrizinol from different sunscreen formulations without any interference of the pharmaceutical base.

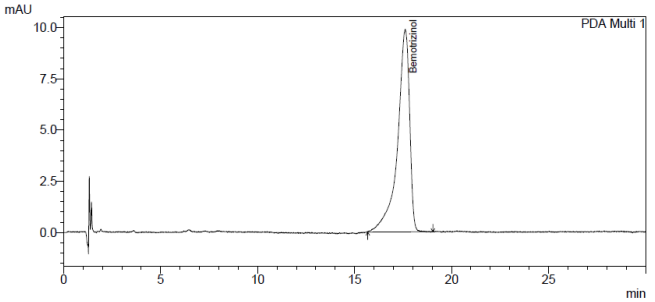

Figure 2: Chromatogram of bemotrizinol in standard solution

HPLC Method Validation and Optimization

Column and mobile phase selection were done simultaneously. The development was started with BDS Hypersil (4.6 × 250 mm, 5-micron particle size) column with acetonitrile 100% as mobile phase at a flow rate of 2.5 mL per minute. Under this condition, resolution, and symmetry of the peak was not satisfactory, so the mobile phase was changed to methanol 100%. Peak broadening with less plate count of the peak was observed. Further trial was carried out with different columns, such as, HyperClone (4.6 × 250 mm, 5-micron particle size) keeping the mobile phase and rest of chromatographic conditions was the same, and peak broadening with less plate count of the peak was observed. The next trial column was µBondapack (3.9 × 300 mm, 10-micron particle size) and the symmetrical peak was well resolved with good peak area by using 100% methanol, which provides the best chromatographic response for further studies.

Method Validation

System Suitability

To verify the system, method, and column performance the system suitability parameter was measure and which was suitable for the intended application. Retention time, tailing factor, and theoretical plates were the measured parameters. For bemotrizinol, the measured peak area was varied lesser than 2%, retention time was 17.599 minutes, and % RSD = 0.33. Theoretical plates were 4,680.915 (more than 2,000), and the tailing factor was 0.958. The results of the system suitability parameters are presented in Table 3. The proposed method offers high sensitivity and bemotrizinol can be detected accurately.

Precision

The intra-day precision of repeatability was studied by replicate analysis. The precision also studied in terms of intra-day changes in the peak area of the solution on the

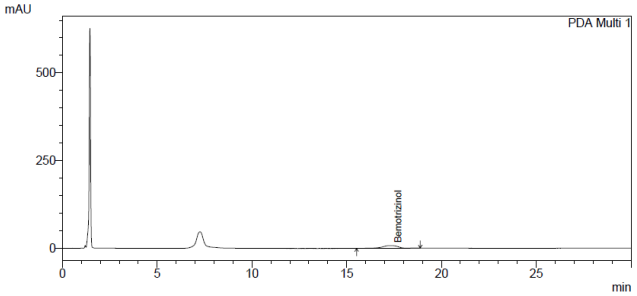

Figure 3: Chromatogram of bemotrizinol in sample solution

Table 2: Assay % of two different brands

Different brands	*Percentage label claim (% w/w)*
Sample-A (Bioderma)	99.36
Sample-B (Palsons Derma Pvt. Ltd.)	99.54

same day and inter-day precision was performed on three different days, i.e., day-1, 2, and 3, over a period of 1-week. The precision was determined by repeatability (intra-day precision) and intermediate precision (inter-day precision) of bemotrizinol of the standard and sample solution. The intra-day and inter-day variation were calculated in terms of percentage relative standard deviation. The % RSD range was obtained as 1.0858 (sample-A), 0.8859 (sample-B), and 0.9921 (sample-A), 0.9670 (sample-B) for intra-day and inter-day precision, respectively, as shown in the Tables 4 and 5.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results for the assay method which is prepared from stock solution at seven concentration levels of the assay analyze concentration (70 to 130%), which are directly proportional to the concentration (amount) of analyte in the sample solution. 20 µL of each solution was injected into the HPLC system and the peak area of the chromatogram obtained was noted. The linearity of the calibration curve in pure solution was checked over a concentration range and the curve was linear over the concentration range 70 to 130 µg/mL in three replicates. The calibration curve was constructed by plotting the average peak area of bemotrizinol *vs.* bemotrizinol concentration and the regression equation was computed. The concentration *vs.* peak area was analyzed with the least-squares linear regression. The y-intercept and slope of calibration curve were reported. The regression equation for the calibration curve was found to be Y = 7,715X – 15,320 (R^2 = 0.996), Y = 7,686X – 14,910 (R^2 = 0.996), and Y = 7,632X – 13,392 (R^2 = 0.996), respectively. The correlation coefficient (R^2) value is shown in Figure 4.

Limit of Detection (LoD) and Limit of Quantification (LoQ)

The LoD and LoQ were calculated by using the standard deviation of the response (σ) and slope of the calibration curve of analyte (S) as described in the International Conference on Harmonization guideline Q2 (R1). Based on the standard deviation of response and slope, the LoQ and LoD value of the drug calculated on the 10 and 3.3 times, respectively. The LoD is calculated by using formula 3.3σ/S and LoQ 10σ/S. The LoQ was found to be 1.32 µg/mL and LoD was found to be 0.44 µg/mL (Table 6).

Table 3: Analytical parameters

Parameters	*Rosuvastatin calcium*
Linear dynamic range	70–130 µg/mL
R^2 value	0.996
Retention time	17.599
Theoretical plates	4,680.915
Tailing factor	0.958
LoD (µg/mL)	0.44
LoQ (µg/mL)	1.32
RSD%	0.33

Table 4: Comparison of intra-day precision

S. No.	*Percentage label claim (% w/w) Sample-A*	*Percentage label claim (% w/w) Sample-B*
Set-1	99.36	99.54
Set-2	101.79	100.85
Set-3	101.67	100.81
Set-4	100.08	99.16
Set-5	100.98	100.01
Set-6	99.39	98.67
RSD%	1.0858	0.8859

Table 5: Comparison of inter-day precision

	S. No.	*Percentage label claim (% w/w) Sample-A*	*Percentage label claim (% w/w) Sample-B*
Day-1	Set-1	99.36	99.54
	Set-2	101.79	100.85
	Set-3	101.67	100.81
	Set-4	100.08	99.16
	Set-5	100.98	100.01
	Set-6	99.39	98.67
Day-2	Set-1	100.91	100.05
	Set-2	101.83	100.77
	Set-3	100.11	102.23
	Set-4	100.12	99.64
	Set-5	99.56	100.3
	Set-6	100.22	100.07
Day-3	Set-1	101.06	100.82
	Set-2	99.35	102.47
	Set-3	98.66	101.05
	Set-4	99.85	101.31
	Set-5	98.69	101.05
	Set-6	99.65	100.79
RSD%		0.9921	0.967

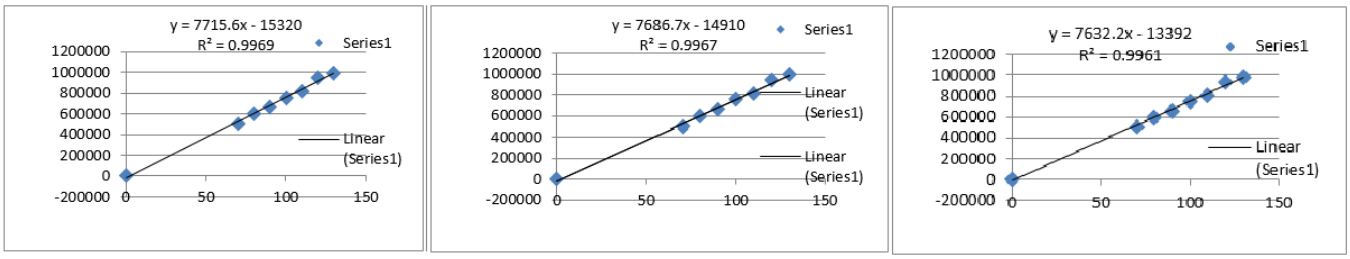

Figure 4: Linearity

Accuracy

The accuracy of the assay method was evaluated with the recovery of the standard from excipients. Recovery studies were carried out by applying the method to drug content present in sunscreen cream form to which a known amount of standard (bemotrizinol) was added at three concentration levels (80, 100, and 120%) and the percentage of recovery in each case was calculated. The technique involves the addition of a standard drug solution to a pre-analyzed sample solution. The resulting sample solutions were injected and chromatograms were recorded and the concentration of the standard drug from sunscreen cream was determined. The results were shown in Table 7.

Robustness

The robustness of an analytical procedure refers to its capacity to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for routine analysis.[16] For this study, the method was evaluated by testing the same sample under different analytical parameters deliberately changing from the original condition. For this study, column temperature set at 25 and 29 (±2°C), the wavelength was set at 252 and 256 (±2 nm), and flow rate was set at 2.25 to 2.75 (±0.25 mL/min). The % RSD of assay value of same sample under robustness condition was less than 2%, which were shown in Table 8, and indicating that the developed method was robust.

Solution Stability

The solution stability of bemotrizinol in the assay method was determined by keeping the sample for short-term stability at room temperature for 12 hours. The same sample solutions were injected and the % RSD of the bemotrizinol peak area was calculated. The % RSD of bemotrizinol peak area from the solution stability was within 2% and which experiments confirm that the sample solution during the assay was stable up to 12 hours at room temperature. The results are displayed in Table 9.

Table 6: Limit of detection **(**LoD) and limit of quantification **(**LoQ)

S. No.	*Slope S*	*y-intercepts*
Set-1	7,715	15,320
Set-2	7,686	14,910
Set-3	7,632	13,392
SD	42.12283625	1,015.6778
Mean	7,677.666667	14,540.667

Limit of detection (LoD) and limit of quantification (LoQ)

$$\text{Limit of detection (LoD)} = \frac{3.3\sigma}{S} = 0.44 \qquad \text{Limit of quantification(LoQ)} = \frac{10\,\sigma}{S} = 1.32$$

Table 7: Comparison of recovery study of two different brand

% target	*Sample-A (% recovery)*	*Sample-B (% recovery)*	*Mean*
80	100	100.43	100.48
	100.52	100.97	
100	94.45	94.63	99.2875
	105.04	103.03	
120	99.95	98.18	99.0675
	100.01	98.13	

Table 8: Change of column temperature (±2°C), flow rate (±0.1 mL/min), and wavelength (±2 nm)

Change of column temperature Column temperature = 25°C	*Percentage label claim (% w/w)*	*Change of column temperature Column temperature = 29°C*	*Percentage label claim (% w/w)*	*RSD*
Sample-A	102.34	Sample-A	99.8	1.6904
Sample-B	99.91	Sample-B	103.18	
Change of flow rate Flow = 2.25 mL/min	*Percentage label claim (%w/w)*	*Change of flow rate Flow = 2.75 mL/min*	*Percentage label claim (%w/w)*	*RSD*
Sample-A	99.36	Sample-A	100.7	0.8936
Sample-B	99.23	Sample-B	100.96	
Change of wavelength Wavelength = 252	*Percentage label claim (% w/w)*	*Change of wavelength Wavelength = 256*	*Percentage label claim (% w/w)*	*RSD*
Sample-A	98.43	Sample-A	97.36	
Sample-B	96.71	Sample-B	99.4	1.21

Table 9: Stability of the solution

Time interval	*Area of sample-A*	*Area of sample-B*	*Acceptance criteria*
2 hours	2,849,015	3,042,639	
4 hours	2,849,752	3,058,136	
6 hours	2,849,069	3,046,770	
10 hours	2,854,218	3,060,287	% of RSD is ≤ 2
12 hours	2,848,123	3,028,654	
Mean	2,850,035.4	3,047,297.2	
SD	2,408.67584	12,803.9072	
% of RSD	0.08	0.42	

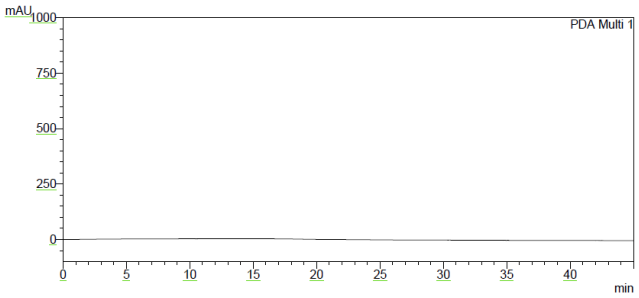

Figure 5: Chromatogram of blank solution

Selectivity/ Specificity

The specificity of the developed method was established by interference and blank detection. As per the test method, the mobile phase was injected (Figure 5). The sample solution and standard solution were prepared as per specification and inject into the chromatographic system. The chromatogram of the sample solution and standard solution were shown in Figures 2 and 3.

DISCUSSION

The proposed method was fast, cost-effective, accurate, precise, very simple, and excluding buffer for qualification, as well as, quantification of bemotrizinol in pharmaceutical cosmetic preparation. The aim of this research was to develop optimal experimental conditions allowing chromatographic separation of bemotrizinol for quantitative analysis from a complex cosmetic matrix. HPLC was the most commonly chromatographic technique for the quantification and qualification of several sunscreen agents in cosmetic preparation. The complete separation of the analyte was accomplished in less than 30 minutes. The symmetrical peak was obtained through experimental trials and quantification was achieved with UV detection at 254 nm (Figure 2). RP-HPLC separation was achieved on a µBond pack (3.9 × 300 mm, 10-micron particle size) column with methanol (100%) as a mobile phase at a flow rate of 2.5 mL per minute.

To determine linearity, calibration graph was obtained by plotting bemotrizinol concentration *vs.* peak area. Linearity was in the concentration range 70 to 130 µg/mL. The regression equation for the calibration curve was found to be $Y = 7{,}715X - 15{,}320$ ($R^2 = 0.996$), $Y = 7{,}686X - 14{,}910$ ($R^2 = 0.996$), and $Y = 7{,}632X - 13{,}392$ ($R^2 = 0.996$), respectively, where X is concentration in µg/mL and Y is the peak area in absorbance units; the correlation coefficient was 0.996. For the determination of intra-day and inter-day precision, the % RSD was obtained as 1.0858 (sample-A), 0.8859 (sample-B), and 0.9921 (sample-A), 0.967 (sample-B), respectively. The LoQ was found 1.32 µg/mL and LoD was found to be 0.44 µg/mL.

This HPLC method was successfully developed and validated for the assay for routine analysis. The method was shown very specific, linear, precise, accurate, and robust for quantification and qualification of bemotrizinol in cosmetic preparation.

CONCLUSION

The proposed new HPLC method was developed and validated for the estimation of bemotrizinol in a different sunscreen formulation. All the validation parameters meet the ICH guidelines and the analytical method was found to be simple, cost-effective, precise, accurate, linear, and specific. The percentage of recovery shows that the method was free from all interference from the sample matrix. Hence, this method may be successfully recommended for reproducible quantitative analysis of routine and quality control analysis of bemotrizinol in sunscreen formulations.

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