ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 17, Issue 1, 2024

Online - 2455-3891
Print - 0974-2441

Research Article

DEVELOPMENT AND VALIDATION OF NOVEL RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF SALICYLIC ACID AND BECLOMETHASONE DIPROPIONATE IN A TOPICAL OINTMENT DOSAGE FORM

VANITA SAWANT, CHINMAYI MALI, VANDANA JAIN

Department of Quality Assurance, Oriental College of Pharmacy, Navi Mumbai, Maharashtra, India.
***Corresponding author: Vandana Jain; Email: vandana.jain@ocp.edu.in**

Received: 23 June 2023, Revised and Accepted: 06 August 2023

ABSTRACT

Objective: The objective of the study is to develop and validate an analytical method to simultaneously estimate salicylic acid (SA) and beclomethasone dipropionate (BD) in a topical ointment formulation.

Methods: The simultaneous estimation of SA and BD in a topical ointment formulation was carried out by developing and validating a novel, accurate, and economical isocratic reversed-phase high-performance liquid chromatographic (RP-HPLC) method. Separation was achieved by chromatographic technique using a prontosil HPLC C18 (250×4.6 mm) column with a particle size of 5 µm. The mobile phase utilized for this study includes methanol, acetonitrile, and 0.1% orthophosphoric acid in the ratio 50:35:15 v/v/v, respectively. The flow rate of 1 mL/min and column temperature of 28±2°C were set. The detection of the two drugs was carried out at 235 nm using an ultraviolet detector. It was observed that SA and BD were retained at 3.59 min and 6.00 min, respectively.

Results: The RP-HPLC method was found to be linear with excellent correlation between peak areas and concentrations of 30–108 µg/mL for SA and 1–3.6 µg/mL for BD. The observed recovery data was obtained within the acceptance range of 98–102%, which confirmed the accuracy of the developed method. The two drugs, SA and BD, showed good resolution with a short analysis time of 7 min.

Conclusion: The method was successfully created and validated in compliance with the recommendations of the International Conference on Harmonization for specificity, precision, linearity, accuracy, and robustness.

Keywords: RP-HPLC, Salicylic acid, Beclomethasone dipropionate, Simultaneous, Validation.

 DOI: http://dx.doi.org/10.22159/ajpcr.2024v17i1.48682. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

The objective is to develop a reversed-phase high-performance liquid chromatographic (RP-HPLC) method and validate it by simultaneous estimation of salicylic acid (SA) and beclomethasone dipropionate (BD) in a topical ointment formulation. BD is a second-generation synthetic corticosteroid as well as a diester of beclomethasone, which is structurally similar to dexamethasone. BD is used in a variety of inflammatory disorders, such as asthma, allergic rhinitis, and dermatoses, to alleviate symptoms because of its anti-inflammatory, antipruritic, and anti-allergy effects [1]. By β-elimination of water from the D-rings bearing the 1, 3-dihydroxyacetone side chain, which is catalyzed by an alkaline situation, the BD in the medication formulations can produce enol aldehyde. Enol aldehyde is one type of major degradation product that can be further broken down into a number of secondary degradation products depending on the formula and storage conditions [2]. The mechanism of action of BD is observed at the level of the cell nucleus, which interacts with DNA and consequently stimulates or inhibits gene transcription. This drug acts as a transcription factor that alters the expression of target genes in response to a specific hormonal signal [3]. SA is a beta-hydroxy acid, a mono-hydroxybenzoic acid, and a subclass of phenolic acid [4]. SA is the main ingredient in many skin-care products for the treatment of seborrheic dermatitis, acne, psoriasis, calluses, corns, keratosis pilaris, acanthosis nigricans, ichthyosis, and warts. As a comedolytic and bacteriostatic drug, SA causes the epidermis' cells to shed more readily, unclogs clogged pores and kills germs there, reduces pore diameter to prevent clogging again, and creates space for new cell growth [5].

Betasalic ointment (Cipla Ltd.) is a topical ointment, which is a combination of corticosteroid BD and keratolytic SA. SA helps keratin to break in the hardened and thickened skin and aids to shed skin cells from the area to which it is applied. It also enhances the penetration of the BD into the skin more effectively. SA is used for the treatment of psoriasis, eczema, and dry, scaly, inflamed skin conditions [6].

In this paper, the development of a RP-HPLC method for betasalic ointment is reported. This is the first known method that has the capability to separate and quantitate the two active pharmaceutical ingredients simultaneously. This method was validated in accordance with the current International Conference on Harmonization (ICH) guidelines [7].

METHODS

HPLC-grade BD (purity 99%) was procured as a gift sample from Rusi Pharma Pvt. Ltd., Mumbai, India. HPLC-grade SA (purity 99%) was purchased from S. D. Fine-Chem Limited. A betasalic ointment used for analysis was procured from a local market. HPLC-grade solvents were purchased from Thermo Fisher Scientific India Pvt. Ltd., Powai, Mumbai. All other reagents employed in this method were of analytical grade. All chemicals and reagents used for RP-HPLC were filtered through 0.45 µm filter paper. The label claim for SA and BD specified in the commercially available formulation, namely betasalic, was 3% w/w and 0.1% w/w, respectively.

Selection of wavelength

A wavelength suitable for HPLC analysis was determined by generating ultraviolet (UV) spectrums in the range of 200–400 nm for individual drugs, which were then overlapped. These two markers' UV overlain spectra revealed that the medications absorb noticeably around

235 nm; therefore, this wavelength was chosen as the detection one for HPLC analysis (Fig. 1).

Chromatographic conditions
The method was developed using RP-HPLC on a prontosil C18 column (250×4.6 mm, 5 µ). The run time was 7 min. The mobile phase of the gradient system included methanol, acetonitrile, and 0.1% orthophosphoric acid in a ratio of 50:35:15 v/v/v, which were degassed using an ultrasonic bath at a flow rate of 1 mL/min, a column temperature set at 28°C, and a wavelength of 235 nm using a UV detector.

Preparation of solution for orthophosphoric acid (0.1% v/v)
0.5 mL of orthophosphoric acid was taken in a volumetric flask, and the volume was made up to 500 ml with distilled water. This solution was then filtered using a 0.45 µ membrane filter, which was further sonicated before use.

Preparation of a standard solution
About 100 mg each of SA and BD were weighed in two separate 100 mL volumetric flasks, to which 75 mL of methanol was added. The solutions were further sonicated for about 20 min, and the volumes were made up to the mark with the same solvent. These stock solutions were further diluted to obtain working concentrations of 60 µg/mL (SA) and 2 µg/mL (BD), respectively.

Preparation of sample solution
2 g of betasalic cream containing 0.002 mg of BD and 0.06 mg of SA was weighed accurately in a 100 mL volumetric flask to which 70 mL of methanol was added. This solution was sonicated to completely dissolve the active constituents present in the cream, which was then filled up to the mark with the same solvent. A filter paper of 0.45 µm pore size was used to filter the final solution. From this solution, 10 µL of the sample solution was injected into the HPLC instrument using an autosampler.

RESULTS

Optimized chromatographic conditions
The HPLC method for the analysis of drugs was optimized by performing a preliminary study using several mobile phases. Various binary and ternary combinations of solvents in their appropriate proportions were studied to obtain a mobile phase which can separate the analytes.

The estimation of SA and BD was initially performed using a mobile phase containing methanol:0.1% orthophosphoric acid (80:20 v/v),

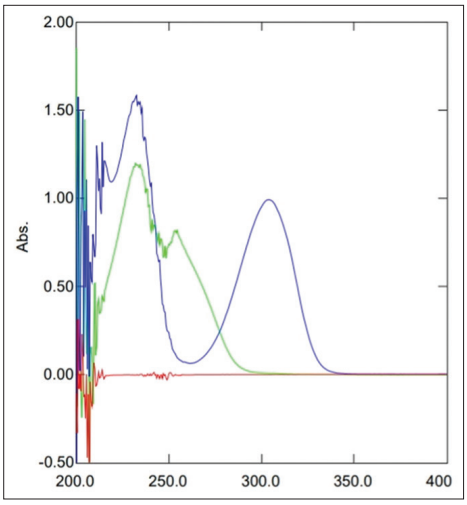

Fig. 1: Ultraviolet (UV) overlay spectrum of salicylic acid 30 µg/mL and beclomethasone dipropionate 30 µg/mL using UV detector and lab solutions software generated within a range of 400–200 nm

where the peaks of SA and BD were resolved but the retention time was late. With a mixture of acetonitrile:0.1% orthophosphoric acid (85:15 v/v), the SA and BD peaks were not resolved properly. After several other trials, a satisfactory result was achieved using a combination of methanol, acetonitrile, and 0.1% orthophosphoric acid in the ratio of 50:35:15 v/v/v. This mobile phase system was degassed using an ultrasonic bath at a flow rate of 1 ml per min, with the column temperature maintained at 28°C and a detection wavelength of 235 nm using a UV detector.

Method development
A novel, RP-HPLC technique was developed considering the system suitability parameters, i.e., resolution factor between peaks, tailing factor, number of theoretical plates, run time, and cost-effectiveness. The developed optimized method resulted in the elution of SA at 3.59 min and BD at 6.00 min. The run time of 7 min was observed.

System suitability
For the purity of working standards, we injected six standard injections, and the % relative standard deviation (% RSD), tailing factor, theoretical plates, and resolution were calculated and given in Table 1. The specified chromatographic settings were suitable for method development and validation, according to system suitability factors.

Method validation
Analytical method validation is a process that establishes laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. The HPLC method was developed and validated in accordance with the ICH guidelines for the validation of analytical procedures [8]. The validation parameters studied in this method were linearity, accuracy, system precision, method precision, and robustness.

Specificity
Figs. 2 and 3 for standard drug solutions and sample chromatograms reveal that the peaks found in the standard solutions and sample solution at working concentrations are only because of the drugs, as blank has no peak at the retention time of SA and BD. Therefore, it was concluded that the developed method was said to be specific [9].

Precision
System precision
Six replicate injections of the standard solution at working concentrations yielded an % RSD value of <2 in relation to the drug's peak area. It designates the adequate reproducibility and hence the accuracy of the system [10]. System precision results are tabulated in Table 2.

Method precision
Method precision was determined by carrying out the analysis of the sample under the test of repeatability at the working concentration. Three injections of the sample from the same homogeneous mixture at working concentration showed % RSD <2 regarding the content of two markers, which indicates that the method developed is precise by

Table 1: Observations of system suitability

Parameters for system suitability	Result		Acceptance criteria
	Salicylic acid	Beclomethasone dipropionate	
Retention Time (min)	3.59	6.00	For information
% RSD for area count of 6 standard replicate injections	0.99	0.85	NMT 2.0
Tailing factor	1.15	0.98	NMT 2.0
Theoretical plates	8485	10397	NLT 2000
Resolution	-	10.65	NLT 2.0

the test of repeatability [10] and therefore can be understood that the method gives consistently reproducible results (Table 3).

Linearity

Standard solutions of SA and BD at different concentration levels were prepared in triplicate. By comparing the concentration level to the matching peak areas for each individual marker, calibration curves were created. The results show an excellent correlation between peak areas and concentration levels within the tested concentration range of 30–108 µg/mL for SA and 1–3.6 µg/mL for BD (Table 4). The correlation coefficients were >0.99 for individual markers, which meet the method validation acceptance criteria, and hence, the method is said to be linear (Figs. 4 and 5).

Accuracy

By measuring the percent mean recovery of each ingredient in the formulation at three different levels (80%, 100%, and 120%), recovery tests were used to determine accuracy. Three estimations were made for each level. The percent mean recovery was calculated as shown in Table 5. The accepted limits of mean recovery are 98–102%, and all observed data were within the required range, confirming the correctness of the devised procedure and showing good recovery values.

Robustness

To determine the robustness of the developed method, the tailing factor and peak area of the system suitability parameter were assessed while the experimental conditions were purposefully changed. According to the test procedure previously mentioned, the solution was produced

Table 2: Result of System precision

S. No	% Assay of salicylic acid	% Assay of beclomethasone dipropionate
1	101.55	99.35
2	99.62	99.54
3	99.53	100.95
4	100.75	99.88
5	100.50	100.79
6	98.35	101.18
Mean	100.05	100.28
SD	1.12	0.78
% RSD	1.12	0.78

Table 3: Result of method precision

S. No	% assay of salicylic acid	% Assay of beclomethasone dipropionate
1	99.65	99.35
2	100.83	99.54
3	99.31	100.95
4	101.38	99.88
5	99.15	100.79
6	99.17	101.18
Mean	99.91	100.28
SD	0.95	0.78
% RSD	0.95	0.78

Table 4: Data of linearity studies

Parameters	Salicylic acid	Beclomethasone dipropionate
Linearity (µg/ml)	30–108	1–3.6
Equation of regression line	y=44350×+15744	y=800.05×+22283
R^2	0.9904	0.9937

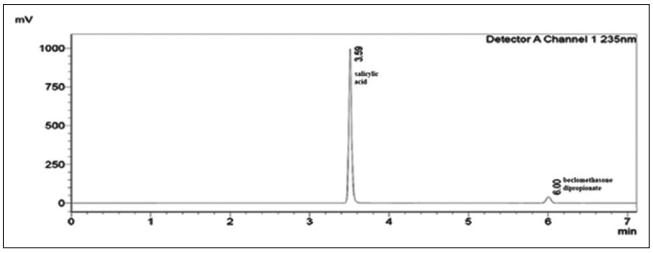

Fig. 2: Typical chromatogram of salicylic acid 60 µg/mL and beclomethasone dipropionate 2 µg/mL standard solution showing peaks at 3.59 min and 6.00 min, respectively, with a run time of 7 min

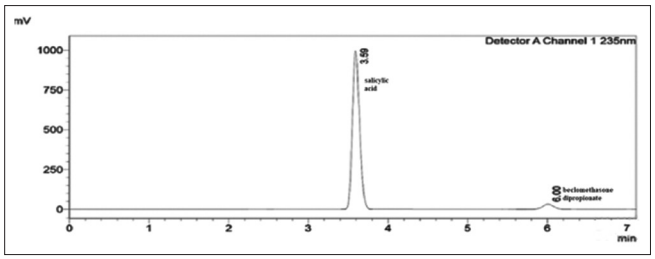

Fig. 3: Typical chromatogram of salicylic acid 60 µg/mL and beclomethasone dipropionate 2 µg/mL sample solution showing peaks at 3.59 min and 6.00 min, respectively, with a run time of 7 min

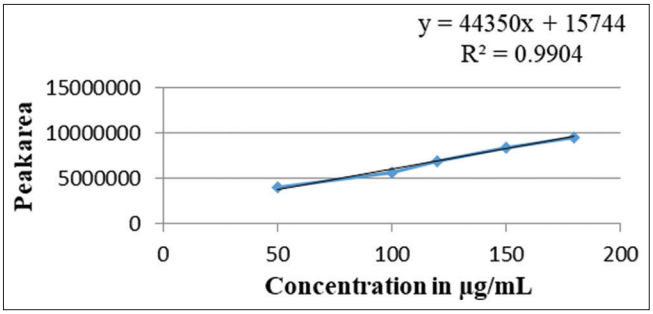

Fig. 4: Standard curve of salicylic acid obtained within a concentration range of 30–108 µg/mL depicting correlation coefficient >0.99 states the method to be linear as per the linear regression equation y=44350× + 15744. ×: concentration, y: absorbance, r2: correlation coefficient

Table 5: Percent mean recovery of salicylic acid and beclomethasone dipropionate

Compounds	Level (%)	Sample (µg/mL)	Standard added (µg/mL)	Total amount	Amount recovered	% Recovery
Salicylic Acid	80	30	24	54	53.997	99.99
	100	30	30	60	60.001	100
	120	30	36	66	65.989	99.9
Beclomethasone	80	1	0.8	1.8	1.794	99.7
Dipropionate	100	1	1	2	1.977	98.8
	120	1	1.2	2.2	2.198	99.9

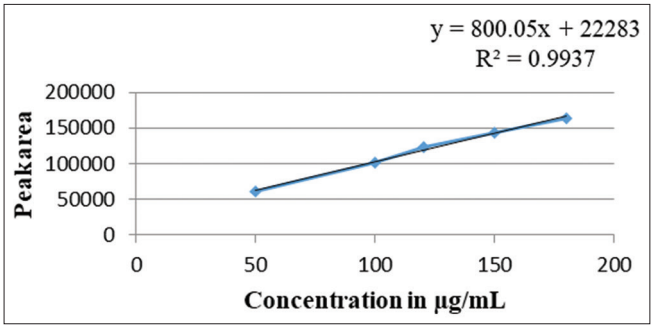

Fig. 5: Standard curve of beclomethasone dipropionate obtained within a concentration range of 1–3.6 µg/mL depicting correlation coefficient >0.99 states the method to be linear as per the linear regression equation y=800.05× + 22283. ×: concentration, y: absorbance, r2: correlation coefficient

Table 6: Result of robustness

Parameters	Deviation	% RSD for peak area	
		Salicylic acid	Beclomethasone dipropionate
Flow Rate	0.8 mL/min	1.04	0.43
	1.2 mL/min	1.59	1.49
Column Temperature	26°C	0.95	1.03
	30°C	1.50	0.10
Wavelength	233 nm	1.47	1.56
	237 nm	1.41	1.00

and then administered under a variety of variable conditions, including column temperature (26°C and 30°C), flow rate (0.8 mL/min and 1.2 mL/min), and detection wavelength (233 nm and 237 nm). The proposed method is robust at tiny but deliberate modifications, according to robustness data. Robustness data are given in Table 6.

DISCUSSION

The major aim of the development of the chromatographic method was to urge a reliable technique for the quantification of SA and BD in pharmaceutical dosage form. For the analysis of SA and BD in pharmaceutical dose form, several chromatographic conditions were used. The analysis's findings fell within the acceptable range. The calibration curves for SA and BD were found to be linear over the concentration ranges of 30–108 g/mL and 1–3.6 g/mL, respectively. The samples were analyzed at 235 nm, the injection volume was 10 µL, and the separation was done by utilizing the prontosil HPLC C18 (250×4.6 mm, 5 µm) column. Retention times and the tailing factor were calculated. The retention times of SA and BD were found to be 3.59 and 6.00 min, respectively. The proposed column was selected, which gave a pointy and symmetrical peak with a 1.15 tailing factor and theoretical plates of 8485 for SA and a 0.98 tailing factor and theoretical plates of 10397 for BD. SA and BD have a 10.65 resolution. Statistics were used to confirm the method's linearity. The accuracy and precision studies yielded RSD values of <2.0%, demonstrating the accuracy and precision of the devised analytical method.

CONCLUSION

The technique has been created for the concurrent determination of SA and BD in topical ointments using RP-HPLC and a UV detector. The two medications' resolution is strong, and their analysis times are under 10. The validated method was found to be linear, selective, reproducible, robust, and accurate, making it useful and versatile for the simultaneous estimation of two drugs in marketed formulations. The given method is suitable for the assays of BD and SA.

ACKNOWLEDGMENTS

We are grateful to Rusi Pharma Pvt. Ltd., for a gift sample of beclomethasone dipropionate and to S. D. Fine-Chem Ltd., for a gift sample of SA. The writers appreciate the support and encouragement they have received from Ms. Pallavi Gadhave and Ms. Anuja Jadhav to complete the research work.

AUTHOR'S CONTRIBUTION

Dr.Vandana Jain oversaw experiments and data analysis. Vanita Sawant and Chinmayi Mali gathered, validated, analyzed, and wrote the information on the subject. The final manuscript was read and approved by all writers.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

REFERENCES

1. Drug Bank, Beclomethasone Dipropionate. Available from: https://go.drugbank.com/drugs/DB00394 [Last accessed on 2022 Dec 12].
2. De Souza ALR, Azevedo C, Kelly M, Moreira S. Validation of a stability-indicating HPLC method with diode array detection for the determination of beclomethasone dipropionate in aqueous suspension for nebulizer. Asian J Biomed Pharm Sci 2013;3:4-9.
3. Chassot JM, Ferreira LM, Gomes FP, Cruz L, Tasso L. Stability-indicating RP-HPLC method for determination of beclomethasone dipropionate in nanocapsule suspensions. Braz J Pharm Sci 2015;51:803-10. doi: 10.1590/S1984-82502015000400006
4. Sharma A, Kumar I, Rana K. RP-HPLC method development and validation for the combination of imiquimod and salicylic acid. Int J Pharm Pharm Sci 2020;12:41-8. doi: 10.22159/ijpps.2020v12i9.38382
5. Patil SA, Khairnar BJ, Mane DV, Chaudhari BR. A validated stability-indicating HPLC related substances method for salicylic acid in bulk drug and dosage form. World J Pharm Sci 2015;3:1025-282.
6. Medicine I; 2016. Available from: https://www.medicineindia.org/pharmacology-for-generic/2016/beclomethasone-dipropionate-salicylic-acid [Last accessed on 2023 Jan 18].
7. Shou M, Galinada WA, Wei YC, Tang Q, Markovich RJ, Rustum AM. Development and validation of a stability-indicating HPLC method for simultaneous determination of salicylic acid, betamethasone dipropionate and their related compounds in diprosalic Lotion®. J Pharm Biomed Anal 2009;50:356-61. doi: 10.1016/j.jpba.2009.05.015. Assessed 20 Feb 2023. PMID 19545962
8. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use. Validation of analytical procedures: Text and Methodology ICH. Vol. Q2(R1); 2005. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf [Last accessed on 2023 Mar 06].
9. Shaikh S, Jain V. Development and validation of novel rp-hplc method for the simultaneous estimation of ellagic acid and quercetin in an ayurvedic formulation. Int J Appl Pharm 2018;10:111-6. doi: 10.22159/ijap.2018v10i4.27011
10. Gholve S, Gangapure S, Birajdar M, Mujewar I, Bhusnure OG. RP-HPLC method development and validation for determination of didanosine in pharmaceutical dosage forms. J Drug Deliv Ther 2019;9:343-7. doi: 10.22270/jddt.v9i4-s.3328