

International Journal of Biomedical Investigation

journal homepage: <https://ijbi.edwiserinternational.com>

Original Article

A Validated Analytical RP-HPLC Method Developed for the Estimation of Salicylic Acid in Bulk and Cosmetic Product

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ARTICLE INFO

Received 10 August 2025
Revised 23 September 2026
Available Online 04 October 2026

Keywords:

RP-HPLC
ICH Q2(R1)
Salicylic acid
Method validation
Cosmetic face serum

ABSTRACT

Objective: To develop and validate a simple, precise, and accurate reversed-phase High-performance liquid chromatographic (RP-HPLC) method for the estimation of salicylic acid in bulk drug and cosmetic face serum formulations, in accordance with ICH Q2(R1) guidelines.

Methods: Chromatographic separation was achieved on a C₁₈ column using a mobile phase of methanol and potassium dihydrogen phosphate buffer (pH 3.5) in the ratio 60:40 (v/v) containing 0.1% orthophosphoric acid, delivered under isocratic elution with UV detection at 296 nm. The method was validated for linearity, precision, accuracy, ruggedness and robustness as per ICH Q2(R1) guidelines.

Results: Salicylic acid eluted at a retention time of 3.683 minutes, with theoretical plates greater than 2000 and a tailing factor below 2.0, indicating good system suitability. The method exhibited excellent linearity over the concentration range of 10–60 µg/mL ($r^2 = 0.9997$), with a limit of detection (LOD) and limit of quantification (LOQ) of 0.00000335 µg/mL and 0.00001017 µg/mL, respectively. Precision studies yielded %RSD values below 2%, and accuracy, assessed by the standard addition method at 50%, 100%, and 150% of label claim, showed recovery values ranging from 98.7% to 101.2%. All ruggedness and robustness parameters complied with ICH Q2(R1) acceptance criteria.

Conclusion: The developed RP-HPLC method is simple, rapid, cost-effective, and environmentally friendly, and is suitable for the routine quality control and quantitative determination of salicylic acid in bulk drug substances and cosmetic formulations.

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<https://doi.org/10.31531/2581-4745.1000183>

Introduction

Salicylic acid (2-hydroxybenzoic acid) is a widely used beta-hydroxy acid valued for its keratolytic, comedolytic, and anti-inflammatory properties, making it a key active ingredient in pharmaceutical and

cosmetic formulations, particularly face serums targeting acne, hyperpigmentation, and exfoliation [1]. Given its concentration-dependent efficacy and safety profile, accurate and reliable quantification of salicylic acid in such formulations is essential to ensure product quality, batch-to-batch consistency, and regulatory compliance [2].

Among the analytical techniques available for quantifying salicylic acid, Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) is preferred for its high sensitivity, specificity, and ability to resolve the analyte from formulation excipients and potential degradation products, an advantage over UV-spectrophotometric methods, which, while simpler, are more susceptible to spectral interference in complex matrices. RP-HPLC also offers superior precision and robustness, making it suitable for routine quality control and stability-indicating applications [3].

In the present study, a simple, precise, and accurate RP-HPLC method was developed and validated for the estimation of salicylic acid in cosmetic face serum formulations. Chromatographic separation was achieved using a mobile phase composed of methanol and potassium phosphate buffer (pH 3.5), delivered under isocratic conditions on a C18 column, with salicylic acid eluting at a retention time of 3.6 minutes. The acidic pH of the buffer was selected to suppress the ionization of salicylic acid ($pK_a \sim 2.97$), ensuring sharp, symmetric peak shape and reproducible retention behavior [4].

The developed method was validated as per International Council for Harmonisation (ICH) Q2(R1) guidelines for parameters including linearity, precision, accuracy, specificity, robustness, and system suitability, with the objective of establishing a reliable analytical tool for the routine quantification of salicylic acid in cosmetic serum matrices.

Materials and Methods

Chemical and Reagents

Salicylic acid was purchased from Loba Chemie Pvt. Ltd. Analytical HPLC Grade water, Potassium dihydrogen phosphate buffer and 0.1% Orthophosphoric acid were used as the mobile phase. The Derma Co. 2% salicylic acid face serum was purchased from a local pharmacy and utilized for this study.

Instrumentation

Shimadzu RP-HPLC Prominence – I LC 2030 plus.

Identification test

Salicylic acid was identified by the Melting point and further confirmed by Fourier Transform Infrared (FT-IR) spectroscopy.

Method

Preparation of buffer

1.36 g of potassium dihydrogen phosphate buffer was dissolved in 1000 ml of HPLC grade water and pH was adjusted 3.5 with Ortho phosphoric acid. The solution was then filtered through a membrane filter.

Preparation of Mobile Phase

The mobile phase was prepared by mixing Methanol: Potassium dihydrogen phosphate buffers the ratio of 60:40 (v/v) containing 0.1% H_3PO_4 , filtered through a membrane filter, and degassed by sonication before use.

Preparation of standard stock solution

An accurately weighed quantity of Salicylic acid (10 mg) was transferred into a 100 mL volumetric flask, dissolved in mobile phase and diluted to the mark. This resulted in a stock solution with a concentration of 100 $\mu\text{g/ml}$.

Preparation of working standard solution

From the standard stock solution, 2 mL was transferred into a 10 mL volumetric flask and diluted to volume with the mobile phase to obtain a working standard solution of 20 $\mu\text{g/ml}$.

Preparation of sample solution

1 ml of Derma co serum was transferred into 50 ml volumetric flask and added a diluent up to the mark (400 $\mu\text{g/ml}$), Sonicated for 10 minutes and 2.5 ml of the above solution was transferred into the 10 ml of volumetric flask and made up to the mark with diluent (100 $\mu\text{g/ml}$), 2 ml of the above solution was transferred into the 10 ml of volumetric flask and made up to the mark with diluent (20 $\mu\text{g/ml}$).

System Suitability

System suitability was assessed by injecting the standard solution under optimized conditions. Salicylic acid produced a sharp, symmetric peak at 3.683 minutes. Theoretical plates were >2000 , and the tailing factor was <2.0 . These values indicate good column efficiency and peak symmetry. All parameters complied with ICH acceptance criteria.

Linearity procedure

For the linearity study, aliquots of 1, 2, 3, 4, 5 and 6 ml of the standard stock solution (100 $\mu\text{g/mL}$) were each transferred into a separate 10 mL volumetric flask and diluted to volume with the mobile phase to obtain

concentrations of 10 - 60 µg/ml. Each solution was injected, and calibration curves were constructed by plotting peak area against concentration.

Formulation procedure

1 ml of Derma co serum was transferred into 50 ml volumetric flask and added a diluent up to the mark (400 µg/ml) and sonicate for 10 minutes, 2.5 ml of the above solution was transferred into the 10 ml of volumetric flask and made up to the mark with diluent (100 µg/ml), 2 ml of the above solution was transferred into the 10 ml of volumetric flask and made up to the mark with diluent (20 µg/ml). This procedure was repeated for six times.

Recovery procedure

The accuracy of the developed RP-HPLC method was assessed by the standard addition spike-recovery technique at three levels, corresponding to 50%, 100%, and 150% of the label claim concentration of salicylic acid in the Derma co serum formulation.

In each case, 1 ml of the Derma co serum was taken in 50 ml of volumetric flask and diluted with diluent up to the mark and to get concentration of solution (400 µg/ml) and 2.5 ml from the above solution was taken in the 10 ml volumetric flask and diluted to 10 ml with diluent up to the mark and to get a concentration solution (100 µg/ml).

Separately, 10 mg Salicylic acid raw material was added into a 100 ml volumetric flask and 100 ml of diluent made up to the mark (100 µg/ml)

For 50% recovery, 1 ml of the standard solution (100 µg/ml) was added to 2 ml of the formulation solution (100 µg/ml) and the volume made up to 10 ml with mobile phase, get a final concentration of 30 µg/ml.

For 100% recovery, 2 ml of the standard solution (100 µg/ml) was added to 2 ml of the formulation solution

(100 µg/ml) and the volume made up to 10 ml with mobile phase, get a final concentration of 40 µg/ml.

For 150% recovery, 3 ml of the standard solution (100 µg/ml) was added to 2 ml of the formulation solution (100 µg/ml) and the volume made up to 10 ml with mobile phase, get a final concentration of 50 µg/ml.

Each of the three solutions was prepared in triplicate and analysed by the proposed RP-HPLC method, and the percentage recovery of salicylic acid was calculated at each level to confirm the accuracy of the method.

Ruggedness

1 ml of Derma co serum was transferred into 50 ml volumetric flask and made up to the mark with diluent (400 µg/ml) and sonicated for 10 minutes, 2.5 ml of the above solution was transferred into the 10 ml of volumetric flask and made up to the mark with diluent (100 µg/ml), 2 ml of the above solution was transferred into the 10 ml of volumetric flask and made up to the mark with diluent (20 µg/ml).

Robustness

1 ml of Derma co serum was transferred into 50 ml volumetric flask and made up to the mark with diluent (400 µg/ml) and sonicated for 10 minutes, 2.5 ml of the above solution was transferred into the 10 ml of volumetric flask and made up to the mark with diluent (100 µg/ml), 2 ml of the above solution was transferred into the 10 ml of volumetric flask and made up to the mark with diluent (20 µg/ml). Deliberately parameter tested at a time:

1. Flow rate (± 0.1 ml / min)
2. Mobile phase ratio ($\pm 1\%$)
3. Column oven temperature ($\pm 5^\circ\text{C}$)

Results and Discussion

Optical Characteristic

Table 1: Optical characteristic of Salicylic acid.

Parameters	Salicylic Acid
Beer's law (µg/ml)	10-60
Regression equation ($y=mx + c$)	$Y = 11059x + 19716$
Slope	11059.2
Intercept	19715.8
Correlation coefficient (r^2)	0.9997
Standard error	0.0039
Limit of detection (µg/ml)	0.00000335
Limit of quantification (µg/ml)	0.00001017

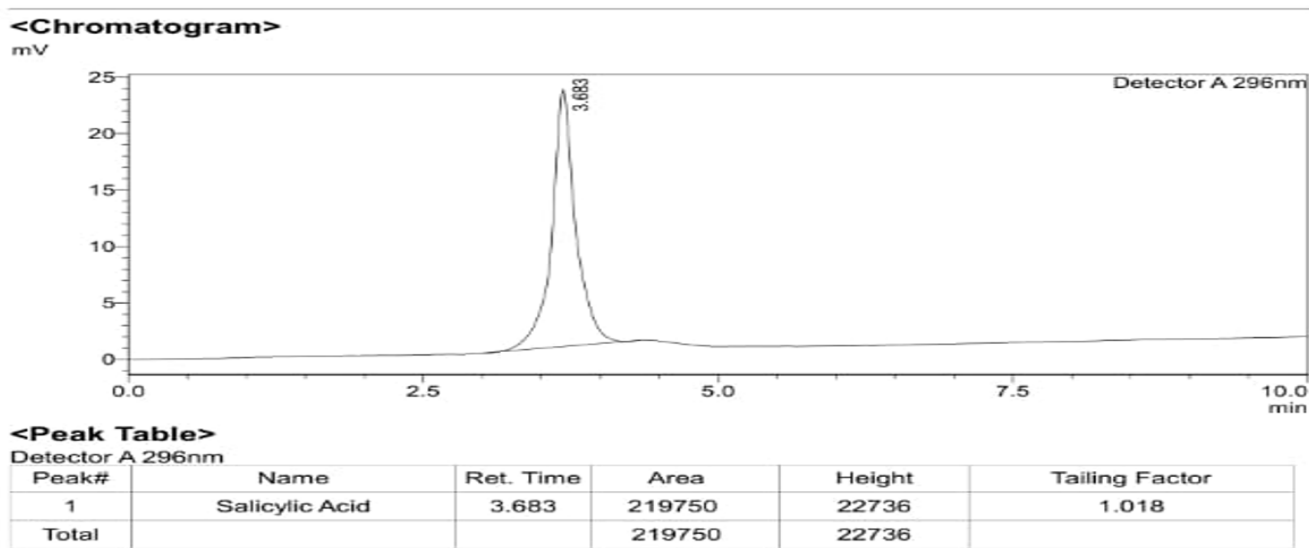


Figure 1: Optimized peak of Salicylic acid by RP-HPLC.

Linearity

The stock solutions of salicylic acid in the concentration range of 10–60 µg/mL were prepared in the mobile phase and injected into the column [5]. The

chromatograms were recorded and a calibration curve was constructed by plotting peak area against concentration, which showed excellent linearity with a correlation coefficient of 0.9997.

Table 2: Linearity of salicylic acid.

Concentration (x-axis)	Area (y-axis)
10	135772
20	230449
30	351656
40	458294
50	569916
60	688560

Mean of 6 Observation

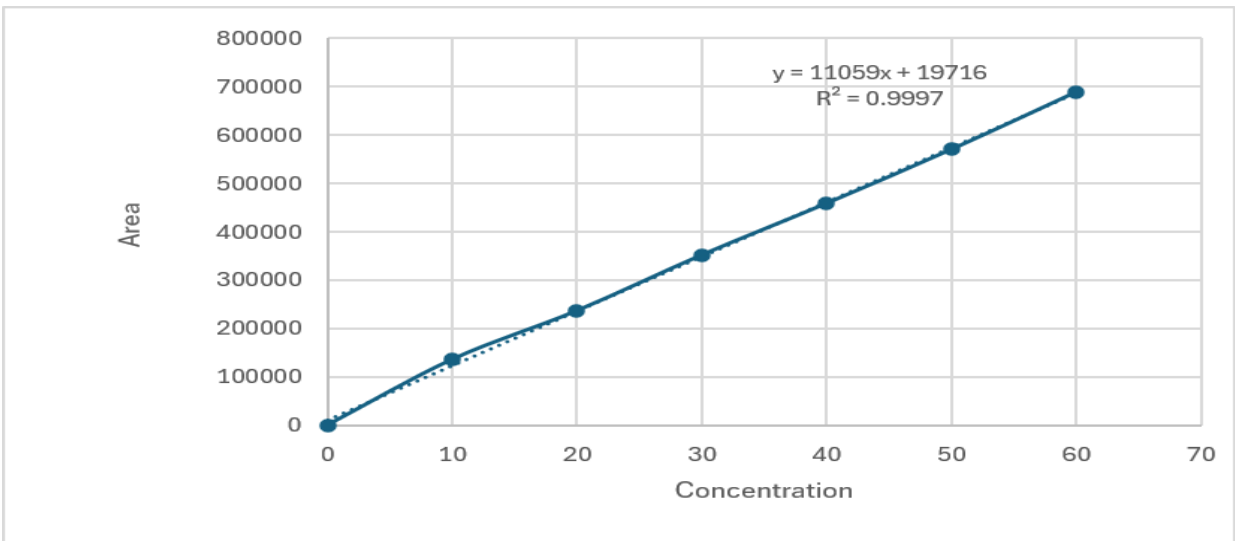


Figure 2: Calibration Graph of Salicylic acid

Formulation

The precision of the developed analytical method was assessed through repeatability (intra-day precision) and intermediate precision (inter-day precision) studies. Six replicate analyses were performed under identical

experimental conditions, and the mean, standard deviation (SD), and percentage relative standard deviation (%RSD) were determined [6]. The %RSD values obtained were below 2%, indicating that the method exhibits good precision, with satisfactory consistency and reproducibility of the analytical results.

Table 3: Quantification of Formulation by RP- HPLC method.

Precision	Label claim	Amount found (mcg/ml)	% purity	Average %	S. D	% RSD
1	20 µg/ml	20.10	100.52	100.74	0.2756	0.2735
2		20.15	100.75			
3		20.11	100.55			
4		20.24	101.22			
5		20.18	100.92			
6		20.10	100.50			

* Mean of 6 Observation

Recovery

The accuracy of the proposed method was assessed using the standard addition technique at three concentration levels, corresponding to 50%, 100%, and 150% of the target concentration. Known quantities of

the standard drug were added to the pre-analysed sample, and the resulting mixtures were analysed using the proposed method [7]. The percentage recovery values ranged from 98.7% to 101.2%, indicating good accuracy of the developed method.

Table 4: Recovery study of Salicylic acid by RP- HPLC method.

Sample	%	Sample amount (µg/ml)	Amount spiked (µg/ml)	Estimated Amount * (µg/ml)	Recovered Amount * (µg/ml)	Average % Recovery *	SD	% RSD
Salicylic acid	50	20	10	30.12	10.12	101.25	1.2752	1.2735
	100	20	20	39.75	19.75	98.75		
	150	20	30	50.12	30.12	100.41		

*Mean of 3 Observations

Ruggedness & Robustness

Ruggedness refers to the ability of an analytical method to produce consistent and reproducible results when the same sample is analyzed under different routine conditions, such as by different analysts, on different instruments, on different days, or using different reagent lots or laboratories. It indicates the reliability of the method when minor variations occur during normal laboratory operations [8].

Robustness is an essential parameter of analytical method validation and indicates the capability of a

method to withstand small and deliberate changes in experimental conditions without significantly affecting the analytical results. In the present study, the robustness of the developed method was evaluated by deliberately varying the detector wavelength within a predefined range. The effect of these variations on the analytical results was assessed, and the obtained %RSD values were found to be below 2%. These results confirm that the developed method is sufficiently robust and capable of providing reliable results despite minor changes in the experimental conditions.

Table 5: Ruggedness of Salicylic acid by RP-HPLC.

Sample	Condition	% obtained *	SD	%RSD
Salicylic acid	Analyst-1	100.65	1.2641	1.2555
	Analyst-2	100.42	0.6102	0.6123

* Mean of 3 Observations

Table 6: Robustness of Salicylic acid by RP-HPLC.

Parameter	Amount Found (µg/ml)	% Purity*	SD	%RSD
Flow Rate				
0.9 ml/min	20.37	101.85	1.164	1.152
1 ml/min	20.18	100.93	0.532	0.531
1.1 ml/min	19.89	99.34	1.782	1.767
Column Oven Temperature				
35°C	19.82	99.11	0.414	0.417
40°C	20.12	100.62	0.257	0.265
45°C	19.74	98.73	1.331	1.356
Mobile Phase Composition				
Methanol: Potassium dihydrogen phosphate buffer 59:41 (0.1% H_3PO_4)	20.43	102.15	0.979	0.968
Methanol: Potassium dihydrogen phosphate buffer 60: 40(0.1% H_3PO_4)	20.19	100.95	0.782	0.782
Methanol: Potassium dihydrogen phosphate buffer 61: 39 (0.1% H_3PO_4)	19.75	98.75	1.523	1.540

* Mean of 3 Observation

Conclusion

A simple, rapid, accurate and economical, friendly RP-HPLC method was successfully developed and validated for the quantitative estimation of salicylic acid in bulk drug and cosmetic formulations. The method was validated in accordance with ICH guidelines and demonstrated satisfactory performance in terms of linearity, accuracy, precision, specificity, robustness, and ruggedness. The calibration curve showed excellent linearity over the selected concentration range, with a high correlation coefficient. Accuracy was confirmed through recovery studies, while the low %RSD values obtained in precision studies indicated good repeatability and intermediate precision.

The method exhibited no significant interference from commonly used cosmetic excipients, demonstrating its specificity and suitability for routine analysis. Overall, the developed RP-HPLC method is simple, reliable, cost-effective, and environmentally friendly, making it

a suitable analytical approach for quality control and routine determination of salicylic acid in bulk drug substances and cosmetic formulations.

Acknowledgements

The authors are thankful to the Management and Principal, Adhiparasakthi College of Pharmacy, Melmaruvathur, for providing the necessary facilities to carry out this research work.

Conflict of Interest

The authors declared no conflict of interest and no funding was required to conduct these review data.

Availability of Data and Materials

The data supporting this study findings will be made available upon request.

Funding

The project received no external funding.

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