Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 1s, https://carcinogenesis.com/

Development and Validation of a UV Spectrophotometric Absorbance Correction Method for the Simultaneous Estimation of Salicylic Acid and Niacinamide in bulk and liquid dosage form

Keerthisikha Palur*[1], Sreenivasa Charan Archakam[1], Kummara Vishnu[2], M.G Praveen[2], Chintha Yasmitha[2], Goduguchintha Nandu[2], Kethu Sirisha[2]

[1]Professor, Department of Pharmaceutical Analysis, Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupathi.

[2]B.Pharmacy IV year, Sri Padmavathi school of Pharmacy, Tiruchanoor, Tirupathi.

ABSTRACT

The present study describes the development and validation of a simple, rapid, and accurate UV spectrophotometric method using the absorbance correction approach for the simultaneous estimation of salicylic acid and niacinamide in liquid dosage form. Salicylic acid and niacinamide, commonly used in dermatological formulations, exhibit overlapping UV spectra, which necessitates the use of absorbance correction for precise quantification. The method involves measuring absorbance at selected wavelengths where each drug demonstrates maximal absorption with minimal interference from the other. Validation was performed according to ICH guidelines, assessing linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness. The results demonstrated good linearity and reproducibility for both analytes within the specified concentration ranges. The proposed method is cost-effective, less time-consuming, and avoids complex sample preparation, offering a practical alternative to chromatographic techniques for routine analysis and bioanalytical studies. This method can be employed for routine analysis in quality control laboratories.

Keywords: *Salicylic acid, Niacinamide, UV spectrophotometry, Absorbance correction method, Method validation.*

How to Cite: Keerthisikha Palur, Sreenivasa Charan Archakam, Kummara Vishnu, M.G Praveen, Chintha Yasmitha, Goduguchintha Nandu, Kethu Sirisha, (2025) Development and Validation of a UV Spectrophotometric Absorbance Correction Method for the Simultaneous Estimation of Salicylic Acid and Niacinamide in bulk and liquid dosage form, *Journal of Carcinogenesis*, *Vol.24*, *No.1s*, 36-40.

1. INTRODUCTION

Salicylic acid and niacinamide have garnered significant attention as synergistic active agents in topical dermatological formulations aimed at managing acne vulgaris, hyperpigmentation, and photoaged skin[1,2]. Salicylic acid, a beta-hydroxy acid, promotes exfoliation and unclogs pores through its keratolytic and comedolytic effects. Niacinamide, a form of vitamin B3, supports skin barrier repair, reduces inflammation, and regulates sebum secretion. When used together, these actives provide complementary benefits-enhancing therapeutic efficacy while minimizing irritation. This combination represents a promising approach in modern dermatological and cosmetic formulations aimed at improving overall skin texture and tone[3-8].

A review of the available literature reveals that only a few analytical methods have been reported for the simultaneous estimation of salicylic acid and niacinamide, primarily based on RP-HPLC techniques. However, no UV spectrophotometric method has yet been developed for their combined analysis in biological matrices. Hence, in the present study, a simple, accurate, and reproducible UV spectrophotometric method using the absorbance correction approach was developed and validated for the simultaneous estimation of salicylic acid and niacinamide in serum dosage form. The proposed method is designed to offer a reliable alternative to chromatographic techniques, ensuring cost-effectiveness and suitability for routine laboratory analysis and bioanalytical studies[9-15].

UV spectrophotometry remains one of the most widely employed analytical techniques due to its simplicity, cost-effectiveness, and reliability for quantitative estimation of pharmaceutical compounds. The absorbance correction method, in particular, offers a significant advantage in the simultaneous estimation of multicomponent systems where spectral overlap occurs. By applying mathematical correction based on individual absorbance values at selected wavelengths, this method enables accurate quantification of each component without prior separation. This approach minimizes analytical

complexity, reduces solvent consumption, and provides rapid results with acceptable accuracy and precision, making it highly suitable for routine and bioanalytical applications[16-24].

2. MATERIALS AND METHODS

Chemicals and reagents

Working standards (active pharmaceutical ingredients, APIs) of Salicylic Acid and Niacinamide were kindly gifted by MSN Laboratories, Hyderabad, India. Methanol (analytical grade) was used as the solvent for preparation of standard and sample solutions. All other reagents and chemicals were of analytical grade.

Instruments used in the study

An analytical balance was used for accurate weighing of the drug standards. Absorbance measurements were performed using a Shimadzu double-beam UV-visible spectrophotometer (UV-1800) equipped with UV Probe software (Shimadzu Corporation, Kyoto, Japan). Quartz cuvettes with a 1 cm path length were used for all measurements. Standard laboratory glassware, including volumetric flasks and pipettes, were employed for preparation and dilution of solutions[25].

Preparation of Standard and Sample Solutions

Standard stock solutions of Salicylic Acid and Niacinamide (1000 µg/mL each) were prepared by accurately weighing 25 mg of each drug and dissolving in 25 mL of methanol. The solutions were sonicated for complete dissolution and filtered to remove any insoluble particles. Working standard solutions (100 µg/mL) were prepared by diluting 2.5 mL of each stock solution to 25 mL with methanol. Suitable aliquots of these working solutions were further transferred to 10 mL volumetric flasks and diluted to volume with methanol for UV spectrophotometric analysis.

The sample solution was prepared from the commercial serum formulation. An appropriate volume of the serum was diluted with methanol to obtain final concentrations of 6 µg/mL for Salicylic Acid and 15 µg/mL for Niacinamide. The solution was mixed thoroughly to ensure uniformity, and any insoluble components were removed by filtration or centrifugation as needed. The resulting solution was used for UV spectrophotometric analysis[26].

Determination of λmax

The λmax values of the working standard solutions were determined using the trial and error method. Both Salicylic Acid and Niacinamide working solutions were scanned in a UV-visible spectrophotometer using methanol as a common solvent. The maximum absorbance (λmax) was found to be 232.8 nm for Salicylic Acid (λ_1) and 262 nm for Niacinamide (λ_2). These wavelengths were subsequently used for the absorbance correction method (ACM) for simultaneous estimation[27].

Construction of Calibration curve

The absorption spectra of the working standard solutions of Salicylic Acid and Niacinamide were recorded over the range of 200–400 nm and stored in the instrument memory. For Salicylic Acid, the absorbances at 232.8 nm (λ_1) were plotted against their respective concentrations, showing good linearity over the range of 2–12 µg/mL. Similarly, for Niacinamide, the absorbances at 262 nm (λ_2) were plotted against concentration, demonstrating linearity over the range of 4–20 µg/mL. The resulting calibration curves were used to generate regression equations for simultaneous estimation of the two drugs in mixture solutions.

3. U.V. SPECTROSCOPIC METHOD

Absorbance Correction Method (ACM)

The ACM was employed for the simultaneous estimation of Salicylic Acid (X) and Niacinamide (Y) in a binary mixture exhibiting overlapping spectra. Niacinamide interferes at the λmax of Salicylic Acid (λ_1), while Salicylic Acid does not interfere at the λmax of Niacinamide (λ_2).

The absorption factor of Niacinamide (Y), defined as the ratio of absorbance at λ_1 to absorbance at λ_2 (Abs_1/Abs_2), was first calculated using the pure component. Since Salicylic Acid (X) does not contribute at λ_2, the absorbance of X at λ_1 in the mixture (X + Y) was calculated using:

$$\text{Abs of X at } \lambda_1 = \text{Abs}_{\lambda_1}(\text{X+Y}) - \frac{\text{Abs}_1}{\text{Abs}_2} \times \text{Abs}_{\lambda_2}(\text{X+Y})$$

Where:

- **Abs_1/Abs_2** = absorption factor of pure Niacinamide (Y)
- **Abs λ_1 and Abs λ_2** = absorbance of the mixture at λ_1 and λ_2, respectively

The concentrations of Salicylic Acid (X) and Niacinamide (Y) were then calculated from their respective regression equations obtained by plotting absorbance versus concentration of the zero-order spectra at λ_1 and λ_2[28].

4. RESULTS AND DISCUSSION

The Spectrophotometric method using Absorption Correction Method was successfully developed for simultaneous determination of salicylic acid and niacinamide from binary mixture. All the optimized method parameters are summarized and presented in Table 1 Based on the solubility profile of selected drugs methanol was used as common solvent for both drugs i.e., salicylic acid and niacinamide. The wavelengths selected for the determination of salicylic acid and niacinamide were 232.8nm and 262 nm.

Table 1: Optimization and selection of method parameters

Method parameters	Optimized parameters
Scanning range	200nm to 400 nm
Scan speed	Fast
Analytical wavelength for determination of salicylic acid	232.8 nm
Analytical wavelength for determination of niacinamide	262 nm

Absorption correction method

The zero-order UV absorption spectra of Salicylic Acid and Niacinamide are presented in Fig. 1. As shown, the two compounds exhibit significant spectral overlap in the 230–250 nm region, which can lead to interference in direct estimation if measured individually at a single wavelength. Salicylic Acid shows maximum absorbance at **232.8 nm (λ_1)**, while Niacinamide has maximum absorbance at **262 nm (λ_2)**. At λ_1, Niacinamide contributes to the absorbance of the mixture, whereas Salicylic Acid does not interfere at λ_2. This overlapping behavior necessitated the use of the **absorbance correction method (ACM)** to accurately determine the concentrations of both drugs in the mixture. The figure clearly illustrates the extent of spectral overlap and highlights the importance of applying mathematical correction for simultaneous estimation.

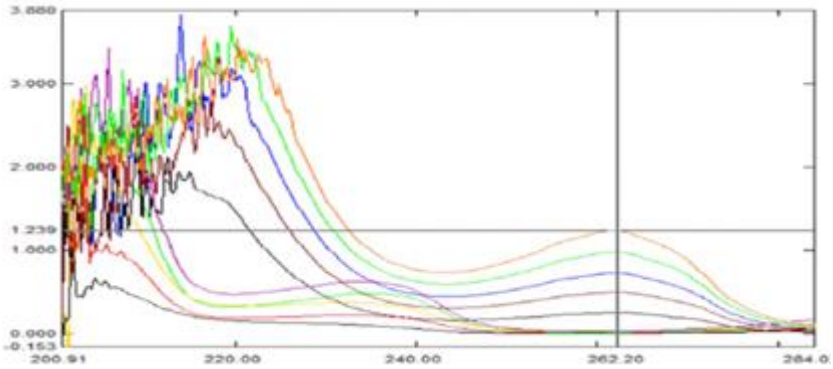

Fig.1. Overlay spectra of salicylic acid and niacinamide

Validation of UV Spectrophotometric method

The developed UV spectrophotometric method was validated according to ICH Q2 (R1) guidelines for parameters including linearity, range accuracy and precision.

Linearity and Range

The proposed UV spectrophotometric method exhibited good linearity for both drugs over the selected concentration ranges. For Salicylic Acid, linearity was observed in the range of 2–10 µg/mL with a correlation coefficient (r) of 1, slope of 0.112, and intercept of 0.760. For Niacinamide, linearity was observed in the range of 4–20 µg/mL with a slope of 0.254, intercept of 1.237, and excellent correlation, demonstrating the method's suitability for simultaneous estimation in this concentration range. The high correlation coefficients indicate strong proportionality between absorbance and concentration, ensuring reliable quantification of both analytes in serum formulations.

Precision

Precision was assessed in terms of intra-day and inter-day variations. Standard solutions at three different concentrations were analyzed multiple times within the same day and on different days. The relative standard deviation (RSD) for both Salicylic Acid and Niacinamide was found to be less than 2%, confirming the reproducibility of the method.

Accuracy

The accuracy of the method was evaluated by the recovery study using the commercial serum formulation. Known concentrations of Salicylic Acid and Niacinamide were analyzed using the developed method, and percent recoveries were

calculated. The mean recovery values for both drugs were within the acceptable range of 98–102%, indicating the method is accurate for simultaneous estimation.

Table 2: Method validation Parameters

Parameter	Salicylic Acid	Niacinamide	Remarks
Linearity Range (µg/mL)	2–10	4–20	Linear over selected ranges
Correlation Coefficient (r)	1.000	0.999+	Excellent linearity
Slope	0.112	0.254	—
Intercept	0.760	1.237	—
Accuracy (% Recovery)	101.2%	100.5%	Acceptable per ICH guidelines
Precision (RSD, %)	Intra-day: 0.806 Inter-day: 0.912	Intra-day: 0.902 Inter-day: 1.024	Highly reproducible, RSD <2%

Assay of Commercial Serum Formulation

The developed UV spectrophotometric method using the absorbance correction method (ACM) was successfully applied to the simultaneous estimation of Salicylic Acid and Niacinamide in the commercial serum formulation. The sample solution was prepared to final concentrations of 6 µg/mL for Salicylic Acid and 15 µg/mL for Niacinamide. The absorbances measured at 232.8 nm (λ_1) and 262 nm (λ_2) were used in the ACM equation to determine the individual concentrations. The assay results indicated that the content of Salicylic Acid and Niacinamide in the serum were 101 % and 103 % of the labeled claim, respectively, which is in excellent agreement with the product specification. These results demonstrate that the method is accurate, precise, and suitable for routine quality control analysis of commercial serum formulations containing these two active ingredients.

5. CONCLUSION

A simple, rapid, and reliable UV spectrophotometric method using the absorbance correction method (ACM) was successfully developed and validated for the simultaneous estimation of Salicylic Acid and Niacinamide in a commercial serum formulation. The method exhibited good accuracy, precision, and linearity within the selected concentration ranges, with assay results closely matching the labeled claims. The spectral overlap between the two drugs was effectively resolved using ACM, making this method suitable for routine quality control and formulation analysis. Its simplicity, cost-effectiveness, and reproducibility make it a practical alternative to more complex chromatographic techniques for simultaneous estimation of these active ingredients in serum-based formulations.

Acknowledgements

The authors sincerely thank MSN Laboratories, Hyderabad, India, for kindly providing the working standards of Salicylic Acid and Niacinamide. We also express our gratitude to the management, and principal of our institute for providing the necessary facilities and support to carry out this research.

Conflicts of Interest

The authors declare that there are no conflicts of interest associated with this work.

REFERENCES

[1] Patel, S.; Patel, D.; Patel, C.; *J. Pharm*. Anal., **2015**, 5(3), 189–194.
[2] Bhatt, P.; Raval, M.; Shah, D.; *Int. J. Pharm. Sci*. Res., **2014**, 5(5), 195–200.
[3] Wankhede, S.B.; Tajne, M.R.; Gupta, K.R.; Wadodkar, S.G.; *Indian J. Pharm. Sci*., **2007**, 69, 298–300.
[4] Glombitza, B.W.; Schmidt, P.C.*; J. Pharm. Sci.*, **1994**, 83(5), 751.
[5] Favre, H.A.; Powell, W.H.; *Royal Soc. Chem*., **2013**, Dec 5.
[6] Davies, M.; Marks, R.; Br. J. *Dermatol*., **1976**, 95, 187–192.
[7] Blessy, M.; Patel, R.; Prajapati, P.; Agrawal, Y.K.; *J. Pharm. Anal*., **2014**, 4(3), 159–165.
[8] Beckett, A.H.; Stenlake, J.B.; *Practical Pharmaceutical Chemistry*, 4th Ed.; CBS Publishers, New Delhi, **2002**.
[9] Patel, P.; Shrivastava, B.; Prajapati, J.; Akhtar, J.; *Indian J. Chem. Tech*., **2013**, 20(3), 185–188.
[10] Snyder, L.R.; Kirkland, J.L.; Glajch, J.L.; Practical HPLC Method Development; Wiley, New York, **1997**, 34–40, 134–140.
[11] Blessy, M.; Patel, R.; Prajapati, P.; Agrawal, Y.K.; *J. Pharm. Anal*., **2014**, 4(3), 159–165.
[12] Sarkis, N.; Sawan, A.; *BMC Chem*., **2022**, 16(1), 1–11.
[13] Jharia, A.N.; Kumar, D.; Parashar, A.K.; Patel, M.; Nema, R.K.; *Curr. Res. Pharm. Sci.*, **2013**, 3(3), 99–101.
[14] Sheikh, S.; Asghar, S.; Patnish, A.; *Int. J. Sci. Res*., **2012**.
[15] Street, K.W. Jr.; Schenk, G.H.; *J. Pharm. Sci*., **1981**, 70(6), 641–646.

[16] Kokot, Z.; Burda, K.; *J. Pharm. Biomed. Anal*., **1998**, 18(4–5), 871–875.
[17] Gamis-Gracia, L.; Luque de Castro, M.D.*; J. Liq. Chromatogr. Relat. Technol*., **1997**, 20(13), 2123–2133.
[18] Ali, S.L.; *J. Chromatogr*. A., **1976**, 126, 651–663.
[19] Konstantinos, D.G.; Ioannou, P.C.; Efstathiou, C.E.; Analyst, **1991**, 116(4), 373–378.
[20] Bundgaard, H.; *J. Pharm. Pharmacol*., **1976**, 28(7), 544–547.
[21] Reed, R.C.; Davis, W.W.; *J. Pharm. Sci*., 1965, 54(10), 1533–1534.
[22] Geeta, N.; Baggi, T.R.; Microchem. J., **1988**, 38(2), 236–240.
[23] Sethi, A.; Systematic Lab Experiments in Organic Chemistry; New Age International, **2003**.
[24] Wesp, E.F.; Brode, W.R.; J. Am. *Chem. Soc*., **1934**, 56(5), 1037–1042.
[25] Mehta, V.N.; Kailasa, S.K.; Wu, H.F.; *New J. Chem*., **2014**, 38(4), 1503–1511.
[26] Mehlig, J.P.; *Ind. Eng. Chem. Anal. Ed*., **1938**, 10(3), 136–139.
[27] ICH Q2(R1); Validation of Analytical Procedures: Text and Methodology; Int. Conf. Harmonisation, **2005**.
[28] ICH Guideline; Stability Testing of New Drug Substances and Products Q1A(R2); ICH Harmonised Tripartite Guideline, **1994**.