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Validated Stability-Indicating RP-HPLC Method for the Determination of Salicylic Acid

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ABSTRACT

The objective of this work was to develop a simple, sensitive, accurate, precise and reproducible high performance liquid chromatography (HPLC) method for the determination of salicylic acid in pharmaceutical dosage forms. Shimadzo Prominance model L20 AD HPLC system equipped with SPD 20A UV-Vis detector was used for the analysis. The separation was done on RESTEX allure C18 column (3 µm, 15 cm × 4.6 mm), for an isocratic elution a mixture of water, methanol and glacial acetic acid (65:35:1, v/v) mobile phase at a wavelength of 254 nm. The flow rate was 1.0mL/min. The RP-HPLC method developed for analysis of salicylic acid was validated with respect to specificity, selectivity, linearity, accuracy, precision and robustness as per the ICH guidelines. The retention time of salicylic acid was 7.575 min. The linearity was established over the concentration ranges of 50-350 µg/mL with correlation coefficients (r^2) 0.999. The percentage accuracy of salicylic acid ranged from 99.76 -101.66%. The relative standard deviation values for intra-day and inter-day precision was lower than 2.0% and the assay result was found to be in the range 99.57-101.32%. Salicylic acid was subjected to stress conditions such as neutral, acidic, alkaline, oxidation and photolysis degradations as per ICH guidelines. The degradation studies revealed that the drug was found to degrade maximum (1.67%) in alkaline degradation conditions and was highly resistant towards neutral, acidic, oxidative and photolytic degradation conditions.

Keywords: Salicylic acid, RP-HPLC, Validation, Stability-indicating, stress degradation, ICH guidelines

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INTRODUCTION

Salicylic acid (SA), is chemically known as 2-hydroxybenzoic acid/ orthohydrobenzoic acid (Figure 1). It is a colorless crystalline organic acid derived from the metabolism of salicin and has molecular formula $C_7H_6O_3$.[1] SAs are one of the key motifs in pharmaceutically and biologically active compounds, useful synthetic intermediates as well as important building blocks in material science.[2-9] Due to the high prominence of SAs, a number of synthetic methods have been developed to synthesize salicylic acid, but the most commonly used synthetic route is the Kolbe-Schmitt reaction.[10-13] SA is widely used for a number of cosmetic formulations because of its abundant biological activities.[14-15] It is successfully used to treat various skin disorders for more than 2,000 years. The ability of SA to exfoliate the stratum corneum makes it a useful peeling agent for patients with acne.[16] SA is also topically used as keratolytic, bacteriostatic, fungicidal, and photoprotective agent. Topical applications of SA are reported to reduce the rate of keratinocyte proliferation, inhibit cholesterol sulfotransferase, an enzyme responsible for cholesterol sulfate formation within keratinocytes and directly solubilize the stratum corneum by dissolving the intercellular cement.[17] The concentrations of salicylic acid beneficial in treatment of warts and localized hyperkeratosis is around 10-40%, while lower concentrations are helpful in treatment of plaque psoriasis, comedonal acne, actinic keratosis, tinea nigra etc.[18]

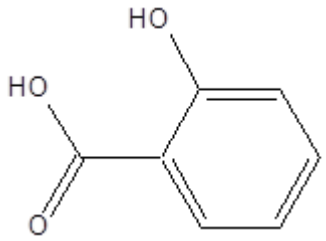


Figure 1: Chemical Structure of Salicylic acid (2-Hydroxy-benzoic acid)

Chemical stability of pharmaceutical molecules has become a matter of great concern as it marks the safety and efficacy of the drug product. Thus International Conference on Harmonization (ICH) has made stability-indicating assay method (SIM) for every drug candidate mandatory. A stability-indicating assay method helps in establishing the intrinsic stability of the drug that assures changes in identity, purity and potency of the product on exposure to various conditions. Hence, it was considered of greater importance for us to study the force degradation studies of salicylic acid by subjecting it to stress conditions viz.: acidic, alkaline, oxidative, dry heat, and photolytic stress.[20-21.]

The literature reported analytical methods used in the quantitative estimation of salicylic acid alone or in combination with other drugs reported includes UV, HPLC, HPTLC, LC-MS/MS, GC and fluorimetric methods. [22-35]

From the literature survey, it is observed that various analytical methods are available for analyzing salicylic acid in laboratory-prepared mixture, pharmaceutical preparation, and biological matrices such as human plasma. Therefore, the aim of our study was to develop a simple, precise, specific, accurate, cost-effective validated RP-HPLC method according to USP and ICH guidelines for the quantitative estimation of salicylic acid in presence of its degradation products or other pharmaceutical excipients. The analytical method developed was applied to study the stress degradation studies of SA as suggested by ICH guidelines.[36-37, 20-21]

MATERIALS AND METHOD

Reagents and Chemicals

Methanol, acetic acid and water used were of HPLC grade (Fisher Scientific, UK). Sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl) were obtained from Scharlau, Spain. Salicylic acid (SA) standard (purity 100%) was kindly gifted by Dabur international Ras Al Khaimah. All the chemicals procured were of analytical grade and used as received.

HPLC Apparatus and Conditions

Chromatographic separation was accomplished using the instrument Shimadzo Prominance model L20 HPLC system equipped with SPD 20A prominence UV-Vis detector, RESTEX allure C18 (3 µm, 15 cm × 4.6 mm) column. Isocratic elution was performed using the solvent system as a mixture of water, methanol and glacial acetic acid (65:35:1, v/v) and UV detection at 254 nm. The overall run time of the analysis was 10 minutes and the flow rate was 1.0 mL/min. 20 µL of sample was injected into the HPLC system. All the analyses were carried out at room temperature. Results were acquired and processed by Shimadzu LC Solution software.

METHOD DEVELOPMENT

Preparation of the mobile phase:

The mobile phase was prepared by mixing water, methanol and glacial acetic acid (65:35:1, v/v). The solution was filtered through 0.45µm nylon filter paper and sonicated for about 5 minutes.

Preparation of Standard Solution:

Standard stock solution of salicylic acid (SA) was prepared using the diluent mixture [methanol, water, and glacial acetic acid (70:30:4)] to obtain a concentration of 1mg/mL. The procedure

involved accurately weighed 10mg of salicylic acid standard sample and transferred into a 10ml volumetric flask, dissolved in 5ml of diluent. The resultant solution was sonicated for about 5 minutes to dissolve the drug completely and finally made the volume up to 10 ml with methanol to get the primary stock solution of 1mg/mL (1000µg/mL). Further, sample solutions were prepared by appropriate dilution of the standard solutions with the diluent. The solution was mixed well and filtered through 0.45µm membrane filter. Aliquots of the suitable salicylic acid working standard solutions were transferred into a series of 10 mL volumetric flasks so that the final concentration was in the range of 50-350 µg/mL. The working standard solution (300 µg/mL) was prepared by taking 3ml of stock solution in 10ml volumetric flask and diluted up to 10ml with methanol. The solution was mixed well and filtered through 0.45µm membrane filter.

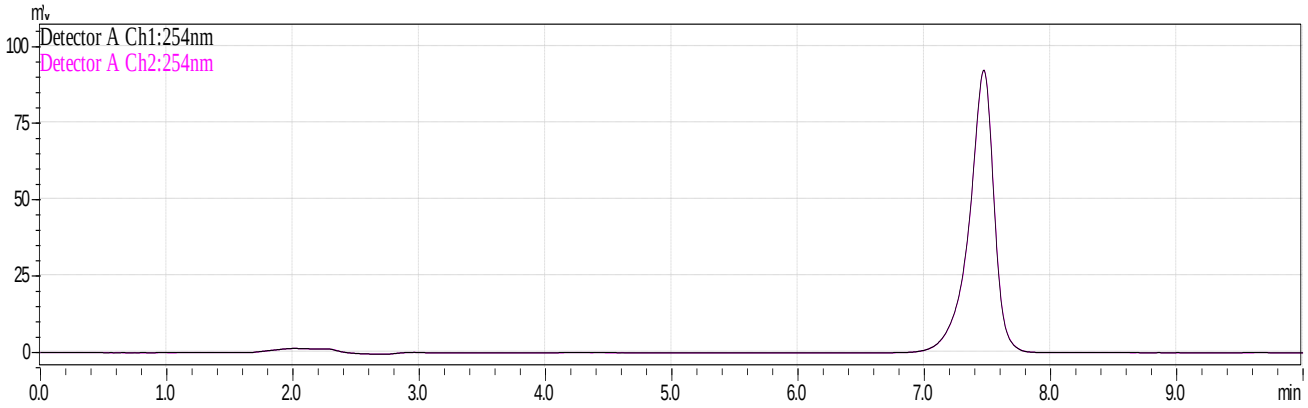
ANALYTICAL METHOD VALIDATION

In the present study, analytical method developed for the estimation of salicylic acid was validated with respect to system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and ruggedness. Later the modified method was used to estimate the percentage content of salicylic acid in different pharmaceutical ointments containing salicylic acid (3% w/w). [36-37]

System suitability

The system suitability studies were performed to confirm that the resolution and reproducibility of the chromatographic system is adequate for the analysis. The assessment for the suitability of the system was performed using six (6) drug replicas at concentration of 300 µg/mL. Various parameters assessed included repeatability, retention time, peak area, capacity factor, tailing factor, theoretical plates of the column. Results of the analysis are summarized in Table 1. A typical chromatogram of salicylic acid is shown in Figures 3A-B.

(A) SA 150 µg/mL



(B) SA 300 µg/mL

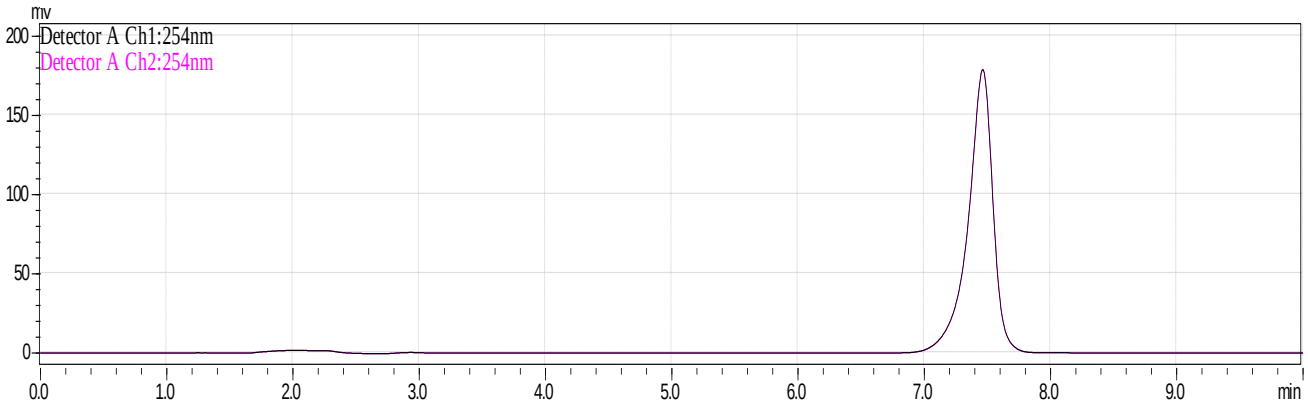


Figure 3: Typical chromatograms of salicylic acid pure drug: A (150 µg/mL); (B) (300 µg/mL).

Table 1: Chromatographic characteristics of system suitability study of salicylic acid (SA)

Sl. No	Parameters	Value (Mean ± %RSD)*
1	Retention time	7.65 ± 0.64
2	Peak area	208786.95 ± 0.057
3	Tailing factor	1.0141 ± 1.595
4	Theoretical plates	2214.68 ± 0.19
5	Capacity factor	3.358 ± 0.513

* Mean and % RSD of six samples of salicylic acid

Linearity

Seven levels of calibration standard solutions of salicylic acid were prepared from the stock solutions in the concentration range of 50-350 µg/mL to encompass the expected concentration in the measured sample. For evaluation of the calibration graph, a weighted linear regression was performed with nominal concentrations of calibration standards against measured peak areas. Calibration graph (concentration *vs.* peak area) was constructed at seven concentrations levels (50-350 µg/mL). The analytical curve was evaluated on three different days. The calibration curve of SA is reported in Figure 2 and the data for linear regression studies is shown in Table 2.

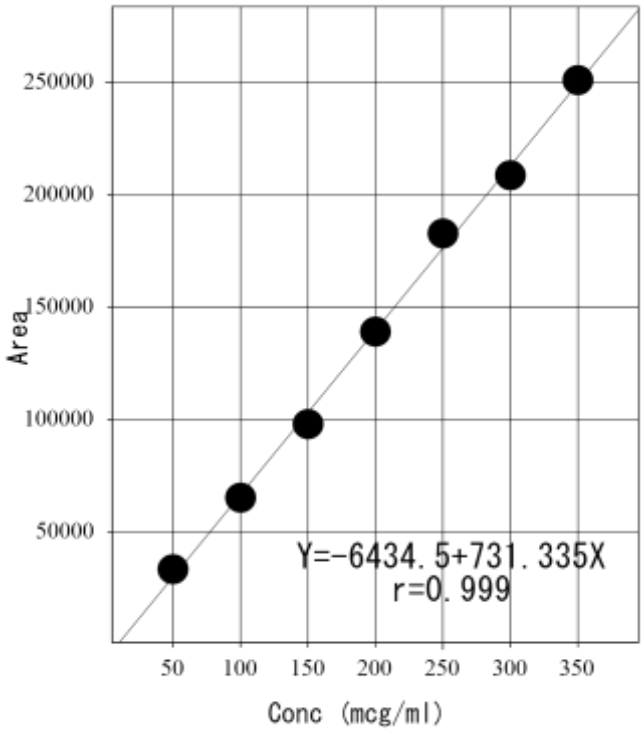


Figure 2: **Calibration curve for salicylic acid (concentration range 50-350 µg/mL)**

Table 2: Parameters of regression analysis data for salicylic acid (SA)

	Statistical Parameters	Values
1	Concentration range	50-350µg/mL
2	Regression equation	-6434.5+731.335x
3	Correlation coefficient (r^2)	0.999

Sensitivity

The sensitivity of salicylic acid was determined in terms of Limit of quantification (LOQ) and Limit of detection (LOD) as per the USP guidelines.[36] LOD was determined by establishing the minimum level at which the analyte can reliably be detected (signal-to-noise ratio is 3:1) while LOQ was determined by establishing the lowest concentration of analyte that can be determined with acceptable precision and accuracy (signal-to-noise ratio is 10:1).

Precision

Precision mainly communicates the variations or reproducibility of the analytical data. Precision was determined by repeatability (intraday precision) and intermediate precision (interday precision) for standard and sample solutions of salicylic acid. Precision was determined in six replicates of salicylic acid solution in the concentration range 50-300 µg/mL on the same day (intra-day precision) and daily for 6 times over a period of three days (interday precision). The results were expressed as %RSD of the measurements.

Intra-day precision

In the intra-day studies, six replicate injections of standard solutions of salicylic acid in the concentration (50-300 µg/mL) were injected into the HPLC system at different time intervals within a day. % RSD was calculated for the each analysis was calculated and summarized in table 3.

Table 3: Results of intraday and interday precision studies for salicylic acid (SA)

Concentration (µg/mL)	Day 1		Day 3	
	* Peak Area (Mean ± SD)	%RSD	* Peak Area (Mean ± SD)	% RSD
50.01	33608.3 ± 213.13	0.63	33838.3 ± 223.64	0.66
100.11	65416.5 ± 353.27	0.54	65675.5 ± 381.86	0.58
150.09	98124.76 ±742.37	0.76	98236.76 ± 779.05	0.79
200.03	139249.26 ± 853.55	0.61	139308.09 ± 917.97	0.66
250.1	182910.98 ± 979.25	0.61	183115.98 ± 998.85	0.66
300.06	208774.77 ± 1069.08	0.54	208875.77 ± 1095.89	0.55

* Mean and % RSD of six samples of salicylic acid

Inter-day precision

In the inter-day studies, six injections of standard solutions of salicylic acid in the concentration (50-300 µg/mL) were injected into the RP-HPLC system at different time intervals over a period of three days. % RSD was calculated for the each analysis was calculated and summarized in table 3.

Accuracy

Accuracy of the method was determined by calculating recoveries of drug by method of standard addition. Known amount of standard drug corresponding to 50%, 100%, and 150% of the label claim was added to prequantified sample solution and the amounts of drug were estimated by measuring peak areas and the results of the study is represented in the Table 4 .

Table 4: Results of accuracy studies for salicylic acid (SA)

Amount added (µg/mL)	*Mean Peak area for SA ± SD	% RSD	*Amount recovered (µg/mL)	% Recovery
150.09	98124.76 ±742.37	0.76	150.97	100.59
300.06	208742.98 ± 1051.11	0.50	302.03	100.66
450.07	307932.29 ± 1189.36	0.386	449.03	99.76

* Mean and % RSD of six samples of salicylic acid

Robustness

Robustness is a measure of method capacity to remain unaffected by slight deliberate changes in chromatographic conditions.. The chromatographic parameters selected were the effect of methanol in the mobile phase composition (63 and 67%), flow rate (0.8 and 1.2 mL/min) and

wavelength (252 and 256 nm). Only one parameter was changed while the others were kept constant. Results of the study are summarized in Table 5.

Table 5: Results of Robustness studies for salicylic acid (SA)

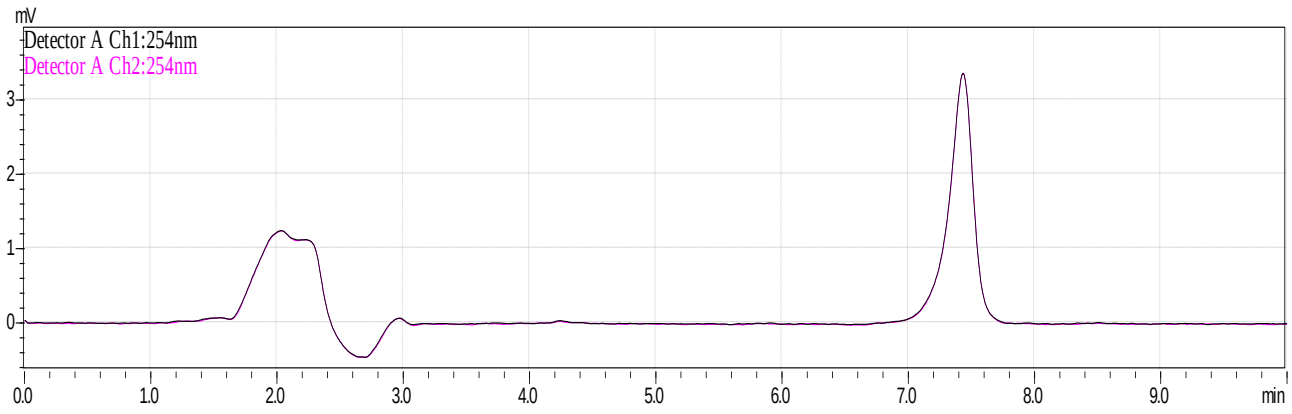
Condition	Modification	*Mean Peak area for SA ± SD	%RSD
Mobile phase composition [Water, methanol and glacial acetic acid (65:35:1, v/v)]	63:37:01	208778.98 ± 1043.15	0.50
	67:33:01	208806.98 ± 1019.1	0.49
Flow rate (1mL/min)	0.8 mL	208786.98 ± 1072.22	0.51
	1.2 mL	208781.23 ± 1051.11	0.50
Wavelength 254nm	252 nm	208779.68 ± 1143.85	0.55
	256 nm	208812.98 ± 1163.89	0.56

* Mean and % RSD of six samples of salicylic acid

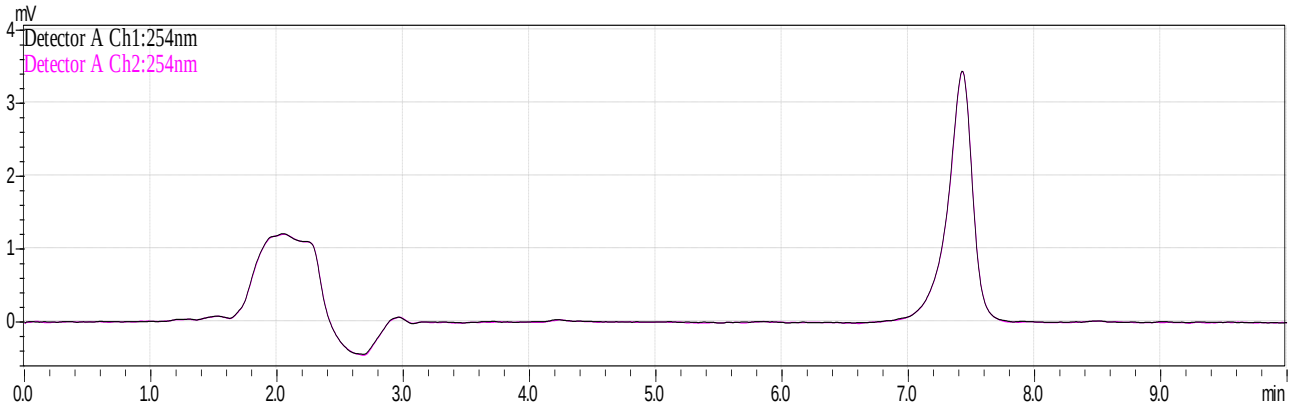
Analysis of Marketed Formulations

Three different brands of salicylic acid ointments (Uclom-S; Clonus-S; Clotech-S; Label claim 3%w/w salicylic acid) were used to determine the drug content. 3 gm each of the above ointments were accurately weighed and transferred carefully into three clean 100 mL volumetric flasks, respectively. The ointment was dissolved in methanol by gentle heating. The solution was filtered through 0.22 µm millipore filter paper and volume was adjusted using methanol. The final concentration of working solution equivalent to 300µ/mL was prepared by appropriate dilution in methanol. The resulting solution was filtered through 0.45 µm millipore filter paper and subjected to chromatographic analysis in triplicate. Typical chromatograms for the formulations are shown in the figures **4A-3C** and the percent drug recovery data is summarized in Table 6.

A. Uclom-S (300 µg/mL)



B. Clonus-S (300 µg/mL)



C. Clotech-S (300 µg/mL)

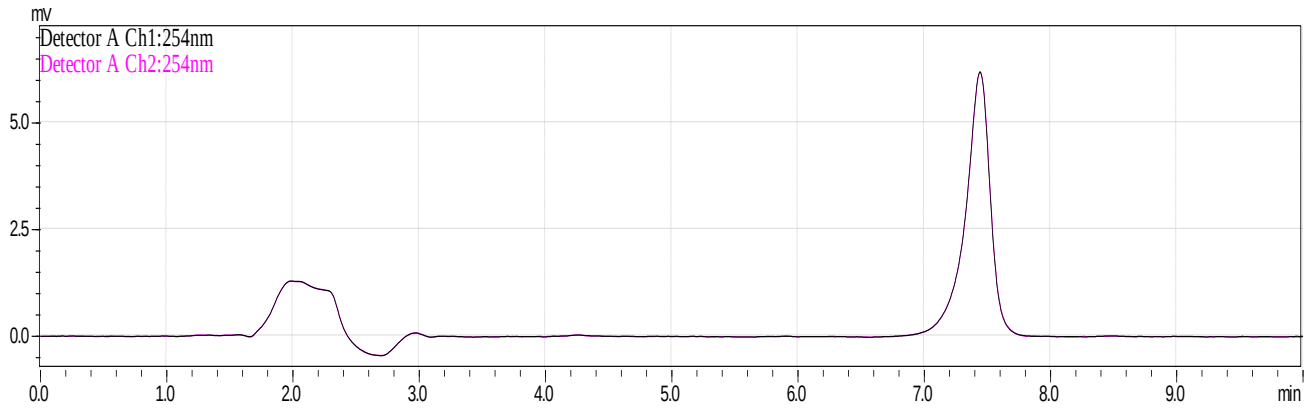


Figure 4: Typical chromatograms for formulations of salicylic acid (300 µg/mL): A. Uclom-S; B. Clonus-S; C. Clotech-S.

Table 6: Determination of salicylic acid (SA) in semisolid dosage form

	Ointment brand names of Salicylic acid (3% w/w)	*Amount added (µg)	*Amount recovered (µg)	% Recovery
1	Uclom-S	300.03	304.02	101.32
2	Clonus-S	300.02	298.75	99.57
3	Clotech-S	300.05	300.99	100.31

* Mean of six samples of salicylic acid

Forced degradation solutions

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method.[20-21] Stability of salicylic acid was determined by subjecting it to oxidative, alkaline, acidic, neutral, and photolytic conditions in order to accelerate conditions auspicious for degradation. The stress solutions of SA at concentration of 300µg/mL were prepared from stock solution of 1 mg/mL using methanol and subjected to heating (80°C). Standard stress

solutions of SA were filtered through 0.45 µm membrane filter paper and injected in to HPLC at regular time intervals. The HPLC chromatograms of the degradation studies are shown in Figures 5A-E and percent drug degraded is displayed in the table 7.

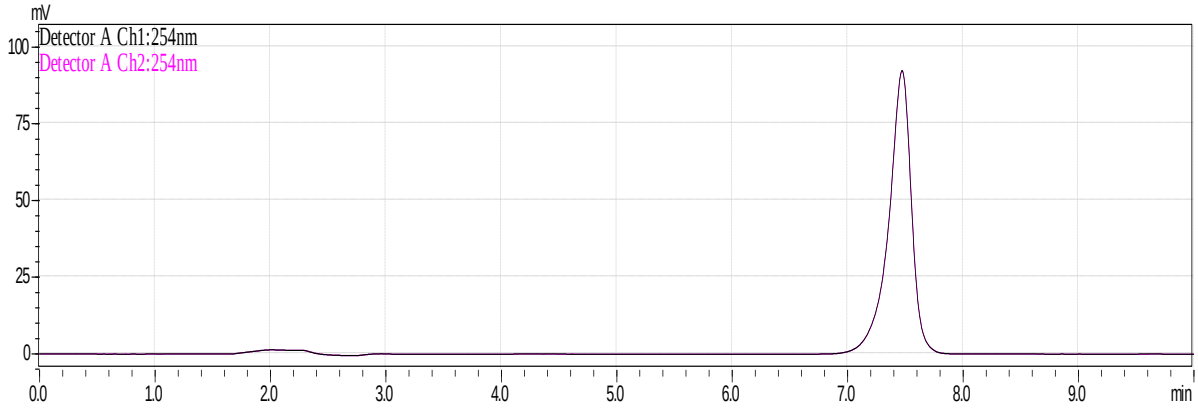


Figure 5A: **HPLC chromatogram of salicylic acid (300µg/mL) after exposure to neutral degradation.**

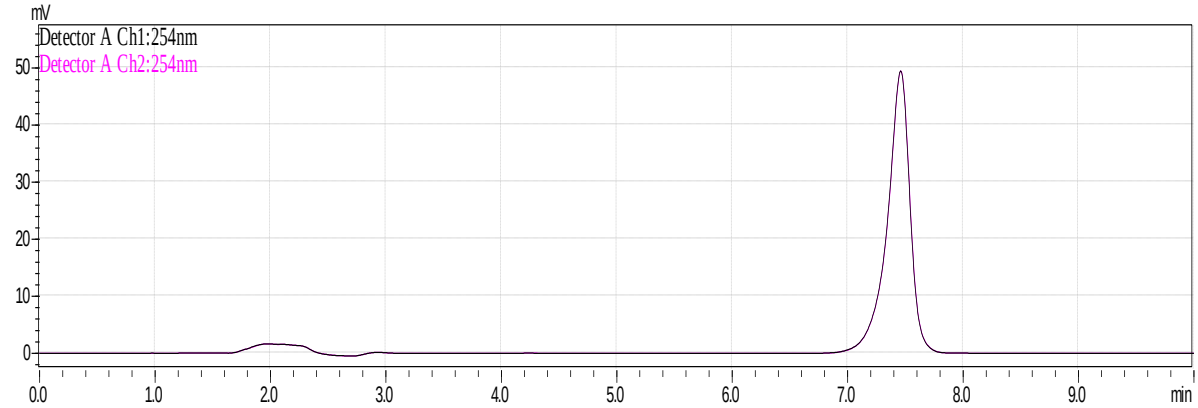


Figure 5B: **HPLC chromatogram of salicylic acid (300µg/mL) after exposure to acid hydrolysis (0.1 N hydrochloric acid for 30 min 80 ºC).**

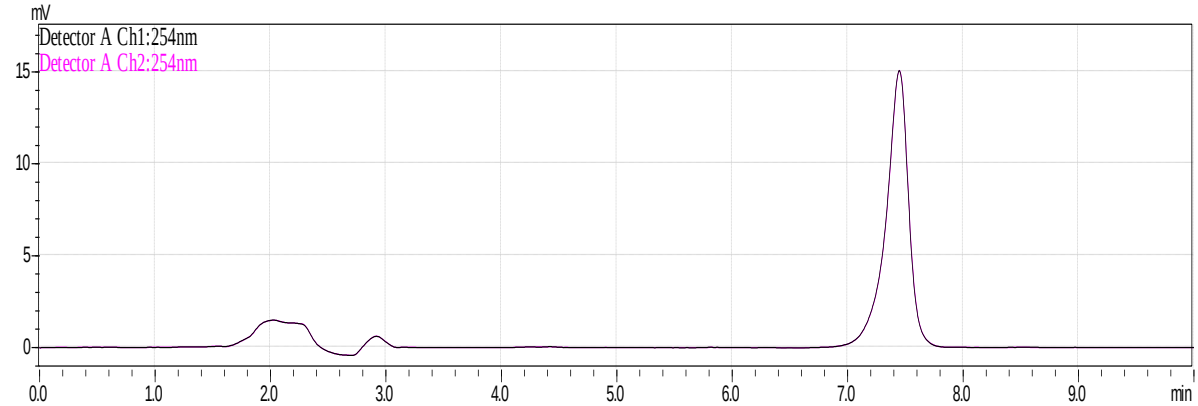


Figure 5C: **HPLC chromatogram of salicylic acid (300µg/mL) after exposure to alkaline hydrolysis (0.1 N sodium hydroxide for 30 min 80.**

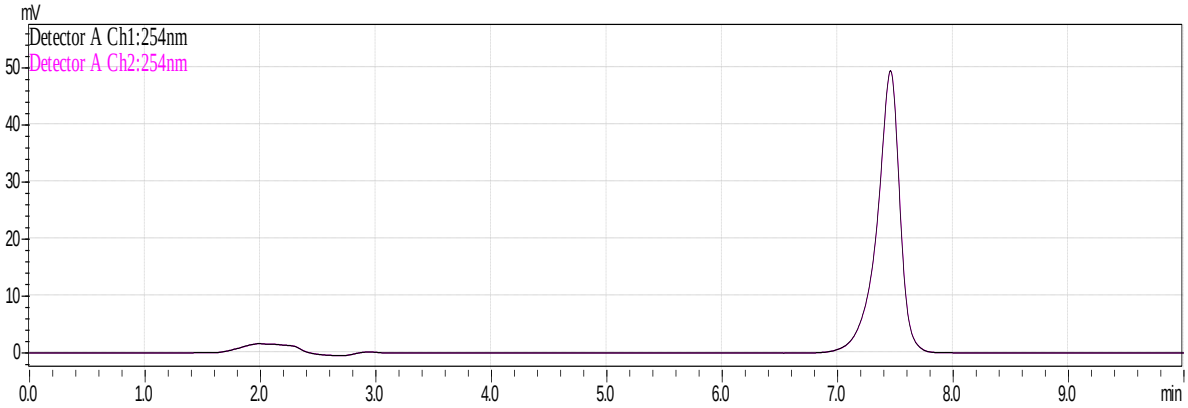


Figure 5D: **HPLC chromatogram of salicylic acid (300µg/mL) after exposure to oxidative degradation (3 % H_2O_2 for 30 min in a thermostat maintained at 80 ºC).**

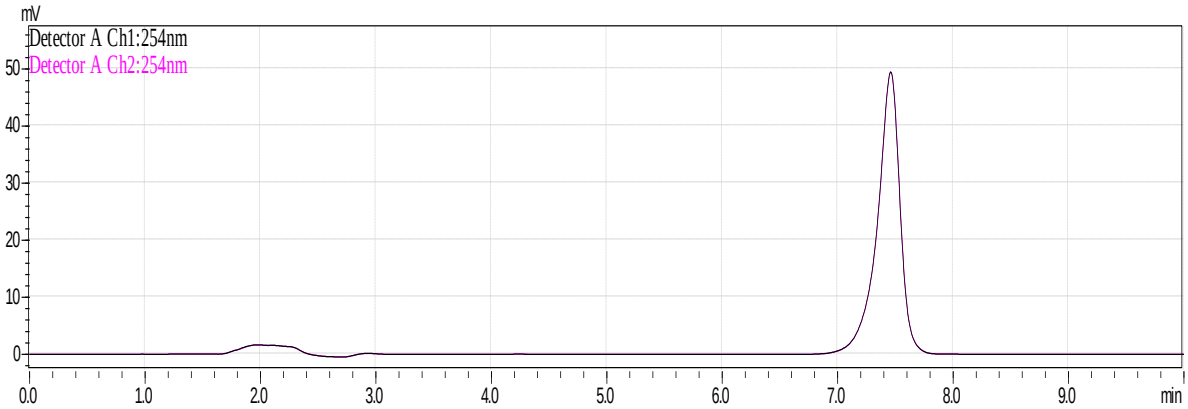


Figure 5E: **HPLC chromatogram of salicylic acid (300µg/mL) after exposure to photolytic degradation.**

Table 7: Results of Stress degradation studies for salicylic acid (SA)

Sl. No	Stress condition	*Mean Peak area for SA ± SD	%Drug recovered	% Drug degraded
1	Neutral	208772.98 ± 1045.53	100	0
2	Acidic	208784.98 ± 1012.12	99.73	0.27
3	Alkaline	208776.53 ± 1053.19	98.33	1.67
4	Oxidative	208791.14 ± 1049.42	99.92	0.08
5	Photolytic	208778.50 ± 1051.33	99.75	0.25

* Mean and standard deviation (SD) of six samples of salicylic acid

Neutral degradation

Salicylic acid sample (300µg/mL) was treated with methanol for about 30 min in a thermostat maintained at temperature of 80 ºC. Later it was cooled to room temperature and diluted with methanol, filtered through 0.45 µm membrane filter paper and injected into HPLC system. At

regular time intervals, 20 µl of SA sample solutions were injected into the HPLC system and the chromatogram recorded is presented in Figure 5A.

Acidic degradation

Acid degradation studies were achieved by treating SA solution (300µg/mL) with 0.1 N hydrochloric acid (0.1N HCl) for about 30 min in thermostat maintained at 80 ºC. Later it was cooled to room temperature neutralized with 0.1N NaOH and diluted with methanol, filtered through 0.45 µm membrane filter paper and injected into HPLC system. The chromatogram recorded is presented in Figure 5B.

Alkaline degradation

Alkaline degradation studies were performed by treating the SA solution (300µg/mL) with 0.1 N sodium hydroxide for about 30 min in a thermostat maintained at 80 ºC. Later it was cooled to room temperature neutralized with 0.1N HCl, diluted with methanol and filtered through 0.45 µm membrane filter paper before injecting into the HPLC system. The chromatogram recorded is presented in Figure 5C.

Oxidative degradation

Oxidative degradation was performed by treating SA solution (300µg/mL) with 3 % H_2O_2 for 30 min in a thermostat maintained at 80 ºC. Later it was cooled to room temperature, diluted with methanol and filtered through 0.45 µm membrane filter paper before injecting into the HPLC system. The chromatogram recorded is presented in Figure 5D.

Photolytic degradation:

Salicylic acid was exposed to direct sunlight for 7 days. Stock solution of SA (1mg/mL) was prepared using the standard procedure described above. The solution obtained was further diluted with methanol to obtain a concentration of 300µg/mL and 20µL was injected into the HPLC system. The chromatogram recorded is presented in Figure 5E.

RESULTS AND DISCUSSION

Method Development

The HPLC method carried out in the present experimental work was aimed at developing a new system capable of eluting resolving salicylic acid and its degradations products. Based on trial and error method, the mobile phase, which gave best possible separation and resolution, was selected and retention time was also taken in to the consideration. During the development of this method, different compositions of mobile phase were tested. The mobile phase was chosen after several trials with methanol, acetonitrile, water and acetic acid in various proportions. Finally, the

mobile phase consisting of water, methanol and glacial acetic acid (65:35:1, v/v) was selected to achieve maximum separation and sensitivity. Flow rates between 1 and 2 mL/min were studied. A flow rate of 1.0 ml/min gave an optimal signal to noise ratio with a reasonable separation time. Using RESTEX allure C18 column (3 µm, 15 cm × 4.6 mm), the retention time for salicylic acid observed to be 7.575 min, respectively. Total time of analysis was 10 min. Detection wavelength of 254 nm was chosen for the analysis. Several preliminary chromatographic runs were performed to investigate the suitability for drug content estimation and cost because of the increasing importance of rapid economic analysis in pharmaceutical analysis to increase the throughput.

System suitability

This test was performed by collection of data from a standard solution containing 300 µg/ml of salicylic acid that was injected six times of standard resolution solution. The parameters measured were tailing factor, capacity factor, theoretical plates, and retention time. %RSD for tailing factor was 1.595, the capacity factor was more than 2 (3.358 ± 0.513) and the theoretical plates were more than 2000 (2214.68 ± 0.19). The average of retention time was 7.65 minutes and peak area was 208786.95 ± 0.057. The results (Mean ± %RSD of six replicates) of the chromatographic parameters are shown in Table 1.Typical chromatograms of salicylic acid pure drug150 and 300 µg/mL is shown in the Figures 3A-B. The method was found to be precise and specific.

Method Validation

HPLC method was validated according to the International Conference on Harmonization Guidelines.[36-37] The method was validated with respect to parameters including linearity, limit of detection (LOD), and limit of quantitation (LOQ), recovery, precision, accuracy, robustness, and specificity.

Linearity

Linearity for detector response was observed in the concentration range 50-350 µg/ml for salicylic acid. The calibration curve for SA was constructed with concentration against peak area (Figure 2). The linear regression data values are shown in Table 2. The regression equation for the calibration curve was found to be y = -6434.5+731.335x and the correlation coefficient (r^2) of 0.999 was obtained. Good linearity was found between the peak area and analyte concentration.

Precision

Precision of the method was determined in relation to repeatability (intra-day) and intermediate precision (interday). It was evaluated by performing six independent determinations of the standard salicylic acid solutions of six different precision (interday). The precision of the

method was evaluated by performing six independent determinations of the standard salicylic acid solutions of six different concentrations (50-300 µg/mL) and calculating RSD (%). For day 1 (one) precision studies, the RSD (%) values for the six samples of SA was observed in the range of 0.54-0.76 while for day 3 (three) precision studies the RSD (%) range was 0.55-0.79. This shows that precision of the method is satisfactory as % relative standard deviation is not more than 2.0%.The results are depicted in Table 3.

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

The limit of detection and limit of quantitation for salicylic acid was calculated from the linearity data using relative standard deviation of the response and slope of the calibration curve. By the analysis of samples with known concentrations of analyte and establishing the minimum level at which the analyte can be reliably detected. We found 5µg/mL of salicylic acid. Limit of quantification is the concentration that can be quantified reliably with a specified level of accuracy and precision. LOQ was found to be 25 µg/mL of salicylic acid. The results indicate that this method is sensitive.

Accuracy

To prove the accuracy of the proposed RP-HPLC method, recovery studies were accomplished by standard addition method at three different concentration levels (50%, 100% and 150%) summarized in table 4. Percent RSD for salicylic acid was found to be in the range 0.386-0.76 and the percentage recovery was 99.76-100.66%. The results of the recovery test indicate that the method is highly accurate.

Robustness

Robustness of the analytical method was determined by consistency of the peak height and peak shape with the deliberate small changes in the experimental conditions. Under all the deliberately altered chromatographic conditions (flow rate, mobile phase and wavelength), peaks were adequately resolved and elution orders remained unchanged which indicate that the developed method for SA is robust. The results are summarized in Table 5.

Analysis of Marketed Formulations

The proposed validated method was applied for the quantification of SA in two different ointment dosage forms (Uclom-S and Clotech-S, Label claim 3%w/w). The results of the assay are shown in Table 6 and HPLC chromatogram for the representative samples are presented in Figures **4A-3C**. The percentage recovery of the drug was observed in the range 99.57 - 101.32. The assay results

indicate that the validated method was sensitive and specific for the quantitative analysis of salicylic acid in the marketed formulation.

Forced Degradation Studies

In order to evaluate the stability indicating properties of the developed method, forced degradation studies were carried out in accordance with ICH guidelines.[20-21] The stability of SA was determined by exposing the pure sample to neutral, acidic, alkaline, oxidative, and photolytic conditions in order to accelerate conditions favorable to degradation. The results and typical chromatograms of the degradation studies are displayed in the table 7 and figures **5A-E,** respectively.

During acid hydrolysis process, (0.1N HCl for 30 min), it was found that 0.27% of salicylic acid content was decreased, but there was no detectable degradation peak(s). The samples submitted to alkaline condition (0.1N NaOH for 30 min) showed 1.67% degradation of salicylic acid content, and also there was no detectable degradation peak(s). In both cases, the peak purity was 99.99%. The samples in presence of neutral, oxidative and photolytic stress degradation conditions displayed the degradation percent for SA as 0, 0.08 and 0.25, respectively. From the chromatograms for the degradation studies for SA, good selectivity and resolution of the compound and absence of degraded products seem to suggest that HPLC is a selective and specific method for the analysis of salicylic acid samples from stability studies

CONCLUSION

The proposed method for the determination of salicylic acid based on the RP-HPLC method with spectrophotometric detection was shown to be reliable, simple, accurate, sensitive and precise. The validated method could be successfully applied for the determination of salicylic acid in pharmaceutical preparations without interference from co-formulated drugs. The good validation criteria of the proposed method allow its use in quality control laboratories as an alternative to the official methods. The detection limit of the proposed method was found to be 5 µg/ml while the quantitation limit was 25 µg/mL. The results demonstrated the ability of the proposed method to be used as a stability-indicating HPLC method for the analysis of salicylic acid.

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