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Original Article

A validated stability-indicating HPLC related substances method for salicylic acid in bulk drug and dosage form

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ABSTRACT

A Reverse Phase High Performance Liquid Chromatography (RP-HPLC) was developed for quantitative determination of salicylic acid in bulk drugs and in pharmaceutical dosage form. The developed method is stability indicating which separates the drugs from its degradation products and is also applicable for related substances determination of salicylic acid in bulk drugs. The chromatographic method separation was achieved on a Kromasil 60-5CN (250 X 4.6) mm, 5 µ column and the mobile phase containing the mixture of mobile phase-A is the mixture of 1.0 mL of trifluoro acetic acid in 1000 mL purified water and mobile phase-B is acetonitrile (70:30, v/v). The detection was carried out at wave length 230 nm and column oven temperature at 30°C. Forced degradation studies were performed for Salicylic acid using acid (0.1 N HCl), base (0.1 N NaOH), Oxidation (2% H_2O_2) and thermal (80 °C) degradation. The degradation was observed for Salicylic acid in Acidic, Basic and Oxidative degraded products were well resolved from main peak. The developed method was validated with respective linearity, accuracy, precision, robustness, and forced degradation studies prove stability indicating power of the method.

Key Words: Salicylic acid, Forced degradation, RP- HPLC, Stability indicating, Validation.

INTRODUCTION

Salicylic acid is known for its ability to ease aches, and pains and reduce fevers. These medicinal properties, particularly fever relief, have been known since ancient times, and it is used as an anti-inflammatory drug. In modern medicine[1], salicylic acid and its derivatives are used as constituents of some rubefacient products. For example, methyl salicylate is used as a liniment to soothe joint and muscle pain, and choline salicylate is used topically to relieve the pain of mouth ulcers. Cotton pads soaked in salicylic acid can be used to chemically exfoliate skin. As with other hydroxy acids, salicylic acid is a key ingredient in many skin-care products for the treatment of seborrhoeic dermatitis, acne, psoriasis, calluses, corns, keratosis pilaris, acanthosis nigricans, ichthyosis, and warts[2,3] The standard treatment for calluses is a 6% aspirin suspension in petroleum jelly, applied on the callus for one hour and then removed with washing. Salicylic acid works as a keratolytic, comedolytic, and bacteriostatic agent, causing the cells of the epidermis to shed more readily, opening clogged pores and neutralizing bacteria within, preventing pores from clogging up again by constricting pore diameter, and allowing room for new cell growth. Because of its effect on skin cells, salicylic acid is used in several shampoos to treat dandruff. Use of concentrated solutions of salicylic acid may cause hyperpigmentation on unpretreated skin for those with darker skin types [4,5] (Fitzpatrick photo types IV, V, VI), as well as with the lack of use of a broad spectrum sunblock. Bismuth subsalicylate, a salt of bismuth and salicylic acid, is the active ingredient in stomach relief aids such as Pepto-Bismol, is the main ingredient of Kaopectate, and "displays anti-inflammatory action (due to salicylic acid) and also acts as an antacid and mild antibiotic [6].

EXPERIMENTAL SECTION

Material and reagents: Salicylic acid was made available from Sigma Aldrich Ltd India (purity ≥ 99.0 %), trifluoro acetic acid from Merck and Acetonitrile (HPLC grade) were obtained from Qualigens fine chemicals, India Ltd. Hydrochloric acid, hydrogen peroxide and sodium hydroxide were obtained from rankem laboratories, India. All

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chemicals and reagent were used as HPLC grades, and Milli-Q- water was used throughout the experiment.

Chromatography Conditions: A chromatographic system (Water, Japan) consisting of quaternary solvent delivery pump, a degasser an auto injector, column oven and UV detector, 10A-VP series with Chromeleon software. The chromatographic column of 250 mm length and internal diameter of 4.6 mm filled with Kromacil-CN C-18 stationary phase with particle size 5µm and pore size 100 A° was used. The instrumental settings were a flow of 1.0 mL/min; the injection volume was 10 µl. column oven temperature 30 °C.

Mobile phase:

Mobile phase preparation: Buffer preparation: 1.0 mL of trifluoro acetic acid in 1000 mL water.

Mobile phase: mixture of Buffer and Acetonitrile (70: 30)

1.0 mL trifluoro acetic acid in 1000 mL of purified water and acetonitrile (70: 30 v/v). The mobile phase was premixed and filtered through a 0.45 µm nylon filter and degassed. Typical chromatograph is shown in Fig.2.

Preparation of Standard stock solution: Standard stock solution 500 ppm of Salicylic acid in mobile phase solution was prepared in volumetric flask.

Sample solution: Ten tablets of Salicylic acid (1300.0 mg) were finely ground using agate mortar and pestle. The ground material, which was equivalent to 50 mg of the active pharmaceutical ingredients, was transferred accurately in to a 100.0 ml calibrated volumetric flask containing mobile phase the drug was extracted from tablet by vortex mixing followed by ultra-sonication for 10 min. The resultant mixture was filtered through 0.45 µ membrane filter, The desired concentration for drug was obtained with diluents [7,8] .

Selectivity: Selectivity is the ability of the proposed method to access unequivocally the analyte in the presence of components which may be expected to be present typically impurities, related substances, these might be includes degradants matrix etc[9] .The specificity of the developed liquid chromatography(LC) method for Salicylic acid was carried out in the presence of its related impurities. Forced degradation studies were also carried out for Salicylic acid to provide an indication of the stability indicating property and specificity of proposed method. Intentional degradation was attempted to stress condition. Acid (1.0 N HCl), base (1.0 N NaOH), oxidation (2 % H_2O_2) and to evaluate the ability of the proposed method to separate Salicylic acid from its degradation products .For acid 4.0 hour, for base 4.0 minutes and for oxidative study period was 3.0 hours . The peak purity test was carried out for Salicylic acid peak by using photo diode array detector (PDA) detector in stress samples. Related substances studies were carried out for stress samples against Salicylic acid standard and the mass balance (% Related substances = % Related substances + % degradation) was calculated[10,11] .

RESULT AND DISCUSSION

Optimization of chromatographic condition: The primary objective in developing this stability-indicating HPLC method was to achieve the resolution between Salicylic acid and its impurity along with degradation products. In C18 and C8 stationary phase using ammonium acetate, phosphate buffer and different ion pairing reagent ,sodium dodecasulphate (SDS), Octane sulfonic acid) at different pH. The resolution between the Salicylic acid and degradation products could not achieve and the retention time of Salicylic acid and degradation product about 10 min by using C18 and C8 stationary phase. It was observed that the analyte has strong interaction with non-polar stationary phases such as octadecyl silane co-valently bound on silica in particular with high carbon loading which results in peak tailing. Mobile phase was selected in terms of its components and proportion. The separation of degradation product and Salicylic acid was achieved on observed at the retention time of main peak and its impurities shown Kromacil CN ODS (250 X 4.6) mm 5 µ column and the mobile phase containing the mixture of mobile phase 1.0 mL trifluoro acetic acid in 1000 mL of purified water and acetonitrile (70 : 30 v/v). Forced degradation study showed the method is highly specific and the entire degradation products were separated from the main peak . The developed method was found to be specific and method was validated in accordance with International Conference on Harmonization (ICH) guidelines.[12] After that method development need to validate as per ICH guidelines, as specificity is passes, no interference was observed at the main peak and impurities shown in table no. I , method is linear correlation coefficient (r) is greater than 0.990 shown in table-II, Method is accurate and accuracy between 98 to 102 % shown in table no.III and IV and method is robust is shown in table No.V.

Result of forced degradation experiments: The Singh and Bakshi[13] suggested that target degradation of 10-20 % when establishing stability indicating properties of analytical methods, because even intermediate degradation products should not interfere with any stage of drug analysis. Although conditions used for forced degradation were adjusted to achieve degradation in this range, this could not be achieved for condition than expose to acid, base and oxidizing agent even after long exposure. Peak purity test result confirmed the Salicylic acid peak was homogeneous under all the % for drug substances and drug products at stress condition tested. The mass balance of Salicylic acid in stress samples was close to 100% and moreover, related substances of unaffected Salicylic acid in the tablets conformed the stability - indicating nature of the method. The result forced degradation studies are summarized in Table-I

Chromatographic peak purity data were obtained from the spectral analysis report. Peak purity greater than 990 is indicative of peak homogeneity. The peak purity for Salicylic acid from degradation studies was in the range 999-1000, indicating homogeneous peaks and thus establishing the specificity of the method. Chromatograms obtained from famciclovir tablets solutions obtained after stress testing under acidic, basic and oxidative conditions are shown in fig. (2b-j) respectively. No peaks co-eluted with the Salicylic acid peaks, suggesting the method which is enabled for specific analysis of famciclovir in the presence of its degraded products and impurity.

METHOD VALIDATION

System suitability: For system suitability the resolution impurity and Salicylic acid were evaluated if the resolution was found greater than 1.5 hence studies six replicate injection of salicylic acid standard. solution were used and the RSD of peak area ratio , and number of theoretical plates of the peak were calculated .The system suitability result are shown in Table II.

Precision: The precision of the related substances method was checked by injecting six individual preparation of Salicylic acid (500 µg mL $^{-1}$) spiked with 1.5 µg mL^{-1} of impurity. The intermediate precision (inter-day precision) of the method was also evaluated using two different analyst, different HPLC system and different days in the same laboratory.

Accuracy (Recovery test): Accuracy of the method was studies by recovery experiment. The recovery experiment were performed by adding known amounts of the drugs in the placebo. The recovery was performed at five levels, 50%, 100% and 150 % of the label claim of the tablet 300 mg . Three samples were prepared for each recovery level, and the percentage recoveries were calculated from amount found and amount added. The accuracy study of impurity was carried out in triplicate at three levels 50%, 100%, and 150% of 0.3% (500 µg mL^{-1}) p-hydroxy benzoic acid and phenol. Three samples ere prepared for each recovery level, and the percentage recoveries were calculated from amount found and amount added. The average recoveries of three levels nine determination from Salicylic acid were 96.0-

Calibration and linearity: Linearity test solution for the method prepared from Salicylic acid impurity stock solution at concentration levels from Limit of detection (LOQ) to 150% of the impurity. Standard solution containing Salicylic acid impurity in each linearity level were prepared . Linearity solution was injected in triplicate. The calibration graphs were obtained by plotting peak area verses the concentration data treated by least-suare linear regression analysis, The calibration graph were found to be in the mentioned concentration the slopes and correlation coeffients are shown in Table-II

Robustness: To determine the robustness of the developed method experimental condition were purposely altered and the resolution between Salicylic acid and its impurity were evaluated the flow rate of the mobile phase was 1.0 mL min $^{-1.}$ To study the effect of flow rate on the resolution, it changed by 0.2 unit from 0.8 to 1.2 mL min $^{-1}$. While the other mobile phase components were held constant as stated in chromatography conditions. .While other mobile phase components were held constant as stated in chromatography conditions. The effect of column oven temperature at 25°C and 35°C instead of 30°C while the other mobile phase component were held constant stated in chromatography condition . The result are shown in Table-V

***Stability of analytical solution*:** The stability of the standard solution and the sample solution was tested at intervals of 0, 12,16 and 24 h .The stability of the solution was determined by comparing results of the related substances of the freshly prepared standard solution . The RSD for the related substances determined up to 48 h for Salicylic acid was 0.40 % .The related substances values were ± 2% after 24 h.

The result indicate that the solution were stable for 24 h at ambient temperature.

CONCLUSION

The developed method was completely validated showing satisfactory data for all method validated parameters tested . The developed method can be conveniently used for the related substances in bulk drugs, pharmaceutical dosage form and used for dissolution of tablets containing Salicylic acid in quality control laboratory.

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Table I: Results of analysis of forced degradation study samples using proposed method, indicating percentage degradation and peak purity of Salicylic acid

Stress conditions/duration	(%) Degradation	Peak purity data*
Acidic/1.0 N HCl/ 4h	0.0006	1.000
Basic/1.0 N HCl/ 4h	0.0010	1.000
Oxidative/ 2% H_2O_2 /4 h	0.0006	0.999
Thermal/80 ^oC/24h	0.0001	0.999

*Peak purity values in the range of 0.990-1.000 indicate a homogeneous peak

Table II: Regression Characteristics and System Suitability Parameters of Proposed RP-HPLC Method

Parameters	**Results**
Retention time (min) salicylic acid	8.5
Tailing factor- Salicylic acid	1.2
Theoretical plates- Salicylic acid	12256
Linearity range-Impurity (ugmL^{-1})	LOQ-150
Limit of detection-Salicylic acid (LOD) (ugmL^{-1})	0.0007
Limit of Quantification- Salicylic acid (LOQ) (ugmL^{-1})	0.002
Slope (m)	13425
Intercept (c)	31
Correlation coefficient (r)	0.999
Method precision (RSD %) (n=6)	0.30

Table III: Recovery result of Salicylic acid

Level of addition	**Amount added (mg)**	**Amount found (mg)**	**% Recovery)**
LOQ	2.97	3.09	104.0
50	5.01	5.01	99.8
100	9.97	9.98	100.2
150	14.69	14.52	98.8

Table IV: Recovery result of Impurity

Level of addition	**Amount added (ug)**	**Amount found (ug)**	**% Recovery**
80	2.0	1.92	96.00
90	2.25	2.21	98.22
100	2.5	2.48	99.20
110	2.75	2.71	98.54
120	3.0	2.92	97.33

Table V: Results of robustness study

Parameter	Variations	Resolutions*
As such sample	-	3.4
Tempreture	28°C	Not applicable
	32°C	Not applicable
Flow Rate	0.8 mL/min	Not applicable
	1.2 mL/min	Not applicable

*Resolution between Salicylic acid and Impurity (Phenol)

Fig.2. (a). Chemical structure of Salicylic acid

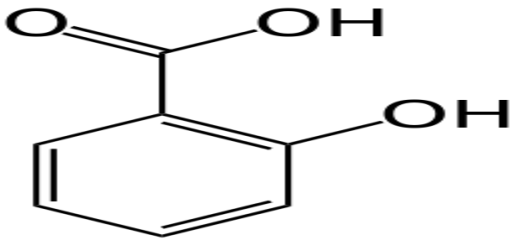


Fig.2. (b). A typical blank chromatogram of the Salicylic acid

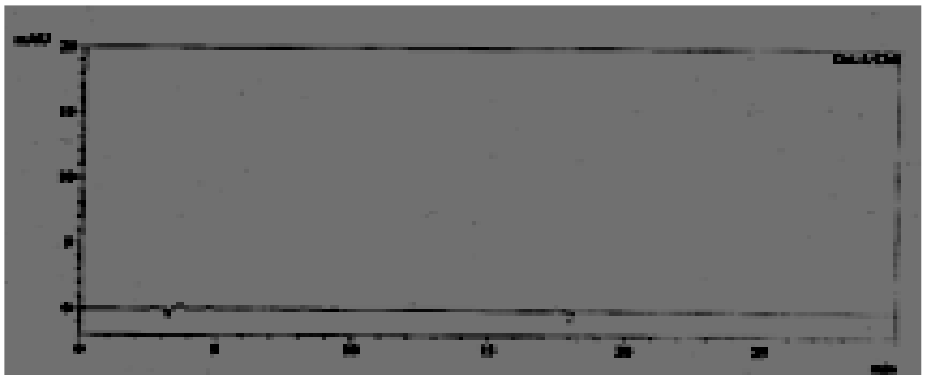


Fig.2. (c). A typical chromatogram of the Salicylic acid standard sample

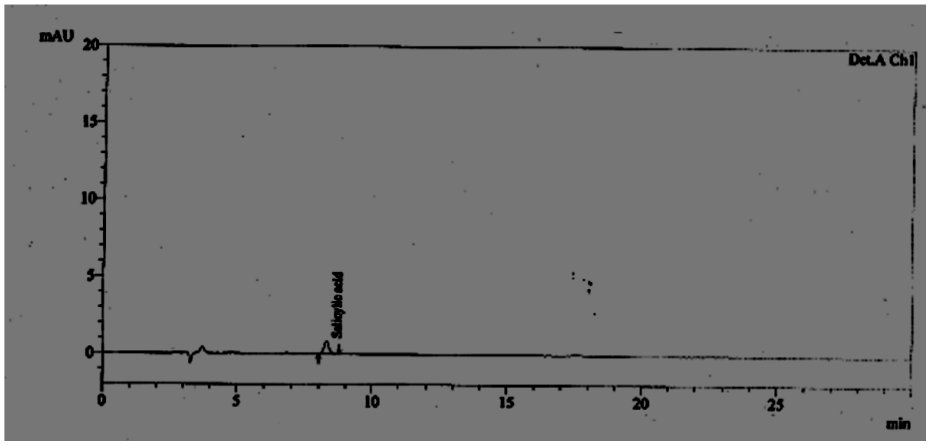


fig.2. (d). A typical Chromatogram of Salicylic acid as such sample

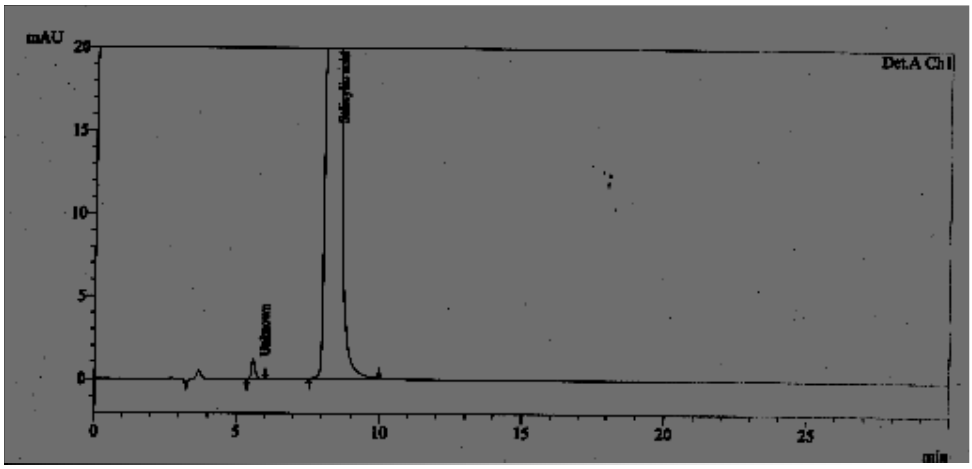


Fig.2. (e). A typical Chromatogram of Salicylic acid in acid degradation (0.1 N HCL)

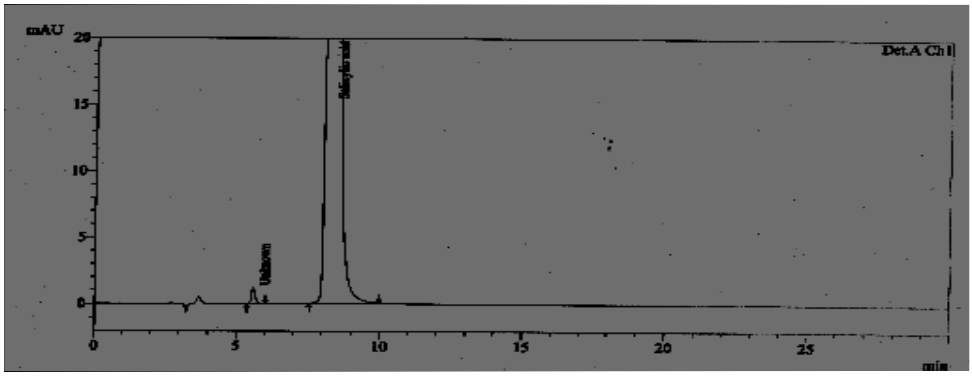


Fig.2. (f). A typical Chromatogram of Salicylic acid in base degradation (0.1 N NaOH)

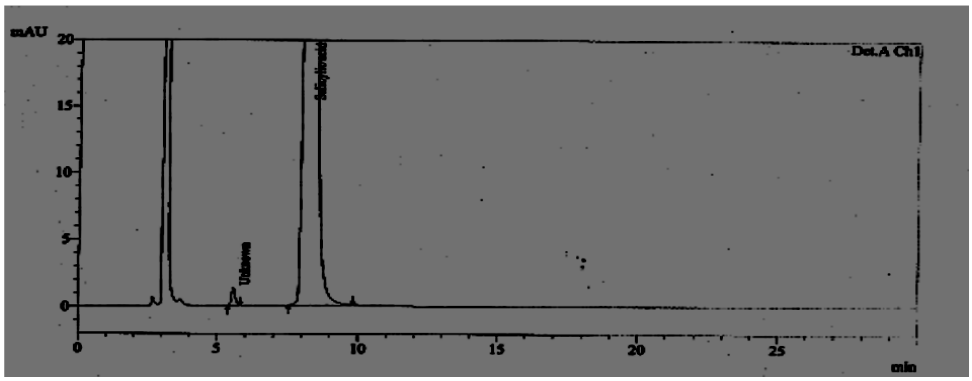


fig.2. (g). A typical Chromatogram of Salicylic acid in oxidative degradation (2.0 % H_2O_2)

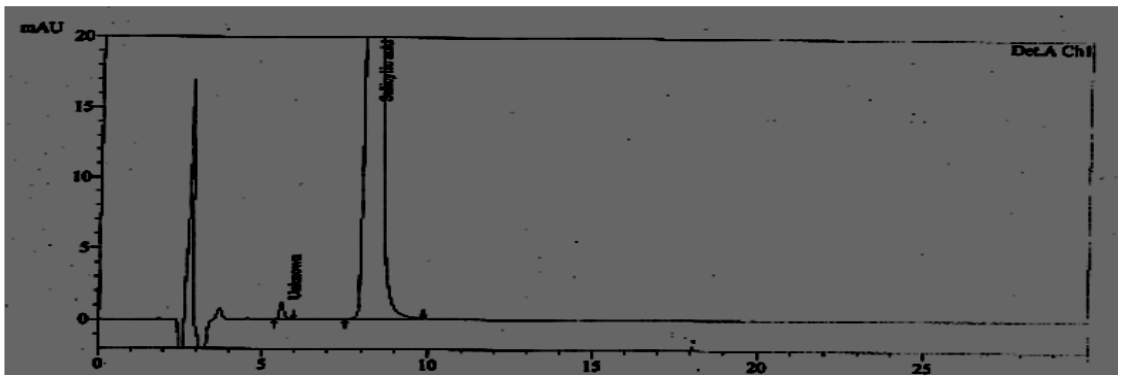
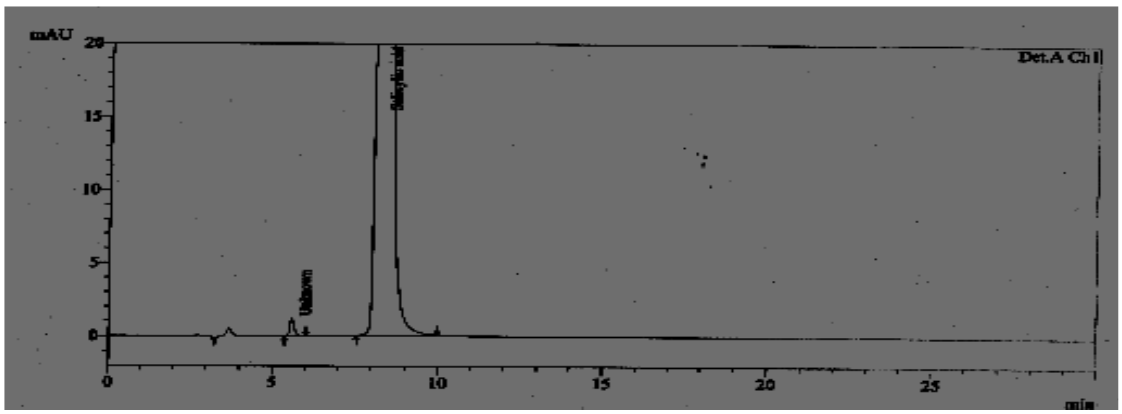


Fig.2. (h). Chromatogram of Salicylic acid in Thermal degradation at 80 °C



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