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Research Article

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VALIDATION AND DEVELOPMENT OF A RP-HPLC METHOD FOR DETERMINATION OF GLYCOSIDE (SALICIN) IN AN AYURVEDIC PREPARATION

Ajay S. Rana, Navneet K. Upadhyay*, Sameer Sapra, Megha Sharma

School of Pharmaceutical Sciences, Shoolini University, Solan HP

Abstract Salicin, a glycoside isolated from *Salix Alba*, has provided us with most potent weapon, Acetyl-salicylic acid for killing pain. Separation of salicin was achieved on Agilent HPLC with Aclipse XDB-C18, 5 µm and 150mm X 4.6mm, injection volume 5 µl at flow rate of 1ml/min by using a binary pump system. The ratio of mobile phase was 80 ml: 20ml (Acetonitrile: Water). The linearity was determined by six different concentrations of Salicin in triplicate and calibration curve was plotted in range of 5-30 µg/ml. The Coefficient of determination (r^2) of salicin was 0.998 for HPLC with best resolution and sensitivity at the absorption maxima of 253nm. The average percentage Recovery of Salicin in Ayurvedic preparation was found to be 100±1%. Specificity of the method was determined and the method was specific for estimation of Salicin in the Ayurvedic preparation. The observations and results obtained for each validation parameter including specificity, method precision (repeatability), intermediate precision (ruggedness), linearity and accuracy lie well within the acceptance criteria.

Keywords Salicin, *Salix Alba*, HPLC, Validation

Introduction

Salicin, (2R, 3S, 4S, 5R, 6S)-2-(Hydroxymethyl)-6-[2-(hydroxymethyl) phenoxy] oxane-3, 4, 5-triol a glycoside related to aspirin. An alcoholic glycoside is salicin, which is found in the genus *salix*. Salicin is an alcoholic β-glucoside (Figure 1).

Salicin is an anti-inflammatory agent, analgesic, anti-inflammatory, and antipyretic [1]. The salicin transformed to salicylic acid and has an action very similar to aspirin [2]. Salicin is closely related in chemical make-up to aspirin. When consumed, the acetalic ether bridge is broken down. The two parts of the molecule, glucose and salicylic alcohol, then are metabolized separately. By oxidizing the alcohol function the aromatic part finally is metabolized to salicylic acid [3]. Various methods have been reported in the literature for determination of salicin by using liquid chromatographic method [4-6], TLC-Spectrophotometric method [7], Densitometry method [8], UHPLC-ESI/TOFMS method [9] and HPLC-UV-MS and micro-HPLC [10]. There is no article concerning complete analysis and validation of salicin in an ayurvedic preparation, so the aim of this study was to establish a method based on RP-HPLC-DAD that is capable of analyzing salicin in ayurvedic prapration. The observations validated for the parameters including Specificity, Method Precision (Repeatability), Intermediate precision (Ruggedness), Robustness, Linearity and accuracy [11].

Material and Methods

Salicin was purchased from international labs, Solan. All the reagents purchased were of analytical grade. Acetonitrile, Methanol and water was purchased from Hi media and was of HPLC grade. All the other chemicals and solvents of analytical grade were used without any further purification. Analytical grade water was obtained using water purification systems ELIX 03 (MILLIPORE, USA).

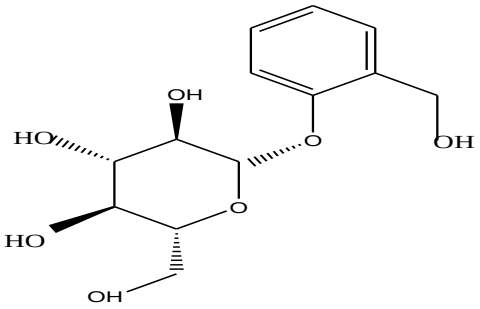

Figure 1: Salicin

HPLC system of Agilent 1200 composed of 515 series pumps combined with Auto-sampler along with photodiode array detector set at wavelength range 190-800 nm with column from Agilent C18 bonded with 5 µm (4.6 x 250 mm) coupled with EZ-Chrom software recording and processing of chromatographic data. Ultrasonic cleaner (Steryl medi-equip systems) and water purification system ELIX 03 (MILLIPORE, USA).

Preparation of standard solutions: The stock solutions of salicin were freshly prepared each day and were stored in the dark at 2-8°C. A stock solution of salicin (1 mg/mL) was prepared by dissolving 10 mg of accurately weighed salicin in same solvent system in a 10 mL volumetric flask.

Chromatographic conditions: For HPLC a number of preliminary trials were conducted with combinations of different organic solvents, compositions, and flow rate to check the retention time, shape, resolution, and other chromatographic parameters. Among all tried experiments, the mobile phase combination of acetonitrile and H_2O in the ratio of (80:20 v/v) with isocratic elution at flow rate of 1.0 ml/min was found to be most suitable. Best resolution and sensitivity of the method were obtained for salicin at 253 nm. Typical chromatogram with optimized condition gives sharp and symmetric peak with retention time of 1.253 min (Figure 2).

Calibration curve for salicin: Dilutions prepared by stock solution and for HPLC Linearity were determined by six different concentrations of salicin in triplicate and calibration curve was plotted in range of 5-30 µg/ml of salicin. Calibration curve was plotted by replicate analysis at all concentration levels and linear relationship was evaluated using the least square method with Microsoft® Excel program. The regression equation and Coefficient of determination (r^2) of salicin for HPLC were Y = 20598 x – 3718, 0.998 respectively.

Determination of Salicin in Ayurvedic Formulations: (sample preparation): An amount of the powdered formulation equivalent to 100 mg of salicin was weighed accurately, and extracted into 3 × 20 ml portions of chloroform with shaking. The residue was filtered using Whatmann No. 42 filter paper. The filtrate was evaporated to dryness under vacuum and the remaining drug was dissolved in methanol and diluted to 100 ml. 20µl of sample solution were used in triplicate on a HPLC and developed, scanned as above. Peak areas were recorded and the amount of salicin was calculated using the calibration plot on HPLC.

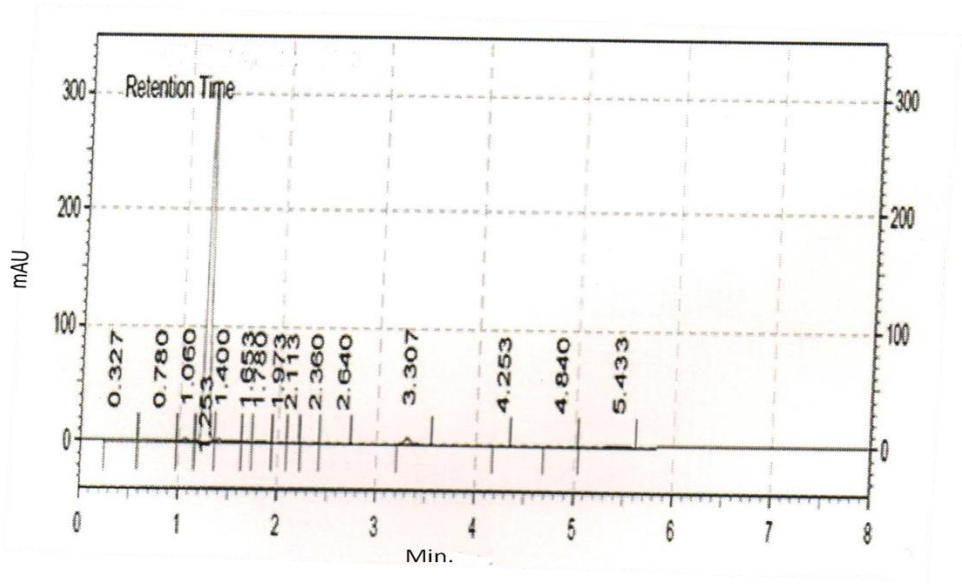

Figure 2: Chromatogram of standard drug

Results and Discussion

Absorption maxima of salicin were detected at 253 nm and area at different concentration showed in Table 1. Linearity graph is shown in Figure 3.

Table 1: Calibration data for analysis of Salicin

Concentration(µg/ml)	Area(mAU)
5	101453
10	209243
15	289001
20	412823
25	511913
30	616003

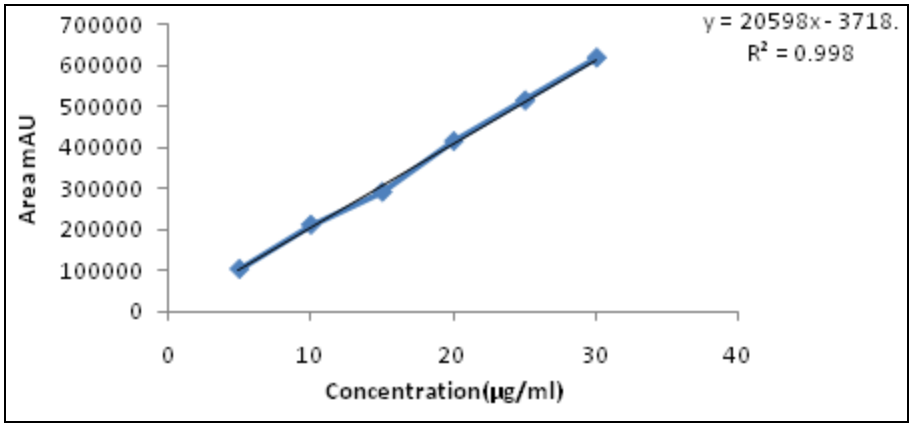

Figure 3: Linearity graph

Chromatographic characteristics data showed in (Table 2), Reproducibility data and Repeatability data for analysis of salicin was according to validation.

Table 2: Chromatographic Characteristics

Parameters	**Value**
λ_{max} (nm)	253
Range (µg/ml)	5 – 30
Correlation coefficient (r^2)	0.998
Regression Equation (y=a+bc)	y = 20598x – 3718
Intercept (a)	3718
Slope (c)	20598
Limit of quantification (µg/ml)	0.9
Limit of detection (µg/ml)	0.29

Recovery studies were done so as to check the accuracy of the method which was mentioned in (Table 3). Results of analysis of salicin in marketed formulation were 100 ± 1% found.

Table 3: Accuracy data for analysis of Salicin (Recovery studies)

Amount of sample taken (µg/ml) (A)	**Amount of standard added (µg/ml) (B)**	**Total Amount (A + B) (µg/ml)**	**Total amount found (µg/ml)**	**Approximately % Recovery**
10	8	18	17.830	99
10	10	20	20.333	101
10	12	22	22.123	100

Conclusion

The developed method is useful due to high tolerance limit for common Recipients found in Ayurvedic formulations. The developed method does not require any elaborate treatment of the drug and tedious extraction procedure. The method which we developed for the validation was studied at 253 nm wavelength. Accuracy and reproducibility was determined by calculating the recovery study that was close to 100 %. The developed method is simple, precise, and accurate and reproducible. Due to high sensitivity and simple sample preparation, the method can be used for routine analysis.

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