ISSN: 2249- 5746
International Journal of Ayurvedic and Herbal Medicine 11:1 (2021) 3949-3953
Journal homepage: http://www.interscience.org.uk
DOI:10.31142/ijahm/v11i1.03
Impact Factor: 6.012

Development of a Novel RP-HPLC Method for Simultaneous Estimation of Aloe Emodin, Rhein, and Piperine in a Polyherbal Formulation

Jain V[1], Jaiswar U[2], Patil R[3]
[1,2,3] Department of Quality Assurance, Oriental College of Pharmacy, Sanpada, Navi Mumbai-400705, Maharashtra, India.

Abstract: The present manuscript describes a rapid, novel, simple, precise, accurate, and robust HPLC method development and validation for simultaneous estimation of aloe emodin, rhein and piperine in a polyherbal formulation. The proposed chromatographic method was carried out isocratically, in a short run time of 10 minutes using Prontosil C_{18} (250 × 4.6 mm) SH 5.0 µm, usingmobile phase acetonitrile and water (0.05% orthophosphoric acid) in the ratio 55:45. Flow rate was set at 1 mL/min, and the UV detection was at 257 nm. Retention time of aloe emodin, rhein and piperinewas found to be 7.064 ± 0.2 min, 7.580 ± 0.2 min and 8.800 ± 0.2 min respectively. Calibration curves were linear over the concentration range of 1 to 30µg/mL.Regression coefficient were 0.998, 0.996, 0.999 for aloe emodin, rhein and piperinerespectively. This study reveals that the developedmethod was well validated, reliable and can be used to for routine analysis of aloe emodin, rhein and piperine in polyherbal formulations, thus conforming to the need of ensuring safety and quality of herbal formulations.

Keywords: Aloe emodin, HPLC, Piperine, Rhein

INTRODUCTION

Medicinal herbs are indispensable part of human society to prevent and cure diseases, from the beginning of civilization[1].Medicinal plants play significant role in world healthcare[2]. According to World Health Organization WHO 80% of the world's population currently uses herbal drugs for major healthcare[3]. Demandfor herbal medicines have increased in developing as well as developed countries for primary health care due to their wide biological activities, medicinal properties, safety and low costs[4]. Standardization of herbal medicines is essential in order to assess quality of products[5]. Analytical method such as high performance liquid chromatography (HPLC) is one of the most valuable tool for quality control and standardization of herbal products[6].

The selected formulationof Constikalp tablets contains *Cassia angustifolia*, *Glycyrrhiza glabra*, *Foeniculum vulgare*, *Piper nigrum*, *Zingiber officinale*, *Cassia fistula*, *Terminalia chebula*, *Terminalia belerica*, *Emblica officinalis*, and *Ricinus communis*. Aloe emodin and rhein belong to class of anthraquinones. The formulation is used for treatment of constipation, the effect of which is mainly attributed to the anthraquinones, while pepper is well documented as a bioavailability enhancer and therefore finds role in many herbal formulations. Apart from purgative property, rhein and aloe emodin are reported to possess hepatoprotective property[7-8].

There are several reported HPLC methods for individual estimation of aloe emodin, rhein, and piperine~~or~~ for simultaneous estimation in combination with other phytoconstituents. Through exhaustive literature survey it was revealed that so far no chromatographic method has been developed for simultaneous estimation of these markers, hence it was thought worthwhile to develop a chromatographic method for simultaneous estimation of these three phytoconstituents in the polyherbal formulation[9-13].

MATERIALS AND METHODS

Procurement of Sample

Marketed formulation of Constikalp(Vaidrishi Laboratories, Delhi) was procured from the local market of Mumbai, Maharashtra, India.

Standards and Reagents

Standards of aloe-emodin, rhein, and piperine were procured from Sigma-Aldrich chemicals Pvt. Ltd, India.HPLC grade, acetonitrile and methanol (Thomas Baker), and other reagents potassium dihydrogen orthophosphate and ortho-phosphoric acid (AR grade) were procured from Thermo Fisher Scientific Pvt. Ltd, India. Demineralised water was used for analysis.

Instrumentation

Analysis was performed on Shimadzu prominence i-3D LC 2030 HPLC consisting of a quaternary low-pressure gradient solvent delivery pump, UV detector and an autosampler. Stationary phase of the column was Prontosil C_{18} (250 × 4.6 mm) SH 5.0 µm, operated at 28°C. Responses of peak area were recorded and integrated using LabSolutions software. The wavelength (λ max) was obtained by using UV-Visible spectrophotometer.

Preparation of standard solution

100 mg of all the three markers i.e. aloe emodin, rhein and piperine were weighed accurately and transferred to a 100 mL volumetric flask, and then volume was made up with methanol to obtain a solution of 1000 µg/mL.

Preparation of sample solution

10 tablets of the formulation were triturated and 1 gmof powder mixture was weighed accurately, this powder was subjected to extractionby refluxing with methanol for 30 minutes. The refluxed solutionwas filtered through 0.45µm filter paper to get an extract. The volume was made up to 100 mL, and this solution was used for analysis.

HPLC METHOD DEVELOPMENT

Selection of wavelength

Suitable wavelength for the HPLC analysis was determined by recording UV spectrums in the range of 200-400 nm for individual drug solutions of aloe-emodin, rhein, and piperine then overlapping as shown in fig 1. UV overlain spectra of the selected API revealed appreciable active absorption at 257 nm, and hence 257 nm was taken as detection wavelength for HPLC analysis.

Selection of mobile phase

The standard solution of aloe emodin, rhein and piperinewere injected into the HPLC system and run in different solvent systems. Initially, trials were carried out using acetonitrile and methanol in different ratios, and acetonitrile and KH2PO4 buffer (potassium dihydrogen phosphate having molarity 0.05) having pH 2.5in the ratio of 45:55, the next trials were done by changing mobile phase composition that is acetonitrile and

orthophosphoric acid water (0.1%). Further changes were done in percentage of OPA water. Finally, acetonitrile and water with 0.05% OPA in the ratio of 55:45 was selected as the mobile phase for analysis, as it gave good resolution and proper peak shape.

Optimized chromatographic condition

The mobile phase finalized was acetonitrile and water with 0.05% OPA (55:45). The flow rate was kept 1.0mL/min, temperature was set at 28°C. Run time was kept 10 min. The detection wavelength was 257 nm and injection volume was 10 µl. Retention time of aloe emodin, rhein and piperine was found to be 7.064 ± 0.2 min, 7.580 ± 0.2 min and 8.800 ± 0.2 min respectively.

RESULTS AND DISCUSSION

HPLC method development and validation

Validation is a process of establishing documented evidence that provides high degree of assurance that a process, procedure, or activity will consistently produce a desired result meeting its predetermined specifications and quality characteristics. The method was validated as per ICH guidelines Q2 (R1)[14].

Specificity

Using the established optimized chromatographic conditions, retention time (RT) of aloe emodin, rhein and piperine was found to be 7.064 ± 0.2 min, 7.580 ± 0.2 min and 8.800 ± 0.2 min respectively. System suitability parameters were within acceptable limits as depicted in Table 1. The developed method was found to be specific as no peak co-eluted with the analytes peak from the blank solution and there was no interference of any other constituents at the retention time of aloe emodin, rhein and piperine. The results are depicted in fig.2 and fig.3.

Linearity

Stock solutions of the three selected markers were diluted into five known concentrations. Graphs are plotted with concentration on x-axis, and area on y-axis. Correlation coefficient (R^2) was found to be 0.998, 0.996, and 0.999 for aloe emodin, rhein, and piperine respectively. Calibration curves were found to be linear over the concentration range of 1-30 µg/mL as shown in Fig 4, 5 and 6. The results are depicted in Table 2.

Accuracy

The accuracy of the proposed analytical method was determined by recovery studies. Known amount of selected standards were spiked into sample solution at 80 %, 100 %, and 120 % recovery levels and injected in triplicate. The percent recovery was calculated against their respective recovery levels**.** The mean percent recovery of aloe emodin, rhein and piperinewas observed to be within the acceptance criterion of 98- 102 %. The percentage of recovery results are tabulated in Table 3.

Quantification of markers

The amount of aloe emodin, rhein and piperinepresent in the formulation was calculated using linear regression analysis. Quantification of the markers was done by performing HPLC analysis of sample solutions. The results obtained were used for further recovery studies. The results are depicted in Table 4.

Precision

Repeatabilityof the proposed method was determined by injecting six replicates of standard and sample solutions for system, intermediate and method precision respectively. The precision was presented as percentrelative standard deviation (% RSD) of the response. The standard analysis of the results proved that

RSD of the peak areas obtained was less than 2% for all the three selected markers hence, the proposed method was found to be precise. The data of precision is presented in Table 5, 6 and 7.

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value.The LOQ of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

LOD expresses as LOD =3.3 σ/S, and LOQ =10 σ/S, where σ is standard deviation of intercepts of calibration curve and S is slope of calibration curve. The linear regression equation was used to determine LOD and LOQ LOD of aloe emodin, rhein, and piperine were found to be 0.20, 0.14 and 0.18 µg/mL and LOQ of aloe emodin, rhein, and piperine were found to be 0.6, 0.42 and 0.54µg/mL.

Robustness

The capability of an analytical method to remain unaffected by slight variation in parameters is termed as robustness. Robustness of the analytical method was carried out with test solution by making deliberate changes in flow rate of mobile phase (± 0.2 mL/min), column temperature (± 1°C), and wavelength (± 1 nm). Results of robustness are tabulated in Table 8. % RSD of peak area response of the test solution in three replicate injections was found to be less than 2.0 %, hence the robustness parameters were found to be acceptable.

Solution Stability

The solution of emodin, rhein, and piperinewere injected at different time intervals for evaluating the stability of solution. The solution was found to be stable over 24 h. These solutions did not show any degradation up till 24 h. % RSDwas calculated for indicating the stability of the solution, and results are depicted in Table 9.

DISCUSSION

Selection of extraction method

Reflux extraction, Soxhlet extraction and Ultrasonic extraction were investigated for this experiment. Reflux extraction was opted owing to its high efficiency, simplicity and rapidity. In this experiment trials for solvent selection were conducted by using different extraction solvents with different extraction times. The results revealed that that the selected markers extracted relatively completely when the sample is extracted by reflux extraction with methanol for 30 minutes.

Selection of detection wavelength

UV spectra recorded shows absorption peaks of aloe emodin and rhein at around 230, 250, and 430. UV spectra recorded shows absorption peaks of piperine at around 235, 260, and 340.A single wavelength of 257 was selected as detection wavelength for absorption of all the markers.

Selection of mobile phase

Mobile phase systems with different proportions of methanol: water and acetonitrile: water was tested with addition of dihydrogen orthophosphate buffer and ortho-phosphoric acid in different percentage to the above proportions. It was found that addition of 0.05% orthophosphoric acid to water can effectively improve sharpness of aloe emodin, rhein and piperine peaks. So acetonitrile and waterin the ratio 55:45 with 0.05% orthophosphoric acid was selected as the mobile phase.

CONCLUSION

Anthraquinones are well documented for their beneficial effect in clinical treatment of constipation, and piles, while piperine is a bioavailability enhancer widely used in variety of herbal and ayurvedic formulations. To the best of our knowledge, so far there has not been any reported method for simultaneous estimation of these bioactive markers (aloe emodin, rhein and piperine). Hence the present work describes the development of a novel RP-HPLC method for simultaneous quantification of selected markers. The developed novel RP-HPLC method is rapid, simple, precise, accurate, and robust and can be used for quantification of aloe emodin, rhein and piperinein herbal formulations. The method is found to be specific as proven by chromatographic comparison. The developed RP-HPLC method can be used to standardize formulations containing aloe emodin, rhein and piperine as markers.

The method is found to be linear within the tested range of 1-30 µg/mL. The correlation coefficient is found to be within the range. The accuracy of the method is calculated by recovery study and the proposed method was found to be accurate as all the parameters of the method complies as per the acceptance criteria. The present validation proves that the developed HPLC method is suitable for the determination of aloe emodin, rhein and piperine in polyherbal formulations.

ACKNOWLEDGEMENT

Authors are grateful to Oriental College of Pharmacy, Sanpada, Navi-Mumbai for providing necessary facility to carry out the study.