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Research Article

HPLC ANALYSIS OF BOERAVINONE B AND EUPALITIN-3-O- β-D-GALACTOPYRANOSIDE FROM PLANT AND FORMULATION OF *BOERHAVIA DIFFUSA* LINN

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ABSTRACT

In the present work a precise, accurate and reproducible HPLC method is developed and validated for simultaneous quantification of Boeravinone B and Eupalitin-3-O-β-D-galactopyranoside from the whole plant of *Boerhavia diffusa* Linn. and from marketed Punarnava® capsules of Himalaya herbal healthcare. There are no methods reported for simultaneous separation and quantification of these markers from any plant matrix. Validation of the method showed response was a linear function of concentration in the range 10–120 µg mL^{-1} for Boeravinone B and 5–60 µg mL^{-1} for Eupalitin-3-O-β-D-galactopyranoside. The method was suitably validated and was found to be precise and robust. This HPLC method can be used as a quality control tool for quantification of these markers simultaneously from raw material as well as marketed formulation.

Keywords: Boeravinone B, Eupalitin-3-O-β-D-galactopyranoside, HPLC, *Boerhavia diffusa* Linn.

INTRODUCTION

Boerhavia diffusa belongs to the family Nyctaginaceae. *Boerhavia diffusa* has been widely used for treatment of edema, dermatopathies, heart disorders, anaemia, renal disorders, hepatic disorders and inflammatory disorders. *Boerhavia diffusa* Linn. has drawn a lot of attention for the biological activities the plant possess. Its leaves have been found to possess anti-diabetic[3] and analgesic effects[4], whilst diuretic[2], immunomodulatory[5,6] anti-lymphoproliferative[7] and hepatoprotective[8] properties have been attributed to the roots. The *Boerhavia diffusa* Linn. plant contains a large number of compounds such as flavonoids, rotanoids, alkaloids, steroids, triterpenoids, quinines, coumarins, proteins[9,10,11,12,13].

The chemical constituents present in the plant include, Punarnavoside[9], Boeravinone A-F[10,11,12], liriodendrin[12], hypoxanthine-9-L-arabinofuranoside[14], Eupalitin-3-O-β-D-galactopyranoside[15], Eupalitin[15], repenone, repenol[16], ursolic acid[2,17], 5,7-dihyroxy-3',4'-dimethoxy-6,8-dimethylflavone[18], β-sitosterol[1,2], stigmasterol, campesterol[10], syringaresinolmono-β-D-glucoside[11], palmitic, heptadecyclic, oleic, strearic, arachidic and behenic acids[12], hentriacontane[2,17], β-ecdysone, triacontanol[1], polysaccharides[19], boerhavisterol, boerhadiffusene, diffusarotenoid, boerhavilanostenyl benzoate[17].

Boerhavia diffusa Linn. is used commercially as an ingredient of several Over the Counter (OTC) herbal formulations, the market samples are often known to be adulterated with *Trianthema portulacastrum* Linn[1,20]. Thus it is important to establish the quality of the plant raw material as well as the finished formulation.

In the present work a precise, accurate and reproducible HPLC method is developed and validated for simultaneous quantification of Boeravinone B and Eupalitin 3-O-β-D-galactopyranoside from the whole plant of *Boerhavia diffusa* Linn. and from marketed formulation Punarnava® capsules of Himalaya herbal healthcare. There are no methods reported for simultaneous separation and quantification of these markers from any plant matrix.

This HPLC method can thus help to check for the adulteration in the raw material, as well as serve as a quality control tool for quantification of these markers simultaneously from raw material as well as marketed formulation.

MATERIALS AND METHODS

Chemical and reagents

The working standards of Boeravinone B (97.30%) and Eupalitin-3-O-β-D-galactopyranoside (97.10%) were obtained from Natural Remedies Pvt Ltd., India. HPLC grade methanol, acetonitrile and water obtained from E. Merck, Mumbai, India were used.

Preparation of standard stock solutions

Standard stock solutions of pure drugs were prepared separately by dissolving 10 mg of each drug in 10 mL of methanol to get concentration of 1000 µg/mL. This stock was further diluted to 200 µg/mL & 400 µg/mL for Boeravinone B and 100 µg/mL & 200 µg/mL for Eupalitin-3-0-β-D-galactopyranoside.

Preparation of calibration and quality levels

For calibration curve, aliquots of 10-120 µg/mL and 5-60 µg/mL were prepared from the above stocks for Boeravinone B and Eupalitin-3-0-β-D-galactopyranoside respectively. Also three quality control levels (LQC, MQC, HQC) each of Boeravinone B (20, 60, 100 µg/mL) and Eupalitin-3-0-β-D-galactopyranoside (10, 30, 50 µg/mL) were prepared for precision, accuracy and ruggedness studies.

Plant Material

The fresh plant of *Boerhavia diffusa* Linn. was collected from Bhayandar, Thane district, Maharashtra in the month of August 2010. The Herbarium of the plant was prepared and authenticated by Agharkar Research Institute, Pune; its voucher specimen no. is 10-172. The plant material was shade dried for five days and was kept thereafter in hot air oven maintained at 45 ± 5°C for fifteen days. The plant material was then powdered, sieved through 85 mesh and was stored in airtight plastic bottle at room temperature for further analysis.

Soxhlet Extraction of phytoconstituents

Plant sample: 0.2 gm of accurately weighed whole plant powder was extracted with 200 ml of Methanol in a Soxhlet apparatus for 14 hours, followed by filtration (5µ syringe filter). This extract was concentrated to 5 ml, followed by transferring its contents to 10 ml standard volumetric flask and volume made up to mark with methanol. This filtrate was then used for HPLC analysis.

Marketed formulation

For analysis of the Punarnava® capsule, contents of twenty capsules were combined and 0.2 gm was accurately weighed was extracted with 200 ml of Methanol in a Soxhlet apparatus for 14 hours, followed by filtration (5µ syringe filter). This extract was concentrated to 5 ml, followed by transferring its contents to 10 ml standard volumetric flask and volume made up to mark with methanol. This filtrate was then used for HPLC analysis.

Chromatographic procedure

The HPLC column used was a C18 column (250 mm X 4.6 mm, 5 µm). The mobile phase was a Gradient mixture of Acetonitrile and water. The mobile phase prepared was filtered through 0.45 µm Millipore filter and degassed by sonication for 30 min. The flow rate was adjusted to 1.0 ml/min. Injection volume was adjusted to 20 µl and detection was made at 270 nm. The Instrumentation and Chromatographic conditions have been presented in Table 1.

RESULT AND DISCUSSION

Method validation

ICH harmonized tripartate guidelines Q2 (R1) were followed for the validation of the developed analytical method (ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1), Nov. 2005).

Selectivity and Specificity

During the UV scan no appreciable difference was found in the spectra of reference standards and the analysed samples. Hence, the method demonstrated a high degree of selectivity. Refer Figure 1 and Figure 2 for HPLC chromatograms of Plant sample and Marketed formulation of *Boerhavia diffusa* Linn. respectively.

System suitability

System suitability tests were used to verify whether the resolution and reproducibility of the chromatographic system were adequate for the analysis. For Boeravinone B and Eupalitin-3-0-β-D-galactopyranoside the %CV values for area and retention time was found to be <2% indicating that the system was suitable to carry out further analysis.

Inter-Day and Intra-Day Precision

Variability of the method was studied by analysing quality control samples of Boeravinone B (20, 60, 100 µg/mL) and Eupalitin-3-0-β-D- galactopyranoside (10, 30, 50 µg/mL) on the same day (intra-day precision) and on different days (inter-day

precision) and the results were expressed as % RSD.

Recovery

Recovery of the method was assessed and the values for all the three components were within acceptable limits (85.0 to 115.0%). This indicated that the method was reliable and accurate.

Ruggedness

Proposed method was not influenced by the factors considered for ruggedness study. Change in flow rate and mobile phase composition affected the retention time of the analytes but the results were satisfactory since % CV was <2%.

Stock solution stability

The stability of the master stocks of all the standards was evaluated by storing the stocks in refrigerator at 2-8°C for 72 hours. This was followed by comparing these concentrations of these stocks against freshly prepared stocks for each standard.

Assay

The assay value for plant sample of *Boerhavia diffusa* Linn. was found to be 0.43 % and 0.1% for Boeravinone B and Eupalitin-3-0-β-D-galactopyranoside respectively, while in the marketed formulation was found to be 0.11% for Boeravinone B. The proposed HPLC method was found to be suitable for qualitative and simultaneous quantitative analysis of Boeravinone B and Eupalitin-3-0-β-D-galactopyranoside.

CONCLUSION

A precise, accurate and reproducible HPLC method is validated for simultaneous quantification of active markers Boeravinone B and Eupalitin-3-O-β-D-galactopyranoside. This HPLC method can aid in confirming adulteration in the raw material as well as serve as a quality control tool for quantification of these markers simultaneously from raw material as well as marketed formulation.

Table 1: Instrumentation and Chromatographic Conditions

HPLC	Shimadzu UFLC Prominence System
Mobile phase	Gradient Acetonitrile : water
Pump	LC – 20 AD binary pumps
Detector	SPD – M 20 A Photo Diode Aray
Column oven	CTO – 20 AC at 40°C
Column	Phenomenex C_{18} column (250 mm X 4.6 mm, 5 µm)
Run time	25 min
Flow rate	1.0 ml/min
Injection volume	20 µl
Detection wavelength	270 nm

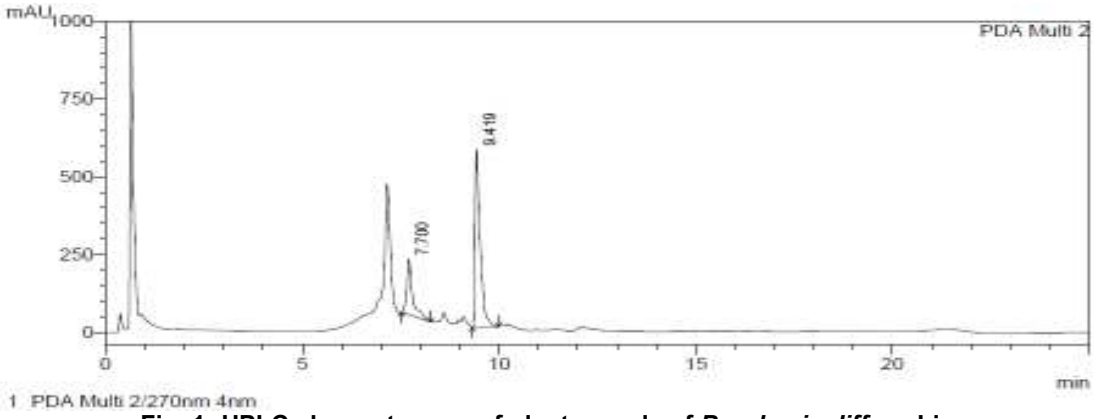

Fig. 1: HPLC chromatogram of plant sample of *Boerhavia diffusa* Linn

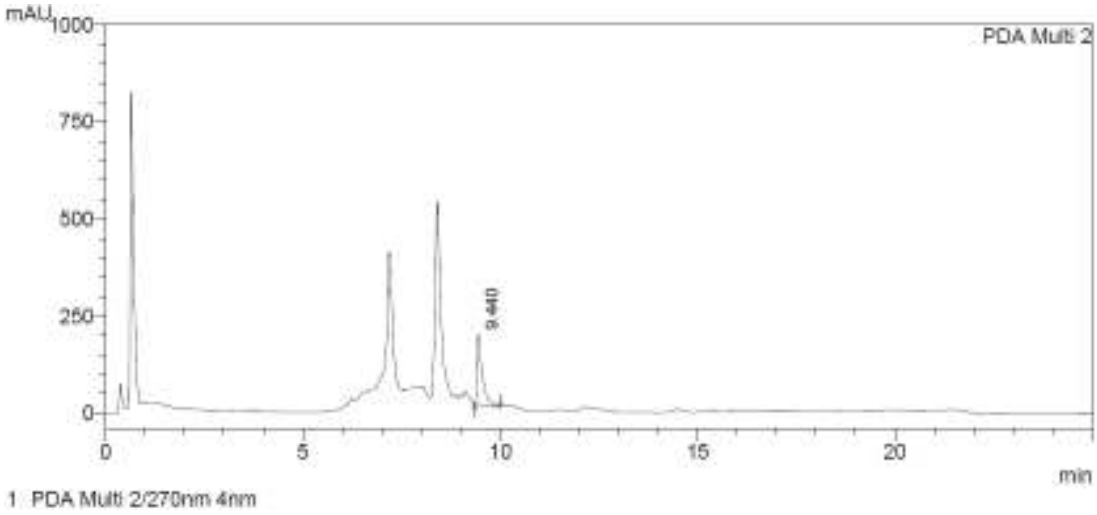

Fig. 2: HPLC chromatogram of formulation sample of *Boerhavia diffusa* Linn

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