HIGH-PERFORMANCE
LIQUID CHROMATOGRAPHIC DETERMINATION
OF BERGENIN IN DIFFERENT *BERGENIA* SPECIES

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SUMMARY

Because previous chemical investigations have revealed the presence of bergenin and gallic acid as important bioactive components of *Bergenia*, a simple, highly precise RP-HPLC method coupled with photodiode-array detection has been developed and validated for simultaneous determination of these compounds in the *Bergenia* species *B. ligulata* (Wall) Eng., *B. ciliata* (Royle) Raizada, and *B. stracheyi* Engl. *B. ciliata* and *B. stracheyi* were found to contain the most bergenin, 3.275 and 3.277%, respectively; *B. ligulata* contained 2.419% bergenin.

INTRODUCTION

Quantification of bioactive principles by use of modern analytical tools is essential for establishing the authenticity, creditability, prescription, and usage of herbal medicines.

Bergenia species (Saxifragaceae) are important medicinal plants distributed in South and East Asia and European countries. In India these plants grow at high altitudes in the Himalayas usually in rocky areas and on cliffs. They are popularly known in the Indian system of medicine as *Pashanbhed* (meaning 'to dissolve the stone'), because the rhizomes have been used for centuries in herbal formulations for dissolution of kidney and bladder stones, and for treatment of leucorrhea, piles, and pulmonary infections [1].

There are many reports of ethnobotanical use of different *Bergenia* species as antilithics, for treatment of boils and blisters [2]; for treatment of urinary calculi and other urinary diseases; as anti-diabetic drugs; for treatment of heart disease, haemorrhoids, stomach disorders, and ophthalmia [3]; and for dissolving kidney stones [4]. Alcoholic extracts of the plants

have been shown to have analgesic, anti-inflammatory, and diuretic properties [5]. Other pharmacological claims include antibacterial [6], anti-inflammatory [7], and anti-tussive activity of *B. ciliata* [8], and anti-viral [9] and antilithiatic [10] activity.

Previous chemical investigations of these plants have revealed the presence of β-sitosterol-D-glucoside [11], bergenin [12], α-afzelechin [13], leucocyanidin, gallic acid [14], methyl gallate, catechin [15], and pashaanolactone [16]. Bergenin is reported to have anti-inflammatory [17], antitussive [18], anti-HIV [19], antiarrhythmic [20], and neuroprotective [21] activity.

Because of their great importance it is important to identify which *Bergenia* species contain most bergenin, so the most potent plant species is used in herbal formulations and has the strongest desired effect. A simple and highly precise RP-HPLC method with photodiode-array (PDA) detection has therefore been developed and validated for simultaneous analysis of the bioactive molecules bergenin and gallic acid in three different *Bergenia* species, viz. *B. ligulata* (Wall) Eng., *B. ciliata* (Royle) Raizada, and *B. stracheyi* Engl. The method can also be used for quality control and standardization of the species, to identify the species used in a formulation.

EXPERIMENTAL

Chemicals

Bergenin and gallic acid were obtained from Sigma–Aldrich (Steinheim, Germany). HPLC-grade acetonitrile, water, and phosphoric acid were from Merck (Darmstadt, Germany).

Plant Material and Extraction

Rhizomes of three different *Bergenia* species, *B. ligulata* (Wall) Eng., *B. ciliata* (Royle) Raizada, and *B. stracheyi* Engl. were collected from Almora (Uttracnhal, India). The specimens were authenticated and a voucher specimen of each (LWG 222437BC; LWG 222438BL; LWG 222439BS, 2004) was deposited in a herbarium. Samples of air-dried (45–55°C) powdered rhizome from the three *Bergenia* species (1.0 g) were extracted with 3 × 10 mL methanol. The extracts from each species were separately combined, concentrated by rotary evaporation (Büchi, USA) at low temperature (45°C), and then freeze-dried (Freezone 4.5; Labconco, USA) under high vacuum (133×10^4 mBar) at -40 ± 2°C to furnish resi-

dues equivalent to 5.0, 8.833, and 13.83%, respectively, of the dry mass of the original plant sample.

To identify peaks present in the blank mobile phase was treated in the same way.

Qualitative and Quantitative HPLC Analysis of Bergenin

Chromatography was performed with Waters (Milford, MA, USA) 515 pumps, an online degasser, a Waters pump-control module, a Rheodyne 7725 injection valve with a 20-μL loop, a Waters 2996 photodiode array detector, and Waters Empower software. Compounds were separated on a 150 mm × 3.6 mm i.d., 5-μm particle, Waters Symmetry column protected by a guard column of the same type.

An HPLC fingerprint profile was established for the phenolic fraction. Elution was performed at a flow rate of 1 mL min^{-1} with a gradient prepared from water–phosphoric acid 99.7:0.3 (*v/v*) (component A) and acetonitrile–water–phosphoric acid 79.7:20:0.3 (*v/v*) (component B). The gradient was: 0–5 min, 88–85% A; 5–10 min, 85–75% A; 10–20 min, 75–70% A. Typical chromatograms obtained under these conditions are shown in Fig. 1.

Calibration

The amounts of the active compounds were determined by use of calibration plots established by chromatography of gallic acid and bergenin standards at seven different concentrations ranging from 2.5 to 200 μg mL^{-1}. Each solution was chromatographed in triplicate. The corresponding peak areas were plotted against the concentration of the phenolic compound injected. Peaks were identified by comparison of retention times and UV absorption spectra with those of standards.

Validation

Selectivity and Peak Purity

Selectivity was checked by using an extract of *Bergenia* species and a mixture of available standards and optimizing separation and detection. The purity of the peaks was checked by acquisition of spectra ($\lambda = 200–400$ nm) by use of the PDA detector and use of multivariate analysis. Spectra were acquired at the upslope, apex, and downslope of each peak, computer normalized, and superimposed. Peaks were considered pure when there was coincidence between the three spectra (match factor ≥98%).
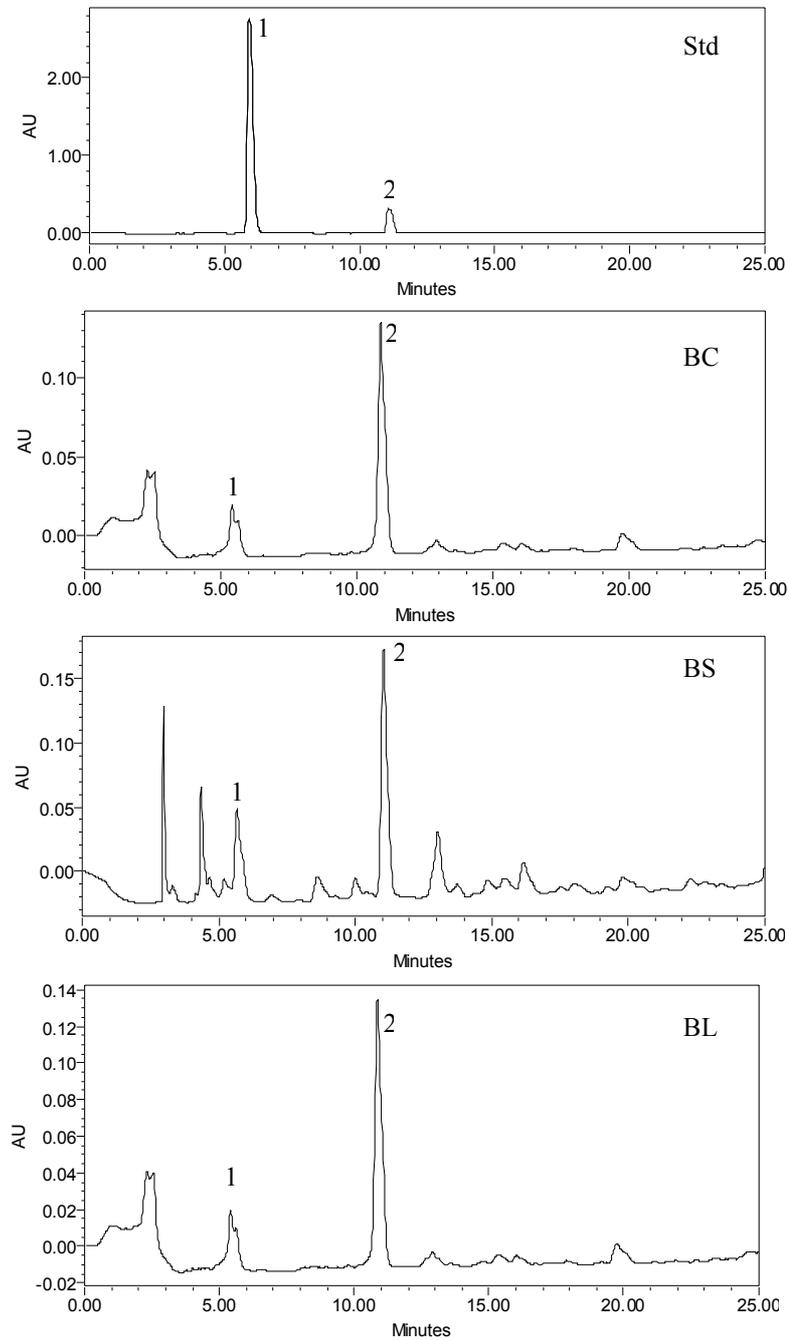
Fig. 1

Chromatograms obtained from *Bergenia* species (Std, standard; BC, *B. ciliate*; BS, *B. stracheyi*; BL, *B. ligulata*) at 272 showing 1, gallic acid; 2, bergenin

Linearity, and Limits of Detection and Quantification

The linearity of the detector response for the standards was assessed by linear regression analysis of the amounts of each standard (μg) and the area of the corresponding peak on the chromatogram. Linearity was also confirmed for extracts. After chromatographic separation, the peak areas obtained were plotted against extract concentration by linear regression analysis.

Limits of detection and quantification were determined as the amounts for which the calculated signal-to-noise ratios were approximately 3:1 and 10:1, respectively.

Statistics

When applicable, one-way or two-way analysis of variance (SPSS 11.0 for Windows) was used to assess the observed differences in phenolic content. Differences were considered to be statistically significant when the *P* value was <0.05.

RESULT AND DISCUSSION

Quantitative analysis of bergenin by HPLC with PDA detection showed that amounts of bergenin were largest in *B. ciliata* and *B. stracheyi*. The results are summarized in Table I. Gallic acid was also shown to be present but quantitative analysis was not performed.

Table I

Amount (%) of bergenin in *Bergenia* species

Species	Amount of bergenin (%)
Bergenia ciliata	3.275
B. stracheyi	3.277
B. ligulata	2.419

The HPLC method was validated by determination of linearity, peak purity, and limits of quantification and detection. For qualitative purposes, the method was evaluated by taking into account retention time precision, peak purity, and selectivity for standards. High retention time repeatability was apparent from *RSD* values below 1.2% for both standards and extracts. Peak purity was studied for major peaks. Impurities or co-

elution were not observed (match factors $\geq 95\%$). Linearity and limits of detection (*LOD*) and quantification (*LOQ*) were evaluated for quantitative purposes (Table II). *LOD* for bergenin and gallic acid were 1.16 and 0.66 µg mL^{-1} and *LOQ* were 3.9 and 4.2 µg mL^{-1}, respectively, implying the method was suitable for quantification of these compound. R^2 values for the compounds were >0.98, confirming the linearity of the method. The method can therefore be regarded as suitable for quality control and standardization of *Bergenia* species.

Table II

Retention times, correlation coefficients and linear range from regression analysis, and limits of quantification (*LOQ*) and detection (*LOD*)

Compound	t_R (min)^a	R^2	Linear range (µg mL^{-1})	*LOD* (µg mL^{-1})	*LOQ* (µg mL^{-1})
Gallic acid	5.91 ± 0.12	0.990	10–200	0.66	4.2
Bergenin	11.06 ± 0.23	0.957	5–50	1.16	3.9

^{a}Means from ten replicates $\pm$ *SD*

These results suggest *B. ciliata* and *B. stracheyi* are better sources of bergenin than *B. ligulata* and that their biological activity will be greater.

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