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Short Communication

Determination of plumbagin by normal-phase high-performance liquid chromatography☆

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ABSTRACT

A simple and rapid normal-phase high-performance liquid chromatographic method for the determination of a pharmacologically important 1,4-naphthoquinone, plumbagin, and a chromene, 2,2-dimethyl-5-hydroxy-6-acetylchromene, in plant extracts was developed. Both compounds were well resolved with maximum recovery (97%) on a μSpherogel column using *n*-hexane–chloroform–2-propanol (30:70:2) as the mobile phase.

INTRODUCTION

Plumbago zeylanica L. is a perennial herb used in Indian medicine for various diseases, *e.g.*, diarrhoea, dyspepsia, piles and anasarea and also in skin diseases including leprotic lesions. The plant is claimed to lead to permanent sterilization [1]. *P. zeylanica* is a rich source of a pharmacologically important 1,4-naphthoquinone, plumbagin. Plumbagin exhibits a wide range of activities, *e.g.*, antifertility [2], mitotic inhibitor [3], antifungal [4], anticoagulant [5] and antibacterial against both Gram-positive and Gram-negative bacteria [4]. In clinical studies plumbagin has been found to be useful in patients with common warts [6]. Owing to the use of *P. zeylanica* in many important Ayurvedic preparations of Indian medicines, a rapid and sensitive method was devised for the determination of its active ingredient plumbagin. The high-performance liquid chromatographic (HPLC) separation of naturally occurring quinones on a cyano-bonded column was reported by Marston and Hostettmann [7], who used *n*-hexane containing 1% acetic acid as the mobile phase.

In this study, we found that the above system [7] is not suitable for the HPLC of *P. zeylanica* as the detection limit of two major constituents of the plant extract, 2,2-dimethyl-5-hydroxy-6-acetylchromene and plumbagin, was very poor. These two constituents have close R_F values in thin-layer chromatography. In this work we developed a method for the determination of plumbagin in *P. zeylanica* roots. To the best of our knowledge, this is the first report of this determination in *P. zeylanica* by HPLC.

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EXPERIMENTAL

Chemicals

The solvents used were of HPLC grade (Spectrochem, Bombay, India) and filtered through a Millipore 0.5-μm filter. Plumbagin and 2,2-dimethyl-5-hydroxy-6-acetylchromene were isolated from the *n*-hexane extract (3.35 g) of *P. zeylanica* roots (500 g) by silica gel column chromatography (400 g, 60–120 mesh; Qualigens Fine Chemicals, Bombay, India) employing *n*-hexane–ethyl acetate (95:5) as mobile phase. Fractions of 50 ml each were collected and monitored by TLC employing benzene (Merck, Bombay, India) as developing solvent and the spots were detected by spraying with 50% H_2SO_4 followed by heating at 80°C. The earlier fractions (28–30) yielded 2,2-dimethyl-5-hydroxy-6-acetylchromene and fractions 31–36 yielded plumbagin. Both compounds yielded crystals in light petroleum (b.p. 60–80°C). The compounds were characterized by ^1H and ^{13}C NMR and mass spectrometry.

The roots of *P. zeylanica* were collected from Ambikapur, Madhya Pradesh, India, in September 1990 and a voucher specimen has been deposited in the Botany Department of this Institute.

Apparatus

A Waters (Milford, MA, USA) modular HPLC system consisting of a U6K injector, M-6000A pump, M-450 variable-wavelength detector and M-730 data system was used. Analysis was performed on μSpherogel column (30 cm × 8.0 mm I.D.; particle size 10 μm) (Altex Scientific, Berkeley, CA, USA).

HPLC conditions

The composition of the mobile phase was optimized by varying the percentage of *n*-hexane, resulting in the following operating conditions: *n*-hexane–chloroform–2-propanol (30:70:2, v/v/v), flow-rate 1 ml/min, column temperature 27°C, detector wavelength 267 nm and detector sensitivity 0.04 a.u.f.s.

Calibration graphs

Known amounts of plumbagin (10 mg in 50 ml) and 2,2-dimethyl-5-hydroxy-6-acetylchromene (10 mg in 50 ml) were prepared in chloroform. Different volumes of these standards were injected using the HPLC conditions described above. The area counts of peaks (y) and the corresponding concentrations (x) were used to plot the calibration graphs. The graphs were linear in the range 0.2–10 μg. The regression equations for plumbagin and 2,2-dimethyl-5-hydroxy-6-acetylchromene were $y = 7.800x + 0.260$ ($r = 0.99$) and $y = 6.600x - 0.000$ ($r = 0.99$), respectively.

Extraction procedure

Four sets of air-dried roots (10 g each) were Soxhlet extracted using *n*-hexane for different time intervals (1, 2, 4 and 6 h). Similarly, another four sets (10 g each) were extracted with chloroform. The extract thus obtained in each extraction was concentrated under vacuum and diluted to 10 ml with chloroform. Samples were filtered through a Millipore sample filtration kit and 1 μl of each extract was injected into the HPLC system under the conditions mentioned above. Three experiments were peformed and average values are reported in Table I. Extraction of plant material with chloroform for 4 h was found to give a maximum yield of plumbagin.

RESULTS AND DISCUSSION

Fig. 1 shows plots of the capacity factor and resolution *versus* percentage of *n*-hexane in the mobile phase. The effect of variation in *n*-hexane concentration on the recovery of plumbagin is shown in Fig. 2. Fig. 1 shows that the capacity factor difference between the two compounds increases with increasing *n*-hexane concentration. The resolution increases similarly. The recovery of both plumbagin and 2,2-dimethyl-5-hydroxy-6-acetylchromene is effected by the composition of the mobile phase. The solvents *n*-hexane–chloroform–2-propanol (75:25:2 to 35:65:2) are not efficient in eluting these compounds completely, and causes peak broadening, baseline shifts and smaller area counts, although a better resolution as is evident from Fig. 1. The mobile phases *n*-hexane–chloroform–isopropanol (10:90:2 to 25:75:2) provided a stable baseline, symmetrical peaks and reproducible

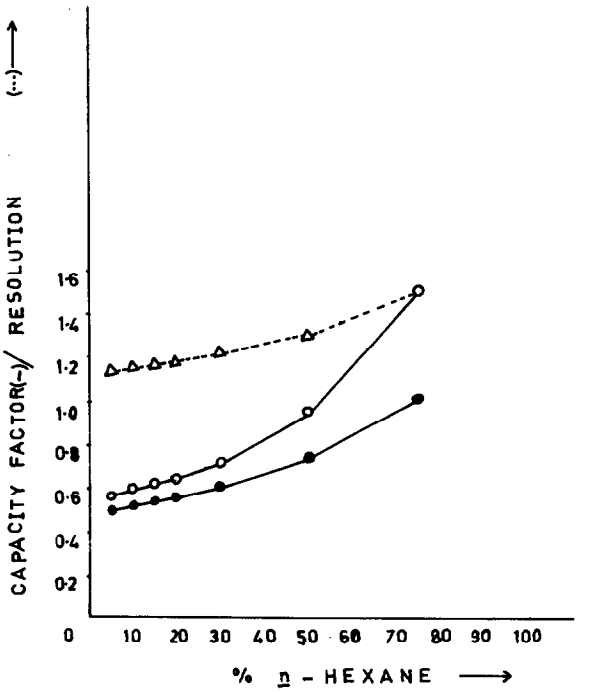

Fig. 1. Effect of *n*-hexane concentration in the mobile phase on the capacity factor (○ = plumbagin; ● = 2,2-dimethyl-5-hydroxy-6-acetylchromene) and the resolution (△).

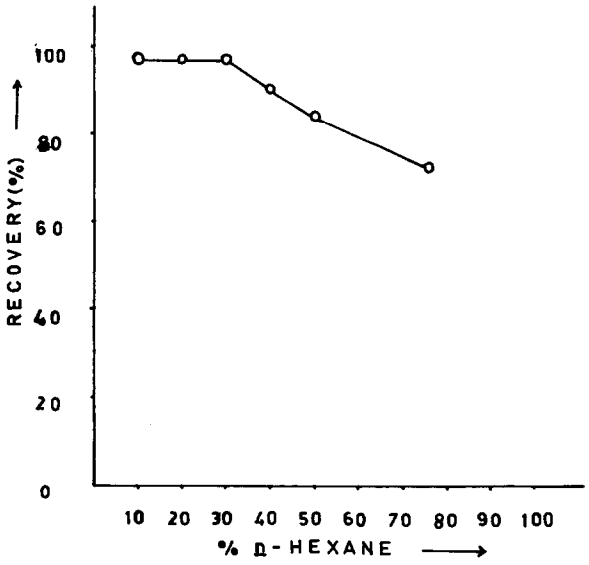

Fig. 2. Effect of *n*-hexane concentration in the mobile phase on the recovery of plumbagin.

area counts with lower resolution. Taking into consideration all these factors, the optimum conditions were fulfilled by using *n*-hexane–chloroform–2-propanol (30:70:2). The recovery of plumbagin was only 72% with *n*-hexane–chloroform–2-propanol (75:25:2), 84% with *n*-hexane–chloroform–2-propanol (50:50:2), 90% with *n*-hexane–chloroform–2-propanol (40:60:2) and the maximum of 97% with *n*-hexane–chloroform–2-propanol (30:70:2). All the recoveries were calculated by injecting a stock solution in each mobile phase.

Table I shows that the extraction of both plumbagin and 2,2-dimethyl-5-hydroxy-6-acetylchromene is not complete when extracted with *n*-hexane, as the amount is less than that extracted with chloroform for the same intervals. This further confirms that an increase in the *n*-hexane concentration in the mobile phase will decrease the recovery of the compounds.

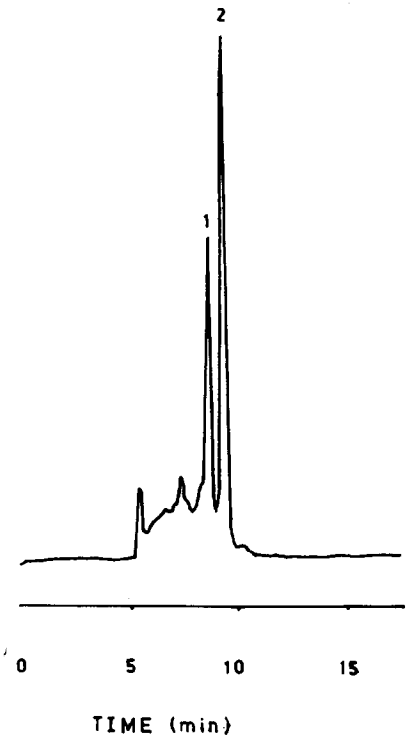

Fig. 3. HPLC separation of plumbagin and 2,2-dimethyl-5-hydroxy-6-acetylchromene from a plant extract on a μSpherogel column. UV detection at 267 nm. Mobile phase, *n*-hexane–chloroform–2-propanol (30:70:2); flow-rate, 1 ml/min. Peaks: 1 = 2,2-dimethyl-5-hydroxy-6-acetylchromene; 2 = plumbagin.

TABLE I

CONTENTS OF PLUMBAGIN AND 2,2-DIMETHYL-5-HYDROXY-6-ACETYLCHROMENE IN *PLUMBAGO ZEYLANICA* EXTRACTED FOR DIFFERENT TIME INTERVALS WITH *n*-HEXANE AND CHLOROFORM

Solvent	Plumbagin (%)				2,2-Dimethyl-5-hydroxy-6-acetylchromene (%)			
	1 h	2 h	4 h	6 h	1 h	2 h	4 h	6 h
n-Hexane	0.010	0.012	0.018	0.018	0.008	0.010	0.014	0.014
Chloroform	0.021	0.024	0.032	0.032	0.012	0.018	0.022	0.022

These observations led us to select the solvent system *n*-hexane–chloroform–2-propanol (30:70:2) for the optimum separation and determination of plumbagin. The separation of the two compounds from a plant extract is shown in Fig. 3. The numbers of theoretical plates for plumbagin and 2,2-dimethyl-5-hydroxy-6-acetylchromene were 3750 and 2240, respectively.

In conclusion, the HPLC method described is efficient and simple for the separation and determination of plumbagin and 2,2-dimethyl-5-hydroxy-6-acetylchromene in plant extracts.

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