ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
22 (2000) 705–709

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Short communication

# LC analysis of hepatoprotective diterpenoids from *Andrographis paniculata*

D.C. Jain *, M.M. Gupta, S. Saxena, S. Kumar

*Central Institute of Medicinal and Aromatic Plants, P.O. CIMAP, Lucknow 226015, India*

Received 26 November 1998; received in revised form 14 September 1999; accepted 15 September 1999

*Keywords:* *Andrographis paniculata*; Andrographolides; Hepatoprotective; Liquid chromatography

## 1. Introduction

*Andrographis paniculata* (Acanthaceae), commonly known as Kalmegh, has been used widely in India for the treatment of hepatitis [1,2]. The plant is reported to possess protective activity against various liver disorders. Andrographolide and related compounds were investigated for their pharmacological properties and all showed at least some degree of antipyretic, antimalarial and antiinflammatory activity [3–5]. During crop improvement, drug analysis and process development, a sensitive and accurate analytical method is required for the quantitation of important diterpenoids, which are present in the plant. Although few methods such as gravimetric [6,7], colorimetric [8], spectrophotometric [9,10], titrimetric [11,12] have been reported for the quantitative estimation of andrographolides, many of these procedures are time consuming, not very precise and require multiple step extraction and purification. Reported HPLC [13] and HPTLC [14] methods are for the quantitation of andrographolide (**2**) only. Here a simple reversed phase liquid chromatographic method is described for the simultaneous determination of the three major andrographolides viz 14-deoxy-11,12-didehydroandrographolide (**1**) andrographolide (**2**), and neoandrographolide (**3**) (Fig. 1) in *A. paniculata* leaf extract with UV detection at 230 nm by employing an isocratic binary mobile phase.

## 2. Experimental

*A. paniculata* leaves were collected from the experimental farm of Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow and Voucher specimen has been deposited in the herbarium of this Institute.

### 2.1. Extraction

*A. paniculata* leaves (500 g) were extracted with methanol and the extract (18 g) was column chro-

* Corresponding author. Fax: +91-522-342666.
*E-mail address:* cimap@technologist.com (D.C. Jain)


PII: S0731-7085(99)00297-6

matographed over silica gel (E. Merck, India). Elutions were carried out with $CHCl_3$ followed by a $CHCl_3$–MeOH mixture with increasing polarity. Fractions (250 ml each) were collected and monitored by TLC. Three pure compounds were isolated from different fractions and identified with the help of spectral data by comparison with that reported in the literature [15,16].

Compound (**1**), isolated from fractions (16–17) of $CHCl_3$–MeOH (97:3), m.p. 196–197°C, was identified as 14-deoxy-11,12-didehydroandrographolide. Compound (**2**), identified as andrographolide, was isolated from fractions (23–25) of $CHCl_3$–MeOH (92:8), m.p. 229–230°C. Compound (**3**), isolated from the fractions (31–35) of $CHCl_3$–MeOH (88:12), m.p. 173–174°C, was identified as neoandrographolide.

### 2.2. Chromatographic instrument and conditions

A Shimadzu (Japan) LC-10A gradient liquid chromatography instrument, equipped with two LC-10 AD pumps and controlled by a model CBM-10 interface, a model 7725 i manual injector (Rheodyne), a 20-μl sample loop and a multidimensional UV-VIS detector (SPD-10 A) were used. Data were collected with the LC-10 workstation equipped with a Pentium computer (Datamini, Singapore) and HP-deskjet printer. Solvents were filtered by using a millipore system and the analysis was performed on a Waters make μ Bondapak $C_{18}$ column (300 × 3.9 mm, I.D. 10 μm). A constant flow rate of 1 ml/min was used during analysis. The composition of the mobile phase was optimized by varying the percentage of acetonitrile in water/methanol, in water. A good resolution of the compounds (**1**–**3**) was achieved in the following operating conditions; acetonitrile–water (70:30, v/v), flow rate 1 ml/min, column temperature 26°C, detector wave length 230 nm, the absorption maxima closed to all the compounds.

### 2.3. Sample preparation

Air-dried and powdered plant material (1 g) was extracted with methanol (4 × 5 ml, 12 h), filtered, completely dried under vacuum and 5 ml of acetonitrile was added. Sample was filtered through a millipore filter (0.45 μm) and a known amount was subject to LC separation. A freshly prepared standard solution of compounds **1**–**3** (1 mg/ml) each were prepared in acetonitrile and different volumes were injected for the preparation of calibration graphs and LC analysis as above. The area count of each peak ($X$) and the corresponding concentration ($Y$) was used to plot the calibration graphs. The content of each andrographolide (**1**–**3**) was calculated using calibration graphs of compounds **1**,**2** and **3**.

## 3. Results and discussion

### 3.1. Selection of mobile phase

The composition of mobile phase was optimized using different proportions of acetonitrite

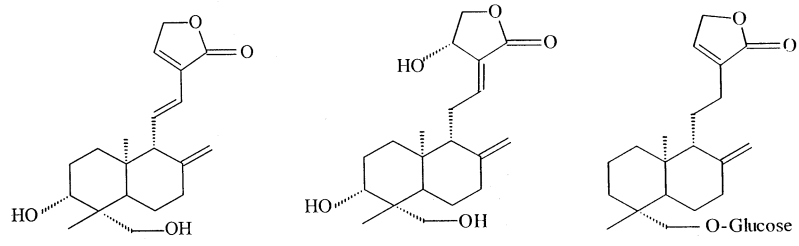


Fig. 1. Structure of three major andrographolides (**1**–**3**) in *A. paniculata* extract.

in water. Fig. 2 illustrates the separation of andrographolides (**1**–**3**) in an artificial mixture of standards and plant sample extract. Peaks corresponding to compounds (**1**–**3**) were symmetrical and base line separation of these peaks has been achieved. Recoveries of compounds **1**,**2** and **3** were 97, 97 and 96%, respectively. For the examination of recovery rates, known amounts of stock solutions of pure compounds (**1**–**3**) were added in the *A. paniculata* plant extract and the quantitation was repeated thrice. Column performance report is given in Table 1.

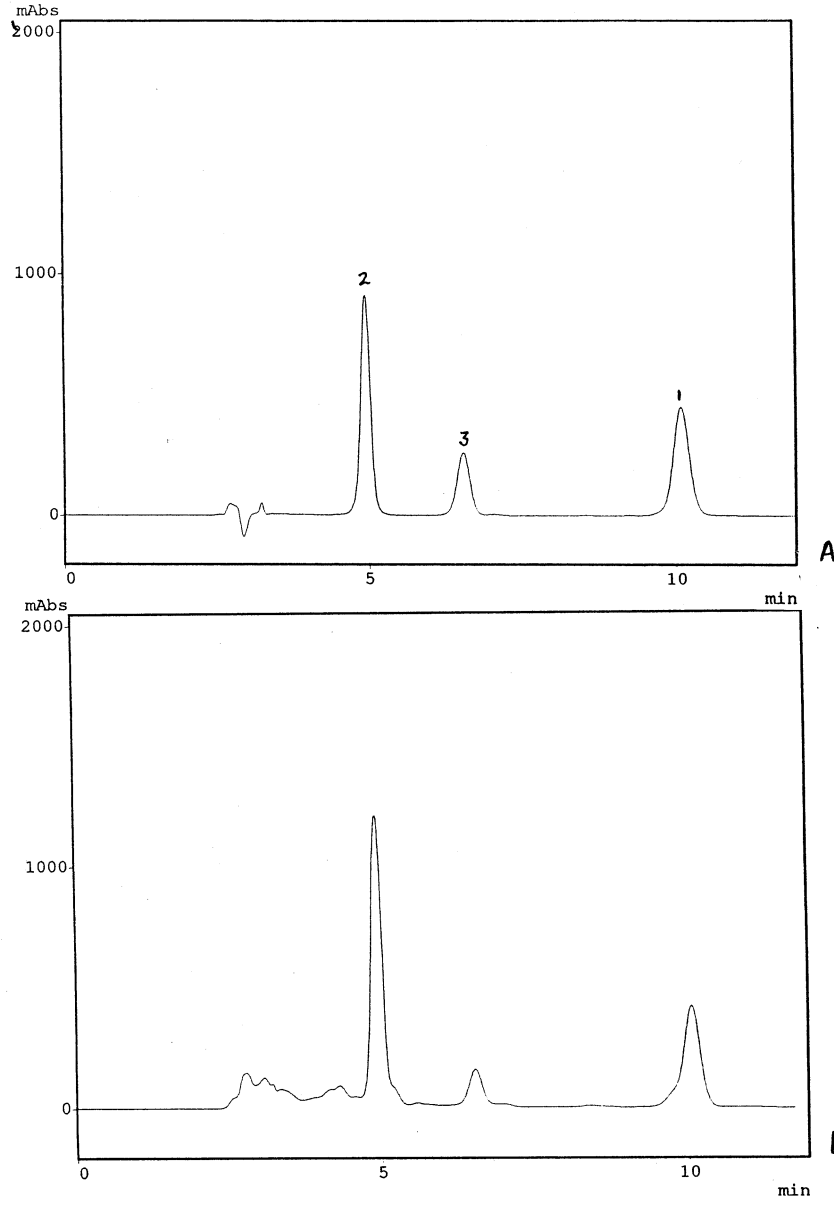


Fig. 2. LC separation of andrographolides in an artificial mixture of pure compounds (A) and an *A. paniculata* plant extract (B). HPLC conditions: μ BondapaK $C_{18}$ column; UV detection at 230 nm; mobile phase, acetonitrile–water (70:30); flow rate 1 ml/min; injection volume, 5 μl; 14-deoxy-11,12-didehydroandrographolide (**1**), andrographolide (**2**) and neoandrographolide (**3**).

Table 1
ODS column performance in the separation of andrographolides (**1**–**3**) from the extract of *A. paniculata*

| Compounds | *tr* (min) | *N* | Capacity factor (*k'*) | Recovery(%) | Resolution |
|---|---|---|---|---|---|
| Andrographolide (**2**) | $4.93 \pm 0.3$ | 4359 | 1.68 | $97 \pm 1$ | 7.88 |
| Neoandrographolide (**3**) | $6.52 \pm 0.06$ | 4378 | 2.55 | $96 \pm 2$ | 4.60 |
| 14-Deoxy-11,12-didehydroandrographolide (**1**) | $10.08 \pm 0.08$ | 6767 | 4.48 | $97 \pm 2$ | 8.04 |

Table 2
Comparison of two determination procedures

| Procedure of estimation | % content ± S.D. of andrographolide derivatives | | |
|---|---|---|---|
| | 14-Deoxy-11,12-didehydroandrographolide | Andrographolide | Neoandrographolide |
| HPTLC | $1.71 \pm 0.03$ | $2.66 \pm 0.04$ | $0.62 \pm 0.02$ |
| HPLC | $1.78 \pm 0.01$ | $2.80 \pm 0.02$ | $0.65 \pm 0.01$ |

Table 2 is a comparison of andrographolides content using previously reported HPTLC [14] method to that of newly developed LC method. Content of andrographolide was 1.78% in LC method in comparison with 1.71% in HPTLC, neoandrographolide was 2.80% in LC in comparison with 2.66% in HPTLC, and 0.65% of 14-deoxy-11,10-didehydroandrographolide in LC in comparison with 0.62% in HPTLC. Recoveries of all the compounds were better in LC method than HPTLC due to less retention of compounds in LC column than HPTLC plates.

### 3.2. Linearity

Linearity was determined by using five concentrations in a working range of 1–20 μg of each component (**1**–**3**). Linear regression equations and correlation coefficients for all the three compounds (**1**–**3**) have been given in Table 3. Calibration plots of peak areas versus concentrations are linear, with *r* values between 0.9903 and 0.9970. These values indicate good linearity in the examined concentration range.

### 3.3. Detection limits

Detection limit was determined by estimating the minimal mass of each compound (**1**–**3**) that can be quatitated. Detection limits in the LC method for the compounds **1**–**3** were 0.20, 0.25 and 0.10 μg/injection, respectively, in comparison with 0.50, 0.40 and 0.48 μg/spot in HPTLC method.

### 3.4. Precision

Precision of the method was measured by repeating the measurements four times. Mean and S.D. values for the retention times and recoveries

Table 3
Linear regressions for andrographolides (**1**–**3**)

| Compounds | Equation [$Y = A \pm \text{S.D.}X \pm C \pm \text{S.D.}$] | *r* |
|---|---|---|
| Andrographolide | $Y = 7.614 \pm 0.192 \times 10^{-7} \times -0.880 \pm 0.04$ | 0.9903 |
| Neoandrographolide | $Y = 1.261 \pm 0.114 \times 10^{-6} \times -0.031 \pm 0.03$ | 0.9955 |
| 14-Deoxy-11,12-didehydroandrographolide | $Y = 2.819 \pm 0.202 \times 10^{-6} \times -0.535 \pm 0.03$ | 0.9970 |

for all the three compounds have been presented in Table 1. S.D. values were $\pm 0.03 - \pm 0.08$ for retention times and $\pm 1 - \pm 2\%$ for recovery of compounds **1**–**3**.

### 3.5. Conclusions

The reported reversed phase LC method using UV detector is suitable for the analysis of hepatoprotective compounds in *A. paniculata* extract and is simple, rapid and precise. A good separation of all the three major diterpenoids has been achieved and could be used for rapid screening of *A. paniculata* plants for their genotypic quality assessment, drug analysis, etc.

## References

[1] A. Sharma, R.T. Singh, V. Sehgal, S.S. Handa, Fitoterapia 62 (1991) 131–138.
[2] W. Tang, G. Eisenbrand, Chinese Drug of Plant Origin, Springer-Verlag, Berlin, 1992, pp. 97–103.
[3] S. Madav, H.C. Tripathi, Tandan, S.K. Mishra, Ind. J. Pharm. Sci., 57 (1995) 121–125.
[4] P. Mishra, N.L. Pal, P.Y. Guru, J.C. Katiyar, V. Srivastava, J.S. Tandon, Int. J. Pharmacogn. 30 (1992) 263–274.
[5] S. Saxena, D.C. Jain, R.S. Bhakuni, R.P. Sharma, Indian Drugs 35 (1998) 458–467.
[6] S.K. Srivastava, P.C. Bose, G.K. Ray, B. Mukherjee, Ind. J. Pharm. 21 (1959) 229–230.
[7] S.P. Sengupta, S. Banerjee, D. Chakravarty, Ind. J. Pharm. 11 (1949) 77–78.
[8] P.C. Maiti, S.K. Kanji, R. Chatterjee, Ind. J. Pharm. 21 (1959) 169.
[9] K.N. Gaind, R.N. Dar, R.N. Kaul, Ind. J. Pharm. 25 (1963) 225–226.
[10] Z. Pinye, P. Nuanbing, J. Weijie, Chin. J. Pharm. Anal. 4 (1984) 34–36.
[11] V. Subba Rao, Ind. J. Pharm. 24 (1962) 134.
[12] P.B. Talukdar, S. Banerjee, P.K. Chatterjee, A.K. Dutta, Ind. J. Chem. 6 (1968) 359–363.
[13] S.S. Handa, A. Sharma, Ind. J. Med. Res. (B) 92 (1990) 276–283.
[14] S. Saxena, D.C. Jain, M.M. Gupta, R.S. Bhakuni, H.O. Mishra, R.P. Sharma, Phytochemical analysis, submitted, (1999).
[15] T. Mastuda, M. Kuroyanagi, S. Sugiyama, K. Umchara, A. Ueno, K. Nishi, Chem. Pharm. Bull. 42 (1994) 1216–1225.
[16] T. Fujita, R. Fujitani, Y. Takeda, Y. Takaishi, T. Yamada, M. Kido, I. Miura, Chem. Pharm. Bull. 32 (1984) 2117–2125.