# An HPLC method for the estimation of andrographolide in rabbit serum

**Article** · April 2003

CITATIONS
21

READS
498

**6 authors**, including:

P. Thirugnanasambantham
Rumi Herbals R& D Centre
**29** PUBLICATIONS **429** CITATIONS
SEE PROFILE

Viswanathan Subramanian
Meenakshi Academy of Higher Education and Research
**67** PUBLICATIONS **1,027** CITATIONS
SEE PROFILE

**Indian Journal of Pharmacology 2003; 35: 109-112** **SHORT COMMUNICATION**

# AN HPLC METHOD FOR THE ESTIMATION OF ANDROGRAPHOLIDE IN RABBIT SERUM

K. SENTHIL KUMARAN*, P. THIRUGNANASAMBANTHAM**,
S. VISWANATHAN**, M. SREE RAMA MURTHY***

*Department of Pharmaceutics, C.L.Baid Metha College of Pharmacy, Chennai-600096.
**Medicinal Chemistry Research Center, Institute of Pharmacology, Madras Medical College, Chennai-3.
***T.T.K. Healthcare Ltd, 5, Old Trunk Road, Chennai-600 043.

Manuscript Received: 17.4.2002 Revised: 27.6.2002 Accepted: 29.6.2002

**ABSTRACT**

***Objective:*** To develop a HPLC method for the estimation of andrographolide in biological fluids.

***Methods:*** Andrographolide was extracted from serum using methanol. A Shimadzu HPLC (Model LC 10 A) with UV- Vis spectrophotometric detector (model SPD 10 AV) and a data processor (model 10 R4 A) were used for andrographolide estimation. A Neuclosil $C_{18}$ octadecyl silane (ODS) column (5m, 250x4.6 mm Hypersil) was used. The mobile phase used was methanol: Water in the ratio of 65:35 v/v at a flow rate of 1.0 ml/min. The elutes were monitored at 223 nm. The precision and accuracy of the method were determined by measuring inter-day and intra-day variations.

***Results:*** The absolute recovery of andrographolide ranged between 97.24% to 99.4%. The inter-day co-efficient of variation was 0.4% to 1.4% and intra-day co-efficient of variation was 1.05% to 2.8%.

***Conclusion:*** The developed HPLC method for estimation of andrographolide in serum is sensitive, reliable and accurate.

**KEY WORDS** *Andrographis paniculata* Nees kalmegh liquid chromatography

## INTRODUCTION

*Andrographis paniculata* Nees (Acanthaceae) is common throughout the plains of India.The plant is used as a bitter tonic and in liver diseases[1-2]. It is also used as antipyretic and anthelmintic. Further this plant has been studied for its antifertility[3], antidiabetic[4] and hypotensive activity[5]. Most of the biological actions of *Andrographis paniculata* have been ascribed to the presence of andrographolide, a diterpene lactone present in this plant. The hepato-protective action of *Andrographis paniculata* has also been suggested to be due to the presence of andrographolide and this has been confirmed by many scientific studies[6]. In the present study an attempt has been made to develop a sensitive and precise method for the determination of andro-grapholide in serum by reverse phase high performance liquid chromatography (HPLC).

**Correspondence: K. Senthil Kumaran**
**e-mail: kskumaran54@rediffmail.com**

## MATERIALS AND METHODS

Andrographolide was isolated from dried *Andrographis paniculata*.The plant material was collected around Chennai, and identified in the Department of Pharmacognosy, Madras Medical College and confirmed by comparison with the reference herbarium specimens. The whole plant material (4 kg) was dried and ground to coarse powder and extracted at room temperature successively with petroleum ether (60-80°C), chloroform and methanol. The methanolic extract was concentrated under reduced pressure and activated charcoal (400 g) was added to it. After 24 h, charcoal was filtered off and the filtrate was concentrated under reduced pressure and left overnight for crystallization. The resulting crystals were collected by filtration[7], and the isolated andrographolide (yield 0.78 %) was compared with an authentic sample (Research-

organics, Chennai) for its purity. Nimesulide was gifted from TTK Health Care Ltd., Chennai. Methanol (HPLC grade, Qualigens) and potassium dihydrogen orthophosphate, sodium hydroxide AR (Glaxo) were the other chemicals used.

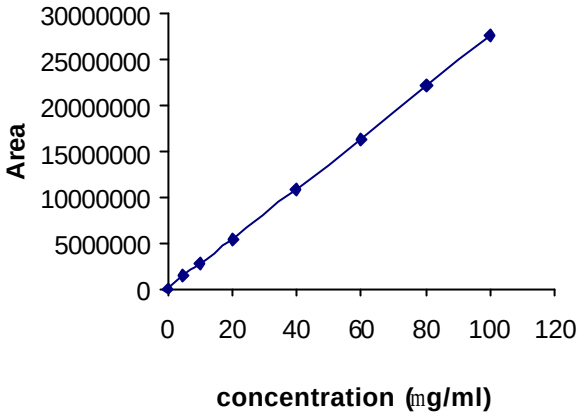
**Preparation of standards:** Stock solution of andrographolide (1 mg/ml) in methanol was prepared. Dilution of this solution was made with methanol to produce working standard solution containing 100 µg/ml of andrographolide. Similarly 1 mg/ml of nimesulide (internal standard) in 0.1 N sodium hydroxide was prepared and the working standard solution contained 100 µg/ml of nimesulide.

**Extraction from serum:** From working standard solution of andrographolide (100 µg/ml) 0, 50, 100, 200, 400, 600, 800, 1000 µl volume of andrographolide standard solutions were placed in screw-capped test tubes and diluted to 1000 µl with methanol. Then these standard solutions were mixed with 0.5 ml of rabbit serum thus providing calibration standards of 0, 5,10, 20, 40, 60, 80 and 100 µg/ml of andrographolide in serum. A 100 µl volume of internal standard equivalent to 100 µg of nimesulide was added to these tubes, which were shaken on a vortex mixer for 1 min. Then 0.5 ml of phosphate buffer (pH 5.8) and 2 ml of methanol were added. The tubes were capped and vortex mixed for 5 min and then centrifuged at 8000 g for 5 min. The supernatant layer was separated.

**Elution by HPLC:** A Shimadzu HPLC system (model LC 10A) with UV-Vis spectrophotometric detector (model SPD 10 AV) and a data processor model (10 R 4A) was used. A Neuclosil $C_{18}$ octadecyl silane (ODS) column (5 µ 250 x 4.6 mm Hypersil, Machery-nagel Dureh Germany) was used. The mobile phase was methanol: water (65:35 v/v). Aliquots of 20 µl of supernatant of the sample extracts were injected into the HPLC system and eluted with the mobile phase at a flow rate of 1.0 ml/min. The elutes were monitored at 223 nm with the detector range setting fixed at 0.1. The two peaks for andrographolide and the internal standard were well separated.

The precision and accuracy of the method were determined by measuring the inter-day (5 samples within a day) and intra-day (3 samples on day 1, 7 and 14th day n=9) variations in the recovery of andrographolide from serum samples. Fresh samples of serum were used every time. The co-efficient of variation was calculated from the above results.

**Figure 1.** Calibration graph for andrographolide.




## RESULTS

**Quantification:** Peak- area ratios of andrographolide to the internal standard were measured. A representative calibration graph of andrographolide was constructed with different concentration of andro-grapholide. (Figure 1) The peak-area versus andrographolide concentration in the range of 5-100 µg/ml resulted in the regression equation, r =0.99.

**Recovery:** The extraction recovery of andrographolide was assessed at concentrations of 5, 10, 20 µg/ml. Serum samples (in triplicate) containing andrographolide and internal standard were extracted and studied. Absolute recovery was calculated by comparing area of the peak for direct injection of andrographolide in methanol with those of biological samples containing same amount of andrographolide. The absolute recovery of andrographolide ranged from 97.24% to 99.4% as estimated using HPLC method.

**Precision:** The precision of the assay was determined by estimating serum samples containing andrographolide at three different concentrations 5, 10 and 20 µg/ml. Endogenous interfering peaks were visible in blank serum. The two peaks were well separated

**Figure 1.** HPLC chromatogram of serum andrographolide and intemal standard (nimesulide).

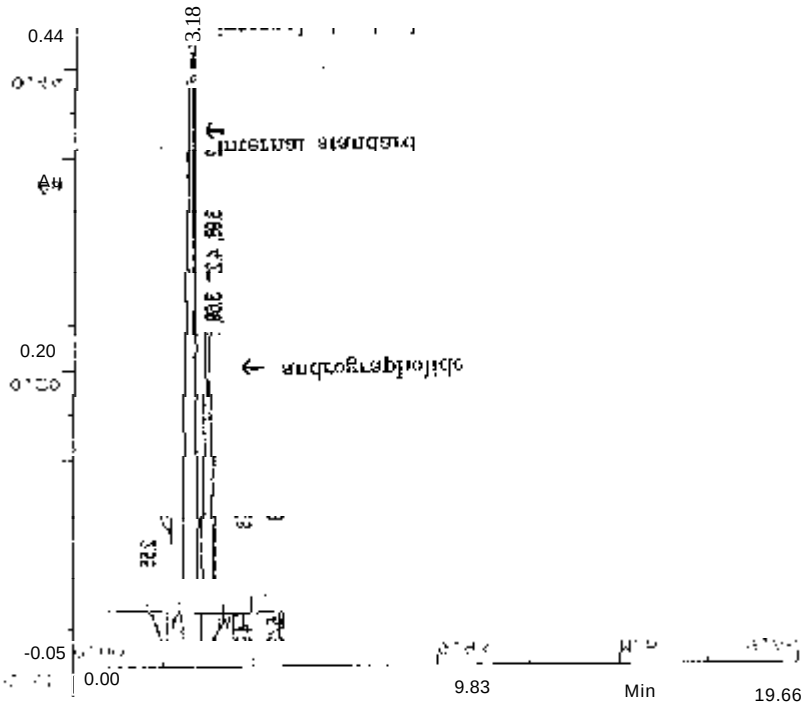



**Table 1.** Precision of determination of andrographolide in serum (HPLC).

| | Intra-day variation ( n= 9 ) | | | Inter-day variation ( n = 5 ) | | |
|---|---|---|---|---|---|---|
| Concentration used mg/ml | Quantity recovered mg/ml | Percent recovery | C.V. % | Quantity recovered mg/ml | Percent recovery | C.V. % |
| 5 | 5.0±0.02 | 100.2 | 0.40 | 5 .02±0.14 | 100.4 | 2.80 |
| 10 | 9.98±0.14 | 99.8 | 1.40 | 10.14±0.16 | 101.4 | 1.60 |
| 20 | 19.94±0.25 | 99.7 | 1.25 | 20.02±0.21 | 100.1 | 1.05 |

Values are mean ± SD, C.V.- Co-efficient of variation.

with the retention time 3.69 and 3.18 min for andrographolide and the internal standard respectively (Figure 2). The intra-day co-efficient of variation ranged between 0.4-1.4%. The inter-day co-efficient of variation varied from 1.05-2.8% (Table 1).

## DISCUSSION

In the present study a HPLC method was developed for estimation of andrographolide in serum. Andrographolide was extracted using methanol.

A Neuclosil $C_{18}$ (ODS) column was used and measurement was done at 223 nm with a mobile phase of methanol:water (65:35). This is a slight modification of the method described by Handa and Sharma (1990) who used a mobile phase of chloroform: methanol (9:1 v/v) and detection at 254 nm. This modification was attempted because of the strong peak and better resolution of the compound at 223 nm. The absolute recovery and the precision of the assay values indicate that the developed HPLC method for estimation of andrographolide in various biological samples is very sensitive, reliable and accurate one. Such a sensitive method is essential for determination of various pharmacokinetic parameters of this useful phytochemical. This method will be very useful while designing future clinical trials with andrographolide in humans. The suitability of this method in estimating andrographolide from other biological samples like urine and feces remains to be elucidated. Our preliminary experiments in rabbits indicate good reproducibility of this method (un published data).

## REFERENCES


1. Nadkarani AK. Nadkarani's Indian Materia Medica. Vol.I, 1st ed. Bombay: Popular Book Depot; 1954.
2. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. 1st ed. New Delhi: Publication and Information; 1956.
3. Akbarsha MA, Manivannan B, Shahulhamid K, Vijayan B. Antifertility effect of *Andrographis paniculata* (Nees) in male albino rat. *Indian J Exp Biology* 1990;**28**:421-6
4. Lal J, Tripathi HC, Tandon SK. Antidiabetic activity of andrographolide. *Indian J Pharmacol* 1986;**18**:58-61.
5. Zhang CY, Tan-BK. Dose dependent hypotensive effect of andrographolide on systolic pressure of spontaneously hypertensive rats and normotensive controls. *Clinical Exp Pharmacol Physiol* 1996;**23**:675-8.
6. Visen PKS, Shukla B, Patnaik BN, Dhawan BN. Androgrpholide protects rat hepatocytes against paracetamol-induced damage. *J Ethnopharmacol* 1993;**40**:131-6.
7. Handa SS, Sharma A. Hepatoprotective activity of andrographolide from *Andrographis paniculata* against carbon tetrachloride. *Indian J Med Res* 1990;**92**:276-83.