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A Simple HPLC Method for the Estimation of Andrographolide

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Abstract

A simple, rapid, selective and quantitative HPLC method has been developed for the determination of Andrographolide. Mobile phase was Water: Acetonitrile (60:40), Flow rate 1.0 ml/min. Retention time of pure Andrographolide was 5.038 minutes. The content of andrographolide in crude (BNPL/AD/003) was 30% and reasonable pure sample (BNPL/AD/006) purity is 82% respectively. The developed method was found to be simple, robust, rugged, and economic for routine use in the herbal drug industry.

***Keywords:** Andrographolide; Andrographis paniculata; HPLC; Kalmegh*

Introduction

Medicinal plants coming from natural & traditional background are used from thousands of years in treatment of many diseases and disorders. Andrographolide is an important active constituent having potential biological activities, obtained from the herb *Andrographis paniculata* and it is available in the form of many ayurvedic dosage formulations. These plants have many active ingredients and thus they are used in preparation of various herbal formulations to treat various disorders. *Andrographis paniculata* (family: *Acanthaceae*) is one of the important traditional herb of India and also known as Kalmegh. Chemically Kalmegh composed of active constituents like Andrographolide Neoandrographolide, deoxyandrographolide etc. Andrographolide is a major active compound (**FIG. 1).** The Andrographilide's raw material constituents have many pharmacological actions. Chemically Andrographolide have bicyclic diterpenoid lactone ring. It is mainly used as antibacterial, antioxidant, hepatoprotective, anti-fungal, anti-inflammatory. Due to wide variety of biological activities it is used in the treatment of many diseases and available in the form of many ayurvedic formulations. Hence the quality control of formulation containing Andrographolide plays an important role in the ayurvedic industries. *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 an antipyretic and anthelmintic. Further, this plant has been studied for its antifertility,

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antidiabetic and hypotensive activity [3-5]. Most of the biological actions of *Andrographis paniculata* have been ascribed to the presence of andrographolide, a diterpene lactone present in this plant. The hepatoprotective 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].

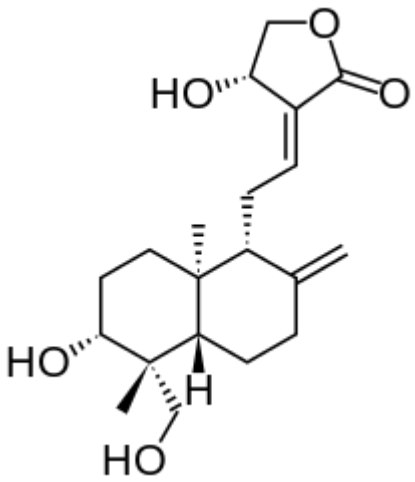

FIG. 1. **Chemical structure of Andrographolide.**

In the present study, a simple method for the determination of andrographolide by reverse phase High Performance Liquid Chromatography (HPLC) analytical procedure was established to analyse the crude and reasonably pure Andrographilides. There are several reports on HPLC methods for the analysis of Andrographilide [7]. However, our method was a simple and giving better resolution with strong peak (**TABLE 1).**

MATERIALS AND METHODS

Andrographolide was isolated from dried *Andrographis paniculata*. The plant raw material (BNPL/RMAD/001) was obtained from the local market and moisture content is 11.2%. Methanol, Water, Acetonitrile (HPLC grade, Qualigens).

Sample Preparation for HPLC

Weighed 50 mg Andrographilide sample accurately and transferred in to volumetric flask. The sample dissolved in methanol and made up accurate volume.

Preparation of HPLC standards

Weighed accurately 50 mg of in-house Andrographilides reference sample (BNPL/RFSTD/002) in to a 50 ml volumetric flask and dissolved completely. Made up volume with methanol solvent.

Preparation of Mobile phase

0.1 v/v percentage phosphoric acid was taken in to beaker and added 60 ml of HPLC grade of water and 40 ml of Acetonitrile and sonicated.

Calculation

$$\frac{\text{Peak Area of the Sample x Con, of the Standard x Purity of the Ref Std}}{\text{Peak area of the Standard x Concentration of the Sample}}$$

HPLC Conditions

TABLE 1. **The summary Of HPLC conditions are depicted below.**

S.no	Item	Conditions
1	Column	Phenomenex C18 5u, (4.6X250mm)
2	Wave length	223 nm
3	Flow rate	1ml/min
4	Volume of injection	20 ul
5	Temperature	250 C +_ 20C
6	System	Isocratic
7	Run time	15 min
8	Mobile phase	0.1 v/v %phosphoric acid in Water : Acetonitrile (60:40)

Results

Since Andrographolide is freely soluble in methanol, the plant materials (BNPL/RMAD/001) was extracted with methanol. Mobile phase for HPLC was Water : Acetonitrile (60:40). First run was of blank to check error from mobile phase. In the present HPLC conditions, the reasonably pure Andrographilide material (BNPL/AD/006) is eluting at retention time 5.038 and some nonpolar impurities were obtained at 6.413, 6.642, and 9.278 retention time respectively. The moisture content of the isolated drug was found to be 0.34% by Karl–Fischer method.

Determination of λ max

The drug was scanned in Shimadzu,-Model-UV-1601PC UV-Spectrophotometer (with methanol as blank). The λmax was found to be 223 nm which matches with standard value of andrographolide.

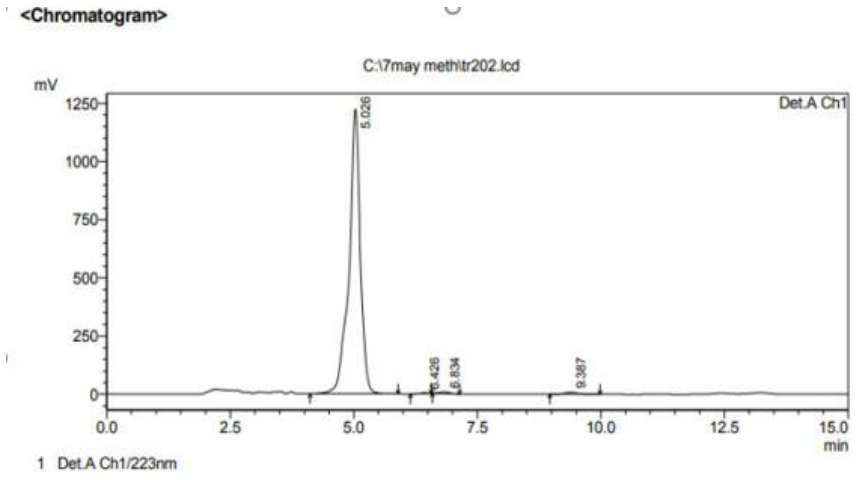

FIG.2. **The HPLC chromatogram of Andrographolide reference standard (BNPL/RFSTD/002)**.

TABLE 2. **HPLC chromatogram of Andrographolide reference standard (BNPL/RFSTD/002)**.

Peak	Ret. Time	Area	Height	Area%	Height%
1	5.026	19410433	1221351	98.547%	98.445%
2	6.426	21948	2616	0.111%	0.211%
3	6.834	105235	7582	0.534%	0.611%
4	9.387	158950	9089	0.807%	0.733%
Total		19696566	1240639	100	100

We have injected the internal reference standard of Andrographilide (BNPL/RFSTD/002) and elueted at 5.026 (**FIG. 2) &(TABLE 2).**

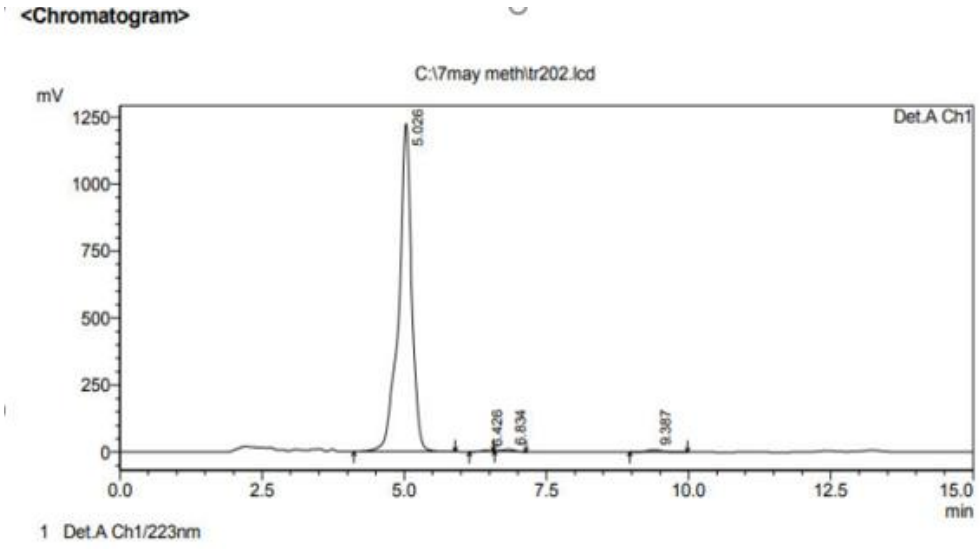

FIG. 3. **The HPLC chromatogram of crude Andrographolide (NPL/AD/003).**

TABLE 3. **HPLC chromatogram of crude Andrographolide (NPL/AD/003).**

Peak	Ret. Time	Area	Height	Area%	Height%
1	5.026	19410433	1221351	98.547%	98.445%
2	6.426	21948	2616	0.111%	0.211%
3	6.834	105235	7582	0.534%	0.611%
4	9.387	158950	9089	0.807%	0.733%
Total		19696566	1240639	100	100

During the process development of Andrographilide at our research lab we have achieved 30% material and it was analysed (**FIG. 3)** & (**TABLE 3**) The crude material was purified using acid base treatment and achieved 82% material.The HPLC chromatogram of pure material (BNPL/AD/006) is depicted at **FIG. 4 & TABLE 4**.

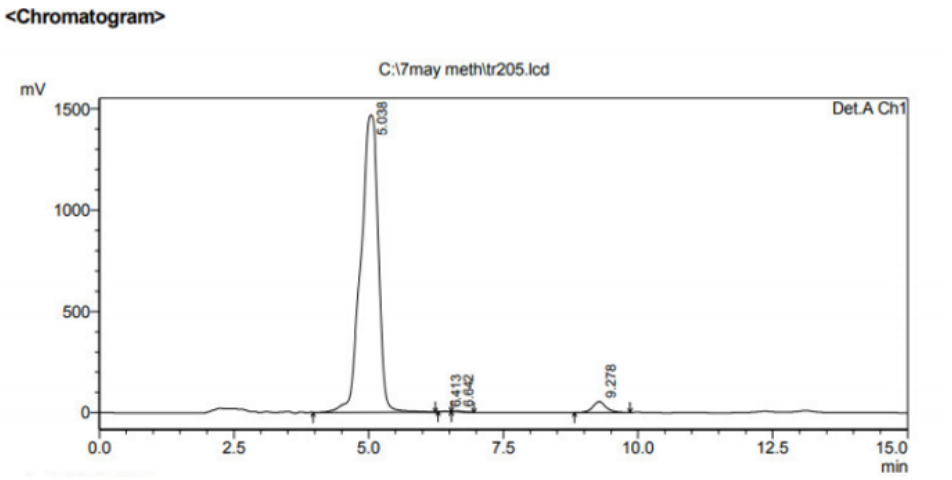

FIG. 4. **The HPLC chromatogram of pure Andrographolide (BNPL/AD/006)**.

TABLE 4. **HPLC chromatogram of pure Andrographolide (BNPL/AD/006).**

Peak	Ret. Time	Area	Height	Area%	Height%
1	5.038	32972827	1466502	97.158%	96.084%
2	6.413	14496	1970	0.043%	0.129%
3	6.642	35653	3998	0.105%	0.262%
4	9.278	914316	53801	2.694%	3.525%
Total		33937293	1526271	100	100

We have developed a robust HPLC method which be used to analyse crude and pure material of Andrographilde. The graphical representation of Andrographilide content in crude (30%) and pure sample (82%) are depicted in **FIG. 5.**

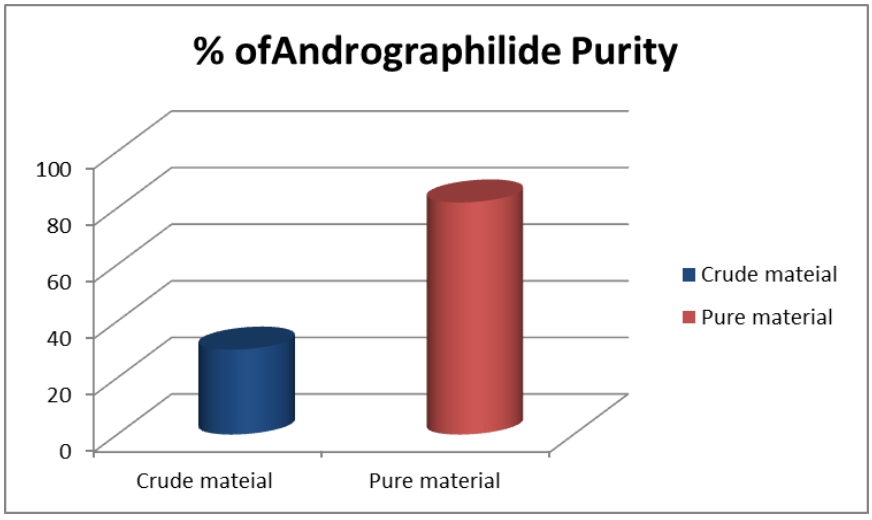

FIG. 5. **Graph of Andrographilide content in crude and pure material.**

The data showed significant variations in andrographolide concentration in the crude and pure sample.

Discussion

In the present study, a HPLC method was developed for estimation of andrographolide in crude and pure material. Andrographolide was extracted using methanol and Phenomenex C185u, (4.6 mm X 250 mm) column was used. Sample measurement was done at 223 nm with a mobile phase of Water: Acetonitrile (60:40). This is a slight modification of the method described by earlier authors who used a mobile phase of chloroform: methanol (9:1 v/v) and detection at 254 nm [7]. This modification was attempted because of the strong peak and better resolution of the compound at 223 nm. The results of the study aimed at the determination of the andrographolide content in crude and pure material. The developed HPLC method for estimation of andrographolide in various crude 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.

Conclusion

The newly developed HPLC analytical method is a simple, specific and selective for the determination of Andrographolide in bulk. The developed method was found to be precise, better resolution, strong peak, robust, stable and economic for routine use in the herbal drug industry.

Acknowledgement

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