



QUANTITATIVE HPLC ANALYSIS OF ANDROGRAPHOLIDE IN ANDROGRAPHIS PANICULATA AT TWO DIFFERENT STAGES OF LIFE CYCLE OF PLANT

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ABSTRACT

A simple, rapid, selective and quantitative HPLC method has been developed for determination of Andrographolide in *Andrographis paniculata* at two different stages of life cycle. Whole plant material at two different stages, one after 110 days of plantation i.e., just before flowering (Sample A) and the other at maturity of the crop i.e., bearing flowers, fully matured seed capsules (Sample B) was studied. Mobile phase was methanol: water (65 : 35), flow rate 1.5 mL/min. Retention time of pure Andrographolide was 2.871 minutes. The average andrographolide content varied from 0.81% to 1.86%.

Key words: Andrographolide, *Andrographis paniculata*, Kalmegh, HPLC, King of Bitters.

INTRODUCTION

Andrographis paniculata (Family Acanthaceae) is available abundantly in south eastern Asia i.e., India, Sri Lanka, Pakistan and Indonesia. It is found in wild through out of plains of India especially in Tamilnadu, Karnataka, Maharashtra, Orissa, Uttar Pradesh and Uttarakhand. It is generally found in all kinds of vegetative lands i.e., in pine, evergreen, deciduous areas, along roads and villages. It is easily cultivated from seeds on all types of soil¹. The herb, *Andrographis paniculata* is the main source of the bitter principle Andrographolide. The extremely bitter and characteristic taste of *A. paniculata* gives it the term “king of bitters”. The aqueous extract of *Andrographis paniculata* showed antimicrobial activity which may be due to combined effect of the isolated arabinogalactan proteins and andrographolides². *Andrographis paniculata* or Kalmegh is one of the most widely used plants in ayurvedic formulations³. *Andrographis paniculata* was recommended in Charaka Samhita dating to 175 BC for treatment of jaundice along with other plants in multi plant preparations⁴. It has also been used traditionally for sluggish liver as antidote in case of colic dysentery and dyspepsia⁵. It has been employed with benefit in case of general debility in convalescence after fever, disorders of liver and advanced stages of dysentery⁶. The juice of fresh leaves is a domestic remedy in the treatment of colic pain, loss of appetite, irregular stools and diarrhea⁷. The bitter principle andrographolide was isolated in pure form by Gorter⁸. Andrographolide is also attributed with some other activities like liver protection⁹, anticancer activity¹⁰, anti-diabetic activity¹¹ and anti-malarial activity¹².

Andrographis paniculata commonly known as Kalmegh is one of the widely used medicinal herb. Whole plant has a wide range of pharmacological activity¹³. Andrographolide is used as standard to analyze Kalmegh. There is a wide variation in the amount and type of chemical constituents in samples of different species, in samples that differ in method and time of collection¹⁴. Thus the potency, quality and purity of drugs have to be evaluated. Active constituents can be analyzed by several methods such as colorimetric, titrimetric, gravimetric, spectrometric and chromatographic techniques. The gravimetric method described in the Indian pharmacopoeia was found to give high values¹⁵. This is due to some yellow coloring substance other than andrographolide which is also soluble in ethyl acetate. The spectrophotometric method proposed by Maiti et al.¹⁶ suffers from the disadvantage that the red color formed with the addition of alcoholic potassium hydroxide to the solution of andrographolide is unstable and fades away quickly. Subbarao has suggested a chemical method involving a lactone titration but the method has been reported to be not suitable for detecting minute quantities. High performance liquid chromatographic methods were reported for estimation of Andrographolide in *Andrographis paniculata*¹⁷⁻²¹ and in rabbit serum²².

In the present study, accurate, simple, specific and reproducible HPLC method has been developed and validated²³ for the determination of andrographolide in *A. paniculata* herb, at two different stages of life cycle.

EXPERIMENTAL

Plant material

Two plants of *Andrographis paniculata* were obtained as a gift from Forest Research Institute (FRI), Dehradun, Uttarakhand, India in the month of June 2009. After one complete life cycle of the plants, fully matured seeds were collected and sown in nursery and the crop was grown as per cultivation practices developed by Pandey and Mandal²⁴. Whole plant material at two different stages, one after 110 days of plantation i.e., just before flowering (Sample A) and the other at maturity of the crop i.e., bearing flowers, fully matured seed capsules (Sample B) was collected. Then plant material was dried under shade, powdered using blender and stored in air tight bottles.

Materials and methods

All solvents were of HPLC Grade and all reagents were of analytical reagent grade. Andrographolide standard 99.8% (Product code A009, Lot No. T11B001) was procured from Natural Remedies, Bangalore. Water used for chromatographic separation was purified using Millipore mill purified system.

Physico-chemical properties of the drug

Melting point of the drug: The melting point of the drug, determined by melting point apparatus, was found to be between 228-229°C, which matches with the value (231°C) of pure andrographolide.

Determination of moisture content by Karl-Fischer method: The moisture content of the isolated drug was found to be 0.112% by Karl-Fischer method.

Determination of λ max: The drug was scanned in Shimadzu, UV-1700 Pharmaspec UV-vis-Spectrophotometer (with methanol as blank). The λ max was found to be 223 nm which matches with standard value of andrographolide.

Sample Preparation for HPLC

Weighed about 2.5 gm of *Andrographis paniculata* plant powder of sample A and B separately into a round bottom flask added about 50 mL of methanol and refluxed on a water bath for 30 min. Cool and

filter, Reflux the residue further with methanol till the last extract turns colorless, cool and filter. The same operation was repeated twice with methanol (2×30 mL) for both sample. Combined all the methanolic extracts of sample A and made up to 50 mL with methanol and filtered through 0.45 μ m membrane filter. Same procedure was for sample B.

Preparation of standard solution

Standard working solution was prepared by dissolving 10mg of Andrographolide [99.8%] with 100mL of methanol to give a concentration of 100 μ g/mL.

HPLC instrumentation

Shimadzu Model-LC2010 CHT, Serial No. C-21254505638 HPLC instrument was used for the chromatographic separation using C 18 column (250 nm x 4.6 nm). Isocratic elution was carried out with methanol at a flow rate 1.5 mL/min. The detection was performed with a D2 lamp at 223 nm wavelength. L C Solution software was used for integration and calibration. Evaluation was via peak areas with linear regression. Mobile Phase 65 Volumes of methanol and 35 Volumes of water were used.

Estimation of andrographolide

To estimate the content of *Andrographis paniculata* plant in samples A and B, 20 μ L aliquots of sample were injected in to HPLC. The HPLC analysis was continued for 15 min, since the retention time of the andrographolide was 2.871 ± 0.004 min. The content of andrographolide was calculated by linear regression and mean percentages were calculated from six replicate experiments.

RESULTS AND DISCUSSION

Since Andrographolide is freely soluble in methanol, the plant materials were extracted with methanol. Mobile phase for HPLC was methanol: water (65 : 35). First run was of blank to check error from mobile phase Fig. 1.

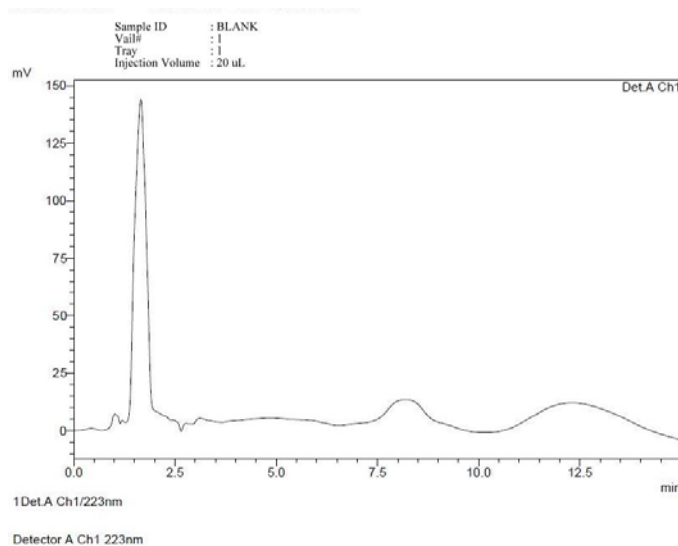


Fig. 1: HPLC Chromatogram of Blank

The standard Andrographolide took 2.871 minutes as retention time Fig. 2. The retention time and peak area were recorded for calculating total Andrographolide content in the sample A (whole plant just before flowering) and Sample B (whole plant after flowering) Fig. 3 and Fig. 4.

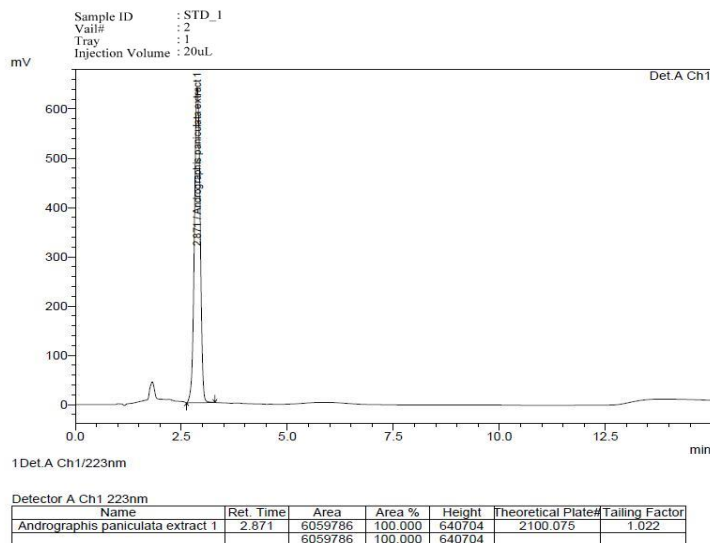
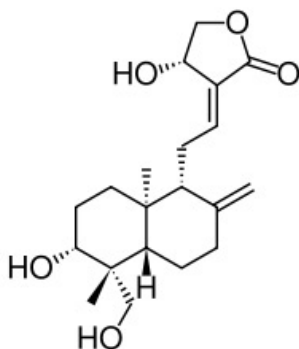


Fig. 2: HPLC Chromatogram of Standard Andrographolide



Mol. Formula: $C_{20}H_{30}O_5$ Formula Weight: 350.454

Structure of Andrographolide

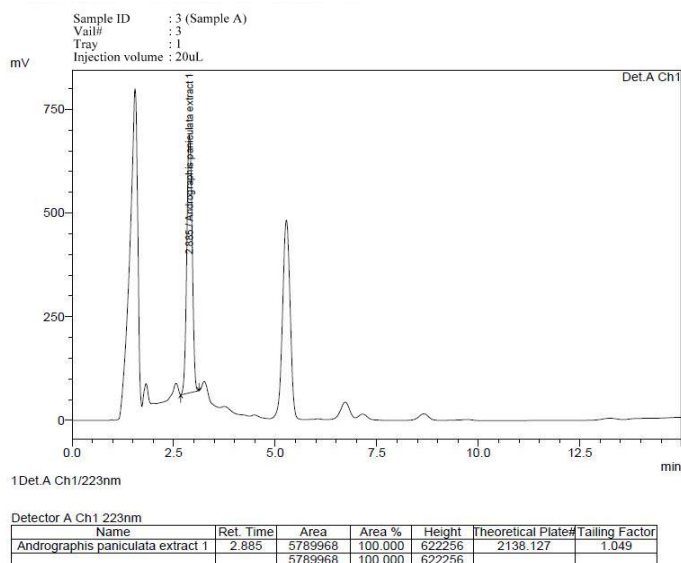


Fig. 3: HPLC Chromatogram of Sample A (just before flowering)

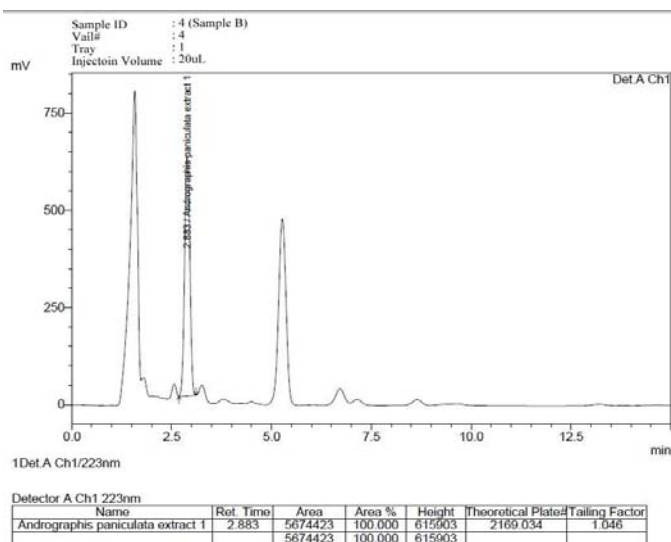


Fig. 4: HPLC Chromatogram of Sample B (after flowering)

The Andrographolide content was varied from 0.81% to 1.86 % in the above two samples of *Andrographis paniculata* Table 1 and Fig. 5.

Table 1: Andrographolide content in Sample A and B

S. No	Sample weight (mg)	% of Andrographolide
1.	Sample A ₁ = 2531.4	1.86%
2.	Sample A ₂ = 2501.0	1.83%
3.	Sample B ₁ = 2512.4	0.81%
4.	Sample B ₂ = 2549.0	0.83%

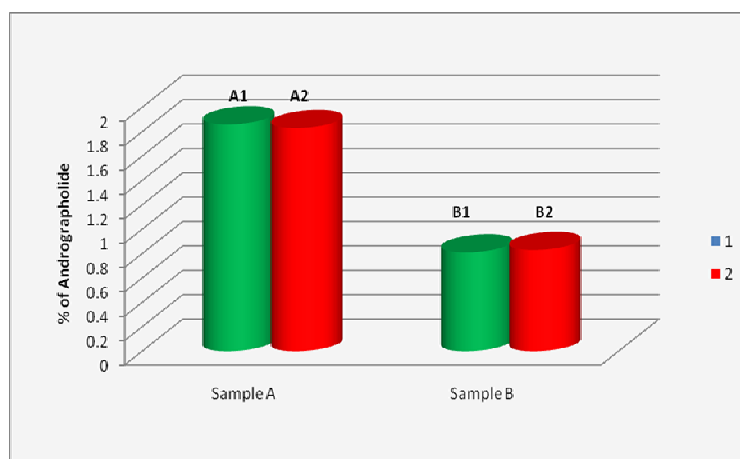


Fig. 5: Graph of Andrographolide content in Sample A and B

The maximum Andrographolide content (1.86 %) was found in the sample A harvested after 110 days of plantation i.e. just before flowering making it ideal harvesting time. The andrographolide content also depends on growing region and season.

CONCLUSION

The developed HPLC method can be utilized for the quantitative determination of Andrographolide in *Andrographis paniculata* herb samples. The method developed is simple, sensitive and statistically validated. Extensive work has been done on this plant, but still it requires more R and D work for drug development.

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