International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 2, 2011

Research Article

QUANTITATIVE HPTLC ANALYSIS OF ANDROGRAPHOLIDE IN *ANDROGRAPHIS PANICULATA* OBTAINED FROM DIFFERENT GEOGRAPHICAL SOURCES (INDIA)

MAMATHA A[1]

K.L.E.S University's College of Pharmacy, Rajajinagar II Block, Bangalore 560002. Karnataka, India Email: mamathasmitha@gmail.com

Received: 16 Oct 2010, Revised and Accepted: 22 Nov 2010

ABSTRACT

A simple, sensitive and accurate HPTLC method has been developed and validated for the quantitative estimation of Andrographolide in Kalmegh. The method employed TLC Aluminium plates precoated with silica gel 60 F254 as stationary phase with mobile phase as Chloroform:Methanol::7:1v/v. Andrographolide showed mean Rf value of 0.41 with λ max at 231nm. The method was validated in terms of linearity (100 – 500ng), precision, and accuracy (100.03% recovery). Limit of detection and limit of quantification were found to be 30ng and 100ng respectively. The validated method was applied for quantification of Andrographolide in methanolic extracts of Kalmegh samples obtained from various geographical sources. The proposed method is simple, sensitive and precise; it can be used for routine analysis.

Keywords: *Andrographis paniculata,* HPTLC, geographical sources.

INTRODUCTION

Andrographis paniculata Nees belonging to family Acanthaceae, commonly known as Kalmegh is one of the widely used medicinal herb. It is an important drug in ancient system of medicine[1,2]. Whole plant has a wide range of pharmacological activity[3]. Andrographolide is used as standard to analyse Kalmegh[4-7].

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 collection[8]. 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. Recent methods are analysis by HPLC and HPTLC.

High Performance Thin Layer Chromatography is one of the modern sophisticated technique that can be used for wide diverse applications. It is a simple and powerful tool for high-resolution chromatography and trace quantitative analysis is made possible. It is most widely used for quick and easy determination of quality, authenticity and purity of the crude drugs and market formulations.

Literature survey reveals that there are very few validated HPTLC methods for the estimation of Andrographolide. Hence an attempt was made to develop and validate HPTLC methods for evaluation of Andrographolide.

The validated method was proposed to be applied for estimation of the marker in extracts prepared from samples of Kalmegh, obtained from various geographical sources to study the variations in samples.

MATERIALS AND METHODS

Andrographolide standard was procured from Natural Remedies, Bangalore. Silica Gel 60 F254 Aluminium plates (Merck) was used as stationary phase. Chloroform: Methanol:: 7:1v/v was used as mobile phase. Methanol was used as solvent. Kalmegh plants were procured from four different geographical sources. Karnataka and Tamil Nadu samples were procured from Natural Remedies, Bangalore. Andhra Pradesh variety was collected from Tirupathi Ayurvedic college and Kerala sample was procured from Ayur Pentacare, Bangalore.

A CAMAG HPTLC system (Switzerland) comprising of CAMAG Linomat 5 applicator, CAMAG TLC Scanner 3, CAMAG winCATS software, version 1.3.3, Hamilton syringe (100µl), CAMAG Reprostar 3, Shimadzu weighing balance, CAMAG TLC Plate heater, CAMAG uv cabinet were used for the study.

Preparation of standard and sample drug solution

Standard: 10 mg of Andrographolide was diluted with 100ml of methanol to give a concentration of 100µg/ml.

Sample: 100g of coarsely powdered samples were subjected to defatting by refluxing for 4 hours with petroleum ether. The material was dried and extracted by refluxing with 250ml methanol for 12 hours. The extract was concentrated over water bath, labelled and stored.

HPTLC method and chromatographic conditions

The chromatographic estimation was performed using the following conditions, stationary phase was precoated silica gel 60 F_{254} aluminium sheets (10 x 10 cm) and the mobile phase used was Chloroform:Methanol::7:1 v/v. The chamber saturation time employed was 10 minutes and the developing distance was 9cm. Scanning wavelength of 231nm with a slit dimension of 5.0 x 0.45mm and scanning speed of 10 mm/s were employed.

Method validation[9-11]

The developed method was validated in terms of linearity, intra day precision, inter day precision and accuracy. The limit of Quantification and limit of detection for Andrographolide were also determined.

Linearity of detector response: 10mg of standard Andrographolide was dissolved in 100ml methanol to give a concentration of 100ng/µl. From this stock solution, 1-5µl was spotted as sharp bands on the precoated TLC plate. The plate was developed in the chamber, previously saturated for 10 minutes with mobile phase. The mobile phase was allowed to travel up to 90mm. The plate was removed from the chamber and dried in hot air. Scanning was done at 231nm in absorbance mode. Data peak area of each band was recorded.

Accuracy of the analysis was evaluated by carrying out a recovery study. For that, a known concentration of standard in three different levels was added to preanalysed sample. And average recovery was calculated.

The intra-day precision was determined by analyzing standard Andrographolide in the concentration of 200ng/spot for 6 times on the morning and after noon of the same day, while inter day precision was determined by analyzing a concentration of 200ng/spot for 6 times on two consecutive days. In both the cases % CV (Co-efficient of variation) were calculated.

Application of validated HPTLC method to quantify Andrographolide in Kalmegh

100mg extract of each sample was dissolved in 10ml methanol. This was filtered through Whatmann no.1 filter paper and stored in an air

tight labeled container. 10µl of each of the sample solutions were spotted along with 2 & 4µl of the standard solution on the chromatoplate. The plate was developed in a previously saturated twin trough chamber and scanned at 231nm. The peak areas of standard and samples were compared and the amount of Andrographolide present in each sample was calculated.

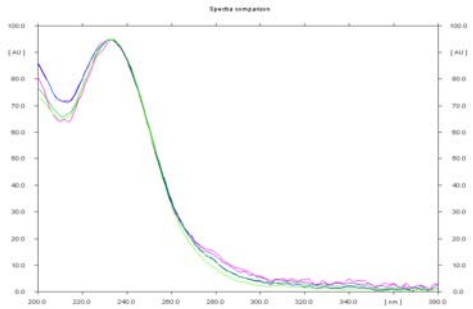

Fig. 1: Spectrum showing λ max at 231nm

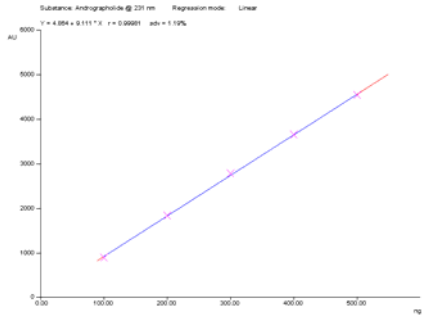

Fig. 2: Linear graph of Andrographolide for Andrographolide at 231nm

Table 1: Analysis of Andrographolide

Parameters	Kalmegh standard - Andrographolide
Stationary Phase	Merck TLC plates silica gel 60 F 254 (10x10 cm)
Software	win CATS –version 1.3.3
Application instrument	CAMAG Linomat 5
Band Length	6mm
Mobile Phase	Chloroform:methanol (7:1)
Development Chamber	Twin Trough Glass Chamber
Development Distance	9 cm
Tank saturation time	10 minutes
Development time	15 minutes
λ max	231nm
Scanning instrument	CAMAG TLC SCANNER 3
Documenting instrument	CAMAG REPROSTAR 3
Derivatising agent	10% sulphuric acid

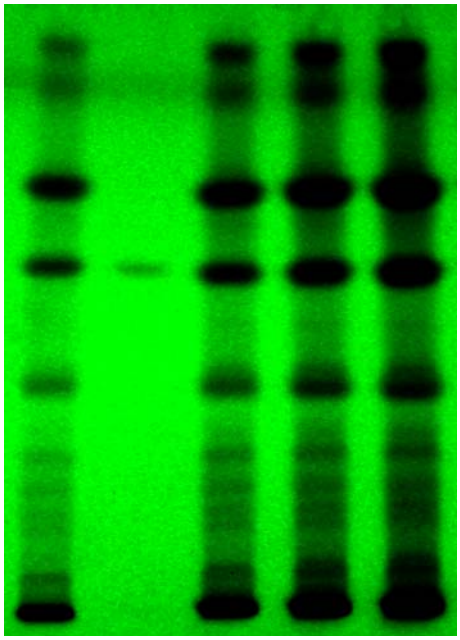

Fig. 3: HPTLC pattern for Kalmegh methanolic extract along with *s*tandard Andrographolide

Table 2: Amount of Andrographolide in Kalmegh extracts by HPTLC

Sample Identity	Methanolic extract	
	100 mg	%
Sample 1 Karnataka	569.04µg	1.19%
Sample 2 Tamil Nadu	446.60µg	0.85%
Sample 3 Andhra Pradesh	430.86µg	0.70%
Sample 4 Kerala	556.56µg	0.99%

Table 3: Results of HPTLC method validation

Sl.No	Parameter	Andrographolide
1.	Limit of Detection	30 ng
2.	Limit of Quantification	100 ng
3.	Linearity	100-500ng
	Correlation coefficient (r)	0.99981
	Standard Deviation	1.19%
	Y value	4.864 + 9.111*X
	Precision (cv)	
4.	n = 6	1.14% -1.58%
	Intra day variation	
	Inter day variation	1.14% -1.25%
5.	Accuracy	100.03%
6.	Specificity	Specific

RESULTS AND DISCUSSION

Literature survey indicated that various methods have been reported for analysis of Andrographolide in Kalmegh. Most of them are either by colorimetry, gravimetry or spectrometry which are time consuming and require multiple steps of extraction[12,13]. The above method may turn out to be simple, fast and non cost effective for the routine analysis purposes such as assay for comparison of samples from various geographical sources.

Since Andrographolide is freely soluble in methanol, the plant materials were extracted with methanol. Combination of Chloroform and Methanol offered optimum migration (mean R_f = 0.41) and resolution of Andrographolide from other components. (Table 1). λ max of Andrographolide was found to be at 231nm. (Fig. 1).

Linearity of Andrographolide was found to be in the range of 100 – 500ng (Fig. 2) with a correlation efficient of 0.99981. The limit of detection and limit of quantification was found to be 30ng and 100ng respectively. These values show that the method is highly selective. (Table 2).

The amount of Andrographolide in Kalmegh samples collected from various geographical sources were spotted, developed (Fig. 3), and calculated by comparing peak area of standard and sample solutions. % of Andrographolide was found to be in the range of 0.70 – 1.19% (Table 3). Kalmegh sample obtained from Karnataka was found to be the best with highest content of Andrographolide.The cv values for intra day variation and inter day variation were found to be in the range of 1.14% -1.58% and 1.14%-1.25%. Lower values of coefficient of variation in the analysis indicate that the method is precise. Average % recovery was found to be 100.03% (Table 2) which indicates that the method is reproducible.

Different validation parameters for the proposed HPTLC method for determination of Andrographolide in Kalmegh have been summarized in Table 3. The proposed HPTLC method was found to be rapid, simple, specific, sensitive, precise and accurate. Thus it can be employed for the routine quality control analysis of Andrographolide in Kalmegh.

REFERENCES

1. Chopra IC, Handa KL, Kapur LD. Chopra's Indigenous drugs of India, Calcutta: Academic Publishers; 1994.
2. Longman O. Indian Medicinal Plants – a compendium of 500 species. Chennai: Orient Longman Pvt. Ltd; 2002.
3. Nandan KJ. Feature Article: Andrographis paniculata: Kalmegh. Phytopharm 2004: 2 –19.
4. Mukherjee KP. Analytical Profiles of selected medicinal Plants Quality Control of herbal drugs, New Delhi: Business Horizon; 2002.
5. Rajpal V. Standardization of Botanicals. New Delhi: Eastern Publishers; 2002.
6. AgroIndia, Medicinal Aromatic and other Plants of commercial use, Mumbai: R.K.Buisness International; 1998.
7. Indian Herbal Pharmacopoeia. A joint publication of Regional Research Institue (Jammu Tawi) and Indian Drug Manufacturer's Association (Mumbai); 1999.
8. Kleipool RJC. Constituents of *Andrographis paniculata* Nees. Nature, 1952 January; 4288: 33-34.
9. ICH Harmonised Tripartite Guidelines. Validation of Analytical procedures: Definition and Terminology/Methodology Q2A/Q2B, Geneva; 1996.
10. United States Pharmacopoeia. The National Formulary 24, Validation of compendial methods. USA: Asian Edition. Mack Publishing; 1998.
11. Ferenczi KF, Vegh Z, Turak AN, Renger B, Zeller M. Validation and Quality Assurance of Planar Chromatographic procedure in Pharmaceutical analysis. JAOAC 2001; 84 Suppl 4: 1265.
12. Talukdar PB, Datta AK. Quantitative Estimation of Andrographolide by TLC. Indian J.Appl. Chem. 1969; 32 Suppl 1: 228-229.
13. Talukdar PB, Banerjee S, Chatterjee PK, Datta AK. Studies on estimation of Andrographolide in *Andrographis paniculata* Nees (Kalmegh). Indian J. Chem.1968; 6: 359-363.