Research Article

DETERMINATION OF ANDROGRAPHOLIDE IN *ANDROGRAPHIS PANICULATA* EXTRACTS WITH AND WITHOUT HUMAN SERUM BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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DOI: 10.7897/2230-8407.041209**ABSTRACT**

Andrographis paniculata (Acanthaceae) commonly known as Kalmegh is used both in Ayurvedic and Unani system of medicines for a number of ailments. Kalmegh contains bitter principles – Andrographolide, 14-deoxy-11, 12- didehydroandrographolide, neoandrographolide, 14-deoxy-11, oxyandrographolide, 14-deoxy-andrographolide, and flavonoids. HPTLC analysis indicates that the maximum amount of andrographolide was present in extracts of *Andrographis paniculata* prepared by refluxation. Amount of Andrographolide in all extracts with and without serum along with the % recovery is compared and the variation was found to be < 15 %. The HPTLC method developed for estimation of andrographolide is simple, sensitive and accurate.

Keywords: *Andrographis paniculata*, Andrographolide, HPTLC, Heat reflux extraction, Soxhlet extraction.

INTRODUCTION

Andrographis paniculata (Fam - Acanthaceae) also known as “Kalmegh” a well known medicinal plant of Ayurveda has been used for centuries in Asia¹. There are three more species of *Andrographis paniculata* available in India. They are *Andrographis lineate*, *Andrographis viscosula* and *Andrographis alata*. *Andrographis lineate* is an erect herb found widely in Deccan, Carnatic, Yercaud and Nilgiris of South India². *Andrographis viscosula* is found in the hills of Tinnevely, South India³. *Andrographis alata* is another species also found in South India⁴. The extracts and constituents exhibit a wide spectrum of biological activities including anti-inflammatory, immune stimulatory, hepatoprotective, anticancer⁵ etc. Andrographolide, the major diterpenoid is used as marker compound. Long known in traditional Asian medicine as an immune system booster, *Andrographis* has demonstrated significant activity in fighting common cold, flu and upper respiratory infection⁶. The antimicrobial activity (antibacterial and antifungal activity) of aqueous extract, andrographolides and arabinogalactan proteins from *Andrographis paniculata* was reported and the activity was found to be significant in comparison to some known antibiotics⁷. HPTLC method was reported to be the most suitable method for the estimation of active constituents of extracts, plant species (raw material) or poly herbal formulations⁸. Pharmacokinetic study of herbal drugs involves analysis of marker compound in serum. Literature survey reveals that not many methods are available for analysis of marker compound in serum. Several methods such as HPTLC, HPLC, liquid chromatography-electrospray ionization tandem mass spectrometry etc have been reported for the determination of various drugs in Serum. In recent years HPTLC has gained importance since it allows reliable quantification of analytes at micro and even nanogram levels⁹. Lamotrigine, an antiepileptic drug in human serum has been estimated by HPTLC method¹⁰. HPTLC was also developed to study the Dog serum concentration of Pentoxifylline¹¹. HPTLC determination of 5-methoxypsoralen in serum necessary for the therapeutic survey of patients treated with Puvotherapy was reported.

This assay range was adequate for analyzing human serum, as it corresponded to Psoralen concentrations measured in serum from patients treated with Psoralen and UV A against Psoriasis and Vitiligo. The limit of detection was found to be 15 ng per mL¹².

MATERIALS AND METHODS**Extraction Procedure**

Drug was dried at 60°C, powdered and sieved through mesh size #44, 22 and 12 to obtain 3 samples A1, A2 and A3 respectively. All the samples were defatted using petroleum ether for 8 h.

Refluxation

It was performed in Round bottomed flask using water and methanol separately as solvents for 12 h by taking 10 g of defatted powdered material of 3 samples.

Soxhlation

It was performed in Soxhlet apparatus using methanol as solvent for 12 h by taking 10 g of defatted powdered material of 3 samples.

HPTLC Analysis**Development of HPTLC method for estimation of Andrographolide****Instruments**

CAMAG Linomat 5 sample applicator, CAMAG TLC Scanner 3, CAMAG Reprostar 3, CAMAG TLC plate heater, Twin trough chamber, win CATS software –version 1.3.3.

Stationary Phase

Merck TLC plates silica gel 60F254

Mobile phase

Chloroform: Methanol in the ratio of 7:1v/v

Standard preparation

1 mg/ml standard Andrographolide in methanol

Application

1 μ l – 5 μ l of the standard solution was applied as bands on the plate.

Development and scanning

The spotted plates were developed up to 90 mm and scanned at 200 nm to 500 nm. λ max was determined, and then the chromatogram was scanned at λ max.

Linearity

1 mg/ml of standard Andrographolide in methanol was prepared. 1 μ l – 5 μ l were spotted. The chromatogram was developed and scanned at 231 nm.

Estimation of Andrographolide in extracts by HPTLC method**Sample preparation**

10 mg/ml solution of extracts in methanol was prepared

Standard preparation

1 mg/ml solution of standard andrographolide in methanol

Application

1 μ l, 3 μ l and 5 μ l of the standard solution and 5 μ l of all sample solutions

Development of HPTLC method for estimation of Andrographolide in Human serum

Different trials were carried out to establish a method for the estimation of Andrographolide in presence of Human serum using the method validated in our study out of which mixing of sample in human serum and extracting with acetonitrile was found to be simple and sensitive since the method gave good separation. Sample, standard preparation and application is similar as given in 2. [B]. The λ max for Andrographolide was found to be 231 nm.

Estimation of Andrographolide in extracts by HPTLC method in presence of human serum

Methanol and Water extracts were mixed with human serum, extracted using acetonitrile. The resultant extracts were spotted with standard Andrographolide. The chromatogram was scanned at 231 nm.

Table 1: Percentage yield in Kalmegh sample by conventional method

Sample Identity	Water Reflux	Methanol Reflux	Soxhlet
A1	19.060	19.210	18.350
A2	19.055	19.063	18.162
A3	19.004	18.396	18.114

A1: Aerial powder passed through mesh size # 44, A2: Aerial powder passed through mesh size # 22 and A3: Aerial powder passed through mesh size # 12

Table 2: Area and amount of Andrographolide by Refluxation and Soxhlation Method

S. No.	Sample identity	Area	Amt. μ g	Amt. in extract	% Andrographolide
1	WR-A1	9562.03	4.298	8.59	1.6335
2	WR-A2	6462.9	3.357	6.714	1.279
3	WR-A3	3658.13	2.090	4.18	0.796
4	MR-A1	4003.8	4.304	8.608	1.654
5	MR-A2	5385.10	3.291	6.582	1.254
6	MR-A3	7144.5	1.421	2.842	0.523
7	MS-A1	2009.042	2.55	5.1	0.936
8	MS-A2	2761.78	0.351	0.702	0.127
9	MS-A3	6638.12	1.154	2.308	0.416

Volume Applied: 5 μ L

Table 3: Amount and percentage of Andrographolide with and without Human serum in refluxation and soxhletion

S. No.	Extracts	Amt. μ g	% Andrographolide without human serum	Amt. μ g	% Andrographolide with human serum	% Recovery
1	WR-A1	4.298	1.6335	3.180	1.2118	74.1842
2	WR-A2	3.357	1.279	3.017	1.146	89.6012
3	WR-A3	2.090	0.796	1.981	0.7281	91.4698
4	MR-A1	4.304	1.654	3.233	1.2421	75.0967
5	MR-A2	3.291	1.254	2.531	0.9724	77.5438
6	MR-A3	1.421	0.523	1.337	0.4919	94.0535
7	MS-A1	2.55	0.936	1.831	0.671	71.6880
8	MS-A2	0.351	0.127	0.203	0.0737	58.0314
9	MS-A3	1.154	0.416	0.735	0.2662	63.9903

Vol. applied: 5 μ l

Figure 1: Percentage yield by conventional extraction methods
A] Development of HPTLC method for estimation of Andrographolide

Figure 2: Spectrum showing λ max at 231 nm For Andrographolide

Figure 3: Linear graph for Andrographolide at 231 nm

Figure 4: Yield of methanol extracts obtained from Refluxation and Soxhlation

Figure 5: Percentage Extraction of Andrographolide, w/w obtained From Refluxation and Soxhlation
C] Development of HPTLC method for estimation of Andrographolide in Human serum

Figure 6: Spectrum showing λ max at 231 nm For Andrographolide

Figure 7: Linear graph for Andrographolide at 231 nm

RESULTS AND DISCUSSION

Comparison of the results indicated that Refluxaion with methanol yields highest extractive value. However the sieve size did not show any significant influence on the extractive value. The highest yield with methanol refluxation was 19.21 % and lowest yield with soxhletion was 18.114 %.

HPTLC Analysis

HPTLC method was found to be significant and sensitive in the linearity range of 1 µg - 5 µg, regression value of 0.99995. The linearity graph is in the useful concentration range for quantification of andrographolide in *Andrographis paniculata*. Methanol was found to be a better solvent compared to water and refluxation was found to be better method than soxhlation for extraction of Andrographolide. The amount of andrographolide with serum of andrographolide was found to be 58.0314 % to 94.0535 % of andrographolide without human serum in extraction methods. The HPTLC method developed for the estimation of Andrographolide was proved to be simple, sensitive and accurate.

CONCLUSION

Reflux method of extraction was found to be good in terms of yield and amount. The proposed HPTLC method can be successfully used for estimating Andrographolide. Amount of Andrographolide in all extracts with and without serum along with % recovery was compared and the variation was found to be < 15 %. Hence it can be concluded that the method developed was simple and useful for analysis of Andrographolide in human serum.

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