HPTLC Analysis of Hepatoprotective Diterpenoid Andrographolide from *Andrographis Paniculata* Nees (*Kalmegh*)

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A rapid, accurate and simple high performance thin layer chromatography method for quantitative estimation of andrographolide in *Andrographis paniculata* is described here. The assay combines separation of andrographolide

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on silica gel 60 F_{254} HPTLC plates followed by scanning of the spots at 232 nm using a Camag Scanner 3. Thirty germplasm collections of *Andrographis paniculata* were evaluated for andrographolide content by this method. Andrographolide content ranged from 1.14% to 2.60%.

Andrographis paniculata Nees (Acanthaceae) popularly known as "*Kalmegh*" is a well known drug in the Indian system of medicine and is widely cultivated in India. It is used as a wonder drug in traditional Sidha and Ayurvedic system of medicine as well as in tribal medicine in India for multiple clinical applications[1]. It is a component of over 50% of the multi-ingredient herbal formulations available in India for the treatment of liver ailments[2]. The plant has been reported to possess antipyretic, antihepatotoxic, analgesic, antifertility, antibacterial, antiinflammatory and immuno suppressive properties due to its bitter content[3-6]. Andrographolide ($C_{20}H_{30}O_5$; MW 350.44), a bicyclic diterpene lactone is the main active bitter principle of *kalmegh*. In addition it possesses neoandrographolide and deoxy andrographolide.

During crop improvement and drug analysis, a sensitive and accurate analytical method is required for the quantitation of important diterpenoids, which are present in the plant. Although few methods such as gravimetric[7,8], colorimetric[9], spectrophotometric[10] methods have been reported for the quantitative estimation of andrographolides, many of these procedures are time consuming, not very precise and require multiple step extraction and purification.

Sexena *et al.*[11] have described a HPTLC method using benzene as one of the mobile phase solvents. Benzene being carcinogenic in nature is not safe for routine analysis. Further their method requires post chromatographic derivatisation for detection and quantification whereas our method does not require any post chromatographic derivatisation and hence is less time consuming. The present HPTLC procedure offers a sensitive, accurate and reliable method for routine detection and quantification of andrographolide present in *Andrographis paniculata* extract. Quantitative variation in andrographolide content in thirty germplasm collections of *Andrographis paniculata* existing at NBPGR, New Delhi was studied using this method.

Thirty accessions of *Andrographis paniculata* collected from various agro-climatic regions were grown under identical conditions at Issapur farm of NBPGR, New Delhi. They were harvested at 105 days of growth. The leaves were separated and dried and kept in a polythene pack for chemical analysis. Dry leaf powder (1g) was extracted with methanol (3×20 ml for 6 h each time) in a Soxhlet apparatus. Extracts were concentrated under vacuum, dissolved in methanol, filtered, re-concentrated and made up to 1 ml in methanol for HPTLC analysis. Samples together with standards (andrographolide 1 mg/ml) prepared in methanol, were applied to the silica gel HPTLC plate (60 F_{254}, E. Merck, Germany, 20×10 cm) as 6 mm wide bands with an automatic TLC applicator Linomat V with N_2 flow (Camag, Switzerland), 10 mm from the bottom.

The HPTLC plates were developed using a CAMAG twin trough glass tank. A solvent combination of chloroform: methanol (7:1) with presaturation for 15 min was found to give best TLC resolution. Each plate was developed to a height of 8 cm. The HPTLC runs were made in the laboratory conditions of 25±5° and 50% relative humidity. After development the plates were withdrawn and dried and spots were visualized in UV light (UV cabinet, Camag, Switzerland). Andrographolide content was quantified using Camag TLC scanner 3 equipped with Wincat software under the following conditions: slit width 5×0.45 mm, wavelength 232 nm UV (deuterium lamp) absorption-reflection detection mode. Different amounts (0.2-1 µg) of stock solution of standard andrographolide were applied in duplicates on HPTLC plate. A calibration curve was constructed by plotting concentration versus spot area of the compound. A known amount of plant extract was spotted on TLC plate and concentration of andrographolide in *Andrographis paniculata* leaf extract was determined using above calibration curve.

Different compositions of the mobile phase were tested and a good resolution was achieved by using chloroform:methanol (7:1) as mobile phase. Andrographolide showed Rf value of 0.35 in plant extract, which was verified by comparing with Rf of the standard samples. Other lactones such as neoandrographolide showed Rf of 0.15 and andrographoside 0.03. Thus they do not interfere

TABLE 1: PROPERTIES OF THE STANDARD CURVE FOR ANDROGRAPHOLIDE

AMOUNT OF ANDROGRAPHOLIDE (µg)	PEAK AREA (n=3) (AU)	RSD (%)
0.2	980.34	0.75
0.4	1802.79	1.41
0.6	2704.43	1.02
0.8	3531.99	0.89
1.0	4337.36	0.91

Correlation coefficient r = 0.99983, $y = a_0 + a_1x$, intercept, a_0=138.41; slope, a_1=4221.61; sdv=1.07%

with the quantification of andrographolide. A spectrum of andrographolide exhibited maxima at 232 nm UV. Comparing the spectra of standard and sample tracks did peak purity test. The calibration graph of andrographolide was linear in the range of 0.2 to 1 µg (Table 1). The calibration plots were $y= a_0+a_1x$ type, where y is the response and x is the amount of andrographolide, a_0 is the intercept of the plot on the y-axis, a_1 is its slope. The regression equation obtained was Y= 138.411+4221.617×X, r=0.99983, sdv=1.07%. For the examination of recovery of andrographolide, known amount of stock solution of pure andrographolide was added to *Andrographis paniculata* leaves extract and quantitative analysis were repeated three times. The average recovery of andrographolide was 96%.

Thirty accessions of *Andrographis paniculata* were analyzed by this method. Results showed mean andrographolide content of 1.56% in dry leaves whereas andrographolide content ranged from 1.14% to 2.60% amongst these collections. Sabu *et al.*[12] reported andrographolide content 0.73% to 1.47% in leaves of 12 accessions collected from southern regions of India and 3 accessions collected from other tropical Asian countries. In our studies we have identified promising accessions containing andrographolide content above 2% were IC 342134 (2.60%), IC 471913 (2.17%) IC 111287 (2.11%), and IC 342138 (2.05%). Hence, these can be cultivated to obtain drug of uniform potency and to increase *Andrographis* supply for meeting requirements of drug industry.

This HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Simultaneously a large number of samples along with the standard can be analyzed in one TLC plate and solvent requirement is also very negligible, thus making it inexpensive compared to HPLC. In addition it requires very small amount of sample and can detect active principle concentration in nanograms level. Thus this method can be conveniently adopted for routine quality control analysis.

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