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ESTIMATION OF ANDROGRAPHOLIDE IN HERBAL POWDER AND POLYHERBAL ASAVA BY HPTLC

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ABSTRAT

Herbal powder and polyherbal formulation containing Andrographis peniculata were standardized and validated by high performance thin layer chromatographic method. Andrographolide in sample was identified and amount was estimated densitometrically. This method involves separation of compounds by TLC on pre-coated silica gel 60F 254 plates with the solvent system of benzene: ethyl acetate (5:5) and scanned using densitometric scanner in UV reflectance photomode at 220 nm. The linearity was observed in the range of 360ng-660 ng /spot. The andrographolide content of 237.2 µg/ 100 mg in powder and 41.80 mg/ 5ml. was observed in the test sample. The average percentage recovery value of 97.68 was obtained. The proposed method being precise and sensitive can be used for the detection, monitoring and quantification of andrographolide in Andrographis peniculata.

KEYWORDS

Andrographis peniculata, Andrographolide, HPTLC

INTRODUCTION

Standardization of ayurvedic drug and plant materials is the need of the day. Several pharmacopoeias containing monographs on plant material describes only the physico-chemical parameter. Andrographis peniculata Nees (Acanthaceae), commenly known as kalmegh, is widely used in the traditional system of Indian medicine in the treatment of hepatitis. The drug is mainly for its diterpenoid andrographolide and related compound.[1] The plant is reported to possess protective activity against various liver disorders.[2] Andrographolide and related compound were investigated for their pharmacological properties and all shows varying degrees of anti pyretic, anti-malarial, anthelmintic, stomachic, antityphoid, antibiotic activity.[3]Among the complex mixture of biologically active compound in the plant, andrographolide can be used as an analytical marker compound to determine the quality of plant material of different sources.[4]

During crop improvement and drug analysis, a sensitive and accurate analytical method is

required for the quantitation of important compound like andrographolide, which is present in the plant. Although few methods such as gravimetric, colorimetric, spectrophotometric methods have been reported for the quantitative estimation of andrographolide.[5,6]Many of these methods are time consuming , not very precise and required multiple step extraction and purification.[7] The present HPTLC method offers a sensitive, accurate and reliable method for routine detection and quantification of andrographolide present in andrographis peniculata.[8]

MATERIALS AND METHOD

Standard solution

The andrographolide was isolated by using following procedure. An accurately weighed quantity of of std. andrographolide (1 mg) was dissolved in methanol (5-7 ml) and sonicated for 5 min. The volume was made up with methanol (10 ml) to give conc. (100 ng/µl). From this solution, 3 ml was further diluted to 10 ml to get final conc. (30 ng/ul).

Sample solution

The kalmegh powder (0.5 g) was weighed accurately and dissolved in methanol (7 ml). The solution was sonicated for 5 min and filtered though Whatman filter paper (No.01). The volume was made up to with methanol (10 ml). The std. solution (30 ng/µl) and the sample solution (50 µg/µl) were applied as 10 µl and 5 µl on the TLC plate, respectively. The plate was then developed with the mobile phase in the twin trough chamber and scanned.[9] The sample solution (5 ml) was accurately pipette out and diluted with water (10 ml). The diluted sample was extracted with diethyl ether (3 X 20 ml).The separated ether layer was combined and filter though the bed of sodium sulphate. The filtrate then evaporated and reconstitute with methanol (5 ml).[10] From this, 20 µl (565 µg/µl) of sample solution and 5 µl (30 ng/µl) of std. solution were applied on the TLC plate, respectively. The sample solution (5 ml) was accurately pipette out and diluted with water (10 ml). The diluted sample was extracted with diethyl ether (3 X 20 ml).The separated ether layer was combined and filter though the bed of sodium sulphate. The filtrate then evaporated and reconstitute with methanol (5 ml). From this, 20 µl (565 µg/µl) of sample solution and 5 µl (30 ng/µl) of std. solution were applied on the TLC plate, respectively. The chromatogram was developed and evaluated in the scanner.

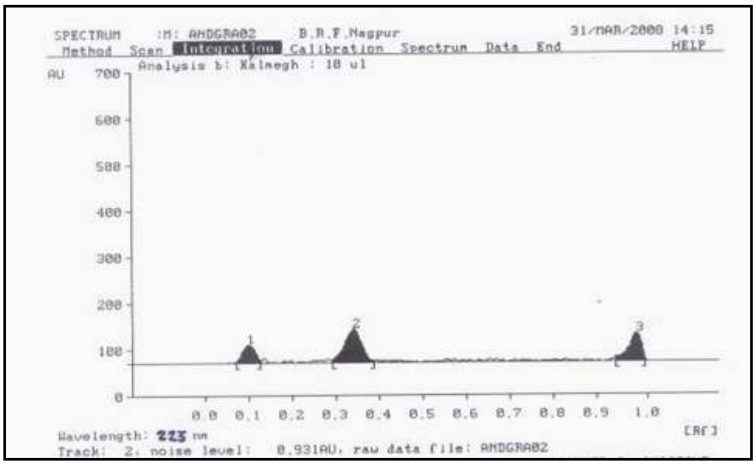
RESULT AND DISCUSSION

Different compositions of the mobile phase were tested and a good resolution was achieved by using benzene: ethyl acetate (5:5) as mobile phase. Andrographolide showed Rf value of 0.10 in plant extract, which was verified by comparing with Rf of the standard samples [Figure - 1]. Thus they do not interfere with the quantification of andrographolide. A spectrum of andrographolide exhibited maxima at 222 nm UV. Comparing the spectra of standard and sample tracks did peak purity test [Figure - 2]. The calibration graph of andrographolide was linear in the range of 360 to 600 ng. The calibration plots were $y= a_0 +a_1$ x 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=1.8%. For the examination of recovery of andrographolide, known amount of stock solution of pure andrographolide was added to preparation and quantitative analysis were repeated three times. The average recovery of andrographolide was 97.68% [Table - 1].Results showed mean andrographolide content of 237.2 (µg) per 100 mg in dry powder whereas andrographolide content ranged from 41.80 (µg /5 ml) in the preparation.

This HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Simultaneously a large number of samples

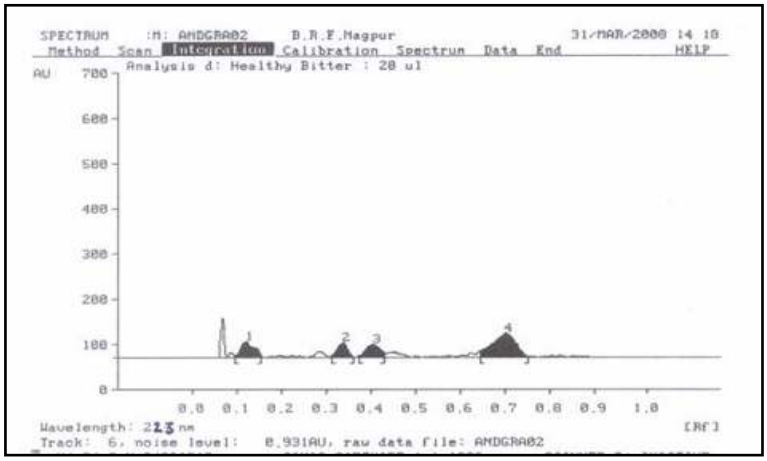
along with the standard can be analyzed in one TLC plate and solvent requirement is also very negligible, thus making it inexpensive compared to HPLC. In addition it requires very small amount of sample and can detect active principle concentration in nanograms level. Thus this method can be conveniently adopted for routine quality control analysis.

Figure 1
Chromatogram of kalmegh powder containing andrographolide



µl = microliter , Rf = Retention factor

Figure 2
Chromatogram of asava containing andrographolide



µl = microliter , Rf = Retention factor

Table 1
Statistical data for recovery study of andrographolide

Track No.	Sample applied (µg) [A]	Andrographolide present in[A] (ng) [B]	Std added to [A] (ng) [C]	Total andrographolide applied (ng) [D]	Total andrographolide recovered (ng) [E]	% Recovery E/D x 100 [F]
1	100	379.55	100	479.55	255.11	94.70
2	100	379.55	150	529.55	512.87	96.87
3	100	379.55	----	379.55	379.55	100
4	----	----	600	600	----	----
					Mean	97.68
					S.D.	1.801
					R.S.D.	1.080

(n= 3) , µg = microgram, ng = nanogram

CONCLUSION

It can be concluded that the proposed HPTLC method is rapid, simple and accurate for quantitative monitoring of andrographolide from *andrographis peniculata*.

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