TABLE 2: STATISTICAL ANALYSIS OF ESTIMATION OF THE CHLOROQUINE

Sample	Labeled amount	Standard Deviation*	Coefficient of variation*	t_{cal}*	t_{tab}*
Tablet 1	500	0.4082	0.0816	1.2733	
Tablet 2	250	0.4921	0.1972	1.5480	2.132
Tablet 3	500	0.3399	0.0679	1.3250	
Tablet 4	250	1.080	0.4328	0.8013	

Average of three determinations based on label claim, t_{tab} = Tabulated value or Theoretical value.

and coefficient of variation were satisfactorily low, indicates the reproducibility of the method. Results obtained with analysis of commercial formulations have also been subjected to statistical evaluation using student's 't' test to further evaluate the proposed method. The 't' values were found to be less than 't' theoretical with 4 degrees of freedom at 5% level of significance indicating that there is no significant difference between proposed method and the reference method. The proposed spectrophotometric method was found to be simple, precise, accurate and less time consuming. Therefore, it may be preferably used for routine analysis of chloroquine phosphate in pharmaceutical formulations.

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HPTLC Standardization of *Gymnema Sylvestre* R. Br. Using Gymnestrogenin as Reference

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A simple and reproducible high performance thin layer chromatography method for the determination of gymnestrogenin in *Gymnema sylvestre* was developed and is described. This method involves separation of compounds by TLC on pre-coated silica gel 60F 254 plates with a solvent system of chloroform: methanol (9:1) and scanned using densitometric scanner in UV reflectance photomode at 293 nm. The linearity was observed in the range of 4 to 10 μg. The gymnestrogenin content of 1.11 % w/w was observed in test sample. The average percentage

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recovery value of 99.1±0.27 was obtained. The proposed method being precise and sensitive can be used for detection, monitoring and quantification of gymnestrogenin in Gymnema sylvestre.

Standardization of ayurvedic drugs and plant materials is the need of the day. Several Pharmacopoeias containing monographs on plant materials describe only the physico-chemical parameters. Hence, modern methods describing the identification and quantification of active compounds in the plant material may be useful for proper standardization of herbs and their formulation. *Gymnema sylvestre* R. Br. (Asclepiadaceae) is one such plant which is widely used in indigenous system of medicine[1]. The leaves are used as stomachic, diuretic and a remedy for cough and eye pain. The crude saponin fraction of this plant named gymnemic acid is believed to be effective for diabetics. Many antisweet saponins in gymnemic acid were found, the gymnestrogenin was among the one[2,3]. Among the complex mixture of biologically active compounds in the plant, gymnestrogenin can be used as analytical markers to determine the quality of plant material of different sources. Extensive literature survey reveals that the plant is well researched for its therapeutic action but lacks in the use of high performance thin layer chromatographic (HPTLC) methods for the standardization. A suitable sensitive and reliable quantitative HPTLC method has been developed for the first time for quality control determination of gymnestrogenin from *Gymnema sylvestre*. Presently this technique is extensively being used for quantification of plant extracts because of its simplicity, accuracy, cost effectiveness and rapidity.

The leaves of *Gymnema sylvestre* were collected from our campus and identified in the Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi where a voucher specimen is maintained.

Around 5 g of air dried sample was ground to pass through 20 mesh S.S. sieve and 1 g from it was accurately weighed and cold macerated with 50 ml mixture of ethanol (95%) and water (1:1) for about 72 h. The resulting solutions were filtered, concentrated under vacuum; redissolved in methanol (25 ml) with warming on a water bath and the final volumes were made up to 50 ml. This solution was used for further HPTLC analysis as per the procedure mentioned below.

A Camag HPTLC system equipped with a sample applicator Linomat IV, twin rough plate development chamber, TLC scanner III and integration software CATS 4.0 was used. An aluminium plate (10x10 cm) precoated with silica gel 60F 254 (E. Merck) was used an adsorbent. Chloroform and methanol in the ratio of 9:1 was used as a mobile phase. The solvent was allowed to run up to 80 mm and the chromatograms were scanned at 293 nm. A 1 mg/ml solution of gymnestrogenin reference standard was prepared in methanol as a stock solution.

The test sample was shaken well; 25 µl of it were applied on TLC plate along with 4, 5, 6, 7, 8, 9 and 10 µl of standard gymnestrogenin. Likewise three such plates were prepared. The plates were developed up to 80 mm under chamber saturation condition. After air-drying the solvent, the plates were scanned at 293 nm in UV reflectance mode. The amount of gymnestrogenin present was determined using the calibration curve plotted between concentration and area of standard. The regression equation was found to be, Y=108.17x-140.39 with a correlation coefficient of 0.9995.

The method was validated and recovery studies were performed using the following Procedure[4]. A varying known amount of gymnestrogenin standard was added to about l g of fine powdered test sample in which the contents of gymnestrogenin had been estimated previously by proposed method. The samples were cold macerated and analyzed separately as per the procedure mentioned above. The contents of gymnestrogenin were quantified and percentage recoveries were calculated (Table 1).

Sample preparation and development of suitable mobile phase of solvent system are two important steps in developing the analytical procedure which becomes more significant for herbal drugs because of their complexity of chemical compounds and their affinity towards various solvents. By trying different composition of mobile phase the desired resolution of gymnestrogenin with symmetrical and reproducible peaks were achieved by using chloroform: methanol (9:1). Using the proposed HPTLC method the R_f of gymnestrogenin was about 0.37. The content of gymnestrogenin was found to be 1.11% w/w in test sample. The chromatograms of standard and test sample are shown in figure 1 and 2, respectively.

The calibration curve was linear in the range of 4 to 10 µg for gymnestrogenin. Further a correlation coefficient 0.9995 indicates good linearity between concentration and area. The method allows reliable quantification of gymnestrogenin and provides good resolution from other constituents of *Gymnema sylvestre*. To ascertain the purity

TABLE 1: RECOVERY OF GYMNESTROGENIN FROM *GYMNEMA SYLVESTRE*

Sample	Quantity of sample (mg)	Amount found in A (mg)	Amount added to A (mg)	Total amount taken B+C(mg)	Total gymnestrogenin found (mg)	% Recovery mean[a]±S.D
	A	B	C	D	E	
G. sylvestre	1000	11.1	1	12.1	12.0	99.5±0.22
G.sylvestre	1000	11.1	2	13.1	12.9	99.0±0.27
G. sylvestre	1000	11.1	3	14.1	13.9	98.9±0.24

[a]n=3, Average percentage recovery is 99.11±0.27.

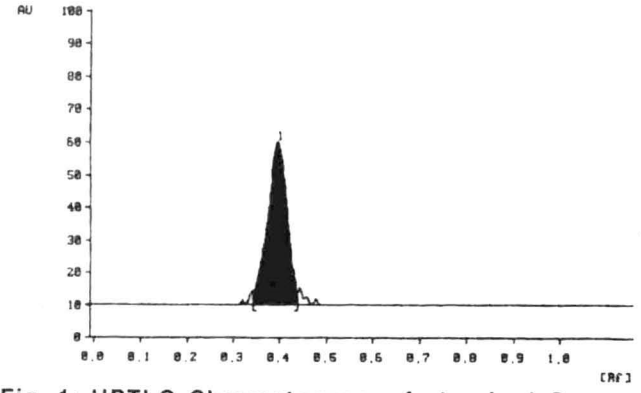

Fig. 1: HPTLC Chromatogram of standard Gymnestrogenin.

1- Gymnestrogenin (Standard)

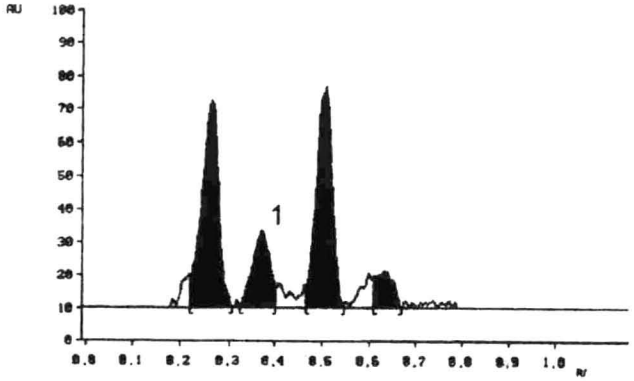

Fig. 2: HPTLC Chromatogram of *Gymnema sylvestre*.

1- Gymnestrogenin (Test sample).

of peak in test sample, its *in situ* reflectance spectrum was compared with standard, which provides clear super impossibility indicating the purity of peak. The recovery values 98.9 to 99.5% (average of 99.1±0.27), which were obtained indicate excellent reliability and reproducibility of the proposed method. It can be concluded that the proposed HPTLC method is rapid, simple and accurate for quantitative monitoring of gymnestrogenin in *Gymnema sylvestre*.

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