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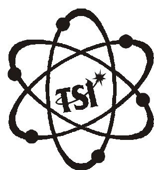

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Determination Of Bergenin In *Caesalpinia Digyna* By HPTLC

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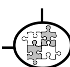

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ABSTRACT

A sensitive, rapid, simple and accurate high performance thin layer chromatographic method has been developed to standardize the root of *Caesalpinia digyna* (Family: Leguminosae) using bergenin as an analytical marker. Precoated HPTLC silica gel GF_{254} plates were used as stationary phase and ethyl acetate-methanol-glacial acetic acid (8:1.5:0.2, v/v) was used as mobile phase. Rf value of bergenin was 0.22. Detection and quantification were performed by densitometry at 275 nm. The calibration plot was linear in the range of 0.12 μg to 2 μg of bergenin and the correlation coeffecient, 0.999 was indicative of good linear dependence of peak area on concentration. The method permits reliable quantification of bergenin in *Caesalpinia digyna*. The developed HPTLC method was found to be reproducible, accurate and precise.
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KEYWORDS

HPTLC;
Standardization;
Caesalpinia digyna;
Bergenin.

INTRODUCTION

Caesalpinia digyna is a large, scandent, prickly shrub or climber, growing wild in the scrub forests of the eastern himalayas. The plant is one of the ingredients of an indigenous drug preparation, 'Geriforte', which has been used for curing senile purities with excellent result. The drug is also reported to exhibit antifatigue effect in rats[1-2]. The roots have marked astringent and antipyretic properties. They also have an intoxicating effect and are given internally in pthisis and scrofula[3]. The ethanol water extract of roots inhibits the growth of *Mycobacterium tuberculosis*[4]. The plant contains caesalpinine A, cellallocinnine, ellagic acid, gallic acid, pipecolic acid, bergenin and tannins[5-9]. Because of its widespread

use in various geographic regions, and to detect its adulteration with roots from younger trees and other lower quality roots, it is important to standardize the root of *Caesalpinia digyna*. Therefore, we have devised a HPTLC method for standardization of its extract using bergenin as marker compound.

EXPERIMENTAL

Plant material

The root of *Caesalpinia digyna* was purchased from Abirami Botanicals, Tuticorin, Tamilnadu, India and identified by Dr. D.Suresh Baburaj, Survey of Medicinal Plants and Collection Unit, Ootacamund, India. The root was cut into small pieces and ground to a powder in a mill. The powder was stored in a closed vessel for further use.

Reagents

Analytical grade ethyl acetate, methanol and glacial acetic acid were obtained from Qualigens Fine Chemicals, Mumbai, India.

Analytical marker

Bergenin was isolated from the root of *Caesalpinia digyna* and was characterized by spectral studies[10].

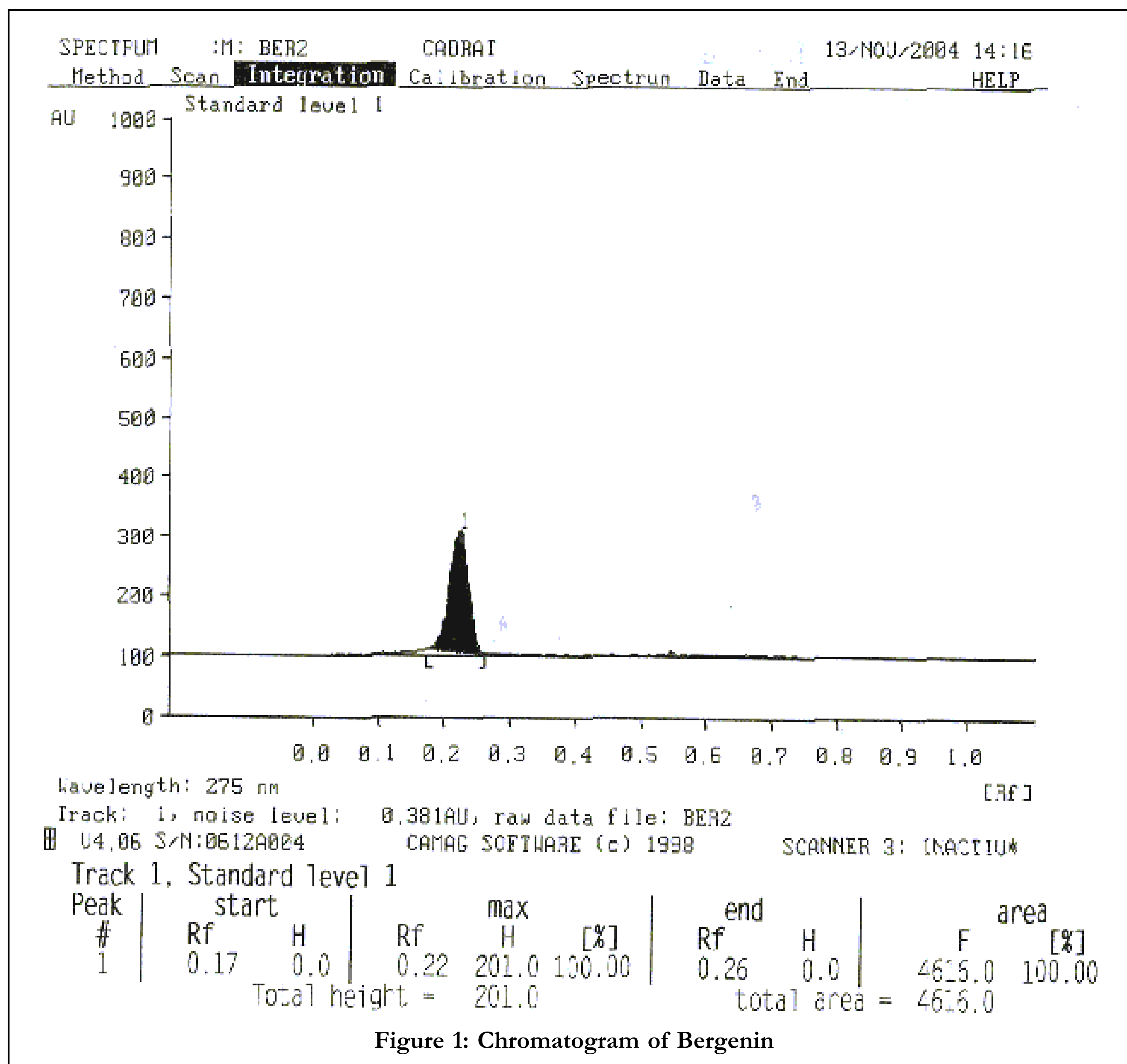

Figure 1: Chromatogram of Bergenin

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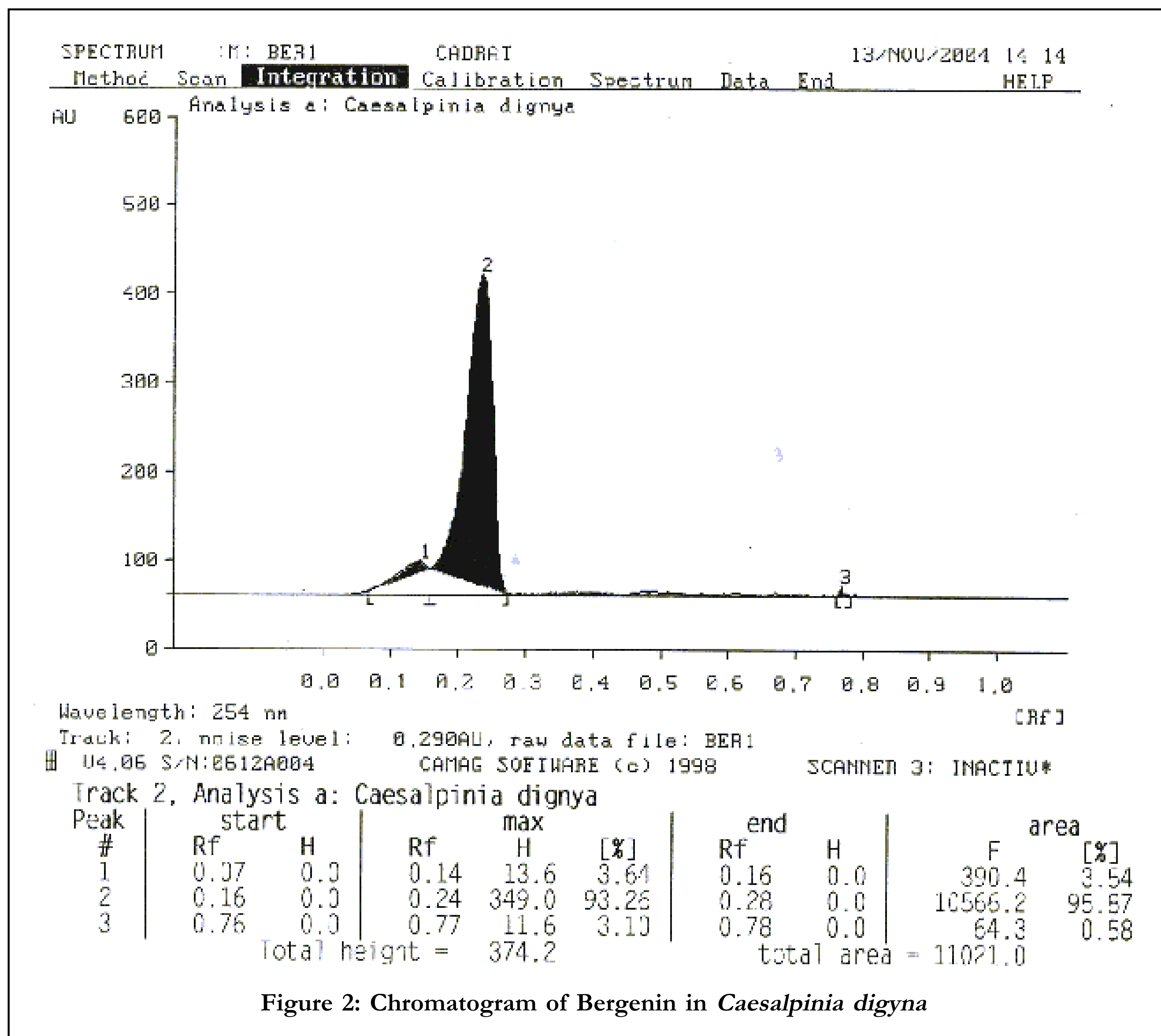

Figure 2: Chromatogram of Bergenin in *Caesalpinia digyna*

Standard and sample solutions

10 mg of bergenin was dissolved in 5 ml of methanol in a 10 ml volumetric flask, and the volume was made up to 10 ml with the same solvent (stock solution). Various concentrations were prepared from the stock solution.

1 g of *Caesalpinia digyna* root powder was extracted with 20 ml of methanol by heating for 15 min. The extract was filtered through whatman filter paper. The operation was repeated twice. The combined extract was evaporated to 10 ml. This solution was used for the HPTLC analysis.

Chromatography

Chromatography was performed on aluminium backed HPTLC silica gel 60 GF_{254} plates (E Merck, Mumbai, India). Various concentrations of standard and sample solutions were applied by means of Camag Linomat IV sample applicator onto the HPTLC plates as 8 mm bandwidth. The chromatogram was developed under chamber saturation conditions with ethyl acetate-methanol-glacial acetic acid (8:1.5:0.2, v/v) in a Camag twin trough chamber. Evaluation of both standard and samples were performed by scanning at 275 nm with a Camag TLC scanner III controlled by CATS V.406 software. The peak areas were recorded for all the peaks. The amount of bergenin was computed from peak areas.

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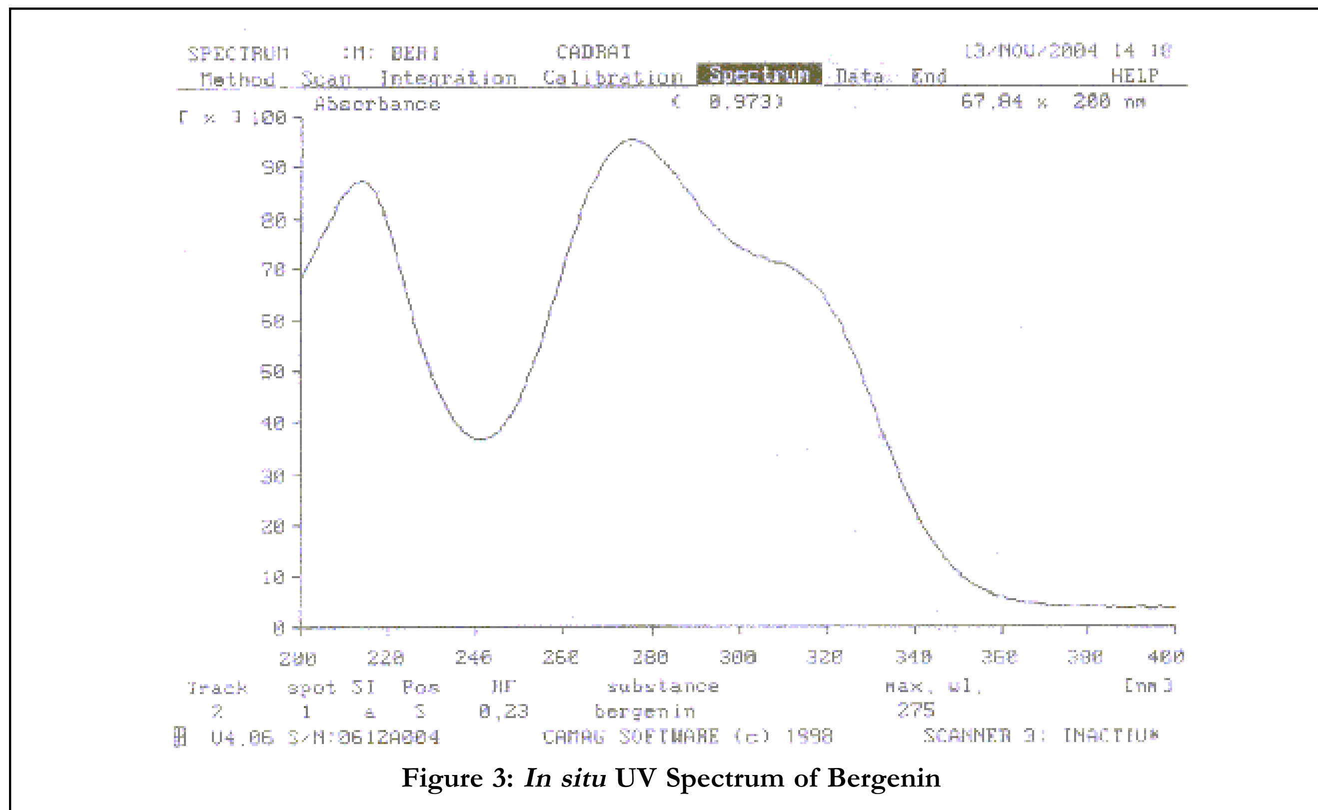

Figure 3: *In situ* UV Spectrum of Bergenin

RESULTS AND DISCUSSION

Chromatography

The mobile phase resolved the bergenin efficiently from other components of *Caesalpinia digyna.* The chromatograms of bergenin and methanol extract of root are shown in figure 1 and figure 2, respectively. The Rf value of bergenin was 0.22. The *in situ* UV spectrum of bergenin is shown in figure 3. The wavelength 275 nm was selected for detection of bergenin in standard and samples. The results are shown in TABLE 1. The low RSD values are indicative of the high accuracy and precision of the method.

TABLE 1: Results of HPTLC analysis of bergenin in the extract of *Caesalpinia digyna*

Plant extract	Constituent	Amount found by proposed method [%w/w]	RSD (%) (n=3)
Caesalpinia digyna root extract	Bergenin	0.430	0.20

System suitability

System suitability studies were performed on freshly prepared standard solutions of bergenin to ascertain the effectiveness of the developed method.

1. Linearity and limits of quantifications and detection

Calibration plot of peak area against concentra-

TABLE 2: Results of recovery analysis

Plant extract	Amount of bergenin present in (ng) A	Amount of bergenin Added to A (ng) B	Total bergenin taken (A+B) (ng) C	Total bergenin found (ng) D	% Recovery D/C) x 100 (mean)
Caesalpinia digyna root extract	430	200	630	615	98.26
		400	830	834	
		600	1030	996	

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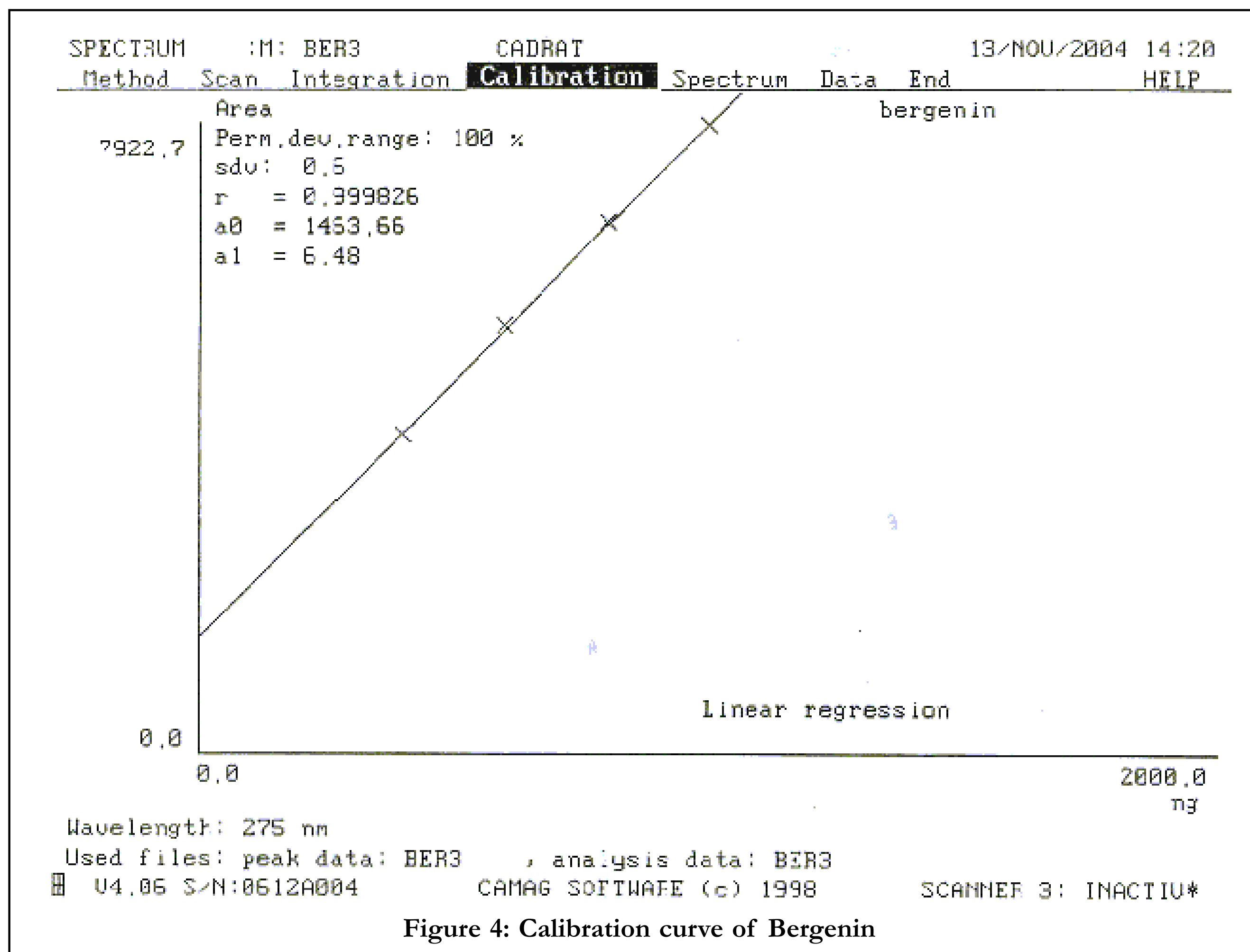

Figure 4: Calibration curve of Bergenin

tions were linear in the range of 0.12 μg to 2μg for bergenin (Figure 4). The calibrations line were represented by the linear equations $Y_{ber} = 6.48\ x + 1463.6$ for this equation the co-relation coefficient, r, was 0.999. The LOD and LOQ were calculated by use of equation,

LOD = 3 X N/B and LOQ = 10 X N/B,

Where N is the standard deviation of the peak areas of the drug (n=3), taken as measure of the noise, B is the slope of the corresponding calibration curve.

The limit of quantifications was 120 ng and limit of detection was 40 ng.

2. Accuracy and precision

The accuracy and precision of the method were studied by performing experiments by standard addition technique. Three different levels of standards were added to the previously analyzed samples, each level being repeated thrice. The amount (μg) of drug found by the method (Y-axis) was plotted against the amount of standard drug (X-axis). The intercept on the Y-axis indicates the amount of drug (μg)

TABLE 3: Results from ruggedness studies

Analyst	Percentage of bergenin in *Caesalpinia digyna* root extract
I	96.87
II	97.19
III	97.08
Mean	97.04

TABLE 4: Results from robustness studies

Development distance (cm)	Bergenin assay (%) from *Caesalpinia digyna* root extract
7	99.4
7.5	99.0
8	98.3

present in the extracts. The percentage recovery was calculated from the amount of drug found. The recovery obtained for bergenin was 98.26 as shown in TABLE 2. This shows that there is no interference from the other constituents in the extract.

3. Ruggedness and robustness

The results of ruggedness testing are reported in TABLE 3. Robustness studies are shown in TABLE 4.

CONCLUSION

The HPTLC method proposed for determination of bergenin in the plant extract are accurate, precise, rapid and selective. It can, therefore, be easily and conveniently adopted for routine quality control analysis.

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