

Quantitative Determination of Corosolic acid in *Lagerstroemia Speciosa* Leaves, Extracts and Dosage Forms

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Abstract: *Lagerstroemia speciosa* extracts and their active ingredient, corosolic acid exhibits significant anti-diabetic activity in humans. A reliable and reproducible high performance liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC) methods have been developed for the quantitative determination of corosolic acid in *Lagerstroemia speciosa* leaves and extracts. The methods developed have demonstrated good precision and recovery with % RSD less than 2%. The contents of corosolic acid determined by HPLC and HPTLC methods are in good agreement. The methods developed have also been employed for quantitative determination of corosolic acid in *Lagerstroemia speciosa* leaves and extracts. HPLC method has been found suitable for the quantitative determination of corosolic acid in dosage forms also.

Keywords: Corosolic acid, *Lagerstroemia speciosa*, dosage forms, HPLC, HPTLC

1. Introduction

Diabetes mellitus is a chronic condition associated with abnormally high levels of glucose in the blood. The global incidence of diabetes is increasing at an alarming rate and the studies indicate that there will be about 300 million people suffering from diabetes by the year 2010. Non – insulin dependent diabetes mellitus (Type – 2) is the common form of diabetes.

Lagerstroemia speciosa L. (Family Lythraceae), popularly known as Banaba, is an ornamental plant grows widely in the Philippines, India and South East Asian Countries. The tea from the leaves of Banaba has traditionally been used in the Philippines, as a folk

medicine for the prevention and treatment of diabetes [1]. In addition Banaba extracts also known to possess weight loss [2] and antioxidant effects [3]. Corosolic acid, the active ingredient of Banaba extracts displayed potential anti-diabetic activity [4 - 7]. Banaba extracts standardized to 3% corosolic acid have shown statistically significant anti-diabetic activity in a human clinical study.

Corosolic acid has attracted much attention for its beneficial health effects, particularly with respect to its potential anti diabetic activity. In view of the therapeutic importance of corosolic acid, development of analytical methods for the determination of corosolic acid in plant materials is required. A high

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performance liquid chromatographic (HPLC) method was [8] reported for estimation of corosolic acid.

In the present study, accurate, simple and reproducible HPLC and HPTLC methods have been developed and validated [9] for the determination of corosolic acid in *Lagerstroemia speciosa* leaves, extracts and dosage forms.

2. Experimental

2.1. Materials:

Standard corosolic acid was provided by the phytochemistry division of Laila Research Centre, Vijayawada, India. Identity and purity (>95%) of isolated compound was confirmed by chromatographic (TLC, HPLC) and the spectral (IR, Mass & NMR) methods. Methanol, acetonitrile were of HPLC grade, analytical grade phosphoric acid, chloroform and diethyl ether were purchased from M/s Qualigens (Mumbai, India). Ultra pure water generated by the Barnstead Nanopure System (Model 3750, USA) was used. Methanol was used as a solvent for the preparation of standards and samples, Acetonitrile and 0.1% (v/v) phosphoric acid in water (75:25, v/v) were used as mobile phase for HPLC analysis. Acetonitrile, methanol and diethyl ether (1:1:1) mixed solvent used as a solvent for preparation of standards and samples, chloroform and methanol (9:1) used as a mobile phase for HPTLC analysis. All solutions used for HPLC analysis were filtered through 0.45µm membrane filter using Millipore Swinnex type filtration unit. *Lagerstroemia speciosa* leaves and extracts were provided by M/s Laila Impex, Vijayawada, India and dosage forms capsules and tablets containing corosolic acid were procured from U.S.A.

2.2. Sample preparation for HPLC analysis

2.2.1. *Lagerstroemia speciosa* leaves

Weighed about 5 g of dried leaf powder of *Lagerstroemia speciosa* in to a round bottom flask, added about 30 ml of methanol and refluxed on boiling water bath for about 30 min. Filtered and repeated the same operation (2 x 30 ml) with methanol. Combined all the alcoholic extracts and made up to 100 ml with methanol and filtered through 0.45 µm membrane filter.

2.2.2. *Lagerstroemia speciosa* leaf extracts

Weighed accurately about 500 mg of extract, dissolved in methanol and made up to 50ml with methanol and filtered through 0.45 µm membrane filter.

2.3. Dosage forms

The average weights were determined by weighing 20 capsules / tablets. In case of capsules, hard gelatin capsules were removed and the contents were finely powdered. 1-5 g of powdered samples were weighed and dissolved in methanol, added 10.0 ml of standard corosolic acid stock solution (1mg/ ml) and made up to 100 ml with methanol and filtered through 0.45 µm membrane filter.

2.4. Sample preparation for HPTLC analysis

2.4.1. *Lagerstroemia speciosa* leaves

Weighed about 8 g of dried powder of *Lagerstroemia speciosa* in to a round bottom flask, added about 30ml of methanol and refluxed on a boiling water bath for 30 min. Filtered and repeated the same operation (2 x 30 ml) with methanol. Combined all the methanolic extracts and concentrated under reduced pressure. The obtained residue was dissolved in 100ml of mixed solvent, acetonitrile : methanol : diethyl ether (1:1:1) and filtered through Whatman no. 1 filter paper.

2.4.2. *Lagerstroemia speciosa* leaf extracts

Weighed about 1 g of extract, dissolved in mixed solvent, made up to 50 ml with mixed solvent and filtered through Whatman no.1 filter paper.

2.5. Calibration curve of standard corosolic acid – HPLC method

1 mg/ml corosolic acid standard solution was prepared in methanol (stock solution). Standard working solutions were prepared by diluting standard stock solutions with methanol in the concentration range 100 – 600 µg/ml. 20 µl from each working standard solution was injected in six replicates. Calibration curve was generated by linear regression based on peak areas.

2.6. Calibration curve of standard corosolic acid – HPTLC method

1 mg/ml corosolic acid standard solution was prepared in a mixed solvent (stock solution). Standard working solutions were prepared by diluting standard stock solution with mixed solvent in the concentration range 200 – 1200 µg/ml. 10µl from each working standard solution was spotted on the TLC plate to obtain final concentration range 2.0 – 12.0 µg / spot. Each concentration was spotted six times on TLC plates. Calibration curve was generated by linear regression based on the peak areas.

2.7. HPLC instrumentation

The HPLC system, supplied by M/s Shimadzu comprising LC-10AT VP pumps, SCL-10A VP auto injector and Phenomenex Luna C₁₈, 5 µ, (250 X 4.6 mm) column was used at ambient temperature. Isocratic elution was carried out with acetonitrile : 0.1% (v/v) phosphoric acid in water (75 : 25, v/v) at a flow rate of 1ml / min, detection was at 210 nm using SPD – M10 AVP photodiode array

detector. Class VP software was used for integration and calibration. Evaluation was via peak areas with linear regression.

2.8. HPTLC instrumentation

The samples were spotted with a Camag microlitre syringe on a pre-coated silicagel aluminum plates 60 F – 254 (20 nm x 10nm) with 250 µm thickness, (E. Merck, Darmstadt, Germany) using a Camag Linimat IV (Muttentz, Switzerland) applicator. Linear ascending development was carried out in 20 cm x 10 cm twin trough glass chamber (Camag Muttentz, Switzerland) using mobile phase consisting of chloroform: methanol (9 : 1). The length of the chromatogram run was 8 cm. After air drying, the TLC plates were scanned with a Camag TLC scanner-III in absorbance mode at 210 nm, controlled by Cats software 4.03 version. Evaluation was via peak areas with linear regression.

2.9. Estimation of corosolic acid

To estimate the content of corosolic acid in *Lagerstroemia speciosa* leaves and extracts samples, aliquots of 10 µl were subjected to HPTLC and aliquots of 20 µl were injected in to HPLC. The HPTLC plates were developed to a distance of 8 cm from the point of application, dried and scanned at 210 nm. The HPLC analysis was continued for 20 min, since the retention time of the corosolic acid was 9.4 ± 0.4 min. The content of corosolic acid was calculated by linear regression and mean percentages were calculated from six replicate experiments. In case of dosage forms, mean mg / capsule or tablet calculated from six replicate experiments.

3. Results and discussion

Because of the complexity of the chemical composition of herbal extracts, quality of the herbal extracts can only be assured by the use of validated analytical methods for identifica-

tion and quantification of the active ingredients. The HPLC and HPTLC methods for the quantitative estimation of corosolic acid were validated with regard to their specificity, precision, accuracy and linearity.

3.1. HPLC method validation

The development of suitable mobile phase is an important step in devising an analytical procedure. The composition of the HPLC mobile phase was optimized to achieve good resolution. The best resolution and peak shape were obtained by acetonitrile: 0.1% (v/v) phosphoric acid in water (75:25, v/v) as mobile phase. The compound with a retention time 9.4 ± 0.4 min was identified as corosolic acid (Figure 1). Specificity was ascertained by comparing the standard and sample peak purity. The peak corresponding to corosolic acid in the sample was confirmed by comparing the spectrum obtained by photodiode array detector, which was completely in agreement with the standard (Figure 2)

Precision is a measure of either reproducibility or repeatability of the analytical method. Intermediate precision express the laboratory variations, by intra- and inter-day variation. Six determinations of three concentrations of standard corosolic acid on the same day (intra-day) and on different days (inter-day) were carried out and expressed as percent relative standard deviation (% RSD). The results noted in Table 1, reveal that no significant intra- and inter-day variations. The % RSD for intra- and inter-day analysis was found to be in the range of 0.02 – 0.08 which are less than 2%.

The accuracy of the method was determined from recovery studies. The pre-analyzed sample was spiked with three different concentrations of standard corosolic acid and the mixtures were analyzed by the proposed method. The recoveries are in the range of 95.98 – 100.16% reported in the Table 2. The average recovery percentage value was found to be $98.58 \pm 1.85\%$.

The linearity of the standard curve was evaluated by determining six standard working solutions containing 100 – 600 $\mu\text{g/ml}$ corosolic acid. Peak area and concentrations were subjected to least square linear regression analysis to calculate the calibration equation and correlation coefficient. Linearity was obtained over a concentration range of 2.0 – 12.0 μg per injection with a correlation coefficient of 0.9981 ± 0.0004 . The linearity of calibration graph and adherence of the system to Beer's law was validated by high value of correlation coefficient.

The limit of detection (LOD) and limit of quantification (LOQ) were estimated to check the sensitivity of the method. The signal to noise ratio for LOD was considered as 3 : 1 and 10 : 1 for LOQ. LOD and LOQ were experimentally verified by diluting the known concentration of corosolic acid until the average responses were approximately three or ten times the standard deviation of the responses for six replicates determinations. The LOD and LOQ were found to be 0.016 and 0.048 μg per injection, respectively.

3.2. HPTLC method validation

The composition of the mobile phase for TLC was optimized by testing different solvent mixtures of varying polarity. The best results were obtained using chloroform : methanol (9:1). The selected mobile phase produced highly symmetrical peaks showing good resolution (Figure 3). The compound with a R_f value of 0.40 ± 0.03 was identified as corosolic acid.

The specificity of the method was ascertained by analyzing standards and samples. The spot for corosolic acid in the sample was confirmed by comparing the R_f value. Spectral studies revealed that the peaks obtained from both standard corosolic acid and test samples were identical, because they had similar pattern as shown in Figure 4.

The precision of the method was studied by applying six replicates of the three different

concentration of the standard. The results in Table 1 showed that the % RSD for intra- and

Inter - day analysis was found to be in the range 1.16 – 1.78, that is less than 2%.

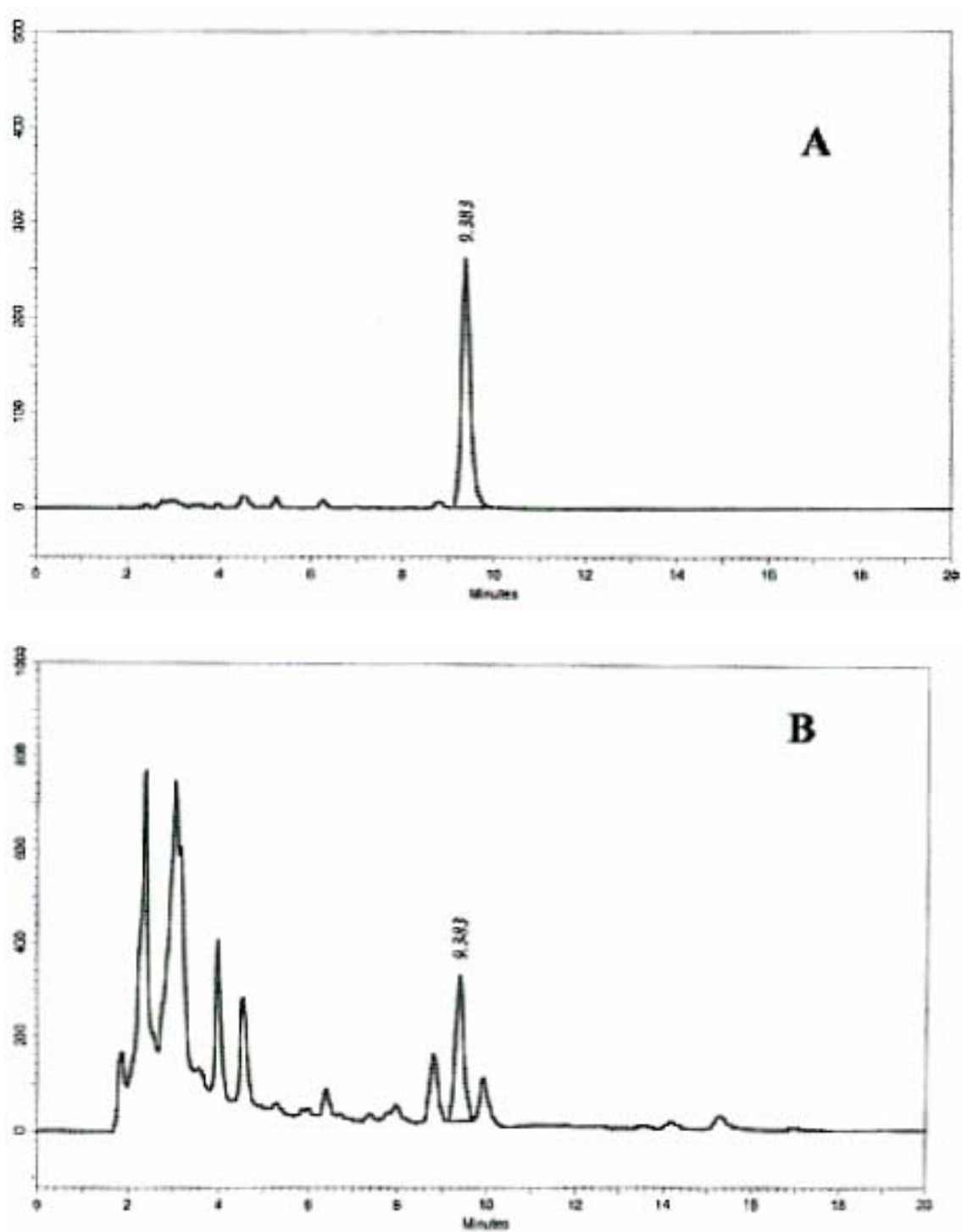


Figure 1. HPLC chromatograms of A) Corosolic acid standard B) *Lagerstroemia speciosa* leaf extract

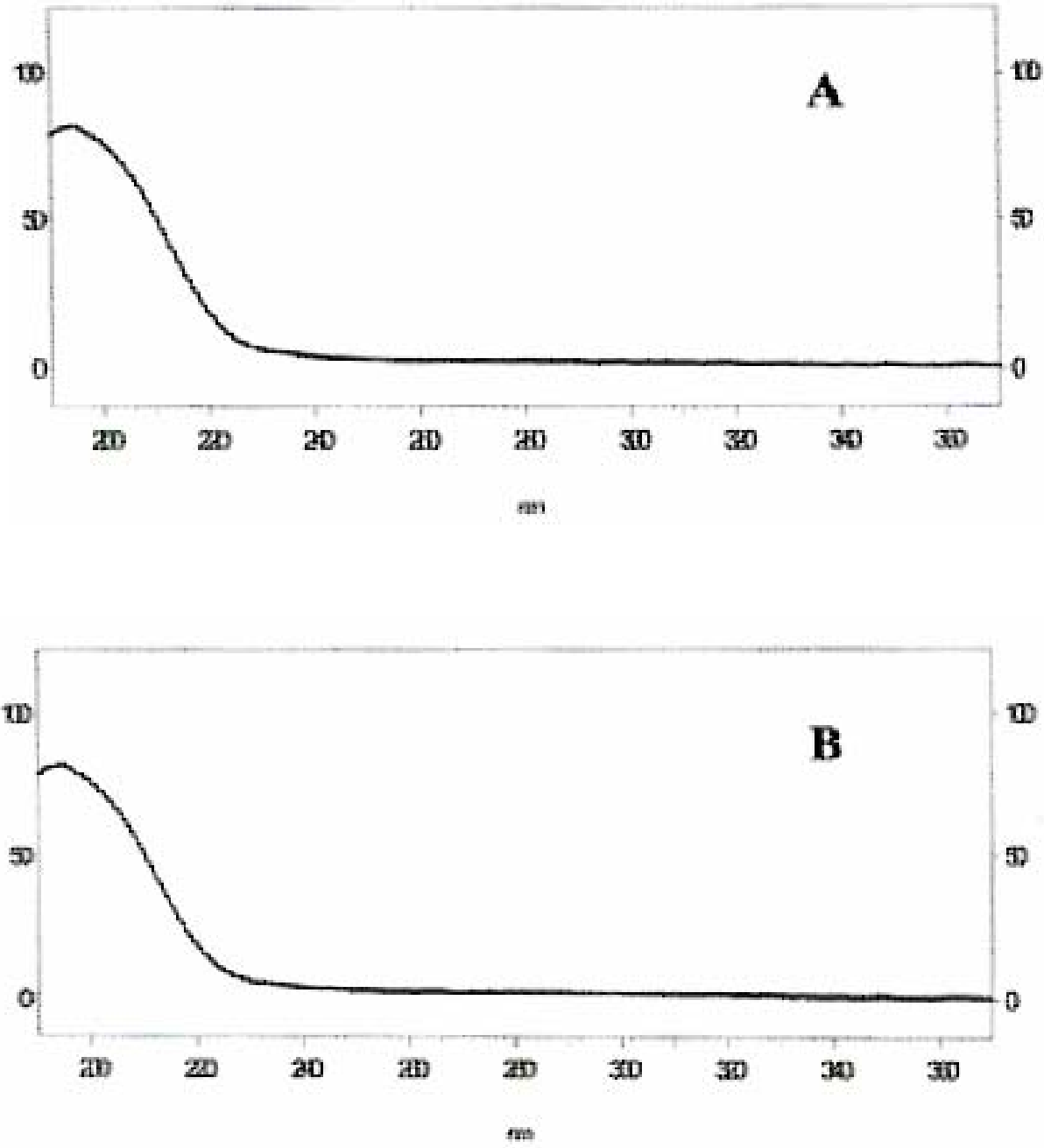


Figure 2. Spectra of Corosolic acid obtained by photodiode array detector.A) Corosolic acid standard.B) *Lagerstroemia speciosa* extract

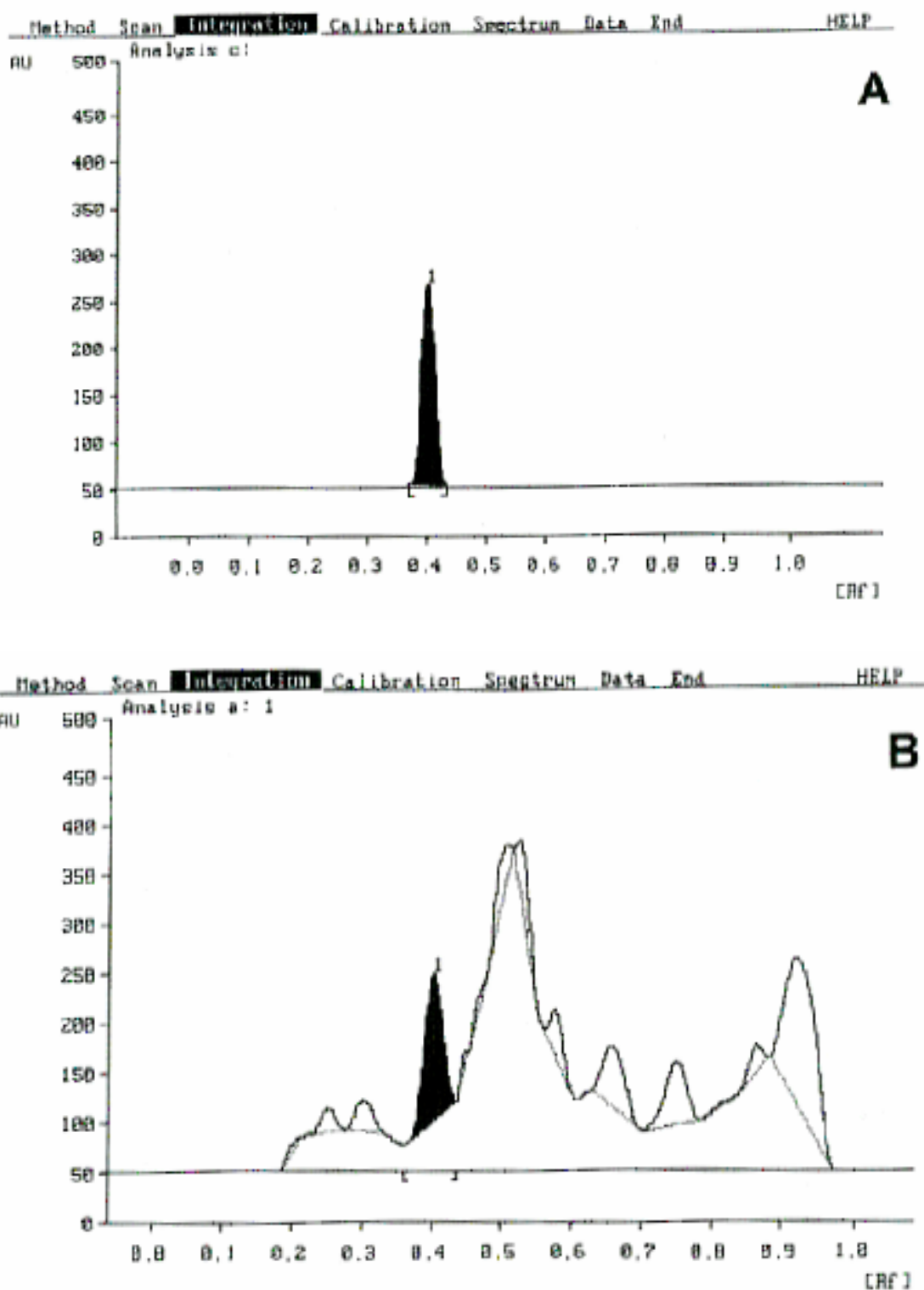


Figure 3. HPTLC chromatograms of A) Corosolic acid standard B) *Lagerstroemia speciosa* leaf extract

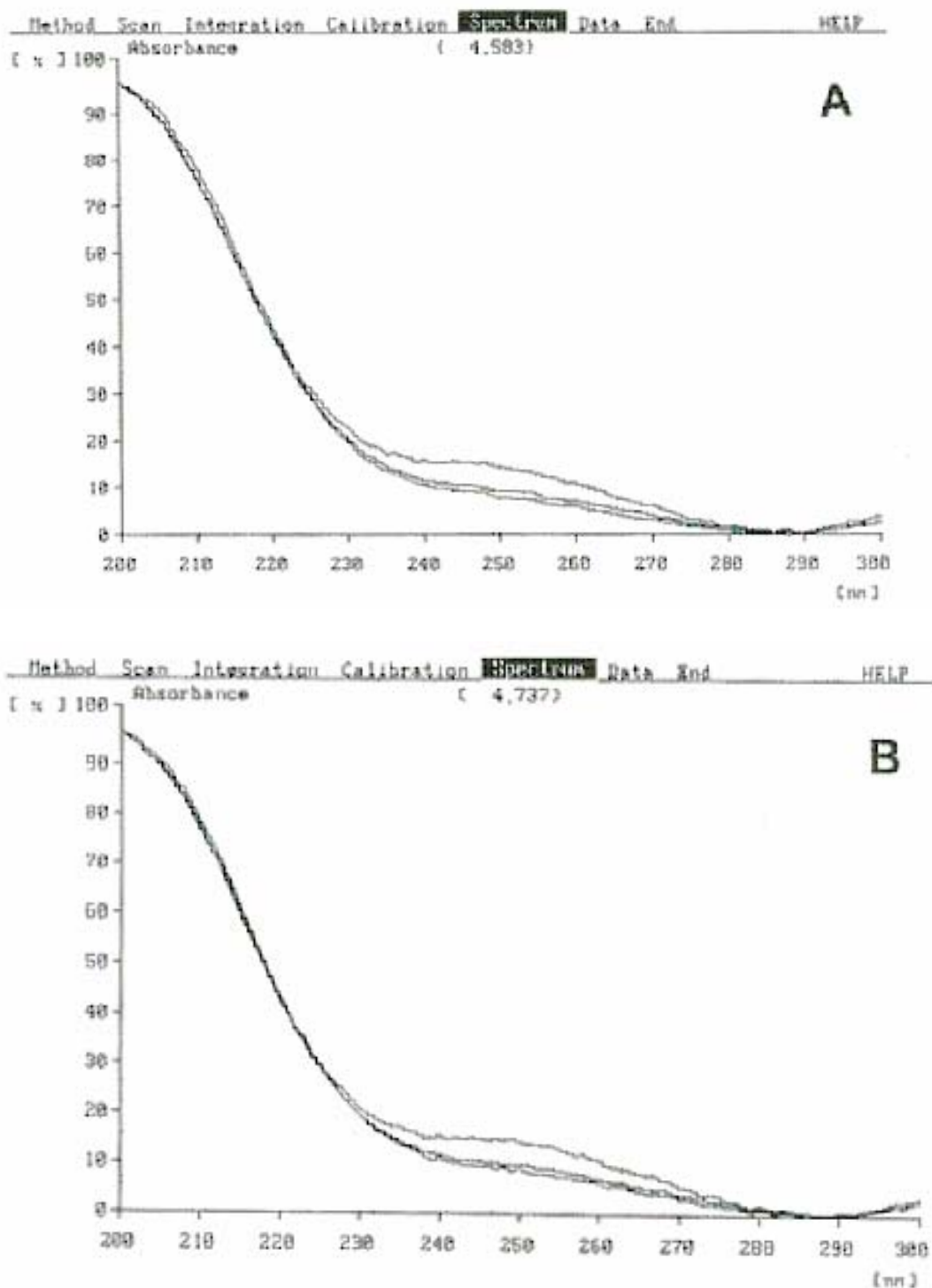


Figure 4. Over lay spectra of A) Corosolic acid standard B) Sample at peak start, peak maximum and peak end in absorbance mode in the UV range, taken on the Camag TLC scanner III

Table 1. Intra- and Inter-day precision of HPLC and HPTLC methods (n=6)

HPLC method				
Corosolic acid (μg)	Intra-day		Inter-day	
	Corosolic acid (μg)	% RSD	Corosolic acid (μg)	% RSD
4.0000	4.0255 \pm 0.0006	0.02	4.0326 \pm 0.0241	0.59
8.0000	7.7160 \pm 0.0677	0.88	8.0246 \pm 0.0027	0.03
12.0000	12.1031 \pm 0.0334	0.28	11.9322 \pm 0.0196	0.16
HPTLC method				
4.0000	3.9568 \pm 0.0531	1.34	3.8975 \pm 0.0451	1.16
8.0000	7.8320 \pm 0.1062	1.36	7.9050 \pm 0.0965	1.22
12.0000	11.9560 \pm 0.2020	1.69	12.0155 \pm 0.2150	1.78

Table 2. Recovery study (n = 6)

HPLC method		
Amount of corosolic acid added (μg)	Amount of corosolic acid recovered (μg)	% Recovery
2.8450	2.7309	95.98
5.6900	5.6992	100.16
9.4832	9.4440	99.59
HPTLC Method		
2.0231	2.0178	99.73
4.0462	4.0840	100.93
8.0923	8.0040	98.91

The accuracy of the method was determined from recovery studies. A known but varying amounts of standards of corosolic acid was added to the pre-analyzed sample and analyzed according to the procedure. The results reported in Table 2. The average recovery percentage value was found to be $99.86 \pm 0.831\%$.

Linearity was evaluated by determining six standard working concentrations containing 200 – 1200 $\mu\text{g/ml}$ of corosolic acid. Peak area and concentration were subjected to least square linear regression analysis to calculate the calibration equation and correlation coefficient. Linearity was found over the concentration range 2 – 12 $\mu\text{g / spot}$ with a correlation coefficient of 0.9735 ± 0.0014 . The linearity of the calibration curve and adherence to the system to Beer's law was validated by a high value correlation coefficient.

In order to estimate the limit of detection (LOD) and limit of quantification (LOQ), blank, mixed solvent (acetonitrile: methanol : diethyl ether, 1:1:1) was spotted six times. LOD and LOQ were determined based on the standard deviation of the response of the

blank and slope estimated from the calibration curve of the corosolic acid. The LOD and LOQ were found to be 0.004 and 0.013 μg per spot for corosolic acid.

3.3. Application of HPLC and HPTLC methods

The HPLC method developed was applied for the estimation of corosolic acid in two different *Lagerstroemia speciosa* leaves, extracts and dosage forms, three different capsules and one tablet. Apart from *Lagerstroemia speciosa* extract, the dosage forms contain vitamins, minerals, other herbal extracts and excipients. Since the content of corosolic acid is very minute, we adopted a standard addition method for estimation of corosolic acid in the dosage forms. The results obtained are presented in Table 3. The % RSD values were found to be less than 2%, which shows that the precision of the method is reasonably good. Analysis of the dosage forms revealed that the presence of vitamins, minerals, herbal extracts and excipients does not interfere in the estimation of corosolic acid.

Table 3. Estimation of Corosolic acid by HPLC and HPTLC methods (n = 6)

Sample	HPLC method		HPTLC method	
	Estimated amount	% RSD	Estimated amount	% RSD
RM-1	0.3150 ^a	1.74	0.3087 ^a	1.90
RM-2	0.3800 ^a	1.95	0.3754 ^a	1.85
EM-1	1.7880 ^a	0.65	1.7815 ^a	1.67
EM-2	11.3400 ^a	1.01	11.1780 ^a	1.50
FM-1	0.0480 ^b	1.95	-	
FM-2	0.1350 ^b	1.80	-	
FM-3	0.1800 ^b	1.95	-	
FM-4	0.0200 ^b	1.75	-	

RM: *Lagerstroemia speciosa* leaf

EM: *Lagerstroemia speciosa* leaf extract

FM-1: Fat Burner Extreme capsules – High Power Nutrition, Idaho 83704.

FM-2: Cortisol capsules – Advanced Nutrient Science Institute, Largo, FL 33777.

FM-3: Cortislim capsules – Window Rock Enterprises Inc. Brea, CA 82823.

FM-4: Suvaril Tablets – Berkeley Premium Nutraceuticals Inc. Cincinnati, Ohio 45242.

a: Expressed as mg/100mg

b: Expressed as mg/Capsule or Tablet

The HPTLC method developed was applied for the estimation of corosolic acid in two different *Lagerstroemia speciosa* leaves and extracts. The results obtained were presented in Table 3. The % RSD values were found to be less than 2%. Corosolic acid in dosage forms cannot be estimated, because of the interference of the other matrices. However this method can be utilized for corosolic acid estimation in *Lagerstroemia speciosa* leaves and extracts.

4. Conclusions

The developed HPLC method can be utilized for the quantitative determination of corosolic acid in *Lagerstroemia speciosa* leaves, extracts and dosage forms. HPTLC method also can be utilized for quantitative determination of corosolic acid in *Lagerstroemia speciosa* leaves and extracts. The methods developed are simple, sensitive and statistically validated for linearity, accuracy and precision.

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