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QUANTIFICATION OF ASPARTIC ACID BY HPTLC METHOD IN *COCCINIA INDICA* CRUDE DRUG, LAB EXTRACT AND COMMERCIAL EXTRACT

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ABSTRACT

The aim of the present study is to quantify the amount of aspartic acid in *Coccinia indica* crude drug, lab extract and commercial extract by HPTLC method. The stationary phase was precoated aluminium silica gel G F $_{254}$ Plates. The mobile phase for aspartic acid was n-Butanol: Glacial acetic Acid: Water (50: 10: 40). The methanolic solution of standard aspartic acid (1 mg/ ml concentration) 5 µl, crude drug methanolic solution 7 µl (100 mg/ ml concentration), lab extracts methanolic solution 7 µl (100 mg/ ml concentration) and commercial extracts methanolic solution 7 µl (100 mg/ ml concentration) were applied as 7mm babd width using CAMAG Linomat IV applicator. The mobile phase was n-Butenol: Glacial acetic Acid: Water (50: 10: 40) and scanned by CAMAG Scanner III CATS (4.06) software at wavelength of 544 nm. The R_f of the sample *Coccinia indica* crude drug, lab extract and commercial extract were matching with the standard R_f of marker compound aspartic acid. The amount of aspartic acid was calculated by comparing the peak area of standard and the same in the crude drug, lab extract and commercial extract. The content of aspartic acid was found to be 0.2778 % w/w, 0.9205 % w/w & 0.3057 % w/w for *Coccinia indica* crude drug, lab extract and commercial extract respectively. The calibration curve of standard aspartic acid was prepared by method of linear regression, using 1 mg/ml standard solution. The obtained data was subjected to statistical analysis using linear regression analysis and the correlation coeffiecient were determined. The correlation coeffiecient of aspartic acid was found to be 0.9899. In order to obtain precission and accuracy the recovery study were performed and result obtained with mean value 99.89 % for aspartic acid, which prove reproducibility of the result. The mean of % RSD value was found to be 1.54 for aspartic acid. This estimation technique is very much useful for the estimation of aspartic acid present in the various medicinal plants.

KEY WORDS: Accuracy, Aspartic acid, Precission, *Coccinia indica*, HPTLC.

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INTRODUCTION

Coccinia indica belongs to family of Cucurbitaceae. This plant traditionally used as wound healer, anti-inflammatory,appetizer, laxative, liver diseases, jaundice, blood purifier and antidiabetic. The main active chemical constituents present in the plant are flavonoids, steroids and amino acids (Aspartic acid, γ-amino butyric acid, alanine, methionine, threonine and valine, cephalandrol, tritriacontane, β-sitosterol, cephalandrine A and B [2, 4, 6. 7]. HPTLC is emerging as a versatile, high throughput & cost-effective technology that is uniquely suited to assessing the identity and quality of botanical materials. The main objective of the present study is to quantification of one of the active constituent aspartic acid using HPTLC method [1, 2, 3, 4].

MATERIALS AND METHODS

Plant material

Coccinia indica leaf material was collected at Ooty and authentified by Dr. S. Rajan, Field Botanist, Medicinal Plant Collection and Survey Unit, Department of Ayush, Emerald, Ooty, (T.N.) India. Commercial extract of *Coccinia indica* was obtained from Amsar Pvt. Ltd., Indore, (M.P.) India. The marker compound (aspartic acid) was obtained from Natural Remedies Pvt. Ltd., Bangalore, India.

Preparation of the plant extract

Coarse powder of the dried material of *Coccinia indica* seed extraction was carried out by maceration method (For 7 days) by using ethanol 70% as a solvent.

Method development of HPTLC

Standard preparation

5 mg of aspartic acid was dissolved in 5 ml of methanol (1mg/ml concentration).

Sample preparation (extracts)

Crude drug preparation

1000 mg of powdered *Coccinia indica* crude drug was dissolved in 10 ml of methanol and slightly warmed on water bath and filtered through whatman filter paper, and the same solution was used for HPTLC analysis (100 mg/ml concentration).

Extract preparation

1000 mg of lab extract was dissolved in 10 ml of methanol and slightly warmed on water bath and filtered through Whatman filter paper, and the same solution was used for HPTLC analysis (100 mg/ml concentration). The same procedure was followed for the preparation of commercial extract.

Chromatographic conditions for determination of aspartic acid

Stationary phase : Precoated Silica gel F $_{254}$ Plates (MERCK)
Mobile phase : n-Butanol: Glacial acetic Acid: Water (50: 10: 40)
Saturation : 60 mins
Development chamber : CAMAG twin trough development chamber
Applicator : CAMAG Linomat IV applicator
Scanner : CAMAG Scanner III CATS (4.06), Switzerland
Mode of scanning : Absorption (deuterium)
Detection wavelength : 544 nm
Volume applied (standard) : 5 µl
Volume applied (sample) : 7 µl each sample

Procedure

Before spotting, the plates were pre-washed with methanol. Standard and samples solutions were applied to the plates as sharp bands by means of CAMAG Linomat IV applicator. The spots were dried in a current of air. The mobile phase (20 ml) was poured into a twin trough glass development chamber was left to equilibrate for 30 minutes and the plate was placed in the chamber [10, 11]. The plate was then

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developed until the solvent front had travelled at a distance of 75 mm above the base of the plate. The plate was then removed from the chamber and dried in a current of air. Detection and quantification was performed with CAMAG Scanner III at a wavelength of 544 nm [5, 6, 7].

Linearity

Linearity was performed by applying standard solution at different concentration range from 100 to 500 ng/spot aspartic acid, on 20 x 20 cm HPTLC plates, precoated silica gel G F $_{254}$ Plates (Merck) in the form of sharp 7 mm bands; the distance between two adjacent band was 8 mm. the plates were developed in a solvent system of n-Butenol: Glacial acetic Acid: Water (50: 10: 40) for aspartic acid, up to a distance 75 mm, at room temperature [9, 11]. The plates were dried in air. The detector response for aspartic acid was measured for each band at wavelength of 544 nm, using CAMAG TLC Scanner and winCat software [10]. The peak area of aspartic acid was recorded for each concentration. The linearity curve of aspartic acid was obtained by plotting agraph of peak area of aspartic acid *vs* applied concentration of aspartic acid (ng).

Method validation

The method was validated for precision, repeatability and accuracy. The precision was checked by repeated scanning of same spot of aspartic acid (250 ng), three times and was expressed as relative standard deviation (% RSD). The repeatability of the method was confirmed by analyzing 100 ng, 250 ng and 500 ng/spot and 500 ng, 1500 ng and 2500 ng/spot of standard aspartic acid, solution (n = 3) and was expressed as % RSD. The precision of the method was studied by analyzing aliquots of standard solution of aspartic acid (100 ng, 250 ng and 500 ng/spot) on the same day (intra-day precision) and on different days (inter-day precision) and the results were expressed as % RSD. Study the accuracy, the recovery experiment was performed by the method of standard addition. The recovery of the added amount of standard was analyzed at three different levels. Each level of addition was repeated three times on three different days and the recovery of the add amount of standard was calculated. Limit of detection and limit of quantitation was also calculated by the proposed method. [8, 9]

RESULTS AND DISCUSSION

The amount of aspartic acid present in the crude drug, lab extract and commercial extract of *Coccinia indica* were estimated by using HPTLC technique by comparing with the peak area of standard and sample. The results are given in table 1. The results reveals that the R_f of the sample *Coccinia indica* crude drug, lab extract and commercial extract were matching with the standard R_f of marker compound aspartic acid and the amount of marker compound present in the samples were calculated (Fig. 1, 2 & 3). The content of aspartic acid was found to be 0.2778% w/w, 0.9205% w/w & 0.3057% w/w in *Coccinia indica* crude drug, lab extract and commercial extract. The calibration curve was linear in the range of 100 to 500 ng/spot aspartic acid and the correlation coefficient was determined. The correlation coefficient was found to be 0.9899 for aspartic acid.

The limit of quantification was found to be 120 ng for aspartic acid and the limit of detection was 40 ng. The method was validated in terms of precision and reproducible expressed as % RSD which were found to be less than 2% for aspartic acid. The recovery values obtained were 99.49 to 100.24 % showing accuracy of the method for aspartic acid. The average percentage recovery was found to be 99.88 % for aspartic acid given in table 2.

Table 1
HPTLC quantification of aspartic acid in Coccinia indica

S.No.	Sample	Standard R_f values	Sample R_f values	Amount of Marker Compound (%w/w)
1	*Coccinia indica* raw material	0.26	0.24	0.2778
2	*Coccinia indica* lab extract	0.26	0.27	0.9205
3	*Coccinia indica* commercial extract	0.26	0.25	0.3057

Table 2
Validation parameters for quantification of aspartic acid by HPTLC

Parameters	Aspartic acid
Precission (% RSD)	< 2 %
Linearity	100 to 500 ng/spot
Limit of detection	40 ng/spot
Limit of quantification	120 ng/spot
Accuracy	99.49 to 100.24 %

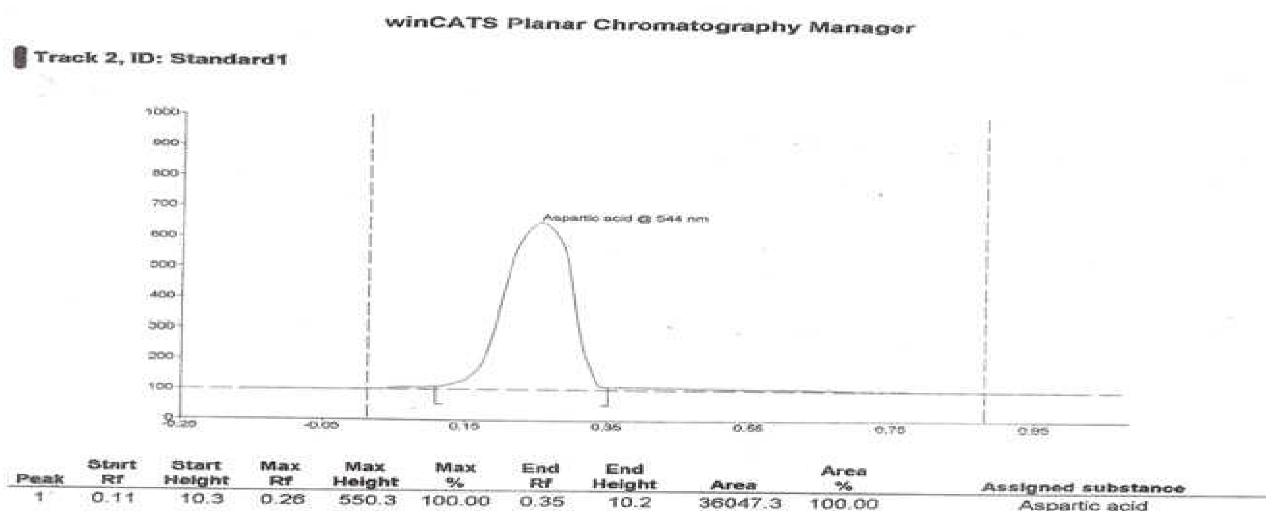
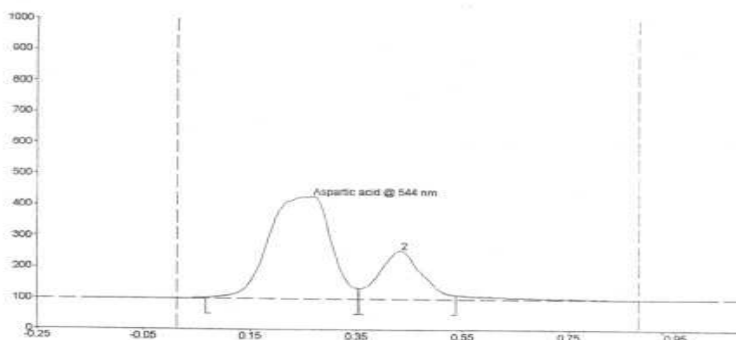


Figure 1
HPTLC Chromatogram of standard aspartic acid

Track 7, ID: C. Indica Lab



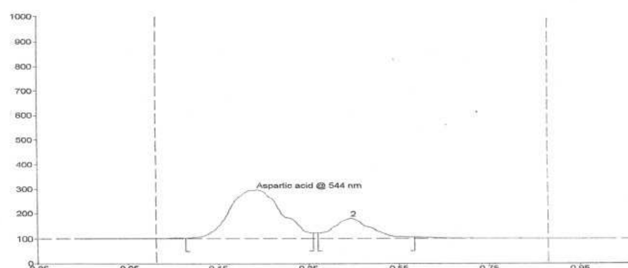
winCATS Planar Chromatography Manager

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.07	2.6	0.27	328.2	67.94	0.35	34.1	26546.0	75.16	Aspartic acid
2	0.35	34.2	0.44	154.8	32.06	0.54	13.6	8773.2	24.84	unknown *

Figure 2
HPTLC Chromatogram of Coccinia indica lab extract

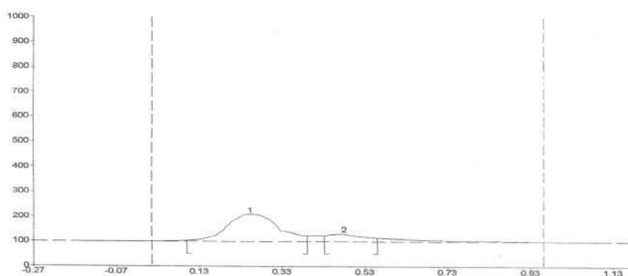
winCATS Planar Chromatography Manager

Track 9, ID: C. Indica Raw material



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.08	2.0	0.24	197.7	70.75	0.36	22.3	16026.3	76.48	Aspartic acid
2	0.37	22.6	0.45	81.7	29.25	0.59	7.2	4928.7	23.52	unknown *

Track 8, ID: C. Indica Commercial



winCATS Planar Chromatography Manager

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.10	4.7	0.25	110.9	77.47	0.39	24.5	8818.2	83.09	unknown *
2	0.43	26.6	0.47	32.2	22.53	0.56	15.0	1794.3	16.91	unknown *

Figure 3
HPTLC Chromatogram of Coccinia indica raw material (Track No. 9) and commercial extract (Track No. 8)

CONCLUSION

The developed HPTLC method was utilised for estimation of aspartic acid in *Coccinia indica* could be used as a valuable analytical tool in the routine analysis. Aspartic acid can be used as one of the appropriate analytical markers present in the various medicinal plants.

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