Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Issue 4, 2012 ISSN - 0974-2441

Research Article

QUANTIFICATION OF (-) EPICATECHIN BY HPTLC METHOD IN *CASSIA FISTULA* CRUDE DRUG, LAB EXTRACT AND COMMERCIAL EXTRACT

PRAVEEN PATIDAR*, DARSHAN DUBEY [1] AND KAMLESH DASHORA [2]

[1, 2] **Institute of Pharmacy,Vikram University,Ujjain, India.Email:** praveenpatidar86@gmail.com

Received:18 july 2012, Revised and Accepted:21 September 2012

ABSTRACT

A simple sensitive HPTLC method developed for the Quantification of (-) epicatechin in the plant *Cassia fistula* crude drug, lab extract and commercial extract. The stationary phase was precoated aluminium silica gel G F $_{254}$ Plates. The mobile phase was chloroform: acetone: formic acid (75:16.5: 8.5). The plate was scanned and quantified at 364 nm for (-) epicatechin. The amount of (-) epicatechin was estimated by the comparing the peak area of standard and the same were present in the crude drug, lab extract and commercial extract. The content of (-) epicatechin were found to be 2.63% w/w, 5.85% w/w & 3.75% w/w for *Cassia fistula* crude drug, lab extract and commercial extract respectively. The method was validated in terms of linearity, accuracy and specificity. This estimation technique is very much useful for the estimation of (-) epicatechin present in the various medicinal plants.

Keywords: *Cassia fistula,* (-) epicatechin, Estimation, HPTLC

INTRODUCTION

Cassia fistula belonging to family Caesulpinaceae is native to India, Amazon and Sri Lanka, and is now widely cultivated worldwide. It is having moderate laxative, liver disorder, Jaundice, antidiabetic and lowers cholesterol activity. *Cassia fistula* leaf contains (-) epiafzelechin, (-) epiafzelechin-3-O-glucoside, (-) epicatechin, procyanidin B2, rhein, rhein glucoside, sennoside A & B, chrysophanol, physcion [1, 2 3, 4, 5, 6]. High Performance Thin Layer Chromatography (HPTLC) is emerging as a versatile, high throughput & cost-effective technology that is uniquely suited to assessing the identity and quality of botanical materials [7, 8].

The aim of the present work is to develop a method for estimation of (-) epicatechin by HPTLC Technique simultaneously.

MATERIALS AND METHODS

Plant materials

Cassia fistula 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. Commercial extract of C*assia fistula* was obtained from Amsar Pvt. Ltd., Indore, (M.P.).The marker compound was obtained from Natural Remedies Pvt. Ltd., Bangalore, India.

Preparation of the plant extract

Coarse powder of the dried material of *Cassia fistula* leaf extraction was carried out by maceration method (For 7 days) by using ethanol 90% as a solvent.

Method development of HPTLC

Standard preparation

5 mg of (-) Epicatechin was dissolved in 5 ml of methanol (1mg/ml concentration).

Sample Preparation (extracts)

Crude drug preparation: 1000 mg of powdered *Cassia fistula* 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 Condition

Stationary phase (Merck)	:	Precoated Silica Gel G F $_{254}$ Plates
Mobile phase	:	Chloroform: Acetone: Formic acid (75:16.5: 8.5)
Saturation	:	40 mins
Development chamber chamber	:	CAMAG twin trough development
Applicator	:	CAMAG Linomat IV applicator
Scanner	:	CAMAG Scanner III CATS (4.06), Switzerland
Mode of scanning	:	Absorption (deuterium)
Detection wavelength	:	364 nm
Volume applied (Standard)	:	8 µl
Volume applied (Sample)	:	10 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 minits and the plate was placed in the chamber. The plate was then 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 364 nm[9, 10, 11].

Linearity

Linearity was performed by applying standard solution at different concentration range from 1 to 5 µg/spot 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 chloroform: acetone: formic acid (75:16.5: 8.5), up to a distance 75 mm, at room temperature [1]. The plates were dried in air. The detector response for (-) epicatechin was measured for each band at wavelength of 364 nm, using CAMAG TLC Scanner and winCat software. The peak area of (-) epicatechin were recorded for each concentration. The linearity curve of (-) epicatechin was obtained by plotting agraph of peak area of (-) epicatechin *vs* applied concentration of (-) epicatechin (µg).

Method validation

The method was validated for precission, repeatability and accuracy. The precission was checked by repeated sacannig of same spot of (-) epicatechin (2 µg) three times each and was expressed as relative

standard deviation (% RSD). The repeatability of the method was confirmed by analyzing 1 µg, 2 µg and 5 µg of standard (-) epicatechin solution (n = 3) and was expressed as % RSD. The precision of the method was studied by analyzing aliquots of standard solution of (-) epicatechin (1 µg, 2 µg and 5 µg/spot) on the same day (intra-day precision) and on different days (inter-day precision) and the results were expressed as % RSD [13, 14].

To 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 alsocalculated by the proposed method.

RESULTS AND DISCUSSION

The amount of (-) epicatechin present in the crude drug, lab extract and commercial extract of *Cassia fistula* 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 *Cassia fistula* crude drug, lab extract and commercial extract were matching with the standard R_f of marker compound (-) epicatechin and the amount of marker compound present in the samples were calculated. The content of (-) epicatechin was found to be 2.63 % w/w, 5.85% w/w & 3.75% w/w in *Cassia fistula* crude drug, lab extract and commercial extract respectively.

The calibration curve was linear in the range of 1 µg to 5 µg/spot and the correlation coefficient was determined. The correlation coefficient was found to be 0.9964. The limit of quantification was found to be 3µg and the limit of detection was 1 µg. The method was validated in terms of precission and reproducible expressed as % RSD which were found to be less than 2%. The recovery values obtained were 98.28 to 100.4%, showing accuracy of the method. The average percentage recovery was found to be 99.12%.

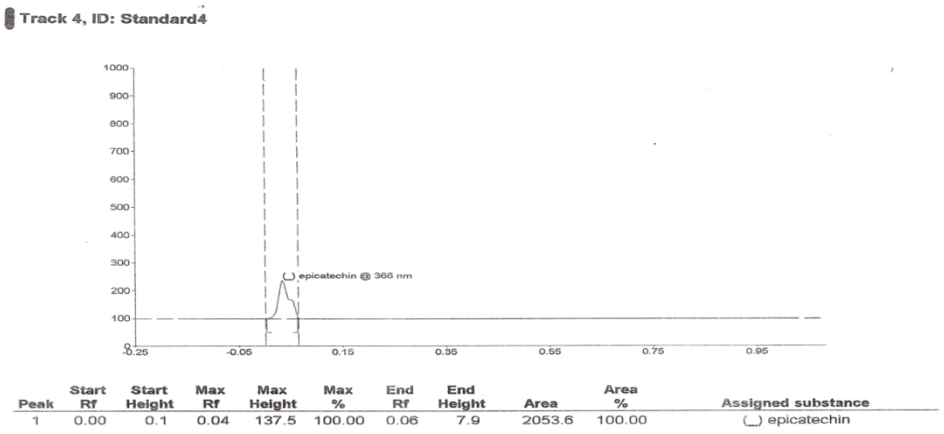
In conclusion the developed HPTLC method was simple accurate, precise, economic and can be utilised for the routine analysis and quantitative determination of (-) epicatechin in *Cassia fistula.*

Table No. 1. HPTLC quantification of (-) Epicatechin in *Cassia fistula*

S.No.	Sample	Standard R_f values	Sample R_f values	Amount of Marker Compound
1	*Cassia fistula* crude material	0.04	0.04	2.63 %
2	*Cassia fistula* lab extract	0.04	0.04	5.85 %
3	*Cassia fistula* commercial extract	0.04	0.04	3.75 %

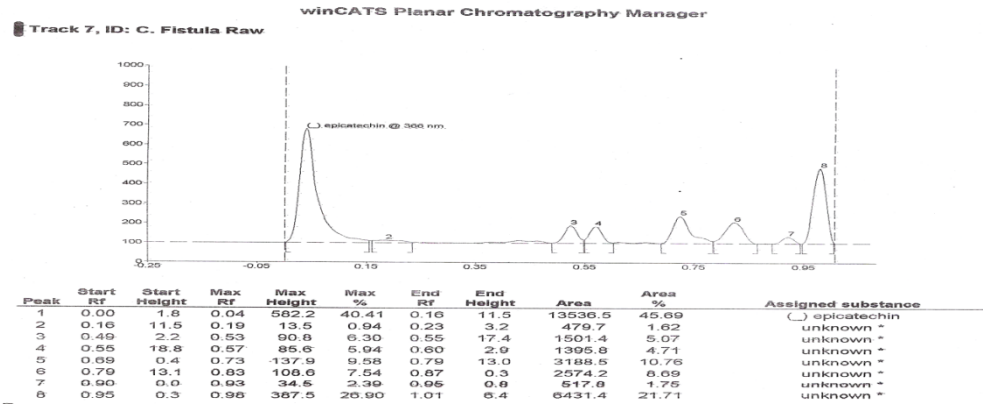
Table No. 2. Validation parameters for quantification of (-) epicatechin by HPTLC

Parameters	Results
Precission (% RSD)	< 2 %
Linearity	1 to 5 µg/spot
Limit of detection	1 µg/spot
Limit of quantification	3 µg/spot
Accuracy	98.28 to 100.4 %



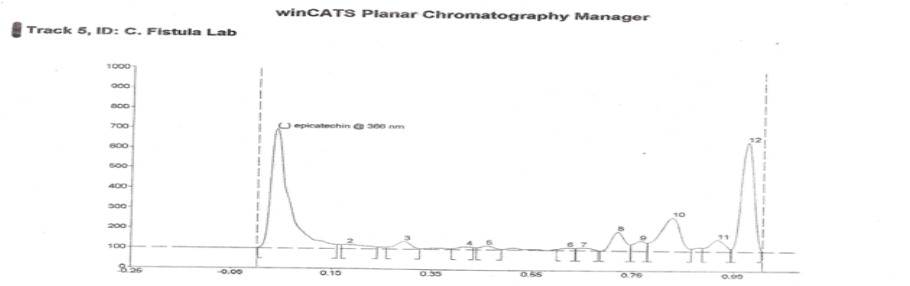
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	0.1	0.04	137.5	100.00	0.06	7.9	2053.6	100.00	(_) epicatechin

Fig. 1:HPTLC Chromatogram of standard (-) Epicatechin



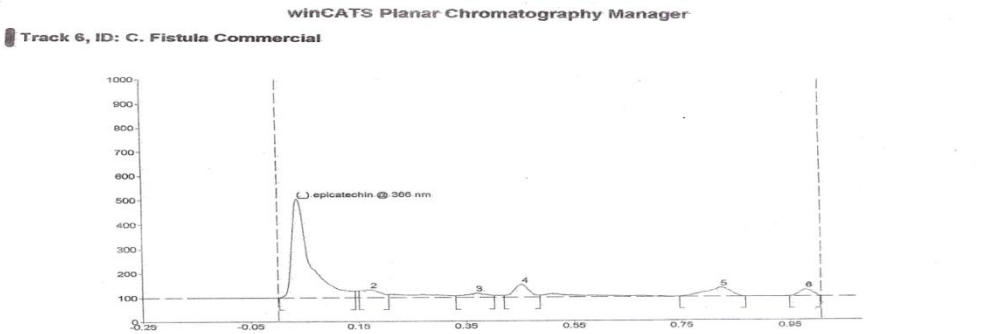
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	1.8	0.04	582.2	40.41	0.16	11.5	13536.5	45.69	(_) epicatechin
2	0.16	11.5	0.19	13.5	0.94	0.23	3.2	479.7	1.62	unknown *
3	0.49	2.2	0.53	90.8	6.30	0.55	17.4	1501.4	5.07	unknown *
4	0.55	18.8	0.57	85.6	5.94	0.60	2.9	1395.8	4.71	unknown *
5	0.69	0.4	0.73	137.9	9.58	0.79	13.0	3188.5	10.76	unknown *
6	0.79	13.1	0.83	108.6	7.54	0.87	0.3	2574.2	8.69	unknown *
7	0.90	0.0	0.93	34.5	2.39	0.95	0.8	517.8	1.75	unknown *
8	0.95	0.3	0.98	387.5	26.90	1.01	5.4	6431.4	21.71	unknown *

Fig. 2:HPTLC Chromatogram of *Cassia fistula* raw material



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	1.2	0.04	597.5	36.44	0.16	20.6	15035.9	41.69	(-) epicatechin
2	0.17	20.4	0.18	22.0	1.34	0.24	10.4	753.0	2.09	unknown *
3	0.26	10.4	0.29	39.7	2.42	0.33	4.6	875.0	2.43	unknown *
4	0.39	6.0	0.42	15.4	0.94	0.43	12.5	321.9	0.89	unknown *
5	0.44	11.7	0.46	20.1	1.22	0.49	4.7	425.0	1.18	unknown *
6	0.60	6.1	0.62	13.3	0.81	0.64	12.1	257.2	0.71	unknown *
7	0.64	12.3	0.65	13.8	0.84	0.68	0.8	293.0	0.81	unknown *
8	0.69	0.1	0.72	95.0	5.80	0.75	31.6	1781.0	4.94	unknown *
9	0.75	32.1	0.77	49.4	3.02	0.78	42.7	868.1	2.41	unknown *
10	0.78	42.8	0.83	168.5	10.28	0.87	14.8	4757.7	13.19	unknown *
11	0.89	19.6	0.92	59.2	3.61	0.95	14.0	1289.2	3.57	unknown *
12	0.95	14.2	0.98	545.7	33.28	1.01	6.3	9406.9	26.08	unknown *

Fig. 3:HPTLC Chromatogram of *Cassia fistula* lab extract



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	0.7	0.04	406.7	70.53	0.14	27.5	9641.5	69.29	(-) epicatechin
2	0.15	28.1	0.17	33.7	5.84	0.21	14.2	885.8	6.37	unknown *
3	0.33	7.2	0.37	17.7	3.08	0.40	9.0	546.4	3.93	unknown *
4	0.42	8.8	0.46	53.3	9.25	0.49	8.7	1090.3	7.84	unknown *
5	0.75	1.7	0.82	36.8	6.39	0.87	0.6	1244.1	8.94	unknown *
6	0.95	1.8	0.98	28.4	4.92	1.01	1.4	507.0	3.64	unknown *

Fig. 4: HPTLC Chromatogram of *Cassia fistula* commercial extract

CONCLUSION

The developed HPTLC method was utilised for estimation of (-) epicatechin in *Cassia fistula* could be used as a valuable analytical tool in the routine analysis. (-) epicatechin can be used as one of the appropriate analytical markers present in the various medicinal plants.

REFERENCES

1. Agrawal Ram K, V Ravichandran, Prateek K Jain, Aman D Kaur, 2008, High-performance thin layer chromatography method for estimation of conessine in herbal extract and pharmaceutical dosage formulations, *Journal of Pharmaceutical and Biomedical Analysis,* 46; 391–394.
2. Anne Schibli and Eike Rich, 2007, High-performance thin layer chromatography for the analysis of medicinal plants, Thiene Medicinal Publishers, Inc., New York, p. 227-232.
3. Bahorun, Reergheen, Okezie, Aruoma, 2005, Phytochemical constituents of *Cassia fistula*, *African Journal of Biotechnology*, 4 (13); 1530-1540.
4. Barthakur, Arnold, Allii, 1995, The Indian laburmum (*Cassia fistula* L) fruit: an analysis of its chemical constituents, *Plant foods for human nutrition*, 47; 55-62.
5. Bhakta, Mukherjee, Kakali, Banerjee, Mandal, Maity, Saha, 1999, Evaluation of hepatoprotective activity of *Cassia fistula* leaf extract, *Journal of Ethanopharmacology* , 66; 277-282.
6. Bhakta, Mukherjee, Kakali, Banerjee, Mandal, Maity, Saha, 2003, Hepatoprotective activity of *Cassia fistula* leaf extract, *Phytomedicine*, 8 (3); 220-224.
7. Chaudhary RD, 2006, Herbal Drug Industries, Fourth Reprint, Eastern Publishers, New Delhi, p. 71, 473-474.
8. Merforta Irmgard, Wagnera Steffen, Ure˜nac Abraham, Reichb Eike, 2008, Validated HPTLC methods for the determination of salicin in *Salix* sp. and of harpagoside in *Harpogophytum procumbens, Journal of Pharmaceutical and Biomedical Analysis* 48; 587–59.
9. Mukherjee K Pulok, 2002, Quality control of herbal drugs, 1st edition, Business Horizones, p. 493-515, 583-584.
10. Nagarsenkar Mangal S and Tayade Nitin G, 2007, Validated HPTLC method of analysis for artemether and its formulation, *Journal of Pharmaceutical and Biomedical Analysis*, 43; 839-844.
11. Sethi PD, 1996, HPTLC – Quantitative analysis of pharmaceutical formulations, CBS Publishers and Distributors, New Delhi, p. 1-30.
12. Wagner H and Bladt S, 1996, Plant Drug Analysis, 2nd edition, Springer Verlag, Berlin, p. 224-230.
13. Yoganarasimhan SN, 1996, Medicinal Plants of India, Vol. I Karnataka, Interline Publisher Pvt. Ltd., Banglore, p. 101, 130, 499.
14. Yoganarasimhan SN, 2000, Medicinal Plants of India, Vol. II Tamil Nadu, Interline Publisher Pvt. Ltd., Banglore, p. 111-2, 147, 579-580.

\