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RESEARCH ARTICLE

Quantification of (-) Epicatechin by HPTLC Method in Developed Polyherbal Tablet Formulation

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ABSTRACT

A simple sensitive HPTLC method developed for the Quantification of (-) epicatechin in the plant raw material tablet formulation. Traditional system of medicine recommends various hepatoprotective agents and preparations to treat hepatic disorders. Polyherbal crude drugs formulation (tablet) was developed by using well documented medicinal plants, *Cassia fistula, Coccina indica* and *Vigna mungo* for treatment of liver disorders by exploiting the knowledge of Traditional system of medicine. The stationary phase was precoated aluminium silica gel G F_{254} Plates. The mobile phase for 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 was present in the raw material tablet formulation. The content of (-) epicatechin was found to be 1.22% w/w in polyherbal crude tablet formulation. The calibration curve was linear in the range of 1 µg to 5 µg/spot and the correlation coefficient was found to be 0.9964. The limit of quantification was found to be 3 µg/spot and the limit of detection was 1 µg/spot. 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%. This estimation technique is very much useful for the estimation of (-) epicatechin present in the various medicinal plants and formulations.

Keywords: (-) epicatechin, Polyherbal, Estimation, HPTLC.

1. INTRODUCTION

A great deal of research has been carried out to evaluate scientific basis for the claimed hepatoprotective activity of herbal agents as in the form of formulation. The selected plant materials; *Cassia fistula* (family- Caesalpinaceae), *Coccinia indica* (family- Cucurbitaceae) and *Vigna mungo* (family- Papilionaceae) reported to have hepatoprotective activity [1, 2, 3, 4, 5, 6, 7, 8]. The polyherbal crude tablet formulation contains the crude raw materials of *Cassia fistula*, *Coccinia indica* and *Vigna mungo*. *Cassia fistula* leaf contains (-) epiafzelechin, (-) epiafzelechin-3-O-glucoside, (-) epicatechin, procyanidin B2, rhein, rhein glucoside, sennoside A & B, chrysophanol, physcion [9, 10, 11, 12, 13]. 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 [14,15].

The aim of the present work is to develop a method for estimation of (-) epicatechin by HPTLC technique and formulate the polyherbal tablet formulation simultaneously.

2. MATERIALS AND METHODS

Plant materials

All the three plants crude materials *Cassia fistula* (leaf material), *Coccinia indica* (leaf material) and *Vigna mungo* (seed material) were collected in and around of Nilgiri District, Tamilnadu and authenticated by Dr. S. Rajan, Field Botanist, Medicinal Plant Collection and Survey Unit, Department of Ayush, Emerald, Ooty, (T.N.), India.

Preparation of formulations

Polyherbal tablet formulation contains the crude raw materials of *Cassia fistula*, *Coccinia indica* and *Vigna*

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mungo prepared by wet granulation (aqueous) method [16] using suitable excipients like Starch, Sodium benzoate, Starch, Gelatin, Primlose, Sodium starch glycolate, Talc and Magnesium stearate [Table 1].

Physical evaluation

The prepared polyherbal crude tablet formulations were subjected to determination of various physical parameters like disintegration time, hardness, thickness, friability and weight variation test as per the standard procedures [17].

Method development of HPTLC

Standard preparation

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

Formulation preparation: 2000 mg of crushed crude tablet formulation 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 (200 mg/ml concentration).

Chromatographic Condition

Stationary phase	:	Precoated Silica Gel G F $_{254}$ Plates (Merck)
Mobile phase	:	Chloroform: Acetone: Formic acid
Saturation	:	40 mins
Development chamber	:	CAMAG twin trough development chamber
Applicator	:	CAMAG Linomat IV applicator
Mode of scanning	:	Absorption (deuterium)
Detection wavelength	:	364 nm
Volume applied (Standard)	:	8 µl
Volume applied (Sample)	:	10 µl

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 III at a wavelength of 364 nm [9, 10, 18, 19].

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. 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 scanning 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 [18, 19]. 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 was also calculated by the proposed method.

3. RESULTS AND DISCUSSION

Formulation development

The prepared formulations was subjected to determinations of various physical evaluations like disintegration time, hardness, thickness, friability and weight variation test and pass the Indian Pharmacopoeia standards (Table 2).

HPTLC Estimation

The amount of (-) epicatechin present in the polyherbal crude drugs formulation was estimated by using HPTLC technique by comparing with the peak area of standard and sample. The results are given in table 3. The results reveals that the R_f of the sample polyherbal crude drugs formulation was matching with the standard R_f of marker compound (-) epicatechin and the amount of marker

compound present in the samples was calculated. The content of (-) epicatechin was found to be 1.22 % w/w in polyherbal crude drugs formulation. (Fig. 1 & 2).

Validation

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%. Result was given in table 4.

Sr.NO	INGREDIENTS	PER TABLET (mg)
1	*Cassia fistula*	450
2	*Coccinia indica*	125
3	*Vigna mungo*	175
4	Starch (diluent)	110
5	Sodium benzoate (preservative)	1
6	Starch (binding agent)	20
7	Gelatin (binding agent)	15
8	Primlose (super disintegrating agent)	20
9	Sodium starch glycolate (super disintegrating agent)	10
10	Starch (disintegrating agent)	19
11	Talc (glidant)	10
12	Magnesium stearate (lubricating agent)	5
	Total weight	960

Table 1: Composition of polyherbal crude tablet formulation

S.No	Quality control tests	Formulation (Crud drugs)
1	Physical appearance	Dark brown coloured tablets
2	Weight variation test	960 ± 2.15
3	Hardness test	3.0 kg/sq.cm
4	Friability test	0.89 %
5	Thickness	6.90 mm
6	Disintegration time test	11.4 min

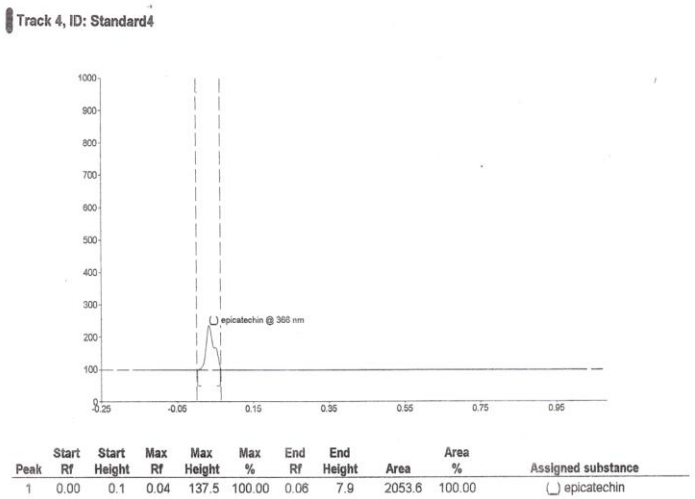
Table 2: Physical evaluation of polyherbal crude tablet formulation

S.No.	Standard R_f values	Sample R_f values	Amount of Marker Compound
1	0.04	0.05	1.22 %

Table No. 3. HPTLC quantification of (-) Epicatechin in polyherbal crude tablet Formulation

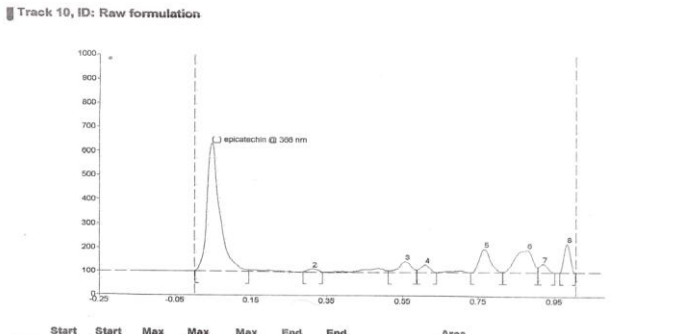
Parameters	Results
Precission (% RSD)	< 2 %
Linearity	1 to 5 µg/spot
Limit of detection	1 µg/spot
Limit of quantification	3 µg/spot
Accuracy	98.28 to 100.4 %

Table No. 4. Validation parameters for quantification of (-) epicatechin by HPTLC



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 (Track No. 4)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	2.2	0.05	533.1	53.95	0.14	6.1	12572.9	57.65	epicatechin
2	0.29	1.9	0.31	13.8	1.39	0.34	0.3	235.1	1.08	unknown *
3	0.51	7.5	0.56	47.5	4.81	0.59	10.7	1081.1	4.96	unknown *
4	0.59	10.8	0.61	32.7	3.31	0.64	1.3	571.7	2.62	unknown *
5	0.73	0.3	0.77	101.4	10.26	0.81	3.6	2100.1	9.63	unknown *
6	0.82	3.8	0.88	97.2	9.84	0.91	24.3	3205.6	14.70	unknown *
7	0.91	25.3	0.92	38.2	3.86	0.95	0.3	595.4	2.73	unknown *
8	0.97	0.9	0.99	124.2	12.57	1.01	0.4	1447.9	6.64	unknown *

Fig. 2. HPTLC Chromatogram of polyherbal raw Formulation (Track No. 10)

4. CONCLUSION

The developed HPTLC method was utilised for estimation of (-) epicatechin in polyherbal tablet formulation could be used as a valuable analytical tool in the routine analysis. (-) epicatechin can be used as one of theappropriate analytical markers present in the various medicinal plants and formulations.

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Conflict of Interest: None Declared

