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HPTLC METHOD VALIDATION OF FLAVONOIDS IN *CASSIA AURICULATA* LINN-A HIGH VALUE MEDICINAL PLANT

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ABSTRACT

A simple high performance thin layer chromatographic (HPTLC) method has been developed for the simultaneous determination of the pharmacologically important active Flavonoids viz., rutin, ferulic acid, caffeic acid, isoquercitrin, quercitrin in *Cassia auriculata* Linn. The assay combines the separation and quantification of the analytes on silicagel 60 GF_{254}. HPTLC plates with visualization under UV and scanning at 540nm. The method is rapid, simple and precise.

INTRODUCTION

Cassia auriculata (family: Caesalpiniaceae) is a valuable plant drug in ayurvedic and modern system of medicine for the treatment of pitta, kapha, skin diseases, ulcers, diabetes, diarrhea, conjunctivitis and noctural emission.[1] HPTLC method has been developed in the present work for flavonoids from methanolic extract of dried leaves of *Cassia auriculata.* International Organization like ISO, ICH, AOAC and IUPSC have published guidelines for method validation techniques keeping in view of the above, present study we are reporting a HPTLC method validation data for flavonoids *in Cassia auriculata*[2].

MATERIALS AND METHODS

Collection of plant material

The leaves of *Cassia auriculata* was collected from Thennilai, near Karur District in Tamilnadu. The sample was dried in the shade, finely powdered and the powder was passed through 80 mesh sieve and stored in airtight container at room temperature .About 300gm of the powder was taken in a soxhlet extractor and extracted with methanol[3].The solvent recovered by distillation. The residue was concentrated, dried and stored in the desiccator for further experiment and analysis.

Instrumentation and chromatographic conditions

HPTLC was performed on 20cm*10cm aluminum backed plates coated with silica gel $60GF_{254}$ (Merck, Mumbai, India) .Sample solution were applied to the plates as bands 8.0mm wide,30.0mm apart and 10.0mm from the bottom edge of the chromatographic plate by use of a camac (Muttenz, Switzerland) Linomat V sample applicator equipped with a 100-µl Hamilton (USA) syringe.

Ascending development to a distance of 80mm was performed at room temperature (28 ± 2^0C),with methanol, toluene:ethyl acetate,formicacid,5:4:0.2(V/V/V),as mobile phase, in a camag glass twin-trough chamber previously saturated with mobile phase vapour for 20 minutes. After development, the plates were dried with a hair dryer and then scanned at 540nm with a camag TLC scanner with WINCAT software, using the deuterium lamp. The method was validated according to the ICH guidelines[4].

System suitability

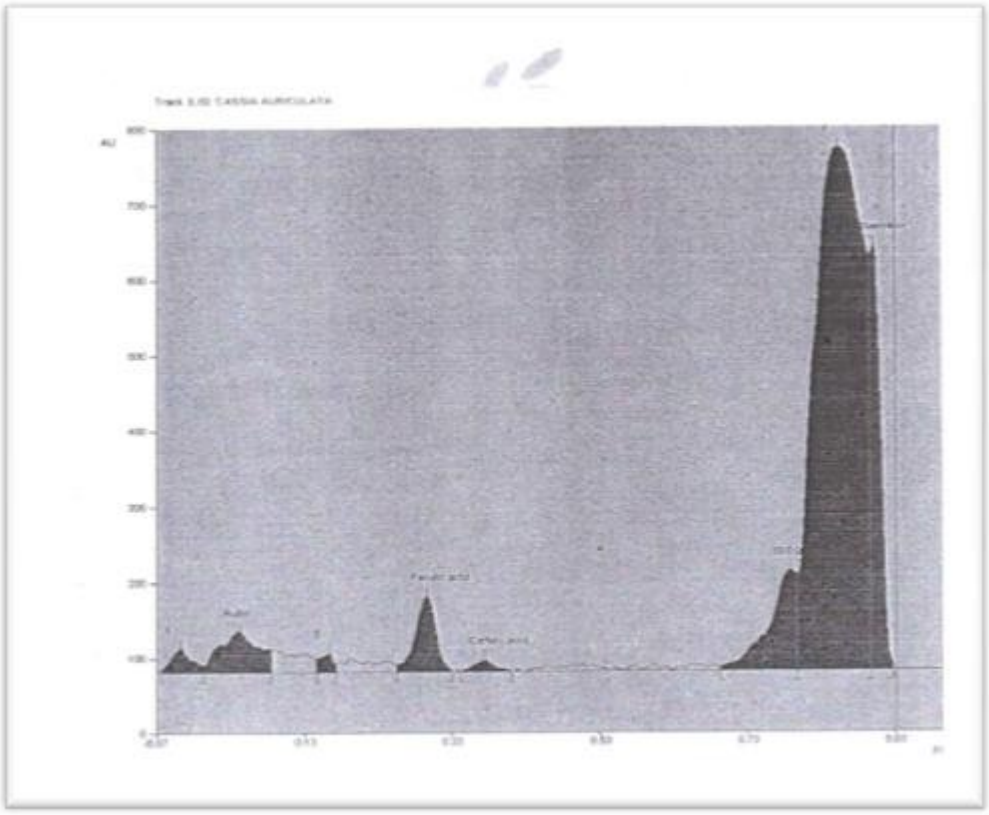
System suitability tests are performed to verify whether resolution and repeatability were adequate for the analysis. System suitability was determined by applying freshly prepared standard solution and next applying sample solution. Five times to the same chromatographic

plate. The plate was developed under the optimized chromatographic conditions then scanned and densitograms were recorded[5,6,7,8].The measured peak area for flavonoids and their retention factors were noted for each concentration of flavonoids(Table-1).

Table 1:HPTLC Method validation of flavonoids in *Cassia auriculata* leaf extract.

Sl.No	Flavonoids*	Rf	Peakarea	Concentration µg/g
1.	Galllic acid	Not Detected	Not Detected	Not Detected
2.	Rutin	0.05	2540.006	5.87
3.	Ferulic acid	0.30	2373.79	1.66
4.	Caffeic acid	0.38	341.52	0.09
5.	Isoquercitrin	0.79	4334.84	0.89
6.	Quercitrin	0.91	7535.6	2.57

Fig 1: Chromatogram of leaf extract of *Cassia auriculata* diagram



RESULTS AND DISCUSSION

Different compositions of the mobile phase for HPTLC were tested and the desired resolution of compounds, together with symmetrical and reproducible peaks, was achieved using methanol, toluene:ethylacetate, formic acid (5:4:0.2) as the mobile phase (fig 1).Peak purity tests of all

flavonoids compounds were performed by comparing the spectra at (540nm) of each in both the standard and the sample tracks[9,10,11] .

Different solvents of varying polarity have been applied for the extraction and methanol was found suitable for the most efficient extraction *of Cassia auriculata* derivatives. The method is particularly suitable for the analysis of a large number plant samples for the improvement of *Cassia auriculata* drug for these major and biologically important components[12,13].Results of the analytical programme will be presented elsewhere.

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