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HPTLC METHOD ANALYSIS OF FLAVONOIDS IN *DATURA STRAMONIUM* LINN. LEAF

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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., Gallic acid, rutin, ferulic acid, quercitrin in *Datura stramonium* Linn. The assay combines the separation and quantification of the analytes on silica gel 60 GF_{254} HPTLC plates with visualization under UV and scanning at 540nm. The method is rapid, simple and precise.

INTRODUCTION

Datura stramonium is a member of *solanaceae* family. The higher plants are among the most prominent natural resources. They provide nutrients, fiber, wood and many chemical compounds such as alkaloids[1]. The cell, tissue and organ cultures lead to changes in the production of chemical compounds, since the botanical organs and cells have the required capacity for the production of different secondary metabolites[2].

Among *solanaceae* plants *Datura stramonium* highly regarded by the workers, since it has a great resource of tropane alkaloids[3,4]. Botanical alkaloids are one of the important botanical products and form the major part of medicinal compounds[5].

MATERIALS AND METHODS

Collection of plant material

The leaves of *Datura stramonium* was collected from K. Paramathy, 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[6].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[7].

System suitability

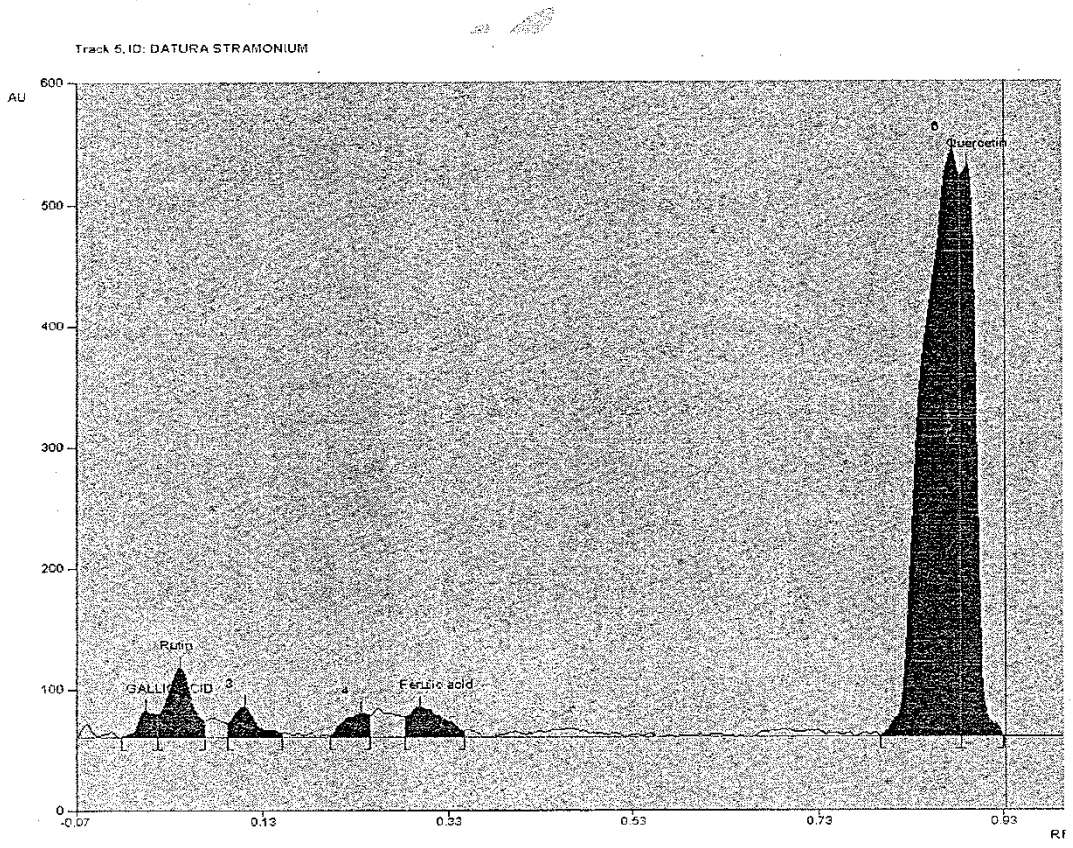
System suitability tests are performed to verify whether resolution and repetability were adequate for the analysis. System suitability was determined by applying freshly prepared standard

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[8,9,10,11].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 *Datura stramonium* leaf extract.

Sl.No	Flavonoids*	Rf Peak area	Concentration µg/g
1. Galllic acid	0.01	344.63	7.53
2. Rutin	0.05	1344.883	3.11
3. Ferulic acid	0.30	841.48	0.59
4. Caffeic acid	Not Detected	Not Detected	Not Detected
5. Isoquercitrin	Not Detected	Not Detected	Not Detected
6. Quercitrin	0.90	6923.3	2.36

Fig 1:Chromatogram of leaf extract of *Datura stramonium* 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[12,13,14] .

Different solvents of varying polarity have been applied for the extraction and methanol was found suitable for the most efficient extraction *of Datura stramonium* derivatives. The method is particularly suitable for the analysis of a large number plant samples for the improvement of *Datura stramonium* drug for these major and biologically important components[15,16].Results of the analytical programme will be presented elsewhere.

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