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CAFFIEC ACID FROM THE LEAVES IN *TECOMA STANS*: QUANTIFCATION AND VALIDATION BY USING HPLC

Sushmitha, Naira Nayeem*

Krupanidhi College of Pharmacy, Bangalore-35, India.

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***Correspondence for Author:**

Dr Naira Nayeem
Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Bangalore, India.
naira_64@yahoo.co.in

ABSTRACT

The aim of current study was to develop and validate a simple, reliable and fast analytical procedure for the determination of caffiec acid in *Tecoma stans* using high-pressure liquid chromatography (HPLC).Reverse phase Phenomenex (US) C18 Column 250 x 4.6mm I.D, 5µ Particle Size was used for the development. The mobile phase was prepared separately with 0.1N Potassium dihydrogen Phosphate buffer (mobile phase A) and methanol (mobile phase B). The pH of the mobile phase (A) was maintained at 5.2 with prior correction with 10% phosphoric acid. The flow rate was maintained at 1.0 ml/min with an injectable volume of 20 micro l. The method was validated for linearity accuracy and robustness. The retention time for caffiec acid was found to be 2.915. The method produced linear responses in the concentration range of 50-150%. The amount of caffiec acid was found to be 4.9mg/gm. Hence a rapid, simple, accurate and specific HPLC method was validated for quantitative estimation of caffiec acid present in the dried leaves of *Tecoma stans.*

Key words: *Tecoma stans*, caffiec acid, HPLC, Phytoconstituents.

INTRODUCTION

Tecoma stans which is commonly known as ginger thomas ,yellow bignonia, yellow cedar, yellow elder, yellow trumpet tree(English) and piliya(Hindi)[1]. *Tecoma stans* is a shrub or small tree, 5-7.6 m in height, often with a woody base, commonly found as a weed of waysides, on roadsides. It has many medicinal properties and is particularly used as anti-bacterial ,anti-inflamatory, anti proliferating activity[2,3]. The leaves of plant contains flavanoids like quercetin, rutin, alkaloids like tecostanine ,tecomine,tecominine. Phenolic acids like gallic acid, the other compounds like caffiec acid and vanillic acid[4,5].

However there are few reports pertaining to quantification and validation of the phytoconstituents. WHO has recognized quality control problem and has published guidelines to ensure the reliability and repeatability of research on herbal medicines like International standards and specification for identity, purity, strength and manufacturing practices[6] .Hence it was found worthwhile to carry out quantitative phytochemical analysis of plant. Caffeic acid is a hydroxy cinnamic acid which consists of phenolic and acrylic functional groups. Caffiec acid and its derivatives have been reported to possess a number of pharmacological activities like cancer cell proliferation, Neuroprotective, against nephrotoxicity ,anti-inflammatory, Antihyperglycemic and Antioxidant[7-13].

Hence a HPLC method has been developed in the present work for quantification of caffiec acid from methanolic extract of dried leaves of *Tecoma stans*.

MATERIAL AND METHODS

Reagents and Standards: Quantification of Betaine by HPLC includes the use of following materials, instruments and chemicals i.e.Methanolic extract of *Tecoma stans,* Potassium dihydrogen phosphate, methanol, caffiec acid was procured from Sigma Aldrich, Hyderabad, India. HPLC (Shimadzu), Phenomenex (US) C18 Column 250 x 4.6mm I.D, 5µ Particle, SPD-M20A Diode Array Detector.

Plant Material: Fresh *Tecoma stans* leaves were collected from botanical gardens of Krupanidhi College of Pharmacy. The Leaves were dried in the shade and powdered .About 200 gm of the powder was taken in a Soxhlet extractor and extracted with methanol . The solvent was recovered by distillation. The residue was concentrated, dried and stored in the desiccator for further experiment and analysis.

PREPARATION OF STOCK SOLUTIONS

Preparation of caffiec acid standard solution

Accurately about 10mg of caffiec acid was taken working as the working standard and transfered into 100ml volumetric flask. Dissolved and diluted to 100ml with mobile phase. From the above solution 1 ml was taken into 10 ml volumetric flask and the volumewas made up with mobile phase.

Preparation of sample solution

About 10 mg of plant extract and taken and transferred into 100ml volumetric flask. this was

then dissolved and diluted to 100ml with mobile phase. From the above solution 1 ml was taken into 10 ml volumetric flask and the volume was made up with mobile phase.

Chromatographic Condition

The mobile phase was prepared separately with 0.1N Potassium dihydrogen Phosphate buffer (mobile phase A) and methanol (mobile phase B) and mobile phase was eluted as per following gradient programming. The pH of the mobile phase (A) was maintained at 5.2 with prior correction with 10% phosphoric acid. The prepared buffer was filtered through a Millipore 0.45 µm membrane filter and ultrasonically degassed prior to use. The detection wavelength was set at 247 nm. The elution was done at a flow rate of 1.0 ml/min under ambient condition.

METHOD VALIDATION [14,15]

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Repeatability of precision was evaluated by analysis of six replicates of sample solution of same concentration.

Robustness of the method

By introducing small changes in the mobile phase composition, mobile phase volume and duration of mobile phase saturation, the effects on the results were examined. Robustness of the method was done in four replicates and % R.S.D peak area was calculated.

Specificity

The specificity of the method was ascertained by analyzing the standard drug and extract. Caffiec acid in the sample was confirmed by comparing the Rf values with that of the standard. The specificity of the method was evaluated with regard to interference due to presence of blank and any other excipients.

System suitability

The standard stock solution of caffiec acid and vanillic acid was injected six times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard Chromatograms obtained, by calculating the % RSD of retention times, tailing factor,

theoretical plates and peak areas from six replicate injections. The RSD for peak areas from six replicate injections of caffiec acid was found to be and0.3 %.

Accuracy

Accuracy is a measure of the closeness of agreement between the value which accepted either as convectional true value & the value found. Assay was performed in triplicate for various concentrations of Caffiec acid equivalent to 50%, 100 %,and 150 % of the standard amount was injected into the HPLC system per the test procedure. The average % recovery of caffiec acid was calculated.

Linearity and Range

The linearity of calibration curves (analyte to peak area ratio Vs concentration) in pure solution was checked over the concentration ranges of about 50%-150% (Assay concentration in mcg/ml) for caffiec acid.The total eluting time was less than 25min.

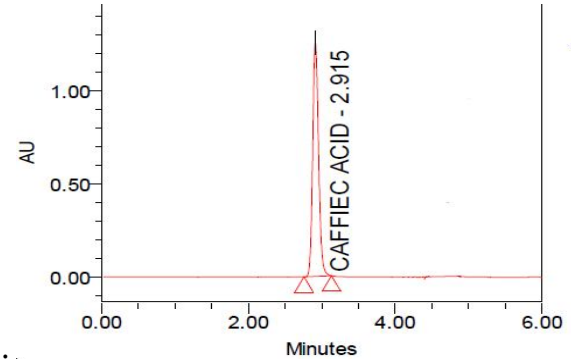
RESULTS AND DISCUSSION

The developed method was validated using different parameters like linearity accuracy and robustness. The best result was achieved by using Phenomenex (US) C18 Column 250 x 4.6mm I.D, 5µ Particle Size and mobile phase consisting of potassium dihydrogen phosphate:methanol with a flow rate of 1.0ml/min with SPD-M20A Diode Array Detector set at 247nm for caffiec acid. The retention time for caffiec acid was 2.915. The method produced linear responses in the concentration range of 50-150%. The amount of caffiec acid was found to be 5mg/gm. Results show that drug was clearly separated from blank and its excipients. Thus, the HPLC method was found to be selective. The method was performed and validated for accuracy, precision, linearity, and robustness as per ICH guidelines. The results of the Method development and validation of caffiec acid are as shown in the following tables and chromatograms.

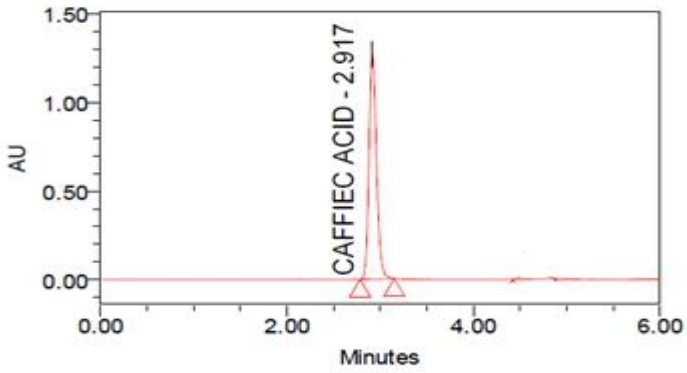
Table 1: Retention times in methanolic extract sample

PEAK	RET.TIME	AREA	HEIGHT	AREA%	HEIGHT%
1	2.917	16843	2773	2.683	5.467
2	4.646	1548007	43874	87.290	86.496
3	5.808	191068	2215	3.356	4.367
4	10.813	943	14	0.150	0.027
5	13.808	203248	1082	3.888	2.133

6		182025	766	2.633	1.511
TOTAL		2142134	50723	100.000	100.000

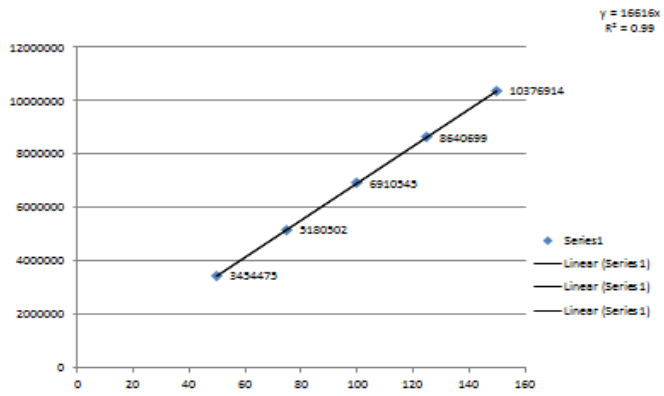


CHROMATOGRAM OF CAFFIEC ACID IN STANDARD



CHROMATOGRAM OF CAFFIEC ACID IN SAMPLE

LINERITY: The concentration, peak area and retention time for linearity of caffiec acid was calculated by regression analysis; the calibration curves were linear in the studied range. Linearity concentration was in the range of 0.32mg/ml-0.48mg/ml and r_2 values were found to be 0.995.



LINEARITY FOR CAFFIEC ACID

Accuracy

The accuracy of the method was determined by recovery studies. The recovery studies were performed by standard addition method at 50% for three times, 100% for three times,150% for three times. The method was found to be accurate as %RSD was well within the limits, which indicates that the method is accurate.

Table no 2: Data showing % recovery results for caffiec acid

Sample No.	Spike Level	% Recovery	% Mean Recovery	%RSD
1.	50%	99.86%		
	50%	99.86%	99.87%	0.02%
	50%	99.89%		
2.	100%	100.06%		
	100%	100.13%	100.10%	0.04%
	100%	100.13		
3.	150%	100.65%		
	150%	100.13%	100.27%	0.03%
	150%	100.03%		

Robustness

Slight changes in the flow rate and temperature did not have any effect on the method. It was observed that there were no significant changes in the retention time and area of the chromatograms which proved that the RP HPLC method developed was robust.

Table no 3: Data for robustness

S. No	Parameters	Normal Range	Changes
1	Flow Rate	1.0ml/min	±0.1ml
2	Temperature	25	±0.5°C

Table no 4: System suitability parameters for caffiec acid were as follows

Validation parameters	Caffiec acid
Mobile phase	Potassium dihydrogen phosphate :methanol
Flow rate	1ml/min
Detection wave length	247
Rt	2.917
Run time	30mts
linearity	R2=0.995
precision	%RSD<2

CONCLUSION

A rapid, simple, accurate and specific HPLC method for quantitative estimation of caffiec acid present in the dried leaves of *Tecoma stans* has been developed and validated. The data could be used as a QC standard. The validated parameters indicate that the developed method is quick, selectivity and economic. Hence the developed method is suitable for the estimation of caffiec acid in multicomponent herbal formulation

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