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Quantification and Validation of Vanillic acid in *Tecoma stans* Leaves by HPLC

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ABSTRACT

A simple, accurate, rapid and precise High performance liquid chromatographic (HPLC) method was developed and validated for the quantification of vanillic acid in *Tecoma stans*. Reverse phase Phenomenex (US) C18 Column 250 x 4.6mm I.D, 5µ Particle Size was used for the development. The mobile phase used was 0.1N potassium dihydrogen phosphate:methanol (60:40) with a flow rate of 1.0ml/min with SPD-M20A Diode Array Detector set at 247nm . The pH was maintained at 5.2. The retention time for vanillic acid was found to be 4.647. The method produced linear responses in the concentration range of vanillic acid 50%-150%. The amount of vanillic acid was found to be 5.1mg/gm. The developed method was then validated by using various validation parameters like accuracy, precision, linearity and robustness as per ICH guidelines. The method was found to be simple, specific, selective, and robust.

Keywords: *Tecoma stans, Vanillic acid, HPLC, quantification, validation.*

Introduction

Tecoma stans is commonly referred to as yellow bells, yellow cedar and yellow elder. It is an evergreen shrub 6-15 feet tall and 6-10 feet wide ,it consists of bright yellow, trumpet-shaped flowers [1,2].It has many medicinal properties and has been reported to show antidiabetic,anti-spasmodic,Antifungal,anti-bacterial, wound healing, anti-inflammatory, anti-proliferating activity, lipoxygenase, xanthine Oxidase and acetylcholinesterase inhibitory activity [3-7]. The leaves of plant contain flavonoids like quercetin, rutin, alkaloids like tecomine, tecominine, Phenolic acids like gallic acid, caffiec acid and vanillic acid [8-11].However there are few reports pertaining to quantative phytochemical analysis. Hence it was found worthwhile to carry out quantitative phytochemical analysis of plant. Vanillic acid is a dihydroxybenzoic acid derivative. Vanillic acid has been reported to possess antinociceptive, anti-inflammatory, hepatoprotective activity and also has beneficial effect on Ulcerative Colitis[12-16]. Hence a HPLC method has been developed in the present work for quantification of vanillic acid from methanolic extract of dried leaves of *Tecoma stans*. WHO 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 to solve the quality control problems [17].

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Materials and Methods

Reagents and Standards:
Methanolic extract of *Tecoma stans*, Potassium dihydrogen phosphate, methanol, standard vanillic acid which was procured from Sigma Aldrich, Hyderabad, India.

Plant Material: Fresh *Tecoma stans* leaves were collected from the botanical garden of Krupanidhi College of Pharmacy, Bangalore. The plant was authenticated by T.Manjulatha, HOD ,Department of Botany, JMJ College, Guntur. 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 analysis.

METHOD DEVELOPMENT:

Preparation of vanillic acid standard solution:

10 mg of standard vanillic acid was accurately weighed and transferred into 100ml volumetric flask, dissolved and diluted to 100ml with mobile phase. From the above solution 1 ml was transferred to 10 ml volumetric flask and make up the volume with mobile phase.

Preparation of sample solution:

Accurately weighed about 10 mg of plant extract and transferred into 100ml volumetric flask dissolved and diluted to 100ml with mobile phase. From the above solution take 1 ml into 10 ml volumetric flask and make up the volume 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) is 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 [18]:

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same sample under the conditions prescribed. Precision is usually considered at three levels: repeatability, intermediate precision and reproducibility. In this study repeatability of precision was evaluated by analysis of six replicates of sample solution of same concentration.

Robustness of the method

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

Specificity

Vanillic acid in the sample was confirmed by comparing the Rf values with that of the standard to ascertain the specificity. 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 vanillic acid was injected six times into HPLC system as per test procedure. The % RSD of retention times, tailing factor, theoretical plates and peak areas were evaluated from standard chromatograms for evaluating the system suitability parameters from six replicate injections.

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 vanillic acid equivalent to 50 %, 100 %, and 150 % of the standard amount, which were injected into the HPLC system per the test procedure. The average % recovery of vanillic acid was calculated.

Linearity and Range

The linearity of calibration curves in pure solution was checked over the concentration ranges of about 50 % - 150 % (Assay concentration in mcg/ml) for vanillic acid.

Results and Discussion

A new method was developed and validated using various parameters like accuracy, robustness, linearity and range.

The best result was achieved by using Phenomenex (US) C18 Column 250 x 4.6 mm I.D, 5µ Particle Size and mobile phase consisting of potassium dihydrogen phosphate: methanol

Figure 1: Chromatogram of vanillic acid in sample

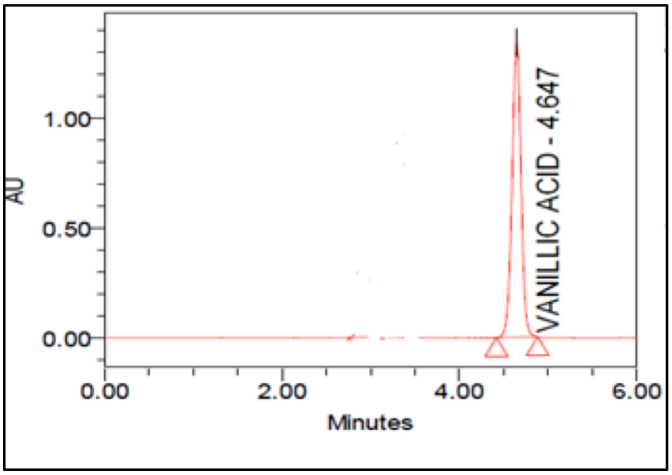
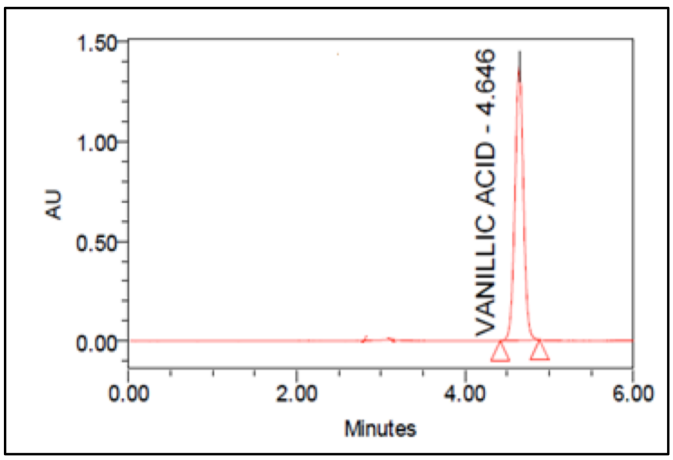


Figure 2: Chromatogram of vanillic acid in standard



(600:400) with a flow rate of 1.0 ml/min with SPD-M20A Diode Array Detector set at 247nm for vanillic acid. The retention time for vanillic acid is 4.647. The method produced linear responses in the concentration range of 50 % - 150 %. The amount of vanillic acid was found to be 5.1 mg/gm. The developed method was then validated by using various validation parameters. The figure shows that drug was clearly separated from blank and its excipients. The RSD for peak areas from six replicate injections of vanillic acid was found to be 0.2 %. The total eluting time was less than 25 min. The following tables and chromatograms depict the results of the method development and validation of vanillic acid.

Specificity and selectivity: This was evaluated by examining the samples for any interfering peaks. The specificity of the method was evaluated taking into consideration, any interference due to presence of blank and any other excipients. The figure shows no interference and that the drug was clearly separated from blank and its excipients. Thus the proposed HPLC method is selective.

Linearity: The concentration, peak area and retention time for vanillic acid was calculated by regression analysis. The method produced linear responses in the concentration range of vanillic acid 50 % - 150 %. The equations of the regression analysis were obtained R2 =0.999 for vanillic acid.

Accuracy: The accuracy of the method was determined by recovery studies which were performed by standard addition method three times each at 50%, 100% and 150%.The method was found to be accurate as %RSD and was within the limits. Hence the method was accurate. The recovery was found to be between 99-100%.

Table 1: Retention times in methanolic extract sample of *Tecoma stans*

PEAK	RET.TIME	AREA	HEIGHT	AREA%	HEIGHT%
1	2.241	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

Table 2: Data showing % recovery results for vanillic acid

Sample No.	Spike Level	% Recovery	% Mean Recovery	%RSD
	50%	101.18%		
1	50%	100.49%	100.34%	0.16%
	50%	100.36%		
	100%	99.98%		
2	100%	99.95%	99.91%	0.09%
	100%	99.82%		
	150%	99.76%		
3	150%	99.74%	99.66%	0.16%
	150%	99.48%		

Table 3: Data for robustness of the method

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

Figure 3: Linearity graph for vanillic acid

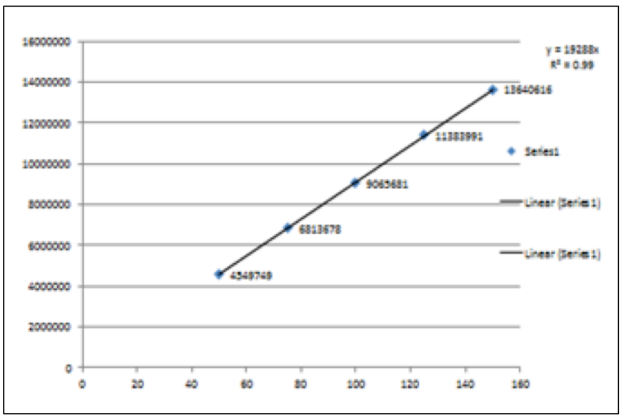


Table 4: System suitability parameters of vanillic acid

Validation parameters	Vanillic acid
Mobile phase	potassium dihydrogen phosphate:methanol
Flow rate	1.0ml/min
Detection wavelength	247
Rt	4.647
Runtime	30mts
Linearity	R2=.995
Precision	%<RSD2
Column temperature	Ambient

Robustness: When slight changes were made in the flow rate and temperature, no significant changes in the retention time and area were observed which shows that the method was robust.

Conclusion

A rapid, simple, accurate and specific HPLC method for quantitative estimation of vanillic acid present in the dried leaves of *Tecoma stans* has been developed and validated according to the ICH guidelines. The data could be used as a QC standard. Hence the developed method is suitable and can be used for the quality control and estimation of vanillic acid in multicomponent herbal formulation or traditional drugs.

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