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Original Research Paper

METHOD DEVELOPMENT AND VALIDATION OF CAPROIC ACID FROM *VANILLA PLANIFOLIA* PODS BY HPLC

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ABSTRACT

The aim of the current study was to develop and validate a simple, reliable and fast HPLC method for the determination of caproic acid in the methanolic extract of *Vanilla planifolia* using reversed phase high pressure liquid chromatography (RP-HPLC). The column used for separation was Agilent C18 Zorbax 150 x 4.6 mm, 5µ particle size. The mobile phase used is phosphate buffer having pH of 2.45 with methanol in the ratio 40 : 60 v/v. Isocratic elution technique was used with a flow rate of 1.0 ml/min and the injection volume was 10µl. Detection was done using a PDA detector at a wavelength of 269 nm. The method was validated in terms of system suitability, accuracy, precision, specificity, linearity range and robustness. The method produced linear responses in the range of 50-150%. Hence a simple, reliable and rapid method was developed and validated for the estimation of caproic acid in the methanolic extract of *Vanilla planifolia*.

Keywords: Caproic acid, *Vanilla planifolia*, Method development, Validation, HPLC.

INTRODUCTION

Vanilla planifolia, is the scientific name for the plant referred to as the "vanilla vine", "vanilla orchid" or as vanilla.[1] It has several medicinal properties recorded in literature these properties have been attributed to the phytoconstituents present in it.[2-5] Vanilla pods are found to contain vanillin, caproic acid, eugenol, phenol ether, vanillic acid, anisaldehyde, hydroxy benzoic acid, lactones, and carbohydrates, aliphatic, B-complex, calcium, potassium, magnesium, manganese, zinc and iron.[6-7] Caproic acid is one of the constituents present in the vanilla pods. It is also known as hexanoic acid. Caproic acid is the carboxylic acid derived from hexane. It is reported to possess anti-fungal, and anti-bacterial.[8-9] Review of literature has revealed that the various constituents of the plant like vanillin, vanillic acid, ethyl vanillin, 4-hydroxybenzyl alcohol, 4-droxybenzoic acid etc. have been analyzed using various techniques such as HPLC, RP LC with UV, LC (UHPLC) etc.[10] There are no reported methods for the quantification of caproic acid in literature. In the present study an attempt was made to develop a method for quantification and validation of the developed method using ICH guidelines.[11,12]

MATERIALS AND METHODS

The apparatus and chemicals used for the experiment were HPLC Water E2695, Photo diode array Detector Waters Model 2998, Sonicator Spectra Lab UBS 20, Weighing balance Sartorious, Methanol HPLC, Phosphate buffer, water HPLC grade were obtained from Sadha chemicals, Hyderabad. While standard Caproic acid was obtained from, Lara drugs Pvt Ltd. Hyderabad.

Collection and Extraction of Plant Material

The pods of Vanilla planifolia were collected from Chikmagalur district, Karnataka. The dried pods of Vanilla planifolia were subjected to repeated extraction and filtered. The filtered extract was then concentrated using a rotary evaporator. The powdered extract was sifted to achieve a uniform particle size.

TLC of Caproic Acid

Caproic acid was estimated by paper partition chromatography on Whatman no 1filter paper. The solvent system used was redistilled n-butanol saturated with an equal volume of aqueous 1.5N NH_3. The indicator solution used, was a solution of 0.04% (w/v) bromocresol purple in a 1: 5 (v/v) dilution of formalin in ethanol. The pH of this solution was adjusted to approximately 5.0 by the addition of 0.1 N NaOH. The Rf value of the plant extract was 0.5 which matched the Rf value of caproic acid standard.[13]

METHOD DEVELOPMENT

Preparation of Mobile Phase

The phosphate buffer was pipetted out (2 ml) and dissolved in 1 L of HPLC grade water. It was degassed using a sonicator. The pH of the resulting buffer was 2.45. HPLC grade methanol was degassed using a sonicator and poured into the solvent reservoir.

Preparation of Stock Solution

The plant extract was accurately weighed (100 mg) and transferred into clean, dry 50 ml volumetric flask and 10 ml of water was added. This was then sonicated for 20 minutes to dissolve the extract. The volume was made up to 50 ml using water. This solution was filtered and 5ml was pipetted out from it, into a 25 ml flask and the volume was made up to 25 ml using water.

Preparation of Standard Solution

The caproic acid reference standard were weighed accurately (50 mg) and transferred into 50 ml volumetric flask and 10 ml of water was added. The volume was made up to 50 ml using water. This solution was then filtered and 5ml was pipetted out from it, into a 25 ml flask and the volume was made up to 25 ml using water.

Chromatographic Conditions

The column used for separation was Agilent C18 Zorbax 150 x 4.6 mm, 5µ particle size. The mobile phase used is phosphate buffer having pH of 2.45 with methanol in the ratio 40 : 60 v/v. Isocratic elution technique was used with a flow rate of 1.0 ml/min and the injection volume was 10µl.

Validation of Developed Method

The developed method was validated according to ICH Q2 (R1) guidelines. HPLC method of analysis for *Vanilla planifolia* extract was carried out to quantify caproic acid. According to the ICH description, the following parameters for method validation were considered; Specificity, System suitability, Accuracy, Linearity, Precision and Robustness.

System Suitability

The standard stock solution of caproic acid was injected five times into HPLC system. The system suitability parameters were evaluated from standard chromatograms obtained, by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas. The RSD for peak areas is obtained from five replicate injections of caproic acid.

Accuracy

Assay was performed in triplicate for various concentrations of caproic acid. Amount equivalent to 50%, 100% and 150% of the standard amount was injected into the HPLC system. The average % recovery of caproic acid was calculated.

Precision

The precision of an analytical procedure expresses the closeness of agreement 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.

Specificity

The specificity of the method was evaluated with regard to interference due to presence of blank and any other excipients. The figure shows that drug was clearly separated from blank and its excipients.

Linearity

The linearity of calibration curves in pure solution was checked over the concentration ranges of about 50%-150% for caproic acid.

Robustness

The robustness of an analytical procedure is tested by measuring its capacity to remain unaffected by small, deliberate variations in the method parameters and provides an indication of its reliability during the normal use.

Variation of Flow Rate and Column Oven Temperature

The system suitability solutions were prepared and injected into HPLC system with ±0.5 ml flow and ± 0.5 °C of the column oven temperature used during method. The HPLC system suitability is evaluated by the test method for both flow and column oven temperatures.

RESULTS AND DISCUSSION

The developed method was validated using different parameters like linearity accuracy and robustness. Best results were obtained for separation by useing Agilent C18 Zorbax 150 x 4.6 mm, 5µ particle size. The mobile phase used was phosphate buffer having pH of 2.45 with methanol in the ratio 40: 60 v/v. Isocratic elution technique was used with a flow rate of 1.0 ml/min and the injection volume was 10µl. Detection was done using a PDA detector at a wavelength of 269nm. The method was validated in terms of system suitability, accuracy, precision, specificity, linearity range and robustness. The method produced linear responses in the range of 50-150%. The retention time for caproic acid is 2.915. The developed method for the analysis of caproic acid passed all the system suitability parameters. It showed good retention time for caproic acid. The USP tailing, USP resolution, USP theoretical plates, similarity factor and %RSD were within specified limit. The developed method was found to be accurate, precise, specific, showed linearity and met the acceptance criteria in robustness studies. The results of the Method development and validation of caproic acid are as shown in the following tables and chromatograms.

Method Validation

System Suitability

System suitability test is a Pharmacopoeia requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solution. The method developed for the analysis of caproic acid passed all the system suitability parameters. A good resolution for these compounds was seen with decreased retention time.

Accuracy

Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. Accuracy was performed in three different levels for caproic acid. Spiked known quantity of caproic acid standard at 50%, 100% and 150% level were used for analysis. Analysis of samples was done in triplicate for each level. The mean percentage recovery was within the limits so the method was found to be accurate.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The system precision was checked using caproic acid reference standard to ensure that the analytical system is precise. The retention time and area of six determinations were measured and RSD was calculated. The peak area was used to calculate the percentage assay to determine the precision of the method. The proposed method is precise as the % RSD value was within acceptance limit.

Specificity

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is the procedure to detect quantitatively, the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively, the analyte in presence of components that may be expected to be present in the sample matrix. On the basis of these chromatograms we can say that there is no interference of blank and placebo, it was also seen that there was no other interfering peak around the retention time of caproic acid so the method is specific.

Linearity

The concentration, peak area and retention time for linearity of caproic acid is as given in the table. The calibration curves were linear in the studied range and equations of the regression analysis were obtained: y = 16616 x, R2 = 0.99. The correlation coefficient was in acceptable limit.

Robustness

Robustness studies were done at different column temperatures and at different flow rate conditions and all parameters meet the acceptance criteria. 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.

CONCLUSION

Vanilla planifolia is a plant of medicinal importance. A new HPLC method was developed and validated according to ICH guidelines for the quantitative determination of caproic acid present in vanilla pods with new solvent system. . The developed method was found to be rapid, simple, accurate and specific. Hence the developed method can be used for the estimation of caproic acid the proposed HPLC method can be applied for quality control of several traditional drugs and formulations if caproic acid is present in them.

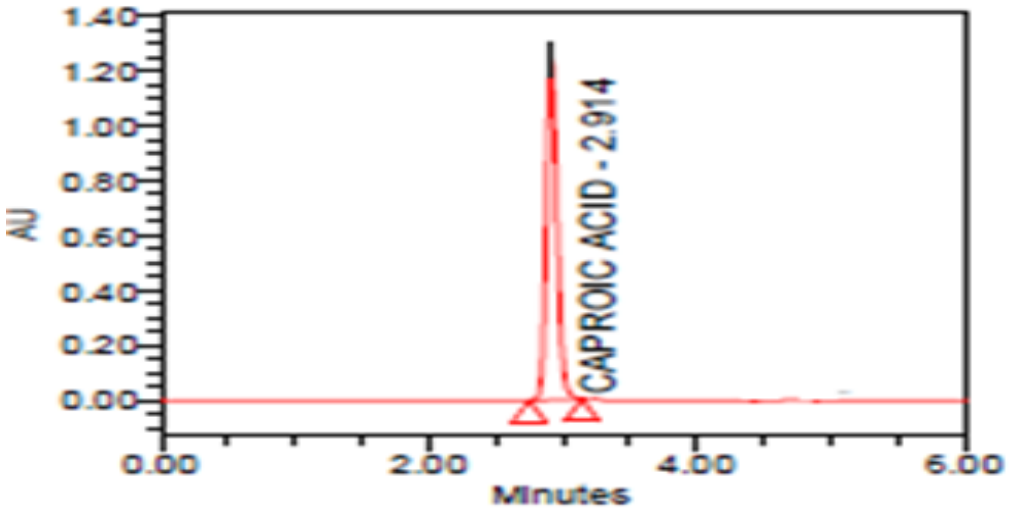

Figure 1: Chromatogram of caproic acid in standard

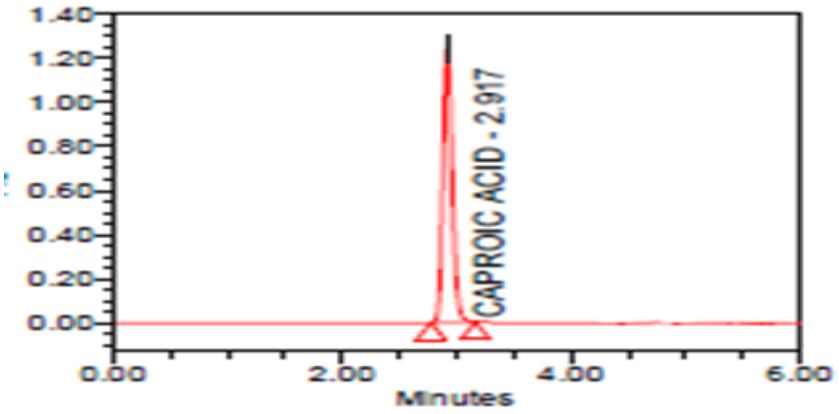

Figure 2: Chromatogram of caproic acid in sample

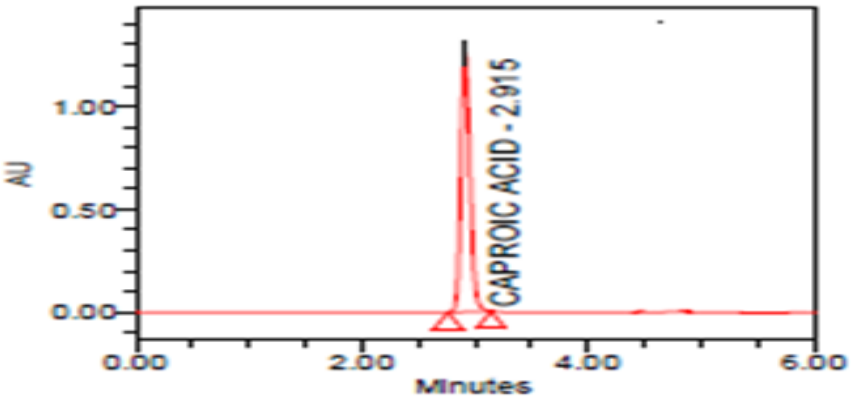

Figure 3: Chromatogram of caproic acid for specificity

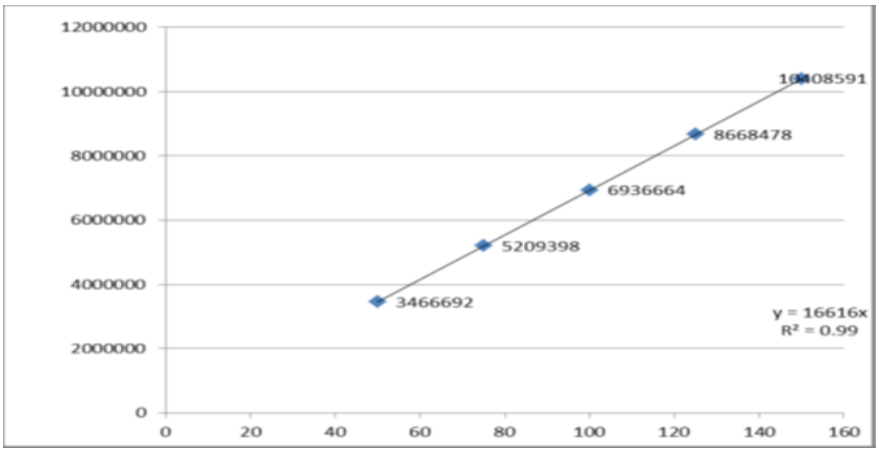

Figure 4: Linearity graph for caproic acid

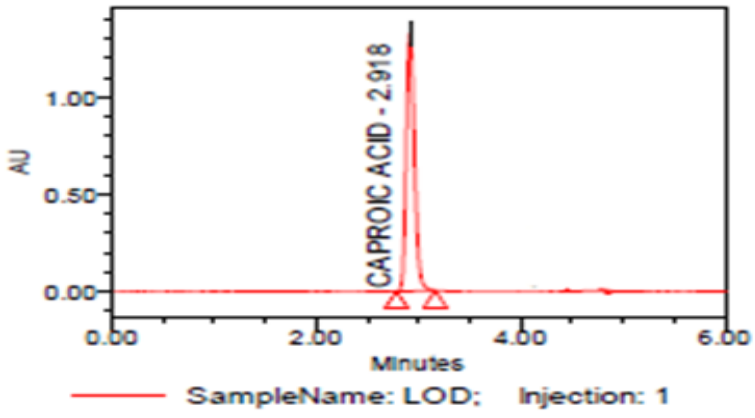

Figure 5: Limit of detection

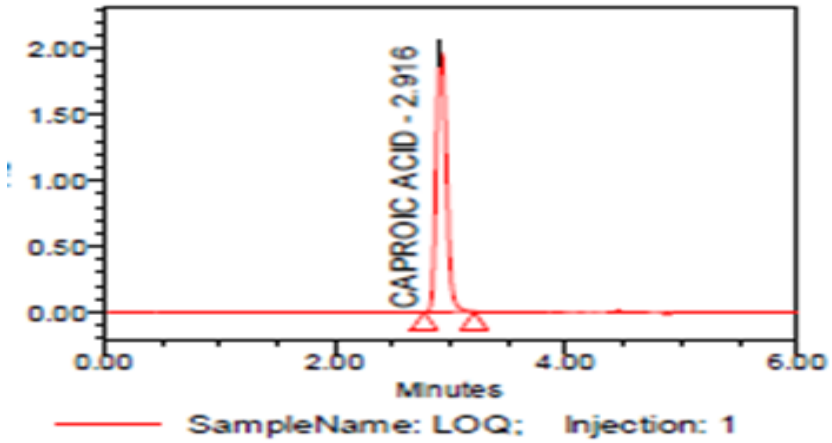

Figure 6: Limit of Quantitation

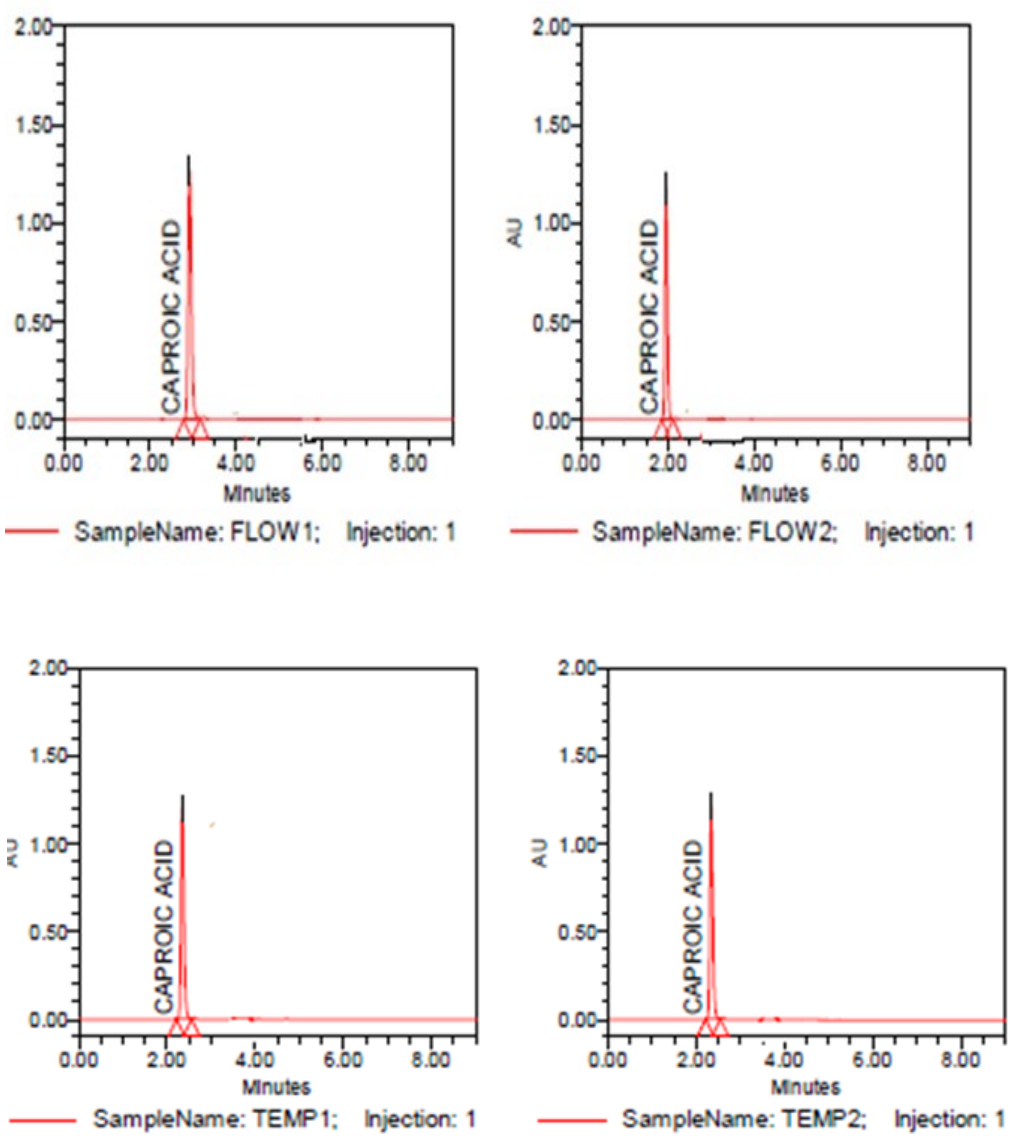

Figure 7: Chromatograms for robustness

Table 1: Retention time of caproic acid

Sample	RT	Area	USP Tailing	USP Plate count
Caproic acid	2.917	6996296	1.168	6478

Table 2: Data for System suitability injection for caproic acid

Trial	Sample Name	Inj	RT	Area	USP Tailing	USP Plate Count	s/n
1	caproic acid	1	2.914	6896926	1.178	6540	1508.829
2	caproic acid	2	2.917	6933111	1.187	6437	1541.403
3	caproic acid	3	2.917	6936639	1.154	6547	1348.177
4	caproic acid	4	2.918	6949486	1.173	6521	1369.738
5	caproic acid	5	2.919	6950509	1.170	6404	1416.055
Mean				6933334			
%RSD				0.3			

Table 3: System suitability parameters

	Validation Parameters	Specification	Results
1	Retention time	Not applicable	2.915
2	Tailing	Not more than 2	1.168
3	Theoritical plates	Not less than 2500	6478
4	Similarity factor	0.98	0.99
5	%RSD %0.3	Not more than 2.0%	0.3%

Table 4: Data for accuracy

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	%Recovery	%Mean
50%	50.00	3469647	99.000	99.19	100.19	
50%	50.00	3467273	99.000	99.12	100.12	
50%	50.00	3461606	99.000	98.96	99.95	
100%	100.00	6935200	198.000	198.25	100.12	
100%	100.00	6930572	198.000	198.12	100.06	100.15
100%	100.00	6931436	198.000	198.15	100.07	
150%	150.00	10407150	297.000	297.50	100.16	
150%	150.00	10402150	297.000	297.36	100.12	
150%	150.00	10466250	297.000	297.19	100.73	

Table 5: Linearity concentration data for caproic acid

Concentration (µg/ml)	Area	Percentage solution	LOD	LOQ
100	3466692	50		
150.00	5209398	57	2.941	9.804
200.00	6936664	100		
250	8668478	125		
300	10408591	150		

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