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Original Article

Validation of newly developed analytical method for standardization of cajanin using RP-HPLC method in prepared extract

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ABSTRACT

In this study, a simple, precise and accurate analytical method was developed and validated for identification of Cajanin using RP-HPLC method in prepared extract. Spectrophotometric determination was performed on a Perkin-Elmer UV-VIS Double Beam Spectrophotometer to know the maximum absorbance of the compounds. Chromatographic separation was performed using merck C18 analytical column (5 µm, 250 mm x 4.6 mm, i.d). Phosphate buffer at pH 3.5 and acetonitrile in the ratio of 30:70 v/v was considered to be suitable solvent system. The flow rate was maintained at 0.8ml/min. The effluents were detected by means of UV detector at 292nm. The calibration curves were linear at a range of 5 - 25µg/ml with significant correlation coefficient of 0.9966. The retention time was found to be at 4.14min. The method was validated according to ICH guidelines and was found to contain the %RSD values below 2% which shows that the method was precise, specific and accurate.

Keywords: Cajanin, Spectrophotometric method, Method development and validation, RP-HPLC method, ICH guidelines

INTRODUCTION

Cajanin is a plant derived compound extracted from *Cajanun*cajan. It is also found in coffee and coffee products. The chemical name is 3-(2,4-dihydroxyphenyl)-5-hydroxy-7-methoxychromen-4-one with a molecular formula of $C_{16}H_{12}O_6$. Cajanin is a very hydrophobic molecule, practically insoluble (in water), and relatively neutral.

MATERIALS AND METHODS

Plant Material and Reagents: The plant parts were collected from the local market and were

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shade dried for 15 days. After shade drying they are separately powdered and stored in well closed airtight containers for further use. Cajanin standard was purchased from Sigma-Aldrich Laboratories Ltd., Bangalore. HPLC grade methanol, acetonitrile and potassium dihydrogen orthophosphoric acid were purchased from E. Merck (India) Ltd., Worli, Mumbai, India.

Extraction: The shade dried and coarsely powdered plant parts were extracted with 80 % aqueous methanol by maceration at room temperature for 72 hrs. After completion of extraction, the extracts were filtered, concentrated to dryness. The residues were then stored in desiccator.

Apparatus and chromatographic conditions: Analysis using UV was performed on a Perkin-Elmer UV-VIS Double Beam Spectrophotometer. Data acquisition was made with software named as lambda 25 and 292nm is used as wavelength for the study. HPLC analysis was performed on a chromatographic system of Waters 2695 equipped with an auto injector with UV/Visible detector (UV-2489). A chromatographic separation was achieved on merck C18 analytical column (5 µm, 250 mm x 4.6 mm, i.d). Data acquisition was made with Empower 3 software. Analytical Balance (BSA224S-CW, Sartorius), Ultra Sonicator (Fast Clean) from Shimadzu were used for the study.

Preparation of standard and sample solutions: As the compound is freely soluble in ethanol standard stock solutions of Cajanin were prepared in ethanol at a concentration of 100µg/ml. The aliquots were prepared by using mobile phase. Accurately weighed and transferred 100mg of the extract into a 100ml clean dry volumetric flask containing 10ml ethanol. The solution was sonicated for about 10mins and then made upto volume with mobile phase. 10ml was pipette out from stock solution into separate 100ml volumetric flask and made upto the mark with mobile phase.

Determination of amount present: Measure the absorbance at 292nm and the amount present and percentage purity was calculated.

Validation of HPLC method [15]: The method was validated as per ICH guidelines for linearity, accuracy, precision, sensitivity, robustness, ruggedness and sensitivity.

Linearity: Linearity was performed by making serial dilutions at a range of 5-25µg/ml. Calibration curve was constructed by plotting concentrations against peak areas. Linearity was assessed by calculating slope, y-intercept and co-efficient of determination. The results obtained were shown in table 1 and figure 1.

Accuracy: The accuracy of proposed method was determined at three concentration ranges (50,100 and 150%). All studies were carried out in triplicates and the results obtained were tabulated in table 2.

Precision: The degree of closeness of agreement between a series of measurements obtained from multiple samplings of the same homogeneous sample under the prescribed condition was determined. The intra-day precision was performed by analyzing six replicate standard solutions on the same day, and inter-day precision was performed by analyzing a series of standard solutions for three consecutive days using the proposed HPLC method. The data obtained was represented in table 3**.**

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by a small, but deliberate variation in the method parameters and provides an indication of its reliability during normal usage. It was investigated under a change of conditions like deliberate changes in the change in flow rate and mobile phase ratio.

Ruggedness: Ruggedness is the degree of reproducibility of results obtained by the analysis of the same sample under a variety of normal test conditions i.e. different analysts, laboratories, instruments, reagents, assay temperatures, small variations in mobile phase, different days etc. (i.e. from laboratory to laboratory, from analyst to analyst).Ruggedness of the method was investigated under a variety of conditions including different analysts.

Sensitivity (LOD & LOQ): Detection limit (LOD) and quantification limit (LOQ) were calculated from standard deviation (σ) and slope values (S) which were obtained from calibration curve. The values were calculated using non instrumental method.

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically these might include impurities, matrix, degradants etc. It is evaluated by injecting the blank, placebo and the control sample solution prepared as per the proposed method to check for the interference of any peak at the retention time of Cajanin.

RESULTS AND DISCUSSIONS

Linearity was determined for Cajanin in the range of 5-25 µg/ml. The correlation coefficient value was 0.9996. The regression equation was found to be y = 17854x + 28991 which is shown in fig. 1 and the results were presented in the table 1. The percentage recovery of Cajanin was found to be 97.02%, 100.139% and 98.623% from 50%, 100% and 150% sample solutions respectively. The percentage recovery was found to be within the range which indicates that the proposed method was more accurate when compared to existing methods. The results were displayed in the table 2. Both inter-day and intra-day precision were carried out and the %RSD was found to be 0.2534% and 0.2817% respectively. The % RSD value indicates a good degree of precision within the specified limit. The results of precision studies were shown in the table 3. The LOD and LOQ value for Cajanin was found to be 0.044 µg/ml, 0.134µg/ml, respectively which resembles that the proposed method was sensitive. The relative standard deviation for the value of Cajanin obtained under deliberately modified chromatographic conditions should be less than 2%. The results obtained in the present study also indicate the method is robust. The results obtained are shown in table 4. Ruggedness of the method was investigated under a variety of conditions including different analysts. The results have shown that there is no significant change which indicates that the proposed method is having ruggedness. The chromatogram of Cajanin was shown in fig. 2. The specificity of the proposed method was illustrated in fig. 3 which shows that there is no interference of any peak at the retention time of Cajanin in the chromatogram of blank solution. Thus the proposed method was specific and selective.

Table 1: Linearity ofCajanin

S.No.	Conc. (ppm)	Peak Area
1	5	118289
2	10	198537
3	15	308833
4	20	388927
5	25	469456

Table 2: Accuracy results for Cajanin

Level	Amount added (ppm)	Total amount (ppm)	Amount found	% recovery
50%	5	15	14.55	97.02
100%	10	20	20.02	100.13
150%	15	25	24.65	98.62

Table 3: Precision results for Cajanin

Parameter	%RSD
Inter-day	0.253
Intra-day	0.281

Table 4: Robustness results for Cajanin

S.No	Parameter	Condition	AUC	%RSD
1	Flow change	0.6ml/min	198565	0.251
		1ml/min	199257	0.254
2	Wavelength	290nm	198537	0.275
		294nm	197532	0.281

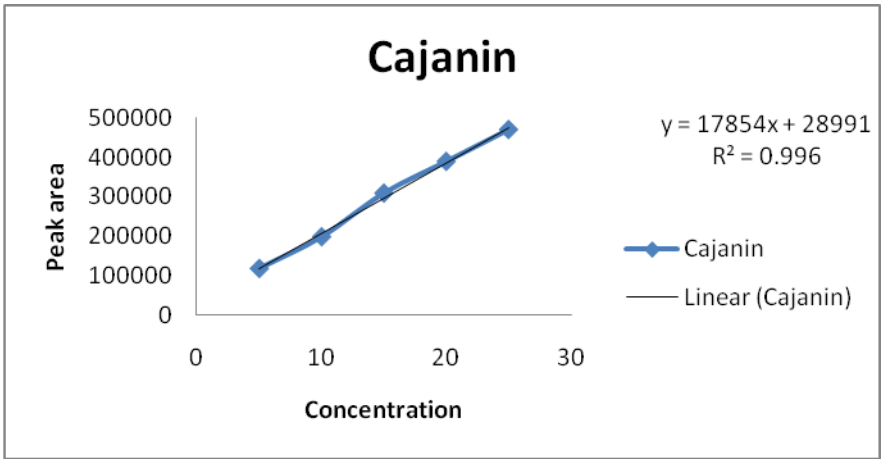

Fig 1: Calibration plot of Cajanin

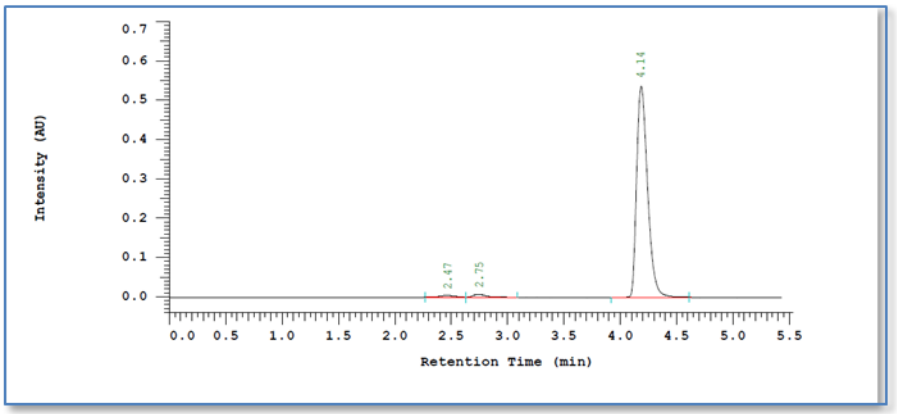

Fig. 2: Typical HPLC chromatogram for Cajanin

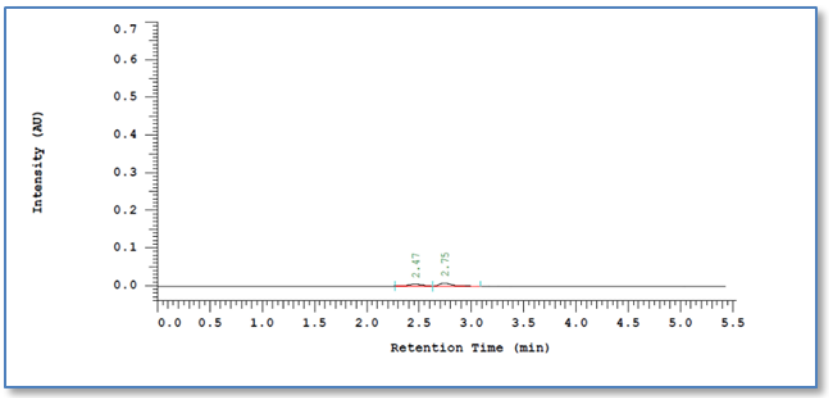

Fig. 3: Typical HPLC chromatogram for blank

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