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Development and validation of HPLC method for estimation of betulinic acid

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ABSTRACT

A simple, specific, and economic HPLC method has been developed to estimate betulinic acid. The λmax of betulinic acid was found to be 210 nm. Linearity in the concentration range of 25-150 µg/ml was found to be exhibiting a good correlation coefficient (R^2 =0.999). The developed method was validated statistically to demonstrate linearity, accuracy, precision, LOD as well as LOQ. The validation parameters were selected as per the ICH [Q2 (R1)] guideline. The results of the study proved the applicability of the present method in routine analysis of betulinic acid.

Keywords: Betulinic acid, HPLC, Triterpene acid, ICH, LOD.

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INTRODUCTION

Betulinic acid is the pentacyclic triterpenoid, which occurred naturally then exhibits potent anticancer activity. It is a 3b-hydroxy-lup-20(29)-en-28-oic acid and broadly dispersed pentacyclic lupane-type triterpene [1, 2].An accurate and sensitive RP-HPLC method was reported for the determination of betulinic acid in white birch bark. The linearity of the drug was recorded and separation was carried out by Diamonsil C18 reversed-phase column (250mm×4.6mm i.d.) with a 5μm pore size column (Dikma Technologies Corporation, Beijing, China). The mobile phase was acetonitrile-water (86:14, v/v). Betulin and betulinic acid were quantified by UV detector at λ = 210 nm. Flow rate and injection volume were 1.0 ml min−1 and 20μl, respectively. HPLC method was described for the simultaneous Quantitation of betulinic acid as well as ursolic acid from the dried stem bark of plant [3-4]. The developed HPLC method was validated by using ICH guidelines. The mobile phase was used acetonitrile: water (88:12). The injection volume was 10μl. The detection was carried out by using a PDA detector at λ = 210 nm [4-5]. In the current study, efforts were made in developing a simple, specific, and economic HPLC method to determine betulinic acid and validate it as per the ICH guidelines.

MATERIALS AND METHODS

Materials: The drug of choice in the current examination, Betulinic acid was attained from Aktin Chemicals (Chengdu, China) as a gift sample. Methanol (purity 99.7%), acetonitrile (purity 99.8%) of HPLC grade were procured from Merck Pvt. Ltd, Mumbai, India. All supplementary chemicals were of analytical grade.

Preparation of standard solution: The standard solution of betulinic acid was prepared by dissolving appropriate weights in methanol to make 1000 μg/ml concentration and stored in the refrigerator. The working solution was prepared freshly every day by an appropriate dissolution of the standard solution in methanol.

Preparation of sample solutions: The standard solution of betulinic acid was prepared by dissolving appropriate weights in methanol to make 1000 μg/ml concentration and stored in the refrigerator. 6 sample solutions were prepared in the range 25-150 μg/ml.

Chromatographic Conditions (HPLC)
HPLC analysis was performed by (Agilent Technologies, 1200 series).A 4.5 × 250 mm C-18 m Qualisil column was used for the chromatographic separation. The injection volume was 50μL. The analysis was performed using a solution of Acetonitrile: Water (70:30) as a mobile phase in a flow rate of 1 mL/min, using a PDA detector at 210 nm. Peak area and retention time of the above solutions was measured.

Method Validation
Linearity and Range
Preparation of calibration curve of betulinic acid: Each standard solution betulinic acid in the concentration range of 25-150 μg/ml was injected in triplicates into the HPLC system, under optimized chromatographic conditions.

System Suitability
LOD & LOQ: Limit of detection (LOD) and Limit of quantitation (LOQ) for the assay was calculated using the following formula:
LOD=3.3× (standard deviation of the y-intercept of the regression line/slope of the calibration curve)
LOQ = 10 × (standard deviation of the y-intercept of the regression line/slope of the calibration curve)

Accuracy: Accuracy of a developed method carried out by performing a recovery study using standard addition method, in which drug was added at three different concentrations.

Precision study: The precision study of the method was performed by intraday and inter-day variation study. The intraday and inter-day precision was performed by determining the absorbance of 3 replicates of different concentrations of drugs at three different periods of the same day and on another day. The result of precision studies was expressed in terms of %RSD.

Ruggedness and Robustness: The ruggedness of the method was determined on carrying out the method by two different analysts and Robustness of the method was determined by change in mobile phase composition Acetonitrile: Water (70:30) to Acetonitrile: Water (69:31) and flow rate change from 1 mL/min to 1.1 mL/min.

RESULTS AND DISCUSSION

Method Validation
Linearity and Range: The λmax of Betulinic acid was 210 nm. It was found to be linear within the concentration range of 25-150 μg/ml and displayed a correlation coefficient of 0.999. The result of regression analysis is given in Table 1.

System Suitability
LOD and LOQ: The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.24μg/ml and 0.74μg/ml respectively (Table 2) which indicates that the proposed UV method is sensitive.

Table 1: Result of Linearity of Betulinic acid

Betulinic acid	Concentration Range	Regression Equation	Regression coefficient
Sample (210 nm)	25-150 µg/ml	y = 5521.x – 2341.	0.999

Table 2: Result of LOD and LOQ of Betulinic Acid

Drug	LOD (µg/ml)	LOQ (µg/ml)
Betulinic Acid	0.24	0.74

Accuracy: Results of the recovery study were within the range of 99.66-100.40 % indicating that the developed method is an accurate method for the determination of betulinic acid.

Precision: The developed method was found to be précised as the % RSD values for intraday and the inter-day precision study was found within acceptance criteria.

Ruggedness and Robustness: It was observed that there were no significant changes in the results, which demonstrated that the developed method is rugged and robust. The resolution of components from the sample solution did not change much due to the change in alteration in methods.

Chromatogram of Betulinic Acid: From below figures 1 and 2, I found that there was no peak at the retention time of betulinic acid. The retention point of betulinic acid was found to be 11.047 from figure 3. The proposed method was found to be precise, economical, and accurate.

CONCLUSION

The developed method was found to be simple, accurate, precise, specific, and rapid. This method can be applied for routine quantitative analysis of Betulinic acid in bulk form. There was no effect of any blank and placebo trials on the retention time of betulinic acid. The validation parameters were validated as per the ICH [Q2 (R1)] guideline.

Table 3: Result Accuracy study of Betulinic Acid

Initial Amount (µg/ml)	Amount of standard drug added (µg/ml)	Amount of standard drug added (%)	Amount Recovery (µg/ml)	% Drug Recovered Mean ± SD	%RSD
75	60	80	69.80	99.66 ± 0.65	0.54
75	75	100	75.12	100.16 ± 0.30	0.58
75	90	120	90.36	100.40 ± 0.70	0.33

Table 4: Result of Intraday Assay of the proposed method

Precision	Amount Taken (µg/ml)	% Amount found (µg/ml) Mean ± SD	%RSD
Intra-day (n=3)	75	173.41 ± 1.31	0.91
	100	231.72 ± 1.42	0.65
	125	290.89 ± 1.55	0.84

Table 5: Result of Interday Assay of the proposed method

Precision	Amount Taken (µg/ml)	% Amount found (µg/ml) Mean ± SD	%RSD
Inter-day (n=3)	75	174.01 ± 1.20	0.84
	100	231.15 ± 1.39	0.80
	125	291.07 ± 1.58	0.81

Table 6: Result of Ruggedness of the proposed method

Analyst	% Amount found (µg/ml) ± SD (n=6)	%RSD
I	73.29 ± 0.48	0.65
II	72.29 ± 0.42	0.55

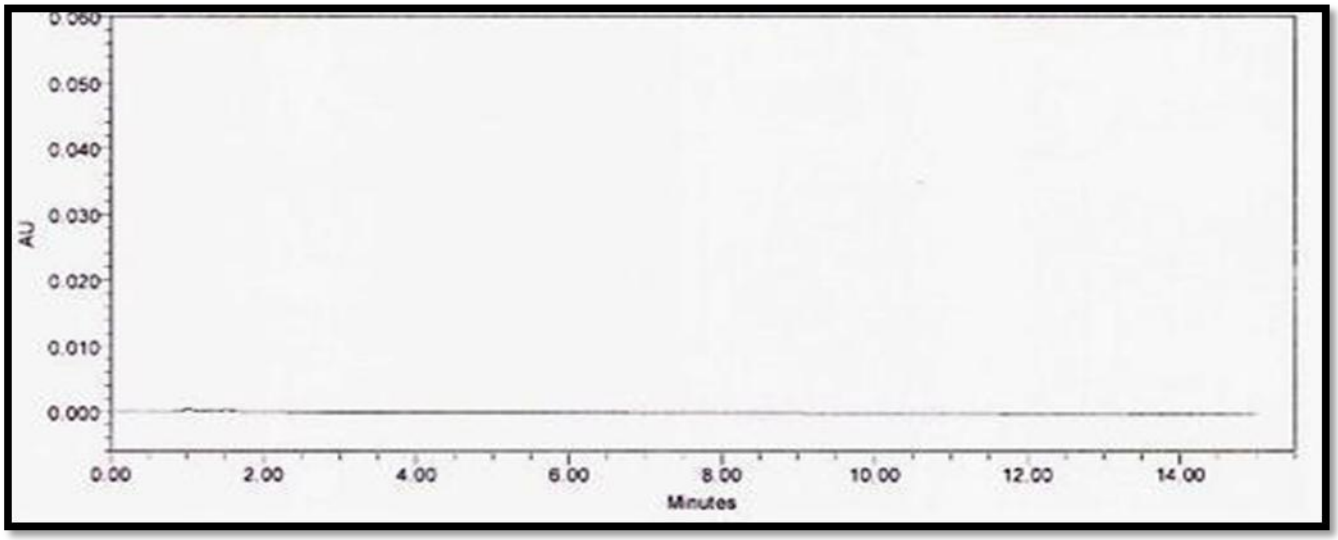

Figure 1: Chromatogram of Blank Preparation

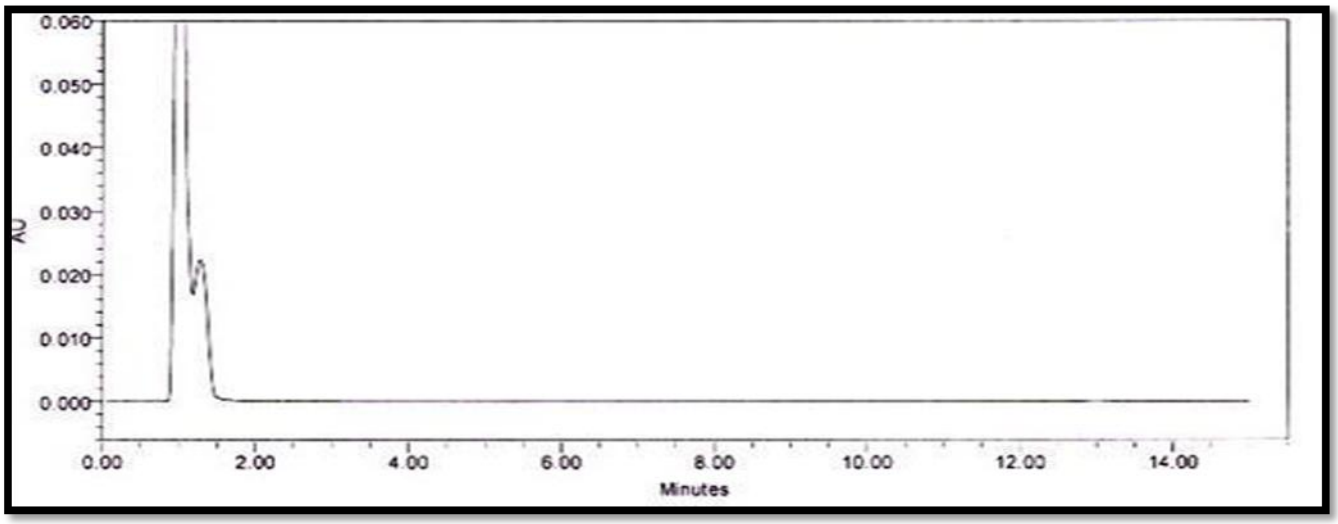

Figure 2: Chromatogram of Placebo Preparation

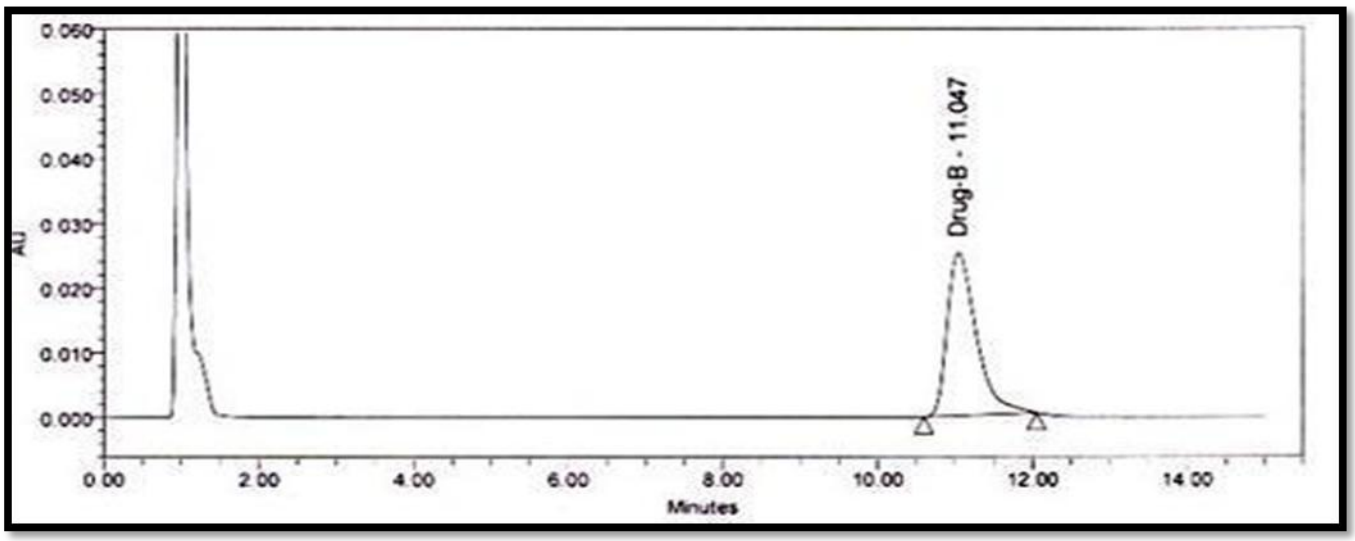

Figure 3: Chromatogram of Sample Preparation (Betulinic Acid)

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