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A SIMPLE AND PRECISE METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF BETA CARYOPHYLLENE

Gajbhiye Prashik O.*, Jawarkar Shailesh G., Manwar Gaurav G., Kalamb Vaishnavi S., Malthankar Akash S.,1
Department of Quality Assurance,
Vidyabharati College of Pharmacy, C K Naidu Road, Camp, Amravati, Maharashtra, India-444 602

Abstract: Beta-caryophyllene (BCP) is, the sesquiterpene hydrocarbon (E) that's one of the maximum studied and promising herbal compounds, a unstable oil determined in lots of ingredients and spices plant life which include clove, cinnamon, cannabis, and black pepper. It is used as meals components and cosmetics and taken into consideration to be safe. As in keeping with taste and extract manufacturers, affiliation and USFDA additionally authorized BCP as a flavoring agent in meals. The developed method was based on the best suitable carrier solvent system consisted of methanol: water: orthophosphoric acid (98:2:0.1 %v/v). Sample solution (3X10-2 M BCP) was prepared in this solvent and injected to the instrumental system at a flow rate of 1 ml/min. The signals were detected by a UV detector at 210 nm RP-HPLC leave-taking and quantification of the drug on Hypersil BDS RP (250 x 4.6 mm, 5µ) column using a mobile phase consisting methanol: water: OPA (98:2:0.1, % v/v) at flow rate of 1 ml/ min. Quantitation was attained with UV detector at 200-400 nm based on peak area with linear calibration curves at concentration ranges 25-75µg/ml for the drug. The retention time of Beta Caryophyllene was 4.08 min. The method had been successively functional to pharmaceutical formulation. No chromatographic intrusion from the ingredients was introduced. The developed method was verified for linearity, recovery and specificity. The intra and inter-day precision and accuracy values had been with inside the recognition variety as consistent with ICH guidelines.

KEYWORDS: Beta-Caryophyllene (BCP), Antioxidant, RP-HPLC, Method Validation.

I. INTRODUCTION[1,2,3]

Beta-caryophyllene (BCP) is, the sesquiterpene hydrocarbon, chemically, (1R,4E,9S)-4,11,11-trimethyl-8-methylidenebicyclo [7.2.0] undec-4-ene is a widely distributed in essential oils of various plants. Several biological activities are attributed to β-caryophyllene, such as anti-inflammatory, antibiotic, antioxidant, anticarcinogenic and local anesthetic activities.

Beta caryophyllene is an odoriferous bicyclic sesquiterpene found in various herbs and spices. it was found that beta-caryophyllene is a ligand of the cannabinoid receptor 2 (CB2). Activation of CB2 will decrease pain, a major signal for inflammatory responses and an endogenous CB2 selective agonist, because of selective binding it does now no longer indicates psychotropic aspect results and additionally displayed pharmacological sports which include antidiabetic, anti-inflammatory/oxidant, anticancer, and additionally display PAR-γ activation that's answerable for insulin- secreting pastime and in combinational remedy it's far extra powerful.

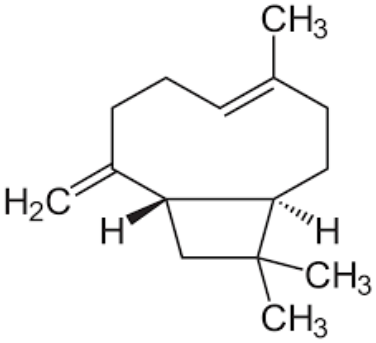

Figure No. 1. Structure of Beta Caryophyllene

There are a few articles to be had describing investigative tactics for BCP. This evaluation paper describes the easy, specific and sensitive HPLC approach that is recuperated and attested(documented) technique for estimation of Beta caryophyllene as in step with ICH (International Conference on Harmonization) guidelines. Here, we evaluation provided novel approach for the decisiveness of beta caryophyllene which makes use of a totally reasonably-priced solvent device on a Hypersil BDS C18 analytical column. This form of device primes to stepped forward preservation, very serrated and proportioned height figures and shows excellent selectivity for Beta Caryophyllene.

Instrumental and non-instrumental analytical methods are two types of methodologies. Spectroscopy, chromatography, mass spectroscopy, Calorimetry, microscopy, electrochemistry, environmental analysis, forensic, crystallography, and other instrumental approaches are only a few examples. Chromatography is used to separate and purify test materials. The mixture to be separated is put on a stationary phase (solid or liquid), and a pure solvent (water or any other gas) is allowed to gently pass across the stationary phase, carrying the components separately based on their solubility in the pure solvent. Chromatography differs from most other physical and chemical separation procedures in that two mutually incompatible phases, one stationary and the other mobile, are brought into contact. After being placed in the mobile phase, a sample is passed through a column (manifold) that contains a dispersed stationary phase. The growing quantity of drugs and drug combinations on the market needs the creation of analytical techniques to check their quality. The methods must be designed so that they take less time to create and deliver the most precise and reliable outcomes feasible. Beta caryophyllene in solid oral dosage forms, which was recently authorized by the USFDA, is the current research dosage form. The purpose of this project is to develop and verify an ICH-compliant RP-HPLC technology for estimating beta caryophyllene in bulk and pharmaceutical dose forms.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

The Beta caryophyllene changed into procured from Natural Aroma Pvt. Ltd. Other analytical reagents encompass orthophosphoric acid (OPA) of HPLC grade changed into bought from the Merck Life Science Pvt. Ltd., Maharashtra. MEOH and water of HPLC grade have been bought from the Finar Pvt. Ltd., Mumbai. Analytical grade reagents and chemical substances have been used on this study.

2.2. Selection of wavelength:

The pattern changed into scanned from 200-400 nm with UV detector. The Wavelength decided on for evaluation selected changed into 210 nm on foundation of suitable depth of Beta Caryophyllene.

2.3. Instrumentation and Chromatographic Conditions:

The analysis of the drug was carried out on HPLC model no acme 9000, Acme pump, UV/vis detector and running autochro-3000 software. A reverse phase Hypercell BDS-RP (250 x 4.6 mm, 5 µ) column equilibrated with mobile phase Methanol: Water: OPA (98:02:0.1% v/v) was used. Mobile phase flow rate was maintained at 1.0 ml/min and effluents were monitored at 210 nm. The temperature of the system was maintained at 300 C. The sample was injected using a 10 µl fixed loop, and the total run time was 10 min.

2.4. Preparation of Standard Stock Solution

Prepare a Standard Stock Solution (SSS-I) of by adding 15 mg of beta Caryophyllene in 50 ml volumetric flask & add diluent (methanol: water: OPA, 98: 2: 0.1 %v/v), mix for 2 minutes and make the volume with diluent. (Conc. of beta caryophyllene = 300 µg/ml). Then add 1.0 ml of ASSS-I in 10 ml volumetric flask and add diluent and vortex and make up the volume with diluent. (Conc. of Beta caryophyllene = 30 µg/ml).

2.5. Calibration of standards:

The mobile phase and stationary phase were allowed to equilibrate until baseline was achieved. Pipette 10 mg of beta caryophyllene into a 10 ml volumetric flask from the freshly made standard stock solution. It was then diluted with the mobile phase. To reach the final concentration, 0.5, 1.0, 1.5, 2.0 and 2.5 of the solution were pipette out into a 10 ml volumetric flask, and volume was brought up to 10 ml with the mobile phase. Beta caryophyllene (50, 100, 150, 200 and 250 g/ml). Samples were injected and peaks were recorded at 210 nm, as shown in the graph plotting drug concentration versus peak area.

2.6. Validation of A Method for Analysis of Beta Caryophyllene:

Specificity & Assay:

Individual samples of Beta Caryophyllene were prepared of 300 µg/ml and peaks were for identified from Retention Time. Blank was injected to ensure there is no blank peak interfering with the main analyte peaks. (Table No. 1)

Linearity:

The linearity of an analytical technique is determined by the mathematical treatment of test results obtained from sample analysis with analyte concentrations across a given range. Visual representation of area as a function of analyte concentration. Percentage curve fittings are determined.

Accuracy (recovery):

The accuracy is measured using a known amount of analyte supplied to the system. The accuracy of the test findings is calculated using the proportion of analyte recovered by the assay.

Precision:

Intra-day precision:

Sample solutions containing 10 mg of beta caryophyllene three different concentration (80 µg/ml, 100 µg/ml, 120 µg/ml). beta caryophyllene were determined three times on the same day and %R.S.D. was calculated.

Inter-day precision:

Sample solutions containing 10 mg of beta caryophyllene three different concentration (80 µg/ml, 100 µg/ml, 120 µg/ml). Beta caryophyllene were determined three times on the same day and %R.S.D. was calculated.

Detection Limit:

By using the below formula, it can be calculated

$$DL = 3.3\sigma/S$$

Where, σ = the S.D. of the y-intercepts of regression lines.

S = the slope of the calibration curve.

The calibration curve can be used to estimate the slope S and S.D. was used should be calculated from the y-intercepts of the regression line in the calibration curve.

Quantitation Limit:

It was calculated by the below formula.

$$QL = 10\sigma/S$$

Where, σ = the S.D. of the y-intercepts of regression lines.

S = the slope of the calibration curve.

The calibration curve can be used to estimate the slope S and S.D. was used should be calculated from the y-intercepts of the regression line in the calibration curve.

3. RESULTS AND DISCUSSION:

3.1. Specificity:

Specificity for the beta caryophyllene was found as mentioned in the following table no. 01 and mentioned figures 02 & 03. The % Assay of beta caryophyllene for this developed technique was found to be 97.50.

Table No. 1: Specificity and Assay:

Sample (API Name)	**Beta caryophyllene**
Standard Solution	831.2832
% Assay	99.52

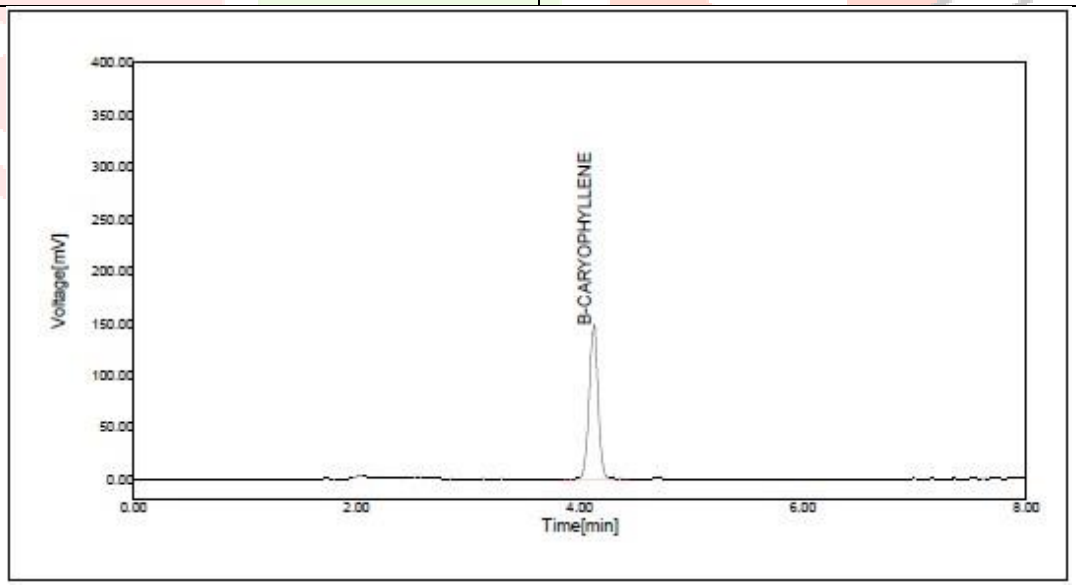

Fig. 02: Chromatogram of Working Standard of β-Caryophyllene

3.2. Linearity:

The data revealed a linear association between peak areas and concentrations in the ranges of 25.05-75.15 ug/mL for Beta caryophyllene. The linear equation for Beta caryophyllene was y = 16.08x – 11.54, where x represents the concentration of the drug and y represents the peak area. The correlation coefficient was 9.000 and the calibration curve of beta caryophyllene is depicted in figure 4. Linearity data for the beta caryophyllene is represented in table no. 02.

Table No. 02: Linearity and range:

% Conc	Conc (ug/ml)	BCA AREA
50%	25.05	406.8234
75%	37.58	617.6392
100%	50.10	830.7294
125%	62.63	1017.2466
150%	75.15	1214.2703

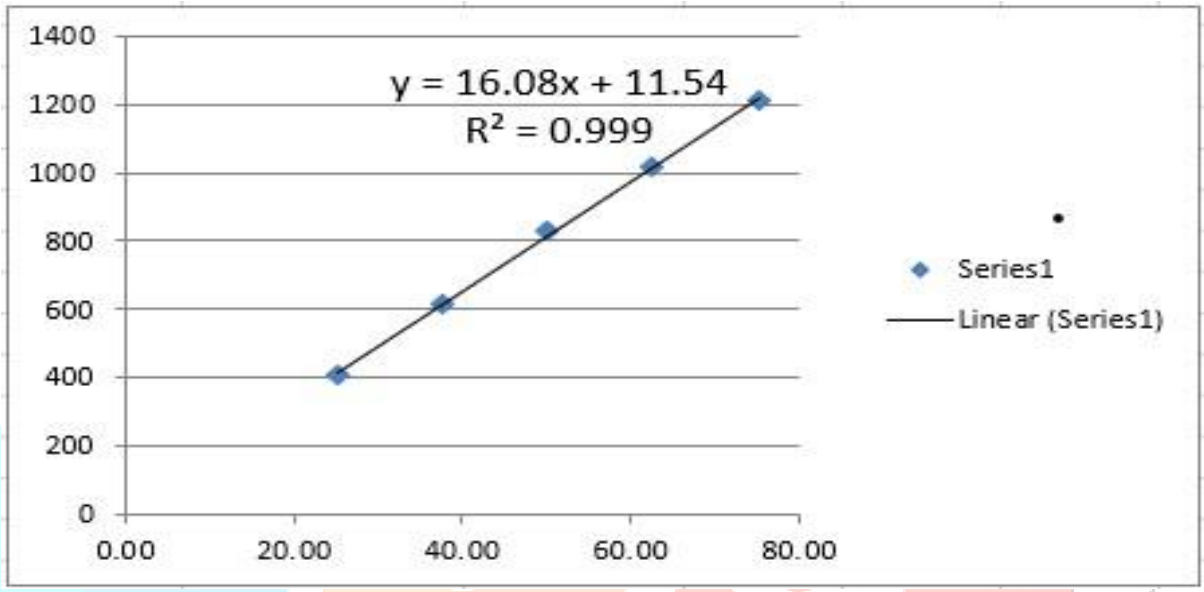

Fig. 04: Calibration curve (Linearity) of Beta caryophyllene

3.3. System Suitability:

The system suitability was assessed by six replicate injections of the mixture containing as internal standard. The resolution, peak asymmetry, number of theoretical plates and HETP were calculated are represented in table no.03.

Table No. 03: System Suitability:

Sr. No.	Parameters	Beta caryophyllene
1	Theoretical Plates	19517
2	Retention Time	4.08
3	Asymmetry factor (TF)	0.93

3.4. Accuracy:

Validation of Recovery Studies which is done statistically is represented in table no. 04 which shows the effects of Beta Caryophyllene. Recovery studies were carried out to ensure that the developed approach was accurate. The solution which is previously analyzed means the specific standard drug concentration 80, 100, and 120 percentages were mixed and then allowed for the recovery analysis. Recovery experiments at various concentration levels are used to verify the accuracy of the RP-HPLC and UV Spectrophotometric methods. The recovery rate was determined to be between 97 and 103%.

Table No. 04: Accuracy of Beta Caryophyllene:

Level of Recovery	Sample No. (ug/ml)	Spiked Amount	Spiked Amount wrt Sample	Area ATV	Amount Recovered (ug/ml)	%Recovery	% RSD
80%	Reps1	40.10	40.10	666.9625	40.32	100.56	0.87
	Reps2	40.20	40.20	671.8127	40.62	101.04	
	Reps3	40.00	40.00	657.2418	39.74	99.34	
100%	Reps1	50.20	50.20	822.4567	49.73	99.06	0.46
	Reps2	50.10	50.10	815.7165	49.32	98.44	
	Reps3	50.10	50.10	813.4127	49.18	98.16	
120%	Reps1	60.00	60.00	985.0048	59.55	99.26	0.92
	Reps2	60.20	60.20	994.4275	60.12	99.87	
	Reps3	60.10	60.10	975.0276	58.95	98.09	

3.5. Precision:

Intraday and inter-day precision investigations on the RP-HPLC method for beta caryophyllene demonstrate high precision percent amounts ranging from 97 to 101 percent, indicating an analytical procedure that was concluded. Table no. 05 shows the results of intraday and inter-day precision experiments on the RP-HPLC technique for beta caryophyllene.

Table No. 05: Precision for beta caryophyllene

Instrument Precision	Peak Area
Parameter	**ATV**
Rep 1(50 ug/ml)	836.7568
Rep 2(50 ug/ml)	826.5165
Rep 3(50 ug/ml)	822.8553
Rep 4(50 ug/ml)	810.2636
Rep 5(50 ug/ml)	846.8294
Average	828.6443
SD	13.8960
%RSD	1.68

3.6. Limit Detection:

Depending on the standard deviation of response and slope, the limit of detection means LOD is detected. The LOD is the lowest limit that can be detected. The value of LOD of Beta caryophyllene was observed as 1.976 (ug/mL), the analytical method that concluded.

Limit of detection = 3.3X 9.6327/16.0838 = 1.976 (ug/mL)

3.7. Limit Quantification:

The LOQ is that the lowest concentration which will be quantitatively measured. The value of LOQ for Beta caryophyllene was observed as 5.989 (ug/mL) for the concluded method.

Limit of Quantitation = 10X 9.6327/16.0838=5.989 (µg/mL).

4. Conclusion:

The developed new method proved to be simple in procedure and it produced more accurate results. Hence, the methods effective for the routine analysis of beta caryophyllene. The simple, accurate and sensitive validated RP-HPLC method for simultaneous determination of Beta caryophyllene has been developed. The method may be recommended for routine and quality control analysis of the investigated drugs in pharmaceutical formulations. The data demonstrate that the RP-HPLC method we have developed showed acceptable linearity, specificity, accuracy, precision and LOD & LOQ in the concentration range of 25-75 µg/ml for Beta caryophyllene as per the requirement of ICH guidelines. In this study, stability of drug was established according to ICH-recommended stress conditions. The approach devised can be regarded a useful tool for ensuring quality control of a viable alternative to chromatography.

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