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METHOD DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-HPLC METHOD FOR DETERMINATION OF BETA CARYOPHYLLENE

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Abstract: An easiest, precise, accurate and isocratic RP-HPLC stability-indicating method was developed and validated for determination of Beta caryophyllene. RP-HPLC separation was achieved on Hypersil BDS RP (5µ, 250 mm X 4.6 mm) column using mobile phase comported of methanol: water: orthophosphoric acid (98:02:0.1, % v/v) at flow rate of 1 ml/ min. Forced declination studies were performed on bulk sample of Beta caryophyllene using acid (1 N Hydrochloric acid), base (1 N Sodium hydroxide solution), oxidation (3% Hydrogen Peroxide Solution), Dry heat (80˚C). Good resolution between the peaks corresponds to declination products. The estimation angles of Beta caryophyllene showed good linearity in the attention range 25-75 µg/ ml with UV discovery (210 nm). The correlation portions were in the range 0.999. With limit of discovery and quantification 1.976 ug/ml and 5.989 µg/ml, independently. The system has the needful delicacy, selectivity, perceptivity and perfection to assay declination products performing from the stress studies didn't intrude with assay is therefore stability- indicating.

Keywords: Stability indicating method, Beta Caryophyllene (BCP), Recovery studies, stress studies.

I. INTRODUCTION

BCP is a sesquiterpene hydrocarbon with the chemical formula (1R,4E,9S)-4,11,11-trimethyl-8-methylidenebicyclo [7.2.0] Undec-4-ene is found in a wide variety of plant essential oils. Anti-inflammatory, antibacterial, antioxidant, anticarcinogenic, and local anaesthetic effects are among the biological actions attributed to -caryophyllene.

Beta caryophyllene is an odorous bicyclic sesquiterpene that can be found in a variety of herbs and spices. Beta-caryophyllene was discovered to be a cannabinoid receptor 2 ligand (CB2). CB2 activation reduces pain, a major signal for inflammatory responses and an endogenous CB2 selective agonist; because of selective binding, it no longer indicates psychotropic aspect effects and additionally displayed pharmacological sports which include antidiabetic, anti-inflammatory/oxidant, anticancer, and additionally displayed PAR-γ activation, which is accountable for insulin-secreting pastime, and it's far more powerful in combinational remedy.

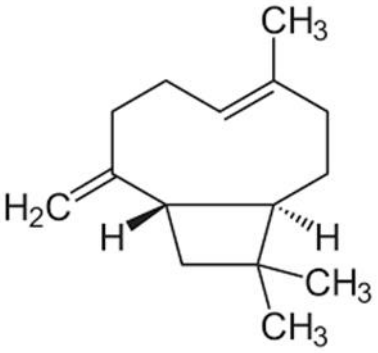


Figure 1: Structure of Beta Caryophyllene

All medications must be tested by a stability-indicating assay technique before announcement, according to current good manufacturing procedures (GMPs). Stress degradation studies of therapeutic elements can be useful in identifying the likely waste products, as well as establishing the degradation pathways and fundamental constancy of the molecule, and validating the analytical system's stability-indicating power. The type of stress study depends on the specific medicinal ingredient and the type of drug product being tested. Given Beta caryophyllene's sensitivity to diverse conditions, it was thought that an RP-HPLC method of analysis that separated the drug from the secondary products produced under ICH guideline settings (hydrolysis, oxidation, and thermal stress) would be of broad interest. These tests provide valuable information on the drug's inherent strength and aid in the

verification of analytical systems to be utilised in drug stability testing. It was attempted to build a single stable indicating HP-LC method [9] that could be utilised to estimate related compounds as well as the assay of bulk Beta caryophyllene samples. Stress testing on the drug substance should be undertaken to determine stability characteristics and support the felicity of the proposed analytical system, according to the International Conference on Harmonization (ICH) guideline Q1A (R2) for parent drug (API) stability testing. [1, 11,12]

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. Forced Degradation Studies:

Forced degradation or accelerated degradation may be a process whereby the natural degradation rate of a product or material is increased by the applying of additional stress. Forced degradation studies are want to identify reaction which will occur to degrade a processed product. Forced degradation is sometimes conducted before final formulation and it's done by applying external stress conditions and rapidly checked for material stabilities.

To determine whether the analytical approach was stability indicating, Beta Caryophyllene of active pharmaceutical ingredient (API) was tensed under numerous situations to accomplish forced degradation studies. Purposeful declination was struggled to pressurize conditions of acidic hydrolysis (using 1 N HCl), alkaline hydrolysis (using 1N NaOH), oxidative degradation (using 3.0% H2O2) and dry & wet thermal treatment (heated at 80 °C and 45 °C) with reflux condition respectively. After completion of the degradation procedures, the resolutions were neutralized and diluted with mobile phase. Stress degradation conditions were categorical on the idea of unobjectionable pH range of the column. The resolution peak within drug and its degradants should be quite 1.5. Attempt become made to decompose 10.30% of the drug by introducing drug to strained situations then milder conditions were used. This is done to reduce the degradation interval.

2.6.1. Acidic Degradation

50 mg of BCP was weighed in a 10 ml volumetric flask and 1 ml of 1 N Hydrochloric acid was added and kept at room temperature for 6 hr. Later 5 ml of diluent was added and sonicated for 2 minutes and made up to the volume with diluent.

2.6.2. Alkaline Degradation

50.4 mg of BCP was weighed in a 10 ml volumetric flask and 1 ml of 1 N Sodium hydroxide solution was added and kept at room temperature for 6 hr. Later 5 ml of diluent was added and sonicated for 2 minutes and made up to the volume with diluent.

2.6.3. Oxidative Degradation

50.9 mg of BCP was weighed in a 10 ml volumetric flask and 1 ml of 3% Hydrogen Peroxide Solution was added and kept at room temperature for 6 hr. Later 5 ml of diluent was added and sonicated for 2 minutes and made up to the volume with diluent.

2.6.4. Photolytic:

49.9 mg of BCP was weighed in a 10 ml volumetric flask and kept at 254 nm in UV Cabinet for 3 hours. Later 5 ml of diluent was added and sonicated for 2 minutes and made up to the volume with diluent.

2.6.5. Thermal Stress Studies/ Dry heat Degradation:

50.3 mg of BCP was weighed in a 10 ml volumetric flask and kept at 45˚C in Hot Air Oven for 3 hours. Later 5 ml of diluent was added and sonicated for 2 minutes and made up to the volume with diluent.

3. RESULTS AND DISCUSSION

3.1. Optimization of analytical conditions:

Different columns having different sizes and chemical natures have been attempted for separation and resolution. The Hypersil BDS (250mm X 4.5mm, 5u) column have become extra tremendous over the opposite columns. Individual drug solution turned into injected into column, each elution sample and determination parameters studied as a characteristic of pH, as a function of mobile phase component and their ratio. To develop an acceptable HPLC method for estimation of beta caryophyllene, different mobile phases were employed to realize the simplest separation from degradant peaks. The chosen and optimized mobile phase was methanol: water: orthophosphoric acid (98:02:0.1 % v/v) and conditions optimized have been: flow rate (1.2 ml/min), detector wavelength (210 nm), run time was 4.17 min. Here the peaks were separated and confirmed higher resolution, theoretical plate count and asymmetry was found as 0.94 for Beta caryophyllene. The proposed chromatographic conditions have been observed applicable and suitable for the quantitative determination of the drugs.

3.2. Results of forced degradation studies

Acidic declination of Beta caryophyllene was executed in 50 mg of drug in 0.1 N hydrochloric acid at temperature 30°C. Hydrolytic degradation of Beta Caryophyllene was observed to be very gradual and less, for that reason better compelled conditions have been attempted to boost degradation process. Then in addition degradation meted out within the mixture of methanol and hydrochloric solution having strength of 1 N HCl with influx at 60°C. Drug were given degraded into degradants having RT of 4.12 and 3.13 min and the 13.16 % of drug degraded in 6 hr. In case of alkaline degradation, process was carried out for 6 hours at ambient temperature in 1 N NaOH and gave one minor degradant peak height showing RT of 4.03 min. Approximately 17.53% of drug was degraded in 6 hr. Progress of decay can't be judged, right here in oxidative declination beta caryophyllene is veritably labile to experimental condition and degradant peak further suffer degradation thus incessantly degradant Rt changes. Initially retention time was obtained of 2.50 then change to 4.02 and approximately 11.53% of drug was degraded in 6 hr. Thermal degradation was carried out as dry heat. Dry heat shows minimal degradation among all forced condition, as 2.75% of drug was degraded in 3 hours with degradant peaks as RT at 4.62 and 4.00 minutes. In case of photolytic degradation, roughly 0.97% of drug was degraded in 3 hour and the degradants peaks at retention time of 4.00 minutes. Degradants concentrations increase as time passes and the degraded products were well resolved from the parent drug for chromatogram vs time in min. The order of stability for beta caryophyllene was found to be photolytic < dry heat < H2O2 < acid < alkali. Developed RP-HPLC method is able to separate all degradants, produced from all stress condition from drug peak by resolution. Analytical data for evaluation are given in Table no. 01 and figures 01, 02, 03, 04 and 05 shows acidic, basic (alkaline), oxidative, photolytic and thermal (Dry Heat) degradation respectively.

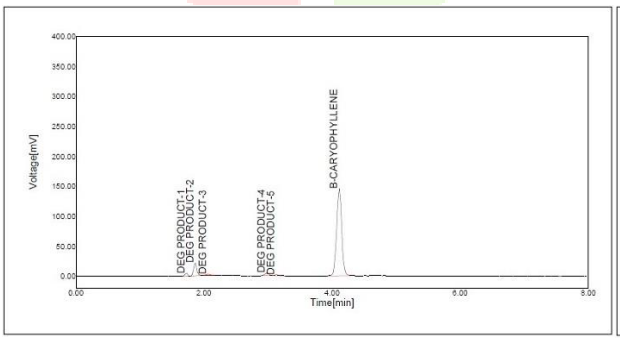


Fig. 01: Acidic Degradation of BCP

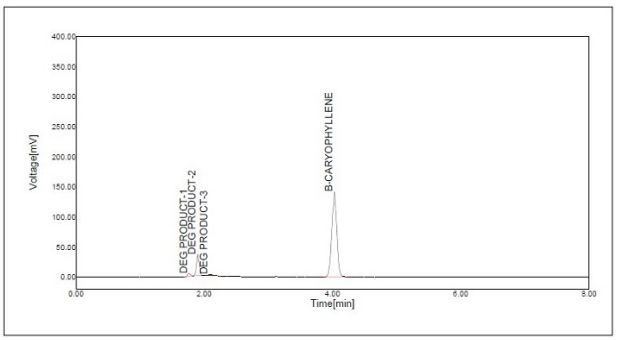


Fig. 02: Basic Degradation of BCP

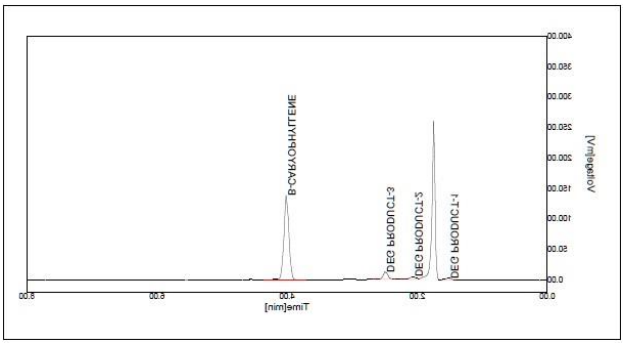


Fig. 03: Oxidative Degradation of BCP

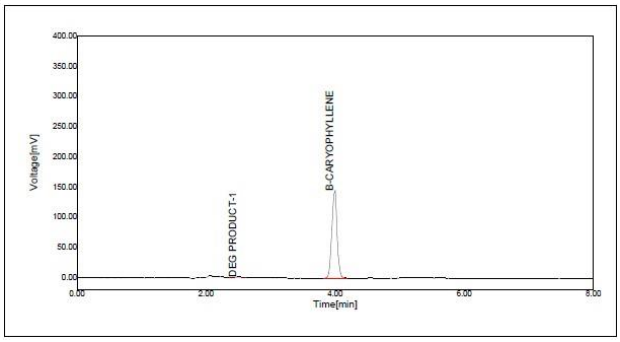


Fig. 04: Photolytic Degradation of BCP

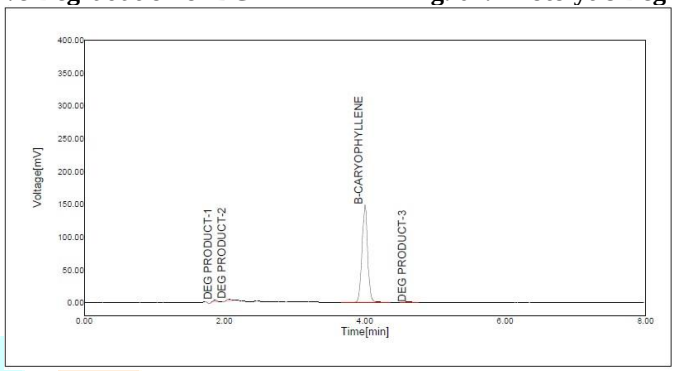


Fig. 05: Dry Heat Thermal Degradation of BCP

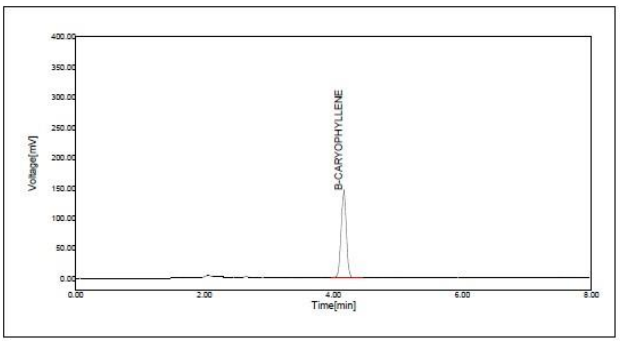
Table No. 01: Analytical Data for Evaluation of Various Stress Degradation Conditions

Sample ID	Area	% Assay	% Deg
Acidic Degradation, 1 N HCl (reflux at 30°C)	799.8875	86.84	13.16
Alkaline Degradation, 1 N NaOH *(At ambient temperature)*	757.8424	85.47	17.53
Oxidative Degradation 3% hydrogen peroxide *(At ambient temperature)*	746.9797	88.47	11.53
Dry heat Degradation (at 80 ^{o}C)	822.9200	97.25	2.75
Photolytic Degradation	814.0703	99.03	0.97

4. METHOD VALIDATION

4.1. Specificity:

Specificity for the beta caryophyllene was found as mentioned in the following table no. 02 and mentioned figures 06 & 07. The % Assay of beta caryophyllene for this developed technique was found to be 99.52.



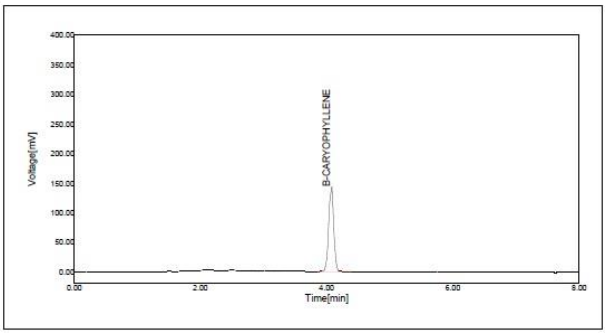


Fig. 06: Chromatogram of Working Standard Fig. 07: Chromatogram of Test Solution

Table No. 02: Specificity and Assay:

Sample (API Name)	Beta caryophyllene
Standard Solution	831.2832
Test Solution	830.0306
% Assay	99.52

4.2. Linearity:

The data revealed a linear association between peak areas and concentrations in the ranges of 25-75 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 0.999 and the calibration curve of Beta caryophyllene is depicted in figure 8. Linearity data for the Beta caryophyllene is represented in table no. 03.

Table No. 03: Linearity and range:

% Conc	Conc (ug/ml)	BCP 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

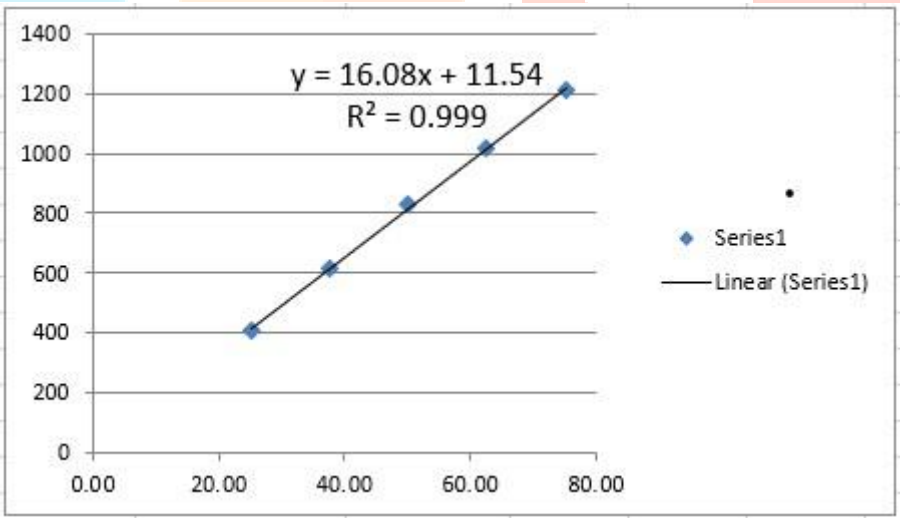


Fig. 08: Calibration curve (linearity) of Beta caryophyllene

4.3. 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. 04.

Table No. 04: System Suitability:

Sr. No.	Parameters	Beta caryophyllene
1	Theoretical Plates	19517
2	Retention Time	4.08
3	Asymmetry factor	0.94

4.4. Accuracy:

Validation of Recovery Studies which is done statistically is represented in table no. 05 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 98 and 102%.

Table No. 05: 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	

4.5. Precision:

Intraday and inter-day precision investigations on the RP-HPLC method for Beta caryophyllene demonstrate high precision percent amounts ranging from 98 to 101 percent, indicating an analytical procedure that was concluded. Table no. 06 shows the results of intraday and inter-day precision experiments on the RP-HPLC technique for Beta caryophyllene.

Table No. 06: 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

4.6. 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 as1.976 (ug/mL), the analytical method that concluded.

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

4.7. 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)

Conclusions:

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 necessity of ICH guidelines. In this testing, firmness of drug was conventionally according to ICH-recommended stress conditions. There was no finding of interference any mortified products or excipients in the estimation of the API. In conclusion, the projected system could be regularly used for the analysis of API in pharmaceutical dosage form.

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