BETA CARYOPHYLLENE OXIDE QUANTIFICATION BY UV SPECTROSCOPY: METHOD DEVELOPMENT AND OPTIMIZATION BY ICH GUIDELINES

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Abstract

UV-Spectrophotometric method was carried out for profiling of phytochemicals .The method of analysis of Beta Caryophyllene oxide (BCO) in the lab has been developed. BCO exhibited absorption maximum of 285 nm. Analytical parameter including linearity and range, precision, accuracy, robustness and ruggedness were established following the International Conference on Harmonization (ICH) guidelines. The calibration curve showed a linear response according to Beer-Lambert's law for BCO concentration ranging from 0.1 to 0.9 mg/ml, with a correlation coefficient of 0.9963. Accuracy ranged from 99.78 to 102.24, while precision, expressed as relative standard deviation (% RSD) was 1.1164 % (Intraday) and 0.6948% (Interday) across nine sample preparations. The method's sensitivity was demonstrated by a limit of detection (LOD) of 0.0378 mg/ml and limit of quantification (LOQ) of 0.1147 mg/ml. Ruggedness, assessed by two analysts, showed %RSD values 0.801% and 0.751%. Robustness was evaluated at three wavelengths (284 nm, 285 nm, and 286 nm) yielding values of 1.705, 1.581, and 1.695, respectively. The study concluded that developed method was linear, accurate, precise, and cost-effective. It is suitable for routine analysis of BCO formulations.

Keywords: *ICH guidelines, standard curve, Beer-Lambert's Law, Accuracy, Precision*

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1. Introduction

In nature, β-caryophyllene (BCP) is a bycyclic sesquiterpenes. Trans caryophyllene is the predominant form. Its oxidation product is β-caryophyllene oxide (BCO), which oxidises instantly when exposed to air and consumes over half of the original chemical after five weeks. Skold et al. (2006) Black pepper (*Piper nigrum L*.) Geddo et al. (2019), cinnamon (*Cinnamomum spp*.), oregano (*Origanum vulgare L*.), basil (*Ocimum spp*.), mint (*Mentha piperita*), thyme (*Thymus vulgaris*), sage (*Salivia officinalis*), black caraway (*Nigellia sativa*), lavender (*Lavandula angustifolia*), and cloves (*Syzygium aromaticum*) naturally occur in essential oils. Fidyt et al. (2016). It was also discovered in the leaves of Annona cherimola, citronella, pine trees, plants belonging to the genera Copaifera, Murraya, Spiranthes, Ocimum, and Croton. It was discovered that BCO has anti-inflammatory, antioxidant, antifungal, anticarcinogenic, and analgesic qualities. Skold et al. (2006). BCP and BCO are utilised as food and cosmetic ingredients and have a pronounced woody aroma. The European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA) have authorised these natural compounds as flavouring agents. Both compounds are poorly soluble in water. Aqueous media, like biological fluid, impede their uptake by the cell. Nonetheless, the ability of both BCP and BCO to interact with artificial lipid bilayers clearly suggests their affinity for the cell membrane. Sarpietro et al. (2015)

A review of the literature found that a number of analytical techniques, including gas chromatography Park et al. (2021), Raman spectroscopy Boaroto et al. (2018), GC-MS method Ibrahim et al. (2019), and high performance liquid chromatography (HPLC) method Borges et al. (2013), were used.

Nevertheless, thorough research on the evaluation and validation of UV techniques for BCO for routine in vitro sample analysis is lacking. For parameters like linearity and range, precision, accuracy, robustness, and ruggedness, the suggested approach is validated.

By analysing how they absorb UV and visible light, phytochemicals are analysed using UV-visible spectroscopy. This method aids in the purity, concentration, and presence of certain functional groups as well as the characterisation, identification, and authenticity of phytochemicals.

Thus, the goal of the current work is to create and evaluate a technique for BCO analysis using a UV-visible spectrophotometer that can be readily modified for routine use in quality testing labs. In addition to addressing the inaccuracy brought on by partial extraction and the application of internal standards, this has allowed us to shorten the overall analysis time.

2. Drug Profile

Table1: Drug profile of BCO

Parameters	**BCO**
Synonyms	Caryophyllene oxide (-)- Caryophyllene oxide beta-Caryophyllene oxide
Molecular formula	$C_{15}H_{24}O$
Molecular weight	220.35g/mol
Description	Caryophyllene oxide is an epoxide. It acts as a metabolite.
IUPAC Name	(1R,4R,6R,10S)-4,12,12-trimethyl-9-methylidene-5-oxatricyclo[8.2.0.0$^{4.6}$]dodecan
Appearance	White crystalline powder
Boiling point	279-280℃
Melting point	60-62℃

3. Beer-Lambert's Law

According to Beer-Lambert's law, one may determine the concentration of a solution by measuring the absorbance of a specific sample. As long as the species is known to absorb light at a specific wavelength, it is possible to detect the concentration of a specific chemical species in a solution.

$$A=\varepsilon \times l \times c$$

where, A = Absorbance, l= path length, and c = concentration of the absorbing chemical species when the path length is measured in centimeters, and the concentration of the absorbing species is measured in Molarity, the proportionality constant is called the Molar Absorptivity, ε, having units of $M^{-1}cm^{-1}$. Hardesty et al. (2010), Yadav et al. (2016)

4. MATERIALS AND METHODS

4.1 Materials

Spectral measurements were performed using a double beam UV Spectrophotometric (Labtronics LT-2201, India) with 10 mm quartz cuvettes. BCO was acquired from Sigma-Aldrich in India. Merck Chemicals in India supplied all of the analytical-grade chemicals and reagents utilised. Standard class A borosilicate volumetric glassware was used for all dilutions. Water from Millipore (Millipore Direct Q® 3UV)

4.2 Methods

4.2.1 Preparation of phosphate buffer

Phosphate buffer solution (pH7.2) was prepared according to the standard method given in Indian Pharmacoepia.

4.2.2 Preparation of standard stock solution

The concentration of standard stock solution is 1mg/ml

4.2.3 Determination of Absorption maxima wavelength (λ_{max})

1 ml of standard stock solution was pipette out into a 10 ml volumetric flask. The capacity was made up to 10 ml through phosphate buffer solution. The concentration of final solution was 0.1 mg/ml and scanned in the range of 200-400 nm wavelengths to establish the λ_{max}.

4.2.4 Preparation of Standard Calibration Curve

Volumetric flasks were used to generate serial dilutions in the range of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 mg/ml. The calibration curve was created by plotting the measured absorbance (A) against the matching drug concentration at the absorption maxima. At λmax 285 nm, the absorbance was measured. Singh et al. (2017)

4.2.5 Analytical Method Validation

A high level of confidence that a chosen activity can systematically deliver the desired result or product fulfilling its predetermined specification and quality attributes is provided by validation, which is specified as ICH principles creating document proof. A crucial component of a quality assurance program is validation, which aims to show that the analytical method is safe to use and appropriate for the intended proposition. The ICH specifications for linearity, accuracy, precision, detection limit, and quantification limit were followed when validating the method. These measures are thought to be the most crucial for assay-type analytical technique validation.

4.2.6 Linearity and Range

The ability of the result to stay exactly proportionate to the analyte concentration is known as linearity. The linearity of the concentration range across which Beer-Lambert's law is followed. An analytical procedure's range is the range between the highest and lowest analyte concentrations or quantities in the sample for which it demonstrates an appropriate degree of linearity, precision, and accuracy. ICH et al. (2005)

4.2.7 Precision

Precision, as delineated by ICH, refers to the degree of concordance among quantity values acquired by repeated measurements of a quantity under designated conditions. Accuracy was assessed for both repeatability (intraday precision) and intermediate precision (interday precision). The accuracy of intraday measurements was established by doing the trials many times throughout the same day. Interday precision was assessed by doing the experiment many times across two separate days to achieve repeatability for each technique. The accuracy was articulated in % RSD and computed using the subsequent equation. Rapalli et al. (2020)

$$\%RSD=\frac{\text{Standard deviation of values}}{\text{Mean value}}\times 100$$

4.2.8 Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated with the help of following equation:

$$LOD = 3.3\sigma/S \text{ and}$$

$$LOQ=10\sigma/S$$

Where S=Slope of calibration curve, σ= residual standard deviation calculated using calibration curve.

4.2.8.1 Accuracy

Accuracy is characterised by the proximity of concordance between the actual value and the analytical value. Employing the standard addition technique, a specified quantity of standard stock solution was included at varying levels of 80%, 100%, and 120% into the BCO solution. The resolution was examined using the suggested technique. The concentration of samples was re-evaluated in triplicate using the linearity curve. Jain et al. (2011) The accuracy analysis is presented in Table 5.

4.2.8.2 Robustness

The ICH characterizes resilience as the ability to stay stable despite slight, intentional alterations in the technique's parameters. Developing methods that include expected alterations in the separation parameter is the paramount aspect of resilience. The resilience of a technique is evaluated by altering parameters, including detector wavelength variations (284, 285, and 286nm), within a sensible range and quantifying the effect of these factors. If the parameter's impact falls within a certain tolerance, it is deemed to be within the method's

robustness range, the absorbance is computed, and the %RSD is determined to be acceptable, remaining below 2%, as outlined in Table 6. Chakure et al. (2018)

4.2.8.3 Ruggedness

The Ruggedness was determined by doing the experiment under consistent conditions by two distinct analysts (analyst 1 and analyst 2). The technique was evaluated by analysis conducted by two analysers at the identical dose of 0.4 mg/ml. The result was determined to be ≤0.80% % RSD The analysis of Ruggedness is presented in Table 7.

5. RESULT AND DISCUSSION

5.1 Analytical wavelength

The absorption maxima for BCO were determined at wavelength of 285 nm (Figure 1).

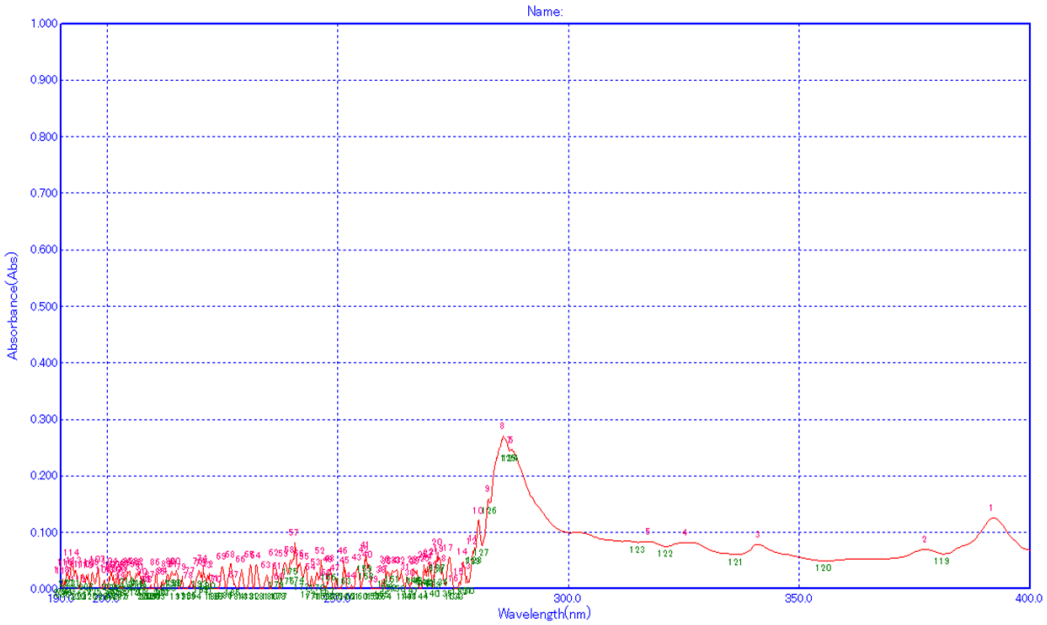


Fig.1: UV Spectrum graph of BCO at 285nm

5.2 Calibration Curve

The results of concentration vs absorbance were graphed, revealing that the technique exhibited linearity over the generated concentration spectrum, as shown by the standard equation y=0.6345 + 0.0215. The regression coefficient was found to be 0.9963. The calibration results revealed that the regression coefficient was below 1, according to the parameters of Beer-Lambert's law (Figure 2) (table 2).

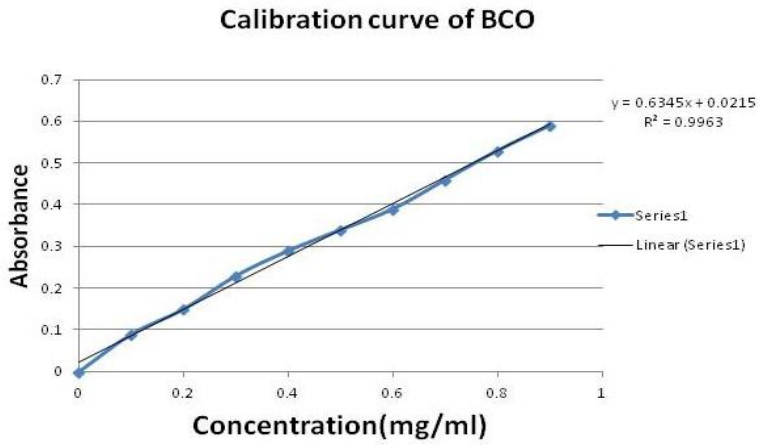


Fig 2: Standard calibration curve of BCO at different concentration expressed as mg/ml.

Table 2: Optical characteristic of BCO

Validation Parameter	**Result Obtained**
Beer´s law limit (mg/ml)	0.1- 0.9 mg/ml
Correlation coefficient	0.9963
Regression equation	Y=0.6345x+ 0.0215
Slope (a)	0.6345
Intercept (b)	0.0215

5.3 Validation of the proposed methods was carried out.

5.3.1 Precision

The intra-day and inter-day assessments were conducted on entirely separate days, and the results indicated no major discrepancies between them. The findings indicate a %RSD of less than 2%, demonstrating the reliability of the suggested technique. The accuracy outcomes for intra-day and inter-day are displayed in Tables 3 and 4 correspondingly. The percentage of relative standard deviation (% RSD) was likewise computed.

Table 3: Intra-day precision study for BCO

Concentration (mg/ml)	**Calculated concentration(mg/ml)**			**Mean**	**SD**	**%RSD**
	Trial 1	**Trial 2**	**Trial 3**			
0.1	0.1252	0.1205	0.1221	0.1226	0.0024	1.9625
0.2	0.2198	0.2167	0.2151	0.2172	0.0024	1.1082
0.3	0.3301	0.3317	0.3349	0.3322	0.0024	0.7245

0.4	0.4215	0.4278	0.4247	0.4247	0.0031	0.7421
0.5	0.5051	0.5019	0.5035	0.5035	0.0015	0.3129
0.6	0.5949	0.5776	0.5981	0.5902	0.0110	1.8691
0.7	0.6989	0.6973	0.6989	0.6984	0.0009	0.1302
0.8	0.8014	0.7998	0.8408	0.8140	0.0232	2.8520
0.9	0.9101	0.9133	0.9070	0.9101	0.0031	0.3463
Average of %RSD						1.1164

Table 4: Inter-day precision study for BCO

Concentration (mg/ml)	**Calculated concentration (mg/ml)**			**Mean**	**SD**	**%RSD**
	Day 1	**Day 2**	**Day 3**			
0.1	0.1012	0.102	0.10	0.1010	0.0010	0.9960
0.2	0.2245	0.2198	0.2230	0.2224	0.0024	1.0820
0.3	0.3305	0.3349	0.3317	0.3322	0.0024	0.7245
0.4	0.4375	0.4357	0.4373	0.4368	0.0009	0.2083
0.5	0.5066	0.5051	0.5051	0.5035	0.0009	0.1807
0.6	0.5949	0.5981	0.5933	0.5954	0.0024	0.4042
0.7	0.6989	0.6926	0.6958	0.6958	0.0031	0.4530
0.8	0.7698	0.8014	0.7856	0.7856	0.0157	2.0060
0.9	0.9101	0.9133	0.9101	0.9112	0.0018	0.1997
Average % RSD						0.6948

5.3.2 Accuracy

The precision was determined and expressed as the percentage recovery of the medication from the formulated combination. The mean recoveries for the specified concentrations of 80%, 100%, and 120% were determined to be 102.24%, 100.29%, and 99.78%, respectively, demonstrating the great precision of the established methodology, which is less than 2%.

Table 5: Accuracy reading of BCO

% Recovery level	Original concentration (mg/ml)	Absorbance	Calculated Concentration (mg/ml)	% Recovery	Mean % Recovery	SD	%RSD
80% (0.24 mg/ml)	0.3	0.196	0.28	101.86	102.24	0.00090	0.32
	0.3	0.197	0.28	102.44			
	0.3	0.197	0.28	102.44			
100% (0.6 mg/ml)	0.6	0.401	0.60	99.68	100.29	0.0039	0.65
	0.6	0.403	0.60	100.21			
	0.6	0.406	0.61	101.00			
120% (1.08mg/ml)	0.9	0.647	0.99	99.58	99.78	0.0050	0.51
	0.9	0.652	0.99	100.37			
	0.9	0.646	0.98	99.42			

5.3.3 Robustness:

The %RSD was determined to be within the permissible threshold, not above 2%. The findings from the robustness assessment of the established assay technique demonstrated that the assay values of the test preparation solution were stable across all variation situations and corresponded with the anticipated values.

Table 6: Results showing robustness

Sample No.	Absorbance at 284 nm	Absorbance at 285 nm	Absorbance at 286 nm
1	0.416	0.398	0.398
2	0.395	0.399	0.394

3	0.410	0.397	0.400
4	0.410	0.401	0.411
5	0.409	0.399	0.395
6	0.408	0.414	0.407
Mean	**0.408**	**0.401**	**0.400**
SD	**0.006**	**0.006**	**0.006**
% RSD	**1.705**	**1.581**	**1.695**

5.3.4 Ruggedness

The inter-day recovery studies have given satisfactory result with an average percentage recovery of 0.208. The % RSD was calculated by analyst 1 and analyst 2 on six consecutive day's ≤0.801% for all concentration.

Table 7: Result of ruggedness

Analyst	**Days**	**Concentration (mg/ml)**	**Absorbance**	**Statistical Analysis**
Analyst 1	1	0.4	0.339	Mean-0.341 SD-0.0027 %RSD-0.801
	2	0.4	0.342	
	3	0.4	0.340	
	4	0.4	0.346	
	5	0.4	0.343	
	6	0.4	0.339	
Analyst 2	1	0.4	0.341	Mean-0.341 SD-0.0025 %RSD-0.751
	2	0.4	0.341	
	3	0.4	0.345	
	4	0.4	0.343	
	5	0.4	0.338	
	6	0.4	0.339	

5.4 LOD and LOQ

LOD and LOQ values were found to be 0.0378 mg/ml and 0.1147 mg/ml respectively. Summary of validation of BCO is given in the table 8.

Table 8: Summary of validation of BCO

S.NO.	Parameter	Result
1.	Linearity indicated by correlation coefficien	0.9963
2.	Linear regression equation	Y= 0.6345x+ 0.0215
3.	Range	0.1- 0.9 mg/ml
4.	Inter-day precision (%RSD)	0.6948
5.	Intra-day precision(%RSD)	1.1164
6.	Limit of detection(LOD)	0.0378 mg/ml
7.	Limit of quantification(LOQ)	0.1147 mg/ml
8.	Robustness indicated by % RSD	< 2
9.	Ruggedness indicated by % RSD	0. 0.801& 0.751

6. Conclusion

The UV spectrophotometric method is cost effective, rapid and non destructive used in pharmaceutical analysis, drug development and quality control. This method is suitable for the estimation of BCO in laboratory. Therefore it can be concluded that the developed analytical UV method is specific, precise, linear, accurate, and robust, making it well-suited for the in-vitro assay of BCO.

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8. Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this review.

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