MOLEKUL
eISSN: 2503-0310

Articles
https://doi.org/10.20884/1.jm.2025.20.3.15690

Development and Validation of Zerumbone Analysis Method Using Ultraviolet-Visible Spectrophotometry

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Received May 07, 2025; **Accepted** November 11, 2025; **Available online** November 20, 2025

ABSTRACT. Analytical method development ensures that an analytical procedure is suitable for the identification and quantification of compounds of interest. This study aimed to develop and validate a simple, cost-effective analytical method for the determination of zerumbone using UV-Vis spectrophotometry. Method validation was conducted according to ICH Q2(R2) guidelines, including tests for specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision. Zerumbone exhibited a maximum absorption wavelength (λmax) of 212 nm when dissolved in dimethyl sulfoxide (DMSO). The method demonstrated excellent linearity with a determination coefficient (R^2) of 0.9991 across a concentration range of 10-50 µg/mL. Accuracy, evaluated via recovery studies, ranged from 91,00% to 110,45%. Precision testing results in a % RSD of 0.58%. The calculated LOD and LOQ were 1.55 µg/mL and 4.70 µg/mL, respectively. This validated method is simple, rapid, and economical, making it a promising alternative for zerumbone analysis in quality control laboratories.

Keywords: Zerumbone, Method Development, UV-Vis Spectrophotometry, Validation, ICH Guidelines

INTRODUCTION

Analytical methods are essential in pharmaceutical and natural product research, ensuring the quality, safety, and efficacy of active compounds. Method development aims to establish whether an analytical procedure can reliably identify and quantify an analyte in a given matrix (ICH, 2022). Zerumbone (*2,6,9,9-tetramethyl-[2E,6E,10E]-cycloundeca-2,6,10-trien-1-one*) is a sesquiterpene predominantly found in the *Zingiber zerumbet* rhizomes (Hwang, et al., 2020), exhibiting pharmacological activities such as anticancer, antioxidant, anti-inflammatory, and antibacterial effects (Wahab, et al., 2022). Due to these benefits, accurate and practical analytical methods for zerumbone quantification are essential for herbal medicine standardization.

Advanced techniques such as HPLC, GC-MS, and UHPLC have been widely employed for zerumbone determination (Kongkiatpaiboon et al., 2023; Kshirsagar et al., 2018). However, these methods require expensive instruments, highly trained personnel, and longer analysis times. In contrast, UV-Vis spectrophotometry offers a rapid, cost-effective, and widely accessible alternative. Despite these advantages, limited studies have explored UV-Vis methods for zerumbone quantification, particularly using optimized solvent systems that influence spectral properties. However, these methods require expensive instruments, highly trained personnel, and longer analysis times. In contrast, UV-Vis spectrophotometry offers a rapid, cost-effective, and widely accessible alternative. Despite these advantages, limited studies have explored UV-Vis methods for zerumbone quantification, particularly using optimized solvent systems that influence spectral properties.

This study introduces a simple and validated UV-Vis spectrophotometric method for zerumbone analysis using dimethyl sulfoxide (DMSO) as the solvent. DMSO was selected due to its ability to enhance zerumbone solubility and produce distinct absorption spectra. The novelty of this research lies in its systematic validation according to ICH Q2(R2) guidelines and its potential application for routine quality control in herbal industries where access to sophisticated instruments is limited.

EXPERIMENTAL SECTION

Material

Zerumbone standard (purity ≥90%) was purchased from MarkHerb®). DMSO (analytical grade) was obtained from Supelco®. All other reagents were of analytical grade. Certified reference material (CRM) for zerumbone was not available; therefore, a high-purity reference standard was used.

Instrumentation

UV-Vis spectrophotometer (Genesys 10S Thermo Scientific®) equipped with a 1 cm quartz cuvette was used. Spectral scanning was performed at a resolution of 1 nm in the range of 200 – 400 nm.

Preparation of Standard Stock Solution

A 1000 µg/mL zerumbone solution was prepared by dissolving 10 mg of zerumbone in 2.5 mL of DMSO and adjusting the volume to 10 mL with the same solvent. The solution was sonicated for 3 minutes to ensure complete dissolution.

Determination of λmax

A working solution (10 µg/mL) was scanned in the 200-400 nm range against DMSO as the blank to determine the λmax.

Operating Time

The absorbance of the 10 µg/mL zerumbone solution was measured at λmax at one-minute intervals until stabilization.

Method Validation

Specificity

Dimethyl sulfoxide (DMSO), essential oil from *Zingiber zerumbet*, and a 10 µg/mL zerumbone solution were prepared. The absorbance of each solution was measured at the λmax absorption wavelength and compared.

Linearity

Five series of zerumbone solution concentrations were prepared by diluting the standard stock solution to 10, 20, 30, 40, and 50 µg/mL. Absorbance measurements were performed three times (triplicate) using the UV-Vis spectrophotometer, with DMSO as the blank. The data were then processed using Microsoft Excel to obtain a linear curve, y = bx + a, with r^2 as the determinant of linearity.

LOD dan LOQ

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were statistically calculated from the linear regression line of the calibration curve. The standard deviation (SD) of the response and the slope of the calibration curve were used in the following equations.

$$SD = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}$$

Where SD = Standard Deviation; n = Number of data points; x = Value of each measurement; $\bar{x}$ = Mean of the data

$$\text{LOD} = 3.3\, x \left(\frac{\sigma}{S}\right)$$

$$\text{LOQ} = 10\, x \left(\frac{\sigma}{S}\right)$$

Where σ = SD of response; S = Slope of the calibration curve.

Accuracy

The Reference Material Comparison method was used by measuring the absorbance of three zerumbone concentration series (20, 30, and 40 µg/mL) using the UV-Vis spectrophotometer at the maximum wavelength, with DMSO as the blank. Measurements were performed in triplicate, and the recovery percentage was calculated using the following formula.

$$\%\, recovery = \left(\frac{Concentration\ obtained}{Actual\ concentration}\right) x\ 100\%$$

Precision

Samples were measured in triplicate on three different days (interday) and on the same day (intraday). Absorbance measurements were performed intraday and interday on zerumbone solutions with concentrations of 20, 30, and 40 µg/mL. The % relative standard deviation (RSD) was then calculated using the following formula.

$$\%\ \text{RSD} = \left(\frac{\text{SD}}{\text{Average}}\right) \text{x } 100\%$$

Where RSD = relative standard deviation; SD = standard deviation

RESULTS AND DISCUSSION

Zerumbone contains α,β-unsaturated carbonyl groups, which contribute to its characteristic absorption in the UV region. In this study, the initial step involved preparing a stock solution at a higher concentration, which was subsequently diluted to obtain a working solution for determining λmax, as well as for evaluating specificity, linearity, precision, and accuracy. The determination of the λmax was essential to achieve optimal sensitivity, as measurement near λmax ensure compliance with the Lambert-Beer Law and minimum potential errors during repeated measurement (Fatimah et al., 2018). As shown in **Figure 1**, the maximum absorption wavelength of zerumbone was observed at 212 nm with peak absorbances of 0.420, which represents a distinctive feature of its UV spectrum.

This finding differs from previous reports, where 2018)the λmax of zerumbone in methanol was recorded at 245 nm (Manandhar et al., 2024). The difference can be explained by the hypsochromic (blue) shift observed when using DMSO as the solvent, which is attributed to differences in solvent polarity. This phenomenon, known as solvatochromism, occurs because the polarity of the solvent influences the energy gap between ground and excited states, thereby altering the wavelength of maximum absorption (Christian et al., 2014) (Gandjar & Rohman, 2015).

The purpose of determining the operating time is to identify the time at which the zerumbone absorption reading becomes stable (Sukmawati et al., 2018). Based on the research results, the absorbance stabilized in less than 1 minute, which is attributed to the volatile nature of zerumbone. According to the ICH guidelines, Validation of Analytical Procedures (2023), parameters that can be used to assess the validity of an analytical method include specificity, linearity, LOD (Limit of Detection),

LOQ (Limit of Quantification), accuracy, and precision.

Specificity refers to the ability of the method to accurately measure the analyte of interest in the presence of other components within the matrix (Christian et al., 2014). In this study, the specificity test demonstrated that zerumbone and *Z. zerumbet* essential oil produced distinct absorption peaks at 212 nm, whereas the DMSO blank showed no interference (**Table 1**). These findings confirm that the proposed method is selective for zerumbone and capable of distinguishing it from potential matrix components.

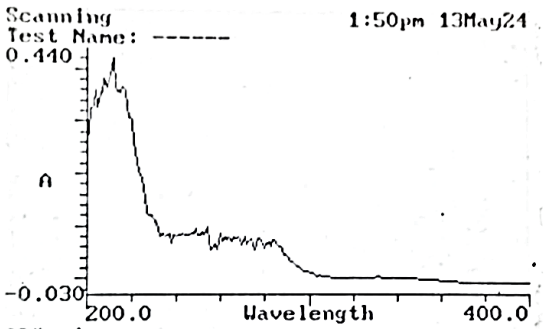

Figure 1. UV spectrum of zerumbone in DMSO showing λmax at 212 nm

Table 1. Specificity test results for zerumbone, essential oil, and DMSO

Maximum Wavelength (nm)	Sample	Replicate	Absorbance	Average
212 nm	zerumbone 10 µg/mL	I	0.429	0.427
		II	0.439	
		III	0.412	
	Essential Oil of *Zingiber zerumbet*	I	0.114	0.110
		II	0.111	
		III	0.105	
	DMSO	I	-0.005	-0.004
		II	-0.009	
		III	0	

Table 2. Linearity test result

Concentration (µg/mL)	Absorbance			
	*R1	R2	R3	Average
10	0.461	0.472667	0.467	0.466889
20	0.527333	0.527333	0.525667	0.526778
30	0.588	0.585667	0.587667	0.587111
40	0.641667	0.641667	0.641667	0.641667
50	0.722	0.697667	0.708333	0.709333

*R=Replicate

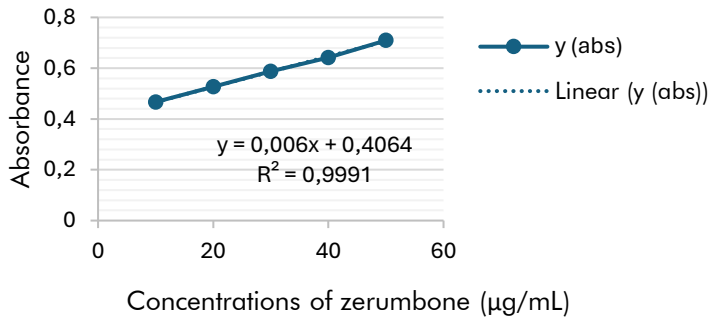

Figure 2. Calibration curve of zerumbone (10–50 µg/mL) in DMSO.

Linearity assessment evaluates the method's ability to produce results directly proportional to the analyte concentration within a specified range (Maddeppungeng, et al., 2022). The calibration curve constructed for zerumbone (10-50 µg/mL) exhibited excellent linearity with the regression equation y = 0.006x + 0.4064 and correlation coefficient (R^2) of 0.9991 (**Figure 2**). This high degree of linearity indicates that the method is highly reliable for quantitative determination (ICH, 2022) of zerumbone across the tested range.

The sensitivity of an analytical method is expressed in terms of its limit of detection (LOD) and limit of quantification (LOQ) (**Table 3**). Based on statistical calculation using the standard deviation of the response and the slope of the calibration curve (Fatimah et al., 2018), the LOD and LOQ values were determined to be 1.553 µg/mL and 4.706 µg/mL, respectively. These low values demonstrate the method's capability to detect and accurately quantify even small amounts of zerumbone, making it suitable for application in herbal extract analysis.

Accuracy indicates how close the measured values are to the true value (Kemenkes, 2020). In this study, recovery studies were conducted by analysing spiked samples at different concentrations. The recovery percentage ranged from 91.00% to 110.45% (**Table 4**), which falls within the acceptance criteria recommended by ICH (80-120%) (ICH, 2022). These results confirm that the proposed method provides accurate measurement with minimal deviation from the true concentration.

Precision reflects the consistency of the method under normal operating conditions and was evaluated in terms of repeatability (intra-day) and intermediate precision (inter-day) (Asma, et al., 2022). The relative standard deviation (%RSD) obtained for all tested concentrations was 0.58%, which is well below the maximum limit of 2% recommended by ICH (ICH, 2023). This demonstrates the high reproducibility and reliability of the UV-Vis spectrophotometric method.

Table 3. LOD and LOQ test result

Concentration/x (µg/mL)	Absorbance/ y'	y	y - y'	(y-y') 2
10	0.466	0.466	-0.00049	2.39012E-07
20	0.527	0.526	-0.00038	1.42716E-07
30	0.587	0.586	-0.00071	5.05679E-07
40	0.641	0.646	0.00473	2.24044E-05
50	0.709	0.706	-0.00293	8.60444E-06
SD		0.0028		
LOD		1.553 µg/mL		
LOQ		4.706 µg/mL		

Table 4. Accuracy test result

Concentration (µg/mL)	Replicate	Absorbance	Concentration obtained (µg/mL)	Recovery (%)
10	I	0.461	9.10	91.00
	II	0.472	11.04	110.45
	III	0.467	10.10	101.00
20	I	0.527	20.16	100.78
	II	0.527	20.16	100.78
	III	0.525	19.88	99.39
30	I	0.588	30.27	100.89
	II	0.585	29.88	99.59
	III	0.587	30.21	100.70
40	I	0.641	39.21	98.03
	II	0.641	39.21	98.03
	III	0.641	39.21	98.03
50	I	0.722	52.27	104.53
	II	0.697	48.54	97.09
	III	0.708	50.32	100.64
Accuracy				91.00 – 110.45

Table 5. Precision test result

Concentration (µg/mL)	Days	Replicate	Absorbance	Average	SD	RSD (%)
20	1	I	0.527	0.523	0.0032	0.62
		II	0.527			
		III	0.525			
	2	I	0.526			
		II	0.519			
		III	0.523			
	3	I	0.519			
		II	0.524			
		III	0.519			
30	1	I	0.588	0.585	0.0036	0.62
		II	0.585			
		III	0.587			
	2	I	0.578			
		II	0.581			
		III	0.588			
	3	I	0.582			
		II	0.587			
		III	0.590			
40	1	I	0.641	0.642	0.0031	0.49
		II	0.641			
		III	0.641			
	2	I	0.647			
		II	0.641			
		III	0.644			
	3	I	0.642			
		II	0.635			
		III	0.646			
					Average	0.58%

Although HPLC and other advanced chromatographic methods provide high sensitivity and specificity for zerumbone analysis, they are often associated with high cost, lengthy analysis times, and the need for complex instrumentation (Saputri & Muchtaridi, 2018). In contrast, the UV-Vis method developed in this study is simple, rapid, and cost-effective, requiring minimal sample preparation and enabling completion of analysis within five minutes. This makes the method highly practical for routine quality control in resource-limited laboratories and small-scale herbal industries.

CONCLUSIONS

The developed analytical method for zerumbone using UV-Visible spectrophotometry has been successfully validated in accordance with ICH Q2(R2) guidelines. The method demonstrated excellent specificity, linearity (R^2 = 0.9991), sensitivity (LOD = 1.553 µg/mL, LOQ = 4.706 µg/mL), accuracy (91.00–110.45%), and precision (%RSD = 0.58%). These findings confirm that the method is reliable, reproducible, and suitable for quantitative analysis of zerumbone. Given its simplicity, cost-effectiveness, and rapid execution, this method holds strong potential for routine quality control of zerumbone in pharmaceutical formulations, herbal preparations, and natural product-based industries. Furthermore, it may serve as a practical alternative analytical approach in resource-limited laboratory settings.

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