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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATIVE ESTIMATION OF METHYL EUGENOL

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Abstract:

Methyl Eugenol is an allyl chain-substituted guaiacol and finds variety of applications. Thus development of a validated UV spectrophotometric method will always be advantageous as the method is simple and rapid. The method was validated according to International Conference on Harmonization (ICH) guidelines Q2(R1) with respect to linearity and range, precision, accuracy, detection limit (DL) and quantitation limit (QL). The detection limit (DL) and quantitation limit (QL) were determined as per the ICH guidelines and were found to be 0.82 and 2.48 µg mL^{-1} respectively. The method is expected to be useful in a variety of industries where Methyl eugenol finds its application.

Key Words: *Accuracy, Detection limit, ICH, Linearity, Precision, Quantitation limit, Range*

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INTRODUCTION:

Methyl Eugenol ($C_{11}H_{14}O_2$; 1,2-Dimethoxy-4-(prop-2-en-1-yl)benzene), is an allyl chain substituted guaiacol. Methyl Eugenol oil possesses anti-inflammatory and anesthetic properties along with its recognized antioxidant, antimicrobial, antiviral and antifungal activities. Thus Methyl eugenol has been recognized as a potent pharmacologically phytochemical[1] and good number of methyl eugenol delivery systems have been reported for a variety of applications.[2-8] Methyl Eugenol has its applications in the fragrance and flavoring industries also.[9] Methyl Eugenol is a significant phytochemical biomarker compound of ayurvedic and other marketed herbal formulations. It shows anti-inflammatory, anti-bacterial and anti-tubercular activity. 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. Thus development of a validated UV spectrophotometric method will always be advantageous as the method is simple and rapid. Till date no studies have been reported a validated UV spectrophotometric assay method for the estimation of methyl eugenol in methanol. Towards this objective of quantification of methyl eugenol efforts have been made towards the development and validation analytical method by UV spectrophotometry.

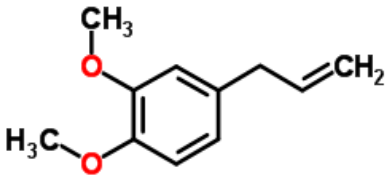

Fig.1. Chemical structure of methyl eugenol

MATERIALS AND METHODS:

Materials

Methyl Eugenol (pure) was purchased from Central Drug House, Delhi, India. Methanol was purchased from S D Fine-Chem ltd, Mumbai, India. Reagent grade I water (Millipore, Molsheim, France) was used for the study.

UV spectrophotometry

A Shimadzu UV – 1601 (Shimadzu Corp, Kyoto, Japan) spectrophotometer was employed in the study. The method was validated according to ICH guidelines, Q2(R1)[10] with respect to linearity and range, precision, accuracy, detection limit (DL) and quantitation limit (QL).

Preparation of standard solutions

Methyl Eugenol (100 mg) was dissolved methanol in a 100 mL volumetric flask and then the volume was made up with methanol. The dilutions of this stock solution were made by diluting the required aliquot with methanol to obtain standard solution in the range of 5- 50 µg mL^{-1}. The absorbance of the resultant solutions was determined at the λ_{max} of 282 nm.

Linearity and range

The calibration curve was plotted using the concentration range of 5 - 50 µg mL^{-1}. The absorbance of the solutions was determined at 282 nm. A calibration curve was constructed by plotting absorbance vs. concentration of standard solution and the regression equation was determined. The experiment was carried out in triplicate.

Accuracy as recovery

Accuracy was determined by recovery studies using standard addition method. The pre-analyzed samples were spiked with extra 50, 100 and 150% of the standard eugenol and the mixtures were analyzed by the proposed method. The experiment was conducted in triplicate.

Precision

Three concentrations of eugenol solution (10, 25 and 40 µg mL^{-1}) were prepared. The precision of the method was assessed by analyzing eugenol for repeatability and intermediate precision.

(a) Repeatability

Repeatability (intraday) was assessed by analyzing eugenol in three different concentrations (10, 25 and 40 µg mL^{-1}) of three times a day. The % RSD was calculated for absorbance thus obtained, to obtain the intra-day variation.

(b) Intermediate precision

Intermediate precision (inter-day) was established by analyzing three different concentrations (10, 25 and 40 µg mL^{-1}) of eugenol for three different days. The % RSD was calculated for absorbance thus obtained, to obtain the inter-day variation.

Detection and quantitation limits

The detection limit (DL) is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated. The quantitation limit (QL) is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. The limit of quantification and limit of detection were determined based on the technique of signal-to-noise ratio[10] using the equations (1) and (2).

$$QL = 10\,\sigma / S \quad \text{---------- (1)}$$

$$DL = 3.3\,\sigma / S \quad \text{---------- (2)}$$

Where, σ is the standard deviation of the intercept of the calibration plot and S is the slope of the calibration curve.

RESULTS AND DISCUSSION:

UV spectrophotometry

The UV method for the estimation of eugenol in methanol was validated. Fig. 2 shows the UV spectrum of eugenol in methanol with absorption peaks at 210, 226.0 and 281.5 nm.

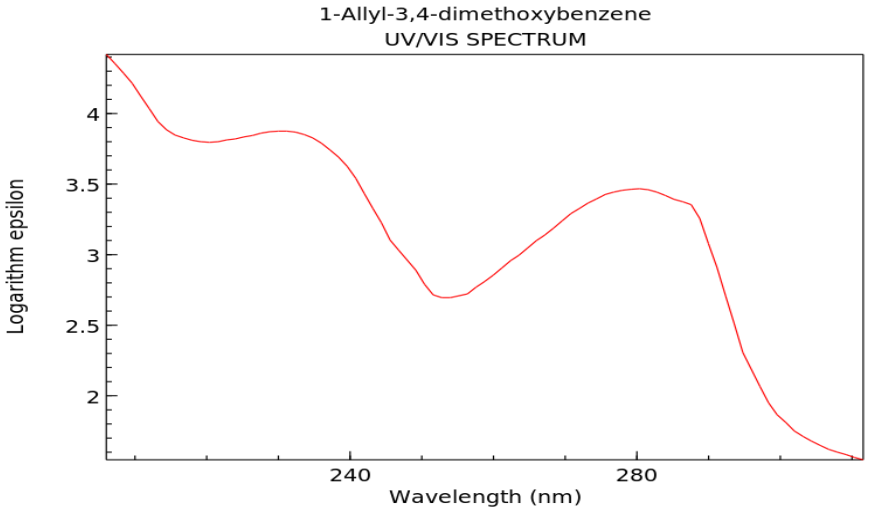

Fig. 2. UV spectrum of Methyl eugenol in methanol

Linearity and range

The absorbance of the prepared standard solutions (5-50 µg mL^{-1}) was determined at 282 nm. The mean absorbance was found to be 0.1100 – 1.0182. The plot of absorbance versus concentration (Fig. 3) obeyed Beer-Lambert's law in above concentration range with regression coefficient of 0.9973.

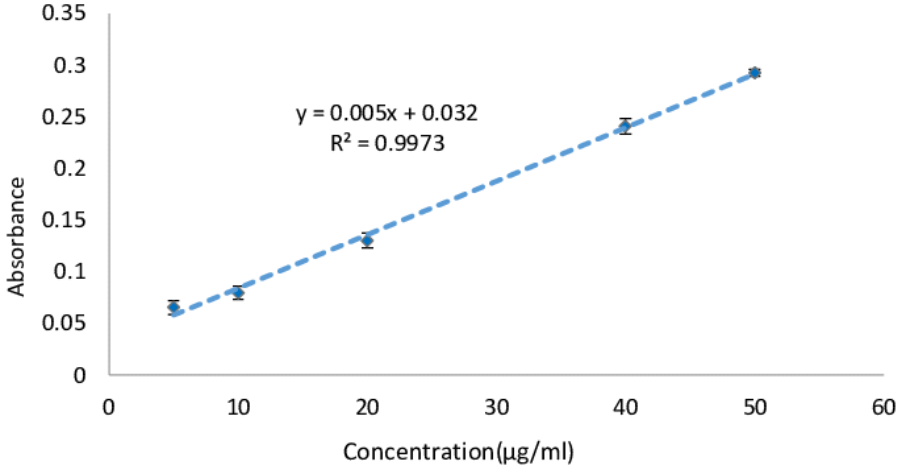

Fig. 3. Calibration curve of methyl eugenol in methanol by UV spectrophotometry (282 nm)

Accuracy as recovery

Accuracy was investigated by analyzing three concentrations of standard drug solution previously analyzed using standard addition technique. The recovery studies were carried out to check the sensitivity of the method to estimate methyl eugenol. The standard addition technique was carried out by adding 50, 100 and 150% of the eugenol concentration in the sample. The % recoveries of the three concentrations were found to be 99.96 – 101.74 %, indicative of high accuracy. The values of % recovery and % RSD are displayed in Table 1. The mean % recovery values, close to 100%, and their low % RSD values indicated high accuracy of the analytical method.

Table 1- Recovery data for the accuracy of the UV method in Methyl eugenol in methanol

Excess of methyl eugenol added (%)	Concentration of sample (µg mL^{-1})	Theoretical concentration of spiked sample (µg mL^{-1})	Concentration of spiked sample ± SD (µg mL^{-1}) (n=3)	Recovery ± SD (%)	% RSD
50	20	30	29.99±0.48	99.96± 1.60	1.60
100	20	40	40.70±0.56	101.74± 1.39	1.37
150	20	50	49.95±0.54	99.89± 1.07	1.08

Precision

The precision method was assessed by analyzing methyl eugenol in three different concentrations as 10, 25 and 40 µg mL^{-1} of eugenol.

(a) Repeatability

Repeatability (intra-day) was assessed by analyzing methyl eugenol in three different concentrations (10, 25 and 40 µg mL^{-1}) of eugenol three times a day. The % RSD was calculated for absorbance thus obtained, to obtain the intraday variation and is given in Table 2.

(b) Intermediate precision

Intermediate precision (inter-day) was established by analyzing three different concentrations (10, 25 and 40 µg mL^{-1}) of methyl eugenol for three different days. The % RSD was calculated for absorbance thus obtained, to obtain the inter-day variation and is given in Table 2.

Table 2- Repeatability and intermediate precision

Concentration (µg mL^{-1})	Repeatability (n=3)		Intermediate precision (n=3)	
	Mean absorbance at 282 nm ±SD	% RSD	Mean absorbance at 282 nm ±SD	% RSD
10	0.2119±0.0039	1.83	0.2125±0.0033	1.56
25	0.5183±0.0043	0.82	0.5145±0.0053	1.03
40	0.8343±0.0078	0.93	0.8331±0.0061	0.73

The low values of % RSD for repeatability and intermediate precision suggested an excellent precision of the developed UV spectrophotometric method.

Detection and quantitation limits

The detection limit (DL) and quantitation limit (QL) were determined as per the ICH guidelines and were found to be 0.82 and 2.48 µg mL^{-1} respectively.

Table 3- Optical, linear regression and validation data (n = 3)

Parameter	Data (Mean ± SD)
Optical characteristics	
$E_{1\%, 1cm}$	204.64±2.54
Regression analysis	
Slope	0.0206±0.0003
Intercept	0.0063±0.0051
Regression coefficient (R^2)	0.9987±0.0005
Validation	
Range (µg mL^{-1})	5 – 50
Detection limit (DL) (µg mL^{-1})	0.82
Quantitation limit (QL) (µg mL^{-1})	2.48

Table 3 summarizes the optical, linear regression and validation data of UV spectrophotometry for the quantification of Methyl eugenol in methanol.

CONCLUSION:

The UV spectrophotometric method using methanol as solvent for the quantification of methyl eugenol was successfully developed and validated. The method was validated in terms of linearity and range, accuracy and precision. The detection limit (DL) and quantitation limit (QL) were determined as per the ICH guidelines and were found to be 0.82 and 2.48 μg mL^{-1} respectively. The method is expected to be useful in a variety of industries where Methyl eugenol finds its application.

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