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SIMULTANEOUS ESTIMATION OF EUGENOL IN UV METHOD

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ABSTRACT

Eugenol in therapeutic doses has not been implicated in causing serum enzyme elevations or clinically apparent liver injury, but ingestions of high doses, as with an overdose, can cause severe liver injury. Eugenol is used as a component of several dental materials. They are reported to be widely used in dentistry as temporary filing materials, cavity liners for pulp protection, capping materials, temporary cementation of fixed protheses, impression materials and major ingredients of endodontic sealers. In addition, eugenol has been used in dentistry for disinfecting root canals. In vitro, eugenol has been shown to have antibacterial, antifungal, antioxidant and antineoplastic activity. This UV-spectrophotometric technique is quite simple, accurate, precise, reproducible, and sensitive. The UV method has been developed for quantification of Eugenol in tablet formulation. The validation procedure confirms that this is an appropriate method for their quantification in the formulation. It is also used in routine quality control of the formulations containing this entire compound.

Key words: Eugenol, UV method, Estimation, Direct.

INTRODUCTION

Eugenol, also called clove oil, is an aromatic oil extracted from cloves that is used widely as a flavoring for foods and teas and as an herbal oil used topically to treat toothache and more rarely to be taken orally to treat gastrointestinal and respiratory complaints. Eugenol in therapeutic doses has not been implicated in causing serum enzyme elevations or clinically apparent liver injury, but ingestions of high doses, as with an overdose, can cause severe liver injury. Eugenol is used as a component of several dental materials (e.g., dental cements, impression pastes and surgical pastes). Such products are principally combinations of Zinc oxide and eugenol in varying ratios. They are reported to be widely used in dentistry as temporary filing materials, cavity liners for pulp protection, capping materials, temporary cementation of fixed protheses, impression materials and major ingredients of endodontic sealers. In addition, eugenol has been used in dentistry for disinfecting root canals. Cloves are now grown in several tropical regions and the spice sold as intact flower buds or as a ground powder. Eugenol is the most abundant ingredient in clove oil and is thought to be responsible for its aromatic as well as both beneficial and harmful effects. In vitro, eugenol has been shown to have antibacterial, antifungal, antioxidant and antineoplastic activity.

CHEMICAL MATERIAL

Eugenol was a gift sample from Aarovin Pharmaceuticals, Chennai. All chemicals (distilled water, methanol) and reagents used were of analytical grade and purchased from Qualigens Fine Chemicals, Mumbai, India [2-3].

INSTRUMENTATION

A Labindia UV–visible spectrophotometer (UV-T60-India) was used for all absorbance measurements with matched quartz cells.

METHOD DEVELOPMENT

Preparation of standard stock solution

Accurately weighed 10 mg of eugenol was transferred to a 100 ml volumetric flask, dissolved in 20 ml distilled water by shaking manually for 10 min. The volume was adjusted with the same up to the mark to give the final strength, i.e. 100 µg/ml.

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Selection of wavelength for analysis of Eugenol

Appropriate volume 0.5 ml of standard stock solution of eugenol was transferred into a 10 ml volumetric flask, diluted to a mark with distilled water to give concentration of 5 µg/ml(and also 10, 15µg/ml). The resulting solution was scanned in the UV range (200–400 nm). In spectrum eugenol showed absorbance maximum at 275 nm [4-5].

Validation of the method

The method was validated in terms of linearity, accuracy, precision, and ruggedness. Linearity study Different aliquots of eugenol the range 0.5–3 ml were transferred into series of 10 ml volumetric flasks, and the volume was made up to the mark with distilled water to get concentrations 5, 10, 15, 20, 25, and 30 µg/ml, respectively. The solutions were scanned on a spectrophotometer in the UV range 200–400 nm. The spectrum was recorded at 275 nm. The calibration plot was constructed as concentration vs. absorbance [6-7].

Accuracy & Precision studies

To the pre analysed sample solutions, a known amount of standard stock solution was added at different levels, i.e. 50%, 100%, and 150%. The solutions were reanalyzed by the proposed method [8-9]. Precision of the method was studied as intraday and interday variations. Intraday precision was determined by analyzing the 10, 15 and 20 µg/ml of eugenol solutions for three times in the same day. Interday precision was determined by analyzing the 10, 15, and 20 µg/ml of eugenol solutions daily for 3 days over the period of week [11-14].

Sensitivity

The sensitivity of measurements of eugenol by the use of the proposed method was estimated in terms of the limit of quantification (LOQ) and limit of detection (LOD). The LOQ and LOD were calculated using equation LOD = 3 × N/B and LOQ = 10 × N/B, where 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve [15-16].

Repeatability & Ruggedness

Repeatability was determined by analyzing 20 µg/ml concentration of eugenol solution for six times. Ruggedness of the proposed method is determined for 20 µg/ml concentration of eugenol by analysis of aliquots from a homogenous slot by two analysts using same operational and environmental conditions [17].

RESULTS & DISCUSSION

Selection of wavelength for analysis of eugenol

During the development phase, the use of ethanol as the diluent resulted in preferable outcome in UV analysis. The pre-determined wavelength of maximum absorption (λmax) was 275 nm.

Table 1: Solubility data of the formulation

S. No	Solvent	Amount (mcg/ml)
1	Pet ether	2.78
2	Chloroform	2.34
3	Methanol	1.17
4	Ethnaol	0.86
5	Acetone	0.81
6	Distilled water	0.63

Table 2: Recovery, Assay studies

Parameter	eugenol
Amount used	54mcg
Amount recovered	51.23mcg
Percentage recovered	100.16%
Label Claim	700mg
Estimated amount	500.32mg
Percentage of assay	99.5%

Table 3: Precision and accuracy data of the formulation

S. No	Parameters	values
1	max(nm)	275
2	linearity range	23-47µg/ml
3	regression equation	Y=0.0912X-0.0936

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4	correlation coefficient	3.651
5	slope	0.0978
6	intercept	0.0899
7	Limit of detection(µg/ml)	0.9637
8	Limit of quantification(µg/ml)	8.9143
9	Intra day	0.9987±0.0810
10	Interday	0.9671±0.0472

CONCLUSION

This UV-spectrophotometric technique is quite simple, accurate, precise, reproducible, and sensitive. The UV method has been developed for quantification of Eugenol in tablet formulation. The validation procedure confirms that this is an appropriate method for their quantification in the formulation. It is also used in routine quality control of the formulations containing this entire compound.

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