

Spectrophotometric determination of the synthetic attractant methyl eugenol

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Abstract

A simple, rapid and sensitive colorimetric method for quantitative determination of methyl eugenol (ME), a synthetic attractant for oriental fruit fly (OFF). On the other hand, in pure form and in commercial preparations is described. Oxidative coupling of ME with 3-methyl benzothiazoline-2-one hydrazone (MBTH) in presence of ferric chloride produces a blue colored product, which absorbs maximally at 619 nm. The color is linear in the range 2.5-10 $\mu\text{g mL}^{-1}$, with mean percentage recovery of 100.01 ± 0.58 and correlation coefficient of 0.9998 ($n=7$). Various parameters affecting the reaction pathway have been optimized and method could be successfully applied to determine ME in both pure form and in the commercial preparation without interferences from diluents. The method needs neither extraction nor heating. The results were in good statistically agreement with those obtained by applying a reported method. Limit of detection (LOD) and limit of quantification (LOQ) are reported.

Key words: Spectrophotometry; 3-Methyl benzothiazoline-2-one hydrazone; MBTH; Methyl eugenol (ME); Oriental fruit fly (OFF); LOD and LOQ

1. Introduction

Methyl eugenol (ME) is an extremely effective attractive kairomone lure for biological control of male oriental fruit fly. Fruits, vegetables, and nuts are important as essential building blocks of any diet. They are loaded with vitamins and minerals which are essential for healthy living (Prior *et al*, 2000). Oriental Fruit Fly (OFF), *Bactrocera dorsalis* (Hendel), is considered one of the most serious of the world. fruit fly pests due to its potential economic harm (Shelly *et al*, 2000). Male oriental fruit flies are strongly attracted to (ME), a naturally occurring compound reported from ten different plant families (Shelly *et al*, 2000, Shelly *et al*, 2008). ME is the most environmentally friendly and least intrusive fruit fly eradication strategy available (YongYue *et al*, 2006, Vagas *et al*, 2000, Cornelius *et al*, 2001). ME could be determined by gradient HPLC method using [Inertsil column C18, 47% acetonitrile as a mobile phase and UV-detector at 230nm (Graves *et al*, 1995). Gas chromatography (GC) /MS (Siano *et al*, 2003), have been suggested for the determination of ME in food products.

3-Methyl benzothiazoline-2-one hydrazone has been used as a sensitive chromogenic reagent, in presence of oxidizing agents, for spectrophotometric determination of phenols, aromatic amines, heterocyclic bases to form highly colored products (Gasparie *et al*, 1977, El-Gendy *et al*, 2001, Ahmed *et al*, 2008, Alarfaj *et al*, 2009). The purpose of this work is to determine methyl eugenol in commercial preparations using rapid, simple, precise, accurate and low-cost method. The proposed procedure is based on the formation of a stable blue colored oxidative coupling product using MBTH in presence of ferric chloride.

2. Experimental

2.1. Apparatus

Shimadzu UV-1601 PC, dual-beam UV vis spectrophotometer (Kyoto-Japan), with matched 1cm quartz cells, connected to an IBM-compatible PC and an HP-600 inkjet printer. Bundled, UV-PC personal spectroscopy software version 3.7 was used to process the absorption.

2.2. Reagents and solvents

All reagents used throughout this work were of analytical pure grade, and solvents were of spectroscopic grade. (A) 3-Methyl benzothiazoline-2-one hydrazone (MBTH) aqueous solution, 0.35% (w/v), This solution is freshly prepared by dissolving an adequate weight of MBTH, obtained from E. Merck (Darmstadt-Germany), in 0.1M HCl. (B) Ferric chloride solution (FeCl_3) 4% (w/v). It is prepared by dissolving an adequate weight of the salt in 0.1M HCl. (c) 0.1M hydrochloric acid solution obtained from Prolabo (Briare Le Canal-France).

2.2.1. Pure standard: Standard Methyl Eugenol (ME): product of Aldrich Chem. Co. (WI-USA), kindly supplied by the Ministry of Agriculture (MARC), Central Agricultural Pesticides Laboratory (CAPL), Dokki, Giza. Its purity was found to be $99.63 \pm 0.67\%$.

2.2.2. Commercial sample: It is a liquid formulation supplied by the Ministry of Agriculture (MALRC), Central Agricultural Pesticides Laboratory (CAPL), Dokki, Giza. It was claimed to contain 98g of pure ME in each 100mL.

2.2.3. Standard stock and working solutions

2.2.3.1. Standard stock solutions of methyl eugenol (1mg mL⁻¹): Methyl eugenol standard stock solution (1mgmL⁻¹) is prepared by complete dissolving of 100mg of standard ME in an equi-mixture of methanol and 0.1N HCl (i.e., 1:1 by volumes) in 100mL calibrated volu-

metric flask.

2.2.3.2. Working standard solution of methyl eugenol (25 µg mL⁻¹): It was prepared by transferring 2.5 mL of methyl eugenol standard stock solution (1 mg mL⁻¹) into 100-mL calibrated volumetric flask and completing the volume to the mark with methanol 0.1 N HCl solvent mixture (1:1, v/v).

2.3. General procedures of analysis:

2.3.1. Spectral characteristics of methyl eugenol solution: Aliquot of 3 mL of ME-working standard solution (representing 75 µg) was transferred into a 10-mL calibrated volumetric flask and the volume was completed with methanol. The prepared solution was scanned in the range (200-800 nm) against methanol as a blank.

2.3.2. Spectral characteristics of the colored product of methyl eugenol and MBTH: Aliquot of 3 mL of ME-working standard solution (representing 75 µg) was transferred into a 10-mL calibrated volumetric flask, and 2.5 mL of MBTH & 1.5 mL FeCl₃ were added and left for 35 minutes. The volume was completed with distilled water, and the developed color was scanned in the UV-visible region (200-800 nm) against a reagent blank.

2.3.3. Construction of calibration curve of methyl eugenol: Aliquots of 1, 1.5, 2, 2.5, 3, 3.5 & 4 mL of ME working standard solution (equivalent to 25 µg mL⁻¹) were accurately transferred into a series of 10 mL calibrated volumetric flasks. Complementary volumes of solvent mixture [methanol-0.1 N HCl (1:1, v/v)] were added to adjust the volume to 4 mL, then 2.5 mL of MBTH-solution (0.3%, w/v) were added, followed by 1.5 mL of ferric chloride. The solution was kept for ~35 minutes at room temperature (25 °C) and was diluted to the mark with distilled water. The absorbance was measured at 619 nm against a reagent blank done parallel with the experiment. The calibration graph was obtained by plotting the absorbance versus the corresponding ME-concentration (µg mL⁻¹) and the regression equation was computed.

2.3.4. Application to the commercial preparation containing methyl eugenol: ME liquid formulation was diluted by using a mixture of methanol and 0.1 N HCl (1:1, v/v) to get ME concentration of ~25 µg mL⁻¹. An aliquot equivalent to ~50 g of ME was accurately transferred from the prepared solution into calibrated 10-mL measuring flasks and the volume was adjusted complementary to ~4 mL with the same solvent mixture. Then 2.5 mL of MBTH solution (0.3%, w/v) were added, followed by 1.5 mL of ferric chloride. The solution was kept for ~35 minutes at room temperature (25 °C) and diluted to the mark with distilled water. The absorbance was measured at 619 nm against a reagent blank simultaneously with the experiment. The ME-concentration (µg mL⁻¹) could be calculated from the computed regression equation.

3. Results and discussion

3.1. Reaction involved

The reaction of MBTH with phenols is carried out in acidic or alkaline media and in presence of an oxidizing agent. It was shown that phenols react in the *o* and *p*-position to the hydroxyl group via oxidative coupling (Gasparie *et al*,

1977). Methyl eugenol in a mixture of methanol and 0.1 N HCl (1:1, v/v) exhibits absorption maxima located at 230 nm. The addition of aqueous solutions of ferric chloride, MBTH to the drug solution produced a new characteristic peak at (619 nm). The blank solution was prepared similarly as the sample solution except for the presence of the drug, which does not show any peak at 619 nm. The reaction of MBTH with Methyl eugenol in the presence of oxidant ferric chloride proceeds via oxidative coupling. MBTH loses two electrons and one proton on oxidation with the oxidizing agent (i.e., ferric chloride) to form the electrophilic intermediate, which is the active coupling species (El-Gendy *et al*, 2001). The intermediate would be expected to attack carbon atom with high electron density to form the blue colored product. To study the stoichiometry of the reaction, the continuous validation method was applied. It was confirmed that ME interacts with MBTH in the 1:3-ratio.

3.2. Optimization of variables

The conditions for the production of the most intense and stable color, namely, effect of MBTH-concentration, iron (III) concentration, acid concentration, reaction time, and the effect of diluting solvent, were studied.

3.3. Effect of MBTH concentration

When various concentrations of MBTH solutions were added to a fixed concentration of ME, 2.5 mL of 0.3% solution was found to be sufficient for maximum color intensity. Increasing concentrations did not affect the color intensity.

3.4. Effect of iron (III) concentration

The optimum concentration of ferric chloride solution for maximum color development was found to be 1.5 mL of 4% FeCl₃-solution per 10 mL of reaction mixture. Higher concentrations of oxidant did not affect the absorption intensity of the color. Several other oxidants were investigated, e.g. ammonium ferric sulfate, potassium iodate and hydrogen peroxide. Only iron (III)-chloride gave the characteristic color with ME with MBTH.

3.5. Effect of hydrochloric acid concentration

Different concentrations of hydrochloric acid (0.01-0.2 M) used as a solvent for MBTH and FeCl₃, have been tested. Maximal color intensity could be achieved by using 0.1 M strength. Higher acidities did not affect the developed color intensity.

3.6. Effect of diluting solvent

Methanol, distilled water, 0.1 M HCl and acetonitrile were utilized as development solvents; where water gave the best color intensity and stability.

3.7. Effect of reaction time

Development time of 35 minutes, in all the tested diluting solvents, at 25 °C was found optimum for the maximal absorption intensity of the colored product, which was stable for at least 2 hours.

3.8. Quantification and method validation

A linear correlation was found between the absorbance at 619 nm and concentration in the 2.5-10 µg mL⁻¹-range. Correlation coefficient, intercept and slope for the

calibration data of ME are presented in table 1.

The linear Beer's law plot of the investigated drug can also be used for computing the regression equation and calculation of the concentration. The apparent molar absorptivity of the resulting colored product was found to be $\sim 16200 \text{ mol}^{-1} \text{ cm}^{-1}$ ($\log = 4.2092$). The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the current ICH guidelines (ICH Q2A 1994, ICH Q2B 1996) as the ratio of 3.3 and 10 standard deviations of the blank, respectively, and the slope of the calibration line (table 1). To examine the intra. day accuracy and precision of the method, solution containing two different concentrations (within the working limits) of ME were prepared and analyzed in nine replicates.

The relative standard deviation (0.11-0.55 %) indicates the high intra-day precision of the method. The inter-day precision was evaluated by performing replicate analyses on pure ME solution at two concentration Levels over a period of three successive days by preparing all solutions fresh at each day. The intra-day RSD-values (0.58-0.93%) and the low RSD (%) -values (2.0%) reflect a good precision of the proposed method (table 1).

Table 1: Assay parameters of the reaction between ME and MBTH.

*aThe inter-day (n=9) and *bThe intraday (n=9) relative standard deviations of samples of concentrations (5 & 7.5 $\mu\text{g mL}^{-1}$) for Methyl eugenol.

3.9. Application to commercial preparation

In order to demonstrate the usefulness of the proposed method, ME was determined in commercial preparation. The results agreed with the nominal contents (recovery 98.18-99.32%) (Table 2). The validity of the method was further confirmed by standard addition technique. To a fixed and known quantity of pre-analyzed Commercial preparation, pure ME was added at three different concentration levels, The total concentration was found by the proposed method. The experiment was repeated three times at each level. The percent recoveries of the pure drug added (97.81- 98.91%) revealed that there is no interference of excipients and additives in the determination (table 3).

Parameter	Methyl Eugenol
λ_{max}	619nm
Linearity Range ($\mu\text{g mL}^{-1}$)	2.5-12
Molar absorptivity $\text{mol}^{-1} \text{ cm}^{-1}$	16200
Regression equation	
Slope	0.093
Intercept	+0.1682
Correlation Coefficient (r^2)	0.9994
Recovery \pm SD (%)	100.01 \pm 0.58
LOD ($\mu\text{g mL}^{-1}$)	0.386
LOQ ($\mu\text{g mL}^{-1}$)	1.121
RSD (%) *a	0.932-0.578
RSD (%) *b	0.910-0.553

Table 2: Determination of methyl eugenol in its commercial preparation by the proposed MBTH colorimetric method.

*Average of three determinations, #Claimed ($\mu\text{g mL}^{-1}$): 5

Table 3: Application of the standard addition technique to methyl eugenol in its commercial preparation using the Proposed MBTH colorimetric method.

*Average of three determinations, #Claimed ($\mu\text{g mL}^{-1}$): 5

4. Statistical analysis of the results in comparison with the reported method

The results of the proposed method were statistically analyzed (Hiangetal, 2003) and compared with those obtained by applying the reported HPLC method (Graves et al, 1995). Table 4: shows that at 95% confidence level, the calculated t-&F-values are less than the theoretical ones. Therefore, there is no significant difference between the proposed method and the reported one indicating that the proposed method is as accurate and precise as the reported method.

Table 4: Statistical comparison of the results obtained

Preparation	Found# ($\mu\text{g mL}^{-1}$)	Recovery * (%)
Methyl Eugenol	4.964	98.18
Formulation	5.023	99.32
Formulation	4.964	98.38
Mean \pm SD		98.63 \pm 0.68

by the proposed MBTH colorimetric method and those of the reported method for the analysis of pure samples of methyl eugenol.

5. CONCLUSION

Preparation	Added($\mu\text{g mL}^{-1}$)	Found # ($\mu\text{g mL}^{-1}$)	Recovery * (%)
Methyl Eugenol	2.5	2.492	98.91
Formulation	5.0	4.993	98.21
Formulation	7.5	7.394	97.81
Mean \pm SD			98.31 \pm 0.68

In conclusion the proposed method has several advantages over other methods, these advantages are:

It is simple and specific for methyl eugenol.

The method can be used as spot-testing for identify-

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Parameter	Proposed method	Reported method
Mean recovery	100.01	99.63
SD	0.58	0.67
RSD	0.580	0.672
n	7	5
Variance	0.336	0.450

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