Application of 3-methyl benzothiazoline-2-one hydrazone as a chromogen for the spectrophotometric determination of oriental fruit fly's synthetic attractant

Ezzat M.Abdel-Moety[1], Hayam M.Lotfy[1]*, Nasr S.Khalil[2], Yasmine Rostom[1]

[1]Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini-11562, Cairo, (EGYPT)
[2]Central Laboratory of Agricultural Pesticides, Agricultural Research Centre, Ministry of Agriculture-12618, Dokki, Giza, (EGYPT)
E-mail : hayamlotfyhm@hotmail.com
Received: 15th November, 2009 ; Accepted: 25th November, 2009

ABSTRACT

A simple, rapid and sensitive colorimetric method for quantitative determination of methyl eugenol (ME), a synthetic attractant for oriental fruit fly (OFF), 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 2.5-10μg 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 the method could be successfully applied to determine ME in both pure form and in the commercial preparation without interferences from excepients and diluents. The method needs neither extraction nor heating. The obtained results were in good statistically agreement with those obtained by applying a reported method. Limit of detection (LOD) and limit of quantitation (LOQ) are reported. © 2010 Trade Science Inc. - INDIA

INTRODUCTION

Methyl Eugenol (ME) is an extremely effective attractive kairomone lure for biological control of male oriental fruit fly Structure of ME is shown in figure 1.

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[1]. Oriental Fruit Fly (OFF), *Bactrocera dorsalis* (Hendel), is considered one of the most serious of the world's fruit fly pests due to its potential economic harm[2]. Male oriental fruit flies are strongly attracted to
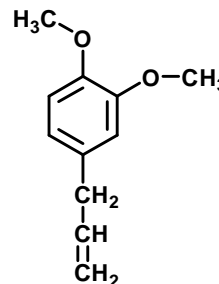
C$_{11}$H$_{14}$O$_2$ (MW: 178.2)
Figure 1 : Methyl eugenol

Full Paper

(ME), a naturally occurring compound reported from ten different plant families[3,4]. ME is the most environmentally friendly and least intrusive fruit fly eradication strategy available[5-8].ME could be determined by gradient HPLC method in rodent plasma using [Inertsil column C_{18} ($5\mu m$, 15cm×4.6mm, *i.d.*)], 47% acetonitrile/ acetonitrile as a mobile phase and UV-detector at 230nm[9]. Gas chromatography (GC)/MS[10] 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[11-17]. 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.

EXPERIMENTAL

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.

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).

Samples

Pure standard

Standard Methyl Eugenol (ME): A product of Aldrich Chem. Co. (WI-USA), kindly supplied by the Ministry of Agriculture (*MARC*), located at Dokki, Giza. Its purity was found to be 99.63±0.67 % as checked by applying the reported method[9].

Commercial sample

It is a liquid formulation (BNo.:1465), manufactured and distributed by Production Unit of Pesticide Alternatives in Sendion were claimed to contain 98g of pure ME in each 100mL, it was kindly supplied from the Ministry of Agriculture (*MARC*), located at Dokki, Giza.

Standard stock and working solutions

Standard stock solutions of methyl eugenol (1mg mL^{-1})

Methyl eugenol standard stock solution (1mg mL^{-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 volumetric flask.

Working standard solution of methyl eugenol ($25\mu g$ mL^{-1})

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

GENERAL PROCEDURE OF ANALYSIS

Spectral characteristics of methyl eugenol solution

An aliquot of 3mL of ME-working standard solution (representing $75\mu g$) was transferred into a 10–mL calibrated volumetric flask and the volume was completed with methanol. The prepared solution was scanned in the range 200-800nm against methanol as a blank.

Spectral characteristics of the colored product of methyl eugenol and MBTH

Aliquot of 3mL of ME-working standard solution

Full Paper

(representing 75µg) was transferred into a 10-mL cali-
brated volumetric flask, and 2.5mL of MBTH & 1.5mL
FeCl$_3$ 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 -
800nm) against a reagent blank. Figure 2 shows the
UV-visible (200 -800nm) spectra of methyl eugenol
solution and the colored reaction product of methyl
eugenol with MBTH measured against the correspond-
ing reagent blanks.

Construction of calibration curve of methyl eugenol

Aliquots of 1, 1.5, 2, 2.5, 3, 3.5 & 4mL of ME-
working standard solution (equivalent to 25µg mL^{-1})
were accurately transferred into a series of 10mL cali-
brated volumetric flasks. Complementary volumes of
solvent mixture [methanol-0.1N HCl (1:1, v/v)] were
added to adjust the volume to 4mL, then 2.5mL of
MBTH-solution (0.3% , w/v) were added, followed
by 1.5mL of ferric chloride. The solution was kept for
~35 minutes at room temperature (20±5°C) and was
diluted to the mark with distilled water. The absorbance
was measured at 619nm against a reagent blank done
parallel with the experiment. The calibration graph was
obtained by plotting the absorbance *versus* the corre-
sponding ME-concentration (µg mL^{-1}) and the regres-
sion equation was computed.

Application to the commercial preparation contain-ing methyl eugenol

ME liquid formulation was diluted by using a mix-
ture of methanol and 0.1N HCl (1:1, v/v) to get ME-
concentration of ~25µg mL^{-1}. An aliquot equivalent to
~50µg of ME was accurately transferred from the pre-

**Figure 2 : The Absorption spectrum of pure methyl eugenol
(7.5 µg mL $^{-1}$) in a mixture of methanol and 0.1 N HCl (1:1, v/
v); (- -) and the final colored product (7.5 µg mL $^{-1}$) (—) by
applying the proposed procedure**

pared solution into calibrated 10–mL measuring flasks
and the volume was adjusted complementary to ~4mL
with the same solvent mixture. Then 2.5mL of MBTH-
solution (0.3 %, w/v) were added, followed by 1.5 mL
of ferric chloride. The solution was kept for ~35 min-
utes at room temperature (20±5°C) and diluted to the
mark with distilled water. The absorbance was mea-
sured at 619 nm against a reagent blank simultaneously
with the experiment. The ME-concentration (µg mL^{-1})
could be calculated from the computed regression equa-
tion.

RESULTS AND DISCUSSION

Reaction involved

The reaction of MBTH with phenols is carried out
in acidic or alkaline media and in presence of an oxi-
dizing agent. It was shown that phenols react in the *o-*
and *p*-position to the hydroxyl group via oxidative cou-
pling[11]. Methyl eugenol in a mixture of methanol and
0.1N HCl (1:1, v/v) exhibits absorption maxima lo-
cated at 230nm. The addition of aqueous solutions of
ferric chloride, MBTH to the drug solution produced
a new characteristic peak at 619 nm (Figure 2). The
blank solution was prepared similarly as the sample
solution except for the presence of the drug, which
does not show any peak at 619nm.

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[17,18]. The inter-
mediate 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 (Scheme 1).

Optimization of variables

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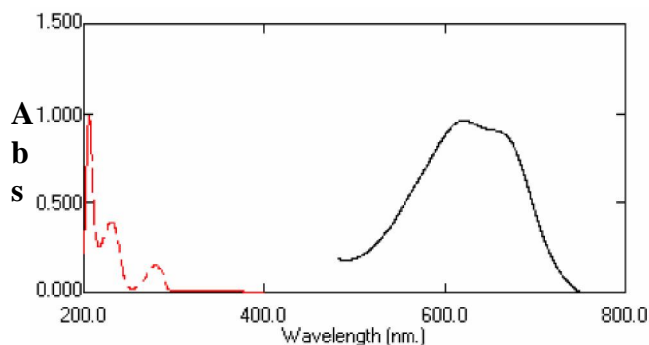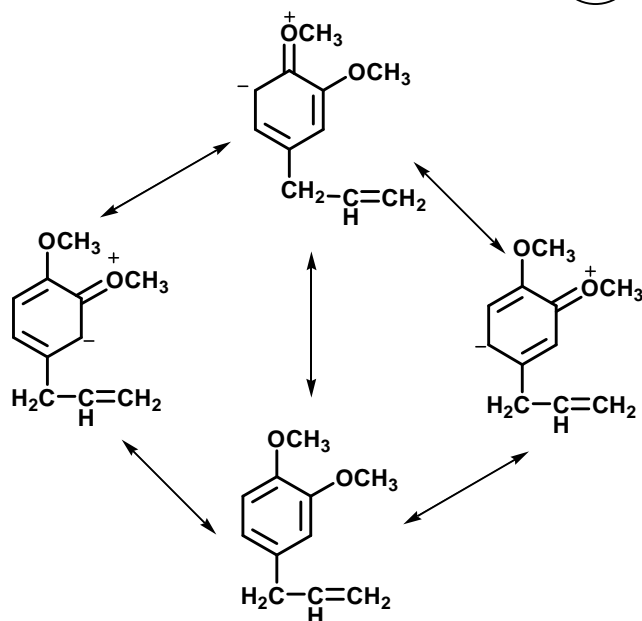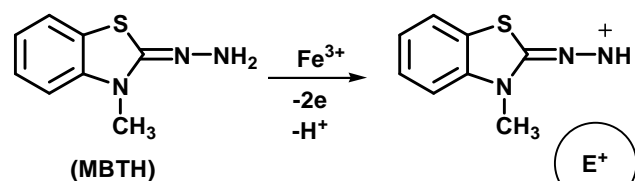reaction time, and the effect of diluting solvent, were
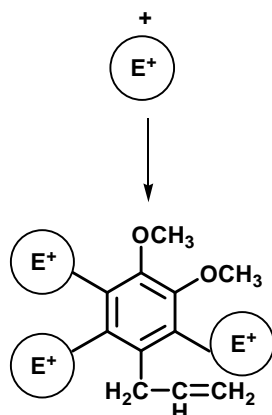studied.

Full Paper

(MBTH) $\xrightarrow[\substack{-2e \\ -H^+}]{Fe^{3+}}$ E⁺

OCH₃ / OCH₃ / CH₂—C=CH₂ / H

(Methyl Eugenol)

+

E⁺

OCH₃ / OCH₃ / E⁺ / E⁺ / H₂C—C=CH₂ / H

Blue colored reaction product
(1:3)
Scheme 1

Effect of MBTH concentration

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

Effect of iron (III) concentration

The optimum concentration of ferric chloride solu-

tion for maximum color development was found to be 1.5mL of 4% $FeCl_3$-solution per 10mL of reaction mixture. Higher concentrations of oxidant did not affect the absorption intensity of the color. Several other oxidants were investigated, *e.g.* ammonium cerric sulfate, potassium iodate and hydrogen peroxide. Only iron(III)-chloride gave the characteristic color with ME with MBTH.

Effect of hydrochloric acid concentration

Different concentrations of hydrochloric acid (0.01-0.2M) used as a solvent for MBTH and $FeCl_3$, have been tested. Maximal color intensity could be achieved by using 0.1M strength. Higher acidities did not affect the developed color intensity.

Effect of diluting solvent

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

Effect of reaction time

A development time of 35 minutes, in all the tested diluting solvents, at 20±5°C was found optimum for the maximal absorption intensity of the colored product, which was stable for at least 2 hours.

Quantification and method validation

A linear correlation was found between the absorbance at 619nm and concentration in the 2.5-10µg 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~16200mol⁻¹cm⁻¹ (logε = 4.2092).

The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the current ICH guidelines[19,20] 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 rela-

Full Paper

TABLE 1: Assay parameters of the reaction between ME and MBTH

Parameter	Methyl Eugenol
λ_{max}	619nm
Linearity Range (μg mL^{-1})	2.5-10
Molar absorptivity mol^{-1} cm^{-1}	16200
Regression equation	
Slope	0.091
Intercept	+0.1672
Correlation Coefficient (r^2)	0.9998
Recovery $\pm$ SD (%)	100.01 $\pm$ 0.58
LOD (μg mL^{-1})	0.374
LOQ (μg mL^{-1})	1.132
RSD (%) *[a]	0.932-0.578
RSD (%) *[b]	0.110-0.553

*[a]The interday (n=9) and *[b]The intraday (n=9) relative standard deviations of samples of concentrations (5 & 7.5μg mL^{-1}) for methyl eugenol.

tive 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 inter–day RSD-values (0.58-0.93%) and the low RSD(%)-values ($\leq$ 2.0 %.) reflect a good precision of the proposed method (TABLE 1).

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 99.28-100.46%) (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 (98.59–99.86%) revealed that there is no interference of excipients and additives in the determination (TABLE 3).

Statistical analysis of the results in comparison with the reported method

The results of the proposed method were statisti-

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

Preparation	Found[#] (μg mL^{-1})	Recovery * (%)
Methyl Eugenol	4.964	99.28
Formulation	5.023	100.46
B.No:1465	4.964	99.28
Mean $\pm$ SD		99.67 $\pm$ 0.681

*Average of three determinations, [#]Claimed (μg mL^{-1}): 5

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

Preparation	Added (μg mL^{-1})	Found[#] (μg mL^{-1})	Recovery * (%)
Methyl Eugenol	2.5	2.492	99.69
Formulation	5.0	4.993	99.86
B.No:1465	7.5	7.394	98.59
Mean $\pm$ SD			99.38$\pm$ 0.689

*Average of three determinations, [#]Claimed (μg mL^{-1}): 5

TABLE 4: Statistical comparison of the results obtained by the proposed MBTH colorimetric method and those of the reported method[a] for the analysis of pure samples of methyl eugenol

Parameter	Proposed method	Reported method[9]
Mean recovery	100.01	99.63
SD	0.58	0.67
RSD	0.580	0.672
n	7	5
Variance	0.336	0.450
F-value	1.339 (4.53)	
Student's t-test	0.178 (2.228)	

[a]Figures in parentheses are the corresponding theoretical t- and F-values (P= 0.05)

cally analyzed[21,22] and compared with those obtained by applying the reported HPLC method[9]. 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.

CONCLUSION

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 identifi-

Full Paper

cation and/or for quantitative analysis.

- It can be recommended for routine analytical laboratories when sophisticated expensive equipments are unavailable.

REFERENCES

[1] R.L.Prior, G.Cao; HortScience, **35**, 588-592 **(2000)**.

[2] T.E.Shelly; Ecological-Entomology, **25(1)**, 109-114 **(2000)**.

[3] T.E.Shelly, J.Edu; Florida-Entomologist; **91(3)**, 388-393 **(2008)**.

[4] T.E.Shelly, J.Edu, E.Pahio, Wee-SukLing, R.Nishida; Entomologia-Experimentalis-et-Applicata, **128(3)**, 380-388 **(2008)**.

[5] L.YongYue, Z.Ling, L.GuangWen, L.JinTian, Y.Xin, X.YiJuan; Chin., Bull.Entomol., **43(1)**, 123-126 **(2006)**.

[6] T.E.Shelly, S.Resilva, H.Bignayan; Florida Entomologist, **79(4)**, 481-488 **(1996)**.

[7] R.I.Vargas, J.D.Stark, M.H.Kido, H.M.Ketter, L.C.Whitehand; J.Econ.Entomol., **93(1)**, 81-87 **(2000)**.

[8] M.L.Cornelius, J.J.Duan, R.H.Messing; J.Entomol. Sci., **36(3)**, 259-267 **(2001)**.

[9] W.Graves, S.Runyon; J.Chromatogr.B, Biomed. Appl., **663(2)**, 255-262 **(1995)**.

[10] F.Siano, C.Ghizzoni, F.Gionfriddo, E.Colombo, L.Servillo, D.Castaldo; Food Chem., **81(3)**, 469-475 **(2003)**.

[11] J.Gasparič, D.Svobodová, Pospišilová; Microchimica Acta, **67(3-4)**, 241-250 **(1977)**.

[12] N.A.El-Ragehy, S.S.Abbas, El-Khateeb; J.Pharm. Biomed.Anal., **25**, 143-151 **(2001)**.

[13] N.A.Alarfaj, S.A.Altamimi, L.Z.Almarshady; Asian J.Chem., **21(1)**, 217-216 **(2009)**.

[14] Z.Ahmed, Y.N.Manohara, K.P.Channabasawaraj, M.Majumdar; E-Journal of Chemistry, **5(4)**, 713-717 **(2008)**.

[15] B.Hiremath, B.H.M.Mruthyunjayaswamy; Acta Pharm., **58**, 275-285 **(2008)**.

[16] N.Rahman, Y.Ahmed, S.Azmi; Canad.J.Anal. Sci.Spectr., **50(3)**, 116-129 **(2005)**.

[17] A.E.El-Gendy, M.G.El-Badricy, H.M.Loutfy, M.F.El-Tarras; Spectr.Letters, **34(2)**, 221-234 **(2001)**.

[18] E.Sawicki, T.W.Stanely, T.R.Hauser, W.Elbert, J.L.Noe; Anal.Chem., **33**, 722 **(1961)**.

[19] ICH(Q2A) Note for Guidance on Validation of Analytical Methods: Definition and Terminology. International Conference on Harmonisation, IFPMA, Geneva, **(1994)**.

[20] ICH(Q2B) Note for Guidance on Validation of Analytical Methods Methodology. International Conference on Harmonisation, IFPMA, Geneva, **(1996)**.

[21] J.D.Hinchen; Practical Statistics for Chemical Research, Methuen and Science Paperbacks, London, England, 110,111,176,177 **(1969)**.

[22] C.L.Hiang; 'Statistical Method of Analysis', World Scientific Publishing Co, USA, **(2003)**.