UV, HPLC Method development and quantification of eugenol isolated by preparative paper chromatography from Alcoholic extracts of different species of Ocimum

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Received on: 20-06-2010; Revised on: 16-07-2010; Accepted on:15-09-2010

ABSTRACT

Around 2 ml of 99% eugenol was taken in a clean and dry test tube. The standard eugenol was spotted on a whatman filter paper having dimension 4 X 20 cm. 95% of methanol was mixed with 5% of chloroform by ultra sonication and taken around 100 ml in a chromatographic chamber. and its (Rf) value was calculated. 5mg of each extract was drawn and dissolved in 5 ml of solvent (Meoh:Chloroform 95:5) then 0.1 ml from the above solution was applied to the paper in the form of band and all the procedure was maintained as like as standard. After complete development the paper was removed out and allowed to dry. Measure the Rf value as compared with the standard eugenol. Then the portion which resemble with the standard Rf value was cut out and dissolved in 5ml of the above solvent. The solution then taken for UV analysis. By trial and error, the method having 70 parts of methanol, 20 parts of water and 10 parts of Acetonitrile was fit strong for eugenol in an Isocratic system having column C-18 and this method was developed for both standard eugenol and eugenol present in alcoholic extracts of different species of Ocimum, and subjected for HPLC analysis.

Keywords: Isolation of eugenol by preparative paper chromatography; UV, HPLC method development; Quantification against standard; Ocimum.

INTRODUCTION

In Ayurveda Tulsi has been well documented for its therapeutic potentials. Although the traditional medical practitioners in India have been widely using this medicinal plant for management of various disease conditions1. In last few decades several studies have been carried out by Indian scientists and researchers to suggest the role of essential oils & eugenol in therapeutic potentials of Tulsi. Eugenol is a phenolic compound and major constituent of essential oils extracted from different parts of Tulsi plant4-5,7. Sophisticated chromatographic and spectrophotometric techniques are used for isolation, qualitative and quantitative estimation of eugenol from extracts of different species of Tulsi2-4.

MATERIALS AND METHODS

Materials
Sonicator, heating mantle, soxhlet extractor, electrical balance (Dhona), U.V, Spectrophotometer (Thermo Scientific- UV–10, Shimadzu), HPLC (Shimadzu LC– 20AT, SPD- 20A), were supplied by the Department of Pharmaceutical Analysis & Quality Assurance, Jeypore College of Pharmacy, Rondapalli, Jeypore. Methanol for HPLC, Acetonitrile for HPLC, Water for HPLC, Eugenol extra pure (Merck Pvt. Ltd. Mumbai & Loba Chemie Pvt, Ltd, Mumbai) and other chemicals and reagents were procured from authorized suppliers.

Plant material
The leaves of Ocimum species (O. gratissimum linn. O. americanum linn. O. Sanctum linn. and O. basilicum linn.) were collected from the local area of south eastern Odisha (India) in the month of January 2010. The plant material were identified and authenticated by Botanical Survey of India, Shibpur, Howrah vide Letter No. CNH/I-I/49/2009/Tech.II/168 dated 05.03.2010. The collected leaves were shade dried under normal environmental condition, powdered, stored in a closed container for further use.

Preparation of Extract
The powdered leaf of each Ocimum species (750 g) was extracted with methanol by using Soxhlet extraction apparatus. The extract was filtered and concentrated by distilling of the solvent to obtain the crude extract. Then it was dried by rotary evaporator and stored for further study.

Preparation of Mobile Phase:
95% of methanol was mixed with 5% of chloroform by ultra sonication and taken around 100 ml in a chromatographic chamber. The chamber was saturated for 30 mins to avoid edge effect.

Method
After chamber saturation the spotted paper was placed in the chamber by the help of a thread tied at its upper part. Then the mobile phase was allow to run, the pre sent eugenol in the spot according to its affinity towards mobile phase moved and its retardation factor (Rf) was calculated.

Preparation of sample for preparative paper chromatography.
5mg of each extract was drawn and dissolved in 5 ml of solvent (Meoh:Chloroform 95:5) then 0.1 ml from the above solution was applied to the paper in the form of band and all the procedure was maintained as like as standard. After complete development the paper was removed out and allowed to dry. Measure the Rf value as compared with the standard eugenol. Then the portion which resemble with the standard Rf value was cut out and dissolved in 5ml of the above solvent. The solution then taken for UV analysis.

UV analysis:
Optimization of eugenol by UV spectroscopic Method
Preparation of Mobile Phase:
Solvent was prepared by mixing 95 parts of methanol with 5 parts of chloroform with proper sonication. Since standard eugenol contains 99%, hence 99 gm in 100ml and 1000 µg contained in 1.01 ml.

1.01ml-----dissolved up to 10 ml of solvent to give--100 µg / ml of eugenol.

From the above 10ml contains 100 µg of eugenol 1ml was drawn and again dissolved up to 10 ml of solvent to give 10 µg / ml of eugenol. Again From the above 10ml contains 10 µg of eugenol 1ml was drawn and dissolved up to 10 ml of solvent to give 1 µg / ml of eugenol.
The prepared three concentrations (1 µg / ml, 10 µg / ml, 100 µg / ml) of eugenol was then carried out for determination of ‘t’max.

Calibration of Eugenol
From 99% eugenol stock solution was prepared. that is 10.1 ml of solution contains 99% was dissolved in 10 ml of solvent to give 1000µg. from that stock solution different Eli

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RESULTS AND DISCUSSION

Quantification of eugenol isolated by paper chromatography from alcoholic extracts of different species of Ocimum.

UV analysis of eugenol contained in Ocimum basilicum l.

By comparing the Rf value of standard eugenol from paper chromatography, the band of eugenol obtained from extract of Ocimum basilicum was identified and cut it out. The paper piece containing eugenol was thoroughly mixed with 5 ml of solvent and filtered. Then the solution was taken for UV Analysis.

UV analysis of eugenol contained in Ocimum gratissimum l.

By comparing the Rf value of standard eugenol from paper chromatography, the band of eugenol obtained from extract of Ocimum gratissimum was identified and cut it out. The paper piece containing eugenol was thoroughly mixed with 5 ml of solvent and filtered. Then the solution was taken for UV Analysis.

UV analysis of eugenol contained in Ocimum Sanctum l.

By comparing the Rf value of standard eugenol from paper chromatography, the band of eugenol obtained from extract of Ocimum sanctum was identified and cut it out. The paper piece containing eugenol was thoroughly mixed with 5 ml of solvent and filtered. Then the solution was taken for UV Analysis.

UV analysis of eugenol contained in Ocimum americanum l.

By comparing the Rf value of standard eugenol from paper chromatography, the band of eugenol obtained from extract of Ocimum americanum was identified and cut it out. The paper piece containing eugenol was thoroughly mixed with 5 ml of solvent and filtered. Then the solution was taken for UV Analysis.

Quantitative estimation of eugenol by HPLC method.

Development of method

After all trial and error, the following developed method having 70 parts of methanol, 20 parts of water and 10 parts of acetonitrile was fit strong for eugenol in a Isocratic system and this method was developed for both standard eugenol and eugenol present in alcoholic extracts of different species of ocimum.

Calibration of eugenol by HPLC

1.01ml of 99% v/v eugenol when diluted up to 10 ml of solvent (MeOH: H2O: ACN, 70:20:10) to give 100 µg / ml of eugenol. From this stock solution aliquots of 25, 50, 75, 100, 125µmol gram per ml of eugenol were prepared. After sufficient 30 min sonication and filtration through 0.45 micron filter paper those solutions were injected to HPLC for Calibration.

Quantitative estimation of eugenol isolated by paper chromatography from alcoholic extracts of different species of ocimum.

By putting the peak areas of eugenol which isolated by paper chromatography from alcoholic extracts of different species of ocimum obtained at retention time 6.04, in the standard calibration curve the concentrations can be determined.

RESULTS AND DISCUSSION

UV spectrum of standard Eugenol.

From the above spectrum it was found that the rmax of eugenol (Peak-4) is 281 nm.

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<tr>
<th>Table 1. Calibration Table of Eugenol</th>
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</table>

UV analysis of Ocimum basilicum l. and Ocimum gratissimum l. at rmax 281nm and having absorbance 0.419 and 0.929 A respectively.

Quantification

Ocimum basilicum linn

350 mg of Extract was dissolved in 10ml of solvent. So 1 ml of solution Contains 35 mg of extracts. This 1ml was taken as a band in paper (For paper Chromatography). The developed Eugenol band was cut out & dissolved in again 5ml of Solvent and taken for UV Analysis. It was found from calibration curve 6.75 µg of Eugenol present in 1ml of Solvent. Hence 5ml of solution contains = 6.75×5=33.75 µg =0.033mg So 1ml of Solvent which Contains 35 mg of extract contains 0.033 mg of Eugenol. Hence 5000mg (5gm) of Extract contains = 0.033/35×5000 mg of Eugenol = 4.71mg of Eugenol. Hence % of Eugenol contains in Ocimum basilicum = 4.71/5000 × 100 = 0.09%.

Ocimum gratissimum linn

(5×15) = 75 µg =0.075mg =0.075/35×5000. = 10.71 mg of Eugenol. Hence % of Eugenol contains in Ocimum gratissimum = 10.71/5000 × 100 = 0.21%

Ocimum sanctum linn

(5×25) = 125 µg =0.125mg = 0.125/35×5000 = 17.85 mg of Eugenol. Hence % of Eugenol contains in Ocimum sanctums = 17.85/5000 × 100 = 0.35%.

Ocimum americanum linn

(5×20) =100 µg =0.1mg =0.1/35×5000= 14.28 mg of Eugenol. Hence % of Eugenol contains in Ocimum americanum = 14.28/5000 × 100 = 0.28%.

CONCLUSION

Hence from that UV analysis it was conclude that the Ocimum basilicum linn contains 4.8 mg (0.09%) of Eugenol. Ocimum gratissimum linn contains 10.71 mg (0.21%) of Eugenol. Ocimum sanctum linn contains 17.85 mg (0.35%) of Eugenol. Ocimum americanum linn contains 14.28 mg (0.28%) of Eugenol From 5gm of Extracts.

Quantitative estimation of eugenol by HPLC method

Figure 1. UV spectrum of eugenol

From the above spectrum peak no 2 is the peak of eugenol of Ocimum sanctum l. and Ocimum americanum l at rmax 281nm and having absorbance 1.555 A and 1.239 A respectively.
Figure 8. HPLC Chromatogram of Alcoholic extract of Ocimum basilicum linn.

Figure 9. HPLC Chromatogram of Ocimum gratissimum linn.

Figure 10. HPLC Chromatogram of Ocimum sanctum linn.

Figure 11. HPLC Chromatogram of Ocimum americanum linn.

Figure 12. HPLC Calibration curve of Eugenol.

Calibration Table

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<tr>
<th>Concentration(ng/ml)</th>
<th>Peak Area</th>
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<td>25</td>
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<tr>
<td>50</td>
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<td>75</td>
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<td>100</td>
<td>5356</td>
</tr>
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<td>125</td>
<td>6678</td>
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</table>

Quantification:

Ocimum basilicum linn
350 mg of Extract was dissolved in 10ml of solvent. So 1 ml of solution Contains 35 mg of extracts. This 1ml was taken as a band in paper (For paper Chromatography). The developed Eugenol band was cut out & dissolved in again 2ml of Solvent and taken for sonication and filtration then from this 2ml 2µl was injected to HPLC. It was found from calibration curve, 30ng of Eugenol present in 2 µl of Solution. Hence 2ml of solution contains = 0.03 mg So 1ml of Solvent which Contains 35 mg of extract contains 0.03mg of Eugenol.Hence 5000ng (5gm) of Extract contains = 0.03/35x5000 mg of Eugenol = 4.28 mg of Eugenol. Hence % of Eugenol contains in Ocimum basilicum = 4.28/5000 x 100 = 0.08%.

Ocimum gratissimum linn
1ml of Solvent which Contains 35 mg of extract contains 0.073mg of Eugenol. Hence 5000mg (5gm) of Extract contains = 0.073/35x5000 mg of Eugenol= 10.42 mg of Eugenol Hence % of Eugenol contains in Ocimum gratissimum = 10.42/5000 x 100 = 0.20%

Ocimum sanctum linn
1ml of Solvent which Contains 35 mg of extract contains 0.122mg of Eugenol. Hence 5000mg (5gm) of Extract contains = 0.122/35x5000 mg of Eugenol = 17.42 mg of Eugenol Hence % of Eugenol contains in Ocimum sanctum = 17.42/5000 x 100 = 0.34%.

Ocimum americanum linn
1ml of Solvent which Contains 35 mg of extract contains 0.098mg of Eugenol. Hence 5000mg (5gm) of Extract contains = 0.098/35x5000 mg of Eugenol = 14.0 mg of Eugenol Hence % of Eugenol contains in Ocimum americanum = 14.0/5000 x 100 = 0.28%.

CONCLUSION
Hence from that HPLC analysis it was conclude that the Ocimum basilicum linn contains 4.28 mg (0.08%) of Eugenol.Ocimum gratissimum linn contains 10.42 mg (0.20%) of Eugenol.Ocimum sanctum linn contains 17.42 mg (0.34%) of Eugenol.Ocimum americanum linn contains 14.0 mg (0.28%) of Eugenol From 5gm of Extract.

ACKNOWLEDGEMENT
Authors wish to thank to local people of south eastern odisha and Botanical Survey of India, Shibpur, Howrah, West Bangle, India, for providing valuable information about the plant and its identification. The author wish to express their gratitude Jeyppore College of Pharmacy, Rondapalli, Jeyppore, Koraput, Odisha.

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Source of support: Nil, Conflict of interest: None Declared