EVALUATION ON EUGENOL CONTENT IN SOME THAI TRADITIONAL MEDICINE SAMPLES BY REVERSE-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC)

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Abstract— A sensitive RP-HPLC method with ultra-violet (UV) detection has been developed for determination of eugenol in commercials of Thai traditional medicine. Analysis of samples were performed on RP-C18 column and eluted with isocratic system by methanol and water (63:37, v/v) as mobile phase system and at 1.0 mL/min flow rate. The eluate was detected at 280 nm. This method was applied to analyse eugenol content in 10 commercials of Thai traditional medicine. The results showed that eugenol peak was detected at 9.6 minute. The linearity of calibration curve was in the range of 0.17-95.84 µg/mL. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.02 µg/mL and 0.005 µg/mL respectively. A simple, precise and accurate HPLC-UV method has been developed for the determination of eugenol in 10 commercials of Thai traditional medicine samples. Its revealed that eugenol could be detected in Thai traditional medicine in the range of 0.17-95.84 µg/mL.

Keyword— Eugenol, High Performance Liquid Chromatography, Thai traditional medicine.

I. INTRODUCTION

Traditional medicine has been used for thousands of years in some countries for its therapeautic properieties, containing inherent active ingrediants that has properties to heal sores and relieve pain[1]. The Kingdom of Thailand has its own system of traditional medicine and it had been a means of national health care for the Thai people until the early 20th century[2]. Some herbs and plants used in traditional medicine and some herbs store their precious essential oil within their leaves, root, rinds, seed and other plant parts. Some essential oils of plant were phytochemicals and the features of traditional medicine is highly dependent of chemical phytoconstituents in their extracted final product [1]. Eugenol (4-allyl-2-methoxyphenol) (fig.1) is a semivolatile compound. It is a major component of essential oil isolated from clove and found in some herbs such as basil, cinnamon, fennel, marjoram, nutmeg and anise[3]. It possesses as an antioxidant, anti-inflammatory and antifungal[4]. Eugenol has been widely used as herbal drug to treat dyspepsia, acute/chronic gastritis, diarrhea and dentistry. Furthermore eugenol is also an important flavoring agent in cosmetic and food products[5]. There have many works report about eugenol in many plants, however, there was no report about this volatile oil in Thai traditional medicine. This work aim to analysis eugenol in some Thai traditional medicine that sold in Thai market to support the quality of traditional medicine. There are many methods for analysis eugenol such as HPLC [5-7] and GC [8-9]. This work describes a simple, sensitive and accurate HPLC-UV method for the determination of eugenol.

II. MATERIALS AND METHODS

Reagents and standards
Eugenol (99%, C10H12O2) was purchased Sigma-Aldrich (Steinheim, Germany). HPLC grade methanol was procured from Carlo Erba Reagents (Italy). Sample of eight commercially traditional medicine that sold in Bangkok, Thailand: stomachic mixture (3 samples: S1, S2, S3) cough medicine (7 samples: S4, S5, S6, S7, S8, S9, S10) were purchased from drug store in Bangkok, Thailand: stomachic mixture (3 samples: S1, S2, S3) cough medicine (7 samples: S4, S5, S6, S7, S8, S9, S10)

Apparatus
Chromatographic analysis was performed with an Agilent Technologies HPLC system including a 1260 Quat Pump VL pumps and 1260-TCC detector, an on-line solvent vacuum degasser and manual sample injector fitted with a 20 µL injection loop. The column used was a VertiSep GES C18 column (4.6 mm × 250mm, 5 µm). A mixture of 63% methanol and 37% water for 12 min at a flow rate 1.0 mL min-1 were used as a mobile phase and the detection was performed at the wavelength of 280 nm.

Preparation of sample solution
1.xxxx g sample was exactly weighted and 10.0 mL of methanol as extraction solvent were added. Follow by sonication for 15 minutes in an ultrasonic bath at room temperature. Extracted sample solution was filtered with polytetrafluoroethylene syringe filters no. 0.45 µm. The filtrate was collected in polypropylene tubes and stored at 4°C until further analysis.

III. RESULTS AND DISCUSSION

Method optimization
HPLC condition were optimized for separation of eugenol, methanol: water (63:37, v/v) was successfully. The retention time of eugenol was found to be 9.6 minute and the eluate was detected at 280 nm. (Fig. 2)
Evaluation on Eugenol Content in Some Thai Traditional Medicine Samples by Reverse-Phase High Performance Liquid Chromatography (RP-HPLC)

Calibration curve
The linearity of peak area response versus concentration for eugenol was studied between concentration ranges of 1-100 µg mL⁻¹. The regression equation and correlation coefficient was \( y = 24.745x + 30.66 \) (Fig 2.). Fig 3 shows the obtained chromatograms of standards at concentration level of 10 µg/mL.

![Fig. 2: Calibration curve of eugenol](image)

Fig. 2: Calibration curve of eugenol

Limit of detection (LOD) and limit of quantitation (LOQ) The limit of detection (LOD) was signal to noise ratio of 3 and limit of quantitation (LOQ) was signal to noise ratio of 10. The LOD and LOQ were found to be 0.02 µg/mL and 0.005 µg/mL respectively. The LOD and LOQ were obtained from the following equation:

\[
\text{LOD} = 3 \sigma/s \\
\text{LOQ} = 10 \sigma/s \\
\sigma = \text{Standard deviation of response} \\
S = \text{Slope of calibration curve}
\]

Analysis of sample
Thai traditional medicine samples were purchased from Thai market in Bangkok, Thailand; stomachic mixture (3 samples: S1, S2, S3) cough medicine (7 samples: S4, S5, S6, S7, S8, S9, S10) and stored at room temperature. The results for samples were found at the concentration range of 96-102%. The results are show in table 2. Fig 4 shows the obtained chromatograms of cough medicine sample (S8).

![Fig. 3: shows the obtained chromatograms of standards of eugenol](image)

Fig. 3: shows the obtained chromatograms of standards of eugenol

![Table. 1: Quantitative result of eugenol from commercially traditional medicine thai drugs.](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear range (µg/mL)</td>
<td>0.1-1000</td>
</tr>
<tr>
<td>LOD (µg/mL)</td>
<td>0.02</td>
</tr>
<tr>
<td>LOQ (µg/mL)</td>
<td>0.005</td>
</tr>
<tr>
<td>Regression equation</td>
<td>( y = 24.745x + 30.66 )</td>
</tr>
<tr>
<td>( r^2 )</td>
<td>0.9982</td>
</tr>
<tr>
<td>RSD% (intra-day)</td>
<td>0.97%</td>
</tr>
<tr>
<td>RSD% (inter-day)</td>
<td>2.77%</td>
</tr>
</tbody>
</table>

![Table. 2: Concentration of eugenol in commercially thai traditional medicine samples.](image)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Found mean ± SD (µg/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>n.d.</td>
<td>96.72</td>
</tr>
<tr>
<td>S2</td>
<td>0.71 ± 1.14</td>
<td>102.14</td>
</tr>
<tr>
<td>S3</td>
<td>n.d.</td>
<td>98.02</td>
</tr>
<tr>
<td>S4</td>
<td>n.d.</td>
<td>96.40</td>
</tr>
<tr>
<td>S5</td>
<td>32.58 ± 2.83</td>
<td>97.39</td>
</tr>
<tr>
<td>S6</td>
<td>95.84 ± 1.65</td>
<td>98.72</td>
</tr>
<tr>
<td>S7</td>
<td>n.d.</td>
<td>101.58</td>
</tr>
<tr>
<td>S8</td>
<td>85.34 ± 0.68</td>
<td>98.84</td>
</tr>
<tr>
<td>S9</td>
<td>3.15 ± 1.75</td>
<td>101.17</td>
</tr>
<tr>
<td>S10</td>
<td>0.17 ± 0.13</td>
<td>100.16</td>
</tr>
</tbody>
</table>

* n.d. = not detected

Accuracy
Accuracy of the method is determined by performing the recovery studies. Recovery study was performed by addition of known amount of standard in real sample and the results are show in table 1.

![Fig. 4: shows the obtained chromatograms of cough medicine sample (S8).](image)

Fig. 4: shows the obtained chromatograms of cough medicine sample (S8).
CONCLUSION

The HPLC-UV method developed in this study was successfully for determination of eugenol and this method can be applied for determination of thai traditional medicines containing eugenol. It concludes that the developed method is sample, accurate, sensitive and precise. The results of study indicate that eugenol was found in traditional medicine, which dependent of ingredients in traditional medicine and cough medicine sample (S8) was found highest. This will aid in identification of bioactive compounds that possess therapeutic activity of the traditional medicine which are major constituents of plant materials.

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