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Quantitative Determination of Eugenol in Eugenia Caryophyllata Thunb by Three Wavelength Spectrophotometry

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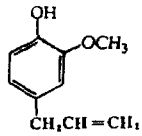
Abstract: Three wavelength spectrophotometry was used to determine the content of eugenol in Eugenia Caryophyllata Thunb. Using this method could effectively eliminate the deviation of background absorption caused by the change of concentration and the error of quantitative analysis caused by asymmetric peaks, and at the same time the leaning degree of base line was corrected. This method was simple, the recovery ratio was 90.05%—116.94% and the coefficient of variation was 3.5%.

Keywords: Spectrophotometry, Three wavelength, Eugenia Caryophyllata Thunb, Eugenol.

1. Introduction

Eugenia caryophyllata thunb is myrtaceae in classification of the plant system. Eugenia caryophyllata thunb is a good crude drugs. It has a efficiency of antibiosis, analgesia and is

good for the stomach. The main chemistry constituents of eugenia caryophyllata thunb is eugenol. Eugenol costitutional formula is



when quantitative determination of eugenol was carried out using traditional spectrophotometry method, baseline shift and asymmetric absorption peak occur on the solution absorption curve. Three wavelength spectrophotometry can eliminate the absorbance error of baseline shift and asymmetric absorption peak. It is more accurate than traditional spectrophotometry method.

Principle of the method

Principle of three wavelength spectrophotometry is shown in Fig. 1. Three Wavelength points λ_1, λ_2 and λ_3 of absorbance determinations can be selected;

$m=\lambda_2-\lambda_3 \quad n=\lambda_1-\lambda_2$

$\because$ in $\Delta\lambda_3 P\lambda_1 \quad N\lambda_2 // P\lambda_1$

$$\therefore \frac{N\lambda_2}{P\lambda_1}=\frac{\lambda_3\lambda_2}{\lambda_3\lambda_1}=\frac{m}{m+n}$$

$\because P\lambda_1=A_1$

$$\therefore N\lambda_2=\frac{mA_1}{m+n}$$

at the same time

$\because$ in $\Delta P\lambda_3 R, MN // R\lambda_3$

$$\therefore \frac{MN}{P\lambda_3}=\frac{PN}{P\lambda_3}=\frac{\lambda_1\lambda_2}{\lambda_1\lambda_3}=\frac{n}{n+m}$$

$\because R\lambda_3=A_3$

$$\therefore NM=\frac{nA_3}{m+n}$$

$$\therefore \Delta A=A_2-(N\lambda_2+MN)$$

$$=A_2-\frac{mA_1+nA_3}{m+n}$$

$$=\left(a_{\lambda_2}-\frac{ma_{\lambda_1}+na_{\lambda_3}}{m+n}\right)b\cdot c \qquad (1)$$

In equation (1) a is the molar extinction coefficients of component determinated at various wavelength points; b is the path length of the absorbing solution; c is the concentration in mole/litre.

Equation (1) states that value ΔA is proportional to the concentration of component determinated.

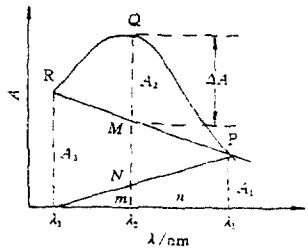


Fig. 1 Principle of three wavelength spectrophotometry

From Fig. 1 we know if selective three wavelength points are corresponding to absorption spectrum three points at one straight line, ΔA value determinated must be zero. Therfore, when absorption spectrum of interfering components is one straight line which produces the interference, selective three wavelength points are determinated. Values ΔA are independent of interfering components concentrations.

2. Experimental

2.1 Reagents

Distilled water, eugenol were prepared by

Chemistry Department of Liaoning University.

2.2 **Apparatus**

A Hitachi U－3400 Autometic recording spectrophotometer equipped with 1－cm quartz cells was employed.

2.3 **Procedure**

Transfer a portion of the standard eugenol solution 12.8μg/mL to a 1－cm cell and measure the absorbance *vs.* methanol blank by scaning from 200－400nm with computer program RER WL SCAN of U－3400, the spectrum is plotted automatically. It is shown in Fig. 2. Three wavelength point were selected by graphic calculating method, that is, $\lambda_1=302$ nm, $\lambda_2=280.6$ nm and $\lambda_3=253.4$ nm.

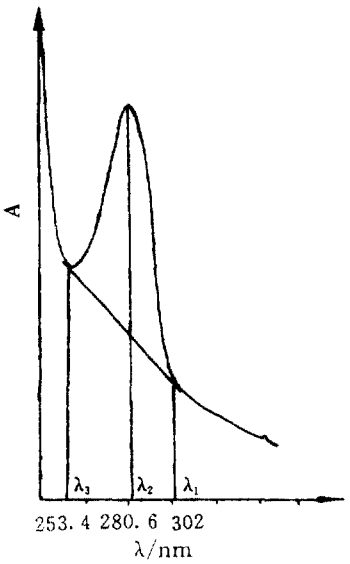


Fig. 2 Eugenol absorption spectrum

Transfer the samples eugenia caryophyllata thunb extraction solution and measure the absorbance values A and ΔA at $\lambda_1=302$ nm, $\lambda_2=280.6$ nm and $\lambda_3=253.4$ nm, by computer program SELECT OPERATING MODE and then determine the concentration of each sample solution.

3. Results and Discussion

Prepare a calibration curve by diluting a set of six standard eugenol solutions as above mentioned. Plot the absorbance. Measure the absorbance at $\lambda_1=302$ nm, $\lambda_2=280.6$ nm and $\lambda_3=253.4$ nm respectively. Calculate the ΔA values $\Delta A=A_2-\frac{mA_1+nA_3}{m+n}$. The results are listed in Table 1.

Table 1. Relationship between eugenol concentrations and ΔA

Eugenol conc. (μg/mL)	3.2	4.8	6.4	8.0	9.6	11.2	12.8
ΔA	0.0789	0.1284	0.1879	0.2229	0.2742	0.3250	0.3774

The data were analyzed on－line with a microcomputer by a linear least squared method, the regression equation of concentration *vs* ΔA is:

$\Delta A=-0.0172+0.0307C$

Relation coefficent $r=0.09940$

The above results demonstrate that eugenol concentration in the range of 3.2－12.8μg/mL. Linearly changed with ΔA when the absorbance was measured at wavelength 302 nm, 280.6 nm and 253.4 nm.

Experimental results show that when the samples were left for more than 8h, the absorbance were not changed.

Take five standard eugenol solutions with different concentration, measure the absorbances at 302 nm, 280.6 nm and 253.4 nm respectively. The results are listed in Table 2.

Table 2. Recovery ratio and precision

No	Sample quanty (μg/mL)	Add. standard quanty (μg)	Nine measure values (μg/mL)	Average values (μg/mL)	Average recovery ratio (%)	Coef. of var. (%)
1	3.2	1.6	4.7296—4.7557	4.7427	96.42	3.5
2	4.8	1.6	6.6515—6.6840	6.6710	116.94	3.6
3	6.4	1.6	7.8046—7.8567	7.8408	99.05	3.2
4	8.0	1.6	9.4723—9.5212	9.4919	93.24	2.8
5	9.6	1.6	11.1042—11.1889	11.1466	96.67	3.0

Table 2 shows that the recovery ratio is 90.05%～116.94%, the coefficient of varition is 3.5%.

Acknowledgment

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