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Simultaneous Determination of Diosmin and Hesperidin in *Cissus quadrangularis* Capsules by Ultraviolet-Visible Spectroscopy

Sutima Friedman[1], Prapasinee Soraphum[1], Parawan Ramanandana[2] and Phurit Thanarangsarit[2]*
[1]Pharmacy Student, Faculty of Pharmaceutical Sciences, Huachiew Chalermprakiet University, Thailand
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Huachiew Chalermprakiet University, Thailand
*Corresponding Author's E-mail: stamphurit@hotmail.com

Rationale

Cissus quadrangularis L. (Vitaceae) or commonly known in Thailand as "Phet Sang Khat" is a medicinal plant that has been used in Ayurveda for very long time. Many scientific studies reported various potential benefits by using of *C. quadrangularis* stem powders or extracts for the treatment of irregular menstruation, bone fractures, back pain, and hemorrhoids. Interestingly, the active constituents that play a crucial role for the treatment of hemorrhoids are supposed to be diosmin and hesperidin, which are generally found in 9:1 ratio and should be standardized.

Previous studies have introduced different analytical methods, such as high-performance liquid chromatography (HPLC), and UV-visible spectroscopy for concomitant analysis of diosmin and hesperidin in beverages, food supplements, and pharmaceutical dosage forms, but no reports for *C. quadrangularis* powders or extracts.

Research Objectives

To develop and validate an analytical method for determination of diosmin and hesperidin in *C. quadrangularis* capsules, using UV-visible spectroscopy based on simultaneous equations (Vierodt's method).

Methodology

Preparation of stock standard solutions

Diosmin and hesperidin were weighed accurately about 5 and 12 mg, respectively, into 50-mL volumetric flask, separately. Dissolved and adjusted to the volume with 0.2 N NaOH to obtain diosmin and hesperidin stock standard solution at concentration of 100 and 240 µg/mL, respectively.

Preparation of standard solutions

Diosmin and hesperidin standard solutions were prepared by diluting the *Stock standard solutions* with 0.2 N NaOH to get series of diosmin standard solution at concentrations of 1, 2, 3, 4, and 5 µg/mL. Similarly, a series of hesperidin standard solution at concentrations of 4.8, 9.6, 14.4, 19.2, and 24 µg/mL was also prepared. These solutions were scanned to determine the λ_{max} and measured the absorbances at corresponding wavelengths.

Preparation of sample solution

The capsule powder was weighed accurately equivalent to 125 mg of *C. quadrangularis* into a 50-mL volumetric flask. The powder was dissolved in 20 mL of 0.2 N NaOH, shook for 5 min, adjusted to the volume with the same solvent, and mixed. The mixture was filtered through a 0.45-µm filter paper and discarded the first 5 mL of filtrate. Pipetted 2.0 mL of the filtrate into a 50-mL volumetric flask and adjusted to the volume with 0.2 N NaOH to obtain 0.1 mg/mL of *C. quadrangularis* powder.

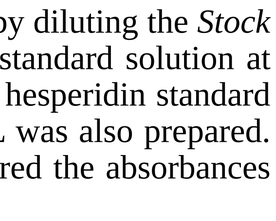
Method validation (ICH and AOAC guideline)

Specificity
- UV spectra of single and combined standard solution, including sample solution were obtained in range of 200 – 400 nm.
- **Characteristics of UV spectra and λ_{max} are resemble**

Linearity
- Calibration curves of 5 concentrations of either diosmin (1 – 5 µg/mL) or hesperidin (4.8 – 24 µg/mL) were constructed at corresponding wavelengths.
- **Correlation coefficient (r) > 0.995**

Accuracy
- Standard addition method at the 100% level (n = 6).
- 5.0 mL of standard diosmin (90 µg/mL) and hesperidin (10 µg/mL) were added into 25-mL volumetric flask containing 5.0 mL of *Sample solution*.
- **%Recovery = 97 – 103**

Precision
- Same as *Accuracy*.
- **%RSD ≤ 2.7**



Assay of *C. quadrangularis* capsules

Pooled powder from 20 capsules were accurately weighed and prepared by a stepwise procedure for *Sample solution*, then diluted with 0.2 N NaOH to obtain 0.02 mg/mL of *C. quadrangularis* powder. The absorbances of the resulting solution were measured at the corresponding wavelengths.

Simultaneous equation method

The absorbances of sample mixtures were measured at corresponding λ_{max} of diosmin and hesperidin. The concentration of diosmin (x) and hesperidin (y) in the mixtures were calculated by simultaneous equation using the following formula:

$$C_x = \frac{A_2ay_1 - A_1ay_2}{ax_2ay_1 - ax_1ay_2} \qquad C_y = \frac{A_1ax_2 - A_2ax_1}{ax_2ay_1 - ax_1ay_2}$$

Where C_x = concentration of diosmin (µg/mL)
C_y = concentration of hesperidin (µg/mL)
A_1 = absorbance of sample solution at λ_{max} of hesperidin (284.0 nm)
A_2 = absorbance of sample solution at λ_{max} of diosmin (267.5 nm)
ax_1 = absorptivity of diosmin at 284.0 nm
ax_2 = absorptivity of diosmin at 267.5 nm
ay_1 = absorptivity of hesperidin at 284.0 nm
ay_2 = absorptivity of hesperidin at 267.5 nm

The absorptivity (a) values of diosmin and hesperidin were obtained from the slope of their calibration curves.

Results

Method validation

All validation results are displayed in Table 1 and Figure 1.

Table 1 ***Validation results of diosmin and hesperidin***

Parameters	Acceptance criteria	Diosmin	Hesperidin	Conclusion
Specificity	Characteristics of UV spectra and λ_{max} are resemble	λ_{max} = 267.5 nm (Figure 1)	λ_{max} = 284.0 nm (Figure 1)	Failed
Linearity	r > 0.995	267.5 nm y = 0.0498x + 0.003 r = 0.9997 284.0 nm y = 0.034x + 0.0027 r = 0.9997	267.5 nm y = 0.0194x - 0.0063 r = 0.9999 284.0 nm y = 0.0304x - 0.0078 r = 0.9999	Passed
Range	Concentration range that gives a linear response	1 – 5 µg/mL	4.8 – 24 µg/mL	-
Accuracy	%Recovery = 97 – 103	97.47	103.9	Failed
Precision	%RSD ≤ 2.7	0.16	4.73	Failed

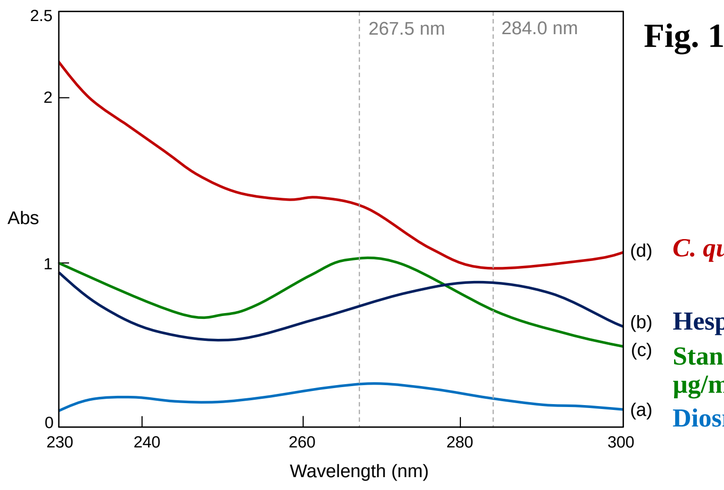
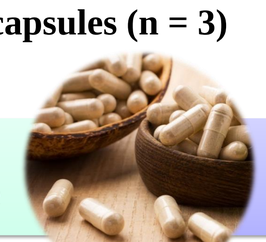


Fig. 1 ***UV overlay spectra of diosmin and hesperidin standard and C. quadrangularis sample solution.***

Assay of *C. quadrangularis* capsules (n = 3)

Diosmin
29.88 ± 0.08 mg/capsule



Hesperidin
3.176 ± 0.45 mg/capsule

250-mg *C. quadrangularis* capsules
(contained 125 mg of *C. quadrangularis* powder)

Discussion and Conclusion

This study was adapted from Srilatha, *et al.* (2013) which introduced the quantitative analysis of diosmin and hesperidin in Daflon® 500 mg (each tablet contains diosmin 450 mg and hesperidin 50 mg). For method validation, the results of diosmin met the criteria in terms of linearity, accuracy, and precision. On the other hand, the validation results of hesperidin seemed to be unsatisfied especially for specificity and precision. This may due to a very low proportion of hesperidin which usually occurred in combination with diosmin in 1:9 ratio, and the effect of other compounds in the matrix that possibly interfered the measured absorbances. Therefore, an appropriate extraction process must be applied for sample preparation to primarily separate the interferences, resulting to an increase of method specificity.

Our findings showed the concept of simultaneous analysis to determine the quantity of diosmin and hesperidin in *C. quadrangularis* capsules without the separation of each compound. Although this analytical method was found to be simple and easy to operate, the presence of other components in the analyte still affected the specificity as mentioned above.

In conclusion, the analytical method in this study was considered to be simple, accurate and precise, especially for the quantification of diosmin. However, the application of the developed method for simultaneously determination of diosmin and hesperidin in *C. quadrangularis* capsules still had some limitations. Thus, separation technique with qualitative and quantitative analysis, such as high-performance liquid chromatography (HPLC) could be a specific, accurate, and precise method to estimate the content of diosmin and hesperidin in multicomponent formulations for further study.

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