Rapid Determination of Resveratrol and Piceid in Wine by High-Performance Liquid Chromatography

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Abstract. In this study, a solid-phase extraction column dedicated to the detection of resveratrol was directly used for purification after adjusting the pH of the sample, without resorting to a new method for sample extraction. The compounds cis-resveratrol, trans-resveratrol, cis-piceid, and trans-piceid were thus detected in wine simultaneously by high-performance liquid chromatography (HPLC). This method offers rapid and easy operation and involves low consumption of organic solvents, paving the way for efficient determination of the variety and other parameters of grapes used in wine samples.

1. Introduction

Resveratrol is a plant hormone that offers health benefits and is mainly found in grape, polygonum cuspidata, and other plants. It mainly exists in the form of monomers and glycosides. Both of its cis and trans isomers can combine with glucose to form cis- or trans-resveratrol glycosides [1]. Resveratrol and its glycoside isomers are bioactive substances that affect human health [2,3]. Grapes and wine contain higher levels of resveratrol and its glycosides than other plants and products.

Under normal conditions, most methods cannot simultaneously determine all the four isomers mentioned above; therefore, the results obtained using such techniques cannot truly reflect the content of each isomer of resveratrol in wine [4,5]. In addition, the sample pretreatment process takes a long time for extraction and consumes high amounts of organic solvents [6,7,8]. Thus, the purpose of this study was to establish a simple high-performance liquid chromatography (HPLC) method for the simultaneous determination of these four isomers wherein sample extraction is not necessary. After adjusting the pH, the solid-phase extraction column dedicated to the detection of resveratrol was directly used for purification. The procedure is simple and consumes low amounts of organic solvents, which minimizes their exposure to the human body and improves the efficiency of the technique simultaneously.

Fig. 1 Structures of resveratrol isomers
Fig. 2 Structures of piceid isomers

2. Materials and methods

2.1 Materials
Great Wall wine used for the experiment was produced in Zhangjiakou and purchased from the local supermarket.

2.2 Instruments and reagents
Instrumental analysis was conducted with an LC-20A HPLC system equipped with a UV detector (Shimadzu, Japan). Nylon micropore filters with pore diameters of 0.22 μm were obtained from JIN TENG Technical Company (Tianjin, China). Analytes were extracted by solid-phase extraction using a ProElut BLC column (6 mL, 30/pk, Dikma, China). Chromatographic separation was performed using a Platsil ODS HPLC column (250 mm × 4.6 mm ID, 5 μm particle size).

Stock solutions of 1000μg/mL of 4 compounds prepared in methanol were obtained from the Agricultural Environmental Protection Institution in Tianjin, China. HPLC-grade acetonitrile and methanol were supplied by Sigma-Aldrich (St. Louis, USA). Ammonia (analytical grade) was obtained from Shanghai Haoshen Chemical Reagent (Shanghai, China). HPLC-grade water was obtained from a Millipore water purification system (Burlington, MA, United States).

2.3 Standard solutions
Stock sample solutions:
Stock solutions of 1000 μg/mL for the trans isomers of resveratrol and piceid were prepared in methanol and stored at −20 °C in darkness. Then, these trans-isomers were converted to their cis forms under ultraviolet irradiation in methanol.

Mixed standard solution of cis- and trans- piceid:
A 1 mL aliquot of the 1000 μg/mL stock solution of trans-resveratrol glycoside was placed in a covered 10 mL transparent glass tube and irradiated for 2 h under mixed wavelengths of only 254 nm and 365 nm. Then, 40 μL of the resulting solution was diluted with 30% acetonitrile in water to 1 mL. A 40 μg/mL trans-resveratrol glycoside solution was obtained as the single standard compound and prepared for HPLC analysis. Using the external standard method to calculate the peak area, the concentrations of trans- and cis-resveratrol glycosides solution irradiated were determined to be 295 and 705 μg/mL, respectively.

Mixed standard solution of cis- and trans-resveratrol:
A 1 mL aliquot of the 1000 μg/mL stock solution of trans-resveratrol was introduced into a covered 10 mL transparent glass tube and irradiated for 2 h under mixed wavelengths of only 254 nm and 365 nm. Then, 40 μL of the solution was diluted with 30% acetonitrile in water to 1 mL. The 40 μg/mL trans-resveratrol solution was obtained as the single standard compound and prepared for HPLC analysis. Using the external standard method to calculate the peak area, the concentration of trans-resveratrol solution irradiated was determined to be 405 μg/mL, and that of cis-resveratrol was 595 μg/mL.

Mixed standard solution of four compounds:
The same volumes of the standard solution of cis/trans-resveratrol glycosides and cis/trans-resveratrol were taken and mixed to obtain the mixture of the four compounds, and then the concentration of the four compounds were 147.5 μg/mL of trans-resveratrol glycosides, 352.5 μg/mL of cis-resveratrol glycosides, 202.5 μg/mL of trans-resveratrol glycosides and 297.5 μg/mL of cis-resveratrol glycosides.

2.4 Sample preparation
About 50 mL of the wine was placed in a 100 mL beaker, and the pH was adjusted to 6.0 with ammonium hydroxide, and then 5 mL of the resulting solution was taken out for purification (if the recovery test was needed, the mixed standard solution was added in this step).

For sample purification, first, the sample was activated, 5 mL of methanol and 5 mL of water were added to the ProElut BLC column, and the effluent was discarded. Then, 5 mL of the solution was added to be purified and the effluent was discarded. Around 5 mL of 30% methanol in water was added during leaching, the effluent was discarded, and the column was dried. Then, 5 mL of methanol was added during elution, and the effluent was collected. During redissolution, the collected effluent was blown with nitrogen to a volume less than 1 mL, and adjusted to 5 mL using the mobile phase. Then, it was passed through a 0.22-μm microporous filter membrane and collected in a 2-mL automatic sample bottle for HPLC analysis.

2.5 Chromatographic analysis
Chromatographic separation was carried out using an isocratic elution of (A) water and (B) acetonitrile as the mobile phase in a 70:30(v/v) ratio at a flow rate of 1.0 mL min⁻¹. HPLC analysis was performed with a Platisil ODS column at 30 °C. The UV detector was set to 305 nm for all compounds. The injection volume was 10 μL and total run time was 20 min.

3. Results and discussion
3.1 The separation of standard compounds
The analytical method described in this paper resulted in good separation of the mixed standard of the four compounds (Figure 3).

![Fig. 3 Standard liquid chromatography of 4 compounds](image)

3.2 Accuracy and precision
Accuracy measurement:
50 mL samples were taken in 100 mL beakers and analyzed in parallel for 6 times. The content was determined in accordance with the method described in Section 2.4. The RSD measured for the accuracy of the method as determined by the six runs was 4.610%.

Precision measurement:
The precision of the instrument was measured by 6 consecutive injections of the same sample and the RSD obtained was 1.378%.

The results thus showed good accuracy of the experimental method and precision of the instrument.
3.3 Stability experiments
In 100 mL beakers, 50 mL samples were taken and treated according to the method described in Section 2.4. The contents of the samples were measured every 2 h for 24 h and the experiments were run for a total of 13 times. The results showed no significant change in the peak area of resveratrol and resveratrol glycosides in the wine for 24 h. The RSDs for cis- and trans-piceid and cis- and trans-resveratrol were 2.16%, 2.88%, 2.83%, and 3.12%, respectively. The results showed good stability of resveratrol and resveratrol glycosides extracted from the samples.

3.4 Recovery experiments
In this work, the matrix matching–standard solution–external standard method was used for quantitative analysis. Four mixed standard solutions were added to the matrix of a known concentration of Great Wall wine for the recovery test. First, about 50 mL wine solution was added to a 100-mL beaker and the sample pH was adjusted to 6.0 with ammonia. Then, for 5 mL of the sample with cis- and trans-piceid added at 28.2 mg/L and 11.8 mg/L respectively, the cis-resveratrol and trans-resveratrol concentrations were 23.8 mg/L and 16.2 mg/L, respectively. In accordance with the methods described in Sections 2.4 and 2.5 for sample purification and instrumental analysis, respectively, the experiment was repeated 3 times (see Figure 4 and Figure 5).

Table 1 shows the recovery and relative standard deviation (RSD) of the wine for the investigated addition level. The results show that this method offers good recovery, which can meet the analysis requirements.

![Fig. 4 Liquid chromatography results obtained for wine (blank sample)](image)

![Fig. 5 Liquid chromatography results obtained for wine (sample with added standard)](image)

| Component            | Blank (mg/L) | Added (mg/L) | Found (mg/L) | Recovery (%) | RSD (%) |
|----------------------|--------------|--------------|--------------|--------------|---------|
|                      |              |              | 1   | 2   | 3   |               |         |
| Cis-Piceid           | 4.74         | 28.2         | 32.1 | 32.61 | 32.99 | 101.34        | 1.37    |
| Trans-Piceid         | 4.43         | 11.8         | 15.98 | 16.33 | 16.28 | 100.28        | 1.17    |
| Cis-Resveratrol      | 0.66         | 23.8         | 24.55 | 24.41 | 24.61 | 99.73         | 0.42    |
| Trans-Resveratrol    | 0.98         | 16.2         | 17.25 | 17.15 | 17.66 | 98.94         | 1.56    |

4. Conclusions
In this study, a HPLC method was used for the simultaneous determination of cis/trans-resveratrol and cis/trans-resveratrol glycosides in wine. The accuracy, precision, and stability of the method and the recovery it offered were investigated, and the results were satisfactory. This method offers the
advantages of simple operation, low consumption of organic solvents, accuracy, and rapid analysis, and is therefore an efficient detection method for the grape variety used in wine.

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References
[1] J. Zhang, X.Y. Huang, T.L. Xie, Y. Yang, J.X. GUAN, L.J. Xie, G Cheng, Liquor-making Science&Technology, 8, 124-127+131 (2017)
[2] D. Wu, Y.R. Mu, Z.H. Liu, L.L. Guo, R. Yu, XY. Lv, Modern Instruments, 3, 28-29 (2008).
[3] Y.P. Liu, D.W. Wen , Z Chen, Y.P. Liao, H.W. Liu, Chinese Journal of Chromatography, 22, 583-588, (2004).
[4] R. Flamini, P. Traldi, Mass spectrometry in grape and wine chemistry(John Wiley & Sons, 2009)
[5] L. Zhou, Y.H. Dong, C.L. Hu, C.D. Chu, Z.X. Jin, Acta Nutrimenta Sinica, 32, 86-87(2010)
[6] C. Tian, H.P. Hou, Liquor-making Science & Technology, 7, 65-69 (2018)
[7] G.M. Han, F. Chen, M. Hou, H. Wang, Food science, 32, 180-183, (2011)
[8] H.Z. Weng, T.X. Yue, Y.F. Cheng, China Brewing, 3, 164-166(2009)