HPLC-ELSD determination of triterpenoids and triterpenoid saponins in *Ilex pupurea* leaves

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Abstract: **Aim** To establish an HPLC-ELSD method for the quantification of pedunculoside, ziyuglycoside I and rutundic acid, in the leaves of *Ilex purpurea* Hassk. **Methods** By optimizing the chromatographic conditions of HPLC and the parameters of ELSD to study the methodology. Column: Allsphere ODS-2 (250 mm × 4.6 mm ID, 5 μm), mobile phase: methanol-water (59∶41), flow rate: 1.0 mL·min^{-1}, drift tube temperature: 59 ℃, gas flow rate: 2.38 L·min^{-1}. **Results** The calibration curves were linear in the range of 2.56 - 25.60 μg for pedunculoside, 1.64 - 16.40 μg for ziyuglycoside I and 3.74 - 37.40 μg for rutundic acid. The average recovery of pedunculoside was 96.3 %, RSD 1.59 % (n = 5), ziyuglycoside I 97.3 %, RSD 3.82 % (n = 5), rutundic acid 97.7 %, RSD 2.11 % (n = 5). All of RSDs of the precision were less than 4 % (n = 6), and the reproduciblities less than 5 % (n = 6). **Conclusion** The method is simple, accurate, effective and feasible. It can be used for the determination of the contents of pedunculoside, ziyuglycoside I and rutundic acid in leaf of *Ilex purpurea* Hassk.

Key words: *Ilex purpurea*; triterpenoids; triterpenoid saponins; HPLC-ELSD

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HPLC-ELSD法测定四季青中三萜及其皂苷的含量

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摘要: 目的 建立同时测定四季青中3种三萜及其皂苷——长梗冬青苷、地榆皂苷I和救必应酸含量的HPLC分析方法。方法 C_{18}柱;流动相:甲醇-水(59∶41);流速1.0 mL·min^{-1};检测器:Sedex 55蒸发光散射检测器。结果 线性范围为长梗冬青苷2.56 ~ 25.60 μg(r = 0.999 2),地榆皂苷I 1.64 ~ 16.40 μg(r = 0.998 2)和救必应酸3.74 ~ 37.40 μg(r = 0.999 4)。平均加样回收率为长梗冬青苷96.3 %, RSD 1.6 %(n = 5);地榆皂苷I 97.3 %, RSD 3.8 %(n = 5);救必应酸97.7 %, RSD 2.1 %(n = 5)。3个化合物的精密度RSD(n = 6)均 < 4 %;重现性RSD(n = 6)均 < 5 %。结论 本方法简便、有效、可行,可用于四季青三萜及其皂苷的含量测定。

关键词: 四季青; 三萜; 三萜皂苷; HPLC-ELSD

Ilex purpurea Hassk. is a plant of Genus *Ilex* L., which is distributed in the south area of the Yangtze river. In China, its leaves (Sijiqing) has been used as a remedy (when given internally) for bronchitis, pneumonia and ulceration and as an external treatment for scald, chilbliain, *etc*[1].

There are some known compounds[2,3] in the plant, among them, triterpenoids and triterpenoid saponins are regarded as the active anti-inflamatory constituents. Thus it is essential to establish an analytical method for the triterpenoids and triterpenoid saponins to control the

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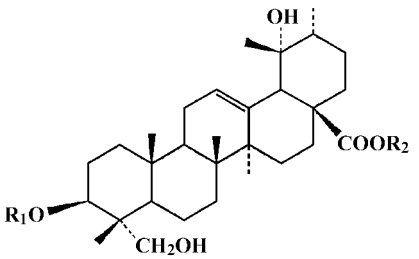
quality of Sijiqing. Most of the triterpenoids and triterpenoid saponins are non-chromophoric, the use of direct UV detection without pre- or post-column derivatization is impossible. The published results demonstrated that evaporation light scattering detector (ELSD) is an universal detector for the analysis of non-chromophoric and non-volatile compounds[4,5]. In this paper, a simple and effective analytical method using HPLC-ELSD for the simultaneous determination of pedunculoside (PC), ziyuglycoside I (ZG) and rutundic acid (RA) in Sijiqing was established, and the ZG was isolated from this plant for the first time.



Pedunculoside: R_1 = H, R_2 = β-*D*-glu; Ziyuglycoside I: R_1 = β-*D*-ara, R_2 = β-*D*-glu; Rutundic acid: R_1 = H, R_2 = H

Figure 1 Structures of three triterpenoids and triterpenoid saponins

Materials and methods

Chemicals and materials Methanol was HPLC grade. Redistilled water was made in our own lab. Various leaves of *Ilex purpurea* Hassk. were collected in Anhui and Jiangsu province, and authenticated by professor Li Ping. PC, ZG and RA were isolated from the leaves of *Ilex purpurea* Hassk., their HPLC purity was over 99 %.

Apparatus and conditions The HPLC system consisted of LC-10AD pump (Shimadzu, Tokyo, Japan), Sedex 55 ELSD system (France) and a Nitrox nitrogen generator. An Allsphere ODS-2 analytical column (250 mm × 4.6 mm ID, 5 μm) coupled with a C_{18} guard column (20 mm × 4.0 mm ID, 5 μm) was used at a column temperature of 25 ℃. The chromatographic data were recorded and processed by HS Chromatographic Work Station software (Yingpu Technical Development Co. Ltd, Hangzhou). The mobile phase consisted of methanol-water (59∶41) at a flow rate of 1.0 mL•min^{-1}. Temperature for the detector drift tube was set at 59 ℃. The nitrogen flow was 1.06 SLPM (standard liters per minute) with the pressure of nebulizing gas of 0.2 MPa. Injection volume was 20 μL.

Results

1 Calibration curves

A mixed stock solution consisting of PC (1.28 g•L^{-1}), ZG(0.82 g•L^{-1}) and RA(1.87 g•L^{-1}) was prepared. 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of the stock solution was transfered into a 1 mL volumetric flask and diluted to volume with methanol. 20 μL of the above solutions were accurately injected for analysis. The correlation coefficients of PC, ZG and RA were 0.999 2, 0.998 2 and 0.999 4, respectively (Table 1).

Table 1 Calibration curve of the three compounds

Compound	t_R/ min	Calibration curve	Correlation coefficient	Linear range /μg
Pedunculoside	13.42	Y=2.693 X-3.606	0.999 2	2.56-25.60
Ziyuglycoside I	20.07	Y=2.345 X-1.159	0.998 2	1.64-16.40
Rutundic acid	27.29	Y=2.075 X+0.006	0.999 4	3.74-37.40

X: ln of contents of injected solution; Y: ln of peak areas

2 Precision, reproducibility and recovery

The precision was determined with the same sample solution consisting of PC (0.768 g•L^{-1}), ZG (0.498 g•L^{-1}), and RA (1.122 g•L^{-1}). The relative standard deviation (RSD) values (n=6) of PC, ZG and RA were 2.0 %, 3.8 % and 1.4 %, respectively.

In order to test the reproducibility, six sample solutions were prepared. Each solution was injected twice. The contents of PC, ZG and RA were calculated and the RSD were as follows: 4.6 % (PC), 4.0 % (ZG) and 3.4 % (RA).

The recovery experiment was carried out to evaluate the accuracy of the method. Five sample solutions of the plant materials were prepared with different contents of the three compounds, and a certain amount of the three standards were added. Each solution was injected twice. The content of each compound was calculated and compared to the expected content. The recoveries of the three compounds were all between 95 % and 100 %, and RSDs of PC, ZG and RA were 1.5 %, 3.8 % and 2.1 %, respectively (Table 2).

Table 2 Recoveries of three compounds (n=5, $\bar{x}\pm s$)

Compound	Contained/ mg	Added/ mg	Found/ mg	Recovery/ %
Pedunculoside	4.33±0.10	3.23±0.17	7.46±0.16	96.3±1.5
Ziyuglycoside I	2.38±0.07	1.52±0.07	3.86±0.12	97±4
Rutundic acid	3.20±0.05	3.33±0.25	6.44±0.23	97.7±2.1

3 Limit of detection

Dilute the stock solution to provide a concentration ranges of (0.1-10) mg•L^{-1}. 20 μL of the solution were injected for analysis. The limit of detection for each

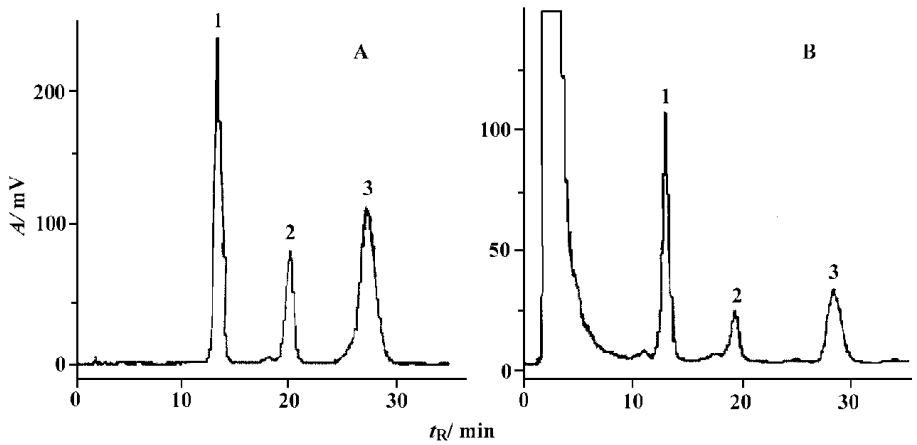
analyte was determined when the ratio of peak area of the analyte to noise was greater than three . The results were as follows : PC 0.02 μg , ZG 0.01 μg , RA 0.05 μg .

4 Analysis of three triterpenoids and triterpenoid saponins in the crude drug materials

2.0 g crushed sample of leaf of *Ilex purpurea* was extracted in Soxhlet′s extractor with methanol for 10 h . The methanol was transferred to a 50 mL volumetric flask and diluted to volume with methanol . The solution was filtered through a filter (0.45 μm) and 20 μL of the filtrate was injected for analysis . The content of each compound was calculated by the correspouding calibration curve. The retention time (min) of pedunculoside was 13.42 , ziyuglycoside I was 20.07 and rutundic acid was 27.29 (Figure 2) . The results showed that there were some difference in the contents of pedunculoside and rutundic acid (Table 3) .

Table 3 Contents of pedunculoside , ziyuglycoside I and rutundic acid

Source	Contents/ %		
	Pedunculoside	Ziyuglycoside I	Rutundic acid
Jinzhai , Anhui	2.22	1.06	1.27
Jurong , Jiangsu	2.84	1.12	1.36
Nanjing , Jiangsu	2.30	0.93	1.88



1 : Pedunculoside (t_R 13.4 min) ; 2 : Ziyuglycoside I (t_R 20.07 min) ; 3 : Rutundic acid (t_R 27.29 min)

Figure 2 HPLC chromatograph of the standard(A) and samples(B)

Discussion

For simultaneous determination of triterpenoids and triterpenoid saponins in the leaves of *Ilex purpurea* , three compounds , pedunculoside , ziyuglycoside I and rutundic acid were selected . Representative chromatograms of the extract of Sijiqing samples and authentic samples were shown in Figure 2 , and their contents were summarized in Table 3 . The results showed that the contents of pedunculoside in Sijiqing were higher than others analyzed and the developed HPLC-ELSD method was a feasible , effective and suitable analytical method for triterpenoids and triterpenoid saponins in *Ilex purpurea* .

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