APPLICATION BRIEF

Liquid Chromatography

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HPLC Analysis of Oleanolic Acid and Ursonic Acid in *Eriobotrya Japonica* Leaf

Introduction

Eriobotrya japonica leaf is included in the Chinese Pharmacopoeia and is widely used as a medicinal material in traditional treatment of a variety of chronic diseases in China and other East Asian countries. Phytochemical investigations showed that its main components are essential oil, triterpenes, sesquiterpenes, flavonoids, tannins, and megastigmane glycosides. Among them, triterpene acids are considered as the key pharmacological components. To date, more than twenty triterpene acids in *Eriobotrya japonica* leaf have been identified, including the major four triterpene acids, that is, ursonic acid, corosolic acid, oleanolic acid, and maslinic acid, which belong to ursane type and oleanane type.[1]

In the 2020 Edition of the Chinese Pharmacopeia, two marker compounds, oleanolic acid (Figure 1a) and ursonic acid (Figure 1b) are assayed by using an HPLC-UV method, which is important for quality control of this traditional Chinese medicine (TCM).[2]

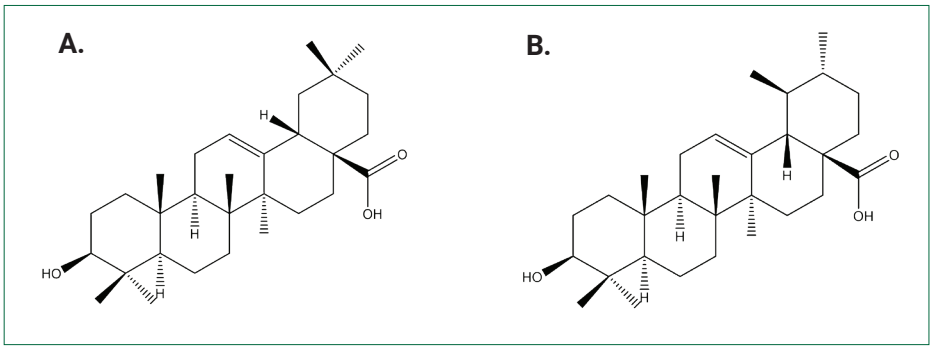

Figure 1: Chemical structures of a) oleanolic acid and b) ursonic acid (ChemDraw®).

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In this work, analysis of the two marker compounds in accordance with the Chinese Pharmacopoeia was conducted using a PerkinElmer LC 300 with a PerkinElmer Epic™ C18 column and consumables, and SimplicityChrom™ software (Figure 2). A regulatory quality monitor workflow for quantification of oleanolic acid and ursonic acid was established.

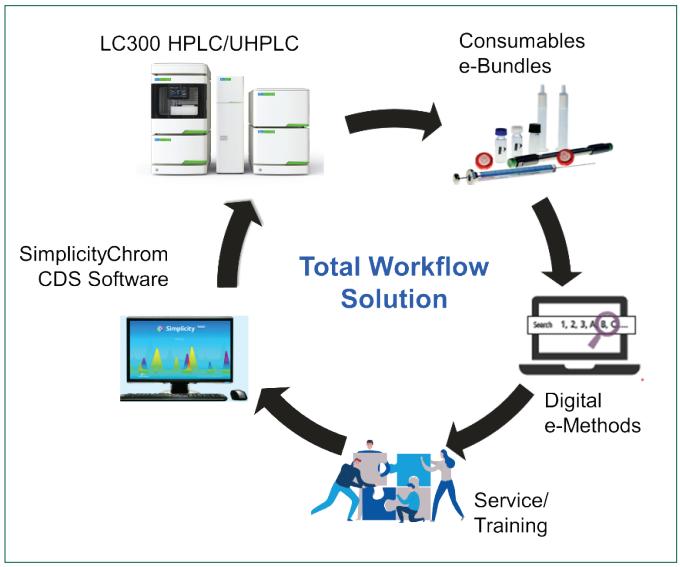

Figure 2: The schematic diagram of the LC total workflow solution.

Experimental Conditions

Hardware/Software

The chromatographic separation was conducted by a PerkinElmer LC 300 HPLC/UHPLC system and detection was achieved using a PerkinElmer LC 300 PDA detector. All instrument control, data acquisition and data processing were performed using SimplicityChrom software.

Method Parameters

The LC parameters are shown in Table 1.

Table 1: LC method parameters.

Column	Epic C18 150×4.6 mm, 3 µm (P/N: 135191-EC18)
Mobile Phase	0.5% (w/v) NH_4Ac-methanol-acetonitrile (21:12:67)
Flow Rate	1.0 mL/min
Oven Temperature	30 °C
Detector Wavelength	Analytical Wavelength: 210 nm (Bandwidth: 4 nm) Reference Wavelength: 360 nm (Bandwidth: 4 nm)
Sampling Rate	5 pts/sec (Hz)
Injection Volume	10 µL, Partial Loop Injection Mode

Solvents, Standards and Samples

Oleanolic acid (OA) and ursonic (UA) standard reference materials were purchased from National institutes for food and drug control (NIFDC), China. The herbal plant was purchased from a qualified pharmacy store. All solvents were LC/MS grade. All other chemicals and reagents were of the highest grade available.

Preparation of sample solution: transfer 1g dried herbal sample powder to a 100 mL flat bottom conical flask, add 50 mL of ethanol. Sealed and sonicated (Frequency: 50 kHz, Power: 250 W) for 30 min. The solution was filtered with 0.22 µm PTFE syringe filters (P/N: 02542884).

Preparation of standard solution: dissolve oleanolic acid and ursonic acid standard materials and dilute with ethanol to make a concentration of 50 µg/mL and 200 µg/mL for OA and UA respectively.

Results and Discussion

Based on the chromatographic conditions and system suitability specification in the Chinese Pharmacopoeia, the standard solution and sample solution were analyzed using a PerkinElmer LC 300 with an Epic C18 column.

Figure 3 shows that Epic C18 has excellent separation capability on the two identified peaks with good retention and peak shape. The specification of plate number (not less than 5000 N) for ursonic acid was met, which was observed as a value of 18460 N.

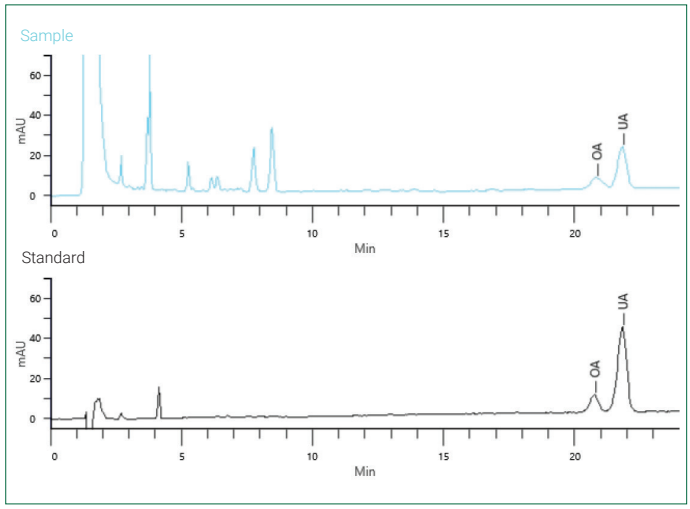

Figure 3: The chromatograms of sample (top) and standard (bottom).

Six consecutive standards were analyzed and the retention and area response generated excellent repeatability, as shown in Table 2, with relative standard deviations (RSD%) below 2.0%.

Table 2: Repeatability results of six injections of standard (10 µg/mL).

	Oleanolic acid Time (min)	Peak Area	Ursonic Acid Time (min)	Peak Area
RSD %	0.07	1.76	0.06	1.10

Conclusion

- A robust and reproducible LC-UV workflow for analysis of oleanolic acid and ursonic acid in Eribotorya leaf has been developed by coupling a PerkinElmer LC 300 system with an Epic C18 column.
- This method can be applied for assay determination of oleanolic acid and ursonic acid in accordance with the Chinese Pharmacopoeia.
- The Epic C18 column shows excellent separation capability and peak shape due to the superior base deactivation.

Reference

1. Feng Li, Yijia Li, Qingxian Li, Xianai Shi, Yanghao Guo, Evidence-Based Complementary and Alternative Medicine, vol. 2017
2. Vol. 1, Chinese Pharmacopoeia 2020.

Consumables

Component	Description	Part Number
Columns	Epic C18 150 x 4.6mm, 3 µm	135191-EC18
HPLC Vials	2 mL Amber 9 mm Screw Top Vial with Write-on Patch and Fill Lines (100/Pack)	N9307802
HPLC Vials Caps	9 mm Screw Top Blue (Polypropylene) Cap with PTFE/ Silicone Pre-Slit Septa (100/Pack)	N9306203
Peek Fittings	Finger-Tight Fittings, PEEK, 5.5K psi Max (5/Pack)	N9307822
Stainless Steel Fittings	Ti Hybrid w/Flat Wrench Ferrule/Nut	N9306301
Syringes	Syringe 1 mL BD Luer-Lok Disposable (100/Pack)	02542890
Syringe Filters	0.22 µm PTFE (Hydrophobic) Syringe Filter, 17 mm	02542884

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