



Liquid Chromatography

AUTHORS

Bin Yuan

Yanchao Shi

PerkinElmer, Inc.

Shanghai, China

Suma Ramagiri

PerkinElmer, Inc.

Waltham, MA USA

Chlorogenic Acid Analysis in Children Throat-Reliever Granule by LC-UV

Introduction

Children's throat-reliever granules (Xiaoer Yanbian Keli) are a traditional Chinese formulated medicine, comprised of the *Lonicera Japonica* herbal plant. It is used for the treatment of throat inflammation when children have cough or cold.¹ For implementation of quality control processes, Chinese Pharmacopoeia 2020 has finalized the assay of a method in which the specification of the assay of chlorogenic acid (Figure 1) is not less than 3 mg per bag (size: 4 g/bag).

In this work, quantification of the marker compound chlorogenic acid (CA) in a formulated drug, based on Chinese Pharmacopoeia, is presented using a PerkinElmer LC 300 UHPLC with SimplicityChrom™ software, PerkinElmer Epic™ C18 column, and additional chromatography consumables. A workflow for regulatory quality monitoring for the quantification of pill formulation is established herein, and outlined in Figure 2.

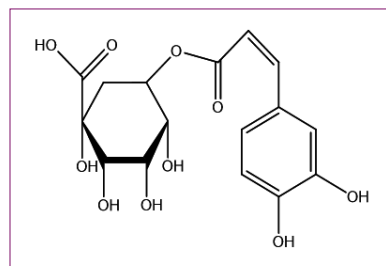


Figure 1: Chemical structure of chlorogenic acid (ChemDraw).

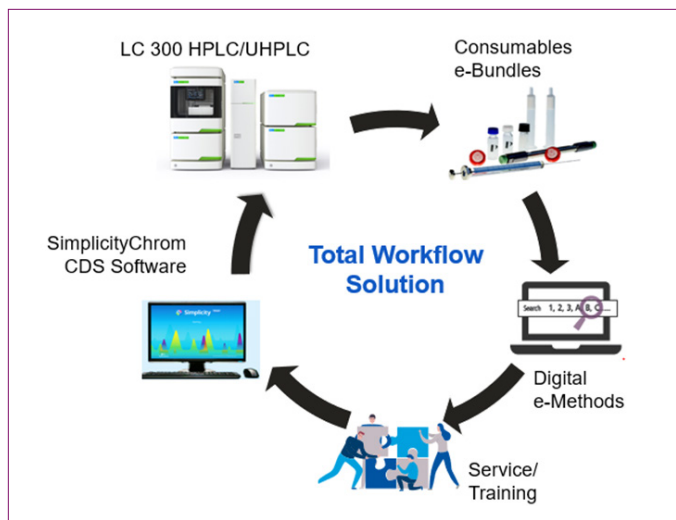


Figure 2: Schematic diagram of the LC total workflow solution.

Experimental

Hardware/Software

Chromatographic separation was conducted utilizing a PerkinElmer LC 300 UHPLC system, with subsequent detection achieved using a PerkinElmer LC 300 PDA detector. All instrument control, data acquisition and data processing were performed using SimplicityChrom software.

Method Parameters

LC parameters are shown in Table 1.

LC Parameters	
Column	Epic C18 150×4.6 mm, 3 μm, (P/N: 135191-EC18)
Mobile Phase	0.4% H ₃ PO ₄ -Acetonitrile (88:12)
Flow rate	1.0 mL/min
Oven Temperature	30 °C
Detection	327 nm
Injection Volume	10 μL (partial loop)

Table 1: LC Parameters.

Solvents, Standards and Samples

A chlorogenic acid reference standard was purchased from National Institutes for Food and Drug Control (NIFDC), China. All solvents were LC-MS grade. All other chemicals and reagents were of the highest grade available.

The sample solution was prepared by transferring 0.5 g of the sample powder into a 100-mL flat-bottom conic flask and then adding 50 mL of a 50% methanol aqueous solution. The flask was sealed and sonicated for 30 minutes (power: 250 W, frequency: 20 kHz). The solution was then filtered with 0.22 μm PTFE syringe filters (P/N: 02542884).

The standard solution was prepared by dissolving the chlorogenic acid standard material in a 50% methanol aqueous solution to make a concentration of 20 μg/mL.

Results and Discussion

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

Figure 3 illustrates that the Epic C18 column has excellent separation capability with good retention and peak shape. The specification of plate number (not less than 2000 N) was met, which was observed as a value of 13,300 N. No interference existed in the extract solvent and blank diluent.

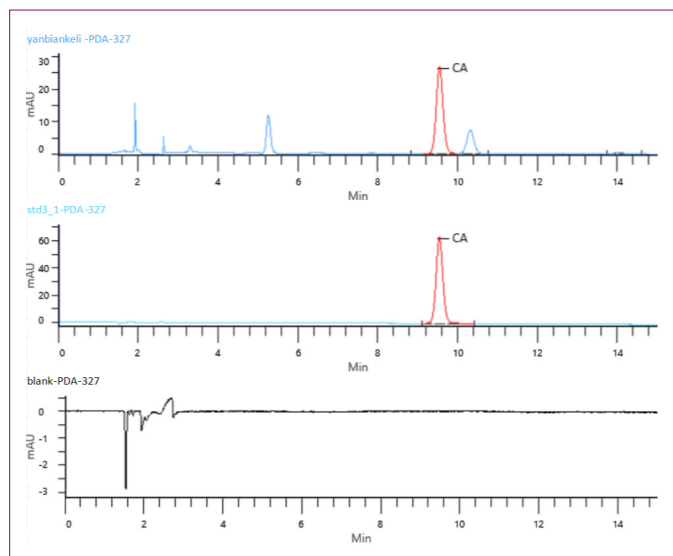


Figure 3: Chromatograms of the sample (top), standard (middle) and blank (bottom).

Five consecutive replications of the standard were analyzed, and the retention and area response generated excellent repeatability, below 0.25%, as shown in Table 2.

No	Time (min)	Area
1	9.549	764.4646
2	9.542	768.1037
3	9.537	766.8568
4	9.538	768.6210
5	9.538	766.6764
RSD%	0.05	0.21

Table 2: Repeatability results of 5 consecutive injections of the standard (20 μg/mL).

The standard stock solution was diluted to concentrations of 2, 4, 20, 40, and 80 µg/mL. Each solution was then injected, and the area response determined. The concentrations and area responses were analyzed using a linear regression analysis, which shows a good linearity ($R^2=0.9996$) in the above range, as displayed in Figure 4.

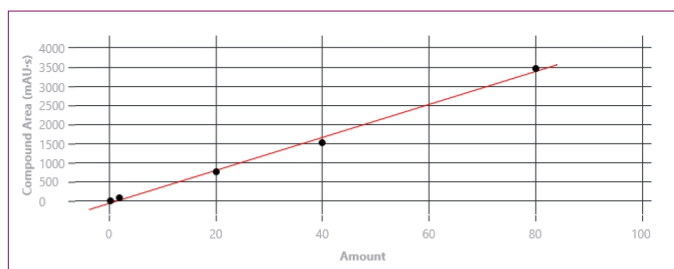


Figure 4: The linearity and range plot.

Three batches of commercially formulated drugs were treated as per the Chinese Pharmacopoeia procedure, and analyzed simultaneously. The assay results are shown in Table 3, and demonstrate that the result met the accepted label-claim value (NLT: 3 mg/bag).

Consumables

Component	Description	Part NO
Columns	Epic C18, 3 µm, 4.6 x 150 mm	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 (pkg. 5)	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

Name	Sample 1 (mg/g)	Sample 2 (mg/g)	Sample 3 (mg/g)	Mean (mg/g)	Mean (mg/bag)	RSD (%)
CA	0.896	0.898	0.898	0.897	3.6	0.13

Table 3: Sample assay results.

Conclusion

A robust and reproducible LC-UV workflow for quantification of chlorogenic acid in commercial children's throat-reliever granules has been developed by coupling a PerkinElmer LC 300 system with an Epic C18 column. This method can be applied for assay determination of chlorogenic acid in accordance with the Chinese Pharmacopoeia. The Epic C18 column shows excellent separation capability and peak shape due to the superior base deactivation.

References

- Handbook of clinic traditional formulated drugs (2019): Peoples' Health Publishing House.
- Vol. 1, Chinese Pharmacopoeia 2020.