

## Application Note

# Isolation of a Natural Product from Echinacea Extract Using the Prep 150 LC

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## Abstract

In this study, we focus on the feasibility of using the Prep 150 LC System as a tool for isolating cichoric acid from Echinacea purpurea extract. The Prep 150 LC System is controlled by ChromScope Software, intuitive software that enables rapid user success in isolating compounds. The principles outlined here are applicable to any isolation where the compounds have a UV chromophore.

## Benefits

- The Prep 150 LC System – an affordable, highly reliable system for preparative chromatography – is suitable for compound isolation from natural product extracts.
- The Prep 150 LC System is controlled by ChromScope Software, an intuitive and easy-to-use software that enables users to quickly purify compounds, reducing the amount of time required for training.
- The Prep 150 LC System, with its straightforward design and uncomplicated software control, facilitates users to more efficiently process samples and thereby increase productivity.

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## Introduction

Echinacea, or purple coneflower, an herbaceous flowering plant in the daisy family, is a perennial that can withstand dry climates.<sup>1,2</sup> One of the major compounds in Echinacea purpurea flowers is cichoric acid, a phenylpropanoid and a caffeic acid derivative.<sup>3,4</sup> A recent literature search on cichoric acid indicated that over 50% of the published research on this compound is related to its medicinal uses, including the treatment of upper respiratory infections, cancer, and the improvement of immune responses to different stimuli.<sup>5,6,7</sup> Many factors, including location, growing conditions, and sample handling, contribute to how much cichoric acid is found in different natural product extracts. Isolating enough of a target compound to effectively perform other experimental studies often requires several injections of the crude mixture. In this study, we focus on the feasibility of using the Prep 150 LC System as a tool for isolating cichoric acid from Echinacea purpurea extract. The Prep 150 LC System is controlled by ChromScope Software, intuitive software that enables rapid user success in isolating compounds. The principles outlined here are applicable to any isolation where the compounds have a UV chromophore.

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## Experimental

### LC conditions

Preparative LC system:	Prep 150 LC with 2545 Binary Gradient Module, Prep Inject manual injector module, 2489 UV/Visible Detector, and Waters Fraction Collector III
Analytical column:	Atlantis T3, 5 $\mu$ m, 4.6 x 50 mm (p/n 186003744)
Preparative column:	Atlantis T3 OBD Prep 5 $\mu$ m, 19 x 50 mm, (p/n 186003696)

Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	Acetonitrile with 0.1% formic acid
Gradient:	Reported in figures
Column temp.:	Room
Sample temp.:	Room
Injection vol.:	Reported in figures
Flow rate:	Reported in figures

## UV conditions for Preparative System

Detector:	2489 UV/Visible
Wavelength mode:	Single
Wavelength:	330 nm
Sampling rate:	5 points/sec
Filter time constant:	Normal

## Data management

ChromScope Software

## Sample description

A total of 5.3 g of Echinacea purpurea powdered organic root (StarWest Botanicals, Sacramento, CA 95838) was

extracted with 20 mL of 70:30 methanol/water with shaking for approximately 3 hours in each of two 50 mL centrifuge tubes (containing 2.66 g and 2.64 g powdered Echinacea, respectively). The tubes were then centrifuged and the supernatant was filtered with several 13 mm GHP Acrodisc syringe filters. The methanol was evaporated from the extract to an orange-brown gum. The gum was dissolved in 14 mL of 95:5 water/acetonitrile.

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## Results and Discussion

Although there are at least four caffeic acid derivatives in Echinacea extracts, cichoric acid is one of the most abundant, and its usefulness in medicinal herb products arises from its immunostimulating properties.<sup>8</sup> Analytical chromatography on the crude Echinacea extract before methanol evaporation indicated that cichoric acid was the most abundant compound present (Figure 1A). Cichoric acid is well resolved from its neighboring peaks in the crude extract using the fast screening gradient. To increase peak resolution even more, the gradient was focused<sup>9</sup> and a loading study was performed on the analytical column. As shown in Figure 1B, an injection volume of 35  $\mu$ L of crude extract was an acceptable load on the analytical column.

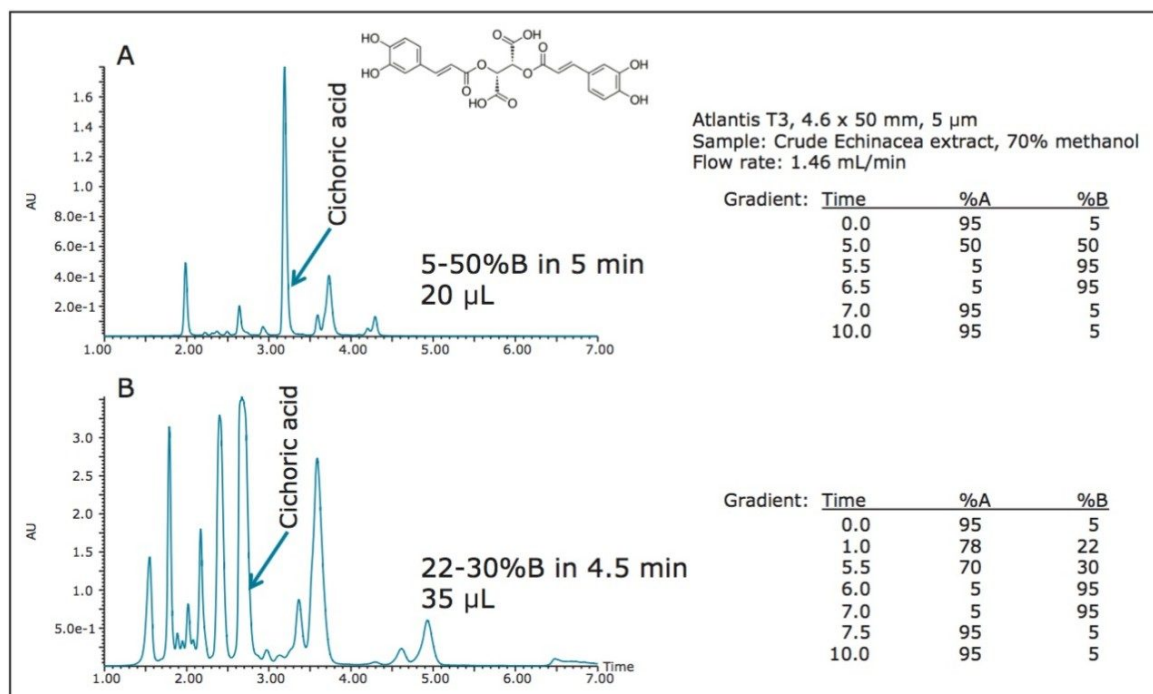


Figure 1. A: Cichoric acid in the crude echinacea extract. B: Focusing and loading on the 4.6 x 50 mm column.

Because large volume injections of strong solvent can distort preparative chromatography, the methanol in the crude extract was evaporated to a gum with a rotary evaporator. The residue was then dissolved in 14 mL 95:5 water/acetonitrile. Using the integrated Prep Calculator tool in ChromScope, geometric scaling of the flow rate and injection volume from the analytical column to the preparative column resulted in the preparative chromatography shown in Figure 2. The gradient method is conveniently displayed with the chromatogram in the injection information tab. Alternatively, fraction collection information can be viewed, eliminating the need to search for tube collection volumes (Figure 3).

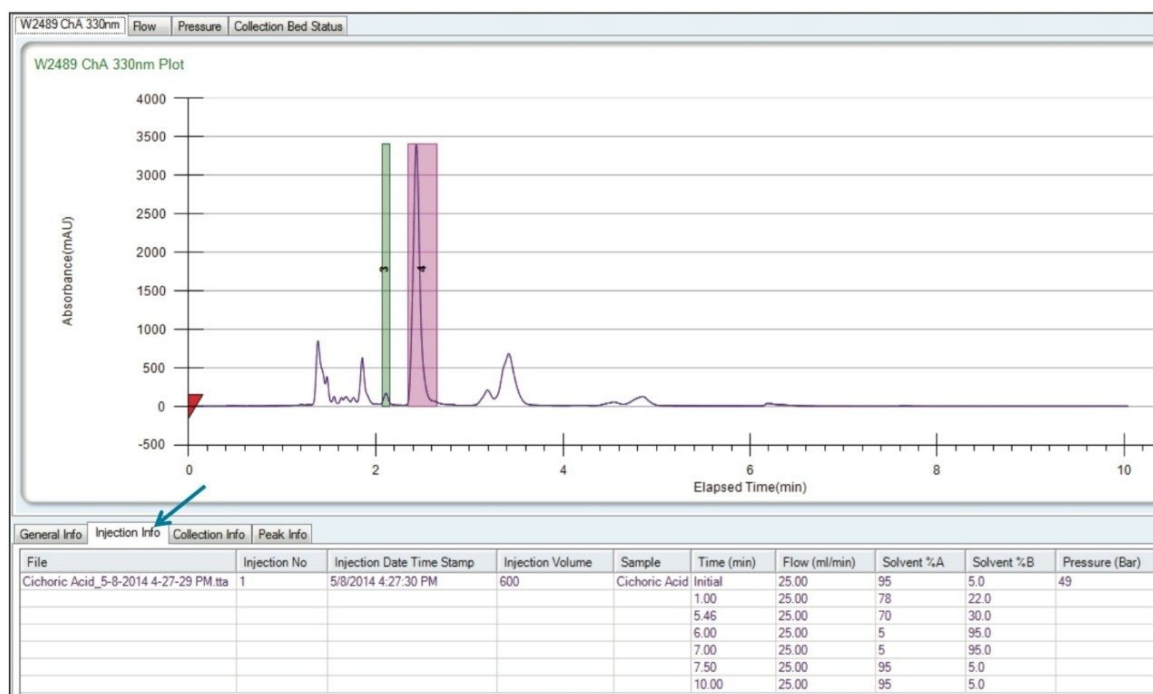


Figure 2. Preparative isolation of cichoric acid (4) and a minor impurity (3), an isomer of cichoric acid, from the crude echinacea extract.

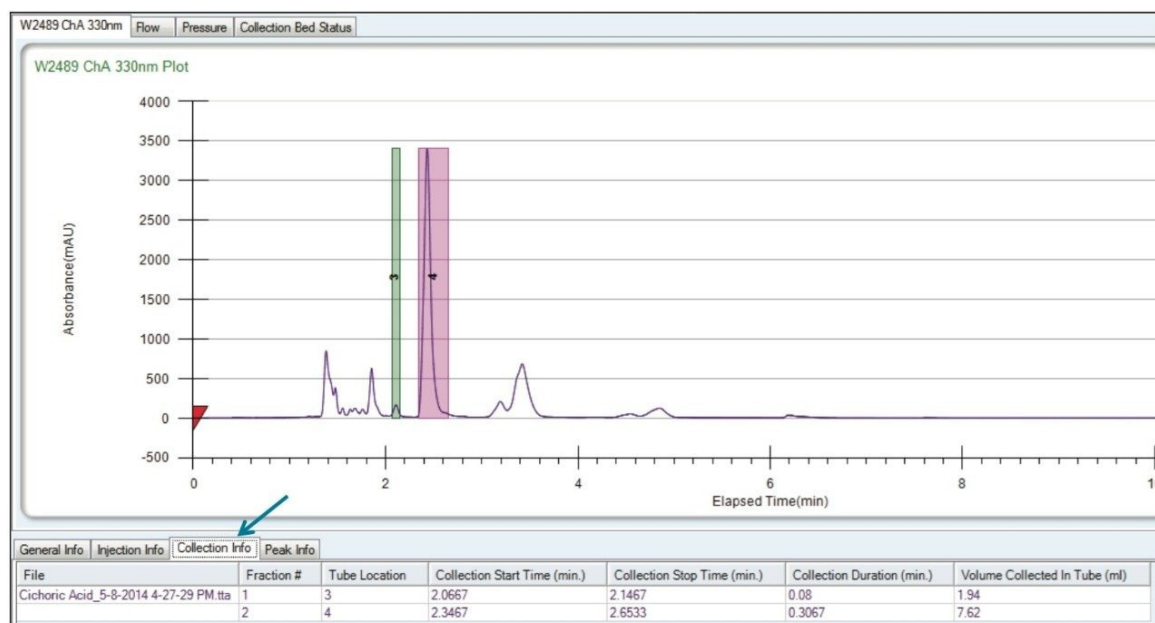


Figure 3. Fraction collection information is conveniently accessible for viewing with the chromatogram.

Multiple injections on the Prep 150 LC System showed very good reproducibility. A total of six preparative injections of the reconstituted echinacea extract were performed. Fraction analysis on the pool showed excellent purity for the isolated product (Figure 4).

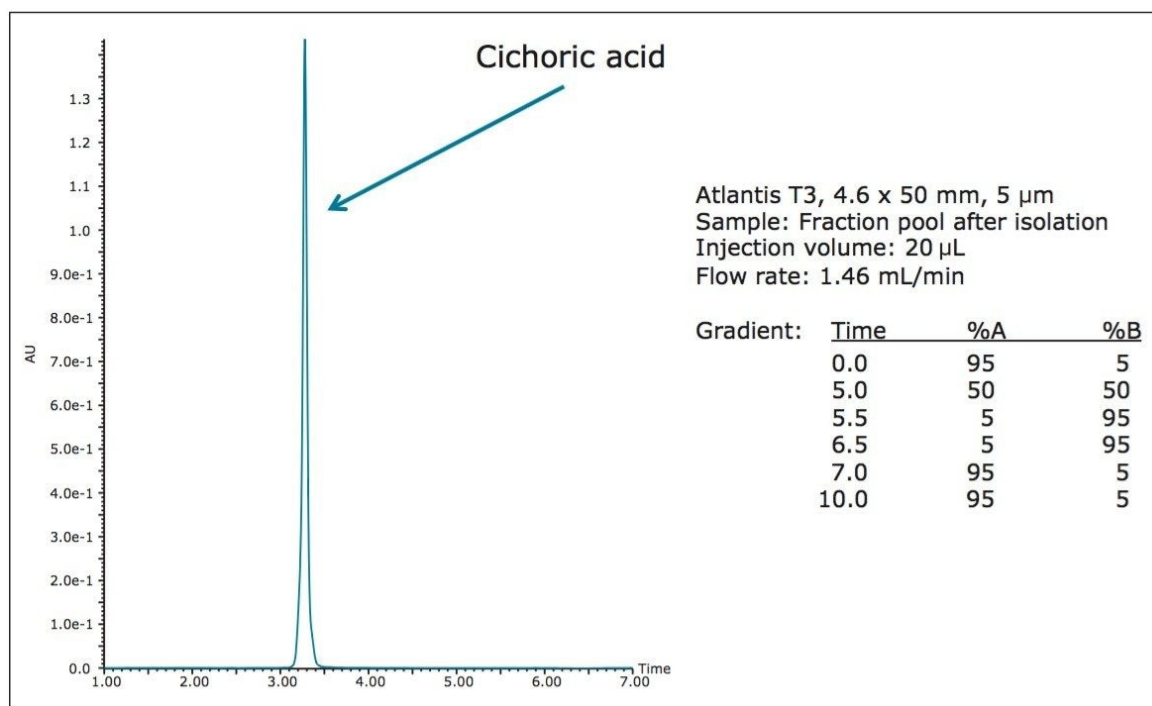


Figure 4. Analysis of the fraction pool after isolation.

## Conclusion

- The affordable, robust Prep 150 LC System is ideal for synthetic chemists who require their own conveniently accessible purification instrumentation, improving sample turnaround time.
- ChromScope, the intuitive Prep 150 LC System control software, is easy for users to learn and use, reducing the investment in time required for training.
- The integrated Prep Calculator Tool simplifies scaling and saves time by eliminating the need to manually calculate prep gradients.
- Highly-visible, colored fraction collection bars on the chromatogram inform the user of the location of the isolated product, reducing errors in sample handling and workup.
- The Prep 150 LC System enables users to rapidly isolate and purify compounds, improving productivity.



- The Prep 150 LC System is suitable for compound isolation from natural product extracts and from any sample mixture that has UV-absorbing compounds.

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