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Isolation of Flavonoids from Ginkgo Biloba Leaf using the Waters Prep 150 LC System

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APPLICATION BENEFITS

- A preparative HPLC system that provides high performance with intuitive, easy-to-use software
- Total software control of all system components with ChromScope™ Software

WATERS SOLUTIONS

Prep 150 LC System

ACQUITY UPLC® H-Class System

ChromScope Software Version 1.4

XBridge® Columns

KEY WORDS

Preparative HPLC, Ginkgo biloba, ChromScope, Prep 150 LC, XBridge

INTRODUCTION

Ginkgo biloba leaf has a long history of medicinal use for the treatment of numerous conditions, going back thousands of years.[1] Extracts of Ginkgo biloba leaves contain a wide variety of active compounds and are a particularly rich source of flavonoids, primarily quercetin, kaempferol, and isorhamnetin (Figure 1).

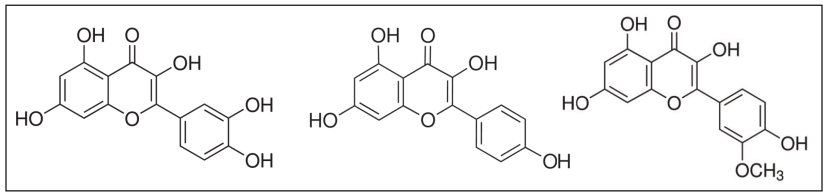

Figure 1. Chemical structures of quercetin, kaempferol, and isorhamnetin.

Often, the goal in natural product purification is to isolate individual component compounds that may have biological activity. Sufficient amounts of these compounds are isolated for a wide variety of purposes, such as preparation of standards, or for use in other studies (e.g. clinical trials, bioassays). Any isolated compound needs to be of as high purity as possible and needs to be obtained quickly and efficiently. Many techniques for extraction, isolation, and purification of natural products have been previously described.[2] Preparative reversed-phase high performance liquid chromatography (RP-HPLC) is a separation technique that is widely used in this kind of endeavor. It is considered to be a rapid, reliable, and robust technique that has wide applicability over many classes of compounds. This application note describes the isolation of the flavonoids from Ginkgo biloba leaf powder using a Waters® Prep 150 LC System (Figure 2).

Figure 2. Waters Prep 150 LC System.

EXPERIMENTAL

Separations

Preparative chromatographic separations were carried out using the Prep 150 LC System (Figure 2) consisting of the following Waters components:

Pump:	2545 Binary Gradient Module
Detector:	2489 UV/Visible Detector with Semi-Prep TaperSlit™ Flow Cell
Injector:	Preparative Injector configured with a 5 mL loop
Collector:	Fraction Collector III
Software:	ChromScope Version 1.4

Analytical chromatographic separations (for method development and final purity checks) were carried out using an ACQUITY UPLC H-Class System equipped with an ACQUITY PDA Detector controlled with Empower® 3 Software. Two initial analytical scale separations were developed (one gradient for purity checks and one isocratic for purification) with the conditions described below.

Analytical gradient conditions (for purity checks)

System:	ACQUITY UPLC H-Class
Column temp.:	50 °C
Flow rate:	0.50 mL/min
Mobile phase A:	0.2% Formic acid in water
Mobile phase B:	Acetonitrile
Gradient:	85:15 to 30:70 over 13 minutes, then to 5:95 13 to 15 minutes
Detection:	UV @ 371 nm
Column:	ACQUITY UPLC BEH C_{18} Column, 130Å, 1.7 µm, 2.1 mm x 100 mm

Analytical Isocratic Conditions (for prep scale up)

System:	ACQUITY UPLC H-Class
Column temp.:	Ambient
Flow rate:	1.4 mL/min
Mobile phase A:	0.2% Formic acid in water
Mobile phase B:	Acetonitrile
Composition:	73% A and 27% B
Detection:	UV @ 371 nm
Column:	XBridge BEH C_{18} Column, 130Å, 5 µm, 4.6 mm x 100 mm

The preparative separation was geometrically scaled from the analytical method to the 19 x 100 mm XBridge Column and is described below.

Preparative Isocratic Conditions

System:	Prep 150 LC
Column temp.:	Ambient
Flow rate:	24.8 mL/min
Mobile phase A:	0.2% Formic acid in water
Mobile phase B:	Acetonitrile
Composition:	73% A and 27% B
Injection vol.:	0.25 mL
Detection:	UV @ 371 nm
Column:	XBridge BEH C_{18} OBD™ Prep Column, 130Å, 5 µm, 19 mm x 100 mm

Extraction

Ginkgo biloba leaf powder (20 g) was added to 100 mL of methanol and sonicated for 60 minutes. Following the addition of 40 mL of 3N hydrochloric acid, the mixture was brought to a boil and allowed to reflux for 90 minutes. Using this reflux technique provided hydrolysis of the flavone glycosides to the respective flavonoids. After cooling to room temperature, this extract passed through a Whatman #1 filter paper and was used without further treatment.

RESULTS AND DISCUSSION

Gradient UPLC® analysis of the prepared extract indicated quercetin and kaempferol concentrations of 0.088 and 0.104 mg/mL, respectively (a quantitative standard of isorhamnetin was not available), and an approximate overall purity of 11%, 14%, and 5% for the 3 compounds by UV (371 nm) area percent (Figure 3). Using a previously developed analytical isocratic HPLC method (Figure 4), the flow rate for the preparative separation was scaled to a 19 mm I.D. column using the Analytical to Prep Gradient Calculator built into the ChromScope Software (Figure 5). The Analytical to Prep Gradient Calculator is an easy-to-use tool that aids in analytical-to-preparative scaling calculations providing both flow rate and gradient time calculation information. Proper scaling of analytical methods to prep allows for method development at an analytical scale, preserving sample and lowering solvent usage during method development. Scaled preparative chromatography will be very similar to the analytical chromatography, increasing confidence in the ability of the preparative method to collect peaks of interest. A previously published application note[3] describes in greater detail these scaling calculations.

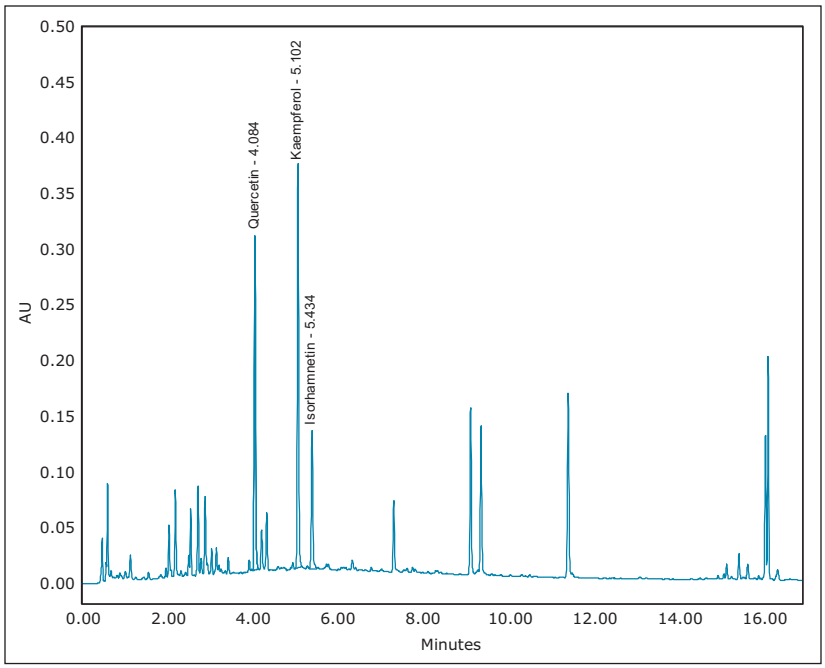

Figure 3. UPLC chromatogram of Ginkgo biloba leaf powder extract.

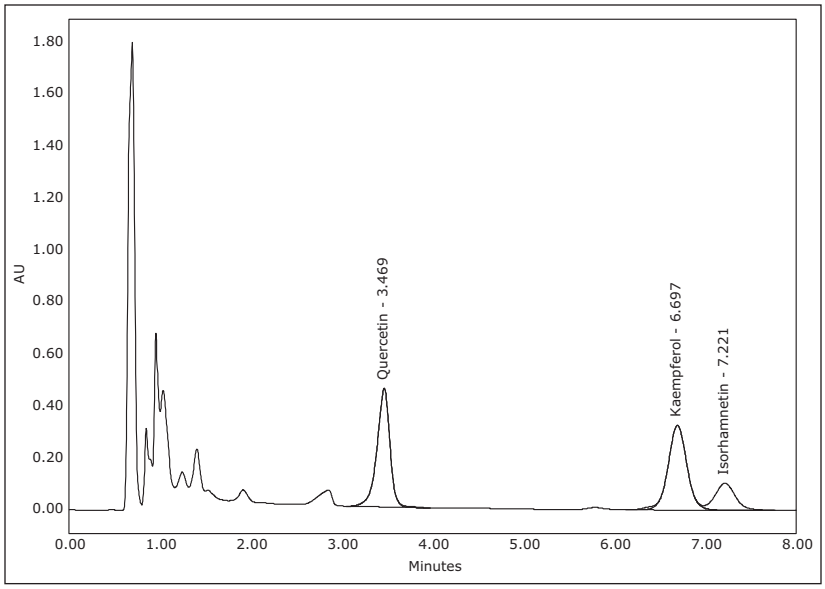

Figure 4. Analytical scale isocratic HPLC chromatogram of Ginkgo biloba leaf powder extract.

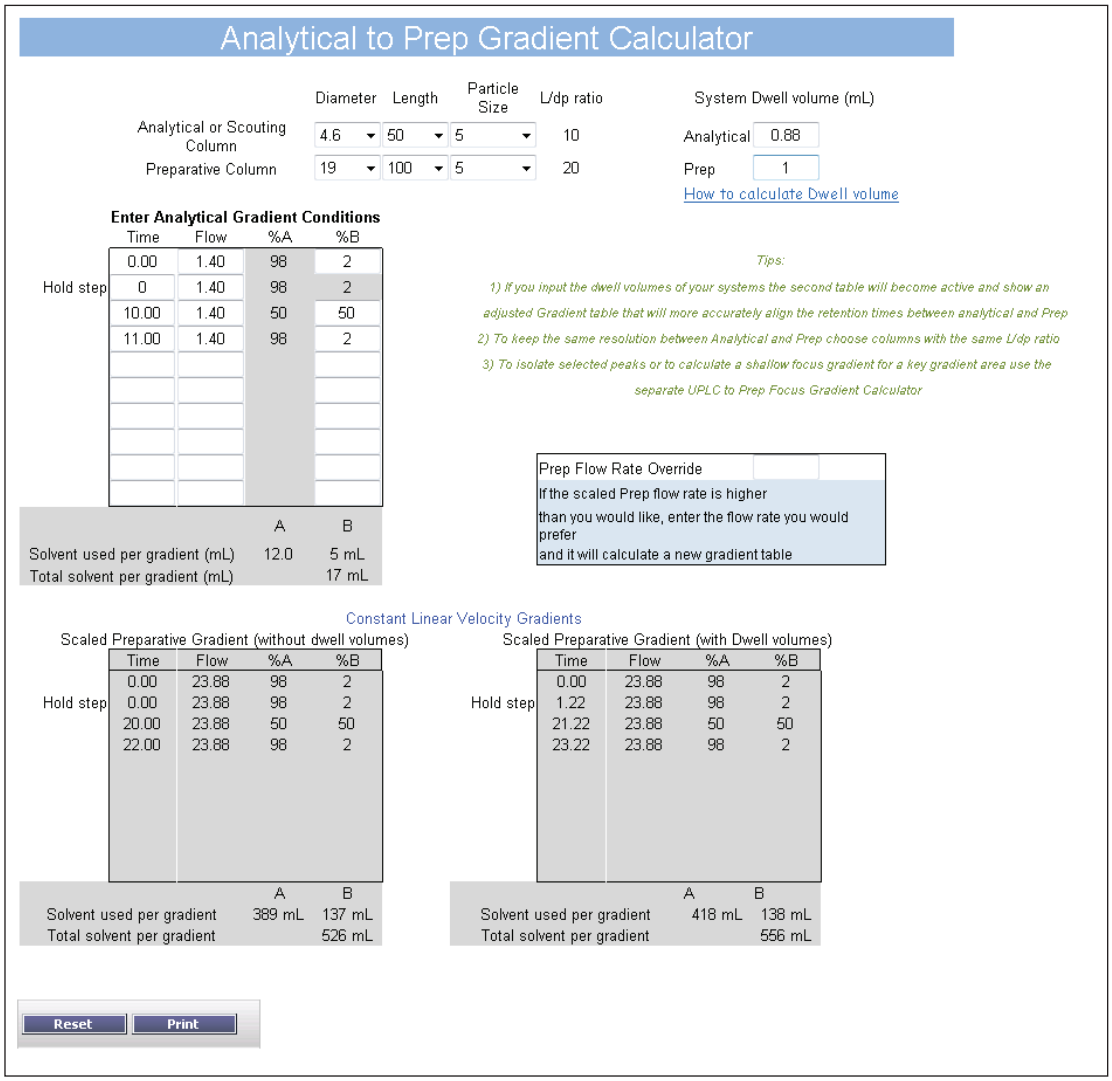

Figure 5. Waters ChromScope Analytical to Prep Gradient Calculator.

Using a 0.25 mL injection, acceptable preparative separations were achieved (Figure 6) with the isocratic purification separation method and threshold based fraction collection method. Fraction collection was easily set up through the Chromscope Software for threshold collection within windows (Figure 7). In this example, a pair of collection windows is defined (3.0 to 4.0 and 6.0 to 7.5 minutes), and any peaks that eluted outside of that window are not collected. To be collected, peaks eluting within the window must meet peak threshold criteria (in this case, 25 mAU for the first window, 15 mAU for the second window). Purity analysis of the collected fractions showed UV area% purity of 87, 96, and 85% for the three compounds, respectively, based on UPLC analysis (data not shown).

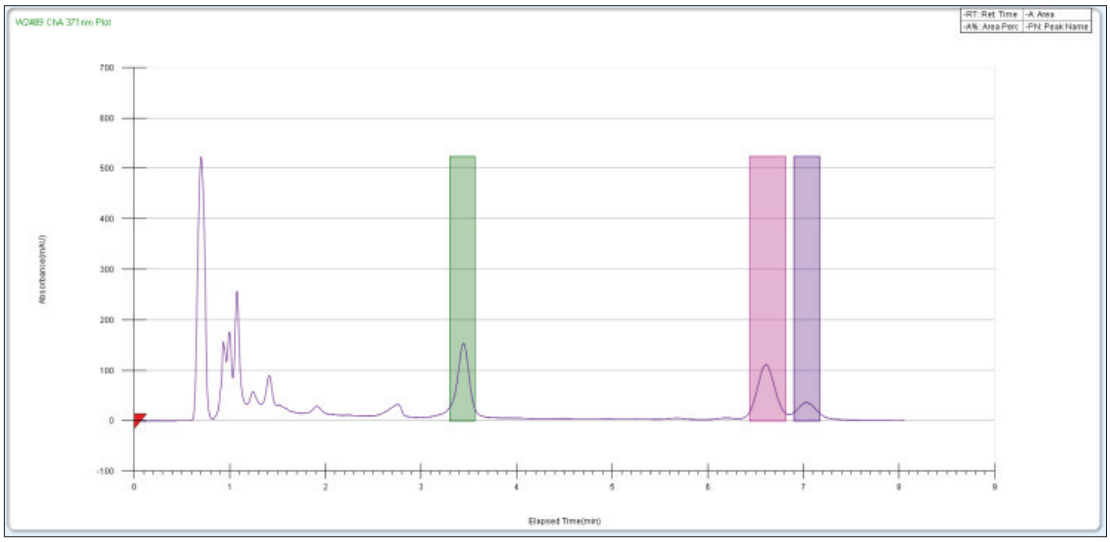

Figure 6. ChromScope Preparative HPLC chromatogram showing three collected peaks.

Fraction Collection Parameters

Current selected window: 2

	Window #	Single Fraction	Collection Type	Select Detector Signal	Threshold Start (mAU)	Threshold Stop (mAU)	Time Window Start (min.)	Time Window Stop (min.)	Collect waste within time window	Fraction is Inverted
	1	☐	Signal Threshold	(W2489) 2DChannel_1	25.0000	25.0000	3.000	4.000	☐	☐
▶	2	☐	Signal Threshold	(W2489) 2DChannel_1	15.0000	15.0000	6.000	7.500	☐	☐
✳		☐							☐	☐

Figure 7. ChromScope Fraction Collection Method.

CONCLUSIONS

- Using a Waters Prep 150 LC preparative HPLC system, material from an extract of Gingko biloba leaf powder was isolated and purified.
- Total system software control through Waters ChromScope software (Version 1.4) provided system flexibility by allowing easy modification of all method parameters.
- Separations were developed analytically and then geometrically scaled to a 19 x 100 mm preparative column on the Prep 150 LC System using the Analytical to Prep Gradient Calculator.
- Easy-to-set up fraction collection allowed for precise collection of target compounds.
- The initial extract contained 15% quercetin and 18% kaempferol. Each component was purified to greater than 87% on this easy-to-use system.

References

1. *PDR for Herbal Medicines*, 4th Ed., 2007, Thompson Healthcare Inc, Montvale NJ, U.S.A.
2. *Natural Products Isolation* 2nd edition, edited by S.D. Sarker, Z. Latif, A.I., 2006 Humana Press Inc, Totowa, NJ, U.S.A.
3. "Analytical HPLC to Preparative HPLC: Scale Up Techniques using a Natural Product Extract." Andrew Aubin and Ronan Cleary. Waters Application Note 720003120en, 2009

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