

## Amazonian Bark Extract

Wide Spectrum of  
Retention for Polar and  
Non-Polar Compounds

### Extended Application Note

## Introduction

The Amazon rainforest is the most biodiverse region in the world. Over one quarter of natural medicines have been found in rainforests, and new species are constantly being discovered. Therefore, there is a need for analytical study of the indigenous flora and fauna in developing new pharmaceuticals.

In some cases, natives of the region have traditionally consumed these natural products for their therapeutic effects but the active ingredients themselves may still be undetermined. One example is the “rose of Venezuela” tree (*Brownea grandiceps*), in which the bark is added to boiled water and the resulting extract is consumed as a tea. Among other beneficial effects, the beverage is believed to exhibit hemostatic and antidiabetic properties.

In order to identify some of its chemical components, a *Brownea grandiceps* bark extract was analyzed with the Cogent Diamond Hydride™ and Cogent Bidentate C18™ columns. Together these two columns can cover retention for a wide spectrum of polar and nonpolar compounds. In a complex natural product such as a bark extract, they are an excellent column choice for a thorough analytical investigation. The columns can be used in Reversed Phase (RP) and Aqueous Normal Phase (ANP) modes to obtain separation for the various compounds that may be found in the extract. In addition to being sold individually, they are also available together in convenient Cogent™ Metabolomics Kits.

## Experimental

### Materials

*Brownea grandiceps* tree bark was obtained from the Amazon rainforest in Brazil. Formic acid LC-MS ultra-grade was from Sigma-Aldrich (St. Louis, MO, USA). Deionized water (DI H<sub>2</sub>O) was prepared on a Milli-Q™ purification system from Millipore (Bedford, MA, USA). Acetonitrile (HPLC grade) was obtained from GFS Chemicals, Inc. (Powell, OH, USA).

### Instrumentation

An Agilent (Little Falls, DE, USA) 1200SL Series LC™ system, including degasser, binary pump, temperature-controlled autosampler, and temperature-controlled column compartment was used. The mass spectrometer system was an Agilent (Santa Clara, CA, USA) Model 6210 MSD TOF with a dual sprayer electrospray source (ESI). The analytical columns were Diamond Hydride™ (DH) and Cogent Bidentate C18™ (BDC18) stationary phases (MicroSolve Technology Corporation Leland, NC, USA), 2.1 x 150 mm (DH) and 2.1 x 100 mm (BDC18), 4µm, 100Å. Mobile phase A was DI H<sub>2</sub>O + 0.1% formic acid and mobile phase B was ACN + 0.1% formic acid. The DH was used with Gradient 1 and the BDC18 was used with Gradient 2 (see tables on left).

### Sample Preparation

Two pieces of the bark were boiled for 5 min in DI water, then filtered (0.45µm, Nylon AQ™ Brand Syringe Filters) and injected using the LC-MS conditions described above.

#### Gradient 1:

Time (min.)	%B
0	80
2	10
5	10
6	80

#### Gradient 2:

Time (min.)	%B
0	20
2	80
5	80
6	20

Post Time: 5 min

Flow Rate: 0.4 mL/min

Injection Volume: 1.0µL

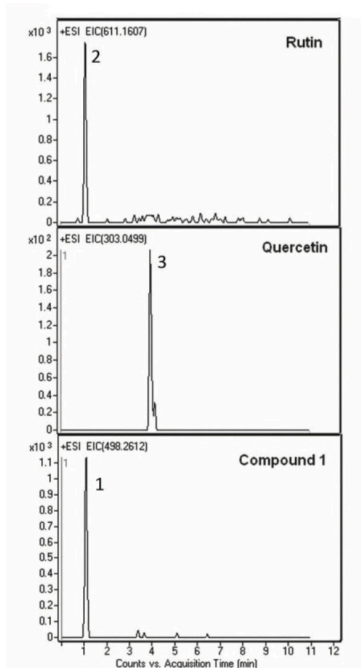


Figure 1

## Results and Discussion

The first investigations were carried out in ANP mode with the Cogent Diamond Hydride™. Three compounds were identified in the EICs, shown in **Figure 1**. Quercetin (peak 3) was the most strongly retained and was well chromatographically separated from the others. Two other compounds were also observed (rutin and 6-beta-O-2',3'-dihydrocinamonyl-12- hydroxy-(13)-15-en-16,12-olide-18-cassaneic acid ("compound 1")). Preliminary research suggests that quercetin may have antiviral, anti-cancer, and anti- inflammatory properties. Likewise, rutin has been reputed to have health benefits as well such as anti-oxidant properties, lowered risk of heart attack or stroke, and others.

Natural products such as this bark extract can be very complex in terms of the number of compounds so it is often advisable to perform runs in both RP and ANP to encompass the full range of polarity of compounds. Hence the Bidentate C18™ was used in reversed phase next. **Figure 2** shows the chromatograms of two new compounds that were retained and identified by their m/z values.

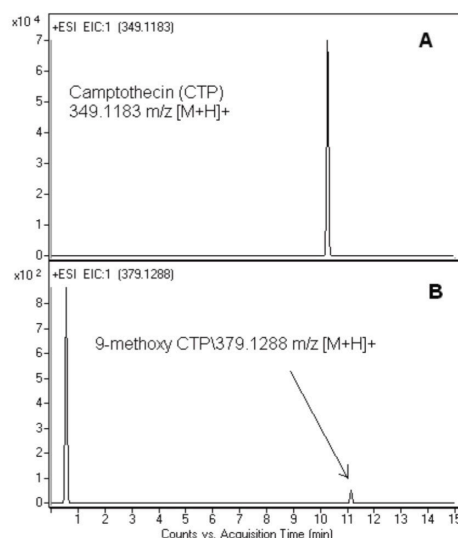
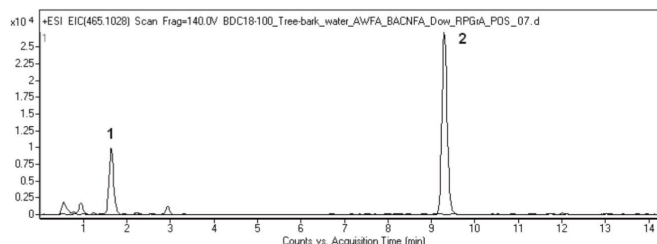


Figure 2

Camptothecin (CPT) has shown significant anticancer activity in preliminary clinical studies. 9-Methoxy CTP, a camptothecin analog, was observed as well in much lower abundance. The peak shapes for both compounds were very sharp and symmetrical.

**Figure 3** illustrates the orthogonality of using both ANP and RP approaches. Here, Quercetin (peak 1) is less retained than with the ANP data discussed earlier, but its 3- O-glucoside (isoquercetin, peak 2) is highly retained.



**Figure 3**

1. Quercetin  $m/z$  303.0499  $[M + H]^+$
2. Isoquercetin  $m/z$  465.1028  $[M + H]^+$

The extract contains many more compounds that have yet to be identified. For instance, other peaks were observed in the EICs at  $m/z$  215. These could potentially play a role in the extract's therapeutic properties as well.

## Conclusion

Together, the Cogent Diamond Hydride™ and Cogent Bidentate C18™ columns can be useful tools in the study of natural products. Analytical investigations of the *Brownea grandiceps* bark may lead to new insight into its therapeutic mechanism of action as well as to potential pharmaceuticals.