

Analysis of Antioxidants in Foods and Dietary Supplements Using HPLC With Post-Column Derivatization

Antioxidants protect cells from the damaging effects of free radicals and offer numerous benefits for human health. Many phenolic compounds found in plants, as well as some vitamins, exhibit antioxidant activity. Several colorimetric assays exist to measure the total antioxidant capacity, typically expressed as Trolox or Gallic acid equivalents.

An increased interest in antioxidants has created a demand for methods that are not only capable of determining the total antioxidant activity of the sample, but also are able to identify and quantify individual compounds known for their biological benefits. Our method abstract demonstrates that the well-known colorimetric reagents, such as Folin-Ciocalteu and 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonate (ABTS), can be successfully used for analysis of antioxidants in foods and dietary supplements by HPLC with post-column derivatization.

Reagents Overview

Folin-Ciocalteu Reagent (FCR) reacts with reducing compounds to form a chromophore that can be detected using UV/Vis detection. Phenolic and non-phenolic antioxidants, including Vitamin C, Vitamin B1 and Folic acid, can be analyzed using this reagent with high sensitivity. Many antioxidants can be easily detected in solution at levels as low as 1 ug/mL.

The ABTS reagent is used in the Trolox-equivalent antioxidant capacity (TEAC) assay. The reaction is based on suppression of the absorbance of radical cations by antioxidants, and can be detected by UV/Vis detection as negative peaks on the chromatograms. This indirect detection method allows for analyzing common antioxidants at levels between 40 and 200 ug/mL.

Using both reagents, it is possible both to identify and quantify specific target compounds, as well as to calculate the total antioxidant capacity of the sample by comparing the total area of all the peaks on the chromatogram with Trolox or Gallic Acid calibration curves.

Method

Sample Preparation

- **Fresh Fruit:** Homogenize 10 g of fresh fruit with 20 mL of water/methanol (50:50) extraction solution for 5 min. Centrifuge and filter through 0.45 um Nylon filter
- **Dried Fruit:** Mix 4 g of finely ground dried fruit with 20 mL of 100% Methanol. Shake for 1 h on a mechanical shaker. Centrifuge and filter through 0.45 um Nylon filter.
- **Dietary Supplements:** Mix 100 mg of finely ground sample with 10 mL of 100% Methanol. Shake for 30 min on a mechanical shaker. Centrifuge and filter through 0.45 um Nylon filter.
- **Dry Tea Leaves:** Homogenize 1 g of dry tea leaves with 25 mL of water/methanol (50:50) extraction solution for 5 min. Centrifuge and filter through 0.45 um Nylon filter. Alternatively, mix 1 g of dry tea leaves with 25 mL of hot water, stir on a hot plate for 5 min. Cool down, centrifuge and filter through 0.45 um Nylon filter.
- All extracts can be diluted if needed with water/methanol (50:50).

Analytical Conditions

Column: Reversed-phase C₁₈ column, 4.6x150 mm
Temperature: 40 °C
Flow Rate: 1 mL/min
Mobile Phase: 4.8% Acetic acid in water, Methanol
Injection Volume: 10-50 uL

Post-Column Conditions

Post-column System: Onyx PCX, Pinnacle PCX or Vector PCX
Reactor Volume: 1.4 mL
Reactor Temperature: 130 °C

Reagents: Option 1: 40% of Folin-Ciocalteu reagent in water
 Option 2: 15% of ABTS stock in Methanol. ABTS stock solution: 7 mmole/L of 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonate is dissolved in a 2.45 mmole/L solution of Sodium Persulphate in water. Stock solution should be stored in the dark for at least 12 h before making a working reagent.

Flow Rate: 0.1 mL/min

Detection: UV/Vis 635 nm

HPLC Gradient		
Time	Acetic Acid / water %	Methanol %
0	95	5
3	95	5
25	40	60
30	40	60
30.1	95	5
40	95	5

Calculated Total Antioxidant Capacity of Selected Samples		
Matrix	Trolox Equivalent, ABTS Reagent	Gallic Acid Equivalent, Folin-Ciocalteu Reagent
Fresh Raspberries	1.03 mg/g	0.63 mg/g
Fresh Blueberries	0.66 mg/g	0.35 mg/g
Dry Goji berries	2.39 mg/g	3.30 mg/g
Green tea	-	50.0 mg/g
Antioxidant Dietary Supplement	477.24 mg/g	120.0 mg/g

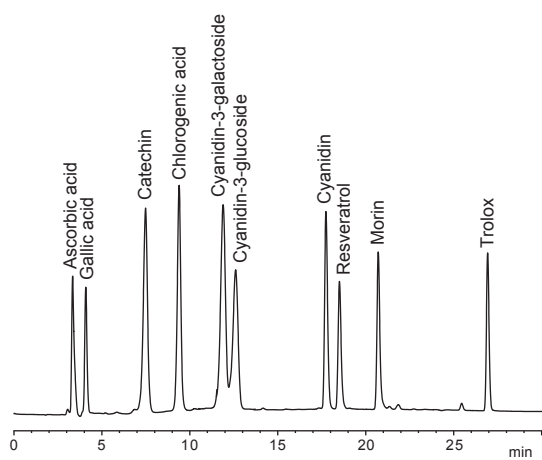


Fig 1. Mixture of 10 common antioxidants, analyzed using Folin-Ciocalteu reagent

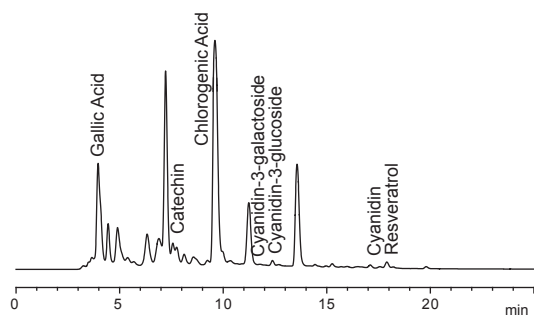


Fig 3. Hot water extract of green tea, analyzed using Folin-Ciocalteu reagent

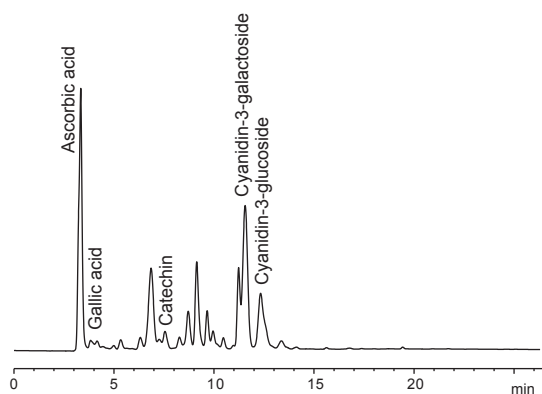


Fig 2. Fresh raspberries extract, analyzed using Folin-Ciocalteu reagent

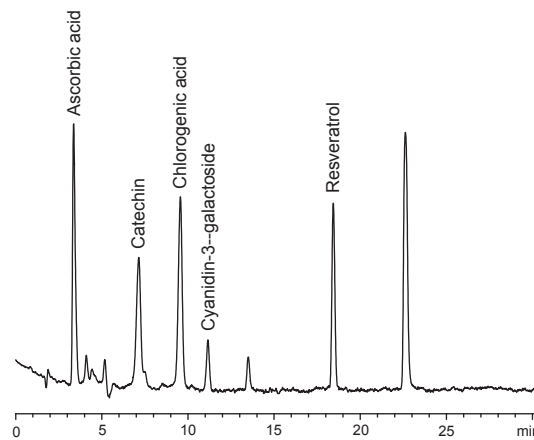


Fig 4. Extract of dietary supplement, analyzed using ABTS reagent

References:

Barbara Kuznierewicz, Anita Piasek, Agnieszka Bartoszek, Jacek Namiesnik, "The Optimization of Analytical Parameters for Routine Profiling of Antioxidants in Complex Mixtures by HPLC Couples with Post-column Derivatization," *Phytochem. Anal.* 2011, 22, 392-402