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Determination of Flavonoids (Catechins) by HPLC-ECD

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Introduction

Flavonoids are ubiquitous in the plant kingdom and are very efficient antioxidants. High-performance liquid chromatography (HPLC) with electrochemical detection (ECD) is a sensitive, simple and selective method for determination of flavonoids. Figure 1 shows typical HPLC chromatograms and the legend gives details of the method of preparation of HPLC samples of flavonoids (catechin) from the plasma of rats given green-tea extracts. The major flavonoids (catechins) in green-tea extracts are the four epicatechins: (-)-epicatechin, EC; (-)-epicatechin gallate, ECG; (-)-epigallocatechin, EGC; and (-)-epigallocatechin gallate, EGCG. They were separated on a ODS C18 reversed-phase column by isocratic elution with 85:15 0.1% phosphoric acid-acetonitrile solution containing 0.1 mM Na2EDTA [1].

Protocol

1. Add ethyl gallate (0.1-50 μg/mL, 50 μL; internal standard) to plasma (200 μL).
2. Add metaphosphoric acid solution (30% w/v, 200 μL).
3. Mix for 1 min.
4. Sonicate at 20 kHz for 30 s.
5. Leave to stand in an ice bath for 10 min.
6. Centrifuge at 3000 rpm for 10 min.
7. Dilute with HPLC mobile phase and pass through 0.45-μm filter.
8. Take 10-20 μL of sample for analysis by HPLC with electrochemical detection (ECD)

HPLC was performed with a Shimadzu (Kyoto, Japan) LC-10AT pump and a Shiseido (Tokyo Japan) Nanospace Sl-1/2005 ECD with the applied voltage set at 600 mV. Compounds were separated on a 4.6 mm i.d. x 250 mm TSK gel ODS80Ts reversed-phase column (Tosoh, Tokyo, Japan) maintained at 30°C in a Shimadzu CT0-10 AC column oven. The mobile phase was 85:15 0.1 M phosphoric acid-acetonitrile containing 0.1 mM Na2EDTA at a flow rate of 1.0 mL/min. The limits of detection for epicatechins are approximately 1 ng/mL (signal-to-noise ratio, S/N = 3) in plasma or bile.

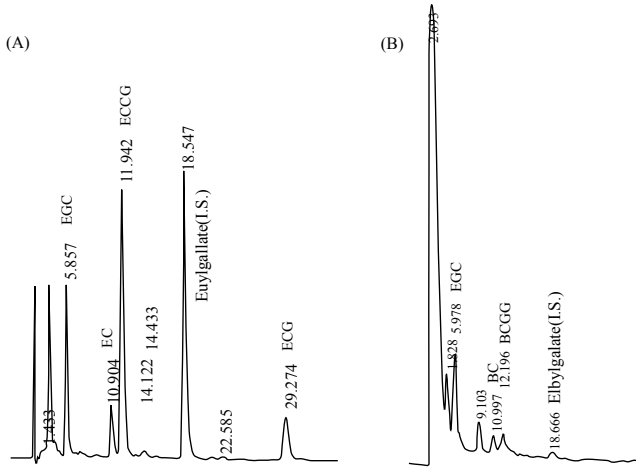

Figure 1: HPLC-ECD chromatogram of flavonoids (catechins). Sample A was prepared by adding a mixture of tea catechins (50 mg/ml) to rat plasma. Sample B was a plasma sample (200 μL) prepared from blood obtained 1 h after oral administration of the catechin mixture (100 mg/kg). The catechin mixture contained EC 5.81%, GC 1.44%, ECG 12.51%, EGC 17.57% and EGCG 53.90%.

Discussion

Aqueous solution of flavonoids (catechins) are readily oxidized and polymerized under alkaline conditions, but are fairly stable under acidic conditions. Oxidation is accelerated in the presence of metal ions such as Fe^{2+}, Fe^{3+}, and Cu^{2+}. Plasma samples for HPLC analysis should be stored in the presence of an antioxidant and metal chelator. Epicatechins in rat or human plasma are relatively stable when mixed 1:50 (v/v) with VitaminC-EDTA solution (0.4 M NaH_2P04 buffer containing 20% vitamin C and 0.1% Na_2EDTA, pH 3.6) and stored at −80°C [2].

Free epicatechins are detected in plasma and bile 1-2 h after oral administration of tea catechin or green tea powder to rats or man [3-5]. Epicatechins are also present in the conjugated form (e.g. glucuronide and sulfate) in plasma and bile, and hence, whole catechins can be detected after pretreatment with β-glucuronidase (Sigma G-7896) and sulfatase (Sigma S-9754) [2]. When EGCG, a major green tea catechin, is incubated with rat plasma or bile at 37°C, three small peaks arising from three dimers can be detected in both fluids by following the disappearance of EGCG [5-9].

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