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HPLC Analysis of Extracted Coenzyme Q (Coq) Homologues from Animal Tissues

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Introduction

Coenzyme Q (CoQ) plays an important role in ATP synthesis as an electron-carrying component of the mitochondrial respiratory chain. In recent years, increasing attention has focused on the reduced form of CoQ homologues (CoQ_nH_2) as antioxidants.

During lipid peroxidation in disease states it is important to detect the decrease in endogenous antioxidants and to measure the increase in lipid peroxides. Measurement of CoQ homologues in animal tissues by High Performance Liquid Chromatography (HPLC) is described.

Protocol

Extraction procedure

1. Animal tissue (approx. 300 mg) is stored in liquid nitrogen.
2. Add ice-cold distilled water (8 vol.).
3. Homogenize tissues by means of a Polytron^R under a stream of nitrogen for 20 s at 4°C. A sample of the homogenate is used for protein assay.
4. Place homogenate (1 ml) in individual 10-ml test tubes with a screw cap.
5. Add HPLC-grade ethanol (2 ml) to each tube.
6. Extract each tube with HPLC-grade n-hexane (3 × 5 ml, shaking vigorously for 10 min during each extraction). After each extraction centrifuge at 750 g for 5 min, remove the upper n-hexane layer carefully and pour into 60-ml dark-brown centrifuge tubes with stoppers, previously filled with nitrogen gas.
7. Evaporate the combined n-hexane layers (15 ml) under a stream of nitrogen.
8. Re-dissolve the residue in ice-cold ethanol (0.5-1.0 ml) and pass the solution through a 0.45 µm filter.
9. Analyse by HPLC.
10. HPLC was performed with a JASCO 880-PU pump and an injector. Compounds were separated on a 250 mm × 4.6 mm i.d., 7 µm particle, Chemcosorb ODS-H column. The mobile phase was 0.7% (w/v) $NaClO_4.H_2O$ in 700:300;1 HPLC-grade ethanol-HPLC-grade methanol-70% $HClO_4$ at a flow rate of 1.2 ml/min. The injection volume was 10 µl. A JASCO 870-UV detector (operated at 275 nm) was used for determination of oxidized CoQ_n and a JASCO 840-EC electrochemical detector (ECD) (+700 mV relative to Ag^+ /AgCI) for CoQ_nH_2. The chart speed was 2 em/min.

Preparation of CoQ_n homologue standards

Standard solutions of CoQ_9 and CoQ_{10}: Chromatographically pure CoQ_9 and CoQ_{10} from Eisai (Tokyo, Japan) were dissolved in HPLC-grade ethanol at a concentration of 550 µg/mL and diluted to 5.5 µg/mL with ethanol. When stored in dark-brown vials at −80°C these standard solutions are stable for approximately 1 year.

Standard solutions of CoQ_9H_2 and $CoQ_{10}H_2$: Standard solutions of CoQ_9 and CoQ_{10} were reduced with $NaBH_4$. CoQ_n stock solution (200 µL) was vortex-mixed with a mixture of $NaBH_4$ (0.3%, 40 µL) in water and 160 µL HPLC-grade ethanol. $CoQnH_2$ standards should be freshly prepared before use.

Results and Discussion

Typical UV and ECD detected HPLC chromatograms of CoQ homologues from the liver of a three-week-old rabbit are shown in figure 1.

The ECD is connected to the outlet of the UV detector. ECD is sensitive to electrical noise and the baseline on the ECD chromatogram is not always stable. This seems to be because of dirt in the HPLC system, including the column, and because of mechanical and electrical noise. The working electrode should be cleaned when the sensitivity of the electrochemical detector falls.

1n some animal species, and tissues, an unknown peak co-elutes with the CoQ_nH_2 such as α-tocopherol peak on the chromatogram if the applied potential is +700 mV (Figure 1). If this happens, selectivity is improved by use of a setting near +600 mV. It has previously been reported that electrochemical detection can be performed with an applied potential of +500 mV [1,2].

For UV detection in figure 1, to avoid interference of determination of the interest peaks, solid phase extraction (SPE) is quite recommendable and the automated SPE is commercially available and significant separation results were reported [3-5].

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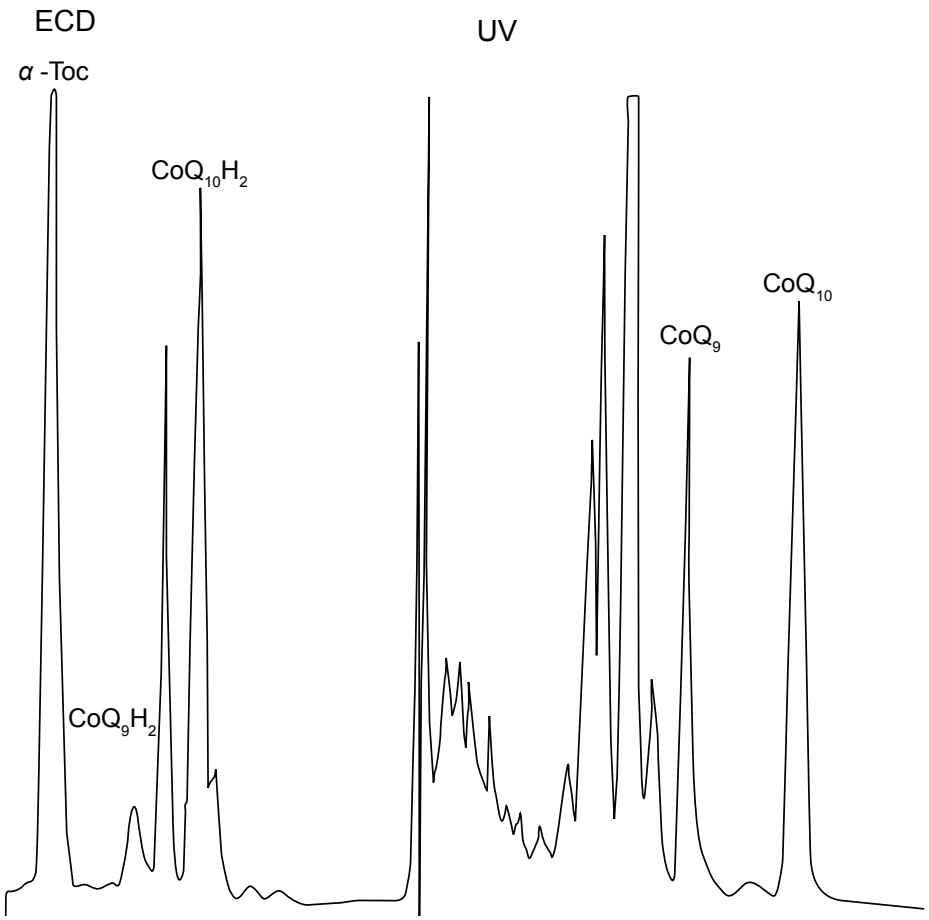

ECD w as +700 mV application and UV detection was 278 nm.
From both chromatograms, it can be found ECD was more selective and sensitive. IN ECD at 700 mV application, α-tocopherol was co-eluted.

Figure 1: Typical HPLC chromatogram of liver CoQ derivatives.