CAPSULES notes and applications from BASi

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September 2001

Coenzyme Q10

Purpose

Determination of coenzyme Q10 by dual-series LCEC detection.

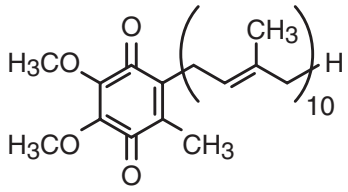

Figure 1. Structure of coenzyme Q10.

Coenzyme Q10 (F1) is a reversibly reducible quinone with a long isoprene side chain. The length of the side chain varies in nature, providing analogous compounds (Q6-Q9) in other organisms. The ubiquinones function as electron acceptors in mitochondrial oxidative phosphorylation. The reduced forms are strong antioxidants. For this reason, ubiquinones have created a lot of interest as nutraceuticals. Proponents claim effectiveness for treating stroke, heart disease, and cancer.

Existing Methods

Liquid chromatography with UV detection, which suffers from lack of sensitivity. Chemical reduction followed by oxidative LCEC, which is time consuming.

Conditions

System: BAS-200b Liquid Chromatograph
Electrode: Dual 3 mm glassy carbon (MF-1000)
Potential: -500 mV upstream, +800 mV downstream (vs. Ag/AgCl)
UV Wavelength: 275 nm (when used)
Column: 2 x 100 mm C_{18} (MF-8957)
Mobile Phase: 388 mL methanol, 7 mL acetic acid, 7 mL isopropanol, 64 mL hexanes, 3.2 g sodium acetate trihydrate
Flow Rate: 0.8 mL/min
Injection Volume: 10 µL

Notes

Separation of 100 pg standard oxidized coenzyme Q10 is shown in F2. The upstream (reductive) electrode responds to the oxidized form of the compound, while the downstream (oxidative) electrode responds to the reduced form. Thus, the upstream electrode can be used to quantify only the oxidized form, while the downstream electrode can quantify the total amount (endogenous reduced compound plus the amount produced by reduction upstream). Endogenous reduced coenzyme Q10 (if any) could be quantified by using only the downstream electrode.

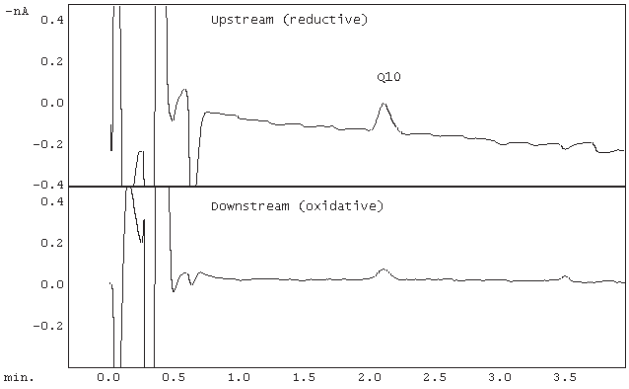

Figure 2. Separation of 100 pg oxidized coenzyme Q10.

Oxygen, which is reducible, must be removed from the mobile phase and sample for best results. The BAS-200b chromatograph is designed for helium sparging of the mobile phase to remove oxygen. Instructions for removing oxygen from the sample can be found in [1].

Retention time can be shortened or lengthened by increasing or decreasing (respectively) the amount of hexanes in the mobile phase.

These samples were diluted in methanol. As F3 shows, diluents higher in the elutropic series may adversely affect peak shape.

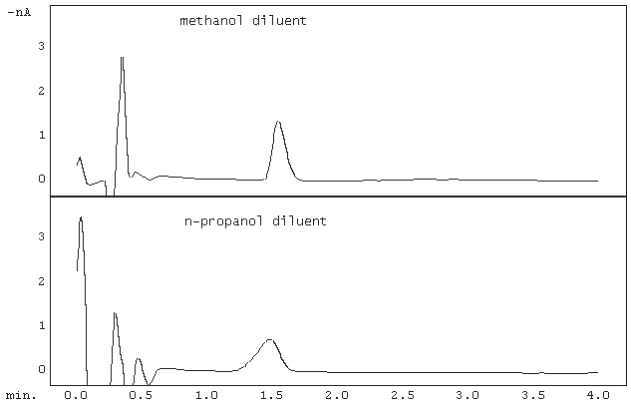

Figure 3. Effect of sample diluent on peak shape.

Results were linear between 50 pg (the detection limit at S/N = 3) and 1 ng injected coenzyme Q10 (F4).

The detection limit for a UV detector under the same chromatographic conditions was 400 pg.

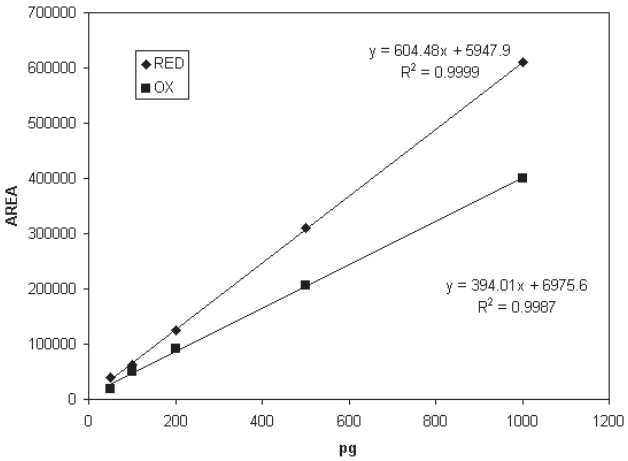

Figure 4. Linearity of the responses at the upstream reducing (RED) and downstream oxidizing (OX) electrodes.

References

1. B.P. Solomon, Current Separations 14 (1996): 110-112.