



Analysis of Quinones such as CoQ10 and Vitamin K

AN09-0815

Background

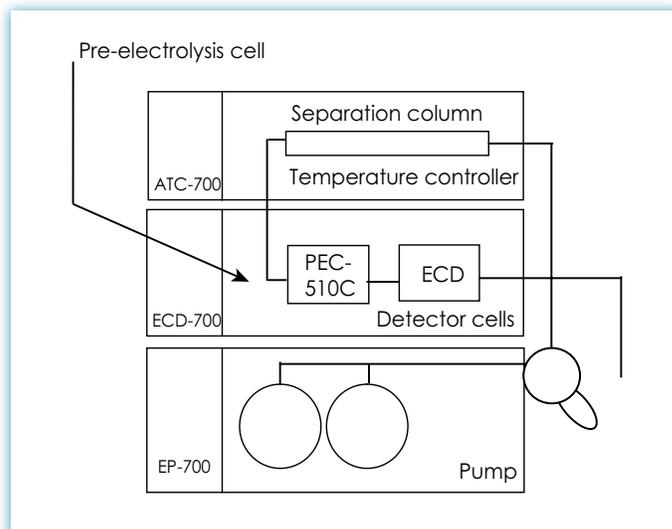
Coenzyme Q10 and vitamin K represent a class of compounds known as quinones. As a class, quinones act as important electron donors and acceptors in a variety of biological processes. Electrochemical detection (ECD) provides a very sensitive method for analyzing this class of molecules. Eicom's HPLC-ECD systems support the following convenient, sensitive, and stable application for quinone detection.

Previous ECD methods relied on an expensive and disposable platinum reduction column, situated between the separation column and detector cell. The chemical nature of the metallic packing material introduced unnecessary error into the analysis. Over time, the reactive surface of the metal particles declined, inevitably leading to a loss of sensitivity. Often, this decline could be so rapid as to cause significant variations between the first and last sample when a large number of samples had to be run during one experiment. Moreover, the validity of comparing single experiments in a series of experiments that were conducted over longer periods of time was a constant concern.

Our solution does away with the reducing column entirely. Instead, we use a post-column pre-electrolysis cell (the PEC-510) to reduce the CoQ10 before it enters the electrochemical detector. The pre-electrolysis cell is significantly less prone to age-induced decreases in sensitivity and accuracy. Furthermore, should any decrease in sensitivity be observed, the performance of the pre-electrolysis cell can be easily recovered by using the appropriate applied voltage. Using an electrolysis cell, instead of the platinum column, affords the user an additional measure of confidence in peak identification because the pre-electrolysis cell also functions as an auxiliary detector. The current can be monitored simultaneously with the signal from the main detector cell. For signals that correspond to the target compounds, a negative, or mirror image, peak coincides with the main detector's peak.

Instrument Set-up

Eicom's 700 series HPLC-ECD system



Analytical Conditions

HPLC-ECD	Eicom 700 series system equipped with Pre-electrolysis cell PEC510C
Separation column	Eicompak SC-50DS 2.1 x 150 mm
Flow rate	230 µl/min
Temperature	25°C
Applied potential	+700 mV vs AgCl ₂ for CoQ10 +450 mV vs. AgCl ₂ for vitK
Working electrode	Graphite WE-G
Detection limit	vitK1 1 pg (2 fmol) at S/N=2, or 20 µl of 0.1 nM CoQ10 2 pg (2 fmol) at S/N=2, or 20 µl of 0.1 nM
Linear range	10 ⁴ , 2 pg to 10 ng

Simple Sample Preparation

CoQ10 and related compounds can easily be extracted from any biological material with the appropriate organic solvent (such as isopropanol, hexane, etc)

Sample data: CoQ10 and Vitamin K

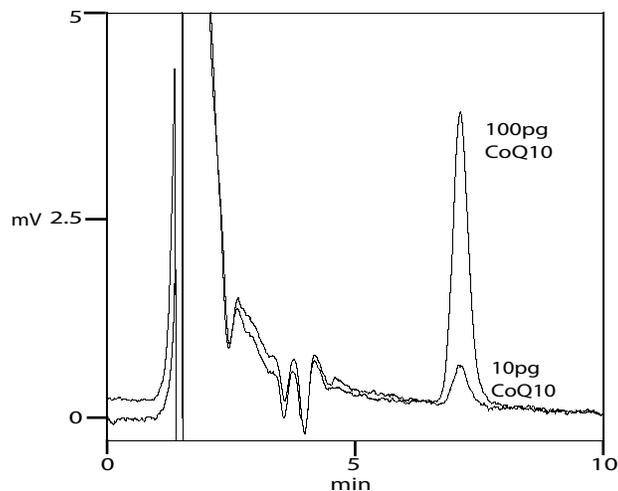


Fig 1. Coenzyme Q10 standards Figure 1. Coenzyme Q10 standards

Comments

1. Simultaneous detection of CoQ10 and CoQ9 possible.
 2. Simultaneous detection of CoQ10 and vitamin K possible
 3. Detection of tocopherols (vitamin E) is also possible.
- Please inquiry about these and other quinones.

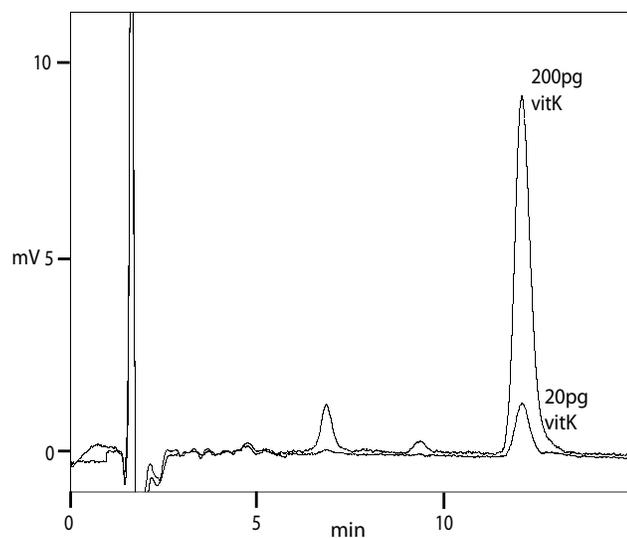


Fig 2. Vitamin K standards