# Comparison of In-Line Connected Diode Array and Electrochemical Detectors in the High-Performance Liquid Chromatographic Analysis of Coenzymes $Q_9$ and $Q_{10}$ in Food Materials

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In-line connected electrochemical (EC) and diode array (DAD) detectors were compared in the reversed-phase high-performance liquid chromatographic (RP-HPLC) analysis of coenzymes $Q_9$ and $Q_{10}$ in some food materials (beef steak, beef heart, Baltic herring fillet, and rye flour). Coenzymes $Q_9$ and $Q_{10}$ were extracted from the samples using a 5:1 *n*-hexane−ethanol mixture. Coefficient of variation (CV%) of quadruplicate or quintuplicate determined samples for coenzymes $Q_9$ and $Q_{10}$ was <10 by both EC detector and DAD. Responses of the detection systems were linear in the range evaluated, 10−200 ng/injection, and had correlation coefficients exceeding 0.999. Recoveries of added coenzymes $Q_9$ and $Q_{10}$ varied 73−105% for DAD and 74−103% for EC detector, respectively. Detection limits for coenzymes $Q_9$ and $Q_{10}$ using the DAD system were 4 and 6 ng/injection, respectively, and 0.2 and 0.3 ng/injection by EC detection. Results derived from the two detection systems were generally similar. However, although EC detector was 20-fold more sensitive, the selectivity was, in some cases, poorer than that of DAD.

**Keywords:** *Coenzyme $Q_9$; coenzyme $Q_{10}$; ubiquinone; HPLC; electrochemical detection; diode array detection; food analysis*

## INTRODUCTION

Coenzyme Q is a lipid-soluble compound found in plants and animals in two redox forms and varying length of the isoprenoid tail. The predominant form of coenzyme Q in humans and animals is coenzyme $Q_{10}$, containing 10 isoprenoid units in the side chain. Besides its activities in the electron-transport chain, coenzyme Q (in reduced form) has also been implicated as the only endogenously synthesized lipid-soluble antioxidant protecting cellular membranes, particularly those of mitochondria, and plasma lipoproteins from free-radical damage (Beyer, 1992; Frei et al., 1990; Ernster and Dallner, 1995).

It is not known to what extent diet contributes to the tissue concentration of coenzyme $Q_{10}$, but there is convincing evidence from a number of sources (e.g. Mohr et al., 1992; Kaplan et al., 1995; Weber et al., 1994) that administration of coenzyme $Q_{10}$ to humans produces an increase in the blood level of the coenzyme (partly present in reduced form). Elevated levels of coenzyme Q in blood may have several important functions. Among these are an enhanced protection of LDL from oxidation, prevention of free-radical damage caused by neutrophils in inflammatory diseases, and prevention of oxidative injury by endothelial cells resulting from ischemia−reperfusion (Ernster and Dallner, 1995).

Despite the presumption that dietary intake of coenzyme Q may have beneficial effects on human health, to our knowledge only two studies have focused on the coenzyme Q contents in foods during the past two decades (Kamei et al., 1986; Weber et al., 1997). In these studies contents of coenzymes $Q_9$ and $Q_{10}$ were determined in several food items using HPLC coupled with UV detection. HPLC methods employing EC detection have become widely used in the determination of coenzyme Q in biological samples (plasma, tissue, etc.) due to improved selectivity and sensitivity (e.g. Okamoto et al., 1988; Wakabayashi et al., 1994; Laaksonen et al., 1995). In addition, coupled EC−UV detections have been used to determine oxidized (with UV) and reduced (with EC) coenzyme Q in biological samples (e.g. Ikenoya et al., 1981; Lang et al., 1986, 1987; Podda et al., 1996). To our knowledge, EC detection has not been applied in food analysis. In light of the high applicability of EC detection in clinical studies, the present study sought to compare the advantages of EC and DAD detections for food-borne coenzyme Q analysis. Food-borne coenzyme Q may have antioxidative and other beneficial effects for humans. Hence, it is essential to be able to quantify the contents of these compounds in foods reliably.

## MATERIALS AND METHODS

**Samples.** Samples of beef steak (0.5 kg), pork heart (0.3 kg), Baltic herring fillet (0.5 kg), and rye flour (1 kg) were purchased from local retail stores. All samples, except rye flour, were homogenized using a blender (Bamix, Switzerland), packed into plastic containers in 50-g portions, and stored at −20 °C until analysis. Rye flour was stored at room temperature.

**Standards.** Coenzyme $Q_9$ and $Q_{10}$ standards were obtained from Sigma Chemicals. Coenzyme $Q_9$ solution was prepared by dissolving 2 mg in 20 mL of ethanol (99%) and coenzyme $Q_{10}$ by dissolving 10 mg in 100 mL of ethanol (99%). Concentrations of the standard solutions were confirmed by measuring absorbance at a wavelength of 275 nm and by reference to known coefficients ($E^{1\%}_{1cm}$ 185 for coenzyme $Q_9$ and 165 for coenzyme $Q_{10}$; Hatefi, 1963).

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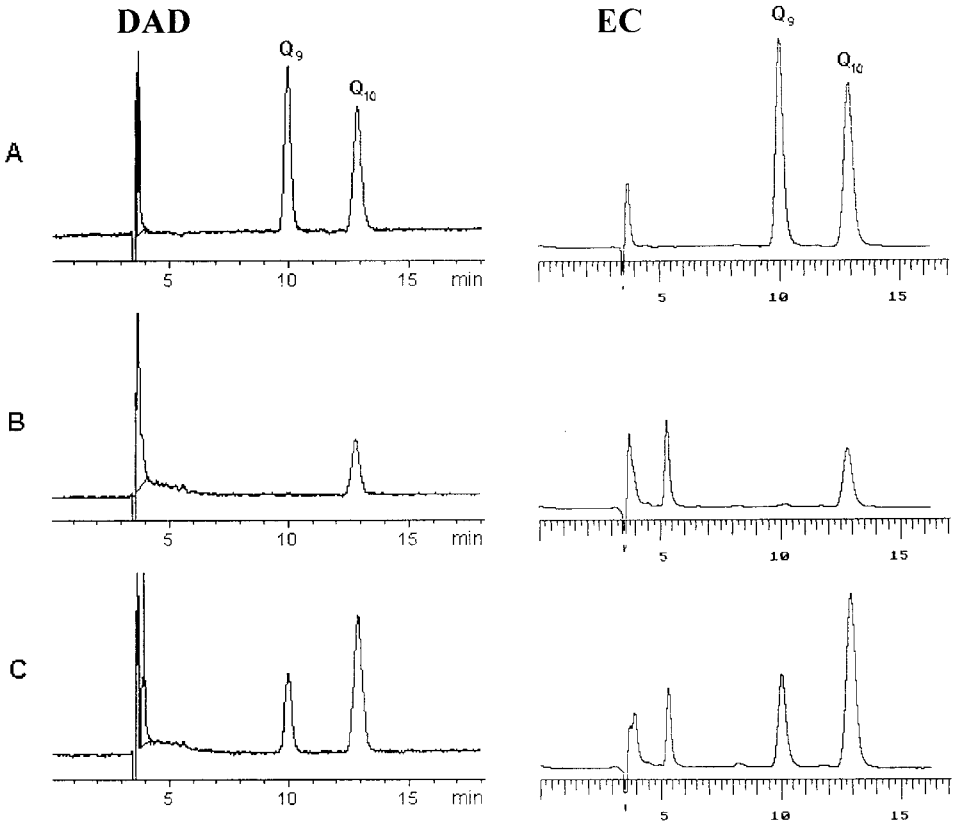


**Figure 1.** HPLC chromatograms of beef steak sample by DAD and EC detection systems: A, standard mixture of coenzymes $Q_9$ and $Q_{10}$; B, beef steak sample; C, beef steak sample with added coenzymes $Q_9$ and $Q_{10}$.

**Extraction.** Extraction of the samples was performed according to Ikenoya et al. (1981), Okamoto et al. (1988), and Weber et al. (1997) with some modifications. A 1-g sample of rye flour or defrosted and homogenized beef steak, pork heart, and Baltic herring fillet was weighed into an extraction tube and homogenized in 5 mL of 0.15 M NaCl (saline) with a Heidolph Diax 600 homogenizer (Heidolph, Germany); 5 mL of ethanol (99%) was added and the mixture was rehomogenized. After that, 25 mL of *n*-hexane (HPLC-grade) was added into a tube and mixed vigorously for 8 min using a Heidoplh Promax 2020 shaker (Heidolph, Germany). The extraction was repeated three times with 5 mL of ethanol and 25 mL of *n*-hexane. The combined *n*-hexane layer was washed with 30 mL of saline, dried with anhydrous $Na_2SO_4$ (p.a.), and filtered. The organic solvent was then removed by rotary evaporation and the residue dissolved in 5 mL of 2-propanol.

**HPLC Analysis.** The analytical HPLC system consisted of a Hewlett-Packard 1090 series II high-performance liquid chromatograph equipped with a diode array detector (Hewlett-Packard) and a coulometric multielectrode (eight channel) electrochemical detector (CEAS, Esa Inc.). HPLC and DAD instrumentation was controlled by the HP 3D Chem Station computer program revision A.03.04. EC detector was controlled by the ESA CoulArray version 1.001 computer program. Temperature of EC cells was set at 35 °C.

The coenzymes were separated at room temperature using a Vydac 201TP54 column (5 $\mu$m, 25 cm × 4.6 mm; The Separations Group). Mobile phase consisted of methanol, ethanol, 2-propanol (all HPLC grade), and 1 M ammonium acetate buffer, pH 4.4 (53:24:21:2), at flow rates of 0.7 mL/min (rye flour) and 0.8 mL/min (other samples). Injection volume of the samples was 10 $\mu$L. Quantification was done using an external standard method. With DAD the quantification was based on peak area and the wavelength used was 275 nm. In the case of the EC detection the quantification was based on peak height and the detector was operated in the redox mode. Coenzymes $Q_9$ and $Q_{10}$ were reduced at the upstream electrode (channel 1; electrode potential −1000 mV), and the quantification was based on reoxidizing the compounds at the downstream electrode (channel 2) set at 500 mV. Channels 3−8 were not used.

**Method Reliability Tests.** Linearity ranges of the standard curves for coenzymes $Q_9$ and $Q_{10}$ as well as detection limits of the coenzymes were tested. Recovery tests were carried out by spiking coenzymes (18−60 $\mu$g) into samples before extraction. To study repeatability of the methods 4−5 separate determinations were performed for all samples and coefficients of variation calculated. Results obtained by the DAD and EC detections were compared statistically using the *t*-test for paired observations.

## RESULTS AND DISCUSSION

Two basic methods have been employed for the extraction of coenzymes Q in biological tissues and foods. The first involves saponification before extraction (Abe et al., 1978; Kamei et al., 1986), and the second is based on direct solvent extraction (Ikenoya et al., 1981; Lang et al., 1986; Lang and Packer, 1987; Okamoto et al., 1988; Weber et al., 1997). In the present study the samples were prehandled using direct solvent extraction with ethanol−*n*-hexane. This extraction procedure was chosen because it was simple to perform and, according to previous studies, efficient for tissue and food samples. For example, Ikenoya et al. (1981) examined several solvents and extraction systems and found the ethanol−*n*-hexane mixture to be the most efficient for tissues and mitochondrial fractions. In addition, according to Weber et al. (1997) direct ethanol−*n*-hexane solvent extraction was effective for food samples other than fats and oils and gave comparable results with the saponification procedure.

It is well-known that ubiquinols are not stable and oxidize easily in air into ubiquinones (Ikenoya et al., 1981; Lang and Packer, 1987; Edlund, 1988). However,

**Table 1. Contents of Coenzymes $Q_9$ and $Q_{10}$ in Different Food Samples Using DAD and EC Detection**

| | $Q_9$ ($\mu$g/g) | | $Q_{10}$ ($\mu$g/g) | |
|---|---|---|---|---|
| sample | DAD | EC | DAD | EC |
| Baltic herring | nd[a] | nd | 10.56 | 12.61 |
| | nd | nd | 10.55 | 12.57 |
| | nd | nd | 11.80 | 14.92 |
| | nd | nd | 11.87 | 15.27 |
| | nd | nd | 11.42 | 14.03 |
| *mean* | | | *11.2* | *14* |
| *SD* | | | *0.65* | *1.3* |
| beef | nd | qi[b] | 15.41 | 14.86 |
| | nd | qi | 19.02 | 16.00 |
| | nd | qi | 17.10 | 16.95 |
| | nd | qi | 16.78 | 16.28 |
| | nd | qi | 18.14 | 16.64 |
| *mean* | | | *17* | *16.1* |
| *SD* | | | *1.4* | *0.80* |
| pork heart | <2 | qi | 66.17 | 67.05 |
| | <2 | qi | 68.28 | 65.25 |
| | <2 | qi | 56.28 | 56.11 |
| | <2 | qi | 62.91 | 65.74 |
| *mean* | | | *63* | *64* |
| *SD* | | | *5.2* | *5.0* |
| rye flour | 3.14 | qi | nd | nd |
| | 2.91 | qi | nd | nd |
| | 3.57 | qi | nd | nd |
| | 3.38 | qi | nd | nd |
| | 3.23 | qi | nd | nd |
| *mean* | *3.2* | | | |
| *SD* | *0.25* | | | |

[a] nd, not detected. [b] qi, quantification impossible.

when quantifying total coenzyme $Q_{10}$ and $Q_9$ contents employing the present detection systems, it must be confirmed that all the coenzymes are in the oxidized form. Weber et al. (1997) observed no reduced coenzyme Q after ethanol−*n*-hexane extraction; thus all coenzyme Q was converted into the oxidized form. In the present study, in addition to using a previous extraction procedure, also an electrochemical reactor was employed to convert all coenzyme Q into the oxidized form. Hence, determination of the contents of total coenzyme Q was possible at least with the EC detector. Similarities of the beef steak and pork heart results obtained by DAD and EC detection indicated that only oxidized coenzyme Q was present in these samples before HPLC analysis. In the case of Baltic herring it was possible that the sample also contained small amounts of reduced coenzyme $Q_{10}$ after extraction (see below and Table 1).

Because EC detector was used (in-line with DAD) the mobile phase had to contain ions. Hence, some compromises had to be made when the composition of the mobile phase was developed to prevent possible precipitations and ensure the conductivity necessary for electrochemical reactions. For example, the use of *n*-hexane (in which the lipophilic coenzymes Q are very soluble) was impossible. The mobile phase containing methanol, ethanol, 2-propanol, and ammonium acetate buffer was well-suited for both EC detector and DAD, and analysis time was fast, with retention times of 9−10 min for coenzyme $Q_9$ and 12−13 min for coenzyme $Q_{10}$, respectively.

The coenzymes $Q_9$ and $Q_{10}$ separated well from each other, but their separation from the sample matrix varied according to the food sample and detection system in question. Coenzyme $Q_{10}$ in Baltic herring, pork heart, and beef steak separated well from the matrix and could be quantified using both DAD and EC detection (Figures 1 and 2). On the other hand, coenzyme $Q_9$ could not be quantified in beef steak, pork heart, and rye flour by EC detection due to interfering compounds eluting at the same retention time as the studied compound. With DAD, no interfering compounds were found, but coenzyme $Q_9$ was not detected in beef steak and the content in pork heart was below the limit of determination. However, quantification of coenzyme $Q_9$ in rye flour was successful using DAD when the flow rate of the mobile phase was lowered from 0.8 to 0.7 mL/min (Figure 3).

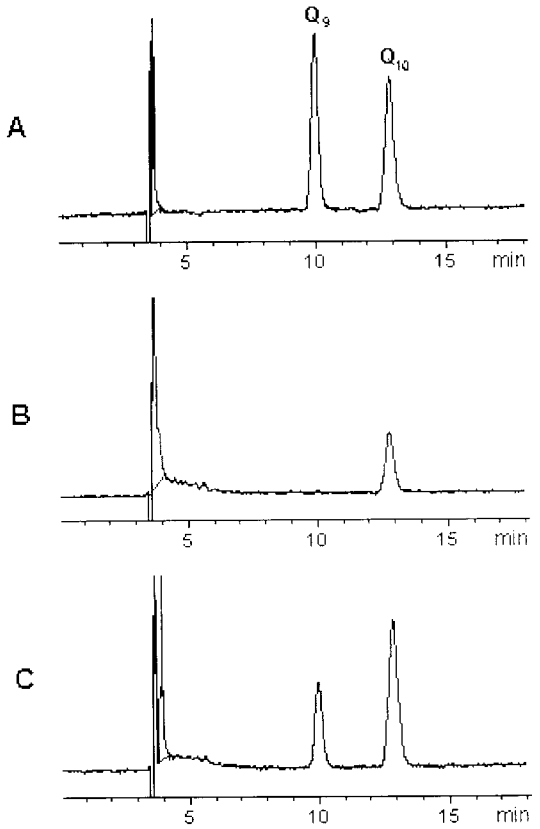


**Figure 2.** HPLC chromatograms of Baltic herring sample by DAD: A, standard mixture of coenzymes $Q_9$ and $Q_{10}$; B, Baltic herring sample; C, Baltic herring sample with added coenzymes $Q_9$ and $Q_{10}$.

DAD results for coenzyme $Q_{10}$ in Baltic herring, pork heart, and beef steak correlated well ($r = 0.996$) with those obtained by the EC detection method. Furthermore, no statistically significant difference was observed between the two detection methods in beef steak and pork heart ($p = 0.0922$ and 0.9210, respectively). In the case of Baltic herring, however, the EC detection gave slightly higher results than DAD ($p = 0.0007$). Repeatability of the methods was tested by analyzing all the samples in quadruplicate or quintuplicate. Acceptable repeatability was obtained for coenzymes $Q_9$ and $Q_{10}$ using both DAD and EC detection, with a coefficient of variation (CV%) < 10. Responses of the detection systems were linear in the range examined, 10−200 ng/injection, with correlation coefficients exceeding 0.999. Recovery tests for the coenzymes were made on each food item; the recoveries of added coenzymes $Q_9$ and $Q_{10}$ varied 73−105% when using DAD and 74−103% employing the EC detection system. These recoveries were similar to the previously reported values (Weber et al., 1997).

Detection limits for coenzymes $Q_9$ and $Q_{10}$ were 4 and 6 ng/injection, respectively, using DAD and 0.2 and 0.3

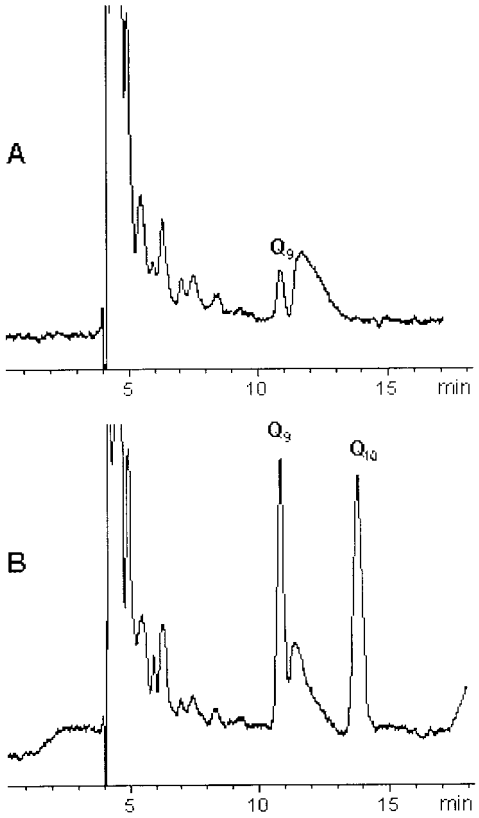


**Figure 3.** HPLC chromatograms of rye flour sample by DAD: A, rye flour sample; B, rye flour sample with added coenzymes $Q_9$ and $Q_{10}$.

ng/injection by EC detection. Hence, the EC detection system was 20-fold more sensitive for the analysis of coenzymes $Q_9$ and $Q_{10}$ than DAD. As mentioned above, the selectivity of the EC detector was, however, poor in some cases, especially when low levels of coenzyme $Q_9$ had to be quantified, and the advantage of the high sensitivity could not be utilized. It appears that, to be able to exploit the sensitivity of the EC detection, more efficient sample purification is needed.

Contents of coenzymes $Q_9$ and $Q_{10}$ in Baltic herring, beef steak, pork heart, and rye flour are shown in Table 1. The contents of the coenzymes in Baltic herring have not been reported earlier. However, according to Weber et al. (1997) and Kamei et al. (1986) different fish species contained coenzyme $Q_{10}$ 4.3−27 $\mu$g/g (processed samples) and 5.5−64.3 $\mu$g/g (raw samples), respectively. Kamei et al. (1986) detected coenzyme $Q_9$ in two fish species, while five species did not contain this compound. Weber et al. (1997) did not detect coenzyme $Q_9$ in fish. These earlier results are in agreement with the results obtained in this study (Table 1). Moreover the previous coenzyme Q results reported for cereals (Weber et al., 1997; Kamei et al., 1986) are similar to the result obtained for rye flour in this study. On the other hand, lower coenzyme Q contents were found in beef steak and pork heart (Table 1) than reported earlier (31 and 151−282 $\mu$g/g, respectively; Weber et al., 1997). However, the results given in Table 1 have not been corrected by observed recovery and the determinations were performed from raw samples, in contrast to the study of Weber et al. (1997) which employed recovery corrections and fried samples in the analysis. In addition, the sampling was not representative in this study because the aim was to compare two different detection systems, not to produce food composition data.

## CONCLUSIONS

Both DAD and EC detection systems were well-suited for the analysis of coenzyme $Q_{10}$ in Baltic herring, beef steak, and pork heart. Results obtained from the two detection systems were generally similar. Although EC detection was 20-fold more sensitive than DAD, that advantage was not significant in the analysis of scant contents of coenzyme $Q_9$ by EC detection due to poor selectivity. To exploit the sensitivity of the EC detection system, it appears that a more efficient sample purification is needed.

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