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Analysis of ubidecarenone (CoQ_{10}) aqueous samples using reversed phase liquid chromatography

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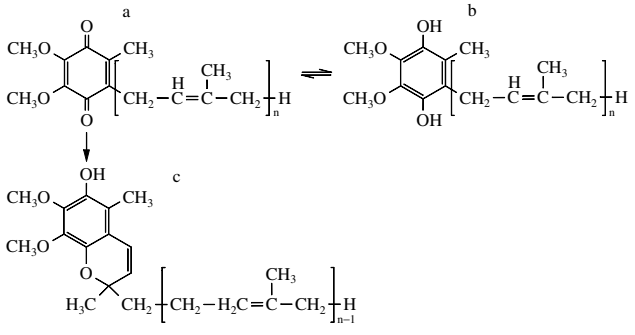
A novel method was developed for the quantitative determination of ubidecarenone (CoQ_{10}) in aqueous media using non aqueous reversed phase liquid chromatography. Standards were prepared by melting the compound in Cremophor EL followed by dilution with distilled water. Samples were then analyzed by a Reverse Phase HPLC method using a Waters Novapak C18, 3.9 × 150 mm column. The mobile phase used was methanol : n-hexane (9 : 1) at a flow rate of 1.5 ml/min. Response of the detector to the analyte was linear (r: 0.999) over the range of 2.5–100 μg ml^{-1}, with a limit of detection of 0.17 μg ml^{-1}. Acetone used to solubilize CoQ_{10} in the surfactant and Cremophor EL did not interfere with sample analysis. This method provided a convenient and alternative approach to the existing methods that require organic solvent extractions prior to analysis. Besides, this method enabled the separation of major photolytic decomposition products of CoQ_{10}.

1. Introduction

Ubidecarenone (2,3-dimethoxy-5-methyl-6-multiprenyl-1, 4-benzoquinone) or coenzyme Q10 (Scheme) is a lipid soluble compound available in the United States as a nutritional supplement. It is comprised of a redox-active quinoid nucleus and a hydrophobic side chain containing a number of monounsaturated *trans*-isoprenoid units [1]. The main function of CoQ_{10} in biology is to act as a redox component of transmembrane electron transport systems, such as the respiratory chain of mitochondria. This compound is present, however, in all cellular membranes. It has been proposed that CoQ_{10} participates in electron transport reaction in both the Golgi apparatus and plasma membranes. Increased attention has been focused on this redox lipid after the finding that the reduced form of CoQ_{10} has an antioxidant role, i.e., inhibiting lipid peroxidation [1].

Several chromatographic methods have been developed for the quantitative determination of CoQ_{10} in plasma [2, 3] and in nutritional formulations [4]. Such methods require extraction of samples in organic solvents prior to analysis. It is more practical, however, to evaluate their behavior in aqueous samples resembling physiological gastric and intestinal fluids. The method reported here provides an alternative approach for direct analysis of aqueous CoQ10 samples using non-aqueous reversed phase liquid chromatography.

Scheme (a) Chemical structure of CoQ10, oxidized form, n = 10 (b) Chemical structure of CoQ10, reduced form, n = 10 (c) Chemical structure of ubichromenol, n = 10)



2. Investigations and results

The isocratic reversed-phase LC conditions described allowed the separation of CoQ_{10} within a run time of less than 10 min. No interfering peaks were observed in the chromatograms. For the given standards, the response of the detector to the analyte was linear ($r^2 = 0.999$) over the range of 2.5–100 μg ml^{-1}, with a limit of detection of 0.17 μg ml^{-1} based on a signal to noise ratio of approximately 5. The calibration curve was $Y = 32442X - 2119.9$, where Y is the peak area ratios and X is the sample concentration. Relative standard deviation of the slope was 1.3%. The intra- and inter- run validation data for CoQ_{10} is reported in Tables 1 and 2, respectively. Both intra- and interrun errors were < 9%. The precision of the assay is demonstrated by a RSD of < 5%. (Tables 1 and 2).

CoQ_{10} melting or the use of a cosolvent, facilitates mixing the compound with Cremophor® EL. This is critical to ensure complete micellar solubilization of CoQ_{10} in water. Accuracy and precision for the assay where acetone was used as a cosolvent in the preparation of the stock solution is given in Table 3. Inter run accuracy (% error) and precision (RSD) were < 8% and < 4% respectively. To evaluate possible interference of Cremophor EL concentration with the analysis of CoQ_{10}, stock solution with 1% surfactant was prepared. Accuracy of this assay given as % error was ≤ 4% and the RSD was < 2% (Table 4). Cremophor EL (1%) chromatogram is given in Fig. 1. The chromatogram of a CoQ_{10} sample given in Fig. 2 shows the separation of the oxidized and reduced forms of the compound [4] with retention times of 4.4 and 6.1 min. As an application to stability studies, the major light degradation product, ubichromenol [4], for the same sample stored in light stability chamber for 48 h is shown in Fig. 3. As shown in the Fig., ubichromenol elutes with a retention time of 4.8 min.

3. Discussion

The HPLC method reported here provides an alternative approach for a direct analysis of aqueous CoQ_{10} samples without the need for extraction or purification. Solubilized ubidecarenone samples in water were separated with excellent accuracy and precision. Furthermore, this method enabled the separation of oxidized and reduced CoQ_{10} and

Table 1: Intra-run accuracy and precision of the assay

Added concentration (μg/ml)	Calculated concentration (μg/ml)	Error (%)	RSD (%)
2.5	2.29	−8.27	2.58
10	9.82	−1.85	3.99
50	49.6	−0.81	0.36
100	99.8	−0.2	1.79

* (n = 6)

Table 2: Inter-run accuracy and precision of the assay

Added concentration (μg/ml)	Calculated concentration (μg/ml)	Error (%)	RSD (%)
2.5	2.38	−5	4.04
10	9.32	−6.83	4.26
50	49.98	−0.03	1.43
100	99.95	−0.05	0.8

* (n = 6)

Table 3: Inter-run accuracy and precision of the assay using cosolvent

Added concentration (μg/ml)	Calculated concentration (μg/ml)	Error (%)	RSD (%)
2.5	2.7	8	3.7
10	10.1	1	1.98
50	46.77	−6.47	1.42
100	98.03	−1.97	2.09

* (n = 3)

Table 4: Inter-run accuracy and precision of the assay, Cremophor effect

Added concentration (μg/ml)	Calculated concentration (μg/ml)	Error (%)	RSD (%)
2.5	2.73	0.33	4.22
10	10.4	4	1.92
50	50.1	0.2	1
100	99.7	−0.3	1.31

* (n = 3)

its major photolytic degradation product, ubichromenol, which helps in evaluating sample integrity in any given experimental variable. The use of a cosolvent to facilitate CoQ_{10} mixing with surfactants, does not affect the analysis of the compound. This could be advantageous in the instances where high temperatures are unfavored and the use of organic solvent is permitted. Similarly, higher Cremophor EL concentration may enhance the solubilizing power of the surfactant without interference with the assay and the separation of CoQ_{10}. The chromatographic method reported here would be of particular advantage when evaluating dissolution profiles and release patterns of CoQ_{10} from dosage forms including superior emulsified systems, and is a subject for future investigation.

4. Experimental

4.1. Chemicals

Ubidecarenone (CoQ_{10}) was a generous gift from Kyowa Hakko USA (New York, NY). Cremophor® EL was obtained from BASF Corp. (Mount Olive, NJ). HPLC grade methanol, acetone, and n-hexane were purchased from VWR Scientific (Minneapolis, MN). All the chemicals were used as received.

4.2. Chromatography

Coenzyme Q_{10} and its degradation and/or impurity products were analyzed at ambient temperature utilizing a C18, 3.9 mm × 150 mm reverse phase chromatography column (Nova-Pak; Waters, Milford, MA). The mobile phase consisted of methanol : n-hexane (9 : 1) and was pumped at a flow rate of 1.5 ml min^{-1}. The HPLC instrument consisted of a 510 pump (Waters), 712 WISP autosampler (Waters), and a 490E UV detector (Waters) set at a wavelength of 275 nm. The chromatographic data was managed using Star 5.3 software (Varian, Walnut Creek, CA).

4.3. Standard solutions

Stock solution of CoQ_{10} (0.1 mg ml^{-1}, 0.1% Cremophor EL) was prepared by melting 100 mg of the compound in 1 g of Cremophor EL at 55 °C and the volume was made to 1 l with distilled water. The standards (0, 2.5, 5, 10, 20, 30, 40, 50, 80, and 100 μg ml^{-1}) were prepared by diluting the stock solution with distilled water. In 4 ml amber colored HPLC vial, 1 ml of the standard was diluted with 1 ml of methanol and analyzed. Calibration curve was constructed by plotting the peak areas against the concentration.

4.4. Assay validation

The intra- and inter-run precision and accuracy of the assay were determined by RSD and % error, respectively (n = 6), based on reported guidelines [5]. Briefly, quality control samples were run along with calibration standards at the lowest concentration (2.5 μg ml^{-1}), middle of the curve

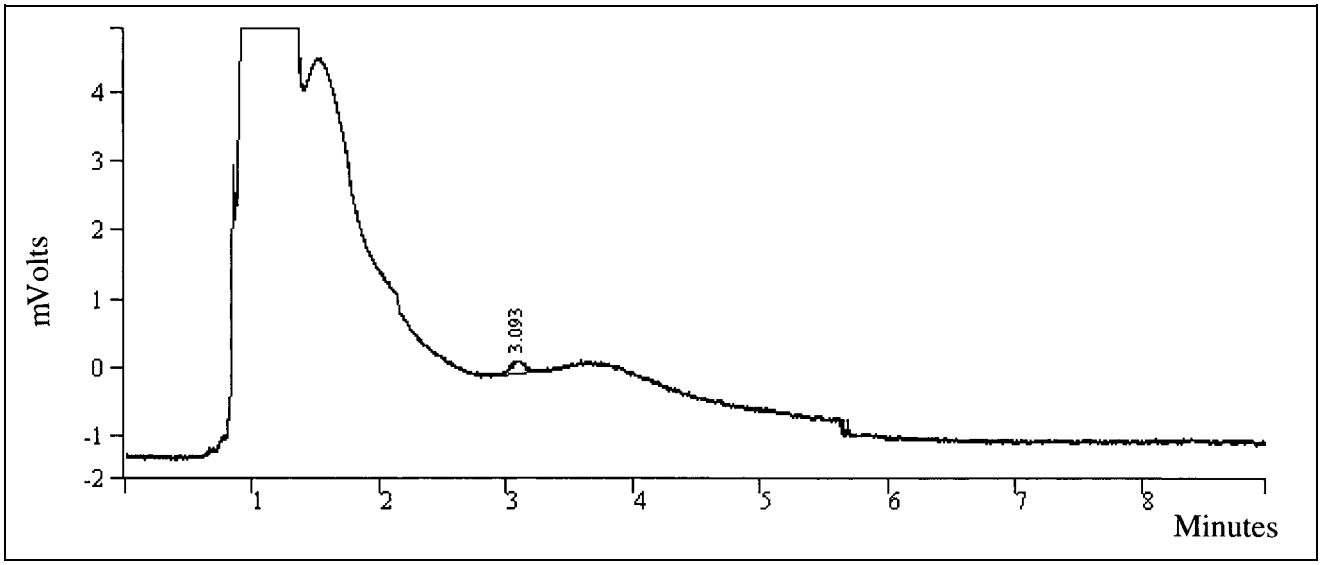


Fig. 1: Cremophor EL (1%) chromatogram

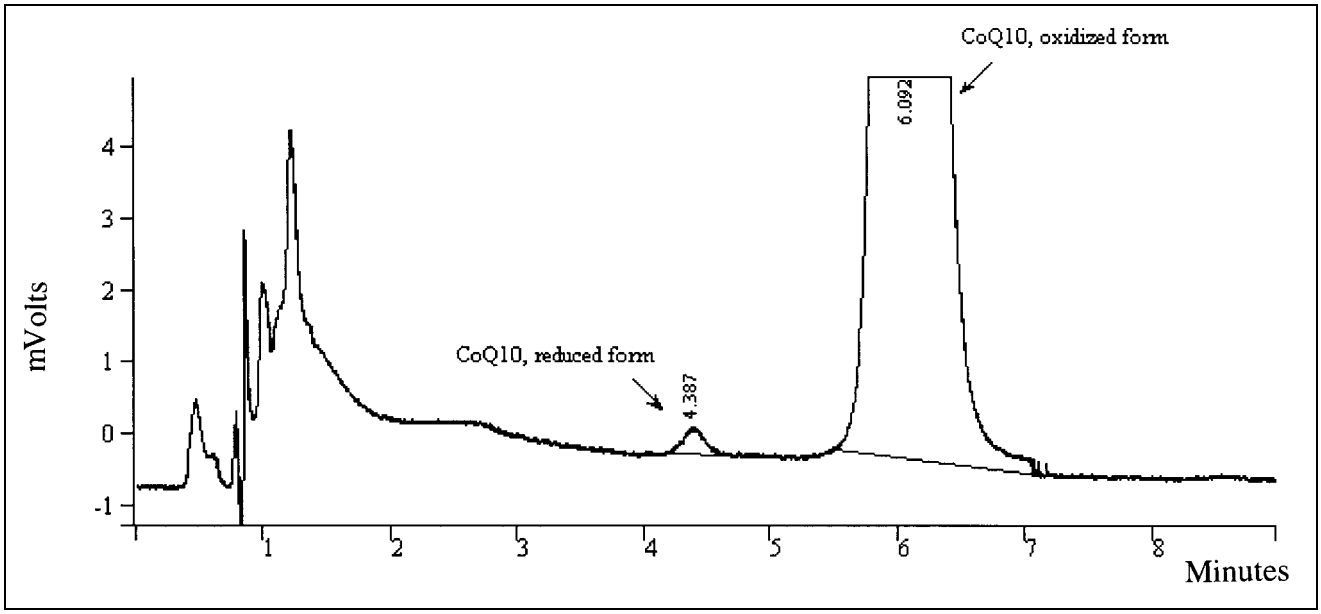


Fig. 2: CoQ10 chromatogram

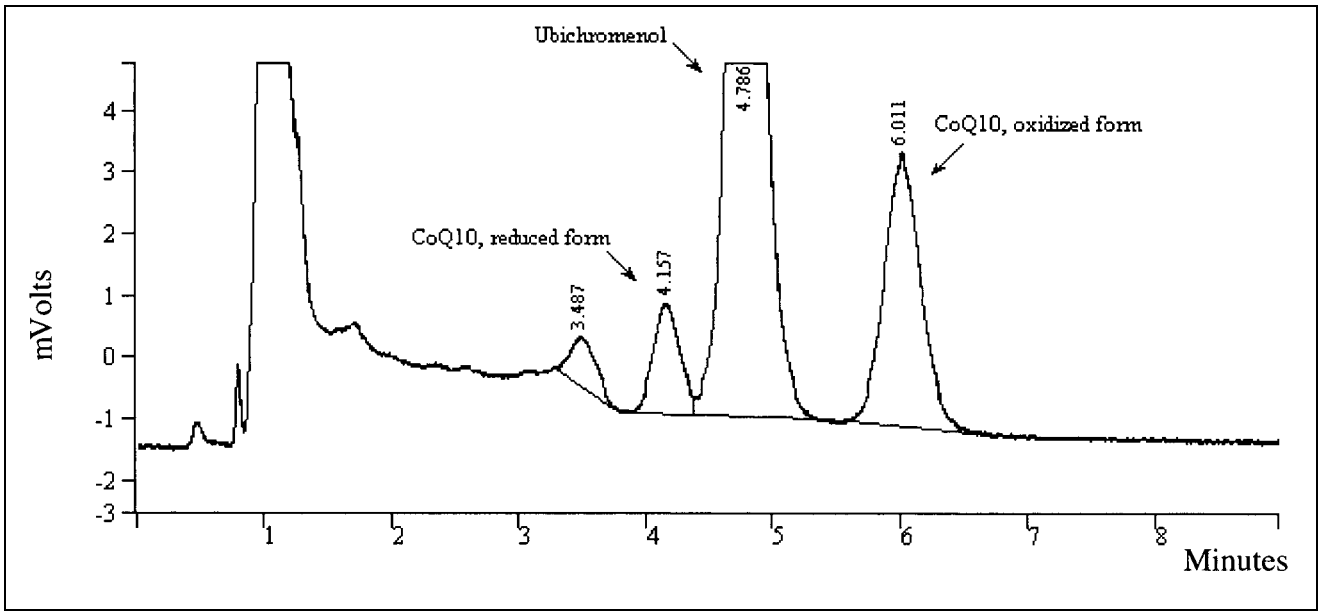


Fig. 3: CoQ10 degradation products

(10 and 50 μg ml^{-1}), and highest concentration (100 μg ml^{-1}) in the calibration curve. The concentrations of the quality control samples were then determined against the calibration curve and used for calculating RSD and % error.

4.5. Effects of cosolvent and surfactant concentration on assay validation

To eliminate the effect of temperature on the integrity of CoQ_{10}, stock solution (0.1 mg ml^{-1}, 0.1% Cremophor EL) was prepared by dissolving 100 mg of CoQ_{10} in 3 ml of acetone. One gram of Cremophor EL was added and mixed with the solution and subsequently diluted to 1 l with distilled water. Similarly, to evaluate the effect of Cremophor EL concentration on CoQ_{10} analysis, a stock solution (0.1 mg ml^{-1}, 1% Cremophor EL) was prepared by mixing solubilized CoQ10 in acetone with 10 g of Cremophor EL. Assay validation was performed as described above.

4.6. Application to stability studies

Coenzyme Q_{10} was melted in 1 g of Cremophor EL and dissolved in 100 ml distilled water at a concentration equivalent to 35 μg ml^{-1}. The solution was then kept in an Erlenmeyer flask and stored at 25 °C in a 600 foot candle light stability chamber. Samples were taken after 48 h and analyzed with the HPLC.

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