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Short communication

A simplified chromatographic method for quantitative determination of coenzyme Q_{10} in dog plasma[1]

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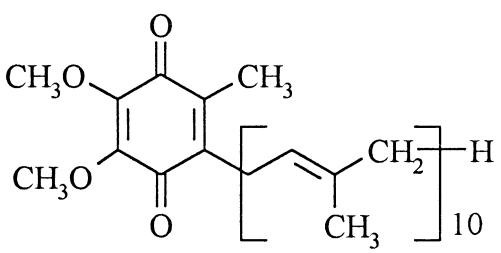
1. Introduction

Coenzyme Q_{10} (CoQ_{10}), also known as ubiquinone or 2,3 - dimethoxy - 5 - methyl - 6 - decaprenyl-1,4-benzoquinone, is a lipid soluble compound that occurs in the mitochondria of human cells [1]. CoQ_{10} functions as a cofactor in the electron transport of oxidative phosphorylation and is essential for production of cellular energy in the form of ATP. Since mitochondria are very abundant in myocardial cells and because of their huge energy needs, a deficiency of CoQ_{10} could have a particularly severe effect on myocardial function [2]. CoQ_{10} is beneficial for preventing cellular damage during myocardial ischemia and reperfusion. It has been used orally to treat various cardiovascular disorders which include angina pectoris, hypertension, and congestive heart failure [3].



Coenzyme Q_{10}

Various analytical methods have been reported for quantitative determination of CoQ_{10} in human plasma [4,5]. But they require extraction and purification by TLC where recovery and accuracy are poor. Grossi et al. reported a systematic study for quantitative determination of CoQ_{10} in human

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plasma involving sample preparation by solid phase extraction [6]. This method could not be adopted for the analysis of CoQ_{10} in dog plasma due to poor recovery. For the purpose of bioavailability studies of various formulations of CoQ_{10} in Beagle dogs, a RP-HPLC method was developed by using a simple, solid phase extraction method for sample preparation.

2. Experimental

2.1. Materials

Coenzyme Q_{10} and trichloro acetic acid were purchased from Spectrum Chemicals (Gardena, California). The internal standard, Coenzyme Q_9 was a generous gift from Eisai Co (Tokyo, Japan). Dog plasma was kindly provided by Hemopet (Irvine, California). HPLC grade methanol and *n*-hexane were purchased from Fisher Scientific (Fair Lawn, New Jersy). Sep-Pak silica (100 mg) solid phase extraction cartridges were purchased from Waters (Milford, Massachusetts). Since the solutions of CoQ_{10} are susceptable to photodegradation, studies were carried out under the illumination of yellow light. All the containers used were wrapped with aluminium foil.

2.2. Sample preparation

Frozen plasma samples were thawed at room temperature in the dark just prior to analysis. The extractions were performed in 115×10 mm screw top glass test tubes. To 1 ml of plasma, 100 μl of standard solution of CoQ_{10} (in hexane) and 50 μl of internal standard (CoQ_9, 7.5 μg ml^{-1} in hexane) were added and vortex mixed. Then the plasma was deproteinized with 1 ml of 10% trichloro acetic acid. To this sample, 2 ml of hexane was added, vortex mixed for 5 min, and centrifuged at $2000 \times g$ for 5 min. The hexane phase was transferred to 100 mg silica-solid phase extraction cartridge, previously activated with 5 ml of hexane and mounted on vacuum manifold system. The extraction was repeated twice in similar fashion and the hexane layer was collected on silica cartridge. The total volume of hexane (6.0 ml) was passed through the cartridge. The cartridge was dried under vacuum for 2 min and eluted with 0.75 ml of methanol:hexane (85:15 v/v) mixture. A 100 μl volume of the eluted fraction was injected to the RP-HPLC column for separation and UV detection.

2.3. Chromatography

The LC system consisted of Isco-2350 pump, Isco-V^4 variable absorbance detector. The eluate was monitored for absorbance at 275 nm and the detector out put was recorded using Isco-Chemresearch (Ver 2.4) Software. Then 100 μl of the sample was injected onto a reversed phase column (Nova-Pak C18, 4 μ, 150×3.9 mm i.d., Waters, Milford, Massachusettes) preceded by a guard column (Alpha Bond C18, 7.5×4.6 mm i.d., Alltech, San Jose, CA). The isocratic mobile phase was methanol: *n*-hexane (98:2 v/v). The flow rate was 1.0 ml min^{-1}.

3. Results

3.1. Analytical recovery

The absolute recoveries of CoQ_{10} were estimated by comparison of the peak-area ratios after extraction from plasma with the peak-area ratios obtained after direct injection of a solution containing CoQ_{10} in methanol: *n*-hexane (85:15 v/v). The analysis was repeated four times at each level. The mean recoveries of the CoQ_{10} from plasma samples are shown in Table 1. Interday assay precision was determined by the analysis of the same solutions on different days. The R.S.D. values of interday analysis are shown in Table 2 and were ranged from 1.7 to 4.5%.

3.2. Specificity and reproducibility

The isocratic reversed-phase LC conditions described allowed the separation of CoQ_{10} and the internal standard within a run time of less than 25 min. Typical chromatograms obtained from the analysis of blank dog plasma and the plasma

Table 1
Recovery of the Coenzyme Q_{10} by the analytical method

Intended Conc. (μg ml^{-1})	Measured Conc. Mean ± S.D.	Percentage recoveries Mean ± S.D.	R.S.D. (%)
0.5	0.48 ± 0.01	95.6 ± 2.6	2.7
1.0	0.93 ± 0.01	93.3 ± 1.1	1.2
1.5	1.4 ± 0.02	93.7 ± 1.2	1.3
2.0	1.9 ± 0.04	92.8 ± 2.1	2.3
2.5	2.4 ± 0.04	94.3 ± 1.5	1.6
3.0	2.8 ± 0.04	93.9 ± 1.2	1.3
3.5	3.3 ± 0.04	92.8 ± 1.4	1.5
4.0	3.7 ± 0.08	93.3 ± 2.0	2.1

$n = 4$.

supplemented with CoQ_{10} and internal standard are shown in Fig. 1. Coenzyme Q_{10} and internal standard were resolved with retention times of 16.6 and 26.7 min, respectively. No interfering peaks were observed in the chromatograms. The reproducibility of retention times of CoQ_{10} and internal standard were determined for six consecutive injections of CoQ_{10} (7.5 μg ml^{-1}) and internal standard (5.0 μg ml^{-1}) in methanol: *n*-hexane (85:15 v/v). The R.S.D. values were found to be 0.47 and 0.73% for CoQ_{10} and internal standard, respectively.

3.3. Limit of detection

The minimum concentration of CoQ_{10} that could be detected in plasma was 0.05 μg ml^{-1}. This was determined based on signal to noise ratio of approximately 5.

3.4. Linearity

Calibration curve was constructed in the concentration range of 0.5–5.0 μg ml^{-1}. The peak-area ratios of CoQ_{10} and internal standard were plotted as a function of the concentration of CoQ_{10}. The calibration curve was $Y = 0.04096 + 0.35506X$, where Y = Peak area ratios and X = sample concentration ($r = 0.998$). The endogenous CoQ_{10} in

Table 2
Interday precision

Intended Conc. (μg ml^{-1})	Measured Conc. Mean ± S.D.	R.S.D. (%)
0.5	0.44 ± 0.02	4.5
1.0	0.92 ± 0.03	3.3
2.0	1.8 ± 0.05	2.8
3.0	2.7 ± 0.06	2.2
4.0	3.6 ± 0.06	1.7

$n = 3$.

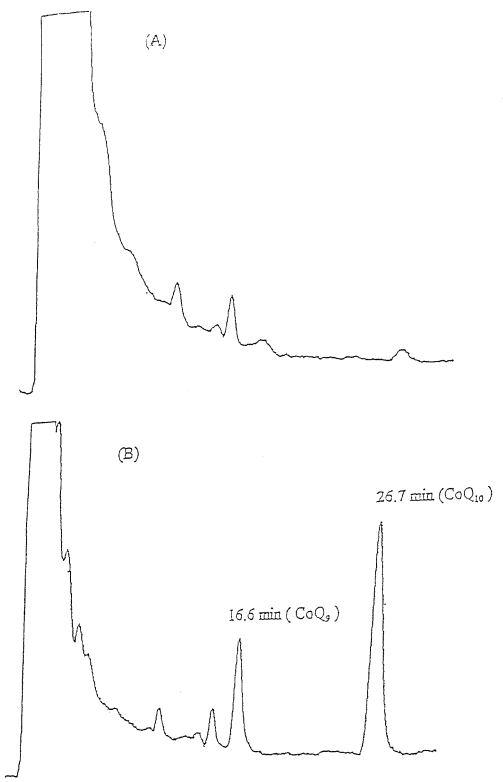

Fig. 1. Chromatograms of (A) blank plasma; (B) plasma spiked with CoQ_{10} (3.0 μg ml^{-1}) and internal standard CoQ_9 (0.75 μg ml^{-1}).

the dog plasma used in this present study is almost negligible.

4. Discussion

CoQ_{10} is very lipophilic compound, freely soluble in *n*-hexane and almost insoluble in methanol. It has a low melting point (49°C) and the solutions are extremely sensitive to light. The reduced form of CoQ_{10}, ubiquinol, is also present in plasma. Ubiquinol is unstable at room temperature and quickly oxidises to ubiquinone during sample treatment allowing the determination of total CoQ_{10} [6]. Coenzyme Q_{10} is extensively bound to plasma proteins, and therefore it was necessary to deproteinize the plasma samples. Initially an attempt was made to deproteinize the plasma with methanol as reported by Grossi et al. [6]. However, the loading step on silica cartridge was adversely affected by methanol due to partial miscibility of methanol in *n*-hexane. Therefore, trichloro acetic acid (10% w/v) was used for deproteinizing the plasma. The mixure of methanol: *n*-hexane (85:15 v/v) was used to elute CoQ_{10} from silica cartridge since methanol (100%) was unable to elute the CoQ_{10} completely from silica cartridge. These two observations are in contrast to the earlier reported study where the method was developed for the analysis of CoQ_{10} in human plasma. The observed differences could be due to difference in compositions of human and dog plasma.

5. Conclusions

A simple, rapid and specific HPLC method for the determination of CoQ_{10} in dog plasma is described. This method is currently being used in the bioavailability studies of CoQ_{10} formulations in Beagle dogs.

Acknowledgements

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