

Development of a new high performance liquid chromatography method for measurement of coenzyme Q10 in healthy blood plasma

Omar F. Abdul-Rasheed, MD, PhD (Clinical Chemistry), Yahya Y. Farid, MD, PhD (Clinical Chemistry).

ABSTRACT

الأهداف: تطوير طريقة جديدة لقياس مستوى التميم الإنزيمي (CoQ10) (Q10) في بلازما دم الإنسان باستخدام كروماتوغرافيا السائل عالي الأداء (HPLC).

الطريقة: تم إجراء هذه الدراسة في قسم الكيمياء والكيمياء الحياتية - كلية الطب - جامعة النهرين - بغداد - العراق خلال الفترة من سبتمبر 2004 حتى يوليو 2005. اشترك في هذه الدراسة 60 متبرع صحي تتراوح أعمارهم ما بين 28.51±7.30 عام. كان جميع المشتركين غير مدخنين ولم يتلقوا أي دواء لمدة 3 أيام وصاموا لمدة 12 ساعة قبل التحليل. تم قياس مستوى التميم الإنزيمي (CoQ10) بطريقه مطوره لكروماتوغرافيا السائل عالي الأداء من النوع أحادي الطور المتحرك بعد خطوة استخلاص واحده بالبروبانول البارد.

النتائج: أعطت الطريقة المطورة (HPLC) واكتشاف الأشعة فوق البنفسجية مدى خطيه للقياس تتراوح بين 0.1-4.0 ملل غرام/ لتر. تتراوح نسبة التباين لمستوى التميم الإنزيمي للطريقة خلال اليوم ما بين 0.2-3.90%. كانت نسبة الاسترجاع التحليلية بين 95.5-101.30%، أما نسبة الاستعادة للتميم الإنزيمي (CoQ10) للطريقة المتبعة فكانت 0.959%.

خاتمة: تم تطوير طريقه جديدة لكروماتوغرافيا السائل (HPLC) عالي الأداء بسرعة ودقه وحساسية عاليه لقياس مستوى التميم الإنزيمي CoQ10 في تحليل نماذج بلازما الدم.

Objectives: To improve a new high performance liquid chromatography (HPLC) method for coenzyme Q10 (CoQ10) measurement in human plasma.

Methods: We conducted this study in the Chemistry and Biochemistry Department, College of Medicine, Al-Nahrain University, Baghdad, Iraq from September 2004 to July 2005. Sixty healthy volunteers aged 28.51±7.30 years were participated in this study. All participants were non-smokers and under no any treatment for the last 3 days and fast for 12 hours before analysis. Coenzyme Q10 levels were measured

by improved isocratic reversed phase HPLC technique after one-step ice-cold 1-propanol extraction step.

Results: The ultra violet (UV) detection, developed HPLC method gives a good linearity range between 0.1-4.0 mg/L. The coefficients of variations (CVs%) of within-day precision for CoQ10 in human plasma were 0.2-3.90%. Analytical recoveries were 95.5-101.30%, whereas the average CV% of CoQ10 reproducibility was 0.959%.

Conclusion: A new improved rapid, precise, and sensitive HPLC method for the quantitation of CoQ10 is suitable for analysis of blood plasma samples.

Saudi Med J 2009; Vol. 30 (9): 1138-1143

From the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Al-Kadhmiya, Baghdad, Iraq.

Received 25th April 2009. Accepted 15th July 2009.

Address correspondence and reprint request to: Dr. Omar F. Abdul-Rasheed, Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Al-Kadhmiya, PO Box 70027, Baghdad, Iraq. Tel. +964 (790) 1885283. E-mail: dr.omar_rasheed@yahoo.com/omar_rasheed39@yahoo.com

Coenzyme Q10 (2,3-dimethoxy-5-methyl-6-decaprenyl benzoquinone), also known as ubiquinone, is a lipid soluble component of all eukaryotic cells.^{1,2} It is an endogenously synthesized provitamin serves as a lipid soluble electron carrier in the mitochondrial electron transport system,^{1,6,7} as well as an important intracellular antioxidant. Coenzyme Q10 protects phospholipid and mitochondrial membrane proteins from peroxidation, and protects DNA against the oxidative damage that accompanies lipid peroxidation.⁶ Coenzyme Q10 exists in several forms, ubiquinone (oxidized form; CoQ10), ubiquinol (reduced form; CoQ10 H₂), which is formed from

the reduction of ubiquinone with 2 equivalents, and semiquinone radical (free radical form, Co Q10 H \cdot) which is formed by the reduction of oxidized form with one equivalent.³ It is prevalent in humans, with high endogenous concentration found in the heart, liver, kidney, retina, and pancreas.³⁻⁵ Presence in the inner mitochondrial membrane, CoQ10 serves as an electron transport carrier during the process of respiration and oxidative phosphorylation, and it involves the manufacturing of ATP. Coenzyme Q10 directly involved in the mitochondrial electron transport chain by the regulation of NADH and succinate dehydrogenase activities enabling reversible reactions between these enzymes.^{3,7,8} Coenzyme Q10 also may prevent the depletion of metabolites necessary for the synthesis of ATP.³⁻⁵ It must be reduced to wield its antioxidative function, and supplementation may inhibit lipid oxidizability.^{3,5} During supplementation with CoQ10, the level of lipid peroxidation decreased in healthy subjects, yet levels of another antioxidant, and vitamin E, remained constant. It appears CoQ10 exerts a sparing effect on vitamin E and perhaps more efficiently prevents lipids peroxidation by inhibiting both its initiation and propagation steps, whereas vitamin E inhibits only propagation step. The membrane, stabilizing properties of CoQ10 emerge from the reduction of free radicals that may cause damage to structural proteins and lipids found in cell membranes.^{3,5} Other mechanisms of action may include stabilization of calcium dependent slow channels, inhibition of intracellular phospholipases, and alteration of prostaglandin metabolism.⁹ Also CoQ10 acts as a potent antioxidant by its action in decreasing the formation of free radicals. It improves the transport of electrons and protons and thus prevents the formation of superoxide radicals. In the other word, it is a strong acting endogenous antioxidant by its action as a substrate for free radicals and accepts the free electrons from free radicals; gets converted to reduced CoQ10 (ubiquinol), thus rendering free radicals into harmless compounds.^{3,6} Recent reports suggest that the percentage of reduced CoQ10 in total plasma concentration of CoQ10 may be lower in patients with many diseases and this ratio may be a useful biomarker of oxidative stress.⁴ The accurate and effective measurement of CoQ10 is important for clinical diagnosis. To date, reduced CoQ10 stability has created problems with its measurements, since it is easily oxidized when exposed to air and thus, susceptible to pre-analytical degradation and analytical error. Investigation of CoQ10 H $_2$ in clinical studies has been hampered by the instability during sample handling, storage, and processing.^{10,11} According to several investigators, the concentration of CoQ10 H $_2$ decreases rapidly within one hour after phlebotomy.^{12,13} At room temperature, it is oxidized at a

rate of 3 nmole/minute. in the hexane extract of human plasma.¹² This is very impractical for analyzing even small numbers of biological specimens. Therefore, in this study a simple, and rapid high performance liquid chromatography (HPLC) procedure was developed for simultaneous determination of total CoQ10 in human plasma in which CoQ10 was first extracted with 1-propanol and the supernatant then was injected immediately to improved the isocratic reversed phase HPLC system with UV detection.

Methods. This study was conducted in Chemistry and Biochemistry Department, College of Medicine, Al-Nahrain University, Baghdad, Iraq between September 2004 to July 2005. After obtaining the approval of the Research and Ethics Committee of Al-Nahrain Medical College and written consent from all participants, 60 male healthy volunteers aged 28.51 \pm 7.30 years participated in this study. Exclusion criteria included smoking, diabetes, hypertension, hyperlipidemia, nephrotic syndrome, acute or chronic renal failure, stroke, and ischemic or coronary heart disease, and those taking medications especially statin drugs. All individuals signed a consent form to allow the use of their blood samples in this study.

Measurement of CoQ10 maximum wavelength (λ max). A standard CoQ10 with 43.7 μ mole/L concentration dissolved in absolute ethanol was prepared and placed in a sample beam quartz cuvette against ethanol in the reference beam and the absorption spectrum was recorded in 210-340 nm wavelengths range.

Preparation of plasma samples. All sample handling was performed in shaded light to avoid photochemical decomposition of CoQ10. Overnight fasting blood samples were taken from the antecubital vein, 5 mL of blood was transferred to a heparinized tube and mixed by gentle inversion 5-6 times. The tube was not opened to ambient air and was placed in ice before processing. Blood samples were processed within one hour after collection and centrifuged at 2,000 x g for 10 minutes at 4°C. The blood plasma was processed immediately.

Preparation of calibrators. All calibrators were prepared under a dim light to avoid photochemical decomposition of CoQ10.¹⁴ A stock solution was prepared by dissolving 10 mg of pure CoQ10 (Sigma-Aldrich Company) in 10 mL of hexane and diluted this solution to 100 mL with 1-propanol. The solution was thoroughly vortex mixed until complete dissolution. A working calibrator was then prepared by dilution with 1-propanol to 5 mg/L. The exact concentration of the working solution was then calculated by reading the absorbance in a spectrophotometer (at 275 nm wavelength, 1 cm quartz cuvette) using a molar

absorptivity (ϵ) of 14,200. A series of calibrators was then prepared with the appropriate volume of 1-propanol to final CoQ10 concentrations of 0.1 mg/L, 0.5 mg/L, 2 mg/L, 3 mg/L, and 4 mg/L. The calibrators were then stored in 1.8 mL polypropylene tubes at -20°C until use. A standard curve was plotted between CoQ10 standard concentrations against area under peak (AUP) of each standard concentration.

Choice of the most appropriate mobile phase polarity.

Two sets of experiments were performed; the first was carried out with the use of perchloric acid/sodium perchlorate mixture while the second set was performed without the addition of perchloric acid/sodium perchlorate mixture. In each experiment, the elution polarity was changed to obtain the most appropriate mobile phase for CoQ10 measurement.

The polarity or mobile phase strength was expressed as polarity index ($P'_{A,B}$). In mixtures with 2 components A and B, the polarity index was calculated according to the following equation:

$$P'_{A,B} = \Phi A P'_{A} + \Phi B P'_{B}$$

Where: A = 1-propanol solvent, B = methanol solvent, ΦA = fraction of 1-propanol in 1-propanol/methanol mixture, P'_{A} = Polarity index of 1-propanol component, ΦB = Fraction of methanol in 1-propanol/methanol mixture, and P'_{B} = Polarity index of methanol component.

Sample analysis. Coenzyme Q10 concentrations were determined by modifying the HPLC method of Edlund¹⁵ and Wahlqvist et al.¹⁶ Coenzyme Q10 was extracted from plasma by extraction with 1-propanol. A 300 μL of plasma sample was placed on a 1.8 mL capped polypropylene tube. All tubes were kept in an ice bath during all processing steps. The sample was then mixed with 1 mL of cold 1-propanol. All tubes were vortex mixed for 5 minutes and centrifuged for 10 minutes at $21,000 \times g$ and 4°C . The resultant supernatant (1-propanol extract) was separated from the precipitate. Three sets of tubes were prepared for each sample to determine CoQ10 level. The first set contains 1-propanol extract only, the second set was prepared by mixing equal volumes of 1-propanol extract and CoQ10 standard (2mg/L), and the third set consists of CoQ10 standard (2 mg/L) only. A 20 μL of each set was injected immediately and separately into an automated HPLC system. All tubes were kept in an ice bath during all processing steps.

The mobile phase used was 1-propanol: methanol (60:40, by volume) containing 89.5mM perchloric acid and 57mM sodium hydroxide with a flow rate of 1.0mL/ minute. Detection was performed by ultra violet (UV) absorption at 275nm. Quantification was done

by comparing the area under peaks (AUP) of samples with those obtained for standards. The measurement of CoQ10 depends on the standard addition method.¹⁷ The HPLC-UV-VIS system used was Shimadzu (Kyoto, Japan) model LC-10ATVP solvent delivery module equipped with a double plunger-reciprocating pump, an SPD-10AVP model UV-VIS detector and 2 cells (pre- and post- column) and an analytical cell.

The reversed-phase chromatography with isocratic elution was carried out on an analytical column packed with Econosil C18 5U, with 250mm length and 4.6 mm internal diameter (ID) in conjunction with a guard column to protect the analytical column. Two pneumatic 6-port valves (Model 7125 Rheodyne, Cotati, CA, USA) injector was set at a needle height of 1.5 mm, and the injection volume was set at 20 μL .

Peak height and area measurements for each injection were obtained by the Shimadzu Chromatopac C-R1B model (Kyoto, Japan).

Coenzyme Q10 recovery. Two pools of healthy plasma were prepared as baseline specimens. Quadruplicate assays are performed. The amount recovered is determined by subtracting the amount originally present in the respective pools from the amount measured or "found".

Reproducibility of CoQ10. To verify the reproducibility of the CoQ10 analysis, human plasma samples from 10 healthy individuals were examined (5 replicates each).^{20,21}

Statistical analysis. The data were analyzed by computer software program Statistical Package for Social Sciences (SPSS for windows, version 17, Chicago). Data were expressed as mean \pm SD.

Results. Figures 1 shows the UV spectrum of CoQ10 in the 210-340 nm region. From this figure the λ_{max} of CoQ10 was found to be 275 nm. To optimize the separation conditions of CoQ10, 2 sets of experiment were performed, the first one is by

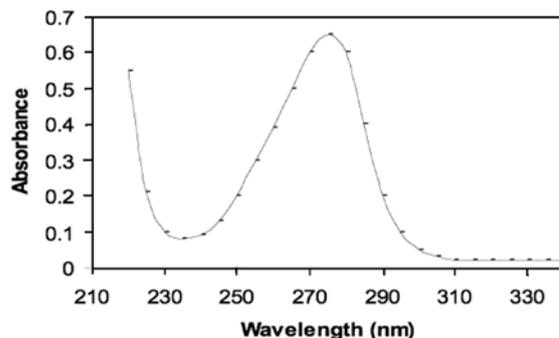


Figure 1 - UV spectrum of 43.7 μM coenzyme Q10 (CoQ10) standard dissolved in absolute ethanol.

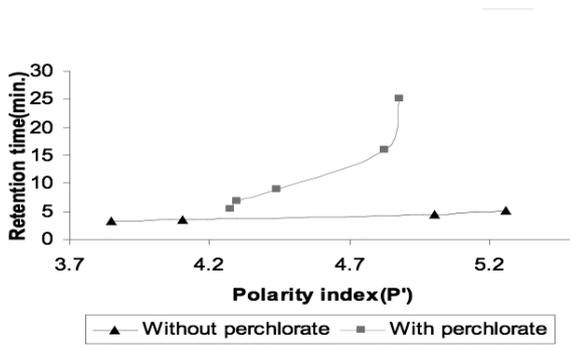


Figure 2 - Retention time of coenzyme Q10 (CoQ10) separation by high performance liquid chromatography (HPLC) method versus mobile phase polarity index in the presence and absence of perchlorate.

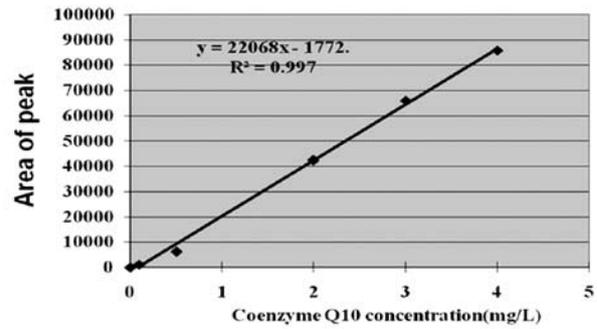


Figure 4 - Coenzyme Q10 (CoQ10) calibration curve (linear regression line, $y=22068x - 1772$).

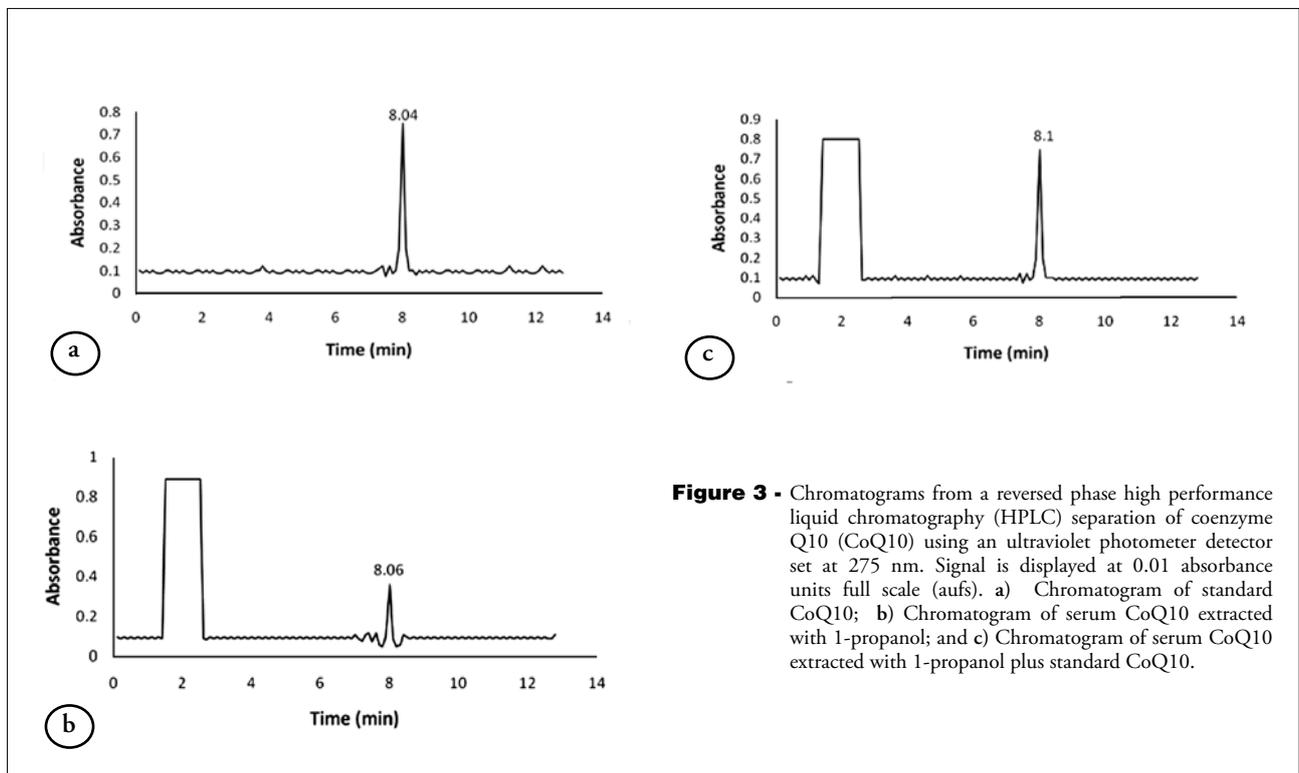


Figure 3 - Chromatograms from a reversed phase high performance liquid chromatography (HPLC) separation of coenzyme Q10 (CoQ10) using an ultraviolet photometer detector set at 275 nm. Signal is displayed at 0.01 absorbance units full scale (aufs). a) Chromatogram of standard CoQ10; b) Chromatogram of serum CoQ10 extracted with 1-propanol; and c) Chromatogram of serum CoQ10 extracted with 1-propanol plus standard CoQ10.

increasing the mobile phase polarity index without the addition of perchlorate, while the second set was carried out by increasing the polarity index with the addition of perchlorate, Figure 2. Each chromatographic system was evaluated by measuring chromatographic criteria in addition to retention time value. From these experiments, it was found that the most suitable mobile phase used for the measurement of CoQ10 in human plasma was 60% 1-propanol, 40% methanol, 57mM sodium hydroxide and 89.5mM perchloric acid. To evaluate the separation method, some important chromatographic criteria were measured accordingly^{18,19} as listed in Table 1. Figure 3a shows the chromatogram

of standard CoQ10 (2 mg/L) only, Figure 3b illustrates the chromatogram of extracted plasma CoQ10 while Figure 3c shows the chromatogram of extracted plasma CoQ10 plus standard CoQ10 (2 mg/L). Triple analyses were performed in optimized conditions with different CoQ10 concentrations and averaged to establish the calibration curve shown in Figure 4. An excellent linear relationship was observed between the peak areas versus CoQ10 concentration. Coenzyme Q10 was found to be linear in the concentration range from 0.1 mg/L to 4.0 mg/L according to the developed HPLC method, and the lower detection limit was found to be 5 µg/L. The analytical recoveries of CoQ10 in human plasma

Table 1 - Chromatographic parameters of coenzyme Q10 (CoQ10) separation on Econosil C18 column with 60% 1-propanol, 40% methanol, 57 mM sodium hydroxide and 89.5 mM perchloric acid mobile phase.

Component	A _s	t _R (minute)	R _s	Selectivity (α)	N	HETP
CoQ10	1.001	9.0	5.16	1.525	2025	0.0123

t_R - retention time (minutes), A_s - peak asymmetry, R_s - resolution
N - number of theoretical plates, HETP - height equivalent to a theoretical plate.

Table 2 - Coenzyme Q10 (CoQ10) recovery results

Plasma pool	Intended CoQ10 concentration (mg/L) mean±S.D	Measured CoQ10 concentration (mg/L) mean±S.D	Recovered CoQ10 concentration (mg/L)	C.V (%)	Mean recovery (%)
A	0.0	0.076 ± 0.0030	-	3.90	101.30
B1	0.0	0.430 ± 0.0011	-	0.20	95.50
B2	1.20	1.600 ± 0.0200	1.17	1.25	97.50
B3	3.00	3.520 ± 0.0660	2.99	1.80	99.60
Average: 98.5%					

Table 3 - Reproducibility of the coenzyme Q10 (CoQ10) analysis in human plasma.

Sample	CoQ10 concentration (mg/L) (mean ± S.D)	C.V (%)
1	0.812 ± 0.003	0.37
2	0.843 ± 0.008	0.95
3	0.876 ± 0.005	0.57
4	0.765 ± 0.007	0.91
5	0.640 ± 0.009	1.40
6	0.896 ± 0.004	0.44
7	0.696 ± 0.005	0.72
8	0.88 ± 0.009	1.02
9	0.750 ± 0.008	1.06
10	0.834 ± 0.018	2.15
Average: 0.959%		

controls are shown in Table 2. Analytical recoveries range was 95.5-101.3%. These individual recoveries are averaged to give an estimate of 98.5% recovery, which corresponds to a 1.5% proportional error or actual errors of 0.75 mg/L at 50 mg/L and 0.015 mg/L at 1 mg/L. These errors are less than the allowable error and thus do not invalidate the analytical procedure.^{20,21} The reproducibility of the CoQ10 analysis is presented in Table 3. The CVs for the total CoQ10 were ≤1%, which shows the excellent reproducibility of analysis. The CoQ10 concentration (mean ± SD) was measured in blood plasma of healthy volunteers and was found 0.822±0.050 mg/L.

Discussion. Coenzyme Q10 is a very lipophilic crystalline compound with good solubility in

hydrocarbons. It is slightly soluble in ethanol, almost insoluble in methanol and completely insoluble in water.¹⁵ From the CoQ10 UV spectrum curve (Figure 1), it was found that the λ_{max} of this compound is approximately 275 nm, this result is in accordance with previous investigators.^{15,22} Various HPLC-EC (electrochemical detection) methods have been described in the past that attempted to measure CoQ10. Historically, many methods are used for the CoQ10 measurement. These methods used chemical reagents to avoid the CoQ10 oxidation and to preserve the photosensitive compound from photodegradation. Previous methods failed in obtaining a reliable method because of the multistep extraction methods usually provide poor yield of CoQ10 measured. Intensive care is required to avoid the oxidation process by ambient oxygen. Preparation of calibrators and controls is particularly problematic to protect CoQ10 H₂ from oxidation to CoQ10.¹⁴ 1-propanol is the most lipophilic alcohol that is miscible with water. It was a slightly more effective extraction medium than 2-propanol and much more effective than acetonitrile and lower alcohols.¹⁵ The current method utilizes one step 1-propanol extraction method to minimize the photodegradation of CoQ10 and the artifactual oxidation of CoQ10 H₂ that frequently occurs during the chemical processing steps. The plasma proteins were precipitated during extraction and were separated by centrifugation. The present method uses the UV detection instead of the electrochemical detection used previously. Analytical recoveries were 95.5-101.3%. Although the current procedure requires 300 μL of sample, the sample size could be further reduced to 200 or 150 μL depending on the detection of trace amounts of CoQ10. The

other positivity of the current method is that the lower retention time of CoQ10 which is about 8 minutes and this will permit us to analyze a lot of samples within a short period of time. The linearity of the present method over a wide concentration range from 10µg/L to 4 mg/L was in accordance with results obtained by previous authors.¹⁴ This improvement is important for providing rapid, specific, sensitive, and reliable method for use in both clinical routine work and research laboratories. Edlund (1988)¹⁵ reported that the normal CoQ10 level mean in serum was 0.818 µg /mL. Kaplan et al (1996)¹⁷ found that the level of human plasma CoQ10 was 0.47±0.18 mg/L. On the other hand, Grossi et al²³ stated that the average level in healthy subjects was 0.80 mg/L with the UV detection method. Recently, many investigators reported that the normal plasma CoQ10 level was 1.02±0.30 µmole/L,⁶ whereas Burke et al¹ reported that the normal level of this coenzyme in human plasma was 0.49±0.14 mg/mL. In this study, the mean ± SD plasma level of healthy controls was 0.822±0.050 mg/L and this value was in consistence with the previous reports.

The limitation of this study is the capability of the method to measure the total CoQ10 rather than oxidized and reduced forms, this needs further future work.

In conclusion, a simple rapid HPLC method was developed using a one extraction step. This improved method gives excellent sensitivity, precision, and accuracy in human plasma; therefore, it is suitable for both research and clinical testing purposes.

References

- Burke BE, Neuenschwander R, Olson RD. Randomized, double-blind, placebo-controlled trial of coenzyme Q10 in isolated systolic hypertension. *South Med J* 2001; 94: 1112-1117.
- Ernster L, Dallner G. Biochemical, physiological and medical aspects of ubiquinone function. *Biochim Biophys Acta* 1995; 1271: 195-204.
- Langade D, Tarapore P, Jagtap A, Arora P, Anand S. Coenzyme Q10 (Ubiquinone): Implications in Clinical Practice. *Bombay Hospital Journal* 2005; 47: 2.
- Qu J, Kaufman Y, Washington I. Coenzyme Q10 in the human retina. *Invest Ophthalmol Vis Sci* 2009; 50: 1814-1818.
- Kimura I, Kimura M, Tsuneki H, Sasaoka T, Koya S. Can Coenzyme Q10 lead to improvement of essential hypertension? A long-term case study. *J Health Sci* 2008; 54: 571-575.
- Niklowitz P, Sonnenschein A, Janetzky B, Andler W, Menke T. Enrichment of coenzyme Q10 in plasma and blood cells: defense against oxidative damage. *Int J Biol Sci* 2007; 3: 257-262.
- Quinzii C, Naini A, Salviati L, Trevisson E, Navas P, Dimauro S, et al. A mutation in para-hydroxybenzoate-polyprenyl transferase (COQ2) causes primary coenzyme Q10 deficiency. *Am J Hum Genet* 2006; 78: 345-349.
- Ogasahara S, Engel AG, Frens D, Mack D. Muscle coenzyme Q deficiency in familial mitochondrial encephalomyopathy. *Proc Natl Acad Sci U S A* 1989; 86: 2379-2382.
- Greenberg S, Frishman WH. Co-enzyme Q10: a new drug for cardiovascular disease. *J Clin Pharmacol* 1990; 30: 596-608.
- Takada M, Ikenoya S, Yuzuriha T, Katayama K. Simultaneous determination of reduced and oxidized ubiquinones. *Methods Enzymol* 1984; 105: 147-155.
- Lang JK, Packer L. Quantitative determination of vitamin E and oxidized and reduced coenzyme Q10 by HPLC with in-line ultraviolet and electrochemical detection. *J Chromatogr* 1987; 385: 109-117.
- Yamashita S, Yamamoto Y. Simultaneous detection of ubiquinol and ubiquinone in human plasma as a marker of oxidative stress. *Anal Biochem* 1997; 250: 66-73.
- Kaikkonen J, Nyyssönen K, Salonen JT. Measurement and stability of plasma reduced, oxidized and total coenzyme Q10 in humans. *Scand J Clin Lab Invest* 1999; 59: 457-466.
- Tang PH, Miles MV, DeGrauw A, Hershey A, Pesce A. HPLC analysis of reduced and oxidized coenzyme Q(10) in human plasma. *Clin Chem* 2001; 47: 256-265.
- Edlund PO. Determination of Coenzyme Q10, α -tocopherol and cholesterol in biological samples by coupled-column liquid chromatography with coulometric and ultraviolet detection. *J Chromatogr* 1988; 425: 87-97.
- Wahlqvist ML, Wattanapenpaiboon N, Savige GS, Kannar D. Bioavailability of two different formulations of Coenzyme Q10 in healthy subjects. *Asia Pac J Clin Nutr* 1998; 7: 37-40.
- Kaplan P, Sebestianová N, Turiaková J, Kucera I. Determination of coenzyme Q in human plasma. *Physiol Res* 1996; 45: 39-45.
- Ullman MD, Bowers LD, Burtis CA. Chromatography/ Mass spectrometry. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. USA: W.B. Saunders Company; 1999. p. 164-170.
- Vasudevan DM, Sreekumari S. Textbook of Biochemistry (for medical students). 4th ed. New Delhi (IN): Jaypee Brothers; 2005. p. 472-474.
- Garber CC, Carey RN. Evaluation of methods. In: Kaplan LA, Pesce AJ, Kazmierczak SC, editors. Clinical chemistry theory, analysis, correlation. 4th ed. USA: Mosby; 2003. p. 402-425.
- Koch DD, Peters T. Selection and evaluation of methods. In: Burtis CA, Ashwood ER, editors. Tietz textbook of clinical chemistry, 3rd ed. USA: W.B. Saunders Company; 1999. p. 320-335.
- Mancini A, Marinis L, Oradei A, Hallgass ME, Conte G, Pozza D, et al. Coenzyme Q10 concentration in normal and pathological human seminal fluid. *Journal of Andrology* 1994; 15: 591-594.
- Grossi G, Bargossi AM, Fiorella PL, Piazzini S. Improved high-performance liquid chromatographic method for the determination of coenzyme Q10 in plasma. *J Chromatogr* 1992; 593: 217-226.