A rapid and non-invasive fluorescence method for quantifying coenzyme Q10 in blood and urine in clinical analysis

Xuechao Fei1 | Yan Yu2 | Yalan Di1 | Li Ai1 | Dandan Yao1,3 | Shangying Bai1,4 | Shengjie Zhao2 | Jihui Lyu3 | Xiang Cai4 | Rongqiao He1,5 | Zhiqian Tong1

1Laboratory of Alzheimer's Optoelectric Therapy, Alzheimer's disease Center, Beijing Institute of Brain Disorders, Capital Medical University, Beijing, China
2Chinese Institute of Rehabilitation Science, China Rehabilitation Research Center, Beijing Key Laboratory of Neural Injury and Rehabilitation, Beijing, China
3Center for Cognitive Disorders, Beijing Geriatric Hospital, Beijing, China
4The Department of Physiology, Southern Illinois University School of Medicine, Carbondale, Illinois
5State Key Laboratory of Brain & Cognitive Science, Institute of Biophysics, CAS Key Laboratory of Mental Health, University of Chinese Academy of Sciences (UCAS), Beijing, China

Correspondence
Zhiqian Tong, Alzheimer's disease Center, Beijing Institute for Brain Disorders, Capital Medical University, Beijing 100069, China. Email: tzqbeida@ccmu.edu.cn

Funding information
the Scientific Research Common Program of Beijing Municipal Commission of Education, Grant/Award Number: KM201510025014; the Major Projects Fund of Beijing Institute for Brain Disorders, Grant/Award Number: ZD2015-08; Beijing Natural Science Foundation of China, Grant/Award Number: 7172022; the startup fund of Advanced Innovation Center for Human Brain Protection, Grant/Award Number: 1172120205; the Special Fund for Fundamental Scientific Research Business of Central Public Research Institutes, Grant/Award Number: 2019CZ-4

Abstract

Background: Coenzyme Q10 (CoQ10) supplementation can improve cognition in patients with Alzheimer's disease (AD) and AD transgenic model mice. To ameliorate the discomfort that patients with AD suffer after several blood extractions, a non-invasive method for detecting urine CoQ10 levels needs to be established.

Methods: Here, we developed a new technique of fluorescence spectrophotometry with ethyl cyanoacetate (FS-ECA), on the basis of the principle that the chemical derivative obtained from the interaction between CoQ10 and ECA was detected by a fluorescence detector at λex/em = 450/515 nm. As a standard reference method, the same batches of the clinical samples were analyzed by high-performance liquid chromatography with an ultraviolet detector (HPLC-UV) at 275 nm.

Results: The limits of detection (LOD) and limits of quantization (LOQ) (serum: 0.021 and 0.043 mg/L; urine: 0.012 and 0.025 mg/L) determined by the FS-ECA method were similar to that obtained through HPLC-UV (serum: 0.017 and 0.035 mg/L; urine: 0.012 and 0.025 mg/L). More importantly, this new FS-ECA technique as well as the conventional HPLC-UV method could detect a marked difference in urine CoQ10 levels between AD and controls.

Conclusion: Our findings suggest that this non-invasive method for quantifying urine CoQ10 potentially replaces HPLC to detect blood CoQ10.

KEYWORDS
Alzheimer's disease (AD), coenzyme Q10 (CoQ10), ethyl cyanoacetate (ECA), fluorescence spectrophotometry (FS), high-performance liquid chromatography (HPLC)
1 INTRODUCTION

Coenzyme Q10 (CoQ10), a hydrophobic molecule containing ten isoprenoid units in the side chain, is a lipid-soluble endogenous compound located in the mitochondrial membranes. It plays an important role as an electron carrier in the respiratory chain and as a powerful antioxidant. Coenzyme Q10 functions as a cofactor in the electron transport during oxidative phosphorylation and is essential for production of cellular energy in the form of adenosine triphosphate. The reduced form of CoQ10 (ubiquinol) can inhibit lipid peroxidation in biological membranes and protects the body from damage caused by harmful molecules. A deficiency of this antioxidant in one's diet has been related to several diseases, such as heart failure, obesity, cancer, chronic pain, and neurological disorders. Notably, plasma CoQ10 concentrations are significantly influenced by dietary uptake. Hence, patients with CoQ10 deficiency can benefit from early diagnosis and treatment with exogenous CoQ10 supplementation.

Alzheimer’s disease (AD) is the most common neurodegenerative disease, leading to progressive cognitive decline. Deposition of amyloid β (Aβ)-mediated senile plaques and neurofibrillary tangles are the pathological hallmarks of AD. However, over the past decades, targeting Aβ and/or tau through antibodies, vaccines, or small-molecule drugs have not resulted in desirable clinical efficacy. Recently, mitochondrial dysfunction is considered to be involved in AD pathology. The mitochondrial defects are found to be predominantly observed in complex I, IV, and V of the electron transport chain, which leads to the generation of free radicals. Endogenous CoQ10 is part of the electron transport chain. Notably, Aβ-induced CoQ10 deficiency has been found in AD transgenic animal models and patients with AD. CoQ10 supplementation (a nano-micelle containing soluble CoQ10) has been identified to contribute to the treatment of ischemia model mice, AD model mice, and patients with dementia. Therefore, it is vital to rapidly and easily quantify CoQ10 concentrations in plasma and/or urine.

Various analytical methods have been developed for the determination of CoQ10 in a variety of biological, drug, and food samples. The first approach for detecting CoQ10 levels in human blood was based on a principle that it can have a colorimetric reaction with ethyl cyanoacetate (ECA) and the blue derivative obtained can be quantified by the thin-layer chromatography (TLC) on silica gel. The average serum CoQ10 levels are ~1 mg/L with a range from 0.4 to 1.8 mg/L. The most regularly utilized technique for CoQ10 detection is high-performance liquid chromatography (HPLC), which can be used in combination with various detection systems using either ultraviolet (UV), fluorescence (FR), mass spectrometry (MS), or electrochemical (EC) detectors. However, this HPLC technique requires sample preparation, use of a bulky instrument, high purchase cost, maintenance costs, and specially trained professionals. Therefore, a simple, sensitive, and efficient method for determining trace amounts of CoQ10 is urgently needed.

In this study, we have established a new method of fluorescence spectrophotometry with ECA (FS-ECA, λex/em = 450/515 nm) to detect CoQ10 levels in the serum and urine of patients with AD. Further, we compared the accuracy of these two methods. Our results suggest that this non-invasive technique of FS-ECA may replace HPLC-UV to detect blood CoQ10 concentrations.

2 MATERIALS AND METHODS

2.1 Ethical disclosure

This clinical investigation (2014SY39) was approved by the Ethics Committee at the Capital Medical University, China.

2.2 Participants

The study was registered at the Chinese Clinical Trial Registry (http://www.chictr.org.cn, Unique Identifier: ChiCTR-OOC-14005576) and conducted between March 2008 and December 2014. We recruited participants from representative regions in Beijing, China. The mean age of 100 individuals in this cross-sectional survey was 77.31 ± 2.49 years. Participants who refused to provide urine samples, or had a life-threatening illness, or were unable to participate in the assessment, were excluded from the entire survey. Information on medical history and medications for each participant were obtained from primary healthcare records or provided by each participant or by their guardians.

2.3 Clinical evaluation

The cognitive status of patients/participants was assessed by neurologists using the activities of daily living (ADL), Clinical Dementia Rating (CDR), and Mini-Mental State Examination (MMSE). A MMSE score ≤20 (adjusted for education level of the participants from rural regions) was defined to be cognitive impairment. The MMSE is widely applied to assess the cognitive ability of patients suffering from memory decline. Patients with Alzheimer’s disease (AD) were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (fourth edition) revised (DSM-IV-R) criteria, as described previously.

2.4 Morning blood and urine samples

Venous bloods of all participants were routinely sampled by the nurse. Morning urine samples from participants were collected and immediately placed on ice, before being stored at ~70°C until analyzed. After centrifugation (8000 xg, 4°C, 10 minutes), the supernatant fractions of urine were subjected to CoQ10 analysis.
2.5 | Chemical reagents

Standard pure CoQ10 (Molecular weight: 863.36) was donated by Novacon Life Biotechnology Co. Ltd, China, and produced by AQUANOVA AG, Germany. The chemical reagents, including hexane (No. HB11141; Shanghai Macklin Biochemical Co. Ltd), ethyl cyanoacetate (ECA, No. B15529; Shanghai Hanhong Scientific Co. Ltd), and dimethyl sulfoxide (DMSO, NO. 1096780; Sigma-Aldrich), were analytical grade. The organic solvents including methanol (No. A452-4) and ethanol (No. A4995-4) were HPLC grade and purchased from Fisher Chemical, Pennsylvania, Pittsburgh, USA. Deionized water was obtained from the Milli-Q system.

2.6 | Detection of CoQ10 by fluorescent spectrophotometer (FS-ECA)

This newly established method for detecting CoQ10 was based on the principle that the chemical derivative between CoQ10 and ethyl cyanoacetate (ECA) could be detected by a fluorescence spectrophotometer (FS-ECA) at $\lambda_{ex/em} = 450/515$ nm, because one methoxy group of CoQ10 can be replaced by a moiety of ECA.\(^{23,24}\)

To prepare the detected samples, 60 μL standard CoQ10 or clinical samples were added into the solutions containing 60 μL ethanol and 120 μL hexane. Then, these mixed solutions were vortexed for 1 minutes and centrifuged at 2800 g for 2 minutes. The supernatants were transferred into 120 μL hexane solution and extracted again. The twice-extracted supernatants were combined and dried using nitrogen. These precipitants were resolved by 60 μL ethanol, and the mixture was incubated in dark for 30 minutes. Then, the fluorescent intensities ($\lambda_{ex/em} = 450/515$ nm) of the derivatives were quantified by using FS-ECA (Multi-Mode Microplate Reader, SpectraMax i3, Molecular Devices).

To determine the optimal temperature for the interaction between CoQ10 and ECA to occur, 20 μL of CoQ10 solutions at different concentrations (0.00, 0.05, 0.07, 0.35, 0.70, 1.75, and 3.50 mg/L) was added to seven vials with 220 μL ECA-sensitive reaction system and the mixture was incubated in dark at 25°C or 35°C for 30 minutes. Then, the sample solutions were examined by FS-ECA.

To explore the optimal volume of ECA in the ECA-sensitive reaction system for the reaction between CoQ10 and ECA to occur, ECA solutions at different volumes (0, 2, 5, 10, 20, 40, 80, and 120 μL) were added to eight vials with an ECA-sensitive reaction system and the mixture was incubated in dark at 25°C for 30 minutes and immediately examined by FS-ECA.

To obtain calibration curves, CoQ10 standards were prepared with concentrations ranging from 0.00, 0.055, 0.109, 0.218, 0.437, to 1.750 mg/L. For routine analysis, a one-point calibration in duplicate was prepared daily and used for quantitative calculation of the samples.

2.7 | Specificity and selectivity of FS-ECA method

Previous studies showed that one methoxy group of CoQ10 can be specifically replaced by a moiety of ECA.\(^{23,24}\) The interference from other CoQs such as CoQ1 (#C7956), CoQ4 (#C2470), and CoQ9 (#27597, Sigma-Aldrich) was also investigated. A possible "interfering peak" has been considered as a peak which was further examined by the FS-ECA method at $\lambda_{ex/em} = 450/515$ nm.

2.8 | Accuracy, precision, and calibration of FS-ECA method

Inter-day precision and accuracy were determined by assaying serum and urine samples in double replicates and six different validation sessions. Intra-day precision was evaluated in five intra-day replicates for each CoQ10 level. Accuracy was calculated as the percent deviation from the nominal concentration. Inter-day and intra-day precisions were expressed as the relative standard deviation (RSD) for each CoQ10 concentration. Calibration curves were obtained by FS-ECA through peak values.

2.9 | Limit of quantification for FS-ECA method

The limit of detection (LOD) in plasma was defined as the concentration that yields a signal-to-noise ratio of 3:1. Percent deviation from the nominal concentration (measure of accuracy) and RSD (measure of precision) of the concentrations considered as the limit of quantification (LOQ) had to be <20%, and it was considered as the lowest calibration standard as previously described.\(^{34-36}\)

2.10 | Recovery of FS-ECA method

Recovery calculation of the method was obtained in triplicate at three final concentration levels (3.50, 1.75, and 0.7 mg/L) from a detector response of the analyte added to and extracted from the serum and urine and compared with the detector response of the spiked analyte after extraction into fractions.

2.11 | Detection of CoQ10 by HPLC-UV

To prepare the detected samples, 450 μL of standard CoQ10 or clinical samples were added into the solutions containing 200 μL ethanol and 650 μL hexane. Then, these mixed solutions were vortexed for 1 minutes and centrifuged at 3000 rpm for 2 minutes. The supernatants were transferred into 650 μL hexane solution and extracted again. The twice-extracted supernatants were combined and further dried with nitrogen (No. MTN-2800N, AutoScience Co. Ltd). These precipitants were resolved by 100 μL ethanol and filtered by 13 mm × 0.22 μm hydrophobic filter...
membranes (No. AA-56312; Shanghai Xingya Purifying Materials Factory). Then, the residues were redissolved in 200 μL of mobile phase which has methanol-ethanol (1:4; 1 mL/min; volume temperature: 50°C), and 100 μL of this sample solution was examined by HPLC-UV at 275 nm (HPLC, No. LC-20A; Shimadzu) as previously reported.25

2.12 | Statistical analysis

Statistical analyses were performed using IBM SPSS software for Windows (version 19.0; SPSS Inc). Correlation analysis between two groups of data was performed using Pearson's correlation coefficient (parameter). Graphs were generated using GraphPad Prism version 5.01 (GraphPad Software Inc). The clinical characteristics and CoQ10 concentrations were compared using the Chi-square statistic for categorical variables and analysis of variance for continuous variables. The levels of CoQ10 in samples from patients with AD and age-matched controls were compared using Student’s t test. Differences between the groups were considered statistically significant when the P value was < .05.

3 | RESULTS

3.1 | Optimization of the reaction conditions of FS-ECA

To obtain high sensitivity from the FS-ECA method, we investigated the experimental parameters including reaction temperature, ECA volume, and reaction time (Figure 1A).

3.1.1 | Optimization of reaction temperature

Previously, some studies have shown that under an alkaline condition with 0.5% KOH, there is the formation of a rapid blue derivative due to the interaction between CoQ10 and ECA (Figure 1B).23,24 Therefore, we controlled the reaction condition of 240 μL ECA-sensitive reaction system (20 μL CoQ10 + 40 μL ECA + 40 μL 0.5% KOH + 140 μL ethanol) in dark for 30 minutes at 25 ± 1°C or 35 ± 1°C. The results showed that the fluorescence intensity of CoQ10 (Ex/Em = 450/515 nm) was dose-dependently increasing at 25°C (Figure 1C) and 35°C (Figure 1D), and the fluorescence values obtained from the condition of the former (25°C) was higher than the latter (35°C) (Figure 1E). Especially, CoQ10 at 0.7 mg/L (the physiological concentrations of human serum: 0.62 ~ 1.14 mg/L37) at 25°C had a higher value than that at 35°C. Notably, the fluorescence peaks of CoQ10 at 35°C (red triangle) were not evenly distributed at 515 nm. These data indicate that the results obtained at room temperature (25°C) are more reliable than at 35°C for detecting the CoQ10 in this ECA-sensitive reaction system.

3.1.2 | Optimization of ECA volume

Based on the available literature, it was clear that CoQ10 can chemically interact with ECA,23,24 but we wanted to know the optimal volume of ECA required for CoQ10 to interact and produce an optimal signal (Figure 2A). We followed the methodology as previously described, wherein we incubated the mixture of varying volumes of ECA with CoQ10 in dark for 30 minutes at 25 ± 1°C. We found that ECA could induce a dose-dependent increase in the fluorescence (Figure 2B, C). Notably, although ECA at 80 and 120 μL induced higher fluorescence values than at 40 μL (Figure 2C), the peaks produced by 40 μL were ideal as they were shifted toward the left (Figure 2D, red arrow). These results indicated that ECA over 40 μL affects the accuracy of FS-ECA method. Meanwhile, we found that ECA at 40 μL could induce a standard peak of fluorescence (red triangle) (Figure 2D). These data suggest that 40 μL ECA is an optimal condition in 240 μL ECA-sensitive reaction system.

3.2 | Optimization of reaction time

Previous studies have stated that the optimal time required for the formation of chemical derivative between CoQ10 and ECA is within 30 minutes.23,24 Hence, we explored whether this incubation time is the optimal time period to detect the fluorescence produced. The results showed that the fluorescence values produced by CoQ10 rapidly increased by 30 minutes, whereas it gradually elevated by 60 minutes (Figure 2B). These data indicate that 30-minutes reaction time is an optimal incubation time in this ECA-sensitive reaction system at 25°C.

3.3 | CoQ10 levels in the serum and urine detected by FS-ECA

Then, we used this new method of FS-ECA to examine the concentrations of CoQ10 in serum and urine of patients with AD and age-matched controls (Table S1). The results showed that there was a linear correlation between the fluorescence values and CoQ10 concentrations (R² = 0.9973) (Figure 3A, B). The serum CoQ10 levels in 12 patients with AD were significantly lower than 14 age-matched controls (0.412 ± 0.031 vs 0.757 ± 0.025, P < .0001) (Figure 3C). Moreover, the urine CoQ10 levels in 50 patients with AD were markedly lower than 50 age-matched controls (0.031 ± 0.003 vs 0.065 ± 0.008, P = .0003) (Figure 3D). These data demonstrate that the method of FS-ECA can be used to examine CoQ10 concentrations in the serum and urine of patients with AD.

3.4 | CoQ10 levels in the serum and urine detected by HPLC-UV

The technique of HPLC-UV is widely accepted to be a standard reference method for detecting CoQ10 contents25; therefore, the
The same batches of clinical samples were analyzed by this conventional method. The results showed that there was a linear correlation between the peak areas of HPLC and CoQ10 concentrations ($R^2 = .9926$) (Figure 4A-C). Moreover, we observed lower urine CoQ10 levels in 12 patients with AD than 14 age-matched controls (0.399 ± 0.088 vs. 0.732 ± 0.030, $P < .0001$) (Figure 4D). The urine
**FIGURE 3** The CoQ10 concentrations detected by FS-ECA in the serum and urine of patients with Alzheimer’s disease (AD). A and B, The standard curve and fluorescence values of CoQ10. C and D, The CoQ10 levels in the serum and urine of AD patients and controls.

**FIGURE 4** The CoQ10 concentrations detected by HPLC-UV in the serum and urine of patients with Alzheimer’s disease (AD). A-C, The HPLC spectrums, standard curve, and fluorescence values of CoQ10 at different concentrations. D and E, The CoQ10 levels in the serum and urine of AD patients and controls.
CoQ10 levels in 50 patients with AD were markedly lower than 50 age-matched controls (0.051 ± 0.002 vs 0.092 ± 0.003, P < .0001) (Figure 4D). These results obtained from HPLC-UV method were consistent with the data obtained from FS-ECA method.

3.5 | Accuracy and stability of the comparative methods

3.5.1 | LOD and LOQ

Next, we determined the limits of detection (LOD) and the limits of quantization (LOQ) of CoQ10 levels analyzed in the serum and urine samples by these two methods. Our results showed that the LOD and LOQ (serum: 0.020 and 0.040 mg/L; urine: 0.012 and 0.024 mg/L) obtained from the FS-ECA method were similar to that of the HPLC-UV method (serum: 0.017 and 0.034 mg/L; urine: 0.014 and 0.028 mg/L) (Table 1). These data indicate that the new FS-ECA method as well as HPLC-UV method accurately detects CoQ10 concentrations in these clinical samples.

3.5.2 | RSD and recovery

Ten replicates of these clinical samples were analyzed at various time points in a day to evaluate within-day variations. The same samples of serum and urine were also analyzed on different days to evaluate day-to-day variations. The data of variations observed within-day are summarized in Table 1. The results of variation obtained from day-to-day experimentation showed that the average relative standard deviations (RSD) detected by HPLC-UV and FS-ECA were 4.08 and 3.30 (RSD < 8%) in the serum, and 3.26 and 1.23 (RSD < 8%) in the urine. The average recoveries examined by these two methods were 98.16% and 96.97% in the serum, and 99.01% and 96.53% in the urine (Table 1). Hence, this FS-ECA method can effectively and steadily determine CoQ10 levels similar to that of the HPLC-UV method.

3.6 | Comparison of the changing trend of two groups of data

If these two methods accurately examine CoQ10 levels in the clinical samples, these two groups of data should have the similar changing trend. To address this possibility, the Pearson’s product correlation coefficient (parameter) was calculated with the use of IBM SPSS software. The results showed that there was a high correlation (Pearson R = .805, P = .003 < .01) in serum CoQ10 values (Figure 5A), and a similar trend in the urine CoQ10 contents (Pearson R = .253, P = .047 < .05) in patients with AD (Figure 5B). Meanwhile, we found that the HPLC-UV method could detect a marked difference in the levels of urine CoQ10 between AD and controls (Figure 5C,D); however, a lower concentration of urine CoQ10 can be recognized and

### Table 1 The accuracy and stability of comparative methods

| Samples | Test items      | Mean ± SD   | RSD (%) | Recovery (%) | LOQ (mg/L) | LOD (mg/L) |
|---------|----------------|-------------|---------|--------------|------------|------------|
| Serum   | Q10 (HPLC-UV)  | 0.732 ± 0.030 | 4.08    | 98.19        | 0.035      | 0.017      |
|         | Q10 (FS-ECA)   | 0.757 ± 0.025 | 3.30    | 96.97        | 0.043      | 0.021      |
| Urine   | Q10 (HPLC-UV)  | 0.092 ± 0.003 | 3.26    | 99.01        | 0.029      | 0.014      |
|         | Q10 (FS-ECA)   | 0.065 ± 0.008 | 1.23    | 96.53        | 0.025      | 0.012      |

### Figure 5 The accuracy and stability of comparative methods. A and B) Comparison of the changing trend of two groups of data analyzed by SPSS. C and D) The HPLC spectrums of urine CoQ10 of AD patients and controls
quantified easily by FS-ECA than HPLC-UV method (Figure 5B). These data demonstrate that the technique of FS-ECA for detecting urine CoQ10 levels is relatively easier and faster compared with the HPLC-UV method.

4 | DISCUSSION

In this study, we have developed the non-invasive FS-ECA method to examine urine CoQ10 in the clinical samples of patients with AD, which potentially replaces HPLC to detect blood CoQ10 concentrations. This new technique has some advantages when compared to the HPLC and other methods for quantifying blood CoQ10 invasively.

Firstly, FS-ECA method for detecting CoQ10 in blood and urine has the similar LOD and LOQ when compared with the standard HPLC-UV method, suggesting that urine CoQ10 examined by FS-ECA may replace the conventional HPLC-UV method to detect blood CoQ10. Secondly, this detection method could quantitatively identify urine CoQ10 with relative advantages over the HPLC-UV approach, including easier preparation and faster detection speed, while with the similar accuracy and specificity. Thirdly, the cost of FS-ECA is lesser than the HPLC method or the ELISA kit for CoQ10, while with the similar accuracy and specificity. Fourthly, FS-ECA is lesser than the HPLC method or the ELISA kit for CoQ10, while with the similar accuracy and specificity. Thirdly, the cost of FS-ECA is lesser than the HPLC method or the ELISA kit for CoQ10, while with the similar accuracy and specificity.

Alternatively, there are some limitations to this FS-ECA method. For example, obtaining the derivative from ECA and urine CoQ10 interaction still involves some steps. The more specific and stable derivative agents that could be derived from CoQ10 needs to be further investigated.

In conclusion, this high accuracy and precision of this assay, taken together with the good and stable recovery, make this method eligible for use in clinical studies for the non-invasive determination of CoQ10 in urine of patients with AD. It may also be a useful tool for monitoring CoQ10 metabolism after oral CoQ10 supplementation.

ACKNOWLEDGMENTS

This study was funded by the Beijing Natural Science Foundation of China (7172022); the Special Fund for Fundamental Scientific Research Business of Central Public Research Institutes (2019CZ-4); the Scientific Research Common Program of Beijing Municipal Commission of Education (KM201510025014); the Major Projects Fund of Beijing Institute for Brain Disorders (ZD2015-08); and the startup fund of Advanced Innovation Center for Human Brain Protection (1172120205).

REFERENCES

1. Mortensen SA, Rosenfeldt F, Kumar A, et al. The effect of coenzyme Q10 on morbidity and mortality in chronic heart failure: results from Q-SYMBIO: a randomized double-blind trial. JACC Heart Fail. 2014;2(6):641-649.
2. Ernst L, Dellner G. Biochemical, physiological and medical aspects of ubiquinone function. Biochim Biophys Acta. 1995;1271(1):195-204.
3. Paliakov EM, Crow BS, Bishop MJ, Norton D, George J, Bralley JA. Rapid quantitative determination of fat-soluble vitamins and coenzyme Q-10 in human serum by reversed phase ultra-high pressure liquid chromatography with UV detection. J Chromatogr B Analyt Technol Biomed Sci. 2009;877(1-2):89-94.
4. Bentinger M, Tekle M, Dellner G. Coenzyme Q-biosynthesis and functions. Biochem Biophys Res Commun. 2010;396(1):74-79.
5. Rom-Pizarro V, Fernández-Romero JM, Gomez-Hens A. Automatic determination of coenzyme Q10 in food using cresyl violet encapsulated into magnetoliposomes. Food Chem. 2017;221:864-870.
6. Weber C, Bysted A, Hilmér G. The coenzyme Q10 content of the average Danish diet. Int J Vitam Nutr Res. 1997;67(2):123-129.
7. Ly PT, Wu Y, Zou H, et al. Inhibition of GSK3beta-mediated BACE1 expression reduces Alzheimer-associated phenotypes. J Clin Invest. 2013;123(1):224-235.
8. Zhang Y, Song W. Islet amyloid polypeptide: Another key molecule in Alzheimer's pathogenesis? Prog Neurobiol. 2017;153:100-120.
9. Zhang S, Cai F, Wu Y, et al. A presenilin-1 mutation causes Alzheimer disease without affecting Notch signaling. Mol Psychiatry. 2018. https://doi.org/10.1038/s41380-018-0101-x
10. Zhang S, Wang Z, Cai F, et al. BACE1 cleavage site selection critical for amyloidogenesis and Alzheimer’s pathogenesis. J Neurosci. 2017;37(29):6915-6925.
11. Cummings J, Lee G, Ritter A, Zhong K. Alzheimer’s disease drug development pipeline: 2018. Alzheimers Dement (NY). 2018;4:195-214.
12. Singh S, Brocker C, Koppaka V, et al. Aldehyde dehydrogenases in cellular responses to oxidative/electrophilic stress. Free Radic Biol Med. 2013;56:89-101.
13. Schapira AH, Reichmann H. Electron transport chain defects in Alzheimer’s disease. Neurology. 1995;45(3 Pt 1):599-600.
14. Ma D, Stokes K, Mahgar K, Domazet-Damjanov D, Sikorska M, Pandey S. Inhibition of stress induced premature senescence in presenilin-1 mutated cells with water soluble Coenzyme Q10. Mitochondrion. 2014;17:106-115.
15. Hyun DH, Mughal MR, Yang H, et al. The plasma membrane redox system is impaired by amyloid beta-peptide and in the hippocampus and cerebral cortex of 3xTgAD mice. Exp Neurol. 2010;225(2):423-429.
16. McManus MJ, Murphy MP, Franklin JL. The mitochondria-targeted antioxidant MitoQ prevents loss of spatial memory retention and early neuropathology in a transgenic mouse model of Alzheimer’s disease. J Neurosci. 2011;31(4):15703-15715.
17. Isobe C, Abe T, Terayama Y. Increase in the oxidized/total coenzyme Q-10 ratio in the cerebrospinal fluid of Alzheimer’s disease patients. Dement Geriatr Cogn Disord. 2009;28(5):449-454.
18. Dumont M, Kipiani K, Yu F, et al. Coenzyme Q10 decreases amyloid pathology and improves behavior in a transgenic mouse model of Alzheimer’s disease. J Alzheimers Dis. 2011;27(1):211-223.
19. Sikorska M, Lanthier P, Miller H, et al. Nanomicellar formulation of coenzyme Q10 (Ubisol-Q10) effectively blocks ongoing neurodegeneration in the mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model: potential use as an adjuvant treatment in Parkinson’s disease. Neurobiol Aging. 2014;35(10):2329-2346.
20. Muthukumaran K, Kanwar A, Vegh C, et al. Ubisol-Q10 (a nanomicellar water-soluble formulation of CoQ10) treatment inhibits alzheimer-type behavioral and pathological symptoms in a double

ORCID

Xuechao Fei https://orcid.org/0000-0002-0511-0386
transgenic mouse (TgAPEswe, PSEN1dE9) model of Alzheimer’s disease. J Alzheimers Dis. 2018;61(1):221-236.

21. Spindler M, Beal MF, Henchcliffe C. Coenzyme Q10 effects in neurodegenerative disease. Neuropsychiatr Dis Treat. 2009;5:597-610.

22. Salehpour F, Farajdokht F, Mahmoudi J, et al. Photobiomodulation and coenzyme Q10 treatments attenuate cognitive impairment associated with model of transient global brain ischemia in artificially aged mice. Front Cell Neurosci. 2019;13:74.

23. Redalieu E, Nilsson IM, Farley TM, Folkes K, Koniuszy FR. Determination and levels of coenzyme Q10 in human blood. Anal Biochem. 1968;23(1):132-140.

24. Ye CQ, Folkes K, Tamagawa H, Pfeiffer C. A modified determination of coenzyme Q10 in human blood and CoQ10 blood levels in diverse patients with allergies. Biofactors. 1998;14(4):303-306.

25. Lunetta S, Roman M. Determination of coenzyme Q10 content in raw materials and dietary supplements by high-performance liquid chromatography-UV: collaborative study. J AOAC Int. 2008;91(4):702-708.

26. Korany MA, Gazy AA, Khamis EF, Ragab MAA, Kamal MF. Analysis of closely related antioxidant nutraceuticals using the green analytical methodology of ANN and smart spectrophotometric methods. J AOAC Int. 2017;100(1):8-17.

27. Nohara Y, Suzuki J, Kubo H. Determination of ubiquinone in blood by high-performance liquid chromatography with post-column fluorescence derivatization using 2-cyanoacetamide. J Fluoresc. 2011;21(6):2093-2100.

28. Arias A, Garcia-Villoria J, Rojo A, Bujan N, Briones P, Ribes A. Analysis of coenzyme Q10 (CoQ10) in lymphocytes by HPLC-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci. 2012;908:23-26.

29. Niklowitz P, Doring F, Paulussen M, Menke T. Determination of coenzyme Q10 tissue status via high-performance liquid chromatography with electrochemical detection in swine tissues (Sus scrofa domestica). Anal Biochem. 2013;437(1):88-94.

30. Reisberg B, Doody R, Stoffler A, Schmitt F, Ferris S, Mobius HJ. Memantine in moderate-to-severe Alzheimer’s disease. N Engl J Med. 2003;348(14):1333-1341.

31. Fillenbaum GG, Peterson B, Morris JC. Estimating the validity of the clinical Dementia Rating Scale: the CERAD experience. Consortium to establish a registry for Alzheimer’s disease. Aging (Milano). 1996;8(6):379-385.

32. Woodford HJ, George J. Cognitive assessment in the elderly: a review of clinical methods. QJM. 2007;100(8):469-484.

33. Bleich S, Wiltfang J, Kornhuber J. Memantine in moderate-to-severe Alzheimer’s disease. N Engl J Med. 2003;349(6):609-610; author reply 609-610.

34. Meier PC, Zund RE. Statistical Methods in Analytical Chemistry, 2nd edn. New York: Wiley-Interscience: 2000:424.

35. Shrivastava A, Gupta VB. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chronicles Young Scientists. 2011;2(1):21-25.

36. Uhrovck J. Strategy for determination of LOD and LOQ values—some basic aspects. Talanta. 2014;119:178-180.

37. 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(4):257-262.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Fei X, Yu Y, Di Y, et al. A rapid and non-invasive fluorescence method for quantifying coenzyme Q10 in blood and urine in clinical analysis. J Clin Lab Anal. 2020;34:e23130. https://doi.org/10.1002/jcla.23130