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Application of derivative spectrophotometry for determination of coenzyme Q_{10} in pharmaceuticals and plasma

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Abstract

The use of derivative spectrophotometry is proposed in this work for determination of coenzyme Q_{10} in formulations and in human plasma. The spectrophotometric procedure is simpler and less expensive than chromatographic techniques commonly used for the analysis of coenzyme. The active compound can be determined in the range 0.25–10 ppm for standard solutions and pharmaceuticals and 0.05–1.5 ppm in plasma. The proposed method was applied for coenzyme determination in real samples. The results agree well with declared value and with these obtained by HPLC. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Coenzyme Q_{10} is a physiologicically important compound. It acts as an electron shuttle in mitochondrial respiratory chain. Furthermore, it works as a stabilizing agent in cellular membranes. Since its discovery by F.L. Crane [1] in 1956, many scientists have examined its role in metabolic pathway. As the result of intensive investigations, we have a better understanding of coenzyme Q_{10} significance in living organisms. It is assumed that some diseases such as parathondose or cardiac disorders are linked (among others) to Q_{10} deficiency. Due to this, the knowledge of Q_{10} level in plasma or tissues, and its correlation with patients age and condition, is very important.

The average level of this compound in healthy subjects is 0.8 ± 0.20 mg l^{-1} [2]. Due to the low concentration of coenzyme Q_{10} in plasma, the method used for its determination should be selective and sensitive. The number of methods reported for determination of coenzyme in biological samples is very limited. The most popular tools used for this purpose are HPLC methods with UV [2–5] or electrochemical [2,6–8] detection. Also, in the literature are described: capillary zone electrophoresis [9], voltammetric [10], chemiluminescent [11], potentiometric [12], fluorimetric [13] and spectrophotometric [14] methods. Although, these methods, especially the chromatographic one, provide high sensitivity, most of

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them are complicated and time-consuming. This led us to search for a simple, rapid, reliable and specific method for the determination of coenzyme Q_{10} level in plasma and its contents in pharmaceutical formulation. The classical UV spectrophotometric methods give severe problems with plasma background, which makes coenzyme Q_{10} determination impossible in this matrix.

An alternative method applied to this problem could be derivative spectrophotometry. This approach is based on mathematical transformation of spectral curve [15,16] into derivative spectra. The derivatisation of spectra allows to obtain more information by discovering the information included in zero-order spectra, especially in the case of multicomponent mixture analysis. Mathematical treatment of spectra eliminates the influence of background or matrix. The use of derivative spectrophotometry in many cases results in the increase of selectivity and sensitivity of the determination. Considering the profits given by application of mathematical approach into chemical analysis and easy access to analytical apparatus, the authors examined the usefulness of the above mentioned method to coenzyme Q_{10} analysis. Derivative spectrophotometry is usually used for resolution of multicomponent mixtures. In the present work, it is applied to eliminate the influence of a complicated matrix. The generation of derivative spectra has been done using the Savitzky-Golay algorithm [17]. The aim of this work is to develop a direct and simple UV-spectrophotometric method for the determination of coenzyme Q_{10} in pharmaceuticals and in plasma.

2. Experimental

2.1. Apparatus

A Hewlett-Packard HP-8452A diode array spectrophotometer coupled to the User Data version software was used for the acquisition and storage of spectra. The 0.5 cm quartz cuvette were used for spectral analysis.

A PC computer equipped with Excel 4.0 for Windows 95 was used for mathematical treatment of data.

As a reference method HPLC method [4] was used. Chromatographic analyses were performing using the HPLC system, Merck, Germany, consisting of: pump type L-6200A Intelligent Pump, a reversed-phase analytical column, Lichrospher 100 RP-18 250×4 mm (5 μm) with a guard column 4×4 mm (5 μm) (Merck, Germany), detector DAD, type L-4500 (Merck, Germany) (the peaks were recorded at 270 nm). For manual injection, a injection valve with 20 μl sample loop was used. All system was managed by interface HPLC-Manager, D-6500. The results were recorded on a PC computer supplied with DryLab Windows G/plus programme (LC Resources Inc.).

The mobile phase consisted of 85% methanol and 15% n-hexane at flow rate 1.5 ml min^{-1} [4].

2.2. Reagents

All reagents used were of chromatographic grade.

Coenzyme Q_{10} was from Sigma-Aldrich, Germany; Coenzyme Q_{10} capsules from Vita Care, Jeno-pharm A/S Stege, Denmark; each capsule contained 10 mg of coenzyme Q_{10} mixed with soya lecithin, glucose syrup, aerosil and magnesium stearate; methanol was from Merck, Germany; n-hexane from Merck, Germany; and 5% human albumin solution for injections from BIOMED, Poland

Two different standard solutions of Q_{10} were prepared as follows:

1. The standard solution of Q_{10} (100 ppm) was prepared by dissolving appropriate amount of compound in n-hexane. Working solutions (0.5–20 ppm) were prepared by the appropriate dilutions of the stock standard solution with n-hexane. The working solutions were prepared freshly every day. The stock solution was stored in brown bottles at 4°C.
2. Synthetic plasma standard was obtained by adding 0.5 ml of commercial albumin solution for injection (BIOMED, Poland) into coenzyme Q_{10} standard solutions (0.15–1.5 ppm). The standard solution were prepared as follows: 0.5 ml of 0.5% human albumin was transferred into 10 ml polypropylene cen-

trifuge tubes. Next 1 ml of methanol and 0.5 ml of hexane Q_{10} standard solutions were added. Next the tubes were mixed for 1 minute and centrifuged for 5 min at $2000 \times g$ to separate the layers. The n-hexane phase was transferred to small vial and extraction was repeated again with 0.5 ml portion of n-hexane. The hexane extracts were dried under nitrogen atmosphere at room temperature. The residue was dissolved in 0.5 ml of n-hexane. The blank solution was prepared in the same way but without addition of coenzyme Q_{10}.

Obtained aliquots of extracts were analysed immediately at $\lambda = 284$ nm.

2.3. Assay of coenzyme Q_{10} in commercial capsules

The content of one capsule was quantitatively transferred into separating funnel. Next 10 ml of deionized, distilled water and 10 ml of n-hexane were added. The mixture was shaken vigorously and organic layer was transferred into a 50-ml calibration flask. The extraction was repeated twice. The hexane extract was adjusted up to 50 ml with n-hexane. The working solutions were prepared by the appropriate dilutions.

2.4. Assay of coenzyme Q_{10} in plasma samples.

First, 0.5 ml of pooled human plasma was pipetted in triplicate into 10 ml polypropylene centrifuge tubes. Next, 1 ml of methanol and 0.5 ml of hexane were added. The tubes were mixed for 1 min and centrifuged for 5 min at $2000 \times g$ to separate the layers. The n-hexane phase was transferred to small vial and extraction was repeated with 0.5 ml portion of n-hexane. The hexane extracts were dried under nitrogen atmosphere at room temperature. The residue was dissolved in 0.5 ml of n-hexane.

Obtained aliquots of extracts were analysed immediately.

The extracts prepared in the same way were used for chromatographic assay of coenzyme Q_{10}.

Blank solution was prepared in the same way using 0.5 ml of human albumin solution for injection. The spectrophotometric measurements were done at $\lambda = 284$ nm.

3. Results and discussion

Application of derivative spectrophotometry requires three stages of optimalization:

1. Optimalization of chemical parameters: concentration of reagents, time of reaction etc.
2. Optimalization of apparatus parameters: spectral bandwidth, speed of spectrum scan, integration time;
3. Optimalization of mathematical parameters like order of derivative, polynomial degree and width of 'derivatisation window' ($\Delta\lambda$).

All spectrophotometric measurements were carried out using diode array spectrophotometer supplied with series of 328 individual photodiodes. This type of apparatus does not allow to change measurement parameters, so the working conditions of the spectrophotometer were: integration time 1 s, spectral bandwidth 2 nm, spectrum scan 0.1 s.

For developing a derivative-spectrophotometric method, the Savitzky-Golay algorithm has been used. The application of this procedure demand to optimize such mathematical parameters, like $\Delta\lambda$, order of derivative and polynomial degree. For this purpose, the spectrum of 5 ppm coenzyme Q_{10} in n-hexane solution has been recorded and then subjected to mathematical treatment.

Using base-to-peak technique, following parameters were chosen: $\Delta\lambda = 14$ nm, first order of derivative, and fifth polynomial degree.

As can be seen from Fig. 1, the zero-order absorption spectra of ubiquinone shows two absorption bands at 217 and 270 nm. The first-order derivative spectrum exhibits four peaks at 198, 204, 256 and 284 nm.

The peaks at lower wavelengths were ignored, because of possible matrix interferences. The negative peak at 284 nm has been chosen for the quantitative determination.

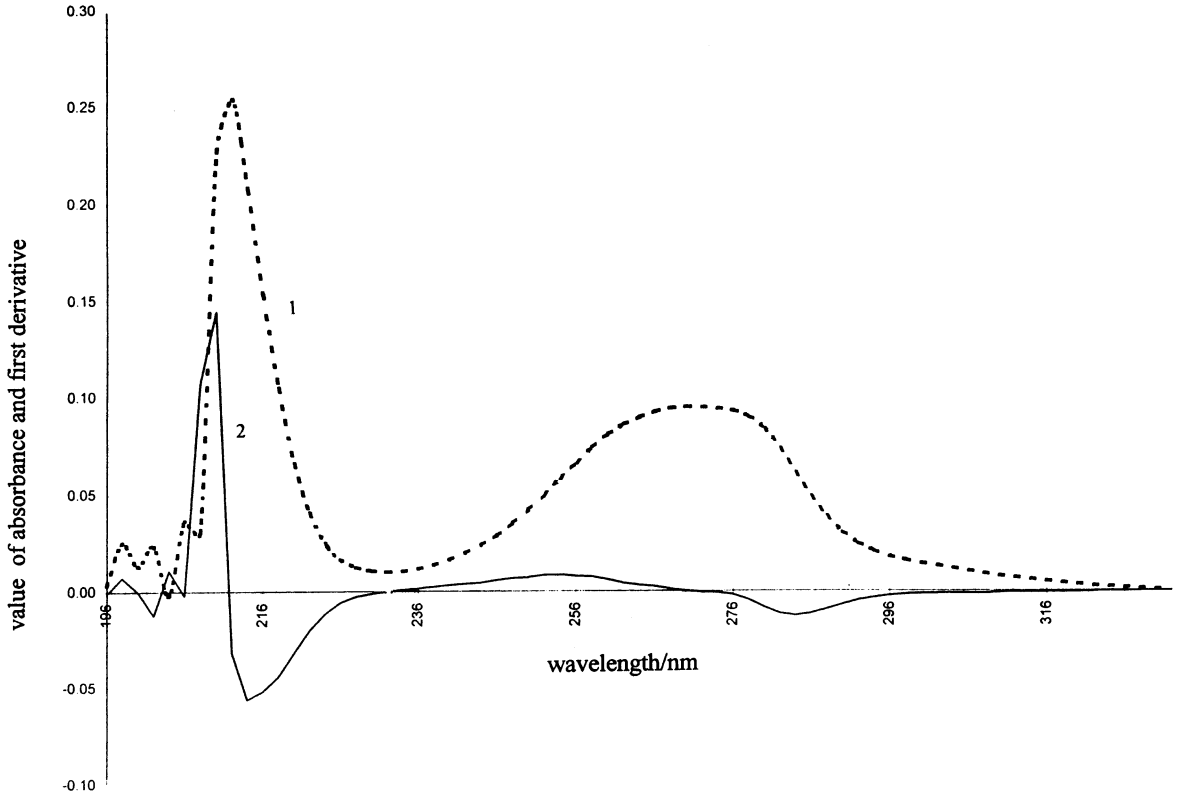

Fig. 1. Zero-order and first derivative spectra of coenzyme Q_{10} solution (5 ppm). 1, zero-order spectrum; 2, first-derivative spectrum.

3.1. Statistical analysis of data

Under the experimental conditions described above, the relationship between $^1D_{284}$ and Q_{10} concentration was examined. The linearity for standard solutions in the range 0.25–10 ppm was obtained. The regression equation and statistical study are given in Table 1. The derivative spectrophotometric methods are very susceptible to influences of matrix contents. The influence of some matrix components and the way of solutions preparation on linearity and repeatability of results were checked. For this purpose, two additional calibration curves were constructed. The first was prepared using standard solutions consisted pharmaceutical preparation ingredients, such glucose, soya lecithin and others. In the presence of excipients, the spectral analysis became very complicated. The simplification of matrix appeared to be necessary. The applied extraction procedure, removed only water dissolved ingredients. In this way, the matrix was simplified by extraction procedure but non-polar substances (soya-lecithin) was extracted with Q_{10} into n-hexane phase. The examined derivative spectrophotometry method allowed to determine coenzyme contents in pharmaceuticals in presence of extracted excipients in the range 0.5–10 ppm of Q_{10}.

The method was validated for determination of Q_{10} level in human plasma samples. This task demanded to construct calibration curve using standard solutions supplemented with 0.5 ml of 5% human albumin. Such approach allowed to eliminate the influence of random errors connected with sample preparation procedure. Because, the physiological level of coenzyme Q_{10} do not exceed 1.5 ppm [2,4], the calibration curve

Table 1
Analytical appraisals of presented method

Method	Linearity ppm	Regression equations	Correlation coefficient	RSD	Limit of detection[a] (u)
Standard solution	0.25–10	$^1D = 2.8 \times 10^{-3}x + 4 \cdot 10^{-4}$	0.9992	4.1% (at 0.5ppm) 0.36% (at 5ppm)	0.2 ppm
Determination in capsules	0.25–10	$^1D = 2.2 \times 10^{-3}x + 2 \cdot 10^{-4}$	0.9999	4.5% (at 0.5 ppm) 0.5% (at 5 ppm)	0.04 ppm
Determination in plasma	0.05–1.5	$^1D = 1.4 \times 10^{-2}x + 2 \cdot 10^{-4}$	0.9997	3.3% (at 0.1 ppm) 0.5% (at 1 ppm)	0.02 ppm

[a] The limit of detection was calculated using formula:

$$u = \frac{S_{x/y}}{a}$$

where $S_{x/y}$ is deviation of regression, and a is slope of regression line.

was constructed only up to 1.5 ppm. Equations and statistical estimation of those are shown in Table 1.

3.2. Application

The described methods were applied to some real samples: the pharmaceutical preparation of coenzyme Q_{10} 'Vita Care' and the pooled human plasma. The obtained results were compared in the first case with declared value. In the case of Q_{10} determination in plasma, the results were compared with those obtained by HPLC method. The results are assembled in Tables 2 and 3.

Table 2
Determination of coenzyme Q_{10} contents in Vita Care capsules ($n = 5$).

Declared value (mg)	Determined by proposed method (mg)	Relative error[a] (%)
10	9.950	−0.50
10	9.950	−0.50
10	10.065	+0.64
10	10.065	+0.64
10	9.950	−0.50

[a] In relation to declared value.

3.3. Conclusions

From the presented results, it appears that the proposed method can be an alternative method for coenzyme Q_{10} determination. The main advantage of derivative method is significantly shortening of analysis times, low cost of analysis, and widespread access to apparatus. The derivative spectrophotometry provides results comparable with those obtained by HPLC method. It can be applied in every laboratory. The relatively large volume of sample, especially in plasma determinations, seems to be the main disadvantage of described method.

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Table 3
The determination of coenzyme Q_{10} in human plasma

Value obtained by proposed method (ppm) ($n = 3$)	Value obtained by HPLC method (ppm) ($n = 3$)	Relative error[a] (%)
0.207	0.210	−1.40
0.061	0.060	−1.70
0.177	0.180	−1.70

[a] In relation to HPLC method.

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