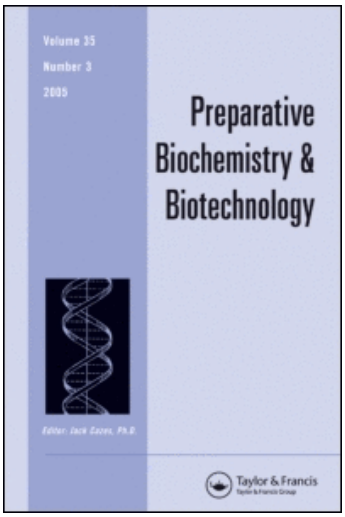


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# FT-IR Spectrophotometric Analysis of Coenzyme Q10 (CoQ10) and its Pharmaceutical Formulations

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# FT-IR Spectrophotometric Analysis of Coenzyme Q10 (CoQ10) and its Pharmaceutical Formulations

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**Abstract:** A Fourier transform infrared (FT-IR) spectrometric method was developed for the rapid, direct measurement of coenzyme $Q_{10}$ ($CoQ_{10}$) in different pharmaceutical products. Conventional KBr spectra were compared for the best determination of active substance in drug preparations. Lambert-Beer's law and two chemometric approaches, partial least squares (PLS) and principal component regression (PCR+) methods, were used in data processing.

**Keywords:** FT-IR analysis, Coenzyme $Q_{10}$, Drug analysis, Chemometric approaches

## INTRODUCTION

Infrared spectrophotometry (IR) provides a useful way for the identification of drugs.[1–4] However, the traditional techniques employed to obtain the IR spectra, such as alkali halides disks, mulls, and thin films, are not always

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adequate for quantitative analysis, but the help of Fourier Transform (FT-IR) permits continuous monitoring of the spectral baseline and simultaneous analysis of different components of the same sample.[5,6].

Coenzyme $Q_{10}$ ($CoQ_{10}$; 2,3-dimethyoxy-5-methyl-6-decaprenyl-1,4-benzoquinone, Fig. 1, also known as ubiquinone, is a lipid soluble compound that is mainly located in the mitochondria and acts as an electron carrier in the electron transport chain,[7] so it is essential for the production of cellular energy in the form of phosphate adenosine triphosphate (ATP). Moreover, $CoQ_{10}$ has been studied as an antioxidant agent and, together with other lipophilic antioxidants, $CoQ_{10}$ plays an intrinsic role in protecting circulating lipoproteins against oxidative damage.[8] Therefore, its concentration in lipoproteins and plasma may be a useful marker of oxidative stress and antioxidant defense. Since $CoQ_{10}$ can be used as a food supplement or as an adjunctive therapy in several diseases,[9,10] it is necessary to assay plasma levels of $CoQ_{10}$ to monitor the bioavailability of orally administered $CoQ_{10}$.

Many analytical methods have been reported for quantitative determination of $CoQ_{10}$ in human plasma, such as spectrophotometric,[11,12] voltammetric,[13] and chemiluminescent.[14] Among these techniques, the most popular tools used for assaying is HPLC,[15–21] because of the complexity of biological fluid specimens which usually require samples to be purified before injection. Thin layer chromatography (TLC) and the solid phase extraction (SPE) pre-treatment method have been described in the published literature.[17–20] However, these manual purification methods are time consuming and expensive, and they are unsuitable for routine determination, especially for clinical chemistry laboratories.

The purpose of the present study is to use FT-IR spectrophotometry to investigate the possibility of quantifying $CoQ_{10}$, in pharmaceutical preparations. Two different pharmaceutical formulations were used, namely: Leiner Health - sample A, containing about 50 mg $CoQ_{10}$ per tablet, and Super Bio-Quinone $Q_{10}$ - sample B, containing about 30 mg $CoQ_{10}$ per tablet. The main objective of this work was to develop a chemometric procedure for the fast and accurate determination of $CoQ_{10}$ in commercial pharmaceutical formulations, using Lambert-Beer's law and/or PCR+ and PLS approaches, thereby reducing the sample pre-treatment and providing an FTIR measurement.

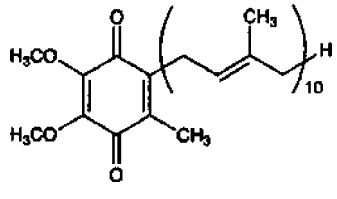


***Figure 1.*** Structure of coenzyme$Q_{10}$.

## EXPERIMENTAL

### Apparatus

Data acquisition was performed using a Spectrum 1000 FT-IR spectrometer equipped with Spectrum for Windows v.5.01 (Perkin Elmer Co., Beaconsfield, Bucks, UK). The commercial software used to generate analysis for the principal component analysis were QUANT+ expert v.4.51 and Spectrum Beer's law v.2.01 (Perkin Elmer Co. UK).



### Reagents and Materials

Coenzyme $Q_{10}$ was purchased from Sigma Chemical Company, St. Louis, MO, USA. The pharmaceutical formulations were obtained and manufactured by Leiner Health Products Inc., Carson, CA, USA. (sample A), from Pharma Nord - Romania and manufactured by Quisser Pharma Germany (sample B), respectively. The principal excipients used in the tablet formulation are maltodextrin (MDex), for sample A, and soy(a) bean (*Soja hispida*) oil (SBO) for sample B. Other excipients are gelatin, magnesium stearate, d-$\alpha$-tocopherol, talc, glycerol, and ferric oxide as colorant for sample B.

### Recommended Procedures

FT-IR spectra were recorded with different resolutions. The spectra were scanned between 4,000 and 400 $cm^{-1}$, by averaging 64 scans for each sample, with a resolution of 4 $cm^{-1}$ (data point resolution/interval 1 $cm^{-1}$) and with a resolution of 8 $cm^{-1}$ (data point resolution/interval 2 $cm^{-1}$), respectively. Accordingly, two sets of spectra were obtained for each sample. Background spectra were obtained for each experimental condition.

Experimental parameters, such as resolution and calibration method, (PCR+, PLS1 or PLS2, respectively) were compared and recommendations for the best options in $CoQ_{10}$ the analysis were made.

Conventional fused KBr disk spectra were recorded with a DTGS detector from samples prepared by compressing a 0.3% mixture of standard substance with spectral grade KBr. Using a conventional fused KBr disk for calibration, the spectra were recorded with a DTGS detector from samples prepared by compressing the standard substance – $CoQ_{10}$ (0.2 mg, 0.4 mg, 0.6 mg and 1.0 mg, respectively) in spectral grade KBr.

For the pharmaceutical dosage formulations, the spectra were recorded in two different ways:

1. Conventional fused KBr disk spectra were recorded with a DTGS detector from samples prepared by compressing 2.0 mg of drug sample with

spectral grade KBr, while the background was spectral grade KBr ($CoQ_{10}$_KBr - for sample A);

2. Conventional fused KBr disk spectra were recorded with a DTGS detector from samples prepared by adding a drop of drug sample onto a fused KBr disk, while the background was spectral grade KBr ($CoQ_{10}$_KBr_SBO - for sample B).

## RESULTS AND DISCUSSION

Figures 2 and 3 present the spectra of $CoQ_{10}$ as standard (Fig. 2, 2 mg) and for the pharmaceutical preparations.

Determination of the major component in drugs with FT-IR spectrometry provides an enormous amount of spectroscopic information for a sample. Chemometric methods, such as principal component regression (PCR+, Improved Principal Component Regression) and partial least squares (PLS2, Multicomponent Partial Least Squares) analyses are commonly used to extract the specific information relevant to the analyte of interest from the full spectrum.[1,22] These two techniques yield more accurate calibration models, compared with multiple linear regression (MLR), where a restricted set of absorption bands is used in the calibration. A good introduction to the partial least squares (Projection to Latent Structures, PLS) method is given by Geladi and Kowalski.[23]

In PCR and PLS2, the spectra are modeled by one set of factors; each property is modeled by relating the concentration values to those factors.

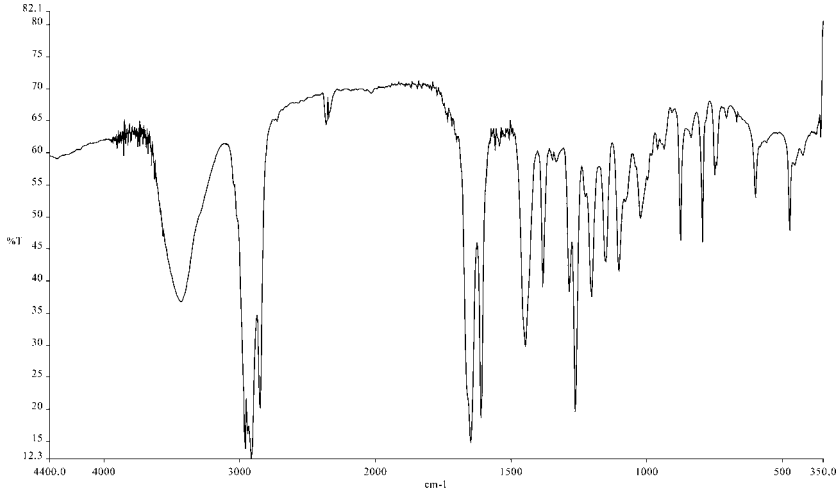


***Figure 2.*** FT-IR spectrum of $CoQ_{10}$ standard in KBr-disk.

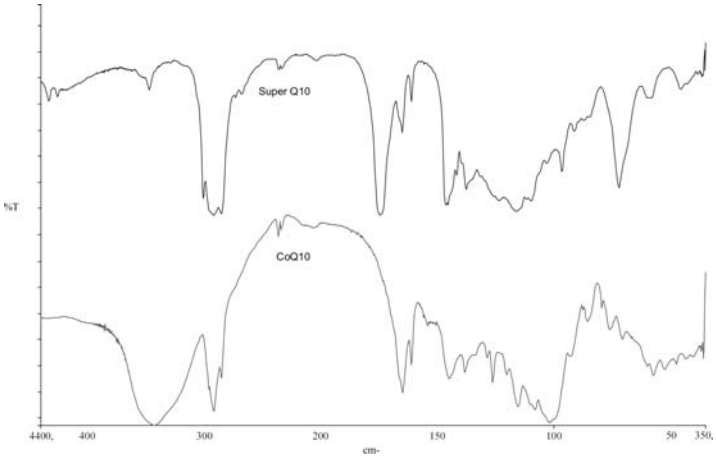


***Figure 3.*** FT-IR spectra of pharmaceutical preparations containing $CoQ_{10}$ (in KBr-disk). (upper) Leiner Health ($CoQ_{10}$, containing 50 mg active ingredient per tablet); (lower) Super Bio-Quinone $Q_{10}$ (Super $Q_{10}$, containing 30 mg active ingredient per tablet).

In PLS1, the spectra are modeled by a different set of factors for each property and the concentration values are modeled by the respective factors. Hence, PLS1 really contains *n* separate calibrations, where *n* is the number of properties in the method.

Our intent was to develop a quantitative method for $CoQ_{10}$ determination in pharmaceutical products using PCR+ and/or PLS approaches failed. The results obtained are much higher than the expected values, taking into account that the determination was made possible by using Lambert-Beer's law.

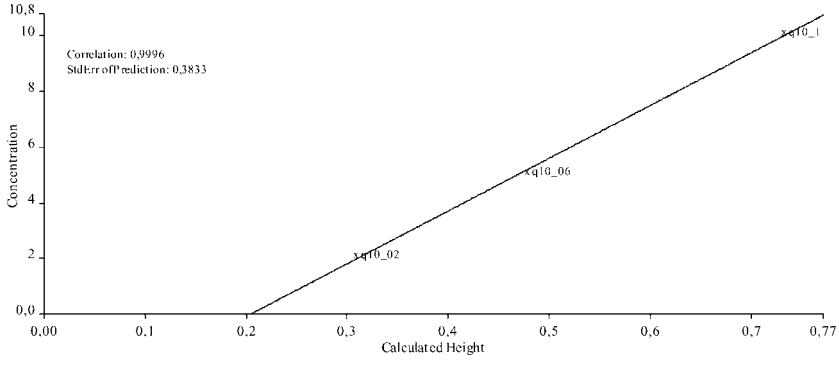


***Figure 4.*** Lambert-Beer's law calibration curve for $CoQ_{10}$ quantitative determination in pharmaceutical preparations.

***Table 1.*** Comparison of the $CoQ_{10}$ determination in tablets using FT-IR Lambert-Beer's law

| | |
|---|---|
| | Sample A |
| Content (mg/tablet) | 47.475 |
| RSD (%) (n = 5) | 3.85 |
| | Sample B |
| Content (mg/tablet) | 28.775 |
| RSD (%) (n = 5) | 2.94 |
| Calibration parameters | |
| Slope | 18.9521 |
| Intercept | − 3.8774 |
| Correlation coefficient | 0,9996 |

We studied the possibility of using the Lambert-Beer's law for quantitative determination of $CoQ_{10}$ in pharmaceutical products. The measurements, carried out under the above mentioned conditions, provide a typical calibration line which corresponds to:

$$A = -3.8774 + 18.9521C_{(mgCoQ10)}$$

with a regression coefficient, R, of 09996.

Figure 4 presents the calibration curve for coenzyme $Q_{10}$, while Table 1 includes the results.

## CONCLUSIONS

It is clear that FT-IR spectrophotometry is capable of providing the direct determination of $CoQ_{10}$ in its pharmaceutical formulations. With the commercial software, involving chemometric approaches, Lambert-Beer's law, the proposed method is simple, precise, and is not time consuming when compared to the chromatographic methods. Quantification could be done in about 5-10 minutes, including the sample preparation and spectral data acquisition.

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