

ORIGINALES

## Coenzyme Q10 stability in pediatric liquid oral dosage formulations

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### Abstract

**Objective:** To study the stability of Coenzyme Q10 (CoQ10), used as therapeutic agent in treatment of cardiovascular and mitochondrial diseases, in three liquid formulations: two soybean oil solutions (50 mg/ml), one of them with the addition of vitamin E and an O/W emulsion (20 mg/ml) for pediatric use. Furthermore, optimize and validate a stability-indicating HPLC method for the analysis of CoQ10 in the studied formulations.

**Method:** All samples were stored at 25°C. CoQ10 content of each formulation was analyzed in duplicate using fast microbore high performance liquid chromatography (Micro HPLC) at 0, 3, 6, 15, 30, 60 and 110 days.

**Results:** All formulations stayed stable at 25°C during the 110 days of the study. However, the oil solutions presented greater content variations through all the study period.

**Conclusions:** The CoQ10 emulsion can be stored for at least 110 days at 25 °C and it has proven to be safer when narrow dose adjustment is required. The proposed analytical method was suitable for the study of stability of different formulations meeting the validation parameters according to international guidelines.

### KEYWORDS

Coenzyme Q10; Pediatric; Formulation; Stability; HPLC

### Estabilidad de Coenzima Q10 en formulaciones líquidas orales para pediatría

### Resumen

**Objetivo:** Estudiar la estabilidad de Coenzima Q10 (CoQ10), utilizada como agente terapéutico en el tratamiento de enfermedades cardiovasculares y mitocondriales en 2 soluciones con aceite de soja como vehículo (50 mg/ml), una de ellas con el agregado de vitamina E y una emulsión O/W (20 mg/ml) para uso pediátrico. Asimismo, optimizar y validar un método indicativo de estabilidad por HPLC aplicado al análisis de CoQ10 en las respectivas formulaciones estudiadas.

**Método:** Todas las muestras fueron almacenadas a temperatura ambiente (25 °C) y su contenido fue analizado utilizando Micro HPLC. Cada muestra fue analizada por duplicado a los 0, 3, 6, 15, 30, 60 y 110 días.

**Resultados:** La coenzima Q10 se mantuvo estable en las formulaciones durante los 110 días a una temperatura de 25°C. Sin embargo, se detectó una mayor variación en las concentraciones obtenidas para las dos soluciones en aceite de soja.

**Conclusiones:** La emulsión O/W de CoQ10 puede ser almacenada por al menos 110 días a 25°C y demostró ser más segura cuando se requiere ajustar la dosis. El método analítico propuesto fue adecuado para realizar el estudio de estabilidad de las distintas formulaciones cumpliendo con los parámetros de validación acorde a las guías internacionales.

### PALABRAS CLAVE

Coenzima Q10; Formulación; Pediátrica; Estabilidad; HPLC

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## Introduction

Availability of a wide range of dosage forms and concentrations of pharmaceutical drugs seems not to be a problem when it comes to treating adult patients. However, the administration of these pharmaceutical formulations is not always possible when the affected patients are children. Most pharmaceutical forms are usually intended to be consumed by adults, and therefore pediatric patients, addressing the same health problems, probably will not tolerate high drug concentrations since there is a significant difference in body weight and hormonal activity. Using solid dosage forms is not the best option because most children find them hard to swallow and when the dose needs to be adjusted by body weight, it is more likely to fail to administer the exact amount of active, so the development of extemporaneous oral liquid formulations, which are commonly accepted by children, is usually the chosen path<sup>1,2</sup>.

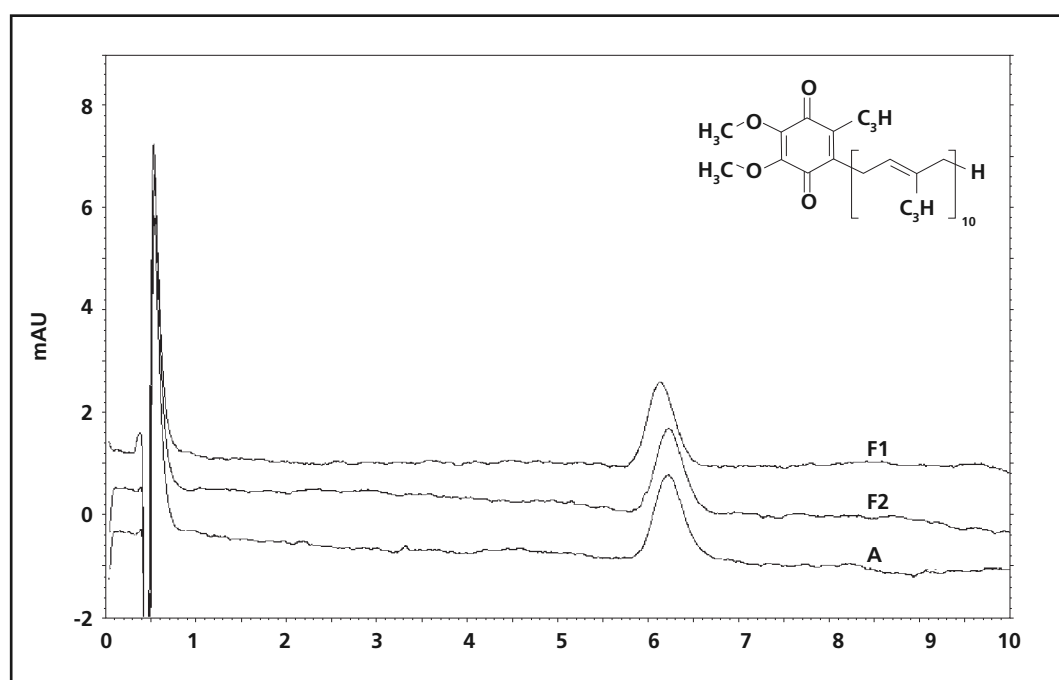
Coenzyme Q10 (CoQ10), also known as ubiquinone or ubidecarenone (Figure 1), is a lipophilic molecule classified as a fat soluble quinone<sup>3</sup>. It is the only lipid soluble antioxidant synthesized endogenously and is present in all cellular membranes and in blood, both in high- and in low-density lipoproteins. It plays an important role in cellular metabolism, participating as an electron carrier in both mitochondrial and extra mitochondrial membranes, and also protects membranes and lipoproteins from protein oxidation and lipid peroxidation<sup>4</sup>.

CoQ10 deficiency is involved in cardiomyopathies and degenerative muscle and neuronal diseases. Most patients with these deficiencies have shown clinical improvement with oral CoQ10 supplementation. Oxidative stress has

also been implicated in many disorders, diabetes, cancer and cardiovascular diseases as chronic heart failure and hypertension, suggesting that oral CoQ10 might be a viable antioxidant strategy for these diseases<sup>4</sup>.

CoQ10 is a compound soluble in organic solvents and lipids but practically insoluble in water. It is poorly absorbed from gastrointestinal tract and its slow absorption can be explained by its high molecular weight and poor solubility. Thus, to improve CoQ10 solubility and bioavailability, a variety of formulations have been developed. Most commonly formulations are based on the powder, tablets, two-piece capsules or softgel capsules containing an oil suspension. Currently, crystalline CoQ10 powder, oil emulsions, solubilisates of CoQ10 and nanoparticulate formulations are available<sup>5-7</sup>.

Extemporaneous or magistral preparation describes the manipulation by pharmacists of various drugs and chemical ingredients using traditional compounding techniques to produce suitable medicines when no commercial form is available. The use of these techniques is widespread in pediatric pharmacy practice<sup>1</sup>. Pediatric practice requires a range of dosage forms that are acceptable at different ages and weight and a range of strengths or concentrations allowing administration of the correct age-related dose<sup>2</sup>. Oral liquid formulations can be prepared by dilution of existing liquid dosage forms but only if parameters such as excipients and pH are suitable orally<sup>8</sup>. Another way to prepare them is to use raw materials/chemicals, choosing the necessary excipients and vehicles to assure solubility, homogeneity and no adverse effects, as well as protection of actives during shelf life from degradation. Glass BD and Haywood A performed a research on stability studies of liquid dosage forms and found out that of the liquid



**Figure 1.** Chemical structure of CoQ10 and chromatograms of CoQ10 standard and formulations, placed on the same pair of axis. A: CoQ10 standard (2 µg/ml), F1: CoQ10 (2 µg/ml) oil solutions, F2: CoQ10 (2 µg/ml) O/W emulsion.

dosage forms reviewed in the literature, stability was considered to be unfavorable for only 6 of 83 dosage forms considered - a small percentage, illustrating that there is minimum risk associated with these dosage forms and that pharmacists taking cognizance of various factors such as drug stability, mechanisms and routes of degradation, and potential interactions with excipients in the tablets and/or capsules utilized in the formulation are further able to minimize the risk involved<sup>9</sup>.

The aim of this study was to develop two oil solutions, one of them with the addition of vitamin E and one O/W emulsion of CoQ10 at different concentrations, optimize and validate a stability-indicating HPLC method for the CoQ10 analysis using microHPLC and then study the chemical stability of CoQ10 in the proposed extemporaneous formulations stored at room temperature over 110 days.

## Material and methods

### Material

Two CoQ10 oil solutions (50 mg/ml) were prepared by solubilising CoQ10 powder (Prest S.A batch: AHK-1115) in soybean oil (Gersoja, batch: LENV141210) at 40°C. It was then added 0.05% w/v of Vitamin E (Rosenco S.R.L batch: 71543224UO) to one of the oil solutions. The vehicle of the O/W emulsion was prepared with xanthan gum 0.25% w/v (Magel S.A. batch: 585/2007), soybean oil 45%, syrup 30%, distilled water 23.85%, methylparaben 0.08% (Magel S.A. batch: IA2011), propylparaben 0.02% (Chutrau, batch:L11814), sodium saccharin 0.3% (Van Rossum, batch:80118) and orange essence 0.5% (Prest S.A., batch:9206). The first step was to solubilize the CoQ10 powder in soybean oil and in second place the orange essence. Then, xanthan gum and syrup were added to the oil solution to obtain a primary emulsion by mixing with an automatic mixer for 5 minutes. Methylparaben, propylparaben were solubilized in distilled water at 90°C. When the solution reached room temperature, sodium saccharine was dissolved. This resultant solution was added to the primary emulsion. Finally, everything was mixed with an automatic mixer for 15 minutes. The final CoQ10 concentration of the O/W emulsion was 20 mg/ml. All the trial formulations were stored in amber glass vials and kept at controlled room temperature (25°C).

### Analytical method

The chromatographic system consisted of an isocratic solvent delivery pump (Thermo Scientific SpectraSystem P4000), with a 50 mm × 2.1 mm reverse phase micro column C18 particle diameter 3.5 µm (Waters X-Terra). Methanol was used as mobile phase with a flow of 0.4 ml/min. The column temperature was set at 25°C. Ten microliter of each sample was introduced into the

column using an automatic injector (Thermo Scientific SpectraSystem AS3000). The column effluent was monitored with a dual wavelength ultraviolet detector (Thermo Scientific SpectraSystem UV2000) set at 275 nm<sup>10-11</sup>. Two aliquots were collected from each container on days 0, 3, 6, 15, 30, 60 and 110. These were diluted with ethanol, sonicated for 30 minutes and finally diluted with mobile phase to obtain a concentration of 2 µg/ml, the resultant solutions were immediately analyzed. A standard solution was prepared solubilizing CoQ10 in ethanol to obtain a concentration of 1 mg/ml, then an appropriate dilution was made in blank of excipients of each formulation and finally in mobile phase. In all cases, the final concentration of the CoQ10 working standard solutions was 2 µg/mL.

## Results and Discussion

### CoQ10 Formulations

The development of pharmaceutical formulations for pediatric patients is a unique challenge. The proper design and formulation of dosage form requires consideration of the physical, chemical and biological characteristics of the active drugs and pharmaceutical excipients. Pharmaceutical actives and excipients must be compatible with one another to produce formulations effective, stable, well tolerated, easy to administer and with good palatability. Thus, liquid formulations rather than solid dosage form are preferred for oral administration to children<sup>12-13</sup>.

In the case of the active drugs with physicochemical characteristics of poor aqueous solubility, high hydrophobicity and consequently poor absorption like CoQ10, various formulation strategies are used. In this sense, oil solution formulation is a simple and good alternative<sup>5</sup>. Moreover, oil-in-water lipid emulsions are utilized as carriers of highly lipophilic drugs<sup>14</sup>. Thus, two oil-solutions and an O/W emulsion were developed to vehiculize CoQ10 for pediatric patients.

CoQ10 is soluble in different oils such as peanut oil, corn oil, soybean oil. However, Weiss et al tested the bioavailability of different formulations containing CoQ10, and a suspension of CoQ10 in soybean oil showed the highest bioavailability<sup>15</sup>. Moreover, soybean oil has been used in emulsions as vehicle for oral and parenteral administration of actives such as diazepam, steroids and vitamins<sup>16,17</sup>. Although other oils such as olive or peanut have been used to vehiculize drugs, soybean oil is preferred because is associated with fewer adverse effects<sup>4</sup>.

The other oil solution has in its formulation vitamin E, because it has been demonstrated that the association between CoQ10 and other antioxidants like vitamin E in subtherapeutic levels increases the absorption of CoQ10 by the body<sup>18</sup>.

Considering the O/W emulsion, xanthan gum is used as emulsifying agent. USPNF 32 describes the xanthan gum as a high molecular weight polysaccharide. It contains D-glucose and D-mannose as the dominant hexose units, along with the D-glucuronic acid. It is widely used in oral and topical pharmaceutical and cosmetic formulations as suspending and stabilization agent. Xanthan gum is non-toxic, compatible with most other pharmaceutical ingredients, and has good stability and viscosity properties in a wide range of pH and temperature<sup>17,19</sup>.

Saccharin is an intense sweetener used in beverages, food products, tabletop sweeteners and oral hygiene products such as toothpastes and mouthwashes. In oral formulations is used at a concentration of 0.02 to 0.5% w / w. Saccharin can be used to mask unpleasant tastes or improve the taste of different systems. Its sweetening power is approximately 500 times greater than sucrose<sup>17</sup>. Parabens are widely used as antimicrobial preservatives in cosmetics, foodstuffs and pharmaceutical formulations. They are used alone or in combination with parabens or other antimicrobial agents. Parabens are effective over a wide pH range and have a broad spectrum of antimicrobial activity. The antimicrobial activity increases as the length of the alkyl chain is greater, but decreases the aqueous solubility, therefore a mixture of parabens is often used to achieve an effective preservation<sup>17,20</sup>.

## Analytical method

Different analytical methods have been applied to the analysis of CoQ10 in different matrices like human plasma using HPLC coupled to the electrochemical detector (ECD), UV-detector or mass spectrometry<sup>21-24</sup>. Although, HPLC-ECD seems to be the most common method use to the analysis of CoQ10 due to its high sensitivity and selectivity, these methods are too time consuming or require several steps during the operation of the equipment, making them unsuitable for the routine analysis.

In previous studies, we have developed a micro HPLC method using a special column with reduced diameter and length and hybrid particulate filler to determine the content of CoQ10 in pharmaceutical and cosmetic formulations as well as in biological fluids and tissues. The proposed method was chosen because it requires shorter analysis times together with less solvent use and dramatic reductions of sample requirements with faster separations compared to columns of conventional diameter and length<sup>10-11</sup>.

The chromatograms corresponding to all three formulations are presented in Figure 1. CoQ10 retention time is 6.3 minutes. No degradation products were observed under the established conditions in all cases.

We evaluated the linearity of the method proposed by establishing a relationship between the concentrations and areas on the standard chromatogram. This is shown by linear regression models obtained for each of the two standard preparations. Linearity was verified at 5 concentration levels (1.00, 1.50, 2.00, 2.50 and 3.00 µg/mL)

**Table 1.** Parameters of validation of method of analysis of CoQ10

Parameter	Oil solutions	O/W Emulsion
Linear range (µg mL <sup>-1</sup> )	1.00 - 3.00 (y= 17369x + 1074.4)	1.00 - 3.00 (y=23336x - 3847.3)
r	0.9911	0.9965
LOD (µg mL <sup>-1</sup> )	0.01	0.01
LOQ (µg mL <sup>-1</sup> )	0.06	0.06
<i>Precision (%RSD)</i>		
Intra-day (n=6)		
Migration time	0.5	0.2
Peak area	2.1	1.5
Inter-day (n=18)		
Migration time	1.0	0.4
Peak area	2.9	2.2
<i>Accuracy</i>		
Spiked levels		
80%	93.9	102.9
100%	96.3	110.6
120%	90.7	106.2

**Table 2.** Mean percentage of initial CoQ10 concentration at 25°C for all three formulations (n=2)

Time (days)	A		B		C	
	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
0	100.0	0.3	100.2	0.7	100.1	0.5
3	101.8	0.7	103.0	3.1	93.0	3.7
6	94.9	1.14	99.3	5.1	101.9	7.4
13	104.9	0.74	85.1	0.1	90.4	1.5
29	102.3	0.05	109.9	1.4	104.5	2.9
57	97.7	0.45	111.5	1.5	105.0	3.0
110	98.5	3.13	97.6	6.8	102.9	6.8

A: CoQ10 O/W emulsion, B: CoQ10 oil solution, C: CoQ10 oil solution with Vitamin E

of CoQ10, prepared in blank of excipients for each formulation, and these were analyzed in duplicate in three separate runs. Limits of detection (LOD) and quantification (LOQ) were determined. Precision was evaluated for intraday (n=6) and inter-day assays (n=18) and it was expressed as %RSD for retention times and areas. Accuracy was evaluated from recovery studies of samples of CoQ10 from their matrix. Placebo samples prepared with all the excipients contained in each one of the different pharmaceutical formulations at concentration levels of 80, 100 and 120% (w/v) of the nominal values were spiked with CoQ10 (Table 1). All parameters were determined for each formulation.

Table 2 shows the stability results for each formulation, all of them stored at room temperature (25°C), expressed as mean percentage of initial CoQ10 concentration. Figure 2 is a graphical representation of these results. The oil solutions show significant variations of CoQ10

concentrations (up to 26%), whereas the O/W emulsion stays on a 10% range. At the end of the study (day 110), the amount of CoQ10 in all three formulations was between 97 and 103%.

## Conclusions

On this particular study, we found significant content variations through all the study period for the oil solutions, probably due to the vehicle chosen. This may potentially modify the amount of CoQ10 delivered to the patient, making them not suitable for pediatric practice in which more reliable dose adjustment is required. Moreover, no differences were found between the two oil solutions, with and without vitamin E.

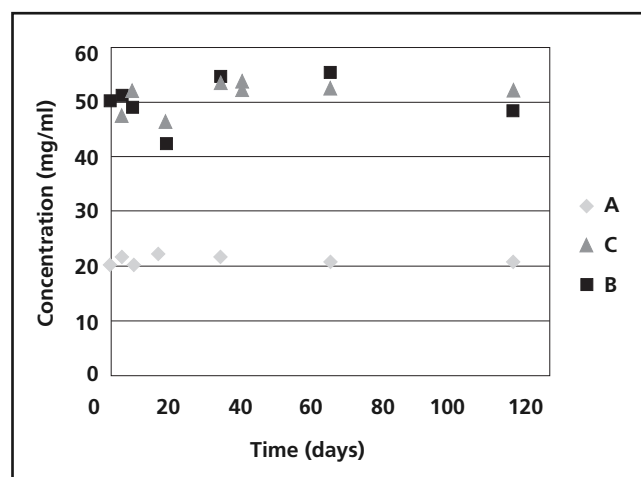
The O/W emulsion content instead was not significantly affected, making it the formulation of choice for pediatric administration of CoQ10. Stability data at controlled room temperature is very important since there is no need of using special storage conditions. In conclusion, the CoQ10 emulsion can be stored for at least 110 days at 25°C and it has proven to be safer when narrow dose adjustment is required.

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**Figure 2.** Graphical representation of CoQ10 content through all the study period. A: CoQ10 O/W emulsion, B: CoQ10 oil solution, C: CoQ10 oil solution with Vitamin E.

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