Ubidecarenone Quantification in Food Supplements
A new HPLC method

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Coenzyme Q10 has a powerful antioxidant effect because it protects cells against free radicals and plays an important role in metabolism. Although controversial some preliminary evidence suggests that Coenzyme Q10 can help prevent or treat treatment of diseases of the cardiovascular and musculoskeletal systems and liver problems. Age increase and medical conditions are associated with lower Coenzyme Q10. There are evidences that diet supplementation with supplements containing Coenzyme Q10 may be beneficial. This paper aims to develop an HPLC method for identifying and dosing Ubidecarenone in food supplements. Using this technique, several supplements containing varying Ubidecarenone, ranging from 76 to 103% of the stated amount per dosage form were tested.

Keywords: Ubidecarenone, HPLC method, dietary supplements

According to WHO Constitution, Governments are responsible for the citizen’s health condition and the highest level of health is a fundamental right of every human being [1]. At the moment, severe pathologies (cancer, cardiovascular, metabolic, neurodegenerative, dermatologic or infectious diseases etc.) have worldwide a higher prevalence [2-4]. Fortunately, today, people are more educated and have many information sources in order to improve their health status. Using different health products, people incline to obtain a better life quality or to prevent the risk of illness [2]. On the other hand, many consumers prefer natural, even organic products. In this respect, the responsible manufactures have established themselves to market safety and efficacy products [5-8]. Also, they use more and more natural and simple, common known molecules, whether we refer to dietary supplements, cosmetics, medical devices and even to medicines (coenzyme Q10, Aloe vera, resveratrol, hyaluronic acid, lipoic acid, omega-3, Ginkgo biloba, Echinacea, Hypericum, Sillybum marianum etc.) [2, 9, 10]. Additionally, producers use modern technologies to prepare pharmaceutical products and also, they have to elaborate and respect strict rules to assure the product’s quality. It isn’t neglected the analytical quality control which is made during production and to the end of process and also based on advanced techniques [11-15].

Coenzyme Q10 (CoQ10 or Ubidecarenone) is a biologically active compound that is similar in chemical structure to menaquinones (Vitamin K2). Part of a family of quinone compounds known as coenzyme Q, CoQ10 is characterized by a quinone ring attached to a repeating series of side-chain isoprene units (Fig. 1).

The number of isoprene units is denoted by the coenzyme-X designation. In the case of CoQ10, there are 10 repeating isoprene units [16, 17]. CoQ10 is synthesized in mitochondria by a set of at least 12 proteins that form a multiprotein complex. The efficiency of coenzymeQ10 depends on the level of glutathione in the body [18]. Beside the antioxidant capacity of CoQ10, other beneficial effects include:
- the prevention of lipid peroxidation initiation in plasma membranes
- the improvement of antihypertensive functions
- the prevention of low-density lipoprotein oxidation
- the treatment of migraine headache
- the treatment of cardiovascular diseases
- the treatment of neurodegenerative disease (Parkinson’s Disease) [19-24].

Coenzyme Q10 has multiple uses due to its antioxidant role. It is used in dietary supplements in combination with other active principles, in anti-aging cosmetics, in improving the blood cardioplegia solution to compensate its flaws and to offer maximum security in case of myocardial protection [25-32].

To estimate the Ubidecarenone content from foods and foods supplements a lot of methods were developed over the years. Many papers were published describing quantitative determination of Ubidecarenone in different matrices (pharmaceutical dosage forms, biological fluids, tissues, foodstuff, etc): HPLC with different detection [32-43], zero-order and second-order derivative spectrophotometry [44, 45], voltammetry [46], 1H NMR spectroscopy [47], FT-NIR spectroscopy [48] and X-ray diffraction [49].

High performance liquid chromatography is a modern method used to assay a lot of active principles in food supplements and also to determine the content of active substances in drug products [50, 51]. In this study a HPLC method for Ubidecarenone assay was developed.

Experimental part
Materials, reagents and standards
For the assay of Ubidecarenone there were analysed five food supplements. A large number of CoQ10 products are available on the market. These products are soft gel
A small number of products use 2-piece gelatine hard shells or CoQ10 in a tablet formulation. The products analysed were soft gel capsules and hard gelatine capsules purchased from Romanian market. The samples were coded as follows:

- PB00A - is presented as hard gelatine capsules containing 10 mg of Q10 coenzyme/caps., 128.25 mg proanthocyanidines from grape seeds extract, 800 µg retinol, 8 mg Tocopherol and 50 mg/caps. of selenium;
- PB00B - is presented as soft gelatine capsules containing 60 mg/caps. of Q10 coenzyme, 150 mg/caps. of magnesium, 15 mg/caps. of Resveratrol and 3 mg/caps. of pyridoxine;
- PB00C - is presented as hard gelatine capsules containing 60 mg of Q10 coenzyme/caps and 95 mg proanthocyanidines from grape seeds/caps. and 50 mg Resveratrol/caps.;
- PB00D - is presented as soft gelatine capsules containing 60 mg of Q10 coenzyme/caps.;
- PB00E - is presented as hard capsules containing 10 mg of Q10 coenzyme/caps. and 300 mg/caps. lipoic acid.

**Sample and standard solutions preparation**

The average mass was performed on 20 capsules from each product. The sample solution for hard and soft gelatine capsules was prepared using 25 mg of content from 20

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**Figure 2. Retention times of Ubidecarenone for samples and standard chromatograms**
capsules which was transferred into a 20 mL volumetric flask and dissolved with ethanol on a Julabo TW12 water bath at 50°C for 2 min.

The standard solution was prepared in the same manner using 5 mg of Ubidecarenone CRS (99.6%) from EDQM. The standard and sample solutions were previously filtered using 0.45 micron filters before injection.

The standard solution was injected six times and each sample solution was prepared in triplicate and injected once. The solvents used for solutions and for mobile phase preparation were of HPLC analytical purity from VWR.

**Equipment and chromatographic conditions**

The identification and assay of Ubidecarenone was performed using a HPLC method developed on the basis of the European Pharmacopoeia.

The separation was achieved using a Zorbax SB-C18, 150 x 4.6 mm, 100 Å, at 25°C temperature with an isocratic mixture of ethanol and methanol in the ratio of 40:60 % V/V at a flow rate of 2 mL/min and UV detection at 275 nm. The volume of injection used was 20 µL. The retention time of Ubidecarenone was around 11 minutes.

Chromatographic separation was performed by HPLC using an Agilent 1200 HPLC system consisting of a quaternary pump, degassing device, autosampler, diode array detector (PDA), and Agilent ChemStation software.

**Results and discussions**

Identification of Ubidecarenone in sample solutions was made by comparison of retention times and by spectral data of PDA with those obtained for the standard solution as is shown in Fig. 2 and Fig. 3.

The results of system suitability parameters such as tailing factor, symmetry and number of theoretical plates are indicated satisfactory results and tabulated in the table 1.

Quantification of Ubidecarenone in each product was performed on the basis of the external standard method against the average of 6 peak areas of standard solution chromatogram. The results are shown in Table 2.

The content of Ubidecarenone in the analyzed samples was found between 76 and 103 % from the claimed label potency. According to this data, the content of Ubidecarenone in samples PBO0A, PBO0C, PBO0D and PBO0E was close to the label content and in the sample PBO0B the content of Ubidecarenone was less than claimed.

The precision of Ubidecarenone was determined as the variation coefficient (RSD %) for six successive injections of the standard solution which was 0.032.

**Conclusions**

An adequate method was developed for the assay of Ubidecarenone in raw materials and finished products as soft gels and hard gelatin capsules. The proposed method is accurate, simple, cost-effective and less time-consuming.

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**References**
