[Frontiers in Bioscience, Scholar, 8, 321-330, June 1, 2016]

Coenzyme Q10 analytical determination in biological matrices and pharmaceuticals

Silvia Lucangioli[1,2], Manuela Martinefski[2], Valeria Tripodi[1,2]

[1]Consejo Nacional de Investigaciones Científicas y Tecnologicas, CONICET, Argentina, [2]Departamento de Tecnología Farmaceutica, Universidad de Buenos Aires, Buenos Aires, Argentina

TABLE OF CONTENTS

1. ABSTRACT

In recent years, the analytical determination of coenzyme Q10 (CoQ10) has gained importance in clinical diagnosis and in pharmaceutical quality control. CoQ10 is an important cofactor in the mitochondrial respiratory chain and a potent endogenous antioxidant. CoQ10 deficiency is often associated with numerous diseases and patients with these conditions may benefit from administration of supplements of CoQ10. In this regard, it has been observed that the best benefits are obtained when CoQ10 deficiency is diagnosed and treated early. Therefore, it is of great value to develop analytical methods for the detection and quantification of CoQ10 in this type of disease. The methods above mentioned should be simple enough to be used in routine clinical laboratories as well as in quality control of pharmaceutical formulations containing CoQ10. Here, we discuss the advantages and disadvantages of different methods of CoQ10 analysis.

2. INTRODUCTION

Coenzyme Q10 (ubiquinone, CoQ10) is a molecule composed of a 1,4-benzoquinone ring substituted with 3-methyl and 5,6-methoxy groups and a decaprenyl tail located at position 6 (Figure 1) and it is classified as a fat-soluble quinone. It is integrated into the inner membrane of the mitochondria acting as an electron carrier from complex I (NADH ubiquinone reductase), complex II (succinate ubiquinone reductase) and flavoprotein oxidoreductase to complex III (ubiquinol: cytochrome c reductase) in the mitochondrial respiratory chain (1).

CoQ10 is also considered as a potent endogenous lipophilic antioxidant that protects biological membranes and mitochondrial DNA from oxidative damage induced by free radicals as well as an effective inhibitor of lipoprotein peroxidation. Taking into account the important functions of CoQ10, decreased levels of this coenzyme affects brain, muscle and myocardial functions, primarily. However, the clinical importance of CoQ10 deficiency has only been recently considered. The causes of this deficiency have been classified as primary or secondary, the latter being much more frequent (2). Low levels of CoQ10 have been reported in a number of disorders including phenylketonuria (3), asthma (4), migraine (5), Friedreich's ataxia (6), cystic fibrosis (7), congestive heart failure (8), preeclampsia 9), eosinophilia gastrointestinal disease (10) and male infertility (11, 12).

Despite there is no wide consensus over the administration of CoQ10 in the treatment of mitochondrial disorders being accepted, clinical and biochemical improvements in the health of these patients have been observed. It is reasonable to hypothesize that if defects in energy metabolism and oxidative damage play a role in the pathogenesis of neurodegenerative diseases, a treatment with CoQ10 could exert beneficial therapeutic effects in cases of deficiency (13).

For these reasons, the detection and quantification of CoQ10 in different matrices such as plasma, muscle, platelets and fibroblasts are extremely

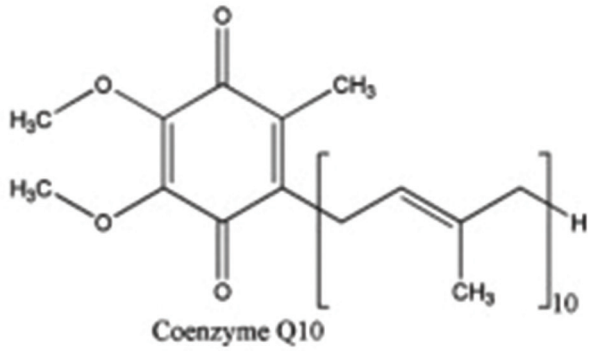

Figure 1. Structure of coenzyme Q10.

important tools not only for diagnosis but also for its after treatment monitoring. Today, the detection and quantification of CoQ10 in the abovementioned matrices have been included in a routine analysis of clinical chemistry laboratories.

CoQ10 physicochemical features like high molecular weight, high hydrophobicity and easy oxidation reactivity along with its low concentrations in biological matrices, make it analytically challenging. Furthermore, taking into account that CoQ10 has a rather complex chemical structure, pharmaceutical formulations must be prepared with special care in order to obtain an acceptable product in terms of bioavailability and efficacy. Product quality and stability as well as potential interactions of drug and excipients that could affect the manufacturing process must be thoroughly studied. A suitable analytical method must be highly sensitive and specific in order to detect low concentrations of the coenzyme molecule in complex matrices surrounded by a universe of interferences. The analytical method must also be simple, fast, inexpensive and appropriate for routine practice. Sample preparation must guarantee sufficient chemical stability with minimum losses. Several analytical methodologies for the determination of CoQ10 in different matrices have been updated in the last years, and they will be discussed in this chapter.

3. ANALYTICAL PROCEDURES

3.1. Sample preparation for CoQ10 extraction

Firstly, sample preparation methods required tedious isolation and purification steps to remove interfering compounds. CoQ10 is a lipophilic molecule, insoluble in water but soluble in hydrocarbons, ethanol, 2-propanol or 1-propanol. Addition of sodium dodecylsulfate (SDS) and subsequent extraction with ethanol, 2-propanol and hexane (14), repeated extractions with ethanol-hexane (15-17), methanol-hexane (18, 19) or 1 or 2-propanol-hexane (20) have been reported. It was observed that the extraction efficiency was improved by using 2-propanol or 1-propanol instead of ethanol. Although liquid-liquid extractions have always been taken as reference methods, a list of drawbacks could be outlined in the implementation of these techniques: tedious sample preparation, excessive solvent consumption and a broad solvent front that could interfere with chromatographic peak detection. In addition, when samples are not completely clear other undesirable events could take place like frit clogging, and pre- and analytical columns reduced lifetime. Sometimes, sample injection automatization is not possible due to sample precipitation. At last, limits of detection and quantitation achieved under the abovementioned conditions are not desirable. The extraction of CoQ10 from plasma samples using different organic solvents was tested by Jiang *et al* (21). They concluded that the use of methanol, acetonitrile and 10% trichloroacetic acid was not suitable because of the strong polarity of the mixture showing recoveries close to zero. On- and off- line solid phase extractions using silica or C18 cartridges have also been used instead of solvent exchange, clean up and concentration step (16, 22). These methods remove interferences but the sample clean up requires protein precipitation steps and they are time consuming and expensive. Other methods used 1-propanol in a single step achieving high recovery in a direct injection (21, 23-26). However, the successful direct injection depends on the sensitivity of the detector used, so an evaporation step should be added if volume reduction is desired. In that case, the evaporation under a stream of nitrogen or argon is highly recommended. Low pressure evaporation procedures, rotator evaporation, should be restricted to large volumes in order to preserve analyte stability.

Measurement of CoQ10 in biological samples has been hampered by analyte instability during sample handling, storage and processing due to its redox behavior. This problem could be minimized by shortening the time between sample collection and storage to less than 30 min (17). Otherwise, the sample could be supplemented with 1,4-benzoquinone solution to completely oxidize CoQ10 before protein extraction to ensure only one oxidation state (25). Another similar strategy is to collect the sample in the presence of EDTA. Finally, if the simultaneous determination of both oxidized and reduced forms of CoQ10 is needed, heparin should be added to the sample (27).

Another anticoagulant like sodium citrate could be used to analyze CoQ10 in plasma and platelets (28, 29) although CoQ10 measurements in serum samples have also been reported (30).

Measurement of CoQ10 in tissues is well known. The hallmark in the diagnosis of CoQ10 deficiency syndrome is a decreased CoQ10 concentration in muscle and/or fibroblast. CoQ10 tissue measurement could follow the same two extraction steps using methanol-hexane as it was previously described (31). In spite of

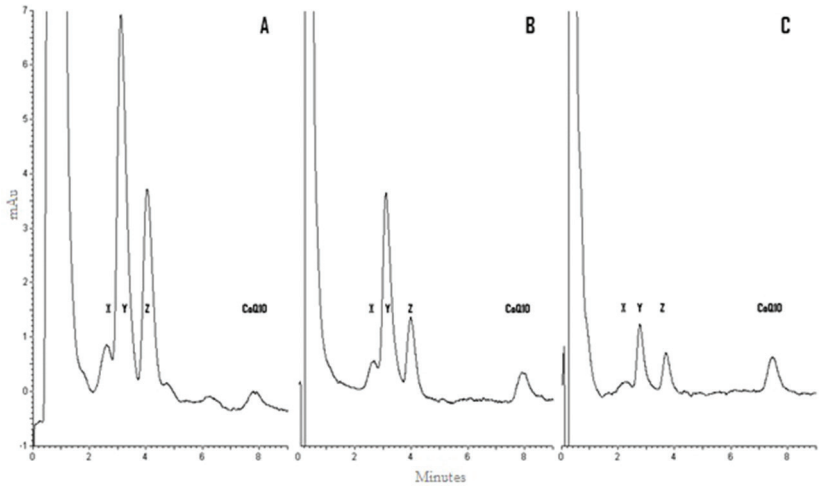

Figure 2. Shows chromatograms obtained of liver extract A After treatment with 1-propanol and direct injection. B C18 treatment. C MISPE procedure. X, Y and Z are endogenous contaminants. Reproduced with permission from ref 34.

being more sensitive, these sampling techniques are not only highly invasive but also too much traumatic if periodic clinical monitoring is needed. Plasmatic and platelet CoQ10 measurements might provide a useful estimation of CoQ10 deficiency (32). However, the relationship between plasma and tissue CoQ10 levels is not clearly comprehended and the way in which these samples are obtained is still invasive, especially for infants undergoing continuous monitoring of their treatment. In a recent work we demonstrated that the CoQ10 determination in buccal mucosa cells (BMC) is an alternative, non-invasive, and simple technique, with a significant correlation with CoQ10 plasma levels (33). The proposed method, based on the use of 1-propanol precipitation and a standardized cell count, is amenable to be applied, especially in infants. Sampling can be easily performed by parents or by a non-trained person. It means a significant advantage and it contributes to ameliorate the discomfort that children suffer after several blood extractions. However, further studies are needed to assess whether this determination in BMC could replace the traditional diagnostic methodology.

Although nowadays the method of choice to pretreat plasma and BMC samples is protein precipitation and extraction with 1-propanol because of the simplicity and excellent analytical recoveries, tissue samples however, are not completely cleaned (Figure 2A) and it is necessary to enhance extraction selectivity and specificity. New selective materials involving molecular recognition mechanisms like immunosorbents and molecular imprinting polymers (MIP) have been recently developed by our group (34).

Low cost and reduced sample pretreatment make MIPs techniques much more accepted than immunosorbents in the last years. MPIs are synthetic polymers containing specific binding sites (cavities) with complementary size, shape and functional groups adapted to recognize analytes with template properties (35). The advantages of MIPs over antibodies include stability, easy preparation, low cost and reutilization (36) that lead to its wide use in place of sorbent, named molecularly imprinted solid phase extraction (MISPE). Recently, the first CoQ10 MISPE procedure has been successfully developed by our group, which cleans tissue samples with high recoveries, great selectivity and low interference compared to traditional sample tissue preparation like C18 pretreatment (Figure 2B and C) (34). In order to avoid the laborious manual extraction, to improve precision, reproducibility and to allow rapidness and automation, some authors described an on-line sample purification by column-switching in a HPLC system: a short precolumn retained both polar and compounds strongly attached to the analyte, achieving a profile free of interferences (21).

3.2. Separation and other analytical Systems

3.2.1. HPLC systems

Many methods have been reported for the analysis of CoQ10 in different matrices like plasma, tissues, platelets, BMC, pharmaceuticals and food formulations. By far, the methodology of choice is HPLC coupled to electrochemical detection (ECD) (14-16, 20, 23, 24), UV-detector (14, 16, 21, 25, 33) or mass spectrometer (HPLC-MS) (37-41). Commonly, CoQ10 is analyzed in a reverse-phase mode using C8 or C18-column, sometimes with a percentage of hexane or isopropanol as components of the mobile phase. HPLC-ECD seems to be the most common method for the analysis of CoQ10 especially in biological matrices, due to its high selectivity and sensitivity (the limit of detection –LOD- reported ranged from 1 to 10 ng/mL), but it is time consuming or require several steps during equipment operation, making it unsuitable for routine analysis (42).

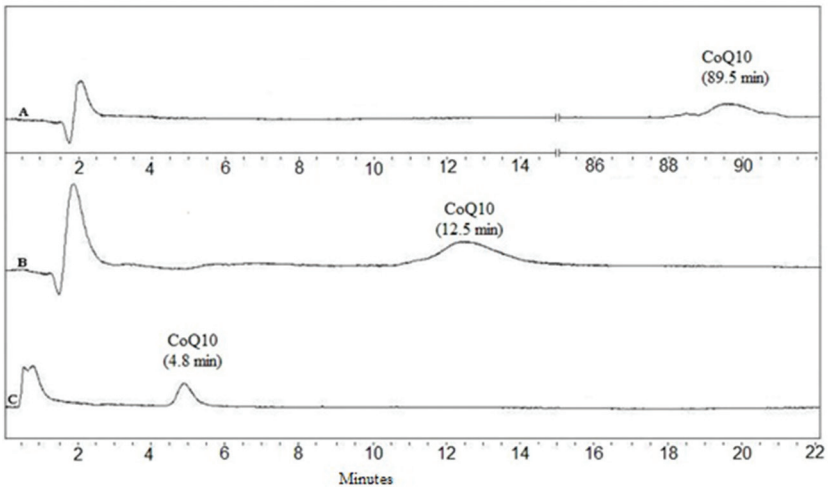

Figure 3. Comparison of analysis of CoQ10 standard using different HPLC columns. Retention time in parentheses. (A) Traditional C18-column (15.0.cm_4.6.mm i.d.). Mobile phase: methanol: water (98:2, v=v), flow rate: 1.0.mL=min. CoQ10 standard: 2.0. mM. (B) Microbore C18-column (15.0.cm, 2.1.mm i.d.). Mobile phase: methanol: water (98:2, v=v), flow rate: 0.3.mL=min. CoQ10 standard: 2.0. mM. (C) XTerra C18 microcolumn (50mm_2.1.mm i.d.). Mobile phase: methanol: water (98:2, v=v), flow rate: 0.3.mL=min. CoQ10 standard: 1.0. mM. Reproduced with permission from ref 48.

The determination of CoQ10 by HPLC-MS and tandem mass spectrometry detection (MS/MS) (41) is a highly sensitive methodology with LOD of 1 ng/mL. However, it requires qualified personnel and expensive installations not usually available in a clinical laboratory. The employment of HPLC coupled to fluorescence detector has been reported for the quantitation of CoQ10 in biological samples like blood (43), human serum and liver homogenate (44). Fluorescence detector possesses high sensitivity and selectivity but the molecule under study must exhibit fluorescent properties. In the case of CoQ10, the introduction of a fluorophore group is necessary because ubiquinone molecule does not exhibit fluorescence per se. For this purpose a post-column derivatization with ethylcyanoacetate in basic medium could reach LOD values in the range of 9-30 ng/mL (43, 44). HPLC with chemiluminescent detection method has also been presented in plasma samples based on luminal chemiluminescence detection of a superoxide anion generated by the redox cycle reaction between CoQ10 and dithiothreitol. An LOD of 26 ng/mL for CoQ10 in plasma samples was obtained, free of interferences (45).

In the last time, instrumentation miniaturization by means of reducing the diameter and length of column and particle size is one of the major current trends in the method separation improvement, in the clinical and pharmaceutical analytical area. This modification of the column design allows a reduced analysis time, less solvent consumption, low sample volume injection, with high resolution, sensitivity and robustness (46, 47). Micro HPLC methods using a special column with reduced diameter and length and hybrid particulate matrix have been developed to determine the content of CoQ10 in pharmaceutical and cosmetic formulations as well as in biological fluids and tissues (41, 48, 49). This method also allows shorter analysis time together with less solvent consumption and a dramatic reduction of sample requirements with faster separations compared to macroscale columns employed (Figure 3). LOD is approximately 17 ng/mL though this value decreases to 3 ng/mL if sample concentration is used as pretreatment (48, 49). In the case of HPLC systems using UV-detection and conventional columns, the LOD increases to 50 ng/mL (16).

3.2.2. Capillary electrophoresis

Capillary electrophoresis (CE) is a powerful technique with relevant characteristics of performance such as simplicity, very high resolution in short time of analysis and low cost of operation. It became an alternative methodology in the analysis of several compounds in different matrices (50, 51). Electrokinetic chromatography (EKC), is a mode of CE, with high selectivity accomplished by the partition of the analytes between a mobile phase, usually a buffer, and a pseudostationary phase like micelles, vesicles, polymers or microdroplets (51). A new microemulsion system (MEEKC) has been developed for the quantification of CoQ10 in plasma samples, based on a double tensioactive agent like sulfosuccinate and cholic acid, (52) (Figure 4). In this case, an LOD of 1 µg/mL has been reported, about 300 times less sensitive than the micro HPLC with UV detection of for CoQ10 in human plasma. The MEEKC system was also employed for the simultaneous determination of CoQ10, ascorbic acid and nutraceuticals (53) using another microemulsion based on SDS as the tensioactive agent.

3.2.3. Spectrophotometry

Spectrophotometry possesses the advantages of simplicity, low cost of operation and easy sample

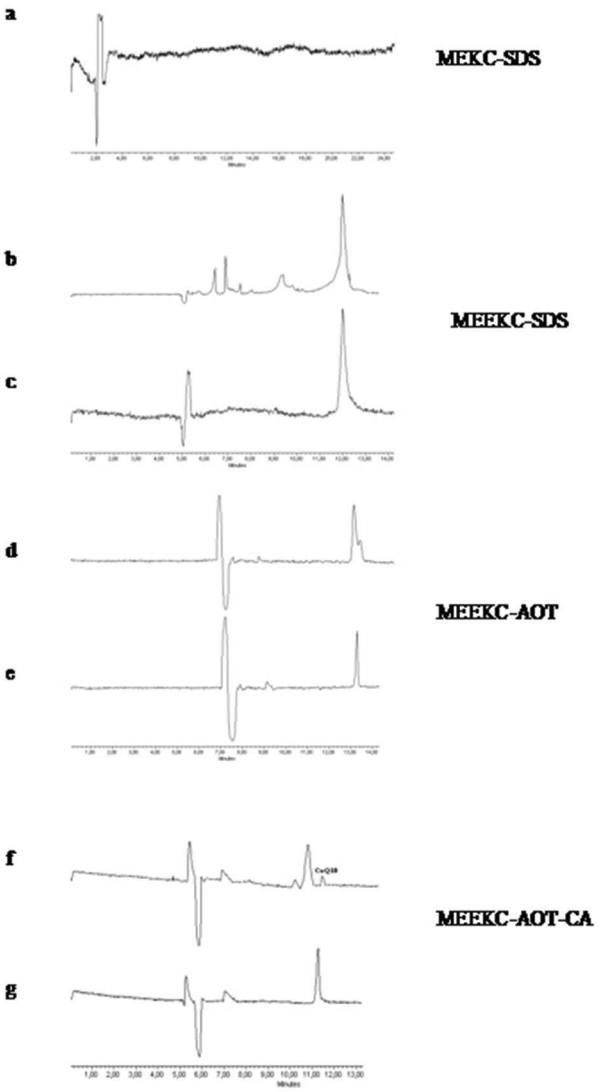

Figure 4. Electropherograms of (a) CoQ10 standard by MEKC-SDS, (b) plasma blank spiked with CoQ10 standard by MEEKC-SDS, (c) CoQ10 standard by MEEKC-SDS, (d) plasma blank spiked with CoQ10 standard by MEEKC-AOT, (e) CoQ10 standard by MEEKC-AOT, (f) plasma blank spiked with CoQ10 standard by MEEKC-AOT-CA and (g) CoQ10 standard by MEEKC-AOT-CA. Reproduced with permission from ref 52.

preparation but it is less sensitive and selective than other separation techniques like liquid chromatography. Therefore, few applications of UV-Vis spectrophotometry for quantitation of CoQ10 are presented in the literature. CoQ10 quantification in pharmaceuticals was described as a fast methodology while applications in biological samples were only reported in urine and blood (18, 54-56).

3.2.4. Electroanalytical methods

Electroanalytical methods are not frequently used for quantitative determination of CoQ10. However, a voltammetric method has been developed for the analysis of CoQ10 in a pharmaceutical formulation. The quantification of CoQ10 was based on differential pulse voltammetry using a glassy carbon electrode in a solvent containing a mixture of acetic acid and acetonitrile. The limits of detection and quantitation were 0.014 mM and 0.046 mM, respectively. No pretreatment was required and no excipient interference was found (57). Polarography, a subclass of voltammetry, is a method applied to the determination of CoQ10 using a beta-cyclodextrin (β-CD) and iodinate system. The stability of CoQ10 to light exposure was improved by the formation of an inclusion complex between CoQ10 and β-CD and the sensitivity of the method was improved by addition of iodinate to the abovementioned inclusion complex. In this case, a detection limit of 0.01 μM was reported (58).

3.3. Quantitative methods and expression of the results

CoQ10 in biological systems coexists in two redox status: the reduced form (ubiquinol, $CoQ10H_2$) and the oxidized form (ubiquinone, CoQ10). Therefore, CoQ10 quantitation in biological samples could be expressed as total CoQ10, that it is the sum of ubiquinol and ubiquinone, or as the two individual redox forms. Both expressions have an important clinical significance. In evaluation of treatment evolution, the ubiquinol status is more valuable because treatment efficacy is evaluated by the increment of ubiquinol/ubiquinone rate. Therefore, the result expression of CoQ10 quantification should be clearly set beforehand as it affects sample preparation. Ubiquinol oxidizes to ubiquinone while samples are being processed. To ensure complete oxidation of the molecule, the use of benzoquinone is required (59). If quantitation of both redox forms is required, the method should be fast and contain as few steps as possible to minimize oxidation conditions (60). *Tang et al* demonstrated that ubiquinol is stable in 1-propanol tissue extracts in contact with ice for 4 h. In plasma samples, the extraction process should be faster because the concentration of ubiquinol rapidly decreases within 1 h after phlebotomy. At room temperature, it is oxidized at a rate of approximately 3 nmol/L per min if human plasma is extracted in hexan (24). If solvent extraction is not employed, CoQ10 decreases around 30% per day at -20°C. Freezing the plasma at -80 ° C, is necessary to preserve all the endogenous ubiquinol; for this reason, some authors state that the determination of ubiquinol percentage in clinical diagnostic test is not useful (32). Nevertheless, in the case of biological samples the analyst should also select the quantitation method to be used. Traditionally, the quantification of CoQ10 is carried out using a five or six point calibration curve prepared in solvent. In biological samples this procedure is not appropriate, but it could be improved if low, middle and high internal controls are also prepared in pooled plasma samples to maintain precision and accuracy throughout several days. Other authors preferred to use coenzyme Q9 (CoQ9), as internal standard to this purpose. However, since CoQ9 is endogenously found in human plasma, it

is not convenient to employ this compound as internal standard (32). However, if the sample preparation is achieved with a high recovery, the use of an internal standard is unnecessary.

4. CONCLUSIONS

Significant progress has been made in the field of coenzyme Q10 determination in biological samples. However respect to analytical methods and their evolution, in most cases, it is difficult to determine the limits of knowledge**.** New matrices, miniaturized systems, automatization, selectivity improvement and sensitivity are the main goals when biological samples are the materials of study.

5. ACKNOWLEDGEMENTS

The authors thank University of Buenos Aires and CONICET for financial support and Dr Cecilia Dobrecky, Dr Jorge Muse and Prof. Dr Viviana Mouriño for her kindly language assistance.

6. REFERENCES

1. B.A. Barshop, J.A Gangoiti. Analysis of coenzyme Q in human blood and tissues. Mitochondrion, 7 Suppl: S89-93 (2007)
DOI: 10.1016/j.mito.2007.04.002
2. C.M Quinzii, M. Hirano. Coenzyme Q and mitochondrial disease. Dev Disabil Res Rev, 16 (2): 183-8. (2010)
DOI: 10.1002/ddrr.108
3. R. Artuch, M.A. Vilaseca, J. Moreno, N. Lambruschini, F.J. Cambra, J. Campistol. Decreased serum ubiquinone-10 concentrations in phenylketonuria. Am J Clin Nutr, 70 (5): 892-5 (1999)
No DOI Found
4. F. Gazdik, A. Gvozdjakova,M. Horvathova,S. Weissova, J. Kucharska, M.R. Pijak, K. Gazdikova. Levels of coenzyme Q10 in asthmatics. Bratisl Lek Listy, 103 (10): 353-6 (2002)
No DOI Found
5. A.D. Hershey, S.W. Powers, A.L. Vockell, S.L. Lecates, P.L. Ellinor, A. Segers, D. Burdine, P. Manning, M.A. Kabbouche. Coenzyme Q10 deficiency and response to supplementation in pediatric and adolescent migraine. Headache, 47 (1): 73-80 (2007)
DOI: 10.1111/j.1526-4610.2007.00652.x
6. J.M. Cooper, L.V. Korlipara, P.E. Hart, J.L. Bradley, A.H. Schapira. Coenzyme Q10 and vitamin E deficiency in Friedreich's ataxia: predictor of efficacy of vitamin E and coenzyme Q10 therapy. Eur J Neurol, 15 (12): 1371-9 (2008)
DOI: 10.1111/j.1468-1331.2008.02318.x
7. T.A. Laguna, M.K. Sontag, I. Osberg, J.S. Wagener, F.J. Accurso, R.J. Sokol. Decreased total serum coenzyme-Q10 concentrations: a longitudinal study in children with cystic fibrosis. J Pediatr, 153 (3): 402-7 (2008)
DOI: 10.1016/j.jpeds.2008.03.028
8. S.L. Molyneux, C.M. Florkowski, P.M. George, A.P. Pilbrow, C.M. Frampton, M. Lever, A.M. Richards. Coenzyme Q10: an independent predictor of mortality in chronic heart failure. J Am Coll Cardiol, 52 (18): 1435-41(2008)
DOI: 10.1016/j.jacc.2008.07.044
9. E. Teran, M. Racines-Orbe, S. Vivero, C. Escudero, G. Molina, A. Calle. Preeclampsia is associated with a decrease in plasma coenzyme Q10 levels. Free Radic Biol Med, 35 (11): 1453-6 (2003)
DOI: 10.1016/j.freeradbiomed.2003.08.004
10. M.V Miles, P.E. Putnam, L. Miles, P.H. Tang, A.J. DeGrauw, B.L. Wong, P.S. Horn, H.L. Foote, M.E. Rothenberg. Acquired coenzyme Q10 deficiency in children with recurrent food intolerance and allergies. Mitochondrion,11 (1): 127-35 (2011)
DOI: 10.1016/j.mito.2010.08.010
11. A. Mancini, G. Balercia. Coenzyme Q(10) in male infertility: physiopathology and therapy. Biofactors, 37 (5): 374-80 (2011)
DOI: 10.1002/biof.164
12. G. Balercia, F. Mosca, F. Mantero, M. Boscaro, A. Mancini, G. Ricciardo-Lamonica, G. Littarru. Coenzyme Q(10) supplementation in infertile men with idiopathic asthenozoospermia: an open, uncontrolled pilot study.Fertil Steril, 81 (1): 93-8 (2004)
DOI: 10.1016/j.fertnstert.2003.05.009
13. R. Matthews, L. Yang, S. Browne, M. Baik, M. Beal. Coenzyme Q10 administration increases brain mitochondrial concentrations and exerts neuroprotective effects. Proc. Natl. Acad. Sci, 95, 8892-97 (1998)
DOI: 10.1073/pnas.95.15.8892
14. J.K. Lang, L. Packer. Quantitative determination of vitamin E and oxidized and

reduced coenzyme Q by high-performance liquid chromatography with in-line ultraviolet and electrochemical detection. J Chromatogr, 385:109-17 (1987)
DOI: 10.1016/S0021-9673(01)94626-6

15. T. Okamoto, Y. Fukunaga, Y. Ida, T. Kishi. Determination of reduced and total ubiquinones in biological materials by liquid chromatography with electrochemical detection. J Chromatog, 430 (1): 11-9 (1988)
DOI: 10.1016/S0378-4347(00)83129-1

16. G. Grossi, P. Bargossi, L. Fiorella, S. Piazzi. Improved high-performance liquid chromatographic method for the determination of coenzyme Q10 in plasma. J. Chromatogr, 593, 217-226 (1992)
DOI: 10.1016/0021-9673(92)80289-7

17. T. Menke, P. Niklowitz, S. Adam, M. Weber, B. Schlüter, W. Andler. Simultaneous detection of ubiquinol-10, ubiquinone-10, and tocopherols in human plasma microsamples and macrosamples as a marker of oxidative damage in neonates and infants. Anal Biochem, 282 (2): 209-17 (2000)
DOI: 10.1006/abio.2000.4579

18. J. Karpińska, B. Mikołuć, J. Piotrowska-Jastrzebska. Application of derivative spectrophotometry for determination of coenzyme Q10 in pharmaceuticals and plasma. J Pharm Biomed Anal;17 (8): 1345-50 (1998)
DOI: 10.1016/S0731-7085(98)00003-X

19. M. Takada, S. Ikenoya, T. Yuzuriha, K. Katayama. Studies on reduced and oxidized coenzyme Q (ubiquinones) II. The determination of oxidation-reduction levels of coenzyme Q in mitochondria, microsomes and plasma by high-performance liquid chromatography.Biochim Biophys Acta, 679 (2): 308-14 (1982)
DOI: 10.1016/0005-2728(82)90301-2

20. Q. Wang, B.L Lee, C.N. Ong. Automated high-performance liquid chromatographic method with precolumn reduction for the determination of ubiquinol and ubiquinone in human plasma. J Chromatogr B Biomed Sci App,726 (1-2): 297-302 (1999)
DOI: 10.1016/S0378-4347(99)00067-5

21. P. Jiang, M. Wu, Y. Zheng, C. Wang, Y. Li, J. Xin, G. Xu. Analysis of coenzyme Q(10) in human plasma by column-switching liquid chromatography J Chromatogr B Analyt Technol Biomed Life Sci, 805 (2): 297-301 (2004)
DOI: 10.1016/j.jchromb.2004.03.008

22. M. Battino, L. Leone, S. Bompadre. High-performance liquid chromatography-EC assay of mitochondrial coenzyme Q9, coenzyme Q9H2, coenzyme Q10, coenzyme Q10H2, and vitamin E with a simplified on-line solid-phase extraction. Methods Enzymol, 378: 156-62 (2004)
DOI: 10.1016/S0076-6879(04)78012-X

23. P.O. Edlund. Determination of coenzyme Q10, alpha-tocopherol and cholesterol in biological samples by coupled-column liquid chromatography with coulometric and ultraviolet detection. J Chromatogr, 425 (1): 87-97 (1988)
DOI: 10.1016/0378-4347(88)80009-4

24. P.H. Tang, M.V. Miles, A. DeGrauw, A. Hershey, A. Pesce. HPLC analysis of reduced and oxidized coenzyme Q(10) in human plasma. Clin Chem,47 (2): 256-65 (2001)
No DOI Found

25. F. Mosca, D. Fattorini, S. Bompadre, G.P. Littarru. Assay of coenzyme Q(10) in plasma by a single dilution step. Anal Biochem, 305 (1): 49-54 (2002)
DOI: 10.1006/abio.2002.5653

26. G.P. Littarru, F. Mosca, D. Fattorini, S. Bompadre, M. Battino. Assay of coenzyme Q10 in plasma by a single dilution step. Methods Enzymol, 378: 170-6 (2004)
DOI: 10.1016/S0076-6879(04)78014-3

27. P. Niklowitz, T. Menke, W. Andler, J.G. Okun. Simultaneous analysis of coenzyme Q10 in plasma, erythrocytes and platelets: comparison of the antioxidant level in blood cells and their environment in healthy children and after oral supplementation in adults. Clin Chim Acta,342 (1-2): 219-26 (2004)
DOI: 10.1016/j.cccn.2003.12.020

28. M.V. Miles, P.H. Tang, L. Miles, P.E. Steele, M.J. Moye, P.S. Horn. Validation and application of an HPLC-EC method for analysis of coenzyme Q10 in blood platelets. Biomed Chromatogr, 22 (12): 1403-8 (2008)
DOI: 10.1002/bmc.1072

29. P. Niklowitz, A. Sonnenschein, B. Janetzky, W. Andler, T. Menke. Enrichment of coenzyme

Q10 in plasma and blood cells: defense against oxidative damage. Int J Biol Sci, 3 (4): 257-62 (2007)
DOI: 10.7150/ijbs.3.257

30. Q. Lü, H. Wang, T. Hu, M. Zhu. Study on changes of vitamin nutrition status of population in a high-risk area of esophageal cancer. Wei Sheng Yan Jiu,36 (6): 719-21 (2007)
No DOI Found

31. R. Montero, J.A. Sánchez-Alcázar, P. Briones, A.R. Hernández, M.D. Cordero, E. Trevisson, L. Salviati, M. Pineda, A. García-Cazorla, P. Navas, R. Artuch.Analysis of coenzyme Q10 in muscle and fibroblasts for the diagnosis of CoQ10 deficiency syndromes. Clin Biochem,41 (9): 697-700 (2008)
DOI: 10.1016/j.clinbiochem.2008.03.007

32. S.L. Molyneux, J.M. Young, C.M. Florkowski, M. Lever, P.M. George. Coenzyme Q10: is there a clinical role and a case for measurement? Clin Biochem Rev, 29 (2): 71-82 (2008)
DOI: 10.1016/j.jacc.2008.07.044

33. M. Martinefski, P. Samassa, S. Lucangioli and V. Tripodi. A novel non-invasive sampling method using buccal mucosa cells for determination of coenzyme Q10. Anal. Bioanal. Chem.
DOI 10.1.0.0.7/s00216-015-8696-0, (2015)

34. M. Contin, S. Flor, M. Martinefski, S. Lucangioli, V. Tripodi. The use of coenzyme Q0 as a template in the development of a molecularly imprinted polymer for the selective recognition of coenzyme Q10. Anal Chim Acta, 807: 67-74 (2014)
DOI: 10.1016/j.aca.2013.11.030

35. V. Pichon, Selective sample treatment using molecularly imprinted polymers. J. Chromatogr. A, 1152: 41-53 (2007)
DOI: 10.1016/j.chroma.2007.02.109

36. P. Jiang, M. Wu, Y. Zheng, C. Wang, Y. Li, J. Xin, G. Xu, Analysis of coenzyme Q(10) in human plasma by column-switching liquid chromatography. J. Chromatogr. B, 805: 297-301 (2004)
DOI: 10.1016/j.jchromb.2004.03.008

37. J. Ruiz-Jiménez, F. Priego-Capote, J.M. Mata-Granados, J.M. Quesada, M.D. Luque de Castro. Determination of the ubiquinol-10 and ubiquinone-10 (coenzyme Q10) in human serum by liquid chromatography tandem mass spectrometry to evaluate the oxidative stress. J Chromatogr A, 1175 (2): 242-8 (2007)
DOI: 10.1016/j.chroma.2007.10.055

38. K. Teshima, T. Kondo. Analytical method for ubiquinone-9 and ubiquinone-10 in rat tissues by liquid chromatography/turbo ion spray tandem mass spectrometry with 1-alkylamine as an additive to the mobile phase. Anal Biochem, 338 (1): 12-9 (2005)
DOI: 10.1016/j.ab.2004.12.007

39. G. Hansen, P. Christensen, E. Tüchsen, T. Lund. Sensitive and selective analysis of coenzyme Q10 in human serum by negative APCI LC-MS. Analyst, 129 (1): 45-50 (2004)
DOI: 10.1039/b308690a

40. R. Rodríguez-Acuña, E. Brenne, F. Lacoste. Determination of coenzyme Q10 and Q9 in vegetable oils. J Agric Food Chem, 56 (15): 6241-5 (2008)
DOI: 10.1021/jf800103e

41. Z. Tang, S. Li, X. Guan, P. Schmitt-Kopplin, S. Lin,Z. Cai. Rapid assessment of the coenzyme Q10 redox state using ultrahigh performance liquid chromatography tándem mass spectrometry. Analyst, 139 (21): 5600-4 (2014)
DOI: 10.1039/C4AN00760C

42. V. Tripodi, S. Flor, M. Contin, S. Lucangioli. Simple, Highly Sensitive Micro HPLC Method for the Determination of Coenzyme Q10 and its Major Related Substances. J Liq Chromatogr Relat Techol, 32: 860–873 (2009)
DOI: 10.1080/10826070902768138

43. Y. Nohara, J. Suzuki, H. Kubo. Determination of ubiquinone in blood by high-performance liquid chromatography with post-column fluorescence derivatization using 2-cyanoacetamide. J Fluoresc, 21(6): 2093-100 (2011)
DOI: 10.1007/s10895-011-0908-1

44. K. Abe, K. Ishibashi, M. Ohmae, K. Kawabe, G. Katsui. Determination of ubiquinone in serum and liver by high-speed liquid chromatography. J Nutr Sci Vitaminol (Tokyo), 24(6): 555-67 (1978)
DOI: 10.3177/jnsv.24.555

45. N. Kishikawa,N. Ohkubo, K. Ohyama, K. Nakashima, N. Kuroda. Selective determination of ubiquinone in human plasma

by HPLC with chemiluminescence reaction based on the redox cycle of quinone. Anal Bioanal Chem, 400 (2): 381-5 (2011)
DOI: 10.1007/s00216-011-4662-7

46. G. Rozing, E. Nägele, P. Hörth, M. Vollmer, R. Moritz, B. Glatz, A. Gratzfeld-Hüsgen. Instrumentation for Advanced Microseparations in Pharmaceutical Analysis and Proteomics. J. Biochem. Biophys. Methods, 60, 233-263 (2004)
DOI: 10.1016/j.jbbm.2004.01.005

47. M. Ascenio Ramos, J. Hernandez Borges, A. Rocco, S. Fanali. Food Analysis: A Continuous Challenge for Miniaturized Separation Techniques. J Sep Sci, 32 (21), 3764–3800 (2009)
DOI: 10.1002/jssc.200900321

48. M. Contin, S. Lucangioli, M. Martinefski, S. Flor, V. Tripodi. Miniaturized HPLC-UV method for analysis of coenzyme Q10 in human plasma. J Liq Chromatogr Relat Techol 34, 2485-94 (2011)
DOI: 10.1080/10826076.2011.591028

49. M. Contin, M. Martinefski, S. Lucangioli S. V. Tripodi. Sistema cromatográfico miniaturizado para la determinación de coenzima Q10 en plasma, músculo y plaquetas. Acta Bioquim Clín Latinoam 45 (2):273-278 (2011)
No DOI Found

50. N.T. Guzman, T. Phillips. 2008. Immunoaffinity capillary electrophoresis as a powerful strategy for the quantification of low-abundance biomarkers, drugs, and metabolites in biological matrices. Electrophoresis, 29 (16): 3259-78 (2008)
DOI: 10.1002/elps.200800058

51. V. Tripodi, S. Flor, A. Carlucci, S. Lucangioli. Simultaneous determination of natural and synthetic estrogens by EKC using a novel microemulsion. Electrophoresis, 27 (22): 4431-8 (2006)
DOI: 10.1002/elps.200600280

52. S. Lucangioli, S. Flor, M. Contin, V. Tripodi. A capillary electrophoretic system based on a novel microemulsion for the analysis of coenzyme Q10 in human plasma by electrokinetic chromatography. Electrophoresis,30(11):1899-905 (2009)
DOI: 10.1002/elps.200800783

53. G. Piepel, B. Pasquini, S. Cooley, A. Heredia-Langner, S. Orlandini, S. Furlanetto. Mixture-process variable approach to optimize a microemulsion electrokinetic chromatography method for the quality control of a nutraceutical based on coenzyme Q10. Talanta, 15;97:73-82 (2012)
DOI: 10.1016/j.talanta.2012.03.064

54. S. Michalkiewicz. Anodic oxidation of oxidized forms of coenzymes Q(10) and Q(0) on carbon electrodes in acetic acid solutions. Bioelectrochemistry, 82(2):103-11 (2011)
DOI: 10.1016/j.bioelechem.2011.06.003

55. S. Michalkiewicz. Cathodic reduction of coenzyme Q10 on glassy carbon electrode in acetic acid-acetonitrile solutions. Bioelectrochemistry, 70 (2):495-500 (2007)
DOI: 10.1016/j.bioelechem.2006.08.003

56. E. Redalieu, I.M. Nilsson, T.M. Farley, K. Folkers, F.R. Koniuszy. Determination and levels of coenzyme Q10 in human blood. Anal Biochem, 23(1):132-40 (1968)
DOI: 10.1016/0003-2697(68)90018-3

57. S. Michalkiewicz. Voltammetric determination of coenzyme Q10 in pharmaceutical dosage forms. Bioelectrochemistry, 73(1):30-6 (2008)
DOI: 10.1016/j.bioelechem.2008.04.004

58. H.Y. Yang, J.F. Song. High-sensitive determination of coenzyme Q(10) in iodinate-beta-cyclodextrin medium by inclusion reaction and catalytic polarography. Anal Biochem, 348(1):69-74 (2006)
DOI: 10.1016/j.ab.2005.10.016

59. M. Contin, S. Flor, M. Martinefski, S. Lucangioli, V. Tripodi. New analytical strategies applied to the determination of Coenzyme Q10 in biological matrix. Methods Mol Biol, 1208:409-20 (2015)
DOI: 10.1007/978-1-4939-1441-8_29

60. P.H. Tang, M.V. Miles, L. Miles, J. Quinlan, B. Wong, A. Wenisch, K. Bove. Measurement of reduced and oxidized coenzyme Q9 and coenzyme Q10 levels in mouse tissues by HPLC with coulometric detection.Clin Chim Acta, 341(1-2):173-84 (2004)
DOI: 10.1016/j.cccn.2003.12.002

Abbreviations: Coenzyme Q10 (ubiquinone, CoQ10), sodium dodecylsulfate (SDS), buccal mucosa cells (BMC), molecular imprinting polymers (MIP), molecularly imprinted solid phase extraction (MISPE), electrochemical detector (ECD), mass

spectrometry (HPLC-MS), limit of detection (LOD), Capillary electrophoresis (CE), Electrokinetic chromatography (EKC), microemulsion electrokinetic chromatography (MEEKC), beta-cyclodextrin (β-CD)

Key Words: Coenzyme Q10, HPLC, Capillary Electrophoresis, Chromatography

Send correspondence to: Valeria Tripodi, Department of Pharmaceutical Technology, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina, 54 11 4964 8262, Junin 956, 5th floor (1113), Tel/Fax: +541152874510, E-mail: vtripodi@ffyb.uba.ar