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STRUCTURE OF CHEMICAL COMPOUNDS, METHODS OF ANALYSIS AND PROCESS CONTROL

BIOPHARMACEUTICAL ANALYSIS OF COENZYME Q10 (UBIDECARENONE)

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The literature on the most frequently used methods for quantitative determination of coenzyme Q_{10} in biological materials was reviewed. HPLC in combination with electrochemical detection had the advantage among spectrophotometric, electrochemical, and chromatographic methods owing to high selectivity and sensitivity and the availability of modern detectors.

Keywords: ubidecarenone, coenzyme Q_{10}, ubiquinone, ubiquinol, spectrophotometric method, electrochemical method, HPLC.

Drugs capable of correcting metabolic dysfunctions must be discovered because of the increasing incidence of diseases caused by them.

One such drug is ubidecarenone (coenzyme Q_{10}, CoQ_{10}, 2-[(2*E*,6*E*,10*E*,14*E*,18*E*,22*E*,26*E*,30*E*,34*E*)-3,7,11,15,19,23, 27,31,35,39-decamethyltetraconta-2,6,10,14,18,22,26,30,34, 38-decaenyl]-5,6-dimethoxy-3-methylcyclohexa-2,5-diene-1 ,4-dione). The chemical structure of CoQ_{10} comprises a benzoquinone and 10 isoprene groups in a side chain (Fig. 1).

The lipophilic side chain in CoQ_{10} is responsible for its hydrophobic properties. For this reason, CoQ_{10} is practically insoluble in H_2O, soluble in Me_2CO, and very poorly soluble in EtOH [1]. CoQ_{10} is sensitive to chemical factors because the benzoquinone ring is easily oxidized.

The present work reviewed the literature on analytical approaches that are used most widely in biopharmaceutical research of CoQ_{10}.

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Sample preparation of CoQ_{10}

Sample preparation precedes quantitative analysis and is performed in order to separate the analyte from associated components. The analysis of CoQ_{10} in biological materials is rather complicated because the compound is highly sensitive to physicochemical factors [2].

Tissues must be thoroughly homogenized in order to determine CoQ_{10} in them. The recommended homogenized sample mass is 0.5 – 1.0 g [3]. Sample preparation in a dark room is recommended because the analyte is light-sensitive [4]. Several researchers suggest preparing samples at low temperature (4 – 5°C) in order to reduce the decomposition rate of CoQ_{10} [5, 6]. Various solvents, e.g., H_2O [7], normal saline [8], and extractants such as 2-PrOH [3] and a MeOH—hexane mixture [9] were used to prepare a homogenate of the required consistency. Samples should be protected from oxygen because of the high propensity of CoQ_{10} to oxidize. For this reason, antioxidants such as 2,6-di-*tert*-butyl-4-methylphenol (BHT) or sodium borohydride were added to the solution [9, 10].

The next step in sample preparation is deproteination of the homogenate or biological fluids (blood, serum, plasma) because proteins in them have a negative effect on chroma-

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tography columns. Proteins are precipitated by EtOH, MeOH, 2-PrOH, or their mixtures [11]. EtOH is used most often to deproteinize blood [10, 12 – 15] and homogenates of various tissues [5 – 7, 9, 16] because it is available and mildly toxic.

The next step after protein precipitation is separation of CoQ_{10} from other matrix components. Such substances are removed by liquid—liquid extraction using extractants such as PrOH, petroleum ether, 2-PrOH, hexane, or their mixtures with MeOH, EtOAc, and EtOH. These extractants provide selective solubilization of the analyte and highly efficient extraction. For example, *n*-hexane was used to extract CoQ_{10} from blood plasma [17 – 19]. 2-PrOH was used to isolate CoQ_{10} from rat blood serum [20]. A mixture of petroleum-ether—EtOAc—MeOH (1:1:1) extracted CoQ_{10} from multivitamin dietary supplements [21]. Also, *n*-PrOH was recommended for deproteination and extraction of CoQ_{10} from blood plasma [22]. Plasma and tissue homogenates were extracted by an EtOH—*n*-hexane mixture (1:2.5) [23, 24] with modifications [25]. The extraction procedure was performed twice, adding *n*-hexane after collecting the first extract. The extracts were combined, evaporated to dryness, and dissolved in an aliquot of EtOH.

The final sample-preparation step removed traces of extractant. For this, an inert gas was used as the drying agent [12, 26]. The procedure was carried out at room temperature. Evaporation at low pressure was used for most of the extract.

Methods for quantitative determination of CoQ_{10}

Physicochemical analytical methods are used for quantitative determination of CoQ_{10}. The conjugated double-bond structure in ubidecarenone has a characteristic absorption in the UV region with the analytical wavelength at 275 nm.

Spectrophotometric methods

Spectrophotometric (SP) methods were used primarily for routine determination of CoQ_{10} in pharmaceutical preparations [1]. These methods have the advantages of availability, rapidity, avoidance of toxic solvents, and low analytical costs. The selectivity and sensitivity of SP methods can be increased by preparing and analyzing CoQ_{10} derivatives. Thus, a colorimetric method was used to determine CoQ_{10} in human urine after preliminary nucleophilic substitution of a methoxy by cyanoethylacetate [27]. The reaction produced a blue compound. Later, this reaction was used to determine CoQ_{10} in human blood [13].

Important deficiencies of SP methods are the low sensitivity and selectivity and the ability of ballast substances to affect the analytical signal.

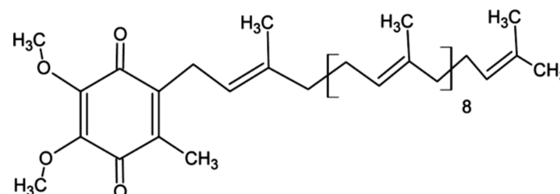

Fig. 1. Structural formula of CoQ_{10} according to *European Pharmacopoeia* 2005, ver. 5.2; $C_{59}H_{90}O_4$, mol. mass 863.34.

Electrochemical methods

The electrophilic groups in ubiquinone can be used for electrochemical analytical methods. Polarography was used to determine CoQ_{10} in medicines and biologically active additives [28].

A voltammetric method for quantitative determination of CoQ_{10} in medicines could also be used [29]. The drawbacks of electrochemical methods are the use of toxic solvents and the lengthy labor-intensive sample preparation, during the course of which the ubiquinone—ubiquinol ratio can change.

HPLC methods

However, HPLC methods combined with various detectors have become the most common. HPLC with electrochemical [30], spectrophotometric [31], and mass-spectrometric detection [32] is the most common method for quantitative analysis of CoQ_{10} in biological samples. Highly selective and sensitive fluorescence detectors were also used to analyze biological samples. Fluorescent groups were added to CoQ_{10} in order to analyze it because CoQ_{10} itself does not fluoresce [33].

Reversed-phase (RP) HPLC was recommended for ubidecarenone determination [34]. This method has several advantages over normal-phase HPLC, i.e., better reproducibility and high selectivity.

HPLC with spectrophotometric and electrochemical detection

The European Pharmacopoeia (7th Ed.) gives an HPLC method with spectrophotometric detection at 275 nm. RP-HPLC using EtOH—MeOH (20:80) mobile phase was used for the analysis [1]. An RP-HPLC method for determining CoQ_{10} in biological samples such as urine, organ tissues, and plasma was developed [35]. An internal standard, i.e., a solution of CoQ_{10} in hexane, was used during the analysis. The mobile phase was a mixture of MeOH and hexane (3:1). The ubiquinone signal was detected at 275 nm. The detection limit was 10 ng/mL. A method for joint determination of α-tocopherol, CoQ_{10}, and retinol in human plasma was developed [12]. The mobile phase was MeOH—hexane

(70:30). Detection was made at 276 nm. The detection limit for ubiquinone was 0.83 μM.

An HPLC method with electrochemical detection was reported [23]. The analysis used a Coulochem II electrochemical detector with a model 5011 cell (ESA, USA) and a Phenomenex Luna C18 column (150 × 4.6 mm, 5 μm). The mobile phase included NaCl solution (0.3%, w/v) in a mixture of EtOH—MeOH—$HClO_4$ solution (7%) (975:15:10 ratio). The oxidized species was converted to the reduced form by adding an alcoholic solution of $NaBH_4$ to the plasma extract before placing it on the column. Ubiquinol was detected under oxidizing conditions at potentials of –50 mV/+350 mV on the first and second electrodes, respectively.

Electrochemical and SP detection were compared for analyzing CoQ_{10} in plasma [36]. The matrix was purified beforehand over a silica-gel cartridge (Bond Elut Si) in order to diminish the effects of interfering components. Then, the eluate was passed over a Bond Elut C18 cartridge with elution by 2-PrOH and analysis using a RP Ultrasphere XL C18 column (4.6 × 70 mm). The eluent for SP detection was MeOH—2-PrOH (4:1); for electrochemical detection, a mixture of acetate buffer (50 mM) and 2-PrOH—MeOH (24:450:1435). The detection limit was 50 ng/mL for SP and 5 ng/mL for electrochemical detection. The supporting electrolyte in most studies was lithium perchlorate. Ammonium formate was selected as the best supporting electrolyte because the corrosive perchlorate in $LiClO_4$ shortened the service life of the instrument [37]. CoQ_{10} in urine was determined quantitatively using HPLC with electrochemical detection [38]. An electrochemical Coulochem II detector with a model 5010 cell (ESA, USA) and Nucleosil 100 C-18 column (250 × 4 mm, 5 μm) was used in the work. The mobile phase consisted of $LiClO_4$ in a MeOH—EtOH mixture (65:35). Detection was made at potentials –600/+600 mV.

HPLC with mass-spectrometric detection

The advantages of the method are the high sensitivity and selectivity and reliability, which allow CoQ_{10} to be determined in complicated matrices. Furthermore, the oxidized and reduced forms of the coenzyme can be determined by using a simple sample-preparation procedure. These advantages are considerable for CoQ_{10} analysis in various biological samples. One study focused on HPLC determination of CoQ_{10} using different detectors, i.e., electrochemical and mass-spectrometric with an ion trap [39]. The ionization sources used electrospray and chemical ionization at atmospheric pressure. The effects of the different ionization types on the detection of negative and positive ions were studied. The best results were obtained in negative-ion mode. The detection limit was 1 ng/mL. A method for determining the oxidized and reduced forms of CoQ_{10} in human blood plasma was developed using UPLC in combination with tandem mass spectrometry. The determination was made using electrospray-ionization mass spectrometry in positive-ion mode. The detection limits for the oxidized and reduced species were 10 and 5 ng/mL, respectively [40]. Thus, although SP methods are the most popular and utilized for pharmaceutical analysis of CoQ_{10}, the complicated matrices of biological samples argue in favor of more selective and sensitive methods, i.e., HPLC in combination with SP, electrochemical, and mass-spectrometric detectors. SP detectors do not always have sufficient selectivity and sensitivity. Despite the obvious advantages, HPLC with mass-spectrometric detection has not yet received wide acceptance because of high equipment costs. Disadvantages of electrochemical detection methods include the use of toxic solvents and labor-intensive sample preparation, during the course of which the ubiquinone—ubiquinol ratio can change. Nevertheless, HPLC with electrochemical detection is currently the optimal choice for biopharmaceutical analysis of CoQ_{10} owing to the combination of high selectivity and sensitivity and available modern detectors.

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REFERENCES

1. *European Pharmacopoeia 7.0*, Vol. 2, Council of Europe, Strasbourg (2011).
2. H. W. Moore and K. Folkers, *J. Am. Chem. Soc.*, **87**(6), 1409 – 1410 (1965).
3. H. Kubo, K. Fujii, T. Kawabe, et al., *J. Food Compos. Anal.*, **21**(31), 199 – 210 (2008).
4. Q. Wang, B. L. Lee, and C. N. Ong, *J. Chromatogr. B: Biomed. Sci. Appl.*, **726**(1 – 2), 297 – 302 (1999).
5. H. Wakabayashi, S. Yamato, M. Nakajima, et al., *Biol. Pharm. Bull.*, **17**(10), 997 – 1002 (1994).
6. S. Ikenoya, M. Takada, T. Yuzuriha, et al., *Chem. Pharm. Bull.*, **29**(1), 158 – 164 (1981).
7. K. Teshima and T. Kondo, *Anal. Biochem.*, **338**(1), 12 – 19 (2005).
8. Y. Yoshida, M. Hayakawa, Y. Habuchi, et al., *Biochim. Biophys. Acta*, **1760**(10), 1558 – 1568 (2006).
9. C. Leray, M. D. Andriamampandry, M. Freund, et al., *J. Lipid Res.*, **39**(10), 2099 – 2105 (1998).
10. B. Finckh, A. Kontush, M. Burdelski, et al., *Anal. Biochem.*, **232**(2), 210 – 216 (1995).
11. M. J. Turkowicz and J. Karpinska, *BioFactors*, **39**(2), 176 – 185 (2013).
12. J. Karpinska, B. Mikoluc, R. Motkowski, et al., *J. Pharm. Biomed. Anal.*, **42**(2), 232 – 236 (2006).
13. T. A. Laguna, M. K. Sontag, I. Osberg, et al., *J. Pediatr.*, **153**(3), 402 – 407 (2008).
14. B. L. Lee and C. N. Ong, *J. Chromatogr. A*, **1216**(15), 3131 – 3137 (2009).
15. M. Morita and K. Folkers, *Biochem. Biophys. Res. Commun.*, **191**(3), 950 – 954 (1993).
16. K. Katayama, M. Takada, T. Yuzuriha, et al., *Biochem. Biophys. Res. Commun.*, **95**(3), 971 – 977 (1980).

17. J. Ruiz-Jimenez, F. Priego-Capote, J. M. Mata-Granados, et al., *J. Chromatogr. A*, **1175**(2), 242 – 248 (2007).
18. L. Li, D. Pabbisetty, S. Zhu, et al., *J. Chromatogr. Sci.*, **46**(3), 215 – 219 (2008).
19. C. Weber, A. Bysted, and G. Holmer, *Mol. Aspects Med.*, No. 18, 251 – 254 (1997).
20. L. Li, D. Pabbisetty, P. Carvalho, et al., *J. Pharm. Biomed. Anal.*, **46**(1), 137 – 142 (2008).
21. D. E. Breithaupt and S. Kraut, *Eur. Food Res. Technol.*, **222**, 643 – 649 (2006).
22. F. Mosca, D. Fattorini, S. Bompadre, et al., *Anal. Biochem.*, **305**(1), 49 – 54 (2002).
23. E. I. Kalenikova, E. V. Kharitonova, E. A. Gorodetskaya, et al., *Biomed. Khim.*, **61**(1), 125 – 131 (2015).
24. A. Lass and R. S. Sohal, *Free Radical Biol. Med.*, **27**(1 – 2), 220 – 226 (1999).
25. E. I. Kalenikova, E. A. Gorodetskaya, E. G. Kolokolchikova, et al., *Biochemistry (Moscow)*, **72**(3), 332 – 338 (2007).
26. R. Rodriguez-Acuna, E. Brenne, and F. Lacoste, *J. Agric. Food Chem.*, **56**(15), 6241 – 6245 (2008).
27. F. R. Koniuszy, P. H. Gale, A. C. Page, et al., *Arch. Biochem. Biophys.*, No. 87, 298 – 305 (1960).
28. H. Y. Yang and J. F. Song, *Anal. Biochem.*, **348**(1), 69 – 74 (2006).
29. S. Michalkiewicz, *Bioelectrochemistry*, **73**(1), 30 – 36 (2008).
30. P. Niklowitz, F. Doring, M. Paulussen, et al., *Anal. Biochem.*, **437**(1), 88 – 94 (2013).
31. D. Orozco, J. Skamarack, K. Reins, et al., *J. AOAC Int.*, **90**(5), 1227 – 1236 (2007).
32. Z. Tang, S. Li, X. Guan, et al., *Analyst*, **139**(21), 5600 – 5604 (2014).
33. Y. Nohara, J. Suzuki, and H. Kubo, *J. Fluoresc.*, **21**(6), 2093 – 2100 (2011).
34. R. Pauls, *J. Chromatogr. Sci.*, **23**(4), 181 – 184 (1985).
35. T. Okamoto and K. Fukui, *J. Chromatogr.*, **342**(1), 35 – 46 (1985).
36. R. A. Hagerman, R. A. Willis, and A. E. Hagerman, *Anal. Biochem.*, **320**(1), 125 – 128 (2003).
37. C. Morisco, B. Trimarco, and M. Condorelli, *Clin. Invest. (Berlin, Ger.)*, **71**, Suppl. 8, S134-S136 (1993).
38. D. Yubero, R. Montero, M. Ramos, et al., *Biofactors*, **41**(6), 424 – 430 (2015).
39. Y. Zu, C. Zhao, C. Li, et al., *J. Sep. Sci.*, **29**(11), 1607 – 1612 (2006).
40. A. J. Claessens, C. K. Yeung, L. J. Risler, et al., *Ann. Clin. Biochem.*, **53**(2), 265 – 273 (2016).