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A Rapid and Sensitive UPLC-MS (APCI) Method for the Determination of CoQ10

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Abstract

CoQ10 is a fat-soluble vitamin-like substance present in every cell of the body and serves as a coenzyme for

several of the key enzymatic steps in the production of energy within the cell. CoQ10 has become a valued dietary supplement and is linked to the treatment of heart disease (especially heart failure), gum diseases, and breast cancer. It is sold around the world as a nutritional supplement. This study describes a highly sensitive, selective, fast, and simple quantification method for CoQ10 using a Photo Diode Array (PDA) detector and single quadrupole MS detection for confirmation of identity.

Benefits

Increased speed, reduced solvent usage, and reduced cost of solvent disposal

Introduction

Coenzyme Q10 (CoQ10 or Ubiquinone) was first discovered in 1957 by Dr. Frederick Crane[1], Ph.D. and its chemical structure was determined the following year by Dr. Karl Folkers.[2]

CoQ10 is a fat-soluble vitamin-like substance present in every cell of the body and serves as a coenzyme for several of the key enzymatic steps in the production of energy within the cell.

CoQ10 is comprised of a quinone ring and a hydrocarbon side chain made up of 10 isoprene units (Figure 1). This side chain is synthesized from acetyl-CoA through the mevalonate pathway (the mevalonate pathway is used for the first steps of cholesterol biosynthesis). The quinone ring is synthesized from the amino acids (tyrosine or phenylalanine) and is responsible for CoQ10 having such powerful antioxidant activity.

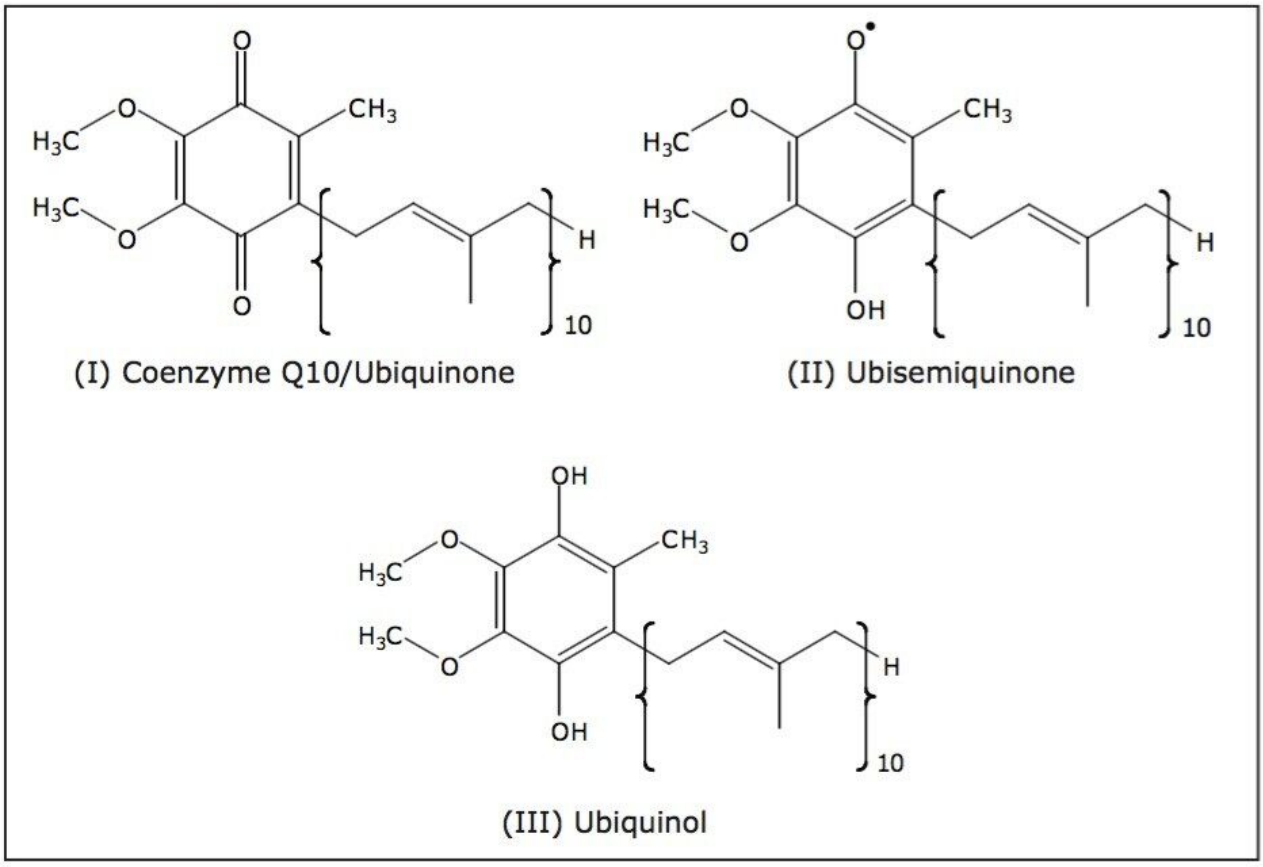

Figure 1. Structures of coenzyme Q10 (I: oxidized form, II: ubisemiquinone, denoted as QH, and III ubiquinol, denoted as QH2).

The reduced form of CoQ10 is able to scavenge free radicals that may cause damage to the body's DNA, proteins, and lipids, reducing the risk to various diseases including cardiovascular disease, and neurodegenerative diseases such as Alzheimer's or Parkinson's.[3, 4]

CoQ10 has become a valued dietary supplement and is linked to the treatment of heart disease (especially heart failure), gum diseases, and breast cancer.[5]

Sources of CoQ10 are limited and in the past few years the demand for CoQ10 has increased dramatically, especially since Japan's decision to grant CoQ10 Foods for Specified Health Use (FOSHU) status. It is now sold around the world as a nutritional supplement. Up until 2001, it was prescribed as a drug in Japan. Japan manufactures 99% of all CoQ10 for distribution worldwide.

Where is it found?

CoQ10, which is essential to the production of cellular energy, can be derived from dietary sources, synthesized in the body, or manufactured.

The dietary sources of CoQ10, include meat, poultry, fish, and soy oil but these sources only contain a small amount of CoQ10.[6] CoQ10 can be synthesised in the body, although this process relies on other essential nutrients to be present. Synthesis within the body can be influenced by many factors such as strenuous exercise, illness or intake of pharmaceutical drugs so that production of CoQ10 does not always meet the body' s requirements.

There are currently two different manufacturing techniques being used to commercially produce CoQ10: (1) Solanesol method, which uses extract (solanesol) from tobacco leaves, where both *trans*- and *cis*- forms of CoQ10 are formed; and (2) Microbiological fermentation method, where selected strains of microbes are placed in a fortified molasses-based carbohydrate medium to produce CoQ10 (as well as CoQ6, CoQ7, CoQ9, and CoQ11). Only *trans*- isomer form is present and this is the form made by the human body.

Presently, determination of CoQ10 is mainly performed using high performance liquid chromatography (HPLC) with ultraviolet (UV) detection[7] or electrochemical (EC) detection[8] for purity and quality control purposes. However, these methods lack sensitivity and they are relatively non-specific. Mass spectrometry will provide excellent sensitivity for quantification in complex matrices with added confirmation of identity.

This study describes a highly sensitive, selective, fast, and simple quantification method for CoQ10 using a Photo Diode Array (PDA) detector and single quadrupole MS detection for confirmation of identity.

The SQ detector (SQD) is compatible with both Waters Empower and MassLynx Software. The MS set-up parameters are made easy with the new functionality of IntelliStart, which is incorporated into both software packages.

Figure 2. ACQUITY PDA SQ detector.

Experimental

LC Conditions

LC system:	ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18} Column 2.1 x 50 mm, 1.7 µm
Column temp.:	30 °C
Flow rate:	700 µL/minute
Mobile phase A:	Methanol:2-propanol (4:1)
Gradient:	Isocratic
Injection volume:	5 µL

PDA Conditions

PDA System:	ACQUITY UPLC PDA
Wavelength:	275 nm

MS Conditions

MS System:	ACQUITY SQD
Ionisation mode:	APCI positive
Corona voltage:	5 µA
Cone voltage:	45 V
Desolvation temp.:	400 °C

Desolvation gas:	800 L/Hr
Source temp.:	130 °C
SIR:	*m/z* 864.4

Acquisition and processing methods

The data were acquired using Waters MassLynx Software version 4.1. Incorporated into MassLynx, is the IntelliStart technology which is designed specifically for the SQ and TQ detectors. IntelliStart automatically optimizes MS parameters for your sample and also monitors the health of the MS system, reducing the time for operatorintensive troubleshooting and upkeep. The data were processed using TargetLynx Application Manager (quantification package capable of automating quality control checks such as, calculating ion ratios, flagging analytical results above/below thresholds set by the user, plus other features).

Samples

CoQ10 from dietary supplements in the form of capsules was diluted with the mobile phase and filtered prior to analysis.

Results and Discussion

A standard CoQ10 solution was continuously infused in to the mass spectrometer and IntelliStart automatically tuned the sample for the optimum MS settings. It was found that positive APCI gave the best response for CoQ10.

The protonated ion *m/z* 864.4 ($[M+H]^+$) was used for quantification using selected ion recording (SIR), as it gave the best selectivity and sensitivity. Figure 3 shows the chromatogram of CoQ10 (t_R = 1.40 min) at 100 ppb using SIR mode. The peak width at the base is approximately 10s.

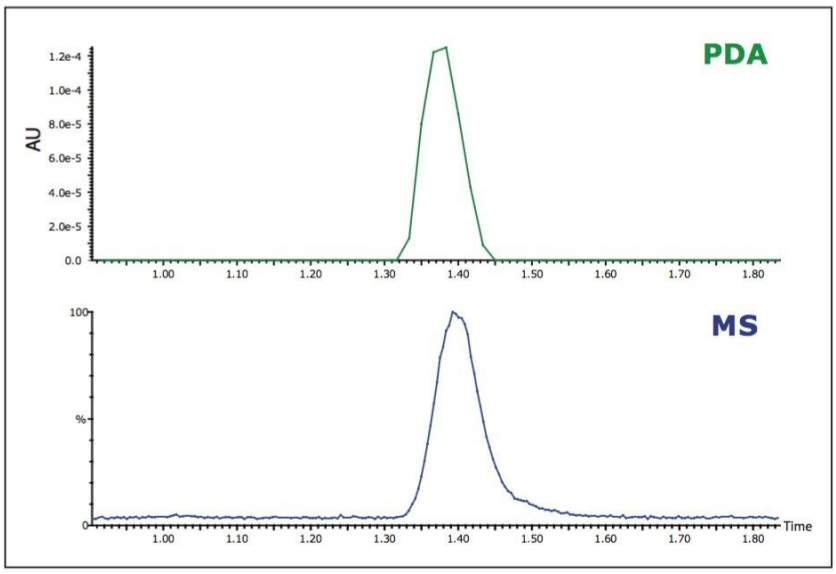

Figure 3. Chromatogram of CoQ10 in a solvent standard at 100 ppb, using both PDA and MS detection.

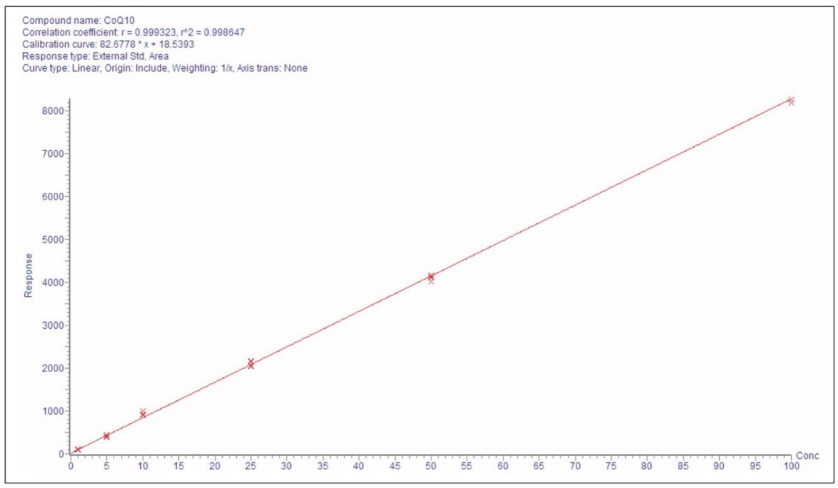

Figure 4. Calibration curve for CoQ10 solvent standards across the range of 1 to 100 ppb, using ACQUITY PDA SQD.

Quantification of CoQ10

The response for CoQ10 using APCI-MS detection was linear ($r^2 = 0.999$) over the range of 1 to 100 ppb.

The method using APCI-MS detection allowed confident identification of CoQ10 in a diluted dietary supplement (Figure 5).

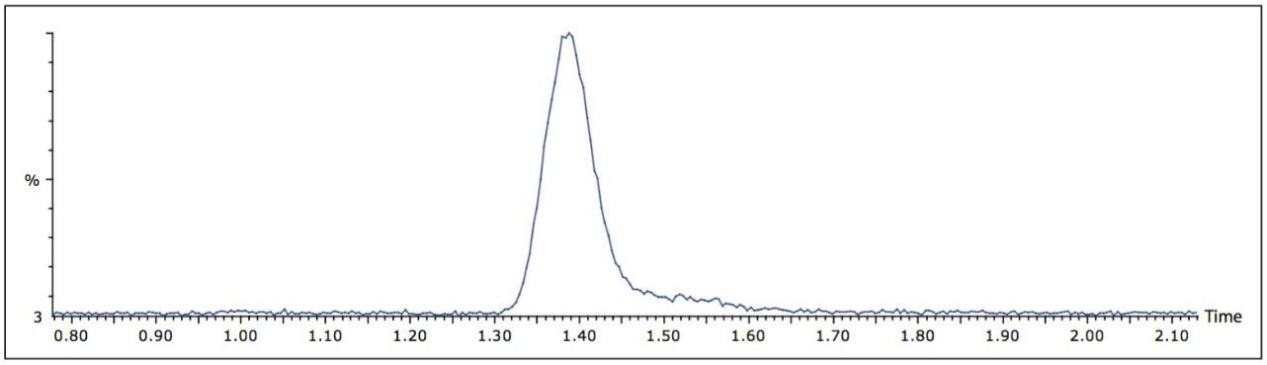

Figure 5. Chromatogram of CoQ10 in a dietary supplement.

Conclusion

A method based on UPLC with PDA and MS detection has been developed for the analysis of CoQ10 in dietary supplements.

The ACQUITY UPLC delivers a fast identification of this fat soluble antioxidant, with a run-time of three minutes. The benefits of ACQUITY UPLC for a revenue conscious laboratory are increased speed, reduced solvent usage, and reduced cost of solvent disposal.

The single quadrupole mass spectrometer (SQD) offers extra confidence in the confirmation of identity of CoQ10, with increased sensitivity for quantification of this coenzyme in dietary supplements.

References

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