Original Article

Evaluation of pharmacokinetic parameters of ubiquinol acetate, ubiquinone and ubiquinol in male Sprague-Dawley rats – A comparative study

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ABSTRACT

Objectives: The objective of this study was to evaluate single-dose oral comparative pharmacokinetics studies of ubiquinol acetate (EnQ10), ubiquinone and ubiquinol in male Sprague-Dawley (SD) rats.

Materials and Methods: Oral suspension formulations of ubiquinol acetate (EnQ10), ubiquinone, and ubiquinol at 300 mg/kg body weight (equivalent dose of ubiquinone) were prepared in 0.1% (v/v) Tween 80 and 15% (w/v) hydroxypropyl-β-cyclodextrin. Six animals per group for each compound were dosed with oral suspension formulations of EnQ10, ubiquinone, and ubiquinol. Blood samples were collected at time points of 1, 2, 4, 6, 8, 10, 24, 30, and 48 h and plasma samples were analysed using liquid chromatography with tandem mass spectrometry for the analyte's ubiquinone and ubiquinol.

Results: In EnQ10 dosed animals, the plasma mean concentration maximum, Cmax (347.83 ng/mL) of ubiquinol was found to be 2.52 times higher versus ubiquinone dosed animals (137.90 ng/mL). Furthermore, in EnQ10 dosed animals, the observed plasma exposure (AUC$_{last}$) (4808.94 h*ng/mL) for ubiquinol was found to be 3.96 times higher versus. Ubiquinone dosed animals (1214.42 h*ng/mL). One-way ANOVA (Analysis of Variance) was performed for the C$_{max}$ and AUC$_{last}$ of ubiquinol. There was a significant increase ($P < 0.05$) in the C$_{max}$ and AUC$_{last}$ of ubiquinol in animals dosed with EnQ10 compared to the animals dosed with ubiquinone.

Conclusion: The findings from this study indicated that ubiquinol acetate (EnQ10) showed better oral bioavailability compared to ubiquinone (CoQ10) when administered orally (300 mg/kg body weight equivalent dose of ubiquinone) in the male in male SD Rats.

Keywords: Coenzyme Q10, CoQ10, EnQ10, Ubiquinol, Ubiquinone, Ubiquinol acetate

INTRODUCTION

Coenzyme Q10 (CoQ10) is a natural, lipid soluble and vitamin-like endogenous antioxidant. It is majorly present in organs such as heart, liver and kidney. In elder age, level of CoQ10 synthesis will be reduced. Depletion levels of CoQ10 in body are associated with aging and several age-related pathologies. Hence, CoQ10 has been used as a dietary supplement in humans. CoQ10 is proved to be a medical agent for treating cardiovascular diseases based on its fundamental role in mitochondrial function and cellular bioenergetics.[1,2] CoQ10 mainly present in two forms ubiquinone (inactive, oxidised form of CoQ10) and ubiquinol (active, reduced form of CoQ10). Human body converts ubiquinone into the active form, ubiquinol which is body's preferred form of CoQ10.[3,4]

Most of CoQ10 supplements contain ubiquinone because ubiquinol is unstable and easily converts back into ubiquinone when exposed to air.[5] Ubiquinol acetate (EnQ10) is a stable novel antioxidant developed in recent days. Recent studies have proved that EnQ10 is more stable and biologically active. Furthermore, synthesised ubiquinol acetate showed better stability than that of ubiquinol at the end of 3 months.[6] Hence, understanding the oral pharmacokinetics (PKs) of ubiquinol acetate (EnQ10) is required in comparison with ubiquinone and ubiquinol.

MATERIALS AND METHODS

Test compounds

The test compounds, ubiquinol acetate (Source: Anthem Biosciences Pvt. Ltd.; Batch No.: A21600497; Purity: 99.1%) ubiquinone (Source: Kingdomway; Batch No: 51-175102; Purity: 100.10%) and ubiquinol (Source: Anthem Biosciences Pvt. Ltd.; Batch No.: A011809898; Purity: 98.42%) used in the present study were collected from Department of Chemistry, Anthem Biosciences Pvt. Ltd, Bengaluru, India.

Materials and chemicals

Hydroxypropyl-β-cyclodextrin (catalogue no. H0979; TCI chemicals); Tween 80 (catalogue no. P1754; Sigma-Aldrich); isoflurane USP (Raman and Weil Pvt Ltd); dipotassium ethylenediaminetetraacetate (catalogue no. RANKE2014; Rankem); tert-butyl methyl ether (catalogue no. RANKM2500; Rankem); formic acid (catalogue no. F0507; Sigma Aldrich) and 2-propanol (catalogue no. RANKP0792; Rankem).

Test system

Male Sprague-Dawley (SD) weighing around 250–300 g obtained from Hylasco Biotechnology (India) Pvt Ltd was used for this study. This study was approved by the Institutional Animal Ethical Committee (IAEC) with approval number which is ABD/IAEC/PR/89-17-20.

Rats were freely accessible to food and water during the quarantine period. Rats were fasted for 12 h and allowed free access to water before the experiments. All rats were processed according to suggested ethical guidelines for the care of laboratory animals.

Formulation preparation

Ubiquinol acetate, ubiquinone, and ubiquinol oral formulations were prepared using 0.1% (v/v) Tween 80 and 99.9% (v/v) of 15 % (w/v) hydroxypropyl-β-cyclodextrin in water. The formulations were prepared by trituration using mortar and pestle to obtain uniform suspension formulations of 60 mg/ mL strength (Dose: 300 mg/kg body weight, equivalent dose of ubiquinone; Dose volume: 5 mL/kg body weight).

PK study

All SD rats were randomly divided into three groups (G1, G2, and G3) with 6 animals per group. Six fasted animals per group for each compound ubiquinol acetate (G1), ubiquinone (G2), and ubiquinol (G3) were dosed with oral suspension formulations (60 mg/mL equivalent to ubiquinone). The blood samples were collected in K_2EDTA tubes and plasma samples were harvested at time points of 0 (pre-dose), 1, 2, 4, 6, 8, 10, 24, 30, and 48 h. Then, the plasma samples were analysed using API 3200 Q-trap liquid chromatography with a tandem mass spectrometry system for the analyte's ubiquinone and ubiquinol.

Instruments and conditions

A sensitive high-performance liquid chromatography (HPLC) coupled with a triple-quadrupole mass spectrometry method was developed for the quantification of ubiquinone and ubiquinol in rat plasma using the liquid-liquid extraction (LLE) method.

Liquid-liquid extraction

Plasma samples (100 μL) were added with 10 μL of internal standard working solution (10 μg/mL) in extraction tubes. About 2.5 mL extraction solvent; an equal mixture of isopropyl alcohol and tertiary butyl methyl ether (1:1) was added and kept for mixing for 10 min in a vibromax shaker and then centrifuged at 10,000 rpm at a set temperature of 10°C. Exactly, 2 mL of supernatant was evaporated (45°C) to dryness using a nitrogen turbo evaporator. The dried samples were reconstituted with 200 μL of Mobile phase (1:1 2-Propanol (IPA): 5mM ammonium formate in methanol). Chromatographic separation was achieved on a Venusil XBP (broad pH range) C_{18} (4.6 mm × 50 mm, 3.0 μm) column with 0.1% formic acid in isopropyl alcohol and 5 mM ammonium formate in methanol as mobile phase with isocratic elution. The flow rate was set at 0.700 mL min^{-1}. The detection was accomplished by a triple-quadrupole tandem mass spectrometry in multiple-reaction monitoring scanning through an electrospray ionization source in the positive mode. The optimised mass transition ion pairs (m/z) for quantitation were m/z 880.698/197.100 for ubiquinone, m/z 882.700/197.000 for ubiquinol, and m/z 376.165/165.100 for internal standard (Haloperidol).

Linearity curve in rat plasma

The calibration range for ubiquinone and ubiquinol in rat plasma was prepared according to the concentrations

LC parameters	
Column	Venusil XBP C_{18} (4.6 mm×50 mm, 3.0 μm)
Mobile phase	0.1% Formic acid in IPA: 5mM ammonium formate in methanol (45:55% v/v)
Separation mode	Isocratic
Flow rate	0.700 mL/min
Injection volume	10 μL
Autosampler temperature	10°C
Column oven temperature	40°C
MS parameters	
LC-MS	API 3200 Q Trap
Source	Turbo ion spray
Polarity	Positive
m/z of Ubiquinone [M+NH4]+	880.698→197.100
m/z of Ubiquinol [M+NH4]+	882.700→197.000
m/z of IS (Haloperidol)	376.165→165.100

LC-MS: Liquid chromatography-mass spectrometry, API: Atmospheric pressure ionization

anticipated in the plasma samples to be determined. The calibration curve range of 10.937–4999.995 ng/mL for ubiquinone and 17.699–2265.500 ng/mL for ubiquinol in rat plasma were prepared by spiking blank rat plasma with respective aqueous analyte standards.

PK analysis

The non-compartmental PK model was carried out using the Phoenix WinNonlin 6.3 software. The PK parameters including the area under the curve (AUC) of plasma concentration versus time, the maximum concentration (C_{max}) recorded, the time taken to reach C_{max} (T_{max}), the half-life of elimination ($t_{½}$), and the mean residential time were determined.

Statistical analysis

Statistical analysis was performed on the results by one-way analysis of variance (ANOVA) to compare the C_{max} and AUC_{last} of ubiquinol (analyte) in all dosed groups using GraphPad Prism 5.0 statistical software package. The significance level was chosen at $P < 0.05$ for all statistical analyses in comparison to all dosed groups.

RESULTS

In this study, calibration plots were linear over the range of 10.937–4999.995 ng/mL for ubiquinone [Figure 1] and 17.699–2265.500 ng/mL for ubiquinol [Figure 2] in rat plasma. The linearity coefficient (r) for ubiquinone and ubiquinol was >0.98 and lower limit of quantification level was 10.937 ng/mL for ubiquinone and 17.669 ng/ml for ubiquinol. This developed method was successfully used in the PK studies for the identification of ubiquinone and ubiquinol in rat plasma.

The plasma concentrations of ubiquinone and ubiquinol over the time course after oral administration of ubiquinol acetate, ubiquinone, and ubiquinol are shown in [Graph 1 and Graph 2]. The summary of PK parameters of ubiquinone and ubiquinol are shown in [Table 1]. The summary of statistical analysis results are shown in [Table 2 and Graph 3].

DISCUSSION

Ubiquinol is an essential antioxidant required for human body for various health benefits. CoQ10 in the form of
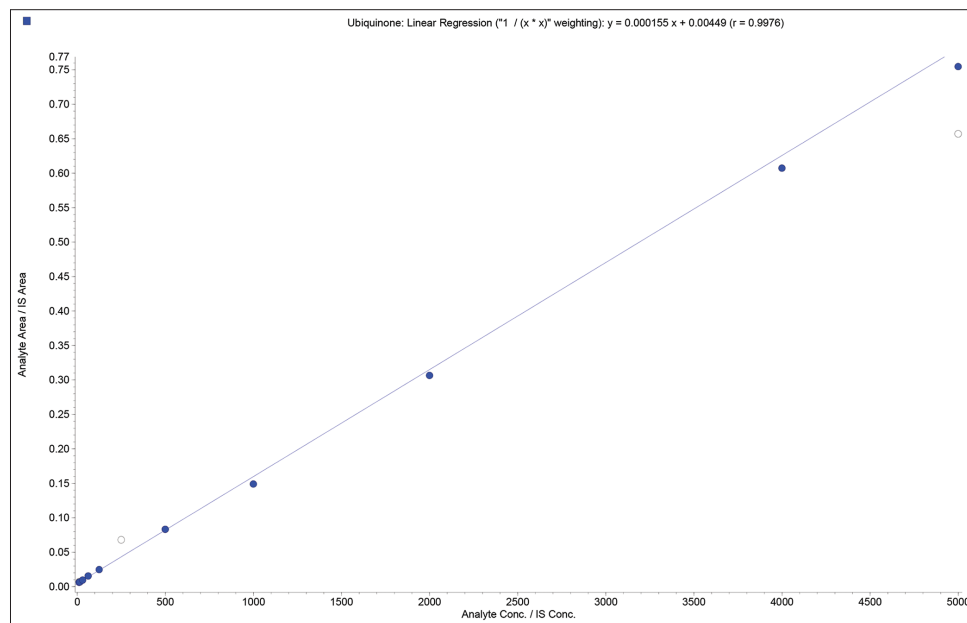

Figure 1: Calibration curve for ubiquinone in rat plasma (10.937–4999.995 ng/mL). PK: Pharmacokinetics, IS: Internal standard.

Ubiquinol : Linear Regression ("1 / (x * x)" weighting): y = 0.000242 x + -0.0033 (r = 0.9890)
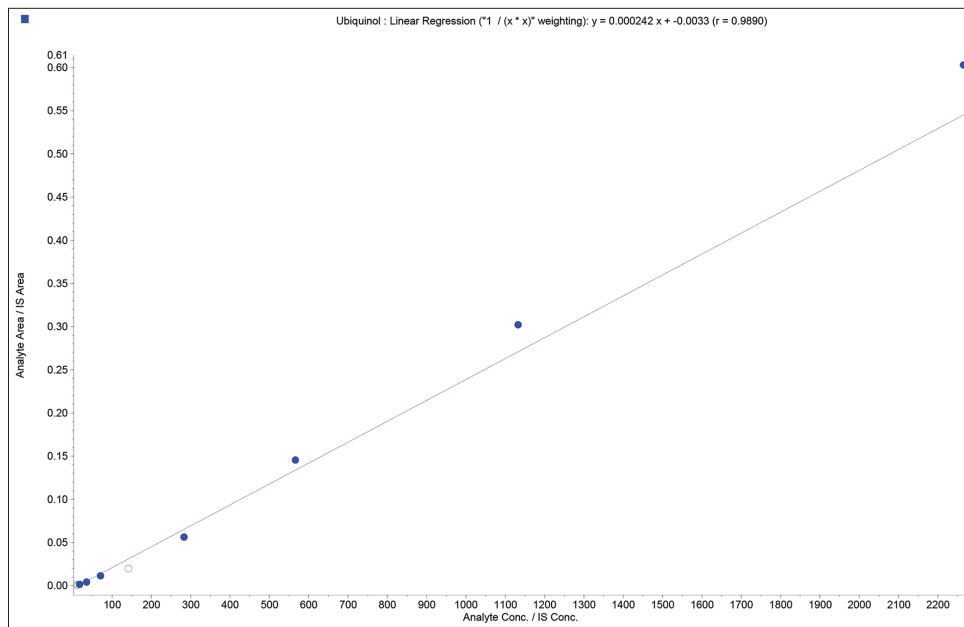
Figure 2: Calibration curve for ubiquinol in rat plasma (17.699–2265.500 ng/mL). PK: Pharmacokinetics, IS: Internal standard.
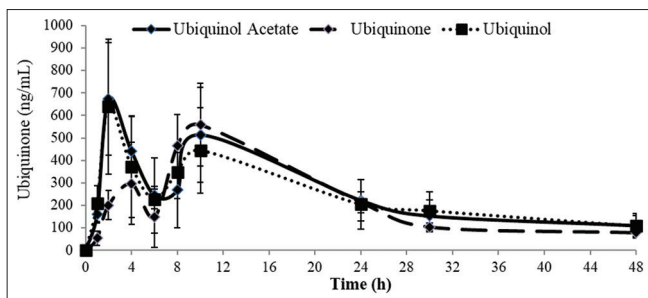
Graph 1: Time-course of plasma ubiquinone after oral administration of ubiquinol acetate, ubiquinone and ubiquinol in male Sprague-Dawley rats.
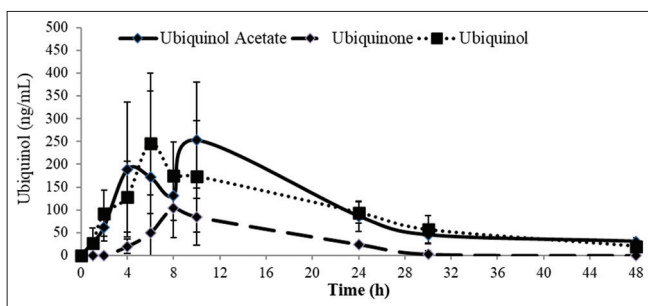
Graph 2: Time-course of plasma ubiquinol after oral administration of ubiquinol acetate, ubiquinone, and ubiquinol in male Sprague-Dawley rats.

ubiquinone and ubiquinol is available in various brands in the market. Ubiquinone (CoQ10) has been on the market for closer to 40 years, whereas ubiquinol has only been around since 2007.[10]

Studies suggest that ubiquinol has superior bioavailability than ubiquinone (CoQ10) because it accounts for 90% of the CoQ10 that's in the blood. Although ubiquinol has more health benefits as an antioxidant, it is known for its chemical instability and is rapidly oxidised to ubiquinone in atmospheric oxygen.[6] Hence, in recent years, efforts have been made to develop a stable form of ubiquinol. Recent studies have proved the chemical stability of novel antioxidant ubiquinol acetate (EnQ10).

Many bioanalytical techniques have been developed for the quantification of ubiquinone and ubiquinol in plasma/serum by HPLC, ultra-performance liquid chromatography, and liquid chromatography-mass spectrometry methods. These methods showed good linearity, precision, accuracy, and recovery in biological matrices.[7-9]

Based on the study results, oral PK studies of ubiquinol acetate, ubiquinone, and ubiquinol in male SD rats showed that the C_{max} (347.83 ng/mL) for ubiquinol (active form) was found to be 2.52 times higher in the animals dosed with ubiquinol acetate (EnQ10) compared to animals dosed with ubiquinone (137.90 ng/mL). The mean plasma exposure AUC_{last} (4808.94 h*ng/mL) for ubiquinol was found to be 3.96 times higher in the animals dosed with ubiquinol acetate compared to animals dosed with ubiquinone (1214.42 h*ng/mL). Statistical analysis was performed by one-way ANOVA for ubiquinol C_{max} and AUC_{last} in animals dosed groups.

CONCLUSION

Based on the obtained results, it was concluded that Ubiquinol acetate (EnQ10) showed better oral bioavailability

Table 1: Summary of pharmacokinetic parameters of ubiquinone and ubiquinol after oral administration ubiquinol acetate, ubiquinone and ubiquinol in male SD rats. Values represent the mean±standard deviation (*n*=6).
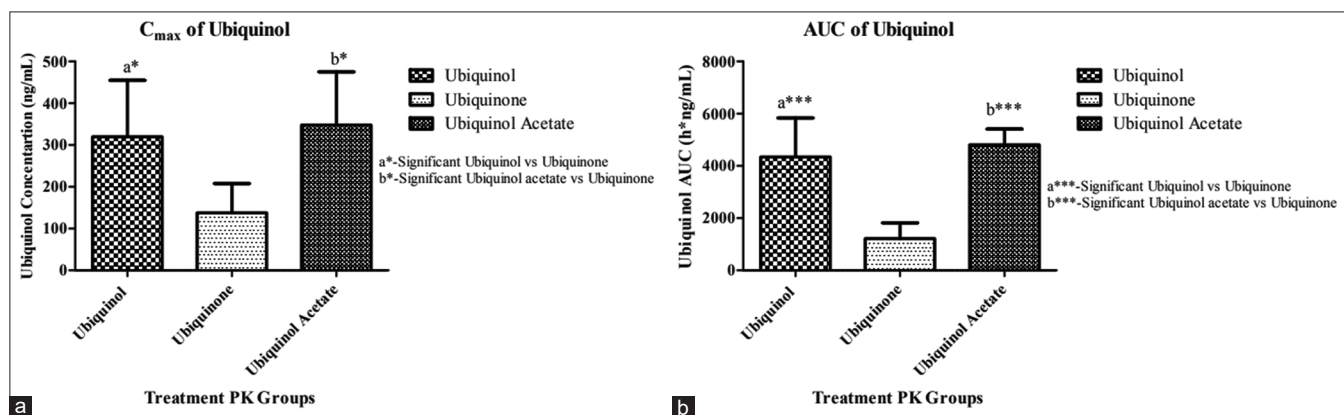
Parameters	Ubiquinone (analyte)			Ubiquinol (analyte)		
	Ubiquinol acetate	Ubiquinone	Ubiquinol	Ubiquinol acetate	Ubiquinone	Ubiquinol
C_{max} (ng/mL)	800.27±136.70	600.63±173.16	656.44±283.29	347.84±127.21	137.91±70.42	319.68±134.97
T_{max} (h)	4.67±4.13	9.33±1.03	3.33±3.27	7.67±2.66	8.333±1.51	7.67±1.97
AUC_{last} (h*ng/mL)	12248.01±1697.68	10746.57±2235.79	11755.37±3966.81	4808.94±602.45	1214.42±608.39	4344.55±1489.48
AUC_{inf} (h*ng/mL)	15687.254±3686.53	12284.54±2518.40	14884.39±5319.77	5397.06±653.41	1700.00±381.47	4985.73±1623.48
AUC_{extrap} (%)	20.36±9.72	12.64±2.47	20.70±4.55	10.93±2.74	32.00±23.510	13.20±5.00
$t_{1/2}$ (h)	20.55±5.63	13.57±1.24	19.93±3.46	13.35±1.610	13.61±7.561	13.98±1.53
MRT_{last} (h)	17.14±1.58	16.87±0.97	17.67±2.30	16.04±2.32	12.00±1.76	15.49±2.61

SD: Sprague-Dawley, AUC: Area under curve (h*ng/mL), C_{max}: Concentration maximum (ng/mL), *n*: Number of animals, T_{max}: Peak Plasma Time (h), t1/2: Half-life (h), MRTlast: Mean residence time (h)

Table 2: Statistical analysis of C_{max} and AUC_{last} of ubiquinol acetate, ubiquinone and ubiquinol in male SD rats.

PK parameters	Ubiquinol acetate		Ubiquinone		Ubiquinol	
	Ubiquinone	Ubiquinol	Ubiquinone	Ubiquinol	Ubiquinone	Ubiquinol
C_{max} (ng/mL)	800.27	**347.84[b*]**	600.63	**137.90[a*]**	656.44	319.68
AUC_{last} (h*ng/mL)	12248.01	**4808.94[b***]**	10746.57	**1214.42[a***]**	11755.37	4344.55

a* the C_{max} (ng/mL) in bold value was found to be statistically significant (*P*<0.05) compared to of ubiquinol versus ubiquinone. b* the C_{max} (ng/mL) in bold value was found to be statistically significant (*P*<0.05) compared to of ubiquinol acetate versus ubiquinone. a*** the AUC_{last} (h*ng/mL) in bold value was found to be statistically significant (*P*<0.05) compared to of ubiquinol versus ubiquinone. b*** the AUC_{last} (h*ng/mL) in bold value was found to be statistically significant (*P*<0.05) compared to of ubiquinol acetate versus ubiquinone, SD: Sprague-Dawley, AUC: Area under curve (h*ng/mL), C_{max}: Concentration maximum (ng/mL), PK: Pharmacokinetics

Graph 3: Statistical analysis by one-way analysis of variance for ubiquinol C_{max} and AUC_{last} after dose administration of ubiquinol acetate, ubiquinone, and ubiquinol in male Sprague-Dawley rats. (a) C_{max} of ubiquinol and (b) AUC_{last} of ubiquinol. PK= Pharmacokinetics, AUC=Area under curve (AUC, h*ng/mL), Cmax = Concentration maximum (Cmax, ng/mL).

compared to Ubiquinone (CoQ10) when administered orally in male SD Rats.

Declaration of patient consent

The authors certify that they have obtained all appropriate consent.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The author(s) confirms that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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