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Bioavailability of water-soluble CoQ10 in beagle dogs

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ABSTRACT

The bioavailability of a novel water-soluble inclusion complex of CoQ10, prepared in our laboratory was determined and compared with the bioavailability of commercially available oil-based form of CoQ10. Experimental work consisted of single dose comparative bioavailability study on seven beagle dogs, with a 14-day washout period between treatments. Identification and quantification of CoQ10 was done with HPLC–MS method using positive APCI ionization and SIM mode, M^+ m/z 863.4. The bioavailability results confirm that the water-soluble formulation has nearly three times higher $AUC_{(0-48\,h)}$, two times higher C_{max}, and T_{max} is shortened from 6 to 4 h.

1. Introduction

Coenzyme Q10 (CoQ10), also known as ubiquinone 50, is a fat-soluble molecule produced in the majority of living cells. It plays a key role in energy metabolism as an integral part of electron transport system [1]. Its lipophilic nature is based on a very long polyisoprene tail. The functional group of the molecule is the quinone ring. Its reduction of the quinone to quinol form allows CoQ10 to be an essential electron carrier in the mitochondrial respiratory chain and its participation in the transport of protons and electrons through different membranes of cells and organelles. As such, it is especially important for proper functioning of muscle tissue, especially the heart muscle [2]. CoQ10 is also recognized as a powerful systemic radical scavenger (antioxidant) that blocks oxidative injuries to DNA, lipids, proteins and other essential molecules and is also capable of functioning synergistically with other antioxidants [3].

CoQ10 is mainly produced in the body, but genetic mutations, ageing, cancer and drugs may cause a decrease of coenzyme Q10 in serum or tissue. In these cases, supplementation of CoQ10 with an exogenous source is necessary. In this case the most convenient method for supplementation is enriched food.

Although CoQ10 is classified as a lipophilic compound, its degree of solubility in lipids is limited, while it is practically insoluble in aqueous solutions. Due to its high molecular weight and poor aqueous solubility it is poorly and slowly absorbed from the gastrointestinal tract [4]. The importance of a soluble CoQ10 formulation was recognized during the development of different CoQ10 preparations. Since then, chemists have endeavored to develop a formulation for oral administration with a better water-solubility and therefore better bioavailability.

Shulz et al. reported that a novel solubilizate formulation CoQ10 (Solu^{TM} Q10) was clearly superior to oily dispersions and crystalline CoQ10 in their overall bioavailability [5]. The non-covalent complex of water-soluble polyoxyethanyl-*f*-tocopheryl sebacate (PTS) and CoQ10 at molar ratio of 2:1 indicates the therapeutic potential of antioxidants for treatments of ischemia/reperfusion injuries and PTS-CoQ10 complex is particularly appropriate for the application in acute conditions, such as stroke or cardiac arrest [6]. Ankola et al. improved the oral bioavailability of CoQ10 by delivering it as nanoparticulate formulation based on biodegradable polylactide-*co*-glycolide (PLGA) and indicated the potential of nanotechnology in improving the therapeutic value of molecules like CoQ10 [7]. In 2006, Bhagava and Chopra reviewed available data on the absorption, metabolism and pharmacokinetics of CoQ10. They represented its fundamental role in cellular bioenergetics and its important role as an antioxidant. Furthermore, in many cases soluble form of CoQ10 show enhanced bioavailability, and its beneficial effects on cardiovascular and neurodegenerative diseases were proved [8].

It is known that cyclodextrins (CDs) are used extensively as pharmaceutical excipients to increase the solubility of poorly water-soluble compounds, bioaccessibility and stability of the preparations, and to produce new, more convenient medical forms.

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There is no evidence that CDs are harmful for the human health. Non-toxic effect level for oral administration of β-cyclodextrin was established to be 0.7–0.8 g/(kg day) in rats and about 2 g/(kg day) in dogs [9].

On the basis of these findings we developed a water-soluble CoQ10 in a form of a water-soluble paste, based on molecular encapsulation of CoQ10 into the β-cyclodextrin's lipophilic cavity [10,11]. Our previous unpublished experiments showed that this product could be easily and uniformly mixed with many food products. In addition when enriched food was placed into the ambient with a pH value below 3, the inclusion complex was disintegrated and CoQ10 was released from the β-cyclodextrin carrier. These findings have proven that the water-soluble paste with 7–10% of CoQ10 could be a very convenient formulation for animals and humans which dislike or cannot swallow relatively big capsules.

Several methods have been reported for determination of CoQ10 either in oxidized form (ubiquinon-10) or reduced form (ubiquinol-10) using reverse-phase high-performance liquid chromatography (RP-HPLC), equipped with UV or electrochemical detector (ECD) for ubiquinone or ubiquinol detection, respectively [12–15]. Mosca et al. described a method for determining total CoQ10 in plasma based on oxidation of CoQ10. The method was based on oxidation of CoQ10, which was performed with *para*-benzoquinone followed by extraction with 1-propanol and direct injection into reverse phase high-performance liquid chromatography apparatus (RP-HPLC) equipped with photodiodide array detector for UV spectrum analysis [16]. In the present paper, we describe a HPLC–MS method based on APCI ionization mode.

The aim of this study, performed in the Veterinary clinic for small animals (University of Ljubljana, Slovenia), was to collect information about the intestinal absorption of the inclusion complex of CoQ10 with β-cyclodextrin. For this purpose the bioavailability profiles of this water-soluble CoQ10 formulation and a commercially available oil-based CoQ10 in the form of soft-gel capsules were compared. The bioavailability was determined by measuring the periodical plasma CoQ10 levels after administration to a group of beagle dogs. The different preparations were compared in the same subjects using the crossover method.

2. Materials and methods

2.1. Materials

Methanol, ethanol, 2-propanol, 1,4-dioxane, acetonitrile, hexane, perchloric acid and acetic acid (LC grade) were supplied by Merck (Darmstadt, Germany). CoQ10 standard was purchased from Sigma–Aldrich (Steinheim, Germany). β-Cyclodextrin (food grade) was supplied by Xi'an Hong Chang Pharmaceuticals Co. (China), and CoQ10 (pharmaceuticals grade) by Linyi Tianliheng Trade Co. (China).

For exogeneous CoQ10 administration, two formulations were selected. The reference product, commercially available 30 mg CoQ10 soft-gel capsules were obtained from a local pharmacy store while the water-soluble paste with 7.5% of CoQ10 in a form of inclusion complex with β-cyclodextrin was synthesized in our laboratory (Laboratory for Food Chemistry, National Institute of Chemistry, Slovenia) according to previously filed patents [10,11].

2.2. Standard solutions

Stock solution was prepared by dissolving 10 mg of CoQ10 in 20 mL of 2-propanol. The stock solution was stable for 1 month stored in the dark at 4 °C. From stock solution, eight different calibration standard solutions were prepared in concentrations from 0.05 to 10 mg/L. These solutions were used for quantification and linearity test. For LOD and LOQ determination the solutions were further diluted down to 0.01 mg/L.

2.3. Sample preparation

Dog blood plasma (400 μL) was denatured with 200 μL of 10% perchloric acid in ethanol (v/v). Samples were extracted three times with 2 mL of *n*-hexane. The combined organic extracts were evaporated on a rotary evaporator (Rotavapor R-144 equipped with a water bath B-480, Büchi, Flawil, Switzerland). The dry residue was redissolved in 200 μL of 2-propanol and analyzed with HPLC/MS.

2.4. Instrumentation

Separation and quantitative determination of CoQ10 was performed with a Surveyor LC system (Thermo Finnigan, Riviera Beach, CA, USA) equipped with LCQ ion-trap mass spectrometer (Finnigan MAT, San Jose, CA, USA).

Analyses of CoQ10 were done by a gradient separation with two mobile phases. The phase A was a mixture of 1,4-dioxan, methanol, ethanol and acetic acid (5:30:65:0.1, v/v/v/v); and phase B was 100% acetonitrile. The starting condition of 30% A and 70% B was held for 1 min, then the amount of the mobile phase A was linearly increased to 100% in a period of 11 min and held at 100% for next 3 min. At the end of a run the mobile phase composition was reverted to the initial conditions and kept for 2 min. The HPLC column was a Gemini C18 with dimensions 100 mm × 4.6 mm, particle size 3 μm (Phenomenex, Torrance, CA, USA). The flow rate was constant at 1.0 mL/min, injection volume was 10 μL, and column temperature was 45 °C. MS identification and quantification was done in positive APCI ionization mode. Ionization discharge current was 5.0 μA and source temperature was 450 °C. Capillary voltage was 23.0 V, tube lens offset was 10.0 V, capillary temperature was 250 °C, sheath gas pressure was 2.7 bar, and auxiliary gas flow was 3 L/min. Data processing was done with Xcalibur 1.3 software (Thermo Finnigan Corporation, USA). The chromatograms were obtained in SIM mode, with molecular mass of CoQ10 M^+ m/z 863.4, and the retention time was 10.6 min.

2.5. Study protocol

The experiment consisted of a single-dose comparative bioavailability study of two CoQ10 preparations with a 14-day washout period between treatments. The study protocol was approved by Ministry of Agriculture, Forestry and Food and the Slovenian Veterinary Administration (VURS) under the code: 323-02-818/2005.

Seven beagle dogs were recruited for the study. The dogs weighted between 16.5 and 22.6 kg, with the average body weight of 19.5 kg. The subject in study were given 30 mg of CoQ10 each, taken orally with food. Venous blood samples (3 mL each) were collected at time 0, 0.33, 0.67, 1.0, 2.0, 4.0, 6.0, 10.0, 24.0, 48.0 h after administration. Blood was collected in heparinized tubes and rapidly centrifuged. Plasma was separated and immediately frozen at −70 °C until needed for analysis. In the first trial, an oil-based capsule containing 30 mg of CoQ10 was given to each dog. After 14 days the experiment was repeated with water-soluble 7.5% CoQ10 paste (second trial). Each dog received a single dose of water-soluble paste. Four hundred milligrams of paste, equivalent to 30 mg of CoQ10, were added into the food.

Plasma samples were combined with quality control (QC) standards at two concentration ranges (0.25 mg/L) and at high (2.5 mg/L) obtained by spiking empty plasma with CoQ10 standard. The overall time needed for the presented study was four analytical days and was done in three sequences. Each sequence consisted of

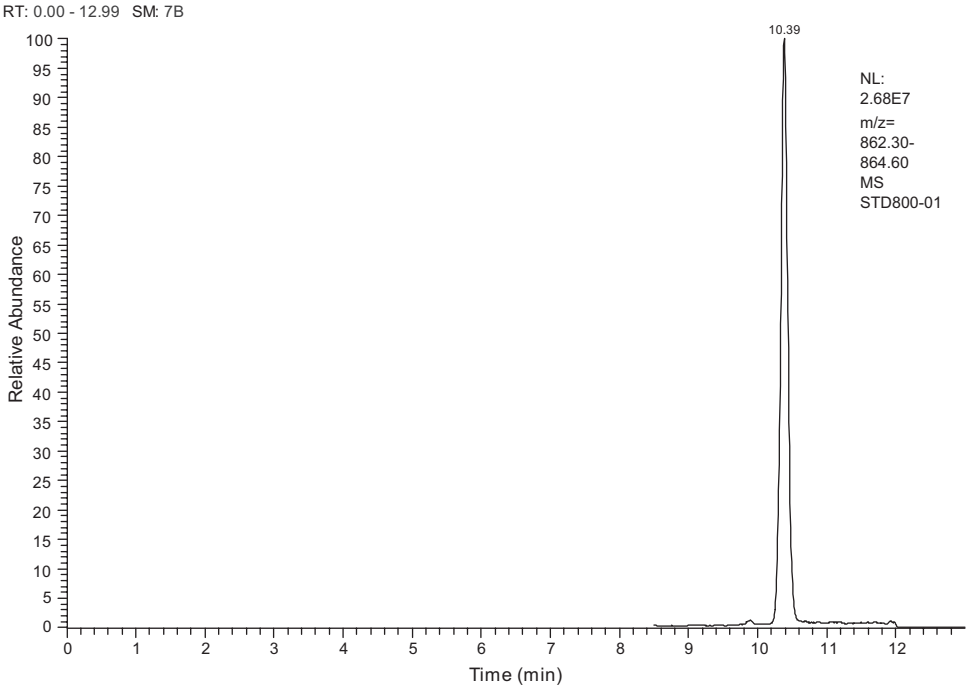
system suitability test solutions (SST, $n = 6$), calibration standards (CS, $n = 9$), plasma samples ($n = 50$), and quality control (QC, $n = 14$) samples.

2.6. Recovery, accuracy and precision of the method

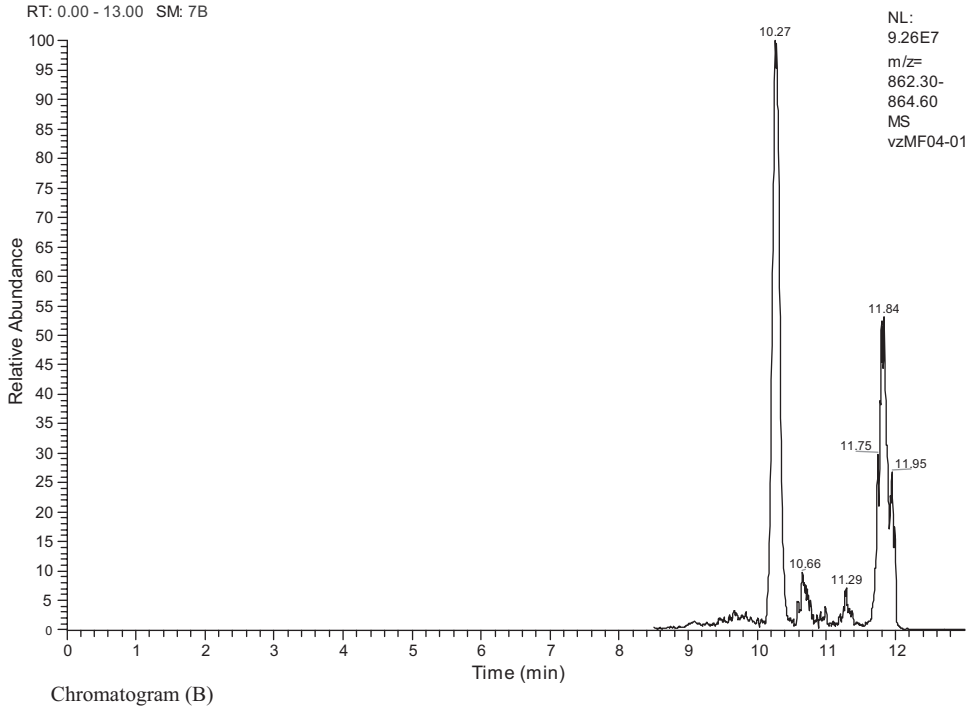
Recovery of CoQ10 was based on a comparison between the peaks obtained by spiking empty plasma on two concentration levels, 0.25 and 2.50 mg of CoQ10/L, with 20-fold determinations for each concentrations, and the corresponding peaks of the standard. Within run precision and accuracy were determined using six samples at two different concentration level (0.25 and 2.50 mg of CoQ10/L) and each level was assayed six times for the intra-day precision and accuracy test over 3-day period.

2.7. Data analysis

All statistics were run under Statgraphic plus ver.4. Analysis of variance (ANOVA) and Student's *t*-test were employed to evaluate differences between groups with respect to plasma CoQ10 levels



Chromatogram (A)



Chromatogram (B)

Fig. 1. Chromatogram of standard CoQ10 (A), and chromatogram of CoQ10 in plasma sample (B) acquired in a SIM mode, m/z 863.4 ± 1 and mass spectrum of CoQ10 standard (C).

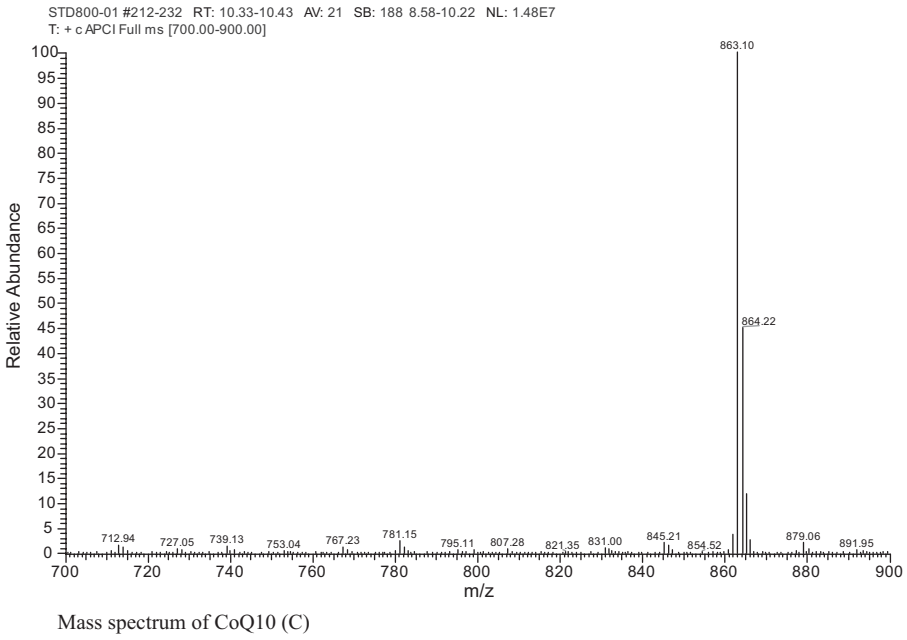


Fig. 1. (*Continued*).

at baseline concentrations, and following time intervals and area under the curve (AUC).

2.8. Molecular modeling

The most probable structure of the inclusion complex of water-soluble β-cyclodextrin and folded CoQ10 was determined using the ChemDraw® Ultra (Chemical Structure Drawing Standard) by Cambridge Soft Corporation, Cambridge, MA 02140, USA. For graphical demonstration of the proportions of the complex, data from the literature was obtained [17]. However, the investigation using molecular modeling, while allowing the construction and three-dimensional manipulation of the molecular complex, is insufficient to indicate the positioning of the whole CoQ10 molecule in relation to β-cyclodextrin and for describing the real structure of this complex.

3. Results and discussion

Typical, representative chromatograms acquired in a SIM mode, m/z 863.4 ± 1, of standard and plasma sample, and mass spectrum of CoQ10 standard are shown in Fig. 1. Peak in plasma sample has the same retention time as the CoQ10 standard. Enclosed chromatograms also show, that there are no peaks generated from the sample, which may interfere with the peak of CoQ10.

Spiking of empty plasma sample with 0.25 and 2.50 mg of CoQ10/L yielded a recovery of 90.5 ± 4.4% and 98.5 ± 2.91%.

Calibration curves constructed using stock solution of CoQ10 as described under Section 2 showed linearity over a concentration range of 0.05–10 mg of CoQ10/L. Correlation coefficients (r^2) for 3 calibration curves obtained over 3-day period ranged from 0.9997 to 0.9999. The limit of quantization was 0.05 mg of CoQ10/L with a precision of 20%.

Within run (intra-day) precision (R.S.D.) for the six standard solutions with 1.0 mg of CoQ10/L was 1.55%, while average day-to-day (inter-day) precision over 3-day period was 1.63%. Inter-day accuracy, determined as deviation from nominal values was 1.02 ± 0.009%.

The results obtained on two different samples stored 3 days at room temperature showed that CoQ10 was practically stable.

The developed HPLC–MS method with APCI ionization proved to be selective, robust and linear and therefore suitable for the quantitative determination of CoQ10 in the blood samples.

It has been assumed for a long time that the shape of CoQ10 molecule is strictly linear, with same possible rotation of isoprenoid tail. According to these conclusions, β-cyclodextrin is not appropriate molecule for preparation of an inclusion complex with CoQ10 [18]. On other side predictions and results of some research groups showed that folded conformations of CoQ10 exist in the nature and have in reduced and oxidized forms the lowest energy levels [19–23]. From the molecular data obtained in the literature [21] we concluded, that folded form of CoQ10 could be easily included into β-cyclodextrin. We found that volume of not folded CoQ10 molecule is 682 Å^3, length is 50 Å, diameter is 4.0–4.5 Å and the volume of the quinol head is nearly 60 Å^3. As the volume of the cavity inside β-CD is 373 Å^3, we found the only chance is folded form of CoQ10 molecule with effective volume of 352 Å^3, length of 21 Å, and the diameter between 4.7–5.2 Å. The groundwork of our success was the fact that we set up conditions, after several experiments, in which more than 50 Å long molecule of CoQ10 was successively folded before it was built in β-cyclodextrin [24]. We also attempted to graphically demonstrate the proportions of our complex, and the results of our calculation are shown in Fig. 2.

Prior to each dosing the baseline plasma levels were determined. Obtained concentrations were found between 0.16 and 0.49 mg/L. Fig. 3 shows the plasma concentration profile for soft-gel formulation and concentration profile for inclusion complex formulation. High standard deviations were explained with the fact that selected dogs previously participated in different studies. Nevertheless, the group of seven dogs was accepted, because the differences between the basic zero concentrations of individual dogs were practically identical in both trials. In Table 1, mean values for base line plasma concentrations, maximum plasma concentration (C_{max}), time of maximum plasma concentration (T_{max}), area under the plasma concentration ($AUC_{0-48\,h}$), elimination half life ($t_{1/2}$), for both formulations are shown. The advantage of the water-soluble CoQ10 in comparison with commercially available soft-gel capsules is seen

Table 1
Pharmacokinetic parameters of CoQ10, and mean plasma values ± S.D. for some bioavailability parameters for soft gel capsules (**1**) and inclusion complex of CoQ10 with β-cyclodextrin (**2**)

	Basline CoQ10 (mg/L)	C_{max} (mg/L)	T_{max} (h)	$AUC_{0-48\,h}$ (mg/L h)	AUC (%)	$t_{(1/2)}$ (h)
1	0.35 ± 0.13	0.62 ± 0.22	6.0 ± 0.5	6.70 ± 0.26	100	20 ± 4
2	0.36 ± 0.09	1.21 ± 0.48	4.0 ± 0.5	17.43 ± 0.33	260	16 ± 2

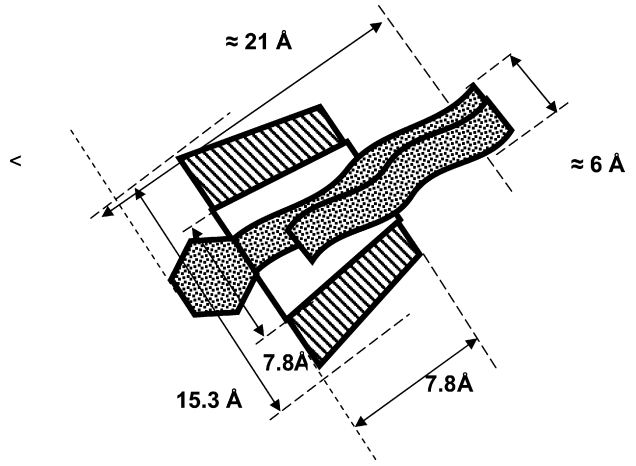


Fig. 2. Anticipated configuration of the β-cyclodextrin–CoQ10 inclusion complex.

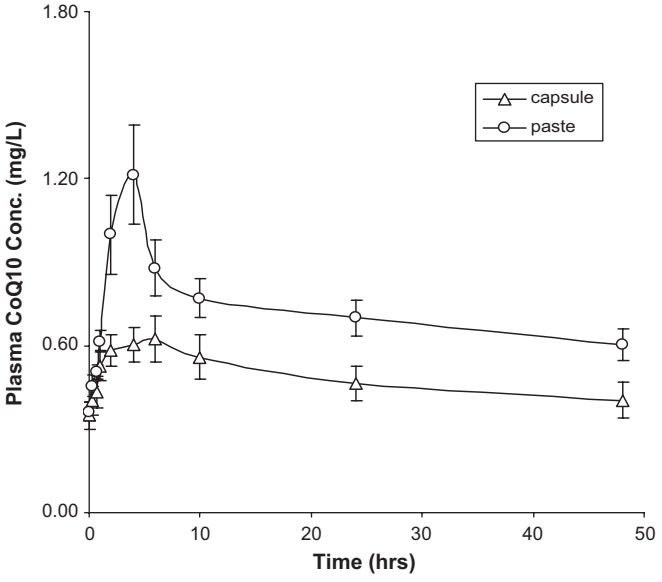


Fig. 3. Comparison of CoQ10 plasma concentration–time curves (mean ± S.E.M.) after an oral dose of 30 mg soft gel capsule and 375 mg of paste (equivalent to 30 mg CoQ10) to each dog.

in nearly three times higher $AUC_{(0-48\,h)}$, nearly two times higher C_{max}, and a shortened T_{max} from 6 to 4 h.

Pharmacokinetic parameters are in a good correlation with results of other studies, but most of them were done with different formulations and in most cases with human volunteers [25,26]. In the study on beagle dogs reported by Reddy at al. very similar plasma concentration profiles with two very recognizable elimination slopes, and C_{max}, and T_{max} values were obtained, nevertheless higher concentrations and different formulations were tested [27]. The concentrations of CoQ10 in soft-gel capsules (30 mg) were probably too low to produce a very high absorption maximum and two step elimination slopes otherwise noticed in previously mentioned study and also in our experiment with water-soluble paste.

4. Conclusion

Obtained result shows a significant advantage of inclusion complex of CoQ10 and β-cyclodextrin over oil-based formulations expressed with higher $AUC_{(0-48\,h)}$, and C_{max}, and shortened T_{max}.

The HPLC–MS method originally developed and validated in our laboratory for quantitative determination of CoQ10 in plasma is a reliable, selective, sensitive and robust analytical tool and therefore capable to demonstrate that the newly developed inclusion complex of CoQ10 with β-cyclodextrin expresses better bioavailability than the selected commercially available formulation.

The results show that water-soluble formulation presents a strongly improved absorption and we expect that the inclusion complex will soon be used as a basic food and fodder additive, especially in products with reduced amount of fat.

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