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An improvement of separation and response applying post-column compensation and one-step acetone protein precipitation for the determination of coenzyme Q10 in rat plasma by SFC-MS/MS

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ABSTRACT

Coenzyme Q10 (CoQ10) solid dispersion was prepared to improve its oral bioavailability due to the poor solubility of CoQ10. To evaluate the pharmacokinetic behaviors of CoQ10 solid dispersion, a simple, rapid, sensitive and environment friendly method for the determination of CoQ10 in rat plasma was developed. In this study, samples were prepared by one-step protein precipitation with acetone and then the supercritical fluid chromatography-electrospray ionization tandem mass spectrometry (SFC-ESI–MS/MS) method was used. The separation was achieved by an ACQUITY UPC^{2TM} BEH 2-EP column (100 mm × 3 mm, 1.7 μm) maintained at 35 °C, using carbon dioxide (≥99.99%) and methanol (85:15, *v/v*) as the mobile phase at a flow rate of 1.0 ml/min. To improve the response and sensitivity, the compensation solvent of methanol with 2 mM ammonium acetate at a flow rate of 0.2 ml/min was used and the total analysis time was only 1.5 min for each sample. The detection was carried out on a tandem mass spectrometer with electrospray ionization (ESI) source and the mass transition ion pair was *m/z* 881.0 → 197.0 and 285.1 → 193.0 for CoQ10 and diazepam, internal standard (IS), respectively. Calibration curve was linear over the concentration range of 2.00–500.00 ng/ml ($r^2 \geq 0.998$) with a lower limit of quantification of 2.00 ng/ml. The intra- and inter-day accuracy and precision were below 15% for all quality control samples. The proposed method was rapid, accurate and reproducible, which was suitable to compare the pharmacokinetic behaviors in rats after a single oral dose of 100 mg/kg CoQ10 solid dispersion or tablets.

1. Introduction

Coenzyme Q10 (Fig. 1a), also named ubiquinone, is a lipid-soluble benzoquinone which plays an important role in the electron transport [1]. In the past few years, coenzyme Q10 has been widely applied in clinical therapy, such as mitochondrial disease [2], breast cancer [3], diabetes [4] and neurodegenerative diseases [5] due to its anti-oxidation effect and enhancing immunity effect. Coenzyme Q10 is insoluble in water, very slightly soluble in ethanol and soluble in acetone and trichloromethane. Therefore, the poor bioavailability was caused by its extremely low solubility in water (<0.25 μg/ml) which limited the clinical development of coenzyme Q10 despite its versatility [6]. Solid dispersion, which is an effective strategy to enhance the solubility of insoluble drugs, has been used as a formulation of CoQ10 to improve its oral bioavailability in the previous studies [7,8]. However, reports on systematic methods for the determination of CoQ10 solid dispersion were minimal. Therefore, a simple, rapid and sensitive analytical method was needed to investigate the pharmacokinetic behaviors of CoQ10 solid dispersion.

Currently, several methods have been developed to determine the CoQ10 concentrations in biological matrices, including liquid chromatography-tandem mass spectrometry (LC–MS/MS) [9–12], ultra-performance liquid chromatography mass spectrometry (UPLC–MS) and UPLC–MS/MS [13,14] methods. Nevertheless, plenty of organic reagents were used for the mobile phase due to the very weak polarity of CoQ10 and these methods are time-consuming which is against the high-throughout determination. In addition, the pretreatment methods were complicated particularly. Li et al. described a UPLC–MS method for analysis of CoQ10 in rat serum, but the lower limit of quantitation (LLOQ) is 50 ng/ml, which may be inadequate for the pharmacokinetic study in vivo. To enhance the mass spectrometric response of CoQ10 with

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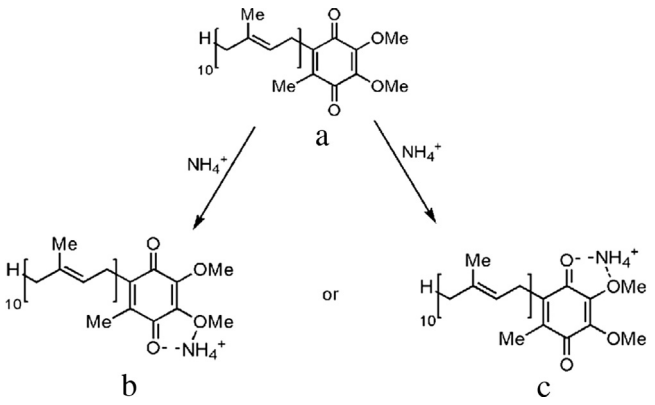

Fig. 1. Chemical structures of CoQ10 and $[M+NH_4]^+$.

UPLC–MS/MS, Tang et al. used the methanol containing ammonium acetate as the mobile phase, however, the whole run time was about 6 min for each sample with large amount of organic solvents. Therefore, a novel and green method which can improve the response of ions as much as possible based on the high-efficiency separation should be developed. In this paper, a simple one-step acetone protein precipitation method was applied to the pretreatment and supercritical fluid chromatography (SFC) was considered for the analysis of CoQ10 due to the weak polarity of CO_2.

In recent years, SFC technique has attracted widespread attention because of its application in separation and analysis especially in biological samples [15–17]. A supercritical fluid is a substance whose temperature and pressure exceed the critical point–has certain properties including high diffusivity and low viscosity that makes it suitable for use as a mobile phase [17,18]. Among them, supercritical carbon dioxide ($ScCO_2$) is commonly used because of its non-toxic, non-flammable behavior and relatively cheap availability. Therefore, SFC-MS/MS is highly suitable for the analysis of lipid-soluble drugs due to non-polar characteristic of CO_2 which can reduce the use of organic solvents tremendously. Besides, a small amount of organic modifier is generally added to the supercritical CO_2 to increase the mobile phase polarity and extend the range of compounds [18,19]. In recent years, many hydrophobic drugs including 3-*n*-butylphthalide (NBP), lacidipine, bifendate were studied by SFC-MS/MS in our laboratory and preferable results were obtained [20–22]. In addition, the $[M+H]^+$ sensitivity of CoQ10 is very low possibly due to its instability [23]. In the proposed study, post-column compensation is used to improve the response of CoQ10 as compared to traditionally analytical methods. The post-column compensation of methanol containing 2 mM ammonium acetate can provide a large number of $[NH_4]^+$ to form a stable 5-membered chelating ammonium cation with CoQ10 (Fig. 1 b, c) after separation, which can significantly improve the mass spectrometric response of CoQ10. The aim of this study is to develop a novel, rapid, efficient, sensitive and environment friendly analytical method for the determination of CoQ10 in rat plasma using supercritical fluid chromatography with tandem mass spectrometry (SFC-MS/MS).

2. Experimental

2.1. Chemicals and materials

CoQ10 (purity > 99.8%) was obtained from Beijing Jiakangyuan Co. Ltd (Beijing, China) and diazepam (purity > 99.9%, IS) was purchased from the National Institutes for Food and Drug Control (Beijing, PR China). Commercial CoQ10 tablets were obtained from Eisai Pharmaceutical Co. Ltd (Shenyang, Liaoning, China). CoQ10 solid dispersion was self-prepared. HPLC-grade methanol, ethanol and ammonium acetate were offered by Fisher Scientific (Pittsburgh, PA, USA). CO_2 (≥99.99%) was supplied by Shenyang Qianzhen Chemical Gas (Shenyang, Liaoning, China). High-purity nitrogen (99.99%) was also obtained from Shenyang Qianzhen Chemical Gas (Shenyang, Liaoning, China).

2.2. Equipments and conditions

An ACQUITY UPC^{2TM} system (Waters, Milford, MA, USA) including binary solvent manager, sample manager, column manager and convergence chromatography manager was used. Chromatographic analysis was performed on the ACQUITY UPC^{2TM} BEH 2-EP column (100 mm × 3 mm 1.7 μm). The mobile phase was carbon dioxide and methanol (modifier) (85:15, *v/v*), while the post-column compensation solvent was composed of methanol containing 2 mM ammonium acetate with 0.2 ml/min to increase the response of the ion. The flow rate was set at 1.0 ml/min and 5 μl was injected into the system. The backpressure of the system was maintained at 2000 ψ and the column temperature was maintained at 35 °C.

An ACQUITY UPC^{2TM} system was coupled to a Waters Triple Quadrupole mass spectrometer fitted with an ESI source interface. The optimized parameters were as follows: capillary voltage, 3 kV; cone voltage, 35 V; desolvation temperature, 350 °C; source temperature, 150 °C; collision energy, 25 V and 27 V for CoQ10 and IS respectively. The mass spectrometer was operated in positive ionization mode. The trasitionstransitions of CoQ10 and IS were *m/z* 881.0 → 197.0 and 285.1 → 193.0, respectively. MassLynx^{TM} NT 4.1 software with QuanLynx^{TM} program (Waters, Milford, MA, USA) was used for data acquisition and analysis.

2.3. Standard solution preparation

Stock standard solution of CoQ10 (50 μg/ml) was prepared as follows, 5 mg of CoQ10 was weighed accurately and placed into a 100 ml volumetric flask, dissolved with ethanol. The solution was diluted with methanol to the volume. The IS stock solution was prepared by dissolving approximately 5 mg diazepam into 100 ml with ethanol, and then diluted to 150 ng/ml. All the solutions were stored at −20 °C until analysis.

2.4. Sample preparation

10 μl of IS solution (150 ng/ml) and 20 μl methanol was added to 100 μl plasma sample. The mixture was vortexed for 1 min and deproteinized with 300 μl acetone. The solution was centrifuged for 10 min at 11222*g* after vortexed for another 3 min, and then the supernatant layer was transferred to an Eppendorf micro tube. The final solution was obtained through additional 5 min centrifugation at 11222*g*. 5 μl of the solution was injected into the SFC-MS/MS system.

2.5. Method validation

2.5.1. Selectivity

To verify the selectivity of the method, the chromatograms of six different batches of blank plasma, corresponding blank samples spiked with analyte at LLOQ level and IS at a concentration of 150 ng/ml and plasma samples after oral administration of CoQ10 solid dispersion were compared.

2.5.2. Linearity and sensitivity

Calibration standards were prepared by spiking 100 μl blank rat plasma with 20 μl of the appropriate standard solutions to make the effective concentrations of 2, 10, 25, 50, 100, 250 and 500 ng/ml