Electronic Supplementary Material (ESI) for Analyst

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Rapid assessment of coenzyme Q_{10} redox state using ultrahigh performance liquid chromatography tandem mass spectrometry

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Method validation

The optimized UPLC–ESI-MS/MS method was evaluated in accordance with US FDA guidelines for bioanalytical method validation. [1] The following parameters were tested:

Linearity and calibration curve

Linearity was determined by linear regression with a 1/x weighting factor. Acceptable linearity was achieved when the coefficient of determination was at least 0.990. The linear range for each form of coenzyme Q_{10} was evaluated using serially diluted working standard solutions with a constant amount of internal standard. Calibration curves were built using the peak area ratio of the analyte and the internal standard (analyte/IS), plotted versus the corresponding concentration. (Fig. S1 and Fig. S2)

Accuracy, precision and recovery

Accuracy and precision were assessed at three different concentration levels. The accuracy of the method in terms of recovery was tested by calculating the measured value with the amount of standards spiked to the liver tissue extract. The precision was expressed as the coefficient of variation of the measured value. Within-day accuracy and precision were assessed by preparing and analyzing 6 replicates on the same day. For between-day precision, one replicate was analyzed at three consecutive days. Wihtin-day and between-day precision and accuracy were required to be within ± 20%. (Table S1)

Selectivity

The method selectivity was verified through comparison of the chromatogram of blank sample matrix with the chromatograms of the reference standard solution and the solvent blank. No obvious interference peak from surrogate matrix was detected at the retention time of coenzyme Q_{10}. (The blank matrix was prepared by stripping liver tissue extract with activated charcoal, thus target analytes were removed.)

Sensitivity

Sensitivity was evaluated by measuring the limit of detection (LOD) and limit of quantification (LOQ) for each analyte. The LOD and LOQ were assessed from the signla to noise ratio (*S/N* = 3:1 for LOD, *S/N* = 10:1 for LOQ). (Table S2)

Carryover and matrix effect

Carryover was evaluated by injecting solvent blank immediately after liver tissue sample analysis. Carryover was not observed as evidenced by no analyte peak in the blank. (Fig. S3) Matrix effect was evaluated through post-column infusion experiment as suggested by Matuszewski et al. [2] Both forms of coenzyme Q_{10} are not affected by matrix effects.

Analyte stability

Six months of long-term stability demonstrate CoQ_{10} stock solution in methanol is stable at − 20 °C. And CoQ_{10} stock solution (in brown glass volumetric flask) was very stable in methanol at room temperature with no degradation found in 3 days (data not shown). $CoQ_{10}H_2$ was stable up to 3.5 hours on the autosampler without any significant oxidation (Fig. S4), allowing for more than 20 samples to be analyzed simultaneously with in a single chromatographic run.

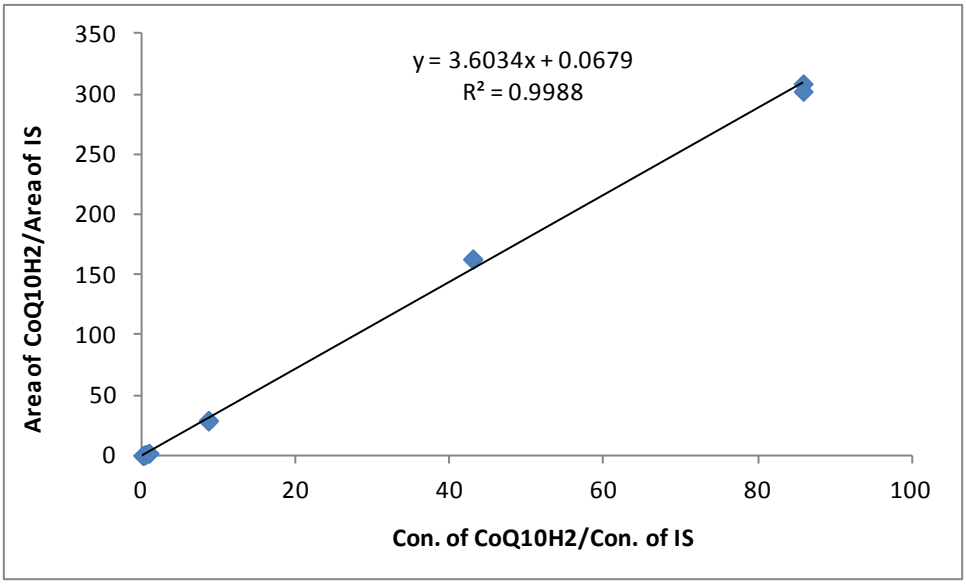

Fig. S1 Calibration curve for UPLC–ESI-MS/MS analysis of $CoQ_{10}H_2$ with Dipropoxy-CoQ_{10} (DP-Q_{10}) as internal standard.

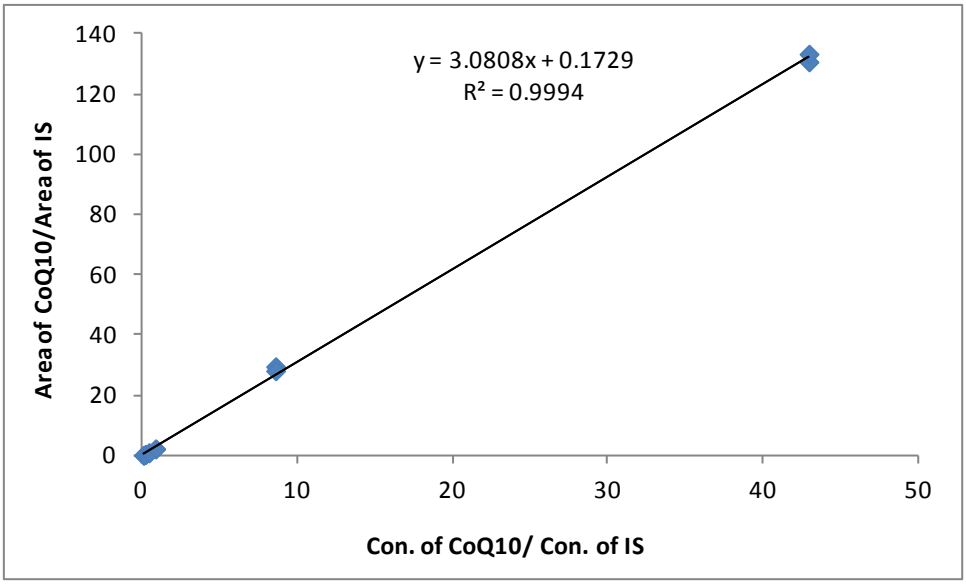

Fig. S2 Calibration curve for UPLC–ESI-MS/MS analysis of CoQ_{10} with Dipropoxy-CoQ_{10} (DP-Q_{10}) as internal standard.

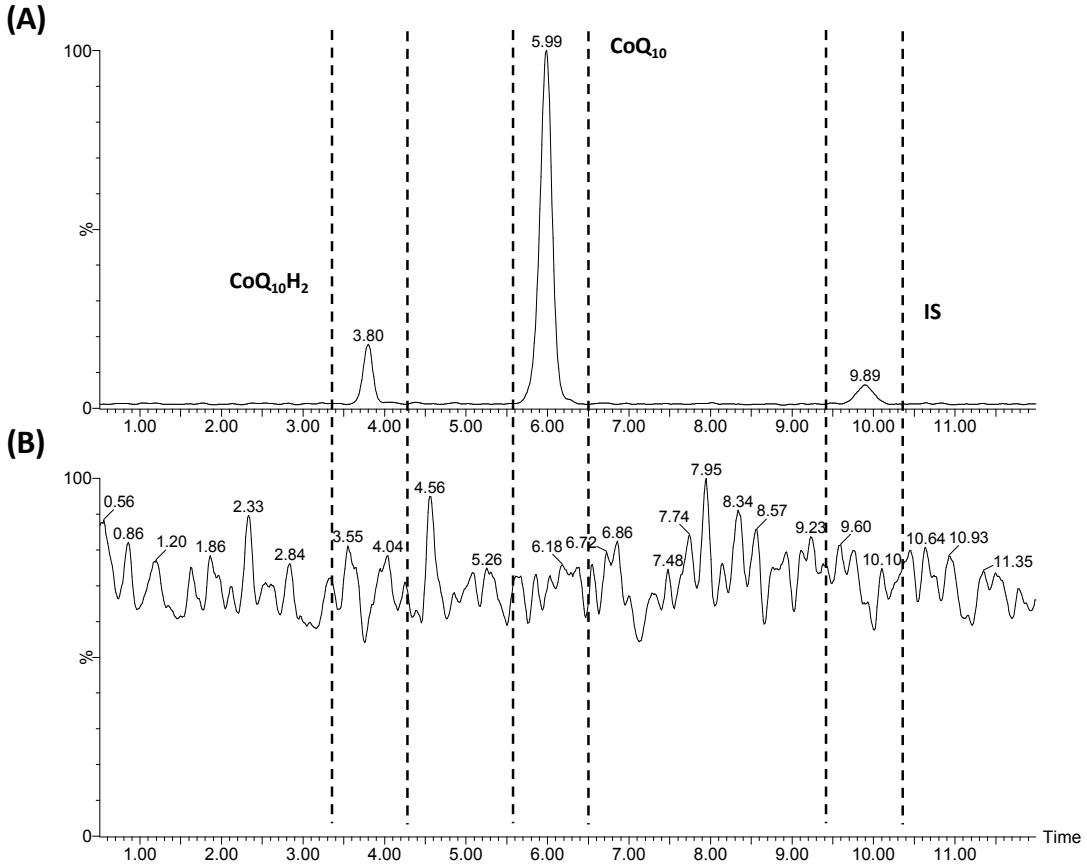

Fig. S3 Sample carryover test. Carryover was not observed as evidenced by no analyte peak in the solvent blank (B) analyzed immediately after tissue sample analysis (A).

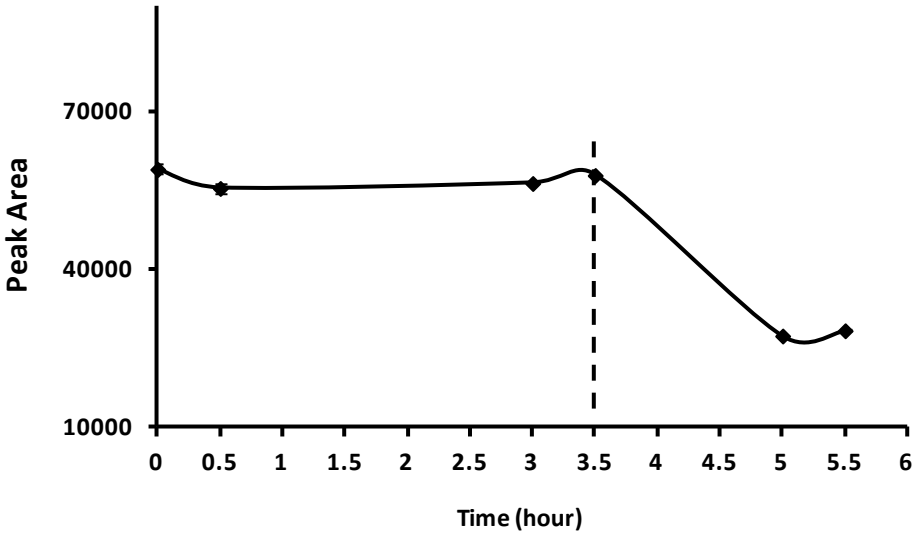

Fig. S4 Stability test of $CoQ_{10}H_2$ on the autosampler. $CoQ_{10}H_2$ at the concentration of 1.0 μg mL^{-1} was stable up to 3.5 hours on the autosampler without any significant oxidation, allowing for more than 20 samples to be analyzed simultaneously with in a single chromatographic run.

Analyte	Concentration (ng mL^{-1})	Precision	
		Within-day RSD (%) (n=6)	Between-day RSD (%) (n=9)
$CoQ_{10}H_2$ [a]	85.8	8.3	8.5
	214.6	5.7	4.4
	858.8	3.6	2.9
CoQ_{10}	85.8	6.9	7.3
	214.6	3.4	3.2
	858.8	2.3	2.5

Table S1 Within- and between-day assay variability. For $CoQ_{10}H_2$ & CoQ_{10} at each level, the repeatability results were satisfactory, as indicated by both within-day and between-day variability not exceed 10.0 %. [a] Because of the instability of $CoQ_{10}H_2$, its solutions were prepared immediately prior to the analysis, and freshly prepared each time, and the test was conducted within 3.5 hours.

Analyte	Spiked concentrations (ng mL^{-1})						LOD	LOQ
	45		180		900			
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	(ng mL^{-1})	(ng mL^{-1})
$CoQ_{10}H_2$	109	8.8	93	6.9	97	3.5	7.0	15.0
CoQ_{10}	79	7.6	91	3.1	95	2.6	1.0	5.0

Table S2 Percentage recovery of the reduced and oxidized coenzyme Q_{10} ($CoQ_{10}H_2$ & CoQ_{10}) in freeze dried liver tissue sample by standard addition method.

References

1. U.S. Food and Drug Administration, Center for Drug Evaluation and Research, Guidance for Industry: Bioanalytical Method Validation, 2001. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf

2. B. K. Matuszewski, M. L. Constanzer, and C. M. Chavez-Eng, *Anal. Chem.*, 2003, **75**, 3019-3030.