Determination of Coenzyme Q10 in human muscle homogenates by HPLC

Principle- The strongly uv-absorbing quinones can be detected at their uv maximum at 275 nm. Quantitation is based on peak areas, using external standardization.

References-

1. Lang et al- Analytical Biochemistry 157, 106-116 (1986)
2. Lopez, Di Mauro et al- The American Journal of Human Genetics Volume 79, 1126-1129 (2006)

Method- Coenzyme Q10 was extracted from the homogenate prepared for measurement of mitochondrial enzyme activity, within a half hour of homogenization (1:10) in 50 mM Tris ph 7.4, 150 mM KCl. 50µl of the homogenate was mixed with 450µl of ice cold propanol. An internal standard- Coenzyme Q9 was added at this point (1.5µl of a 0.3µgm/ml solution). After vortexing, the sample was left on ice for 5 minutes, then spun down in a tabletop centrifuge for 2 minutes. The supernate was filtered through a 0.22µ filter, and kept on ice.
The prepared samples are analyzed with an eluant composition of methanol/ reagent alcohol 1/9, 20 mM lithium perchlorate. The column is Waters YMC AQ, 4.6 mMx250mM. The flow rate is 0.5ml/min. The injection volume is 50µl.
Coenzyme Q10 standards are prepared at 15ng/50µl and 150ng/50µl. Peak areas are measured both in the standards and samples and CoQ10 is calculated as follows

µgm/gm wet weight=
Standard conc/area x 1/0.5mg x sample peak area x 100(final dilution)

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