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, to calculate the 1% and 5% (v/v) concentration limits of the assay. These limits were considered acceptable because both were

located in the linear range of the curve, indicating that the assay is sufficiently sensitive for use in biological samples.Table 2Limit of detection and limit of quantification (in µg/g) for the 1 and 5% (v/v) ethanol solutions of the CPT II assay.SampleLODQLCK_1% (v/v)QLCK_5% (v/v)QLCK_1% (v/v)QLCK_5% (v/v)Human liver15.611.08.68.6Rat brain2.41.41.21.2Rat liver5.23.41.31.3Horse brain0.40.40.40.4 Robustness of the assay {#Sec20} ---------- To determine the robustness of the assay, the following changes were introduced to the assay procedure. The standard solution was replaced with PBS and the assay procedure was repeated. Liver samples from rats treated with 6.6 µmol/kg and 12 µmol/kg of CPT II were diluted with PBS to obtain the following concentrations: 1 µg/g; 5 µg/g; 10 µg/g; 20 µg/g; 40 µg/g; and 60 µg/g. Assay robustness was also evaluated by using different extraction solvents for the extraction of the sample. Three different solvents were used in the following manner: the standard solution was replaced with extraction solvent (1:10) for 20 min at 37 °C and centrifuged at 13,200×*g* for 10 min at 4 °C. The supernatant was obtained after incubation for 30 min at 37 °C and assayed by LC--MS/MS. Results and discussion {#Sec21} ========== LC--MS/MS methodology for CPT II {#Sec22} ---------- The CPT II assay was developed and validated for the first time and the final assay is a liquid chromatographic assay coupled to an atmospheric pressure chemical ionization (APCI) mass spectrometer. The chromatographic analysis was performed with a Kinet 82157476af