A quantitative phenytoin GC-MS method and its validation for samples from human ex situ brain microdialysis, blood and saliva using solid-phase extraction

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This study describes the development and validation of a gas chromatography-mass spectrometry (GC-MS) method to identify and quantitate phenytoin in brain microdialysate, saliva and blood from human samples. A solid-phase extraction (SPE) was performed with a nonpolar C8-SCX column. The eluate was evaporated with nitrogen (50řC) and derivatized with trimethylsulfonium hydroxide before GC-MS analysis. As the internal standard, 5-(p-methylphenyl)-5-phenylhydantoin was used. The MS was run in scan mode and the identification was made with three ion fragment masses. All peaks were identified with MassLib. Spiked phenytoin samples showed recovery after SPE of $\geq$94%. The calibration curve (phenytoin 50 to 1,200 ng/mL, n = 6, at six concentration levels) showed good linearity and correlation (rš >0.998). The limit of detection was 15 ng/mL; the limit of quantification was 50 ng/mL. Dried extracted samples were stable within a 15% deviation range for $\geq$4 weeks at room temperature. The method met International Organization for Standardization standards and was able to detect and quantify phenytoin in different biological matrices and patient samples. The GC-MS method with SPE is specific, sensitive, robust and well reproducible, and is therefore an appropriate candidate for the pharmacokinetic assessment of phenytoin concentrations in different human biological samples.
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