A Quantitative Phenytoin GC-MS Method for samples from Human ex Situ Brain Microdialysis, Blood, and Saliva Using Solid Phase Extraction and its Validation

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Abstract

Therapeutic drug monitoring of phenytoin (PHT) is important in critically ill neurological patients. In the present work, development and validation of an analytical GC-MS method is described, which can be used to quantitate drug concentrations at the site of action (in brain microdialysate) as well as in body fluids (saliva, blood). Prior to the GC-MS analysis a solid phase extraction (SPE) with a non-polar C_8 column was performed and verified by using artificial CSF, blood, and saliva samples. The SPE cartridges were equilibrated with 1mL acetonitrile, pH adjusted with 1mL citric buffer pH 5.0 prior to application of samples. This was followed by a wash step with 1mL citric buffer, and pH adjustment by 1mL of acetic acid 0.01M. PHT was eluted with 2 x 1mL acetone and then evaporated with nitrogen at 50°C. The derivatization was performed with 50µL trimethylsulfone hydroxide before GC-MS analysis. As internal standard 5-(p-Methylphenyl)-5-Phenylhydantoin was used. The dried extracted samples were stable within a 15% deviation for more than 4 weeks at room temperature and at least for 33 hours on the auto sampler tray with a 5% concentration variation. 5-(p-Methylphenyl)-5-Phenylhydantoin was evaluated as internal standard and found to be appropriate. The calibration curve from 50 to 1200ng/mL with 12 samples at 6 different concentration levels showed good linearity and correlation (r^2> 0.995). Spiked PHT samples showed a recovery after SPE of more than 94%, independent of the used matrices. The limit of detection in artificial CSF, blood and saliva was found to be 15ng/mL and the limit of quantification was 50ng/mL. The PHT concentration in brain microdialysate was presumed to be 10% of the lower serum value (i.e. > 50ng/mL), corresponding to free PHT. The temperature program of the GC-MS System was set to 120°C for 1 min then raised by 10°C/min to 300°C, held for 6 min. The retention time of PHT was about 15 minutes, the retention time of the IS was 16 minutes. The carrier gas was high purity helium (99.95%). The MS instrument was run in scan mode. All chromatographic peaks were analyzed with MassLib™. To investigate the reproducibility and robustness, the analytical method was transferred to a second GC-MS instrument. The method for brain microdialysate, blood, and saliva met the ISO17025 standards and is therefore suitable to be used for (human) PHT

determination. The method was confirmed by exploratory true patient samples. The analytical procedure is robust and reproducible and therefore also appropriate for a comparative pharmacokinetic assessment of PHT in biological samples (e.g., from ex situ brain microdialysis) of neurosurgical patients.

Key words

GC-MS, Phenytoin, Biological Samples, Microdialysate, ISO 17025 Validation