A DRIED BLOOD SPOT MICROSAMPLING-LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR THERAPEUTIC DRUG MONITORING OF PHENOBARBITAL IN NEWBORNS

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Introduction: Phenobarbital (PB), a barbiturate and nonselective central nervous system depressant, acts at $GABA_A$ receptors in neurons. In preterm infants, PB is used for treatment and prevention of seizures. However, more than 50% of newborns are unresponsive to recommended dosage and need additional loading doses, which might be responsible for respiratory depression. Therapeutic Drug Monitoring (TDM), a laboratory practice for measurement of serum/plasma drug levels, is needed for dose adjustment to minimize PB side-effects. TDM requires frequent blood drawing not feasible in preterm infants. Microsampling techniques, such as dried blood spot (DBS), can minimize sample volume needed for analysis and could be a good sampling choice for TDM in newborns. Here, we developed and optimized a DBS microsampling - liquid chromatography tandem mass spectrometry (LC-MS/MS) method for measurement of PB in preterm infants.

Materials and methods: Blood was mixed with various concentrations of PB and 20 µL of sample were spotted on a DBS, air-dried for 1h, cut and re-hydrated with 50 µL of ultra-pure water supplemented with the internal standard primidone (ISTD) at 100 ng/mL. After incubation at +37°C for 10 min, samples were treated with 200 µL of 80% methanol and sonicated for 10 min. Supernatants were transferred to a new tube, centrifuged, and 100 µL of clear supernatants were transferred to appropriate vials for acquisition. LC–MS/MS was carried out on a Thermo Scientific TSQ Endura triple quadrupole mass spectrometer coupled to Dionex UltiMate 3000 UHPLC system. Injecting volume was of 20 µL and separation was performed with a Phenomenex Luna Omega C18column (50 x 2.1mm; 1.6µm particle size). The mobile phase system was composed of solvent A (0.1% of formic acid in water) and B (0.1% of formic acid in acetonitrile). The composition changed from 35% to 49.5% of B at 1min. The column was rinsed with 90% of B and re-equilibrated to starting conditions, at 35% of B, for a total runtime of 3.5min. PB and ISTD were measured in selected reaction monitoring and electrospray negative or positive mode, respectively. The following precursor (m/z) and products (m/z) were obtained: PB, 230.8à 188, 230.8; and ISTD, 219.9à 163. Results. The method was validated by following the EMA and FDA guidelines. Lower limit of quantization (LLOQ) of PB was 1µg/mL. Selectivity was evaluated by analyzing blank DBS samples which showed signals <20% of the LLOQ for PB precursors and <5% for ISTD. The absence of carry-over was confirmed by injecting blank samples after the highest PB concentration with signals <20% or <5% of the LLOQ for PB precursors or ISTD, respectively. Extraction recovery after methanol precipitation was 65-80% for PB, and ≥95% for ISTD. Linearity was assessed by analyzing calibration curves at 1, 5, 12.5, 25, 50, and 75µg/mL concentrations. R^2value was greater than the accepted cut-off of 0.99(0.9982). Within-run and between-run precision and accuracy were evaluated by analyzing supernatants from DBS samples after methanol precipitation with PB at 3, 20, and 70 µg/mL and at various time points (0, 3, 6h). Aliquots were kept at +4°C and re-run at 24or 48h and three times per day. CV values were <15% for all concentrations. Relative standard error for accuracy evaluation was 24-26% for PB at 70 µg/mL. Stability of PB at 3, 20, and 70 µg/mL was estimated in DBS specimens kept at +4°C right after spotting or after a 2h air-drying and analyzed at 24/48h, and after 15days of +4°C storage. After 15days, we documented a significant decrease in PB signals.

Discussion and conclusions: High plasma levels of PB, a barbiturate for treatment and prevention of seizures in preterm infants, can cause respiratory depression and death. For this reason, TDM is highly recommended and DBS sampling may reduce sample volume needed. Here we developed a rapid and selective DBS sampling-LC-MS/MS method for measurement of PB in newborns.