FULLY AUTOMATED DIRECT EXTRACTION AND ANALYSIS OF DRIED BLOOD SPOTS FOR THE DETERMINATION OF FOUR ANTI-EPILEPTIC DRUGS AND TWO ACTIVE METABOLITES

Sofie Velghe and Christophe P. Stove
Laboratory of Toxicology, Faculty of Pharmaceutical Sciences, Ghent University, Belgium

Dosage adjustment of anti-epileptic drugs by therapeutic drug monitoring is very useful, especially for children. Considering the benefits of dried blood spots (DBS), this matrix could be an alternative to conventional venous sampling for this purpose. Since manual punching and off-line extraction slow down DBS analysis, an automated direct extraction and analysis of DBS can be advantageous. A method for quantifying four anti-epileptic drugs (AEDs) and two active metabolites was developed, including carbamazepine, valproic acid, phenytoin, phenobarbital, carbamazepine-10,11-epoxide and oxcarbazepine. To that end, we used a prototype on-line DBS-SPE device (Spark Holland) coupled to liquid chromatography (Shimadzu) and tandem mass spectrometry (AB SCIEX QTRAP® 5500) (LC-MS/MS).
For the LC-MS/MS method, a Chromolith® reversed phase (RP)-18 endcapped 100x4.60 mm column equipped with a 0.5 µm Krudkatcher classic HPLC in-line filter was chosen as it gave the best results in terms of compound separation. A mobile phase consisting of 5 mM ammonium acetate (A) and 5 mM ammonium acetate in acetonitrile/water (95/5, v/v) (B) at a flow rate of 1.0 ml/min turned out to be the best option, with the following proportions of solvent B in the 8 minute gradient elution program: 20% for 0.34 min, linearly increased to 49% in 3.21min, followed by a short isocratic period of 49% for 0.55 min, a fast increase to 95% in 0.4 min, maintained for 1.0 min and finally, reversal to starting conditions. The QTRAP® 5500 was equipped with an ESI source (TurboIonSpray®) and detected all compounds, using an optimized multiple reaction monitoring (MRM) algorithm, operating in negative mode for valproic acid, phenytoin and phenobarbital and in positive mode for the other three compounds. For the on-line DBS-SPE system a HySphere resin GP, 7µm, 2x10 mm internal diameter cartridge (Spark Holland) was best suited for sample clean-up. The following SPE conditions turned out to be the best option: (1) preconditioning with 1 mL of methanol (MeOH); (2) equilibration with 1 mL of water; (3) elution of the DBS sample from the paper card directly to the SPE cartridge using 1 mL of water; (4) washing the cartridge with 1 mL of 5% MeOH in water and (5) elution of the sample from the cartridge using the LC pump gradient. In a next step, the eluent is directed to the Chromolith® column for LC-MS/MS analysis.
Preliminary experiments on real-life patient samples readily demonstrated the applicability of the method. In a next step, the developed method will be validated based on U.S. FDA and European Medicines Agency (EMA) guidelines for bioanalytical method validation. This will encompass the evaluation of selectivity, carry-over, matrix effect, linearity, precision, accuracy, stability, recovery and hematocrit-effect. Carry-over will be investigated by analyzing blank blood DBS cards after the analysis of the highest calibrator and QC sample. Matrix effect and recovery will be tested by analyzing blood of different hematocrit levels. Accuracy and precision will be evaluated using QC samples at four concentration levels, analyzed on different days. Stability will be investigated by storing QC samples at four concentration levels at room temperature for 2 and 4h before spotting. To establish the stability on the DBS cards, QC samples at four concentration levels will be spotted onto DBS cards, stored at three different temperatures (room temperature, 4°C and -20°C) and tested on different days for several months. After completing the validation, the method will be applied on patient samples originating from developing countries, to demonstrate the benefits of DBS sampling of AEDs in pediatrics in developing countries.
Automated direct extraction and analysis of DBS can open up new ways for TDM of AEDs, certainly in clinical routine.