Poster

[P26-1] P26-1: Anticonvulsant drugs

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Tue. Sep 26, 2017 12:30 PM - 1:30 PM Annex Hall (1F)

(Tue. Sep 26, 2017 12:30 PM - 1:30 PM Annex Hall)

[P26-1-2] Development and validation of a dried blood spot method for routine therapeutic drug monitoring of common antiepileptic drugs

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Keywords: dried blood spot, therapeutic drug monitoring, antiepileptic drugs, liquid chromatography tandem mass spectrometry

Background

Monitoring of antiepileptic drugs in children with epilepsy require several visits at a clinic for blood collection. Dried blood spot (DBS), an alternative way of collection, performed at home by self-collection may save time and costs for this patient group. The aim was to develop and validate a DBS method for carbamazepine, lamotrigine, levetiracetam and valproic acid with the requirements of using equipment and material adapted to a routine laboratory setting.

Methods

A 4.7 mm disc was punched out into a 96 well plate with an automated puncher with barcode reading. Extraction solution (200L), 65:35(v/v) methanol/water with isotopically labeled internal standards was added to each well followed by extraction on a microplate shaker for 30 minutes. Extracts were transferred to new wells and vacuum centrifuged for 30 minutes followed by reconstitution. A 6 L aliquot was injected on to the LC-MS/MS system. Chromatographic separation was achieved on a RP column, Acquity UPLC BEH, C18, 2.1 x 50 mm, 1.7 m with mobile phases A (aqueous 10 mM ammonium formate and 0.15% formic acid) and B (MeOH). Validation was performed according to the guidelines for bioanalytical validation from European Medicines Agency and additional DBS specific validation.

Results

Calibration curves had R2 values >0.994. Quantification ranges for carbamazepine and lamotrigine were 2.5-80 mol/L, for levetiracetam 5-400 mol/L, valproic acid 20-1000 mol/L. Within and-between run precision was 1.97 to 10.50 %. For lamotrigine, levetiracetam and carbamazepine volumes of 15 to 50 L in the hematocrit range of 0.3 L/L to 0.55 L/L was within accuracy limits of ±15 %. For valproic acid the hematocrit range was 0.35 to 0.50 L/L. Spiked capillary samples at low and high concentration were comparable with spiked venous samples.

Conclusions

A DBS method based on a 96-well format was developed for four commonly prescribed antiepileptic drugs. The method was thoroughly validated and traceability in sample preparation by barcode reading makes it suitable for the clinical laboratory. Requirements of using the same materials and equipment as for routine plasma methods were achieved.