**THE THERAPEUTIC DRUG MONITORING (TDM) OF ANTIEPILEPTIC DRUGS – MULTIDRUG ANALYSIS USING UPLC/MS/MS**

**ABSTRACT**

The preparation of a flow injection analysis (FIA) system for the determination of 24 antiepileptic drugs (AEDs) has been described. The method used for all analyses (Figure 1) is a flow injection analysis to produce a single profile AEDs were analysed on a UPLC/MS/MS system using the simple sample extraction and dilution process. The calibration curves prepared in solvent were used to check compliance and indicate a patient has taken an AED. Linear correlation was observed for all the compounds under investigation. A method capable of analysing a panel of AEDs and, where appropriate, the active metabolite, simultaneously has proved to be an extremely useful means of assisting in the individualisation of treatment regimen. The main advantage of this technique is the ability to achieve lower limits of detection compared to other methods. The material to produce mixed serum samples was not available so the serum samples were used to determine that the AEDs contained were detectable, linear and precise using this extraction procedure.

**INTRODUCTION**

- The desired therapeutic effect of most anti-epileptic drugs (AEDs) is achieved within a specific concentration range. If levels are too low, patients may not respond at all, or may be subject to side-effects. If levels are too high, the side-effects may be undesirable or toxic.
- AEDs are a diverse group of compounds including acids and bases of widely varying chemical properties, so a range of ionisation efficiencies.
- Some methods are available for the determination of AEDs in serum/plasma using a single protocol. These analyses are usually achieved by immunoassay, GC and LC spectrometry method (UPLC/MS/MS) to quantitate a panel of AEDs. A is injected matrix, B is injected water. C dem-

**METHODS**

**Mass Spectrometry**

- The solvent used was water 1000 µL (B) containing 0.1% formic acid (A). The calibration curve was linear to 1000 µg/mL of each AED with R² = 0.996.
- The solvent calibration curve produces a response at the detector. However, when fragmented in the collision cell the major product ion is at m/z 182 and there is also a minor product ion at m/z 180.
- Mixed analyte calibration curves were prepared in water from calibrators and quality controls. The correlation coefficient is related to the linearity of the calibration curve. Calibrators and quality controls were accurately detected above the limit of detection for each AED. The calibration curves for the 24 AEDs prepared in water and analysed on the UPLC/MS/MS system produced quantitative data with good limits of detection, linearity and reproducibility in a single extraction. This is facilitated by the speed and chromatographic resolution of UPLC and the UPLC/MS/MS system capable of simultaneous quantitation of the compounds of interest using multiple reaction monitoring (MRM).
- Experiments
- Preliminary investigations into the linearity, limit of detection and reproducibility of the analytes in solvent and serum matrix have been performed. Different mobile phase modifiers were considered whilst developing the chromatographic separation. These involved lowering and raising the pH as well as lowering the concentration of formic acid. As AEDs are a diverse group of compounds, their stability will vary depending on the type of polarities of the Quattro Premier XE.
- **RESULTS**

- Using ESI and ES+ modes it was possible to analyse 24 AEDs in 12 minutes, with no need to separate isomers, in an ion suppression approach to the same acidic moiety and only one MRM transition in total.
- **DISCUSSION**

- AEDs are a diverse group of compounds including acids (e.g. valproic acid, weak bases (e.g. carbamazepine) and benzodiazepines (e.g. diazepam). Different mobile phase modifiers were considered whilst developing the chromatographic separation. These involved using a diverse group of compounds, their stability will vary depending on the type of polarities of the Quattro Premier XE.
- **Conclusions**

- • Different mobile phase modifiers were considered whilst developing the chromatographic separation. These involved using a diverse group of compounds, their stability will vary depending on the type of polarities of the Quattro Premier XE.
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