Extraction of Antiepileptic Drugs from Human Urine, Serum and Oral Fluid Using Supported Liquid Extraction (ISOLUTE® SLE+) in 96-Well Plate Prior to LC-MS-MS

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Introduction

Antiepileptic drugs are prescribed to suppress seizures in epilepsy patients. A variety of different types of AEDs have been synthesized to pharmacologically address different types of epilepsy. The ability to therapeutically monitor these drugs in patients is necessary for maintaining optimal medical care and managing any adverse effects of the drug. A fast and clean extraction method is needed that works in a variety of biological matrices and affords a high throughput workflow. Here ISOLUTE SLE+ is demonstrated as an effective way to extract AEDs from serum, oral fluid and urine with good efficiency and recovery. The method was developed on a 96 well plate format to facilitate a high throughput workflow model.

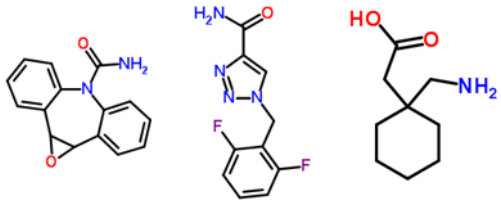

Figure 1. *Structures of Carbamazepine Epoxide, Ruginamide, and Gabapentine*

Experimental

Reagents

Acetonitrile, Methanol, Ammonium Acetate, Trifluoroacetic Acid, Formic Acid and Methyl tert-Butyl Ether were purchased from Sigma-Aldrich Co. (Atlanta, GA.) The antiepileptic analyte standards (Table 1) were purchased from Cerilliant (Round Rock TX.)

Sample Preparation- Neutral Analytes in Serum, Urine and Oral Fluid

The samples were processed on a 400uL supported liquid extraction 96 well plate format (ISOLUTE SLE+). An aliquot (100uL) of matrix (urine or serum spiked with internal standard) was pretreated with 250uL of 5mM ammonium acetate (pH 2.9). The entire sample was loaded into the designated well. A slight pulse of vacuum or positive pressure was applied to facilitate flow of sample onto sorbent bed. Sample was allowed to sit for 5 minutes. The sample was eluted with two aliquots of methyl tert-butyl ether with 1% triflouroacetic acid (2 x 700uL) into a collection tube. The sample was dried down and reconstituted in 300uL of aqueous mobile phase. Note: Oral fluid was collected using the Orasure Intercept and Immunalysis Quantisol collection kits.

Sample Preparation- Neutral and Zwitterionic Analytes in Serum, Urine and Oral Fluid

The samples were processed on a 400uL supported liquid extraction 96 well plate format (ISOLUTE SLE+). An aliquot (100uL) of matrix (urine or serum spiked with internal standard) was pretreated with 100uL of 50% aqueous formic acid. The entire sample was loaded into the designated well. A slight pulse of vacuum or positive pressure was applied to facilitate flow of sample onto sorbent bed. Sample was allowed to sit for 5 minutes. The sample was eluted with two aliquots of methyl tert-butyl ether with 1% triflouroacetic acid (2 x 700uL) into a collection tube. The sample was dried down and reconstituted in 300uL of aqueous mobile phase.

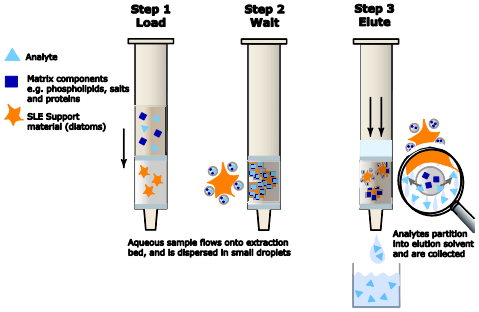

Figure 2. *Illustration of LOAD-WAIT-ELUTE strategy for ISOLUTE SLE+ (Supported Liquid Extraction)*

Liquid Chromatography-Mass Spectrometry Analysis

An optimized Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) analytical method for detection of the antiepileptic drugs was developed on a standard C_{18} column (150mm x 4.6mm, 5µm). Detection of the target analyte was achieved using an Applied Biosystems /MDS Sciex 4000 Q-Trap triple quadrapole mass spectrometer (Applied Biosystems, Foster City, CA.) equipped with a Turbo Ionspray® interface. Chromatographic separation was accomplished using the Agilent 1200 Liquid Handling System (Agilent Technologies, Berkshire, UK). A gradient method of 5mM ammonium formate with 0.1% Formic Acid (**A**) and acetonitrile:methanol (50:50) (**B**) was developed. The gradient starts at 70% **A** to 30% **A** in 4 minutes at 1.0 ml/min flow rate. Figure 3 shows typical chromatograms obtained for the target analytes.

Scan	Analyte	MRM Transition	Declustering Potential (DP)	Collision Energy (CE)	Cell Exit Potential (CXP)
1	Gabapentine	172 > 154	40	25	16
2	Felbamate	178 > 117	40	25	16
3	Rufinamide	239 > 127	40	25	16
4	Oxcarbazepine	253 > 208	40	25	16
5	Tiagabine	376 . 247	40	25	16
6	Vigabatrine	130 > 70.9	40	25	16
7	Carbamazepine Epoxide	253 > 180	40	25	16

Table 1. *MRM transitions for antiepileptic drugs.*

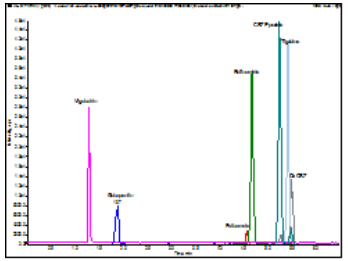

Figure 3. *Typical extracted ion chromatogram for Antiepileptic drugs.*

Results/Discussion

All of the antiepileptic drugs were spiked into blank biological matrices at concentrations ranging from 20ng/mL to 100ng/mL. It was observed that the initial pretreatment strategy of 5mM ammonium acetate (pH 2.9) worked well for the neutral analytes but was less effective for the zwitterionic analytes (i.e. gabapentin and vigabatrine). Hence a second pretreatment strategy was investigated. The effect of 50% formic acid is not fully understood, but theoretically it is proposed that the strong acid strength can effectively neutralize the acidic moiety of the zwitterionic species making it more amenable to the organic elution solvent. Recoveries observed under the first pretreatment strategy (Figure 4a) were over 70% for the neutral analytes in both urine and serum. A substantial improvement was observed for the gabapentin using the second elution strategy (Figure 4b) while the same conditions seemed to have an adverse effect on the vigabatrine. Similar results are observed for samples extracted from oral fluid using ammonium acetate elution strategy (Fig 5). The neutral analytes were extracted with good efficiency while the zwitterionic analytes demonstrated poor recovery. A comparative performance between the neat oral fluid and the Orasure and Immunalysis collection kits was observed. Overall the extraction methodologies proved effective to a diverse array of biological matrices. Further optimization can be employed to address the zwitterionic analytes.

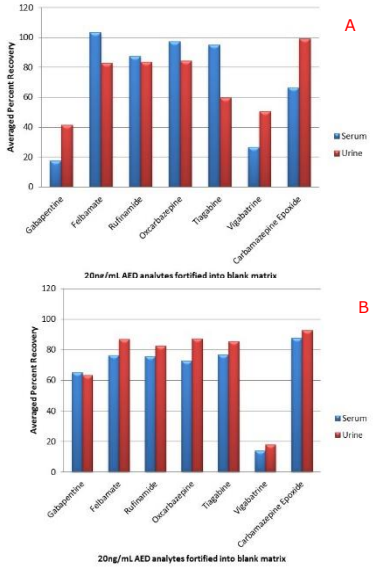

Figure 4. *Recoveries of spiked antiepileptic suite into serum and urine pretreated with 5mM ammonium acetate (A) and 50% aqueous formic acid (B).*

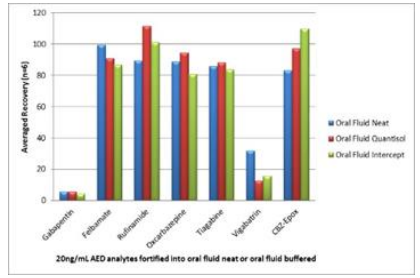

Figure 5. *Recoveries of spiked antiepileptic suite into oral fluid using Quantisol and Intercept oral fluid collection kits.*

Conclusion

- ISOLUTE® SLE+ is a fast sample preparation alternative for biological fluids.
- The extraction methodologies employed here demonstrate the utility of supported liquid extraction as a viable extraction sorbent for antiepileptic drugs.
- The authors of this poster would like to thank Dean Fritch for his assistance with the Orasure Intercept kits.