DM-MAP: Designing a Drug and Metabolite Microanalytical Platform for Detection and Quantification of Molecules in Pre-Clinical Studies: Initial Studies with Lamotrigine

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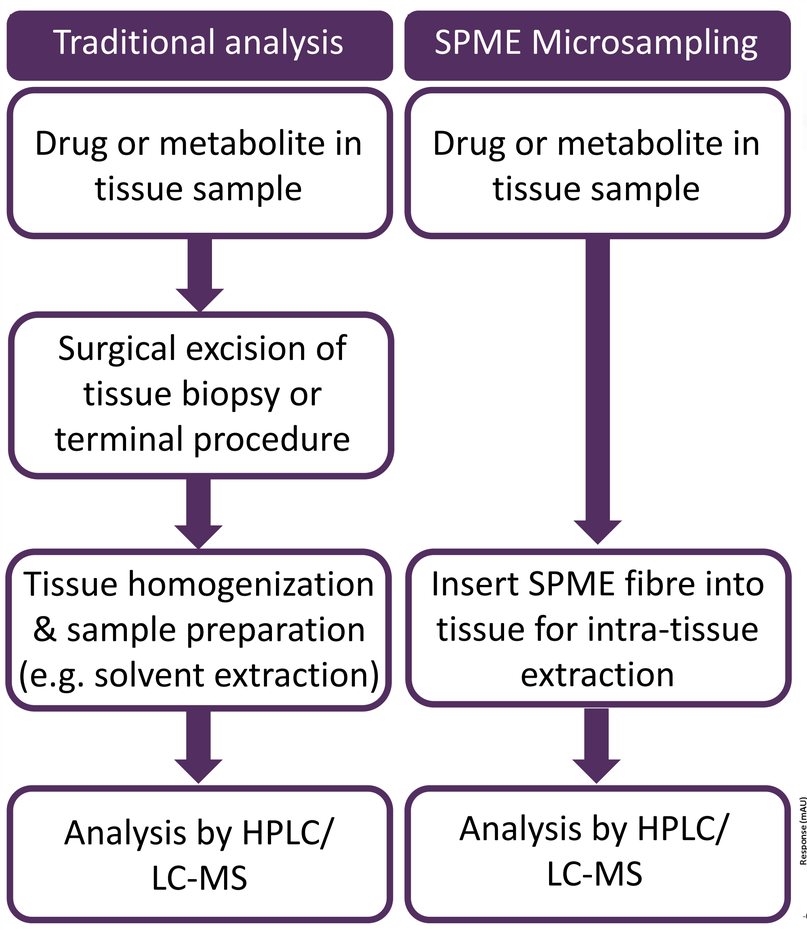
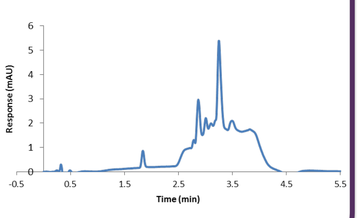
Introduction

- Drug development requires a stage of preclinical testing whereby animals are dosed with novel compounds to assess pharmacological, toxicological and disposition properties.
- It is not only the concentration of drug and metabolites in blood that is of interest, but also measurement of their distribution to tissues and organs from the bloodstream.
- Tissue and organ sampling restrictions require multiple animal sacrifice to assess local drug concentrations and novel sampling methods that can be used *in vivo* would be of benefit.
- Solid Phase Micro Extraction (SPME) is a minimally invasive technique using a stainless steal fibre coated with extraction phases, to which drug adsorbs from sample matrices.
- SPME has characteristics suited to *in vivo* drug metabolism, pharmacokinetic and toxicokinetic (DMPKT) testing: amenable to miniaturisation, the ability to sample independently of sample volume (i.e. tissue sites); and extracting small fractions of total sample concentration, to leave the tissue's drug concentration largely unchanged for later time points.



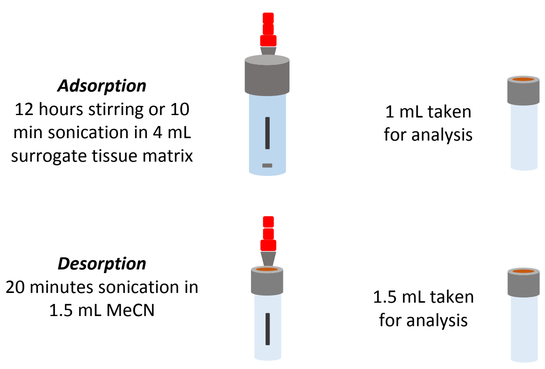






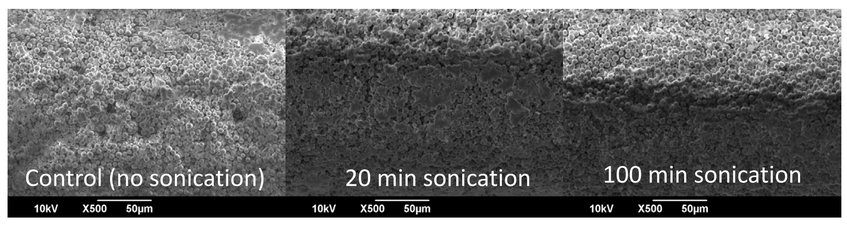
Methodology

- Lamotrigine adsorption phase: C18-SPME fibres were used to extract lamotrigine from a saturated solution of PBS (4 mL) as model interstitial fluid (130 µg/mL). Overnight stirring at 37°C was compared with 10 min sonication. The effect of temperature rise during sonication on adsorption was also studied.
- Analyte desorption phase: SPME fibres were sonicated for 20 min in 1.5 mL MeCN in order to recover analyte from the fibres.
- Detection: uHPLC-UV analysis was performed using a C18 (50 mm x 2.1 mm, 1.8 µm) at 35˚C, flow rate 0.5 mL min^{-1}, 1 µL injection volume, 270 nm detection. A gradient method from 90% acidified water/10% MeCN to 30% water over 4 min was employed.



Results

- The use of sonication to enhance adsorption compared to stirring was investigated to overcome poor mixing in tissue matrices. Sonication did not alter the integrity of the SPME coating when examined by electron microscopy.



- Lamotrigine recovery was lower following sonication. This may have arisen from temperature rises during sonication; recovery was lower at higher compared to lower sonication temperatures.

Adsorption type	Amount Recovered (µg)	S.D ±
12 hr stirring	1.25	0.63
10 min sonication	0.95	0.28

Sonication temperature	Amount Recovered (µg)	S.D ±
30˚C	1.44	0.18
60˚C	0.27	0.27

- Using stirring for adsorption and sonication for desorption resulted in efficient extraction, but with inter-SPME variability.

Fibre Type	Amount Recovered (µg)	S.D	% mass balance	S.D
C18	0.98	0.06	100.95%	15.46%

Aims and NC3Rs Impact

- The aim of the current work was to develop an approach for selecting an appropriate fibre phase for sampling from diverse sample matrices. In addition the optimization of the SPME adsorption/desorption processes for poorly stirred media was investigated, including a determination of the variability to expected due to the fibre sampling process.
- Refinement – SPME protocols could provide a more animal-friendly manner of sampling that causes less distress to an animal, for example, avoiding skin punch biopsies.
- Reduction – The avoidance of tissue removal protocols can have immediate benefit in reducing the numbers of animals sacrificed during tissue and organ drug concentration determination.

Conclusions and Future Work

- SPME tissue extraction protocols were investigated. Sonication to achieve rapid adsorption was impractical due to altered equilibrium, but will be suitable to improve desorption kinetics.
- Inter-fibre variability of extraction performance was an issue and a number of fibre types will be screened to improve this.
- Utilising available control tissue/study animals (3R's), proof-of-concept tissue concentration SPME studies will be evaluated in a step wise manner i.e. tissue homogenates to isolated organ perfusions, prior to pre-clinical evaluation.

Acknowledgements

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National Centre for the Replacement Refinement & Reduction of Animals in Research