Development of a Microsampling Platform for Determination of Drug Molecules in Pre-Clinical Studies: Initial *in vitro* Investigations using Metoprolol and Tissue Homogenates

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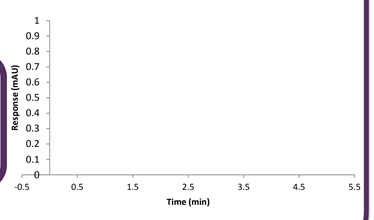
Introduction

- 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 steel 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, sampling is independent of sample volume (i.e. tissue sites); and small fractions of total sample concentration are extracted leaving the tissue's drug concentration largely unchanged for later time points.

Traditional analysis	SPME Microsampling
Drug or metabolite in tissue sample	Drug or metabolite in tissue sample
Surgical excision of tissue biopsy or terminal procedure	
Tissue homogenization & sample preparation (e.g. solvent extraction)	Insert SPME fibre into tissue for intra-tissue extraction
Analysis by HPLC/ LC-MS	Analysis by HPLC/ LC-MS



Aims

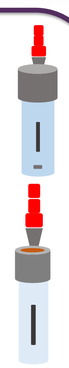
- To investigate metoprolol drug adsorption to different SPME fibre chemical phases from models of hydrophilic/hydrophobic tissue compartment (PBS and corn oil) in order to find the optimal fibre type for drug adsorption on to fibre.
- To use one of the optimal fibre types for method development for extraction of metoprolol by SPME from tissue homogenates (liver and skin).
- To investigate loading of an internal standard onto the SPME fibre, to allow for SPME-calibration in future studies; thus increasing method precision.

Methodology

- Metoprolol adsorption phase: SPME fibres were inserted into different sample volumes, matrices, and concentrations.
- Metoprolol desorption phase: SPME fibres were sonicated for 20 min in 1.5 mL MeCN in order to recover analyte from the fibres.

Adsorption
Fibre inserted into sample and agitation applied to increase speed to reach equilibrium

Desorption
20 minutes sonication in 1.5 mL MeCN

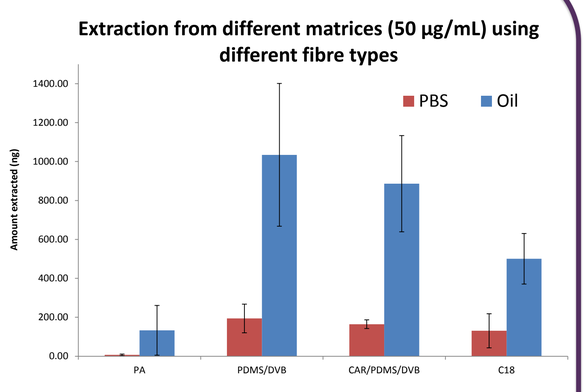


Matrix	Concentration (µg/mL)	Agitation
PBS + Corn Oil	50	Stirring
PBS	150	Stirring/Shaking
Liver/Skin homogenate	50	Stirring/ Shaking
PBS	50	Stirring/Shaking/Sonication

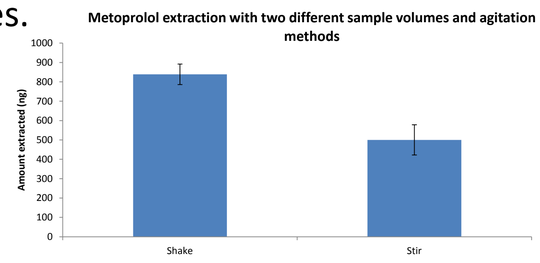
- Experimental parameters are shown; tissue homogenate was made up 10 %w/v with PBS
- Detection: uHPLC-UV/LC-MS analysis was performed using a C18 column (50 mm x 2.1 mm, 1.8 µm) at 35˚C, flow rate 0.5 mL min^{-1}, 1 µL injection volume, 270 nm detection, or *m/z* detection *268* >> *116*. A gradient method from 90% acidified water/10% MeCN to 30% water over 4 min was employed.

Results

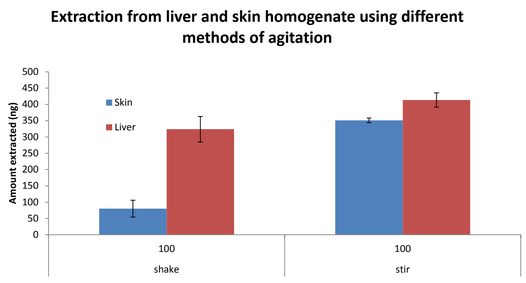
- Screening of fibre types took place from simulated hydrophilic & hydrophobic tissue compartments.
- Extraction from PBS showed no difference between fibre types; from oil PDMS/DVB was optimal but with high RSD



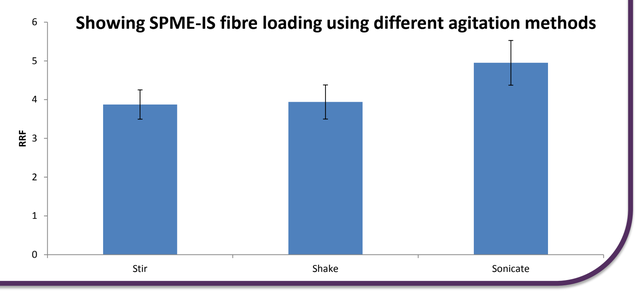
- Different agitation methods (shaking and stirring) were investigated: Shaking showed larger amounts of drug extracted vs stirring and was considered in future studies.



- Extraction from skin and liver homogenates as tissue matrices using two different methods of agitation: Stirring was shown to extract more drug from tissue, however less drug was extracted from skin; indicative of greater drug-tissue binding.



- Loading of an internal standard onto fibre for SPME calibration by extraction from an IS solution – sonication was shown to give a higher RRF.



Conclusions and Future Work

- SPME was evaluated by *in vitro* studies with simplified model media, to select an optimum fibre type (C18, PDMS/DVB).
- C18 fibres were chosen to study extraction from tissue homogenates. The effect of stirring and tissue type on recovery was identified, and the need for SPME-calibration was revealed.
- SPME detection of drugs will be evaluated in a step-wise manner in animal dosing studies; using homogenates, whole tissues, isolated perfused organ, and *in vivo* animal studies.

Acknowledgements

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