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Plasma protein binding study of Diclofenac and Salicylic acid in human plasma using Rapid Equilibrium Dialysis (RED) and UPLC-MS/MS

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Introduction

The binding of drugs to plasma proteins is an important parameter in drug development [1-2]. To determine the plasma protein binding of Diclofenac and Salicylic acid in human plasma Rapid Equilibrium Dialysis (RED) was performed. The samples were analyzed using UPLC-MS/MS.

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Experimental (I)

RED device; 8 kDa MWCO (ThermoFisher, product code 90006)
Thermomixer (Eppendorf)
Pooled human plasma (EDTA, Bioreclamation)
Diclofenac reference substance and internal standard
Salicylic acid reference substance and internal standard

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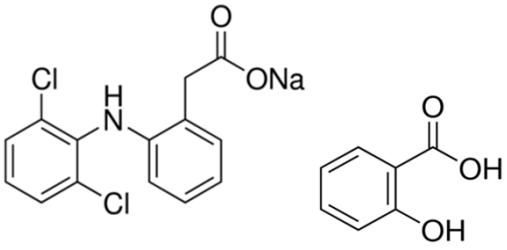
Experimental (II)

UPLC: Acquity (Waters)
Mass spectrometry: XEVO-TQS (Waters)
Ionization: electrospray (positive and negative)



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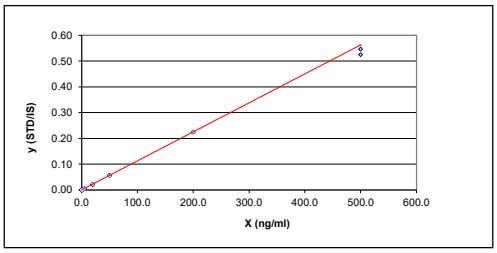
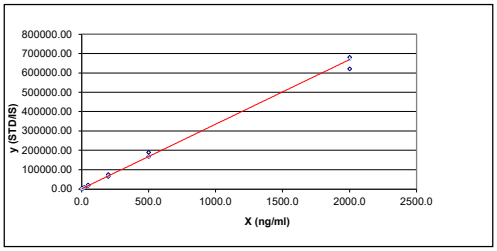
Calibration results

Diclofenac:
Calibration range 0.2-500 ng/ml



Salicylic acid:
Calibration range 2-2000 ng/ml



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Plasma protein binding results of Diclofenac

Parameter	Diclofenac conc (ng/ml) in plasma Before RED	Diclofenac conc (ng/ml) Donor compartment After RED	Diclofenac conc (ng/ml) Acceptor compartment After RED	Free fraction (%)	Bound fraction (%)
Replicate 1	1070	1005	2.6	0.2	99.8
Replicate 2	1100	1095	2.5		
Replicate 3	1120	1130	2.9		
Average (ng/ml)	1097	1077	2.6		
CV (%)	2.3	6.0	7.8		

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Plasma protein binding results of Salicylic acid

Parameter	Salicylic acid conc (ng/ml) in plasma Before RED	Salicylic acid conc (ng/ml) Donor compartment After RED	Salicylic acid conc (ng/ml) Acceptor compartment After RED	Free fraction (%)	Bound fraction (%)
Replicate 1	1135	885	80	9.0	91.0
Replicate 2	1125	945	85		
Replicate 3	1105	950	85		
Average (ng/ml)	1122	927	83		
CV (%)	1.4	3.9	3.5		

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Conclusions

The protein bound fraction of Diclofenac and Salicylic acid in human plasma was 99.8 % and 91.0 %, respectively. The repeatability of the experiments, expressed as the coefficient of variation (CV), was within 10%, which was fit for purpose.

The results obtained were in close agreement with results reported earlier [1].

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References

[1] F. Zheng et al. Compilation of 222 drugs' plasma protein binding data and guidance for study designs. *Drug discovery today*, (2012) 17(9-10), 475-485

[2] B. Buscher et al. Bioanalysis for plasma protein binding studies in drug discovery and drug development: views and recommendations of the European Bioanalysis Forum, *Bioanalysis,* (2014) 6(5), 1-10

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