Extraction of Drugs from Mitra® Micro-sampling Devices: Overcoming a New Haematocrit Effect

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The promise of microsampling, delivered

Introduction

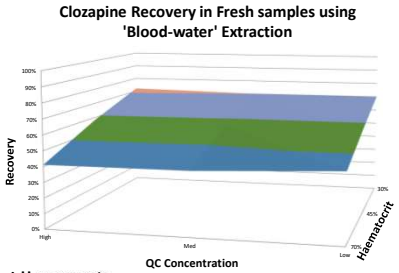
The analysis of dried blood spots is an established methodology in neonatal and paediatric screening but this technique has a number of shortcomings when looking at bioanalysis of therapeutic drugs.

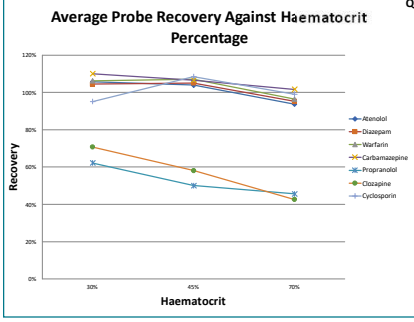
Mitra® Microsampling Devices offer to overcome some of these shortcomings, however, extraction efficiencies are analyte dependant and most bioanalytical laboratories are time-limited when optimising methods for novel therapeutics.

The aim of this study was to establish a generic workflow for optimising LC-MS/MS methods using these devices by assessing a selection of drug probes, chosen based on differing physicochemical and biochemical properties.

1 A New Haematocrit Effect

Initial protocols involved mixing dried samples for 30 minutes in water to produce 'blood-water' before a further protein precipitation extraction and analysis by LC-MS/MS and illustrated:





- Out of the 7 probe compounds Clozapine and Propranolol demonstrated significant differences in recovery across haematocrits.
- No major differences were seen across the range of concentrations tested.

2 Reagent Screen

Following the "blood-water" test, analytes that demonstrate inconsistent recovery are then put through a screen of extraction reagents using whichever haematocrit/drug concentration value gave the lowest recovery.

Extraction Reagent	Atenolol	Diazepam	Warfarin	Carbamaze-pine	Propranolol	Clozapine	Cyclosporin
0.1% FA (aq)	96%	97%	94%	96%	83%	88%	116%
0.1% NH_3 (aq)	95%	95%	100%	96%	76%	89%	109%
50:50 ACN:H_2O	93%	97%	99%	98%	87%	83%	101%
0.1% NH_3 (50:50 IPA:H_2O)	96%	93%	96%	93%	91%	85%	101%
Water	100%	101%	107%	104%	46%	58%	127%
0.1% NH_3 (50:50 ACN:H_2O)	93%	94%	96%	93%	89%	86%	93%
50:50 MeOH:H_2O	85%	94%	98%	96%	66%	82%	115%
0.1% NH_3 (MeOH)	92%	98%	76%	98%	100%	75%	75%
0.1% FA (50:50 MeOH:ACN)	83%	95%	88%	95%	82%	77%	79%
0.1% FA (50:50 ACN:H_2O)	92%	94%	95%	96%	85%	86%	46%
0.1% FA (MeOH)	83%	91%	83%	93%	87%	72%	81%
0.1% FA (50:50 IPA:H_2O)	60%	82%	78%	88%	84%	73%	71%
0.1% NH_3 (50:50 MeOH:H_2O)	93%	91%	99%	92%	82%	47%	52%
0.1% NH_3 (50:50 MeOH:ACN)	88%	95%	67%	94%	94%	77%	47%
0.1% FA (50:50 MeOH:IPA)	74%	87%	76%	88%	81%	70%	79%
50:50 MeOH:ACN	72%	98%	68%	97%	86%	84%	34%
MeOH	75%	93%	67%	93%	83%	79%	42%
0.1% NH_3 (50:50 MeOH:IPA)	76%	83%	42%	85%	88%	66%	40%
0.1% FA (50:50 MeOH:H_2O)	83%	65%	55%	72%	70%	64%	47%
0.1% FA (50:50 IPA:ACN)	58%	57%	48%	73%	66%	47%	27%
0.1% FA (ACN)	40%	47%	38%	54%	55%	29%	20%
0.1% NH_3 (50:50 IPA:ACN)	46%	33%	14%	46%	61%	32%	19%
0.1M $ZnSO_4$	70%	13%	13%	41%	31%	25%	22%
0.1% NH_3 (ACN)	33%	29%	15%	34%	48%	27%	10%
0.1% FA (IPA)	22%	23%	19%	25%	39%	20%	17%
ACN	22%	27%	14%	30%	32%	22%	12%
0.1% NH_3 (IPA)	23%	15%	8%	20%	44%	22%	16%
IPA	11%	13%	8%	16%	29%	20%	11%

3 Optimising the Chosen Reagent

Following the reagent screen the chosen extraction solution can be further optimised by altering the % modifier or % organic solvent used.

In the example below, various mixtures of ammonia and acetonitrile were assessed for the 7 analytes in this multi-analyte method.

Reagent	Atenolol	Diazepam	Warfarin	Carbamaze-pine	Propranolol	Clozapine	Cyclosporin
0.1% NH_3 (25:75 ACN:H2O)	105%	96%	99%	100%	*80%*	*76%*	111%
0.1% NH_3 (50:50 ACN:H2O)	91%	95%	96%	105%	*81%*	*73%*	87%
0.1% NH_3 (75:25 ACN:H2O)	104%	99%	92%	107%	91%	*78%*	*76%*
1% NH_3 (25:75 ACN:H2O)	111%	98%	99%	102%	87%	*79%*	116%
1% NH_3 (50:50 ACN:H2O)	111%	98%	99%	102%	99%	*77%*	97%
1% NH_3 (75:25 ACN:H2O)	113%	98%	91%	101%	94%	*75%*	*80%*
5% NH_3 (25:75 ACN:H2O)	116%	96%	105%	101%	91%	103%	109%
5% NH_3 (50:50 ACN:H2O)	108%	100%	101%	103%	104%	*82%*	92%
5% NH_3 (75:25 ACN:H2O)	114%	93%	91%	101%	99%	*77%*	*76%*

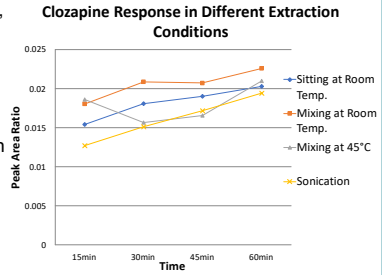
<85% Recovery

4 Optimising the Extraction Procedure

If recoveries remain inconsistent, a number of extraction techniques can be assessed.

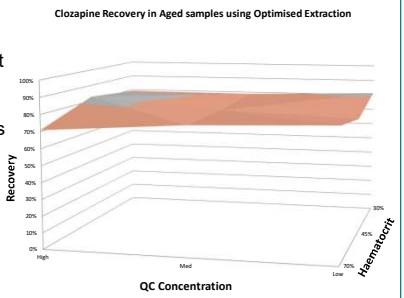
In this study, the time left in solvent combined with mixing gave the largest improvements in recovery.

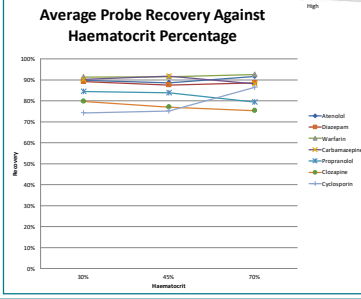
Sonication and increased temperature had less of an effect.



5 Overcoming the New Haematocrit Effect

Combining the optimal reagent and mixing conditions, we can achieve consistent recoveries across a range of haematocrits and concentrations, for clozapine, in combination with the other 6 analytes tested.





Optimisation should be performed on the 'worst case' conditions, which is generally 70% haematocrit at QC low, using aged samples as a more accurate representation of study samples.

Conclusions

Haematocrit recovery bias observed in blood samples dried onto Mitra® volumetric microsampling devices can be overcome by improving the recovery of the extraction.

Aged samples which return the lowest haematocrit and concentration after an initial screen, should be used to optimise the extraction – taking care not to misinterpret stability and extraction related recovery bias.

Mixing conditions can slightly improve efficiency of extraction, but ultimately, the choice of extraction reagent is key to developing an acceptable method for analysing dried blood samples across a range of haematocrit levels.