Development of a LC-MS method for quantification of six common pharmaceuticals in OFM samples

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Analytes

Amitriptyline, clobetasol-17-propionate (CP17), diclofenac, hydrocortisone, lidocaine and metronidazole are common pharmaceuticals with different polarities and protein binding characters.

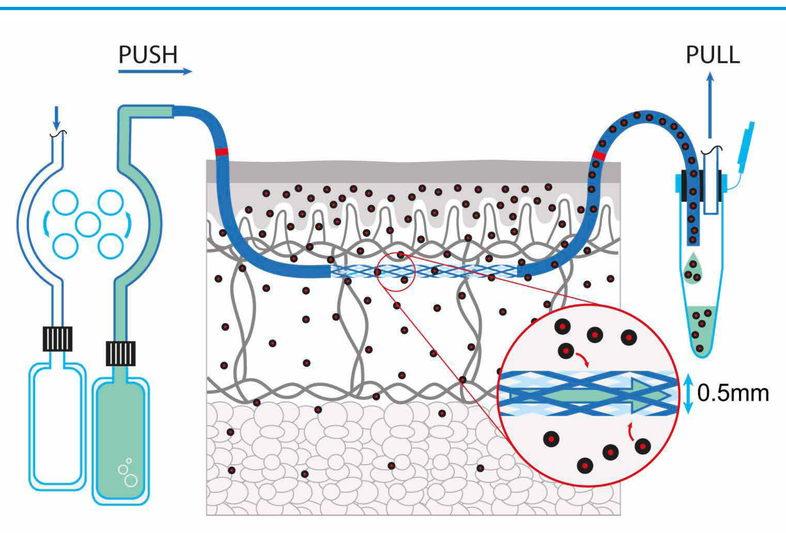
We aimed to develop a fast, robust and sensitive LC-MS method to quantify those analytes in samples collected by dermal open flow microperfusion.

OFM Sampling

Open flow microperfusion (OFM) sampling is used in preclinical and clinical studies and provides diluted interstitial fluid with a volume of only a few µL per sample and thus usually very low analyte concentrations.

Hence it is important to use an analytical method that offers:

- robustness
- high analyte recovery
- good sample clean up
- high sensitivity and selectivity

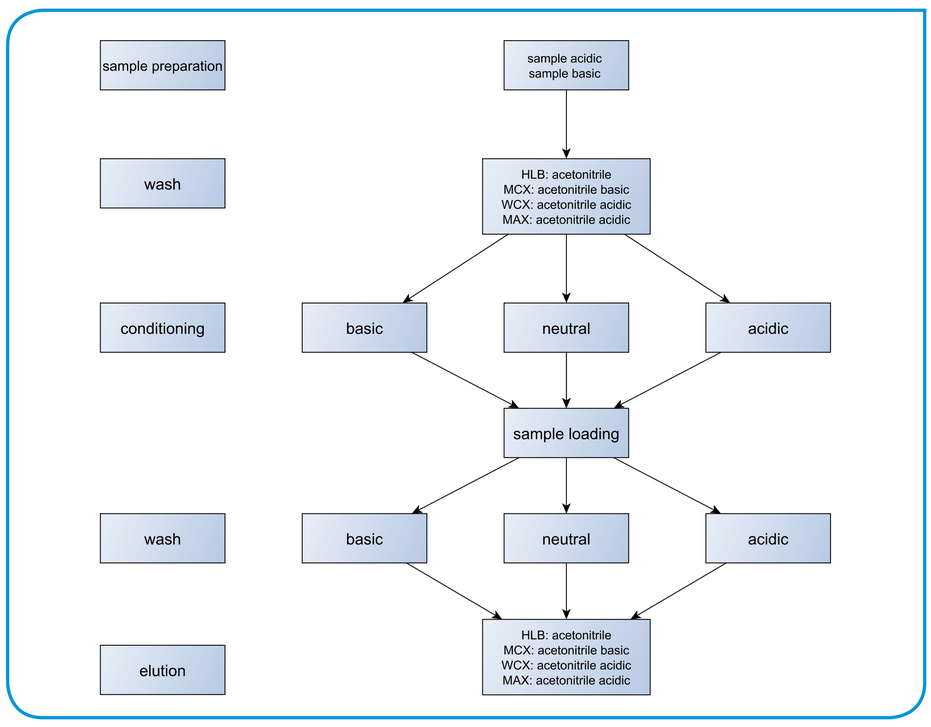


Open Flow Microperfusion (OFM): a membrane-free probe is CE-certified for clinical use in dermal and subcutaneous tissue in humans to continuously sample interstitial fluid for PK/PD studies directly in the target tissue

Aim

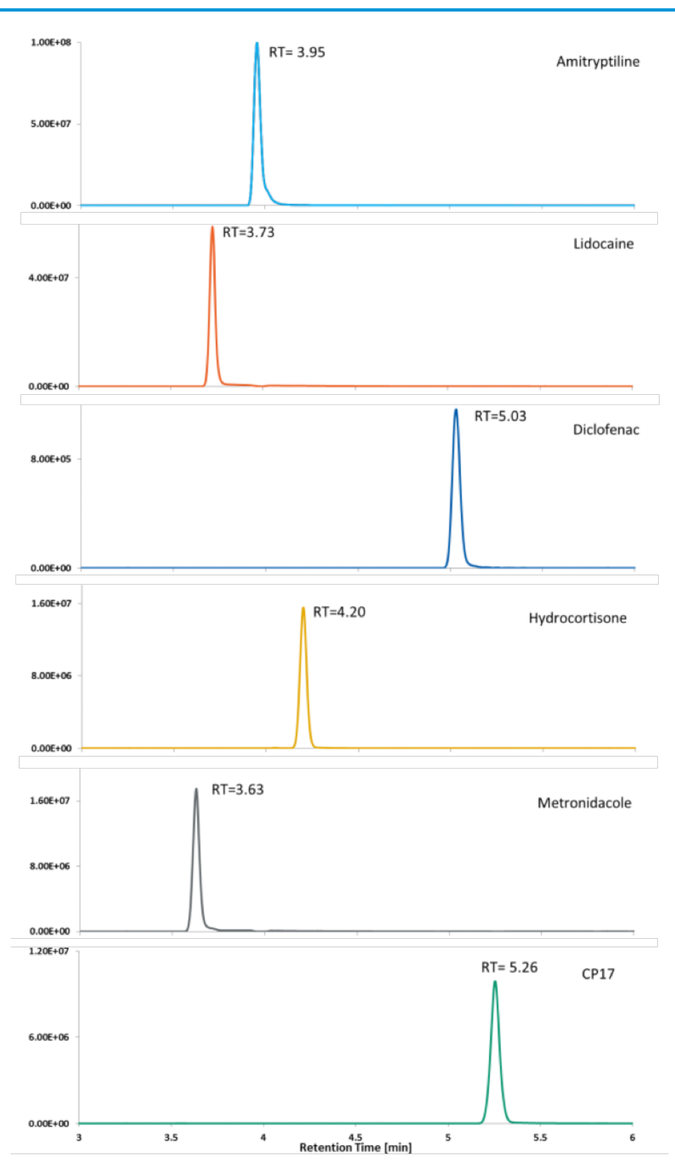
Development of a LC-MS method for quantification of amitriptyline, clobetasol-17-propionate, diclofenac, hydrocortisone, lidocaine and metronidazole in OFM samples.

Sample Preparation



Method

The optimisation of the HPLC method was carried out on a Thermo U3000 with a Waters Atlanis T3 column using gradient elution with 0.1% formic acid in water and 0.1% formic acid in actenotrile as mobile phase. The method allowed the separation of all six analytes within seven minutes. A LTQ-Orbitrap XL mass spectrometer was used as detector using heated electrospray ionisation in positive full scan mode (100-500).



Results

Recoveries of >83% were obtained when using the HLB µ-Elution material. A validation was performed according to US FDA “Guidance for Industry, Bioanalytical Method Validation (May 2001)”.

Analyte	LLOQ	Linearity	Accuracy	precision
Amitriptyline	1 ng/ml	1 – 100 ng/ml	103% – 108%	3.2% – 8.5%
Lidocaine	0.5 ng/ml	0.5 – 100 ng/ml	103% – 109%	1.2% – 4.9%
Diclofenac	5 ng/ml	5 – 100 ng/ml	91% – 103%	3.6% – 9.5%
Hydrocortisone	1 ng/ml	1 – 100 ng/ml	92% – 109%	6.0% – 8.4%
Metronidazole	1 ng/ml	1 – 100 ng/ml	101% – 109%	3.5% – 4.4%
CP17	1 ng/ml	1 – 100 ng/ml	99% – 110%	4.4% – 9.1%

Conclusion

Experiments showed that both sample dilution and conditioning of the SPE material have a great influence on analyte recovery. Furthermore, recovery showed that the ionic interaction had a greater influence on extraction efficiency in hydrophilic analytes compared to hydrophobic analytes.

Results showed that we successfully developed and implemented a new method to simultaneously detect amitriptyline, clobetasol-17-propionate, diclofenac, hydrocortisone, lidocaine and metronidazole in samples with a low volume.

Acknowledgements

This work was supported financially by the Austrian Federal Ministry for Transport, Innovation and Technology (BMVIT, Project PROMES).