Clinical applications of direct coupling of Bio-compatible SPME devices to MS via Open-Port Probe sampling interface

Prof. Janusz Pawliszyn Research Group
METABOLOMICS | SAMPLE PREPARATION | MASS SPECTROMETRY

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Abstract

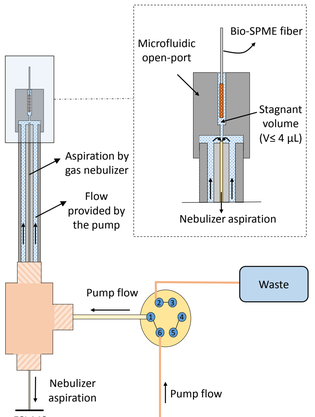
- ✓ We are presenting the advances achieved up to date employing a microfluidic open port that allows the coupling of biocompatible solid-phase microextraction (Bio-SPME) devices through the generation of a stagnant volume follow by a three second switch flow. In this way, an efficient desorption and very sharp peaks (2-3 seconds FWMH) are reached.
- ✓ Towards the monitoring of drugs and biomarkers in the operation room, some applications were developed using this interface such as the monitoring of doxorubicin from perfusate used during in-vivo (IVLP) and ex-vivo (EVLP) lung perfusion to treat lung cancer.
- ✓ Regarding the high-throughput therapeutic drug monitoring, the aim of this work is to push down the limits of detection trying to improve dramatically the total analysis time in high-throughput and the individual turn-around times, as well. Therefore, advances in the development of the quantitation method of testosterone from human plasma is herein presented. The concept of rapid on-fiber derivatization is gathered by using the Amplifex™ keto reagent. Finally, quantitation of immunosuppressive drugs from 100 µL of whole blood is presented. LOQs of sub-ppb levels were achieved for all the compounds with turn-around times of less than 90 minutes.

Instrumental

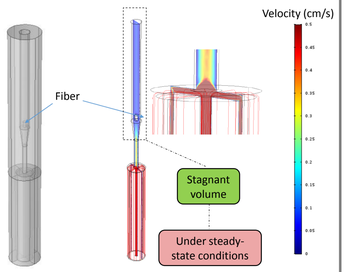
AB API 4000

SCIEX QTRAP 6500+

Set-up and Fundamentals



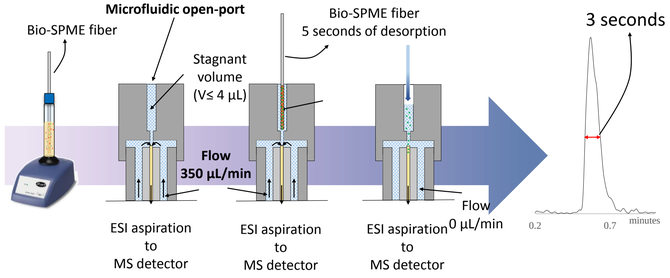
Scheme of the set-up working under steady-state conditions



Scheme of the hydrodynamic modelling of the interface

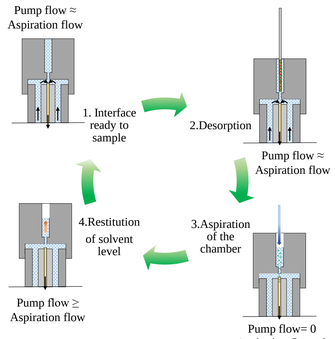
- ✓Desorption volume of 4 µL.
- ✓Any kind of dispositive can be adapted.
- ✓The desorption time can be optimized.
- ✓The fiber can be heated or vibrated in order to increase the desorption speed.

Analytical workflow



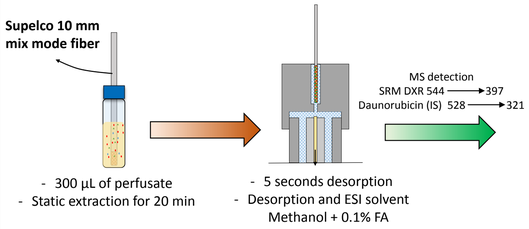
1. Extraction | 2. Desorption | 3.Chamber aspiration | 4. MS detection

Instrumental cycle



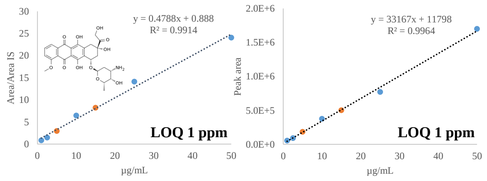
Results

Monitoring of doxorubicin from ex-vivo perfusate from EVLP and IVLP



- 300 µL of perfusate
- Static extraction for 20 min

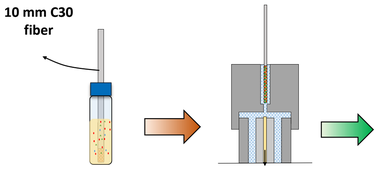
- 5 seconds desorption
- Desorption and ESI solvent Methanol + 0.1% FA



With IS correction

Without IS correction

Quantitation of Testosterone from human plasma



- 200 µL of plasma
- Agitation for 30 minutes

- 5 seconds desorption
- Desorption and ESI solvent Methanol + 0.1% FA

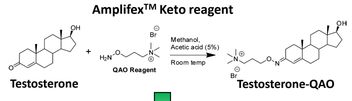
SRM transitions

Compound	SRM transitions
Testosterone	289/109
Testosterone 13C3 (IS)	292/112

LOQ 10 ppb

Strategies of improvement

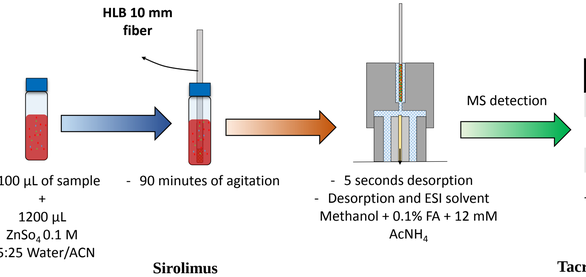
On-fiber derivatization



Off-line improvements between 6-10 times

Estimated LOQ of 1 ppb

Quantitation of immunosuppressive drugs from whole blood

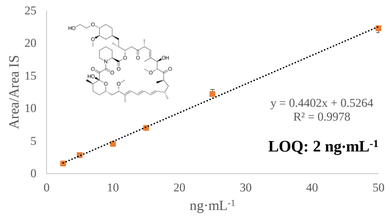


- 100 µL of sample + 1200 µL $ZnSo_4$ 0.1 M 75:25 Water/ACN
- 90 minutes of agitation
- 5 seconds desorption
- Desorption and ESI solvent Methanol + 0.1% FA + 12 mM $AcNH_4$

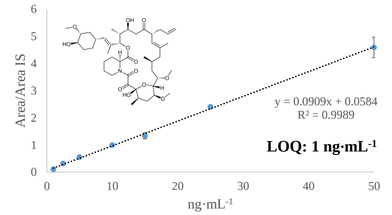
SRM transitions

Compound	SRM transitions
Tacrolimus	821/768
Cyclosporine A	1219/1202
Sirolimus	931/864
Everolimus	975/908

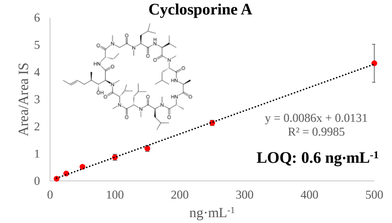
Sirolimus



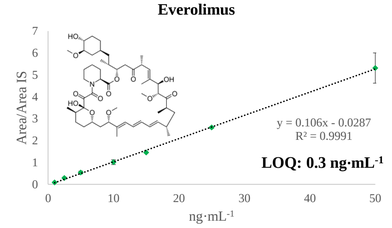
Tacrolimus



Cyclosporine A



Everolimus



Conclusions

- ✓ The new microfluidic open port herein presented showed to be a highly sensitive interface for direct coupling of SPME to MS via ESI ionization.
- ✓ Doxorubicin was successfully quantitated from real perfusate samples from different surgical procedures obtaining a LOQ of 1 ppm.
- ✓ A fast and sensitive method for the determination of immunosuppressive drugs from 100 µL of whole blood was developed. Turn-around times of individual samples is less than 90 min.
- ✓ Testosterone was directly quantitated from human plasma obtaining 10 ppb as LOQ. In order to improve this performance, an on-fiber derivatization procedure is presented showing off-line preliminary results with an LOQ close to 1 ppb.

References

1- Gomez-ríos, G. A.; Liu, C.; Tascon, M.; Reyes-Garces, N.; Arnold, D. W.; Covey, T. R.; Pawliszyn, J. Anal. Chem. 2017, 89 (7), 3805–3809.
2- Star-Weinstock, M.; Williamson, B. L.; Dey, S.; Pillai, S.; Purkayastha, S. Anal. Chem. 2012, 84 (21), 9310–9317.

Acknowledgments

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