Novel method for determining eicosanoids in the interstitial fluid of psoriatic skin

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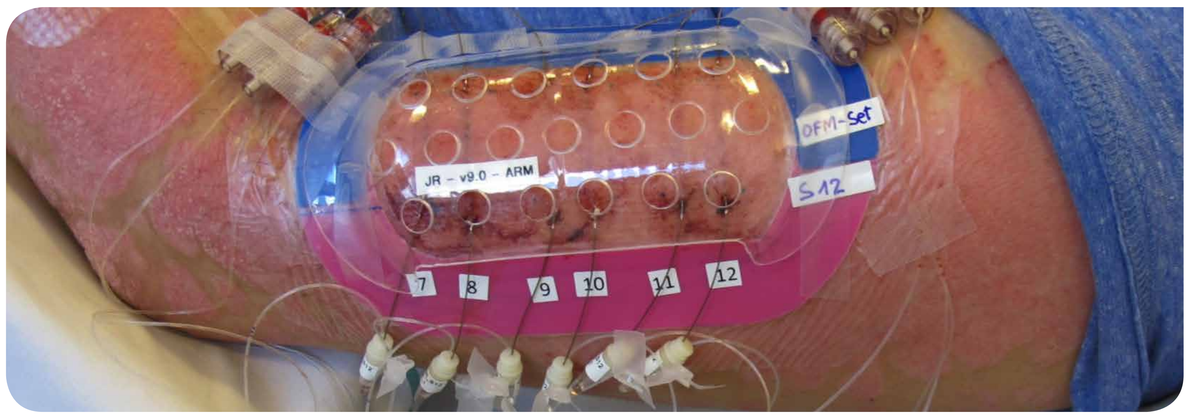
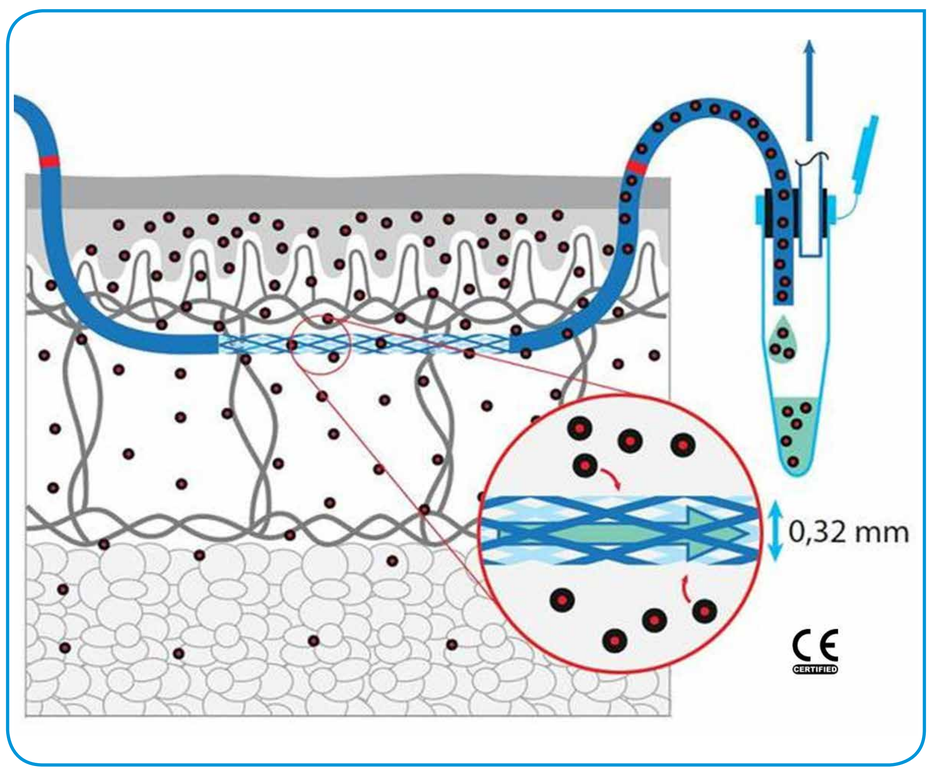


Aim

We developed a multi-analyte-method to measure eicosanoid levels in diluted interstitial fluid by combining high performance liquid chromatography with high resolution mass spectrometry.
This method was tested by comparing eicosanoid levels in dermal interstitial fluid of psoriatic and healthy skin.

Background



Psoriasis

- Complex skin disease with yet unknown etiology. [a]
- 120 – 180 million people affected worldwide. [b]
- Symptoms include dermal inflammation, hyperproliferation of keratinocytes and also altered eicosanoid metabolism.

Dermal open flow microperfusion (dOFM)

- Minimally invasive sampling technique using a flexible membrane-free probe.
- In-vivo sampling of interstitial fluid in the dermis.
- Continuously perfused probe at controlled flow rates in a µl/min range. [c]

Analytical challenges in OFM – analysis

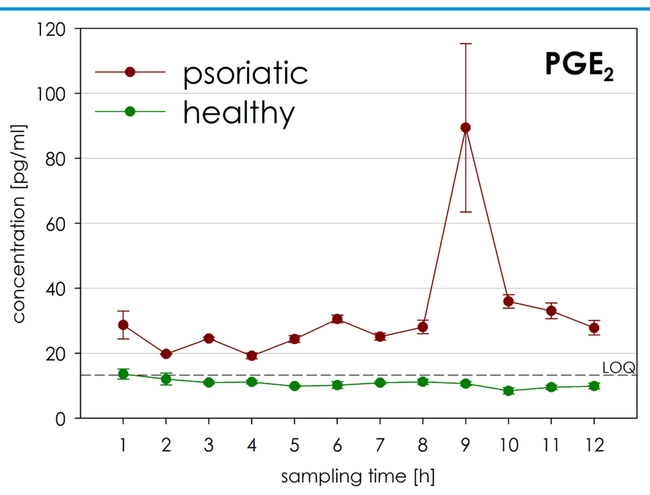
- Small sample volume (60 µl)
- High sample throughput
- High sensitivity & selectivity

Results

Small sample volumes/high sample throughput:
The use of Waters µElution MAX solid phase extraction (SPE) cartridges in 96 well plate format allowed convenient and efficient handling of small sample volumes.

High sensitivity & selectivity:
Detection with the HR Thermo Q Exactive™ MS using targeted MS^2 enabled highly accurate detection of fragment ions with low/no background signal.

Time-concentration profiles in psoriatic and healthy skin:
6-keto-$PGF_{1\alpha}$, TXB_2, $PGF_{2\alpha}$, PGE_2, PGD_2, LTB_4, 12-HEPE, 15-HETE, 12-HETE, 5-HETE were analyzed. As an example we display time-resolved levels of a typical inflammation marker PGE_2, which continuously showed higher concentrations in psoriatic dOFM interstitial fluid compared to healthy samples.



Conclusion

The combination of dOFM sampling with our analytical method, including SPE extraction and high resolution HPLC–MS/MS analysis, renders concentration profiles of bioactive substances such as eicosanoids. In the future, this combined method can serve as an important tool to gain information on the release of mediators with time-resolution for in-vivo pharmacodynamic/pharmacokinetic studies.

Experimental

Sample preparation: Anionic exchange SPE with Waters µElution MAX (2 mg sorbent); SPE protocol for 40 µl applied sample volume: wash 1: NH_4OH (5%); wash 2: MeOH; elution: 2% FA in ACN:MeOH (60:40)

HPLC: Column: Waters Atlantis® T3 3 µm 2.1x150 mm; gradient elution at 25°C with (A) ACN:H_2O:FA (63:37:0.02) and (B) ACN:IPA (50:50); flow rate 300 µl/min; injection volume 10 µl

MS/MS: Detection was performed on an ESI-HR Thermo Q Exactive™ MS operated in negative mode and targeted MS^2 with inclusion list. Analytes were quantified via deuterated internal standards. Linearity is given in analyte depending concentration ranges from 0.01 – 100 ng/ml.

References

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