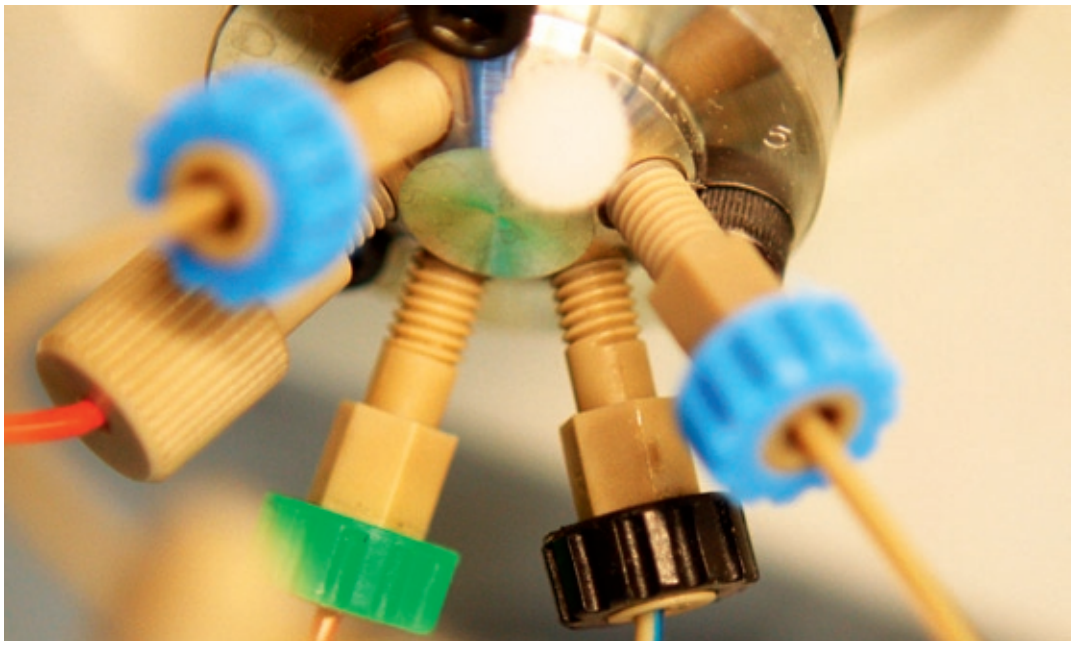
QUANTITATIVE LC-MS/MS ANALYSIS OF COMPOUNDS TO SUPPORT IN VITRO DERMAL ABSORPTION TESTING



TNO triskelion bv

For the investigation of the in vitro dermal absorption of a compound with intended topical use, radio-labeling of the compound is preferred for quantitative analysis. However, in the early discovery phase radio-labeled compounds may not always be available; LC-MS/MS analysis can be used to quantify the test substance in the dermal fractions.

IN VITRO DERMAL ABSORPTION INVESTIGATION

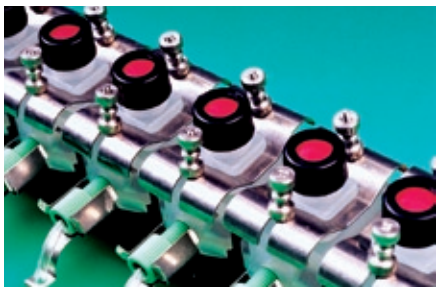
At TNO, in vitro dermal absorption studies can be performed in static Franz diffusion cells or flow-through diffusion cells. Human skin originating from the abdominal or breast region is obtained directly after cosmetic surgery from a local hospital. The test substance is applied in a relevant formulation on top of the skin and receptor fluid is collected at regular intervals during the experiment.

In addition to the amount of test ubstance in the receptor fluid, the residues remaining on and in the skin membranes (i.e. stratum corneum, epidermis and dermis) can be determined. The test substance is analyzed in all relevant dermal fractions: epidermis, dermis, tape strips (stratum corneum), receptor fluid and skin wash solution. Prior to LC-MS/MS analysis the test substance is extracted from the various skin fractions.

Due to the different nature of the skin fractions an adequate sample preparation procedure is developed and applied for each fraction.

Preferably, a structural analogue of the test substance is used as the internal standard. LC-MS/MS experiments are performed using a triple quadrupole mass spectrometer.

Franz cell



Flow-through diffusion cell

QUANTITATIVE LC-MS/MS ANALYSIS OF TEST SUBSTANCE IN DERMAL FRACTIONS

A short pre-validation of the method consists of calibration samples, quality control samples and evaluation of the blank skin fractions. A calibration curve is constructed by the analysis of the test substance in a standard solution (5-6 concentration levels, e.g. 1-500 ng/mL). Quality control (accuracy and precision) is performed by the extraction and analysis of all blank skin fractions spiked with the test substance at two levels (low and high). Blank fractions are extracted and analyzed to investigate the selectivity of the method.

After successful pre-validation, the method is applied to the skin fractions obtained in the in vitro dermal absorption study.

- OECD guideline for the testing of chemicals: Guideline no. 428; Skin Absorption: in vitro method (Paris, April 2004)
- OECD Environmental Health and Safety Publications, Series on Testing and Assessment no. 28. Guidance document for the conduct of skin absorption studies (Paris, March 2004)

WWW.TRISKELION.NL

TNO TRISKELION BV

TNO Triskelion BV is a wholly-owned subsidiary of TNO: Europe's largest independent research institute for technological and strategic research and consultancy. By translating scientific knowledge into practice we optimise the innovative abilities of the industry and government.

VISITING ADDRESS
Utrechtseweg 48
3704 HE Zeist
The Netherlands

POSTAL ADDRESS
P.O. Box 844
3700 AV Zeist
The Netherlands

For more information, please contact us.

THE NETHERLANDS
P +31 88 866 16 20
F +31 88 866 69 70
pharma@tno.triskelion.nl

JAPAN
P +81 3 6234 4195

PHARMA17Q/01-2011