

IN VITRO ASSAYS

In vitro skin permeation assay

In vitro skin permeation assays are performed to assess the transport of a test compound through human skin after topical application of a cosmetic or pharmaceutical formulation. These studies are performed on fragments of human skin using a Vertical Diffusion System (Franz cell). The bioavailability, permeation profile and full mass balance of the test compound through human skin are determined. This test can be carried out with different pharmaceutical presentations including liquid and semi-solid. The assay is conducted according to OECD 428^{1,2}.

Test system: Human skin

Positive control: Benzoic acid.

Skin source: Human abdominoplasty surgery waste.

Skin integrity marker: Phenol red.

Experimental number: Six per group in triplicate.

Diffusion Cell System: Standard open cap ground glass surface.

Receptor Medium: Appropriate receptor medium according to drug solubility and skin compatibility.

Analysis Method: HPLC-DAD

Validation Data

Table 1. Analytical method validation for benzoic acid analysis. The method was developed in an HPLC-DAD system consisting of 2 LC-20AD pumps, CBM-20A controller, SPD-M20A detector and Shimadzu SIL-20A injector. Data acquisition and processing were performed with LC-MS Solution software, version 3.70.390.

| Validated parameters | Benzoic acid |
|--|--------------|
| Limit of detection (ug/mL) | 0,20 |
| Limit of quantitation (ug/mL) | 2,00 |
| Precision | CV% |
| Intraday - t _R - CQB (6,0 ug/mL) | 0,11% |
| Intraday - t _R - CQM (26,0 ug/mL) | 0,24% |
| Intraday - t _R - CQA (42,5 ug/mL) | 0,28% |
| Intraday - area ratio - CQB (6,0 ug/mL) | 0,64% |
| Intraday - area ratio - CQM (26,0 ug/mL) | 0,70% |
| Intraday - area ratio - CQA (42,5 ug/mL) | 1,21% |
| Interday - t _R - CQB (6,0 ug/mL) | 0,64% |
| Interday - t _R - CQM (26,0 ug/mL) | 0,56% |
| Interday - t _R - CQA (42,5 ug/mL) | 0,44% |
| Interday - area ratio - CQB (6,0 ug/mL) | 2,03% |
| Interday - area ratio - CQM (26,0 ug/mL) | 0,73% |
| Interday - area ratio - CQA (42,5 ug/mL) | 0,79% |
| Interday accuracy | %bias |
| CQB (6,0 ug/mL) | 95,08% |
| CQM (26,0 ug/mL) | 105,02% |
| CQA (42,5 ug/mL) | 100,63% |

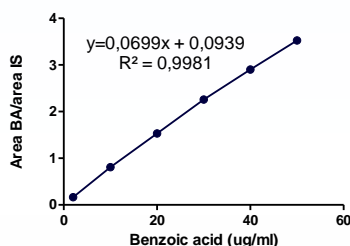


Figure 1. Mean calibration curve for benzoic acid (mean of three different days of analysis). BA – benzoic acid; IS – internal standard.

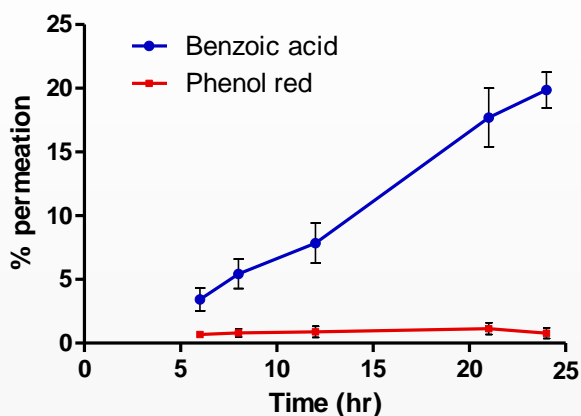


Figure 2: Permeation profile of benzoic acid and phenol red through human skin over 24 hours. Data illustrate the mean ± SEM of 3 independent experiments per group in replicate (n=6).

All experiments are performed in six Franz's cells for each condition and repeated three times. The supply and use of human tissue are fully in line with national ethical guidelines³.

References:

1. OECD Guideline for the Testing of Chemicals 428: Skin Absorption: *In Vitro* Method; April 2004.
2. Hanson, manufacturer's manual. Available in <http://hansonresearch.com/media/99310044VerticalDiffusionCell.pdf>.
3. Ethics Committee on Human Research (CEPSH-UFSC: CAAE 54791316.5.0000.0121/Number 1.691.935