In vivo quantification of triamcinolone acetonide in stratum corneum

C. Pellanda[1,2], V. Figueiredo[1], T. Tassopoulos[1,2], C. Purdon[3], G. Imanidis[2], E.W. Smith[3], C. Surber[1,2]

[1]*Institut für Spital-Pharmazie, Universitätskliniken, Kantonsspital Basel, Basel, Switzerland*
[2]*Institute of Pharmaceutical Technology, University of Basel, Basel, Switzerland*
[3]*College of Pharmacy, University of South Carolina, Columbia, SC, USA*

Introduction

- Tape stripping of the stratum corneum is a non-invasive technique that has been used for bioavailability and dermatopharmacokinetic studies of topically applied drugs (Weigmann et al., 1999[1]).
- Tape stripping is adequate to investigate the phenomenon of drug accumulation in stratum corneum which has been investigated especially in the context of corticosteroid-therapy (Vickers, 1963[2]).
- Triamcinolone acetonide (TACA) is a medium potency topical glucocorticoid often used in dermatology.
- In the present study a set of methods for the *in vivo* quantification of TACA in stratum corneum was used:
  - » **tape-stripping** as a sampling method (standardized technique)
  - » **UV/VIS spectrometry** for the quantification of corneocytes on the tapes (validation by Weigmann et al., 2003[3]).
  - » **HPLC** for the quantification of TACA on the tapes (validation according to ICH guidelines[4,5]).
- The methods were applied to investigate the penetration profile of TACA in the stratum corneum 30 minutes, 3 hours and 24 hours after formulation application.

Methods

1 Sampling of stratum corneum: Tape stripping

- TACA in acetone was applied on the forearm of 3 volunteers on an area of 10.5 cm^2 (100 µg/cm^2).
- Stratum corneum samples were taken 30 minutes, 3 and 24 hours after application by standardized tape stripping procedure.
- A standardized technique is required because the amount of stratum corneum removed is influenced by different factors (as type of tape, applied pressure, vehicle, skin site).
- Stratum corneum was completely removed by tape stripping, which is a prerequisite to determine the thickness of stratum corneum of the individual volunteers.

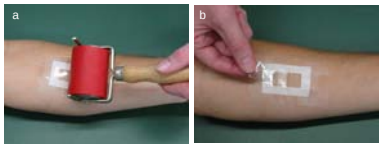

Fig. 1 After application of the formulation, the tape stripping area (5.6 cm^2) is defined by a template. Tapes are uniformly pressed on the skin by a roller (pressure 140 g/cm^2) **(a)** and removed from the skin **(b)**.

2 Corneocytes: UV/VIS-Spectrometry

- The determination of stratum corneum amount adhering to each tape is required for the calculation of the penetration profile.
- The pseudo-absorption of the corneocytes at 430 nm is used to quantify the stratum corneum amount on the tapes.
- The removed tapes with adhering skin were fixed across a slide frame and absorbance at 430 nm was measured.

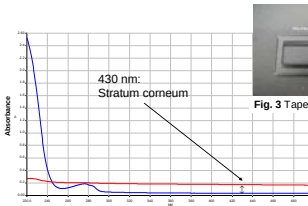

Fig. 2 UV/VIS spectrum of a blank tape (Tesa Multi-Film kristall-klar 19 mm) (blue line) and of a stripped tape (red line). The spectrophotometer was modified to produce a rectangular beam of 1 cm^2.

Fig. 3 Tapes fixed on a slide frame.

3 Triamcinolone acetonide: HPLC

- TACA was quantified by HPLC after individual extraction of tapes with 1.5 mL Methanol 60%.
- Chromatographic conditions
  - » Column: Symmetry Shield™ RP 18 (2.1 x 100mm, 3.5 µm particle size)
  - » Eluent: Methanol 60%
  - » Column temperature: 35°C
  - » Flow Rate: 0.3 mL/min
  - » Injection Volume: 20 µL
  - » Quantification at 240 nm.
- The method was validated according to ICH guidelines.

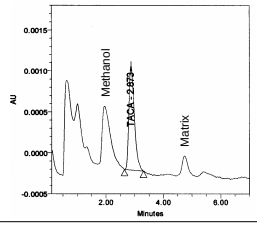

Fig. 4 Chromatogram of a tape with skin, spiked with TACA solution (0.75 µg TACA /tape).

Results

Validation

Regression analysis and test result evaluation were made using appropriate statistical methods (MVA® software, Method Validation in Analytics, and Statgraphics® PLUS 5 software).

Tab. 1 Overview of the validated parameter and of the results of validation (HPLC-method).

✓	Specificity	t_R (TACA) 2.9 min, no matrix interference
✓	LOD	0.035 µg/mL = 9.4 ng/cm^2
✓	LOQ	0.100 µg/mL = 26.8 ng/cm^2
✓	Linearity	y = 35660 x - 573 R = 1.0000 ± 0.00001 linear weighted (1/x) Variances of residues: 4 % RSD of slope: 1.5 %
✓	Range	0.1-20 µg/mL
✓	Accuracy by Recovery	0.5 µg/mL: 86.6% ± 4.4% 5.0 µg/mL: 93.8% ± 1.8% 15.0 µg/mL: 96.1% ± 1.3%
✓	Repeatability	1.96 %
✓	Intermediate Precision	1.96 %
✓	Robustness	0.5 µg/mL: 81.9% ± 9.7% 5.0 µg/mL: 93.0% ± 2.2% 15.0 µg/mL: 96.0% ± 1.2%

- No interference from solvent, skin or tape matrix was recorded at the retention time t_R of TACA, proving the **specificity** of the method.
- Limit of detection **LOD** and limit of quantification **LOQ** were defined as a signal-to-noise ratio of 3:1 and 10:1 respectively.
- For **linearity** assessment of the working **range**, the linear unweighted, the linear weighted (1/x, x = concentration) and the nonlinear regression model were compared. The best fit was achieved with the linear weighted model, which demonstrated the smallest variance of the residues (4% vs. 25% variance at the linear unweighted and 18% at the nonlinear model).
- The **accuracy** was calculated by the recovery of spiked tapes at 3 concentrations. The experimental recovery (87-96%) was within the specified range of 80-120%.
- The **repeatability** was established with the standard deviation (SD) of the peak areas of TACA calculated from 5 measurements at each point of the calibration curve. The **intermediate precision** was established with the SD performed at 3 different days. Both RSD were below 2%.
- The method **robustness** was evaluated by recovery assessment at 8 different conditions (variation ± 5% methanol in the mobile phase, ± 5% flow rate, ± 5% column temperature, ± 2 nm detection wavelength). Data in Tab 1 show an average of the recovery at the 8 different conditions.The robustness of the method was good at mid and high concentration ranges and sufficient at lower range.

Penetration profiles

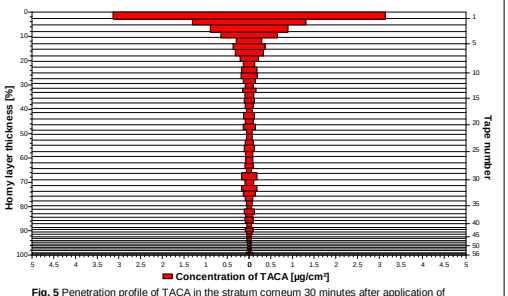

Fig. 5 Penetration profile of TACA in the stratum corneum 30 minutes after application of 100 µg/cm^2 TACA in acetone.

- TACA was detected in all strips including the last tape (lower stratum corneum), and the accumulation in the upper and middle stratum corneum profiles was considerable after 30 minutes and 3 hours.
- After 24 h the amount recovered was minimal and near the LOQ in the deeper layer of stratum corneum.

Conclusions

- We developed and validated a HPLC-method which in combination with tape stripping and UV/VIS-spectrometry allows the *in vivo* quantification of triamcinolone acetonide (TACA) in stratum corneum.
- The validated method proved to be suitable for the investigation of the accumulation of TACA in stratum corneum.
- TACA showed deep penetration into the stratum corneum, and accumulation in the upper and middle stratum corneum was considerable.

References

1 Weigmann H, Lademann J, Meffert H, Schaefer H and Sterry W. Determination of the horny layer profile by tape stripping in combination with optical spectroscopy in the visible range as a prerequisite to quantify percutaneous absorption. *Skin Pharmacology and Applied Skin Physiology* **12**(1-2)**:** 34-45, 1999.

2 Vickers CF. Existence of reservoir in the stratum corneum. Experimental proof. *Archives of dermatology* **88:** 20-23, 1963.

3 Weigmann H, Lindemann U, Antoniou C, Tsikrikas GN, Stratigos AI, Katsambas A, Sterry W and Lademann J. UV/VIS absorbance allows rapid, accurate, and reproducible mass determination of corneocytes removed by tape stripping. *Skin Pharmacology and Applied Skin Physiology* **16**(4)**:** 217-227, 2003.

4 ICH: Q2A Validation of analytical procedures: Text on validation of analytical procedures. U.S. Department of Health and Human Services, Food and Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER); 1994.

5 ICH: Q2B Validation of analytical procedures: Methodology. U.S. Department of Health and Human Services, Food and Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER); 1996.