Methods to Simulate Rubbing of Topical Formulations for In Vitro Skin Permeation Studies
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**Purpose**
Acne vulgaris, a common chronic dermatological condition, affects people of almost every age group. Salicylic acid, a keratolytic agent, is commonly used for the treatment of acne. Rubbing a topical formulation on skin is generally assumed to enhance drug penetration. The aim of our study was to demonstrate different techniques for rubbing a 2% salicylic acid gel on skin and investigate their effect on in vitro permeation of salicylic acid.

**Methods**
The gel was applied on dermatomed porcine ear skin and rubbed for 15 s with a relatively constant pressure of 50 g by different modes such as vertical glass rod, horizontal glass rod, rheometer, and with a gloved finger. The study included evaluation of gel distribution on skin surface after rubbing, skin integrity: pre- and post-rubbing, in vitro drug permeation, drug distribution in skin, skin extraction recovery, and mass balance studies. Normalization study was performed to equalize the amount of gel remaining on skin after rubbing in each test group. Effect of rubbing on skin integrity was investigated using trans-epidermal water loss, skin resistance measurement, and histological evaluation.

**Results**
As per the results of the normalization study, different volumes of gel were applied on skin: 3.1 μL, 4.3 μL, 5.8 μL, 6.0 μL and 9.0 μL for control, vertical glass rod, horizontal glass rod, rheometer and finger test groups, respectively. This ensured insignificantly different amount of drug remaining on the skin surface after rubbing (p>0.05). Results of the gel distribution study revealed that rubbing with a gloved finger resulted in a uniform gel layer with a thickness of 49.61 ± 15.33 μm on skin surface (n=10) (Figure 1). No significant difference between the different test groups was observed in terms of the cumulative amount of drug that permeated in 24 h (n=4, p=0.56). However, rubbing with vertical glass rod resulted in rapid onset of drug permeation at 4h (24.19 ± 3.08 μg/sq.cm) than control (13.95 ± 2.65 μg/sq.cm, n=4, p=0.00) and horizontal glass rod group (14.00 ± 4.50 μg/sq.cm, n=4, p=0.01) (Figure 2). Drug levels in stratum corneum, epidermis, and dermis were also analyzed. Rubbing with finger delivered significantly higher amount of drug into the skin layers as compared to other test groups (p<0.05). Amount of drug extracted from skin was reliably correlated to the actual drug levels in skin (R2=0.991). Considering drug amounts in different compartments such as unabsorbed formulation (cotton swabs), stratum corneum (tape strips), viable epidermis, dermis (skin extraction) and blood circulation (receptor analysis), mass balance ranged from 75.86 ± 2.90 % (vertical glass rod), 76.72 ± 3.73 % (horizontal glass rod), 78.07 ± 5.22 % (control), 78.11 ± 5.24 % (finger) to 80.44 ± 2.99 % (rheometer).

**Conclusion**
Different rubbing methods were demonstrated to simulate rubbing of topical formulation on skin. Also, their effects on skin integrity and in vitro skin permeation of salicylic acid were investigated.