# COMPARATIVE IN VITRO DERMAL ABSORPTION STUDY WITH BENZOIC ACID, TESTOSTERONE AND CAFFEINE USING HUMAN AND RAT SPLIT-THICKNESS SKIN IN A FLOW THROUGH DIFFUSION SYSTEM

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## ABSTRACT

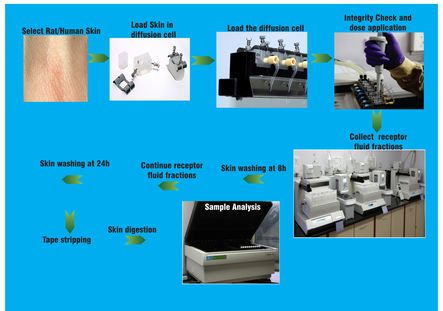
*In vitro* dermal absorption studies offer a valid alternative for *in vivo* studies and are conducted with skin from different species such as human, rat and pig. As these studies are performed on very small pieces of skin in isolation in a sophisticated instrument, mainly flow-through diffusion cells, they require a specific set of technical skills. The authors have optimized the experimental conditions for such studies and the present study was conducted to evaluate and validate comparative *in vitro* dermal absorption of $^{14}C$ labelled benzoic acid, testosterone and caffeine through human and rat skin. These reference compounds cover different physico-chemical properties of Log PoW and molecular weight that can influence absorption. Each test group included eight replicates from four donors (i.e., 2 replicates/donor). Split-thickness skin membranes (300-400 µm) were placed in flow-through diffusion cells with 0.64 $cm^2$ exposure areas. After checking skin integrity, membranes were exposed to reference compounds (4 mg/mL) in independent experiments. The exposure time was 8h with post-exposure sampling for 16h and total study duration of 24h. Mass balance analysis was conducted from samples of receptor fluid, donor and receptor chamber washes, the residues remaining in/on the skin and in the *stratum corneum* by measuring radioactivity using liquid scintillation counting. Residue in different layers of *stratum corneum* was also determined by performing tape stripping. The mean total recovery of benzoic acid was about 93 and 102% in human and rat skin, respectively. The mean total recovery of testosterone was 95% and 97% in human and rat skin, respectively. The mean total recovery of caffeine was 97% and 98% in human and rat skin, respectively. The results of this study indicate that the reference compounds showed different absorption profiles through human and rat skin, which validated our study.

## OBJECTIVE

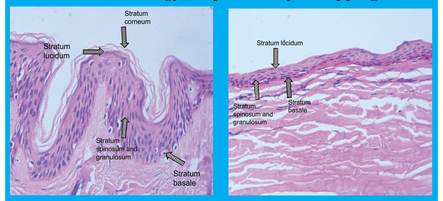
The study was designed for comparative evaluation of dermal absorption of benzoic acid, caffeine and testosterone through human and rat split-thickness skin in an *in vitro* study.

## EXPERIMENTAL PROCEDURE

8 replicates from 4 Rats per group (Wister, JRF Breeding colony) and 8 replicates from 4 Human donors per group (Surgical waste).




### Human Skin Histology: Impact of Tape Stripping




Normal Skin of Human (Without Tape stripping) | Human skin after 15 - Tape stripping

## RESULTS

### Mass Balance – Benzoic Acid

| Parameters | Group - I (Human Skin) | | Group - II (Rat Skin) | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| Receptor Fluid (0-24h) | 55.47 | 12.51 | 42.26 | 6.73 |
| Receptor Compartment Wash | 0.26 | 0.17 | 0.29 | 0.08 |
| Dermis | 1.19 | 0.37 | 19.47* | 4.47 |
| Absorbed dose-I | 56.92 | 12.41 | 62.03 | 3.88 |
| Tape Strips (1-2) | 0.11 | 0.11 | 1.16 | 0.67 |
| Tape Strips (3 to 15) | 0.74 | 0.43 | 2.97 | 1.53 |
| Stratum Corneum | 0.96 | 0.55 | 4.14 | 2.02 |
| Epidermis (without Stratum corneum) | 0.80 | 0.62 | NA | NA |
| Absorbed dose-II | 57.71 | 12.13 | 62.03 | 3.88 |
| Absorbed dose-III | 58.46 | 11.81 | 65.00 | 4.14 |
| Skin Wash at 8h | 30.64 | 4.57 | 29.06 | 2.34 |
| Skin Wash at 24h | 1.93 | 0.89 | 6.47 | 1.32 |
| Donor Compartment Wash | 2.10 | 2.65 | 0.29 | 0.13 |
| Unabsorbed dose | 34.89 | 6.37 | 36.99 | 3.39 |
| Total Recovery | 93.35 | 6.32 | 101.99 | 1.37 |

* = Digested Skin (Dermis+Epidermis without SC)

### Mass Balance – Caffeine

| Parameters | Group - I (Human Skin) | | Group - II (Rat Skin) | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| Receptor Fluid (0-24h) | 4.78 | 1.26 | 19.96 | 3.71 |
| Receptor Compartment Wash | 0.04 | 0.01 | 0.17 | 0.04 |
| Dermis | 0.62 | 0.31 | 15.04* | 4.45 |
| Absorbed dose-I | 5.08 | 1.30 | 32.56 | 10.20 |
| Tape Strips (1-2) | 0.06 | 0.04 | 0.35 | 0.22 |
| Tape Strips (3 to 15) | 0.40 | 0.17 | 4.33 | 3.15 |
| Stratum Corneum | 0.51 | 0.24 | 5.03 | 3.39 |
| Epidermis (without Stratum corneum) | 0.36 | 0.23 | NA | NA |
| Absorbed dose-II | 5.44 | 1.40 | 32.56 | 10.20 |
| Absorbed dose-III | 5.84 | 1.53 | 36.89 | 8.61 |
| Skin Wash at 8h | 86.50 | 4.25 | 52.24 | 5.05 |
| Skin Wash at 24h | 2.40 | 0.64 | 5.52 | 0.65 |
| Donor Compartment Wash | 2.62 | 4.77 | 0.08 | 0.09 |
| Unabsorbed dose | 91.64 | 2.56 | 58.54 | 5.10 |
| Total Recovery | 97.47 | 1.91 | 98.05 | 3.82 |

### Mass Balance – Testosterone

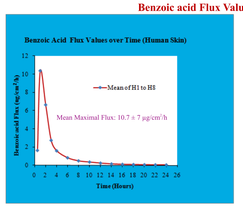
| Parameters | Group - I (Human Skin) | | Group - II (Rat Skin) | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| Receptor Fluid (0-24h) | 3.81 | 1.23 | 13.10 | 2.62 |
| Receptor Compartment Wash | 0.18 | 0.10 | 0.32 | 0.11 |
| Dermis | 0.83 | 0.75 | 11.22* | 2.15* |
| Absorbed dose-I | 4.82 | 1.82 | 24.64 | 3.78 |
| Tape Strips (1-2) | 0.88 | 0.53 | 0.38 | 0.22 |
| Tape Strips (3 to 15) | 1.72 | 1.17 | 3.37 | 2.07 |
| Stratum Corneum | 3.48 | 1.85 | 4.14 | 2.18 |
| Epidermis (without Stratum corneum) | 1.45 | 0.92 | NA | NA |
| Absorbed dose-II | 6.27 | 2.04 | 24.64 | 3.78 |
| Absorbed dose-III | 7.99 | 2.09 | 28.01 | 4.30 |
| Skin Wash at 8h | 80.93 | 3.83 | 60.82 | 5.23 |
| Skin Wash at 24h | 3.89 | 1.26 | 7.11 | 1.71 |
| Donor Compartment Wash | 0.88 | 0.44 | 0.13 | 0.04 |
| Unabsorbed dose | 87.46 | 3.02 | 68.83 | 4.55 |
| Total Recovery | 95.45 | 2.00 | 96.84 | 0.88 |

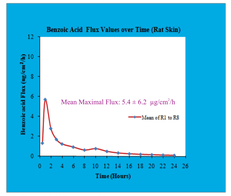
### Ratio of Dermal Absorption of Benzoic Acid, Caffeine and Testosterone between Rat and Human Skin

| Species | Parameters | Benzoic Acid | Caffeine | Testosterone |
|---|---|---|---|---|
| | | Mean±SD | Mean±SD | Mean±SD |
| Human | Absorbed dose I[1] (%) | 56.92±12.41 | 5.08±1.30 | 4.82±1.82 |
| | Absorbed dose II[2] (%) | 56.71±12.13 | 5.44±1.4 | 6.27±2.04 |
| | Absorbed dose III[3] (%) | 58.46±11.81 | 5.84±1.53 | 7.99±2.09 |
| | Maximal flux [µg/cm2/h] | 10.74±7 | 0.18±0.09 | 0.2±0.12 |
| Rat | Absorbed dose I[1] (%) | 62.03±3.88 | 32.56±10.20 | 24.64±3.78 |
| | Absorbed dose II[2] (%) | 62.03±3.88 | 32.56±10.20 | 24.64±3.78 |
| | Absorbed dose III[3] (%) | 65±4.14 | 36.89±8.61 | 28.01±4.30 |
| | Maximal flux [µg/cm2/h] | 5.4±6.23 | 0.83±0.47 | 0.63±0.24 |
| Rat/ Human Ratio | Absorbed dose I[1] (%) | 1.09 | 6.41 | 5.11 |
| | Absorbed dose II[2] (%) | 1.07 | 5.99 | 3.93 |
| | Absorbed dose III[3] (%) | 1.11 | 6.32 | 3.51 |
| | Maximal flux [µg/cm2/h] | 0.50 | 4.61 | 3.15 |

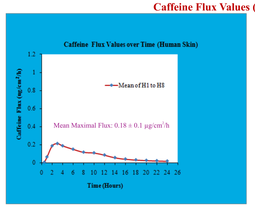
[1]**Absorbed dose I** is calculated from the amount recovered in receptor fluid, the receptor compartment wash, and the vascular dermis.
[2]**Absorbed dose II** is calculated from the absorbed dose I, plus the non-vascular epidermis (without stratum corneum). The absorbed dose II can be considered conservative.
[3]**Absorbed dose III** is calculated from the absorbed dose II plus the dead stratum corneum (tape strips 3 to last). The absorbed dose III can be considered highly conservative.
[1]**Unabsorbed dose** is calculated from amount recovered from skin washings at 8h and 24h, donor compartment wash and first two tape strips

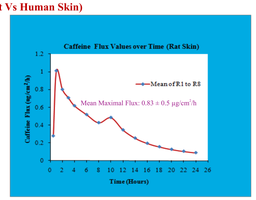
### Benzoic acid Flux Values (Rat Vs Human Skin)



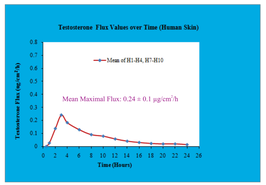



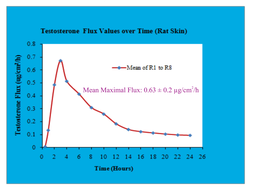

### Caffeine Flux Values (Rat Vs Human Skin)



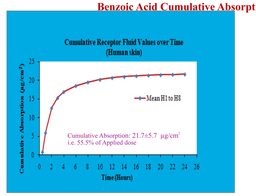



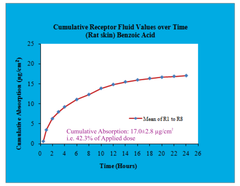

### Testosterone Flux Values (Rat Vs Human Skin)



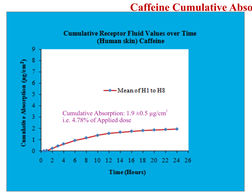



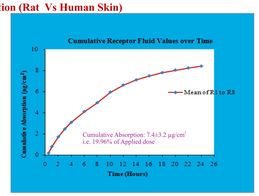

### Benzoic Acid Cumulative Absorption (Rat Vs Human Skin)



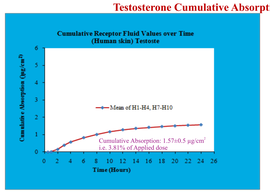



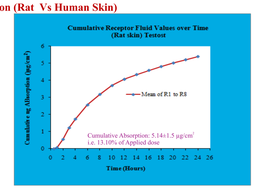

### Caffeine Cumulative Absorption (Rat Vs Human Skin)




### Testosterone Cumulative Absorption (Rat Vs Human Skin)




## CONCLUSION

The results of this study indicate that the reference compound has shown different absorption profile through human and rat skins which validated our study. With this GLP compliant validation study JRF has developed capabilities to conduct technically challenging and complicated in vitro dermal absorption studies as per OECD and EFSA criteria's.

## REFERENCES

EFSA Panel on Plant Protection Products and their Residues (PPR), 2012; Guidance on Dermal Absorption. EFSA Journal 2012;10(4):2665, pp. 1-30.

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