Phospholipid Research Center

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# The effect of saturated phospholipids on human skin assessed with shotgun lipidomic analysis

**Dorothea Gutekunst [1*], Christian Klose [2], Christoph Heidecke [3], Peter van Hoogevest [1]**
[1] Phospholipid Research Center, Germany; *dgutekunst@phospholipid-institute.com; [2] Lipotype GmbH, Germany; [3] Lipoid GmbH, Germany

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## Introduction

**Phospholipids (PLs), as natural and well-tolerated excipients, are widely used in dermal drug delivery systems.** Unsaturated PLs have a transition temperature ($T_m$) below 0 °C, whereas the $T_m$ of saturated PLs is much higher. When applying PLs topically, the temperature of skin dictates the physical state and influences the penetration as well as delivery properties of phospholipid-based formulations. Saturated PLs (e. g. PHOSPHOLIPON® 90 H ) are in a gel-like state on the skin, caused by their $T_m$ around 50 °C [1].

Regarding their rigid structure it can be assumed, that saturated PLs accumulate in upper epidermal layers. Until now, this behaviour is not well investigated for semi-solid phospholipid-based formulations, like oil-in-water (o/w) emulsions, and especially not with respect to structural properties of PLs. One reason might be the lack of suitable analytical methods to identify and quantify the exogenous PLs besides endogenous skin PLs and to follow the metabolism of the PLs in the skin.

## Aim of the Study

The purpose of the study was to investigate the penetration properties of a hydrogenated phospholipid-based dermal formulation using the Lipotype shotgun lipidomics technology. A special focus was placed on the detection of characteristic PLs of the applied formulation, especially phosphatidylcholine (PC), and the distinction from endogenous lipids in human skin.

## Materials & Methods

### TEST FORMULATION

**o/w cream with 6 % PHOSPHOLIPON® 90 H**

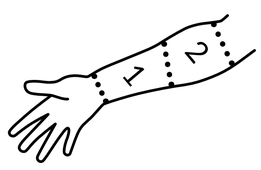
PHOSPHOLIPON® 90 H composition:

| | |
|---|---|
| Phosphatidylcholine (hydrogenated) | ≥ 90 % |
| ∑ (C16:0, C18:0) | ≥ 98 % |
| ∑ (C18:1, C18:2, C18:3) | ≤ 2 % |

### IN-VIVO PILOTSTUDY

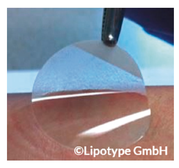
This study was performed in accordance with the Ethical Consideration for cosmetic products. The test formulation was manufactured in compliance with cosmetic Good Manufacturing Practice and can therefore be considered as safe for human testing.

**Performance:**




**Healthy,** female volunteers (18 – 45 years of age)

**Application** 2x/d 2 weeks



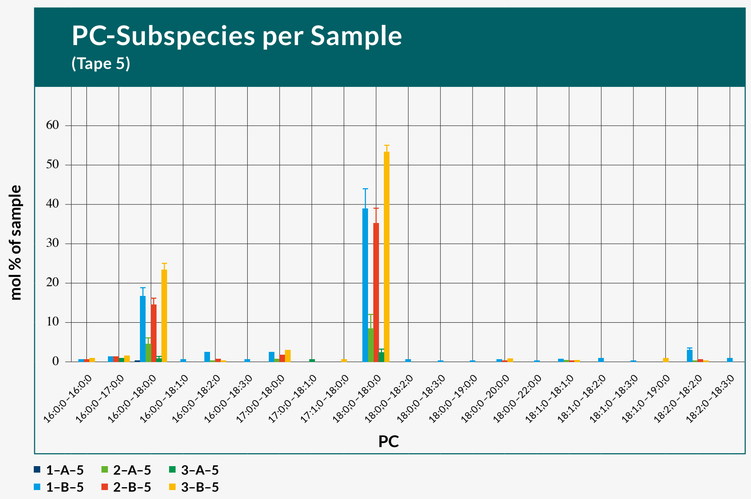

**Tape stripping** Collection of ten D101-D-Squame Standard Sampling Discs

Tapes five and ten were analysed with Lipotype shotgun lipidomics technology, according to the following workflow [2]:

Sample preparation → Sample infusion → MS analysis → Lipid identification (lipotype XPLORER) → Data analysis (lipotype ZOOM)

## Results

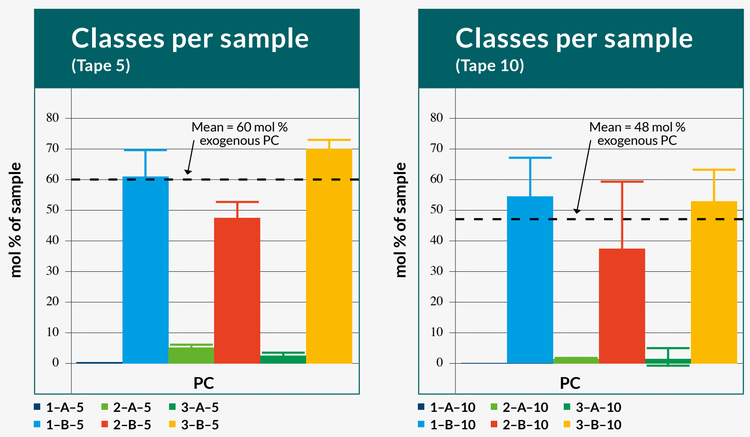
### RECOVERY OF APPLIED SATURATED PHOSPHOLIPIDS




**Legend: 1–A–5**: (**1** = Volunteer No., **A** = Untreated Skin / **B** = Treated Skin, **5** = Tape No.)

The left graph presents an overview of PC-subspecies characterised by different fatty acid composition. After treatment with PHOSPHOLIPON® 90 H-based formulation, the volunteers (1–3) presented a similar profile of saturated fatty acid species (B). The test formulation induced an increased concentration of characteristic subspecies such as 16:0;0 – 18:0;0 and 18:0;0 – 18:0;0. An unequivocal discrimination between exogenous and endogenous PC was possible because of the small amount of stearic and palmitic acid in skin-derived PC species (A).

### PENETRATION PROPERTIES OF PHOSPHOLIPON® 90 H




**Legend: 1–A–5**: (**1** = Volunteer No., **A** = Untreated Skin / **B** = Treated Skin, **5** = Tape No.)

The two left graphs represent the recovery of applied hydrogenated PC in human skin, expressed as molar fraction of total lipids (in mol %). In consideration of the natural variability of the skin lipid composition, the results show a good reproducibility. On average, 60 mol % of exogenous PC was detected in the fifth skin strip. The detected concentration of exogenous PC decreased with increasing depth. 48 mol % were finally measured in tape 10.

## Discussion & Conclusion

This study successfully confirms that hydrogenated PLs penetrate into the SC. A high amount in upper skin layers and a decrease in deeper layers demonstrate an accumulation of saturated PC in upper epidermal layers. This skin distribution was already observed by Blume (2000) [3] and can be used to support the skin barrier function or to influence the distribution of a co-formulated drug substance.

For the first time the detection of exogenous PLs besides endogenous skin lipids was demonstrated using the Lipotype shotgun lipidomics technology. It offers the opportunity for further investigations and will be used to assess the penetration properties as well as metabolism of (un)saturated PLs.

## References

[1] Dörfler, H.-D., Brezesinski, G., Phasenumwandlungserscheinungen in Lecithin/Wasser-Systemen. Colloid & Polymer Sci 1983, 261, 286–292.

[2] Sadowski, T., Klose, C., Gerl, M. J., Wójcik-Maciejewicz, A., Herzog, R., Simons, K., Reich, A., Surma, M. A., Large-scale human skin lipidomics by quantitative, high-throughput shotgun mass spectrometry. Scientific reports 2017, 7, 43761.

[3] Blume, G., Liposomes = Liposomes? SÖFW Journal 2000, 126.