

In Vitro Human Skin Penetration of the Fragrance Material Linalool

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Abstract

The in vitro human skin penetration of linalool [CAS# 78-70-6] was assessed in three vehicles relevant to fragrance exposure [1:3 Ethanol:diethyl phthalate (1:3 EtOH:DEP), 1:3 Ethanol:dipropylene glycol (1:3 EtOH:DPG) and petrolatum]. The assays were conducted under both occlusive and non-occlusive conditions. Diffusion cells were prepared with epidermal membranes obtained from surgical donors. Permeation of linalool, from a 5 µl/cm² target dose of a 4% (w/v) solution, was measured at twelve time-points over 24 hours, using a phosphate buffered saline receptor phase. At 24 hours, the epidermal membranes were wiped, tape stripped 10 times and the linalool content of the wipes, strips and remaining epidermis determined. The filter paper skin supports were extracted and the diffusion cell donor chambers washed and wiped. Potential evaporative loss of linalool was estimated by measuring the loss from PTFE sheets under the same experimental conditions. Levels of linalool in the epidermis (plus any remaining stratum corneum after tape stripping), filter paper membrane support and receptor fluid were combined to produce a total absorbed dose value. Under non-occlusive conditions 2.71 ± 0.2% of the applied dose was absorbed at 24 hours in 1:3 EtOH:DEP, 2.01 ± 0.2% in 1:3 EtOH:DPG and 3.54 ± 0.4% in petrolatum. Under occlusive conditions 7.33 ± 0.58% of the applied dose was absorbed at 24 hours in 1:3 EtOH:DEP, 12.1 ± 1.5% in 1:3 EtOH:DPG and 10.5 ± 1.1% in petrolatum. Evaporative loss was significant and accounted for the differences in absorption between occluded and non-occluded conditions. From these results, systemic exposure to linalool from use in fragrances would be expected to be low based on currently reported use levels. In addition to providing information to risk assessments, the variety of vehicles will provide comparative data to assist in the development of dermal absorption models for this material.

Introduction

Linalool (CAS# 78-70-6, Figure 1) is a fragrance ingredient appreciated for its light and refreshing, floral woody odor with a faintly citrus note (Arctander, 1969). Linalool is a component of numerous essential oils and is incorporated into a variety of consumer product types. A safety assessment of linalool was conducted by Bickers et al. (2003) and it was concluded that there are no safety concerns for this material based on its current reported use level in perfumery. In order to assist in refining this and future assessments of structurally related materials, an in vitro human skin permeation and distribution study was conducted on linalool.

We previously reported on the skin absorption of linalool in 70/30 ethanol (EtOH)/water, diethyl phthalate (DEP) and dipropylene glycol (DPG) (Lalko and Api, 2007). In the assays reported here, we included DEP and DPG in combination with EtOH as this is more realistic of fragrance exposures. Additionally, clinical patch tests are often conducted in petrolatum and this vehicle was included to determine its effect on skin absorption relative to the EtOH containing vehicles.

Methods and Materials

This study was conducted according to the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) guidelines for in-vitro percutaneous absorption assessment (SCCNFP, 2003). The study utilized radio labeled linalool [(1,2-¹⁴C)linalool, 7.40 MBq (0.2 mCi), supplied by GE Amersham. The three vehicles assessed during the study were 1:3 Ethanol:diethyl phthalate (1:3 EtOH:DEP), 1:3 Ethanol:dipropylene glycol (1:3 EtOH:DPG) and petrolatum.

Briefly, twelve active dosed diffusion cells were prepared (using six donors) plus three control cells. Epidermal membranes were used and their integrity was assessed by measuring the permeation rate of tritiated water over a period of one hour. Permeation of linalool, from a 5 µl/cm² target dose of a 4% (w/v) solution in each vehicle, was then measured at twelve time-points over 24 hours, using a 6% buffered saline receptor phase. At 24 hours, the epidermal membranes were wiped, tape stripped 10 times and the linalool content of the wipes, strips and remaining epidermis determined. The filter paper skin supports were extracted and the diffusion cell donor chambers washed and wiped. All samples were analysed for ¹⁴C by liquid scintillation counting. Potential evaporative loss of linalool was estimated by measuring the loss from PTFE sheets under the same experimental conditions.

Results

The total absorbed dose for each run in the individual vehicle is presented in Table 1. The results for studies previously reported are included for comparison.

The distribution based on vehicle utilized and occlusive conditions is presented in Tables 2 to 4.

Figure 1. Linalool

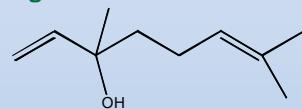


Table 1. Linalool - Percent of applied dose absorbed at 24 hours based on vehicle. (% applied dose, mean ± SE)*

Vehicle	Unoccluded Group	Occluded Group	Unoccluded Group	Occluded Group
70/30 Ethanol/Water	3.57 ± 0.50	14.4 ± 1.2	-	-
DEP	2.77 ± 0.30	5.73 ± 0.34	-	-
1:3 EtOH:DEP	-	-	2.71 ± 0.22	7.33 ± 0.58
DPG	1.80 ± 0.16	7.49 ± 0.55	-	-
1:3 EtOH:DPG	-	-	2.01 ± 0.20	12.1 ± 1.50
Petrolatum	-	-	3.54 ± 0.20	10.5 ± 1.11
Notes	Previously reported studies included for comparison (Lalko and Api, 2007)			

Table 2. Distribution of applied linalool at 24 h for a 25/75 (v/v) ethanol/ DEP vehicle (% applied dose, mean ± SE)

Compartment	Unoccluded group, ethanol/DEP (n=12)	Occluded group, ethanol/DEP (n=12)
Wipe	0.838 ± 0.208	7.97 ± 0.73
Donor chamber/grease wash/wipe	0.881 ± 0.178	14.7 ± 0.9
SC strips 1-10	0.038 ± 0.011	0.239 ± 0.063
Remaining skin	0.049 ± 0.005	0.331 ± 0.046
Filter paper	0.389 ± 0.029	0.942 ± 0.054
Permeated	2.27 ± 0.20	6.05 ± 0.57
Overall recovery	4.46 ± 0.41	30.3 ± 1.2
Absorbed dose*	2.71 ± 0.22	7.33 ± 0.58

*- absorbed dose defined as the combined amounts in the receptor phase, filter paper support and remaining skin (epidermis and any remaining stratum corneum following tape stripping)

Table 3. Distribution of applied linalool at 24 h for a 25/75 (v/v) ethanol/ DPG vehicle (% applied dose, mean ± SE)

Compartment	Unoccluded group, ethanol/DPG (n=12)	Occluded group, ethanol/DPG (n=11)
Wipe	1.14 ± 0.12	1.43 ± 0.24
Donor chamber/grease wash/wipe	0.496 ± 0.113	8.55 ± 1.27
SC strips 1-10	0.012 ± 0.004	0.035 ± 0.004
Remaining skin	0.037 ± 0.006	0.172 ± 0.022
Filter Paper	0.268 ± 0.029	0.913 ± 0.080
Permeated	1.70 ± 0.17	11.0 ± 1.4
Overall recovery	3.65 ± 0.25	22.1 ± 1.5
Absorbed dose*	2.01 ± 0.20	12.1 ± 1.5

*- absorbed dose defined as the combined amounts in the receptor phase, filter paper support and remaining skin (epidermis and any remaining stratum corneum following tape stripping)

Table 4. Distribution of applied linalool at 24 h for a Petrolatum vehicle (% applied dose, mean ± SE)

Compartment	Unoccluded group, Petrolatum (n=12)	Occluded group, Petrolatum (n=12)
Wipe	0.094 ± 0.012	1.07 ± 0.14
Donor chamber/grease wash/wipe	0.711 ± 0.131	7.07 ± 0.68
SC strips 1-10	0.005 ± 0.000	0.013 ± 0.001
Remaining skin	0.033 ± 0.005	0.111 ± 0.020
Filter Paper	0.290 ± 0.029	0.752 ± 0.044
Permeated	3.21 ± 0.42	9.63 ± 1.01
Overall recovery	4.35 ± 0.45	18.6 ± 1.5
Absorbed dose*	3.54 ± 0.45	10.5 ± 1.1

*- absorbed dose defined as the combined amounts in the receptor phase, filter paper support and remaining skin (epidermis and any remaining stratum corneum following tape stripping)

The percent (%) applied dose absorbed vs. time is presented in Figure 2

The evaporative loss expressed as the overall recovery of linalool from PTFE sheets is presented in Figure 3.

Conclusions

As with previous studies in different vehicle, both application conditions and application vehicle had relatively large effects on the permeation of linalool.

Occluded conditions increased permeation of linalool for all vehicles, possibly through a combination of increased skin hydration and reduced evaporative loss of both linalool and volatile vehicles. Low overall recoveries were achieved, even for the occluded groups, due to evaporative loss.

Comparing the results of previously reported studies on similar vehicles, shows that there was essentially no relative difference in absorption due to the addition of ethanol in this system at the level added.

In conclusion, the absorption of linalool through human skin under unoccluded and occluded conditions is low. Systemic exposure resulting from the use of linalool as a fragrance ingredient would be expected to be low based on the results of this study and the currently reported use levels.

References

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Figure 2.

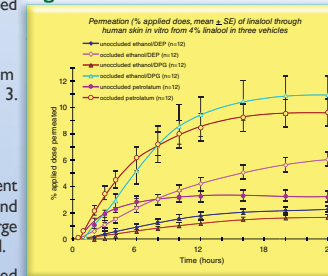


Figure 3.

