

Detection of drug absorption in living skin equivalent models by using MALDI-MSI

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Introduction

Matrix assisted laser desorption ionization (MALDI) mass spectrometry imaging (MSI) is a label free technique able to study intact tissue sections providing ion distribution maps of many species simultaneously. The adoption of MALDI-MSI has contributed to the studies of drug/toxicant absorption in the skin and, recently, there is a growing interest in investigating compounds able to enhance drug penetration.

Although MALDI-MSI has been successfully used for qualitative analysis, its application for quantitative analysis represents one of the major critical challenges.

In this study, a method for quantitative MALDI-MSI has been developed and used to study the effect of the penetration enhancer isosorbide dimethyl (DMI) on the absorption of an antifungal, Terbinafine hydrochloride in Labskin, a living skin equivalent model

Methodology

Sample preparation for MALDI-MS:

Labskin® was treated with Terbinafine hydrochloride at concentration 1% w/w with either 10% or 50% DMI for 24 hours. For analysis by mass spectrometry imaging 12µm tissue sections were cryosectioned (Leica 200 UV, Leica Microsystems, Milton Keynes, UK) and stored at -80°C before matrix application and imaging.

To create a calibration array, different concentrations of Terbinafine hydrochloride mixed with internal standard Terbinafine d7 hydrochloride (50% MeOH) were deposited onto an untreated section of Labskin (12µm) using an acoustic robotic spotter (Portrait 630, Labcyte Inc., Sunnyvale, CA).

Analysis:

MALDI-MSI data was acquired on a Waters Synapt G-2.

MSI data were validated using a Waters Xevo TQD Tandem Mass Spectrometer with ionization mode ESI±.

Conclusion and future work

A method for quantitative analysis by using MALDI-MSI has been determined and the results have been validated using HPLC-MSMS.

The capability of DMI to increase the drug penetration into the upper epidermis of Labskin has been demonstrated.

Enzymes involved in cutaneous xenobiotic metabolism will be investigated.

Results

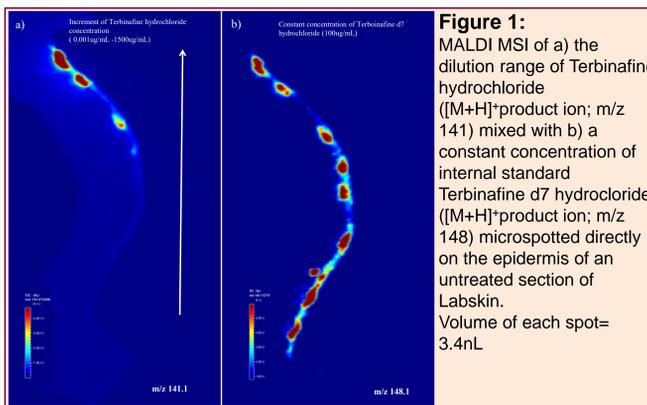


Figure 1: MALDI MSI of a) the dilution range of Terbinafine hydrochloride ([M+H]⁺ product ion; m/z 141) mixed with b) a constant concentration of internal standard Terbinafine d7 hydrochloride ([M+H]⁺ product ion; m/z 148) microspotted directly on the epidermis of an untreated section of Labskin. Volume of each spot= 3.4nL

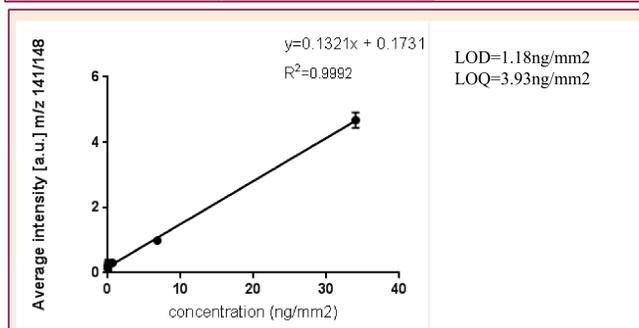


Figure 4: Calibration curves of Terbinafine hydrochloride standards obtained by plotting the intensity ratio of Terbinafine to its internal standard Terbinafine d7 [m/z 141/148] versus the concentration of Terbinafine using MS quant software. Calibration curve derived from 3 repeats.

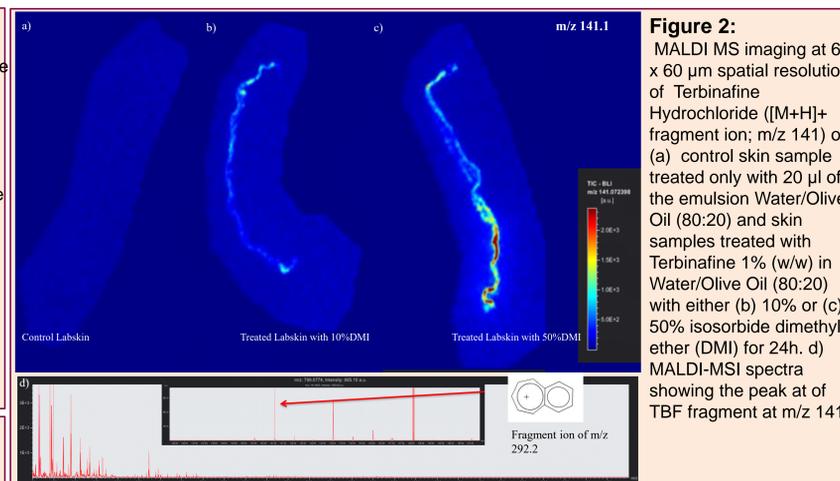


Figure 2: MALDI MS imaging at 60 x 60 µm spatial resolution of Terbinafine Hydrochloride ([M+H]⁺ fragment ion; m/z 141) on (a) control skin sample treated only with 20 µl of the emulsion Water/Olive Oil (80:20) and skin samples treated with Terbinafine 1% (w/w) in Water/Olive Oil (80:20) with either (b) 10% or (c) 50% isosorbide dimethyl ether (DMI) for 24h. (d) MALDI-MSI spectra showing the peak at of TBF fragment at m/z 141.

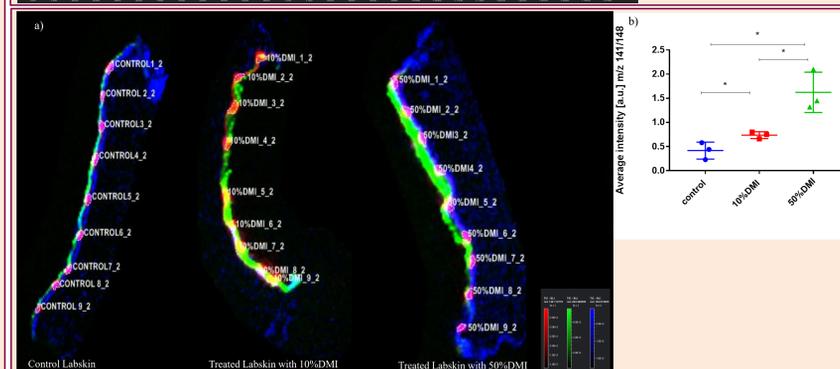


Figure 5: a) ROIs were drawn on the molecular map of the TBF-d7 fragment ion in red (m/z= 148) superimposed with choline head group in blue ([M+H]⁺; m/z 184) and ceramide fragment peak in green ([M+H]⁺; m/z 264.2). The labelled normalisation was selected (m/z 141/148). b) Distribution of intensity values of the ion m/z 141/148 for different Labskin sections.

Discussion

The application of working standards of Terbinafine hydrochloride with its internal standard onto an untreated section of Labskin by microspotting allowed an uniform distribution across the epidermis with minimal lateral diffusion (figure 1).

The spotted untreated Labskin was imaged in the same run with a control skin sample treated only with the emulsion Water/Olive Oil (80:20) and skin samples treated with Terbinafine 1% (w/w) in Water/Olive Oil (80:20) with either 10% or 50% DMI for 24h by using Water Synapt G2 without the ion mobility function enabled (figure 2).

For quantitative investigations, MSI raw data files were converted in imZmL format by using HDI 1.4 software (Waters Corporation, UK) and imported into MSiQuant software. The MSiQuant software allows a number of methods for the definition of regions of interest (ROI) and extraction of peak intensities from them for quantitative analyses. Here the methodology used was to use signals from endogenous species to define the epidermis and stratum corneum of the tissue section (m/z 184 PC to define the tightly packed cells of the epidermis and m/z 264 to define the stratum corneum). Then using the software an average intensity for the signals of the terbinafine and the terbinafine d7 of a ROI located to solely the epidermis for each spot could be extracted (figure 3).

Calibration curves obtained using labelled normalisation can be observed in figure 4.

In order to quantify the amount of drug, ROIs were drawn on the molecular map of the TBF-d7 fragment ion (m/z= 148) in the epidermis area of the control tissue and treated tissues. The labelled normalisation was selected (m/z 141/148) as shown in figure 5.

By comparison of the main intensity of Terbinafine hydrochloride normalised by the internal standard in each treated tissue to calibration curve, the amount of drug was calculated. Quantitative MSI data highlighted that the addition of DMI to the delivery formulation yielded a statistically significant increase of drug permeation into the upper epidermis. The amount of drug in the epidermis was found to rise from 0.35 ± 0.05 mg/g of tissue within the section with 10% DMI to 0.91 ± 0.26mg/g of tissue within the section with 50% DMI.

The results obtained from MSI technique were validated using HPLC-MSMS performed on extracts of the same tissue.

T test was used to determine the significance statistic between two method (figure 6).

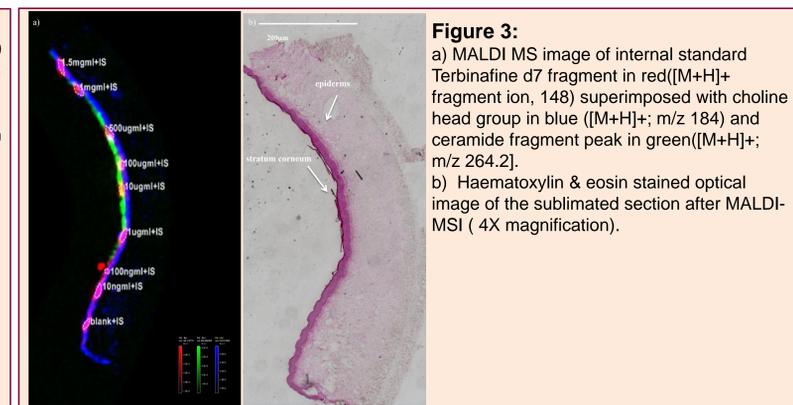


Figure 3: a) MALDI MS image of internal standard Terbinafine d7 fragment in red ([M+H]⁺ fragment ion, 148) superimposed with choline head group in blue ([M+H]⁺; m/z 184) and ceramide fragment peak in green ([M+H]⁺; m/z 264.2). b) Haematoxylin & eosin stained optical image of the sublimated section after MALDI-MSI (4X magnification).

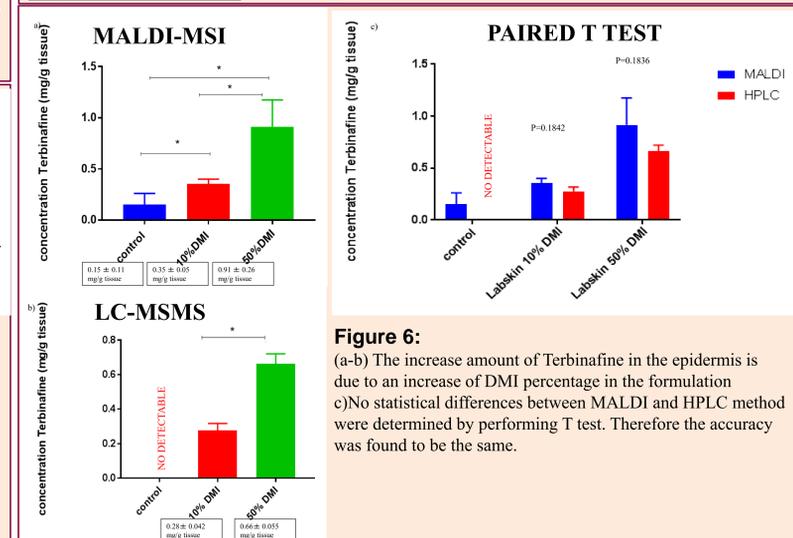


Figure 6: (a-b) The increase amount of Terbinafine in the epidermis is due to an increase of DMI percentage in the formulation (c) No statistical differences between MALDI and HPLC method were determined by performing T test. Therefore the accuracy was found to be the same.

Acknowledgements

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