In vitro model to assess analytical performance of Raman spectroscopy for penetration studies of actives molecules in human skin

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1 Introduction

Confocal Raman Microscopy (CRM) has become a versatile technique that can be applied routinely to monitor skin penetration of active molecules *in vitro* on skin cross sections, *ex vivo* on skin biopsies and *in vivo* [1]. CRM has demonstrated its ability to complement or even surpass conventional approaches, however validating a method requires to estimate the analytical performances according to established criteria by regulatory authorities like EMA [2]. Therefore, in the present study an *in vitro* experimental setup has been developed to determine the limit of detection (LOD) of CRM for the quantification of active molecules in human *stratum corneum.* Using both uni- and multivariate approaches, the sensitivity and accuracy of the analysis are discussed [3].

2 Material and methods

In this study 11 discs of isolated human *stratum corneum* (SC) purchased from Biopredic international (Rennes, France) were exposed to resorcinol solutions prepared in PBS at concentrations 0, 0.5, 1, 2.5, 5, 10, 25 and 50 g/L respectively identified as C1, C2, C3, C4, C5, C6, C7 and C8. Samples were analysed with an Alpha300R Raman microscope (Witec, Ulm, Germany). Additionally, a set of SCs were prepared and analysed by HPLC to provide reference concentrations to construct predictive models. First, Principal Components Analysis (PCA) was used to witness the variability in the data set collected. Second, ratio calculated from Area Under the Curve (AUC) using resorcinol and proteins/lipids bands were used to construct linear regression from the Raman spectra. Third, Partial Least Squares Regression (PLS-R) has been applied with a cross validation protocol to perform quantitative analysis in the fingerprint region. LOD was estimated from results obtained using equations (1) and (2):

$$\boldsymbol{LOD_1 = 3.3 \cdot \frac{S_{C1}}{a}} \quad (1) \qquad\qquad \boldsymbol{LOD_2 = 3.3 \cdot RMSEC} \quad (2)$$

3 Results and discussion

HPLC results demonstrate the linear relationship between the concentrations of solutions used to expose SC samples and the resulting concentration inside the tissues (data not shown). Henceforth, reference concentrations are expressed as mg resorcinol/mg SC. Figure 1a shows the regression plot

constructed using AUC of Raman bands corresponding to resorcinol and lipids/proteins (1400-1500cm^{-1}). Clearly, the intensities of Raman features in the spectra collected are linearly correlated to resorcinol concentrations in SC (R^2 = 0.999) despite a heterogeneity in the distribution of the active molecule in samples (error bars). PLSR results presented in figure 1b display a strong correlation between calculated concentration of resorcinol in SC and the predicted concentrations (slop close to one). R^2 = 0.975 and errors bars account for the heterogeneity encountered in biological samples. The RMSECV = 0.017 mg resorcinol / mg SC corresponds to 13.2% compared to the median concentration of the range studied. For the band ratio analysis, LOD_1 = 0.017 mg resorcinol /mg SC, and LOD_2 = 0.09 mg resorcinol /mg SC. For the PLSR data, the LOD_1 and LOD_2 values were 0.014 and 0.06 mg/resorcinol /mg SC, respectively.

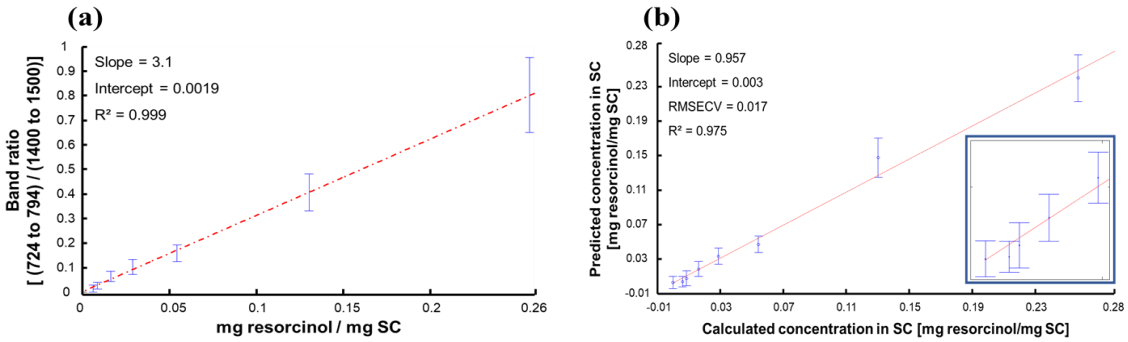

Figure 1 – (a) Band ratio between aromatic resorcinol band and the C-H band of protein / lipids of the SC. (b) PLS-R regression plot for validation set.

Table1: Estimation of analytical performance parameters using PLSR data.

Performance Parameters	***[mg resorcinol /mg SC]***
RMSEC / RMSEP	0.017 / 0.015
RPD	8.2 > **3 (good model)**
BCMSEP	$5.15 \cdot 10^{-5}$
Linearity (R^2)	0.971
Sensitivity (SEN)	18.994
Selectivity (SEL)	0.293

4 Conclusion

While Raman spectroscopy can provide biochemical information about skin samples, the application of CRM for monitoring active ingredients penetration relies on the sensitivity and specificity of the technique. In order to determine key figures of merit to characterize the performance of a CRM, an *in vitro* model delivering precise concentrations (*mg resorcinol /mg SC)* has been thought. Resorcinol can be detected and quantified in isolated *stratum corneum* samples however the accuracy of the analysis strongly depends to the heterogeneity the distribution of resorcinol in skin samples.

5 References

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