

Short Term Scientific Mission (STSM) Report

STSM details

TITLE	Analysis of NMF by HPLC
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Host	Sanja Kezic, Academisch Medisch Centrum (AMC), Coronel Institute of Occupational Health, Amsterdam, the Netherlands
Period	20/07/2015 to 29/07/2015
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Background

The skin barrier has a complex structure designed to protect us from exogenous stressors in the environment including microorganisms, allergens and mechanical trauma. Filaggrin is an important protein in the upper layers of the skin (stratum corneum) and crucial for normal skin barrier functions. Together with its metabolites, they are involved in the intra- and extracellular architecture of the skin, skin hydration, UV-protection, acidification and the defense against microorganisms.

A way to estimate the amount of filaggrin protein in the skin is to do tape stripping and measure the amount of filaggrin's degradation products by High Performance Liquid Chromatography (HPLC).

Aim (purpose) of the STSM

Sanja Kezic and her research team at the AMC and the Coronel Institute of Occupational Health are specialized in the tape stripping method and the use of HPLC to estimate the amount of filaggrin's degradation products. The aim of this STSM was to learn how to analyze tapestrip samples by HPLC. Another goal was to learn basic laboratory skills to make me more secure while working in the lab and to meet other researchers and maybe get some new connections for future work.

Work carried out during the STSM

The first day we prepared labels and vials for all the analyses we would perform during the stay, in total 239 samples. We then prepared the samples for analysis and ran the first batch of 48 samples. One round includes extraction of NMF from the corneocytes by addition of ammonia, shaking for 2 hours and centrifuging with vacuum and heating. After the ammonia has evaporated, water is added and a small proportion of the sample is mixed with something called "Mobile fase" and run through the HPLC. During day two we run the second batch of samples and I took more part in the practical work with pipetting and processing of the samples. The next three days we did one batch of samples each day, and I was shown how to integrate and interpret the HPLC chromatograms. I was also shown how to do the correct calculations in order to obtain the NMF values from the chromatograms in Excel. The last days were spent analyzing the results and make graphs and other statistical calculations.

Main results obtained

Analysis of the tape strips

During my stay, I spent time with a trained laboratory analyst, and together with her I analyzed all the samples that I had brought from Denmark. I took part in all the steps and now know how the analysis is done correctly and why the different parts of the analysis are necessary. During the five days we ran the analyses, I participated more and more, and I am now almost able to analyze the samples by myself. I have learned how to interpret and integrate the chromatograms and to see when there is something wrong and when the analyses need to be repeated.



Figure 1. Labeling the vials before analysis

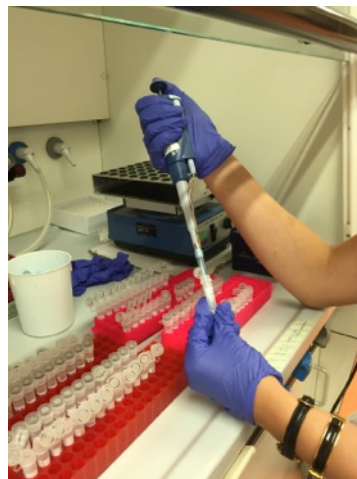


Figure 2. Preparation of the samples by adding ammonia 25%

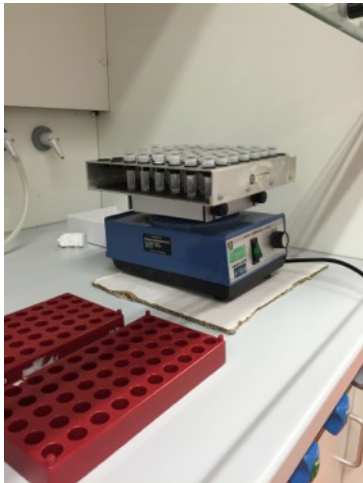


Figure 3. Shaking of the samples



Figure 4. Evaporation of ammonium by centrifuge and vacuum at 60 °C

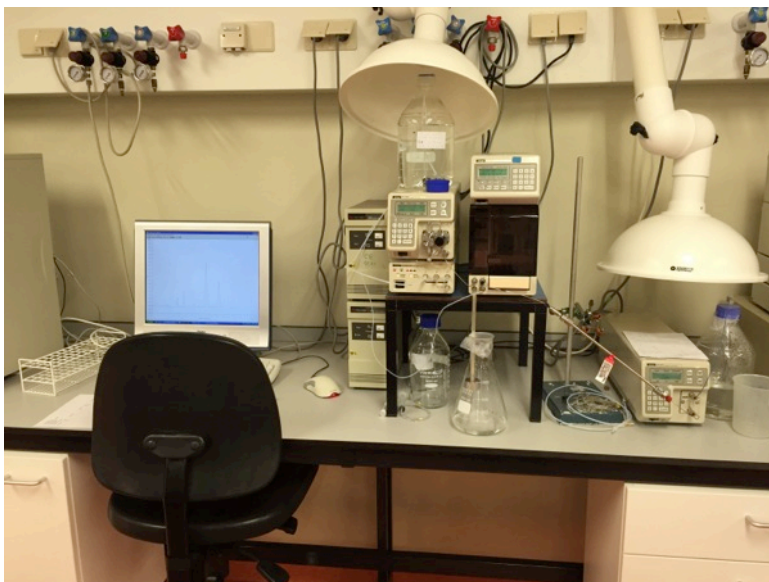


Figure 5. HPLC, used to analyze the amount of NMFs in the tape strips

Laboratory skills and how to behave in a scientific laboratory

The laboratory analysts showed me how to use a pipette correctly and told me how to handle the samples. I was explained the importance of thorough preparation and labeling of the samples, to avoid mix-ups during the analysis. They also showed me around the laboratory and told me what to be aware of to avoid exposure to ammonium that we used during the analyzing process.

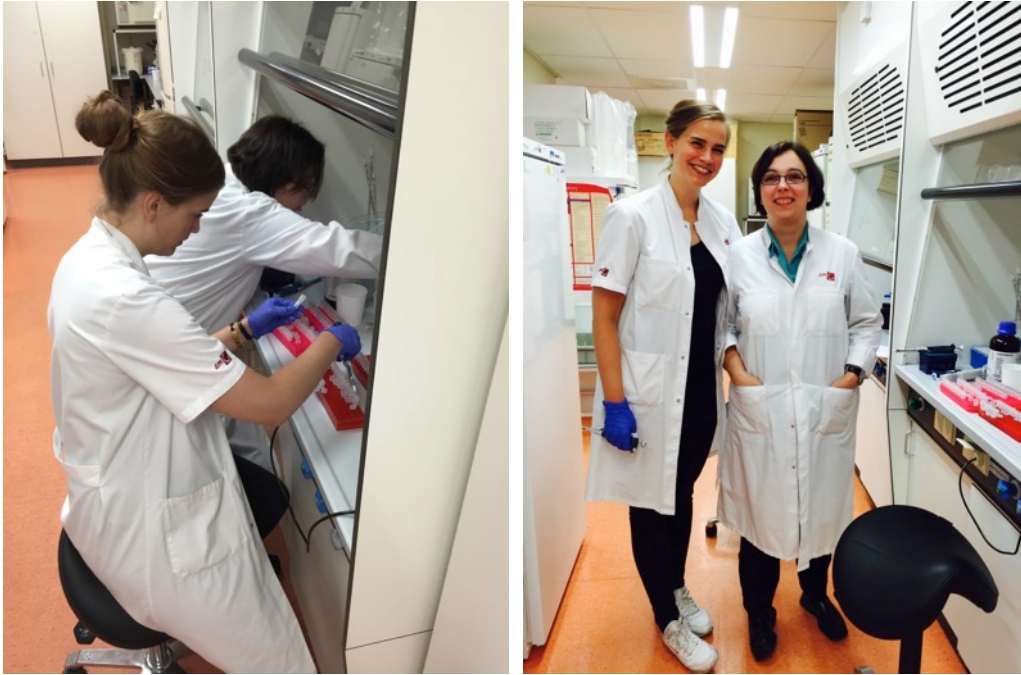


Figure 5. Working in the laboratory and making new friends

Future collaboration with the host institution

We will send all the tape strips from my experimental studies to Sanja and her team at the Coronel Institute for analysis of the degradation products of filaggrin, and because of this, we will have a close collaboration during the rest of my PhD period.

Foreseen publications/articles resulting from the STSM

The results from the analyses carried out during this STSM will be the basis of my first experimental article.

Other comments

I would like to greatly thank Dr. Sanja Kezic and the laboratory analyst for allowing me to visit the AMC and Coronel Institute of Occupational Health and for all the help and support during my stay. I have really enjoyed my stay and I go home with new knowledge and inspiration for future work.

Kristiane Aasen Engebretsen
29/07/15