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Introduction

The evaluation of Eprep Micro Sep cartridges as a medium for on-column derivatisation is presented. Potential physical and chemical variables including mixing of sample and derivatising agent, effect of sample volume and solvents, that are likely to affect the efficiency of the chemical reaction have been optimised using fatty acids as model analytes. In many cases lower limits of detection were obtained using the Eprep Micro Sep cartridges compared to the controls suggesting that the cartridge is functioning as a backbone for the fatty acids during the derivatisation. The optimised technique was applied to olive oil.

Separation

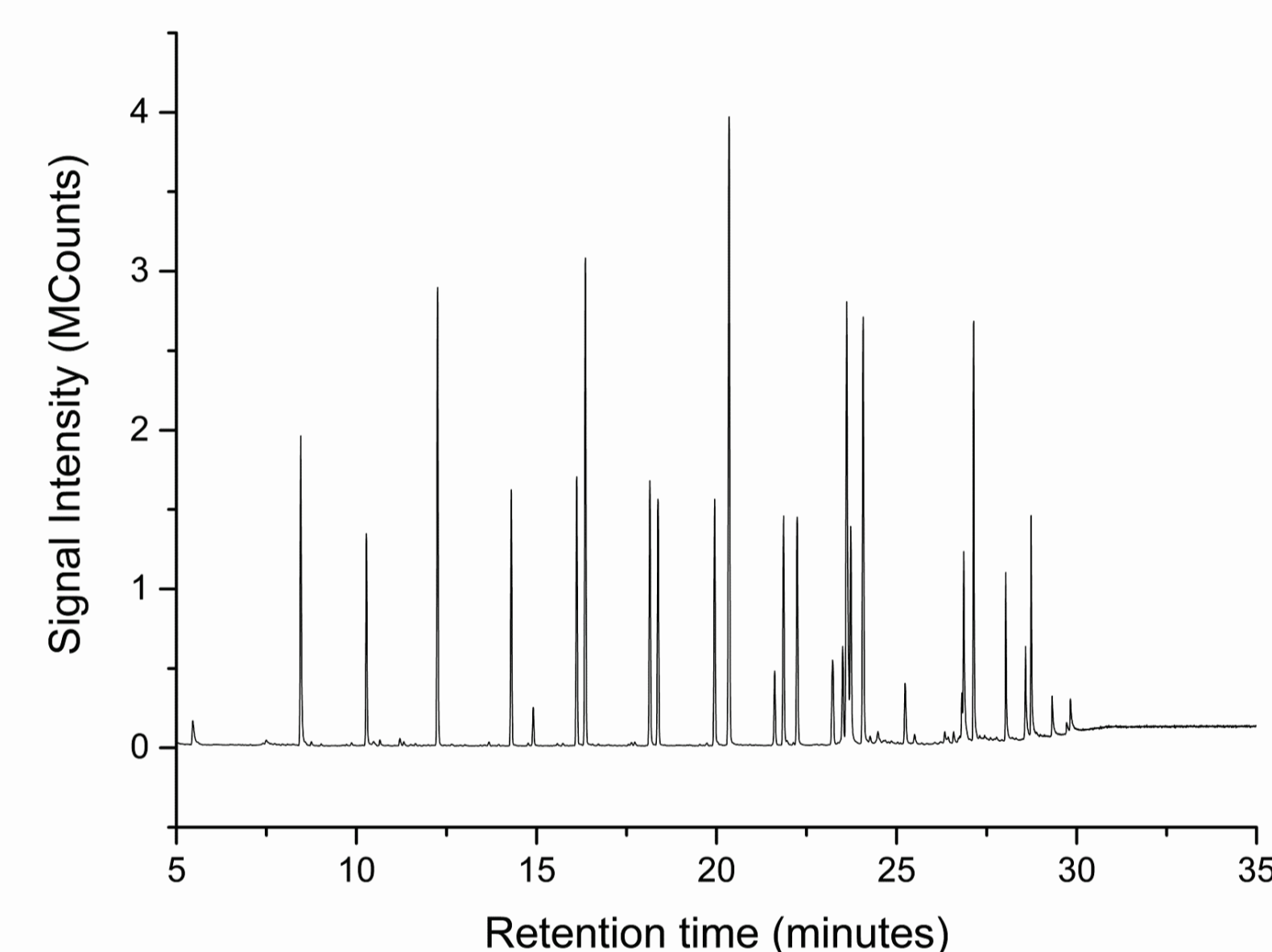


Figure 1 Chromatogram obtained for the separation of 37 FAMES component mix on a GC-MS/MS

Prior to the Eprep testing, a method was developed to separate fatty acids methyl ester (FAMES) using a gas-chromatography ion trap mass spectrometer, as shown in Figure 1. This method was used throughout the experiment to study the performance of the Micro Sep cartridges.

Derivatisation methods

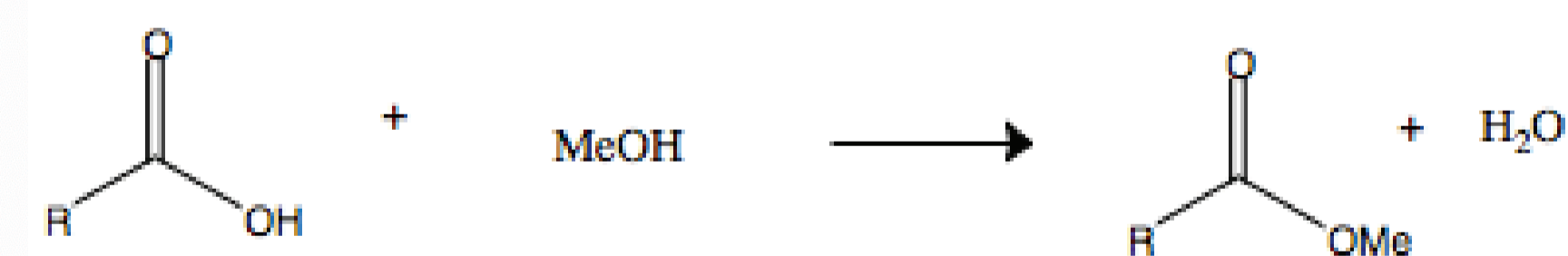
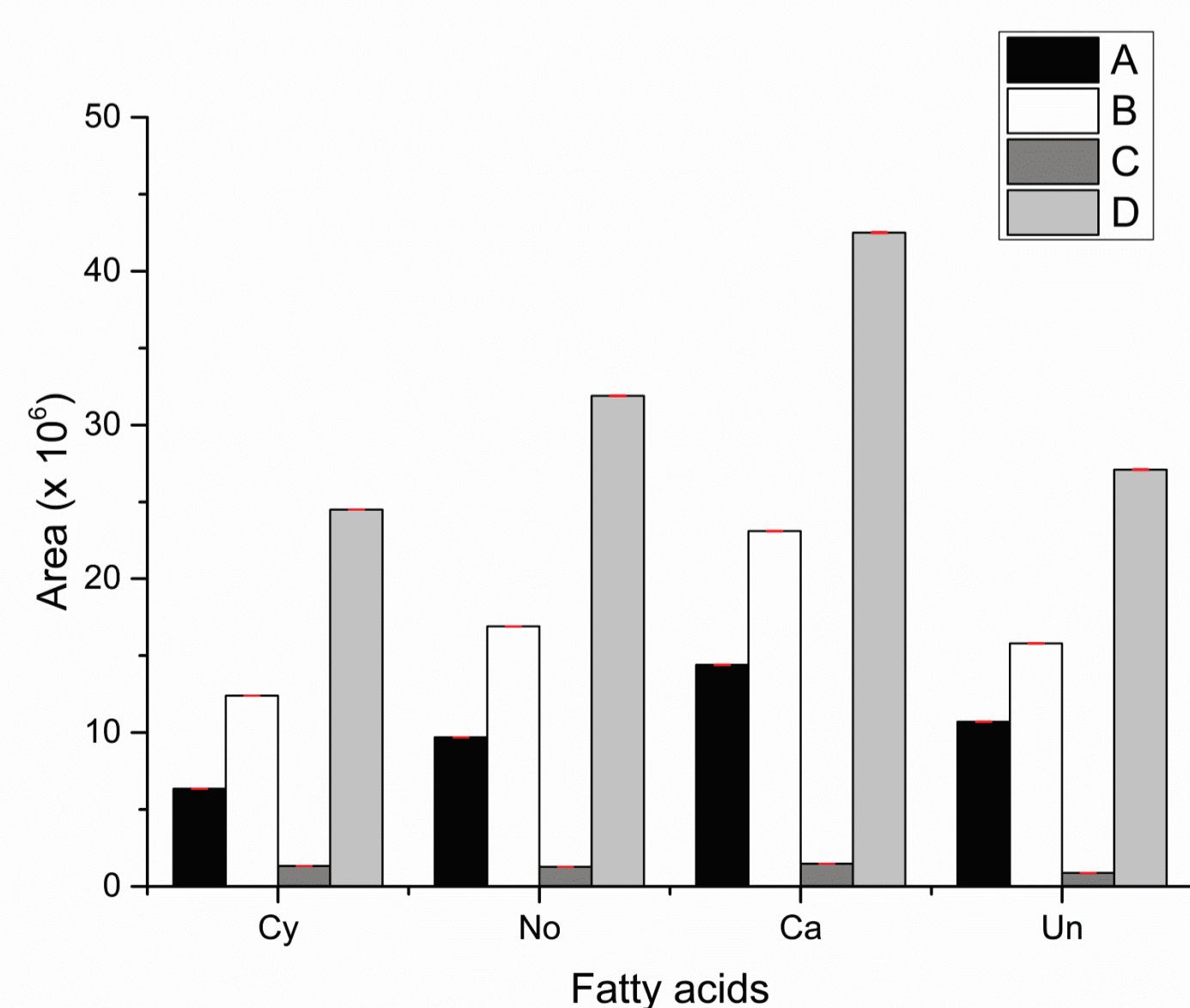


Figure 2 Comparing derivatisation method for caprylic (Cy), pelargonic (No), capric (Ca) and undecyclic (Un) acids using Micro Sep cartridges; (n=3)

- 10 μL fatty acids, the cartridge was left for 1 minute and 10 μL BF₃-MeOH was added followed by incubation
- 10 μL fatty acids, 10 μL BF₃-MeOH, incubation
- withdraw 10 μL BF₃-MeOH and 10 μL fatty acids, inject all through cartridge and incubate.
- 10 μL fatty acids, 10 μL BF₃-MeOH (as experiment B), incubate at 90°C for 10 minutes.

All derivatives were eluted with 150 μL of acetonitrile.

On-column derivatisation

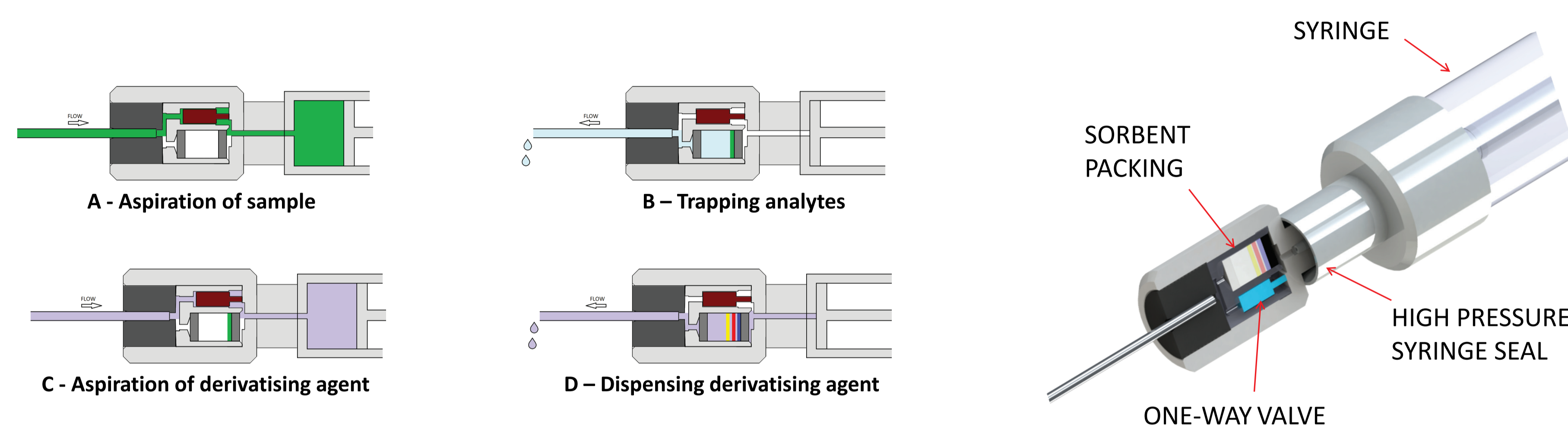


Figure 3 Derivatisation steps using Eprep Micro Sep cartridges

Effect of reagent volumes

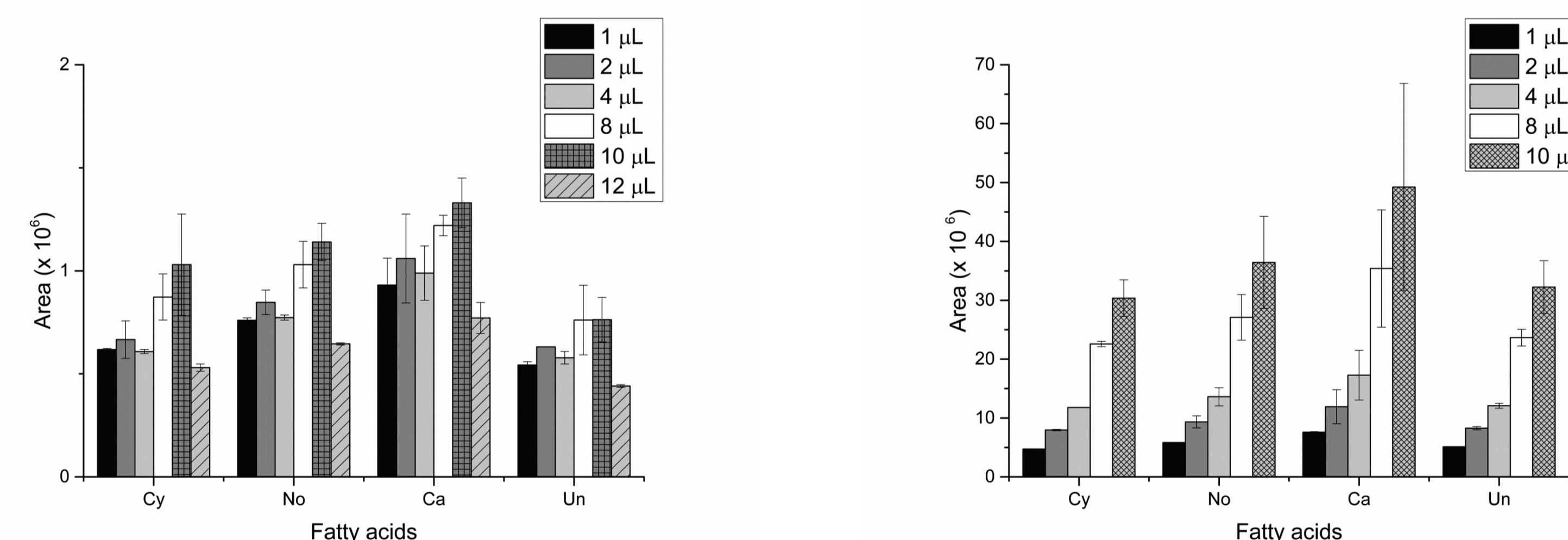
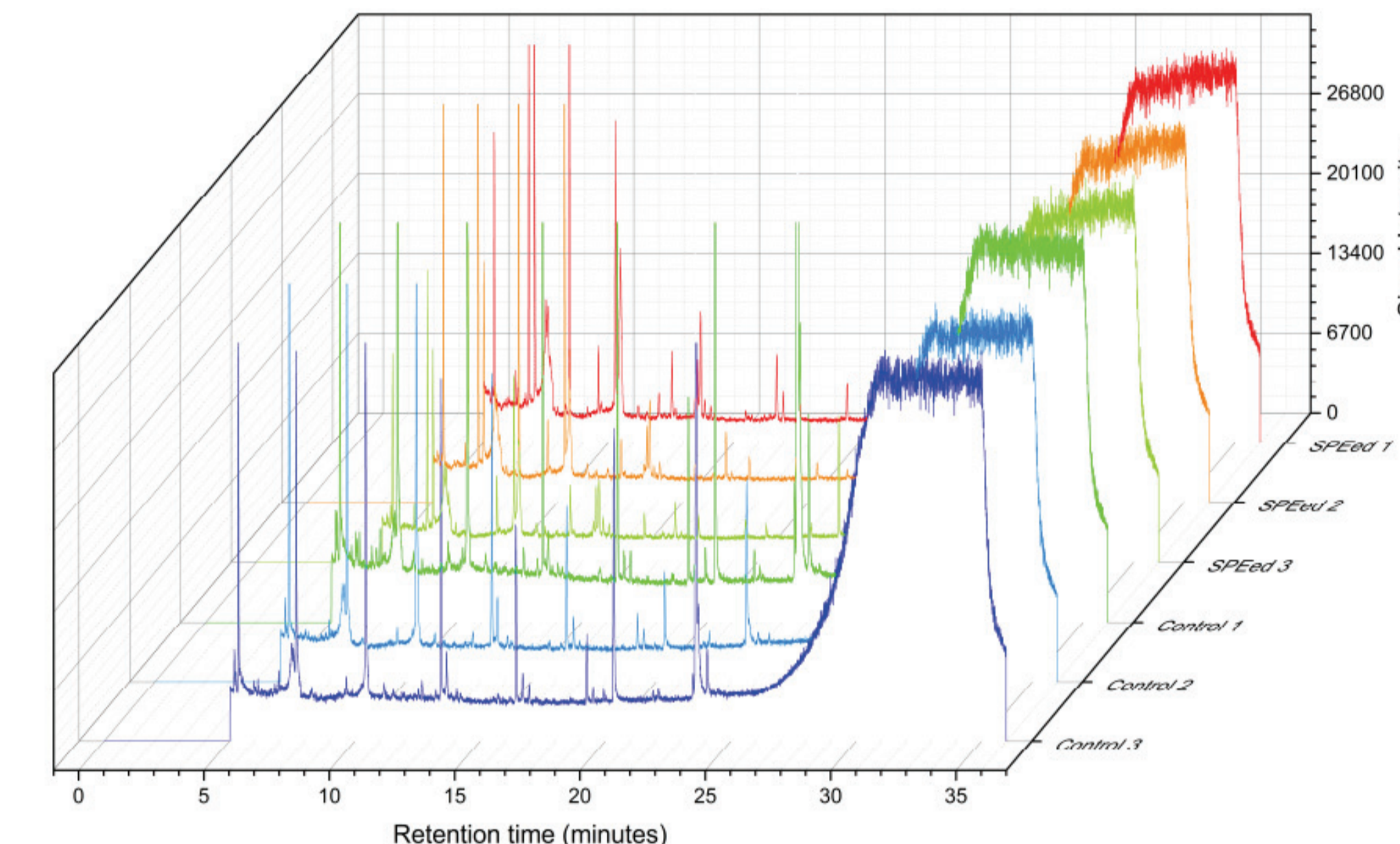


Figure 4 Effect of volume on derivatisation caprylic (Cy), pelargonic (No), capric (Ca) and undecyclic (Un) acids in Micro Sep cartridges (left) and control (right)

The effect of volume of sample and solvents used was also investigated in order to determine the minimum volume required to achieve the maximum conversion of fatty acids to FAMES. Since the volume of the sorbent bed is about 8 μL , the range tested was from 1 to 12 μL . As expected for the control (Figure 4 – bar chart to the right) the amount of FAMES obtained increased with the volume of fatty acids used. This is because the controls were carried out in vials, therefore reaction volume was not a limiting factor. A similar observation can be made with the Micro Sep cartridges from 1 to 10 μL volumes greater than 12 μL however yield less FAMES indicating that 10 μL (of sample and derivatising agent) is the optimum volume. Another point to note is the amount of FAMES. It is believed that the increase in error in these experiments originates from a loss of the valve capability from continuously heating and cooling of the cartridges for short amount of times (about 10 minutes for each step), however further experiments involving repeated derivatisation in a day and a few each day (avoiding the rapid heating and cooling of the cartridge) would need to be carried out to confirm this theory.

Application: Olive oil



Stacked chromatograms of FAMES present olive oil

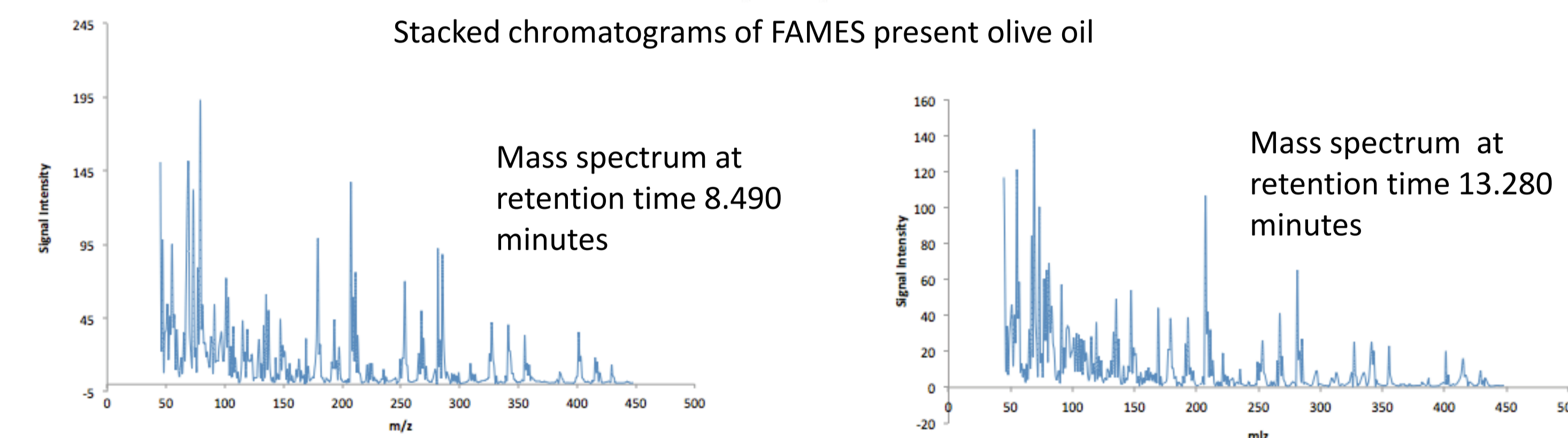


Figure 5 shows the chromatograms obtained from the derivatisation of olive oil on 6 different individual Micro Sep cartridges with the C18 stationary phase. The developed on-column derivatisation method was used to quantify FAMES in olive oil, the concentrations of pelargonic, capric and undecyclic were found to be 54.4, 83.3 and 43.9 ng/mL respectively.

Conclusions

The results presented in this report demonstrate that the Eprep Micro Sep cartridges have a good potential to be used as a tool for on-column derivatisation for fatty acids. Further investigations could be carried out involving other metabolites and application to complex samples.

Acknowledgements

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