

Finding biomarkers for fast detection of *Mycobacterium tuberculosis* in sputum samples using THM – GC-MS: preliminary results

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Introduction:

Automated in-liner thermochemolysis-gas chromatography-mass spectrometry (THM-GC-MS) is a promising approach for fast diagnosis of tuberculosis in an early state. Sputum or bacterial cultures can be injected with a minimum of sample preparation and the detection limits are comparable with those of microscopic tests. Unfortunately, the known biomarkers, for example tuberculostearic acid, are not fully specific for tuberculosis.

In this study, the presences of new potential biomarkers was investigated. This with the aim to reduce the risk of false positives. To find novel markers, a set of cultured bacteria samples were measured followed by chemometric evaluation of the THM-GC-MS data-sets.

Experimental:

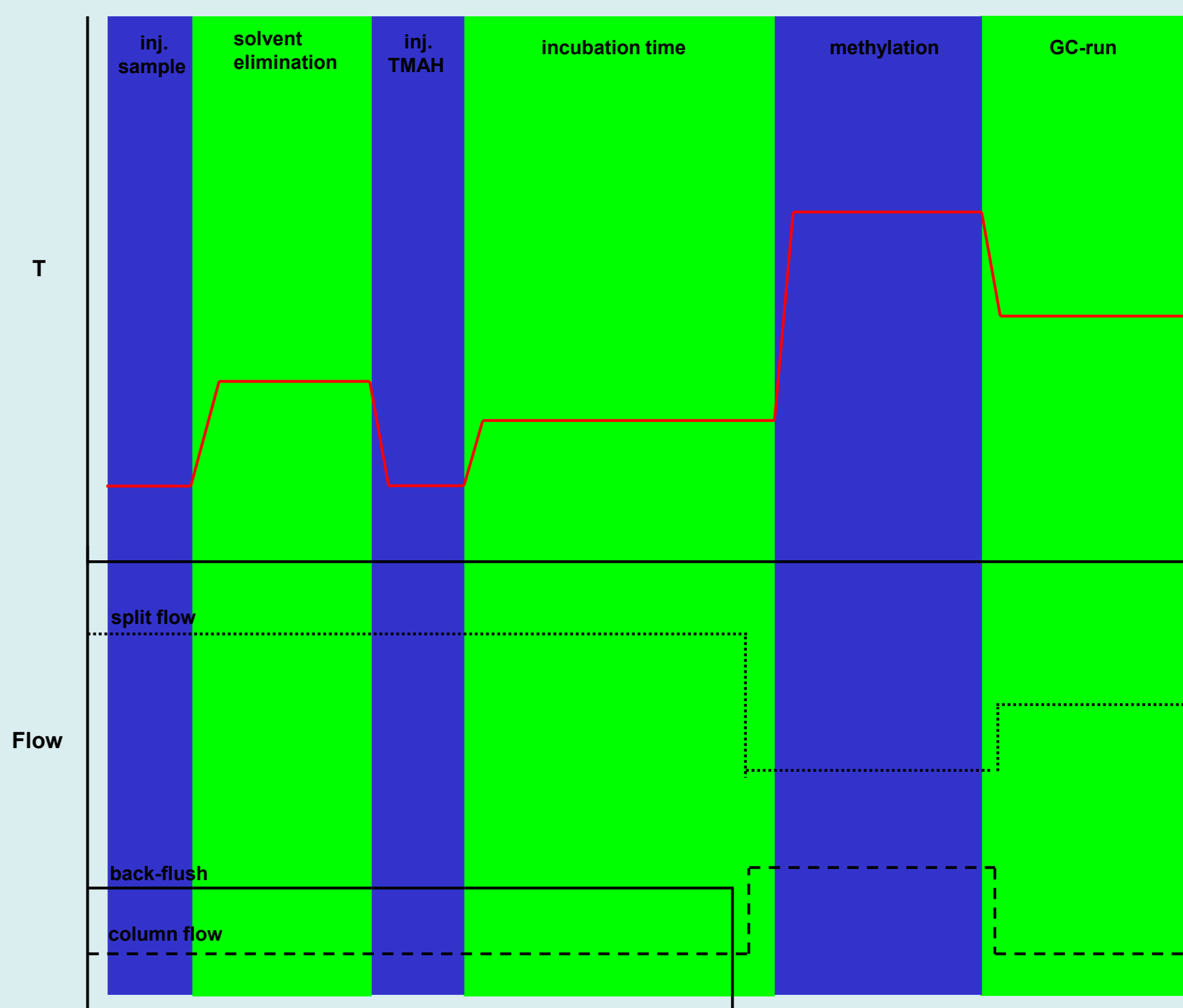
THM-GC-MS

The data set consisted of 43 bacteria samples: 11 *mycobacterium* causing TB and 32 other *mycobacterium* samples. After a simple washing step, the samples were directly injected into the THM-GC-MS. Using the THM-procedure (see schematic figure), the injected sample was first dried before, the reagent was injected. After incubation, the bacterial residues were converted into small, non-polar fragments by rapidly heating the injector. These which were analyzed by GC-MS.

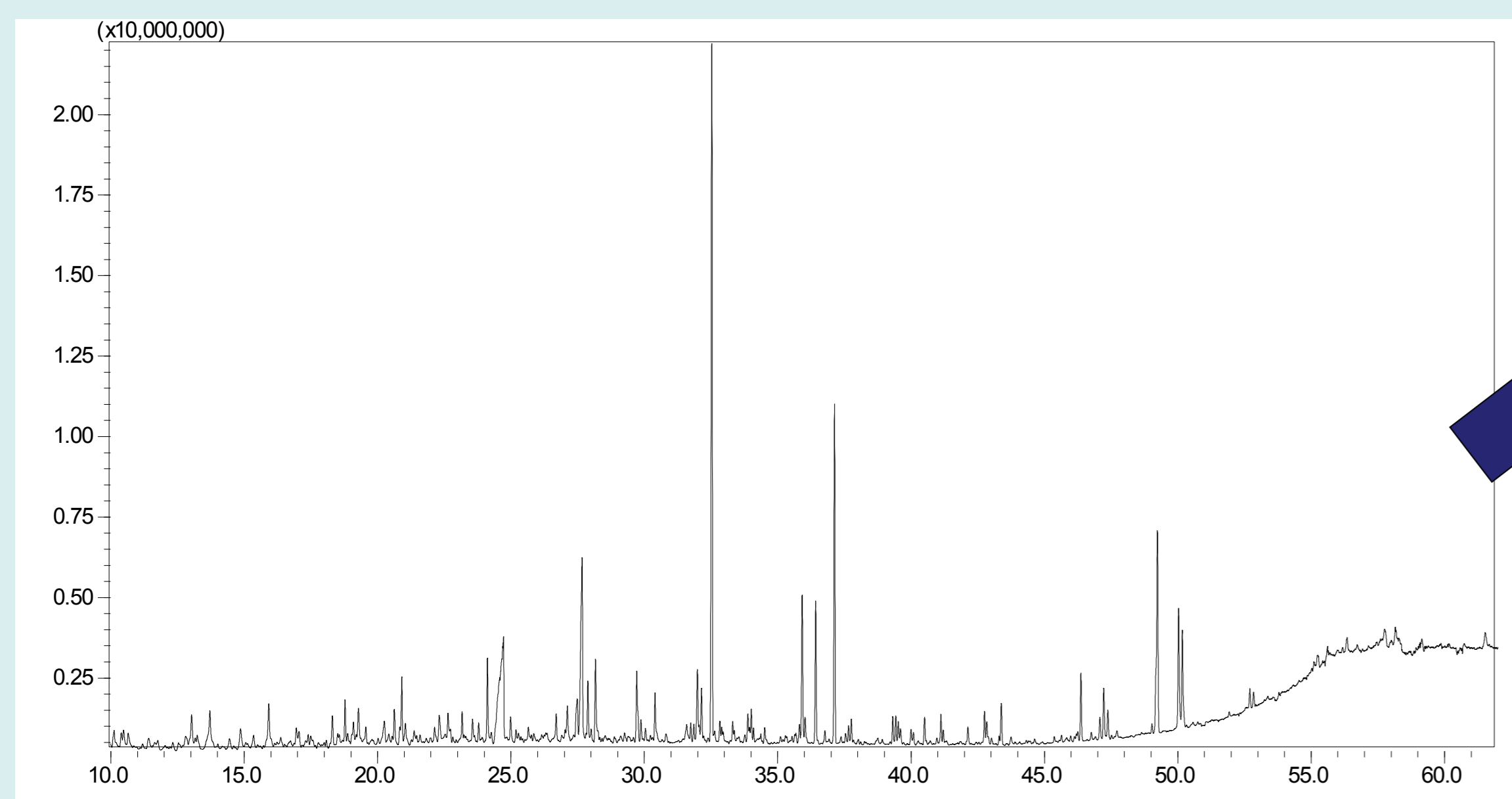
Data-analysis

To find potential biomarkers, first the TIC-chromatograms were corrected for background noise, than the number of data-points was reduced by averaging and finally peak alignments was applied.

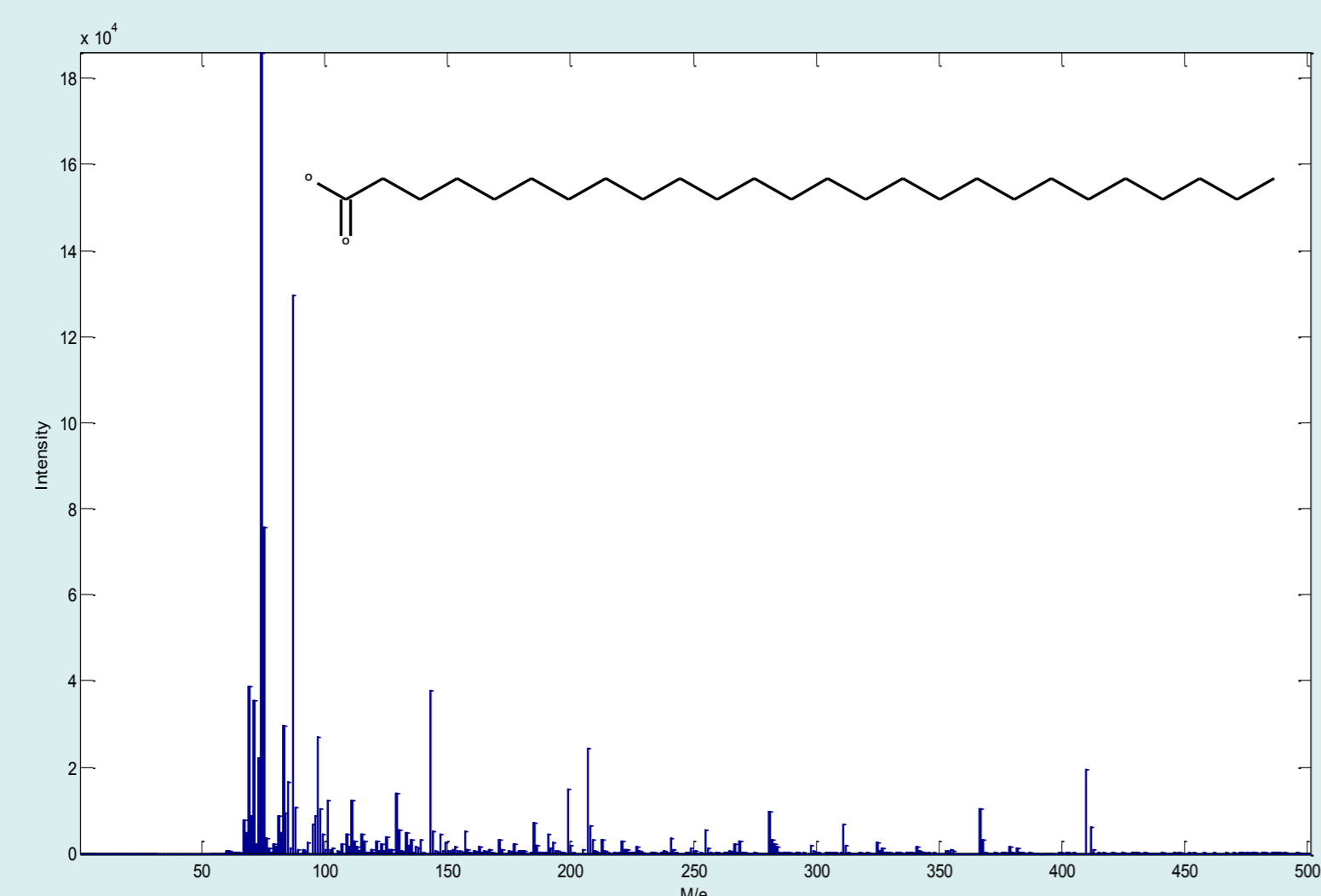
Principal Component Analysis was then applied on the pretreated data set. After that the Wilcoxon rank sum test was performed to assess the actual difference between the two groups.



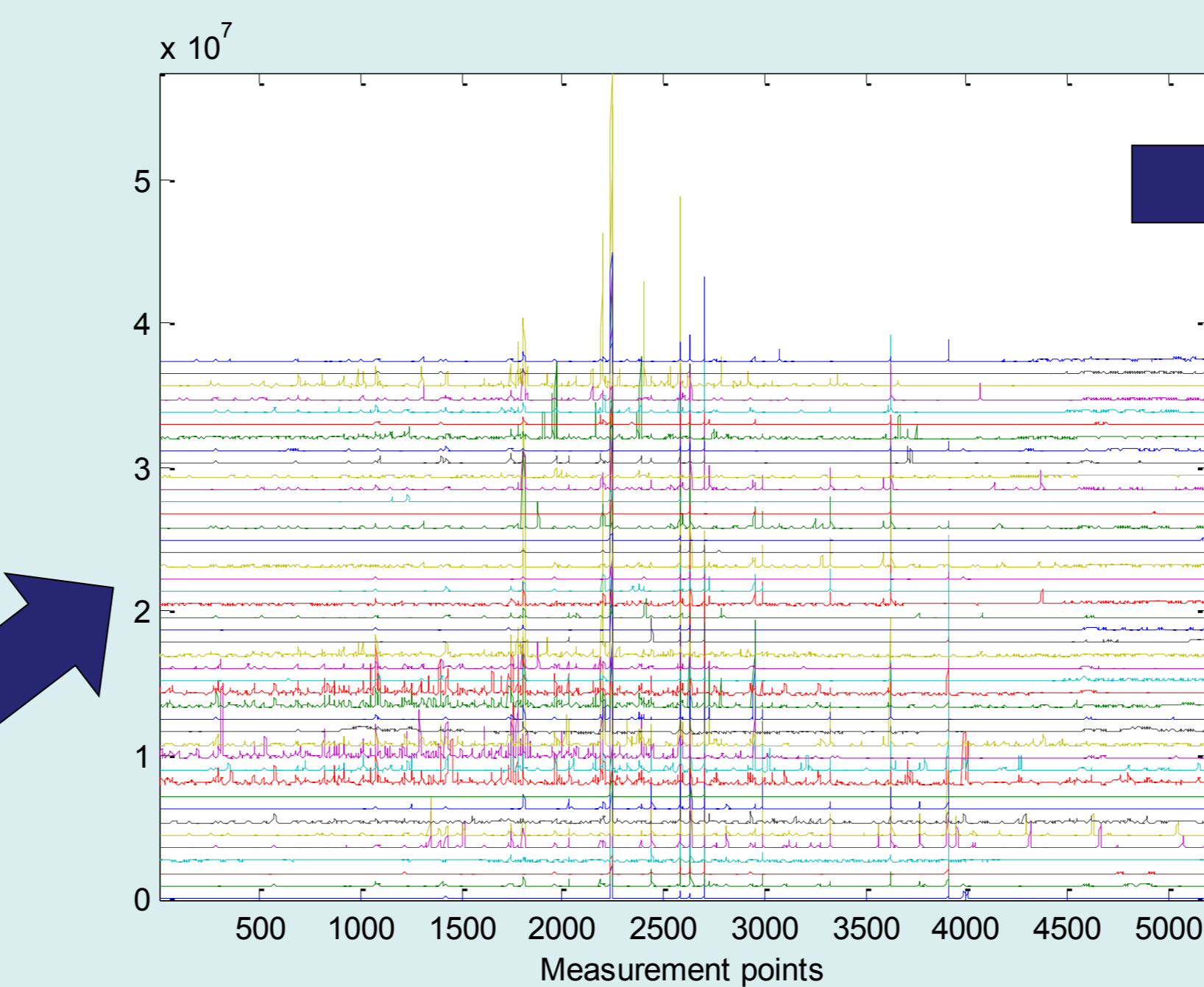
Results:



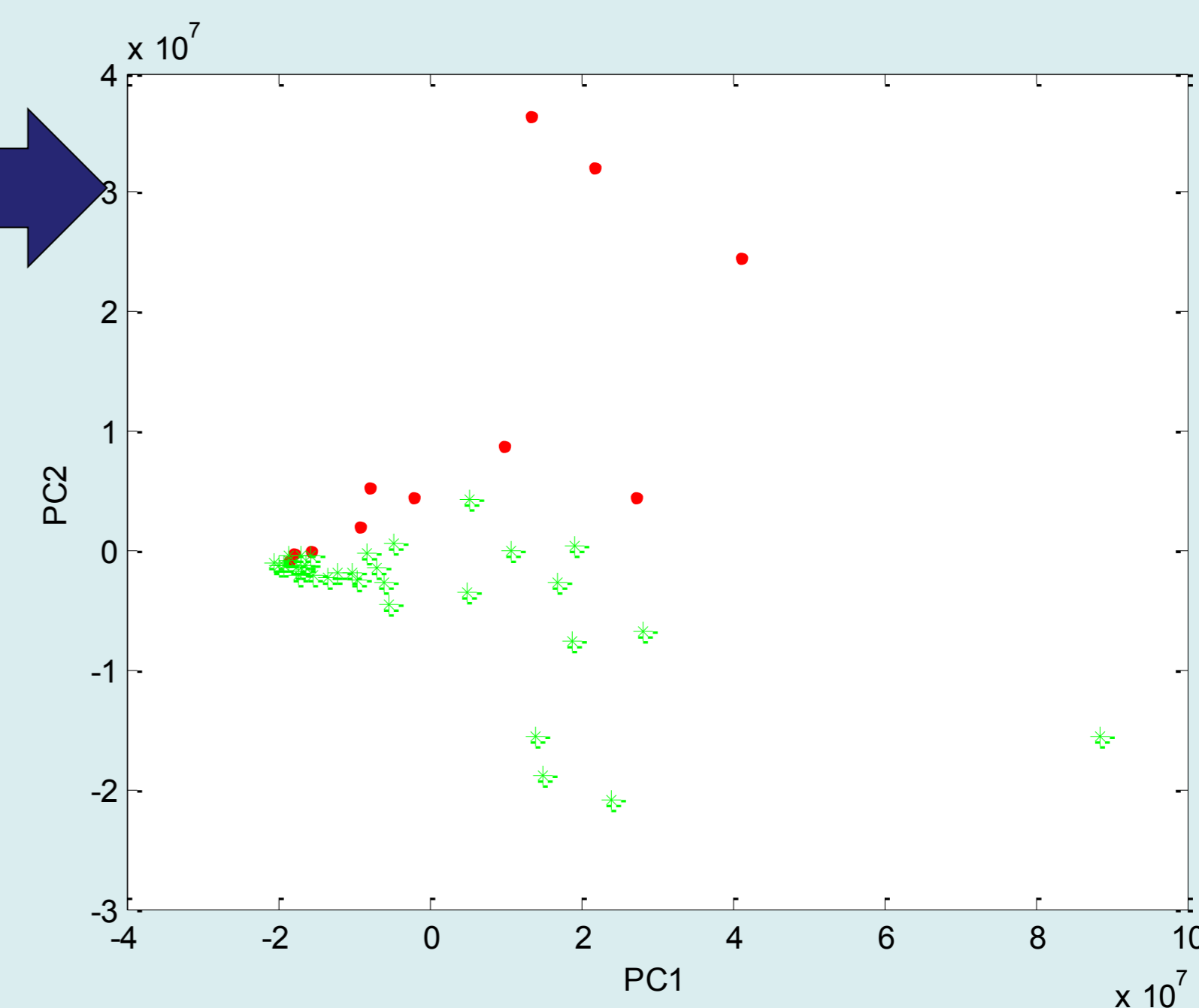
1. TIC-chromatogram of *mycobacteria tuberculosis* after THM-GC-MS.



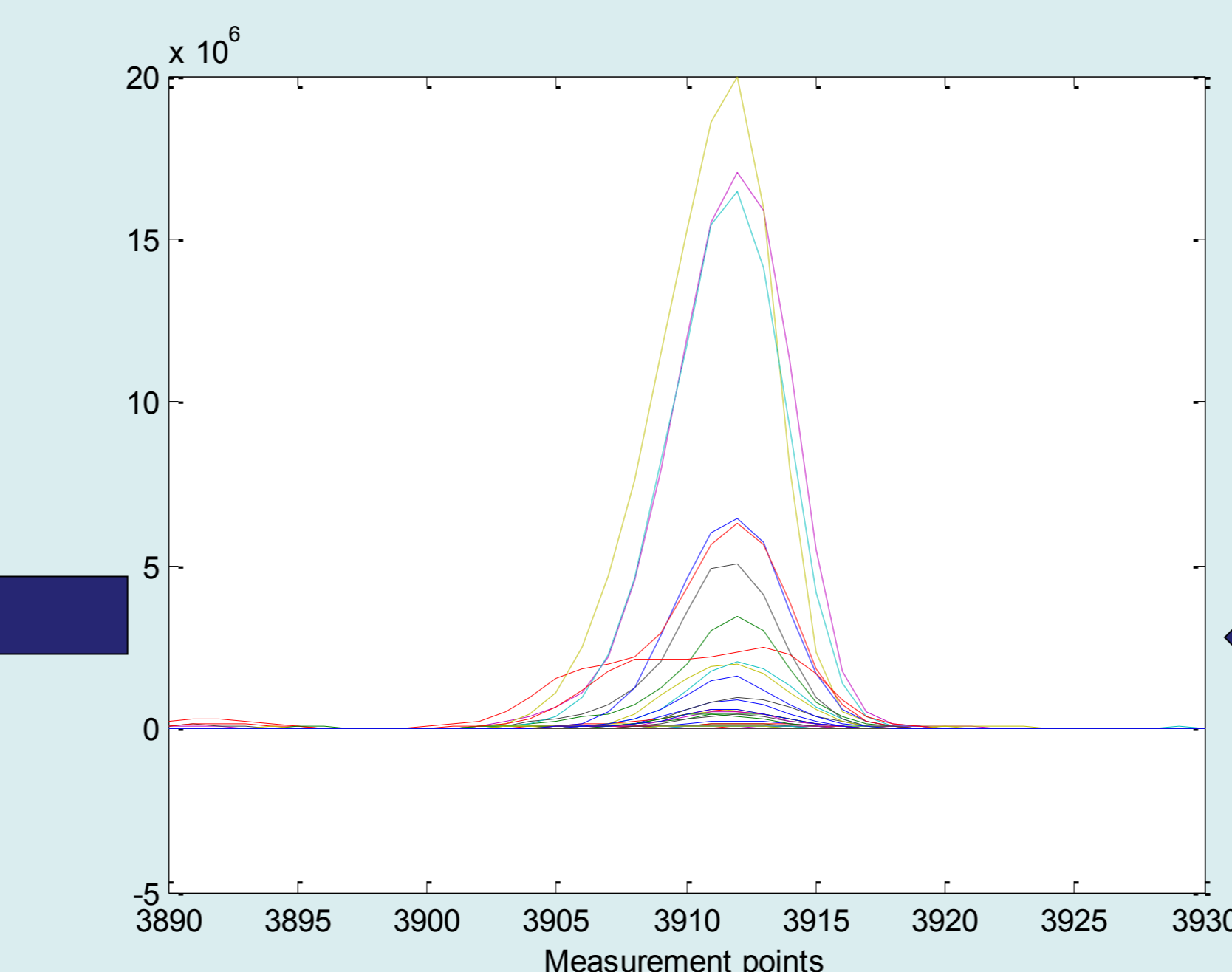
6. The mass spectra of biomarker candidate (elution time 49.1 min) which was identified as hexacosanoic acid.



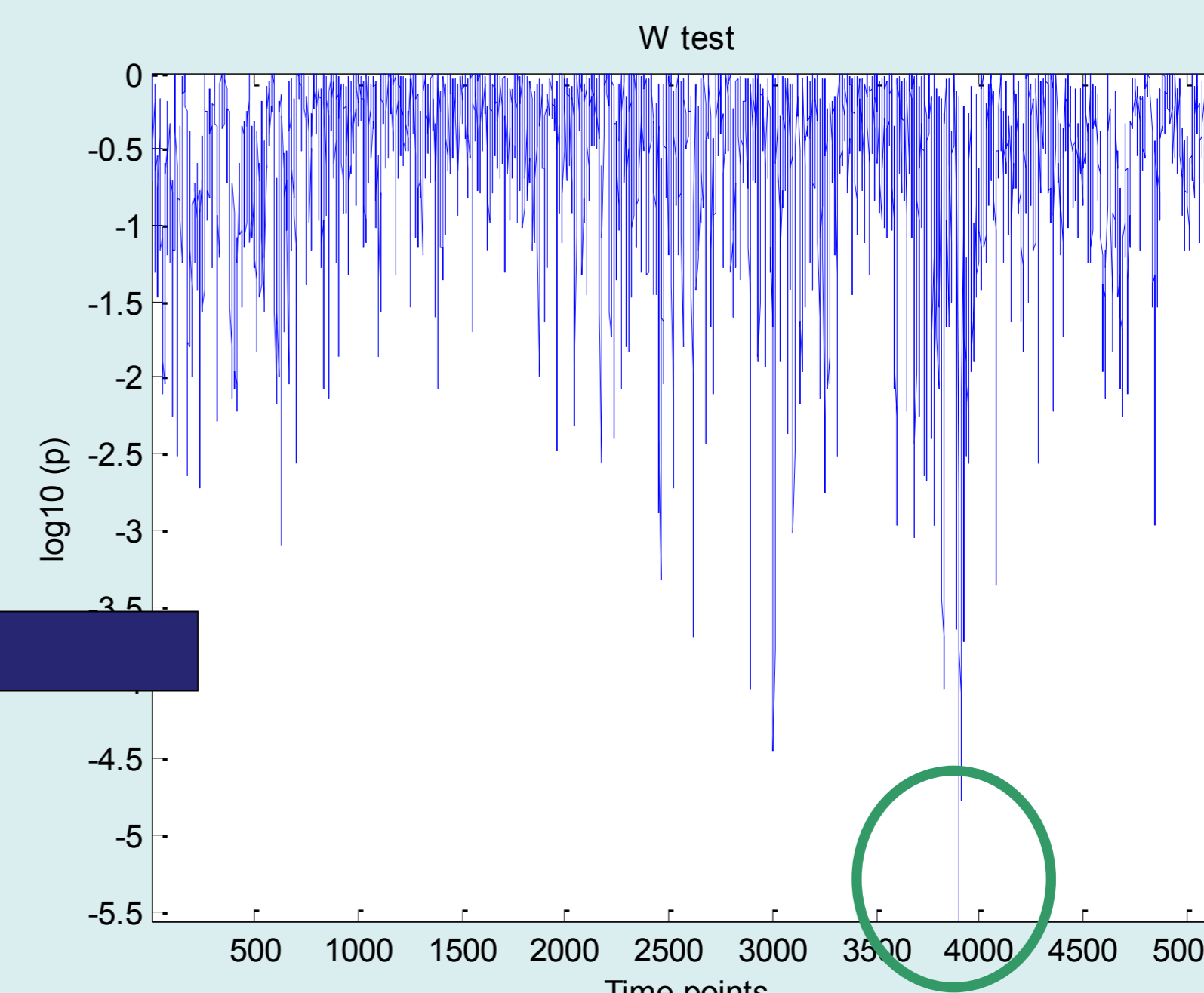
2. All TIC chromatograms after background correction, data averaging and time warping.



3. Score plot of 43 samples. TB sample (red dot). Non-TB sample (green star).



5. The chromatographic profiles of a biomarker candidate in 43 samples.



4. Wilcoxon rank sum test of all time points.

- Conclusions:
- In the PCA plot, there is no obvious discrimination between the two groups.
 - By the W-test, (only) one biomarker candidate was found: hexacosanoic acid, but further validation is needed.
 - Fast diagnosis of tuberculosis in sputum samples using THM-GC-MS is possible and does have potential.

Acknowledgements:

