

# Detection of biomarkers for the diagnosis of tuberculosis by multidimensional GC-MS

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Ngoc A Dang<sup>1</sup>, Arend Kolk<sup>1</sup>, Hans-Ulrich Baier<sup>2</sup>,  
Hans-Gerd Janssen<sup>1,3</sup>

<sup>1</sup>Analytical Chemistry Group, University of  
Amsterdam, P.O. Box 94157, 1090 GD Amsterdam,  
The Netherlands

<sup>2</sup>Shimadzu Europe, Albert-Hahn-Straße 6, 47269  
Duisburg, Germany

<sup>3</sup>Advanced Measurement and Data Modelling,  
Unilever Research, P.O. Box 114, 3130 AC  
Vlaardingen, The Netherlands

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### Introduction

Tuberculosis (TB) is a global emergency caused by *Mycobacterium tuberculosis* (MTB). One third of the world population is infected. Finding biomarker(s) for rapid diagnosis of TB becomes one of the essential requirements for TB control. Single biomarkers or combination of a few biomarkers fail in identification of MTB. Recently we have developed a fully automated GC-MS method using **T**hermally assisted **H**ydrolysis and **M**ethylation (THM) for sputum analysis from TB-suspected patients [1]. In addition,

using chemometrics, we found a list of 20 potential compounds giving better accuracy for diagnosis of TB. Unfortunately, the method still needs further improvement because of the adverse effect of the sputum matrix on the detection limit. Moreover, the diagnosis of TB in HIV positive patients, a high risk group for multidrug resistant TB, remains a problem because their sputum contains fewer mycobacteria than the sputum from HIV negative TB patients.

### Aim

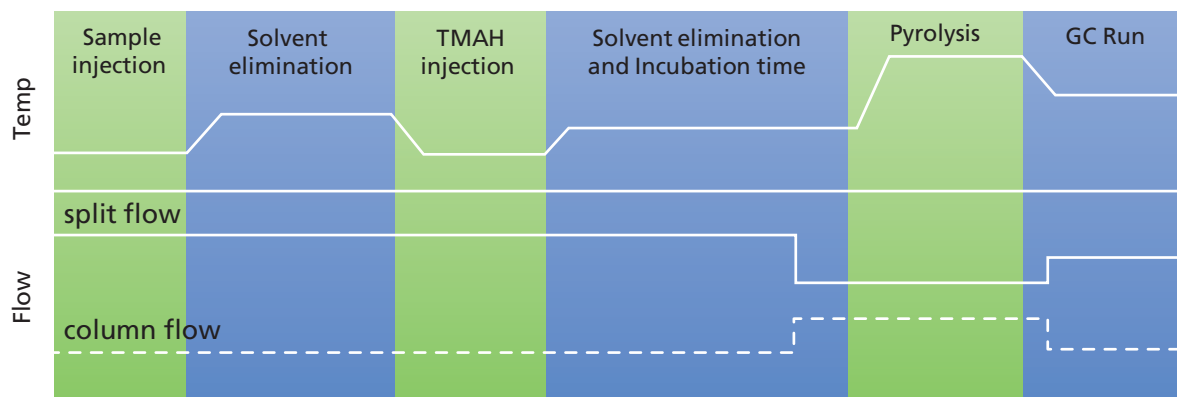
To improve the sensitivity and reduce the negative influence of the sputum matrix, we have developed a

multidimensional heart-cut GC-MS method (MD-GC-MS) and used this for the detection of TB biomarkers in sputum.

### How THM works

Equipment used includes an OPTIC PTV injector (ATAS GL), an autosampler (PAL), a GC-MS or MD-GC-MS (Shimadzu). Fig. 1 shows the THM procedure of decontaminated sputum:

- 1) Inject sputum sample into PTV injector at 40°C.
- 2) Heat injector to 120°C during 3 min for solvent elimination.
- 3) Inject tetra methyl ammonium hydroxide reagent into injector at 40°C.
- 4) Heat injector to 120°C during 1.5 min and incubate material inside the liner for 1 min for methylation.
- 5) Heat injector to 450°C during 3 min for pyrolysis and then cool down injector to 320°C until the end.



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## Biomarker Detection in Sputum by 1D-GCMS

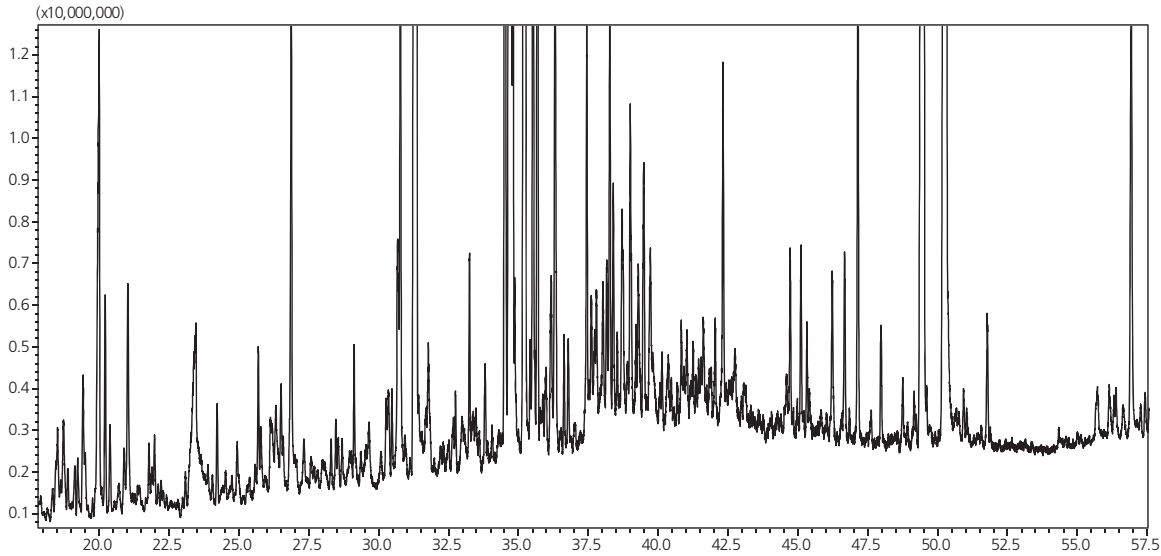


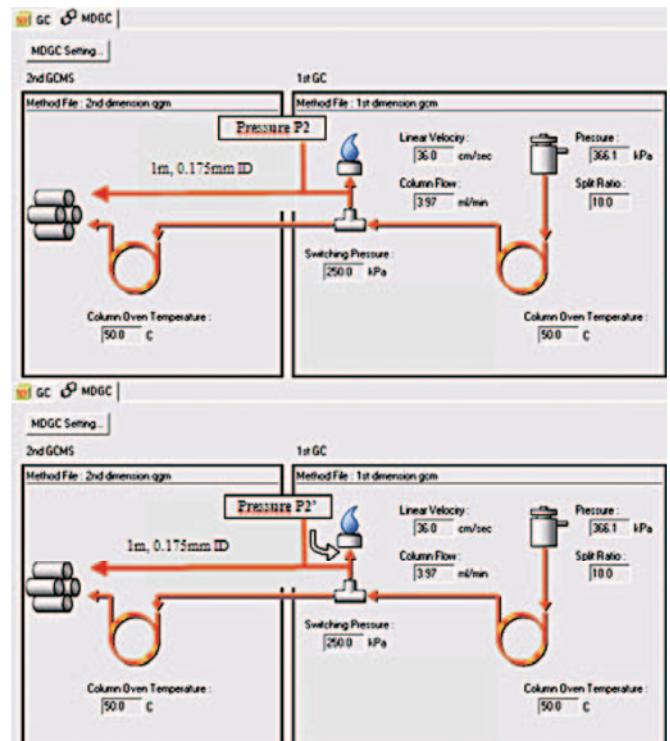
Fig. 2 shows the very complex chromatogram of a sputum sample using 1D-GC-MS. Here, problems encountered include a high background, co-elution with overlapping

peaks, high risk for system contamination and high cost due to the need to frequently change the column.

## Solution by using MD-GC-MS

Flow diagram showing the configuration in standby mode (top) and cut mode (bottom) of MD-GC-MS (Fig. 3):

- 1) All dirt is eliminated in 1<sup>st</sup> dimension, only the peaks of interest are transferred totally (100% recovery) to the second column.
- 2) The transferred peaks are separated in 2<sup>nd</sup> dimension and detected by MS.



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## Sputum Results

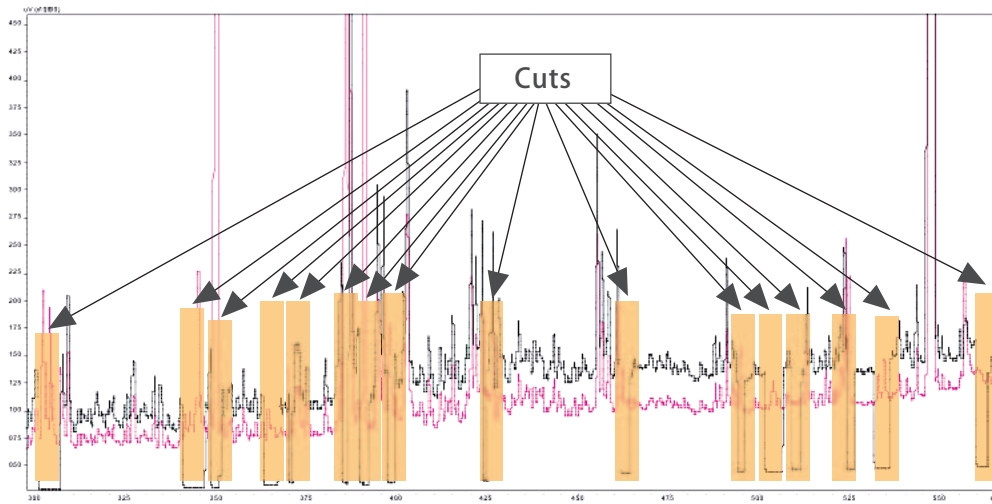


Fig. 4 shows the 1<sup>st</sup> dimension FID chromatogram of sputum sample in standby mode (Pink) and in cut mode with 16 cut positions for 20 target compounds (Black).

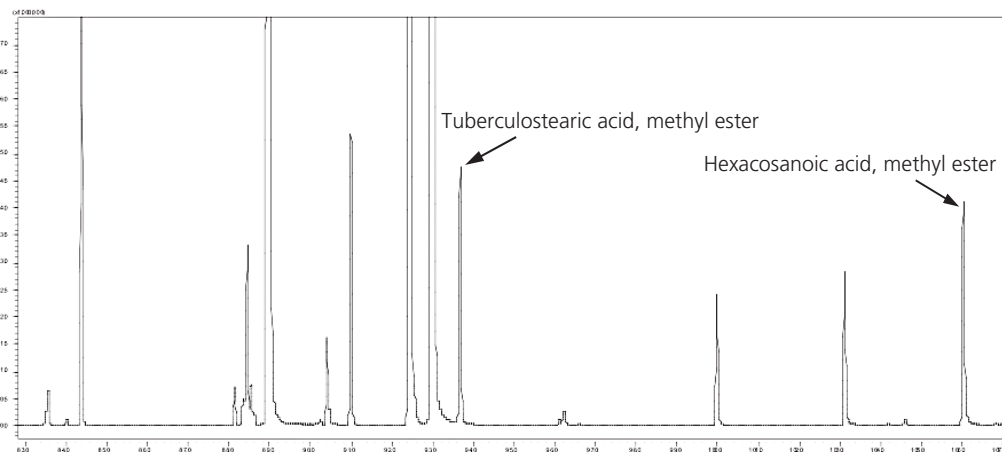


Fig. 5 shows the 2<sup>nd</sup> dimension GC-MS chromatogram of the sputum sample. The clean chromatogram allows accurate quantification of the 20 biomarkers.

## Conclusion

With MD-GC-MS, peaks of interest from the first column can be easily cut and sent to the second column for further separation and identification by MS, while 'dirt' is

effectively eliminated. This method is showing promise for the analysis of complex biological samples, such as our sputum.

## Reference

<sup>1</sup>Kaal E, Kolk AHJ, Kuijper S, Janssen HG J Chromatogr A. 2009 1216(35): 6319-25



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