Paediatric Faecal VOC Analysis: Method Optimisation

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Introduction
Solid phase micro extraction (SPME) – gas chromatography (GC) – mass spectrometry (MS) is a well-established method for volatile organic compounds (VOCs) analysis. VOCs are the end products of complex metabolic networks within an organism and, thus, they may reflect patients’ health state. Therefore, SPME-GC-MS has been applied to analyse biological samples aiming to develop an accurate clinical diagnostic test. To establish a widely used method Van de Kant et al. claimed that background samples and standardization are necessary. Yet, there is a lack of literature evaluating sample preparation steps in direct faecal VOCs analysis; different groups use different methods that ultimately prevent results comparison.

Aims
The project aims to develop a SPME-GC-MS method for direct analysis of VOCs in human faecal samples, prior to a larger study.

Methods
Samples were left on the auto-sampler (Combi PAL, CTC Analytics, CH) for a maximum of 14 hours preceding analysis. They were then pre-incubated at 60°C for 30 minutes prior to fibre exposure for 20 minutes while temperature was kept constant. Compounds were desorbed in the GC-MS (Perkin Elmer, UK) at 220°C. The GC-Oven was set at 40°C, held for 1 minute before increasing to 220°C at a rate of 5°C/min and held for 4 minutes. The MS scan range was set from 10 to 300 m/z.

The following parameters were optimised:
- **Mass**: 50mg, 100mg, 450mg and 700mg
- **Vial volume**: 10ml and 2ml
- **Compound addition**: 0.5ml and 1ml of saturated solution of NaCl; 0.5ml of H3PO4 (0.85% and 1.7%) and 0.5ml of NaOH (5% and 10%)
- **SPME fibres**: CAR/PDMS (85µm) and DVB/CAR/PDMS (50/30µm).

Data as processed using AMDIS in conjunction with the NIST library and the R package Metab (Agglo et al., 2011). All statistics were done in R. P-value < 0.05 were considered as significant.

Results

![Graph 1: Scatterplot comparing the lag of the mean of VOCs intensities in 2 and 10ml for the 3 samples tested (n=group for sample 1 and 2 and n=group for sample 3).](image1)

Sample 1
Sample 2
Sample 3

Vial volume optimisation

![Graph 2: Scatterplot comparing the lag of the mean of VOCs intensities at time 0 and after 14 hours (n=group). The diagonal black segment represents the function area and the grey area represents the 5% tolerance region.](image2)

Sample 1
Sample 2
Sample 3

SPME coating optimisation

![Graph 3: Scatterplot comparing the lag of the mean of VOCs intensities at time 0 and after 14 hours (n=group). The diagonal black segment represents the function area and the grey area represents the 5% tolerance region.](image3)

Sample 1
Sample 2
Sample 3

Salt, acid and base addition

![Graph 4: Bar plot illustrating the influence of the addition of 0.01M of sodium hydroxide 5% (NaOH) and 10% (NaOH) 0.5M of phosphoric acid 0.8% (H3PO4) and 1.7% (H3PO4), 0.5% (H3PO4) and 1% (H3PO4) of saturated sodium chloride solution on the mean of VOCs (±SEM). The control being samples on their own (n/group).](image4)

Sample 1
Sample 2
Sample 3

Discussion
Method optimisation is an essential step to enhance the repeatability of the analytical process. The results show that a sample mass between 450mg and 700mg will be optimal with slightly more compounds identified with 450mg. Masses between 50 and 150mg, more adequate for paediatric studies, have been investigated and show no differences either in the number of VOCs identified or in their intensities. The volume of the vial, the SPME coating and the addition of salt, acid or base shows no difference at 5% significance. Samples are not affected by staying for 14 hours on the auto-sampler, which allow their measurement overnight. The method repeatability has been assessed and meets expected requirement for a metabolomics study. Finally, it has been shown that samples are altered while being analysed more than once.

Conclusion
A reliable method for the direct SPME-GC-MS analysis of human faecal samples has been established, based on the results define above.

![Diagram summarising the optimised method for metabolomics studies using SPME-GC-MS applied on faecal human samples.](image5)

Figure 8: Diagram summarising the optimised method for metabolomics studies using SPME-GC-MS applied on faecal human samples.