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 300m/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 H_3PO_4 (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 (Aggio et al., 2011). All statistics were done in R. P-value < 0.05 were considered as significant.

Results

Mass optimisation

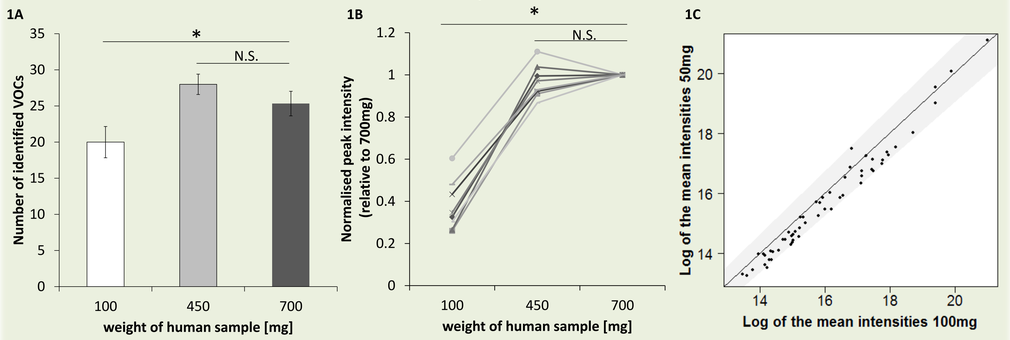

Figure1. (A) Bar plot showing the mean of the number of VOCs identified (± SEM) for the three masses tested (n=3/group)(*p<0.05). **(B)** Mean of the intensities of the 8 identified VOCs present in all nine measurements (n=3/group). **(C)** Scatterplot comparing the mean VOCs' intensities between 50 and 100mg (n=4, N=4). The diagonal black segment represents the function x=y and the grey area represents the 5% tolerance region.

Vial volume optimisation

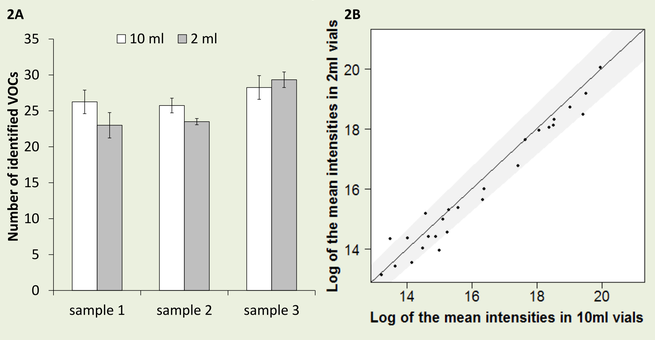

Figure 2. (A) Bar plot showing the mean of VOCs identified (± SEM) in 2 and 10ml for the 3 samples tested (n=4/group for sample 1 and 2 and n=3/group for sample 3). **(B)** Scatterplot comparing the log of the mean of VOCs' intensities identified in 2 and 10ml (n=4, N=3). The diagonal black segment represents the function x=y and the grey area represents the 5% tolerance region.

SPME coating optimisation

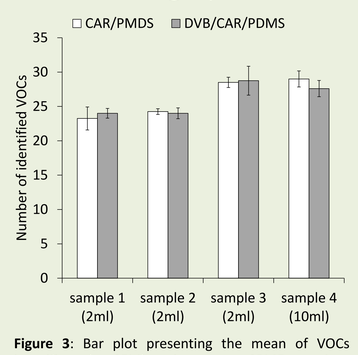

Figure 3: Bar plot presenting the mean of VOCs identified (± SEM) using both SPME coatings investigated (DVB-CAR-PDMS & CAR-PDMS) (n=4/group in 2ml vials; n=5/group in 10ml vials).

Salt, acid and base addition

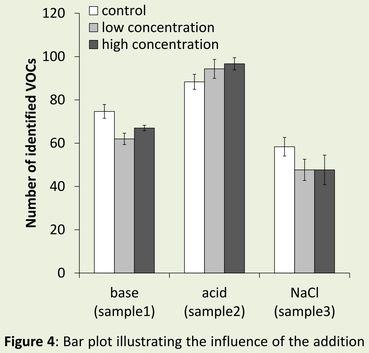

Figure 4: Bar plot illustrating the influence of the addition of 0.5ml of sodium hydroxide 5% (low) and 10% (high), 0.5ml of phosphoric acid 0.85% (low) and 1.7% (high), 0.5 (low) and 1ml (high) of saturated sodium chloride solution on the mean of VOCs (± SEM). The control being samples on their own (n=3/group).

Repeatability

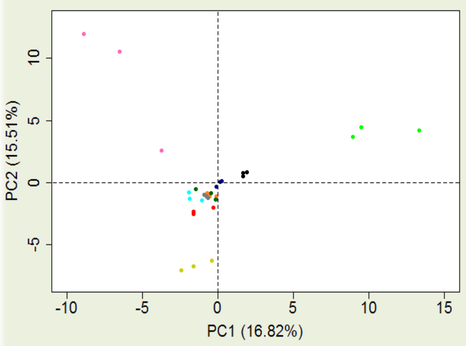

Figure 5: Principal component analysis (PCA) of 10 sets of triplicate used to determine the repeatability of the method.

Table 1: Means of VOCs identified ± SD in each triplicate and the percentage of compounds having a coefficient of variation lower than 30%.

Samples	Number of VOCs ± S.D. (in every triplicate)	VOCs with CV < 30% (%)
Sample 1	24±3 (19)	100
Sample 2	23±1 (18)	100
Sample 3	20±2 (12)	92
Sample 4	48±5 (36)	89
Sample 5	37±4 (26)	81
Sample 6	50±4 (39)	95
Sample 7	32±2 (21)	90
Sample 8	26±4 (18)	67
Sample 9	26±2 (19)	95
Sample 10	27±2 (18)	94

Multiple analysis of the same sample

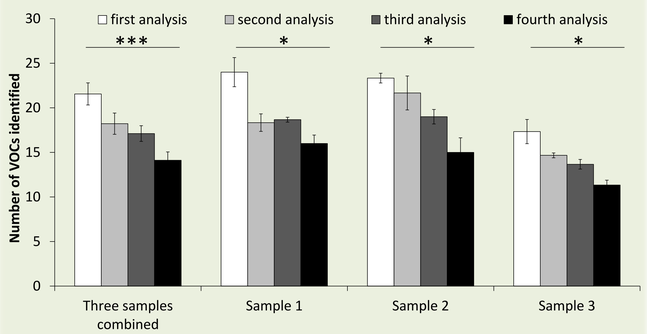

Figure 6: Bar plot showing the mean number of VOCs identified (± SEM) in the 4 analyses performed on 3 sets of triplicates (n=3/group)(*p<0.05; ***p<0.001).

Keeping sample at 1°C for 14 hours

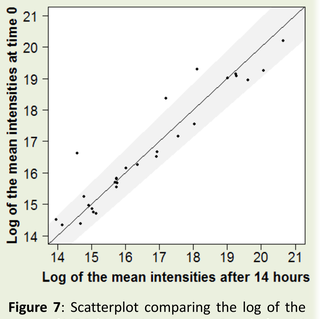

Figure 7: Scatterplot comparing the log of the mean of VOCs' intensities at time 0 and after 14 hours (n=3/group). The diagonal black segment represents the function x=y and the grey area representing the 5% tolerance.

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 100mg, 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.

Acknowledgment

Action Medical Research is thanked for partly funding this work. Rachel Jackson and Elizabeth Simcox and all the staff at the Birmingham Women's Hospital, Royal Wolverhampton Hospital, Sheffield Teaching Hospitals, Birmingham Heartlands Hospital, University Hospitals Coventry and Warwickshire, Liverpool Women University and University Hospitals of Leicester for collecting human samples on-site. Finally the department of Gastroenterology is thanked for their help and advice during the whole project.

References

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Conclusion

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

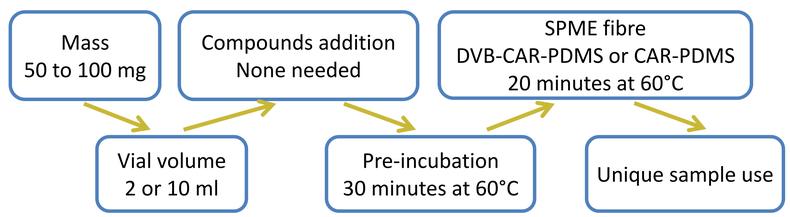

Figure 8: Diagram summarising the optimised method for metabolomics studies using SPME-GC-MS applied on faecal human samples.