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CROSS PLATFORM COMPARISON OF THREE MASS SPECTROMETRY METHODS FOR BREATH ANALYSIS

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INTRODUCTION

The analysis of volatile organic compounds (VOCs) within exhaled breath may offer a non-invasive method for the diagnosis of several diseases including cancer and infectious disease. To meet the requirements of large-scale clinical trials, sample collection and transfer must be secure and efficient, and analysis of VOCs must be fast, accurate and reliable. A lack of standardised practices within the field of breath research has however limited clinical impact, as it has proven difficult validate findings of individual studies. There remains a need to better understand variation in VOC levels detected by different analytical techniques and platforms in order to inform optimal practices.

AIM

The aim of this study was to compare VOC detected by direct injection (DI) PTR-MS and SIFT-MS as well as thermal desorption (TD) PTR-MS

MATERIAL AND METHODS

Breath samples were collected from 20 healthy volunteers who provided informed written consent. Samples were collected by asking subject to exhaled directly into a six-liter Nalophan bag (**Figure 1**).

Using a three-way connector sample bag were connected to PTR-MS and SIFT-MS instruments permitting simultaneously analysis by direct injection. Breath from the same sample bag was subsequently transferred to TD tubes (Tenax TA/Carbograph 5TD, Bio-Monitoring C4-C30, Markes Ltd) using the EasyVOC® manual pump device. In total four TD tubes were loaded with 500ml of breath from each sample. TD tubes were analysed by TD-PTR-MS.

Target VOCs analysed in both online (direct injection) and offline (TD) experiments are presented in **Tables 1** and **2**. Analysis focused on the comparison of (i) direct injection PTR-MS and SIFT-MS and (ii) direct injection PTR-MS and TD-PTR-MS. Data analysis was based on the coefficient correlation with Perason's correlation and Spearman's rho.

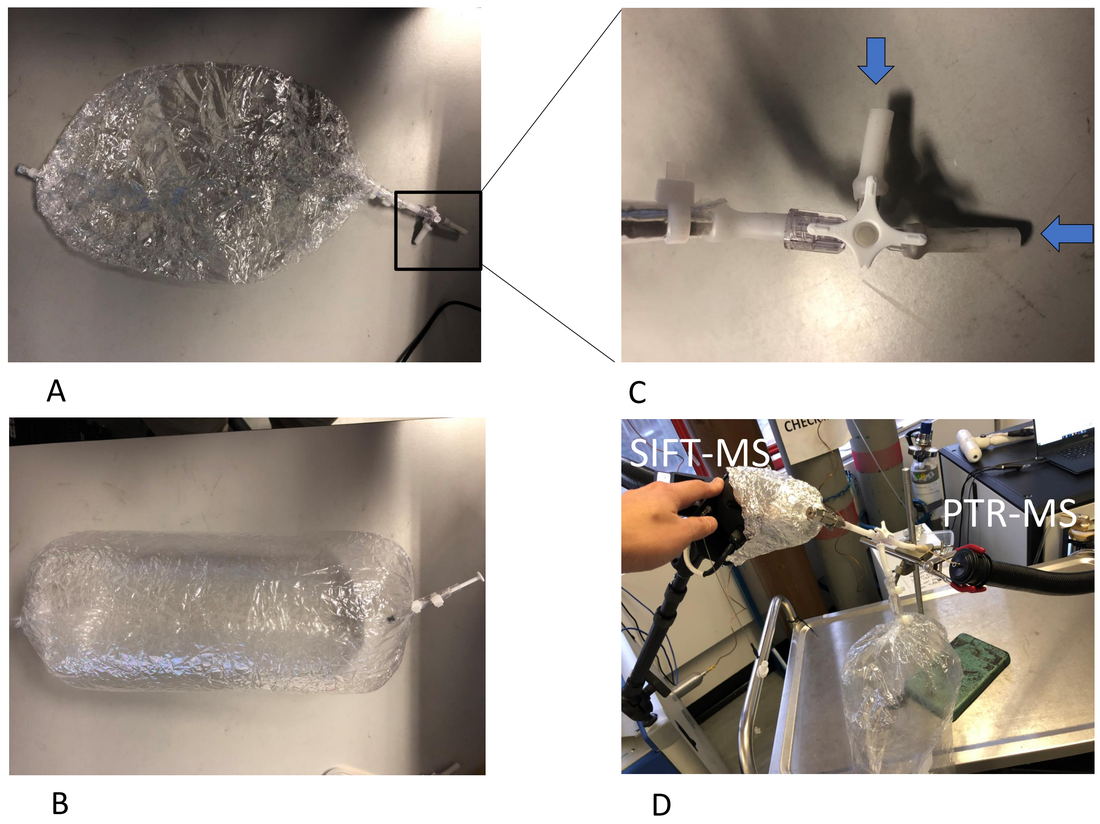


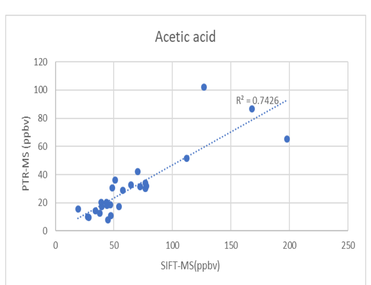
FIGURE 1

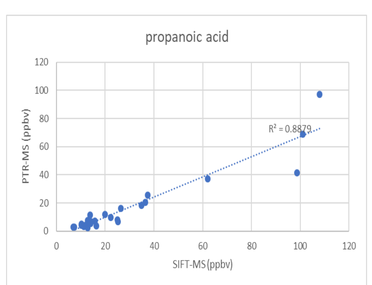
(A and B) Nalophan sample bag used for breath sampling. (C) three way connector (D) used to attach breath sample bags to the inlet of both PTR-MS and SIFT-MS instruments

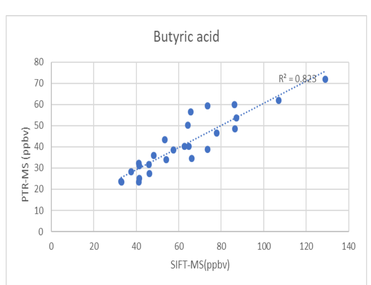
RESULTS

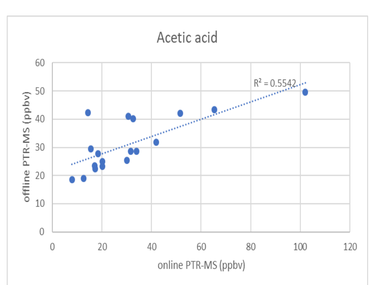
(i) Direct injection PTR-MS vs SIFT-MS: for the abundant compounds acetone and isoprene good correlation was observed between samples analysed by direct injection PTR-MS and SIFT-MS (R^2 >0.89)(**Figure 2, Table 1**). With the exception of pentanoic acid (R^2 = 0.42) volatile fatty acids showed good correlation when analysed by direct injection PTR-MS and SIFT-MS. Correlation between direct injection methods was however improved in patients in whom pentanonic acid levels were >1ppb (n=5; R^2 = 0.916). Phenol showed acceptable correlation between direct injection methods (R^2 = 0.79). Whilst phenol was analysed using H_3O^+ precursor ion in direct injection studies, previous study have shown that the NO+ may be a better choice of precursor ion while using PTR-MS to quantify phenol.

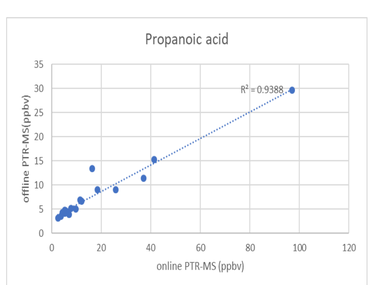
(ii) Direct injection PTR-MS vs TD-PTR-MS: results are similar to direct injection experiments. Generally good correlation could be found in most of the compounds in the study and the results are shown in **Figure 3, Table 2**. For pentanoic acid improved correlation was not found when only patients with higher concentrations of the compound (>1 ppb) were considered.

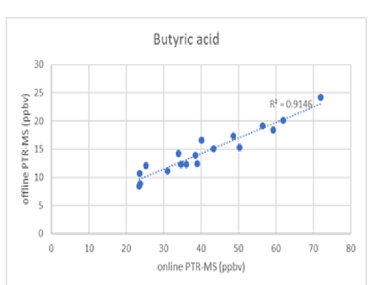


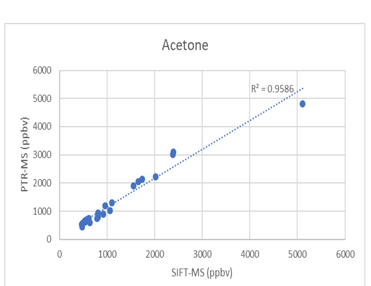


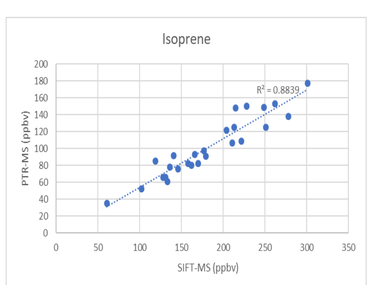


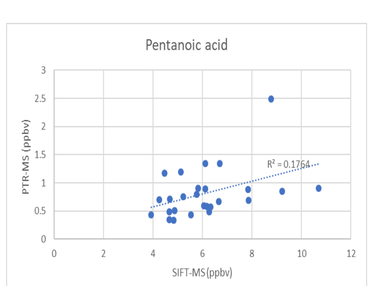


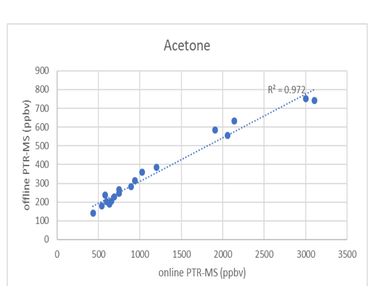


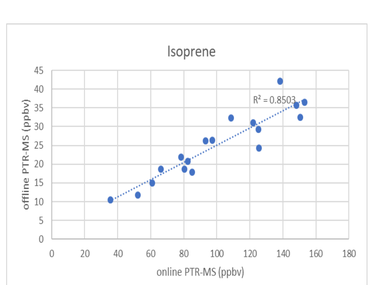












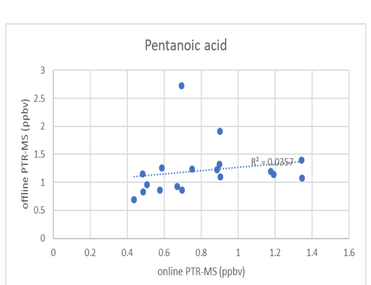


FIGURE 2

Correlation of direct injection samples from PTR-MS And SIFT-MS.

TABLE 1 Correlation of direct injection samples from PTR-MS And SIFT-MS

Compound	Class	MS 1	Precursor ion	MS 2	Precursor ion	Pearson	*P*	spearman's rho	*P*
Acetic acid	Fatty acid	SIFT	H_3O^+	PTR	H_3O^+	0.862**	<0.001	0.854**	<0.001
Propanoic acid	Fatty acid	SIFT	H_3O^+	PTR	H_3O^+	0.942**	<0.001	0.896**	<0.001
Butyric acid	Fatty acid	SIFT	H_3O^+	PTR	H_3O^+	0.907**	<0.001	0.907**	<0.001
Pentanoic acid	Fatty acid	SIFT	H_3O^+	PTR	H_3O^+	0.420*	0.016	0.413*	0.018
Pentanoic acid (2)	Fatty acid	SIFT	H_3O^+	PTR	H_3O^+	0.916*	0.014	0.900*	0.019
Hexanoic acid	Fatty acid	SIFT	H_3O^+	PTR	H_3O^+	0.971**	<0.001	0.729**	<0.001
Hexanoic acid (2)	Fatty acid	SIFT	H_3O^+	PTR	H_3O^+	0.974**	<0.001	0.939**	<0.001
Acetone	Ketone	SIFT	H_3O^+	PTR	H_3O^+	0.979**	<0.001	0.973**	<0.001
Isoprene	Hydrocarbon	SIFT	NO^+	PTR	H_3O^+	0.900**	<0.001	0.893**	<0.001
Methanol	Alcohol	SIFT	H_3O^+	PTR	H_3O^+	0.966**	<0.001	0.903**	<0.001
Ethanol	Alcohol	SIFT	H_3O^+	PTR	H_3O^+	0.983**	<0.001	0.976**	<0.001
Phenol	Aromatic	SIFT	H_3O^+	PTR	H_3O^+	0.794**	<0.001	0.506**	0.004
Phenol	Aromatic	SIFT	NO^+	PTR	H_3O^+	0.683**	<0.001	0.826**	<0.001

(2) Indicates consideration of subjects whose values for this VOC was >1 ppb

FIGURE 3

Correlation of direct injection PTR-MS and TD-PTR-MS

TABLE 2 Correlation of direct injection PTR-MS and TD-PTR-MS

Compound	Class	MS 1	Precursor ion	MS 2	Precursor ion	Pearson	*P*	spearman's rho	*P*
Acetic acid	Fatty acid	DI	H_3O^+	TD	H_3O^+	0.744**	<0.001	0.684**	0.001
Propanoic acid	Fatty acid	DI	H_3O^+	TD	H_3O^+	0.969**	<0.001	0.932**	<0.001
Butyric acid	Fatty acid	DI	H_3O^+	TD	H_3O^+	0.956**	<0.001	0.959**	<0.001
Pentanoic acid	Fatty acid	DI	H_3O^+	TD	H_3O^+	0.189	0.226	0.459*	0.028
Pentanoic acid (2)	Fatty acid	DI	H_3O^+	TD	H_3O^+	0.260	0.370	0.400	0.300
Hexanoic acid	Fatty acid	DI	H_3O^+	TD	H_3O^+	0.905**	<0.001	0.862**	<0.001
Hexanoic acid (2)	Fatty acid	DI	H_3O^+	TD	H_3O^+	0.838**	0.009	0.857**	0.007
Acetone	Ketone	DI	H_3O^+	TD	H_3O^+	0.986**	<0.001	0.973**	<0.001
Isoprene	Hydrocarbon	DI	H_3O^+	TD	NO^+	0.922**	<0.001	0.915**	<0.001
Methanol	Alcohol	DI	H_3O^+	TD	H_3O^+	0.851**	<0.001	0.719**	<0.001
Ethanol	Alcohol	DI	H_3O^+	TD	H_3O^+	0.988**	<0.001	0.967**	<0.001
Phenol	Aromatic	DI	H_3O^+	TD	H_3O^+	0.528*	0.012	0.216	0.195
Phenol	Aromatic	DI	H_3O^+	TD	NO^+	0.543**	0.010	0.647**	0.002

(2) Indicates consideration of subjects whose values for this VOC was >1 ppb

CONCLUSION

For the majority of examined VOCs good correlation was observed between direct injection SIFT-MS and PTR-MS as well as direct injection PTR-MS and TD-PTR-MS. Further data processing based on chemical kinetics may solve the different ratio on each organic compound. Absolute concentrations of VOCs detected using each mass spectrometry method were however different. Further kinetical analysis including investigation of the impact of flow rate and molecular weight may be useful in understanding these observed differences.