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Supplementary information

Rapid ex vivo molecular fingerprinting of biofluids using laser-assisted rapid evaporative ionization mass spectrometry

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Rapid *ex vivo* molecular fingerprinting of biofluids using laser assisted rapid evaporative ionization mass spectrometry

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Supplementary Discussion 1

Direct infusion mass spectrometry (DIMS) experiments

Objective

The experiments that are reported here had the objective to evaluate direct infusion mass spectrometry (DIMS) as an alternative direct mass spectrometry technique for rapid metabolic fingerprinting of biological (semi-)fluids. To this end, three matrix types were considered, *i.e.* saliva, urine, and blood plasma.

Biological samples

For DIMS experiments, a sample pool was considered for each matrix type, which was established by pooling equal aliquots from 10 different biological samples. These samples were from the same sample batch that was used and described for the LA-REIMS method validation experiment in the main manuscript (section 'Anticipated results'). Urine and plasma samples (stored at -80 °C) were not subjected to any pre-treatment and thawed at 4-6 °C shortly before the DIMS experiment was performed. Saliva samples were centrifuged and filtrated before storage (at -80 °C) and thawed at 4-6 °C shortly before the DIMS experiment was performed. Detailed information on the specific saliva preparation can be retrieved in the section 'Sample preparation' in the main manuscript.

Experimental setup

For DIMS, 100 μL of each sample pool (*i.e.* matrix type) was infused at a rate of 200 μL/min (using a syringe pump) into the ESI-source, which was directly coupled to the Waters Xevo G2-XS MS-instrument. The MS parameters (cone and heater bias voltage, and scan time) were kept as specified in the manuscript, in the section 'Equipment set-up'. MS acquisition was attained for 30 s, with 3 technical replicates per matrix type. Hereby, analysis was performed in both the positive and negative ion mode. After each infusion event, the source and infusion lines were cleaned with isopropylalcohol for 10 s. The acquired data files were processed as described in the main manuscript under the section 'LA-REIMS data analysis & classification modelling', steps 1 – 11.

Molecular DIMS fingerprints

As a major parameter to assess the potential of DIMS for rapid fingerprinting of biological fluids, the metabolome coverage was assessed. The total number of detected features per matrix type upon DIMS analysis was 788 (56.6% in negative ionization) for plasma, 2071 (53.1% in negative ionization)

for saliva, and 1600 (44.1% in negative ionization) for urine. It may be noted that these numbers are significantly lower than those obtained by LA-REIMS (section 'Anticipated results'). Indeed, for LA-REIMS analysis, the total number of features was 3015 for plasma (47.2% in negative ionization), 3628 for saliva (52.0% in negative ionization), and 3767 for urine (54.1% in negative ionization). The DIMS-associated molecular fingerprints are presented in Fig. 1. Some degree of similarity can be observed between DIMS and LA-REIMS, especially with respect to the plasma fingerprints. One important reason for the lower metabolome coverage upon DIMS may relate to the fact that all sample constituents (incl. non-volatile residues)[1] are introduced at the same time into the ionization source, whereas for LA-REIMS there is a prior selection of metabolome constituents through the process of laser ablation. Indeed, specific sets of metabolites and mostly lipids are desorbed from the ablated sample, for which there is no or far less interference from macromolecules during ionization. As such, a higher degree of ionization suppression can be presumed for DIMS analysis, resulting in a lower metabolome coverage. In this respect, the usage of nanoelectrospray ionization (nESI) instead of standard electrospray ionization (ESI) may provide a proven strategy to achieve higher ionization efficiency, minimizing ionization suppression or enhancement effects. This is *e.g.* demonstrated by Southam *et al.* (2017)[2], where the coverage for DIMS analysis could be increased significantly by implementing nESI, in combination with spectral stitching.

Practical considerations DIMS versus LA-REIMS

Although various cleaning steps were included to avoid contamination of the ionization source or infusion line, a significant impact of contamination was already observed after a few runs ($n < 30$), with a significant reduction (> 10%) of the signal intensity. This was confirmed by visual inspection of the ionization source (Fig. 2). This of course significantly affects sample throughput as well as it can be presumed that dismantling and deep cleaning of the source will need to be performed at a frequent basis, which implies that sample cohorts need to be separated into more analytical batches with additional issues of inter-batch variability. In this regard, usage of DIMS for biofluid analysis is mostly associated with extraction, dilution or purification of samples[2,3,4], which would even be indispensable for semi-solids such as feces. However, the incorporation of sample pre-treatment and/or removal of the volatile residues through routinely scheduled maintenance of the ion source does not align with the required settings for clinical practice.

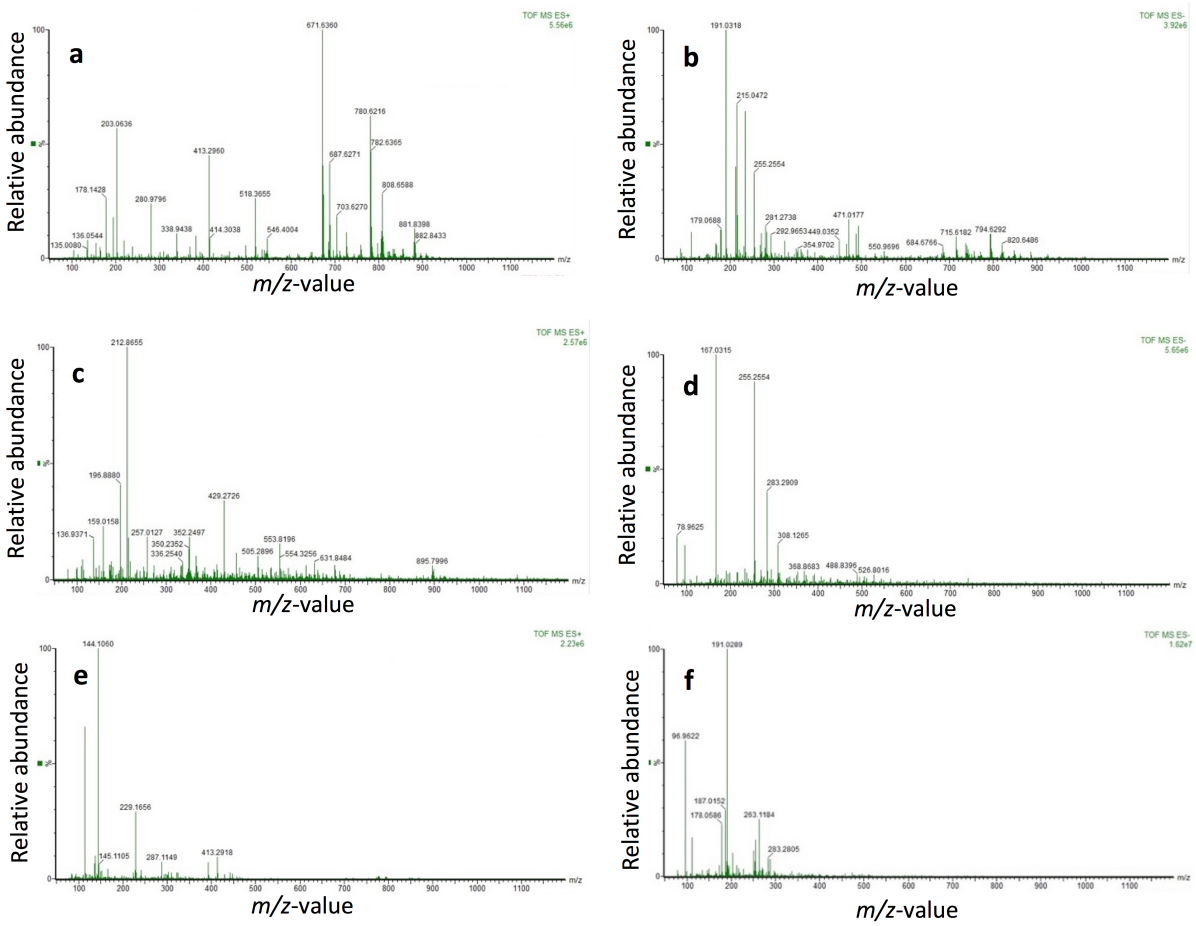

Fig. 1. | Mass spectra and detected features, as obtained by DIMS analysis of plasma (a, positive ionization; b, negative ionization), saliva (c, positive ionization; d, negative ionization) and urine (e, positive ionization; f, negative ionization) (pooled samples, n = 10).

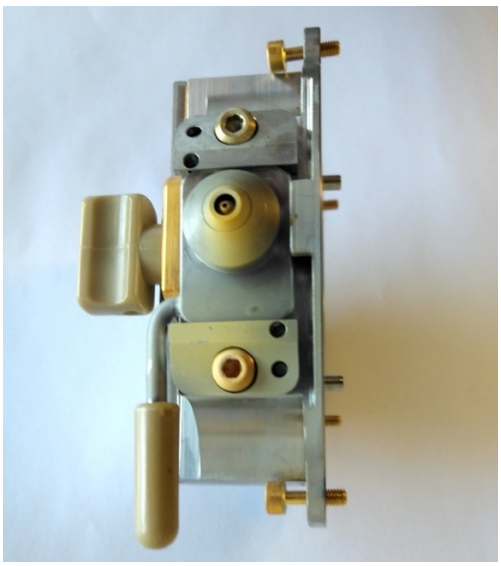

Fig. 2. | ESI-source cone contamination (orange-yellow film) after direct infusion MS-analysis of ca. 30 samples (10 urine, 10 saliva and 10 plasma).

Consulted references

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