Evaluation of Mitra microsampling device for multiple reaction monitoring based measurements of proteins in serum

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1. Introduction

Recently, the use of dried serum spots has gained enormous interest in the context of serum protein analysis. Considering the economics and simplicity associated with transporting dried biofluids, this approach has several advantages over typical liquid sampling.[1] The Mitra© microsampling device is a biological specimen collection method based on Volumetric Absorptive Microsampling technology (VAMS™). The Mitra sampler tip consists of a hydrophilic porous material which was designed to take up a fixed volume (10 µL) biological fluid by capillary action.[2] As such, the Mitra sampler offers advantages for quantitative sampling of serum and subsequent storage compared to the use of conventional methods.

2. Aims and Objectives

Here, we evaluated the use of Mitra microsampling device for sampling serum and subsequent targeted measurement of proteins by multiple reaction monitoring (MRM).

3. Methods

Sampling and sample preparation

Mitra tips (n =3) were dipped into a reference serum sample until fully loaded. The tips were dried overnight (16.5 h) and the Mitra tip heads subsequently transferred to individual wells of 96 well plates. A laboratory standard operating procedure (SOP) was used for the trypsin digestion of serum loaded Mitra tips and liquid serum samples. To ensure complete wetting of the head of the Mitra tips the protocol was modified slightly by increasing the volume of solution used for the initial denaturation step (from 25 µL to 100 µL). Samples were prepared and run in triplicate and identical liquid serum samples used as controls. The experiment was repeated on three separate occasions by the same investigator. Following MRM analysis, mass spectrometry data were imported into Skyline version 3.7.0.10940 for analysis.

LC conditions

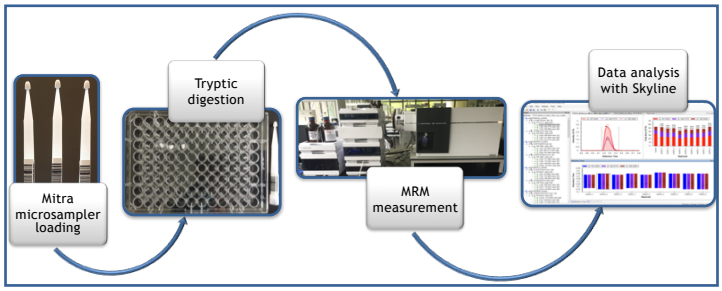
- Agilent 1290 Infinity LC

Mobile phase A	0.1 % formic acid in water
Mobile phase B	0.1 % formic acid in acetonitrile
Gradient elution	13 min starting from 0 to 95 % buffer B (0 min 3 % B, 1 min 8 % B, 8.50 min 28 % B, 9 min 35 % B, 10 min 95 % B, 12 min 95 % B, 13 min 3 % B)
Column	Agilent ZORBAX Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm
Column temperature	50 °C
Flow rate	0.4 mL/min
Injection volume	10 µL

MS conditions

- Agilent 6490 Triple Quadrupole LC/MS with iFunnel Technology
- 16 high abundant serum proteins (HAP) were measured by positive ion mode MRM
- Instrument performance was monitored by measuring proteins in well characterised reference serum digest at the beginning, middle and end of the MRM measurement.

Figure 1: Mitra microsampler workflow.



4. Results

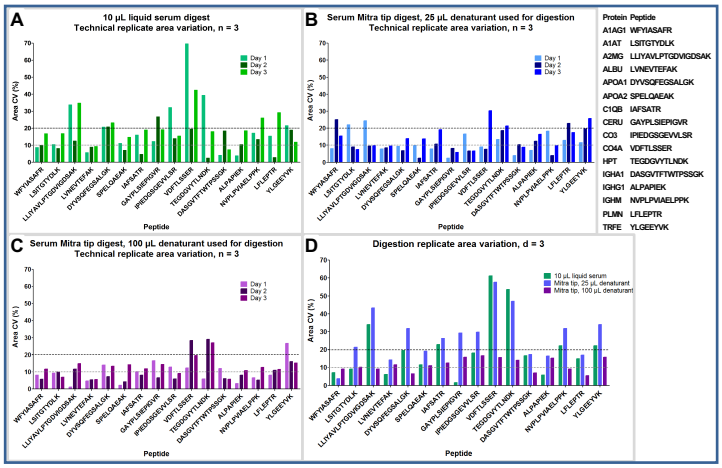
Instrument performance was evaluated by comparing peptide peak areas measured in analytical control samples run at the start (n=3), middle (n = 3) and end (n=3) of each round of analysis. Data revealed no drop in peak areas or increase in % coefficient of variance (% CV) across analytical replicates (n = 9), indicating instrument performance was stable across analysis (data not shown).

Within each batch run peptide peak areas yielded from analysis of Mitra digests vs liquid digests were comparable across the board (data not shown).

Interestingly, it was found that in technical replicate (TR) samples 73 % (liquid digest), 85 % (Mitra digest - 25 µL denaturant) and 92 % (Mitra digest - 100 µL denaturant) of peptides, could be measured with peak area CVs < 20 % (**Figure 2A-C**).

When comparing data acquired on different days (digestion replicate) it was found that 62 % (liquid digest), 37 % (Mitra digest - 25 µL denaturant) and 100 % (Mitra digest - 100 µL denaturant) of peptides, could be measured with peak area CVs < 20 % (**Figure 2D**).

Figure 2: Area CV (%) comparison of three technical replicates from 10 µL liquid serum digests (A) and serum containing Mitra tip digests. For Mitra tip digestion the use of 25 µL (B) vs. 100 µL denaturant solution (C) was evaluated. The area variation of digestion replicates from three different days was compared (D).



The impact of Mitra microsampling on digestion reproducibility was next assessed. It was found that the use of Mitra tips improved the reproducibility of digestions by 29 % (in presence of 25 µL denaturant solution) and by 37 % (in the presence of 100 µL solution). Overall, Mitra tip digests (using 100 µL denaturant solution) produced 43 % lower area CVs for digestion replicates compared to liquid serum digests (**Table 1**).

Table 1: Summary of area CVs (%) of 16 HAP peptides in 10 µL liquid serum digest, serum Mitra tip digest with 25 µL and 100 µL denaturant solution.

		10 µL liquid serum digest	Serum Mitra tip digest, 25 µL denaturant	Serum Mitra tip digest, 100 µL denaturant
Average area CV (%) for 16 peptides	Day 1, TR, n = 3	20.10	11.61	9.66
	Day 2, TR, n = 3	12.39	11.43	10.55
	Day 3, TR, n = 3	20.14	14.49	12.81
	DR, d = 3	20.45	27.58	11.59

5. Conclusions and Future Work

Mitra microsampling devices present a practical and user friendly quantitative device for sampling of serum for subsequent use in targeted protein MRM measurements. Their ease of use and increased level of precision and reproducibility compared to traditional liquid sampling allow for more consistent sample preparation and reduce the opportunity for pre-analytical errors. The data obtained here suggest that the potential use of Mitra devices for sampling, shipping and storing serum prior to measurement of serum protein biomarkers by MRM merits further investigation. Compared to dried serum spots (DSS) this approach is advantageous in that the workflow can be automated, it does not require spot punching and it collects a defined amount of serum.

As an ongoing experiment the stability of proteins in serum loaded Mitra tips will be evaluated over a six month time period. As part of future work, the digests will be analysed by using additional comprehensive MRM assays for detecting > 200 serum proteins. Furthermore, the digestion of Mitra tips on an automated workstation (AssayMAP Bravo, Agilent) will be investigated.

References

1. John, H., Willoh, S., Hörmann, P., Siegert, M., Vondran, A., & Thiermann, H. (2016). Procedures for analysis of dried plasma using microsampling devices to detect sulfur mustard-albumin adducts for verification of poisoning. *Analytical chemistry*, *88*(17), 8787-8794.
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