#H05

Comparison of VAMS and card based microsampling with LC-HRMS analysis to assess cardiovascular drug levels

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Introduction

- Dried blood spot (DBS) cards have been used widely for sample collection due to their low cost, ease of use, easy transportation and storage.
- However, there are regulatory concerns that different hematocrit (Hct) levels may affect the accuracy of the blood volume punched from the DBS sample [1].
- Volumetric absorptive microsampling (VAMS) a microsampling bead technology has been developed specifically to collect fix volume of blood to eliminate the Hct effect.
- This work compares the performance of VAMS and the conventional DBS card for the quantification of 10 cardiovascular drugs levels from volunteers.
- Volunteer feedback on ease of use of the two microsampling methods is presented.
- Adhering to prescriptions helps to achieve therapeutic levels of the drug in the bloodstream (Fig. 1b).
- Evidence suggests that about 50% of cardiovascular patients do not take their medicines as prescribed (non adherent - Fig. 1a) [1, 2].
- Bioanalytical methods developed to infer adherence to medication should detect residual levels of the drug/metabolite up to 24 h after the initial dose.

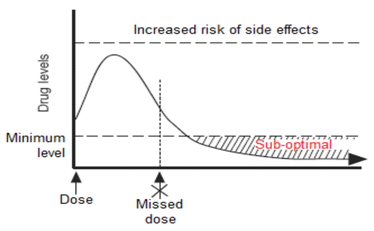

Fig. 1a. Drug concentration verse time profile in non-adherence

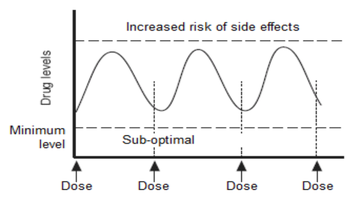

Fig. 1b. Drug concentration verse time profile in adherence

Methods

Blood sample collection

- Calibration blood spot samples were sampled on VAMS and Whatman 903 cards.
- Finger prick blood samples were collected from adult volunteers prescribed with one or more of the cardiovascular drugs.
- Volunteer samples were taken between 2 to 22 hours after dosing on both VAMS and 903 cards.
- A series of blank control samples were taken from healthy volunteers not prescribed any of the CVD drugs.

Analyte solvent extraction from VAMS and 903 cards

- Analytes were extracted with methanol or acetonitrile from VAMs tips and disks punched from 903 cards and analysed (Fig.2).

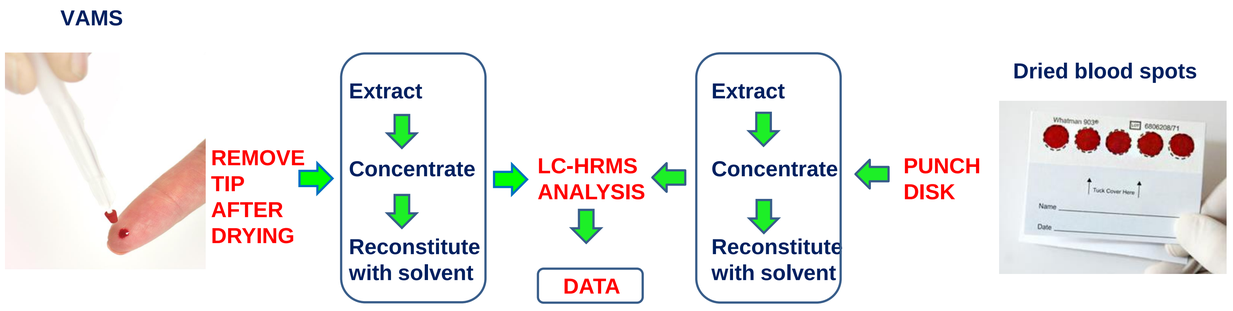

Fig. 2. Analyte solvent extraction from VAMS and 903 cards

LC conditions

- Agilent 1290 Infinity LC

Column:	Zorbax Eclipse Plus C18 2.1x 100mm, 1.8µm pore size
Column temperature:	40ºC
Mobile Phase A:	0.1% Formic acid in water
Mobile Phase B:	0.1% Formic acid in acetonitrile
Flow rate:	0.6ml/min
Gradient conditions:	95:4 to 4:95 in 2.5 mins
Injection volume:	20µl

MS conditions

- Agilent 6530 Accurate Mass QToF mass spectrometer
- Ionization: Electrospray positive
- LC-HRMS with ToF used for amlodipine *(*m/z* 431.1344), atenolol (*m/z* 267.1703), atorvastatin (*m/z* 559.2610), bisoprolol (*m/z* 326.2326), doxazosin (*m/z* 452.1928), lisinopril (*m/z* 406.2336), losartan (*m/z* 423.1695), ramipril (*m/z* 417.2384), simvastatin *(*m/z* 441.2611) and valsartan (*m/z* 436.2343). *$[M+Na]^+$

Results and discussion

Selectivity

- The developed LC-HRMS method showed good selectivity on VAMS and 903 cards.

Linearity and sensitivity

- Method showed good accuracy and linearity on VAMS and 903.
- Precision was typically between ±10% over the quality control (QC) levels for each drug on VAMS, compared to ±15% on 903.
- Back calculated concentrations gave relative errors less than 15%.

Results and discussion

Recovery

- Drug recoveries from 903 card and VAMS were comparable except for valsartan (Fig.3).

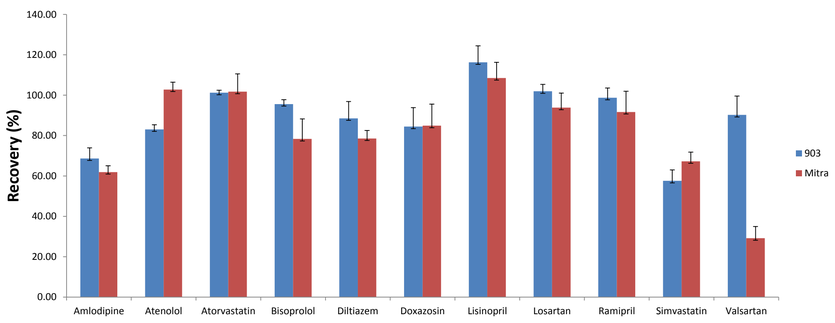

Fig. 3. Comparison of recovery data for 903 card and VAMS

Stability

- All target analytes were stable in VAMS for 8 weeks and on 903 cards at room temperature for 10 weeks.

Analyses of volunteer samples

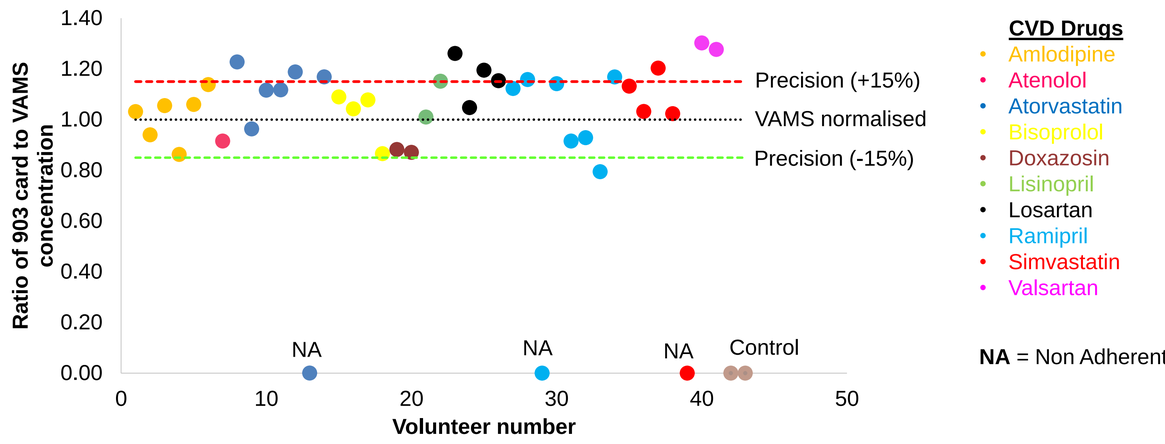

Fig.4. A plot of ratio of measured concentrations from 903 card and VAMS volunteer samples

- 903 cards required sufficient blood to be deposited within marked circles on the card. This made sampling difficult for elderly volunteers. ~17% of spots on 903 cards were rejected for quantification.
- In contrast, VAMS is designed to wick the blood drop from the finger until the substrate is full making it easier to sample. Only one VAMS substrate was rejected as a result of incomplete sample.
- Feedback from volunteers on device usage is summarised in Table 1.

Table 1. Comparison of feedback provided by volunteers on the ease of use of 903 cards and VAMS

903 cards	VAMS
Difficult to drop the blood on the marked circles.	Sampling is easy as the device sucks the blood off the fingertip.
Last spot was small as bleeding stopped after the third spot.	All four samplers were easily filled due to the small size of the tip
Sampling on 903 card was quite complex and required assistance.	Prefer VAMS to card sampling, because it is so easy to use.
A bit complex compared to VAMS.	VAMS is simple and quick to do.
Blood dropped on the 903 card before spotting.	See VAMS as a very easy to use platform.
Took more time to sample on 903 card.	Quick self-sampling on VAMS.
Will need assistance to sample on 903 card.	Can easily do sampling on VAMS without assistance.
Will take several practices to get samples in the marked circles.	Will be willing to give sample again as it is easy.
Directing my finger to the marked circles, was difficult.	Very easy to sample, no need to turn or direct finger after pricking.
Pricked another finger to sample on 903 card because did not bleed well.	Did not bleed well and could still fill the VAMS substrates.

Conclusion

- VAMS and 903 card based microsampling coupled with LC-HRMS analyses has great potential in assessing cardiovascular drug levels.
- Cost of VAMS to 903 cards is 10:1, however volunteers preferred VAMS to 903 cards due to the ease of sampling.
- Information derived from the analyses could serve an evidence base for clinicians to personalise and optimise treatment for individual patient.
- The approach will help clinicians identify non-adherent patients who could then be educated on the need and benefits of adhering to their treatment.
- Such an objective means of assessment will help reduce the burden of non adherence.

References

1. Bernieh D, Lawson G, Tanna S. J Pharm Biomed Anal. 2017 (142), 232-243. https://doi.org/10.1016/j.jpba.2017.04.045

2. Tanna S, & Lawson G. Analytical Chemistry for Assessing Medication Adherence, New York: Elsevier, 2016.