LC-HRMS analysis of 216 patient micro-volume blood samples to allow clinical assessment of medication adherence

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INTRODUCTION

- Cards have been extensively used for dried blood spot (DBS) sample collection.
- But, for some formats, the many advantages of cards including low cost, simple sample collection, ease of transport and automated analyses may be outweighed by regulatory concerns over the 'haematocrit (Hct) effect' leading to variations in the volume of blood sampled from different subjects.
- This problem is avoided by use of a volumetric absorptive micro-sampling (VAMS) sampler which collects a fixed volume of blood regardless of Hct level.
- This project compares side by side data from 903 card and a VAMS micro-sampler to quantitatively determine the levels of 11 selected cardiovascular (CVD) drugs in patient samples as an indicator of adherence to medication prescription.
- Medication non-adherence impacts on patient health, may lead to hospital re-admissions with additional healthcare costs and medicines wastage.

METHODS

Analyte extraction from 903 and VAMS

- The target drugs were extracted in methanol or acetonitrile from VAMS absorptive tips and disks punched from DBS cards and then analysed (Fig. 1).

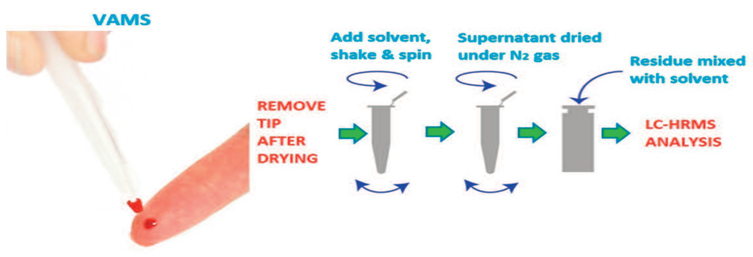

Figure 1. VAMS tip solvent extraction

LC Conditions

- Agilent 1290 Infinity LC

Column:	Zorbax Eclipse Plus C18 2.1x100mm, 1.8μm pore size
Column temperature:	40˚C
Mobile Phase A:	0.1% Formic Acid in water
Mobile Phase B:	0.1% Formic Acid in acetonitrile
Flow rate:	0.6 ml/min
Gradient conditions:	95:4 to 4:95 in 2.5 min
Injection volume:	20 μl

MS Conditions

- Agilent 6530 Accurate Mass QToF mass spectrometer.
- Mass detector operation in electrospray positive ion mode.
- LC-HRMS (± 5ppm) with ToF only used for amlodipine (*m/z* 431.1344*), atenolol (*m/z* 267.1703), atorvastatin (*m/z* 559.2610), bisoprolol (*m/z* 326.2326), diltiazem (*m/z* 415.1686), 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). * = Na^+ adduct

RESULTS

Selectivity

- The developed LC-HRMS method for the simultaneous determination of the target CVD drugs in the extracts demonstrated good selectivity/specificity.

Validation

- Showed good accuracy, precision and good linearity.
- The calibration range and minimum limits of quantification in blood (LoQ S/N = 10) for target drug spiked VAMS standards are detailed in Table 1.

Table 1. Linearity and sensitivity data for the target CVD drugs

	Range (ng/ml)	LoQ (ng/ml)	R^2
Amlodipine	0.1 - 100	1.0	0.989 ± 0.012
Atenolol	10 - 1500	10.0	0.995 ± 0.003
Atorvastatin	0.1 - 100	0.5	0.994 ± 0.003
Bisoprolol	0.1 - 100	0.1	0.999 ± 0.001
Diltiazem	0.5 - 600	0.5	0.998 ± 0.001
Doxazosin	0.1 - 100	0.1	0.994 ± 0.003
Lisinopril	0.1 - 100	0.1	0.990 ± 0.002
Losartan	5.0 - 1000	5.0	0.998 ± 0.002
Ramipril	0.1 - 100	0.1	0.995 ± 0.002
Simvastatin	0.1 - 100	0.1	0.993 ± 0.004
Valsartan	50 - 4000	50.0	0.993 ± 0.002

Stability

- All target drugs stable in 903 and in VAMS for 10 weeks at 23˚C.

RESULTS

Extraction efficiency (recovery)

- Target drug recoveries from VAMS tips were comparable to those obtained from 903 sampling card except for bisoprolol and valsartan (Fig. 2).

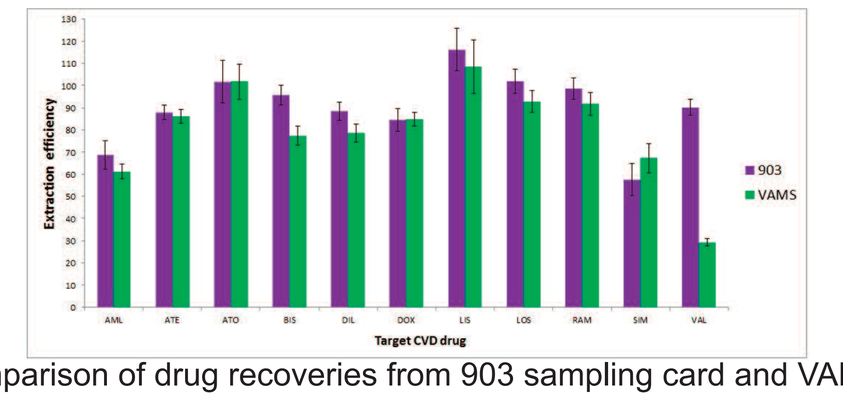

Figure 2. Comparison of drug recoveries from 903 sampling card and VAMS

RESULTS AND DISCUSSION FROM PATIENT SAMPLES

- Both micro-sampling methods lead to successful identification of the correct drug for control patients who were known to be adherent. No false positives were obtained either from patients taking non-target drugs or those taking no medication.
- A prescription regimen should produce therapeutic levels of the particular drug in patient's blood. Achieving this status depends on the pharmacokinetic properties of the drug and the patient adhering to their prescription. Literature values for C_{max}, the maximum concentration in blood for a particular drug dose, provides a reference value for this study.
- In this work the following 3 situations were assumed:
  - Drug concentrations > C_{max} implies non-adherence
  - Drug concentrations between C_{max} and 5% of C_{max} implies adherence
  - Drug concentration < 5% of C_{max} or non-detectable implies non-adherence
- 27% of out-patients versus 17% of control patients were identified as non-adherent to their prescription medication. Non-adherence was not uniform amongst the CVD drugs (Table 2).

Table 2. Results obtained from the analyses of patient samples

CVD drug	No. of patients	Adherent (%)	Non-adherent (%)
Amlodipine	24	25.0	75.0
Atenolol	18	87.5	12.5
Atorvastatin	26	92.0	8.0
Bisoprolol	28	100.0	0
Diltiazem	6	100.0	0
Lisinopril	12	100.0	0
Losartan	20	90.0	10.0
Ramipril	16	88.0	12.0
Simvastatin	12	83.4	16.6
Valsartan	44	64.0	36.0
None - Controls	10	N/A	N/A

- 903 cards require sufficient blood to be deposited within designated areas on the card. This is more difficult for elderly people. In this study ~17% of the spots were unacceptable for quantification. User comments included:
  - So much easier than conventional blood samples
  - Difficult to get the blood into the area marked
  - Finger stopped bleeding/insufficient sample
- The VAMS micro-sampler required only to be held in the blood drop on the finger until the tip was completely full when sampling was completed. Only 1 VAMS sample tip was rejected due to incomplete collection.
- This evidence suggests that VAMS micro-sampling offers the following advantages compared to the conventional DBS sampling card:
  - More 'person friendly' and convenient
  - Easier to enable self-sampling or and/or home sampling

CONCLUSION

- Both micro-sampling methods coupled with LC-HRMS analyses facilitate identification of patients where the prescription apparently failed to produce detectable drug levels in the blood.
- This information should provide the evidence-base for clinicians on how to proceed with the next step of the healthcare process in the event of poor patient progression.

REFERENCE

Tanna S. & Lawson G. In Thomas BF, ed. *Analytical Chemistry for Assessing Medication Adherence.* New York: Elsevier; 2016.