High-resolution mass spectrometry for analysis of selected drugs in dried blood spots

Sangeeta Tanna, Elizabeth Cocks and Graham Lawson

Leicester School of Pharmacy, Faculty of Health and Life Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, UK

OVERVIEW

- This study investigates the potential of high mass accuracy MS (HRMS) to provide the specificity currently associated with multiple reaction monitoring in tandem mass spectrometry (MSMS) techniques for analysis of dried blood spot (DBS) samples from patients.
- DBS are being widely investigated as a tool for micro- sampling to facilitate paediatric pharmacokinetic (PK) studies and for application in preclinical, toxicokinetic and clinical studies.

INTRODUCTION

- In HRMS analyses mass measurement is determined accurately to 1ppm of the chosen mass.
- This *m/z* accuracy provides the specificity of the technique since for organic molecules with an RMM of less than 400 only one structure will fit the accurate mass measurement:

	Accurate Mass (m/z)
Atenolol	276.1703
Caffeine	195.0883
Captopril	218.0845
Dexamethasone	393.2072

- Mass (m/z) accuracy is available from either TOF or Orbitrap systems.
- Data collected per analysis is the total ionisation from the sample which is subsequently "mined" for the corrected mass. The data can be re-interrogated for other information subsequently.
- Quantitative DBS analysis has gained considerable interest in recent years as it offers many advantages including simplicity of sample collection, storage and sample transport.
- Some initial clinical data from DBS for the above drugs is reported.

METHODS

Analyte extraction from dried blood spots (DBS)

- Blood spot samples were collected on Guthrie cards. The cards were pre-treated with 1,4 dithiothreitol (DTT) for captopril.
- The target drugs were solvent extracted from disks punched from the DBS and analysed (Fig. 1).

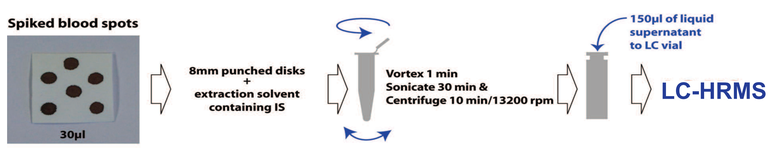

Figure 1. Dried blood spot solvent extraction

MS Conditions

- Agilent 6530 QTOF mass spectrometer, 1290 Infinity LC
- LC HRMS with TOF only used at *m/z* 276.1073 for atenolol, *m/z* 218.0845 for captopril, at *m/z* 195.0883 for caffeine and *m/z* 393.2072 for dexamethasone.
- Mass detectors used with electrospray interface and in positive ion mode.

METHODS

LC Conditions

	Atenolol	Caffeine
Column	Ascentis Express C18 100x2.1mm	Ascentis Express C18 100x2.1mm
Column temperature	30˚C	40˚C
Flow rate	0.2 ml/min	0.5 ml/min
Mobile Phase A	0.1% Formic Acid in water	0.1% Formic Acid in water
Mobile Phase B	0.1% Formic Acid in ACN	0.1% Formic Acid in ACN
Gradient conditions	95:5 to 30:70 in 7 min	90:10 to 0:100 in 5 min
Injection volume	5 µl	5 µl

	Captopril	Dexamethasone
Column	Zorbax Ecplise C18 100x2.1mm	Zorbax Ecplise C18 100x2.1mm
Column temperature	40˚C	40˚C
Flow rate	0.5 ml/min	0.5 ml/min
Mobile Phase A	0.1% Formic Acid in water	0.1% Formic Acid in water
Mobile Phase B	0.1% Formic Acid in MeOH	0.1% Formic Acid in ACN
Gradient conditions	80:20 to 0:100 in 5 min	70:30 to 30:70 in 5 min
Injection volume	10 µl	20µl

RESULTS

Extraction efficiency (recovery)

	Atenolol	Caffeine	Captopril	Dexamethasone
Recovery (%)	96 ± 5	74 ± 6	90 ± 10	98 ± 6

Validation

- Showed good accuracy, precision and good linearity (R^2 > 0.99).
- Minimum limits of quantification (LoQ S/N = 10) for target drug spiked blood spot standards were as follows:

	Atenolol	Caffeine	Captopril	Dexamethasone
Range(ng/ml)	25-1500	100-250000	10-400	7.5-800
LoQ (ng/ml)	50	100	20	15

Selectivity

- Initial TOF data - the total ion chromatogram TIC (Fig. 2a and 3a) shows the complexity of the data available for subsequent analysis.
- Fig. 2b and 3b represents the extracted ion (EIC) data for *m/z* 218.0845 for captopril and for ion of *m/z* 195.0883 for caffeine.

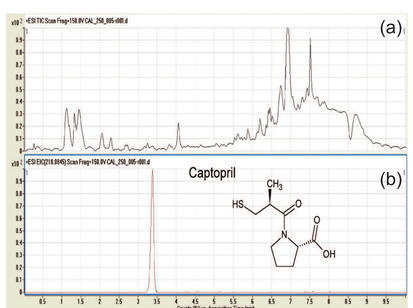

Figure 2. TIC for captopril (a) and EIC for captopril (b)

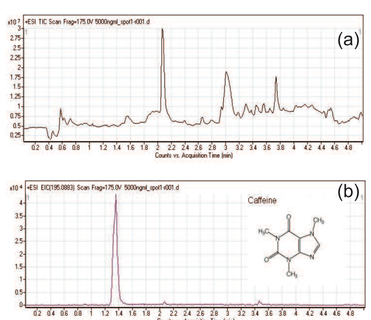

Figure 3. TIC for caffeine (a) and EIC for caffeine (b)

CLINICAL APPLICATION OF DBS TECHNIQUE

- DBS concentration-time profiles for healthy volunteers following the ingestions of a 100mg caffeine tablet, 87.5mg caffeine drink and a 100mg atenolol tablet were plotted.

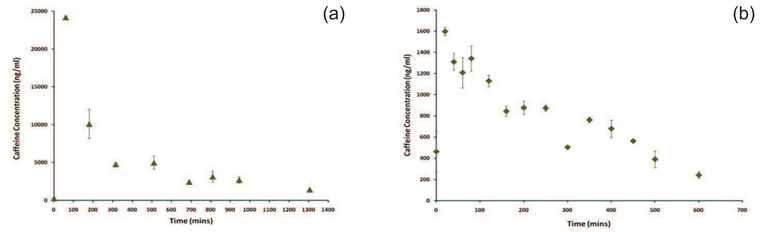

Figure 4. DBS caffeine concentration-time profile for an adult volunteer administered (a) a 100mg caffeine tablet and (b) a caffeine containing drink.

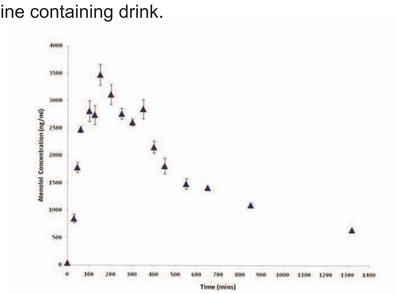

Figure 5. DBS atenolol concentration-time profile for an adult volunteer administered a 100mg atenolol tablet.

CONCLUSIONS

- The TOF gave good data for therapeutic levels of atenolol, captopril, caffeine and dexamethasone and the method demonstrated good selectivity.
- The TOF recorded data may be subsequently re-interrogated for other ions without the need to re-run the sample.
- Figure 4b demonstrates the ability of this methodology to monitor the uptake of additives in food.
- The use of small volume blood samples makes the DBS approach particularly appropriate for use in paediatric PK studies.
- This approach could also facilitate therapeutic drug monitoring.
- DBS sampling is simple and the method could be adapted to other drugs and biomarkers and for monitoring environmental exposure, food safety and forensic toxicology applications.

REFERENCES

[1] Lawson G. et al. *J Pharm. Pharmacol.* 2009. **61(1 Suppl)**: A33.
[2] Patel P. et al. *J Pharm. Pharmacol.* 2009. **61(1 Suppl)**: A112.
[3] Patel P. et al. *J Chromatogr. B* 2010. **878(31)**: 3277-3282.
[4] Tanna S. & Lawson G. *Anal. Methods* 2011. **DOI**: 10.1039/C1AY05160A

ACKNOWLEDGEMENTS

- Sigma Aldrich for donation of LC column