Examples of dried blood spot sampling and analysis to improve paediatric medicine

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OVERVIEW

- Liquid-chromatography mass spectrometry with selected ion monitoring (LC-MS(SIM)) methods were developed and validated to determine captopril and dexamethasone in dried blood spots (DBS).
- This was investigated as a means of measuring target drug levels from neonatal patients to explore the possibility of producing paediatric pharmacokinetic (PK) and bioequivalence data to ensure optimum drug dosing regimens.
- Drug stability in the method was also investigated.

INTRODUCTION

- Current prescribing practice in paediatric care is based on the empirical derivation of the dose from a combination of clinical experience and adult data.
- Optimum drug dosing regimens may be obtained from PK data derived from measured drug levels.
- Paediatric PK studies are not conducted due to the relatively large (5-10ml) volumes of blood required.
- This situation arises from the restriction on blood volume sampled, particularly for neonates, where an acceptable level is ~50µl.
- DBS sampling system used as a means for collecting small volume blood samples on sampling card from paediatric patients.
- Quantitative DBS analysis has gained considerable interest in recent years as it offers many advantages including simplicity of sample collection, storage and sample transport.
- Paediatric clinical data from DBS for the above drugs is reported.

METHODS

Analyte extraction from dried blood spots (DBS)

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

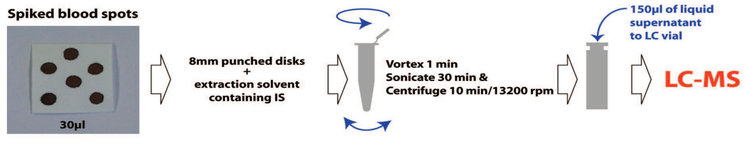


Figure 1. Dried blood spot solvent extraction

MS Conditions

- Mass detectors used with electrospray interface and in positive ion mode.

Captopril:

- Agilent 1100 LC/MSD Ion Trap mass spectrometer
- LC ion trap (IT) MS with Single Ion Monitoring (SIM) at *m/z* 218.0

Dexamethasone:

- Agilent 1100 LC/1200 mass spectrometer
- LC-MS with Single Ion Monitoring (SIM) at *m/z* 393.1

METHODS

LC Conditions

	Captopril	Dexamethasone
Column	Zorbax Ecplise C8 150x3mm	Zorbax EclipsePlus C18 150x2.1mm
Column temperature	35˚C	23˚C
Flow rate	0.5 ml/min	0.2 ml/min
Mobile Phase A	0.1% Formic Acid in water	0.13% Formic Acid in water
Mobile Phase B	0.1% Formic Acid in ACN	ACN
Gradient conditions	60:40 to 0:100 in 3.5 min	60:40 to 20:80 in 6.0 min
Injection volume	25 µl	25 µl

- The following tests were carried out for the target drugs:
  - Optimisation of extraction solvent
  - Determination of maximum DBS extraction efficiency
  - Instrumental calibration versus extracted drug
  - Determination of LoD and LoQ
  - Sample stability studies in DBS

RESULTS

Extraction efficiency (recovery)

- Captopril was 90 ± 10% (with DTT in extraction solvent).
- Dexamethasone was 98 ± 6%.

Sample stability

- Captopril: 91 ± 7% recovery after 12 weeks storage at 23˚C.
- Dexamethasone: Stable in DBS for 7 days at 23˚C and 28 days at 4˚C.

Validation

- Showed good accuracy, precision and good linearity.
- Minimum limits of quantification (LoQ S/N = 10) for captopril and dexamethasone spiked blood standards were as follows:

	Captopril	Dexamethasone
Range	10-400ng/ml	15-800ng/ml
LoQ	50ng/ml	15ng/ml
R^2	0.990	0.985

Selectivity

- No interferences were found at the same retention time as dexamethasone (Fig. 2a).
- The captopril DBS LC-MS method exhibited small interference from sampling card (Fig. 2b).

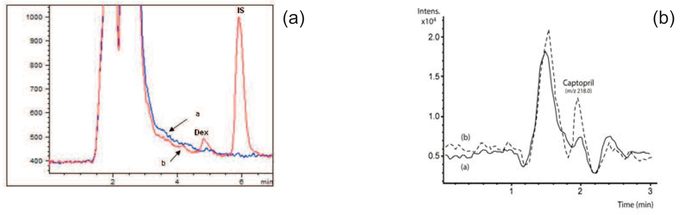


Figure 2 (a). Overlay EICS for dexamethasone at m/z 393.1 of a blank DBS and DBS spiked with dexamethasone. **2(b)**. Overlay EICS for captopril at m/z 218.0 of a blank DBS and DBS spiked with captopril.

APPLICATION TO NEONATE PATIENT DBS SAMPLES

- Heel prick DBS sample taken from neonate patient 1 hour post administration of 1mg/kg captopril orally.
- Captopril levels calculated to be:
  88ng/ml in whole blood ***or*** 1.8ng in DBS ***or*** 7µg/kg body weight.
- Pilot DBS and plasma comparison studies based on a single 25mg oral dose of captopril, in one of three paediatric formulations, were carried out using adult volunteers to assess bioequivalence of formulations (Fig. 3).

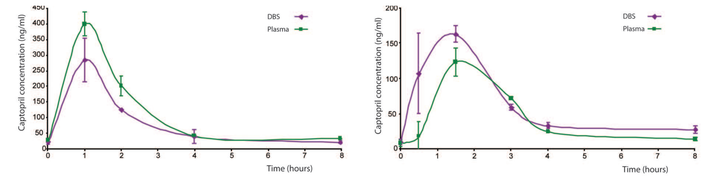
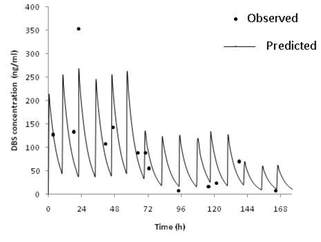


Figure 3. A DBS and plasma comparison of PK plots obtained from adult volunteers administered 25mg captopril as (a) an aqueous solution formulation and (b) a suspension formulation

- A PK profile was generated from DBS samples obtained from a neonate patient administered a 10 day tapering dose of dexamethasone.
- This was compared to the predicted PK profile for dexamethasone in preterm neonates (Fig. 4).



Dexamethasone dosing schedule

1. 250µg/kg twice for 3 days
2. 100µg/kg twice for 3 days
3. 50µg/kg twice for 2 days
4. 25µg/kg twice for 2 days

Figure 4. A DBS PK profile obtained from a neonate patient administered a 10 day tapered dose of dexamethasone and a comparison with the predicted PK profile.

CONCLUSIONS

- The close correlation between the DBS and plasma derived PK plots demonstrates the validity of the DBS technique.
- The use of small volume blood samples makes the simple DBS approach particularly appropriate for use in paediatric PK studies, therapeutic drug monitoring and for assessing bioequivalence of formulations.
- DBS sampling could be used for biomarkers and clinical trials.

REFERENCES

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