

The Use of Dried Blood Spot (DBS) Sampling for the Determination of Drugs in Children and Neonates

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Unlicensed and Off-Label drug use in Children and Neonates

- *Unlicensed and Off-Label drug use in Children and Neonates*
- *Neonatal Screening - Dried blood spots*
- *Drug analysis using Dried blood spots*
- *Determination of metronidazole*
- *Determination of canrenone*
- *Strengths, Weaknesses, Opportunities and Threats*

Unlicensed and Off-Label drug use in Children and Neonates

- *For a number of years we have been investigating the off-label/unlicensed use of drugs in children*
 - *This work involved sparse data analysis [using plasma samples] in order to obtain PK/PD data.*
 - *Samples collected not by design but when samples are being taken for other reasons (e.g. lab tests) we collect an extra amount of blood and obtain plasma*
 - *Drug levels are analysed and PK/PD studies undertaken using population PK/PD methods*
 - *Resulting data can be used to suggest new dosing requirements*

Unlicensed and Off-Label drug use in Children and Neonates

- *In this work we used small volume plasma samples (0.1 - 0.5 mL) and we have developed methods for the analysis of diclofenac, ranitidine, cisapride, midazolam etc*
- *In this work we were mainly dealing with children but following on from this we developed a connection with NICU at which point we needed to develop suitable methods for drug determination in neonates where there were restrictions on quantity of blood available and new sampling methods were required*
- *We have therefore been studying DBS sampling in both neonates and in children*

*Unlicensed and Off-Label drug use in Children
and Neonates*

Dried Blood Spots DBS

What are they and why use them

*Initially used for blood collection for investigating
inborn errors of metabolism*

*Subsequently investigated for sample collection
for drug determination*

Applications of DBS in drug analysis

• *Early Drug Determinations*

- *As far back as 1978 the use of BDS for the determination of theophylline was described §*
- *In 1980 a method for the determination of the AEDs valproic acid, phenobarbitone, primidone and phenytoin was reported **
- *In 1983 chloroquine and its major metabolite was determined using DBS methodology †*

§ *Albani M. Toseland PA. Neuropadiatrie. 9(1):97-9, 1978*

* *Albani M. Oldigs H. Toseland PA. Monatsschr. Kinderheilkd. 128: 371-374, 1980*

† *Patchen LC. Mount DL. Schwartz IK. Churchill FC. Journal of Chromatography. A. 278(1):81-9, 1983*

Applications of DBS in drug analysis

- *A number of newer applications*
 - *determination of anti-malarials when collected at bush hospitals*
 - *determination of retinol in respect of vitamin A deficiency*
 - *determination of testosterone and nandrolone for investigation of steroid pharmacology*
 - *use of DBS has been proposed for the determination of performance enhancing drugs*
 - *determination of benzoylecgonine in DBS has been used to investigate maternal cocaine use*

*Applications of DBS in drug
analysis*

*Applications of DBS in drug analysis in
children and neonates*

Applications of DBS in drug analysis in children and neonates

At the time when we were first beginning our studies in dried blood spot sampling the following application was published

- Oliveira et. al. (2002) have described the use of dried blood spots for the determination of paracetamol plus its glucuronide and sulphate metabolites from DBS in neonates*
- Millership et al (2003) detailed studies into the use of DBS for the determination of diclofenac*

*Applications of DBS in drug analysis in
children and neonates*

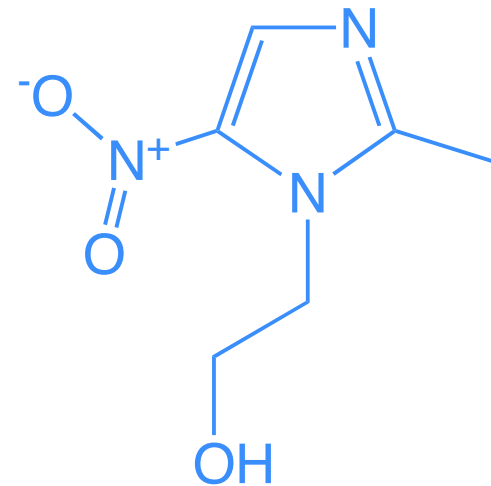
Examples of our investigations

*Applications of DBS in drug analysis in
children and neonates*

Determination of Metronidazole

Application of DBS in the analysis of metronidazole

- *Metronidazole is an antimicrobial used in neonates diagnosed or suspected of having necrotising enterocolitis (NEC).*



Application of DBS in the analysis of metronidazole

- *The manufacturers do not have a licence for the use of the drug in the UK for neonates or children <1 year.*
- *There are discrepancies in the published studies mainly related to the half life $t_{1/2}$ of the drug and its tendency to accumulate in babies <32 weeks gestation*

Application of DBS in the analysis of metronidazole

Aims

- *To optimise and validate a simple microanalytical HPLC/UV method for the measurement of metronidazole in dried blood spots (DBS)*
- *Determine the pharmacokinetics of metronidazole in preterm neonates using modern PK modelling approaches, concentrating on the change in $t_{1/2}$ over time*
- *Design evidence based dosing regimen*

Application of DBS in the analysis of metronidazole

Methodology

- *Spiked whole blood or patient samples (heel pricks) were spotted onto Guthrie cards to prepare the blood standards or samples*
- *The spots were allowed to dry at room temperature in the dark for at least 3 hours before processing*
- *A 6 mm disc (equivalent to 11 μ L) was punched from the centre of each DBS and placed in a 1.5mL Eppendorf tube*
- *The DBS was extracted by adding 975 μ L of water and 25 μ L of the IS solution. The mixture was left for 30 minutes with vortex mixing every 10 minutes*
- *Extract used for analysis*

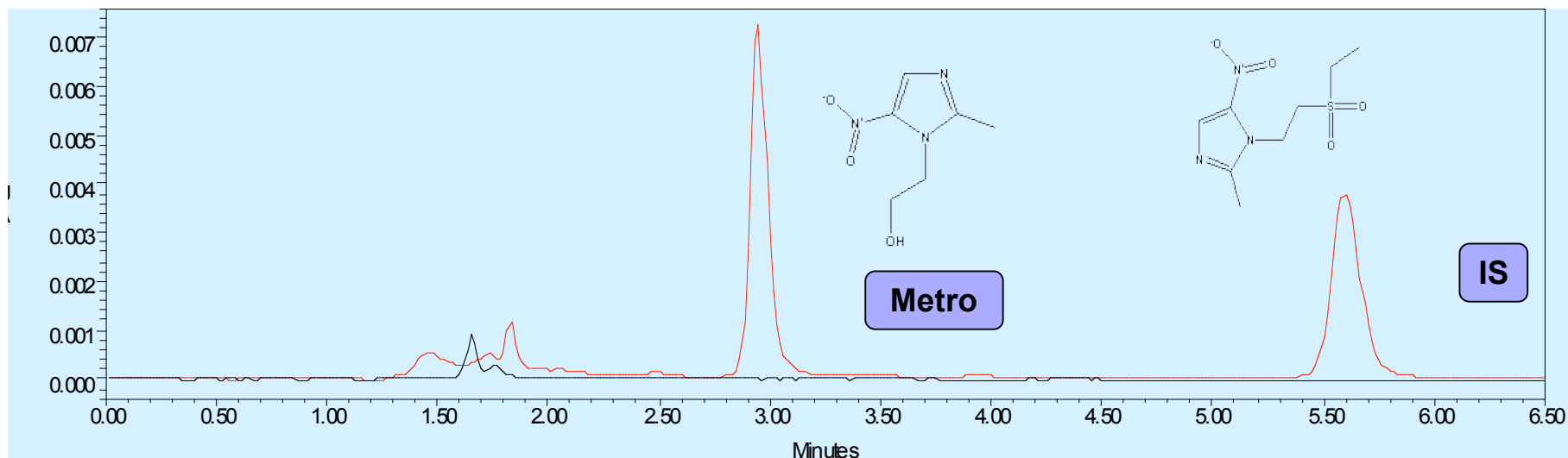
Application of DBS in the analysis of metronidazole

Methodology

- *Symmetry® C18 (5 µm, 3.9 x 150 mm) column from Waters® preceded by a Symmetry® guard column of matching chemistry.*
- *Mobile phase acetonitrile / 0.01 M phosphate solution (KH₂PO₄), pH 4.7, 15:85, v/v. pumped at a flow-rate of 1ml/min.*
- *The UV detection wavelength was 317 nm*
- *80µL injected onto the HPLC system for analysis*

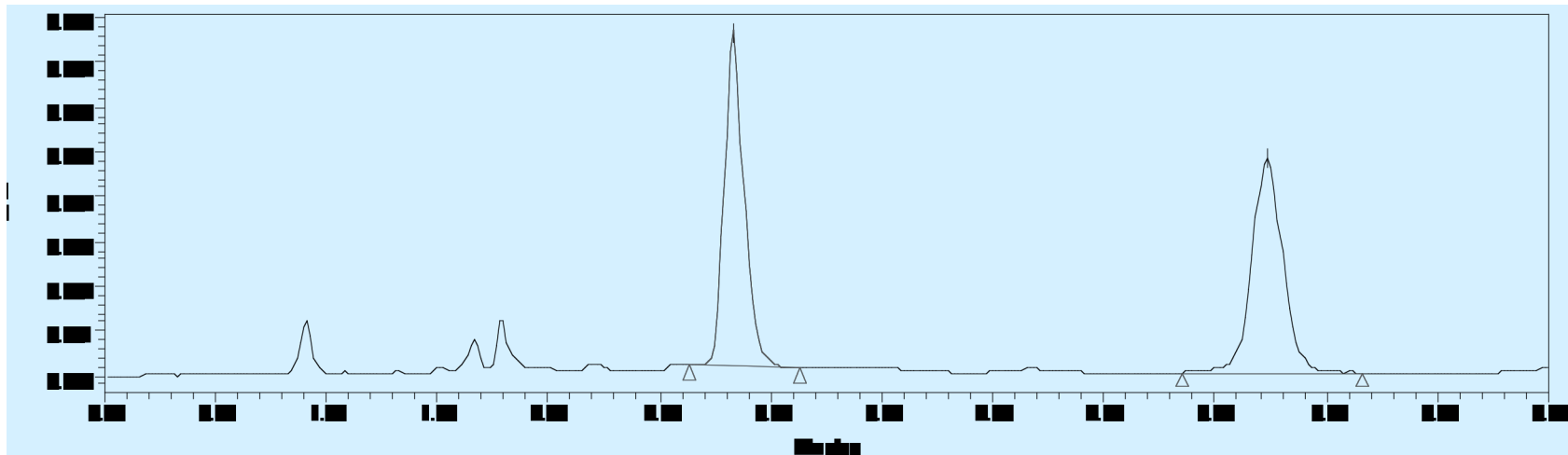
Application of DBS in the analysis of metronidazole

Chromatograms of a blank blood spot sample [—]
and a blood spot spiked with 20 μ g/mL of metronidazole [—].



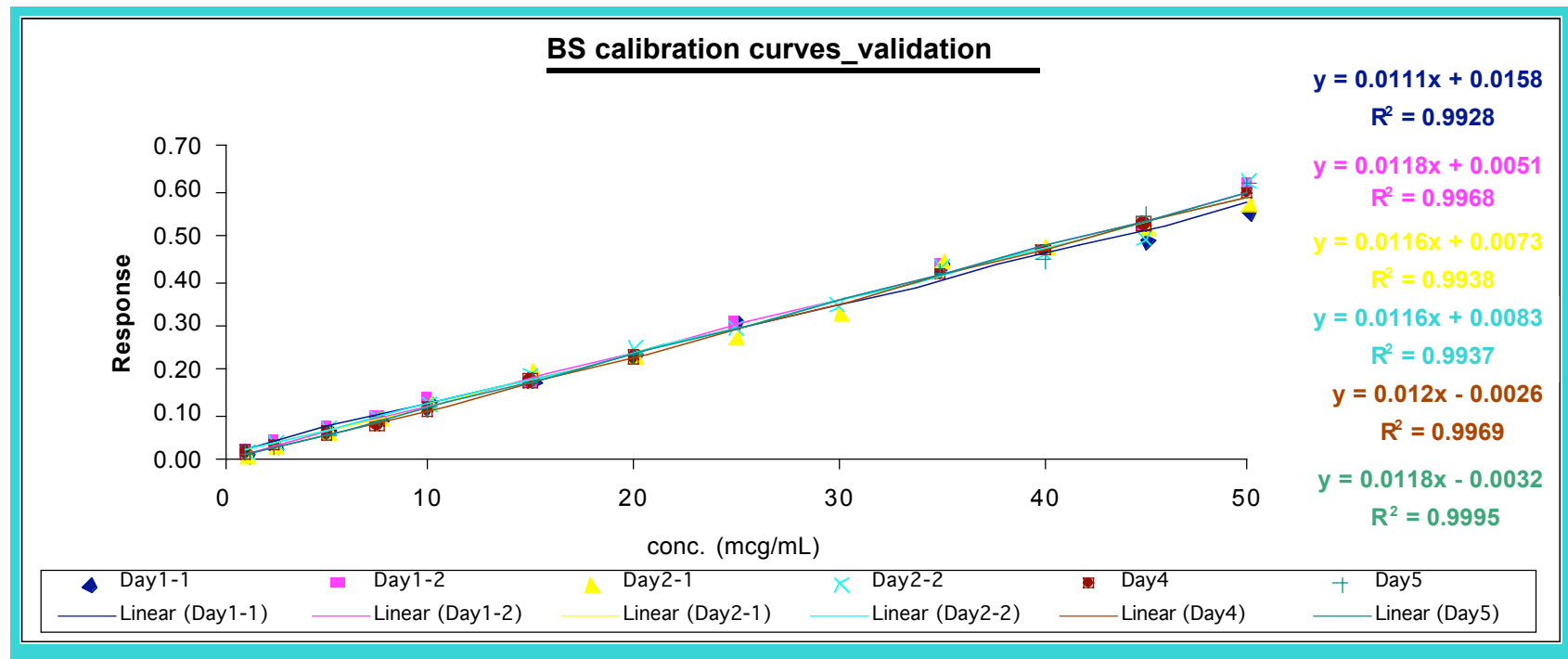
Application of DBS in the analysis of metronidazole

Chromatogram of a blood spot sample taken from neonate after an hour of intravenous administration of 11.4mg of metronidazole (Flagyl®)



Application of DBS in the analysis of metronidazole

- *Validation - 5 day validation calibration curves*



Application of DBS in the analysis of metronidazole

- *Validation - Recovery data*

<i>Spiked Metronidazole Conc. ($\mu\text{g/ml}$)</i>	<i>%Recovery (n=5)</i>	
	<i>(Mean \pm SD)</i>	<i>RSD %</i>
5	79.9 \pm 6.2	7.8
15	78.8 \pm 3.3	4.2
35	78.0 \pm 2.8	3.5
50	78.0 \pm 2.4	3.0

Application of DBS in the analysis of metronidazole

- *Validation - Analysis of Qc samples*

<i>Spiked (nominal) Metronidazole conc. (µg/mL)</i>	<i>Intra-day (n=5)</i>			<i>Inter-day (n=5)</i>		
	<i>Measured (Mean ± SD) (µg/ml)</i>	<i>RSD^a %</i>	<i>Accuracy^b %</i>	<i>Measured (Mean ± SD) (µg/ml)</i>	<i>RSD^a %</i>	<i>Accuracy^b %</i>
<i>LOQ (2.5)</i>	<i>2.3 ± 0.1</i>	<i>2.6</i>	<i>-7.4</i>	<i>2.6 ± 0.3</i>	<i>13.1</i>	<i>2.4</i>
<i>QC_L (5.0)</i>	<i>5.2 ± 0.5</i>	<i>8.8</i>	<i>4.1</i>	<i>5.2 ± 0.2</i>	<i>3.8</i>	<i>3.1</i>
<i>QC_M (15.0)</i>	<i>15.3 ± 0.7</i>	<i>4.4</i>	<i>2.3</i>	<i>14.8 ± 0.5</i>	<i>3.3</i>	<i>-1.4</i>
<i>QC_H (35.0)</i>	<i>36.6 ± 1.3</i>	<i>3.6</i>	<i>4.5</i>	<i>35.7 ± 1.3</i>	<i>3.6</i>	<i>1.9</i>
<i>ULOQ (50.0)</i>	<i>50.7 ± 1.6</i>	<i>3.1</i>	<i>1.3</i>	<i>50.6 ± 1.1</i>	<i>2.1</i>	<i>1.2</i>

Application of DBS in the analysis of metronidazole

- *Validation*
 - *No interference from a range of commonly co-administered drugs was proven.*
 - *The presence of hydroxymetronidazole [the major metabolite of metronidazole] was not significant.*
 - *Method has been applied to the analysis of approximately 200 neonatal samples.*
 - *Preliminary data analysis indicates that weight and gestational age are important.*

*Applications of DBS in drug analysis in
children and neonates*

Determination of Canrenone

*Applications of DBS in drug analysis in
children and neonates*

*Canrenone/Potassium Canrenoate
is used in the treatment of chronic
lung disease*

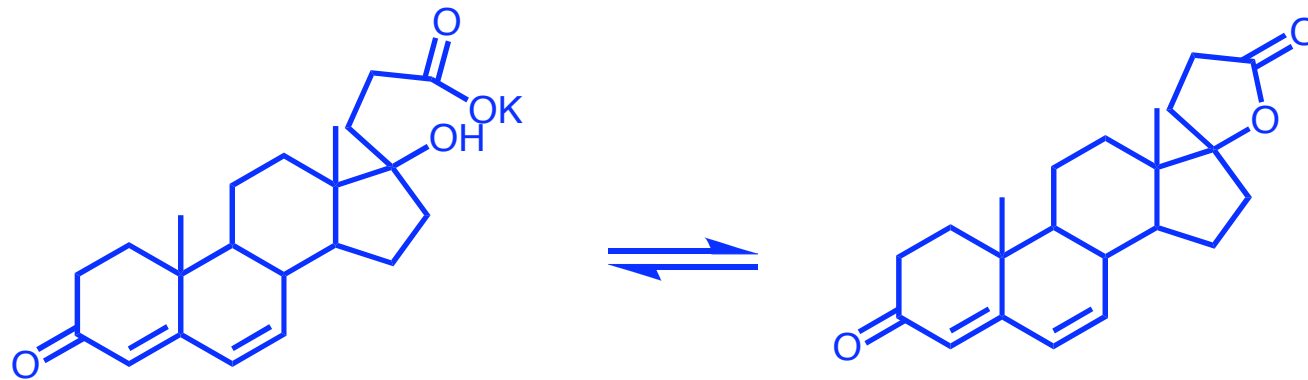
Application of DBS in the analysis of canrenone

Potassium Canrenoate

In the body it is reported that potassium canrenoate is converted to canrenone

Canrenone is thought to be the active compound since it is suggested that the closed lactone ring is required for activity

Application of DBS in the analysis of canrenone



Application of DBS in the analysis of canrenone

The paediatric consultants we are working with along with the FDA and the EMEA have indicated that data on both spironolactone and potassium canrenoate in children and neonates is required

The situation regarding dosing is that neonates in NICU in Belfast are only given canrenoate iv whereas in the RBHSC some are given canrenoate iv and some are given spironolactone orally - this is again concerned with the non availability of age-appropriate formulations

Application of DBS in the analysis of canrenone

We have acquired samples both in the neonatal population and also in children

In neonates only DBS samples were obtained from heel pricks

Application of DBS in the analysis of canrenone

In the first part of the study we have concentrated on the analysis of canrenone from those patients who were administered potassium canrenoate (although substantial studies have been undertaken with patients administered spironolactone, I will only comment briefly on this work)

Application of DBS in the analysis of canrenone

Sample treatment

DBS samples

6mm circle extracted with methanol for 2h

Intermittent shaking every 20 min

Evaporation and reconstitution in 1ml water/AcCN

(9/1)

*SPE using Oasis HLB (wash with 5% methanol, wash
with 60% methanol, elute with methanol)*

Evaporation and reconstitution

Application of DBS in the analysis of canrenone

LCMS method for DBS samples [Dong et al 2006]

Sunfire C18 reversed-phase column (2.1 x 150 mm, 3.5 μm) preceded by a (2.1 x 10 mm, 3.5 μm) guard column of matching chemistry.

The mobile phase used was methanol/water 60:40 (v/v) pumped at a flow rate of 0.3 ml/min.

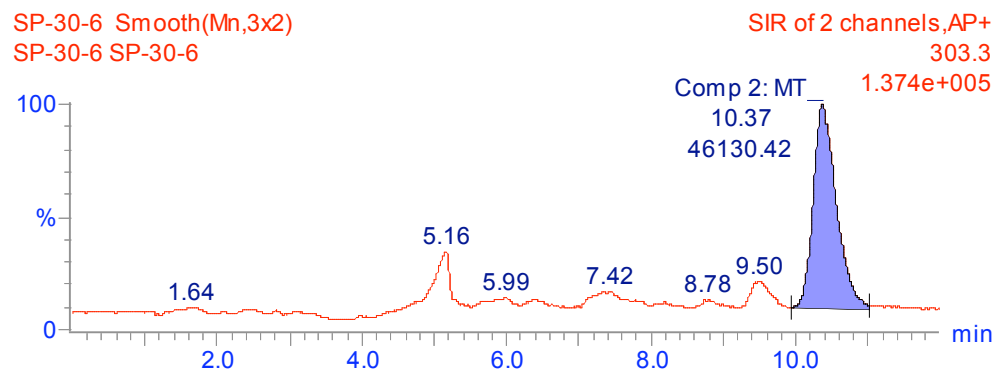
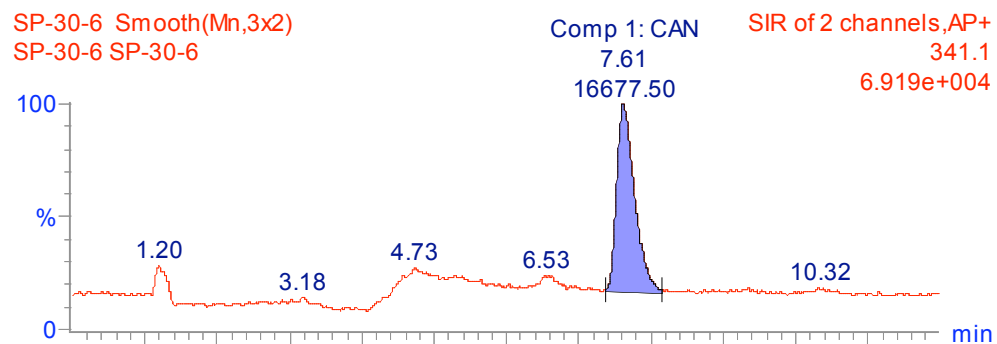
The column temperature was kept at 35°C

Sample injection volume was 40 μl .

Detection involved APCI

Application of DBS in the analysis of canrenone

LCMS traces



Application of DBS in the analysis of canrenone

The method has both been fully validated in a similar manner to that reported for metronidazole

The MS method took time to develop because of considerable difficulties associated with e.g. matrix effects

The developed methods have applied successfully for the analysis of dried blood spots (n=160) samples taken from patients.

Pharmacokinetic analysis is presently underway

Application of DBS in the analysis of canrenone

Through this study we have established that in children and in neonates canrenoate is converted into canrenone

- This has previously been indicated as mainly taking place under physiological conditions therefore these conditions seem to be in existence in children and neonates*

Although not detailed on this work we have established in children, even very young children, treated with spironolactone that metabolism to thiomethylspironolactone and canrenone does occur, this also has not been established previously

Strengths, Weaknesses, Opportunities, and Threats

In terms of our own work

- *Strengths*

- *Easier consent*
- *Ease of sample collection*

- *Weaknesses*

- *Extra analytical sensitivity required*

- *Opportunities*

- *Appears to offer the opportunity to obtain pharmacokinetic data in children and neonates via sparse data or conventional analysis*

- *Threats*

- *Unsuitable method development **ESPECIALLY SAMPLE CLEAN UP** and validation*
- *Unsuitable use of data (DBS v Plasma)*

Acknowledgements

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