The critical assessment of methodologies used in support of paediatric pharmacokinetic studies

A thesis presented for the degree of Doctor of Philosophy in Medicine (Child Health) at the University of Aberdeen

by

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Chapter 8: Stereoselective bioanalysis and pharmacokinetics of intravenous ketorolac in children

Having, evaluated the conduct of the paediatric PK of IV paracetamol as an example of a well known and licensed medicine (Chapters 5-7), the current chapter addresses the PK of intravenous ketorolac as an exemplar of a less commonly used unlicensed medicine in children. Particular issues addressed include the stereoselective quantification of ketorolac assay development and validation. The developed assay will be discussed in the context of a PK study of IV ketorolac in children less than 16 years.

8.1 Background

As discussed in Chapter 3 ketorolac is a potent nonsteroidal anti-inflammatory drug (NSAID), used off label for the control of moderate to severe pain in children. This drug is reported to provide postoperative analgesia in children, with levels of efficacy similar to those provided by morphine and pethidine (Maunuksela et al., 1992) but with the advantage of a longer period of effective analgesia (Rice et al., 1995). Ketorolac has no central nervous system adverse effects, such as sedation and post operative nausea and vomiting, which are largely explained by the drug's poor penetration of the blood brain barrier (Kupulainene et al., 2008). These pharmacological properties favour ketorolac over other analgesics as alternatives to the narcotic analgesics for post-surgical pain control. Although not licensed for use in children less than 16 years old, ketorolac is widely used intravenously in an off label fashion for the management of postoperative pain in children [Forest et al., 1997). However this usage is not supported by robust PK or PD data or by data on safety, and efficacy. Paediatric dose estimation by extrapolations from adult values exposes children to the risk of either under-treatment or toxicity, as experienced in the cases of chloramphenicol and morphine (Sutherland et al, 1959; Weiss et al., 1960).

Although ketorolac is produced as an equal mixture (racemate) of its two chiral (enantiomeric) forms, R (+) and S (-) ketorolac studies of ketorolac

pharmacokinetics have utilised bioassays that do not distinguish between R (+) and S (-) nor any differences between children and adults (Olkkola et al., 1991). The failure to study the two enantiomers is clinically relevant as they are known to have different pharmacological profiles, with the S- enantiomers proven to be the clinically active form (Mroszczak et al. (1996). Furthermore, limited evidence suggests that their respective PKs may differ significantly with age (Hamunen et al., 1999), with one report by Lynn et al (2007) demonstrating a significantly higher clearance of S (-) ketorolac compared to the R (+) enantiomer. Considering these differences between the enantiomeric forms of ketorolac, PK data that are specific for the two forms are clearly required in support of dose determination, and studies of safety and efficacy.

PK studies of racemic mixtures require analytical methods capable of selectively measuring the levels of the enantiomers in the chosen sample matrix. Ketorolac enantiomer concentration may be analysed indirectly on achiral columns after either pre- or post-column derivatization of analytes (Tsina et al., 1996) and most methods have utilised reversed phase C18 columns and ultraviolet detections (Tsina et al., 1996; Nagilla et al., 2007). Although the low cost of these columns reduces overall analytical cost, derivatization of enantiomers prior to detection requires many steps that increase the time of analysis and derivization rather than direct enantiomer measurement, which may introduce errors (Campanero et al., 1998).
Chiral column methods which do not introduce racemisation are more direct and rapid and, have been developed for the measurement of ketorolac enantiomers (Tsina et al., 1996; Lynn et al., 2007). Detection of enantiomers in some of these methods has been by means of UV detectors, which have required the delayed elution of enantiomers up to 10 minutes, leading to prolonged chromatographic run times (Tsina et al. 1996). Ing-Lorenzini and colleagues described a direct mass spectrometric method employing a C18 column in addition to the chiral column. However this combination method requires column switching and consequently a chromatographic runtime well beyond 10 minutes (Ing-Lorenzini et al., 2009). The methods by Lynn et al. (2007), Ing-Lorenzini et al. (2009) and Campanero et al. (1998), require 100, 250 and 1000 µl of plasma respectively for ketorolac extraction which when translated to whole blood might limit multiple and extensive sampling in younger children.

8.2. Aims

In view of the known advantages and disadvantages of existing methods the aims of the present study were to

1. Develop and validate a bioanalytical method to selectively measure the enantiomers of ketorolac using micro volumes of blood.

2 Conduct a stereoselective pharmacokinetic analysis of intravenous ketorolac, using the developed method.

8.3. Bioanalytical method development and validation

8.3.1. Experiment

8.3.1.1. Materials and Methods

R(+) and S(-) ketorolac (98% and 99% enantiomerically pure, respectively) were donated as research compounds by F. Hoffman-La Roche Ltd, Basel, Switzerland. Phenacetin (98% purity), formic acid, hexane, ethyl acetate and ammonium acetate were purchased from Sigma Aldrich (Dorset, England, UK). Acetonitrile was of HPLC grade and purchased from Fisher Scientific (Loughborough, UK).

8.3.1.2. Standards and solutions

8.3.1.2.1 Preparation of internal standard solution

Phenacetin was used as an internal standard. A stock solution was prepared by dissolving 0.005 g of phenacetin powder in 10 ml of acetonitrile and 3 serial dilutions of 1:10 in acetonitrile were made to yield 5 ng/ml solution.

8.3.1.2.2. Preparation of R (+) and S (-) ketorolac plasma standards

A solution of R (+) ketorolac was prepared by dissolving 0.005g of the powder in 10 ml of acetonitrile. A 1:10 dilution of the solution was then made in acetonitrile to yield a 500 µg/ml stock solution of R (+) ketorolac from which spiking solutions

from 0 – 400 µg/ml were prepared (see Table 8.1). The procedure was repeated for S (-) ketorolac. Plasma standard concentrations from 0 – 2 µg/ml were prepared by adding 5µl of each corresponding R (+) and S (-) spiking solution to 990 µl of drug free plasma (Table 8.1).

8.3.1.2.3. Preparation of S (-) and R (+) ketorolac dried blood spots

Dried blood spots (DBS) containing ketorolac enantiomers were prepared by spiking 990 µl of whole blood with 5µl of 400µg/ml of spiking solutions of R (+) and S (-) ketorolac. Thirty microliters of the spiked blood was spotted on Guthrie cards, allowed to dry and stored away from light.

8.3.1.2.4. Preparation of 50mM ammonium acetate buffer (pH 3.0)

From the relationship:

Weight (g) = Number of moles x Molecular mass

$= 50 \times 10^{-3} \times 77.1$ g

3.855 g of ammonium acetate crystals were placed in a 1 litre beaker with a magnetic stirrer and distilled water (Milli-Q Integral) added to the 800ml mark. Concentrated acetic acid was added whilst stirring to buffer the solution at pH 3.0. The resulting solution was transferred to a one-litre volumetric flask and the volume brought up to 1 litre with distilled water.

Table 8.1: preparation of spiking R (-) and S (-) ketorolac solutions and standard plasma solutions

Volume of R (+) ketorolac stock solution (µl)	Volume of acetonitrile (µl)	Spiking R(+) ketorolac concentration (µg/ml)	Standard plasma concentration (µg/ml)
0	1000	0	0.00
20	980	10	0.05
40	960	20	0.10
80	920	40	0.20
200	800	100	0.50
400	600	200	1.00
600	400	300	1.50
800	200	400	2.00

8.3.1.3 Extraction of analytes

Ketorolac enantiomers were extracted from both plasma and dried blood spots by liquid-liquid extraction methods.

8.3.1.3.1 Extraction from plasma

Ketorolac enantiomers were extracted from plasma by liquid-liquid extraction. To 50 µl of plasma standard was added 5 µl of 1ng/ml (10 pg) phenacetin (internal standard), 100 µl of ammonium acetate (50mM, pH 3.0) and 2 ml of hexane-ethyl acetate (50:50, v/v). The mixture was shaken on a rotary mixer for 15 minutes, centrifuged at 4100 *g* for 5 minutes and placed in a -80ºC freezer for 30 minutes to allow freezing of the aqueous layer. The organic layer which contained the analytes was transferred to fresh tubes and evaporated under a stream of nitrogen to dryness. The residue was redisolved in 200 µl of mobile phase and centrifuged at 4100 *g* for 2 minutes. The solution was transferred to a micro Eppendorf tube with a Pasteur

pipette and further centrifuged at 7000 *g* for 5 minutes and 5 µl injected for chromatography.

8.3.1.3.2 Extraction from dried blood spots

To extract ketorolac from the Guthrie cards, paper discs were sampled with a hole puncher of 6mm diameter into a test tube. Five microlitres of the internal standard (10 pg of phenacetin) was added. To this was added 100µl of ammonium acetate buffer (pH 3.0; 50mM, pH 3.0) and vortexed to extract blood from the paper. Five millilitres of ethyl acetated-hexane (50:50, v/v) was added and shaken for 15 minutes, centrifuged at 4100 g for 5 minutes and stored under -80ºC for the aqueous layer to freeze and the organic layer saved. The organic layer was transferred to a fresh tube and evaporated to dryness under nitrogen. The residue was treated as described above for plasma extraction

8.3.1.4. Chromatographic analysis

Quantitative analysis was conducted by liquid chromatography (LC) separation with mass spectrometry (MS) detection. The LC/MS system consisted of a Surveyor series MS pump, autosampler, vacuum degasser and a thermostated column compartment. Enantiomer separation was achieved on a ChiralPak AD-RH column (150mm x 2.1mm I.D., particle size 5µm) from Hichrom Ltd, Reading, UK. The mobile phase was composed of acetonitrile/0.1% aqueous formic acid (50:50; v/v), delivered in isocratic mode at a flow rate of 0.2 ml/min. Detection was by a TSQ Quantum Triple Quadrupole mass spectrometer with an electrospray ionisation (ESI) source operating in a positive ion mode. Xcalibur V. 2.0.6 software was used to control the equipment, acquire and process data. Nitrogen was used as the sheath and auxiliary gas and the collision gas was Argon. ESI optimisation parameters were: spray voltage 3500V; sheath gas 60 (arbitrary units); auxiliary gas 0 (arbitrary units); collision pressure 1.5 mTorr; capillary temperature 375ºC; skimmer offset 0V; and collision energy -20V. Single reaction monitoring (SRM) of ketorolac and phenacetin was performed. The mass transition for ketorolac and phenacetin were: *m/z* 256 → 105 and 180 → 110, respectively.

8.3.1.5. Assay validation

The ICH guidelines (ICH 2001) were used to demonstrate method performance characteristics namely; specificity, standard curve and linearity, accuracy and precision, limit of quantification and limit of detection and recovery.

8.3.1.5.1 Specificity

To 980 µl of drug-free plasma was added 5 µl each of 2 µg/ml R (+) and S (-) ketorolac and 5 µl of 1 µg/ml phenacetin. Drug free plasma was also spiked with phenacetin. The samples were extracted as previously described for plasma (8.3.1.3) and 5 µl injected form chromatography. The chromatograms generated were compared to assess resolution of sample matrix components.

8.3.1.5.2. Standard curve, linearity and sensitivity

Linearity was assessed by constructing a calibration curve using the standard plasma concentrations prepared in section 8.3.1.2.2. Five microlitres (10 pg) of phenacetin was added to 50 µl of each plasma concentration and analyte extraction proceeded as described earlier. Five microlitres of the redisolved residue was injected into the LC/MS system. Two separate standard linear curves were constructed for each enantiomer by regressing peak area ratio [ketorolac enantiomer : phenacetin] on known plasma concentration. From this curve, the slope, the intercept and the coefficient of determination were determined. This procedure was repeated on 3 separate days to give a four day validation period.
Sensitivity was assessed by determining the lower limit of detection (LLOD) and lower limit of quantification (LLOQ) for each enantiomer. LLOD and LLOQ were estimated as the lowest concentrations on the calibration curve, which had a signal-to-noise ratio of 5, and 10 respectively. Precision and accuracy at the LLOQ were also determined, with target CVs% for LLOD and LLOQ of less than 15% and 20% (FDA 2001; ICH 2005).

8.3.1.5.3. Accuracy and precision

Five replicates of quality control (QC) samples containing R (+) and S (-) ketorolac at the high (2 µg/ml), medium (0.5 µg/ml) and low (0.1 µg/ml) concentration of the

standard curve were extracted as previously described. A freshly prepared calibration curve was used in determining the concentrations of the QCs, using the newly developed method. Accuracy was assessed as percentage relative standard errors of the known added amounts of ketorolac enantiomer and precision assessed as the percentage coefficient variation (CV %) for each of the QC samples. Intraday (within assay) precision was evaluated by analysing five sets of QC samples prepared on the same day, whilst a single set of QC samples prepared on three different days were analysed for inter-day (between assay) precision. A CV% less than 20% (FDA 2001; ICH 2005) was accepted as reliable precision.

8.3.1.5.4. Recovery

Five replicate samples of plasma containing R (+) and S (-) ketorolac at the LLOQ were extracted. Five microlitres of the redisolved residue and 5 µl of the same concentration of R (+) and S (-) ketorolac were injected for chromatography and recovery expressed as peak height of extracted ketorolac as a percentage of peak height of ketorolac solution.

$$\% \text{ recovery} = \frac{\text{Peak height of extracted ketorolac enantiomer}}{\text{Peak height of ketorolac enantiomer solution}} \times 100$$

The recovery of the internal standard (phenacetin) was determined in the same way at the concentration (10 ng/ml).

8.3.2. Results

8.3.2.1 Specificity

The full spectra of the mass transition (*m/z* 256 → 105) of ketorolac are presented in Figure 8.1. Figures 8.2 represent chromatograms from extract of blank plasma, Fig 8.3 spiked plasma and 8.4 from plasma obtained 15 minutes post dose from a patient who received 10 mg intravenous racemate ketorolac. There were no interfering peaks from endogenous plasma components with clear resolution of R (+) and S (-) ketorolac and separation from the internal standard (phenacetin). The retention times

were 3.49, 3.8 and 4.9 minutes for phenacetin, R (+) and S (-) ketorolac, respectively enabling the chromatographic run-time to be set at 6 minutes.

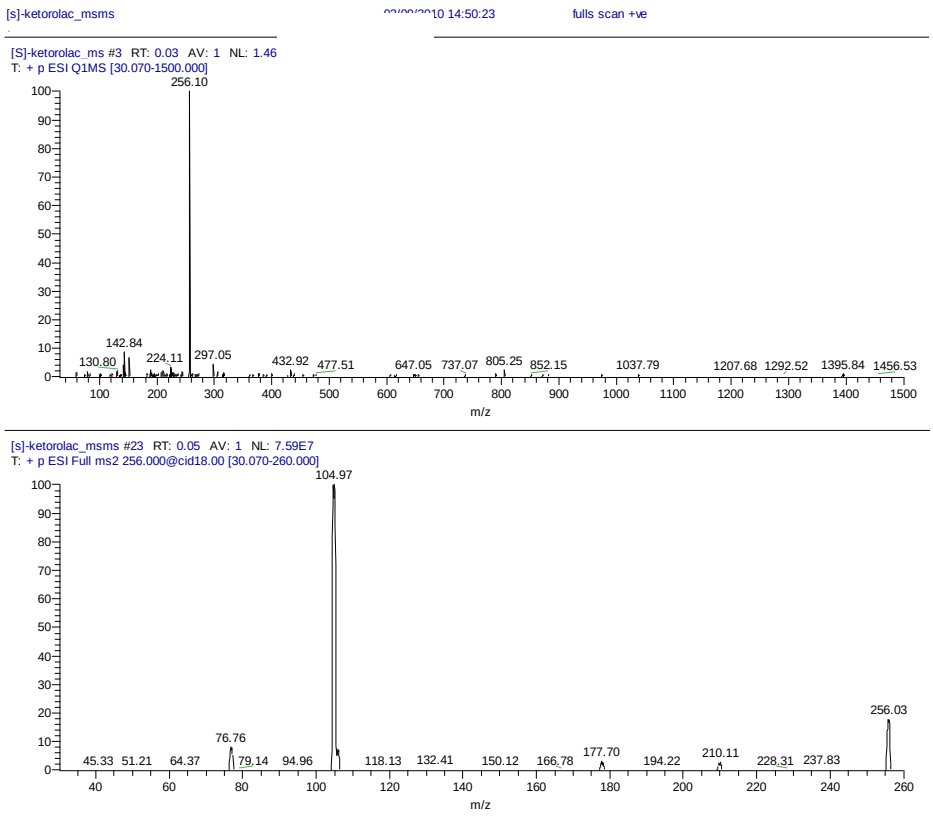


Figure 8.1: Parent ion and product ion spectra for ketorolac

Upper: Parent $[M+H]^+$ at *m/z* 256

Lower: Major ms/ms product ion at *m/z* 105

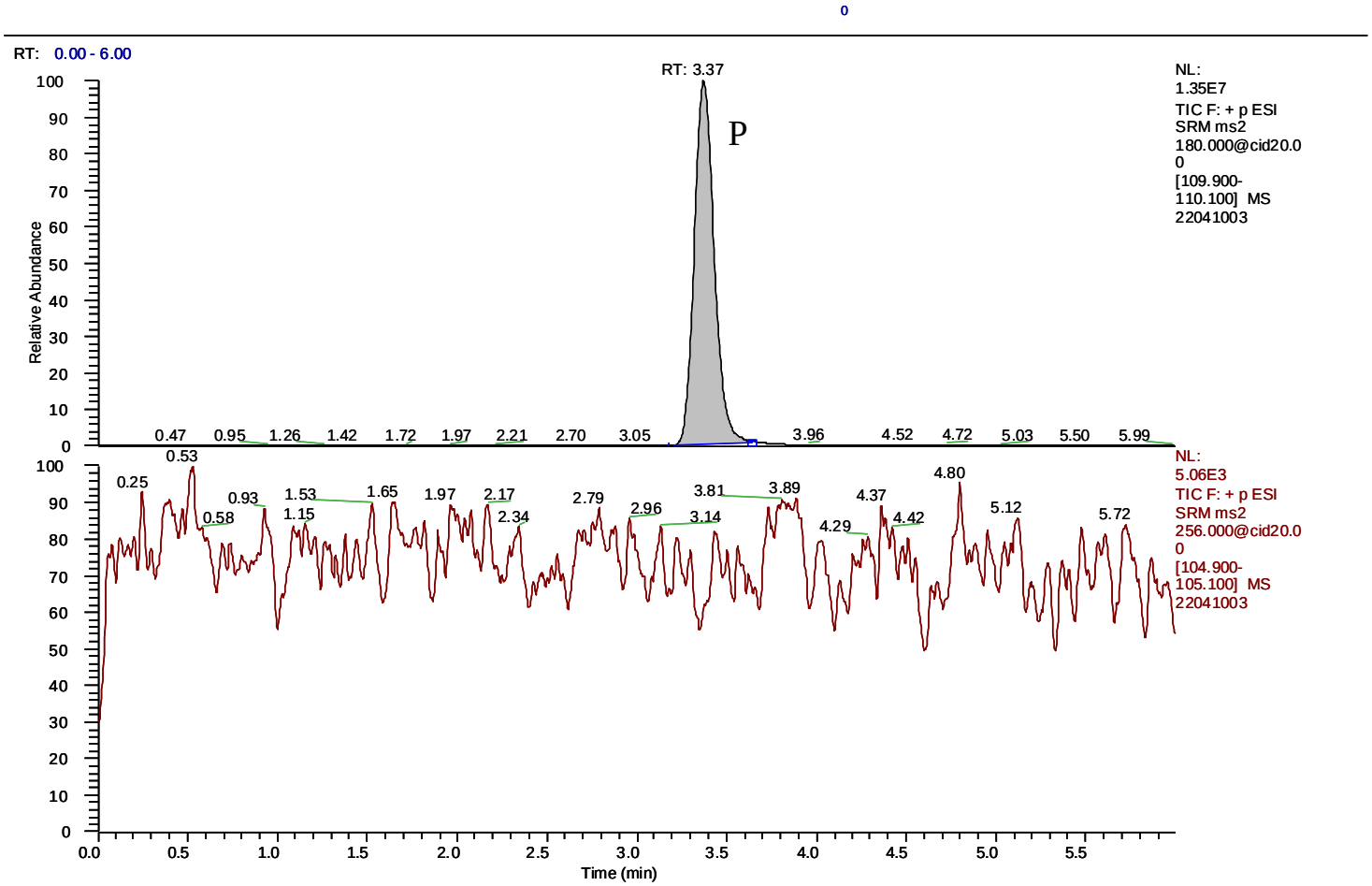


Figure 8.2: Blank plasma spiked with 5 ng phenacetin (**P**)

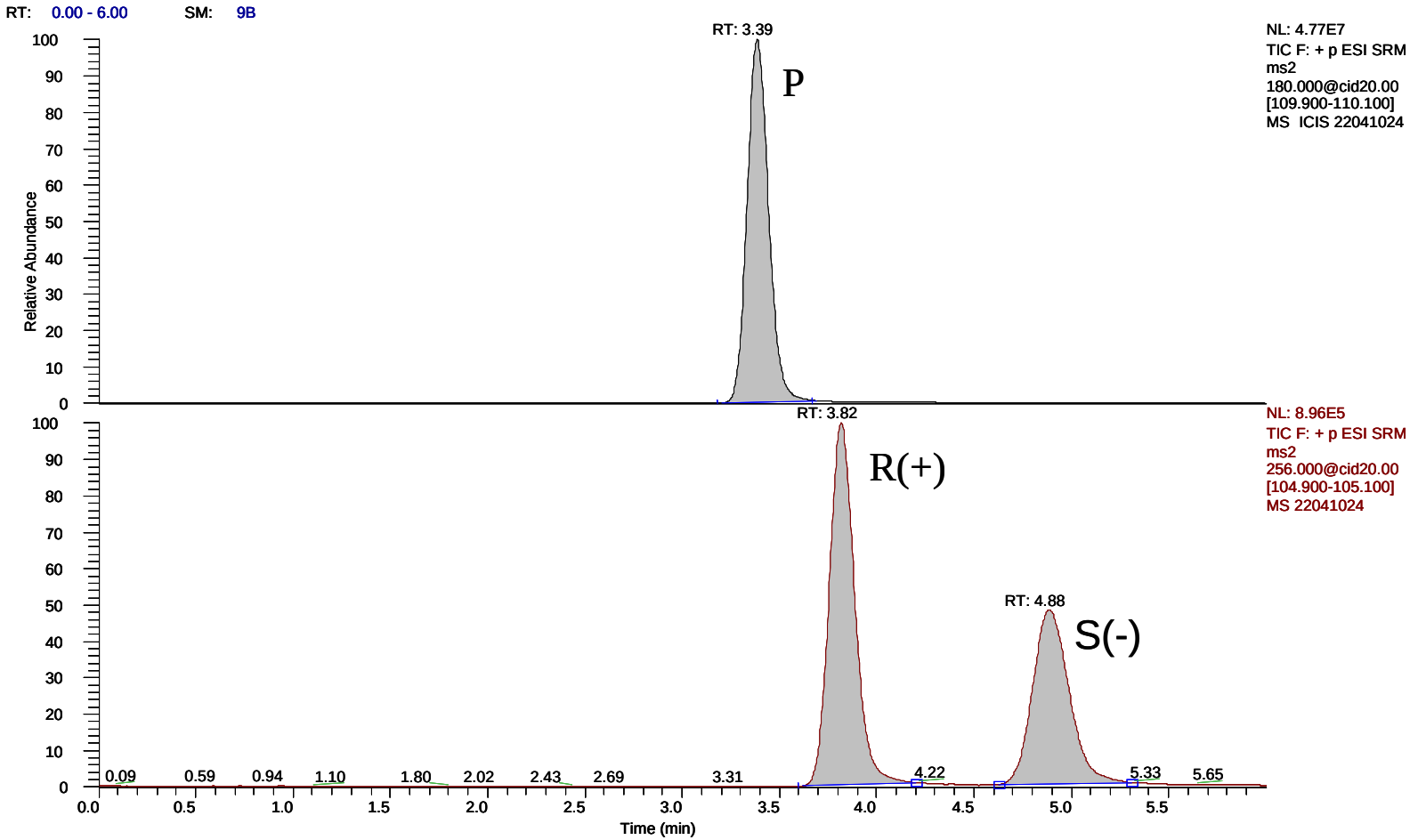


Figure 8. 3: Chromatogram of extracted plasma spiked with5 ng phenacetin (P), 10 ng R (+) and 10 ng S (-) ketorolac

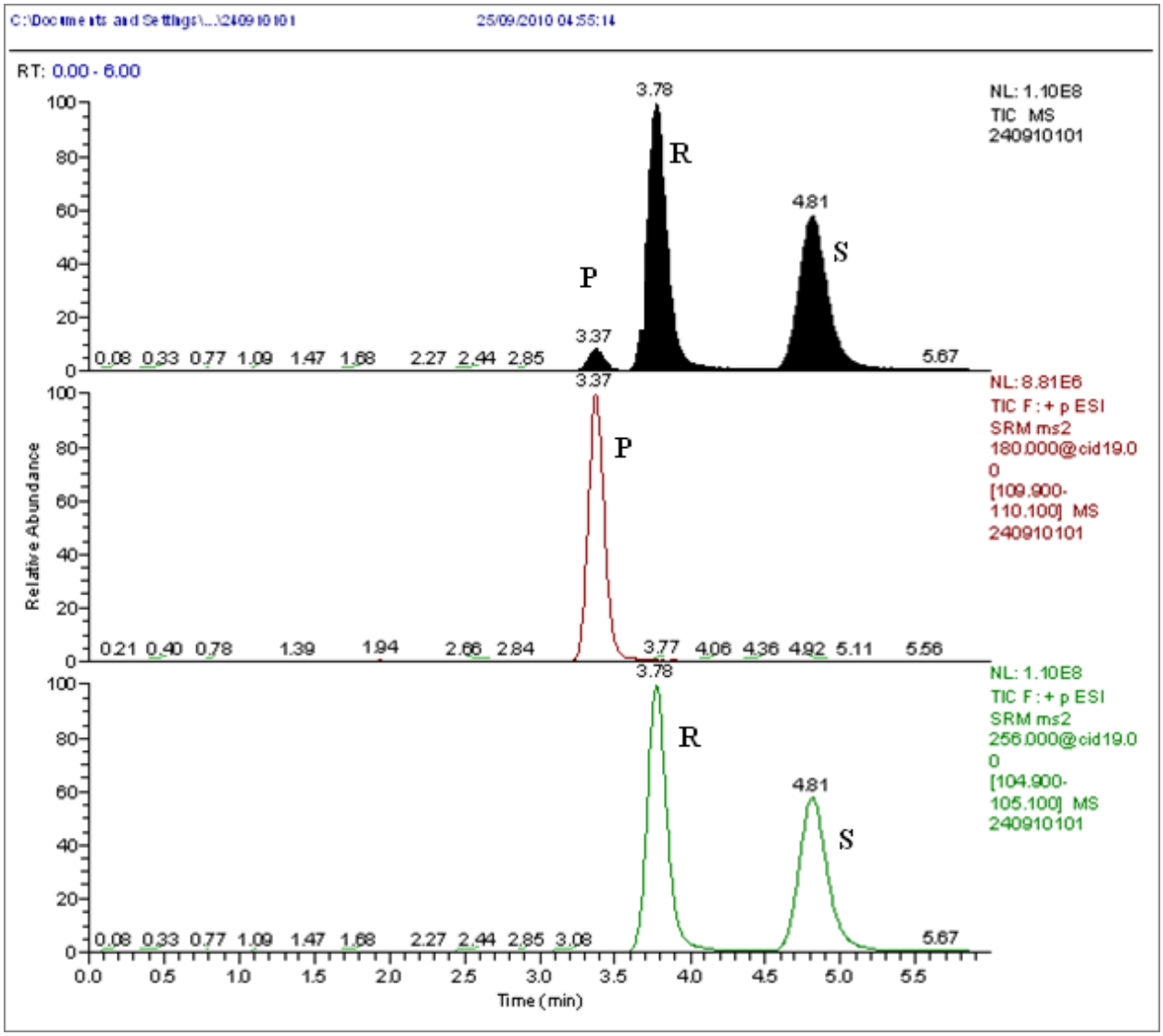


Figure 8.4: Chromatogram of patient sample taken 15 minutes post 10 mg dose of IV ketorolac. Upper panel is at the total line chromatogram showing phenacetin (P), R- and S-ketorolac which are filtered into P (middle panel) and R- and S- ketorolac (lower panel).

8.3.2.2. Standard curve, linearity and sensitivity

The standard curves for R (+) and S (-) ketorolac are presented in Figure 8.5. Each curve was linear in the range 0 – 2000 ng/ml. The linearity was characterized by the equations: $y = 0.0003 + 1.14486e\text{-}005*x$ for R (+) ketorolac; and $y = 0.00019 +$

*7.74816e-006*x* for S (-) ketorolac, where *y* and *x* were the area ratios and ketorolac concentrations, respectively. An assessment of goodness of fit of the curve revealed heteroscedasticity in the data with absolute errors being higher at higher concentrations. The predictive performance of the curve was improved by applying a weighting factor of 1/*x*, where *x* is concentration. The LLOQs of the method were 8 pg on column (31.3ng/ml equivalent) for R (+) ketorolac and 15 pg on column (62.5 ng/ml equivalent) for the S (-) ketorolac.

8.3.2.3. Precision and accuracy

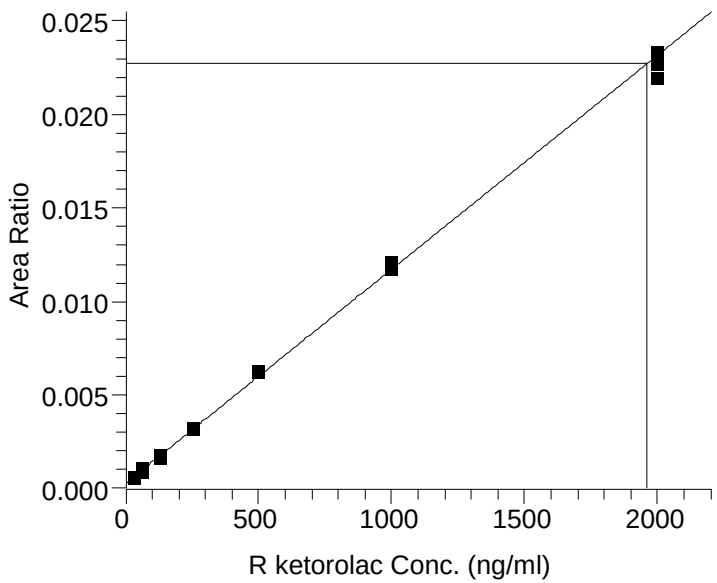
The results for the precision and accuracy of the method are presented in Table 8.2. Intra-day precisions for all the enantiomers were less than the upper limit of 20% recommended by the relevant guide lines (FDA 2001; ICH, 2005). Inter-day precision and accuracy (relative standard error) for all QC samples for both of the enantiomers were also less than 20%.

8.3.2.4. Recovery

The recoveries at the LLOQs were 75.3, 73.9 and 70.0%, for R(+) ketorolac, S(-) ketorolac and phenacetin, respectively. These recoveries were precise as they had respective CVs of 5, 11 and 6%, which were less than the upper limit of 20% recommended by guidelines (FDA 2001; ICH, 2005).

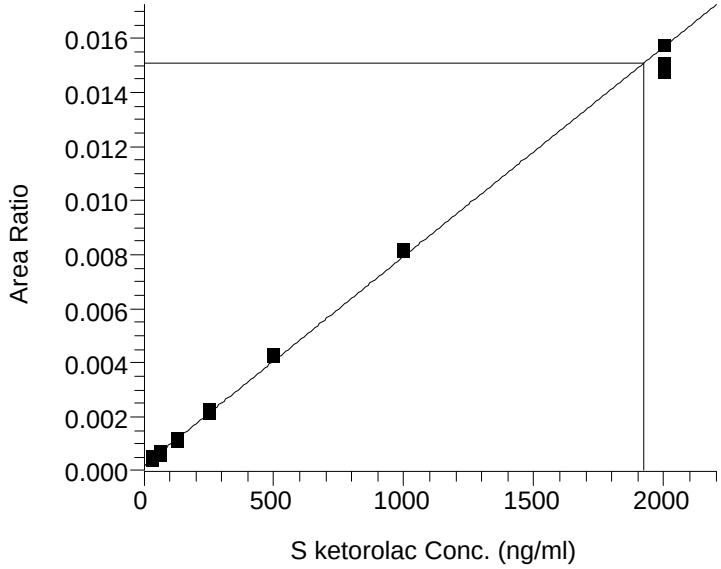
Recovery of analytes from DBS was assessed by analysing a DBS containing 2µg/ml of both R and S ketorolac, and comparing it to the analysis of 11µl of 2µg/ml of plasma; 11 µl was initially established as the volume of blood contained in a 6 mm paper disc sampled with a paper perforator (see chapter 4 section 4.4.1). The results are presented in Table 8.3.

A



Y = 0.000269872+1.14486e-005*X
R^2 = 0.9964, W: 1/X

B



Y = 0.000186606+7.74816e-006*X
R^2 = 0.9983, W: 1/X

Figure 8.5: Representative standard curve for R (+) ketorolac (A) and S (-) ketorolac (B). Data for three 8-point standard curves with threadlines.

Table 8.2: Intra-day and inter-day precision and accuracy

QC sample (ng/ml)	Conc. found mean ± S.D (ng/ml)	Precision (CV %)	Accuracy (% relative error)
Intraday (n =5)			
R(+) ketorolac			
100	113.9 ± 7.6	6.7	13.5
500	537.6 ± 19.4	3.6	7.5
2000	1935.9 ± 132.4	6.8	3.2
S(-) ketorolac			
100	120.0 ± 5.0	4.1	20.0
500	547.4 ± 55.5	9.9	11.7
2000	1919.3 ± 125.0	6.5	4.0
Interday (n= 9)			
R(+) ketorolac			
100	112.1 ± 5.0	9.4	12.1
500	526.9 ± 28.9	5.5	5.4
2000	1932.7 ± 110.0	5.7	3.4
S(-) ketorolac			
100	119.7 ± 10	8.4	19.7
500	543.5 ± 35.6	6.5	8.7
2000	1952.2 ± 113	5.8	2.4

Table 8.3: Comparison of ketorolac enantiomers recoveries from plasma and DBS containing 2µg/ml of both enantiomers

Ketorolac enantiomer	Concentration found (ng/ml)		% difference
	Plasma	DBS	
R(+)ketorolac	506.7	225.1	55.5
S(-)ketorolac	580.0	306.0	47.2

8.4. Stereoselective pharmacokinetics of intravenous ketorolac

8.4.1. Methods

8.4.1.1. Regulatory approval

8.4.1.1.1. Ethics approval

Study site approval was obtained from the Royal Aberdeen Children's Hospital Management, and the study sponsored by the Grampian Research and Development (R&D) office, ref: 2009CH004 and ethics approval granted by the North of Scotland Research Ethics Committees (NREC), ref: 09/S0801/60. All relevant documentation is shown as appendices including the study protocol (Appendix 15), letter of invitation (Appendix 16), Information sheets for parents and guardians and for children (Appendices 17-20), and consent form for parents and guardians (Appendix 21) and Assent forms for children (Appendix 22), and a Peer review of the protocol (Appendix 23). Approvals by R&D and NREC are presented as Appendices 24 and 25, respectively.

8.4.1.1.2 MHRA approval

In line with Directive 2001/20/ER of the European Parliament, a EudraCT number (2009-012512-41) was obtained from the EudraCT Community Clinical Trial System, and an initial application for a clinical trial authorization (CTA) for the conduct of the study was made to the MHRA, UK. The MHRA approval is presented as Appendix 26.

8.4.1.2. Study design

8.4.1.2.1 Setting

The study was conducted within the same setting, the Royal Aberdeen Children's Hospital (RACH), used for the PK study of IV paracetamol presented in Chapter 6. In brief, RACH is a teaching hospital, offering paediatric surgical services in general, ENT, ophthalmology, oral, plastic and orthopaedic surgery. The hospital has three functional operation theatres and a total of 47 beds comprising the Surgical

Ward, Day-case and High Dependency Units. Surgical service at the RACH is lead by 4 paediatric surgeons and 5 paediatric anaesthetists with supporting junior medical, nursing and theatre staff.

8.4.1.2.2 Patients

Following approval by the North of Scotland Research Ethics Committees and authorization by the MHRA-UK, 11 children aged 1.7 to 16 years undergoing elective surgery were recruited to the study. Parents and children were approached when there was an anticipated need for intravenous ketorolac for postoperative pain relief. A child's eligibility for inclusion in the study was decided by the study anaesthetist based on the child's preoperative medical history and current clinical status. Children were excluded if they had liver dysfunction, renal impairment, hypersensitivity to ketorolac or any of the components of the intravenous formulation of ketorolac, a history of asthma, operations with a high risk of haemorrhage or incomplete haemostasis, active peptic ulcer or any history of gastrointestinal bleeding, ulceration or perforation, coagulation disorders, complete or partial syndrome of nasal polyps, previous episodes of angioedema, and use of ketorolac 24 hours prior to study.

Children were identified from elective operation lists and all prospective children so identified were sent an information pack at least a week before they were due for admission to the hospital. The pack contained an age appropriate participant information sheet that fully explained the study, and a letter of invitation that indicated that parental consent and child's assent, where appropriate, would be sought for inclusion in the study (see appendices 20-24). On the day of the surgery, a Good Clinical Practice (GCP) trained research nurse approached the participant in order to verify that they had received the information pack and had sufficient time to decide whether to take part in the study. The research nurse explained the study and what was involved. Willingness to take part was determined through a further brief interview with the parent, guardian and or where appropriate the child. Written informed consent was obtained from the parents, and for children above 7 years old, additional written assent was sought. Once consent was obtained, patient demographic data; age, gender, weight and height were recorded. The body mass

index (BMI) and body surface area (BSA) were calculated using the following formulae:

$$BMI = \frac{\text{Body weight (kg)}}{\text{Height (m)}^2};$$

$$BSA = \text{Body weight (kg)}^{0.425} \text{ X Height (cm)}^{0.725} \text{ x } 71.84 \text{ x } 10^{-4}$$

8.4.1.2.3 Drug administration

General anaesthesia was induced either intravenously with propofol 3.5 $mg.kg^{-1}$ or by facemask with sevoflurane and maintained with isoflurane. After induction of anaesthesia and where medically indicated, ketorolac was administered intravenously at a dose of 0.5 mg/kg (ceiling dose 10 mg) by the study anaesthetist.

8.4.1.2.4 Blood sampling

Blood was sampled from a peripheral line which had been established and maintained with a low volume infusion of 0.9% saline (sodium chloride) during the first 2 hours of sampling. Hepsal (heparin sodium10units/ml) was used when patient was transferred to the ward. After discarding the first 100 µl, 1 ml samples were taken immediately before ketorolac administration and at 15, 30min, 60min, 2hrs, 4hrs, 6hrs and 8hrs and 12 hrs post dose. Where the 8^{th} and 12^{th} hour blood samples were due at times late into the night, they were postponed to the next morning, making sure the last sample was taken not later than 22 hrs post dose. All samples were centrifuged within 2 hours of collection, at 900 g for 15 minutes and the resultant plasma stored at -20^0C in a secured freezer until chromatographic analysis.

8.4.1.3. Stereoselective quantification of intravenous ketorolac

Ketorolac enantiomers were extracted from plasma by the liquid- liquid method described in section 8.3.1.3. Analyte quantification was performed using the

developed and validated method and samples analysed in two batches, using a freshly prepared calibration curve on each occasion.

8.4.1.4. Pharmacokinetic parameter calculations and Statistical Analysis

Individual PK parameters were calculated using non-compartmental pharmacokinetic analysis. The calculations were based on the principle of exponential decay of drug concentration over time defined by the relationship as described in Chapter 6, section6.3.4 of this thesis. Briefly,

$$C_t = C_0.e^{-kel.t}. \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textit{(8.1)},$$

where C_t is the plasma concentration at a given time, t, C_0 the concentration at t = 0 and k_{el} the elimination rate constant. PK parameters were obtained from equation *(8.1)* as follows:

$$\log C_t = \log C_0 - \frac{k_{el}}{2.3} t \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textit{(8.2)}$$

$$k_{el} = 2.3 \text{ x slope} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textit{(8.3)}$$

$$t_{1/2} = \frac{0.693}{k_{el}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textit{(8.4)}$$

where C_1 and C_2 are blood ketorolac concentrations at time t_1 and t_2, respectively. A semi-logarithmic plot of function *(8.2)* was obtained from at least five blood samples, by plotting log-concentration versus time. Equations *(8.3)* and *(8.4)* were used to calculate the elimination rate constant (k_{el}) and the half-life of elimination ($t_{1/2}$), respectively. The clearance (CL) was calculated from the total area under the concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) as follows:

$$\text{CL (L/hour)} = \frac{Dose(mg)}{AUC_{0-\infty}(mg * hour / ml)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (8.5)$$

$AUC_{0-\infty}$ was obtained based on the fundamental relationships:

$$AUC_i = \frac{[C(t_i) - C(t_{i+1})].t}{In[C(t_i) / C(t_{i+1})]} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (8.6)$$

$$\mathrm{AUC}_{0\text{-}t} = \sum_{i=1}^{n} AUC_i \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (8.7),$$

where AUC_i is the area between the times t_i and t_{i+1} at which concentrations were $C(t_i)$ and $C(t_{i+1})$, respectively (Rowland and Tozer, 1995).

$AUC_{0\text{-}\infty}$ was calculated by extrapolating area from last obtainable ketorolac concentration to infinity ($AUC_{last\text{-}\infty}$):

$$\mathrm{AUC}_{last\text{-}\infty} = \frac{C_{last}}{K_e} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (8.8)$$

$$\mathrm{AUC}_{0\text{-}\infty} = \mathrm{AUC}_{0\text{-}t} + \mathrm{AUC}_{last\text{-}\infty} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (8.9)$$

The volume of distribution (V_d) was obtained from CL/k_e. Additionally CL and V_d were normalised to 1 kg body weight of child. Data are presented as medians with ranges.

Due to the small number of children who had adequate plasma concentration-time data that allowed accurate PK parameter estimation by the traditional PK method, the nonparametric equivalent of the student t test (Wilcoxon signed-rank test) was used to compare the PK parameters obtained for R (+) and S (-) ketorolac. The null hypothesis was that, there would be no statistical difference ($P < 0.05$) in the PK values between the parameters for the two enantiomers.

8.4.2. Results

Figure 8.6 is a flow chart of patient recruitment to the study. Between 2nd February and 28th July 2010, 22 prospective participants were approached and of this number, 72 % (16) consented to take part in the research. Five children did not start the study due to difficulty in siting cannula (2), changed medical condition in theatre (2) and prior administration of ibuprofen (1). The demographic data of all children who started the study are presented in Table 8.4. However only five children had blood sampling time-points suitable for classical PK analysis as one of the conditions for ethical approval were that IV cannulae would not be replaced unless clinically

indicated. Cannula failure rate (54.5%) was the single most important factor that prevented adequate blood sampling.

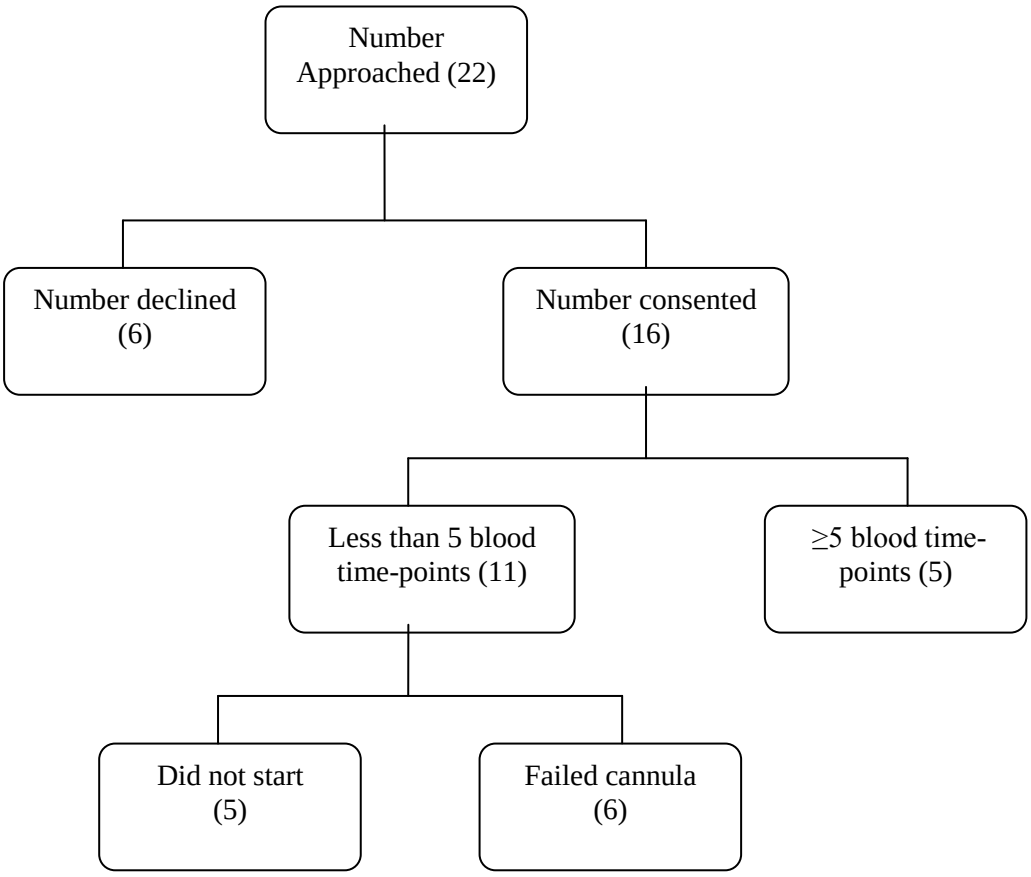


Figure 8.6: Flow chart of patient recruitment on arrival to hospital. A total of 34 participants were invited and those admitted were approached.

A plot of the plasma concentration-time curves for the enantiomers of ketorolac in all the children depicted a steady decline of concentration over time (Figure 8.7). Since the medicine was administered as the racemate (50/50 mixture of the enantiomers), an equal dose of R and S ketorolac was assumed and used for the non-compartmental PK analysis.

Table 8.4: Demographic characteristics of children who started the study

Subject	Gender	Age (years)	Weight (kg)	Height (m)	Dose (mg)	Sample	Comment
1	M	13.4	45.2	1.44	10	4	
2	F	1.7	11.6	0.85	3	4	
3	M	13.0	46.0	1.65	10	8	
4	F	9.4	29.3	1.35	10	7	up to 6 h
5	M	11.6	46.3	1.53	10	9	
6	F	11.0	42.4	1.54	6	4	
7	M	13.0	67.4	1.45	10	5	up to 4 h
8	M	13.8	63.5	1.68	10	8	
9	F	15.2	53.8	1.61	10	8	
10	F	2.3	10.7	0.83	3	5	up to 4 h
11	F	15.6	53.7	1.64	10	5	

Although 5 or more samples were obtained from subjects 4, 7 and 10, sampling times were not beyond 6 h to allow adequate description of the terminal phase of the concentration time curve necessary for non- compartmental PK analysis. This decision was arrived at based on previous estimates of the half life of ketorolac at 4.3 – 6 .5 h (Hamunen et al., 1999; Kauffman et al., 1999)

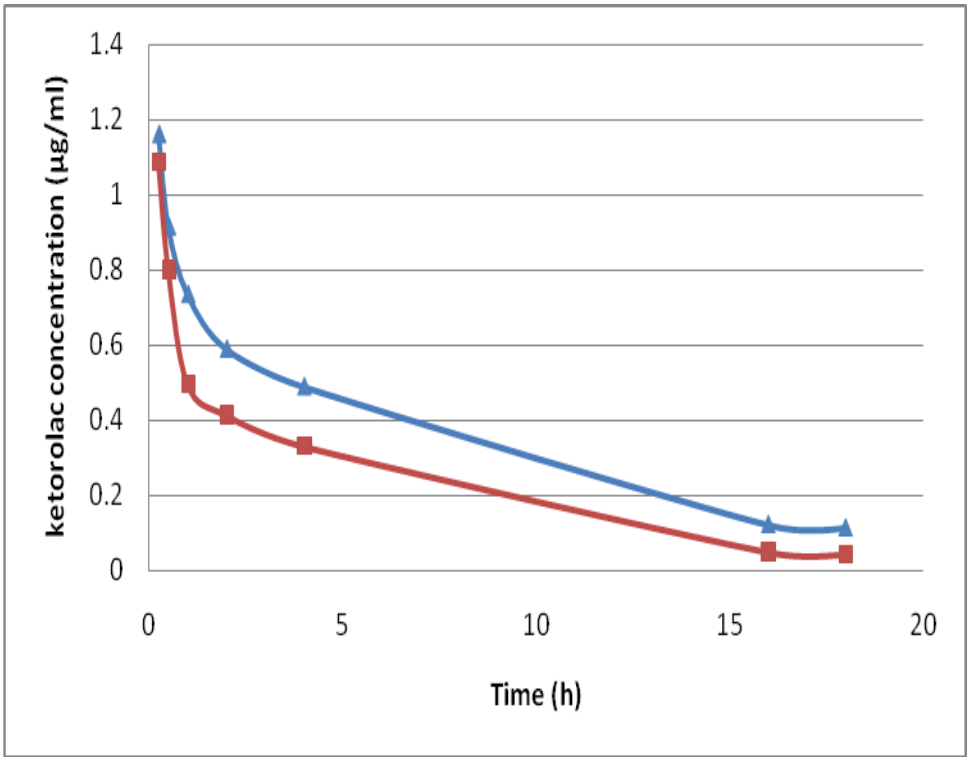


Figure 8.7: Representative plasma concentration- time curve of R (+) ketorolac (triangles) and S (-) ketorolac (squares) for a 13 year old child following administration of 10 mg of intravenous ketorolac.

The patient demographic data along with the PK parameters obtained for individual child are presented in Table 8.5 and where, as can be seen, all parameters were different for the two enantiomers, with V_d and CL of S–ketorolac being higher than those of R. As expected the $t_{1/2}$s of S-ketorolac were shorter than those of R and significantly different (P = 0.043).

Table 8.5: Patient demographic data and individual PK parameters following intravenous administration of 10 mg racemic ketorolac thromethamine to five children

Patient ID	Age (years)	Weight (kg)	Height (m)	BMI (kg/m^2)	BSA (m^2)	R (+) ketorolac			S (-) ketorolac		
						$t_{1/2}$(h)	V_d (l/kg)	CL(l/h/kg)	$t_{1/2}$(h)	V_d (l/kg)	CL(l/h/kg)
1	13.0	46.0	1.65	16.9	1.46	5.7	0.12	0.015	4.2	0.15	0.025
2	11.6	46.3	1.53	19.8	1.42	5.8	0.14	0.017	4.4	0.31	0.049
3	13.8	63.5	1.68	22.5	1.73	2.5	0.07	0.018	1.8	0.17	0.064
4	15.2	53.8	1.61	20.7	1.56	4.1	0.17	0.029	1.8	0.21	0.082
5	15.6	53.7	1.64	20.0	1.57	5.0	0.08	0.012	3.2	0.09	0.020
Mean	13.8	52.7	1.62	20.0	1.65						
(sd)	(1.6)	(7.2)	(0.10)	(2.0)	(0.10)						
Median (maximum – minimum)						5.0	0.12	0.017	3.1	0.17	0.049
						(2.5-5.8)	(0.07-0.17)	(0.12-0.29)	(1.8-4.4)	(0.09-0.31)	(0.02-0.01)
Wilcoxon Signed rank *P*									0.043	0.043	0.043

8.5. Discussion

8.5.1 Bioanalytical method

This is the first report of a microanalytical method for the enantioselective quantification of ketorolac. Although Ketorolac is an NSAID with potential benefit in the management of moderate to severe acute pain in children it has not been licensed for use in children less than 16 years old (BNF, March 2010, SPC, 2009) and data in support of optimisation of therapy is limited. Ketorolac and the chosen internal standard phenacetin were successfully identified using their mass and charge identities, and further resolution of ketorolac enantiomers achieved using a chiral column. All analytes were eluted within 5 minutes and chromatographic run-time was 6 minutes per sample, which enhanced throughput and the use of the chiral column enabled direct stereospecicific analysis of the enanatiomers thus avoiding possible racemisation associated with pre-column derivatization and the need for indirect analysis, with its associated sources of error (Tsina et al., 1996; Brocks, 2006).

The suggested 370 ng/ml plasma concentration for half-maximum (EC_{50}) analgesic effect reported for adults (Mandema and Stanski, 1996) was well within the linear portion (0 - 2000 ng/ml) of the calibration curve of the method. The chosen range was further supported by the highest plasma concentration reported for both enantiomers of ketorolac being1200 ng/ml after a therapeutic dose of 0.5 mg/kg of the racemate (Lynn et al., 2007). With a lower limit of quantification of 31.3 ng/ml for R (+) ketorolac and 62.5 ng/ml for the S (-) ketorolac, the method provided adequate sensitivity to support stereospecific PK analysis, and these sensitivities were achieved with only 50 µl of plasma making the method suitable for PK studies in children of all ages and sizes, and an advance when compared with previously reported methods requiring between 250 and 1000 µl of plasma (Ing-Lorenzini et al., 2009; Campanero et al., 1998). Since less than 150 µl of whole blood is sufficient to obtain 50 µl of plasma the required blood volumes would comfortably conform to the recommendation of 1 ml/kg for studies in children (EMEA, 2006).

Both within-assay (intra-day) and between-assay (inter-day) accuracy for the method, assessed by the percentage relative standard errors, were within the

recommended 20% limits (FDA 2001; ICH, 2005) and acceptable within-assay and between-assay precision was observed, with CVs of less than 10% were found within the calibration range.

The recoveries of all analytes at the lower quantification limit were higher than 70%, and these recoveries had CVs between 5 and 11 %, further demonstrating the reliability of the method. However, only half of the enantiomers were recovered from DBS thus rendering this extraction method unsuitable for PK studies of ketorolac using DBS. There is no published data on the penetration of ketorolac into human red blood cells. However, the high plasma protein binding reported for ketorolac (99.2 % for R and 98.4% for S) (Mroszczak et al., 1996) has the effect of confining the drug to the plasma component, and thus reducing penetration into the red blood cells, which might explain the lower levels measured in DBS. Hence the chosen extraction method was a simple – liquid extraction of microplasma samples. This would effectively preclude the use of DBS and limit the application of the technique to centres with appropriate laboratory support and near patient sample processing. However the DBS technique could be developed in future albeit at the expense of increased analytical time and cost.

8.5.2 Clinical application

The method was successfully applied to a PK study of IV ketorolac in children less than 16 years old and in which the concentration-time profile of the R and S enantiomers showed the exponential decay, typical of a drug delivered as an intravenous bolus. The PK parameters obtained indicated a consistently higher CL and V_d for the S than the R – enantiomers, and consistent with the inverse relationship between $t_{1/2}$ and CL, $t_{1/2}$s were shorter for S than R ketorolac in all the children. All the median differences were statistically significant and in agreement with previous reports (Hamunen et al., 1999; Kauffman et al., 1999; Lynn et al., 2007).

The median CL of 17 ml/h/kg found for R-ketorolac is within the 21.4 ± 5.9 ml/h/kg reported by Hamunen and colleagues (1999) for children of similar age to those in the present study and a clearance for the S-enantiomer at 49 ml/h/kg, similar to the 66.2 ± 17.5 ml/h/kg found in the Hamunen study. However, these values were

lower than the 84 and 372 ml/h/kg for the R and S enantiomers reported by Kauffman and co-workers (1999). The values in this latter reference were for a wider age (3 -18 years) and which may have been influenced by the contribution of younger children who are known to have high values as evidenced by the mean values of 60 and 264 ml/h/kg for R and S- ketorolac being reported for children 0.5 – 1.5 years (Lynn et al., 2007). Although not stereoselectively determined, a higher clearance of 74 ml/h/kg has also been reported for children less than 12 months old in comparison with 34 ml/h/kg for children aged 1- 16 years (Cohen et al., 2009; Dsida et al., 2002).

Whilst the median V_ds obtained of 0.12 l/kg for R ketorolac was close to the lower band of the values found in both the Hamunen et al (1999) and Kauffman et al (1999) studies (0.205 ± 0.05, 0.5 ± 0.34 l/kg), the value for S-ketorolac of 0.17 l/kg was lower than previously reported values for the S (1.29 and 0.89, respectively) from Hamunen et al (1999) and Kauffman et al (1999). Lynn et al, (2007) also reported a lower V_d, 0.37 l/kg for S- ketorolac, albeit in younger children aged 0.5 – 1.5 years old. The higher V_d values reported by Hamunen et al. and Kauffman et al are not consistent with the known plasma binding properties of ketorolac, (99.2 and 98.4% for R and S enantiomers respectively) (Mroszczak et al., 1996; Hayball et al., 1994). Others have found V_d values between 0.113 and 0.26 l/kg using nonstereospecific PK analysis of ketorolac (Olkkola et al., 1991; Gonzalez-Martin et al., 1997; Dsida et al., 2002).

The median $t_{1/2}$ was 5 h for R- and 3.1 h for S- ketorolac, with the values for the former enantiomer agreeing with the 4.3 – 6 .5 h reported earlier for children within the age group studied (Hamunen et al., 1999; Kauffman et al., 1999). A high mean value of 9.5 h was reported for S- ketorolac in the Hamunen et al study, but the wide variation around this value (SD 5.8 h) raises questions as to the generalisability of this figure. The value for S- ketorolac of 5 h found in the present study was longer than the 1.7 h reported by Kauffman et al, which again may be explained by the presence of younger children in this cited study (3 – 18 years), a conclusion also supported by the even shorter $t_{1/2}$s (1.1 h) found in children age 0.5 - 1.5 years (Lynn et al., 2007).

8.5.3 Conclusions

A simple, rapid and reliable bioanalytical method for the measurement of the concentrations of the enantiomers of ketorolac in micro samples of plasma (50 µl) has been successfully developed and applied. Such a microanalytical assay, which selectively quantifies the pharmacologically active S–ketorolac, affords the opportunity to gather PK data in younger children, which would allow effect-concentration analysis in this particular population, in which information is currently limited. The developed method used a chiral column for enantiomer separation, thus avoided the need for pre-derivatization and was therefore less prone to razemization. The PK of the two enanatiomers were significantly different in the children aged around 14 years (13.8 ± 1.6 years), however in this opportunistic study and due to the small number recruited, it was not possible to assess the effect of patient covariates such as age and body size on PK parameters.