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The use of polymeric solid phase extraction and HPLC analysis for the determination of ranitidine in routine plasma samples obtained from paediatric patients

L. G. Hare, J. S. Millership, P. S. Collier, J. C. McElnay, D. J. Carson and M. S. Shields

Abstract

A sensitive HPLC method for the determination of ranitidine in small-volume (0.5 mL) paediatric plasma samples is described. Plasma samples were extracted using a simple, rapid solid phase extraction (SPE) technique developed using disposable copolymer packed SPE cartridges. Chromatographic separation was achieved by reverse-phase HPLC with isocratic elution using a μBondapak C_{18} column and a phosphate buffer (10 mM, pH 3.75)–acetonitrile (87:13 v/v) mobile phase with UV detection at 313 nm. The HPLC system exhibited linearity in the range 8–800 ng mL^{-1}. Intraday % CV and % bias values were in the range 1.28–8.09 % (% bias -4.33 to -0.87) and interday % CV and % bias values were in the range 0.73–15.28 % (% bias -1.80 to $+1.65$). The limits of detection and quantitation obtained were 2 ng mL^{-1} and 8 ng mL^{-1}, respectively, and ranitidine extraction recoveries from plasma ranged from 92.30 to 103.88 %. In this study, the developed HPLC and SPE methodologies have been successfully applied to the determination of ranitidine concentrations in 68 paediatric plasma samples. The sampled population was drawn from patients already receiving the study drug therapeutically. Patients recruited had received ranitidine by two main routes – oral and intravenous. The plasma concentrations of ranitidine encountered in paediatric samples following oral or intravenous administration of a range of prescribed doses are presented graphically. These profiles are based on analysis of the first 68 plasma samples obtained from the first 35 patients recruited to the study receiving ranitidine by the oral or intravenous route.

The School of Pharmacy, The Queen's University of Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK

L. G. Hare, J. S. Millership, P. S. Collier, J. C. McElnay

Department of Child Health, The Queen's University of Belfast, The Institute of Clinical Science, Grosvenor Road, Belfast BT12 6BJ, Northern Ireland, UK

D. J. Carson, M. S. Shields

Correspondence: L. G. Hare, The School of Pharmacy, The Queen's University of Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK

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Introduction

Ranitidine is a histamine H_2-receptor antagonist that effectively inhibits pentagastrin-induced gastric acid secretions (Garg et al 1981). This weakly basic drug is four to five times more potent than its counterpart cimetidine and differs structurally from cimetidine in that it possesses a furanyl nucleus instead of a histamine-related imidazole nucleus (Figure 1) (Mihaly et al 1980).

Ranitidine is commonly used in adults to treat stress ulceration and is also the preferred treatment for Zollinger-Ellison syndrome (Rustum 1988; Prueksaritanont et al 1989). Despite this widespread use in the adult population, ranitidine is only licensed for the oral treatment of peptic ulceration in children (Royal College of Paediatrics & Child Health 1999). However, ranitidine is commonly used outside this licensed indication to treat gastro-oesophageal reflux in hospitalised paediatric patients. Such unlicensed and off-label use of drugs in paediatric patients

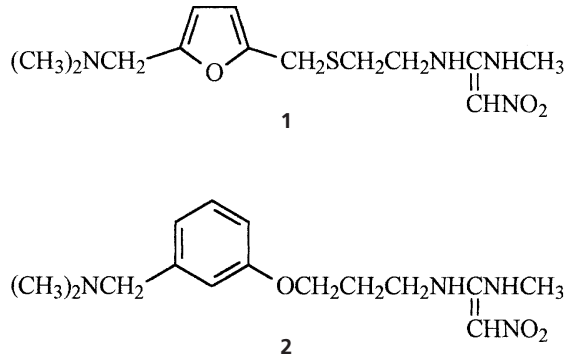

Figure 1 The chemical structures of ranitidine (**1**) and the internal standard AH20480 (**2**).

is currently a topic of governmental concern (Turner et al 1998, 1999; Conroy et al 2000).

In an attempt to address this issue, the present ongoing research programme aims to add to the evidence base for optimising dosage of ranitidine in paediatric patients by carrying out a non-standard pharmacokinetic (PK) paediatric clinical trial using the population PK approach and sparse data analysis. This type of PK study relies on infrequent (or sparse) sampling of blood from a larger patient population than would be used in a standard PK study. The sampled population is drawn from patients already receiving the study drug therapeutically and sampling takes place concurrently with other routine blood sampling. The use of population PK analysis avoids the ethical dilemma of submitting children to the rigours of a normal clinical trial that may involve repeated invasive procedures and is the PK study approach recommended for paediatrics by the FDA (1998a).

The analysis of ranitidine in human plasma is frequently performed using HPLC (Carey & Martin 1979; Mihaly et al 1980; Vandenberghe et al 1980; Boutagy et al 1984; Karnes et al 1987; Prueksaritanont et al 1989; Segelman et al 1990; Al-Khamis et al 1995; Farthing et al 1997; López-Calull et al 1997; Viñas et al 1997; Campanero et al 1998). Although HPLC methods for the determination of plasma ranitidine concentrations vary widely, isocratic reverse-phase HPLC using a μBondapak C_{18} packed analytical column appears to be the most common mode of separation selected (Mihaly et al 1980; Vandenberghe et al 1980; Boutagy et al 1984; Segelman et al 1990; Al-Khamis et al 1995; López-Calull et al 1997). Chromatographic separation of ranitidine from plasma constituents has been achieved using a wide array of mobile-phase conditions. However, in more recent years, a phosphate-buffered acetonitrile mobile phase has frequently been chosen (Karnes et al 1987; Segelman et al 1990; Al-Khamis et al 1995; Farthing et al 1997; López-Calull et al 1997; Viñas et al 1997). Despite such a wide array of HPLC techniques for the quantification of ranitidine in plasma, there are no published articles relating to HPLC analysis of ranitidine in paediatric plasma samples.

The extraction of ranitidine from plasma has been carried out by both liquid–liquid and solid phase extraction means. Liquid–liquid extraction (LLE) techniques have tended to be complex and labour intensive, involving double or back extractions, frequent centrifugation steps and evaporation phases (Carey & Martin 1979; Mihaly et al 1980; Vandenberghe et al 1980; Boutagy et al 1984; Segelman et al 1990; Al-Khamis et al 1995; López-Calull et al 1997; Viñas et al 1997; Campanero et al 1998). Solid phase extraction (SPE) techniques (Karnes et al 1987; Farthing et al 1997) have been simpler and faster than LLE methods but have been solely silica-sorbent based. Silica sorbents are prone to cracking if the sorbent bed dries out and this can lead to poor drug recovery.

This report describes an HPLC method for the determination of ranitidine concentrations in small volume (< 0.5 mL) paediatric plasma samples. In addition, a new method for the extraction of ranitidine from paediatric plasma samples using hydrophilic–lipophilic balanced (HLB) SPE cartridges is described.

Materials and Methods

Materials

All reagents were of analytical grade except where otherwise stated. All water was HPLC grade and was obtained using a Millipore-Q Reagent System (Waters, UK). Ranitidine hydrochloride was purchased from ICN Biomedicals Inc. (Ohio, USA). AH20480, *N*-[3-[5[[(dimethylamine)-methyl]-fenoxy]propyl]]-*N′*-methyl-2-nitro-1,1-ethenediamine (Figure 1), used as the internal standard, was kindly donated by Glaxo-Wellcome (Stevenage, UK). HPLC-grade methanol and acetonitrile were obtained from Lab Scan Analytical Services (Dublin, Ireland). Orthophosphoric acid AR and potassium dihydrogen orthophosphate AR were obtained from BDH (Poole, UK). Filtration of HPLC mobile phases was performed using Gelman FP-450 filters (Pall Gelman Sciences, Northhampton, UK). Screened whole blood, from which drug-free plasma was obtained, was kindly donated to the study by The Northern Ireland Blood Transfusion Service. Plasma was stored at -20°C until required.

Instrumentation

The HPLC system employed in this study consisted of a Shimadzu SCL-10AVP system controller, a Shimadzu SIL-10ADVP autoinjector, a Shimadzu LC-10ATVP pump, a Shimadzu FCV-10ALVP solvent mixer, a Shimadzu DGU-14A degasser, a Shimadzu SPD-10AVP UV detector and a Jones Chromatography model 7990 column heater. Data were acquired and integrated using Shimadzu ClassVP software. The analytical column was a Waters μBondapak C_{18} (300 × 3.9 mm, 10 μm) fitted with a Waters μBondapak C_{18} (3.9 × 20 mm, 10 μm) guard column. UV detection at 313 nm was employed and the column temperature was maintained at 25°C. The injection volume was 40 μL.

Chromatographic conditions

Chromatographic separation was achieved using reverse-phase HPLC with isocratic elution. The system employed was a modification of that described by López-Calull et al (1997) using AH20480 as the internal standard. The mobile phase consisted of acetonitrile–phosphate buffer (10 mM, pH 3.75, 13:87 v/v) filtered and degassed through a 0.45-μm filter. The mobile phase was delivered at a flow rate of 1 mL min^{-1}. Peak area ratios were plotted against ranitidine concentration in the construction of the calibration curve.

Collection of patient plasma samples

Patient blood samples were collected in 5-mL lithium-heparin sample tubes. The sample in the tube was inverted several times to ensure mixing and prevention of coagulation. The blood sample was then centrifuged for 10 min at 3000 *g* to separate the plasma component. Plasma was transferred to a clean sample tube and was stored at −20°C until analysis.

Construction of a standard curve for ranitidine in plasma

Master stock solutions of ranitidine and AH20480 were prepared in methanol at a concentration of 1 mg mL^{-1} and were stored in the dark at 4°C until required. Aqueous working standard solutions of ranitidine (1 μg mL^{-1}) and AH20480 (5 μg mL^{-1}) were prepared from the master stock standards. Plasma standards were prepared from the working ranitidine solution at concentrations of 8, 20, 40, 80, 200, 400 and 800 ng mL^{-1} by serial dilution into drug-free plasma. These ranitidine–plasma standards were then extracted by the SPE procedure that follows, with the internal standard working solution being added as appropriate in the protocol.

Solid phase extraction protocol

A simple one-dimensional SPE protocol was developed for the extraction of ranitidine from plasma using disposable Oasis HLB cartridges (1 mL/30 mg, 30 μm; Waters, UK). The procedure was used in conjunction with a Waters extraction manifold system (20-position manifold with a 13 mm × 75 mm collection rack). The vacuum pressure on the manifold was maintained at 12.7 mmHg throughout the duration of the SPE procedure. Each cartridge was conditioned with methanol (1 mL) and water (1 mL). The plasma sample (calibrant or patient, 0.5 mL), mixed with working internal standard (20 μL of 5 μg mL^{-1} aqueous AH20480), was loaded onto the cartridge. Sample interferents were washed from the cartridge with 5% aqueous methanol (1 mL). Waste solvents were discarded from the manifold system and the collection rack was filled with disposable Pyrex borosilicate glass culture tubes (Corning, NY). Cartridge-retained ranitidine and internal standard were then eluted with 100% methanol (1 mL). Collected eluents were evaporated to dryness at 40°C under a stream of N_2 and residues were reconstituted in 200 μL of mobile phase before injection onto the HPLC system.

Recovery studies

The efficiency of the developed SPE extraction system was assessed by spiking drug-free plasma with known amounts of ranitidine (20, 100 and 750 ng mL^{-1}) and subsequently measuring the amount of spiked ranitidine recovered following HPLC analysis of these samples. A calibration curve for ranitidine in mobile phase, incorporating AH20480 internal standard, was constructed for this purpose. A ranitidine-free plasma sample was also extracted and examined by HPLC to certify that no endogenous material interfered with the assay. In addition, an aqueous sample containing no ranitidine was extracted using the SPE procedure to ensure no interferences from cartridge material.

Linearity, accuracy and sensitivity of HPLC system

The linearity of the developed HPLC system for the determination of plasma ranitidine concentrations was assessed over a concentration range of 8–800 ng mL^{-1}. Plasma–ranitidine standards (8, 20, 40, 80, 200, 400 and 800 ng mL^{-1}) were prepared using drug-free plasma and working solutions of ranitidine and internal standard as previously described. Each extracted standard was injected in triplicate, peak area ratios were calculated and

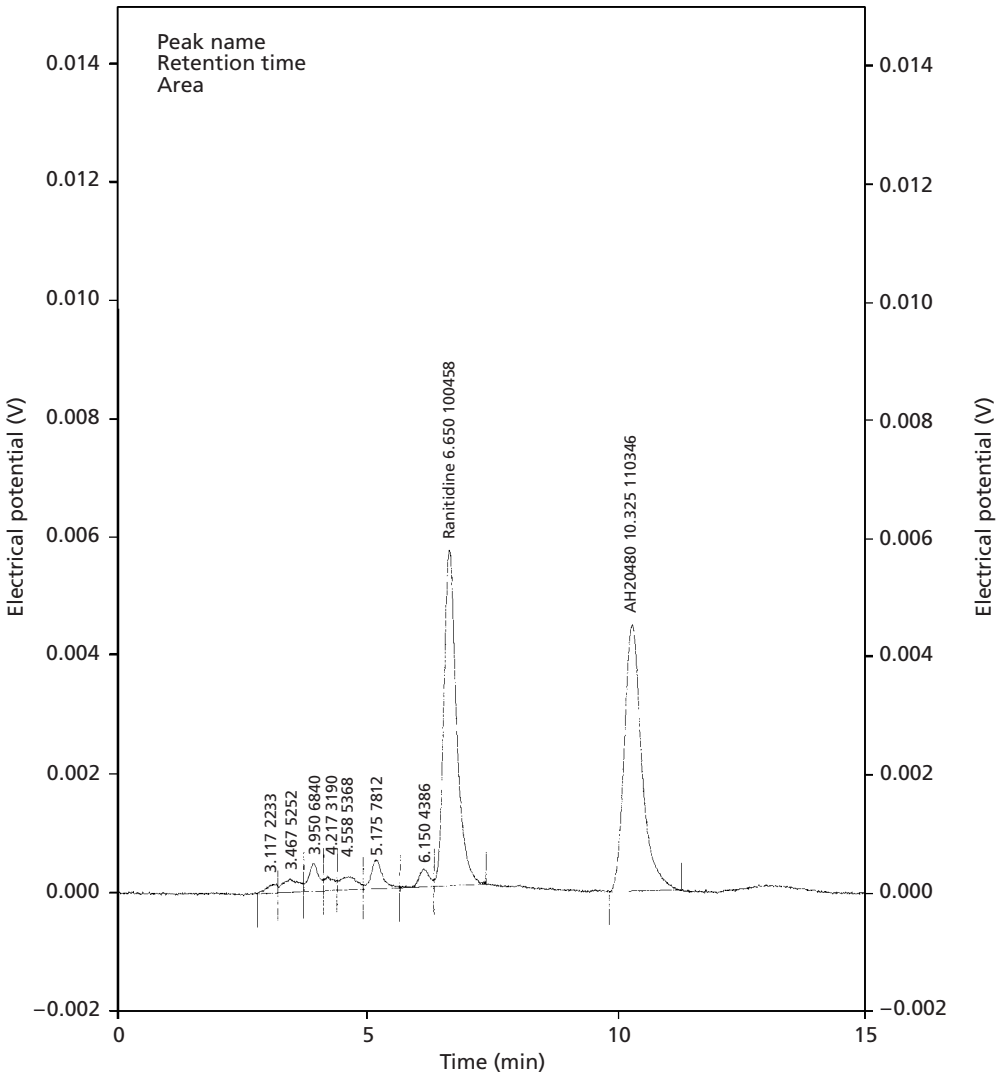

Figure 2 HPLC chromatogram of a plasma sample spiked with ranitidine (200 ng mL^{-1}) and internal standard (20 μL of 5000 ng mL^{-1}) and extracted by SPE.

the calibration curve was constructed accordingly. In this study, the accuracy of the developed HPLC system was determined by measuring system precision and system trueness. The precision of the HPLC system used in this study was evaluated by determination of intra- and interday percentage coefficients of variation (% CV values). Intraday variation was established on a single day by quintuplet estimation of drug-free plasma standards spiked with ranitidine at three different concentrations (20, 200 and 800 ng mL^{-1}). Interday variation was determined over a period of five days using the same methodology. In the latter case, drug-free plasma–ranitidine standards were freshly prepared, extracted and injected in triplicate each day. The trueness of the HPLC system used in the present study is expressed in terms of percentage bias, bias being defined as the positive or negative deviation of the mean analytical result from the known (or assumed) true value (EURACHEM 1998). Limits of quantitation (LOQ) and detection (LOD) were used to evaluate the sensitivity of the developed HPLC system for the determination of plasma ranitidine concentrations. In this study, the LOQ was defined as the lowest concentration point on the ranitidine–plasma calibration curve (EURACHEM 1998; FDA 1998b). The limit of detection (LOD) was defined as the concentration of

ranitidine in plasma that would provide a signal equivalent to three times the signal-to-noise level (S_N).

Results

HPLC method

The isocratic HPLC system used in this study was successful in separating and resolving ranitidine and the internal standard (AH20480) from other extracted plasma components within a run-time of 15 min (Figures 2 and 3). Retention times of 6.6 and 10.3 min were obtained for ranitidine and AH20480 respectively. HPLC analysis of a blank plasma sample extract showed no interference at ranitidine and internal-standard elution times. The HPLC system developed in this study exhibited linearity over the desired ranitidine plasma concentration range (8–800 ng mL^{-1}) with a mean r^2 value of 0.9997 ± 0.0002 ($n = 7$ analytical runs) being obtained. The limit of quantitation (LOQ) established was 8 ng mL^{-1} and the limit of detection (LOD) was determined as 2 ng mL^{-1}. Triplicate estimation of drug-free plasma standards spiked with ranitidine at three different concentrations (20, 200 and 800 ng mL^{-1}) was

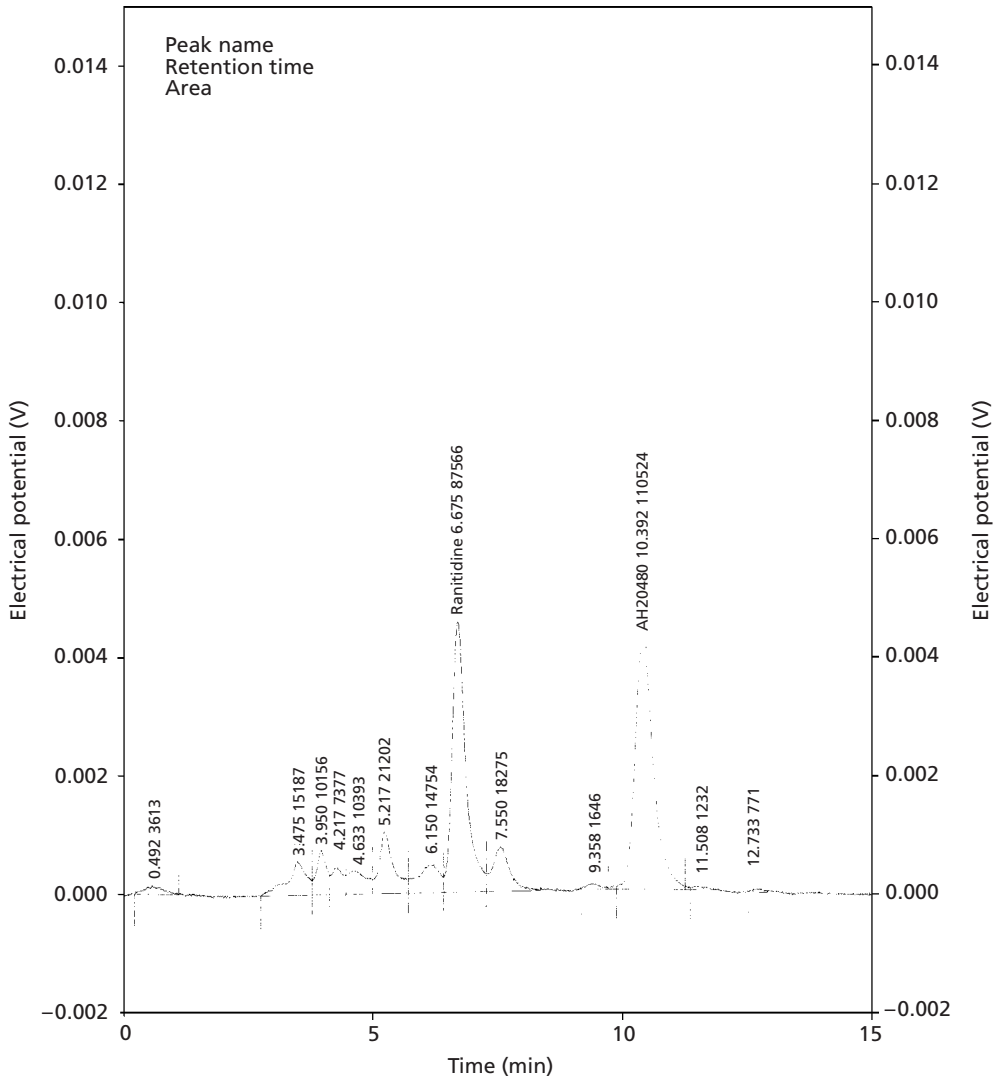

Figure 3 HPLC chromatogram of a paediatric patient plasma sample containing ranitidine and spiked with internal standard (20 μL of 5000 ng mL^{-1}) and extracted by SPE.

Table 1 Precision and bias of HPLC method for the quantification of ranitidine in plasma

Concn of ranititidine (ng mL^{-1})		% CV	% Bias	n
Added to plasma	Quantified			
Intraday				
20	19.27	8.09	−3.65	5
200	191.33	4.19	−4.33	5
800	793.01	1.28	−0.87	5
Interday				
20	20.33	15.28	+1.65	5
200	196.40	4.67	−1.80	5
800	797.63	0.73	−0.30	5

used to evaluate the precision of the HPLC assay used in this study. The intra- and interday coefficients of variation (% CVs) for the assay, ranging between 0.73 and 15.28 %, and corresponding % bias results, ranging from –4.33 to +1.65, are presented in Table 1.

Solid phase extraction procedure

Disposable Oasis HLB cartridges (1 mL) were successfully used in this study to extract ranitidine from paediatric plasma samples. Use of these polymer-packed SPE cartridges, in conjunction with a vacuum manifold, allowed the simultaneous extraction of twenty plasma samples. The efficiency of the SPE extraction system developed in this study was determined using drug-free plasma spiked with known concentrations of ranitidine (20, 100 and 750 ng mL^{-1}). Average ranitidine recoveries (n = 5) were 103.88, 92.30 and 93.25 % (% CV = 7.85, 3.31 and 3.20 %) following SPE extraction of plasma samples spiked with 20, 100 and 750 ng mL^{-1} of ranitidine, respectively. As an internal standard was also being used, its recovery from plasma following SPE extraction was assessed. A consistent concentration of aqueous AH20480 (0.1 μg) was added during the SPE protocol. Recovery of AH20480 from plasma at this concentration was 97.97 % ± 0.85 (n = 9).

Plasma concentrations of ranitidine in paediatric patients

Data obtained following the analysis of 68 clinical samples from 35 paediatric patients are presented in Figure 4. In a 10-h period following intravenous administration of 0.6–2.0 mg kg^{-1} ranitidine to 19 patients aged 0.08–10.42 years, weighing 1.3–25.1 kg, plasma concentrations of 1366–7 ng mL^{-1} were measured. Ranitidine (0.97–5.17 mg kg^{-1}) was administered orally to a further 16 patients and monitored over 13.5 h. Plasma concentrations of 727–7 ng mL^{-1} were obtained in these children who were aged 0.29–11.08 years and weighed 3.6–44.5 kg.

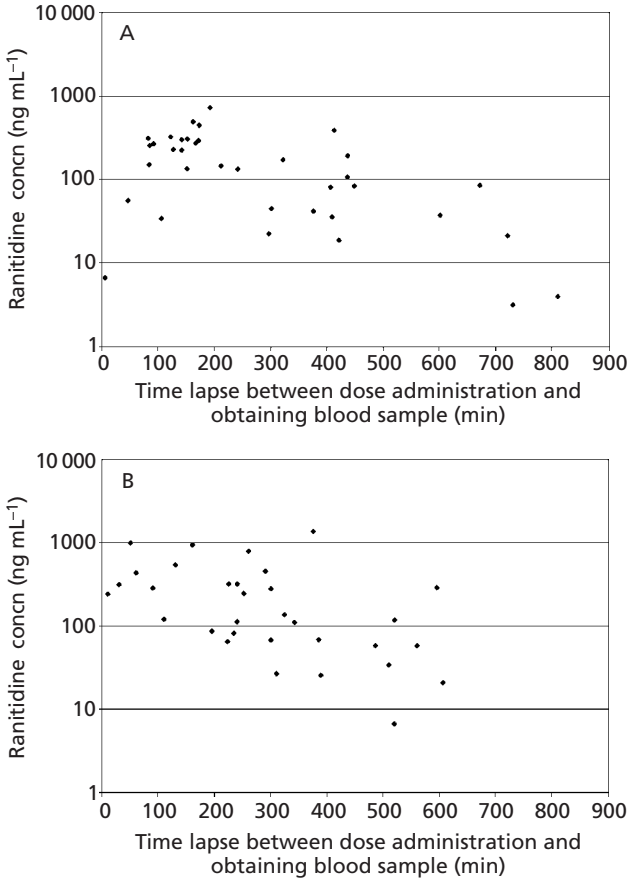

Figure 4 Plasma concentrations of ranitidine encountered in routine clinical paediatric samples obtained at the times indicated following oral administration (A) and intravenous administration (B) of a range of prescribed doses.

Discussion

The chromatographic system employed in this study was a modification of that described by López-Calull et al (1997) for the determination of ranitidine in human serum. A phosphate buffer (10 mM, pH 3.75)–acetonitrile mobile phase (85:15 v/v) was used by López-Calull et al (1997) to achieve separation of ranitidine and AH20480 (the internal standard) from serum samples. However, in this study, use of such mobile-phase conditions to separate ranitidine and AH20480

from plasma components resulted in poor resolution of ranitidine from an unknown plasma component. Adjustment of the mobile phase to phosphate buffer–acetonitrile (87:13 v/v) overcame this difficulty and acceptable ranitidine resolution was achieved (Figures 2 and 3). The HPLC system described by López-Calull et al (1997) was further adapted in this study by the addition of a guard column. As would be expected, the addition of this guard column increased the retention times of ranitidine and the internal standard (6.6 and 10.3 min, respectively) as compared with those reported by López-Calull et al (1997) (4.9 and 6.5 min, respectively).

The HPLC method developed in this study for the determination of ranitidine concentrations in paediatric plasma samples was shown to be both sensitive and precise. The pattern of detection exhibited by ranitidine in plasma was linear over a concentration range of 8–800 ng mL^{-1}. Results obtained during linearity studies compare well with those published previously (Karnes et al 1987; López-Calull et al 1997; Campanero et al 1998). The HPLC method utilised in this study exhibited a high degree of sensitivity and accuracy as displayed by the limits of quantitation (8 ng mL^{-1}) and detection (2 ng mL^{-1}), intra- and interday % CVs and % bias results achieved. The LOQ obtained in this study compares well with those reported previously (7 ng mL^{-1}, López-Calull et al 1997; 10 ng mL^{-1}, Campanero et al 1998). The LOD of 2 ng mL^{-1} achieved for ranitidine in this study is equivalent to the lowest LOD for ranitidine in plasma previously published (López-Calull et al 1997; Campanero et al 1998). Intraday (1.28–8.09 %) and interday (0.73–15.28 %) % CVs obtained in this study compare relatively well with previously published data (Vandenberghe et al 1980; Al-Khamis et al 1995; Farthing et al 1997; López-Calull et al 1997; Campanero et al 1998) and fulfil the essential criteria for HPLC assay precision during the analysis of drugs in biological matrices, as determined by the FDA (1998b).

The majority of previously published methods for the extraction of ranitidine from plasma have been liquid–liquid based. LLE procedures tend, however, to suffer from a number of disadvantages, including time/labour intensive methodologies, large solvent consumption, difficulties involved in achieving optimum conditions required to obtain high analyte distribution coefficients and problems associated with samples which form emulsions during extraction. SPE techniques, on the other hand, are generally fast, efficient at extracting analyte from interferents, use low volumes of organic solvent and can be used to achieve analyte trace enrichment in conjunction with sample purification. Unfortunately, in the past, classical silica-based SPE procedures have also been associated with sorbent drying (cracking of the packing material during extraction), polar analyte breakthrough and strong retention of basic compounds due to interaction between the analyte and silica-silanol groups. Efforts to overcome such difficulties have recently led to the introduction of SPE cartridges packed with novel copolymer materials instead of silica-based sorbents. In this study, paediatric plasma samples were extracted by SPE using disposable polymer-packed SPE cartridges. The particular cartridge packing material used in this study was based on a hydrophilic–lipophilic balanced copolymer derived from vinylpyrrolidone and divinylbenzene. The attributes of this copolymer material include wetting properties such that the material can be allowed to run dry without causing packing-bed cracking. In addition, the material can be used over the full pH range (0–14) and strong retention of basic compounds is avoided as there are no reactive silanol groups present. In this study, excellent ranitidine recoveries of 103.88, 92.30 and 93.25 % were obtained from plasma spiked with ranitidine at 20, 100 and 750 ng mL^{-1}, respectively. There are no articles previously published relating to the extraction of ranitidine from plasma samples using polymer-packed SPE cartridges. However, ranitidine recoveries obtained in this study compare favourably with those obtained by Karnes et al (1987) (93.0 %) and Farthing et al (1997) (90.0 %), both of whom extracted ranitidine from plasma samples using SPE cartridges packed with a silica-based cyano sorbent.

In three of the samples analysed, the initial results indicated that the ranitidine concentrations were above 800 ng mL^{-1}, the highest concentration in the calibration curve. Those samples were therefore diluted appropriately with drug-free plasma and 0.5 mL of the diluted sample re-analysed. Allowance for the dilutions was then made when reporting the ranitidine concentrations in those samples.

Figure 4 presents the plasma concentrations of ranitidine relative to the time of the last dose. However, the graphs should not be regarded as a representation of the pharmacokinetic profile of ranitidine. Although the intravenous and oral data were obtained from paediatric patients who had been prescribed ranitidine as part of their therapy, the patients differed from each other with regard to dose (mg kg^{-1}), frequency of dosing and duration of therapy, age, weight and clinical status. The data presented in Figure 4, however, demonstrate the range of ranitidine concentrations that one is likely to encounter in normal clinical paediatric practice. The assay described within this paper will therefore be of value in determining the population pharmacokinetics

of ranitidine within the paediatric population when samples are obtained as part of routine clinical monitoring. It is for this purpose that the assay is currently being used within our laboratory.

Conclusions

The HPLC and SPE methodologies used in the study display sufficient sensitivity and selectivity for the effective analysis of ranitidine concentrations in small volume paediatric plasma samples. In addition, the developed SPE method offers excellent efficiency in terms of drug recovery and the speed of this method significantly increases daily clinical sample throughput in comparison with that which can be achieved by liquid–liquid extraction methods. Analysis of clinical plasma samples has shown that the assay is suitable for determining ranitidine at concentrations routinely encountered within paediatric patients. It therefore has the potential for use in paediatric pharmacokinetic studies of ranitidine.

References

Al-Khamis, K. I., El-Sayed, Y. M., Al-Rashood, K. A., Bawazir, S. A. (1995) High performance liquid chromatographic determination of ranitidine in human plasma. *J. Liq. Chromatogr.* **18:** 277–286

Boutagy, J., More, D. G., Munro, I. A., Shenfield, G. M. (1984) Simultaneous analysis of cimetidine and ranitidine in human plasma by HPLC. *J. Liq. Chromatogr.* **7**: 1651–1664

Campanero, M. A., Lopez-Ocariz, A., García-Quetglás, E., Maza, A. D. L. (1998) Rapid determination of ranitidine in human plasma by high-performance liquid chromatography. *Chromatographia* **47**: 391–395

Carey, P. F., Martin, L. E. (1979) A high performance liquid chromatographic method for the determination of ranitidine in plasma. *J. Liq. Chromatogr.* **2**: 1291–1303

Conroy, S., Choonara, I., Impicciatore, P., Mohn, A., Arnell, H., Rane, A., Knoeppel, C., Seyberth, H., Pandolfini, C., Raffaelli, M. P., Rocchi, F., Bonati, M., Jong, G., Hoog, M. D., Anker, J. V. D. (2000) Survey of unlicensed and off label drug use in paediatric wards in European countries. *Br. Med. J.* **320**: 79–82

EURACHEM. (1998) *The fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics*. LGC (Teddington) Ltd, Teddington, pp 1–52

Farthing, D., Brouwer, K. L. R., Fakhry, I., Sica, D. (1997) Solid-phase extraction and determination of ranitidine in human plasma by a high-performance liquid chromatographic method using mid-bore chromatography. *J. Chromatogr. B. Biomed. Sci. Appl.* **688**: 350–353

FDA (1998a) In: *Guidance for industry - general considerations for paediatric pharmacokinetic studies for drugs and biological products*. November, pp 1–10

FDA (1998b) In: *Guidance for industry – bioanalytical methods validation for human studies*. December, pp 1–13

Garg, D. C., Weidler, D. J., Baltodano, N., Eshelman, F. N. (1981) Pharmacokinetics of ranitidine, a new histamine H2-receptor blocker. *Clin. Pharmacol. Ther.* **29**: 247–248

Karnes, H. T., Opong-Mensah, K., Farthing, D., Beightol, L. A. (1987) Automated solid-phase extraction and high-performance liquid chromatographic determination of ranitidine from urine, plasma and peritoneal dialysate. *J. Chromatogr.* **422**: 165–173

López-Calull, C., García-Capdevila, L., Arroyo, C., Bonal, J. (1997) Simple and robust high-performance liquid chromatographic method for the determination of ranitidine in microvolumes of human plasma. *J. Chromatogr. B. Biomed. Sci. Appl.* **693**: 228–232

Mihaly, G. W., Drummer, O. H., Marshall, A., Smallwood, R. A., Louis, W. J. (1980) High-pressure liquid chromatographic determination of ranitidine, a new H2-receptor antagonist, in plasma and urine. *J. Pharm. Sci.* **69**: 1155–1160

Prueksaritanont, T., Sittichai, N., Prueksaritanont, S., Vongsaroj, R. (1989) Simultaneous determination of ranitidine and its metabolites in human plasma and urine by high-performance liquid chromatography. *J. Chromatogr.* **490**: 175–185

Royal College of Paediatrics & Child Health (RCPCH) (1999) *Medicines for Children* 1st edn. RCPCH Publications Ltd, London, pp 451–452

Rustum, A. M. (1988) Rapid and sensitive HPLC determination of ranitidine in plasma. Application to pharmacokinetics study. *J. Liq. Chromatogr.* **11**: 2315–2335

Segelman, A. B., Adusumalli, V. E., Segelman, F. H. (1990) Automated liquid chromatographic determination of ranitidine in microlitre samples of rat plasma. *J. Chromatogr.* **535**: 287–292

Turner, S., Longworth, A., Nunn, A. J., Choonara, I. (1998) Unlicensed and off-label drug use in paediatric wards: prospective study. *Br. Med. J.* **316**: 343–345

Turner, S., Nunn, A. J., Fielding, K., Choonara, I. (1999) Adverse drug reactions to unlicensed and off-label drugs on paediatric wards: a prospective study. *Acta Paediatr.* **88**: 965–968

Vandenberghe, H. M., MacLeod, S. M., Mahon, W. A., Lebert, P. A., Soldin, S. J. (1980) Analysis of ranitidine in serum by high performance liquid chromatography. *Ther. Drug. Monit.* **2**: 379–384

Viñas, P., Campillo, N., López-Erroz, C., Hernández-Córdoba, M. (1997) Use of post-column fluorescence derivatization to develop a liquid chromatographic assay for ranitidine and its metabolites in biological fluids. *J. Chromatogr. B. Biomed. Sci. Appl.* **693**: 443–449