Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 824 (2005) 333–340
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Determination of lorazepam in plasma from children by high-performance liquid chromatography with UV detection

Simon N. Muchohi [a,b,*], Kenneth Obiero [c], Gilbert O. Kokwaro [a,b,d], Bernhards R. Ogutu [c], Isaiah M. Githiga [d], Geoffrey Edwards [e,f], Charles R.J.C. Newton [b,g]

[a] *Kenya Medical Research Institute (KEMRI)/Wellcome Trust Research Programme, P.O. Box 43640, 00100 GPO, Nairobi, Kenya*
[b] *Kenya Medical Research Institute (KEMRI)/Wellcome Trust Research Programme, Centre for Geographic Medicine Research (Coast), P.O. Box 230, 80108 Kilifi, Kenya*
[c] *Walter Reed Project/KEMRI Centre for Clinical Research, New Nyanza Provincial General Hospital, P.O. Box 54, Kisumu, Kenya*
[d] *Department of Pharmaceutics and Pharmacy Practice, Faculty of Pharmacy, University of Nairobi, Nairobi, Kenya*
[e] *Molecular and Biochemical Parasitology Research Group, Liverpool School of Tropical Medicine, Pembroke Place, L3 5QA Liverpool, UK*
[f] *Department of Pharmacology and Therapeutics, University of Liverpool, L69 3GE Liverpool, UK*
[g] *Neurosciences Unit, Institute of Child Health, University of London, London, UK*

Received 26 January 2005; accepted 28 July 2005

Abstract

A simple, sensitive, selective, and reproducible reversed-phase high-performance liquid chromatographic (HPLC) method with UV detection was developed for the determination of lorazepam (LZP) in human plasma, using oxazepam (OZP) as internal standard. LZP and OZP were extracted from alkalinized (pH 9.5) spiked and clinical plasma samples using a single step liquid–liquid extraction with a mixture of *n*-hexane–dichloromethane (70:30%; v/v). Chromatographic separation was performed on a reversed-phase *Synergi*® Max RP analytical column (150 mm × 4.6 mm i.d.; 4 μm particle size), using an aqueous mobile phase (10 mM KH_2PO_4 buffer (pH 2.4)–acetonitrile; 65:35%, v/v) delivered at a flow-rate of 2.5 ml/min. Retention times for OZP and LZP were 10.2 and 11.9 min, respectively. Calibration curves were linear from 10 to 300 ng with correlation coefficients (r^2) better than 0.99. The limits of detection (LOD) and quantification (LOQ) were 2.5 and 10 ng/ml, respectively, using 0.5 ml samples. The mean relative recoveries at 20 and 300 ng/ml were 84.1 ± 5.5% (n = 6) and 72.4 ± 5.9% (n = 7), respectively; for OZP at 200 ng the value was 68.2 ± 6.8% (n = 14). The intra-assay relative standard deviations (R.S.D.) at 20, 150 and 270 ng/ml of LZP were 7.8%, 9.8% (n = 7 in all cases) and 6.6% (n = 8), respectively. The inter-assay R.S.D. at the above concentrations were 15.9%, 7.7% and 8.4% (n = 7 in all cases), respectively. Intra- and inter-assay accuracy data were within the acceptance interval of ±20% of the nominal values. There was no interference from other commonly co-administered anticonvulsant, antimicrobial, antipyretic, and antimalarial drugs. The method has been successfully applied to a pharmacokinetic study of LZP in children with severe malaria and convulsions following administration of a single intravenous dose (0.1 mg/kg body weight) of LZP.

Keywords: Lorazepam; High-performance liquid chromatography; Analysis in plasma; Pharmacokinetics; Children

1. Introduction

Acute seizures, particularly if prolonged status epilepticus (SE), are potentially life-threatening medical and neurological emergencies that require prompt treatment. Acute seizures are a clinical feature of severe malaria and other infections in children [1], and benzodiazepines are considered the drugs of choice for rapid termination of acute seizures and SE [2]. In resource-poor countries, diazepam is routinely used as the standard first-line treatment for acute convulsions and SE, since it is widely available, cheap, and rapidly acting. However, it has several disadvantages: First, intravenous (i.v.)

* Corresponding author. Tel.: +254 20 2720163/2710672/2715160; fax: +254 20 2711673.
E-mail address: Sndirangu@wtnairobi.mimcom.net (S.N. Muchohi).

1570-0232/$ – see front matter.
doi:10.1016/j.jchromb.2005.07.040

access is technically difficult in most resource-poor settings, and plasma diazepam concentrations decline rapidly following i.v. administration, leading to recurrence of seizures. Moreover, rectal administration of diazepam, which has been suggested as a practical alternative to i.v. administration under such settings, generally results in erratic absorption and seizure recurrence [3]. Second, administration of multiple doses of diazepam is undesirable, especially in children with severe malaria, due to accumulation and the potential for fatal respiratory depression [4,5]. Third, intramuscular administration of diazepam results in incomplete and erratic absorption [6].

Lorazepam (LZP) is an alternative benzodiazepine that has several advantages over diazepam: following i.v. administration, it has a longer duration of action than predicted from its half-life [7,8]. It prevents seizure recurrence for between 2 and 72 h [2,9]. Therefore, it can be used for both acute treatment and prophylaxis of seizures. It has been shown in a previous study to be better than diazepam for out-of-hospital treatment of SE [10]. It has potent anticonvulsant activity, and is effective in management of SE in both adults [7,11,12] and children [13], including those refractory to phenobarbitone and phenytoin [14]. Although LZP is usually administered i.v., due to its long duration of action, it would help prevent the recurrence of convulsions in hospitalized patients.

The pharmacokinetics of LZP may be altered in children, and a few concentration-dependent side effects such as respiratory depression have been reported in infants and children [15,16]. The pharmacokinetics of LZP have not been described in African children, particularly those with falciparum malaria, a common cause of seizures in sub-Saharan Africa [17]. LZP is extensively metabolized to its inactive glucuronide conjugate, therefore only negligible amounts of free LZP are present in blood and excreted in urine [18]. For pharmacokinetic studies in children with malaria, the ideal analytical method for LZP should be one that is sensitive enough to allow use of small quantities of biological fluid (since large sampling volumes are both impractical and not acceptable to parents/guardians), and fully selective to avoid interference from concurrently administered drugs, such as other anticonvulsants, antimicrobials, antimalarials and antipyretics/analgesics.

Several analytical methods have been published for the quantification of LZP and/or its metabolite(s) in human (or animal) biological fluids or tissue organ extracts. These methods include gas chromatography (GC) with electron-capture detection (GC-ECD) [19–22], nitrogen–phosphorus detection (GC-NPD) [19,23], mass spectrometry (GC–MS) [24–26], and GC with negative ion chemical ionization MS (GC–MS/NICI) [27,28] or selected ion monitoring MS (GC–MS/SIM) [28–30]. High-performance liquid chromatography (HPLC) methods for quantification of LZP in biological fluids have also been reported, with the choice of detectors, including UV (HPLC-UV) or diode array detector (HPLC-DAD) [31–42], and mass spectrometric detection [43]. However, most of these methods have various limitations, including time-consuming sample clean-up and/or derivatization steps [25,26,30]; use of large sample volumes ($\geq$1 ml) [19,21,24,26,29,31,36,41]; inadequate sensitivity [31,32]; and use of expensive solid phase extraction cartridges [25,34–36]. Though the combination of HPLC with MS offers the advantage of the separation power of HPLC with the sensitivity and specificity of MS for analysis of LZP, the technique is expensive and involves expensive equipment, which is not affordable for most non-research laboratories, particularly those in resource-poor countries.

We describe a relatively simple, sensitive and selective HPLC-UV method for the determination of LZP in human plasma samples. The method was successfully applied to a pharmacokinetic study of LZP in children with severe malaria and convulsions following administration of a single i.v. dose (0.1 mg/kg body weight) of LZP.

2. Experimental

2.1. Chemicals and reagents

Lorazepam (lot # 127F08281) was purchased from the Sigma Chemical Company (St. Louis, MO. USA). Oxazepam (internal standard (IS) was a gift from Dr M.S. Lennard (University of Sheffield, UK). HPLC-grade organic solvents (methanol, acetonitrile, dichloromethane, *n*-hexane) and orthophosphoric acid were purchased from BDH Supplies Ltd. (Poole, England). Potassium dihydrogen orthophosphate (AnalaR® grade) was also purchased from BDH. Distilled water was prepared as required using a Manesty Type S 75 water distiller (Manesty Machines Ltd., Liverpool, UK), while deionized water was prepared in-house using Elgastat® C-114 water deionizer cartridges (The Elga Ltd., High Wycombe Bucks, England).

2.2. Standard stock solutions

Stock solutions (1 mg/ml) of LZP and OZP (IS) were prepared by dissolving an appropriate amount of each compound in methanol. The stock solutions were further serially diluted with methanol to make working standard solutions at concentrations of 100, 10 and 1 ng/μl for each compound. All the stock solutions were stored at −20 °C, protected from light (in amber sample vials) and used within three months. A 0.5 M solution of sodium carbonate–sodium hydrogen carbonate buffer (pH 9.5) was prepared by mixing equal volumes of 0.5 M aqueous solutions of sodium carbonate and sodium hydrogen carbonate. Potassium phosphate buffer (10 mM, pH 2.4) was prepared by transferring 12.36 ml of 1 M KH_2PO_4 and 11.16 ml of 1 M H_3PO_4 solutions into a 1000 ml volumetric flask, and making up the volume to the mark with distilled water.

2.3. Chromatographic conditions

Chromatography was performed under isocratic conditions at ambient temperature (about 25 °C). An isocratic delivery system (*Isochrom*® LC; Spectra Physics, San Jose, CA, USA) fitted with a Rheodyne (model 7125; Cotati, CA, USA) valve injector (50 μl loop) was used. Chromatographic separation was achieved with a reversed-phase phenyl column (*Synergi*® Max RP, 150 mm × 4.6 mm i.d., 4 μm particle size; Phenomenex™, Macclesfield, Cheshire, UK) coupled to a guard column (*LiChrospher*® 100 RP-18e, 10 mm × 4.6 mm i.d., 5 μm particle size; Merck, Darmstadt, Germany). The mobile phase consisted of a mixture of potassium dihydrogen orthophosphate (KH_2PO_4) buffer (10 mM, pH 2.4) and acetonitrile (65:35%, v/v), and was delivered in isocratic mode at a flow-rate of 2.5 ml/min, generating an operating backpressure of about 900 psi. Before use, the mobile phase was degassed for 30 min in an ultrasonic bath. The column effluent was monitored with a variable wavelength UV–vis detector (model *SpectraSeries*® UV100; Spectra Physics, San Jose, CA, USA) set at 220 nm. Chromatographic peaks were recorded on a data integrator (*ChromJet*® CH-1; Thermo Separation Products, San Jose, CA, USA).

2.4. Sample preparation

The procedure was validated using spiked plasma aliquots (0.5 ml). Pooled human drug-free plasma was obtained from healthy volunteer blood donors at a local blood bank (National Public Health Laboratories Service, Nairobi, Kenya) and stored frozen at −20 °C. Internal standard (OZP; 200 ng, 20 μl of a 10 ng/μl solution) was added to 0.5 ml aliquot of plasma (blank, standard or patient sample) in a 15 ml borosilicate glass tube. The tubes were vortex-mixed for 10 s. Acetonitrile (1 ml) was added and the tubes vortex mixed for 30 s to precipitate the plasma proteins. Following centrifugation (1500 × *g*; 5 min), the clear supernatant was decanted into another clean tube and basified with sodium carbonate–sodium hydrogen carbonate buffer (pH 9.5; 0.5 ml), followed by vortex mixing for 10 s. The samples were extracted with a mixture of *n*-hexane–dichloromethane (70:30%, v/v; 5 ml) by mechanical tumbling of the mixture (at speed 5) for 30 min on a Stuart *STR4* rotator drive (Stuart Scientific, Bedfordshire, UK), followed by centrifugation (1500 × *g*; 10 min) to separate the phases. The upper organic phase was transferred to a clean glass tube and evaporated to dryness in a water bath (37 °C) under a gentle flow of white spot nitrogen gas (BOC Ltd., Nairobi). The residue was reconstituted in 100 μl of mobile phase, and an aliquot (50 μl) was injected onto the HPLC column.

2.5. Preparation of calibration curves

Calibration curves were prepared by adding various amounts (10, 20, 50, 100, 150, 200 and 300 ng) of LZP to aliquots (500 μl) of pooled drug-free human plasma, and a fixed amount (200 ng) of the IS. The samples were analyzed as described in the extraction procedure (Section 2.4) above. Calibration curves were constructed by plotting peak area ratios (PAR) of LZP to the IS against the known amounts LZP. The results were analyzed by weighted ($1/x$) least-squares linear regression to determine the slope, intercept, and correlation coefficient (r^2). Unknown concentrations of LZP were determined (by interpolation) with reference to the calibration line.

2.6. Method validation

2.6.1. Quality control

Quality control (QC) plasma samples were prepared by spiking drug-free human plasma (5 ml) with various quantities of LZP to yield three different concentrations, corresponding to the low (LQC = 20 ng/ml), medium (MQC = 150 ng/ml) and high (HQC = 270 ng/ml) levels. Samples were aliquoted into cryovials and stored frozen at −20 °C for use with each analytical run. The results of the QC samples provided the basis for accepting or rejecting the run. At least four of the six QC samples had to be within ±20% of their respective nominal value. Two of the six QC samples could be outside the ±20% of their respective nominal value, but not at the same concentration [44].

2.6.2. Assay precision and accuracy

The precision and accuracy of the method were evaluated by assaying QC samples at three different concentrations (Section 2.6.1). Intra-assay precision (*within-day repeatability*) and accuracy were evaluated by analyzing 500 μl aliquots of each of the QC samples ($n = 7$ for each level) on the same day. Inter-assay precision (*day-to-day reproducibility*) and accuracy were assessed by analyzing duplicates of each of the QC samples on seven different days. The concentrations of the LZP in the quality control samples were calculated using the daily calibration curves. Intra- and inter-assay precision were assessed by determining the relative standard deviations (R.S.D.), calculated from the ratio of standard deviation (S.D.) to the mean, and expressed as a percentage.

Accuracy of the assay was determined at each level of the QC samples by comparing the difference between the measured concentration and the corresponding nominal concentration. Accuracy was expressed as percentage error (relative error, R.E.) [i.e., R.E. (%) = [(measured concentration − nominal concentration)/nominal concentration) × 100 (%)]. The following criteria were used to assess the suitability of precision and accuracy: the R.S.D. value determined at each concentration level should not exceed 15%, except at the limit of quantification, where it should not exceed 20%; the accuracy value should be within 80–120% [44].

2.6.3. Analytical recovery

The analytical recovery (extraction efficiency) of the sample preparation procedure for LZP from human plasma was assessed at low and high concentration levels of LZP. Aliquots (500 μl) of drug-free plasma were spiked with LZP (20 or 300 ng/ml) and 200 ng of the IS. The samples were extracted according to the procedure described in Section 2.4. As controls, equivalent amounts of LZP and the IS (200 ng) were added directly into the extracting organic solvent (5 ml), followed by evaporation of the solvent in a water bath (37 °C) under a gentle stream of white spot nitrogen. The residue was then reconstituted in mobile phase (100 μl) and aliquots (50 μl) injected onto the HPLC chromatograph as described above. Recovery was assessed by comparing the chromatographic peak area ratios of LZP to IS of the extracted plasma standards to those obtained from equivalent amounts of LZP and IS spiked directly into the organic phase (corresponding to 100% recovery). The results were expressed as percentage recovery [i.e., recovery (%) = (peak area of extracted analyte/peak area of non-extracted analyte) × 100 (%)]

2.6.4. Selectivity

The selectivity of the method was assessed by evaluating potential interference from various co-administered drugs commonly used in the management of severe malaria and associated complications. Drug-free plasma was spiked with therapeutic concentrations of such drugs which included anticonvulsants (diazepam, midazolam, phenobarbitone, phenytoin, fosphenytoin), antimicrobials (gentamicin, benzyl penicillin, chloramphenicol succinate and chloramphenicol base), antipyretics/analgesics (paracetamol and salicylate) and antimalarials (chloroquine, quinine, proguanil, cycloguanil, pyrimethamine and sulfadoxine), followed by extraction and analysis as described (Section 2.4) above. The retention times for these drugs under the chromatographic conditions for the LZP assay were determined.

2.7. Application of the method in a pharmacokinetic study of LZP in children

The validated method was used to evaluate the pharmacokinetics of LZP in paediatric patients. Children with severe malaria and convulsions were recruited (after obtaining ethical approval from the Kenya Medical Research Institute (KEMRI)/National Ethical Review Committee and informed consent from the parents/guardians), and administered LZP (0.1 mg/kg; *Ativan*®, 4 mg/ml; Wyeth, UK), i.v. (as a slow bolus over 2 min). Venous blood samples (0.75 ml) were collected into heparinized tubes pre-dose and at 10, 20, 30, 40, 60 min, and 2, 4, 6, 8, 12, 24, 36 and 48 h after LZP administration. The plasma was separated by centrifugation (1500 × *g* for 10 min) and stored frozen at −20 °C until analysis for unconjugated LZP. A concentration–time profile for one of the patients is reported.

3. Results and discussion

3.1. Chromatography

A representative chromatogram of an unextracted working solution (in mobile phase) containing 50 ng each of LZP and OZP (IS) is shown in Fig. 1A. Fig. 1B shows the chromatogram of an extracted blank human plasma spiked with LZP (10 ng/ml) and IS (200 ng). Under the described chromatographic conditions, OZP and LZP had retention times of about 10.1 and 11.9 min, respectively. The chromatographic peaks were resolved to baseline throughout the calibration curve range (10–300 ng) studied.

3.2. Sample preparation

The sample preparation step used in this study involved only a single-step, i.e., liquid–liquid extraction with a mixture of organic solvents (*n*-hexane–dichloromethane). This was found to be the most optimal condition for sample preparation as it resulted in a clean chromatogram. The IS corrected for variation in the sample preparation step used.

3.3. Calibration curves

Calibration curves were constructed by plotting peak area ratios of LZP/IS against known amounts of LZP. A weighted ($1/x$) least-squares linear regression was used for calculation of calibration curves to account for unequal variances across the calibration range. Calibration curves for LZP were linear over the range (10–300 ng) studied. The regression equation for LZP was $y = 0.0051x + 0.018$, and correlation coefficient, $r^2 \geq 0.997$ ($n = 11$; R.S.D. = 0.16%). The R.S.D. of the slope of the calibration curves was 6.3%.

3.4. Method validation

3.4.1. Assay precision and accuracy

Intra- and inter-assay precision and accuracy data are shown in Table 1. The intra-assay R.S.D. at 20, 150 and 270 ng/ml of LZP were 7.8%, 9.8% ($n = 7$ in all cases) and 6.6% ($n = 8$), respectively. The inter-assay R.S.D. at the above concentrations were 15.9%, 7.7% and 8.4% ($n = 7$ in all cases), respectively. Accuracy data were also determined and all lay within the acceptance interval of ±20% of the nominal values (Table 1).

3.4.2. Analytical recovery

The mean relative recoveries for LZP at 20 and 300 ng/ml were 84.1 ± 5.5% ($n = 6$) and 72.4 ± 5.9% ($n = 7$), respectively. The mean relative recovery for OZP (IS) at 200 ng was 68.2 ± 6.8% ($n = 14$).

3.4.3. Selectivity

The assay was found to be selective for LZP, and no interfering peaks were observed in the extracts of

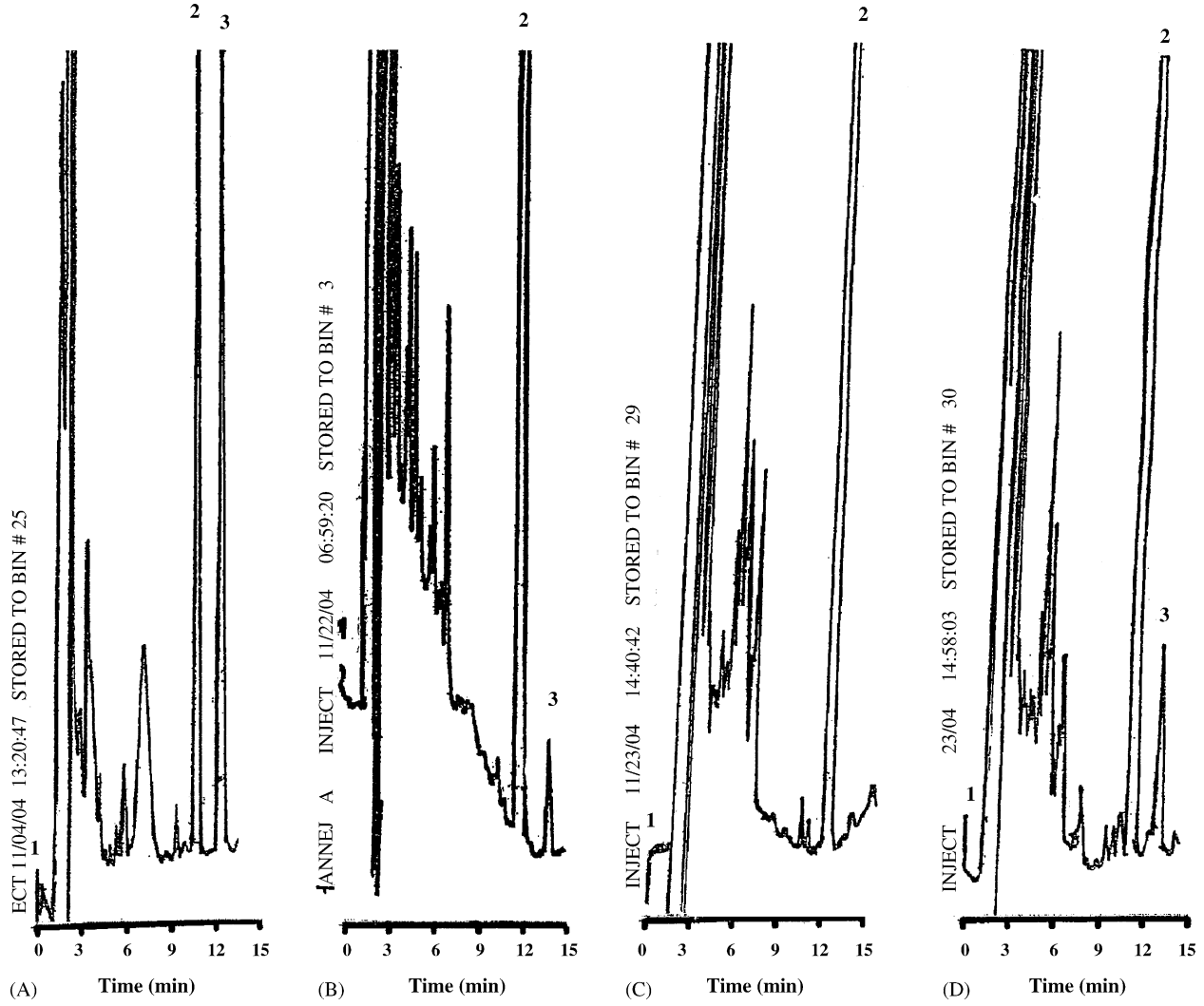

Fig. 1. (A) Chromatograms of standard solutions (in mobile phase) of lorazepam (LZP; 50 ng) and internal standard (oxazepam, OZP; 50 ng). (B) Extracted blank human plasma spiked with LZP (10 ng/ml) and 200 ng of IS. (C) Extracted blank plasma from a child, spiked with 200 ng of IS. (D) Extracted plasma sample obtained after 10 min following intravenous administration of a single dose of LZP (0.1 mg/kg) to a child with severe malaria and convulsions, and spiked with 200 ng IS. The estimated plasma concentration of unconjugated LZP was 54 ng/ml. Peaks: (1) injection event mark; (2) oxazepam, OZP (IS); and (3) lorazepam (LZP).

the different blank or patient plasma samples. Potential interferences by common drugs which are administered concurrently with LZP, such as antimalarials (chloroquine, desethylchloroquine, quinine, pyrimethamine, sulfadoxine, proguanil, cycloguanil, and artesunate), analgesics (acetaminophen and salicylate) or antimicrobials (chloramphenicol and benzyl penicillin), were tested and could be excluded on the basis of different retention times.

Table 1
Intra- and inter-assay precision and accuracy of the assay for lorazepam (LZP) in plasma

Nominal concentration (ng/ml)	No. of replicates (*n*)	Estimated concentration (ng/ml); mean ± S.D.	Precision (R.S.D.%)	Accuracy (R.E.%)
Intra-assay				
20	7	21.3 ± 1.7	7.8	8.7
150	7	167.5 ± 16.5	9.8	13.6
270	8	292.1 ± 19.2	6.6	13.1
Inter-assay				
20	7	21.9 ± 3.5	15.9	17.1
150	7	149.2 ± 11.5	7.7	4.6
270	7	276.6 ± 23.3	8.4	6.4

S.D.: standard deviation; R.S.D.: relative standard deviation; R.E.: relative error, calculated as [(estimated concentration – nominal concentration)/nominal concentration].

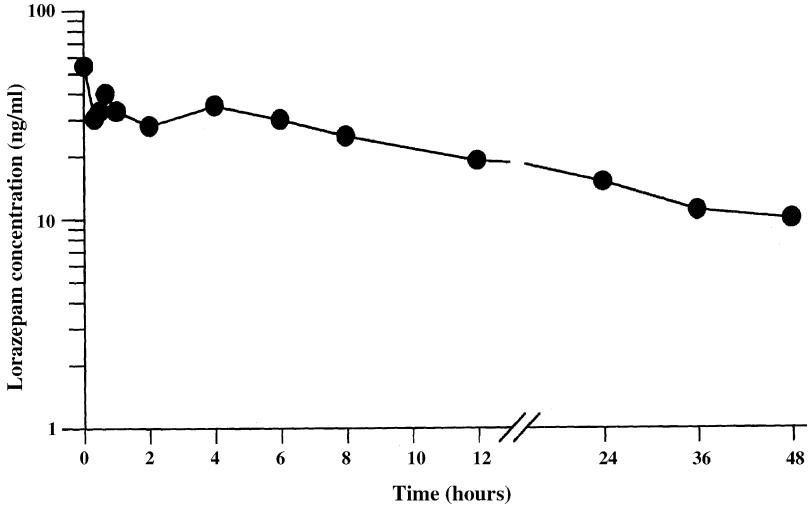

Fig. 2. Semi-logarithmic plasma concentration–time profile of lorazepam (LZP) following administration of a single intravenous (i.v.) dose (0.1 mg/kg) of LZP (*Ativan*®; 4 mg/ml; Wyeth, UK) to a child.

3.4.4. Limits of detection and quantification

The limit of detection (LOD), defined as the lowest concentration yielding a signal-to-noise ratio ≥3, was 2.5 ng/ml The limit of quantification (LOQ), defined as the lowest concentration whose precision and accuracy values were ≤20% ($n = 6$), excluding outliers, was 10 ng/ml.

3.5. Application of the method in a pharmacokinetic study of LZP in children

To demonstrate the clinical applicability of the assay method, we have successfully used the method to quantitatively measure the concentrations of unconjugated LZP in plasma samples obtained from pediatric patients who were administered LZP (0.1 mg/kg) i.v. The chromatogram of extracted plasma sample obtained from a child did not show any interfering peaks (Fig. 1C and D). The semi-logarithmic plasma LZP concentration–time profile for one of the patients is shown in Fig. 2. Maximum plasma LZP concentration of 54 ng/ml was achieved at 10 min after i.v. injection of LZP (0.1 mg/kg).

4. Discussion

LZP is a benzodiazepine that may be useful for termination of acute seizures and SE. Analytical methods for LZP in biological fluids are problematic, due to the high sensitivity required to detect the low unconjugated blood (plasma/serum) LZP concentrations achieved after the recommended doses, and the thermal instability of the 3-hydroxybenzodiazepine ring. Previously published GC methods for the quantification of LZP and/or conjugated glucuronide metabolites in biological matter (whole blood, plasma/serum urine, or brain) include GC-ECD [19–22,45]; GC-NPD [19,23]; GC–MS [25,26] and GC–MS/NICI [27,28] or GC–MS/SIM [24,28,30]. Several HPLC methods with UV or diode array detection [31–41,43] and mass spectrometric detection [43] have also been reported. Although GC methods are more sensitive (with lower limits of quantification ≤2 ng/ml [28,43]) than HPLC methods for measuring LZP, they involve lengthy sample clean-up procedures, and require derivatization steps [25,26,30] to increase the volatility of LZP, which is thermally unstable under GC conditions [22,46]. In addition, another 3-hydroxy benzodiazepine (e.g., OZP) is required as a suitable IS, and thus, making assumption that the thermal-molecular rearrangement that occurs on the column is the same for both LZP and the IS [21], which may lead to poor reproducibility of the assay [22].

We have described a reversed-phase HPLC assay procedure with ultraviolet detection, for the selective, sensitive, accurate, and reproducible quantitative analysis of LZP in human plasma samples. Total run time was about 13 min. A major advantage of the method over previous GC-ECD methods is that this method measures unchanged LZP rather than the product of on-column molecular rearrangement of LZP; therefore, it is subject to less variability. The current method is more sensitive than some of the reported HPLC assays [31,33]. Egan and Abernethy [41] have described a HPLC method for determination of LZP concentrations in plasma that had a sensitivity of 2.5 ng/ml, but it requires a complex extraction procedure. Furthermore, most of the previously reported techniques [21,24,26,36,41,42] that had sensitivities higher than that achieved by the present method required the use large sample volumes (≥1 ml), which is not practical for pharmacokinetic studies in young children, where taking large volumes of blood is ethically unacceptable. Though HPLC–MS technique offers the advantage of the separation power of HPLC with the sensitivity and specificity of MS for analysis of LZP, it involves expensive instrumentation, which may not be affordable for most

non-research laboratories, particularly those in resource-poor countries.

The minimal effective concentration of LZP for termination of seizures is about 30 ng/ml [7]. The maximum plasma concentration of LZP of 54 ng/ml was achieved at 10 min after LZP (0.1 mg/kg) (Fig. 2) and the plasma concentration versus time profile was similar to previous studies [7,47,48]. Thus, following a single i.v. injection of 5 mg LZP in normal adults, Comer and Giesecke [48] found that LZP concentration remained above 30 ng/ml for about 18 h, whilst a mean plasma concentration of 47.6 ± 57.8 ng/ml was achieved within 0.1 (0.08–1.02) h following administration of a single i.v. dose (2 mg ≡ 0.029 mg/kg) of LZP in 11 healthy volunteers [49].

5. Conclusion

The HPLC analytical method for determination of LZP in plasma presented here meets the criteria for routine therapeutic drug monitoring or pharmacokinetic studies. The advantage of the method over previously reported methods is the rapidity, simplicity (single-step sample preparation procedure), high sensitivity (LOQ, 10 ng/ml), and high selectivity (no interferences from endogenous peaks or concurrently administered drugs). The limits of detection and quantification achieved make the assay appropriate for measurement of therapeutic concentrations of LZP in plasma. It is particular useful for pharmacokinetic studies of this anticonvulsant in young and severely ill children, where sample volume must be kept to a minimum. To evaluate its practical utility, we have successfully used this assay method to study the pharmacokinetics of LZP following i.v. administration in children with convulsions associated with severe malaria.

Acknowledgements

This work is published with the permission of the Director of the Kenya Medical Research Institute (KEMRI). We wish to thank Professor Kevin Marsh and Dr. Nobert Peshu, both of the KEMRI/Wellcome Trust Research Programme, Centre for Geographic Medicine Research (Coast), Kilifi, Kenya, for their support. We are indebted to the clinical, nursing and laboratory staff, both in Kilifi District Hospital and the New Nyanza Provincial General Hospital, Kisumu, for their valuable support. We thank Dr. Martin S. Lennard (Molecular Pharmacology and Pharmacogenetics, Division of Clinical Sciences, University of Sheffield, Sheffield, UK) for kindly supplying oxazepam (IS). Professor Gilbert O. Kokwaro was supported by a Research Capability Strengthening Grant from WHO (TDR/MIM grant no. 980074). Simon N. Muchohi is a Ph.D. student in clinical pharmacology supported by the Wellcome Trust of Great Britain. Professor Charles R.J.C. Newton is a Wellcome Trust Senior Clinical Research Fellow (grant no. 070114/Z/02/Z).

References

[1] J. Crawley, S. Smith, F. Kirkham, P. Muthinji, C. Waruiru, K. Marsh, QJM 89 (1996) 591.
[2] S. Shorvon, Status Epilepticus: its Clinical Features and Treatment in Childhood and Adults, Cambridge University Press, Cambridge, 1994, p. 203.
[3] B.R. Ogutu, C.R. Newton, J. Crawley, S.N. Muchohi, G.O. Otieno, G. Edwards, K. Marsh, G.O. Kokwaro, Br. J. Clin. Pharmacol. 53 (2002) 49.
[4] J. Crawley, C. Waruiru, S. Mithwani, I. Mwangi, W. Watkins, D. Ouma, P. Winstanley, T. Peto, K. Marsh, Lancet 355 (2000) 701.
[5] E. Norris, O. Marzouk, A. Nunn, J. McIntyre, I. Choonara, Dev. Med. Child Neurol. 41 (1999) 340.
[6] J.W. Dundee, J.A. Gamble, R.A. Assaf, Lancet 2 (1974) 1461.
[7] J.E. Walker, R.W. Homan, M.R. Vasko, I.L. Crawford, R.D. Bell, W.G. Tasker, Ann. Neurol. 6 (1979) 207.
[8] T.O. Crawford, W.G. Mitchell, S.R. Snodgrass, Neurology 37 (1987) 190.
[9] R. Homan, J. Walker, Clinical studies of lorazepam in status epilepticus, in: A.V. Delgado-Escueta, C.G. Wastterlain, D.L. Treiman (Eds.), Advances in Neurology: Status Epilepticus, Raven Press, New York, 1993.
[10] B.K. Alldredge, A.M. Gelb, S.M. Isaacs, M.D. Corry, F. Allen, S. Ulrich, M.D. Gottwald, N. O'Neil, J.M. Neuhaus, M.R. Segal, D.H. Lowenstein, N. Engl. J. Med. 345 (2001) 631.
[11] I.E. Leppik, A.T. Derivan, R.W. Homan, J. Walker, R.E. Ramsay, B. Patrick, JAMA 249 (1983) 1452.
[12] R.J. Levy, R.L. Krall, Arch. Neurol. 41 (1984) 605.
[13] D.J. Lacey, W.D. Singer, S.J. Horwitz, H. Gilmore, J. Pediatr. 108 (1986) 771.
[14] A. Deshmukh, W. Wittert, E. Schnitzler, H.H. Mangurten, Am. J. Dis. Child 140 (1986) 1042.
[15] C.M. Cronin, Pediatrics 89 (1992) 1129.
[16] P. Vlachos, P. Kentarchou, G. aloupogiannis, Toxicol. Lett. 2 (1978) 109.
[17] C.M. Waruiru, C.R. Newton, D. Forster, L. New, P. Winstanley, I. Mwangi, V. Marsh, M. Winstanley, R.W. Snow, K. Marsh, Trans. R. Soc. Trop. Med. Hyg. 90 (1996) 152.
[18] D.J. Richards, J. Clin. Psychiatry 39 (1978) 58.
[19] P. Lillsunde, T. Seppala, J. Chromatogr. 533 (1990) 97.
[20] R.G. Lister, D.R. Abernethy, D.J. Greenblatt, S.E. File, J. Chromatogr. 277 (1983) 201.
[21] D.J. Greenblatt, K. Franke, R.I. Shader, J. Chromatogr. 146 (1978) 311.
[22] G. de Groot, R.A. Maes, H.H. Lemmens, Arch. Toxicol. 35 (1976) 229.
[23] Z.L. Jiang, J.Y. Tan, L.J. Yao, L.M. Xing, Se Pu 19 (2001) 341.
[24] D. Borrey, E. Meyer, W. Lambert, S. Van Calenbergh, C. Van Peteghem, A.P. De Leenheer, J. Chromatogr. A 910 (2001) 105.
[25] S. Pichini, R. Pacifici, I. Altieri, A. Palmeri, M. Pellegrini, P. Zuccaro, J. Chromatogr. B Biomed. Sci. Appl. 732 (1999) 509.
[26] S. Higuchi, H. Urabe, Y. Shiobara, J. Chromatogr. 164 (1979) 55.
[27] V. Cirimele, P. Kintz, P. Mangin, Int. J. Legal Med. 108 (1996) 265.
[28] E.M. Koves, B. Yen, J. Anal. Toxicol. 13 (1989) 69.
[29] D. Borrey, E. Meyer, W. Lambert, C. Van Peteghem, A.P. De Leenheer, J. Chromatogr. B Biomed. Sci. Appl. 765 (2001) 187.
[30] C. Drouet-Coassolo, C. Aubert, P. Coassolo, J.P. Cano, J. Chromatogr. 487 (1989) 295.
[31] A. Bugey, C. Staub, J. Pharm. Biomed. Anal. 35 (2004) 555.
[32] C. Pistos, J.T. Stewart, J. Pharm. Biomed. Anal. 33 (2003) 1135.
[33] C. Pham-Huy, G. Villain-Pautet, H. Hua, N. Chikhi-Chorfi, H. Galons, M. Thevenin, J.R. Claude, J.M. Warnet, J. Biochem. Biophys. Methods 54 (2002) 287.
[34] M. Segura, J. Barbosa, M. Torrens, M. Farre, C. Castillo, J. Segura, R. de la Torre, J. Anal. Toxicol. 25 (2001) 130.

[35] H. Kanazawa, Y. Kunito, Y. Matsushima, S. Okubo, F. Mashige, J. Chromatogr. A 871 (2000) 181.
[36] T. Kondo, D.C. Buss, P.A. Routledge, Ther. Drug Monit. 15 (1993) 35.
[37] S. Gunawan, N.Y. Walton, D.M. Treiman, Biomed. Chromatogr. 4 (1990) 168.
[38] W.M. Awni, L.J. Bakker, Clin. Chem. 35 (1989) 2124.
[39] S. Gunawan, D.M. Treiman, Ther. Drug Monit. 10 (1988) 172.
[40] C.A. Riley, W.E. Evans, J. Chromatogr. 382 (1986) 199.
[41] J.M. Egan, D.R. Abernethy, J. Chromatogr. 380 (1986) 196.
[42] L.M. Walmsley, L.F. Chasseaud, J. Chromatogr. 226 (1981) 155.
[43] H. Kanazawa, Y. Konishi, Y. Matsushima, T. Takahashi, J. Chromatogr. A 797 (1998) 227.
[44] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, et al., Eur. J. Drug Metab. Pharmacokinet. 16 (1991) 249.
[45] P.J. Howard, J.K. Lilburn, J.W. Dundee, W. Toner, P.D. McIlroy, Anaesthesia 32 (1977) 767.
[46] J.R. Joyce, T.S. Bal, R.E. Ardrey, H.M. Stevens, A.C. Moffat, Biomed. Mass Spectrom. 11 (1984) 284.
[47] J.E. Walker, R.W. Homan, I.L. Crawford, Epilepsia 25 (1984) 464.
[48] W.H. Comer, A.H.J. Giesecke, Semin. Anesth. 1 (1982) 33.
[49] D.P. Wermeling, J.L. Miller, S.M. Archer, J.M. Manaligod, A.C. Rudy, J. Clin. Pharmacol. 41 (2001) 1225.