BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **19**: 202–207 (2005)
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/bmc.436

ORIGINAL RESEARCH

HPLC quantification of metoprolol with solid-phase extraction for the drug monitoring of pediatric patients

Stefanie Albers,[1] Jan-Peer Elshoff,[1] Christina Völker,[2] Andrea Richter[2] and Stephanie Läer[1]*

[1]Institut für Experimentelle und Klinische Pharmakologie, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany
[2]Abteilung für Pädiatrische Kardiologie, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany

Received 3 June 2004; accepted 6 July 2004

ABSTRACT: Although the analytical literature seems abundant for the determination of metoprolol in human plasma, a method using standard equipment providing a sensitive and simple high-performance liquid chromatographic (HPLC) method for limited blood volume, e.g. where 1 mL of blood in a 1 kg infant equals 70 mL of adult blood volume, has rarely been addressed. Therefore, in 500 µL of plasma, metoprolol was extracted using an internal standard and solid-phase extraction columns. Chromatographic analysis was performed on a Spherisorb C_6 column (5 µm particle size) at ambient temperature and fluorimetric detection with an excitation wavelength of 225 nm, and emission wavelength of 310 nm. The mobile phase [30% acetonitrile and 70% 0.25 M potassium acetate buffer (pH 4)] was pumped with 1 mL/min. Metoprolol recovery was determined at 73.0 ± 20.5%, and the limit of quantitation was 2.4 ng/mL. Precision values of intra- and inter-assay were below 15.5% and those for accuracy were between 90 and 110%. This method was developed for monitoring and determination of pharmacokinetic parameters of metoprolol in pediatric patients and therefore metoprolol plasma concentrations in a 2-year-old child with ventricular tachycardia are reported. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: metoprolol; pediatric plasma; fluorescence detection; pharmacokinetic parameters

INTRODUCTION

Metoprolol, a $ß_1$-selective receptor antagonist, is used succesfully for a broad spectrum of cardiovascular disorders (Reiter and Reiffel, 1998; Bristow, 2000). Despite its increasing application to children, no pharmacokinetic data are available for them. To account for the variability in absorption, distribution and elimination of drugs in the pediatric population (Kearns *et al.*, 2003), pharmacokinetic data for metoprolol are needed to develop an age-appropriate dosing regime.

There are more than 100 published methods for determining metoprolol in human plasma. Most of these methods involve relatively laborious and tedious liquid–liquid extraction (Persson *et al.*, 1990; Braza *et al.*, 2002) before HPLC analysis. Other methods differentiate between the enantiomers (Bhatti and Foster, 1992; Mistry *et al.*, 2001), because the metabolism is enantioselective. Detection using mass spectrometry would be a more sensitive approach but is costly and not yet available for every laboratory. However, all these methods are too complex and do not meet the need for the routine analysis of a large sample number.

*Corresponding author: S. Läer, Institut für Experimentelle und Klinische Pharmakologie, Universitätsklinikum Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany.
E-mail: laeer@uke.uni-hamburg.de

Contract/grant sponsor: Kompetenznetzwerk fuer Angeborene Herzfehler, BMBF.
Contract/grant sponsor: Heisenberg Programme, DFG.
Published online 13 October 2004

About 10 methods reported previously overcome this problem by means of a solid-phase extraction (SPE) procedure instead of liquid–liquid extraction (Musch *et al.*, 1989; Herring *et al.*, 1991) and fluorescence detection. SPE is a rapid and simple method which is, like fluorescence detection, feasible in every HPLC laboratory. Regarding the limit of quantitation, there seem to be only one possible method (Mistry *et al.*, 1998). This method requires a blood volume of 2 mL because it is normally applied to determine drug concentrations in adults and is thus not suitable for pediatric patients.

The limited blood volume is one of the major problems of pharmacokinetic investigations in children. Without compromising the infant's clinical state, blood withdrawals well below 5 mL/kg (Warkentin, 1997) seem to be safe and a volume of 2.5 mL drawn at one time is usually recommended for a child of about 3 kg. Several data points are necessary to describe pharmacokinetic parameters and in addition the routine blood analysis has to be performed to define the disease state. Blanchette and Zipursky (1984) reported that 1 mL of blood in a 1 kg infant is equivalent to removing 70 mL from an adult. This demonstrates the critical importance of reducing the needed blood volume for neonates, whereas it will be meaningless for an adult.

Beside the volume limitation there are other technical obstacles like the difficulty of drawing blood from children due to the relative size of their veins. Therefore, techniques like limited data sampling (Panetta

et al., 2003) and subsequent population pharmacokinetic analyses are highly recommended for the pediatric population (Food and Drug Administration, 1998). If on any reason no more than 1 mL of blood from a child is available, which is not uncommon in seriously ill children, plasma concentrations of metoprolol cannot be determined on the basis of the current literature.

Given the clinical importance for this drug in cardiovascular pharmacotherapy of children, we developed a simple and sensitive HPLC method using SPE and requiring 500 µL of plasma. The method represented here uses standard laboratory HPLC equipment and addresses the specific requirements of pediatric patients to achieve widespread use for the drug monitoring of metoprolol in this population.

EXPERIMENTAL

Materials. Metoprololtartrate was provided by Sigma (St Louis, MO, USA). Bisoprololfumarate, the internal standard, was kindly donated and HPLC-grade acetonitrile and methanol were purchased from Merck KGaA (Darmstadt, Germany). Boric acid, *o*-phosphoric acid and potassium hydroxide were supplied by Sigma. All other chemicals were analytical reagent grade.

Standards. Stock solutions of 5.0 µg/mL metoprololtartrate and 0.98 µg/mL bisoprololfumarate were made up in 50% aequous methanol (v/v). By adding dilutions of the metoprolol stock solution to drug-free plasma (Department of Transfusion Medicine, Universitätsklinikum Hamburg-Eppendorf), standard curves of 2.44–195.2 ng/mL metoprolol-free base were obtained, corresponding to the known range of its therapeutic plasma concentrations (Wikstrand *et al.*, 2003). All stock solutions were stored at 4°C until analysis.

Extraction procedure. To an aliquot of 500 µL plasma, 500 µL borate buffer (0.05 M, pH 9), made from 3.09 g boric acid dissolved in 1 L of double-distilled water and adjusted to a pH value of 9.0 with a 3 M aqueous solution of potassium hydroxide, and 100 µL internal standard (bisoprololfumarate 0.98 µg/mL), were added. To quantify the unknown sample, 500 µL drug-free plasma were spiked with metoprolol 48.8 ng/mL and, in the same way as for the unknown sample, 500 µL potassium borate buffer and 100 µL internal standard were added. The samples were vortex-mixed and centrifugated at 4000 rpm for 20 min at a temperature of 4°C. Bond-Elut columns (packed with C_8-bound silica particles of 40 µm, 100 mg/mL of column volume, Analytichem International, Harbor City, CA, USA) had to be prepared by washing them twice with 1 mL of methanol and twice with 1 mL double-distilled water. The supernatant of the centrifugated samples was percolated slowly through the columns under vacuum (Vac-Elut vacuum manifold, Chromatographie Service, Germany; vacuum pump, KNF Neuberger, Germany). After washing again twice with 1 mL double-distilled water, the samples were eluted twice with 400 µL methanol. The eluates were evaporated to dryness under nitrogen at a temperature of 40°C and the residues were dissolved in 200 µL of the mobile phase, consisting of 30% (v/v) acetonitrile and 70% (v/v) 0.25 M potassium acetate buffer pH 4. The buffer was made out of 28.6 mL acetic acid dissolved in 2 L of double distilled water and adjusted to a pH value of 4.0 with an 3 M aqueous solution of potassium hydroxide.

High-performance liquid chromatography. The HPLC system used consisted of an LC Workstation Class LC10 (Shimadzu, Kyoto, Japan) with a SIL-10A auto injector, an LC-10AD liquid chromatograph, an RF-10A spectrofluorometric detector and software provided by the manufacturer. Chromatographic separation was performed on a Spherisorb C_6 column (150 × 4.6 mm i.d., 5 µm particle size; Chromatographie Service, Langerwehe, Germany) with a Spherisorb C_6 guard column (17 × 4 mm i.d., 5 µm particle size) at ambient temperature. The mobile phase consisted of 30% (v/v) acetonitrile and 70% (v/v) 0.25 M potassium acetate buffer (pH 4). The excitation wavelength was set at 225 nm, and the emission was measured at 310 nm. The flow rate was 1 mL/min and the injection volume was 75 µL. Metoprolol was quantified by relating the peak height ratio of metoprolol and the internal standard bisoprolol in the unknown sample to the peak height ratio of a known standard concentration.

Data analysis and statistics. Data were given as arithmetic means. Intra- and interassay variation were determined by replicate analysis of samples at a metoprolol concentration of 2.4, 3.1, 6.1, 12.2, 24.4, 48.8, 97.6 and 195.2 ng/mL, each concentration measured four times at 4 days. The precision was expressed as percentage coefficient of variation (CV). The accuracy was expressed as percentage of the metoprolol concentration measured in each sample referring to the known amount of metoprolol added.

Applications. Metoprolol was determined in plasma samples taken from a 2-year-old child treated with metoprolol for ventricular tachycardia. The patient weighed 12 kg and received a daily dose of 45 mg metoprolol (3.8 mg/kg per day) divided into three equal doses every 8 h. Treatment at this dosing regimen had been performed for a minimum of 7 days and could be assumed as steady state on the basis of pharmacokinetic parameters in adults. Blood samples were drawn to determine the trough (before) and a concentration close to the maximal (assumed 1.8 h after oral application) metoprolol plasma concentration.

In addition two healthy volunteers received a comparable oral dose of metoprolol. One was treated with a single dose of an extended release formulation (Beloc ZOK 95 mg, Astra Zeneca GmbH, Wedel, Germany) and the other one was treated with a single dose of an immediate release one (metoprolol ratiopharm 100 mg, Ratiopharm GmbH, Ulm, Germany). Blood samples were drawn before and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12 and 24 h after oral administration.

RESULTS AND DISCUSSION

The described method provides an HPLC method with high and sufficient accuracy and precision using a

standard laboratory HPLC equipment to measure metoprolol for drug monitoring in small sample volumes from pediatric patients.

Limit of quantitation and recovery

The limit of quantitation (LOQ) of metoprolol was determined at 2.4 ng/mL in 500 µL of human plasma based on a signal-to-noise ratio of 10:1. Recent methods working with solid phase extraction only reach a detection limit of 4 ng/mL in 1 mL of human plasma (Herring *et al.*, 1991), which would have been not sufficient for the drug monitoring of pediatric patients. The recovery rates of metoprolol were 73.0 ± 20.5%. Using the internal standard method, the variation in the recovery rates was balanced by calculating the ratio between the peak heights of metoprolol and the internal standard bisoprolol.

Method development

The intention was to develop a standard and precise HPLC method for metoprolol in small volumes of human plasma. Initially, CN-Bond Elut columns were used for the extraction procedure similar to methods reported previously (Verbesselt *et al.*, 1991; Behn *et al.*, 2001). Starting with an elution solvent at pH 4 in the SPE, as there was no experience using CN-Bond Elut columns for the SPE of metoprolol, we obtained a recovery of about 50%, which was not sufficient. Therefore the effect of the pH of the elution solvents in the SPE was first investigated supposing that metoprolol was adsorbed onto the extraction column but could not be eluted with the chosen buffer, as the investigations of the different fractions during the sample preparation confirmed. Higher pH values should obtain higher recovery values in the assumption that the mechanism of retention during SPE on CN-Bond Elut columns underlies an interaction between the cyano group of the solid phase and the hydroxyl group of metoprolol. The results (Fig. 1) did not confirm this assumption and the recovery could not be increased to a sufficient value. Obviously metoprolol was very strongly adsorbed onto the column and could not be completely released.

Therefore extraction columns were changed to C_8-Bond Elut columns (Laer *et al.*, 2001), where the recovery values increased to approximately 97%. Additionally, by changing to the new columns, we used a nitrogen evaporating process at the end of the sample preparation which enabled the detection of smaller amounts of metoprolol because of the relatively higher concentrated solutions received. Identity was given by adding a standard solution of metoprolol to a metoprolol plasma sample, resulting in one co-eluting peak.

Validation

Intra- and interday assays were performed with eight concentrations covering a range from 2.4 to 195.2 ng/mL metoprolol free base over 4 days with $n = 4$. At LOQ (2.4 ng/mL), metoprolol showed accuracy values of between 90.2 and 98.9% and a precision expressed as percentage coefficient of variation between 3.3 and 15.5% (Table 1). Precision throughout the whole working range was between 0.6 and 15.5%. Precision and accuracy were adequate for pharmacokinetic analyses and were comparable to previously reported methods.

Table 1. Intra- and interday accuracy and precision of metoprolol in 500 µL of human plasma samples

	Nominal concentrations of metoprolol in plasma (ng/mL)							
	195.2	97.6	48.8	24.4	12.2	6.1	3.1	2.4
Concentration found (arithmetic mean value; ng/mL)								
Day 1 ($n = 4$)	183.8	96.2	48.7	24.5	11.3	5.7	2.9	2.2
Day 2 ($n = 4$)	195.1	90.9	47.8	24.1	11.5	5.8	3.4	2.4
Day 3 ($n = 4$)	187.7	99.7	50.4	23.3	12.7	5.9	3.0	2.4
Day 4 ($n = 4$)	190.4	95.8	48.9	25.4	12.1	5.7	3.1	2.3
Interday ($n = 16$)	189.3	95.7	49.0	24.3	11.9	5.8	3.1	2.3
Accuracy (arithmetic mean value; %)								
Day 1 ($n = 4$)	94.2	98.6	99.8	100.2	92.4	93.8	95.1	90.2
Day 2 ($n = 4$)	99.9	93.1	97.9	98.9	93.9	94.9	110.2	98.9
Day 3 ($n = 4$)	96.1	102.1	103.2	95.5	103.9	96.5	99.7	98.0
Day 4 ($n = 4$)	97.5	98.2	100.3	104.2	98.8	93.0	102.4	94.5
Interday ($n = 16$)	96.9	98.0	100.3	99.7	97.2	94.5	101.8	95.4
Precision (arithmetic mean value; CV, %)								
Day 1 ($n = 4$)	1.0	4.6	3.8	4.2	2.3	2.4	4.1	15.5
Day 2 ($n = 4$)	4.3	0.7	3.8	8.2	2.5	4.5	9.5	9.8
Day 3 ($n = 4$)	0.6	2.9	2.5	6.0	7.4	6.5	5.6	3.7
Day 4 ($n = 4$)	0.9	2.9	1.4	6.3	4.5	3.7	3.4	3.3
Interday ($n = 16$)	3.1	4.4	3.4	6.5	6.5	4.3	7.9	9.0

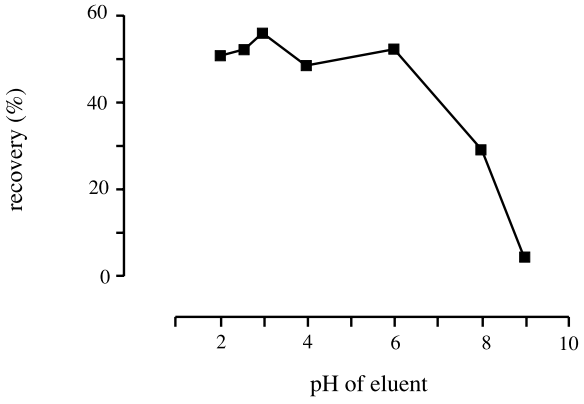

Figure 1. Influence of eluent pH on the recovery of metoprolol.

Application

Using this assay, metoprolol plasma concentrations could be determined in a 2-year-old child at 2.8 ng/mL before and at 38.4 ng/mL 1.8 h after oral application of 15 mg (corresponding to 1.3 mg/kg) immediate release metoprolol under steady-state conditions [Table 2 and see also Fig. 2(C)]. For comparison, Fig. 2(B) shows a representative chromatogram of blank plasma fortified with 48.8 ng/mL metoprolol and bisoprolol as internal standard, Fig. 2(A) is a chromatogram of blank plasma. There were no interfering peaks with other cardiovascular important drugs like amiodarone, digoxin or diuretics.

Typically recommended doses for children with ventricular tachycardia range between 1 and 5 mg/kg

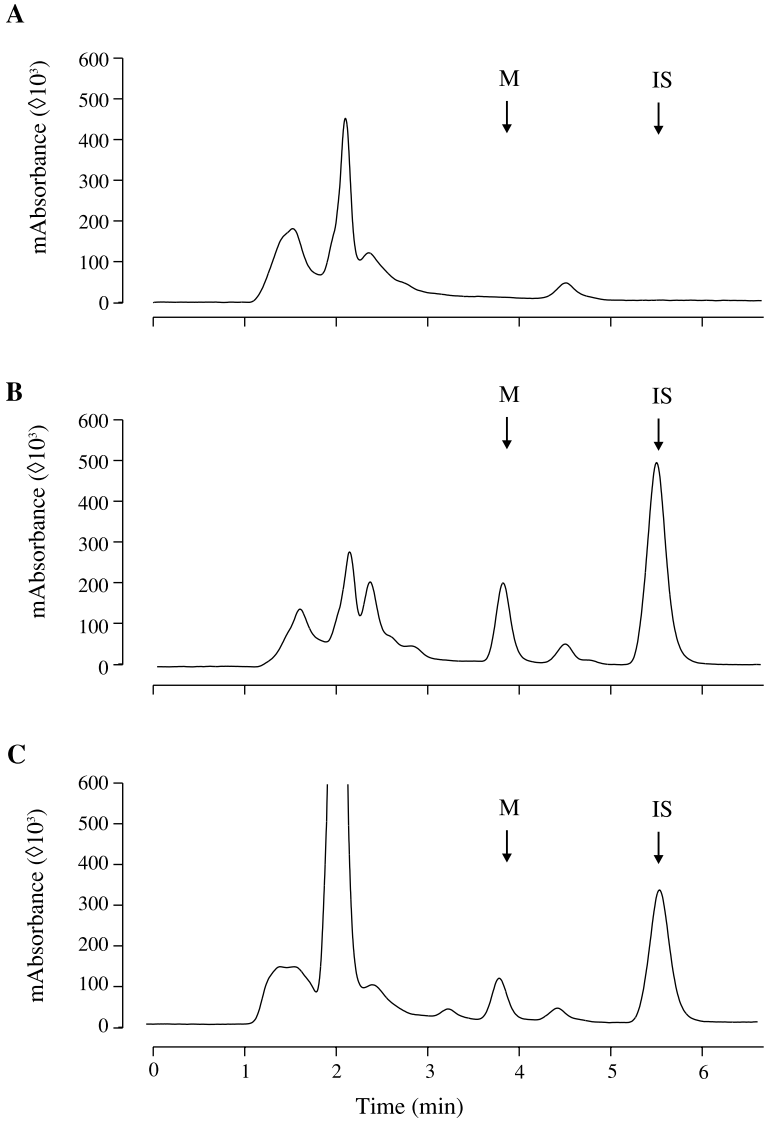

Figure 2. Representative chromatograms of (A) blank plasma, (B) plasma fortified with 48.8 ng/mL metoprolol (M) and 0.98 µg/mL internal standard bisoprolol (IS). (C) Plasma sample from a 2-year-old patient 1.8 h after oral administration of 15 mg metoprolol with a concentration of 38.4 ng/mL metoprolol.

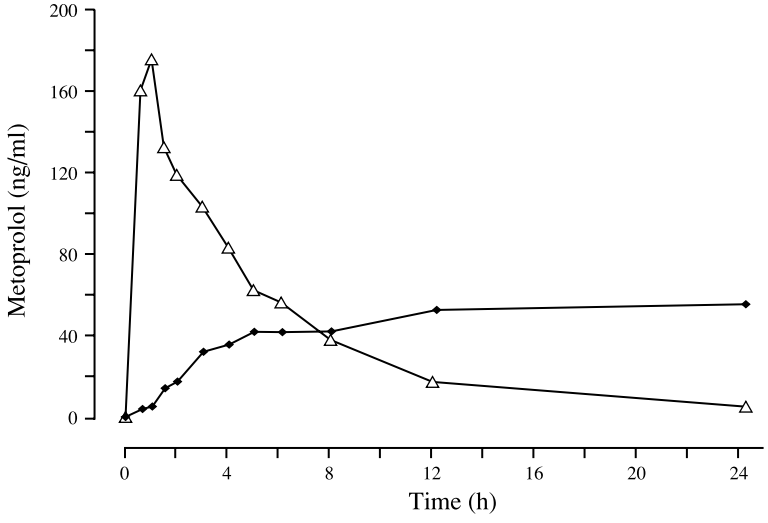

Figure 3. Plasma concentrations of metoprolol in two healthy volunteers. Both were treated with a single oral dose of 78 mg metoprolol-free base. (△) The immediate release formulation, (◆) the extended release formulation.

Table 2. Steady-state plasma concentrations of metoprolol in a 2-year old child

Blood sample	Metoprolol concentration (ng/mL)
Before application	2.8
1.8 h post application of 15 mg Metoprolol	38.4

bodyweight (Illing and Classen, 2003). Therefore, the dose of the child reported here represents a typically used dose. Compared with adults, the maximum daily dose of metoprolol for ventricular tachycardia is 200 mg, corresponding to 2.9 mg/kg for a normal patient weighing 70 kg. For comparison, Fig. 3 shows metoprolol plasma concentrations in two healthy adults following oral application of a single dose of both immediate and extended release drug formulations.

About 1.8 h after oral application of 1.4 mg/kg bodyweight immediate release metoprolol, the metoprolol plasma concentration in the healthy adult volunteer reached 130 ng/mL and was therefore 3.5 times higher than the plasma concentration of the child after 1.3 mg/kg following multiple dose administration. Based on the body weight, we would expect a comparable plasma concentration in both the child and the adult. However, metoprolol is predominantly metabolized by cytochrome P450 subtype 2D6 (Silas *et al.*, 1985). A poor metabolizer status was excluded in the healthy volunteer. Therefore, metabolizing variability between the extensive or ultrarapid metabolizer group or age-dependent differences in absorption, distribution and elimination of metoprolol might explain these different plasma concentrations between the child and the adult. To elucidate all these factors, comprehensive pharmacokinetic investigations will have to be performed in the future.

CONCLUSION

The assay represented here is able to determine precise metoprolol concentrations in small blood volumes under standard HPLC conditions. The application of this assay will enable the evaluation of pharmacokinetic data from metoprolol in the treatment of pediatric patients in the future. This might offer an evidence-based, safe and effective therapeutic regimen in future and might also give a direction about treatment differences between adult and pediatric patients.

Acknowledgements

S.A. is supported by the Kompetenznetzwerk fuer Angeborene Herzfehler, funded by the Bundesministerium fuer Bildung und Forschung (BMBF) of the German Government. S.L. is a recipient of the Heisenberg Programme of the Deutsche Forschungsgemeinschaft (DFG) in Germany.

REFERENCES

Behn F, Laeer S, Mir TS and Scholz H. HPLC quantification of carvedilol in small plasma volumes from children. *Chromatographia* 2001; **53**, 641–644.

Bhatti MM and Foster RT. Stereospecific high-performance liquid chromatographic assay of metoprolol. *Journal of Chromatography* 1992; **579**, 361–365.

Blanchette VS and Zipursky A. Assessment of anemia in newborn infants. *Clinical Perinatology* 1984; **11**, 489–510.

Braza AJ, Modamio P, Lastra CF and Marino EL. Development, validation and analytical error function of two chromatographic methods with fluorimetric detection for the determination of bisoprolol and metoprolol in human plasma. *Biomedical Chromatography* 2002; **16**, 517–522.

Bristow MR. Beta-adrenergic receptor blockade in chronic heart failure. *Circulation* 2000; **101**, 558–569.

Food and Drug Administration. Guidance for Industry: General Considerations for Pediatric Pharmacokinetic Studies for Drugs and Biological Products, 1998; www.fda.gov/cber/gdlns/pedphrm.htm

Herring VL, Bastian TL and Lalonde RL. Solid-phase extraction and direct high-performance liquid chromatographic determination of metoprolol enantiomers in plasma. *Journal of Chromatography* 1991; **567**, 221–227.

Illing S and Classen M. *Klinikleitfaden der Pädiatrie* 6th edn. Fischer: Berlin, 2003; 356.

Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS and Kauffman RE. Developmental pharmacology—drug disposition, action, and therapy in infants and children. *New England Journal of Medicine* 2003; **349**, 1157–1167.

Laer S, Wauer I and Scholz H. Small blood volumes from children for quantitative sotalol determination using high-performance liquid chromatography. *Journal of Chromatography B, Biomedical Science and Applications* 2001; **753**, 421–425.

Mistry B, Leslie J and Eddington NE. A sensitive assay of metoprolol and its major metabolite alpha-hydroxy metoprolol in human plasma and determination of dextromethorphan and its metabolite dextrorphan in urine with high performance liquid chromatography and fluorometric detection. *Journal of Pharmaceutical and Biomedical Analysis* 1998; **6**, 1041–1049.

Mistry B, Leslie JL and Eddington ND. Enantiomeric separation of metoprolol and alpha-hydroxymetoprolol by liquid chromatography and fluorescence detection using a chiral stationary phase. *Journal of Chromatography B, Biomedical Science and Applications* 2001; **758**, 153–161.

Musch G, Buelens Y and Massart DL. A strategy for the determination of beta blockers in plasma using solid-phase extraction in combination with high-performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis* 1989; **7**, 483–497.

Panetta JC, Iacono LC, Adamson PC and Stewart CF. The importance of pharmacokinetic limited sampling models for childhood cancer drug development. *Clinical Cancer Research* 2003; **9**, 5068–5077.

Persson BA, Balmer K, Lagerstrom PO and Schill G. Enantioselective determination of metoprolol in plasma by liquid chromatography on a silica-bonded alpha 1-acid glycoprotein column. *Journal of Chromatography* 1990; **500**, 629–636.

Reiter MJ and Reiffel JA. Importance of beta blockade in the therapy of serious ventricular arrhythmias. *American Journal of Cardiology* 1998; **82**, 9I–19I.

Silas JH, McGourty JC, Lennard MS, Tucker GT and Woods HF. Polymorphic metabolism of metoprolol: clinical studies. *European Journal of Clinical Pharmacology* 1985; **28**(suppl.), 85–88.

Verbesselt R, Tjandramaga TB and De Schepper PJ. High-performance liquid chromatographic determination of 12 antiarrhythmic drugs in plasma using solid-phase column extraction. *Therapeutic Drug Monitoring* 1991; **13**, 157–165.

Warkentin P. *Neonatal–Perinatal Medicine: Diseases of the Fetus and Infant*, Faranoff A and Martin R (eds), 6th edn. St Louis, MO, 1997.

Wikstrand J, Andersson B, Kendall MJ, Stanbrook H and Klibaner M. Pharmacokinetic considerations of formulation: extended-release metoprolol succinate in the treatment of heart failure. *Journal of Cardiovascular Pharmacology* 2003; **41**, 151–157.

