# Controlled Systemic Absorption and Increased Anesthetic Effect of Bupivacaine Following Epidural Administration of Bupivacaine-Hydroxypropyl-β-Cyclodextrin Complex

**Jean Christophe Fréville,[1] Gilles Dollo,[1] Pascal Le Corre,[1,2] François Chevanne,[1] and Roger Le Verge[1]**

*Received March 9, 1996; accepted July 1, 1996*

***Purpose.*** To investigate the influence of complexation between bupivacaine and hydroxypropyl-β-cyclodextrin (HP-β-CD) on the systemic absorption and on the pharmacodynamic effect of bupivacaine following epidural administration in a rabbit model.

***Methods.*** Bupivacaine and bupivacaine-HP-β-CD complex were administered according to a randomized and cross-over design in six rabbits chronically instrumented with an epidural catheter. The plasma concentrations of bupivacaine and the duration and intensity of the motor blockade were evaluated.

***Results.*** Complexation with HP-β-CD led to a decrease in the maximum plasma concentration of bupivacaine. Individual absorption kinetics evaluated by Loo-Riegelman absorption analysis indicated that systemic absorption resulted from two parallel first-order processes. Only the faster absorption phase was slowed by complexation with HP-β-CD. The duration of the motor blockade was increased almost twice but the intensity was not modified.

***Conclusions.*** Complexation with HP-β-CD could be a promising drug delivery system to improve the therapeutic index of bupivacaine.

**KEY WORDS:** hydroxypropyl-β-cyclodextrin; bupivacaine; inclusion complex; epidural administration; systemic absorption; motor blockade.

## INTRODUCTION

Epidural anesthesia using local anesthetics (LAs) is currently increasing for regional anesthesia upon surgery as well as for regional control of acute and chronic pain. However, LAs present short duration of action and major side-effects such as cardiac and neurological toxicity induced by transient high plasma levels resulting from a rapid absorption in the systemic circulation (1). Thus, there is a need for an improvement in the therapeutic index, and for an increase in the duration of the pharmacologic effect (2). This could be obtained by the development of drug delivery systems allowing a controlled release of these LAs. To reach this goal, many formulations have been tested, e.g., lipid solutions or lipid drug carrier (3), liposomes (4), ionic viscous complex with hyaluronic acid (5), microspheres (6). Among the various ways of obtaining such delivery systems, complexation with cyclodextrins (CDs) may also provide an interesting one. Preliminary in vitro studies have shown that complexation between LAs and cyclodextrins is characterized by stability constants values ranging from 20 to 250 $M^{-1}$ (7). Moreover, pharmacodynamic approaches have shown that CDs can modify the effectiveness (increase in both intensity and duration of action) of some spinally administered endogen molecules like endorphinic peptides and opioids (8), as well as spinally or epidurally administered analgesics (9), suggesting interaction between these substrates and CDs. Thus, complexation of LAs with CDs may be an interesting way to investigate in order to obtain a controlled delivery of these drugs.

Cyclodextrins are torus-shaped oligosaccharides containing six (αCD), seven (βCD), or eight (γCD) α-1,4-linked D-glucose units able to entrap drug molecules of appropriate size and polarity in their hydrophobic cavities to form non covalent inclusion compounds. This may improve physical and chemical properties of the inside guest molecule allowing for example the improvement of stability, solubility, dissolution rate and bioavailability (10). βCD has been studied extensively despite a very low aqueous solubility but alkylated derivatives e.g., hydroxypropyl-β-cyclodextrin (HP-β-CD) and more recently sulfoalkyl ether cyclodextrin derivatives (11), have attracted growing interest due to improved complexing ability, greater water solubility and lesser toxicity resulting.

The aim of this investigation was to study the feasibility of using complexes with cyclodextrins as controlled drug delivery systems for local anesthetic drugs. In this respect, we have evaluated the biopharmaceutics and pharmacodynamics of bupivacaine-hydroxypropyl-β-cyclodextrin complex following epidural administration in rabbits.

## MATERIALS AND METHODS

### Reagents

Bupivacaine hydrochloride (B-HCl) was kindly supplied by Astra (Södertälje, Sweden), and hydroxypropyl-β-cyclodextrin (HP-β-CD, MS = 0.59, LAB 1456) by Roquette Frères (Lestrem, France). Bupivacaine (B) was obtained by precipitation following alkalinisation of a saturated solution of B-HCl with a 3% $NH_4OH$ aqueous solution. All other reagents and solvents (E. Merck, Darmstadt, Germany) were of analytical grade. Unless specified otherwise, doses and concentrations of bupivacaine were expressed in term of base equivalent.

### Animals

The study was approved by the local Committee of Laboratory Animal Care in accordance with the rules and guidelines concerning the care and use of laboratory animals. New Zealand albino male rabbits, weighing 3.1–3.4 kg, were housed individually in standard cages on a 12-h light/dark cycle and were given free access to food and tap water. For purpose of comparison between bupivacaine formulations, the animals were chronically instrumented according to the catheterization procedure

[1] Laboratoire de Pharmacie Galénique et Biopharmacie, Faculté des Sciences Pharmaceutiques et Biologiques, Université de Rennes 1, 2 Avenue du Professeur Léon Bernard, 35043 Rennes Cedex, France.
[2] To whom correspondence should be addressed.

**ABBREVIATIONS:** B: bupivacaine; B-HCl: bupivacaine hydrochloride; HP-β-CD: hydroxypropyl-β-cyclodextrin; B-HP-β-CD: bupivacaine-hydroxypropyl-β-cyclodextrin inclusion complex.

0724-8741/96/1000-1576$09.50/0 

described by Malinovsky et al. (12). Animals were studied at least one week after catheter insertion providing that no obvious neurologic deficit was apparent.

### Study Formulations

Two formulations were compared, B-HCl and B complexed with HP-β-CD (B-HP-β-CD), both as solutions containing B at a concentration of 2 mg/ml. The complex between B and HP-β-CD was formed by complete dissolution of 20 mg of B in 10 ml of a solution of HP-β-CD (30% w/v), under conditions derived from phase-solubility study, needed for optimal complexation to occur (7). The mixture was shaken during 48 hours to ensure complete dissolution of B and then stored at 4°C. B-HCl solution was prepared by dissolution in isotonic NaCl solution. Since the affinity constant between B and HP-β-CD was much higher than that between B-HCl and HP-β-CD (95.0 $M^{-1}$ vs 2.0 $M^{-1}$), the complex between B-HCl and HP-β-CD was not studied.

The pH of these solutions were 8.35 and 5.50 for B-HP-β-CD and B-HCl, respectively. All solutions were sterilized by filtration just before administration (over a 0.22 μm filter, Minisart® NML). The lack of non specific adsorption of bupivacaine to the filter membrane has been checked.

### Study Design

The biopharmaceutics and pharmacodynamics of epidural B-HCl and B-HP-β-CD were compared in a group of six animals following a cross-over and randomized administration of a 2 mg dose of bupivacaine with a one-week wash-out period between the administrations. For biopharmaceutic purposes, an intravenous administration of B-HCl (2 mg) was performed one week after the last epidural administration of bupivacaine.

In order to check for a variation in the sensitivity of the animals related to the chronic implantation of the catheter (i.e., the reproducibility of the model), the pharmacodynamics of epidural lidocaine hydrochloride (10 mg), a short-acting local anesthetics, was recorded before the first and after the last epidural administration of bupivacaine.

Before and after each epidural administration (1 ml over a period of 30 sec for all formulations), the catheter was flushed with 0.2 ml isotonic NaCl solution.

### Drug Sampling and Analysis

Blood samples were drawn from a catheter placed in a marginal vein of the ear before the injection and then at 0.5, 1, 1.5, 2, 3.5, 5, 10, 15, 20, 30, 45, 60, 120, 240, 360, 480 minutes. Blood was collected by fractions of 30 sec. during the first five minutes (lower than 1 ml) and then by samples of one ml. After centrifugation, plasma was collected in polypropylene tubes and stored frozen at −20°C until analysis.

Bupivacaine plasma concentrations were determined by a reversed phase liquid chromatographic method with UV detection (13). The accuracy, the within-day and between-day reproducibilities ($n = 10$) at a plasma concentration of 50 ng/ml were 2.7%, 3.9% and 4.7%, respectively. All calibration curves ranging from 2 to 1000 ng/ml were linear ($r^2 = 0.9995$).

### Biopharmaceutics

The maximum plasma concentration of bupivacaine in plasma (Cmax) and the time to reach Cmax (Tmax) were derived from raw data. The area under the plasma concentration-time curves from the time of drug administration up to the last sampling point [AUC (0–480)] following epidural and i.v. administration was computed by the trapezoidal rule. The area under the plasma concentration-time curves from the time of the last sampling point to infinity [AUC ($480 - \infty$)] was calculated by dividing the plasma concentration at 480 min by the apparent elimination rate constant.

Individual plasma concentration data obtained after i.v. administration were analyzed according to an open-system model with first-order elimination from the central compartment. A model was fitted to the data using a least-squares nonlinear regression analysis with the SIPHAR software package (Simed, Créteil, France). The choice of the best weighting scheme and model was based on inspection of standardized weighted residuals versus time plots, and on statistical evaluation of the weighted sum of squared residuals (14).

Individual absorption kinetics after epidural administration of B-HCl and B-HP-β-CD was evaluated by Loo-Riegelman absorption analysis (15). Time corresponding to 10, 50 and 90% absorbed (T10%, T50% and T90%) were derived from raw data and Td (63.2% absorbed) was calculated from the fit of the percent absorbed-time plots using the Weibull equation. The absolute bioavailability of bupivacaine following epidural administration of B-HCl and B-HP-β-CD was determined by the AUC ($0 - \infty$) ratio.

### Pharmacodynamics

Spontaneous motor activity of the animals was evaluated by a blinded experimentation unaware of the formulation studied, by using the Bromage's scale modified for the rabbit model (2) as follows: level 0) free movements with hind limbs without limitation or loss of balance; level 1) limited or asymmetrical movements of the hind limbs in order to support the body and walk; level 2) inability to support the back of the body on hind limbs with detectable ability to move the limbs; level 3) total paralysis of the hind limbs. The intensity of the motor activity was checked continuously and recorded every 5 min until recovery confirmed by three successive measurements of level 0. The duration of motor blockade at each level was derived from raw data.

### Statistics

Biopharmaceutic differences among groups were evaluated by one-way analysis of variance (ANOVA). Following a significant ANOVA, comparisons were made using the Fisher's Least Significant Difference test. Pharmacodynamic differences between the two formulations were checked by a paired t-test. A value of $p < 0.05$ defined statistical significance. Data are presented as mean ± standard deviation (SD).

## RESULTS AND DISCUSSION

### Effect of HP-β-CD on Systemic Absorption of Bupivacaine

The drug clearance from the epidural space results from competitive mechanisms: i) absorption into the systemic circulation responsible for the systemic toxicity and ii) absorption through the dura and arachnoid mater followed by the distribu-

**Table I.** Biopharmaceutic Parameters of Bupivacaine After Epidural Administration of B-HCl and B-HP-β-CD and Intravenous Administration of B-HCl. Each Data Represents the Mean ±SD ($n$ = 6 rabbits)

| Parameter | Epidural Administration | | Intravenous Administration |
|---|---|---|---|
| | B-HCl | B-HP-β-CD | B-HCl |
| Cmax (ng/ml)[a,b,c] | 1180 ± 524 | 595 ± 253 | 2462 ± 564 |
| Tmax (min)[d] | <0.5 | <0.5 | <0.5 |
| AUC (ngxmin/ml) | 19621 ± 4082 | 21757 ± 4567 | 20804 ± 4319 |
| AUC extrapoled (%)[d] | 6.3 | 3.8 | 12.7 |
| $t_{1/2\beta}$ (min)[a,c] | 83.0 ± 15.7 | 110.2 ± 29.6 | 73.0 ± 16.3 |
| Td (min)[e] | 11.0 ± 3.9 | 18.5 ± 3.6 | |
| T10% (min)[e] | 0.2 ± 0.1 | 0.5 ± 0.2 | |
| T50% (min)[e] | 4.4 ± 3.3 | 11.2 ± 3.7 | |
| T90% (min)[a] | 51.7 ± 1.6 | 53.4 ± 0.9 | |

[a] $p < 0.05$ between epidural B-HCl and B-HP-β-CD.
[b] $p < 0.05$ between epidural B-HCl and Intravenous B-HCl.
[c] $p < 0.05$ between epidural B-HP-β-CD and intravenous B-HCl.
[d] Statistical analysis not performed.
[e] $p < 0.01$ between epidural B-HCl and B-HP-β-Cd.

tion in the cerebrospinal fluid (CSF) and absorption through the pia mater allowing to reach the target neural tissue of the spinal cord (16).

The influence of the complexation with HP-β-CD on the biopharmaceutics of bupivacaine is shown in Table I and illustrated in Figure 1. The rapid transfer rate of the drug from the epidural space into the blood stream was highlighted by the low value of Tmax which was lower than 0.5 min. Such a rapid absorption results from the fact that the epidural space is highly vascularized. Compared to B-HCl, the epidural administration of B-HP-β-CD led to smaller Cmax values (595 ± 253 vs 1180 ± 524 ng/ml) suggesting a decrease in the absorption rate in the systemic circulation. Figure 1 (insert) clearly shows that the initial plasma concentrations of bupivacaine were lower following administration of the complex.

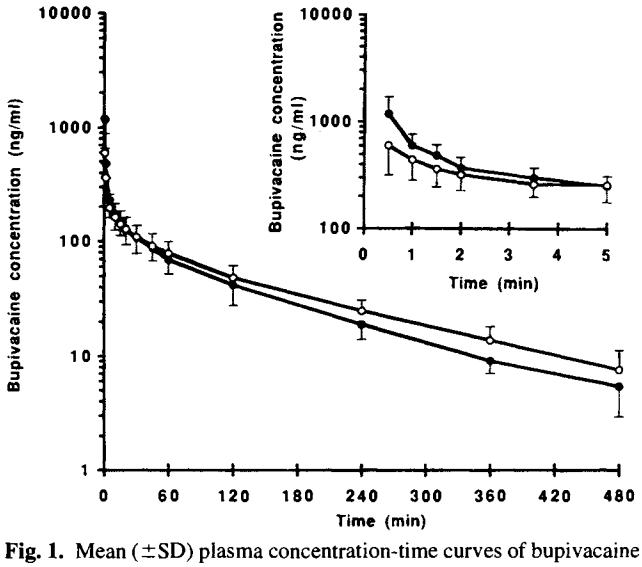


**Fig. 1.** Mean (±SD) plasma concentration-time curves of bupivacaine after single epidural administration in six rabbits of 2 mg of bupivacaine as B-HCl (●) and B-HP-β-CD (○) and expanded view of the initial plasma profiles (insert).

An evaluation of the absorption kinetics was achieved by constructing the individual percent absorbed-time plots following epidural administration of B-HCl and B-HP-β-CD. The pharmacokinetic modeling of plasma concentrations following i.v. administration of B-HCl indicated that the drug behavior was best described by a two-compartment open-system model requiring the percent absorbed-time plots to be constructed according to the Loo-Riegelman method. The mean profiles of the percent drug absorbed into the systemic circulation following epidural B-HCl and B-HP-β-CD showed that the cyclodextrin formulation led to a decrease in absorption rate (Fig. 2). Significant differences were evidenced in time parameters derived from raw data (T10% and T50%) and from fitted parameters (Td) (Table 2). Moreover, the percent remaining to be absorbed versus time displayed a biexponential decay suggesting that epidural drug absorption can be described by two parallel first-order processes (15). Such a biphasic absorption pattern has been evidenced in patients following epidural administration of B-HCl (17). With regard to the rapid phase,

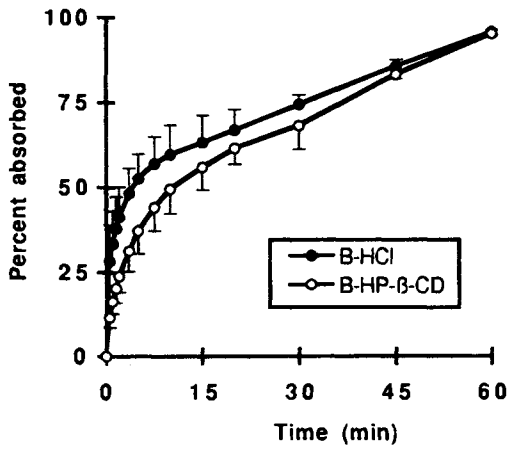


**Fig. 2.** Mean (±SD) percent absorbed-time plots of bupivacaine after single epidural administration of 2 mg of bupivacaine as B-HCl (●) and B-HP-β-CD (○) in six rabbits.

the absorption half-life of bupivacaine following B-HCl was smaller than following B-HP-β-CD (0.62 ± 0.41 min vs 1.21 ± 0.72 min, $p < 0.05$) while no significant difference was observed between absorption half-life of the slower phase (24.1 ± 2.6 vs 21.3 ± 1.1 min). These results suggested that the complexation of bupivacaine with HP-β-CD may serve as a controlled release formulation within the epidural space i.e., that the rate of dissociation of bupivacaine from HP-β-CD may be the rate-limiting step in the absorption into the systemic circulation. The effect of HP-β-CD only concerned the rapid absorption phase which is responsible for the high plasma concentrations. Considering that drug absorption is a first-order process, the decrease in the absorption rate should be attributed to a decrease in the diffusional gradient of free drug across the vascular membrane resulting from drug complexation. Such assumption is likely considering the fact that the epidural space is a fluid-free space. Indeed, the lack of dilution of the complex at the site of administration may prevent its rapid dissociation. Such a feature should be of paramount clinical interest to improve the cardiovascular and neurologic toxicities of local anesthetics resulting from high and transient plasma concentrations. However, complex with higher affinity constants should be of interest to further limit the rate of systemic absorption.

The study design allowed a suitable estimation of the AUC, considering the low percentage of AUC extrapolation, especially following epidural administration (Table 1). Comparison of AUC between epidural and i.v. routes showed that the absolute bioavailability of bupivacaine following epidural administration was complete, and that the complexation of bupivacaine did not apparently modify the extent of bioavailability. Thus, the complexation of bupivacaine with HP-β-CD altered the rate, but not the extent, at which the drug entered the systemic circulation.

Since determination of the drug concentration as a function of time in the CSF was not performed, the influence of complexation on the absorption rate through the dura and arachnoid mater could not be estimated. However, the influence of complexation on this transfer can be assumed indirectly in the light of the increase in the duration of the pharmacodynamic effect observed following administration of B-HP-β-CD compared to B-HCl.

## Effect of HP-β-CD on Pharmacodynamics of Bupivacaine

Basically, the evaluation of the anesthetic action should be based on the measurement of both sensory and motor effects. Although a differential sensory and motor effect may exist, these pharmacodynamic effects share the same molecular mechanism of action and similar profiles of motor and sensitive blockades of LA's have been reported. The epidural anaesthesia evaluation methods based on sensitive effects (sensory blockade) usually performed in rats (tail flick, hot plate) are difficult to bring into operation in rabbits due to anatomical differences, so that, in rabbits, the evaluation of pharmacodynamic effect is only based on the motor effects (motor blockade) (3). Motor blockade is considered as a suitable index for the experimental estimation of the anesthetic action, and has been used in animals and humans. The duration of total motor blockade of bupivacaine following administration of B-HP-β-CD was almost twice compared to the administration of B-HCl (72 ± 19.2 vs 36.2 ± 14.8 min, $p < 0.001$). The difference was not significant for L1 blockade but became significant for L2, which duration raised from 18.3 ± 15.8 to 54.2 ± 21.3 min i.e., the two-fold prolongation of anaesthesia duration was only tied to an increase of L2 blocks duration (Fig. 3). No influence on the intensity of motor blockade was observed. The reproducibility of the model throughout the study was shown by the pharmacodynamics of epidural lidocaine hydrochloride. The duration of total motor blockade of lidocaine hydrochloride obtained before the first and after the last administration of bupivacaine were not significantly different (21.7 ± 2.5 min vs 19.7 ± 5.9 min).

The increase in duration of action should not be attributed to an intrinsic pharmacodynamic activity of HP-β-CD because epidural HP-β-CD (30%, w/v) did not induce any motor blockade in a control group of four rabbits studied with the same catheterization and evaluation procedures. Similarly, it has been shown that intrathecal HP-β-CD (up to 40%, w/v) was apparently devoid of effect on behavior, EEG, nociceptive threshold, spinal reflexes and cardiovascular parameters (8). This increase in duration of motor blockade following administration of the complex suggests that the dissociation of bupivacaine from HP-β-CD could be a rate-limiting step to the transfer through the dura and arachnoid mater.

Such assumption has been raised to explain the increase in the duration of the pharmacodynamic effect of some fentanyl-like opioids administered with HP-β-CD either via intrathecal route (8) or via epidural route in rats (18). This assumption is inconsistent with the ex vivo findings of Bernards (19) who showed that HP-β-CD did not decrease opioids transfer rate through spinal meninges of monkey in a diffusion cell model. He showed that the transfer rate of sufentanil citrate was increased in the presence of a 100-fold higher molar concentration of HP-β-CD. Such a finding was attributed either to a disruption of the arachnoidal cell membrane or a modification of the kinetics of partitioning of the drug between hydrophobic and hydrophilic layers of the membrane. However, the fact that HP-β-CD did not decrease the transfer rates of these opioids should not be so surprising for two reasons. First, the opioids have been used as salts that may not lead to complexes of strong affinity. Indeed, ionization of the drug molecules can reduce their ability to form inclusion complexes because of a

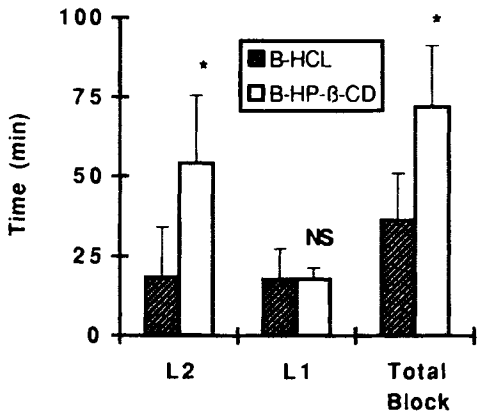


**Fig. 3.** Mean (±SD) duration of motor blockade at level L1, level L2 and total blockade (L1 plus L2) after single epidural administration of 2 mg of bupivacaine as B-HCl (■) and B-HP-β-CD (□) in six rabbits. The asterisk indicates $p < 0.05$. NS indicates not significant.

higher hydrophilicity and of a large number of tightly bound water molecules impeding the inclusion (20). Second, the solutions containing the drug studied and HP-β-CD were significantly diluted (1:100) in the donor compartment at the beginning of the study, so that the complex formed should be almost immediately dissociated.

In summary, the current preliminary findings suggest that complexation with HP-β-CD may be used to improve the epidural delivery of bupivacaine, allowing a slower systemic absorption and a longer pharmacodynamic effect. Additional work is necessary to better define the potential of cyclodextrins in this area i.e., choice of the most appropriate local anesthetic-cyclodextrin complex, and evaluation of the toxicity. From a more general point of view, complexation with cyclodextrins should be of interest for the site-specific delivery of drugs characterized by a rapid systemic absorption producing adverse effects.

## REFERENCES


1. B. G. Covino and D. H. Lambert, In P. G. Barash, B. F. Cullen and R. K. Stoelting (Eds), *Clinical anesthesia*, J. B. Lippincott, Philadelphia, 92, pp. 809–840.
2. T. H. Stanley. *Anaesthesiology*, **38**:665–668 (1988).
3. L. Langerman, E. Golomb and S. Benita, *Anesthesiology*. **74**:105–107 (1991).
4. T. Mashimo, I. Uchida, M. Pak, A. Shibata, S. Nishimura, Y. Inagaki and I. Yoshida, *Anesth. Analg.*, **74**:827–834 (1992).
5. M. M. Doherty, P. J. Hughes, N. V. Korszniak and W. N. Charman, *Anesth. Analg.* **80**:740–746 (1995).
6. P. Le Corre, J. P. Estèbe, F. Chevanne, Y. Mallédant and R. Le Verge, *J. Pharm. Sci.* **84**:75–78 (1995).
7. G. Dollo, P. Le Corre, F. Chevanne and R. Le Verge, *Int. J. Pharm.* **136**:165–174 (1996).
8. J. Jang, T. L. Yaksh and H. F. Hill, *J. Pharmacol. Exp. Ther.* **261**:592–600 (1992).
9. T. F. Meert, P. Putteman and J. Peeters, *Drug Dev. Res.* **28**:28–37 (1993).
10. M. E. Brewster, J. W. Simpkins, M. Singhhora, W. C. Stern and N. Bodor, *J. Parenter. Sci. Technol.* **43**:231–240 (1989).
11. R. A. Rajewski, G. Traiger, J. Bresnahan, P. Jaberaboansari, V. J. Stella and D. O. Thompson, *J. Pharm. Sci.* **84**:927–932 (1995).
12. J. M. Malinowsky, J. M. Bernard, P. Le Corre, J. B. Dumand, J. Y. Lepage JY, R. Le Verge and R. Souron, *Anesth. Analg.* **81**:519–524 (1995).
13. P. Le Guevello, P. Le Corre, F. Chevanne and R. Le Verge, *J. Chromatogr.* **622**:284–290 (1993).
14. H. G. Boxenbaum, S. Riegelman and R. M. Elashoff, *J. Pharmacokin. Biopharm.* **2**:123–148 (1974).
15. M. Gibaldi and D. Perrier, *Pharmacokinetics, 2nd ed.*; Marcel Dekker, New York, 1982.
16. A. G. L. Burm, *Clin. Pharmacokin.* **16**:283–311 (1989).
17. A. G. L. Burm, N. P. E. Vermeulen, J. W. Van Kleef, A. G. De Boer and J. Spierdijk, *Clin. Pharmacokin.* **13**:191–203 (1987).
18. T. F. Meert and W. Melis, *Acta Anaesth. Belg.* **43**:79–89 (1992).
19. C. M. Bernards, *J Pharm. Sci.* **83**:620–622 (1994).
20. F.-Y. Liu, D. O. Kildsig and A. K. Mitra, *Pharm. Res.* **9**:1671–1672 (1992).