Journal of Chromatography, 622 (1993) 284–290
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 7149

Short Communication

High-performance liquid chromatographic determination of bupivacaine in plasma samples for biopharmaceutical studies and application to seven other local anaesthetics

P. Le Guévello, P. Le Corre*, F. Chevanne and R. Le Verge

Laboratoire de Pharmacie Galénique, Biopharmacie et Pharmacie Clinique, Université de Rennes I, 2 avenue du Professeur Léon Bernard, 35043 Rennes Cedex (France)

(First received July 8th, 1993; revised manuscript received September 24th, 1993)

ABSTRACT

A sensitive analytical procedure for bupivacaine dosing in plasma samples by reversed-phase high-performance liquid chromatography is described. After a two-step extraction, the analysis was performed using a C_{18} column and a mobile phase of 0.01 *M* sodium dihydrogen-phosphate (pH 2.1)–acetonitrile (80:20, v/v). The extraction yield of bupivacaine from plasma was 73.5 ± 5.1% (mean ± S.D., $n = 10$). The within-day and between-day reproducibilities at a concentration of 100 ng/ml were 2.1% and 5.6%, respectively ($n = 10$). Calibration curves were linear ($r^2 = 0.9996$) between 5 and 1000 ng/ml. The limit of detection, defined by a signal-to-noise ratio of 3:1, was 2 ng/ml. The accuracy at a concentration of 100 ng/ml was 2.3%. This method could be applied to the plasma analysis of seven other local anaesthetics (articaine, etidocaine, lidocaine, mepivacaine, pramocaine, procaine and tetracaine). The procedure was used in bioavailability studies of bupivacaine-loaded poly(D,L-lactide) (*i.e.* PLA) and poly(D,L-lactide-co-glycolide) (*i.e.* PLGA) microspheres after subcutaneous and intrathecal administrations in rabbits.

INTRODUCTION

Local anaesthetic drugs are widely used for regional anaesthesia and for regional management of major pain, either via central administration (spinal and epidural) or via peripheral administration [1,2]. These techniques are more and more employed to reduce the use of systemic narcotic drugs, which lead to more frequent and severe adverse effects, principally respiratory depression [3]. However, regional administrations of local anaesthetic drugs could be improved by the development of drug delivery systems that would allow controlled release of the anaesthetic drug, leading to a longer duration of action and a lower uptake of these drugs in the systemic circulation resulting in an improvement of the therapeutic index. Recent experimental works with several local anaesthetics highlight the interest of such an approach [4–11]. Biopharmaceutical studies of such delivery systems require sensitive analytical methods for plasma analysis of these drugs.

Several methods have been previously described for plasma analysis of bupivacaine and

* Corresponding author.

0378-4347/93/$06.00

some local anaesthetics, with detection limits ranging between 1 and 50 ng/ml [12–22]. The simultaneous determination of four local anaesthetics (bupivacaine, etidocaine, lidocaine and mepivacaine) by gas chromatography had a limit of detection of 10 ng/ml [14].

This paper describes a high-performance liquid chromatographic (HPLC) method that allows a sensitive assay of bupivacaine, and its application to the evaluation of drug delivery systems based on bupivacaine-loaded microspheres. This procedure could be also used for the plasma determination with an internal standard of seven other local anaesthetics (articaine, etidocaine, lidocaine, mepivacaine, pramocaine, procaine and tetracaine).

EXPERIMENTAL

Chemicals

All the local anaesthetics (Fig. 1) were purchased in hydrochloride form. Articaine was supplied by Laboratoire SPAD (Quetigny, France), bupivacaine, etidocaine, lidocaine and mepivacaine by Laboratoire Astra (Nanterre, France), pramocaine by Laboratoire Abbott (Rungis, France), procaine and tetracaine by Sigma (Paris, France). All other reagents (E. Merck, Darmstadt, Germany) were of analytical grade.

Spectrophotometric method

The UV spectra were obtained between 200 and 300 nm on a Lambda 3B (Perkin Elmer, Norwalk, CO, USA). Except for lidocaine, all the drug solutions were in 0.1 *M* hydrochloric acid at 0.01% (w/v as a base). Owing to its significant UV absorbance, the lidocaine UV spectrum was obtained at 0.001% (w/v).

HPLC analysis

The chromatographic system consisted of a Waters Model 6000A pump (Waters Assoc., Milford, MA, USA) equipped with a Waters Model WISP 710 B automatic injector, an LDC Milton Roy Model Spectromonitor 3100 variable-wavelength detector (LDC Milton Roy, Riviera Beach, FL, USA) set at 205 nm, and a Delsi Model Enica 21 integrator (Delsi,

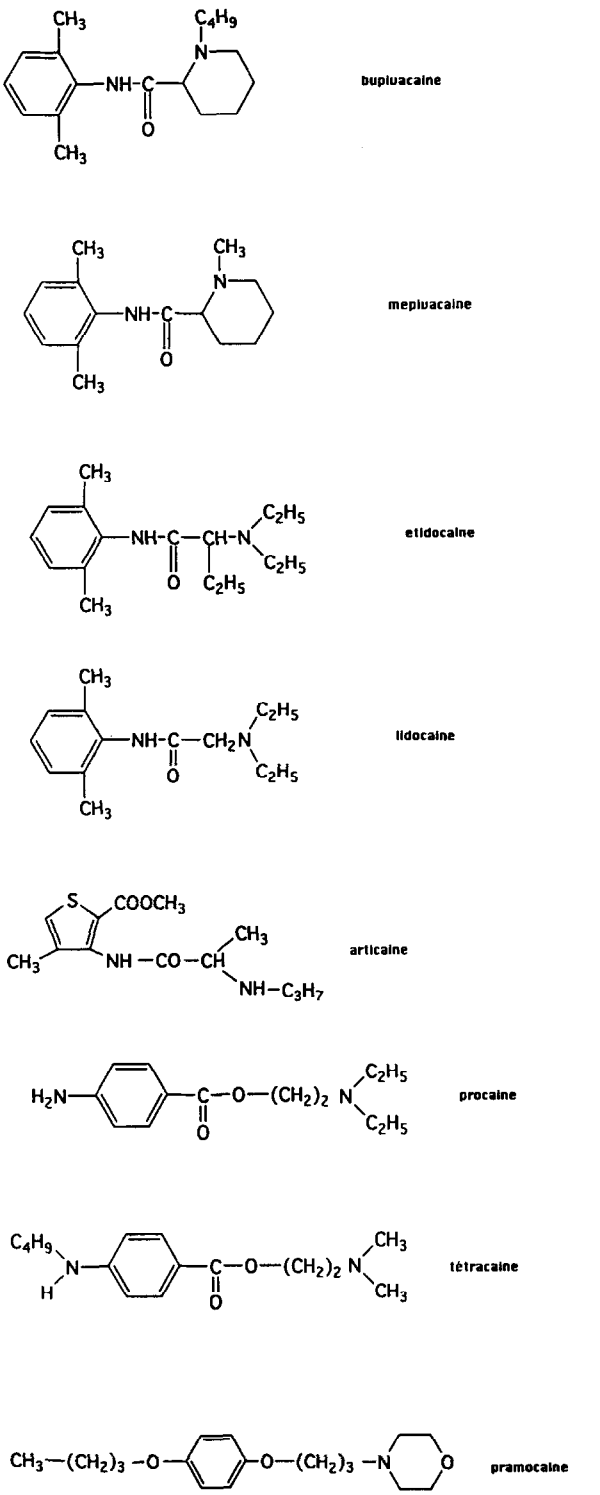

Fig. 1. Structural formulae of the eight local anaesthetics investigated in this study.

Suresnes, France). The analytical chromatography column was a Waters Model μBondapak C_{18} (250 × 4 mm I.D.; particle size 10 μm). The mobile phase was a pH 2.1 mixture of acetonitrile and 0.01 *M* sodium dihydrogenphosphate. The percentage of the organic phase was varied between 5% and 40%, according to the anaesthetic analysed. The flow-rate was 1 ml/min, and the temperature was maintained at 30°C.

General procedure for sample preparation

The same procedure was used for the sample preparation of all eight local anaesthetics. A 1-ml rabbit plasma sample spiked with an aqueous solution of local anaesthetics (100 μl) was alkalinized with 100 μl of 1 *M* sodium hydroxide. The first extraction step was to add 3 ml of heptane–ethyl acetate (90:10, v/v) and shake for 2 min. After centrifugation at 1200 *g* for 10 min, the organic phase was transferred into a conical tube. The second extraction step was carried out after the addition of 50 μl of 0.05 *M* sulphuric acid and shaking for 2 min. After centrifugation at 1200 *g* for 5 min, the organic phase was discarded and 50 μl of the aqueous acid phase were buffered with 820 μg of sodium acetate (50 μl of 0.2 *M* methanolic sodium acetate solution previously dried). A 40-μl aliquot was injected into the chromatograph.

Validation of the method

In order to analyse bupivacaine serially in plasma samples, an internal standard (etidocaine, 100 ng in 50 μl of aqueous solution) was added to plasma samples before alkalinization. For calibration, known amounts of bupivacaine were added to pooled drug-free plasma to obtain concentrations of 5, 10, 20, 50, 100, 200, 500 and 1000 ng/ml by using two bupivacaine standard solutions dosed at 1 or 10 mg/l, respectively. These plasma aliquots were extracted as described above. The linearity was checked by a least-squares linear regression fitting. The extraction yield was determined at a plasma concentration of 100 ng/ml by comparison of the bupivacaine peak area obtained from plasma extracts with those obtained with a 2000 ng/ml bupivacaine standard solution. The within-day and the between-day reproducibilities of the method were evaluated at a plasma concentration of 100 ng/ml ($n = 10$).

For the seven other local anaesthetics, the validation was based on extraction yield, within-day reproducibility and limit of detection.

Pharmacokinetic application

Bupivacaine-loaded microspheres were prepared by the solvent-evaporation technique [23] using two types of biodegradable polymer, *i.e.* poly(D,L-lactide) (PLA), and poly(D,L-lactide-co-glycolide) (PLGA) [24]. In this preliminary comparative bioavailability study in rabbits, we measured plasma concentrations following intrathecal and subcutaneous administrations. Bupivacaine was given as a solution, as PLGA microspheres or as PLA microspheres via the intrathecal route (5, 20 and 20 mg, respectively) or via the subcutaneous route (20, 100 and 30 mg, respectively). Blood samples were collected immediately before administration and then at 0.08, 0.17, 0.5, 0.75, 1, 2, 4, 6, 8 and 24 h. They were centrifuged immediately and then stored at −20°C until assay. Plasma concentration *versus* time curves were fitted with the non-linear least-squares regression computer program SIPHAR (Simed, Créteil, France) [25].

RESULTS AND DISCUSSION

Bupivacaine assay

In order to avoid interference from endogenous plasma components at 205 nm, the wavelength of maximal absorbance, a two-step extraction was carried out. Solvents of different polarities, heptane, diethyl ether and ethyl acetate, were used for extraction, leading to bupivacaine extraction yields of 73.5%, 85.2% and 59.8%, respectively. Heptane was selected because it led to the cleanest extracts based on blank chromatograms. However, to improve the phase separation at the interface between plasma and organic phases during the first extraction step, and thus increase the volume of the collected organic phase, we used ethyl acetate–heptane (10:90, v/v).

Among the local anaesthetics, etidocaine was selected as the internal standard for bupivacaine

analysis owing to its retention time. The separation was optimal with a mobile phase containing 20% acetonitrile (v/v). The capacity factors (k') of etidocaine and bupivacaine were 2.90 and 3.75, respectively. However, tetracaine could be used as the internal standard with a mobile phase containing 25% acetonitrile. In this case, k' for bupivacaine and tetracaine were 2.69 and 4.95, respectively. The chromatographic separation of bupivacaine and etidocaine is illustrated in Fig. 2. The corresponding chromatographic data are listed in Table I. The resolution factor (R_s) was determined by $R_s = 2\,(t_{R_2} - t_{R_1})/(\omega_1 + \omega_2)$ where t_R is the retention time and ω is the base width.

Calibration curves were linear ($r^2 = 0.9996$) between 5 and 1000 ng/ml. The limit of detection, defined by a signal-to-noise ratio of 3:1, was 2 ng/ml. The extraction yield was 73.5 ± 5.1% (mean ± S.D., $n = 10$). The within-day and between-day reproducibilities at a concentration of 100 ng/ml were 2.1% and 5.6%, respectively ($n = 10$). The accuracy at a concentration of 100 ng/ml was 2.3%.

Plasma concentrations *versus* time curves following intrathecal and subcutaneous administration of bupivacaine solution and bupivacaine-loaded microspheres (PLA and PLGA microspheres) are shown in Fig. 3. For comparison, because different doses were administered, the plasma concentration obtained following administration of bupivacaine-loaded microspheres has been corrected for the size of the dose (5 mg for the intrathecal route and 20 mg for the subcutaneous route).

The maximum plasma concentrations (C_{max}) following administration of bupivacaine solution, PLA and PLGA microspheres via the intrathecal route was 470, 44 and 37 ng/ml, respectively.

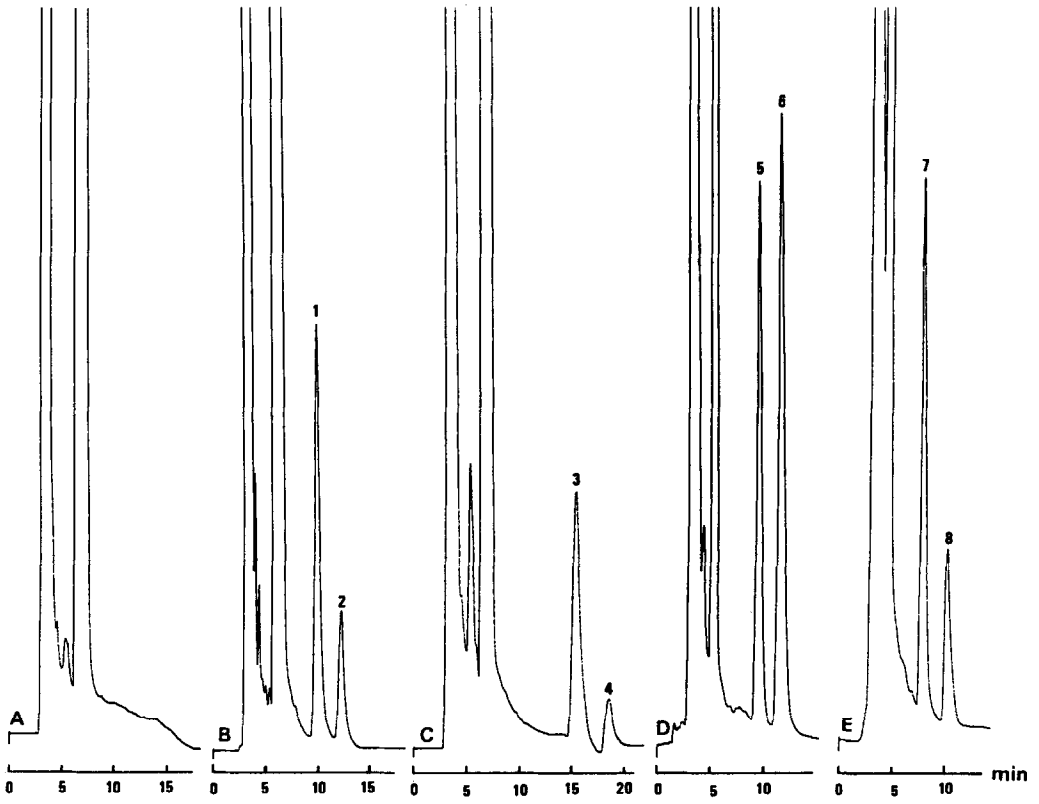

Fig. 2. Chromatograms of extracted plasma samples. (A) Blank plasma sample; (B)–(E) plasma samples spiked with two local anaesthetic drugs (100 ng/ml). Peaks: 1 = procaine; 2 = lidocaine; 3 = mepivacaine; 4 = articaine; 5 = etidocaine; 6 = bupivacaine; 7 = tetracaine; 8 = pramocaine. Conditions as in Experimental. Percentage of acetonitrile in the mobile phase: (B) 5%; (C) 7%; (D) 20%, (E) 35%.

TABLE I

CHROMATOGRAPHIC DATA

Conditions as described under Experimental. k' = Capacity factor; α = separation factor; R_s = resolution factor = $2(t_{R_2} - t_{R_1})/(\omega_1 + \omega_2)$ where t_R is the retention time and ω is the base width.

Acetonitrile (%)	Drug	k'	α	R_s
5	Procaine	3.21	1.78	4.04
	Lidocaine	5.70		
7	Mepivacaine	4.42	1.26	1.85
	Articaine	5.58		
20	Etidocaine	2.90	1.29	1.49
	Bupivacaine	3.75		
35	Tetracaine	2.72	1.39	2.42
	Pramocaine	3.80		

Following subcutaneous administration, C_{max} was 784, 85 and 9 ng/ml, respectively. The time to reach maximum plasma concentration (T_{max}) following administration of bupivacaine solution, PLA and PLGA microspheres was 0.08, 0.75 and 4 h, respectively. Following subcutaneous administration, T_{max} was 0.5, 2 and 24 h, respectively.

These results indicate the suitability of this method for bioavailability studies of bupivacaine-loaded microspheres, which lead to low plasma concentrations.

Assay of the other local anaesthetics

In a preliminary step, we determined the UV spectrum characteristics of the other local anaesthetics that were either unknown or determined in different experimental conditions. Their wavelengths of maximum absorbance are listed in Table II.

A rank correlation was found between the partition coefficients [26] of the charged species and the capacity factor of the amide-type local anaesthetics (lidocaine, mepivacaine, etidocaine and bupivacaine), but not for their uncharged species. This is consistent with the assumed mechanism of chromatography: partition of the charged species between the mobile and stationary phases.

Several authors have studied the relationship between the nature of the mobile phase and the retention in reversed-phase HPLC in order to optimise the mobile phase composition. Various curvilinear or linear relationships between k' and the organic content of the mobile phase have been described [27–30]. Among these, the parameter $R_Q = \log(k'/1 + k')$ defined by Toon

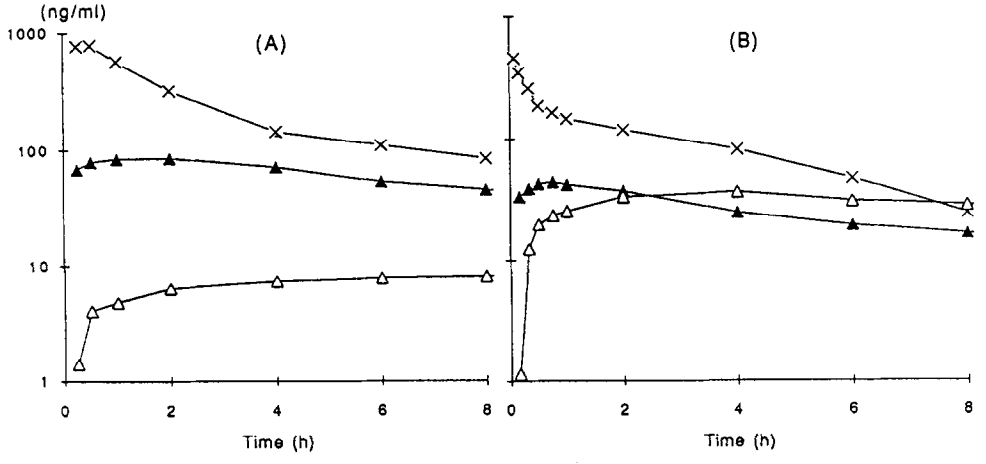

Fig. 3. Bupivacaine plasma concentration–time curves following subcutaneous (A) or intrathecal (B) administration of bupivacaine in rabbits (plasma levels rapported to 20 and 5 mg, respectively): (×) bupivacaine solution; (△) bupivacaine-loaded PLGA microspheres; (▲) bupivacaine-loaded PLA microspheres. Conditions as in Experimental.

TABLE II

EXTRACTION YIELDS AND UV SPECTROPHOTOMETRY DATA

Conditions as described under Experimental. S.D. = standard deviation; S/N = signal-to-noise ratio at 205 nm and at a plasma concentration of 5 ng/ml; log P^0 and log P^+ = partition coefficients of neutral and protonated drug, respectively [24]; $A_{0.01\%}$ = absorbance at 205 nm for $C = 0.01\%$ (w/v); λ_{max} = maximum absorbance wavelength (nm).

Drug	Yield (%)	S.D. (%) ($n = 10$)	S/N	log P^+	log P^0	$A_{0.01\%}$	λ_{max_1}; $A_{0.01\%}$	λ_{max_2}; $A_{0.01\%}$	λ_{max_3}; $A_{0.01\%}$
Amide-type									
Articaine	46.7	2.8	3	–	–	2.780	199; 2.984	272; 3.173	–
Bupivacaine	73.5	5.1	12	1.500	2565	3.076	206; 3.092	262; 0.152	269; 0.119
Etidocaine	72.3	4.5	7	0.480	4900	3.045	205; 3.045	261; 0.138	269; 0.105
Lidocaine	81.1	3.4	5	0.060	304	1.941[a]	199; 3.053[a]	233; 3.273[a]	288; 2.812[a]
Mepivacaine	27.0	0.8	9	0.090	90	3.061	208; 3.068	261; 0.158	269; 0.123
Ester-type									
Procaine	19.6	3.4	2	0.002	81	3.068	207; 3.053	261; 0.155	269; 0.122
Tetracaine	96.7	5.6	14	0.460	3615	2.025	199; 3.053	223; 3.285	300; 3.000
Ether-type									
Pramocaine	39.0	4.2	2	–	–	2.001	200; 2.816	220; 2.804	284; 0.804

[a] For lidocaine, $C = 0.001\%$.

and Rowland [27] gave a linear correlation for acid drugs analysed as the non-ionized form (barbituric acid derivatives). Because a straight-line graph seemed to us to be more convenient to predict the optimal mobile phase composition, we also used this parameter R_Q. We found a linear relationship between R_Q values of local anaesthetics and the concentration of acetonitrile in the mobile phase in the range 5–30% (v/v) (Fig. 4). Thus, this relationship could also be used to optimize the mobile phase selection for the chromatography of basic drugs in their ionized forms.

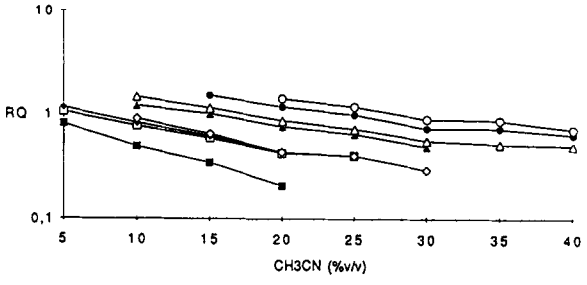

Fig. 4. $R_Q = \log(k'/1 + k')$ of local anaesthetics *versus* the amount of acetonitrile in the mobile phase (%, v/v): (■) procaine; (□) lidocaine; (♦) mepivacaine; (◊) articaine; (▲) etidocaine; (△) bupivacaine; (●) tetracaine; (○) pramocaine.

The method described for bupivacaine was used for the determination of other local anaesthetics in plasma samples. The extraction yields ranged from 19.6% to 96.7% (Table II), indicating that the extraction procedure could be improved for four of these drugs (articaine, mepivacaine, pramocaine and procaine). The signal-to-noise ratio at 205 nm for a drug plasma concentration of 5 ng/ml showed that this method was sensitive for the plasma determination of all the local anaesthetics tested. For a signal-to-noise ratio of 3:1, the limits of detection at 205 nm ranged between 1 and 8 ng/ml for these local anaesthetics (Table II). However, for some of them, the sensitivity could be increased either by performing the detection at the wavelength of maximum absorbance (articaine, lidocaine, pramocaine and tetracaine) or by improving the extraction yield (articaine, mepivacaine, pramocaine and procaine) (Table II).

To allow the plasma determination of these local anaesthetics with an internal standard, four pairs of drugs can be proposed based on op-

timum chromatographic data (k', α and R_s) (Table I).

In conclusion, the HPLC method described here is sensitive enough to allow its application to the study of bioavailability in drug-delivery systems of local anaesthetic drugs leading to low plasma concentrations, and it is effective in the determination of eight local anaesthetic drugs.

REFERENCES

1 G.Levinson, in J.L. Benumof (Editor), *Clinical Procedures in Anesthesia and Intensive Care*, J.B. Lippincott, Philadelphia, PA, 1992, Ch. 31, p. 645.
2 H.B. Howell, in J.L. Benumof (Editor), *Clinical Procedures in Anasthesia and Intensive Care*, J.B. Lippincott, Philadelphia, PA, 1992, Ch. 36, p. 773.
3 J.E.F. Reynolds (Editor), *Martindale*, XIIIth ed., Pharmaceutical Press, London, 1993, p. 995.
4 T. Mashimo, I. Uchida, M. Pak, A. Shibata, S. Nishimura, Y. Inagaki and I. Yoshiya, *Anesth. Analg.*, 74 (1992) 827.
5 L. Langerman, E. Golomb and S. Benita, *Anaesthesiology*, 74 (1991) 105.
6 L. Langerman, E. Golomb and S. Benita, *Anesth. Analg.*, 72 (1991) 635.
7 L. Langerman, G.J. Grant, M. Zakowski, S. Ramanathan and H. Turndorf, *Anaesthesiology*, 77 (1992) 475.
8 L. Langerman, G.J. Grant, M. Zakowski, S. Ramanathan, E. Golomb and H. Turndorf, *Anaesth. Analg.*, 75 (1992) 900.
9 D.B. Masters, C.B. Berde, S. Dutta and R. Langer, *Anaesthesiology*, 75 (1991) A765.
10 F.J. Singelyn and J.M. Gouverneur, *Anaesthesiology*, 73 (1990) A797.
11 F. Legros, H. Luo, P. Bourgeois, N. Lafont and J. Boogaerts, *Anaesthesiology*, 73 (1990) A851.
12 R.L.P. Lindberg, J.H. Kanto and K.K. Pihlajamäki, *J. Chromatogr.*, 383 (1986) 357.
13 U.W. Wiegand, R.C. Chou, E. Lanz and E. Jähnchen, *J. Chromatogr.*, 311 (1984) 218.
14 D.E. Coyle and D.D. Denson, *Ther. Drug. Monit.*, 8 (1986) 98.
15 H.C. Michaelis, W. Geng, G.F. Kahl and H. Foth, *J. Chromatogr.*, 527 (1990) 201.
16 T.B. Vree, A.M. Baars, G.E.C.J.M. Van Oss and L.H.D.J. Booij, *J. Chromatogr.*, 424 (1988) 440.
17 N.W. Sullivan and L. Weiner, *J. Chromatogr.*, 417 (1987) 236.
18 Y.K. Tam, S.R. Tawfik, J. Ke, R.T. Coutts, M.R. Gray and D.G. Wyse, *J. Chromatogr.*, 423 (1987) 199.
19 H. Hattori, S. Yamamoto, T. Yamada and O. Suzuki, *J. Chromatogr.*, 564 (1991) 278.
20 B.J. Clark, A. Hamdi, R.G. Berrisford, S.S. Sabanathan and A.J. Mearns, *J. Chromatogr.*, 553 (1991) 383.
21 A. Ruzafa, M.C. Pastor, J.L. Aguilar and R. Galimany, *J. Liquid Chromatogr.*, 14 (1991) 2937.
22 H. Kasstrissios, M.F. Hung and E.J. Triggs, *J. Chromatogr.*, 577 (1992) 103.
23 R. Bodmeier and J.W. McGinty, *J. Microencapsulation*, 34 (1987) 279.
24 P. Le Guevello, P. Le Corre, F. Chevanne and R. Le Verge, *Compte rendus des 7èmes Journées Scientifiques du Groupe Thématique de Recherche sur les Vecteurs, Paris, December 1992*, p. 77.
25 C. Gomeni and R. Gomeni, in A. Serio and R. O'Moore (Editors), *Proceedings of the 7th International Congress of Medical Informatics, Rome, 1987*, European Federation for Medical Informatics, Rome, 1987, p. 507.
26 G.R. Strichard, V. Sanchez, R. Arthur, R. Chafetz and D. Martin, *Anaesth. Analg.*, 71 (1990) 158.
27 S. Toon and M. Rowland, *J. Chromatogr.*, 208 (1981) 391.
28 J.-P. Thomas, A. Brun and J.-P. Bounine, *J. Chromatogr.*, 172 (1979) 107.
29 J.R. Gant, J.W. Dolan and L.R. Snyder, *J. Chromatogr.*, 185 (1979) 153.
30 P.J. Schoenmakers, H.A.H. Billiet and L. de Galan, *J. Chromatogr.*, 185 (1979) 179.