ELSEVIER

Journal of Chromatography B, 724 (1999) 117–126

JOURNAL OF CHROMATOGRAPHY B

High-performance liquid chromatographic method for the determination of atracurium and laudanosine in human plasma Application to pharmacokinetics

Christine Farenc[a,b], Michel Audran[b], Jean-Yves Lefrant[c], Ingrid Mazerm[a], Françoise Bressolle[a,*]

[a]*Laboratoire de Pharmacocinétique Clinique, Faculté de Pharmacie, 34060 Montpellier Cedex 2, France*
[b]*Laboratoire de Biophysique, Faculté de Pharmacie, 34060 Montpellier Cedex 2, France*
[c]*Fédération d'Anesthésie Réanimation et de l'Urgence, Centre Hospitalier Universitaire, Nîmes Cedex, France*

Received 1 October 1998; received in revised form 27 November 1998; accepted 7 December 1998

Abstract

A high-performance liquid chromatographic method coupled with fluorimetric detection has been developed for the determination of atracurium and its major metabolite, laudanosine, in human plasma. The detection is performed at 240 nm for excitation and 320 nm for emission. Verapamil was used as the internal standard. The proposed technique, involving the direct precipitation of plasma proteins is reproducible, selective and sensitive. Linear detector responses were observed for the calibration curve standards in the range of 40 to 2000 ng/ml. Precision, expressed as C.V., was in the range 1 to 14%. The limit of quantification for both atracurium and laudanosine was 40 ng/ml. The method has been validated and stability tests under various conditions have been performed. This method has been used to determine the pharmacokinetic profile of atracurium and laudanosine in patients with acute respiratory distress syndrome.

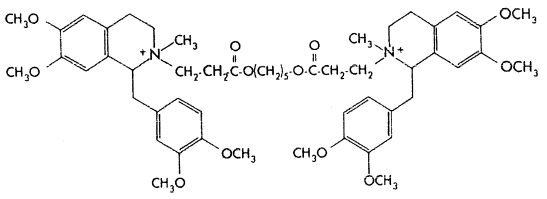
Keywords: Atracurium; Laudanosine

1. Introduction

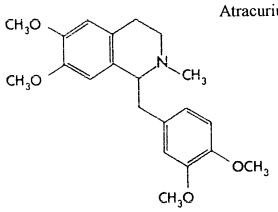
Atracurium besylate, 2,2′-[1,5-pentanediylbis-[oxy(3oxo-3,1-propane diyl)]]bis[1-[(3,4-dimethoxyphenyl)-methyl]-1, 2, 3, 4-tetrahydro-6, 7-dimethoxy-2-methylisoquinolinium] dibenzene sulphonate (Fig. 1) is a highly selective competitive (non-depolarizing) neuromuscular blocking agent used in anesthesiology. Moreover, it is highly suitable for endotracheal intubation, especially where subsequent muscle relaxation is required [1]. This drug is used in 3–17% of critically ill patients with acute respiratory distress syndrome, which necessitates the use of neuromuscular blocking drugs over several days to facilitate mechanical ventilation [2]. In contrast to surgical anaesthesia, the pharmacokinetic/pharmacodynamic profile of atracurium in intensive care unit patients is poorly documented. Nevertheless, in these patients, the volume of distribution is often increased, and the metabolism and elimination processes are often adversely affected.

*Corresponding author. Tel.: +33-4-6754-8075; fax: +33-4-6779-3292; e-mail: fbressolle@aol.com

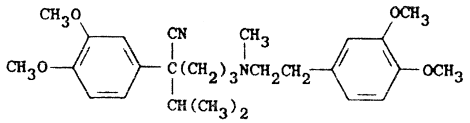
0378-4347/99/$ – see front matter
PII: S0378-4347(98)00576-3



Atracurium



Laudanosine



Verapamil

Fig. 1. Structural formulae of atracurium, laudanosine and verapamil.

Under physiological conditions (temperature and pH), atracurium undergoes relatively rapid hydrolysis by non-specific esterases and by nonenzymatic decomposition (Hoffmann elimination) to give laudanosine (Fig. 1) and pentamethylene-1,5 diacrylate [3–5]. Hoffmann elimination is enhanced by alkalosis and temperature, inhibited by acidosis. A less important route of metabolism (ester hydrolysis) involves the formation of a monoquaternary alcohol and acid, which are eventually degraded to laudanosine by a Hoffmann reaction [3,6]. It is unlikely that the contribution of these metabolites to the neuromuscular blocking effect is of pharmacological importance [7].

Several high-performance liquid chromatographic (HPLC) methods have been reported for the determination of atracurium alone [8,9] or in combination with its metabolites in human plasma [10–13]. According to the authors, strong cation-exchange column [8,11,12] or reversed-phase columns [9,10,13] were used. A method for the determination of atracurium in human plasma by second-derivative UV absorption spectrophotometry was also described [14]. These methods require solid-phase extraction [8,9,11,13,14], liquid–liquid extraction [12] or simple protein precipitation [10]. However, most of the published methods either did not report assay validation [8,11,12] or reported assay validations that were incomplete [9,13]; they could not perform the simultaneous determination of atracurium and laudanosine [8,9] or had difficulties in being adapted as a routine analytical method. Nehmer [15] has studied the influence of the composition (acetonitrile and buffer concentrations) and the pH of the mobile phase on the separation of atracurium from its metabolites and concluded that hydrophobic and silanophilic interactions contribute to the retention of the compounds investigated; however, concentrations greatly exceeding those expected in a clinical setting were used.

The present paper describes an isocratic reversed-phase HPLC method for the rapid separation and simultaneous measurement of atracurium and its metabolite, laudanosine, in human plasma. Verapamil was used as the internal standard. This method was validated with respect to accuracy, precision, selectivity, and limits of quantitation and of detection according to Good Laboratory Practice Guidelines [16–18]. Moreover, stability tests under various conditions have been performed.

2. Experimental

2.1. Chemicals and reagents

Atracurium besylate and laudanosine were kindly supplied by Glaxo-Wellcome Laboratories (Beckenham, UK). These drugs were stored in a refrigerator (about 4°C) and at 20°C with light protection, respectively. The internal standard (verapamil) was obtained from Sigma (St. Louis, MO, USA) and was stored with light protection at room temperature (20°C). The structures of the three compounds are shown in Fig. 1.

Solvents of HPLC grade (acetonitrile and methanol) were purchased from Carlo Erba (Val de Reuil,

France); potassium dihydrogen phosphate, orthophosphoric acid and sulphuric acid were obtained from UCB (Brussels, Belgium), Labosi (Paris, France) and Aldrich (Milwaukee, WI, USA), respectively. All solvents were used without further purification.

The phosphate buffer solution consisted of 0.04 *M* potassium dihydrogen phosphate (5.4 g) in distilled water (1 l).

For validation of the method, human plasma was obtained from pooled blood samples collected from healthy volunteers. Coagulation was prevented by adding EDTA-sodium salt, then the blood was centrifuged at 2000 *g* for 10 min. Blood plasma samples were stored at −30°C before use. Prior to storage or analysis, drug-free plasma, which was used for the preparation of standards and quality control (QC) samples, was acidified (pH 4.2) with 0.5 *M* sulphuric acid (40 μl for 1–ml plasma sample) before the addition of atracurium (and laudanosine).

Blood plasma samples collected during pharmacokinetic studies were acidified before storage, as performed for QC samples.

2.2. Stock solutions and standards

Stock solutions of atracurium and laudanosine (0.2 mg/ml) were prepared in a 6.25×10^{-4} *M* sulphuric acid solution (pH 3.0). These solutions were appropriately diluted extemporaneously in the same acidic medium to obtain working standards. A stock solution of verapamil (internal standard) was prepared at 0.2 mg/ml in acetonitrile–distilled water (1:100, v/v) for solubility reasons. This solution was diluted 20-fold extemporaneously in distilled water before use. All stock solutions were stored at 4°C. Stock solution stabilities were studied over 15 days. For this, these solutions, after appropriate dilution, were repeatedly ($n=3$) injected into the chromatograph immediately after preparation (time 0) and also after every 48 h of storage.

An unextracted working acid solution (pH 3.0) containing the two drugs to be analyzed, at a concentration of 500 ng/ml, and the internal standard, at a concentration of 500 ng/ml, was prepared daily to check the resolution of the chromatographic system.

2.3. Chromatographic system and conditions

The apparatus used for the HPLC analysis was a Hewlett-Packard multisolvent delivery system HP 1100 (Hewlett-Packard, Les Ulis, France) equipped with an autosampler, an oven and a Model 1046A fluorescence detector (Hewlett-Packard) set at excitation and emission wavelengths of 240 and 320 nm, respectively (slit width, 2×2 mm; time constant, 0.03 ms). Data acquisition and treatment was performed with an IBM computer using the ChemStation G2170 AA (Hewlett-Packard). Separation was carried out at 50°C on a C_{18} 'end-capped' Nucleosil column (250×3 mm I.D.; 5 μm particle size; 50 Å pore size, Machery-Nagel, Durën, Germany). A guard column (LiChrospher 100 RP-18, 4×4 mm I.D., 5 μm particle size; Merck, Darmstadt, Germany) was placed just before the inlet of the analytical column to reduce contamination of the analytical column. The HPLC eluent (acetonitrile–methanol–phosphate buffer; 400:43:500, v/v/v) was prepared daily and the final pH was adjusted to 5 with orthophosphoric acid. This mixture was filtered through a 0.45-μm HV filter (Millipore, Bedford, MA, USA). Prior to use, it was degassed by vacuum then with a stream of helium during use. The isocratic separation was performed at a flow-rate of 0.5 ml/min, which corresponds to a pressure of about 11.0 MPa (110 bar).

2.4. Sample processing

To a 5-ml screw-capped glass centrifuge tube, 0.5 ml of acidified plasma sample (unknown or QC samples), 100 μl of internal standard (verapamil, 0.01 mg/ml) and 1 ml of acetonitrile were added and vortex-mixed for 30 s. The tubes were then centrifuged for 15 min at 3000 *g*. The supernatant was drawn off and put into another 5-ml glass tube then acetonitrile was evaporated under a stream of nitrogen at 30°C to a final volume of 0.5 ml. A 100-μl aliquot was injected onto the HPLC column.

2.5. Instrument calibration

Specific volumes of the working solutions were used to spike acidified drug-free plasma samples prior to sample pretreatment. The concentrations

used for the calibration curves were 40, 100, 200, 500, 1000 and 2000 ng/ml plasma for atracurium and laudanosine. The volume added was always smaller than or equal to 2% of the total volume of the samples, so that the integrity of the biological samples was maintained. The assay procedure was performed as described above.

A standard curve, including a blank matrix, was generated for each analytical run and was used to determine the sample concentrations in the unknown samples. For each run, QCs (one QC for five unknown samples) were included and analyzed.

2.6. Data analysis

Calibration curves for atracurium and laudanosine were generated by unweighted least-squares linear regression of the analyte/internal standard peak-area ratio versus the theoretical concentration. The resulting slopes and intercepts were used to obtain concentration values for that day's quality control samples and unknown samples.

The linearity of the method was confirmed by comparing the slopes and the intercepts of the linear calibration curves with zero, and the correlation coefficients with one. Moreover, the Kolmogorov-Smirnov test [19] was used to compare the distribution of the residuals (difference between nominal and back-calculated concentrations) to the expected one [$N(0,1)$].

2.7. Selectivity

The selectivity of the method was verified against endogenous compounds due to the matrices. Several blank plasma from different healthy subjects were tested for the absence of interfering compounds. The retention times of endogenous compounds in the matrix were compared with those of atracurium, laudanosine and the internal standard.

2.8. Precision and accuracy

QC samples were prepared by spiking acidified drug-free plasma samples with atracurium and laudanosine to achieve three different concentrations, the first high (1500 ng/ml), the second middle (800 ng/ml) and the third low (75 ng/ml). Several aliquots of each sample were tested on the same day ($n=6$) to determine the intra-day reproducibility. Aliquots of the same sample were tested once a day on different days ($n=6$) to determine inter-day reproducibility.

The accuracy was expressed as percent error [(mean of measured−mean of added)/mean of added]×100, while the precision was given by the inter-day and intra-day coefficients of variation.

2.9. Determination of the limits of quantitation (LOQ) and detection (LOD)

The LOQ was defined as the lowest concentration that could be determined with an accuracy of between 80 and 120% and a precision ≤20% on a day-to-day basis [16–18]. To determine the analytical error in the LOQ, spiked samples were used.

The LOD was defined as the sample concentration of each analyte that provides a detector response with a signal-to-noise ratio of at least 3:1.

2.10. Recovery study

The extraction recovery was estimated using spiked plasma samples at concentrations of 75, 800 and 1500 ng/ml for atracurium and laudanosine. These samples were treated as described above, except that the internal standard was added to the supernatant before evaporation. In addition, drug-free human plasma was also treated, then the supernatant was supplemented with the two analytes along with the internal standard. The recovery was determined by comparing peak areas from plasma samples spiked with known amounts of drugs before protein precipitation versus peak areas of the same concentrations added to the supernatant after protein precipitation.

The extraction recovery was also determined for the internal standard using spiked plasma samples at a concentration of 2000 ng/ml.

2.11. Stability of the two drugs in plasma

For stability studies, QC samples in acidified plasma, representing the low (75 ng/ml), middle (800 ng/ml) and high (1500 ng/ml) concentrations were used. QC samples were analyzed immediately

after preparation (reference values) and after storage. Each determination was performed in triplicate. Concentrations of each analyte were determined from a calibration curve.

The short-term stability of atracurium and its metabolite was assessed at 1, 2, 4, 6 and 24 h after bench-top storage at ambient laboratory temperature (20°C) and after storage in the refrigerator at 4°C.

The long-term stability of the two analytes was assessed at −30°C after 15, 30 and 60 days of storage. Prior to their analyses, samples were brought to room temperature and vortex-mixed.

The freeze–thaw stability was also determined. Spiked samples were analyzed on a daily basis after repeated freeze/thaw cycles at −30°C on three consecutive days.

The stability of the two analytes in the pretreated samples on the autosampler was studied over a 24-h period at ambient temperature (20°C).

2.12. Pharmacokinetic study

This method was used for the quantification of atracurium and laudanosine in plasma during a pharmacokinetic study. The pharmacokinetic parameters were evaluated in 12 patients with acute respiratory distress syndrome without hepatic (serum bilirubin >100 μ*M*) and/or renal (serum creatinine >150 μ*M*) dysfunction. In these patients, the use of muscle relaxants facilitates mechanical ventilation. Neuromuscular blockage was assessed at the same times as those of plasma sampling. The study protocol was reviewed and approved by the institutional review board. Atracurium was administered as a bolus (1 mg/kg) given over 5 min followed by a 72-h continuous infusion (1 mg/kg/h). Special attention was paid to sample handling. Acidification of plasma samples considerably reduced the degradation of atracurium, but at a pH of about 3 or less, ester hydrolysis could take place [20]. Therefore, blood samples were drawn in EDTA-coated tubes (Venoject®, Terumo, Leuven, Belgium) before drug administration (T0), at the end of the loading dose (T5 min), during the infusion (T1, T24, T48 and T72 h), and at frequent intervals for up to 96 h after the end of infusion. Blood samples were immediately chilled in an ice water bath, then centrifuged at 4°C. A 1-ml volume of plasma was transferred to another tube containing 40 μl of 0.5 *M* sulphuric acid. Pharmacokinetic parameters were calculated using the Pk-fit software [21].

3. Results

3.1. Chromatography

Under the chromatographic conditions used, the number of theoretical plates was approximately 4500. The precolumn was exchanged every 100 sample runs and the column was replaced when the number of theoretical plates had decreased below 3000. Fig. 2 shows chromatograms obtained after pretreatment of the plasma used for the measurement of atracurium and laudanosine concentrations. There was clear resolution of the compounds of interest (separation factors: $\alpha_{1,2}=2.2$; $\alpha_{2,3}=1.9$). Retention times were 5.5 min (inter-day C.V.s=3%) for atracurium, 8.4 min (inter-day C.V.s=4%) for verapamil and 3.6 min (inter-day C.V.s=2%) for laudanosine. The retention factors (k) of the components investigated were 1.65, 3.08 and 0.75, respectively. The total HPLC run time for atracurium and laudanosine was less than 10 min.

In blank plasma samples, no interfering peak was found at the retention times of atracurium, laudanosine and verapamil. Extraction and chromatographic analysis of six separate blank plasma samples confirmed that there were no endogenous substances that interfered with the assay. A representative chromatogram obtained from drug-free plasma is presented in Fig. 2a.

3.2. Linearity

The regression analysis between peak-area ratios of atracurium and laudanosine over the internal standard and plasma concentration revealed that the method is linear. The correlation coefficients (r) for calibration curves were equal to or better than 0.996. Intra-assay reproducibility was determined for calibration curves prepared the same day in replicate ($n=5$) using the same stock solutions. Inter-assay reproducibility was determined for calibration curves prepared on different days (one calibration curve per

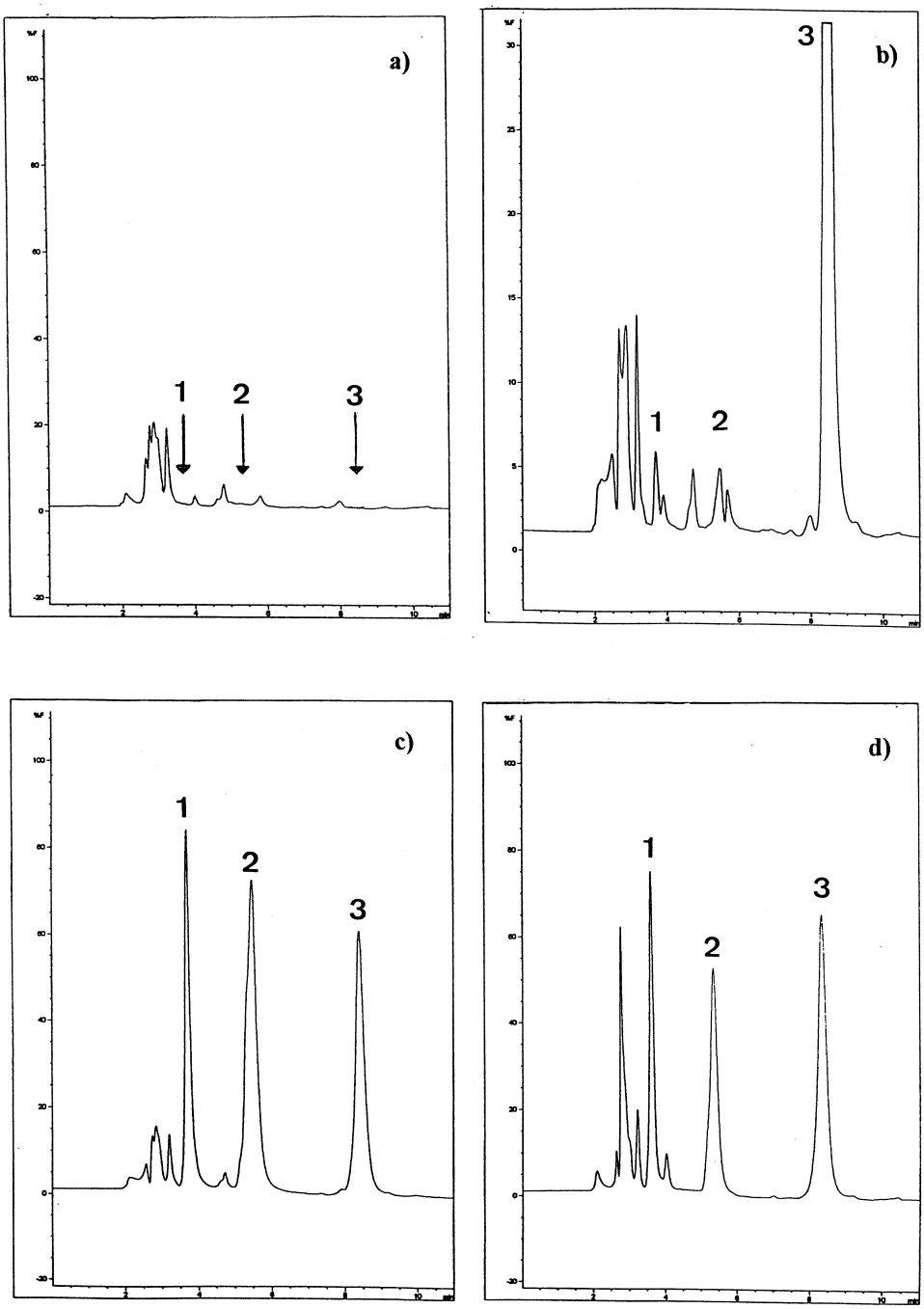


Fig. 2. Chromatograms of blank plasma (a), plasma spiked with 40 ng/ml of atracurium and laudanosine (b), plasma spiked with 2000 ng/ml of atracurium and laudanosine (c) and plasma of a patient treated with atracurium (atracurium, 1875 ng/ml; laudanosine, 1990 ng/ml) (d). Peak 1 is laudanosine, peak 2 is atracurium, peak 3 is verapamil (internal standard). For chromatographic conditions, see Section 2.3.

Table 1
Assay linearity for atracurium and laudanosine

	Atracurium			Laudanosine		
	Correlation coefficient of the linear regression analysis[a] ($r\pm$SD)	*b* Slope±SD	*a* Intercept±SD	Correlation coefficient of the linear regression analysis[a] ($r\pm$SD)	*b* Slope±SD	*a* Intercept±SD
Intra-day reproducibility ($n=5$)	$0.998\pm1.7\times10^{-3}$ C.V.=0.17%	$4\times10^{-4}\pm2.98\times10^{-5}$ C.V.=7.5%	-0.0115 ± 0.0080	$0.997\pm2.2\times10^{-3}$ C.V.=0.21%	$6.6\times10^{-4}\pm1.78\times10^{-5}$ C.V.=2.7%	0.135 ± 0.239
Inter-day reproducibility ($n=6$)	$0.999\pm8.9\times10^{-4}$ C.V.=0.09%	$4\times10^{-4}\pm7.65\times10^{-5}$ C.V.=18%	-0.0057 ± 0.0084	$0.9987\pm1.5\times10^{-3}$ C.V.=0.15%	$6.2\times10^{-4}\pm5.1\times10^{-5}$ C.V.=8.2%	0.009 ± 0.014

r=correlation coefficient.
[a] Linear unweighted regression, formula: $y=a+bx$.

day over six days). The mean regression equations are given in Table 1. For each point on the calibration curve, the concentrations were back-calculated from the equation of the linear regression curves (calculated concentrations) and the coefficients of variation (C.V.%) were computed. Table 2 reports the theoretical and calculated concentrations for each point of the calibration curve in different runs. A linear regression of the back-calculated concentrations versus the nominal ones provided a unit slope and an intercept equal to zero (Student's *t*-test). Moreover, the residuals (difference between nominal and back-calculated concentrations) were normally distributed and centered around zero (Kolmogorov-Smirnov test).

3.3. Precision and accuracy

For concentrations of calibration standards, the precision around the mean value ranged from 0.9 to 14% (Table 2).

For the QC samples, the results of the accuracy and precision (intra-day and inter-day) of the method are given in Table 2, and are ≤13%, which is an acceptable range for validated HPLC methods.

3.4. Extraction recovery, limit of quantitation and limit of detection

The extraction recoveries ($n=9$) averaged 99±11% for atracurium and 98±11% for its metabolite. The extraction efficiency was not statistically different over the range of concentrations studied.

For the internal standard, the extraction recovery was 109±6.5% ($n=4$).

The limit of quantitation was 40 ng/ml for atracurium and laudanosine. The limit of detection was 20 ng/ml for atracurium and 10 ng/ml for laudanosine.

3.5. Stability

All stock solutions were stable at +4°C for at least 15 days without measurable decomposition.

At room temperature (20°C) with light exposure and in the refrigerator (4°C), for all of the concentrations studied (75, 800 and 1500 ng/ml), laudanosine was stable over 24 h in acidified plasma; the percentage recovery was equal to or higher than 94%. At concentrations of 800 and 1500 ng/ml, atracurium was stable over 24 h at 20°C and at 4°C (percentage recoveries, 92–109%); at each time study, no statistical difference appeared by comparison with the reference values. At the lowest concentration (75 ng/ml), atracurium was stable for 6 and 24 h, at 20 and 4°C, respectively. The percentage recovery averaged 80% after 24 h of storage at 20°C.

When stored at −30°C for 60 days in acidified plasma, laudanosine, at concentration of 75, 800 and 1500 ng/ml, and atracurium, at concentrations of

Table 2
Intra- and inter-assay reproducibilities of the HPLC analysis for atracurium and laudanosine[a]

Theoretical concentration (ng/ml)	Atracurium						Laudanosine					
	Intra-assay reproducibility Calculated concentration (ng/ml) (Mean, n=5)	C.V. (%)	Mean recovery (%)	Inter-assay reproducibility Calculated concentration (ng/ml) (Mean, n=6)	C.V. (%)	Mean recovery	Intra-assay reproducibility Calculated concentration (ng/ml) (Mean, n=5)	C.V. (%)	Mean recovery (%)	Inter-assay reproducibility Calculated concentration (ng/ml) (Mean, n=6)	C.V. (%)	Mean recovery (%)
Human plasma												
40	39.9	13.8	99.7	40.6	7.8	102	41.5	12.0	104	40.7	12.0	102
75	80.8	3.5	108	79.1	6.4	105	71.8	3.3	95.7	81.6	8.9	109
100	103	7.8	103	99.7	1.2	99.7	98.3	8.4	98.3	104	9.7	104
200	198	3.2	98.9	204	4.8	102	196	7.1	98.1	208	6.3	104
500	469	6.0	93.8	479	7.1	95.8	499	3.5	99.7	499	3.7	99.8
800	761	12.0	95.1	832	4.6	104	757	0.9	94.7	842	6.3	105
1000	1014	6.1	101	1013	3.2	101	1029	5.6	103	1007	4.9	101
1500	1388	6.2	92.5	1476	7.1	98.4	1464	2.2	97.6	1609	6.4	107
2000	2001	1.4	100	1998	0.9	99.9	2002	1.9	100	1996	1.4	99.8

[a] Calibration standards: 40, 100, 200, 500, 1000 and 2000 ng/ml; quality control samples: 75, 800 and 1500 ng/ml.

800 and 1500 ng/ml, did not reveal any appreciable degradation, with all samples retaining more than 96% of their original concentration values. When frozen at −30°C at a concentration of 75 ng/ml, atracurium was stable for 30 days, with a mean percentage recovery of 95%; after 60 days of storage, relative to the nominal value, the percent recovery averaged 56±1%.

It is of interest that, after sample pretreatment, the analytes were stable at the various concentrations tested for at least 24 h.

At least three freeze–thaw cycles can be tolerated with losses no higher than 10%.

3.6. Pharmacokinetic study

Fig. 3 shows the plasma concentration versus time profile of atracurium and laudanosine in a representative patient. The elimination half-lives were 0.35 h for atracurium (C.V., 4.5%) and 1.6 h (C.V., 14%) for its metabolite. For atracurium; the volume of dis-

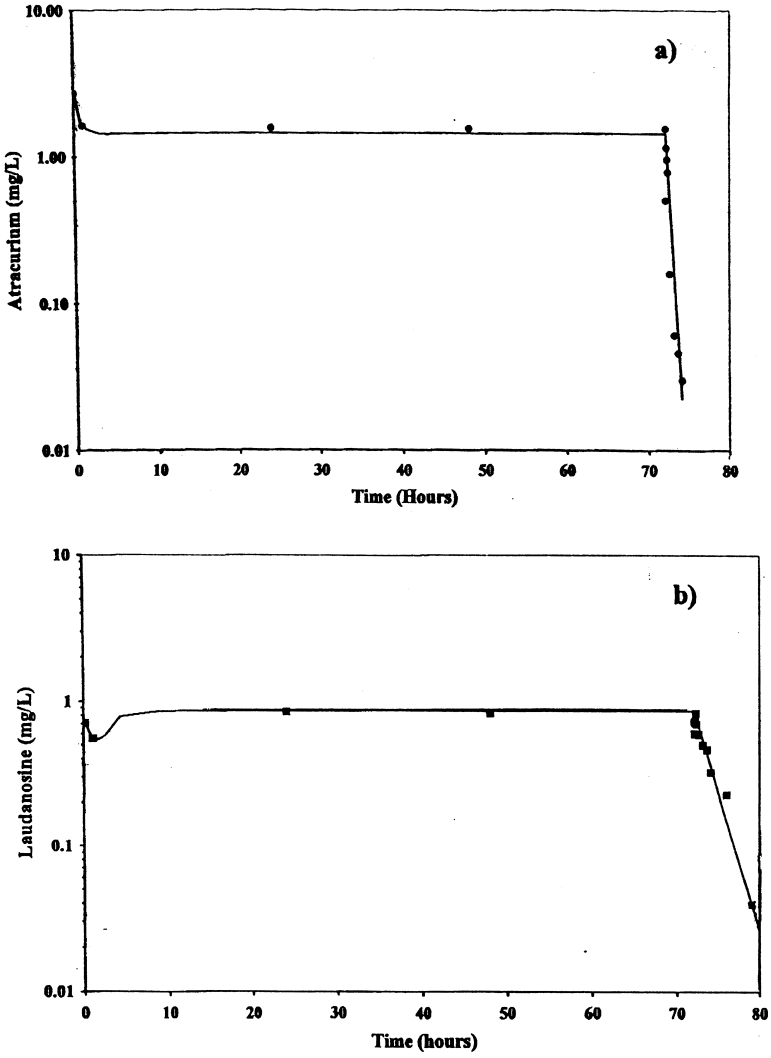


Fig. 3. Plasma concentration–time profile of atracurium (a) and laudanosine (b) after intravenous infusion of atracurium (1 mg/kg given over 5 min followed by a 72-h continuous infusion, 1 mg/kg/h) to a patient with acute respiratory distress syndrome.

tribution was 21.4 l (C.V., 5.3%) and the total clearance was 49 l/h (C.V., 5%).

4. Discussion and conclusion

The HPLC method for atracurium and laudanosine presented here requires small sample volumes (0.5 ml) and enables rapid analysis of these two drugs in plasma. The advantages of the present method are (i) that it requires a simple protein precipitation according to the method published by Varin et al. [10], (ii) its ease and speed of execution without sacrifice of selectivity and (iii) its low cost. Moreover, the chromatographic system uses isocratic elution rather than a gradient. The use of an internal standard allows the control of minor variations in the recovery during the pretreatment step. Since there is no extraction involved in our method, the loss of analytes through degradation is minimized. In contrast, methods involving solid-phase extraction for sample preparation are time-consuming, less easy to perform and are more costly. The method described in this paper may be useful in clinical laboratories.

Quaternary ammonium compounds are irreversibly bound to the free silanol groups of reversed-phase columns; thus, in this study, we used a C_{18} 'end-capped' Nucleosil column to minimize such interactions. Moreover, the use of a mobile phase with an acidic pH enhances separation, stabilizes the analytes and overcomes the retardive or ion-exchange interactions of the quaternary bases with the silanol groups on the C_{18} column. Assay performance was assessed both on the basis of the statistical characteristics of individual calibration lines and from the results of quality control samples.

The method described was found to be suitable for the analysis of all samples collected during pharmacokinetic investigations in humans.

Acknowledgements

We thank Melle B. Marion and M.G. Bougard for their excellent technical assistance.

References

[1] R.T. Owen, Drugs Today 19 (1983) 180.
[2] H.T. Hansen-Plashen, S. Brazinsky, C. Basile, P.N. Lanken, J. Am. Med. Assoc. 266 (1991) 2870.
[3] J.B. Stenlake, R.D. Waigh, J. Urwin, G.H. Dewar, G.G. Coker, Br. J. Anaesth. 55 (1983) 3.
[4] R.A. Merrett, C.W. Thompson, F.W. Webb, Br. J. Anaesth. 55 (1983) 61.
[5] B.J. Carthy, G.H. Hill, Anal. Proc. 20 (1983) 177.
[6] V. Nigrovic, J.B. Pandya, M. Auen, A. Wajskol, Anesth. Analg. 64 (1985) 1047.
[7] D.J. Chapple, J.S. Clark, Br. J. Anaesth. 55 (1983) 11.
[8] E.A.M. Neill, C.R. Jones, J. Chromatogr. 274 (1983) 409.
[9] R.L. Stiller, B.W. Brandom, D.R. Cook, Anesth. Analg. 64 (1985) 58.
[10] F. Varin, J. Ducharme, J.G. Besner, Y. Théorêt, J. Chromatogr. 529 (1990) 319.
[11] R.J. Simmonds, J. Chromatogr. 343 (1985) 431.
[12] R. Bjorksten, G.H. Beemer, D.P. Crankshaw, J. Chromatogr. 533 (1990) 241.
[13] C. Schopfer, A. Benakis, J. Chromatogr. 526 (1990) 223.
[14] G. Carlucci, G. Maurizi, P. Mazzeo, Il Farmaco-Ed. Pr. 43 (1988) 297.
[15] U. Nehmer, J. Chromatogr. 435 (1988) 425.
[16] United States Pharmacopoeia XXXIII, The United States Pharmacopeial Convention, Rockville, MD, 1994.
[17] 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, K.A. Pittman, S. Spector, J. Pharm. Sci. 81 (1992) 309.
[18] F. Bressolle, M. Bromet-Petit, M. Audran, J. Chromatogr. B 686 (1996) 3.
[19] R.R. Sokal, J.R.N. Rohlf, Biometry, W.H. Freeman, San Francisco, CA, 1969.
[20] R.A. Merrett, C.W. Thompson, F.W. Webb, Br. J. Anaesth. 55 (1983) 61.
[21] Pk-fit computer program, version 1.1, RDPP, Montpellier, 1997