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High-performance liquid chromatographic assays with fluorometric detection for mivacurium isomers and their metabolites in human plasma

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Abstract

Two high-performance liquid chromatographic assays coupled with fluorometric detection have been developed for the determination of mivacurium isomers (*trans–trans*, *cis–trans* and *cis–cis*) and their monoester and alcohol metabolites in human plasma. A novel solid-phase extraction procedure allowed good recovery of the mivacurium isomers (mean 98%) and their monoester metabolites (mean 83%), whereas the alcohol metabolites were analyzed after direct precipitation of plasma proteins. For all analytes, these assays proved to be sensitive (LOQ 3.9–15.6 ng/ml), reproducible (C.V. < 15%) and accurate (>94%) over the therapeutic range of concentrations of mivacurium and its metabolites. These two methods were applied successfully to a pharmacokinetic study of mivacurium after a bolus dose of 0.15 mg/kg in anesthetized patients.

1. Introduction

Mivacurium {(*E*)-(1*R*,1′*R*)-2,2′-[4-octene-dioylbis (oxytrimethylene)] bis [1,2,3,4-tetra-hydro-6,7-dimethoxy-2-methyl-1-(3,4,5-trimeth-oxybenzyl)-isoquinolium]-dichloride} is a new short-acting nondepolarizing neuromuscular blocking agent, member of the benzylisoquinolinium family (Fig. 1A). Mivacurium consists of a mixture of 3 stereoisomers: the two most active and equipotent are the *trans–trans* (*tt*) (1*R*1′*R*2*S*2′*S*) and *cis–trans* (*ct*) (1*R*1′*R*2*R*2′*S*) isomers (57 and 37% w/w, respectively) whereas the *cis–cis* (*cc*) (1*R*1′*R*2*R*2′*R*) isomer (6% w/w) has only one tenth the activity of the others in cats and monkeys [1,2]. Rapid hydrolysis of the two most active isomers by plasma cholinesterases explains the short clinical duration of action of mivacurium [3,4]. Renal excretion is a minor elimination pathway; approximately 7% of an administered dose of mivacurium is recovered unchanged in human urine [4]. Hydrolysis of mivacurium by plasma cholinesterases produces two metabolites, namely the alcohols [(*R*,*R*)- and (*R*,*S*)-1,2,3,4-tetrahydro-2-(3-hydroxypropyl)-6,7-dimethoxy-2-methyl-1-(3,4,5-trimethoxy-benzyl) isoquinolinium chloride] and the monoesters [(E)-(1*R*,2*RS*)-2-[3-[(7-carboxy-4-hepten-oyl)oxy]-propyl-1,2,3,4-tetrahydro-6,7-dimeth-oxy-2-methyl-1-(3,4,5-trimethoxybenzyl) iso-

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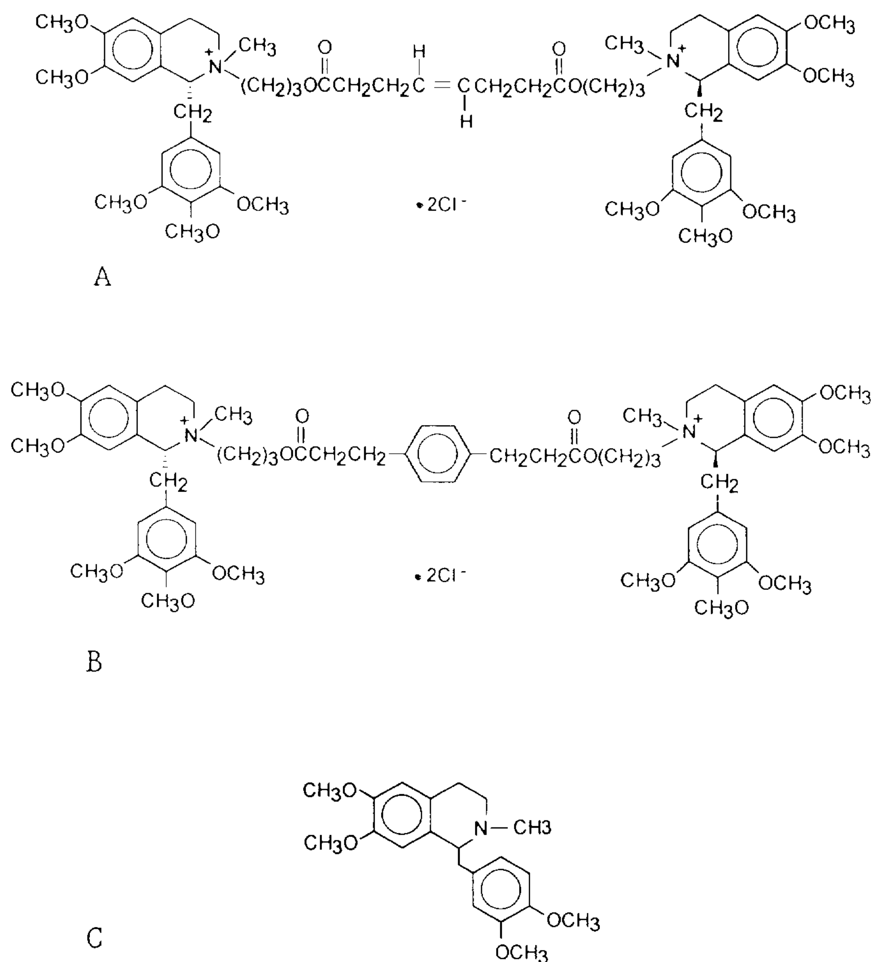

Fig. 1. Chemical structures of (A) mivacurium chloride, (B) BW785U77 (I.S.) and (C) laudanosine (I.S.).

quinolinium chloride, which account respectively for 44 and 46% of the dose in urine [4]. These metabolites are unlikely to be active since after a 5.5-h infusion the recovery times from the neuromuscular block were unchanged [5].

A stereoselective high-performance liquid chromatographic assay with fluorometric detection for mivacurium isomers in human plasma has been recently reported [6]. However this method requires a two-step extraction procedure and has low extraction efficiencies (55, 55 and 54%) for the *tt*, *ct* and *cc* isomers respectively. To our knowledge, separation and quantitation of the *cis* and *trans* isomers of the monoester and alcohol metabolites have not been reported. To determine the plasma concentration–time profile of each individual mivacurium isomer and to follow the formation and elimination of their metabolites in humans, two stereoselective assays were developed which are described in the present report.

2. Experimental

2.1. Chemicals

The three isomers of mivacurium chloride, the monoester and quaternary alcohol metabolites as well as an internal standard (I.S.) {BW785U77; bis-3-[*trans*-1,2,3,4-tetrahydro-6,7-dimethoxy-N-methyl-1-(3,4,5-trimethoxybenzyl) isoquinolin-

ium] propyl-1,3-phenylenedipropionate dichloride)} (Fig. 1B), which was further purified by repeated solid-phase extractions on PrepSep C_{18} cartridges (Fisher Scientific, Fair Lawn, NJ, USA) were kindly supplied by Burroughs Wellcome (Research Triangle Park, NC, USA). All analytes were supplied individually with the exception of the monoester metabolites standard which was supplied as a mixture of the *cis* and *trans* isomers. The relative proportion of the *cis* and *trans* monoester isomers in the mixture was reported to be 26.3 and 66.3%, respectively, by the supplier. All organic solvents were HPLC grade (Anachemia, Montréal, Canada) while environmental grade hydrochloric acid was used (Anachemia). PIC B-8 Reagent (low UV), which consists of a mixture of octanesulfonic acid in water, methyl alcohol and calcium acetate, was purchased from Waters (Milford, MA, USA). Echothiophate iodide, a plasma cholinesterase inhibitor, was kindly supplied by Ayerst Laboratories (Montréal, Canada). Deionized water was purified by a Milli-Q Plus system (Millipore, Bedford, MA, USA) and was filtered through a 0.2-μm membrane (Type HVLP, Millipore) immediately before use.

2.2. Standard solutions

Stock solutions (1 mg/ml equivalent base) of each individual isomer, each alcohol metabolite and the monoester metabolites mixture (corresponding to 0.663 mg/ml and 0.263 mg/ml of the *trans* and *cis* monoesters, respectively), were prepared in a 80:20 mixture of physiological saline (pH 3, adjusted with 0.1 *M* HCl) and methanol, and stored at 4°C. Working solutions of 10 μg/ml containing all three isomers in the same medium were also prepared. For the determination of mivacurium isomers and their monoester metabolites, a stock solution of the internal standard (BW785U77) was prepared similarly but further diluted to give a working solution of 4 μg/ml. Stock and working solutions of laudanosine (0.4 μg/ml) (Sigma, St. Louis, MO, USA) (Fig. 1C) were prepared in 0.005 *M* sulfuric acid and used as the internal standard for the determination of the alcohol metabolites. All solutions were tested weekly for drug degradation.

2.3. Sample preparation

To isolate the three isomers and the *cis* and *trans* monoester metabolites from plasma samples, PrepSep C_{18} solid-phase extraction cartridges containing 300 mg of sorbent were used and conditioned with methanol (3 ml) and distilled water (3 ml). Plasma standard or sample (1 ml), internal standard working solution (100 μl) and water (1 ml) were combined in the reservoir and then aspirated through the sorbent. A vacuum of 50–80 kPa was applied to the manifold of the Vac-Elut chamber (Analytichem International) throughout the extraction procedure. The cartridges were sequentially washed with water (3 ml), acetonitrile (3 ml) and water (3 ml) under vacuum. Analytes were eluted into glass tubes with 2×750 μl of 0.05 *M* ammonium diphosphate (pH 3) in methanol (20:80, v/v), under a small vacuum (<20 kPa). The eluents were then evaporated to dryness using a Speed-Vac concentrator (Model SC210A, Savant Instruments, Farmingdale, NY, USA). The dry residues were dissolved in 300 μl of mobile phase and a volume of 150 μl was injected onto the analytical column. Smaller volumes were used when higher concentrations of mivacurium isomers and their monoester metabolites were injected in order to maintain proper linear detection and integration of the peaks.

For the isolation of the *cis* and *trans* alcohol metabolites from plasma, 250 μl of plasma (sample or standard) and 100 μl of internal standard (40 ng of laudanosine) were added and mixed. Acetonitrile (650 μl) was added, the sample was vortex-mixed for 1 min and centrifuged for 10 min at 2000 *g*. The supernatant was then transferred to a glass tube and evaporated to dryness in the Speed-Vac concentrator. The dry residues were solubilized in 300 μl of mobile phase and 150 μl was injected onto the column.

2.4. Chromatographic apparatus and conditions

The chromatographic system for both assays

consisted of a Constametric 4100 pump (LDC Analytical, Riviera Beach, FL, USA). Samples were injected with a Shimadzu SIL-9A auto-injector (Kyoto, Japan), the peaks were detected with a Kratos fluorescence detector (Ramsey, NJ, USA) at excitation and emission wavelengths of 202 nm and 290 nm, respectively, and integrated with a Shimadzu C-R3A integrator (Kyoto, Japan). Separation of mivacurium isomers and their monoester metabolites was performed on a 5-μm LiChrosphere 60 RP Select B column (125 × 4.6 mm I.D.) combined with a precolumn cartridge (4 mm) (EM Science, Gibbstown, NJ, USA). The mobile phase was acetonitrile–water (37.5:62.5, v/v) containing a final concentration of 0.0052 *M* octanesulfonic acid (PIC B-8 Reagent). The flow-rate was 2.0 ml/min and the mobile phase was allowed to recirculate. For the separation of the alcohol metabolites, a 5-μm Spherisorb C_1 column (125 × 4.6 mm I.D.) (Hichrom, Reading, UK) was used. The mobile phase was acetonitrile–water (12.5:87.5, v/v) containing a final concentration of 0.007 *M* octanesulfonic acid (PIC B-8 Reagent), at a flow-rate of 2.0 ml/min without recirculation. In both assays, the columns were maintained at 35°C with an Eppendorf CH-30 column heater (Madison, WI, USA).

2.5. *Linearity and limit of quantitation*

Cholinesterases in plasma used for the preparation of standard and spiked samples were inhibited by adding 0.001 *M* echothiophate iodide prior to the addition of the analytes. A pool of plasma containing 4000 ng/ml of the three isomers was serially diluted 1:1 with drug-free plasma to give standard plasma concentrations ranging from 3.9 to 2000 ng/ml. This procedure was repeated for the alcohol metabolites (15.6–2000 ng/ml). A pool of plasma consisting of 4000 ng/ml of the monoesters mixture (corresponding to 1052 ng/ml and 2652 ng/ml of the *cis* and *trans* monoesters, respectively) was similarly diluted to give plasma concentrations ranging from 4.1 to 526 ng/ml and 10.4 to 1326 ng/ml for the *cis* and *trans* monoesters, respectively. All biological standards were stored at −20°C. Linearity was assessed by weighted least square regression ($1/x^2$) of the analyte/I.S. peak-height ratio against the corresponding concentration of the analyte in plasma. To be included in the calibration curve, the lowest concentration of standard had to yield a regression-estimated value within 20% of the nominal concentration. This lowest concentration was considered as the lowest limit of quantitation (LOQ).

2.6. *Recovery*

Recovery of mivacurium isomers and their monoester metabolites from human plasma was determined in quadruplicate at three different drug concentrations (31.25, 125, 1000 ng/ml; 41.44, 165.75, 663 ng/ml and 16.44, 65.75, 263 ng/ml for the isomers, the *trans* monoester and the *cis* monoester, respectively). Blank plasma spiked with known amounts of analytes and 100 μl of I.S. were extracted and compared with blank plasma and I.S. extracts subsequently spiked with the same amounts of analytes. The two sets of extracts were then evaporated to dryness using the Speed-Vac system and reconstituted in mobile phase. The recovery was assessed by comparing the peak-height ratios of analyte/I.S. for the two sets of extracts.

2.7. *Precision and accuracy*

The intra-assay precision of the analytical method for the determination of the isomers and their monoester metabolites was determined by using replicate measurements ($n = 4$) of three different concentrations; namely the same samples used for the recovery. For the alcohol metabolites, the intra-assay precision of the method was determined by replicate measurements ($n = 6$) of three different concentrations (31.25, 125, 500 ng/ml). The inter-assay precision for both methods was assessed by using plasma samples from the calibration curve that were independently analyzed on four different days.

To evaluate accuracy, drug-free plasma was spiked with the three mivacurium isomers to give

10 different concentrations ranging from 3.9 to 2000 ng/ml. The samples were assayed blindly and concentrations derived from the calibration curves. Accuracy was evaluated by comparing the estimated concentration with the known concentration of the isomers. This procedure was repeated independently for both the monoester ($n = 10$) and the alcohol ($n = 8$) metabolites respectively.

2.8. Clinical study

These two analytical methods were used to determine the plasma concentrations of mivacurium isomers and their metabolites in patients undergoing elective surgery. An i.v. bolus dose of 0.15 mg/kg mivacurium chloride was administered and arterial blood samples were collected at frequent intervals for up to 4 h. Blood samples were collected into tubes containing echothiophate iodide, centrifuged for 5 min and the plasma was frozen at −20°C until HPLC analysis. The plasma concentration–time profiles of each analyte for one patient are presented.

3. Results and discussion

We have developed two sensitive and selective assays for the quantitation of mivacurium isomers (*tt*, *ct* and *cc*) as well as their monoester and alcohol metabolites. In the first assay, mivacurium isomers and their monoester metabolites are recovered after a single-step solid-phase extraction procedure and separated on a RP-Select B C_8 column, whereas in the second assay, direct precipitation of plasma proteins is carried out before separation of the alcohol metabolites on a Spherisorb C_1 column. An analytical method for dosing mivacurium isomers has been recently reported [6]. However it requires a two-step solid-phase extraction procedure, involving extraction on reversed-phase C_{18} followed by anion-exchange cartridges, in order to achieve adequate purification and could not be applied to the quantitation of mivacurium metabolites since they were eluted from the HPLC column in the solvent front.

By performing a one-step solid-phase extraction on Prep-Sep C_{18} cartridges, which have been found to be reliable for the extraction of other quaternary ammonium products such as doxacurium [7], we were able to efficiently recover mivacurium isomers as well as their monoester and alcohol metabolites. Washing with methanol during the extraction procedure caused the elution of approximately 25% of the

Table 1
Recovery of mivacurium isomers and the monoester metabolites from human plasma, and intra-assay variability

Concentration added (ng/ml)	Recovery (mean ± S.D., $n = 4$) (%)	Coefficient of variation (%)
Mivacurium trans–trans		
31.25	112 ± 2	1.5
125	99 ± 3	2.6
1000	91 ± 1	1.4
Mivacurium cis–trans		
31.25	110 ± 3	2.9
125	96 ± 2	1.6
1000	90 ± 1	1.1
Mivacurium cis–cis		
31.25	107 ± 4	4.0
125	94 ± 2	2.3
1000	88 ± 1	1.3
Monoester trans		
41.44	83 ± 5	6.4
165.75	78 ± 7	8.7
663	85 ± 3	3.9
Monoester cis		
16.44	87 ± 5	5.9
65.75	81 ± 7	9.1
263	86 ± 3	3.4
Alcohol trans ($n = 6$)		
31.25	–	3.8
125	–	6.9
500	–	4.5
Alcohol cis ($n = 6$)		
31.25	–	5.1
125	–	6.6
500	–	4.6

Table 2
Inter-assay variability for mivacurium isomers

Concentration (ng/ml)	Peak-height ratio (mean ± S.D., $n = 4$)					
	Trans–trans	C.V. (%)	*Cis–trans*	C.V. (%)	*Cis–cis*	C.V. (%)
3.9	0.03 ± 0.00	7.2	0.03 ± 0.00	7.9	0.02 ± 0.00	9.9
7.8	0.05 ± 0.00	7.6	0.05 ± 0.00	7.7	0.04 ± 0.00	9.8
15.6	0.10 ± 0.01	7.6	0.09 ± 0.00	2.7	0.07 ± 0.01	9.9
31.25	0.21 ± 0.00	1.6	0.20 ± 0.01	3.8	0.14 ± 0.01	5.2
62.5	0.43 ± 0.01	1.6	0.39 ± 0.01	2.0	0.29 ± 0.01	3.3
125	0.84 ± 0.07	8.7	0.76 ± 0.05	6.3	0.59 ± 0.03	5.8
250	1.81 ± 0.03	1.7	1.58 ± 0.09	5.5	1.20 ± 0.03	2.5
500	3.78 ± 0.11	2.8	3.29 ± 0.16	4.8	2.48 ± 0.12	5.1
1000	8.12 ± 0.81	9.9	6.58 ± 0.28	4.3	4.94 ± 0.25	5.0
2000	15.60 ± 0.31	2.0	13.52 ± 0.45	3.3	9.93 ± 0.24	2.5

Table 3
Inter-assay variability for the monoester metabolites

Concentration (ng/ml)	Peak-height ratio (mean ± S.D., $n = 4$)		Concentration (ng/ml)	Peak-height ratio (mean ± S.D., $n = 4$)	
	Trans	C.V. (%)		*Cis*	C.V. (%)
10.36	0.10 ± 0.00	1.0	4.11	0.04 ± 0.00	3.6
20.72	0.22 ± 0.03	13.0	8.22	0.06 ± 0.00	3.4
41.44	0.41 ± 0.04	8.6	16.44	0.12 ± 0.01	12.0
82.88	0.83 ± 0.03	3.8	32.88	0.21 ± 0.01	5.3
165.75	1.90 ± 0.18	9.5	65.75	0.42 ± 0.04	8.4
331.5	4.02 ± 0.32	7.9	131.5	0.87 ± 0.07	8.4
663	9.34 ± 0.55	5.9	263	2.05 ± 0.17	8.4
1326	18.34 ± 0.45	2.4	526	4.03 ± 0.22	5.5

Table 4
Inter-assay variability for the alcohol metabolites

Concentration (ng/ml)	Peak-height ratio (mean ± S.D., $n = 4$)			
	Trans	C.V. (%)	*Cis*	C.V. (%)
15.6	0.04 ± 0.00	3.1	0.03 ± 0.00	14.7
31.25	0.08 ± 0.00	1.6	0.07 ± 0.00	5.3
62.5	0.18 ± 0.00	1.4	0.12 ± 0.00	3.6
125	0.35 ± 0.00	1.0	0.26 ± 0.01	2.7
250	0.70 ± 0.01	1.4	0.52 ± 0.02	3.6
500	1.37 ± 0.04	2.8	1.02 ± 0.04	3.9
1000	2.52 ± 0.16	6.4	1.70 ± 0.12	7.2
2000	5.13 ± 0.15	2.9	4.11 ± 0.33	8.1

metabolites. A similar observation was reported by Brown et al. [6] for mivacurium isomers in their first extraction step. Thus the methanol wash in our extraction procedure was replaced by a final wash with water. This final wash was deemed necessary to maintain a clean chromatographic separation and to avoid precipitation of the elution phase (ammonium diphosphate 20% in methanol 80%) with the acetonitrile used in the second wash. The mean extraction efficiencies were 101, 99 and 96% for the *tt*, *ct* and *cc* isomers respectively (Table 1) while for the *trans* and *cis* monoesters, the mean extraction efficiencies were 82 and 85%, respectively (Table 1). The lower limits of quantitation in plasma (LOQ) were 3.9, 4.1 and 10.4 ng/ml for mivacurium isomers, the *cis* monoester and the *trans* monoester respectively.

Extraction of a blank plasma sample from an anesthetized patient proved to be free of any interference from endogeneous compounds or perioperative drugs (Fig. 2A). The analysis of a plasma sample taken shortly after the administration of mivacurium chloride to the patient shows good resolution of the isomers and their monoester metabolites (Fig. 2B), corresponding to 381, 116 and 75 ng/ml of the *tt*, *ct* and *cc* isomers, and 478 and 440 ng/ml of the *trans* and *cis* monoesters, respectively. Chromatograms of extracts of drug-free plasma spiked with mivacurium isomers and their monoester metabolites are shown in Fig. 2C, D. The retention times of the *tt*, *ct* and *cc* isomers were 7.1, 7.9 and 8.9 min, respectively, whereas for the *trans* and *cis* monoester metabolites, the retention times were 2.4 and 2.7 min. The total time for

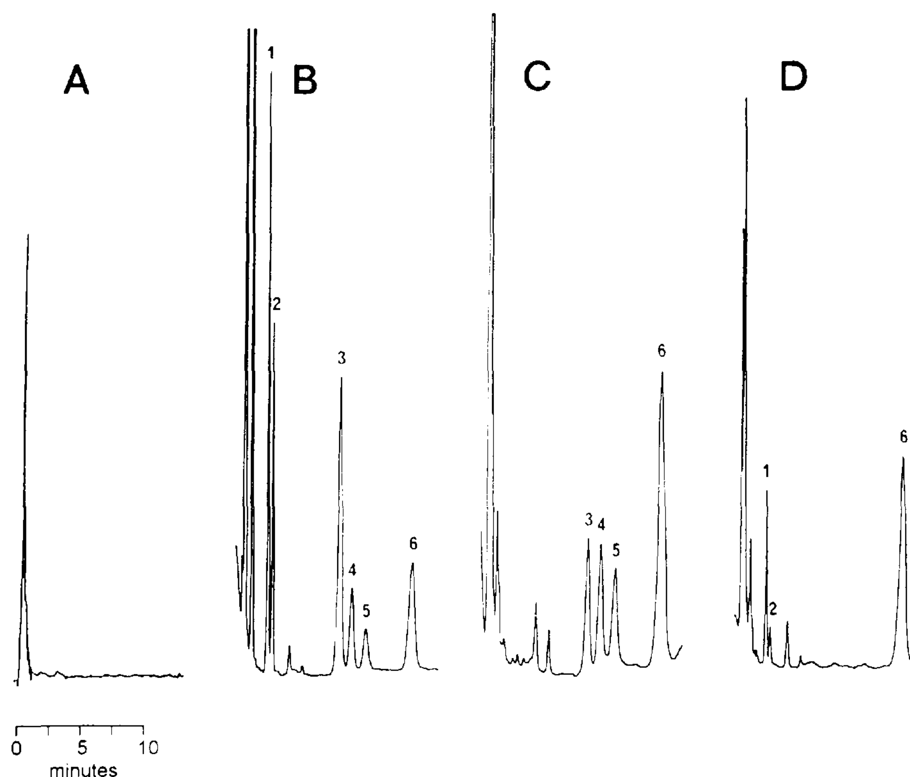

Fig. 2. HPLC chromatograms for: (A) plasma sample collected from a patient before the injection of mivacurium and (B) shortly after an i.v. bolus injection of 0.15 mg/kg mivacurium chloride (50 μl); (C) drug-free plasma spiked with 62.5 ng/ml of each isomers (150 μl); and (D) drug-free plasma spiked with 82.88 and 32.88 ng/ml of the *trans* and *cis* monoester metabolites, respectively (100 μl). Peaks: 1 = monoester *trans*; 2 = monoester *cis*; 3 = mivacurium *trans–trans*; 4 = mivacurium *cis–trans*; 5 = mivacurium *cis–cis*; 6 = internal standard (I.S.).

the chromatographic run was less than 15 min with a retention time of 12 min for the I.S. It was however noted that, although resolution of the peaks was maintained, slight alterations in the percentage of acetonitrile in the mobile phase resulted in an important shift in the retention times.

Attempts were made, for economical reasons, to substitute the PIC B-8 reagent of the mobile phase with octanesulfonic acid at the same concentration (Fisher Scientific, Montreal, Canada). Although resolution of mivacurium isomers was good initially, it deteriorated with time. This leads us to belive that the calcium ions present in the PIC B-8 reagent are necessary and have an important counter-ion effect, critical for proper separation of the peaks.

For the alcohol metabolites, although adequate recoveries were achieved following solid-phase extraction on PrepSep C_{18} cartridges, the *trans* and *cis* alcohols could not be resolved on the RP Select B C_8 column used for the separation of the isomers and the monoesters. However, adequate resolution of the alcohol metabolites was achieved on a more polar column (Spherisorb C_1). Limited equipment facilities and human resources did not allow us to run two HPLC setups (C_8 and C_1 columns) at the same time. Thus for practical and economical reasons, plasma protein precipitation was considered. This method, which has been successfully used for atracurium in similar clinical conditions and concentration ranges [8], was found to be equally acceptable for the alcohol metabolites. Despite the 1:3 dilution factor, the method allowed good sensitivity with a lower limit of quantitation of 15.6 ng/ml.

Blank plasma obtained from one anesthetized patient before the administration of mivacurium showed no interfering peaks from endogeneous compounds or other drugs administered during anesthesia (Fig. 3A). The analysis of a plasma sample taken after the administration of mivacurium chloride to the patient shows good resolution of the *cis* and *trans* alcohol metabolites (Fig. 3B), corresponding to 167 ng/ml and 1122 ng/ml of the *cis* and *trans* alcohols, respectively. A chromatogram of drug-free plasma spiked with the alcohol metabolites is shown in Fig. 3C. The retention times of the *cis* and *trans* alcohol metabolites on the C_1 column, were 5.4 and 6.4 min, respectively, while the I.S. (laudanosine) had a retention time of 4.3 min.

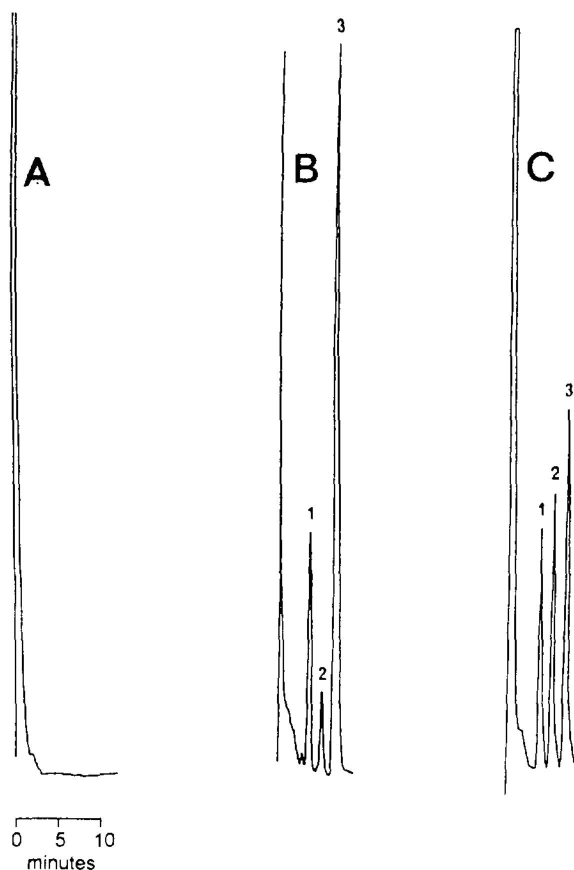

Fig. 3. HPLC chromatograms for: (A) plasma sample collected from a patient before the injection of mivacurium and (B) shortly after an i.v. bolus injection of 0.15 mg/kg mivacurium chloride; and (C) drug-free plasma spiked with 500 ng/ml of alcohol metabolites. Peaks: 1 = laudanosine (I.S.); 2 = alcohol *cis*; 3 = alcohol *trans*.

All calibration curves for each analyte were linear over a wide concentration range. The regression equations for the *tt*, *ct* and *cc* isomers (3.9–2000 ng/ml) were $y = 0.00721x + 0.00197$ ($r^2 = 0.999$); $y = 0.00647x - 0.00096$ ($r^2 = 0.997$); and $y = 0.00500x + 0.00033$ ($r^2 = 0.998$), respectively. For the *trans* and *cis* alcohol metabolites (15.6–2000 ng/ml), the equations were

Table 5
Accuracy of the assay for mivacurium isomers

Spiked concentration (ng/ml)	Estimated concentration of mivacurium (ng/ml)		
	Trans–trans	*Cis–trans*	*Cis–cis*
0 (blank)	0.0	0.0	0.0
10	8.2	9.6	9.4
17.5	19.6	18.7	18.6
37.5	34.7	36.2	36.4
190	178.9	182.0	185.8
250	230.8	237.0	247.8
440	400.5	410.2	420.9
1200	1115.3	1149.2	1158.3
1250	1178.6	1228.2	1250.8
1800	1752.4	1806.8	1815.9
Mean accuracy (%)	92.7	95.7	97.9
S.D. (%)	7.8	4.0	3.6

Table 7
Accuracy of the assay for the alcohol metabolites

Spiked concentration (ng/ml)	Estimated concentration of the alcohols (ng/ml)	
	Trans	*Cis*
0 (blank)	0.0	0.0
40	41.4	37.1
45	43.5	41.3
180	181.8	183.5
240	201.1	206.3
450	391.9	398.7
1250	1070.1	1111.0
1800	1499.0	1565.8
Mean accuracy (%)	93.3	95.5
S.D. (%)	8.6	5.4

$y = 0.00278x - 0.00041$ ($r^2 = 0.998$) and $y = 0.00207x - 0.00043$ ($r^2 = 0.994$), respectively. For the *trans* monoester (10.4–1326 ng/ml), the regression equation was $y = 0.0119x - 0.0305$ ($r^2 = 0.998$), whereas for the *cis* monoester (4.1–526 ng/ml) the regression equation was $y = 0.00741x + 0.00449$ ($r^2 = 0.996$).

Both analytical methods showed good precision with intra-assay variability less than 9% (Table 1) and inter-assay variability less than 15% for all analytes (Tables 2–4). In addition, blindly assayed spiked samples of mivacurium isomers at concentrations covering the standard curves showed a mean accuracy of 93, 96 and 98% for the *tt*, *ct* and *cc* isomers, respectively (Table 5). The mean accuracies for the *trans* and *cis* monoester metabolites were 102 and 109%, respectively (Table 6), while for the *trans* and *cis* alcohol metabolites they were 93 and 96%, respectively (Table 7).

Both analytical methods were applied to de-

Table 6
Accuracy of the assay for the monoester metabolites

Concentration spiked (ng/ml)	Estimated concentration *trans* (ng/ml)	Concentration spiked (ng/ml)	Estimated concentration *cis* (ng/ml)
0 (blank)	0.0	0	0.0
14.6	16.0	5.8	7.0
29.2	27.5	11.6	10.4
56.4	53.4	22.4	21.9
285.1	267.6	113.1	107.2
580.1	641.5	230.1	278.7
613.3	682.8	243.3	295.8
961.4	1022.0	381.4	449.0
1094.0	1124.9	434.0	513.0
1259.7	1240.1	499.7	554.4
Mean accuracy (%)	102.1		108.9
S.D. (%)	7.5		12.6

termine the concentration–time profiles of mivacurium isomers and their metabolites in one patient after the administration of an i.v. bolus of 0.15 mg/kg mivacurium chloride. Arterial blood samples were collected at frequent intervals during 4 h. Perioperative medications given to this patient included thiopental, fentanyl and one dose of cefazoline. The plasma concentration–time profile of the three isomers for this patient is shown in Fig. 4A. Rapid and almost parallel decrease in the concentration of the equipotent *tt* and *ct* isomers is the result of extensive metabolism by plasma cholinesterases, and explains the short clinical duration of action of this muscle relaxant. Plasma concentrations of the *tt* isomer declined rapidly from approximately 1000 ng/ml (C_{max} at 1 min) to 5 ng/ml at 12 min after the bolus injection of the drug, while the concentration for the *ct* isomer declined from 400 ng/ml to 6 ng/ml at 7 min post-injection. Plasma levels of the *cc* isomer (C_{max} = 200 ng/ml), which appears to be metabolised more slowly by plasma cholinesterases, could still be detected 90 min (5 ng/ml) following injection of the drug. Rapid hydrolysis of the *tt* and *ct* isomers gave rise to the formation of monoester and alcohol metabolites which reached peak concentrations within 1 min following the injection of mivacurium chloride and were subsequently slowly eliminated (Fig. 4B, C); one exception being the *cis* alcohol which was only negligibly and transiently present. All plasma concentrations for each analyte were within the limit of quantitation of our two assays.

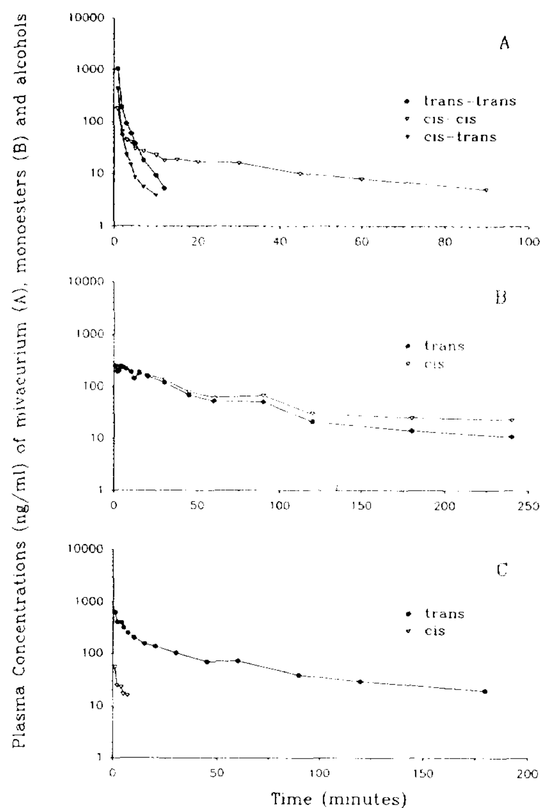

Fig. 4. Concentration–time curves for (A) mivacurium isomers; (B) monoester metabolites; and (C) alcohol metabolites in human plasma following the intravenous injection of 0.15 mg/kg mivacurium chloride in one anesthetized patient.

4. Conclusions

Two stereoselective HPLC assays with fluorescence detection have been developed for the determination of mivacurium isomers and their metabolites. Our one-step extraction procedure offers an improvement which translates into higher recovery efficiencies of the isomers and simultaneous extraction of the metabolites. In addition, our second method for the determination of the alcohol metabolites is simple and rapid, with no extraction procedure required. Both methods proved to be precise, specific and sensitive for the isomers as well as the metabolites of mivacurium and are readily applicable to pharmacokinetic studies in humans.

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