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Review

Rapid method for the quantitation of mivacurium isomers in human and dog plasma by using liquid chromatography with fluorescence detection: Application to pharmacokinetic studies

Sonia I. Lugo[a], Natalie D. Eddington[b,*]

[a]*Department of Pharmaceutical Sciences, School of Pharmacy, University of Puerto Rico, San Juan 00936, Puerto Rico*
[b]*Pharmacokinetics–Biopharmaceutics Laboratory, Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, 100 Penn Street, AHB 540C, Baltimore, MD 21201, USA*

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Abstract

A simple, selective and specific liquid chromatography method was used to simultaneously determine the cis–cis, cis–trans and trans–trans isomers of mivacurium in human and dog plasma. Solid phase extraction was used to separate interfering endogenous products from the compounds of interest. Fluorometric detection was evaluated at excitation and emission maxima of 280 and 325 nm. The calibration curves were found to be linear in the range 50–500 ng ml^{-1}. The method was applied to plasma samples collected from a human and dog study and was found to be satisfactory. Excellent recovery, linearity, accuracy and precision were achieved by the assay for each isomer.

Keywords: Mivacurium; Neuromuscular blockade; Pharmacokinetics

1. Introduction

Mivacurium chloride is a short-acting, non-depolarizing neuromuscular blocking agent [1–3]. It is a bisbenzylisoquinolinium diester, structurally similar to the non-depolarizing neuromuscular blocking agents doxacurium and atracurium. Mivacurium is useful as an adjunct to anesthesia in inducing neuromuscular blockade to permit endotracheal intubation [4]. Mivacurium chloride is a mixture of three stereoisomers, the trans–trans diester, the cis–trans diester and the cis–cis diester.

Pharmacokinetic and pharmacodynamic studies with neuromuscular blocking agents have been hampered by the lack of suitable analytical methods to determine the low drug concentrations encountered in clinical practice and animal pharmacokinetic studies. A number of HPLC methods have been developed to quantitate neuromuscular blocking agents in plasma or serum. The most promising method for the determination of muscle relaxants in biological matrices is HPLC which allows for the simultaneous determination of the

* Corresponding author.

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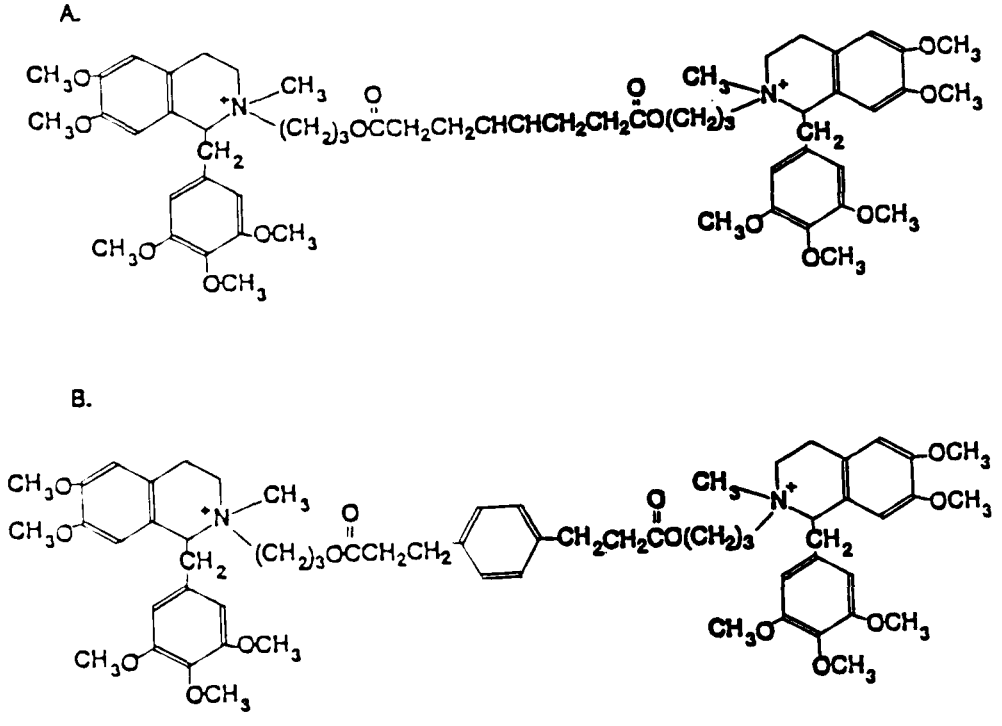

Fig. 1. Chemical structures of mivacurium (A) and the internal standard (B).

intact compound and its metabolites. Fluorometric detection has been used to quantitate vecuronium and related compounds using post-column ion-pair extraction [5]. This method has been used to quantitate other neuromuscular blocking agents such as pancuronium and pipercuronium [6–11]. Fluorometric detection along with solid phase extraction has been used to quantitate atracurium [12].

Assay methods to quantitate mivacurium are scarce; however, Brown et al. [13] developed a fluorometric method for the quantitation of mivacurium and isomers in human plasma. The isomers were extracted from plasma using C_{18} and anion-exchange cartridges. The use of the anion-exchange cartridges added additional steps in the extraction procedure, making it labor-intensive, time-consuming and expensive. The objective of this study was to develop a simple, rugged, sensitive stereospecific analytical method applicable for the analysis of mivacurium in human and dog pharmacokinetic studies.

2. Experimental

2.1. Materials and reagents

Mivacurium, (*E*)-(1*R*,1′*R*)-2,2′-[4-octenedioylbis(oxytrimethylene)]bis[1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-(3,4,5-trimethoxybenzyl)-isoquinolium]dichloride, its three isomers, and the internal standard, bis{3-[trans-1,2,3,4-tetrahydro-6,7-dimethoxy-*N*-methyl-1-(3,4,4-trimethoxybenzyl)isoquinolium]propyl}-1,3-phenylenedipropionate dichloride (BW 785U77), were donated by Burroughs Wellcome Co. (Research Triangle Park, NC). The structures of mivacurium chloride and the internal standard are shown in Fig. 1. Phospholine iodide was purchased from Wyeth–Ayerst Labs (New York, NY). Octanesulfonic acid was purchased from Sigma Chemical Co. (St. Louis, MO). Potassium phosphate monobasic, acetonitrile, and methanol were purchased from J.T. Baker Chemical Co. (Phillipsburg,, NJ). All chemicals and solvents were ACS analytical grade

or HPLC grade. Deionized water was prepared by an ultrapure water system Pyrosystem Plus® (Hydro, Research Triangle Park, NC).

2.2. Instrumentation

The samples were analyzed by an HPLC system consisting of a Waters 6000A solvent delivery system (Waters Associates Inc, Milford, MA), a Perkin-Elmer ISS-100 automatic injector (Perkin-Elmer Corp., Analytical Instruments, Norwalk, CT) and a Perkin-Elmer LC 240 fluorescence detector. The detector was coupled with an HP 3394 integrator plotter (Hewlett-Packard Co., Avondale, PA). A C_{18} column, LiChrosphere 60 RP Select B (12.5 cm × 4.6 mm i.d., 5 μm, EM Science, Gibbstown, NJ), and a guard column (EM Science) containing the same bonded phase were used for the separation of the three isomers and the internal standard. The column was maintained at 35°C with a heating system. The excitation and emission wavelengths were 280 and 325 nm respectively.

The buffer component of the mobile phase was prepared with deionized water and filtered through a 0.45 μm nylon filter. Acetonitrile and octanesulfonic acid were added to the buffer. The final mobile phase consisted of acetonitrile and 0.025 M KH_2PO_4 buffer (40:60, v/v, pH 5.4) containing 0.005 M octanesulfonic acid. The mobile phase was filtered through a 0.45 μm filter and degassed under ultrasound and vacuum for 15 min. The mobile phase was delivered at a flow rate of 1.5 ml min^{-1}.

Preparation of the standards

Standards were prepared containing the three isomers of mivacurium: trans–trans, cis–trans, and cis–cis, in concentrations ranging from 5–500 ng ml^{-1} for human plasma and 5–250 ng ml^{-1} for dog plasma. Standards were prepared from a freshly prepared stock solution of 1 mg ml^{-1}. Phospholine iodide, an esterase inhibitor, was initially added to the plasma at a concentration of 0.33 mg ml^{-1} of plasma to inhibit drug metabolism by pseudocholinesterase. The internal standard (BW 785U77) was prepared at a concentration of 100 ng ml^{-1} in water.

2.3. Extraction procedure

Human and dog plasma standards (1.0 ml) containing 0.33 mg ml^{-1} of phospholine iodide were transferred to 3.0 ml disposable glass tubes. 1 ml of internal standard (100 ng ml^{-1}) was added to each tube. After vortexing for 10 s the samples were loaded onto Sep-Pak C_{18} cartridges (Waters Associates Inc., Milford, MA) and preconditioned with volumes of methanol (2 ml) and distilled water (2 ml). The cartridges were then washed with distilled water (2 ml), 50% methanol (2 ml) and acetonitrile (2 ml). The samples were then eluted with acetonitrile acidified with 0.33% 6 M hydrochloric acid (0.9 ml) and collected into a 10 × 75 mm disposable glass tube. The elutes were evaporated to dryness at 40°C under a gentle stream of nitrogen. The samples were reconstituted with 250 μl of mobile phase and injected onto the HPLC system.

3. Assay validation

3.1. Linearity and range

The linear detector response for the assay was tested as follows. Three determinations ($n = 3$) from a minimum of six concentration levels, ranging from 5–500 ng ml^{-1} or 5–250 ng ml^{-1} were made. Detector response was correlated with analyte concentration by least-squares regression. A weight of $1/y$ was used to determine slopes, intercepts and correlation coefficients. The minimum acceptable coefficient to establish linearity was set at 0.95 a priori.

3.2. Method precision and percent recovery

Precision of the assay was established by analysis of three replicates ($n = 3$) of a standard solution of the analyte at the upper and lower limits (500 and 5 ng ml^{-1} for human plasma; 250 and 5 ng ml^{-1} for dog plasma). To determine intra-day precision of the assay, replicate ($n = 3$) plasma samples of the isomers at six different concentrations were analyzed. To determine inter-day precision, replicate plasma samples ($n = 3$) were analyzed on five different days.

The assay variability contributed by pretreatment extraction and dilution was also determined. Five different samples ($n = 5$) in the concentration range 5–500 ng ml^{-1} (human plasma) or 5–250 ng ml^{-1} (dog plasma) were carried through all pretreatments and analyzed. The percentage relative standard deviations of the assay results were determined.

Extraction efficiency was determined by comparing peak height of extracted plasma samples vs. an aqueous standard. The percent recovery was determined by the following equation:

$$\% \text{ relative recovery} = \frac{\text{PHR of plasma standard}}{\text{PHR of water standard}} \quad (1)$$

3.3. Accuracy

Method standards in the concentration range 5–500 ng ml^{-1} or 5–250 ng ml^{-1} from four different runs performed over several days were used to check for accuracy. The means of the four runs were calculated and compared to the spiked value to determine the percentage difference between the mean and the spiked value (amount added). The percentage relative error was determined as

$$\%RE = [\text{mean-spiked/spiked}] \times 100$$

Between- and within-run accuracies were determined.

Selectivity was evaluated by testing samples to assure that no method interferences were detected. Ruggedness was assessed by checking retention times of the isomers and internal standard using two different batches of mobile phase and plasma. Stability of the samples in plasma stored at room temperature, −80°C and 4°C was determined. Replicate samples ($n = 2$) were analyzed at different times after storage. Stability of the samples after freeze–thaw cycles was also determined by analyzing the samples in triplicate using freshly prepared standards.

3.4. Clinical study

A clinical study was designed to investigate the pharmacokinetics and pharmacodynamics of the isomers of mivacurium. The protocol was approved by the Institutional Review Board (IRB) at the University of Maryland at Baltimore and informed consent was signed prior to enrolment into the study. A female patient (65 kg, 41 years), ASA status I, undergoing elective surgery was recruited for the clinical study after signing the informed consent statement. The patient was administered a bolus dose (0.18 mg kg^{-1}) for tracheal intubation. Serial blood samples (4 ml) were collected into heparinized tubes containing phospholine iodide (0.33 mg ml^{-1}) via an arterial line. Samples were collected prior to dosing and at 1, 2, 4, 6, and 10 min. Plasma was separated by centrifugation and immediately frozen at −80°C, until assayed. The samples were assayed as stated above.

3.5. Dog study

A pharmacokinetic study was designed to investigate the pharmacokinetics and pharmacodynamics of the isomers of mivacurium in beagle dogs. The protocol was approved by the Animal Research Committee of the School of Pharmacy. A male beagle dog (4 kg) was administered a bolus dose (0.015 mg kg^{-1}). Serial blood samples (4 ml) were collected via an arterial line over 12 min into heparinized tubes containing phospholine iodide (0.33 mg ml^{-1}). Plasma was separated by centrifugation and immediately frozen at −80°C, until assayed.

4. Results and discussion

4.1. Resolution

Chromatograms of extracted blank human plasma and a 10 ng ml^{-1} plasma standard of each isomer at 10 ng ml^{-1} of mivacurium are shown in Figs. 2a and 2b respectively. Representative chromatograms of extracted dog plasma are presented in Figs. 3a and 3b. Fig. 3a illustrates a chro-

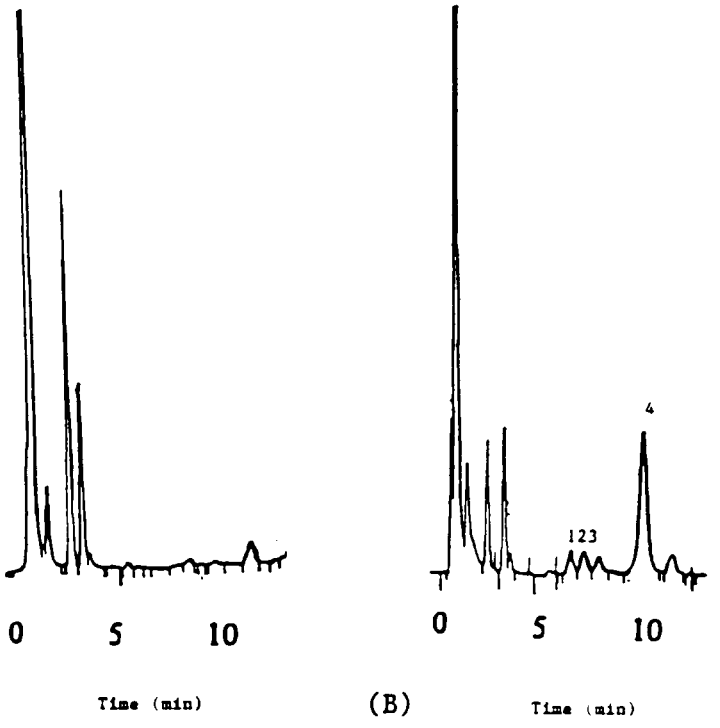

Fig. 2. Extracted human plasma chromatograms: (A) extracted blank human plasma; (B) extracted human plasma standards (10 ng ml^{-1} of each isomer). Peaks 1, 2, 3 and 4 represent the cis–cis, cis–trans, trans–trans isomers and the internal standard respectively.

matogram of extracted blank plasma and Fig. 3b depicts a chromatogram from dog plasma spiked with 10 ng ml^{-1} of each isomer. The assay was found to be specific for the isomers of mivacurium and no interfering peaks from degradation products, phospholine iodide, internal standard or plasma were detected. Moreover, the peaks of the cis–cis, cis–trans and trans–trans isomers and the internal standard were sufficiently separated. Typical retention times for the isomers were 6.5 min (cis–cis), 7.2 min (cis–trans) and 8.1 min (trans–trans) and 10.2 min for the internal standard at a flow rate of 1.5 ml min^{-1}. The lower limit of quantitation for each isomer was 5 ng ml^{-1} of plasma. The retention times of the isomers and the internal standard were not affected by the use of two different batches of plasma and mobile phase.

4.2. Extraction recovery

The optimum extraction of interfering substances and extraction efficiency were achieved with 50% methanol wash. Other methanol washes (100%, 75% and 70%) were also evaluated. 40–60% of the isomers were lost in the methanol wash when the higher percentage methanol washes were used. The relative recoveries for the trans–trans, cis–trans and cis–cis isomers and the internal standard were 73, 72, 70 and 71% respectively for both human and dog plasma.

The extraction method used by previous investigators [13] involves the use of two different types of solid phase extraction cartridges: C_{18} and QMA anion-exchange cartridges. The method presented here requires the use of only C_{18} solid phase cartridges, making the extraction procedures more simple and rapid. The elimination of the extra cartridges reduces the handling of the samples and may contribute to less loss of analyte. The extraction recovery was also improved by using a lower percentage of methanol during the wash. Brown et al. [13] reported recoveries of 55%. They found that a significant proportion of the spiked compound was being lost from the C_{18} cartridge in the methanol wash.

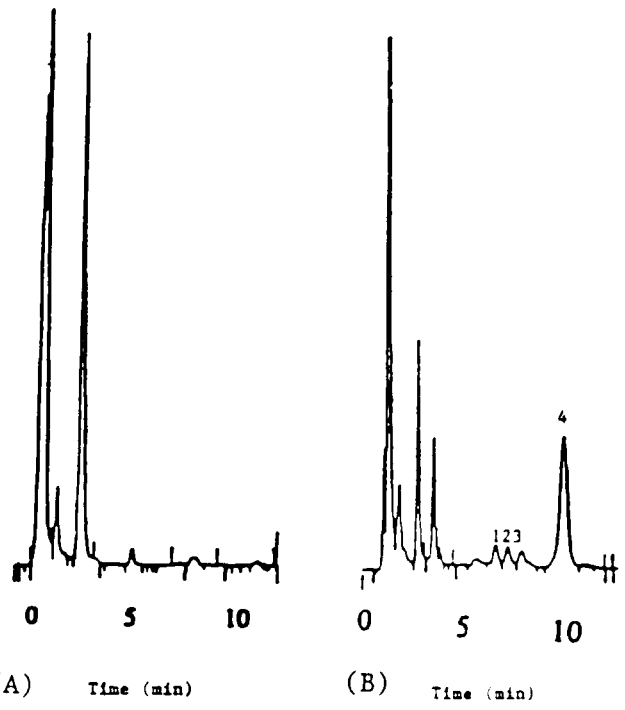

Fig. 3. Extracted dog plasma chromatograms: (A) extracted blank dog plasma; (B) extracted dog plasma standards (10 ng ml^{-1} of each isomer). Peaks 1, 2, 3 and 4 represent the cis–cis, cis–trans, trans–trans isomers and the internal standard respectively.

4.3. Validation assay precision

The results showed excellent correlation coefficients for each of the isomers with a linear range of 5–500 ng ml^{-1} in human plasma and 5–250 ng ml^{-1} in dog plasma. A lower standard curve range was chosen for the dog plasma, since lower levels of mivacurium were expected after drug administration. The standard curves for the three isomers of mivacurium showed linearity over the selected concentration range (5–500 ng ml^{-1} or 5–250 ng ml^{-1}), with consistent slopes and excellent correlation coefficients ($r \geqslant 0.9997$ for human plasma and $r \geqslant 0.9996$ for dog plasma) throughout the validation runs.

The intraday and interday precision data for the isomers of mivacurium in human and dog plasma are listed in Tables 1 and 2 respectively. The intraday and interday precision, as indicated by the RSDs were 7.68% or less for human plasma and 11.65% or less for dog plasma. The intraday and interday accuracy data for the isomers of mivacurium in human and dog plasma are listed in Tables 3 and 4 respectively. The intraday and interday accuracy, as indicated by the relative error, ranged from −8.81 to 4.24% for all the isomers in human plasma and from −11.42 to 11.41 in dog plasma. This is an improvement over the precision data reported by Brown et al. [13]. The precision of the assay, expressed as the percent RSD, was less than 10% at all concentrations except at the lower limit of quantitation (5 ng ml^{-1}), where it went up to 16.2%. The precision of the assay, especially at the lower limit of quantitation (5 ng ml^{-1}), was also improved.

4.4. Stability

The stability of the isomers in plasma stored at −8°C and 4°C was studied. No degradation of the isomers in plasma was detected at the end of 3 months. The isomers were also stable after being stored for 48 h at room temperature. The stability

of the isomers against interconversion was also examined. There were no significant increases in the concentrations of any of the isomers at any point during the analytical method.

4.5. Pharmacokinetic study

A plasma concentration vs. time profile for each isomer following a bolus dose (0.18 mg kg^{-1}) of mivacurium to one patient is shown in Fig. 4. This profile highlights the sensitivity of the assay as well as its utility since it is possible to follow the time course of the mivacurium isomers after a single bolus dose. Fig. 5 displays a concentration vs. time profile for a male beagle dog administered a bolus dose (0.015 mg kg^{-1}) of mivacurium. The time course of the trans–trans, cis–trans and cis–cis isomers was described for the duration of the sampling scheme used in this study. This provides another indication of utility of this analytical method.

Table 1
Intraday and interday assay precision for the stereoisomers of mivacurium in human plasma

Isomer	Concentration (ng ml^{-1})	Intraday (%RSD)	Interday (%RSD)
Trans–trans	500.0	0.44	0.43
	250.0	0.76	1.97
	100.0	2.95	3.46
	50.0	5.55	4.60
	25.0	3.92	4.68
	10.0	6.52	5.25
	5.0	1.17	1.34
Cis–trans	500.0	0.41	0.34
	250.0	0.65	1.16
	100.0	3.57	3.99
	50.0	3.84	3.09
	25.0	4.50	4.02
	10.0	2.87	3.68
	5.0	1.69	2.76
Cis–cis	500.0	0.72	0.59
	250.0	0.64	1.43
	100.0	3.73	3.27
	50.0	4.54	4.46
	25.0	2.19	3.78
	10.0	7.59	7.68
	5.0	1.69	3.66

Table 2
Intraday and interday assay precision for the stereoisomers of mivacurium in dog plasma

Isomer	Concentration (ng ml^{-1})	Intraday (%RSD)	Interday (%RSD)
Trans–trans	250.0	0.72	4.12
	100.0	4.06	3.89
	50.0	9.33	10.39
	25.0	7.24	11.55
	10.0	9.41	11.65
	5.0	7.59	8.22
Cis–trans	250.0	0.25	3.55
	100.0	2.41	2.54
	50.0	8.64	9.16
	25.0	5.69	8.54
	10.0	5.97	6.69
	5.0	4.71	3.67
Cis–cis	250.0	0.42	3.63
	100.0	1.34	1.46
	50.0	9.92	10.20
	25.0	6.26	6.77
	10.0	10.01	10.04
	5.0	9.86	9.73

In contrast to drugs used for ambulatory patients, concentration measurements of drugs used in anesthesia are rarely indicated for monitoring purposes. The maintenance dose of a neuromuscular blocking agent is now determined by direct measurement of muscle relaxation. However, concentration monitoring of neuromuscular blocking agents can be useful for determination of the fate of the drug in the body, to reveal the reasons for an unexpected effect after administration, and for combined pharmacokinetic and pharmacodynamic analysis in order to gain insight into the factors which determine the potency and time course of action of neuromuscular blocking agents.

A sensitive and stereospecific assay for the quantitation of the three isomers of mivacurium in plasma has been reported. The procedure developed is a modification and simplification of the assay developed by Brown et al. [13]. The accuracy and precision data show that the method reported herein is consistent and reliable with low values of relative error and RSD for standards over the entire concentration range (5–500 ng

Table 3
Intraday and interday accuracy for the stereoisomers of mivacurium in human plasma

Isomer	Concentration (ng ml^{-1})	Intraday (%RE)	Interday (%RE)
Trans–trans	500.0	−0.96	−0.59
	250.0	1.43	−0.26
	100.0	0.28	1.59
	25.0	−3.45	0.89
	10.0	−1.13	−0.64
	5.0	−6.63	−8.81
Cis–trans	500.0	−0.59	−0.73
	250.0	−0.30	−0.18
	100.0	1.86	1.32
	25.0	1.11	4.24
	10.0	−6.32	−4.19
	5.0	−8.40	−8.68
Cis–cis	500.0	−0.71	−0.35
	250.0	−0.25	−0.69
	100.0	1.18	0.78
	25.0	2.27	−0.36
	10.0	−6.20	−5.44
	5.0	−3.34	−2.27

Table 4
Relative error of intraday and interday assay precision for the stereoisomers of mivacurium in dog plasma

Isomer	Concentration (ng ml^{-1})	Intraday (%RSD)	Interday (%RSD)
Trans–trans	250.0	−2.19	−5.87
	100.0	7.30	10.26
	50.0	−5.87	1.50
	25.0	6.27	0.79
	10.0	6.50	−2.07
	5.0	−11.42	−5.62
Cis–trans	250.0	−2.84	−6.02
	100.0	9.08	11.15
	50.0	−2.88	4.40
	25.0	3.35	0.50
	10.0	−1.23	−5.04
	5.0	−3.99	−4.31
Cis–cis	250.0	−3.02	−6.19
	100.0	9.90	11.41
	50.0	−3.17	3.76
	25.0	3.12	8.56
	10.0	−1.92	−3.79
	5.0	−3.37	−7.29

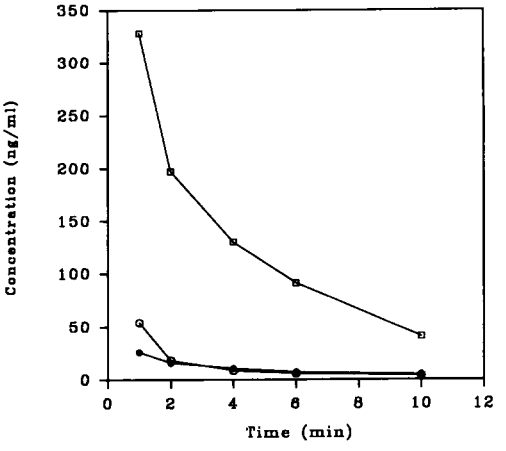

Fig. 4. Plasma concentration vs. time profile from a representative patient following a bolus dose of mivacurium. (□), trans-trans; (○), cis-trans; (●), cis-cis.

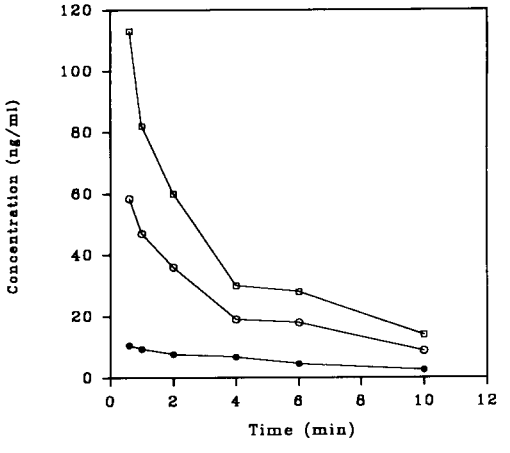

Fig. 5. Plasma concentration vs. time profile from a representative beagle dog following a bolus dose of mivacurium. (□), trans-trans; (○), cis-trans; (●), cis-cis.

ml^{-1}) examined. No interfering peaks from degradation products, phospholine iodide, or plasma were detected. This assay can be adopted to develop new models to monitor neuromuscular blockade in clinical settings and research laboratories.

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