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ORIGINAL RESEARCH

The determination of biperiden in plasma using gas chromatography mass spectrometry: pharmacokinetics after intramuscular administration to guinea pigs†

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ABSTRACT: A gas chromatographic–mass spectrometric (GC-MS) method has been developed for the analysis of the biperiden from plasma. The method utilizes 290 μl of plasma and a simple hexane extraction/clean-up procedure. Standard curves were linear over the range of 1.9–250 ng/mL. The range of correlation coefficients for the individual standard curves was 0.9984–0.9999; the largest coefficient of variation expressed as a percentage (%CV) was 11.5%. Precision and accuracy were examined by assessing between-day and within-day variability. For between-day precision, the %CVs ranged from 2.86 to 5.17%. Accuracy as expressed by percentage error ranging from −2.16 to 5.83%. The study for within-day precision demonstrated %CVs from 0.95 to 5.55% with accuracy from −3.37 to 2.45%. Applicability of the method was demonstrated by examining the pharmacokinetics of intramuscular (i.m.) biperiden as an anticonvulsant treatment in a guinea pig model for organophosphate (OP)-induced seizure activity. Mean pharmacokinetic parameter estimates were similar to literature values; selected mean pharmacokinetic parameter estimates were: apparent volume of distribution, 13.9 L/kg; half-life of elimination, 93 min; time to maximal plasma concentration, 27.4 min; and maximal plasma concentration, 32.22 ηg/mL. The time to maximal plasma concentration was found to be similar to the onset time for terminating OP-induced seizure activity in guinea pigs receiving biperiden as an anticonvulsant treatment. The studies indicate that the method affords the required precision, accuracy and sensitivity to assay biperiden at the doses utilized for these pharmacokinetic studies after i.m. administration to guinea pigs. Copyright © 2001 John Wiley & Sons, Ltd.

INTRODUCTION

Biperiden is an anticholinergic compound that has been utilized clinically for parkinsonism (Standaert and Young, 1996) and also to manage unwanted extrapyramidal effects from neuroleptic drug administration (Friis *et al.*, 1983; McEvoy, 1983; Conti *et al.*, 1984). Additionally, studies from this laboratory have demonstrated the effectiveness of this and other anticholinergic compounds for the treatment of seizure/convulsive activity associated with exposure to multiple lethal doses of organophosphate (OP) cholinesterase inhibitors (Capacio and Shih, 1991; McDonough and Shih, 1993; Shih *et al.*, 1991, 1993). Intoxications with OP compounds produce a progression of signs including hypersecretions, motor convulsions and seizures (Taylor, 1996). The effects are caused by the inhibition of acetylcholinesterase (AChE) and resultant accumulation of acetylcholine. A combined pretreatment/treatment regimen has been suggested as an optimal strategy for managing OP poisoning (Dunn and Sidell, 1989; Sidell, 1992). The US military employs a standard drug regimen consisting of pyridostigmine (PYR) pretreatment and atropine/pralidoxime chloride (2-PAM) as a treatment after exposure. Additionally, the use of diazepam as an anticonvulsant is recommended to control seizure activity.

Compared with the standard anticonvulsant (diazepam) utilized against OP toxicity, cholinolytics offer such advantages as additional antagonism of cholinergic hyperactivity (ie hypersecretions), less sedation, and a quicker onset of action (Anderson *et al.*, 1994a, b, 1997; McDonough *et al.*, 1999, 2000). McDonough *et al.* (2000) have determined the ED_{50} of biperiden for terminating soman-induced seizure activity in guinea pigs receiving PYR pretreatment, soman challenge and

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†In conducting the research described in this report, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals* by the Institute of Laboratory Animal Resources, National Research Council, in accordance with the stipulations mandated for an AAALAC accredited facility. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Abbreviations used: AChE, Acetylcholinesterase; AUC, area under the curve; OP, organophosphate; 2-PAM, pralidoxime chloride; PYR, pyridostigmine.

treatment with atropine and 2-PAM. The efficacy of biperiden was well established in these studies. However, the plasma levels associated with the anticonvulsant effect have not been determined. In this report we demonstrate a modified gas chromatographic–mass spectrometric (GC-MS) method applicable to the determination of biperiden pharmacokinetics in guinea pigs that have also received the above-mentioned drug pretreatment, challenge and treatment regimen. The utility of the method is demonstrated considering other confounding factors such as concomitant administration of drug modalities in the animal model, the dose needed to stop seizure activity, the relatively large volume of distribution of biperiden, and blood sample volume restrictions imposed by using small animals such as the guinea pig. The method facilitated the determination of complete pharmacokinetic profiles from a single animal through the use of serial blood sampling.

MATERIALS AND METHODS

Animals

Male Hartley Crl (HA)BR COBS® guinea pigs (*Cavia porcellus*) weighing 250–300 g obtained from Charles River Laboratories (Wilmington, MA) were utilized for all experiments. Animals were housed in an Association for the Assessment and Accreditation of Laboratory Animal Care International accredited facility and were used in accordance with a protocol approved by the Institutional Animal Care and Use Committee. The study reported here was conducted in accordance with the principles described in the *Guide for the Care and Use of Laboratory Animals*. They were provided commercial certified guinea pig ration as appropriate and tap water *ad libitum*. The guinea pig holding room was maintained at 21 ± 2°C with 50 ± 10% relative humidity. The holding room was ventilated (at least 10–15 complete changes per hour) with 100% conditioned fresh air and was maintained on a 12 h light/dark full spectrum lighting cycle with no twilight.

Chemicals

All chemicals and solvents were of HPLC grade or higher. Dehydrated ethyl alcohol USP (200 proof) was obtained from Pharmco Product Inc. (Brookfield, CT). Pyridostigmine, atropine sulfate, pralidoxime chloride and biperiden were obtained as dry powders from Walter Reed Army Medical Hospital. Trihexyphenidyl for use as internal standard was obtained as a dry powder from Sigma Chemical Co. (St Louis, MO). Soman (pinacolyl methylphosphonofluoridate) was obtained from the US Army Edgewood Chemical and Biological Center (Aberdeen Proving Ground, MD, USA). Biperiden for intramuscular (i.m.) administration to guinea pigs was dissolved in a vehicle containing 40% propylene glycol, 10% ethanol, 1.5% benzyl alcohol, and 48.5% distilled deionized water. All other drugs for animal administration were dissolved in normal saline.

Instrumentation

Gas chromatography (GC). Gas chromatographic separations were performed on a Hewlett-Packard 5890 Series II gas chromatograph. The GC was fitted with a 30 m × 0.25 mm i.d. DB-5MS bonded phase column, 0.25 um film thickness (J&W Scientific, Folsom, CA). Helium was used as the carrier gas at a column head pressure of 12 psi. The oven temperature was held initially at 45°C for 1 min, programed from 45 to 270°C at 20°C min^{-1}, and held at 270°C for 3.75 min. Splitless injections of 1 μL volume were made using a Hewlett-Packard 7673 autosampler. The split delay was set at 1 min, injection port temperature at 250°C, split flow at 60 mL min^{-1}, transfer line temperature at 280°C, and the septum purge at 2 mL min^{-1}. A Hewlett-Packard single-taper borosilicate, deactivated liner with glass wool packing was used in the GC inlet.

Mass spectrometry (MS). Positive-ion electron impact MS analyses were performed on a Hewlett-Packard 5972A mass selective detector interfaced to the GC described above. The MS operating conditions were as follows: ion source pressure approximately 1.5×10^{-5} torr, source temperature 180°C, electron energy 70 eV, electron emission current 50 μA, and electron multiplier voltage +400 V relative to the autotune setting. The mass spectrometer was operated using selected ion monitoring (SIM). Two ions (*m/z* 98 and 218) characteristic for the compounds of interest were monitored at a dwell time of 50 ms each. This resulted in a total scan rate of 6.94 cycles s^{-1}.

Sample preparation

Pooled whole blood from swine (*Sus scrofa*) was obtained commercially (Archer Farms, Belcamp, MD) or from guinea pigs in-house. The whole blood was centrifuged at 2000 ***g*** for 30 min at 5°C. Plasma was decanted into 15 mL aliquots and stored at −70°C until use. Biperiden (7.5 μg/mL) and trihexyphenidyl (1.5 μg/mL) stock solutions for standard curves were made in absolute ethanol and were stored refrigerated (5°C). To all plasma samples (290 μL), trihexyphenidyl (10 μL, 1.5 μg/mL) was added as an internal standard. This was followed by the addition of ammonium hydroxide (20 μL, 1 M) and hexane (2.5 mL). The samples were gently shaken for 1 h. The organic layer was removed and placed in glass vials where it was evaporated under a gentle stream of nitrogen at 30°C. Subsequently the samples were reconstituted with 100 μL ethanol and shaken vigorously for 45 min. The samples were then analyzed by GC-MS under the conditions described.

Standard curves. Six standard curves were prepared and analyzed on six separate days; three experiments were conducted with pig plasma and three with guinea pig plasma. The range of biperiden concentrations utilized in the standard curve was based upon preliminary pharmacokinetic experiments in guinea pigs following intramuscular drug administration. Plasma (870 μL) was spiked with biperiden (30 μL; 7.5 μg/mL). An aliquot (580 μL) was removed and placed in a glass screw top sample vial. From this vial a 290 μL aliquot was removed and serially diluted with an equal volume of clean plasma. Final concentrations of biperiden in 290 μL were 250, 125, 62.50, 31.25, 15.62, 7.81, 3.91 and 1.95 ng/mL. The internal standard, trihexyphenidyl (10 μL, 1.5 μg/mL),

was added to all eight concentrations. The trihexyphenidyl concentration in each sample was 50 ng/ml. These samples were subsequently handled as described under Sample Preparation. For each mass chromatogram, the area under the curve (AUC) was determined for the biperiden and the internal standard peak. A regression line was generated from the mean AUC ratios (biperiden/internal standard) as a function of actual concentration and was used to quantify samples with unknown biperiden concentrations.

Precision and accuracy

Between-day and within-day variabilities were used to measure precision and accuracy. For between-day studies, plasma test samples at five concentration levels (112.50, 56.25, 28.12, 14.06 and 7.03 ng/ml) were prepared and analyzed the same day for a total of 6 days. Precision was assessed by calculating the standard deviation (SD) and percentage coefficient of variation (%CV) at each plasma test sample concentration level. Accuracy was expressed as percentage error by examining the difference between the theoretical and the mean calculated concentration of the sample [(calculated − theoretical)/expected × 100]. For within-day studies, five sets of plasma test samples were made using the same concentration levels as those in the between-day studies. All samples were made and analyzed within one day. Precision and accuracy were expressed as noted in the between-day studies.

Stability

Two stability studies were carried out on ethanol-reconstituted samples: one to determine the effect of repeated storage at −70°C and one to observe the effect of room temperature storage.

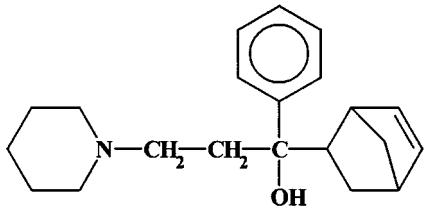
Storage at −70°C. Biperiden plasma test samples at five concentration levels (250, 125, 62.500, 31.250, 15.625, 7.812, 3.910 and 1.950 ng/mL) were freshly prepared as described in Sample Preparation. Samples were analyzed on day 1 and then stored at −70°C between assay days. For five consecutive working days, starting the day after the initial preparation, the samples were warmed to room temperature and reanalyzed.

Storage at room temperature. Two sets of samples at five concentration levels (112.50, 56.250, 28.125, 14.063 and 7.031 ng/mL) were prepared as described in Sample Preparation. Samples were analyzed in duplicate on day 1 and at 6 and 16 days following preparation.

Pharmacokinetic experiments

The assay described above was utilized to determine the pharmacokinetics of biperiden in guinea pigs following i.m. administration of 0.5 mg/kg (0.5 mL/kg). The time-course of biperiden was determined in animals by obtaining serial blood samples for 2 h after administration of the drug. Blood (approximately 600 μL) was collected in heparinized tubes at 0, 10, 20, 30, 60 and 120 min following drug administration. Immediately upon completion of the experiment, plasma was separated from red blood cells and handled as described under Sample Preparation.

A.



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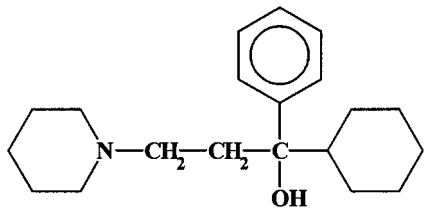

Figure 1. Chemical structures of (A) biperiden and (B) trihexyphenidyl.

Pharmacokinetic analysis

Time-plasma concentration data were fit to standard pharmacokinetic models using WinNonlin (version 1.5, 1997; Scientific Consulting Inc., Cary, NC) non-linear regression software. Choice of the appropriate model was based upon the best fit of the raw data to the mathematical model. The following criteria were utilized as guidelines for determining the appropriate model: minimal sum of squared residuals, high correlation coefficient, small standard deviations of parameter estimates and unbiased distribution patterns of residuals for estimates of observed versus predicted values. The analysis of data from individual animals generated observed *vs* predicted concentrations as a function of time as well as pharmacokinetic parameter estimates. Mean observed concentration–time data ($n = 6$) were plotted with mean predicted values generated by best-fit analysis. Also, the mean of the pharmacokinetic parameter estimates generated for individual animals was calculated.

Statistical analysis

Statistical calculations (t-test and one-way repeated measures analysis of variance; ANOVA) were accomplished with SigmaStat software (version 2.03, 1993; Jandel Scientific, San Rafael, CA). Significance was defined as $p < 0.05$.

RESULTS

The chemical structures for biperiden and trihexyphenidyl are shown in Fig. 1 (A and B). Mass-chromatograms of extracted plasma samples from guinea pigs receiving the pretreatment/treatment drug regimen were free from interfering peaks in the area of biperiden and trihexyphenidyl. The retention times were approximately 14.1

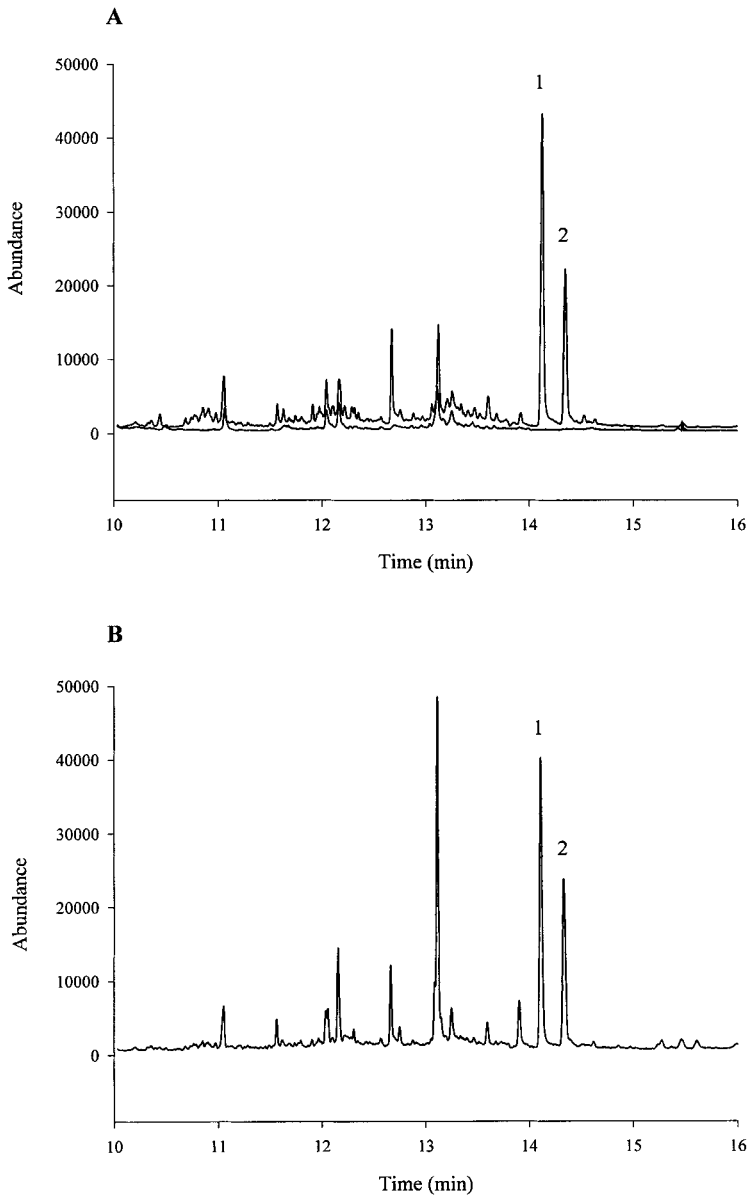

Figure 2. Representative mass-chromatogram of extracted plasma: (A) plasma blank was extracted as described in Sample Preparation, the overlay trace is a similarly treated plasma sample with internal standard (trihexyphenidyl, **1**) and spiked with biperiden (**2**), 31.25 ng/mL. (B) Mass chromatogram from pharmacokinetic studies demonstrating extracted plasma sample obtained at 30 min after biperiden 0.5 mg/kg (intramuscular) administration (internal standard, **1**; biperiden, **2**).

and 14.3 min for trihexyphenidyl and biperiden, respectively. Representative mass-chromatograms of an extracted plasma blank overlaid with extracted plasma containing trihexyphenidyl (50 ng/mL) and biperiden (31.25 ng/mL) are shown in Fig. 2(A). A typical mass-chromatogram representing an extracted plasma sample from guinea pig pharmacokinetic studies obtained at 30 min after biperiden administration is shown in Fig. 2(B). It is worth noting that we did not experience interference from any of the pretreatment/challenge/treatment modalities utilized in this guinea pig model. Mass-chromatograms in the area of trihexyphenidyl and biperiden for the untreated guinea pigs were similar to those receiving the above-mentioned drug regimen.

Standard curves

The AUC ratios for a given concentration were consistent from day to day. The %CV from the mean AUC ratios across concentrations ranged from 4.3 to 11.5%. Standard

Table 1. Precision and accuracy for between-day test samples

Expected concentration (ng/mL)	Mean ($n = 6$) calculated concentration (ng/mL)	Precision		Accuracy %Error
		SD	%CV	
112.50	118.60	3.55	3.00	5.43
56.25	59.53	2.30	3.86	5.83
28.12	29.00	0.83	2.86	3.11
14.06	14.18	0.73	5.17	0.86
7.03	6.88	0.32	4.70	−2.16

Plasma test samples at five concentration levels were prepared and analyzed the same day for a total of 6 days. Precision was assessed by calculating the standard deviation (SD) and percentage coefficient of variation (%CV) at each plasma test sample concentration level. Accuracy was expressed as percentage error by examining the difference between the theoretical and the mean calculated concentration of the sample [(calculated − theoretical)/expected ×100].

curves were linear over the range of biperiden concentrations (1.95–250 ng/mL) utilized in this study. Linear regression analysis indicated that the correlation coefficient for the mean AUC ratios at each concentration level as a function of expected concentration was 0.9999. The range of correlation coefficients for the individual standard curves was 0.9984–0.9999. The regression line for the standard curve is presented in equation (1). Because there were no differences between standard curves constructed with swine and guinea pig plasma, swine plasma, due to its availability, was utilized for all subsequent studies (precision and accuracy, and stability).

$$Y = (0.19)X + 0.004 \quad (1)$$

Precision and accuracy

Between-day variability. Precision and accuracy data for between-day plasma test samples are presented below in Table 1. For precision, the %CV's ranged from 2.86 to 5.17%. Between-day accuracy as expressed by percent error ranged from −2.16 to 5.83%.

Within-day variability. Precision and accuracy data for within-day test plasma samples are presented in Table 2 below. For precision, the %CV's ranged from 0.95 to 5.55%. Accuracy for within-day samples as expressed by percent error ranged from −3.37 to 2.45%.

Stability

Storage at −70 °C. The calculated concentrations of ethanol-reconstituted samples repeatedly stored (days 1–6) at −70°C between days are shown in Table 3. The percentage difference between the determined concentrations on an experimental day relative to the same concentration on day 1 was calculated [(experimental day concentration − day 1 concentration)/day 1 concentration ×100] and is shown in parentheses. The CV's ranged from 0.34 to 1.70%. A one-way repeated-measures ANOVA was conducted comparing means across concentration levels of each assay day (ie mean concentration for days 2–6 were compared with day 1). No statistical difference was detected ($p > 0.05$). In addition, the data did not appear to demonstrate a trend that would suggest a change in concentration over time.

Storage at room temperature. The calculated concentrations of ethanol-reconstituted samples stored at room temperature (21 °C) between assay days are shown in Table 4. A one-way repeated-measures ANOVA was conducted comparing means across concentration levels of each assay day (ie the mean concentration on days 6 and 16 were compared with the mean concentration on

Table 2. Precision and accuracy for within-day test samples

Expected concentration (ng/mL)	Mean ($n = 5$) calculated concentration (ng/mL)	Precision		Accuracy %Error
		SD	%CV	
112.50	114.93	1.66	1.44	2.16
56.25	57.63	0.55	0.95	2.45
28.12	28.35	1.13	3.99	0.79
14.06	13.70	0.76	5.55	−2.65
7.03	6.79	0.36	5.36	−3.37

Five sets of plasma test samples were made using the same concentration levels as those in the between-day studies. All samples were made and analyzed within one day. Precision and accuracy were determined as described in Table 1.

Table 3. Calculated concentrations of samples stored at −70°C between analysis days

	Calculated concentration (ng/mL)							
Day 1	252.82	134.36	63.75	34.87	16.41	9.02	4.07	2.00
% Difference	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Day 2	251.70	133.30	63.39	34.57	15.87	8.93	4.10	2.02
% Difference	(−0.44)	(−0.79)	(−0.57)	(−0.89)	(−3.28)	(−1.06)	(0.78)	(1.35)
Day 3	252.22	133.75	63.81	34.70	16.29	8.92	4.08	2.06
% Difference	(−0.24)	(−0.45)	(0.09)	(−0.49)	(−0.75)	(−1.12)	(0.18)	(3.39)
Day 4	253.81	135.18	64.14	34.63	16.64	8.91	4.19	2.04
% Difference	(0.39)	(0.61)	(0.61)	(−0.71)	(1.35)	(−1.21)	(2.84)	(2.20)
Day 5	253.83	137.65	64.84	34.31	16.36	8.91	4.12	2.01
% Difference	(0.40)	(2.44)	(1.71)	(−1.62)	(−0.32)	(−1.22)	(1.30)	(0.73)
Day 6	253.15	137.22	64.58	34.73	16.34	8.93	4.21	1.97
% Difference	(0.13)	(2.12)	(1.30)	(−0.40)	(−0.45)	(−1.07)	(3.54)	(−1.53)
Mean	252.92	135.24	64.08	34.64	16.32	8.94	4.13	2.02
SD	0.85	1.81	0.55	0.19	0.25	0.04	0.06	0.03
%CV	0.34	1.34	0.85	0.55	1.52	0.47	1.43	1.70

Samples were prepared and analyzed on day 1 and then stored at −70° C between assay days. For five consecutive working days starting the day after the initial preparation, the samples were warmed to room temperature and reanalyzed. Values in parentheses are percent difference in concentration relative to day 1.

Table 4. Calculated concentrations of samples stored at 21°C between analysis days

	Calculated concentration (ng/mL)				
Day 1	125.15	61.40	29.51	14.14	7.07
% Difference	(0)	(0)	(0)	(0)	(0)
Day 6	124.62	60.80	30.05	14.54	7.28
% Difference	(−0.42)	(−0.95)	(1.85)	(2.79)	(3.01)
Day 16	118.58*	58.62*	28.33*	14.02	8.15
% Difference	(−5.24)	(−4.51)	(−4.0)	(−0.90)	(1.18)

Two sets of samples were analyzed in duplicate on day 1 and at 6 and 16 days following preparation. Values in parentheses are percent difference in concentration relative to day 1.
* Indicates a statistically significant difference from day 1 ($p < 0.05$).

day 1). The calculated means from the analysis were 47.45, 47.46 and 45.34 ng/mL for days 1, 2 and 3, respectively. However, no statistical difference was detected ($p > 0.05$). Although the mean concentration on each day did not change significantly, there appeared to be a trend toward a concentration decrease (especially for higher concentration samples) on day 16 relative to the same concentration group assayed on day 1. A paired *t*-test was run to assess differences between day 1 and days 6 and 16 at each concentration level. Three of the five samples assayed on day 16 were found to have a statistically significant difference in concentration relative to day 1 ($p < 0.05$; Table 4). The differences were found at the 125.15, 61.40 and 29.51 ng/mL concentration levels and were −5.24, −4.5 and −4.0% of the concentration determined on day 1 (Table 4).

Pharmacokinetics

The pharmacokinetics of biperiden after im administration to guinea pigs were best described by a one-compartment model with first-order absorption and elimination described by equation (2).

$$C(t) = \frac{D}{V_d}\frac{k_{01}}{k_{01} - k_{10}}(e^{-k10t} - e^{-k01t}) \qquad (2)$$

where C = plasma concentration (ng/mL), t = time (min), D = dose (μg/kg), V_d = apparent volume of distribution

Table 5. Mean pharmacokinetic parameter estimates of biperiden following intramuscular administration to guinea pigs

	Parameter estimate (± SEM)
Volume (L/kg)	13.92 (1.75)
AUC (ng/mL × min)	5076 (566)
$t_{1/2}$- Abs (min)	7.01 (1.70)
$t_{1/2}$- Elim (min)	93.31 (9.52)
t_{max} (min)	27.38 (5.00)
C_{max} (ng/mL)	32.22 (4.64)

The mean plasma concentration–time data ($n = 6$) were fit to standard pharmacokinetic models as described. The data best fit a one-compartment model.

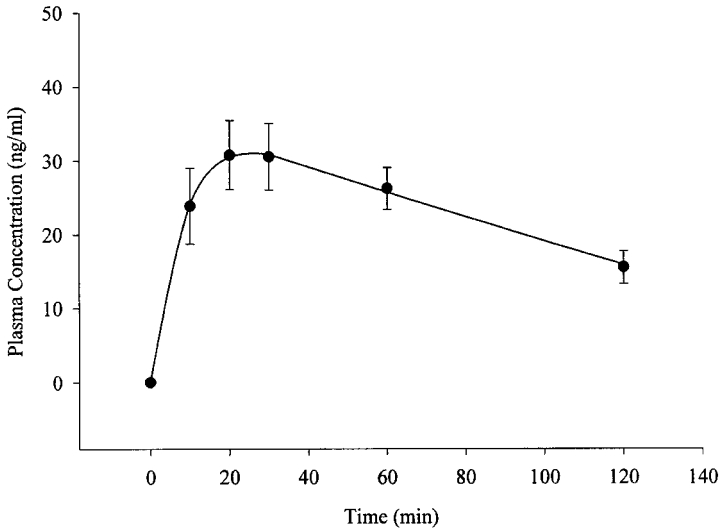

Figure 3. Biperiden pharmacokinetic curves: observed (solid circle; ±SEM) biperiden concentrations are demonstrated along with model-predicted concentrations (solid line) as a function of time. Observed values are mean calculated plasma concentrations from animals ($n = 6$). Predicted values are mean obtained from individually model-fit animals ($n = 6$).

(l/kg), k_{01} = absorption rate constant (min^{-1}), and k_{10} = elimination rate constant (min^{-1}). The following pharmacokinetic parameter estimates were generated: V_d, k_{01}, k_{10}, time to maximum plasma concentration (t_{max}), maximum plasma concentration (C_{max}), half-lives associated with absorption and elimination respectively ($t_{1/2}$-abs and $t_{1/2}$-elim) and area under the time–concentration curve (AUC). The mean pharmacokinetic parameter estimate data were calculated from estimates generated with individually model-fit animals and are presented in Table 5. Observed *vs* predicted plasma concentrations as a function of time are presented in Fig. 3.

DISCUSSION

The determination of plasma biperiden concentrations for pharmacokinetic studies presents a unique set of problems. These are primarily attributable to the dose, relatively large V_d of the drug, and blood volume restrictions inherent in obtaining serial blood samples from small animals such as guinea pigs. Herein we report on the assay and the pharmacokinetics of biperiden at a dose previously shown to terminate ongoing seizure activity. The pharmacokinetic studies were carried out in a guinea pig model used to evaluate efficacy against OP-induced seizure activity.

A number of analytical procedures for the assay of biperiden have appeared in the literature. These methods utilize GC (Ottoila and Taskinen, 1981; Le Bris and Brode, 1985; Yokogawa *et al.*, 1985), GC-MS (Harle *et al.*, 1978; Yisak *et al.*, 1993), capillary electrophoresis (CE; Babackova *et al.*, 1997) and tissue binding radioreceptor assays (Stoll *et al.*, 1989). The GC and GC-MS methods report the lower limit of quantification to be 0.25 and 0.125 ng/mL, respectively. However, these methods report fairly labor-intensive sample extraction procedures and require relatively large volumes of plasma (1.0–2.0 mL) to achieve the reported quantitation limit. Tissue binding assays report a quantitation limit of 200 pg/mL; however, for our purposes, the assay for biperiden would be confounded by the presence of atropine or other anticholinergic compounds utilized as a component of the OP treatment regimen. Capillary electrophoresis, although capable of handling very small sample volumes, has sensitivity limitations; the quantitation limit has been shown to be in the low μg/mL range. We have developed a modified GC-MS method that uses 290 μL of plasma and offers a lower limit of quantification of at least 1.9 ng/mL. The volume requirements and simplicity of this method are ideally suited to serial blood sampling from small animals such as guinea pigs. Additionally, the analysis affords the required sensitivity for the plasma concentrations that are achieved from doses of biperiden utilized against OP-induced seizures.

Compounds structurally related to biperiden (i.e. trihexyphenidyl and procyclidine) have also been shown to display efficacy against OP-induced seizure activity (McDonough *et al.*, 2000). The usefulness of this procedure for the analysis of those compounds for future studies has been briefly considered. As it stands, the

method is directly applicable to trihexyphenidyl (the internal standard). Preliminary studies have indicated that under similar GC conditions procyclidine had a retention time of approximately 0.6 min earlier than trihexyphenidyl with characteristic ions observed at *m/z* 84 and 204 (unpublished results). The resolution of the chromatographic separation observed would enable the simultaneous analysis of procyclidine, trihexyphenidyl and biperiden.

Data from the standard curves demonstrate that the ratios of AUCs were linearly related to theoretical concentration across the range of concentration levels studied. Even though the curves were constructed on different days, the ratios of AUC's for a given concentration level were similar across the 6 days as demonstrated by the %CVs, which ranged from 4.3 to 11.5%. The regression line generated from the standard curve data was used to determine the actual concentrations for the test plasma samples in the precision and accuracy studies as well as the pharmacokinetic studies.

The stability studies were designed to assess the integrity of samples when subjected to temperature conditions commonly expected in the laboratory during analysis and storage. The room temperature studies demonstrate that samples will remain stable for at least 8 days, since initial signs of concentration loss was not observed on day 8 but was apparent on day 16. This is well within the time that samples reside in the automatic liquid sampler while waiting for injection (less than 24 h). Since samples are routinely stored samples at −70°C, stability studies at that temperature were conducted. Although samples were not repeatedly brought to room temperature after storage at −70°C, the results from this study confirm that this can be done with no compromise to integrity. The results of these studies as well as those from the precision and accuracy experiments indicate that samples will remain stable when handled as described and the method will provide a measure of biperiden concentrations with associated variability of less than 10% (Tables 1 and 2).

In our studies, the pharmacokinetics of i.m. biperiden in guinea pigs was characterized by a one-compartment model with first-order absorption and elimination. In the literature, biperiden following intravenous (i.v.) and oral administration to humans has been shown to undergo a biphasic decline with $t_{1/2}$ of 1.5 h (90 min) for the initial rapid distributive phase and 24 h for the slower terminal elimination phase (Grimaldi *et al.*, 1986). This difference in the decline profiles is most likely due to the fact that our experiments were terminated at 2 h and thus we were unable to observe the onset of the terminal elimination phase; species differences can also be a contributing factor. This is further supported by the observation that the $t_{1/2}$ for our studies (93.3 min) is very close to the $t_{1/2}$ reported for the initial rapid distributive phase (90 min), suggesting that we may be observing only the initial decline rate.

Pharmacokinetic studies of biperiden have shown a relatively large V_d in humans (24 L/kg; Grimaldi *et al.*, 1986), rabbits (18.4–18.7 L/kg; Yokogawa *et al.*, 1985, 1986), and rats (14.0 L/kg; Nakashima *et al.*, 1987; Yokogawa *et al.*, 1990) after i.v. administration. The large apparent volume of distribution has been suggested to be a result of marked tissue partitioning (Grimaldi *et al.*, 1986; Brocks, 1999). Data from studies in rats support this notion and report brain/plasma biperiden AUC ratios of 7–12 (Yokogawa *et al.*, 1992). Yokogawa *et al.* (1986) reported a V_d i.m. close to that obtained in the i.v. study after im administration to rabbits (V_d = 15.9 L/kg). These same studies (Yokogawa *et al.*, 1986) have demonstrated that biperiden was completely absorbed from an intramuscular injection site by showing the AUC_{im}/AUC_{iv} close to unity. The V_d determined in our study was 13.9 L/kg and was similar to that reported above for the rats. The data from our experiments suggest that biperiden also has a relatively large V_d in the guinea pig. Additionally, based on the similarity in V_d values between i.m. and i.v. rabbit studies, our investigations suggest that bioavailability from the im injection site in guinea pigs would also be expected to be close to unity.

There are some studies reporting biperiden C_{max} concentrations in humans after oral administration (Hollmann *et al.*, 1984; Le Bris and Brode, 1985; Grimaldi *et al.*, 1986). The investigations administered 4 mg biperiden orally to adult humans and reported C_{max} concentrations in the range of 3.9–6.3 ng/mL (median 5.1 ng/mL). Adjusting for body weight (ie 70 kg) the 4 mg dose corresponds to 0.057 mg/kg. The plasma C_{max} (32.22 ng/mL) in our study was approximately 6-fold greater than those reported in humans; however, our dose was correspondingly greater (8.8-fold). It is difficult to make comparisons due to species and route of administration differences. However, the data suggest that plasma concentrations (adjusting for dosage differences) from our study are in the same range as those reported in the literature.

In our studies, biperiden t_{max} was found to be 27.4 min following injection. The value fits with literature findings where oral administration of biperiden to humans produced t_{max} values in the range of 0.5–2 h after administration (Grimaldi *et al.*, 1986; Hollmann *et al.*, 1984). Moreover, the t_{max} determined in our studies supports the findings of McDonough *et al.* (2000), who have reported the mean time for terminating soman-induced seizures in a similar guinea pig model to be approximately 15 min following i.m. biperiden.

In summary, we have developed a precise and accurate GC-MS method for determining biperiden concentrations in plasma. The method was used to determine biperiden pharmacokinetics in guinea pigs pretreated with PYR, challenged with soman and treated with atropine/2-PAM and biperiden. Plasma samples assayed by this procedure yielded mass chromatograms that were free from

interference of other drug compounds administered during the same time frame as biperiden. The pharmacokinetic data obtained in these experiments are similar to data from the literature in terms of V_d, C_{max}, t_{max} and $t_{1/2}$-elim. Finally, the t_{max} determined in these studies fits well with the time for termination of soman-induced seizure activity in guinea pigs receiving biperiden as an anticonvulsant treatment.

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