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The influence of storage time and temperature on propofol concentrations in canine blood and plasma

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Propofol is an intravenous anesthetic commonly used due to its favorable pharmacokinetic and pharmacodynamic profile. There are discrepancies in the literature about the most appropriate sample for determining propofol concentrations. Although plasma has been used for determining propofol concentrations, whole blood has been the preferred sample because propofol is significantly bound to erythrocytes. There is also a lack of consistency in the literature on the effect of storage time and temperature on propofol concentrations and this may lead to errors in the design of pharmacokinetic/pharmacodynamics studies. The purpose of this study was to determine the difference in propofol concentrations in whole blood versus plasma and to evaluate the influence of storage time (56 days) and temperature (4°C, -20°C, -80°C) on the stability of propofol concentrations in blood and plasma samples. Results from the study indicate that whole blood and plasma samples containing propofol were stable for at least 56 days when stored at -80°C; thus, -80°C is the most appropriate temperature for propofol sample storage out of the three temperatures evaluated. Plasma propofol concentrations were consistently higher than whole blood for all 3 storage temperatures. Consequently, plasma is the most appropriate sample for propofol analysis due to its consistent determinations.

1 The Influence of Storage Time and Temperature on Propofol Concentrations in Canine
2 Blood and Plasma

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30 Abstract.

31 Propofol is an intravenous anesthetic commonly used due to its favorable
32 pharmacokinetic and pharmacodynamic profile. There are discrepancies in the literature
33 about the most appropriate sample for determining propofol concentrations. Although
34 plasma has been used for determining propofol concentrations, whole blood has been
35 the preferred sample because propofol is significantly bound to erythrocytes. There is
36 also a lack of consistency in the literature on the effect of storage time and temperature
37 on propofol concentrations and this may lead to errors in the design of
38 pharmacokinetic/pharmacodynamics studies. The purpose of this study was to
39 determine the difference in propofol concentrations in whole blood versus plasma and to
40 evaluate the influence of storage time (56 days) and temperature (4°C, -20°C, -80°C) on
41 the stability of propofol concentrations in blood and plasma samples. Results from the
42 study indicate that whole blood and plasma samples containing propofol were stable for
43 at least 56 days when stored at -80°C; thus, -80°C is the most appropriate temperature
44 for propofol sample storage out of the three temperatures evaluated. Plasma propofol
45 concentrations were consistently higher than whole blood for all 3 storage temperatures.
46 Consequently, plasma is the most appropriate sample for propofol analysis due to its
47 consistent determinations.

48 INTRODUCTION.

49 Propofol is a short-acting intravenous anesthetic, which is associated with
50 smooth and rapid inductions and recovery and is commonly used in dogs and other
51 veterinary patients (Robertson et al., 1992; Zoran et al., 1993; Mandsager et al., 1995).
52 Propofol is weakly acidic, and drugs of this type are generally considered to bind to

53 albumin in plasma. It is also a lipophilic drug, and despite being highly (98%) bound to
54 serum/plasma proteins (Servin et al., 1988), it is approximately 50% bound to
55 erythrocytes (Mazoit & Samili, 1999). Data from pharmacokinetic/pharmacodynamics
56 studies based on the relationship between blood propofol concentrations and its effects
57 have been used to design propofol dosage regimens for anesthesia (Cuadrado et al.,
58 1998); however, differences in measured propofol concentrations due to the effects of
59 storage time and temperature on plasma and whole blood samples may influence the
60 dosage regimen design. Blood has been the medium of choice for determining propofol
61 concentrations because propofol is significantly bound with the formed components of
62 blood. (Adam et al., 1981; Plummer 1987; Chan & So, 1990). However, plasma (or
63 serum) has been used for pharmacokinetic and pharmacodynamics studies, information
64 comparing propofol concentrations in those fluids with whole blood is scarce: in some
65 studies, a plasma/blood ratio close to 1 has been described (Servin et al., 1988;
66 Coetzee et al., 1995), but ratios between 0.64 and 1 have also been reported
67 (Cuadrado et al., 1998). A ratio of 1 indicates that although propofol is extensively
68 protein bound, there is appreciable binding of the drug to the formed elements in blood,
69 probably to erythrocytes. Whether plasma concentrations reflect the effects of propofol
70 in humans and animals better than whole blood concentrations also remains to be
71 established. Consequently, there is discussion in the literature as to whether propofol
72 concentrations should be determined in whole blood or serum/plasma samples.

73 The method used to store samples may influence the concentration of drugs. It
74 has been suggested that blood samples used for propofol determination should not be
75 frozen (Plummer 1987), and that propofol concentrations in samples stored at 4°C are

76 stable for up to 1 week (Adam et al., 1981), 2 weeks (Cuadrado et al., 1998) or 12
77 weeks (Plummer 1987). Bienert et al. (2005) suggested that blood should not be stored
78 at -20°C because of significant propofol loss, although storage at 4°C is acceptable;
79 however, they recommend that samples should be analyzed as soon as possible. In
80 contrast, plasma samples are stable at 4°C for 60 days, and plasma provides a better
81 matrix for propofol analysis.

82 The pharmacokinetics of propofol have been widely investigated, usually by
83 determination of propofol in whole blood by the use of high performance liquid
84 chromatography. The authors' laboratory started analyzing propofol samples in 2009
85 and, at that time whole blood analysis for propofol seemed to be the most appropriate
86 method because of its interaction with erythrocytes. However, due to recent discussions
87 in the literature it was felt that a re-evaluation of the sample matrix for propofol analysis
88 was warranted. Additionally, samples are stored -80°C in the laboratory and presently
89 there are no data in the literature about sample stability at this temperature. Thus, the
90 purpose of this study was to determine the effect of storage duration and temperature
91 on stability of propofol concentrations in blood and plasma and determine if blood or
92 plasma is an appropriate sample matrix. To achieve the objectives of this study, the
93 following three specific aims were pursued: 1) compared the stability of propofol
94 concentrations between blood and plasma samples. It was hypothesized that plasma is
95 an acceptable sample for propofol studies. 2) Determined the stability of propofol
96 concentrations at 4°C, -20°C, and -80°C storage temperatures in blood and plasma.
97 The working hypothesis was that -80°C is an acceptable storage temperature for
98 propofol studies. 3) Determined the stability of propofol concentrations at various

99 storage lengths (Day 1, 7, 14, 21, 28, 35, 42, 49 and 56) in blood and plasma. It was
100 hypothesized that the stability of propofol would decrease with increased storage
101 duration.

102 **Materials and Methods.**

103 **Equipment.**

104 Propofol was separated on a Waters XBridge C₁₈ (4.6 x 250 mm, 5 µm) column
105 with an XBridge C₁₈ guard column. The mobile phase was a mixture of (A) water
106 adjusted to pH 4.0 with glacial acetic acid and (B) acetonitrile (31:69). The flow rate was
107 1.5 mL/min and the column temperature ambient (24°C). The fluorescence detector
108 was set at an excitation of 276 nm and an emission of 310 nm with the gain at 10x.

109 **Reagents and solutions.**

110 Propofol was purchased from U.S. Pharmacopeia (Rockford, MD, USA), and 2,4-
111 ditert-butylphenol (purity 99%) was purchased from Sigma-Aldrich (Saint Louis, MO,
112 USA). All other reagent grade chemicals were purchased from Fisher Scientific
113 (Pittsburg, PA). Water was obtained from a Barnstead (Dubuque, IA) Nanopure Infinity
114 ultrapure water system.

115 Propofol and 2,4-ditert-butylphenol (internal standard) were dissolved in
116 methanol to produce stock concentrations of 100 µg/mL. Dilutions of the propofol stock
117 solution were prepared to produce 1 and 10 µg/mL working stock solutions. Standards
118 were aliquoted into 2 mL vials to prevent evaporation and cross contamination. All
119 solutions were protected from light in bottles wrapped in aluminum foil and stored at -
120 20°C. Standards were stable for 4 months at this temperature. Standard curves were

121 prepared by fortifying untreated plasma or blood with propofol to produce a linear
122 concentration range of 5-7000 ng/mL.

123 **Sample Collection.**

124 Fifteen adult, male dogs from the UTCVM research colony were determined to
125 be healthy based on results of physical examination, chemistry panel and history were
126 used. Venous blood collected from the jugular vein of each dog was pooled to minimize
127 the impact of individual differences in blood composition (i.e. hematocrit, total protein
128 concentrations) among dogs. The volume of blood collected from each dog did not
129 exceed 1% of body weight in kilograms. The study was approved by the Institutional
130 Animal Care and Use Committee at the University of Tennessee (Protocol number
131 2241-0214).

132 Enough pooled blood was centrifuged to provide plasma for the plasma portion of
133 the study. All blood and plasma samples were immediately spiked with propofol
134 calibration standards (17, 150, 350, 1500, 3500, and 5500 ng/mL) and placed in vials
135 labeled day 1, 7, 14, 21, 28, 35, 42, 49 and 56 for analysis on those dates. Samples
136 were then placed in their respective storage locations (4°C, -20°C, -80°C). Day 1 samples
137 were analyzed immediately after spiking.

138 **Extraction procedure.**

139 Canine plasma and blood samples were analyzed using a reverse phase high-
140 performance liquid chromatography (HPLC) method (Yarbrough et al., 2012). The
141 system consisted of a 2695 separations module, a 2475 fluorescence detector and a
142 computer equipped with Empower software. Propofol was extracted from plasma or
143 blood samples by a liquid extraction method. Briefly, previously frozen samples were

144 thawed and vortexed, and 400 μL were transferred to a clean test tube followed by 10
145 μL of internal standard (100 $\mu\text{g}/\text{mL}$ 2,4-ditert-butylphenol). One milliliter of acetonitrile-
146 methanol (75:25) was added and the tubes vortex mixed, covered and placed in the
147 refrigerator for 10 min. The tubes were vortex mixed for 10 seconds and centrifuged for
148 15 minutes at 1000 x g . The supernatant was removed to a clean tube. The procedure
149 was repeated with an additional 0.5 mL of acetonitrile-methanol, and that supernatant
150 combined. The tubes were centrifuged for 5 min and supernatant was placed in
151 chromatographic vials and 100 μL analyzed.

152 **Statistical analysis.**

153 Actual values and comparative changes in values of the different measured
154 propofol concentrations in blood and plasma samples at varying storage temperatures
155 were used to summarize the effect of storage length on stability of propofol
156 concentration. Graphical representations of some of the above-assessed effects are
157 presented. Validation parameters for plasma were also calculated.

158 **RESULTS.**

159 Samples were analyzed on days 1, 7, 14, 21, 28, 35, 42, 49, and 56.
160 Concentrations were determined with the exception of two blood samples (17 and 150
161 ng/mL) on day 56 stored at -20°C that did not result in any detectable propofol. In both
162 sample media (blood and plasma), samples stored at -20°C resulted in the lowest
163 propofol measurements of all 3 storage temperatures evaluated. Samples stored at -
164 80°C had the highest propofol measurements for both media.

165 Actual values and descriptive statistics (changes in the propofol concentration) of
166 the different measured propofol concentrations in plasma (Table 1) and in blood (Table

167 2) at varying storage temperatures were used to summarize the effect of storage length
168 on the stability of propofol concentration. Due to the large number of graphs generated
169 by this study, graphical representations of the above assessed effect are only presented
170 for two plasma (1500 and 5500 ng/mL) and blood (17 and 3500 ng/mL) concentrations
171 (Figures 1(A & B) and 2 (A & B)).

172 Table 1. Propofol plasma concentrations measured for 56 days at 4°C, -20°C, and -
 173 80°C.

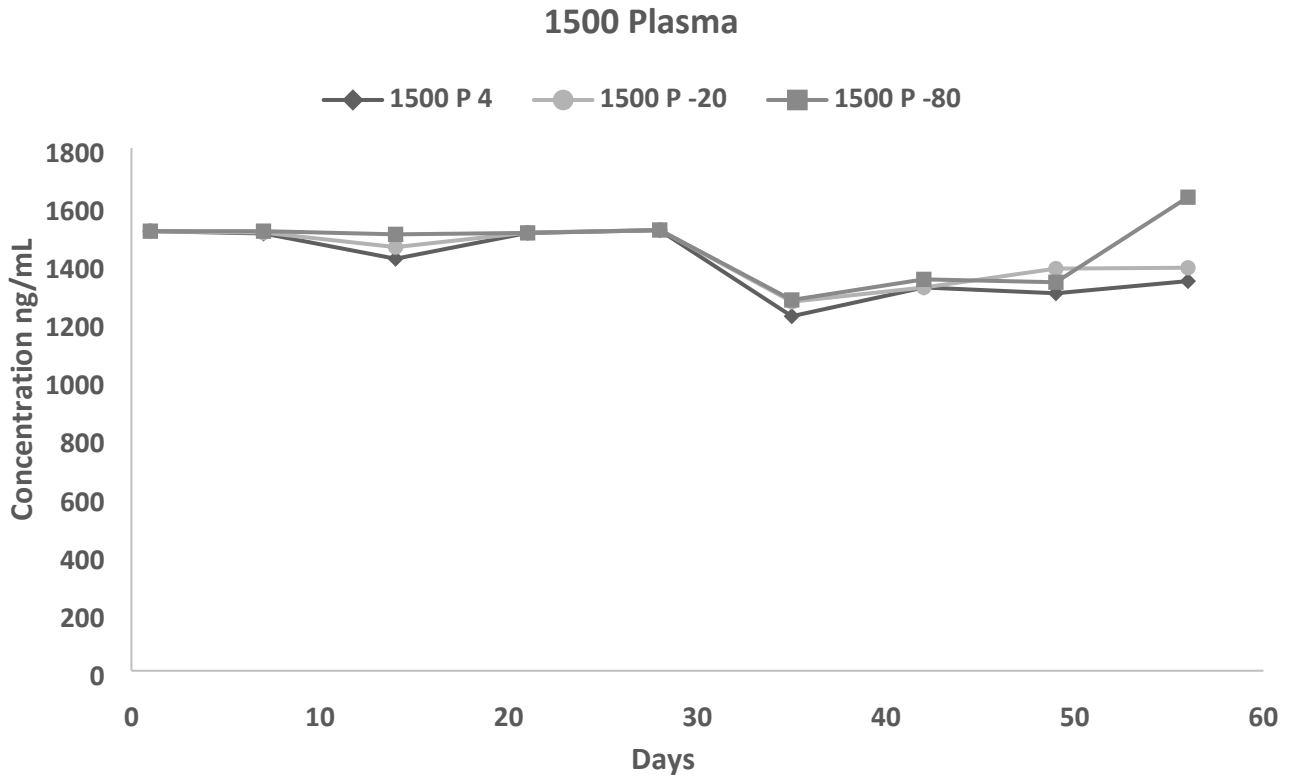
Temp	Concentration at different days of storage (change in concentration from starting concentration)								
Starting conc.	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
4°C									
17	17 (0)	20 (+3)	19 (+2)	13 (-4)	16 (-1)	12 (-5)	16 (-1)	11 (-6)	13 (-4)
150	155 (+5)	155 (+5)	157 (+7)	153 (+3)	169 (+9)	126 (-24)	123 (-27)	153 (+3)	147 (-3)
350	347 (-3)	336 (-14)	310 (-40)	357 (+7)	357 (+7)	313 (-37)	325 (-25)	323 (27)	246 (-104)
1500	1514 (+14)	1506 (+6)	1419 (-81)	1508 (+8)	1517 (+17)	1221 (-279)	1320 (-180)	1300 (-200)	1342 (-158)
3500	3226 (-274)	3396 (-104)	3225 (-275)	3349 (-151)	3439 (-61)	2847 (-653)	2912 (-588)	2940 (-560)	3229 (-271)
5500	5379 (-121)	4414 (-1086)	5033 (-467)	4687 (-813)	5156 (-344)	4541 (-959)	4349 (-1151)	4284 (-1216)	4235 (-1265)
-20°C									
17	17 (0)	12 (-5)	13 (-4)	13 (-4)	10 (-7)	18 (+1)	11 (-6)	17 (0)	14 (-3)
150	155 (+5)	147 (-3)	157 (+7)	154 (+4)	142 (-8)	128 (-22)	137 (-13)	145 (-5)	142 (-8)
350	347 (-3)	351 (+1)	288 (-62)	354 (+4)	356 (+6)	326 (-24)	324 (-26)	261 (-115)	282 (-68)
1500	1514 (+14)	1509 (+9)	1459 (-41)	1509 (+9)	1415 (-85)	1271 (-229)	1320 (-180)	1385 (-115)	1388 (-112)
3500	3226 (-274)	3158 (-342)	3136 (-364)	3283 (-217)	3191 (-309)	2693 (-807)	2533 (-967)	2703 (-797)	2912 (-588)
5500	5379 (-121)	4471 (-1029)	3717 (-1783)	4779 (-721)	5456 (-44)	4036 (-1464)	4176 (-1324)	4407 (-1093)	3886 (-1614)
-80°C									
17	17 (0)	15 (-2)	16 (-1)	16 (-1)	15 (-2)	13 (-4)	13 (-4)	13 (-4)	13 (-4)
150	155 (+5)	169 (+19)	160 (+10)	154 (+4)	153 (+3)	140 (-10)	131 (-19)	140 (-10)	162 (+12)
350	347 (-3)	347 (-3)	346 (-6)	353 (+3)	340 (-10)	313 (-37)	314 (-36)	337 (-13)	357 (+7)
1500	1514 (+14)	1514 (+14)	1503 (+3)	1508 (+8)	1518 (+18)	1277 (-223)	1348 (-152)	1338 (-162)	1631 (+131)
3500	3226 (-274)	3325 (-275)	3271 (-229)	3223 (-277)	3534 (+34)	2827 (-673)	2667 (-833)	2407 (-1093)	2431 (-1069)
5500	5379 (-121)	5156 (-344)	5425 (-75)	5331 (-169)	5526 (+26)	4642 (-858)	4242 (-1238)	3980 (-1520)	4540 (-960)

174 Results reported in ng/mL.

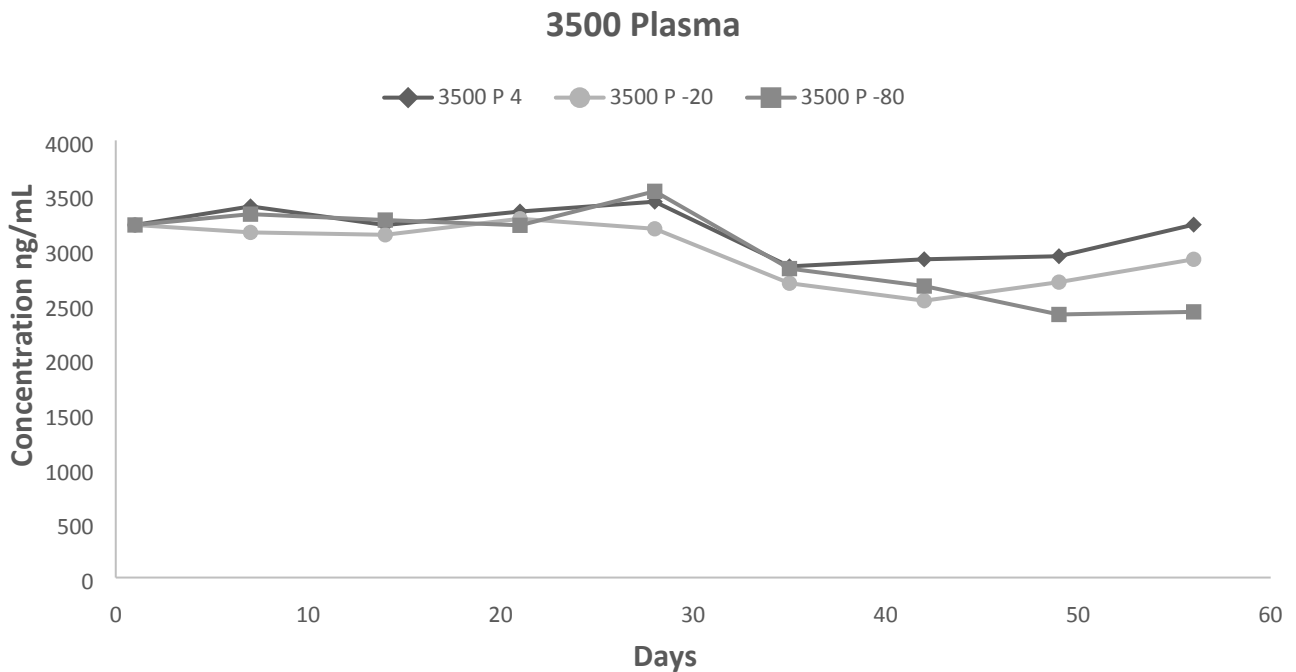
175 Table 2. Propofol blood concentrations measured for 56 days at 4°C, -20°C, and -80°C.

Temp	Concentration at different days of storage (change in concentration from starting concentration)								
Starting conc.	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
4°C									
17	18 (+1)	16 (-1)	16 (-1)	21 (+4)	16 (-1)	19 (+2)	17 (0)	22 (+5)	13 (-4)
150	149 (-1)	145 (-5)	145 (-5)	141 (-9)	140 (-10)	145 (-5)	141 (-9)	133 (-17)	126 (-24)
350	355 (+5)	345 (-5)	344 (-6)	351 (+1)	351 (+1)	227 (-123)	196 (-154)	220 (-130)	179 (-171)
1500	1490 (-10)	1417 (-83)	1314 (-186)	1296 (-204)	1238 (-262)	792 (-708)	913 (-587)	1030 (-470)	825 (-675)
3500	3189 (-311)	2409 (-1091)	2457 (-1043)	2340 (-1160)	2426 (-1074)	1918 (-1582)	1947 (-1553)	1817 (-1683)	1878 (-1622)
5500	4558 (-942)	3974 (-1526)	4074 (-1426)	3878 (-1622)	4246 (-1254)	3972 (-1528)	3431 (-2069)	3512 (-1988)	2713 (-2787)
-20°C									
17	18 (+1)	17 (0)	23 (+6)	26 (+6)	10 (-7)	5 (-12)	5 (-12)	9 (-8)	ND
150	149 (-1)	122 (-28)	163 (+13)	163 (+13)	73 (-77)	53 (-97)	35 (-115)	60 (-90)	ND
350	355 (+5)	190 (-160)	217 (-133)	254 (-96)	280 (-70)	159 (-191)	115 (-235)	115 (-235)	92 (-258)
1500	1490 (-10)	1160 (-340)	1233 (-267)	1217 (-283)	1418 (-82)	1122 (-378)	909 (-591)	1003 (-497)	777 (-723)
3500	3189 (-311)	2257 (-1243)	3156 (-344)	3139 (-361)	2659 (-841)	1788 (-1712)	1262 (-2238)	1301 (-2199)	1007 (-2493)
5500	4558 (-942)	4309 (-1191)	4184 (-1316)	4729 (-771)	4126 (-1374)	3455 (-2045)	2994 (-2506)	3026 (-2474)	2404 (-3096)
-80°C									
17	18 (+1)	16 (-1)	21 (+4)	25 (+8)	19 (+2)	20 (+3)	19 (+2)	21 (+4)	19 (+2)
150	149 (-1)	154 (+4)	133 (-17)	1355 (-15)	142 (-8)	151 (+1)	150 (0)	158 (+8)	155 (+5)
350	355 (+5)	341 (-9)	358 (+8)	345 (-5)	349 (-1)	304 (-46)	357 (+7)	312 (-38)	300 (-50)
1500	1490 (-10)	1201 (-299)	1375 (-125)	1297 (-203)	1488 (-12)	1196 (-304)	1198 (-302)	1293 (-207)	1289 (-211)
3500	3189 (-311)	2286 (-1214)	3122 (-378)	3112 (-388)	2966 (-534)	2430 (-1070)	2422 (-1078)	2388 (-1112)	2225 (-1275)
5500	4558 (-942)	4092 (-1408)	4766 (-734)	4570 (-930)	4528 (-972)	3986 (-1514)	3822 (-1678)	3870 (-1630)	3655 (-1845)

176 ND, no propofol detected in sample; results reported at ng/mL.



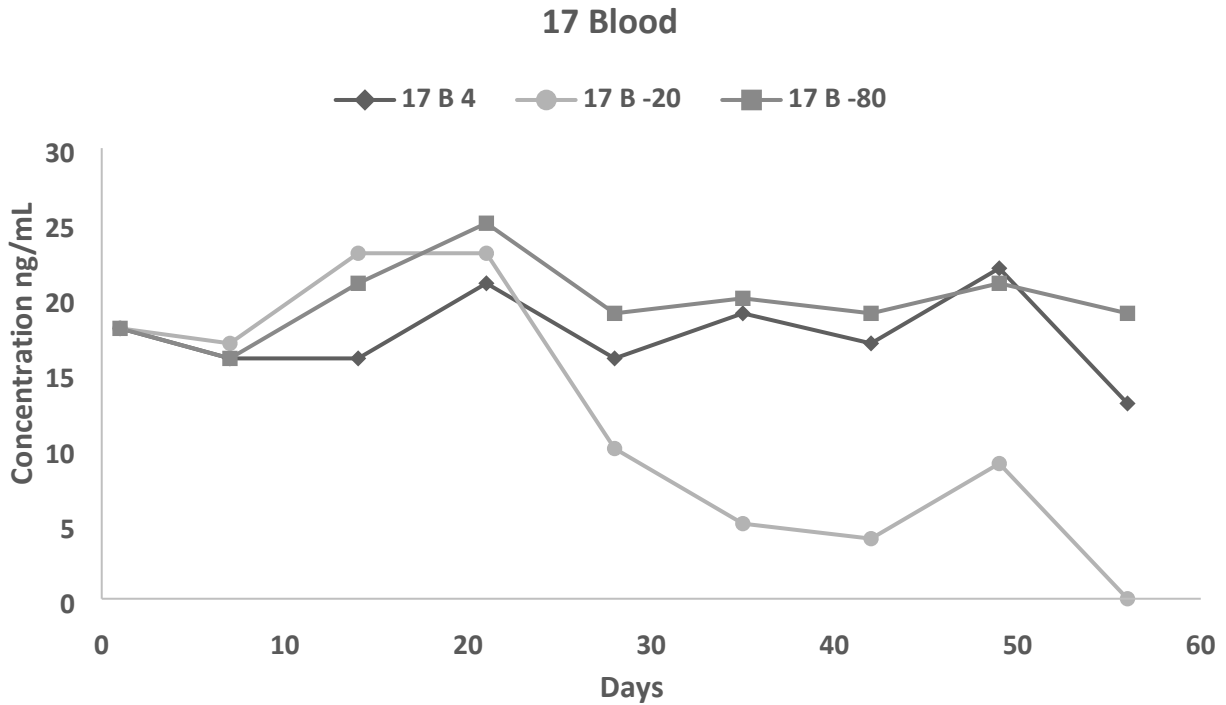
177



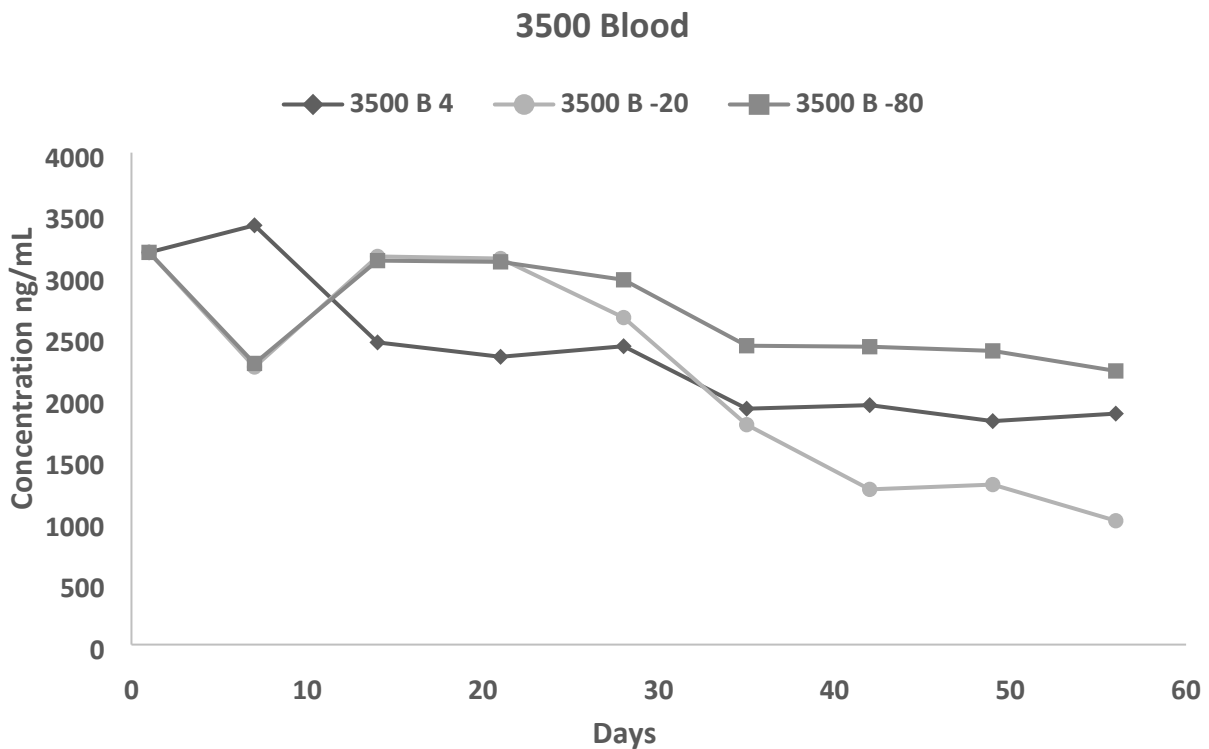
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179 Figure 1. Plasma concentrations spiked with (A) 1500 and (B) 3500 ng/mL of propofol

180 stored at 4°C, 20°C, and -80°C for 56 days.



181



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183 Figure 2. Blood concentrations spiked with (A) 17 and (B) 3500 ng/mL of propofol stored
184 at 4°C, 20°C, and -80°C for 56 days.

185 The same method of analysis that was previously used for propofol in whole
186 blood (Yarbrough et al., 2012) was used to analyze propofol plasma samples. The
187 method of analysis in plasma produced a linear curve over the same concentration
188 range as blood (5-7000 ng/mL) with an r^2 greater than 0.999. The intra and inter-assay
189 variability ranged from 2.8% – 10% and 3.8% – 6.0%, respectively which is very similar
190 to what was observed in the whole blood assay (intra and inter-assay variability ranged
191 from 2.0 to 10% and 0.6 to 11%). The average propofol plasma recovery was 91% while
192 the average recovery for 2,4-ditert-butylphenol was 90%. These recovery values were
193 the same as those for whole blood propofol. The lower limit of quantification was 5
194 ng/mL which is the same as the value for whole blood. Calibration curves were
195 constructed each day of analysis for whole blood and plasma.

196 **DISCUSSION.**

197 In order to determine the optimal conditions required for storing propofol
198 samples, blood and plasma were collected from healthy canines and the samples were
199 pooled in order to reduce the effects of inter-individual variability. A method of analysis
200 which was originally developed for determination of propofol in blood samples was
201 applied and validated for analysis of plasma samples. To determine whether blood or
202 plasma was the appropriate sample matrix and to determine the effect of storage
203 temperature and duration, blood and plasma samples were spiked with various amounts
204 of propofol that fell within a validated linear concentration range, and stored at three
205 different temperatures (4°C, -20°C, -80°C), and analyzed at various times up to 56 days.

206 Propofol was detected in blood for all six concentrations for the entire 56 days
207 except for 17 and 150 ng/mL stored at -20°C on day 56. There was a decrease in

208 propofol concentrations after day 21 for all six concentrations. The impact was greater
209 on samples stored at -20°C , with an overall average loss of 32% from day 21 to 28.
210 There was some propofol loss for the six different concentrations at 4°C (9%) and -80°C
211 (7%) but it is not as dramatic as the loss of propofol in samples stored at -20°C . The
212 overall loss of propofol from day 1 to day 56 ranged from 37%, 73% and 12% for 4°C , -
213 20°C , -80°C , respectively. The majority of the samples stored at -80°C had higher
214 concentrations than those stored at 4°C and -20°C which suggests that -80°C would be
215 an acceptable temperature to store whole blood propofol samples.

216 There were few differences in concentrations among the storage temperatures
217 for plasma samples. Propofol was detected in plasma for all six concentrations for the
218 56-day duration. However, after 28 days there was a decrease in propofol concentration
219 for 150, 350, 1500, 3500 and 5500 ng/mL for all three temperatures. The overall
220 average loss was 18%, 15%, and 14% for 4°C , -20°C , and -80°C , respectively. The
221 overall loss of propofol from day 1 to day 56 ranged from 15%, 15% and 8% for 4°C , -
222 20°C , -80°C , respectively. In general, the concentrations were more consistent in
223 plasma than in blood among the different storage temperatures. However, blood and
224 plasma propofol samples stored at -20°C had lower concentrations than those stored at
225 4°C and -80°C .

226 There were differences between plasma and blood propofol concentrations
227 stored at the three different temperatures. There were large differences in
228 concentrations between plasma and blood at 4°C for 1500, 3500 and 5500 ng/mL and
229 there were also differences in the 350, 1500, 3500, and 5500 ng/mL concentrations
230 stored at -20°C . Similar differences were also detected at -80°C , with plasma having

231 greater concentrations at 17, 1500, 3500, and 5500 ng/mL. Although not all plasma
 232 propofol concentrations were larger than blood concentrations, many of the individual
 233 plasma concentrations were larger than the corresponding blood concentrations, which
 234 suggests that plasma would be suitable for propofol analysis.

235 This method of analysis was applied to samples collected from a study (Reed et
 236 al., 2015) conducted previously in this facility in which canines were anesthetized with
 237 intravenously administered propofol (6 mg/kg loading dose with mean continuous rate
 238 infusion of 0.45 mg/kg/min for 185 ± 32 minutes) (Table 3). Whole blood and plasma
 239 samples were obtained and analyzed within two weeks of collection, after storage at -
 240 80°C . Plasma samples averaged 37% higher concentrations than the same whole blood
 241 samples. This is slightly higher than the average difference between the spiked blood
 242 and plasma samples from the current stability study. Because the samples were
 243 determined with different calibration curves this may have had an impact on the results;
 244 however, any differences should be minor because regression lines and other validation
 245 parameters were similar between the two matrices.

246

247 Table 3. Canine propofol results in blood and plasma samples collected at different time
 248 points during general anesthesia induced with an intravenous administration of 6 mg/kg
 249 and continuous rate infusion of 0.45 mg/kg/min.

Sample	Blood Propofol ng/mL	Plasma Propofol ng/mL
Mitchell W	1355	4143
Mitchell E	3795	7207
Mitchell 60	6194	6799
Quincy W	1395	2388
Quincy E	1952	3209
Quincy 60	5713	7275
Houston W	2497	4073

Houston E	5717	9061
Houston 60	3840	5658

250 Mean anesthesia time (\pm SD) was 185 ± 32 minutes. W, samples collected at walking
251 when animals were able to walk upon recovery from anesthesia; E, samples collected at
252 endotracheal extubation time upon recovery from anesthesia; 60, samples collected at
253 60 minutes after anesthetic induction.

254

255 Although blood has been recommended as the preferred sample for the analysis
256 of propofol concentrations by some authors (Adam et al., 1981; Plummer 1987; Chan &
257 So, 1990) the results of the present study indicate a difference between blood and
258 plasma propofol concentrations, at least under the experimental conditions applied in
259 the study. Moreover, the reproducibility of plasma propofol determinations was clearly
260 better than or equal to the reproducibility of whole blood determinations. Thus, it is
261 important to stress that from an analytical standpoint plasma is the most appropriate
262 sample matrix for propofol analysis. Plasma was also determined to be the sample of
263 choice when compared to blood in a study conducted by Bienert et al. (2005) and found
264 to have more consistent results when compared to blood propofol concentrations in
265 mammalian species (Grossherr et al., 2007). Dawidowicz et al. (2000) also saw a
266 significant decrease in blood propofol concentrations compared to minor changes in
267 plasma samples during 24 days of storage.

268 **Conclusion.**

269 In summary, whole blood and plasma samples containing propofol appear to be
270 stable for at least 56 days when stored at -80°C ; thus, -80°C is an appropriate
271 temperature for propofol sample storage. Plasma propofol concentrations were
272 consistently higher than whole blood for all 3 storage temperatures and from Reed et al.
273 (2015) canine study samples. Consequently, plasma is a more suitable sample matrix

274 than blood for propofol analysis providing consistent determinations. The HPLC method
275 that was developed and validated allows for the determination of whole blood and
276 plasma propofol concentrations and is appropriate for use in pharmacokinetic studies.

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