It has been clearly established that perioperative administration of antimicrobials can decrease the incidence of SSI.\textsuperscript{1–6} Antimicrobials are recommended for procedures associated with high risk of infection or when postoperative infection would have catastrophic consequences on the outcome of surgery.\textsuperscript{3,7–9} In humans and other animals, SSI can be a devastating complication, prolong the duration of hospital stay, and dramatically increase medical costs.\textsuperscript{5,10,11} There is conflicting evidence about the efficacy of prophylactic administration of antimicrobials in veterinary medicine; some investigators detected no effect,\textsuperscript{12} whereas other investigators detected a decrease in the incidence of SSI for routine clean surgical procedures.\textsuperscript{3,13} In a randomized blinded prospective controlled study,\textsuperscript{3} the infection rate for control dogs (15.7%) was significantly higher than the rate for dogs treated perioperatively with antimicrobials (3.8%). In another study,\textsuperscript{13} 347 of 365 (95.3%) dogs that underwent orthopedic surgery received antimicrobials perioperatively. Only 5 of those 347 (0.01%) dogs developed SSI, whereas 3 of 16 (18.7%) dogs that did not receive antimicrobials perioperatively developed SSI. Investigators of other studies\textsuperscript{14–16} have found an overall infection rate between 5.9% and 8.9% for a variety of clean and clean-contaminated procedures, and they have concluded that prophylactic antimicrobial administration was not required for these procedures.

Antimicrobial-resistant bacteria (including multi-drug resistant bacteria), increased risk of hospital-acquired infection, and increased cost of medical care are possible consequences of inappropriate or

**Pharmacokinetics of cefazolin for prophylactic administration to dogs**

**OBJECTIVE**
To evaluate pharmacokinetics of cefazolin after IV injection of cefazolin (22 mg/kg) and after simultaneous IV and IM injections of cefazolin (total dose, 44 mg/kg) to dogs.

**ANIMALS**
12 adult Beagles.

**PROCEDURES**
Dogs (6/group) were assigned to receive a single injection of cefazolin (IV group; 22 mg/kg, IV) or simultaneous injections (IV + IM group; 22 mg/kg, IV, and 22 mg/kg, IM). Interstitial fluid was collected over a 5-hour period by use of ultrafiltration probes for pharmacokinetic analysis.

**RESULTS**
Mean cefazolin concentration in the interstitial fluid at 1, 1.5, 2, 3, 4, and 5 hours after injection was 39.6, 29.1, 21.2, 10.3, 6.4, and 2.7 µg/mL, respectively, for the IV group and 38.3, 53.3, 46.4, 31.7, 19.1, and 8.9 µg/mL, respectively, for the IV + IM group. Mean area under the concentration-time curve extrapolated to infinity, maximum concentration, half-life, and time to maximum concentration was 74.99 and 154.16 h•µg/mL, 37.3 and 51.5 µg/mL, 0.96 and 1.11 hours, and 1.28 and 1.65 hours, respectively, for the IV and IV + IM groups.

**CONCLUSIONS AND CLINICAL RELEVANCE**
Cefazolin concentrations in interstitial fluid of dogs were maintained at > 4 µg/mL for 4 hours after a single IV injection and for 5 hours after simultaneous IV and IM injections. Therefore, simultaneous IV and IM administration of cefazolin 30 to 60 minutes before surgery should provide interstitial fluid concentrations effective against the most common commensal organisms (*Staphylococcus* spp and *Streptococcus* spp) on the skin of dogs for surgical procedures lasting ≤ 4 hours. (Am J Vet Res 2017;78:695–701)
indiscriminate use of antimicrobials. Current guidelines in human medicine include the use of antimicrobials only in clean-contaminated, contaminated, or dirty procedures; administration of the first dose of antimicrobial 1 hour before the first incision; readministration of the antimicrobial during surgery if the procedure is still ongoing after 2 half-lives of the drug have passed; restriction of treatment to the duration of surgery or for 24 hours, except in certain situations (ie, gross contamination or preexisting infection); and avoiding use of newer broad-spectrum antimicrobials. In 1 study, prophylactic administration of antimicrobials to humans was often not consistent with local or national guidelines because a redosing schedule was followed at an appropriate time in only 40% of the patients, although this improved to 68% when an automatic reminder system was used. In another study of humans at a tertiary teaching hospital, an appropriate medication, dose, duration, and redosing schedule in accordance with the hospital guidelines was used for only 3% of the patients. Similarly, there are discrepancies for antimicrobial use and standard recommendations with regard to timing of administration before and during surgery, duration, and antimicrobial prophylaxis for dogs undergoing orthopedic surgery. In 1 study, 16% of the dogs did not receive the antimicrobial within 60 minutes after surgery, 19% received unnecessary repeated doses of the antimicrobial, and 49% received additional doses of the antimicrobial at an incorrect time.

The antimicrobial selected for prophylactic use must be effective against the pathogen or pathogens most likely to cause postoperative wound infection. Antimicrobials with efficacy against commonly encountered pathogens, such as Staphylococcus spp, Streptococcus spp, and sometimes Escherichia coli, are usually recommended for use in veterinary medicine on the basis of the surgery location. Cephalosporins are effective antimicrobials, are tolerated well, and achieve targeted serum and tissue concentrations. Cefazolin has been recommended as the ideal prophylactic antimicrobial for dogs undergoing surgery and has become one of the antimicrobials most commonly used perioperatively because of its spectrum, low incidence of adverse effects, and low cost. For antimicrobial prophylaxis to be effective, an adequate concentration of the drug must be present at the surgical site before the time of contamination and throughout the surgical procedure. The β-lactam antimicrobials (penicillins and cephalosporins) are time-dependent drugs, which means that efficacy is correlated with the amount of time that drug concentration remains above the MIC for a particular pathogen. On the basis of results of pharmacokinetic studies, it has been recommended that time-dependent antimicrobials such as β-lactams should be readministered every 2 half-lives during surgery to maintain targeted plasma concentrations.

In a previous study, it was suggested that the concentration of cefazolin in the interstitial fluid is similar to the concentration in plasma owing to rapid equilibration of cefazolin between serum and soft tissues in a surgical wound. To our knowledge, the concentration of cefazolin in the surgical site has been measured and compared with serum concentrations (by obtaining muscle biopsy specimens and determining the antimicrobial concentration with a modified agar plate diffusion technique) in only 2 studies. However, tissue concentrations may underestimate true surgical site concentrations because the interstitial fluid is diluted with intracellular fluid. An ultrafiltration probe has been used to obtain interstitial fluid in other studies. It has been found that this is a reliable, easily performed, and useful method for the evaluation of drug disposition in dogs, and it eliminates the need for collection of tissue samples or use of tissue cages to estimate concentrations in tissues.

The purpose of the study reported here was to compare the cefazolin concentration in interstitial fluid obtained from dogs receiving a single IV injection of cefazolin and dogs receiving simultaneous IV and IM injections of cefazolin. We hypothesized that the concentration in the interstitial fluid would be higher and more prolonged in the group receiving simultaneous IV and IM injections, compared with results for the group receiving only an IV injection.

Materials and Methods

Animals

Twelve purpose-bred Beagles (6 males and 6 females) were used in the study. All dogs were 1 year old and considered healthy; a physical examination, CBC, and serum biochemical profile were performed to verify health of the dogs. All dogs had an albumin concentration > 3.4 g/dL (range, 3.4 to 4.2 g/dL). All dogs were allowed to acclimatize to the environment before initiation of the study. The study was reviewed and approved by the Institutional Animal Care and Use Committee at Kansas State University.

Implant placement

Dogs were sedated with dexmedetomidine hydrochloride (15 µg/kg, IV). An indwelling percutaneous catheter was placed in a jugular vein, and 2 ultrafiltration probes were placed in the dorsum of each dog. The ultrafiltration probes contained 3 loops with a 12-cm semipermeable membrane. The semipermeable membrane in the loop consisted of pores that allowed water, electrolytes, and low-molecular-weight (< 30 KDa) molecules to diffuse across the membrane, but excluded the passage of proteins, protein-bound drugs, and other high-molecular-weight compounds. For insertion of the ultrafiltration probes, an area (2.5 cm on each side of the midline at the dorsal caudalateral aspect of the scapulae) was shaved and aseptically prepared. One of the insertion sites was infused with a solution of 2% lidocaine hydrochloride.
(1 mg/kg), a stab incision was made through the skin with a No. 11 scalpel blade, and subcutaneous tissues were identified. An introducer needle was inserted in the stab incision, advanced cranially through the subcutaneous tissues for a distance of 10 cm, and exited through the skin; the ultrafiltration probe was then threaded through the needle from a cranial to caudal direction until the tip of the probe was flush with the tip of the introducer needle. The introducer needle containing the probe was then retracted 3 cm so that the 3 loops of the probe remained under the skin in the interstitial space and the nonpermeable portion of the probe remained external to the dog’s skin. The ultrafiltration probe was then secured to the skin with a nonabsorbable nylon suture\(^a\) by use of a fingertrap pattern. A vacuum-vial needle\(^e\) was attached to the ultrafiltration probe tubing, and a collection tube was attached to that needle to apply negative pressure on the probe system for collection of interstitial fluid through the semipermeable membrane. The probe insertion procedure then was repeated for the opposite side. After the ultrafiltration probes were inserted, sedation was reversed by administration of atipamezole hydrochloride\(^f\) (2.4 mg/kg, IM). The initial collection tubes were allowed to remain in place for ≥ 18 hours to equilibrate the system before the initiation of the study.

**Experimental design**

Dogs were assigned to 2 groups (6 dogs/group) by use of randomizing software.\(^g\) At 24 hours after placement of the IV catheter and filtration probes, dogs of one group (IV group) received an injection of cefazolin\(^h\) (22 mg/kg, IV) and dogs of the other group (IV + IM group) received simultaneous IV and IM injections of cefazolin (22 mg/kg, IV; and 22 mg/k g, IM).

**Sample collection**

Interstitial fluid was collected in a microcentrifuge tube\(^i\) inserted in a red top vacuum tube\(^j\) before (time 0) and 1, 1.5, 2, 3, 4, and 5 hours after administration of cefazolin. Samples were immediately placed on ice. All samples subsequently were stored at -70°C until testing was performed.

**UPLC-UV drug analysis**

Concentrations of cefazolin in interstitial fluid were determined by use of UPLC-UV\(^k\) at 271 nm. The mobile phase consisted of 1.7% formic acid in deionized water (solution A) and 1% formic acid in acetonitrile (solution B). The mobile phase gradient started at 90% solution A, decreased to 30% solution A from 0.1 to 2 minutes, decreased to 14.2% solution A at 2.5 minutes, and increased to 90% solution A at 2.51 minutes (total run time, 3.5 minutes). A C18 column\(^l\) maintained at 40°C was used for separation. The sample tray was maintained at 4°C, and injection volume was 5 µL. Interstitial fluid samples were injected directly into the UPLC-UV without prior treatment. Standard curve and quality control samples were created with pooled canine interstitial fluid (linear range, 0.25 to 250 µg/mL). Accuracy of the assay determined by use of 6 replicates each for concentrations of 0.25, 5, and 250 µg/mL was 103%, 104%, and 92% of the actual concentration, respectively. Coefficient of variation for the assay determined by use of 6 replicates each for concentrations of 0.25, 5, and 250 µg/mL was 8%, 9%, and 6%, respectively.

**Statistical analysis**

Statistical analysis of the concentration-time curve was performed with a commercially available software package.\(^m\) Data were tested for equality of variance, and values for individual time points (1, 1.5, 2, 3, 4, and 5 hours) were compared between the 2 groups by use of an independent group means test. Pharmacokinetic analysis of interstitial fluid concentrations was performed with noncompartmental methods by use of computer software.\(^n\) Interstitial fluid pharmacokinetic parameters (AUC\(^{\text{INF}}\) [determined by use of the linear trapezoidal method] and t\(_{1/2}\)) were calculated for each dog, and descriptive statistics (geometric mean, minimum, and maximum values) were reported.\(^o\) Values for C\(_{\text{max}}\) and T\(_{\text{max}}\) were determined directly from the data. Statistical analysis of pharmacokinetic data was conducted with computer software\(^p\) by use of the Mann-Whitney rank sum test.\(^q\) Values were considered significant at P < 0.05.

**Figure 1**—Mean cefazolin concentration-time curve for concentrations in interstitial fluid after administration of a single dose (22 mg/kg, IV) to 5 dogs (IV group [circles]) and simultaneous IV and IM administration (22 mg/kg, IV, and 22 mg/kg, IM) to 6 dogs (IV + IM group [squares]). The MIC\(_{50}\) for *Streptococcus* spp (4 µg/mL [dashed line]) and the MIC\(_{90}\) for *Staphylococcus pseudintermedius* (2 µg/mL [dotted line]) are indicated.
Results

The IV group initially consisted of 3 males and 3 females; however, 1 male was removed from the study because the dog removed the ultrafiltration devices before the initiation of the sample collection period. Thus, data were collected for 5 dogs in the IV group (mean body weight, 8.7 kg; range, 7.4 to 10.8 kg). The IV + IM group consisted of 3 males and 3 females (mean body weight, 9.7 kg; range, 8.7 to 10.7 kg).

Mean concentrations of cefazolin in interstitial fluid were measured for both groups (Figure 1; Table 1). Mean, minimum, and maximum concentrations of cefazolin obtained for the IV and IV + IM groups were plotted (Figure 2).

Comparing the mean cefazolin concentration in interstitial fluid between the IV and IV + IM groups revealed no significant difference at 1 hour (39.6 and 38.3 µg/mL, respectively) and 1.5 hours (29.1 and 53.3 µg/mL, respectively). However, the mean cefazolin concentration in interstitial fluid was significantly lower in the IV group, compared with the concentration in the IV + IM group, at 2 (21.2 and 46.4 µg/mL, respectively; \( P = 0.001 \)), 3 (10.3 and 20.4 µg/mL, respectively; \( P = 0.002 \)), 4 (6.4 and 19.1 µg/mL, respectively; \( P = 0.042 \)), and 5 (2.7 and 8.9 µg/mL, respectively; \( P = 0.003 \)).

Comparing the mean values for pharmacokinetic parameters between the IV and IV + IM groups revealed a significant (\( P = 0.004 \)) difference in AUC\(_{\text{INF}}\) (74.99 and 154.16 h·µg/mL, respectively; Table 2). In addition, the AUC\(_{\text{INF}}\) was dose related. There was no significant difference between the IV and IV + IM groups for Cmax (37.5 and 51.5 µg/mL, respectively), t\(_{1/2}\) (0.96 and 1.11 hours, respectively), and Tmax (1.28 and 1.65 hours, respectively).

Discussion

On the basis of results of the present study, we accepted the hypothesis that doubling the dose of cefazolin (22 mg/kg, IV, and 22 mg/kg, IM) added 1 half-life to persistence of the drug. Although not significant, the half-life was approximately 15% (approx...
10 minutes) longer for the IV + IM group, compared with the expected half-life if the IV dose had been doubled. There is a slightly longer drug exposure with every half-life. This slight increase in exposure time may be explained by a slower absorption rate when the drug is administered IM. After achieving equilibrium between the serum and interstitial fluid as a result of the IV injection of cefazolin, and given the constant elimination rate and low protein-binding capacity of the drug, the IM injection would increase the number of unbound molecules of cefazolin available for distribution for a longer period because of the higher total dose.

In the present study, concentrations of cefazolin in interstitial fluid in all dogs were maintained above 4 µg/mL for 4 hours after a single injection of cefazolin (22 mg/kg, IV) and for 5 hours after simultaneous IV and IM injections of cefazolin (22 mg/kg, IV, and 22 mg/kg, IM; total dose, 44 mg/kg). This concentration should provide antimicrobial activity against the most common methicillin-susceptible commensal organisms on the skin of dogs (Staphylococcus pseudintermedius and Streptococcus spp) for clean surgical procedures. On the basis of these results, cefazolin (22 mg/kg, IV, and 22 mg/kg, IM) administered 30 to 60 minutes before surgery for surgical procedures lasting ≤ 4 hours would achieve and maintain desired interstitial fluid concentrations. Cefazolin (22 mg/kg, IV) administered 30 to 60 minutes before surgery would maintain interstitial fluid concentrations above the MICso for dogs undergoing short (≤ 3 hours) surgical procedures. These recommendations were based on the lowest (minimum) cefazolin concentration, rather than on the mean concentration, to maintain targeted concentrations for all dogs in the present study to reduce the risk of developing an SSI. However, further studies are needed to confirm clinical extrapolation of these data. In the event that surgical time exceeds the aforementioned durations, administration of another dose of cefazolin (22 mg/kg, IV, at 4 hours after initial IV administration and 22 mg/kg, IV, at 5 hours after the initial IV and IM administration) should maintain targeted concentrations in the interstitial fluid.

Total drug exposure (AUCINF) was approximately twice as high after administration of 44 mg/kg (IV + IM group: 145.16 h·µg/mL), compared with the value after 22 mg/kg (IV group: 74.99 h·µg/mL), which suggested a dose-relation phenomenon. Interstitial fluid Cmax was less proportional (51.5 µg/mL after 44 mg/kg and 37.3 µg/kg after 22 mg/kg). The less-than-proportional Cmax most likely was attributable to a delay in Tmax caused by absorption of the IM portion of the dose. However, because cephalosporins are concentration-dependent antimicrobials, the lower-than-proportional Cmax for the IV + IM group would not be detrimental and could be beneficial for decreasing concentration-dependent adverse effects, compared with results after IV administration of 44 mg/kg.

Protein binding is a major factor for tissue distribution of a drug. To predict antimicrobial activity, it is important to know the concentration of the protein-unbound antimicrobial at the site of bacterial contamination (surgical site). Ultrafiltration provides the means for collecting protein-unbound cefazolin in the interstitial fluid by implantation of a semipermeable membrane in the tissue. Investigators of other studies have found that an ultrafiltration device is a reliable and convenient method for collecting interstitial fluid samples from tissues in dogs. This device has become the preferred method for collecting interstitial fluid, rather than collecting tissue biopsy specimens or using tissue cassettes, because of anatomic and physiologic relevance, lack of contamination from intracellular content, ease of insertion, collection of serial samples with the same device, and monitoring drug distribution in unrestrained animals. Furthermore, the ultrafiltration device provides a convenient method for continuous sample collection without residual wounds or lesions after removal of the ultrafiltration probes.

To our knowledge, the study reported here was the first in which an ultrafiltration probe was used to determine the cefazolin concentration in interstitial fluid. By use of this device, we were able to detect a biologically accurate concentration of cefazolin in what we anticipate will be equivalent to the tissue biophase or surgical site, rather than in serum, which is a critical factor for determining the efficacy of agents used for prophylaxis against SSIs.

Timing of antimicrobial administration and redosing schedules are not always in accordance with institution guidelines. In 1 study, investigators found that 78% of dogs received the first antimicrobial dose before surgery; however, only 84% of those dogs received the dose within 60 minutes before the first incision. Twelve percent of dogs were initially treated during surgery (10 to 165 minutes after the
first incision). If a guideline of repeated administration every 90 minutes after the first administration until closure of the surgical site were used, 51% of dogs received the required intraoperative administration, and 19% of dogs that did not require intraoperative administration were treated. In a more recent study, investigators found a redosing incidence of 93.5%, which they considered excellent; however, 28.4% of dogs received antimicrobials late, with the dose being administered more than 30 minutes late in 28% of those dogs. In the study reported here, administration of cefazolin (22 mg/kg, IV, and 22 mg/kg, IM) before surgery resulted in antimicrobial concentrations that should be adequate against the most common skin contaminants for surgical procedures expected to last ≤ 4 hours, and a redosing schedule would not be necessary.

The bacteria most commonly involved in SSIs of dogs and cats are commensal organisms on the skin (gram-positive cocci) and normal flora from the gastrointestinal and other tracts (predominantly gram-negative rods), depending on the surgical procedure. Historically, it has been recommended that time-dependent antimicrobials such as β-lactams be readministered during surgery every 2 half-lives to maintain therapeutic concentrations during surgery. Investigators of other studies have reported that the MIC₉₀ is 0.25 to 2 µg/mL for *S pseudintermedius*, 4 µg/mL for *Streptococcus spp.*, and 16 µg/mL for *E coli*. A more recent study of *E coli* revealed an MIC that inhibited 75% of isolates was 4 µg/mL and the MIC₉₀ was 128 µg/mL. It should be considered that although isolates were collected from 33 infection sites in that study, approximately 70% of the isolates were from the urinary tract, with the ear being a distant second (7.2% of isolates), and no other body system provided more than 4% of isolates. These isolates may have been exposed to various courses of antimicrobials prior to isolation (ie, recurrent urinary tract and otic infections) and may not be representative of bacteria that would typically be found in surgical patients.

The present study had some limitations. We were unable to determine pharmacokinetic parameters of cefazolin in serum because of presumed contamination of the percutaneous catheter with cefazolin during IV administration (data not included). Even if the residual drug in the catheter was 0.1%, it would have biased the data substantially. Injection of the cefazolin and collection of blood samples were conducted through the same catheter; therefore, the measured concentration of the drug in serum was not accurate, especially at early time points. Although we did not determine the pharmacokinetic parameters of cefazolin in serum, this information can be obtained from other studies. Furthermore, the primary site of interest in the present study was interstitial fluid. The AUC for serum after administration of a dose of 20 mg/kg is 135.9 h•µg/mL (8,158 µg•min/mL), and t₁/₂ is 0.91 hours (55.08 minutes). Investigators of another study found the AUC for serum after administration of a dose of 20 mg/kg is 82.5 h•µg/mL and t₁/₂ is 1.53 hours. In the present study, AUC for the interstitial fluid was 74.99 h•µg/mL and t₁/₂ was 0.96 hours after IV administration of a dose of 22 mg/kg, and AUC was 154.16 h•µg/mL and t₁/₂ was 1.11 hours after administration of a dose of 44 mg/kg (22 mg/kg, IV, and 22 mg/kg, IM). These results indicated that exposure to cefazolin after administration was extremely similar in the interstitial fluid and serum, which would indicate good penetration of the antimicrobial into a surgical site.

Purpose-bred research Beagles that were considered healthy were used in the present study. This may not be representative of the general population because there are patient variations associated with breed and size that can alter pharmacokinetics of cefazolin. Patients with underlying conditions or metabolic diseases that may increase glomerular filtration rate may also have increased clearance of cefazolin. Dogs in the present study were not anesthetized and not subjected to surgical conditions. Some operative factors, including prolonged anesthesia time, surgical procedures requiring > 90 minutes for completion, use of certain anesthetic drugs, and hypothermia, can result in a greater risk of SSI, possibly because of increased bacterial contamination, excessive tissue manipulation, and dehydration. It has been suggested that substantial hemorrhage necessitating volume expansion, blood transfusion, and vasopressor or inotropic administration may dilute or increase the clearance of hydrophilic compounds (including cephalosporins), which thereby decreases concentration of the drug.

Data for the present study supported clinical use such that a regimen of a total dose of 44 mg of cefazolin/kg (22 mg/kg, IV, and 22 mg/kg, IM) administered 30 to 60 minutes before surgical procedures expected to last ≤ 4 hours and a single injection of cefazolin (22 mg/kg, IV) administered 30 to 60 minutes before surgical procedures expected to last ≤ 3 hours should provide protection against the most common contaminants on the skin of dogs and cats (*S pseudintermedius* and *Streptococcus spp*). However, if *E coli* or other gram-negative bacteria are suspected, another antimicrobial and dosing regimen should be considered. Further studies are needed to validate these results in clinical settings and to assess interstitial fluid pharmacokinetics of cefazolin in anesthetized patients undergoing surgery.

**Acknowledgments**

This manuscript represents a portion of a thesis submitted by Dr. Gonzalez to the Kansas State University Department of Biomedical Sciences as partial fulfillment of the requirements for a Master of Science degree. Supported by an institutional grant from the Department of Clinical Sciences of the College of Veterinary Medicine at Kansas State University.
The authors thank Kimberly Kalosy, Nathan Kapaldo, Akaterina Davros, Stefanie Campbell, Amanda Schlager, Quwen Kou, and Samantha Belsan for laboratory assistance.

Footnotes

a. Dextranomitor, Zoetis, Florham Park, NJ.

b. Model MF-7023, BASI, West Lafayette, Ind.

c. 2% lidocaine hydrochloride, Hospira Inc, Lake Forest, Ill.

d. Ethilon 3-0, Ethicon Inc, Somerville, NJ.

e. Model MD-1320, BASI, West Lafayette, Ind.

f. Antisedan, Zoetis, Florham Park, NJ.

g. Microsoft Office Excel 2011, Microsoft Corp, Redmond,

h. Wash.

c. Cefazolin, Westward Pharmaceutical, Eatontown, NJ.


j. BD, Franklin Lakes, NJ.

k. Acuity UPLC, Waters Corp, Milford, Mass.

l. Cortecs, 50 X 2.1 mm, 2.7-μm pore size, Waters Corp, Milford Mass.

m. WINKS SDA 6, version 6.0.93, Texasoft Inc, Cedar Hill, Tex.

n. Phoenix 64, Certara, Princeton, NJ.

o. Sigma Plot, version 12.5, Systat Software, Chicago, Ill.

References


33. Tate RS, Akaterina Davros, Stefanie Campbell, Amanda Schlagel, Qiuwen Kou, and Samantha Belsan for laboratory assistance.

34. Akaterina Davros, Stefanie Campbell, Amanda Schlagel, Qiuwen Kou, and Samantha Belsan for laboratory assistance.


