Characteristics of commercially manufactured and compounded protamine zinc insulin

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Objective—To evaluate and compare characteristics of a commercially manufactured protamine zinc insulin (PZI) product and PZI products obtained from various compounding pharmacies.

Design—Evaluation study.

Sample—112 vials of PZI (16 vials of the commercially manufactured product and 8 vials from each of 12 compounding pharmacies) purchased over an 8-month period.

Procedures—Validated methods were used to analyze 2 vials of each product at 4 time points. Appearance, endotoxin concentration, crystal size, insulin concentration in the supernatant, pH, total insulin and zinc concentrations, and species of insulin origin were evaluated.

Results—All 16 vials of commercially manufactured PZI met United States Pharmacopeia (USP) specifications. Of 96 vials of compounded PZI, 1 (1%) contained a concentration of endotoxin > 32 endotoxin U/mL, 23 (24%) had concentrations of insulin in the supernatant > 1.0 U/mL, and 45 (47%) had pH values < 7.1 or > 7.4; all of these values were outside of specifications. Several vials of compounded PZI (52/96 [54%]) did not meet specifications for zinc concentration (0.06 to 0.1 mg/mL for 40 U of insulin/mL, 0.075 to 0.12 mg/mL for 50 U of insulin/mL, and 0.15 to 0.25 mg/mL for 100 U of insulin/mL), and total insulin concentration in 36 [38%] vials was < 90% of the labeled concentration.

Conclusions and Clinical Relevance—Only 1 of 12 compounded PZI products met all USP specifications in all vials tested. Use of compounded PZI insulin products could potentially lead to serious problems with glycemic control in veterinary patients. (*J Am Vet Med Assoc* 2012;240:600–605)

GENERAL

Protamine zinc insulin is a long-acting insulin preparation in which protamine and zinc are complexed with insulin to delay insulin absorption after injection and thus prolong its duration of effect.[1] Insulin is released from the site of injection by the action of proteolytic enzymes that degrade protamine. Zinc is known to inhibit these enzymes, which may explain the extensive duration of action.[2] Proper release of PZI depends on careful and consistent formulation of the product with 3 critical ingredients: protamine, zinc, and insulin. Protamine zinc insulin is a commonly used long acting insulin preparation that was shown to establish good glycemic control in 85% to 90% of diabetic cats in 2 studies.[3,4] Protamine zinc insulin of bovine and porcine origin was originally manufactured by a human pharmaceutical company for human use. It was later manufactured by an animal health pharmaceutical company for treatment of diabetes in cats until production was discontinued in 2008. Currently, an FDA-approved human recombinant PZI product is commercially available for use in cats and has been reported to result in clinical responses comparable to those achieved with PZI of bovine and porcine origin.[4,5] Because of the high cost and intermittent shortages of commercially manufactured PZI, alternative products have been made available to veterinarians by compounding pharmacies. Standards for compounding pharmacies are established by the USP, which is an independent organization that sets standards for the pharmaceutical industry and the practice of pharmacy.[6] Whether compounded PZI products consistently meet USP specifications for PZI[6] has not been previously reported.

The purpose of the study reported here was to evaluate and compare the quality and consistency of several

Abbreviations

cGMP	Current good manufacturing practices
d_{90}	Maximum diameter value that encompasses 90% of particles
d_{100}	Maximum diameter value that encompasses 100% of particles
HPLC	High-performance liquid chromatography
PUVTH	Purdue University Veterinary Teaching Hospital
PZI	Protamine zinc insulin
USP	United States Pharmacopeia

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compounded PZI products and 1 commercially manufactured PZI product that was available for use in cats at the time the study was performed. The hypothesis of the study was that compounded PZI would be less consistent in meeting the USP product standards than the commercially manufactured PZI product.

Materials and Methods

The PUVTH pharmacy ordered 12 compounded PZI products from 12 sources (Park Pharmacy and Compounding, Irvine, Calif; Martin Avenue Pharmacy Inc, Naperville, Ill; Professional Arts Pharmacy, Baltimore, Md; Meds 4 Vets Compounding Pharmacy, Sandy, Utah; Custom Compounding Pharmacies [formerly The Medicine Shoppe], Kingsport, Tenn; Red Oak Drug, Red Oak, Tex; Premier Pharmacy Labs Inc, Weeki Wachee, Fla; BCP Veterinary Pharmacy, Houston, Tex; Stokes Pharmacy, Mount Laurel, NJ; Franck's Pharmacy Compounding Laboratory, Ocala, Fla; Veterinary Pharmacies of America, Houston, Tex; and The Apothecary Shops, Phoenix, Ariz). One commercially manufactured PZI product was ordered from 2 sources.[a,b]

All pharmacies that provided PZI insulin at the time of the study were contacted. The pharmacies were identified by a combination of telephone-call queries, trade-show inquiries, and Web searches. Three 10-mL bottles of PZI (40 U of insulin/mL) were requested from each of these sources at 4 time points. If the requested concentration of PZI was not available from the pharmacy at the start of the study, a higher concentration (50 or 100 U of insulin/mL) was ordered. All orders were placed on a Monday morning, and the vials were shipped out to the laboratory for analysis the following Monday morning. Orders were placed every 2 months over a period of 6 months. Four sets of samples (8 vials of each product) were evaluated for each insulin source.

After receipt of each product at the PUVTH pharmacy, the date, time, condition of the shipment, labeled concentration, species of insulin origin, lot number, and expiration date were recorded, and the insulin was stored in a secure refrigerator at 3.5°C for 1 to 7 days until all orders for that time point had been received. The contents of 1 vial from each source were then repackaged in a glass vial under the direction of a pharmacy technician following a standard operating protocol; these vials were identified only by a single unique letter to mask investigators to product identification. Codes for the masked vials were randomly assigned by drawing lots, and the same code was used for each source throughout the study. All of the masked vials and 1 of each original vial were shipped overnight on ice to the cGMP quality control laboratory of the manufacturer of the commercial PZI product[a] for analysis, and 1 original vial was retained at the PUVTH pharmacy (as a contingency measure for problems that might occur during transport). The key code for masked vials was retained by the primary investigator at PUVTH.

Contents of the vials were analyzed by use of USP methods or cGMP methods that were adapted from USP and validated for use in the manufacture of commercial insulin products at the same quality control laboratory[a] where the tests were performed. Compendial methods were obtained from USP standards for PZI suspension,[6] except for the HPLC assay and evaluation of insulin in the supernatant, which were obtained from the updated monograph for the chemically similar PZI product isophane insulin suspension.[7] All methods were validated for PZI products. The validation reports were reviewed and approved by the Center for Veterinary Medicine branch of the FDA as part of the filing process for a commercial PZI product. The following variables were measured or tested in listed order: appearance, concentration of endotoxin,[8] crystal size,[9] insulin concentration in the supernatant,[7] pH,[10] zinc content,[11] total insulin concentration,[7] and species of insulin origin (identified via HPLC peak retention time). All results were reported to the primary investigator at PUVTH.

In each evaluation (performed by a team of 3 chemists trained in examination of PZI products), the vial contents were visually inspected through the bottle for acceptable suspension characteristics before other tests were performed. Endotoxin analyses were then performed by 1 microbiologist qualified in endotoxin testing to avoid any chance of contamination of the sample and to minimize variability. Samples that had been repackaged to mask commercial laboratory employees to product identification were not tested for endotoxin because of the possibility that the repackaging process could have introduced endotoxin contamination. The endotoxin assay was a kinetic turbidimetric method that was performed in a microtiter plate and analyzed by use of an endotoxin-measuring system[c] with a 96-well plate reader[d] and control software[c] according to the manufacturer's instructions.

The analyses included suitable dilutions of certified endotoxin standard to develop the response curve. Sample dilutions were validated to be in full conformance with the USP requirements[8] and with the instrument manufacturer's recommendation in regard to choosing and verifying prepared concentrations of each sample for accurate measurement without extraneous enhancement, suppression, or other interference.

Crystal size was then determined via microscopic examination. For this analysis, a few drops of each well-mixed product were removed from the vial, placed on a plain glass slide, and examined for crystal size under 500× magnification by use of a microscope equipped with an ocular micrometer (calibrated to National Institutes of Standards and Technology standards) in the eyepiece[e] and a polarized back light source used to enhance visual detection. Crystal sizes were compared with the d_{90} and d_{100} for the FDA-approved commercial product.

Insulin concentration in the supernatant of each product was measured in a 1-mL aliquot from each well-mixed vial. The aliquot was centrifuged at 1,500 × *g* for 10 minutes to obtain the analytic preparation. A pH determination was then performed on the remaining well-suspended contents by use of a pH meter[f] equipped with a microprobe electrode; the equipment was calibrated with standard buffers (pH, 6.0 and 8.0) at the outset and checked with the 6.0-pH buffer between samples. When these tests were completed, the remaining contents were dissolved by acidification with a 20-µL portion of concentrated hydrochloric acid. Total insulin concentration of each suspension was measured in the acidified solution according to the USP

compendial HPLC analysis method,[7] with the adaptation that human insulin, porcine insulin, and bovine insulin reference standards were all used. This adaptation was made because at the outset of analysis, the species of insulin origin in various products was not known, and each product was to be compared with its appropriate reference standard. The method used was validated at the quality control laboratory[a] to be accurate, precise, and specific for bovine, porcine, and human insulin. Desamido compounds were included in the total insulin concentration reported. Zinc concentration was determined on suitable dilutions of the acidified solutions by use of a calibrated atomic absorption spectrometer[g] with an air-acetylene flame. Standards were measured at 4 concentrations ranging from 0.2 to 1.5 mg/L (ppm). Plotted absorbances fell along a well-defined line with very slight absorbance suppression with increasing concentration (a typical pattern in atomic absorption analyses). A second-order calibration curve was fitted to the absorption data to calculate sample concentrations. All standards and samples were read 5 times, and the mean of the 5 readings was accepted if the relative SD of the 5 readings was < 2.0%. Instrument stability was verified by reading a 1-ppm standard after every 5 samples, with a 2.0% agreement criterion. If the criterion was not met, the analysis was rejected and repeated with a new standard curve.

Test results were sorted sequentially according to each variable of interest and were evaluated to determine whether results were affected by masking. The proportion of masked evaluated vials that did not meet the USP specification for each variable was compared with that of vials that were not masked, and no evidence of a difference was detected. Results for products evaluated with and without masking were therefore pooled so that 8 vials of each compounded PZI product (total, 96) were analyzed. For commercially manufactured PZI, results for the 2 sources (8 vials obtained directly from the manufacturer and 8 from a distributor of veterinary products) were further combined so that results for 16 vials of manufactured insulin were evaluated. For each variable, median and range values were calculated for the contents of 8 vials per source for compounded insulin products and of all 16 vials for the commercially manufactured product. The number of vials of each product for which a measured value was outside of the USP specification for that variable was also determined.

Results

The commercially manufactured PZI[a] had a labeled insulin concentration of 40 U/mL. Of the 12 compounded PZI products evaluated, 10 were labeled by the provider as having an insulin concentration of 40 U/mL, 1 had a labeled concentration of 50 U/mL, and 1 had a labeled concentration of 100 U/mL. The manufactured insulin product was labeled as containing insulin of bovine and porcine origin. Of the 12 compounded insulin products, 5 were labeled as containing bovine insulin, and 1 was labeled as containing human recombinant insulin. Six providers did not specify the species of origin for the insulin. Tested vials of the commercially manufactured insulin product were from 6 different lots. Lot numbers were indicated for 8 of 12 compounded insulin products, and vials from 3 to 6 different lots of these products were evaluated during the study. Lot numbers were not indicated for 4 of 12 compounded insulin products. The time to expiration for the manufactured insulin product on the date the product was received ranged from 320 to 873 days. Expiration dates were indicated for 10 of 12 compounded insulin products; for these, time to expiration ranged from 70 to 874 days from the date they were received. Expiration dates were not indicated for 2 of 12 compounded insulin products.

Although there was some variability in appearance among PZI products, the contents of all 112 vials met the criteria of a cloudy white suspension, and none had detectable physical impurities or discoloration. In all 16 vials of the commercially manufactured product, insulin crystals measured < 40 µm (the d_{90} for the FDA-approved commercial product). Insulin crystals measured < 40 µm in most (87/96) vials of compounded products; in 8 other vials, measurements of 1% to 100% of insulin crystals evaluated were between 40 and 100 µm (the d_{100} for the approved commercial product); and in the remaining vial, 70% of the crystals measured < 40 µm, 20% were between 40 and 100 µm, and 10% were > 100 µm. Endotoxin concentration met the USP specification (≤ 32 endotoxin U/mL) in 55 of 56 vials tested, but 1 vial of compounded PZI had a concentration of 160 endotoxin U/mL.

All 16 vials of commercially manufactured PZI had acceptable concentrations of insulin in the supernatant (USP specification, ≤ 1.0 U/mL; **Table 1**). Twenty-three of 96 (24%) vials of compounded PZI had concentrations of insulin in the supernatant above the acceptable limit.

Table 1—Median (range) measured insulin concentrations in the supernatant of 12 compounded PZI products from 12 pharmacies and of 1 commercially manufactured PZI product obtained from 2 sources.[a,b]

Source	Insulin concentration (U/mL)	No. of vials	
		Tested*	Above USP specification
1	17.2 (9.3–18.8)	8	8
2	16.1 (13.8–21.0)	8	8
3	0.1 (0–0.4)	8	0
4	0 (0–0)	8	0
5	0 (0–0)	8	0
6	0 (0–0)	8	0
7	0 (0–0)	8	0
8	0.1 (0–0.2)	8	0
9	0 (0–0)	8	0
10	0 (0–0.1)	8	0
11	0 (0–0)	8	0
12	19.3 (0–28.4)	8	7
13[a] and 14[b]†	0 (0–0)	16	0

Results for individual vials were compared with the USP specification for insulin concentration in the supernatant (≤ 1.0 U/mL). *Two vials of each product were evaluated within 14 days of receipt from the source, including 7 days for repackaging and shipping to the testing laboratory, at 4 time points (approx 2 mo apart); 1 vial of each pair was repackaged according to a standard pharmacy protocol to mask product identification. After verification that test results were not affected by masking, results were combined for each product. †After verification that product values were not different between the 2 sources, results of analysis for the commercially manufactured PZI from 2 sources were combined.

Table 2—Median (range) pH values of the products in Table 1 and comparison of results for individual vials with the USP specification.

Source	pH	No. of vials		
		Tested*	Above USP specification	Below USP specification
1	8.20 (8.10–8.20)	8	8	0
2	7.60 (7.50–7.70)	8	8	0
3	7.10 (7.00–7.20)	8	0	1
4	7.40 (7.40–7.50)	8	3	0
5	7.10 (6.60–7.30)	8	0	3
6	7.50 (7.50–7.60)	8	8	0
7	7.35 (7.30–7.40)	8	0	0
8	7.10 (7.00–7.10)	8	0	3
9	7.30 (7.20–7.40)	8	0	0
10	7.40 (5.10–7.50)	8	1	2
11	7.20 (7.10–7.20)	8	0	0
12	8.20 (8.10–8.40)	8	8	0
13 and 14†	7.30 (7.20–7.30)	16	0	0

The USP specification for pH of PZI is 7.1 to 7.4.
See Table 1 for remainder of key.

The pH values of the contents of all 16 vials of commercially manufactured PZI were within the USP specification (ie, 7.1 to 7.4). In 45 of 96 (47%) vials of compounded PZI, pH values did not meet this requirement; pH was high in 36 (38%) of these and low in 9 (9%). The lowest pH measured in compounded insulin products was 5.1, and the highest was 8.4 (**Table 2**).

In all 16 vials of commercially manufactured PZI, zinc concentrations were within the USP specification (ie, 0.06 to 0.1 mg/mL)[6] for the labeled insulin concentration of 40 U/mL However, zinc concentrations did not meet the specification in 52 of 96 (54%) vials of compounded PZI (**Table 3**). This included 48 of 80 vials that had a labeled insulin concentration of 40 U/mL and 4 of 8 that had a labeled insulin concentration of 50 U/mL (specification for zinc concentration, 0.075 to 0.12 mg/mL). Zinc concentrations were within the specification (ie, 0.15 to 0.25 mg/mL) for all 8 vials of the compounded insulin product that had a labeled insulin concentration of 100 U/mL.

Table 3—Labeled insulin concentrations and measured (median [range]) zinc concentrations of the products in Table 1.

Source	Labeled insulin concentration (U/mL)	Zinc concentration (mg/mL)	No. of vials		
			Tested*	Above USP specification	Below USP specification
1	40	0.02 (0.02–0.02)	8	0	8
2	40	0.02 (0.01–0.03)	8	0	8
3	50	0.07 (0.07–0.08)	8	0	4
4	40	0.19 (0.18–0.20)	8	8	0
5	40	0.08 (0.06–0.08)	8	0	0
6	100	0.20 (0.16– 0.24)	8	0	0
7	40	0.11 (0.11–0.12)	8	8	0
8	40	0.08 (0.07–0.08)	8	0	0
9	40	0.08 (0.08–0.08)	8	0	0
10	40	0.08 (0.07–0.08)	8	0	0
11	40	0.20 (0.19–0.21)	8	8	0
12	40	0.02 (0.01–0.02)	8	0	8
13 and 14†	40	0.07 (0.07–0.07)	16	0	0

Results for individual vials were compared with the USP specification for zinc concentration. This value varies with insulin concentration and is 0.06 to 0.1 mg/mL for 40 U of insulin/mL, 0.075 to 0.12 mg/mL for 50 U of insulin/mL, and 0.15 to 0.25 mg/mL for 100 U of insulin/mL.
See Table 1 for remainder of key.

Table 4—Species of insulin origin, labeled insulin concentration, and measured (median [range]) total insulin concentration of the products in Table 1.

Source	Species	Labeled insulin concentration (U/mL)	Measured insulin concentration (U/mL)	No. of vials		
				Tested*	Above specification	Below specification
1	Human	40	39.45 (36.70–40.90)	8	0	0
2	Human	40	38.60 (35.60–41.00)	8	0	2
3	Bovine	50	42.75 (41.10–45.50)	8	0	7
4	Bovine	40	34.55 (33.80–34.90)	8	0	8
5	Human	40	40.85 (38.30–41.80)	8	0	0
6	Human	100	99.30 (97.10–106.50)	8	0	0
7	Bovine	40	38.35 (38.00–39.00)	8	0	0
8	Bovine	40	33.40 (30.80–35.20)	8	0	8
9	Bovine	40	39.70 (37.50–40.90)	8	0	0
10	Bovine	40	32.95 (30.80–36.20)	8	0	7
11	Human	40	39.15 (37.90–39.90)	8	0	0
12	Human	40	31.70 (24.50–39.20)	8	0	4
13 and 14†	Bovine and porcine	40	40.45 (39.40–41.50)	16	0	0

Results for individual vials were compared with the manufacturer's specification for total insulin concentration (between 90% and 110% of labeled concentration) established for the commercial product.
See Table 1 for remainder of key.

The commercially manufactured PZI contained a median (range) porcine insulin concentration of 14.9% (14.3% to 15.75%) and median (range) bovine insulin concentration of 86.5% (84.5% to 88%). Six of the compounded PZI products contained human insulin, and 6 contained bovine insulin. These were all in accordance with the product label if a species of origin was listed. Median and range total insulin concentrations were determined for each product (**Table 4**). The contents of all 16 vials of the commercially manufactured product were within the manufacturer specification for measured insulin concentration (ie, 90% to 110% of labeled concentration); values for 36 of 96 (38%) vials of compounded products were < 90% of the labeled value. No vial contents had concentrations of insulin > 110% of the labeled value. For compounded insulin products that were labeled as having a concentration of 40 U/mL, the lowest insulin concentration was 24.5 U/mL (61.3% of the labeled value) and the highest was 41.8 U/mL (104.5%). The lowest and highest insulin concentrations of the product labeled as containing 50 U of insulin/mL were 41.1 U/mL (82.2% of the labeled value) and 45.5 U/mL (91.0%), respectively. The lowest and highest insulin concentrations of the product labeled as containing 100 U of insulin/mL were 97.1 U/mL (97.1% of the labeled value) and 106.5 U/mL (106.5%), respectively. For some products, there was marked variability in insulin concentration among different vials. The difference between the maximum and minimum insulin concentrations of individual vials of the same insulin product ranged from 1.0 to 14.7 U/mL.

All 16 vials of commercially manufactured PZI and only 13 of 96 (13.5%) vials of compounded PZI met all USP specifications. Of the 12 sources that supplied compounded insulin products evaluated in this study, only 1 provided a product that met all USP specifications in all 8 vials tested.

Discussion

Protamine zinc insulin is a long-acting insulin formulation designed for SC administration. The prolonged duration of PZI is attributable to the complexing of insulin with zinc and protamine in precise proportions to form a precipitate that is released slowly from the subcutaneous tissues. Onset, duration, and peak activity are strongly influenced by the proportions of insulin, protamine, and zinc in the crystals. Variability in zinc content and the presence of free insulin in the supernatant influence the release kinetics of the insulin. The PZI of porcine and bovine origin has an onset of action of 0.25 to 2 hours, a time to nadir of 1.5 to 20 hours, and a duration of action of 9 to > 24 hours in cats.[12,13] To the authors' knowledge, the pharmacokinetics of human recombinant PZI in cats has not been reported. The long duration of action of PZI in cats is considered important in achieving good glycemic regulation in diabetic cats.

Specifications cited in USP standards for PZI suspension[6] and for isophane insulin suspension[7] were used as appropriate to evaluate the results of various assays in the present study. Protamine zinc insulin is a cloudy white suspension and should contain no physical impurities or discoloration. The USP specification for pH of PZI is 7.1 to 7.4. Insulin suspensions that have a pH outside of this range may cause pain at the time of injection and pathological changes at the site of injection. At the time of the study reported here, the manufacturer specification for PZI insulin concentration was 90% to 110% of the labeled insulin concentration; this specification was later tightened to 95% to 105% of the labeled insulin concentration. This measurement includes insulin in the precipitate and insulin dissolved in the supernatant. Insulin derived from various species (bovine, porcine, and human) and desamido compounds are related compounds that are considered by compendial definition to be equivalent in activity to native insulin and are included in this measurement. Concentration of free insulin in the supernatant is an important characteristic indicative of the amount of insulin that is not complexed in insulin-zinc crystals. The specification is that ≤ 1.0 U of insulin/mL should be free in the supernatant. A PZI product with a higher concentration of free insulin may have properties more similar to regular insulin than to PZI. The long-acting properties of PZI depend upon correct proportions of insulin, protamine, and zinc in the product. Products with high zinc concentrations may release insulin too slowly, and those with low zinc concentrations may release insulin too rapidly. The specification for zinc content of PZI is based on the concentration of insulin. For 40 U of insulin/mL, the specification is 0.06 to 0.1 mg/mL; for 50 U of insulin/mL, it is 0.075 to 0.12 mg/mL; and for 100 U/mL of insulin, it is 0.15 to 0.25 mg/mL These are ranges listed by the USP to reflect the different possible formulations of PZI. The specifications also require that endotoxin concentrations of PZI not exceed 32 endotoxin U/mL. There is no USP requirement for crystal size; thus, in the present study, crystal size was compared with the d_{90} and d_{100} values for the FDA-approved product. In the authors' opinions, it is possible that variations in crystal size could potentially lead to differences in release kinetics of insulin from the tissues.

The commercially manufactured PZI[a] evaluated in the present study included bovine and porcine insulin and is no longer being produced by the manufacturer. Another FDA-approved PZI product is currently available that contains recombinant human insulin rather than insulin of bovine and porcine origin; this PZI is made with the same cGMP manufacturing and quality control standards as the commercially manufactured product tested in the study reported here.

Problems identified in compounded PZI from various sources evaluated in the present study included lack of an expiration date or lot number on the vial, lack of identification of the species of origin (bovine, porcine, or human), unacceptably high endotoxin concentration, pH below or above the recommended range, low total insulin concentration, zinc concentrations below or above acceptable limits, variability in insulin concentration among vials from a single source and among those from different sources that had the same labeled concentration, and unacceptably high concentrations of insulin in the supernatant. The study was not designed to identify the clinical consequences of

these findings; however, such deficiencies could potentially contribute to poor glycemic control in cats treated with compounded PZI. Specifically, variability in zinc content could lead to changes in the onset and duration of insulin action, while excessive concentration of insulin in the supernatant could lead to initial hypoglycemia and decreased duration of insulin effect. Changes in pH could result in discomfort after insulin injection. Variability in insulin concentrations among individual vials could lead to a sudden worsening of glycemic control or to insulin induced-hypoglycemia when a new vial of PZI is used. The presence of excessive endotoxin could raise body temperature and cause anorexia, which could adversely affect glycemic control in diabetic animals.

Although some of these problems could be mitigated by closer monitoring of blood glucose concentrations, this requirement adds to the expense of managing diabetic patients. Moreover, some problems such as presence of endotoxin or an inappropriate pH might not be detected by use of routine blood glucose monitoring. Other problems such as lack of a lot number or expiration date and failure to identify the species of origin for insulin may not have direct clinical consequences; however, these problems reflect a lack of attention to detail and quality control in the compounding process and may make it more difficult to identify the cause of poor glycemic control in individual patients. Results of the present study suggest veterinarians should avoid the use of compounded insulin and recommend the use of manufactured insulin products for treatment of diabetes mellitus.

A thorough discussion regarding legal veterinary compounding is beyond the scope of this report. The FDA, Environmental Protection Agency, USDA, and individual State Boards of Pharmacy all have laws and regulations that impact the use of compounded medications in veterinary patients. The USP requires a compounding pharmacy to have a quality assurance protocol that documents standard operating procedures for potency, sterility, and pyrogen testing. Pharmacies should be able and willing to provide documentation of quality assurance testing. A compounding pharmacy that strictly follows these guidelines for quality assurance testing provided by the USP should be able to detect the problems identified in this study even with intermittent or skip-lot testing.

Testing was conducted at the quality control laboratory of the manufacturer of the commercial PZI product used in the present study because of its status as an approved cGMP laboratory with employees that had extensive experience in testing manufactured insulin. The identity of half of the tested products was masked prior to shipping to the laboratory to eliminate the potential for bias. Only study investigators from PUVTH reviewed the unmasked data and carried out the data analysis.

After the study reported here was completed, the FDA Center for Veterinary Medicine announced that a commercially manufactured porcine insulin suspension for use in veterinary patients was found to be out of specification in regard to stability. The FDA Center for Veterinary Medicine announcement cautioned that practicing veterinarians should watch for possible changes in glycemic control and consider transitioning their diabetic patients to other insulin products. This incident showed that FDA-mandated quality control testing works to detect manufacturing and stability problems in FDA-approved products and helps to assure that such products are in conformance with FDA and USP specifications. No similar system is in place to monitor the quality of compounded products. Finally, practicing veterinarians and pet owners should be reminded that the laboratory analysis of manufactured pharmaceutical products is not the last step in the monitoring process. If any product is observed to have any defect or if it does not perform as expected, this finding should be reported directly to the manufacturer or to the FDA Center for Veterinary Medicine.

a. IDEXX Pharmaceuticals Inc, Greensboro, NC.
b. MidWest Veterinary Supply Inc, Fort Wayne, Ind.
c. Endoscan endotoxin system, Charles River Laboratories, Cambridge, Mass.
d. Tecan Sunrise, Charles River Laboratories, Cambridge, Mass.
e. Zeiss Axiolab microscope with ocular micrometer, Oberkochen, Germany.
f. Beckman 490 pH meter, Charles River Laboratories, Cambridge, Mass.
g. Perkin-Elmer Model 100 atomic absorption spectrometer, Downer's Grove, Ill.

References

1. Scott DA, Fisher AM. Studies on insulin with protamine. *J Pharmacol Exp Ther* 1936;58:78–92.
2. Brange J. *Galenics of insulin, the physico-chemical and pharmaceutical aspects of insulin and insulin preparations*. New York: Springer-Verlag Inc, 1987.
3. Nelson RW, Lynn RC, Wagner-Mann CC, et al. Efficacy of protamine zinc insulin for treatment of diabetes mellitus in cats. *J Am Vet Med Assoc* 2001;218:38–42.
4. Nelson RW, Henley K, Cole C, et al. Field safety and efficacy of protamine zinc recombinant human insulin for treatment of diabetes mellitus in cats. *J Vet Intern Med* 2009;23:787–793.
5. Norsworthy GD, Lynn R, Cole C. Preliminary study of protamine zinc recombinant insulin for treatment of diabetes mellitus in cats. *Vet Ther* 2009;10:24–28.
6. US Pharmacopeial Convention. Protamine zinc insulin suspension. In: *United States pharmacopeia 22–national formulary 17*. Rockville, Md: US Pharmacopeial Convention, 1990;699.
7. US Pharmacopeial Convention. Isophane insulin suspension. In: *United States pharmacopeia 30–national formulary 25*. Rockville, Md: US Pharmacopeial Convention, 2007;2410.
8. US Pharmacopeial Convention. Chapter 85: bacterial endotoxins. In: *United States pharmacopeia 30–national formulary 25*. Rockville, Md: US Pharmacopeial Convention, 2007;110–113.
9. US Pharmacopeial Convention. Chapter 776: optical microscopy. In: *United States pharmacopeia 30–national formulary 25*. Rockville, Md: US Pharmacopeial Convention, 2007;313.
10. US Pharmacopeial Convention. Chapter 791: pH. In: *United States pharmacopeia 30–national formulary 25*. Rockville, Md: US Pharmacopeial Convention, 2007;328–329.
11. US Pharmacopeial Convention. Chapter 591: Zinc determination. In: *United States pharmacopeia 30–national formulary 25*. Rockville, Md: US Pharmacopeial Convention, 2007;219.
12. Marshall RD, Rand JS, Morton JM. Glargine and protamine zinc insulin have a longer duration of action and result in lower mean daily glucose concentrations than lente insulin in healthy cats. *J Vet Pharmacol Ther* 2008;31:205–212.
13. Broussard JD, Peterson ME. Comparison of two ultralente insulin preparations with protamine zinc insulin in clinically normal cats. *Am J Vet Res* 1994;55:127–131.