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ABSTRACT

Compounded trilostane capsules (15 mg, 45 mg, or 100 mg) were purchased from eight pharmacies and assayed for content and dissolution characteristics. Capsules made in-house containing either inert material or 15 mg of the licensed product and proprietary capsules (30 mg and 60 mg) served as controls. Findings were compared with regulatory specifications for the licensed product. Altogether, 96 batches of compounded trilostane and 16 control batches underwent analysis. In total, 36 of 96 (38%) compounded batches were below the acceptance criteria for content. The average percentage label claim (% LC) for each batch ranged from 39% to 152.6% (mean, 97.0%). The range of average % LC for the controls was 96.1–99.6% (mean, 97.7%). The variance in content of the purchased compounded products was substantially greater than for the controls (234.65 versus 1.27; $P < 0.0001$). All control batches exceeded the acceptance criteria for dissolution, but 19 of 96 batches (20%) of purchased compounded products did not. Mean percent dissolution for the purchased compounded products was lower than for controls (75.96% versus 85.12%; $P = 0.013$). These findings indicate that trilostane content of compounded capsules may vary from the prescribed strength, and dissolution characteristics may not match those of the licensed product. The use of compounded trilostane products may therefore negatively impact the management of dogs with hyperadrenocorticism. (*J Am Anim Hosp Assoc* 2012; 48:228–233. DOI 10.5326/JAAHA-MS-5763)

Introduction

Trilostane is a synthetic steroid analog that competitively inhibits 3- β -hydroxysteroid dehydrogenase. This enzyme facilitates the conversion of pregnenolone and 17- α -hydroxypregnenolone to progesterone and 17- α -hydroxyprogesterone, respectively, and is required for the synthesis of cortisol by the adrenal cortex.¹ Trilostane has been widely used in Europe and Australia for many years for the management of dogs with hyperadrenocorticism (HAC) due to either a pituitary adenoma or a functional adrenal cortical tumor and was approved for use in dogs with both forms of HAC in the United States in 2008.^{2–4}

Prior to approval by the US Food and Drug Administration (FDA), compounded trilostane products were marketed to veterinarians through internet sites, at professional conferences, and by direct mailing. Although reformulation of the licensed product is

allowable if specific patients require trilostane in strength or forms that are not commercially available, current FDA regulations do not permit either the importation of trilostane from other countries or the use of products compounded from bulk ingredients.⁵ Numerous pharmacies still market compounded trilostane for veterinary use, and veterinarians and pet owners who purchase those compounded products may not be aware of the source of the trilostane used to produce the compounded products. In addition, there is little to no independent oversight regarding quality control or monitoring of manufacturing standards in facilities that compound trilostane. Uncertainty may therefore arise regarding the content, purity, potency, and stability of compounded trilostane, and inconsistency with drug dosage or uptake may substantially compromise the management of patients receiving compounded trilostane.

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% LC percent label claim; % w/w percent weight/weight; CC compounded controls; FDA United States Food and Drug Administration; HAC hyperadrenocorticism; PC proprietary controls

The goal of this study was to investigate the pharmaceutical properties of commercially available compounded trilostane products and to compare the compounded products with trilostane reformulated in-house from the licensed product, as well as the proprietary product itself. The hypothesis was that clinically significant variations in trilostane content, purity and dissolution characteristics would be documented in products purchased from compounding pharmacies.

Materials and Methods

An internet search was conducted to identify eight pharmacies marketing compounded trilostane products^{a-h}. Two orders for different capsule sizes of trilostane were faxed to each pharmacy every week for 6 wk starting in September 2009. Each request was for 30 capsules containing 15 mg, 45 mg, or 100 mg of trilostane (i.e., sizes that are not currently licensed). Over the study period, each pharmacy supplied four batches of each size. The compounded products were shipped to the pharmacy at Texas A&M Veterinary Medical Teaching Hospital and stored at room temperature until prepared for analysis.

At the end of weeks 2, 4, and 6 of the study, all the shipments received during that time period were inspected. A total of 32 batches were received during each 2 wk interval (i.e., four from each of the eight pharmacies). Two of the authors opened each package and verified that the product label matched the capsule size requested. The 30 capsules were then placed in a standard pharmaceutical vial that was coded with a randomly generated numerical identifier. Correct identification of each batch was verified by both authors.

At the start of the study, one of the investigators (CDN, a licensed pharmacist) prepared 120 capsules containing only inert fillerⁱ and 120 capsules containing 15 mg of the licensed product^j. These capsules were made using a pharmaceutical balance^k and standard pharmaceutical techniques. These were divided into batches (each containing 30 capsules) and packaged and coded in the same manner as the compounded products. The compounded controls (CCs) were used to validate the integrity of the analytic process and to investigate the impact of reformulation on the proprietary drug. Four batches of proprietary capsules (PCs) in 30 mg and 60 mg sizes were similarly packaged and coded.

At the end of every 2 wk interval during the study period, 32 repackaged batches of purchased compounded products together with two or three CC batches (0 mg and/or 15 mg) and two or three PC batches (30 mg and/or 60 mg) were submitted for analysis. Each shipment contained four batches of various sizes from each compounding pharmacy to limit the impact of inter-assay variation. Analysis was performed at a pharmaceutical manufacturing

facility^l compliant with the FDA's current Good Manufacturing Practice regulations and approved by the Medicines and Healthcare products Regulatory Agency of the UK.^{6,7} None of the authors of this report had any involvement in the analytic process, and all personnel at the testing facility were blinded to the source of the capsules. The 0 mg and 15 mg CCs were indistinguishable from the purchased compounded products; however, the 30 mg and 60 mg PCs were easily recognizable due to the imprint on the capsule shells. Information was provided about the label claim for each batch because this was necessary for the analytical process. The 0 mg capsules intentionally mislabeled as containing 15 mg.

Upon receipt at the testing facility, 10 capsules from each batch were emptied and tested for weight variation. The contents of the 10 capsules were subsequently blended together to form a homogeneous composite. Based on the expected total weight of trilostane in the 10 capsule composite, two or three aliquots were weighed out, each with a presumed trilostane content equivalent to the weight of the standard used for comparison. Each sample was then sonicated in methanol in preparation for the analytic process. Trilostane content was determined by a validated reversed-phase high-performance liquid chromatographic^m method using a C18 column and photodiode array detection. The detection wavelengths were selected to optimize the response for each analyte (i.e., trilostane and related substances). The analytical procedure had previously been fully validated as suitable for its intended purpose (i.e., the determination of trilostane and related substances content), per guidelines GL1 and GL2 of the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medical Products. The International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medical Products program harmonizes technical requirements for the registration of veterinary medicinal products in Europe, Japan, and the US. The guidelines listed refer to the validation of analytic procedures regarding definition and terminology (GL1) and methodology (GL2). The accuracy of the analytical method was shown to be 101.7%, 100.7%, and 98.6% at nominal sample solution concentrations of 80%, 100%, and 120%, respectively. The relative standard deviation for the method (an indicator of precision) was 0.75% using 30 mg trilostane capsules and 0.49% using 60 mg trilostane capsules. The coefficient of determination (an indicator of the linearity of a method across the potential range of the analysis, determined on a graph plotting solution concentration against UV detector response) for the analytic method is 0.99993. A value of 1 indicates a perfectly linear relationship (i.e., a straight line) where the response is directly proportional to the amount of analyte within the sample.

Acceptance criteria for trilostane content were set at 90–105% LC, which is consistent with current United States Pharmacopoeia criteria for shelf life specification of a drug product. The 0 mg control batches were removed from further evaluation after determination of 0% trilostane content and were not included in the statistical analysis.

The samples prepared for trilostane determination as described above were also analyzed for determination of related substances (i.e., ketotrilostane) and other impurities using the same chromatography methodology. Findings were expressed as % weight/weight (% w/w), with acceptance criteria set at $\leq 2\%$ w/w for total related substances.

Six capsules from each batch were used for determination of dissolution characteristics. The dissolution method uses the standard United States Pharmacopoeial Apparatus 1 equipment as described in US Pharmacopoeial Convention <711>. The dissolution media is a fully aqueous solution adjusted to pH 8.0 and the samples were analyzed using a ultraviolet spectrophotometric method. The % dissolution was measured every 15 min for a total of 75 min, with minimum acceptance criteria set at $\geq 70\%$ dissolution of the LC by 75 min.

Residual capsules were disposed of appropriately when the analytical process was complete.

Statistical Analysis

Statistical analysis of the data was performed using a commercial software programⁿ. For statistical purposes, the trilostane content results for the two or three assays from each batch were averaged to provide a mean % LC for that batch. The amount of related substances was similarly averaged to provide a mean % w/w for each batch. In the dissolution studies, the results at each time point were averaged to provide a mean % dissolution. The Fischer exact test was used to compare outcomes between compounded and control batches, and an unpaired *t* test was used to compare content and dissolution characteristics between control and compounded batches. An *F* test was used to compare variance in content and dissolution characteristics between control and compounded batches. A one-way analysis of variance was used to compare content and solubility between the three sizes of compounded capsules, with a Bartlett test for equal variances used to identify significant differences.

Results

A total of 96 batches (i.e., 30 simultaneously procured capsules) of compounded trilostane were evaluated. Four batches (each containing 15 mg, 45 mg, and 100 mg capsules) were purchased from the eight pharmacies. Sixteen control batches were evaluated,

comprising eight batches of CC (four batches of 0 mg and four batches of 15 mg) and eight batches of PC (four batches of 30 mg and four batches of 60 mg).

Using an acceptance criterion of 90–105% LC, 36/96 (38%) of the compounded batches failed to meet the target content (Figure 1, Table 1). All of the 15 mg CC batches and all of the 30 mg and 60 mg PC batches met the acceptance criteria for content. All of the 0 mg CC batches had no measurable trilostane. The % LC for each batch of compounded trilostane ranged from 39% to 152.6% (mean, 97.0%). The % LC for the 15 mg, 30 mg, and 60 mg control batches was 96.1–99.6% (mean, 97.7%). The overall variance for the % LC of the compounded product was substantially greater than for the controls (234.65 versus 1.27; $P < 0.0001$). The four batches of 15 mg CC performed as well as the PC, with a mean % LC content of 97.4% (range, 96.8–98.9%).

When the three sizes of compounded capsules were considered separately, similar numbers of batches of each strength met the acceptance criteria (i.e., 18/32 of the 15 mg, 18/32 of the 45 mg, and 23/32 of the 100 mg). However, capsule size did impact the variation in content, with coefficients of variation of 18.7% for the 15 mg size, 19.87% for the 45 mg size, and 7.3% for the 100 mg size. The variances for the three sizes were significantly different (324, 369, and 51, respectively; $P < 0.0001$). The number of failing batches from each pharmacy ranged from 0/12 to 9/12, with a median of 4/12. Only one pharmacy met the acceptance criteria for content in all 12 batches.

The performance of the eight pharmacies (based on 12 batches from each) varied substantially, with coefficients of variation for % LC ranging from 2.74% to 36.48%, with a median of 11%. By comparison, the value for the four batches of 15 mg controls generated at the Texas A&M pharmacy was 1.18%. For

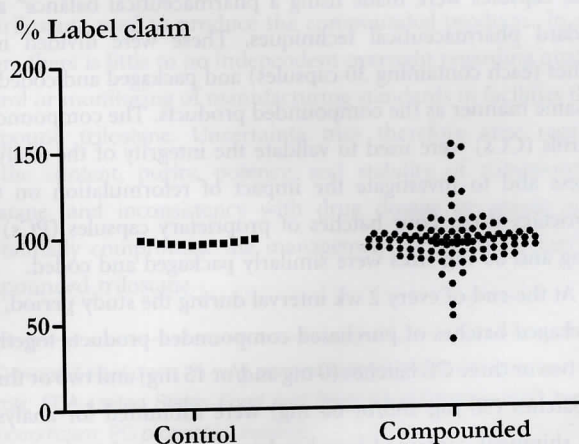


FIGURE 1 Average percent label claim (% LC) for control ($n=12$) and compounded ($n=96$) batches of trilostane.

TABLE 1

Number and Percentage of Compounded Capsules and Controls that Met Each of the Acceptance Criteria

	Trilostane content	Impurity/related substance	Dissolution
Acceptance criteria	90–105%	≤2%	≥70% by 75 min
Compounded capsules	60/96 (63%)	95/96 (99%)	77/96 (80%)
Compounded controls (CC)	8/8 (100%)	8/8 (100%)	8/8 (100%)
Proprietary capsules (PC)	8/8 (100%)	8/8 (100%)	8/8 (100%)

the pharmacy with the most variable % LC, the averaged content for a batch of 45 mg capsules ranged from 39.0% LC to 150.4% LC. Thus, if a patient were prescribed 45 mg of trilostane, the amount of drug provided would average 18 mg in one prescription but 68 mg in a subsequent order.

One of the 96 compounded batches exceeded the acceptance criterion for related substances (set at ≤2% w/w) with an averaged unknown impurity content of 2.733% w/w (Figure 2). The mean related substances content for all the compounded products was 0.624% (range, 0.241–2.733%), which was significantly higher than the mean for the controls (0.392%; range, 0.282–0.468; $P=0.0197$). Again, the results for the CCs were similar to those of the PCs, with mean % impurities of 0.376% (range, 0.282–0.461) and 0.399% (range, 0.319–0.468), respectively.

Using an acceptance criteria of ≥70% dissolution at 75 min, 19/96 (20%) of compounded batches failed the acceptance criteria (Figure 3). Three pharmacies met the dissolution target with all 12 batches, but two failed to meet the target with ≥50% of batches tested. The mean % dissolution for compounded batches was lower than for the controls (75.96% versus 85.12%; $P=0.0130$). In addition, the variance for the dissolution of the

Impurities %w/w

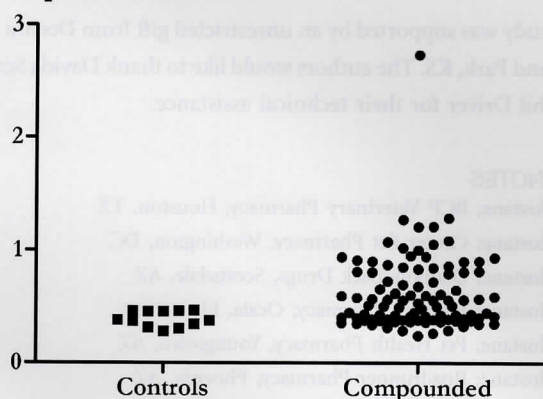


FIGURE 2 Average percent (% weight/weight [w/w]) impurities/related substances for control ($n=12$) and compounded ($n=96$) batches of trilostane.

% Dissolution at 75 minutes

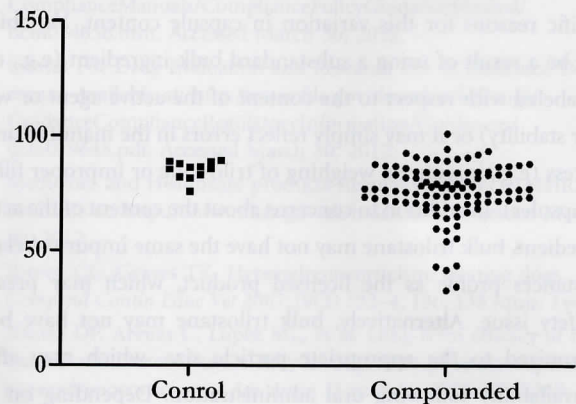


FIGURE 3 Average dissolution based on % LC for control ($n=12$) and compounded ($n=96$) batches of trilostane at 75 min.

compounded products was greater than for the controls (153.51 versus 15.51; $P=0.0002$). All of the control batches met the target for dissolution, with similar results for the CCs and the PCs (range, 82.9–89.3% and 75.8–88.9%, respectively).

Discussion

In the last few years, trilostane has become a standard therapy for dogs with HAC. One product was approved for use in the US in late 2008.⁸ Reformulation of the licensed product is allowable if specific patients require trilostane in strength or forms that are not commercially available, but there is little information regarding the pharmacologic properties of compounded trilostane with respect to content and dissolution characteristics.⁵

A drug is regarded as “conforming to specifications” when it meets the acceptance criteria for specific, critical standards of quality. These standards are proposed and justified by the manufacturer then approved by regulatory authorities as part of the drug approval and licensing process. The FDA mandates quality control for licensed veterinary drugs, and standards must be met regarding the drug’s identity (i.e., does it contain trilostane?), strength (i.e., is it the correct amount of trilostane?), quality (i.e., has the drug been produced by a method that is reproducible and provides bioavailable drug to the patient?), and purity (i.e., do impurities or related substances exceed the allowed amount?).

This study evaluated trilostane products purchased from eight compounding pharmacies and found some of them to vary significantly from the acceptance criteria of the licensed product with respect to drug content and dissolution characteristics. For some of the products evaluated, substantial variation between batch contents was reported, with a >3× increase in actual trilostane dose documented from one batch to the next from the same pharmacy.

It is important to point out that this study did not identify specific reasons for this variation in capsule content. Variability may be a result of using a substandard bulk ingredient (e.g., one mislabeled with respect to the content of the active agent or with poor stability) or it may simply reflect errors in the manufacturing process (e.g., inaccurate weighing of trilostane or improper filling of capsules). In addition to concerns about the content of the active ingredient, bulk trilostane may not have the same impurity/related substances profile as the licensed product, which may present a safety issue. Alternatively, bulk trilostane may not have been micronized to the appropriate particle size, which may affect bioavailability following oral administration. Depending on the excipients used to dilute bulk trilostane or the method of preparing the excipients, capsules may not demonstrate the same dissolution characteristics as the licensed product. As a result, bioavailability could be compromised. Based on the impurity profiles and dissolution characteristics of some of the batches evaluated in this study, it seems likely that bulk agent may have been used in some instances.

The effect of trilostane on adrenal synthesis of cortisol is dose-dependent within an individual animal; therefore, consistent dosing is of paramount importance. Dose adjustments are typically made based on resolution of clinical signs and results of adrenocorticotrophic hormone stimulation testing. Batch-to-batch variability can make individualization of dose difficult. Inadvertent overdose may result in hypocortisolemia and electrolyte derangements, both of which will impact patient well-being and may require medical intervention.⁹ Although these complications are regarded as reversible, some pet owners may decline further treatment following an episode of adrenal compromise due to financial considerations and/or fear of serious complications.¹⁰

Conversely, inadequate trilostane administration will result in persistent signs of HAC and apparent treatment failure. Inappropriately treated patients remain vulnerable to the complications of hypercortisolemia, including infection, thromboembolism, and progressive weakness.¹¹

There are some limitations to this study. First, the pharmaceutical analysis was performed by a company owned by the licensed manufacturer of trilostane. This facility was used because the methods used for trilostane determination are proprietary and the authors were unable to identify an independent analytic facility. To ensure the integrity of their findings, the authors submitted four batches of 15 mg capsules containing licensed product and four batches of 0 mg capsules for blinded analysis. These test samples were submitted alongside the commercially compounded batches and were indistinguishable from the purchased products. The 0 mg CC capsules were correctly identified as containing only inert

material and were therefore removed from further evaluation and excluded from the statistical analysis following determination of content. As the 15 mg CC capsules all met the acceptance criteria for content, presence of related substances, and dissolution characteristics, it seems likely that the analytic process was legitimate. However, it would have been ideal to have an independent laboratory perform the pharmaceutical analysis to avoid any suggestion of bias.

A second limitation was the authors' inability to determine the cause of the observed variations in content and dissolution characteristics. Failure to meet the acceptance criteria may have been due to poor pharmacy practices and/or the use of bulk trilostane. The authors theorize that bulk agent may have been used on occasion, but are unable to verify this conclusion.

This study confirmed that accurate reformulation of the licensed product does not compromise dissolution characteristics and that the target dose can be achieved. Practitioners should consider both the source of the trilostane and the technical proficiency of the pharmacy staff when prescribing nonapproved capsule sizes for their patients.

Conclusion

Reformulation of the licensed trilostane product into a novel capsule size by a trained pharmacist did not affect dissolution characteristics and the target dose could be achieved. However, a substantial proportion of the commercially available compounded trilostane products evaluated in this study failed to meet acceptance criteria for % LC (38%) and/or dissolution characteristics (20%). On the basis of these findings, compounded trilostane products should be used with caution as they may jeopardize the management of dogs with HAC and potentially impact patient safety. ■

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FOOTNOTES

- ^a Trilostane; BCP Veterinary Pharmacy, Houston, TX
- ^b Trilostane; Center Pet Pharmacy, Washington, DC
- ^c Trilostane; Diamondback Drugs, Scottsdale, AZ
- ^d Trilostane; Franck's Pharmacy, Ocala, FL
- ^e Trilostane; Pet Health Pharmacy, Youngtown, AZ
- ^f Trilostane; Roadrunner Pharmacy, Phoenix, AZ
- ^g Trilostane; Steven's Pharmacy, Costa Mesa, CA
- ^h Trilostane; Wedgewood Pharmacy, Swedesboro, NJ
- ⁱ D-(+)-lactose monohydrate; J.T. Baker, Phillipsburg, NJ
- ^j VETORYL Capsules; Dechra Veterinary Products, Overland Park, KS

- ^k CP124S; Sartorius, Elk Grove, IL
^l Dales Pharmaceuticals, Skipton, North Yorkshire, England
^m Agilent 1100 series HPLC; MMM, city, state
ⁿ GraphPad Prism 5 for Windows; GraphPad Software, La Jolla, CA

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