DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY®
Vol. 30, No. 10, pp. 1069–1078, 2004

Investigation of Product Quality Between Extemporaneously Compounded Progesterone Vaginal Suppositories and an Approved Progesterone Vaginal Gel

Vorapann Mahaguna, Ph.D.,* J. Mario McDermott, Feng Zhang, and Felipe Ochoa

PharmaForm, L.L.C., Austin, Texas, USA

ABSTRACT

The purpose of this investigation was to compare quality parameters, including product appearance, content uniformity, pH, weight uniformity, microbial limit testing and preservative effectiveness testing on extemporaneously compounded progesterone vaginal suppositories obtained from 10 randomly chosen compounding pharmacies (90 suppositories each) across the United States, to the Food and Drug Administration (FDA) approved prescription progesterone gel product (Prochieve®/Crinone®) which is manufactured in a cGMP regulated facility. The content uniformity and pH were determined using qualified methods. The microbial limits testing and preservative effectiveness testing were conducted according to compendial methods. Only one pharmacy provided suppositories that were all within the potency limits required for the prescription progesterone gel product. The other pharmacies provided at least some suppositories where progesterone content was either subpotent or superpotent for progesterone. The pH of most of the compounded suppository products was in the range of 4.22 to 7.68 with a median of 6.30 (normal vaginal pH is <5), whereas the gel product was 2.80. For compounded product from one of the pharmacies, microbial limits testing indicated CDC group IVC-2 and *Comamonas acidovorans* were detected. This data indicates that pharmacy compounded delivery systems for progesterone should be used with caution.

Key Words: Progesterone; Suppository; Gel; Vagina; Compounding.

*Correspondence: Vorapann Mahaguna, Ph.D., PharmaForm, L.L.C., 11400 Burnet Road, Bldg 4, Suite 4010, Austin, TX 78758, USA; Fax: (512) 834-2105; E-mail: vmahaguna@pharmaform.com.

DOI: 10.1081/DDC-200040251
0363-9045 (Print); 1520-5762 (Online)

INTRODUCTION

Progesterone is a naturally occurring steroid hormone produced by the corpus luteum, adrenal cortex, and placenta, whose function is critical to the processes of endometrial development, implantation, and subsequent maintenance of early pregnancy. Progesterone has been widely used in the first trimester of pregnancy both to achieve pregnancy as part of Assisted Reproductive Technology (ART) for infertile women with progesterone deficiency and to help support pregnancy in progesterone deficient women through the first trimester,[1] treatment of symptoms associated with premenstrual syndrome (PMS),[2] hormone replacement therapy (HRT) in combination with estrogen to reduce the risk of endometrial cancer in women caused by estrogen therapy alone,[1] and secondary amenorrhea.

Progesterone can be delivered by several routes of administration, including oral, sublingual, injectable (intramuscular and abdominal), or by local application through the use of vaginal or rectal suppositories[1] or vaginal gel. Vaginal delivery is attractive for clinical treatment due to ease of administration, the avoidance of first pass metabolism in the liver resulting in limited metabolic activity, high intercellular permeability, and the large potential surface area available for drug absorption.[3] Systemic delivery of progesterone to the vagina can be accomplished using an emulsified gel or suppository. Progesterone bioadhesive vaginal gel (i.e. Prochieve®/Crinone®) is the only FDA approved product commercially available in 4% (45 mg progesterone) and 8% (90 mg progesterone) strengths. Prochieve®/Crinone® are manufactured according to current good manufacturing practices (cGMP). Other progesterone vaginal products are mostly extemporaneously compounded in pharmacies pursuant to written prescriptions and are compounded to good compounding practices.[4] Even though the compounded product must be used by the patient within a relatively short period of time after compounding, the accuracy of the labeled dose of these products cannot be assured. In addition, the proper physical properties (e.g. those properties influencing the release of drug from the suppository over time) and an acceptable bioburden cannot be assured since these product attributes are not routinely tested after compounding and prior to delivery to the patient. In a survey of compounded pharmacy products conducted by the FDA, 34% of tested products from 12 compounding pharmacies failed analytical testing.[5] The FDA survey included 37 products from five categories: sterile injectables, pellet implants, ophthalmic solutions and ointments, inhalation delivery, and oral delivery. Five progesterone products were included (2 sterile injectables and 3 oral delivery), and 2/5 products failed on potency (1 sterile injectable, 1 oral delivery). It is worth noting that between 1996 and 2002, the FDA tested over 3,000 drug products from commercial manufacturers (cGMP facilities) with a failure rate of less than 2%.[5] Cases of injury to patients from subpotent, superpotent, and contaminated drugs from licensed compounding pharmacies have been publicized in the last few years.

A study that focuses on a specific drug and drug-delivery method from compounding pharmacies has not been published. The objectives of this study were to investigate the consistency in quality of progesterone vaginal suppository products extemporaneously compounded by compounding pharmacists from 10 pharmacies chosen at random from across the United States, and to compare the physical and chemical properties to the control product, Prochieve®/Crinone® vaginal gel. The suppository and gel products were assessed by quantitating pH, uniformity of content, preservative efficacy testing (PET), product appearance and microbial limits testing.

EXPERIMENTAL

Materials

The following materials were used: progesterone USP (Spectrum Chemicals, Gardena, CA); methanol HPLC grade (Mallinckrodt, Hazelwood, MO); HPLC water (in-house). Commercial 8% progesterone vaginal gel product containing 90 mg micronized progesterone Prochieve®/Crinone®; supplied by Columbia Laboratories, Inc., Livingston, NJ). Written prescriptions for progesterone 90 mg vaginal suppositories (e.g. product lots A to J) were filled at retail pharmacies who advertised a specialty in compounding from across the United States. Suppository samples were stored in the refrigerator at 2–8°C when received from each pharmacy. No special instructions about the study were provided to any of the pharmacists. In most instances, the prescriptions were called in to the pharmacist by someone in the physician's office. The types of primary packaging (i.e. directly contacting the product), secondary packaging (i.e. not in direct contact with product), and shelf-life of each product (e.g. as assigned by the compounding pharmacist) used in this study are described in Table 1.

Table 1. Description of progesterone vaginal products acquired and investigated in the study, and instructions provided by the compounding pharmacists.

Progesterone delivery system	Product lot	Primary packaging	Secondary packaging	Shelf-life	Recommended storage
Gel (commercial product)	C03126	Single use vaginal applicator	Sealed foil over wrap	2 yr.	15–30°C (59–86°F)
Pharmacy compounded suppository	A	Sealed individually in white disposable molds	Foil bag	6 mo.	Refrigerator
	B	Sealed individually in white disposable molds	Plastic bag	6 mo.	Away from sunlight, keep in cool, dry place
	C	Tan dispensing sleeves (exposed to air on top)	Cardboard boxes	3 mo.	Refrigerator
	D	Sealed individually in white disposable molds (exposed to air on one side)	Plastic bag	N/A	Refrigerator
	E	No wrapping; all in contact with each other	Partitioned boxes	6 mo.	Refrigerator
	F	Sealed individually in white disposable molds	Plastic bag	3 mo.	Refrigerator
	G	No wrapping; all in contact with each other	Wide mouth, amber plastic bottle	1 yr	Not specified
	H	No wrapping; all in contact with each other	Child-resistant, wide mouth, amber plastic bottle	6 mo.	Refrigerator
	I	Sealed individually in white disposable molds	Plastic bag	N/A	Not specified
	J	Sealed individually in white disposable molds	Plastic bag	6 mo.	Not specified

Appearance

Physical characteristics of each product were determined and reported in terms of color, shape and texture.

pH

Gel Product

A gel sample from 30 applicators was transferred into a 50 mL centrifuge tube and mixed until homogeneous. The pH of the sample was measured by immersing the electrode in the gel. The pH meter was qualified using standard pH buffers.

Suppository Products

One suppository from each product lot was melted in 10 mL of hot water pre-heated to about 60–70°C. The sample was mixed on a stir plate until completely melted. When the sample was cooled to about 38–40°C, the electrode was immersed in the liquid to measure the pH as reported previously.[6]

Uniformity of Content

Sample Preparation

Gel Product

The content uniformity assay was conducted on samples from ten individual applicators. The content of each applicator was dispensed into a 200 mL volumetric flask. About 60 mL of methanol was added to the flask and the sample flask was shaken until all the content was completely dispersed prior to an additional 10-minute sonication. The sample was allowed to equilibrate to ambient temperature prior to diluting to a final volume with methanol and mixed well. A 5-mL aliquot of the stock sample was accurately transferred into a 100 mL volumetric flask and diluted to volume with methanol. The sample was then filtered through a 0.45 μm PTFE syringe filter into a HPLC vial for further analysis.

Suppository Products

The content uniformity assay was conducted on ten[10] individual suppository samples from each product lot. Each suppository was weighed into a 200 mL volumetric flask. The suppository was melted by submerging the sample flask in a 50.0±0.5°C water bath until completely melted. About 160 mL of methanol was added to the flask and the sample flask was shaken until all of the contents were completely dispersed. The sample was allowed to equilibrate to ambient temperature prior to diluting to a final volume with methanol and mixed well. A 5-mL aliquot of the stock sample was accurately transferred into a 100 mL volumetric flask and diluted to volume with methanol. The sample was then filtered through a 0.45 μm PTFE syringe filter into a HPLC vial for further analysis.

Sample Analysis

An isocratic reversed-phase HPLC method was qualified and used for sample analysis. The HPLC chromatographic system (Shimadzu, Columbia, MD) consisted of a system controller (Model SCL-10A VP), a detector (Model SPD-M10A VP, deuterium lamp), a chromatographic data control and acquisition system (Class-VP software), pumps (Model LC-10AT VP), and an auto-injector (SIL-10A). A Waters μBondapak C18, 10 μm, 4.6×250 mm (Waters Corporation, Milford, MA) was used for sample analysis. The mobile phase was composed of methanol and water in a ratio of 75:25 (v/v). Prior to the analysis, the mobile phase was filtered through a 0.45 μm nylon membrane filter (Whatman, 47 mm diameter, Whatman International Ltd., Maidstone, England), and degassed with vacuum sonication for 5 minutes. The flow rate of the mobile phase was 1.5 mL/min and the injection volume was 20 μL. The absorbance was monitored at 275 nm. The run time was 10 minutes. System suitability was established using standards at a nominal concentration of 23 μg/mL.

Efficacy of Antimicrobial Preservative (PET)

Preservative effectiveness testing (PET) was conducted according to the European Pharmacopoeia (Ph. Eur.)<5.1.3> and United States Pharmacopoeia (USP)<51>, Antimicrobial Effectiveness Testing. Test microorganisms included *Aspergillus niger, Escherichia coli, Candida albicans, Staphylococcus aureus,* and *Pseudomonas aeruginosa*.[4,7]

Microbial Limits

Microbial limit testing was conducted according to Ph Eur.<5.1.4> and USP<61>, Microbial Limit Tests. Testing was conducted on total viable aerobic plate count (bacterial plate count), total yeast and mold

count, *Escherichia coli* and Salmonella, Pseudomonas and Staphylococcus, yeast and mold, and Enterobacteriaceae.[4,7]

RESULTS AND DISCUSSION

Commercial progesterone vaginal gel product (Prochieve®/Crinone®), which is manufactured according to current cGMPs, contains micronized progesterone in an oil-in-water emulsion system, packaged in a single use, vaginal applicator, wrapped and sealed in a foil over wrap. Progesterone is partially soluble in both the oil and water phases of the system, with the majority of drug existing as a suspension. The product has 3-year shelf-life and should be stored at 25°C (77°F), with excursions permitted to 15–30°C (59–86°F).

The other products investigated in this study were extemporaneously compounded vaginal suppositories containing 90 mg progesterone in ten randomly chosen pharmacies pursuant to written prescriptions from a licensed physician. Even though compounding is different from manufacturing in terms of GMP requirements, it is the compounding pharmacist's responsibility to ensure that the compounded suppository has the desired strength, quality, and purity, and is dispensed with the appropriate packaging and labeling, all compounding and dispensing activities performed in accordance with Good Compounding Practices (e.g. USP<1075>). Progesterone vaginal suppositories can be prepared using different suppository base excipients, including glycerinated gelatin, cocoa butter, and polyethylene glycol (PEG). Extemporaneous preparation of suppositories usually involves hand molding, fusion, or cold compression. However, the fusion technique is the most commonly employed preparation method to date.[8,9] Price et al. reported that the levels of progesterone in serum achieved after administration of vaginal suppositories containing progesterone was affected by the choice of the suppository base excipient in which progesterone was delivered. The PEG base was superior to the other bases reported in terms of providing the highest peak plasma progesterone concentration during follicular phase of normal subjects, producing a rapid disintegration of the suppository, minimizing leakage, and providing a specific melting point by varying ratios of the low-to-high molecular weight of PEGs in the formulations.[3,10] Therefore, PEG base is commonly used to prepare progesterone suppositories.[2,11] Additionally, liquefaction time, which is measured from the time the suppository was positioned in the dialysis membrane to the time when no visible solid suppository remained, of different types of fatty suppository bases (i.e. Witepsol® H-15 (Riches–Nelson, Inc., Greenwich, CT), Fattibase™ (Paddock Laboratories, Inc., Minneapolis, MN), and cocoa butter (Woltra, New York, NY), was influenced by storage time and temperature. The liquefaction time for most fatty-base suppositories increased with storage time due to gradual, polymorphic changes in the bases during stability storage. Therefore, those suppositories were recommended to be stored under refrigeration to prevent substantial prolongation of liquefaction time.[12]

The recommended packaging of suppositories is individually wrapped or dispensed in the disposable molds in which they are prepared. If they are not packaged properly, they may become deformed, stained, broken, or chipped. From Table 1, six of ten suppository products were properly packaged by using sealed individual disposable molds. This type of packaging is ideal to minimize direct product contact to external environment that may enhance product's instability and to prevent any contaminations. Suppositories should be protected from heat and should be stored in a refrigerator, but should not be frozen. Glycerin and PEG-based suppositories should also be protected from moisture because they tend to be hygroscopic.[8]

Two types of suppository compounded in fatty acid base and PEG base are listed in current USP monograph of Progesterone Vaginal Suppositories. The micronized progesterone content in each suppository can be varied from 25 to 600 mg with no less than 90.0% and not more than 110.0% of the labeled amount of progesterone. For each type of progesterone vaginal suppository, the beyond-use date of ninety days after the day on which they were compounded and the storage under refrigeration are recommended in the current monograph.

Stability

As can be seen in Table 1, the recommended storage condition given on the labels for most compounded suppositories investigated in this study was 5°C (e.g. refrigerator). However, the recommended storage condition of some products (i.e. lots G, I and J) was not specified. It was unclear if any product stability study was properly conducted in those specific formulations to ensure no change in physical-chemical properties of the product throughout the shelf-life, especially when products were stored at high temperature. Additionally, the shelf-life of compounded suppositories varied from a minimum of 3 months to a maximum of 1 year after compounding (Table 1).

Expiration dating of some products (i.e. lots D and I) was not provided on the label, which violated Good Compounding Practices with regards to labeling requirements. USP recommends that the label on the container or package of an official compounded preparation must bear a beyond-use date, based on published data, or appropriate testing, or USP-NF standards. Even though product lot G had the longest shelf-life of 1 year, the product was packaged in a wide mouth, amber plastic bottle without proper individual wrap to prevent any possible contaminations from multiple uses. Stability of this product throughout it's' shelf-life could be questioned since it was not properly packaged and a recommended storage condition was not specified. According to USP<795>, Pharmacy Compounding Practices for nonaqueous solid formulations, a beyond-use date of 25% of the time remaining until the product's expiration date if the product is prepared using a manufactured product or six months, whichever is earlier, can be used. If the product is prepared from USP/NF ingredients, a beyond-use date of six months is appropriate, unless supporting evidence is available to support other dating.[4,9,10]

Comparison of physical and chemical properties of commercial gel product and compounded suppositories was as follows:

Appearance. The physical properties, including product appearance and pH, were included in Table 2. The appearance of the commercial progesterone gel, which was soft and white, is beneficial for providing a coating on the walls of the vagina. Most compounded suppositories were either bullet or cone shaped, which were also suitable for vaginal delivery. The product texture was varied ranging from soft to brittle, depending on the type of suppository base used in the formulation. Since suppositories from most of the pharmacies investigated were properly packaged in individual disposable molds, the product shape was consistently maintained without chips or cracks. However, product lot G, which was improperly packaged, had suppositories that were deformed or chipped.

pH. The normal pH of the vagina is in the acidic range of 3.8 to 4.5. A vaginal pH above 4.5 can be an indication of either infection or menopause.[13] As shown in Table 2, the pH of most of the compounded suppository products was in the range of 4.22 to 7.68. The commercial gel product and some of the compounded suppositories (i.e. product lots A, D, G, and J) were formulated to have the final product pH closer to the vaginal fluid to prevent any irritations that might occur. Suitable product pH will minimize irritation to the vaginal mucosa caused by contact with the product.

Uniformity of Content. According to USP<795>, the compounded preparations are to be prepared to ensure that each preparation shall contain not less than 90.0% and not more than 110.0% of the theoretically calculated and labeled quantity of active ingredient per unit weight or volume, and not less than 90.0% and not more than 110.0% of the theoretically calculated weight or volume per unit of the preparation. For the official monograph of progesterone suppository, the product should contain not less than 90.0% and not more than 110.0% of the labeled amount of progesterone. Additionally, a representative number of suppositories should weigh

Table 2. Appearance and pH of progesterone products investigated in this study.

Progesterone delivery system	Product lot	Appearance	pH
Gel (commercial product)	C03126	Soft, lump free, white gel	2.80
Pharmacy compounded suppositories	A	White, bullet shaped, air trapped in the texture, brittle	4.45
	B	White, bullet shaped	7.68
	C	White, small, cone shaped	7.22
	D	White, bullet shaped	4.28
	E	White, bullet shaped	7.17
	F	White, bullet shaped	7.48
	G	White, small, cone shaped, soft textured (some were deformed or chipped)	4.22
	H	White, bullet shaped, brittle	6.37
	I	White, bullet shaped	6.23
	J	White, bullet shaped	5.56

not less than 90% and not more than 110% of the average weight of all suppositories in the batch.[4]

The release specification for the progesterone content of the cGMP manufactured progesterone vaginal gel product is 95.0%–105.0% of the label claim. From the written prescription used for this study, all compounded suppositories were to contain 90 mg progesterone, however, compounded suppositories from product lot E contained 100 mg as labeled on the package. Thus, the content uniformity of product lot E was calculated based on 100 mg label claim. The results from the weight uniformity and content uniformity testing of the progesterone products investigated in this study are summarized in Table 3.

The USP specification for compounded preparations allows for a wider range of acceptable progesterone content than the GMP release specification for the vaginal gel (90.0%–110.0% vs. 95.0%–105.0%, respectively). The data in Table 3 shows that only 2 pharmacies provided all suppositories within the cGMP specification for the progesterone vaginal gel. Five of the 10 pharmacies provided some suppositories that were within 95.0%–105.0% of label claim and some that were not.

The weight of the compounded suppositories ranged from 1–2 grams with weight variations ranging from 0.4 to 5.6%. For progesterone content in each product lot investigated, the average content uniformity of 10 suppositories from each lot ranged from 76.5 to 113.1% label claim with a RSD ranging from 1.6 to 10.2%. Specifically, progesterone content in the product lots from five of the ten pharmacies investigated (i.e. lots A, C, D, G, and H) were outside the USP specification of 90.0 to 110.0% label claim of progesterone. Even though the average content uniformity of product lot E met the specification, the variation in the product content was too high as indicated by the high RSD of 10.2%. For product lot E, one suppository out of the 10 suppositories tested contained 126.4% label claim of progesterone. This indicated extreme inconsistency in drug content. The average content of suppositories from the other product lots investigated (i.e. lots A, C, D, and G) were below the amount or progesterone claimed on the labels. This may result in ineffective therapeutic treatment. Product lot I had a percent RSD for content uniformity greater than 5% with the progesterone content ranging from 82.6 to 98.7% (93.7% average content). This was caused, in part, by the high percent weight variation of greater than 5% as compared to other product lots of suppositories.

The commercial gel product, prior to being released by the manufacturer for human use, is initially tested and must meet product release specifications for USP or Eur. Ph. for progesterone content, appearance, pH, viscosity, related substance limits, microbial limits and preservative effectiveness testing. The progesterone content in the gel product meets USP requirements. From Table 3, the percent weight variation of the gel product from 10 applicators (i.e. average filled weight) was 7.4%, the average content uniformity was 101.9% of label claim with a low RSD of 1.0%. All results met the approved specifications for the product.

Efficacy of Antimicrobial Preservative (PET). The preservative effectiveness test (PET) or challenge test

Table 3. Weight uniformity and content uniformity of progesterone products investigated in this study.

		Weight (n=10)		Content uniformity (%Label claim, n=10))			
Progesterone delivery system	Product lot	Avg. (mg)	%Wt variation	Avg.	Min.	Max.	% RSD
Gel (commercial product)	C03126	1445.9	7.4	101.9	100.6	103.3	1.0
Pharmacy compounded suppositories	A	1903.3	2.0	79.4	76.4	82.6	2.3
	B	2272.2	1.8	108.2	103.8	119.6	4.0
	C	2022.2	1.2	86.8	84.5	88.4	1.6
	D	2150.7	2.5	81.0	78.2	83.5	2.6
	E	2253.9	0.4	105.7	97.9	126.4	10.2
	F	2013.0	2.1	92.8	89.1	95.6	2.3
	G	1160.6	2.1	76.5	73.4	79.0	2.3
	H	2081.4	2.6	113.1	109.7	118.0	2.5
	I	1913.4	5.6	93.7	82.6	98.7	5.6
	J	1809.0	2.1	97.8	93.9	100.7	2.1

Table 4. Criteria for PET of topical products as listed in the United States Pharmacopoeia (USP) and the European Pharmacopoeia (Ph.Eur.).

Source	Microorganisms	Criteria
USP<51>	Bacteria:	
	• *E. coli*	At 14 days: not less than 2.0 log reduction from initial count
	• *P. aerugenosa*	At 28 days: no increase rom the 14 days' count
	• *S. aureus*	
	Yeast	
	• *C. albicans*	At 14 and 28 days: no increase from the initial count
	Molds	
	• *A. niger*	
Eur.Ph.<5.1.3>	Bacteria:	
	• *E. coli*	At 2 days: not less than 2.0 log reduction from initial count
	• *P. aerugenosa*	At 7 days: not less than 3.0 log reduction from initial count
	• *S. aureus*	At 28 days: no increase from initial count
	Fungi (yeast & molds)	
	• *A. niger*	At 14 days: not less than 2.0 log reduction from initial count
	• *C. albicans*	At 28 days: no increase from initial count

is a procedure to determine whether a drug product is adequately preserved to prevent contamination from the bulk ingredients prior to preparation of the product, during consumer use, and throughout the product's shelf-life. Several factors can influence the preservative activity, such as concentration of preservative, pH, temperature, formulation composition, containers and closures, micro-organisms (i.e. type, nature, and condition), and organic matter.[14] It is not uncommon for suppositories that are extemporaneously compounded to not contain any preservatives or antioxidants, especially when water is excluded from the formulation, a non-oxidizing base is used or the drug is stable in the dosage form for the proposed short shelf-life. However, the accurate shelf-life should be based on stability studies conducted with the drug product stored in the primary dispensing container in a realistic environment.[9] According to USP<51> for category 2 products and Eur. Ph.<5.1.3> for topical preparations, requirements

Table 5. Microbial limits and preservative effectiveness test for the progesterone products investigated in this study.

Progesterone delivery system	Product lot	Microbial limits	PET
Gel (commercial product)	C03126	Complies with the USP/Ph.Eur.	NPD[c] (<10 cfu) complies to the USP/Ph.Eur.
Pharmacy compounded suppositories	A	Complies with the USP/Ph.Eur.	Complies to the USP/Ph.Eur
	B	Complies with the USP/Ph.Eur.	Complies to the USP/Ph.Eur
	C	Complies with the USP/Ph.Eur.	Complies to the USP/Ph.Eur
	D	Complies with the USP/Ph.Eur.	NPD[c] (<10 cfu/supp.) complies to the USP/Ph.Eur
	E	Complies with the USP/Ph.Eur.	Complies to the USP/Ph.Eur
	F	Complies with the USP/Ph.Eur.	Complies to the USP/Ph.Eur
	G	Complies with the USP/Ph.Eur.[a,b]	Complies to the USP/Ph.Eur
	H	Complies with the USP/Ph.Eur.	Complies to the USP/Ph.Eur
	I	Complies with the USP/Ph.Eur.	Complies to the USP/Ph.Eur
	J	Complies with the USP/Ph.Eur.	Complies to the USP/Ph.Eur

[a]Negative for *E. coli* and Salmonella species. However, CDC group IVC-2 and *Comamonas acidovorans* was found.
[b]Negative for enterobacteria. However, *Comamonas acidovorans* was found.
[c]NPD = No Pathogens Detected.

Table 6. Summary of microbial limits for the progesterone products investigated in this study.

Product lot	Bacterial plate count	*E. coli* and salmonella species	Enterbacteria	Pseudomonas and staphylococcus	Yeast and mold count
C03126	None detected <10 cfu/g	Negative	Negative	Negative	None detected <10 cfu/g
A	None detected <10 cfu/g	Negative	Negative	Negative	None detected <10 cfu/g
B	None detected <10 cfu/g	Negative	Negative	Negative	None detected <10 cfu/g
C	None detected <10 cfu/g	Negative	Negative	Negative	None detected <10 cfu/g
D	None detected <10 cfu/g	Negative	Negative	Negative	None detected <10 cfu/g
E	None detected <10 cfu/g	Negative	Negative	Negative	None detected <10 cfu/g
F	None detected <10 cfu/g	Negative	Negative	Negative	None detected <10 cfu/g
G	None detected <10 cfu/g	Negative[a]	Negative[b]	Negative	None detected <10 cfu/g
H	None detected <10 cfu/g	Negative	Negative	Negative	None detected <10 cfu/g
I	None detected <10 cfu/g	Negative	Negative	Negative	None detected <10 cfu/g
J	None detected <10 cfu/g	Negative	Negative	Negative	None detected <10 cfu/g

[a]Negative for *E. coli* and Salmonella species. However, CDC group IVC-2 and *Comamonas acidovorans* was found.
[b]Negative for enterobacteria. However, *Comamonas acidovorans* was found.

for PET testing are summarized in Table 4.[4,7] As can be seen in Table 5, PET results for both the gel and suppository products were in compliance with the USP and Ph. Eur. requirements listed in Table 4. No pathogens were detected (i.e.< 10 cfu) for either the commercial gel product or suppository product lot D. For the other compounded product lots, *A. niger* was detected at a level of greater than 10 cfu per suppository, however the overall results still met the requirements listed in Table 4.

Microbial Limits. Tables 5 and 6 summarizes the results obtained from microbial limit testing as specified in the USP and Eur. Ph. for all products investigated. According to USP<61> and Eur. Ph.<5.1.4> requirements on microbial limits, the bacterial plate count or total aerobic microbial count and total combined molds and yeasts count are quantitative determinations, whereas tests for *E. coli*, Salmonella, Enterbacteria, Pseudomonas, and Staphylococcus are qualitative determinations for the presence or absence of these microorganisms (i.e. positive/negative results).[4,7] The results for bacterial count and total yeast and mold count indicated none were detected for all products. This also indicated that no microbial colonies were recovered from the plates representing the initial 1:10 dilution of the specimen, and the results were expressed as less than 10 colony forming units/gram (<10 cfu/g). For tests of other microorganisms, negative results were reported for all products investigated. However, CDC group IVC-2 and *Comamonas acidovorans* were detected in compounded product lot G, which was packaged in a wide mouth amber plastic bottle without individual wrapping the suppositories.

Iwata et al. reported that the absorption of progesterone in a rabbit model was significantly influenced by the composition of the suppository formulation.[15] The authors found that inclusion of a surfactant in the suppository formulation influenced the release of progesterone from the suppository, depending on the base excipient used (e.g. fatty base with and without ethylene-vinyl acetate copolymers). Depending on exact composition, the area under the plasma concentration –time curve was increased by about 1.6-times with the inclusion of sodium caproate. Cicinelli et al. found that the vaginal route of administration of micronized progesterone in an oil based solution created a preferential distribution of progesterone in the uterus, as measured by determining mean plasma levels of progesterone in the uterine and radial arteries.[16] Since progesterone is not very soluble in the aqueous vaginal fluids, the rate of dissolution of progesterone from the delivery system in the vaginal cavity will be controlled by two factors: first, the rate of release of drug from the dosage form and second, the rate of dissolution of drug particles in the aqueous vaginal fluids.

CONCLUSION

It was clearly demonstrated in this study that the quality of extemporaneously compounded drug delivery systems was inconsistent from pharmacy-to-pharmacy. The preparation of the compounded product is solely the responsibility of the compounding pharmacist to ensure appropriate dose and dosage form being prepared and dispensed for the particular patient since the compounding should be conducted according to current Good Compounding Practices.

Quality control tests should be established for extemporaneously compounded drug delivery systems, including vaginal suppositories, in order to ensure that the highest quality product is dispensed to patients. Product attributes, including content uniformity and potency, pH, preservative effectiveness testing and microbial testing, are each critical factors that must be precisely controlled for reproducible delivery of active pharmaceutical ingredients such as progesterone. Progesterone, having a superpotent or subpotent level of drug in the suppository could result in different and variable plasma levels. Since the exact composition of any of the suppository formulations was not disclosed by the compounding pharmacies used in this study, the potential impact of the excipients on intravaginal delivery and bioavailability of progesterone was not known. It is known that the rate of dissolution is influenced by the particle size of the solid particle of drug, so control of the particle size is important and can affect dissolution and subsequent absorption and bioavailability. It is apparent that many factors will influence the delivery of progesterone and its subsequent bioavailability and therapeutic efficacy. The findings of this investigation are alarming, in light of the published literature, and confirm that compounded progesterone suppositories are not of high quality and cannot guarantee reproducible drug delivery. Semisolid dosage forms, such as suppositories, yielded more inconsistent results when compared to a vaginal gel product. Settling of the active during preparation of the suppository is difficult to control again when compared to the gel product. Consistent product quality of the suppositories was not evident from this independent investigation.

ACKNOWLEDGMENT

The authors acknowledge financial support from Columbia Laboratories, Inc.

REFERENCES

1. American Medical Association. *Family Medical Guide,* 3rd Ed.; Clayman, C.B., Ed.; Random House, Inc.: New York, 1994; 25.
2. Pesko, L.J. Progesterone suppositories. Am. Drug. (USA) **1990**, *201* (1), 52–53.
3. Price, J.H.; Ismail, H.; Gorwill, R.H.; Sarda, I.R. Effect of the suppository base on progesterone delivery from the vagina. Fertil. Steril. **1983**, *39* (4), 490–493.
4. The United States Pharmacopoeia Convention. *The United States Pharmacopoeia XXVII-National Formulary XXII*; The United States Pharmacopoeia Convention, Inc.: Rockville, MD, 2004.
5. *Report: Limited FDA Survey of Compounded Drug Products*; http://www.fda.gov/cder/pharmcomp/survey.htm (accessed Sep 2004).
6. Azechi, Y.; Ishikawa, K.; Mizuno, N.; Takahashi, K. Sustained release of diclofenac from polymer-containing suppository and the mechanism involved. Drug Dev. Ind. Pharm. **2000**, *26* (11), 1177–2283.
7. The Council of Europe. *The European Pharmacopoeia 4.0*; The Council of Europe: Strasbourg, France, 2004.
8. Allen, L.V. The basics of compounding: compounding suppositories, part two- extemporaneous preparation. Int. J. Pharm. Compd. **2000**, *4* (5), 371–373.
9. Duncan-Hewitt, W.; Rains, J. Compounded suppositories and pharmaceutical care. Int. J. Pharm. Compd. **1997**, *1* (2), 93–99.
10. Allen, L.V. The basics of compounding: compounding suppositories, part one- theoretical considerations. Int. J. Pharm. Compd. **2000**, *4* (4), 289–293.
11. Al-Achi, A.; Webster, K.; Greenwood, R.; Nicol, S. Assaying progesterone in extemporaneously prepared suppositories. Int. J. Pharm. Compd. **1997**, *1* (5), 339.
12. Fulper, L.D.; Cleary, R.W.; Harland, E.C.; Hikal, A.H.; Jones, A.B. Liquefaction times of fatty-type suppositories with and without progesterone. Am. J. Hosp. Pharm. (USA) **1990**, *47* (3), 602–603.
13. Caillouette, J.C.; Sharp, C.F.; Zimmerman, G.J.; Roy, S. Vaginal pH as a marker for bacterial pathogens and menopause status. Am. J. Obstet. Gynecol. **1997**, *176* (6), 1270–1275.
14. Russell, A.D. Challenge testing: principles and practice. Int. J. Cosmet. Sci. **2003**, *25*, 147–153.
15. Iwata, M.; Takahashi, Y.; Shirotake, S.; Takayama, K.; Nagai, T. Effect of sodium caprate on the absorption of progesterone from mixed suppositories administered to the vagina of rabbits. Drug Dev. Ind. Pharm. **1997**, *23*, 167–172.
16. Cicinelli, E.; Cignarelli, M.; Sabatelli, S.; Romano, F.; Einer-Jensen, N. Plasma concentrations of progesterone are higher in the uterine artery than in the radial artery after vaginal administration of micronized progesterone in an oil based solution to postmenopausal women. Fertil. Steril. **1998**, *69*, 471–473.