Analytical Method Validation of High-Performance Liquid Chromatography and Stability-Indicating Study of Medroxyprogesterone Acetate Intravaginal Sponges

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ABSTRACT: Medroxyprogesterone acetate is widely used in veterinary medicine as intravaginal dosage for the synchronization of breeding cycle in ewes and goats. The main goal of this study was to develop reverse-phase high-performance liquid chromatography method for the quantification of medroxyprogesterone acetate in veterinary vaginal sponges. A single high-performance liquid chromatography/UV isocratic run was used for the analytical assay of the active ingredient medroxyprogesterone. The chromatographic system consisted of a reverse-phase C18 column as the stationary phase and a mixture of 60% acetonitrile and 40% potassium dihydrogen phosphate buffer as the mobile phase; the pH was adjusted to 5.6. The method was validated according to the International Council for Harmonisation (ICH) guidelines. Forced degradation studies were also performed to evaluate the stability-indicating properties and specificity of the method. Medroxyprogesterone was eluted at 5.9 minutes. The linearity of the method was confirmed in the range of 0.0576 to 0.1134 mg/mL ($R^2 > 0.999$). The limit of quantification was shown to be 3.9 µg/mL. Precision and accuracy ranges were found to be %RSD <0.2 and 98% to 102%, respectively. Medroxyprogesterone capacity factor value of 2.1, tailing factor value of 1.03, and resolution value of 3.9 were obtained in accordance with ICH guidelines. Based on the obtained results, a rapid, precise, accurate, sensitive, and cost-effective analysis procedure was proposed for quantitative determination of medroxyprogesterone in vaginal sponges. This analytical method is the only available method to analyse medroxyprogesterone in veterinary intravaginal dosage form.

KEYWORDS: medroxyprogesterone, intravaginal sponges, HPLC, validation

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Introduction

Medroxyprogesterone acetate also known as 17α-hydroxy-6α-methylprogesterone acetate is a synthetic analogue of the steroid hormone progesterone[1,2] (Figure 1).

Medroxyprogesterone acetate is used as a contraceptive, in hormone replacement therapy, for the treatment of endometriosis in human, and in several other indications.[3,4]

In veterinary medicine, medroxyprogesterone acetate is used as intravaginal dosage form and is indicated for the synchronization of breeding cycle in ewes and goats.[5,6] It is intended to be used to control the timing of oestrus which allows the timing and duration of lambing to be planned. The optimum number of ewes that can be synchronized at one time is determined by the number of fertile rams available at breeding time and the number of lambings that can be handled during the compressed lambing period. Syncro-Breed sponges in conjunction with pregnant mare's serum gonadotropin may be used prior to the normal breeding season to induce oestrus in ewes at a time when they would otherwise be anestrus.[7,8] Breeding at the induced early oestrus advances the time of lambing and produces lambs for the profitable early lamb market.[5]

There are many approved intravaginal sponges in the local and international market.[9,10] To the best of our knowledge, there is no validated analytical method available to quantify medroxyprogesterone and its degradative ingredients, including any possible impurities in veterinary intravaginal sponge dosage forms.

Our main objective of this study was to establish a validated and stability-indicating high-performance liquid chromatography (HPLC) assay method for the determination of medroxyprogesterone acetate in the intravaginal sponges. This study is to establish document evidence in accordance with international guidelines, which provide a high degree of assurance that our developed HPLC assay method for the determination of medroxyprogesterone acetate in the intravaginal sponges is reliable to achieve its intended purpose.

A locally formulated sponge containing 60 mg medroxyprogesterone acetate (Syncro-Breed) was formulated in our research laboratory at Advanced Veterinary Company, Ramallah. An analytical method and stability-indicating study were developed. The developed method was validated in accordance with the requirements of Food and Drug Administration and International Council for Harmonisation (ICH) guidelines.[11–14]

To our knowledge, there is no analytical method in the literature that analyses medroxyprogesterone as intravaginal sponge dosage form. There are few methods in the literature that analyse medroxyprogesterone using HPLC.[15–17] The

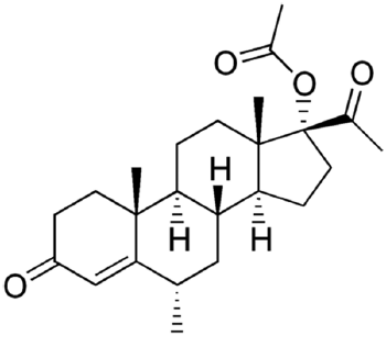

Figure 1. Medroxyprogesterone acetate.

advantage of our developed method compared with that mentioned in the literature is that it is simpler and quicker. Our developed method did not use internal standard (IS), and it used isocratic run which made it easier compared with those mentioned in the literature. In addition, the method has been successfully used for the analysis of drug-excipient compatibility with the formulations and subsequently an in-house stability-indicating study.

The method can be adapted by official pharmacopoeias and can be applied in quality control laboratories for the quantification of medroxyprogesterone in veterinary vaginal sponges. It can be used for purity and degradation evaluation of medroxyprogesterone in formulation as well as raw material.

Methodology

Reagent

Medroxyprogesterone acetate active ingredients were purchased from Taizhou Taifa Pharma Co. Ltd, Zhejiang, China. The medroxyprogesterone United States Pharmacopeia (USP) reference standard (99.8%) was purchased from Sigma-Aldrich, Munich, Germany. The intravaginal sponges containing 60 mg medroxyprogesterone was formulated in our research laboratory. The acetonitrile used was of HPLC grade. The water for HPLC was obtained by double distillation. Other reagents, such as KH_2PO_4, hexanesulphonic acid sodium salt, hydrochloric acid, sodium hydroxide, and hydrogen peroxide, were purchased from Merck-USA, Sigma-Aldrich-Germany, and J.T.Baker-USA reliable commercial sources and were used as such.

Instrumentation

A Dionex-UltiMate 3000 HPLC system equipped with LPG-3400SD pump, WPS-3000SL autosampler, TCC-3000 column oven, and DAD-3000 UV-VIS with diode array detector was used. Chromeleon data system software (Version 6.80 DU10A Build 2826 [171948]) was used for data acquisition and mathematical calculations. The HPLC-grade water was prepared by Aquatron equipment model A4000D. The stationary phase used was LiChrospher 60 RP-Select B, 5 µm, 125 × 4 mm (Merck, USA).

Table 1. High-performance liquid chromatographic conditions.

Flow rate	1.0 mL/min
Wavelength (λ)	243 nm
Temperature	25°C
Injection volume	20 µL

Chromatographic condition

A reverse-phase HPLC chromatography was used. The stationary phase used was LiChrospher 60 RP-Select B, 5 µm, 1254 mm (Merck). The mobile phase consists of 60% acetonitrile:40% buffer (0.02 M). The buffer was prepared by dissolving 1.36 g of KH_2PO_4 and 50 mg of 1-hexanesulphonic acid sodium salt in sufficient water to produce 500 mL; the final pH was adjusted to 5.6. The chromatographic conditions are outlined in Table 1.

Preparation of standard and working solutions

The standard solution was prepared by transferring accurately weighed 60 mg of the USP standard medroxyprogesterone acetate into a 100-mL volumetric flask and dissolving well in ethanol; then, 4 mL of the resulting solution was diluted to 25 mL by mobile phase. The sample solution was prepared by immersing 1 sponge in 20 mL ethanol. The sponge was stirred and pressed well to extract its content, and then, the solution was transferred into a 100-mL volumetric flask. The sponge was extracted 4 times, and the volume was adjusted to 100 mL using the same solvent; 4 mL of the resulting solution was diluted to 25 mL by mobile phase.

Method validation

The method was validated for parameters such as specificity, linearity, range, accuracy, precision, and ruggedness/robustness.

To evaluate the linearity and range of the method, 5 different test concentrations were prepared (based on the original formulation): 60%, 80%, 100%, 120%, and 140%. Ten separate injections were analysed under the same conditions. The accuracy and precision were measured by performing the assay of samples (spiked placebos) prepared at concentration levels of 80%, 100%, and 120% of the test concentration, with 3 replicates for each concentration. The percentage recovery and %RSD were calculated for each of the replicate samples. The limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated based on the standard deviation of the response (σ) and slope approach as defined in ICH guidelines.[18] The LOD was calculated using the formula 3.3*σ/slope, and the LOQ was calculated using the formula 10*σ/slope.

The robustness of the method was determined by performing the same trial using different detection wavelengths and flow rates. The ruggedness (repeatability) was tested by elapsed

Table 2. The applied ruggedness/robustness conditions.

ROBUSTNESS PARAMETER	CONDITION CHECKED
Detection wavelength	241, 243, and 245 nm
Flow rate of the mobile phase	0.8, 1.0, and 1.2 mL/min
Elapsed assay times	The same sample working solution was analysed different times after preparation (*stability of working solution*)
Analyst days	Two analysts analysed the same trial in the same day The same analyst analysed the same trial in 2 different days

Table 3. Conditions followed in the stress study protocol.

	STRESS TYPE	CONDITIONS	TIME
Drug substance	Acid hydrolysis	1 mg/mL in 0.1 N (up to 1N), HCl at RT or higher	1-7 days
	Base hydrolysis	1 mg/mL in 0.1 N (up to 1 N), NaOH at RT or higher	1-7 days
	Oxidative/solution	0.3% (up to 3%) H_2O_2, at RT, protected from light	Few hours to 7 days
Drug product	Thermal	70°C	Up to 3 weeks
	Photodegradation	Fluorescent and UV light	Few hours to 3 days
	Oxidative/solution	0.3% (up to 3%) H_2O_2, at RT, protected from light	Few hours to 7 days

Abbreviation: RT, room temperature.

assay time and by an analyst. The applied ruggedness/robustness parameters are illustrated in Table 2.

Forced degradation study

Forced degradation studies were performed to evaluate the stability-indicating properties and specificity of the method. Intentional degradation was performed by exposing the formulation to 5 stress conditions. The conditions mentioned in Table 3 are the general conditions which are generally followed in the stress study protocol. Stressed samples were analysed periodically, and the presence of related peaks and peak purity for the active ingredients was checked.

Results

The developed method demonstrated linearity within the range of 60% to 140% around the test concentration. The regression line equation was $y = 794.09x - 0.3115$, and the goodness of fit (R^2) was found to be 0.9999, indicating a linear relationship between the concentration of analyte and area under the peak (Figure 2).

The LOD and LOQ were calculated and found to be 1.3 and 3.9 µg/mL, respectively.

The analytical methods were also precise and accurate, the percentage recovery for all the tested samples was in the range of 98% to 102%, and the relative standard deviation (RSD) was less than 2. The analysis of variance test between samples shows no statistical significant difference ($P > .05$). The 1-sample t test of all the assay results showed no significant variation from 100% ($P > .05$). The test results are illustrated in Table 4.

Forced degradation study was conducted by submitting drug product and drug substance to stress conditions of light, heat, acid/base hydrolysis, and oxidation. Solutions of standard, sample, blank, and stressed samples were analysed occasionally; stress testing is terminated if 5% to 20% degradation is obtained or if no degradation is observed after the maximum recommended time. The related peaks were compared and checked for the retention times, area under peaks, peak interference, peak purity, and separation factors (Table 5).

The results demonstrated that the developed method was robust and reproducible (ruggedness) at the mentioned conditions stated in Table 2. All the results of the analysed samples were consistent, and the RSD of all the tests at different conditions was less than 2.

System suitability is used to verify that the system is adequate for the analysis to be performed. Our method showed that all the values for the system suitability parameters are within limits (Table 6 and Figure 3). The column efficiency was about 7150 theoretical plates. The tailing factors are about 1.03, and the resolution is 3.9.

Discussion

The method is selective for medroxyprogesterone acetate because interference between both peaks or between them and any detectable inactive or degradative material peaks were not found. Medroxyprogesterone under the alkaline condition showed significant degradation. The early eluted degraded

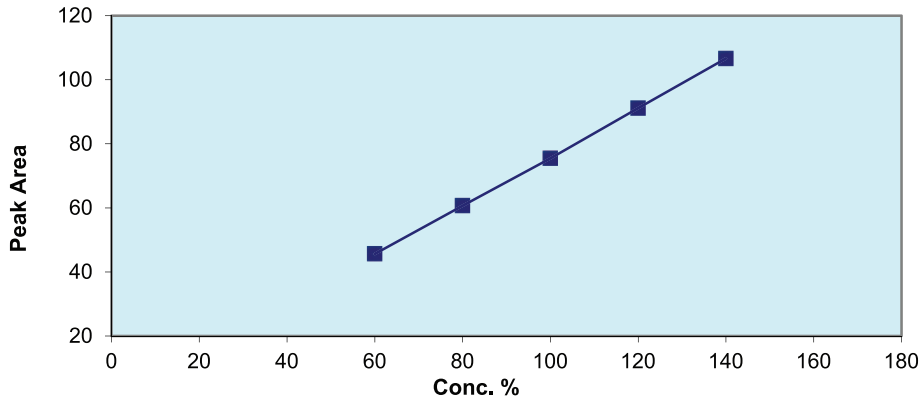

Figure 2. Linearity within the range of 60% to 140%.

Table 4. The accuracy and precision results.

SAMPLE	SAMPLE PEAK AREA			STANDARD PEAK AREA			ASSAY (%)
NO.	INJ. # 1	INJ. # 2	ANOVA TEST (*P* VALUE)	INJ. # 1	INJ. # 2	AVERAGE	
			80%				
1	62.05	61.97					99.92
2	61.25	61.17	0.923	62.23	61.89	62.06	98.63
3	62.30	62.32					100.40
			100%				
1	78.24	78.23					100.92
2	79.51	79.50	0.97	77.48	77.57	77.53	102.55
3	79.81	79.75					102.91
			120%				
1	92.96	92.90					100.91
2	92.92	92.77	0.925	91.99	92.19	92.09	100.82
3	94.41	94.40					102.51
Mean							101.06
SD							1.39
RSD							1.38
% of recovery							101.06

Abbreviations: ANOVA, analysis of variance; RSD, relative standard deviation; SD, standard deviation.

Table 5. Forced degradation results.

	STRESS TYPE	DETECTABLE CHANGES
Drug substance	Base hydrolysis	Degradative (A) of about 17% of the medroxyprogesterone acetate peak
	Acid hydrolysis	No change
	Oxidative/sponges	No change
Drug product	Thermal	No change
	Photodegradation	No change
	Oxidative/sponges	No change

peak is most probably cleaved for the ester bond. The hydroxyl form of the new product will have more polarity than the parent product and will elute early. The early peak shown most probably represents this product. The detected peaks were completely separated from medroxyprogesterone acetate (Figure 4).

Table 6. System suitability results.

PARAMETER	RESULT	ACCEPTANCE CRITERIA
Capacity factor	$K' = 2.1$	>2
Precision/injection repeatability	RSD ⩽1%	RSD ⩽1%
Resolution (R)	3.90	>1.5
Tailing (T)	1.03	⩽2.0
Theoretical plates (N)	7150	⩾1000 plates

Thus, the developed method is qualified and reliable to demonstrate and detect any expected change in the drug product assay during stability studies.

Peak purity for medroxyprogesterone acetate peaks was checked indicating that they are pure from any other excipients or impurities or derivative materials. Thus, the method of analysis is qualified and reliable to demonstrate and detect any expected change in the drug product assay during stability studies. The obtained data were analysed statistically regarding y-intercept and correlation coefficient measurements which demonstrated that the method within the range of 60% to 140% around the test concentration is linear. The obtained data were analysed statistically regarding RSD and percentage of recovery measurements which demonstrated that the method will produce accurate and precise results. According to the data obtained, we conclude that the method is robust enough to reproduce accurate and precise results under different method conditions.

System suitability test is commonly used to verify resolution, column efficiency, and repeatability of a chromatographic system to ensure its adequacy for a particular analysis according

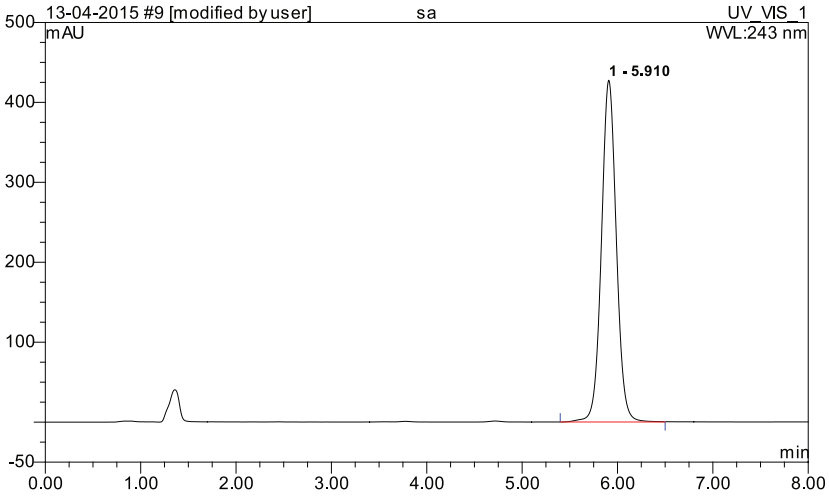

Figure 3. Peak shape of the analysed medroxyprogesterone acetate.

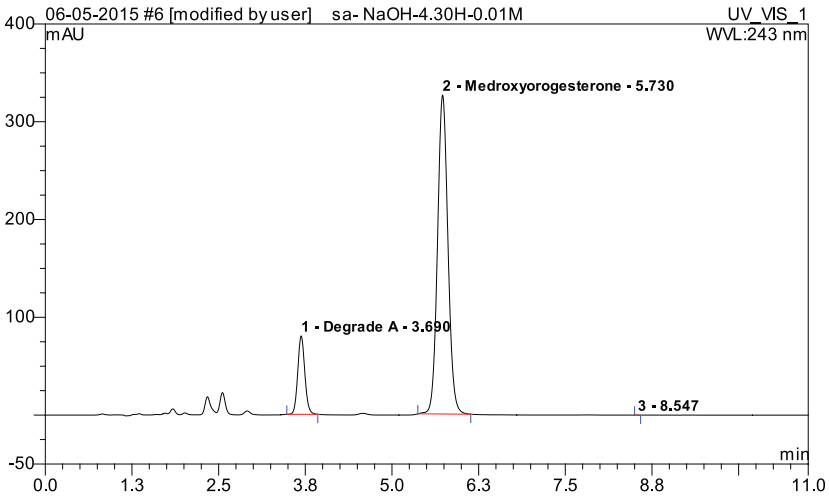

Figure 4. Degradation peaks were separated from medroxyprogesterone acetate.

to the USP and the ICH. Our developed method was optimized to have high theoretical plates (*N*) and symmetrical peak by adding suitable ion pair reagent such as hexane sulphonate.

The advantages of this research project over other published research are that we used simpler method; for example, Burana-osot et al used gradient system, whereas we used isocratic system. Isocratic system is simpler to use especially when the pump system does not have the facility to do gradient. Moreover, in our case, the time of run has been reduced from 15 to 8 minutes. In our developed method, we avoided the use of IS which puts more work burden on the analyst. Omitting the IS from our method did not affect the accuracy of the method, which is proved by the result of the accuracy validation parameter.

Conclusions

This study is the only study that provided a validated method to analyse medroxyprogesterone in veterinary intravaginal dosage form. The developed method was simple and quick. The obtained results were precise, accurate, and sensitive. The developed analytical procedure can be adapted by international pharmacopoeias and can be used by quality control labs.

Author Contributions

This work is an intellectual product of the of the whole team; all members have contributed in various degrees to the research concept, the experiment design and the methods used.

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