Development and Validation of a Stability Indicating HPLC Method for Quantification of Estriol in Compounded Glycerinated Gelatin Troches

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BACKGROUND

- Estriol (E3) is an estrogen hormone that plays an important role in uterine growth and fetal health during pregnancy. E3 is produced by the placenta during fetal development and is detected at high levels around 3 weeks before childbirth. Through hormone replacement therapy, E3 has been studied in clinical trials in the treatment of peri and postmenopausal symptoms such as hot flashes, mood swings, and sleep disturbances as well as benefits for promoting better urogenital health.[1]
- Although there are several FDA approved commercial hormone products to treat peri and postmenopausal symptoms[2], some patients may need a specific dosage form prepared by a specialized compounding pharmacy.[3]
- Compounded HRT can be administered via multiple routes with various dosage forms including: oral troches, vaginal creams/inserts, tablets, and capsules.[3]
- Currently, the United States Pharmacopeia (USP) does not offer an official monograph for determining estriol compounded in glycerinated gelatin.[4]
- It is, therefore, necessary to establish a validated stability indicating method that can quantify estriol in a glycerinated gelatin base.

PURPOSE

The objective of this study was to develop a validated stability indicating HPLC method that quantifies estriol in compounded glycerinated gelatin troches.

METHODS

- A Waters Alliance HPLC system (Model 2965) with a photodiode array detector (Model 2998) and Empower3 software was used for sample analysis.
- The method validation parameters studied were linearity, precision, accuracy, robustness, and system suitability.

- The stationary phase used was a Phenomenex Gemini C18 column (4.6 mm x 15 cm x 3.0 mcm) at a temperature set to 40^{0}C.
- The mobile phase was composed of methanol and water in a ratio of 65:35 (v/v).
- Detection wavelength was 205nm while the flow rate was maintained at 1.00 mL/min for a run time of 15 minutes.

- The forced degradation study examined estriol's stress threshold by subjecting reference standard solutions to acidic and alkaline degradation, oxidative degradation, photodegradation (visible light and UV), and thermal degradation.

RESULTS

Most method validation parameters – linearity, precision, accuracy, robustness, system suitability – met the respective acceptance criteria established by ICH guidelines.

In the forced degradation study, there was significant oxidative, visible light, UV, and thermal degradation but insignificant acidic or alkaline degradation. Additionally, no degradants co-eluted with estriol.

The method will be utilized to quantify estriol in multiple compounded preparations from two different compounding pharmacies.

Degradation	Description of Procedure	Time (hours)	Ave. % Yield
0.1 N HCl	Water bath shaker, 25±5^{o}C, 130 rpm	0.5	96.69 %
0.1 N NaOH	Water bath shaker, 25±5^{o}C, 130 rpm	24	105.03 %
0.1 N H_2O_2	Water bath shaker, 25±5^{o}C, 130 rpm	72	25.89 %
Visible Light	Bench lamp light, 25±5^{o}C	24	81.64 %
UV Light	Hood UV light, 25±5^{o}C	168	81.97 %
Thermal	Digital heatblock, 60±2^{o}C	24	83.80 %

Table 1. Summarized Forced Degradation Results

Parameter	Result	Acceptance Criteria
Ave. Tailing Factor (Tf)	1.76	≤ 2.0
Ave. Asymmetry Factor (As)	1.97 %	≤ 2.0 %
Ave. % RSD Peak Area	0.26 %	≤ 1.0 %
Ave. % RSD Retention Time	0.37 %	≤ 1.0 %
Ave. # Theoretical Plates (N)	2714.37	> 2000
Ave. Height Equivalent to N (HETP)	0.0058 cm	< 0.1cm

Table 2. System Suitability Results

RESULTS, Continued…

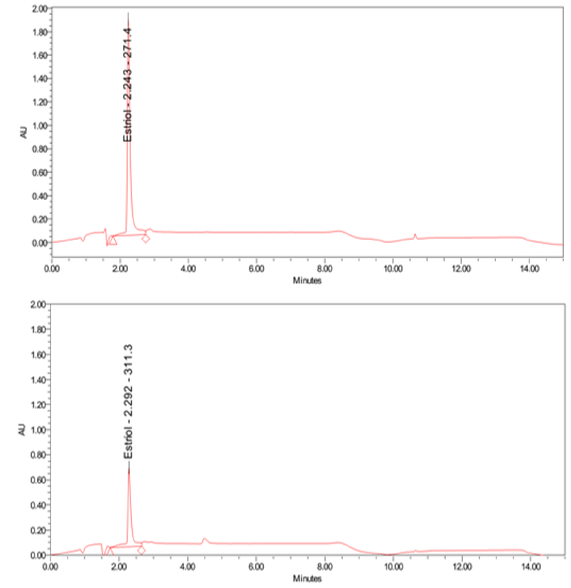

Figure 1. Representative chromatograms comparing 100mcg/mL standard (top) to hydrogen peroxide 3 day forced degradation sample (bottom)

CONCLUSION

This developed and validated method is suitable for both routine potency/strength testing as well as stability testing of estriol in compounded glycerinated gelatin troche dosage forms.

REFERENCES

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