International Journal of Multidisciplinary Research and Growth Evaluation.

To estimate method development and validation of Mestranol by RP- HPLC

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Article Info

ISSN (online): 2582-7138
Impact Factor: 5.307 (SJIF)
Volume: 04
Issue: 05
September-October 2023
Received: 25-08-2023;
Accepted: 15-09-2023
Page No: 478-483

Abstract

A novel stability indicating liquid chromatographic assay method was developed and validated as per ICH guide lines for the quantitative estimation of Mestranol in Tablet formulation. An isocratic reverse phase LC-method was developed using Hypersil ODS C18, 150 x 4.6mm, 5µm column and a mobile phase comprising of a mixture of (0.01M Tri-ethyl amine P^H 2.16± 0.1)Acetonitrile: Phosphate buffer (25:75v/v). The UV-detector set at 224nm with flow rate of 1ml\ min^{-1}. The method is linear between 30µg mL^{-1} to 70µg mL^{-1} with r^2 value as 0.998, the limit of detection (LOD) is 0.001µg ml^{-1} and limit of quantification (LOQ) is 0.003µg ml^{-1}. The Accuracy of the method was found to be in the range of 99.86% to 100.98%. The method precision %RSD were less than 2. Stress degradation studies were done for acid, base, $H_2O_2$and heat. The Proposed method was found to be Linear, precise and accurate for the quantitative estimation of Mestranol in Tablet and can be used for commercial purposes.

Keywords: Mestranol, HPLC, To Estimate method development and validation

1. Introduction

Mestranol is the 3-methyl ether of ethinylestradiol. Ethinylestradiol, is a synthetic derivative of estradiol. Ethinylestradiol is orally bio-active and the estrogen used in almost all modern formulations of combined oral contraceptive pills. It binds to (and activates) the estrogen receptor. Mestranol is a biologically inactive prodrug of ethinylestradiol to which it is demethylated in the liver with a conversion efficiency of 70%.

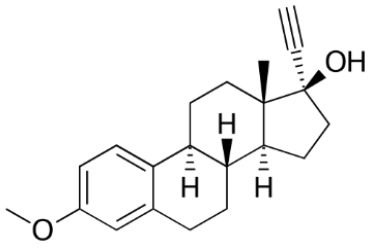


Fig 1: Structure of Mestranol

2. Materials and Methods

2.1. Materials

Pharmaceutical grade working standard Mestranol were obtained from Dr Reddy's Laboratories, All chemicals and reagents were HPLC grade and were purchased from Vijaya chemicals, HYD.

2.2. Methods

2.2.1. Instrumentation

The analysis was performed using HPLC (Waters-717 series) with PDA detector and data handling system EMPOWER2 software, UV-Visible double beam spectrophotometer (ELICO SL-159), analytical balance 0.1mg Sensitivity (SHIMADZU), pH meter (Labindia), ultra sonicator. The column used is Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d. (as Stationary phase) with the flow rate 1.0ml/min (isocratic).

2.3 Sample & Standard Preparation for the Analysis

25 mg of Mestranol standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.1ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

2.4 Method Development

2.4.1 Summary of Optimized Chromatographic Conditions

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Optimized method
Chromatographic conditions

PARAMETERS	METHOD
Stationary phase (column)	: Inertsil -ODS C18 (250 x 4.6 mm, packed with 5 micron)
Mobile Phase	: Methanol and Water (95:05)
Flow rate (ml/min)	: 1.0 ml
Run time (minutes)	: 6
Column temperature (°C)	: Ambient
Volume of injection loop	: 20
Detection wavelength (nm)	: 274nm
Drug RT (min)	: 3.444

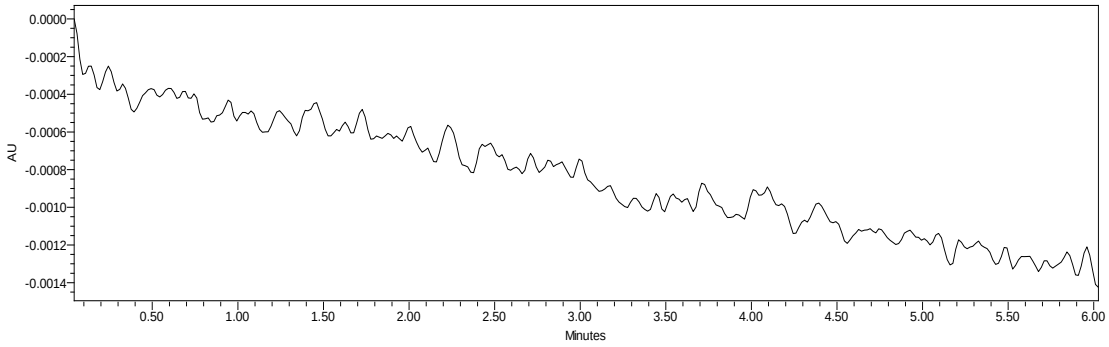


Fig 2: Blank Chromatogram

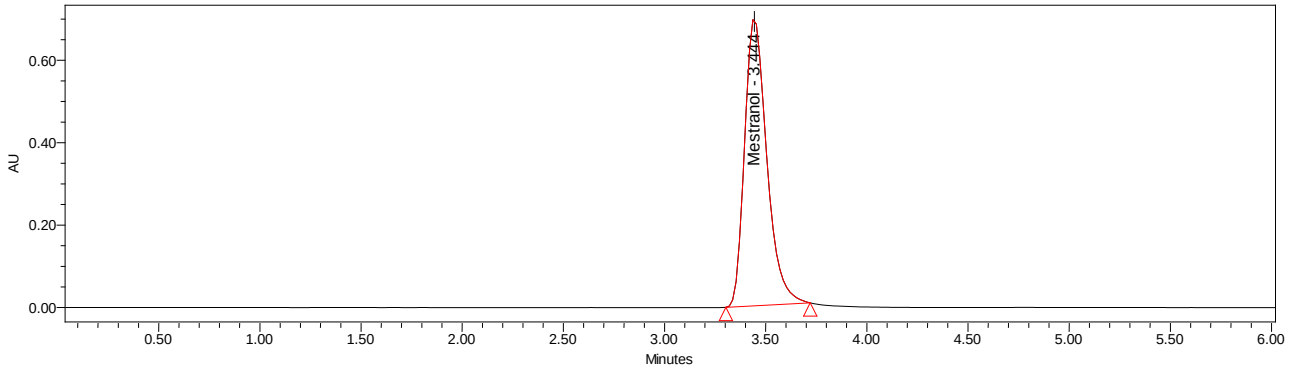


Fig 3: Chromatogram of Mestranol in Optimized Condition

2.5 Method validation

2.5.1 Linearity & Range: Mestranol

Table 1: Data of Linearity

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	17255
20	341644	y-Intercept	-1173
30	512296	Correlation Coefficient	0.999
40	694400		
50	863315		
60	1034451		
70	1204532		

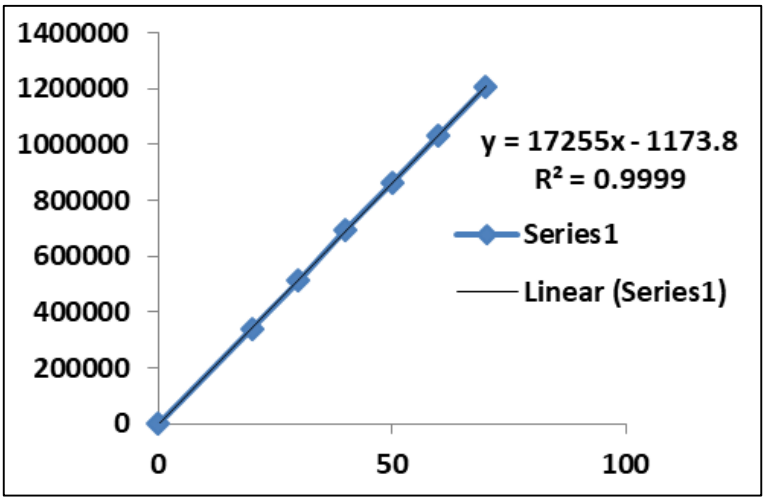


Fig 4: Linearity Plot (Concentration Vs Response)

2.6 Accuracy

Table 2: Accuracy table of Mestranol

Concentration % of spiked level	Area	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	341488	20	19.86	99.293	Mean	99.43
50% Injection 2	341904	20	19.88	99.414		
50% Injection 3	342502	20	19.92	99.587	%RSD	0.14
100% Injection 1	694665	40	40.33	100.82	MEAN	100.72
100% Injection 2	697001	40	40.46	101.16		
100% Injection 3	690442	40	40.08	100.21	%RSD	0.478
150% Injection 1	1036119	60	60.12	100.19	Mean	99.92
150% Injection 2	1031077	60	59.82	99.705		
150% Injection 3	1032948	60	59.93	99.886	%RSD	0.246

2.7 Precision

2.7.1. Repeatability

Table 3: Data of Repeatability (System precision)

	Injection	Peak Areas of Mestranol	%Assay
Concentration	1	694753	100.83
40ppm	2	699261	101.48
	3	695298	100.91
	4	689221	100.03
	5	688636	99.943
Statistical	Mean	693433	100.63
Analysis	SD	4470.41	0.647
	% RSD	0.644	0.643

2.7.2. Intermediate precision

Table 4: Data of Repeatability (Method precision)

	Injection	Peak Areas of Mestranol	%Assay
Concentration	1	696792	101.13
40ppm	2	698360	101.35
	3	699696	101.55
	4	690147	100.16
	5	688127	99.87
	6	692525	100.51
Statistical	Mean	694274	100.76
Analysis	SD	4697.03	0.6805

2.8. Method Robustness

Table 5: Data for Effect of variation in flow rate

	Std Area	*Tailing factor*		Std Area	Tailing factor		Std Area	Tailing factor
Flow 0.8 ml	650145	1.322089	Flow 1.0 ml	690448	1.604878	Flow 1.2 ml	740558	1.285372
	651208	1.331920		693195	1.584354		743510	1.319385
	656044	1.296438		691844	1.543805		746220	1.292055
	652411	1.315454		696955	1.568590		741004	1.304561
	650765	1.326551		699015	1.559986		743099	1.294621
Avg	652114	1.31849	Avg	694291	1.572323	Avg	742878	1.299199
SD	2347.9	0.013728	SD	3583.66	0.023367	SD	2264.48	0.013223
%RSD	0.360	1.04	%RSD	0.5161	1.48	%RSD	0.304	1.01

2.9. LOD & LOQ

The LOD was found to be 0.222µg/ml and LOQ was found to be 0.657µg/ml for Mestranol respectively which represents that sensitivity of the method is high.

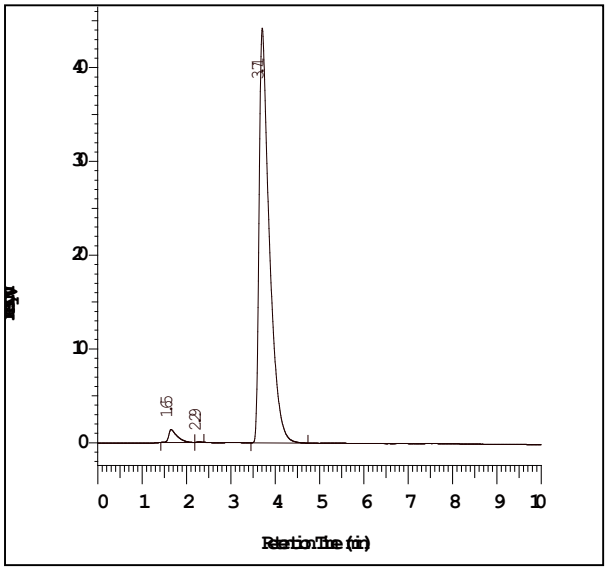
2.1.0 Estimation of Mestranol in Tablet Dosage Form

Assay: Rhodes pharmaceuticals, bearing the label claim Mestranol 0.15mg, Assay was performed with the above formulation. Average % Assay for Mestranol obtained was 99.82% respectively.

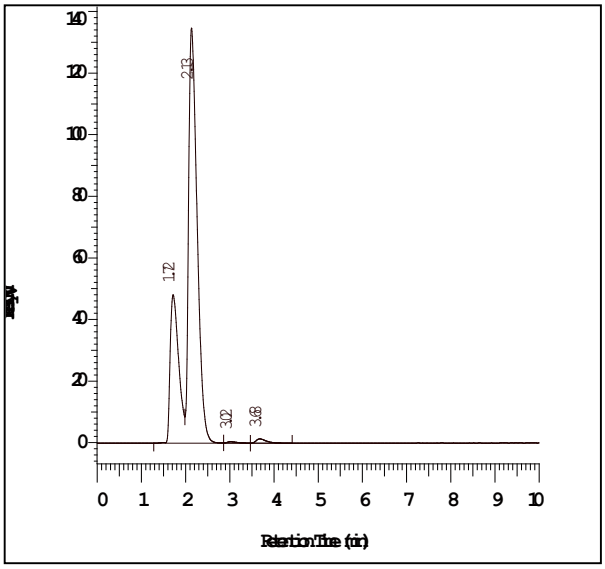
2.1.2 Stability Studies: The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation, photolytic degradation and oxidative degradation.

Table 6: Results of force degradation studies of Mestranol API

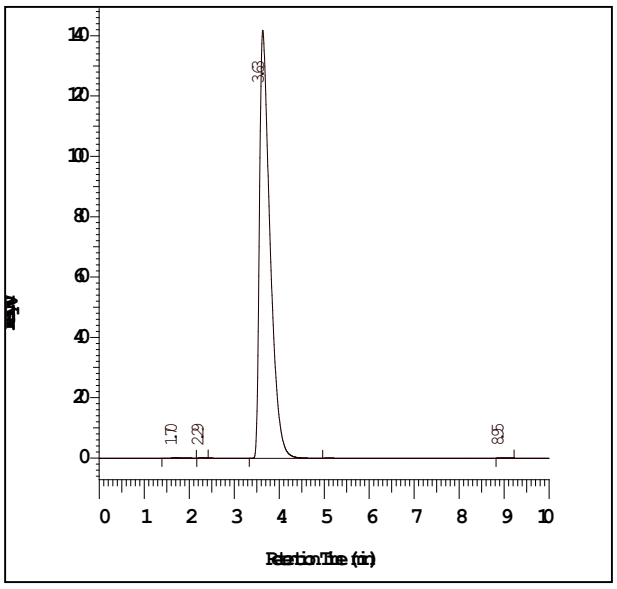
Stress condition	Time	Assay of active substance	Assay of degraded products
Acid Hydrolysis (0.1 M HCl)	24Hrs.	26.78127	73.21
Basic Hydrolysis (0.I MNaOH)	24Hrs.	68.46934	31.53
Thermal Degradation (50 ^{0}C)	24Hrs.	81.8387	18.163
3 % Hydrogen peroxide	24Hrs.	87.44321	12.556



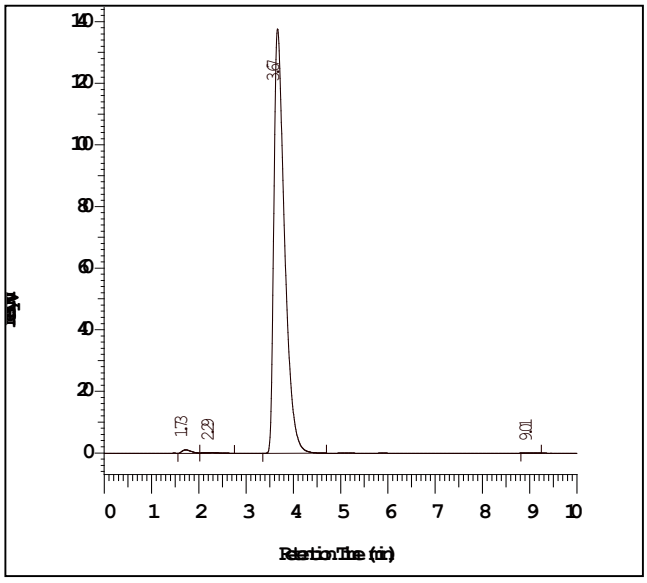
HCL



HYDROLYSIS



THERMAL DEGRADATION



OXIDATION WITH (3%) H_2O_2

3. Results

The results obtained in method validation were

Linearity & Range: Linearity range was found to be 0-70 µg/ml for Mestranol. The correlation coefficient was found to be 0.999, the slope was found to be 17255 and intercept was found to be 1173 for Mestranol.

Accuracy: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.43%,100.72%,and 99.92 for Mestranol respectively.

Repeatability: The Average area, standard deviation and % RSD were calculated for drug and obtained as 0.539186% and for Mestranol. As the limit of Precision was less than "2" the system precision was passed in this method.

LOD & LOQ: The LOD was found to be 0.2µg/ml and LOQ was found to be 0.6µg/ml for Mestranol respectively which represents that sensitivity of the method is high.

Assay: The assay of of Mestranol was found to be 99.82% respectively.

Degradation studies: The results of the stress studies indicated the specificity of the method that has been developed. Mestranol was more stable in thermal and peroxide stress conditions as compare to other stress conditions.

4. Discussion

Stability indicating RP-HPLC method for analysis of Mestrsnol, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Lamivudine it is evident

that most of the HPLC work can be accomplished in the wavelength range of 210-300 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay which can help in the analysis of Mestrsnol, in different formulations.

5. Conclusion

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Mestranol API. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Mestranol indicated that the developed method is specific for the estimation of Mestranol. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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