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A Stability-indicating Reversed-Phase High Performance Liquid Chromatography Method for Simultaneous determination of Ormeloxifene in Pure and Pharmaceutical Formulation

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Abstract: A simple, rapid and accurate and stability indicating HPLC method was developed for the determination of Ormeloxifene in pure and tablet forms. The method showed a linear response for concentrations in the range of 2-12 µg/mL using Methanol: Acetonitrile: Buffer solution (Dissolve 0.02 M potassium di-hydrogen orthophosphate in water. Adjust pH of solution to 6.85 with orthophosphoric acid) in the ratio (45:45:10) as the mobile phase with detection at 232 nm and a flow rate of 1 mL/min and retention time 7.1 min. The value of correlation coefficient, slope and intercept were, 0.9998, 1661.8 and 114.82, respectively. The method was validated for precision, recovery, ruggedness and robustness. The drug undergoes degradation under acidic, basic, photochemical and thermal degradation conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different retention time. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

Keywords: Ormeloxifene, HPLC, Degradation studies.

INTRODUCTION:

Stability indicating methods have become an important aspect of any analytical method validation and a part of US FDA requirements[1]. Chemically, Ormeloxifene is designated as 1-[2-[4-[(3S, 4R)-7-methoxy-2, 2-dimethyl-3-phenyl-chroman-4-yl] phenoxy] ethyl] pyrrolidine[2] is one of the selective estrogen receptor modulators, or SERMs, a class of medication which acts on the estrogen receptor (**Figure: 1**) its action is anti-estrogenic (e.g., uterus, breasts) [3]. It causes an asynchrony in the menstrual cycle between ovulation

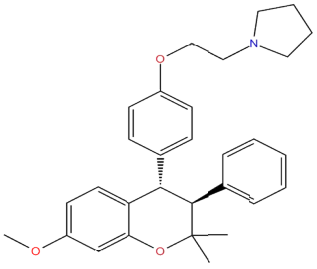

Figure: 1 chemical structure of ormeloxifene

and the development of the uterine lining, although its exact mode of action is not well defined[4]. Very few methods appeared in the literature in the spectrophotometric determination of Ormeloxifene[5, 6, 7]. Therefore, we have made an attempt to develop a new, simple, accurate stability indicating RP-HPLC method for the determination of Ormeloxifene in pure and tablet forms. The proposed method was validated as per ICH guidelines Q2A[8].

MATERIAL AND METHOD

Materials

Ormeloxifene was supplied by Hindustan Latex Ltd. and tablets (Label Claim: 60 mg per tablet, Product Name: Sevista and Manufacturer: Torrent Pharmaceutical Ltd was procured from the market. Methanol, Acetonitrile and water (HPLC grade) were used for analysis.

Instrument used

The HPLC used was a shimazdu HPLC LC-20AT series with SPD-20A UV photodiode array detector and Borwin software, Japan was used for all the experiments. The column used was XTerra® RP18, 250 x 4.6 mm, 5 µ (water Ireland) and Luna C_8 (Octylsilane), 250 x 4.6 mm, 5 µ (Phenomenax, USA). Thermal Stability studies were performed in a dry air oven (Thermo labs, India). Micrositer syringer- 50 µL (Hamilton Company, USA).

Methods

i. Chromatographic conditions

Chromatographic separation was achieved at ambient temperature on a reversed phase column using a mobile-phase consisting of a mixture of Methanol: Acetonitrile (40:60). The mobile phase so prepared was filtered through 0.22 µm nylon membrane filter and degassed by sonication. Flow rate of 1.5 mL / min was maintained. Detection was carried out at 279 nm. The injection volume was 20 µL for assay and degradation level.

ii. Standard preparation

50 mg of Ormeloxifene working standard was accurately weighed and transferred to a 50 mL volumetric flask. Solution was sonicated and diluted up to the mark with mobile phase. A series of standard solutions in the concentration range of 50, 100, 150, 200, 250 and 300ng/ml were prepared followed by a suitable dilution of stock solution with the mobile phase.

iii. Sample preparation

20 tablets were weighed and finely powdered. Blend equivalent to 50 mg of Ormeloxifene was transferred to a 50 mL volumetric flask. About 30mL of mobile phase was added and the solution was sonicated for 15 min and make up to the mark with mobile phase. The solution was further diluted and resulting solution was filtered through 0.22µm nylon membrane filter. The solution was mixed well and centrifuged at 2500 RPM for 10 min.

iv. Preparation of calibration graph

The linearity of response for Ormeloxifene assay method was determined by preparing and injecting solutions with concentrations of about 50, 100, 150, 200, 250 and 300ng/ml of Ormeloxifene.

Method validation

i. Precision

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of the same standard concentration (150 µg / mL for standard application). Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of marketed sample (150 µg / mL for sample application). It showed very low % relative standard deviation (% RSD) of peak area of Ormeloxifene.

ii. Accuracy

Accuracy (Recovery) study was performed by spiking 30, 50 and 70% of Ormeloxifene working standard to a preanalysed sample. The preanalysed sample was weighed in such a way that final concentration is half or 50% of the sample preparation before spiking. The percentage sum level of preanalysed sample and spiked amount of drug should be 80, 100 and 120% of simulated dosages nominal or target concentration of sample preparation. The accuracy of the analytical method was established in duplicate across its range according to the assay procedure.

$$\textbf{\% Recovery} = \frac{\%Amount\,\mathrm{Recovered}}{\%SumLevel} \times 100$$

iii. Ruggedness and robustness of the method

Method robustness and ruggedness was determined by analysing same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, flow rate, instrument and analyst. The robustness and ruggedness of the method was established as the % deviation from mean assay value obtain from precision study is less than ±2.0%.

iv. Analysis of marketed formulation

20 tablets were weighed and finely powdered. Transfer blend equivalent to 100 mg of Ormeloxifene to a 100 mL volumetric flask. Add about 60 mL of mobile phase and sonicate for 15 min and make up volume with mobile phase. Mix well and centrifuge

the solution at 2500 RPM for 10 min. Dilute the solution up to the desired concentration and inject it into the HPLC system.

Forced degradation studies

i. Preparation of acid and based- induced degradation product

Tablet powder equivalent to 100 mg of Ormeloxifene was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 mL of 1 N HCl was added and 5 mL of 1 N NaOH were added separately. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. The acidic forced degradation and the alkaline forced degradation was performed in dark in order to exclude the possible degradative effect of light. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

ii. Preparation of hydrogen peroxide - induced degradation product

Tablet powder equivalent to 100 mg of Ormeloxifene was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 mL of 3.0% H_2O_2 was added. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

iii. Photodegradation product

Tablet powder equivalent to 100 mg of Ormeloxifene (previously kept in UV light for 24 h) was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking and diluted up to the mark with mobile phase. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

iv. Thermal degradation product

Tablet powder equivalent to 100 mg of Ormeloxifene was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC. The specificity degradation study data for the determination of Ormeloxifene and its degradants in pharmaceutical dosage form is given in **Table: 6**. The no stress treatment sample (as control) has been evaluated relative to the standard concentration where as rest of the stressed condition samples **(Figures: 3 to 6)** is evaluated relative to the control sample with respect to the % assay and % degradation. The percentage degradation results are calculated by area normalization method.

v. Detection of the related impurities

Weigh and finely powder not less than 20 tablets. Transfer blend equivalent to 50 mg of Ormeloxifene to a 50 mL volumetric flask. Add about 30 mL of mobile phase and sonicate for 20 min and make up volume with mobile phase. Mix well and centrifuge the solution at 2500 rpm for 10 min. inject the clear supernatant solution into the HPLC system.

Table: 1 Regression characteristics of the proposed HPLC method

S. No	Drug	Ormeloxifene
1.	Range (µg/ml)	50-300µg/ml
2.	Detection wavelength (λ max)	279nm
3.	Mean **'R^2'** value	0.9986
4.	Slope (m)	194.5
5.	Intercept (c)	1073.1
6.	Run time	25min
7.	Retention Time (min)	21.3
8.	Theoretical Plates (N)	6739
9.	Tailing Factor	1.05

Table: 2 Method precision of Ormeloxifene

Sample Preparation	% Assay Ormeloxifene	% Deviation From Mean Assay value Ormeloxifene
1	99.65	-0.23
2	99.36	-0.25
3	100.76	0.88
4	98.87	-1.01
5	100.83	0.95
6	99.82	-0.06
Mean	99.88	
±SD	0.709	
%RSD	0.711	

Table: 3 Ruggedness and robustness of Ormeloxifene

Parameter	Normal (Original)	Changed conditions
Column make	X Terra® RP18 column; 250 x 4.6 mm; 5 µ	Luna C_8 (Octylsilane), 250 x 4.6 mm; 5 µ
Flow rate	1.5 mL/min	1.2 mL/min
Mobile phase Composition	Methanol: Acetonitrile (40:60%v/v) pH adjusted to 3.0-4.5	Methanol: Acetonitrile (50:50%v/v) pH adjusted to 3.0-4.5
Pump	Jasco PU-2080 plus series	Shimazdu LC-20AT
Detector	Jasco UV-2075 plus series	Shimazdu UV-VIS detector
Analyst	Narasimha.V	Kalyan.B
% assay of Ormeloxifene	99.88%	99.83%
% deviation from mean assay value obtained in method precision studies for Ormeloxifene : 0.28%		

Table: 4 Recovery of Ormeloxifene

Sample preparation	% simulated dosage normal	% sum level	% amount recovered	% recovery	Mean % recovery
Preanalysed sample				99.68	
1	80	80.75	81.54	100.97	101.69
2	80	79.86	81.79	102.42	
1	100	100.53	101.87	101.33	101.18
2	100	100.54	101.65	101.04	
1	120	119.62	121.91	101.91	101.43
2	120	120.68	121.84	100.96	

Table: 5 Stressed study data of Ormeloxifene

S. No	Condition	% assay Ormeloxifene	Retention of drug	% Degradation
1	No stress treatment	99.88	21.3	Nil
2	Acid	--	--	--
3	Alkali	--	--	--
4	H_20_2	98.43	15.49, 16.75, 19.65	0.07
5	UV	99.65	18.71	0.21
6	Thermal	99.71	19.58	0.05

Table: 6 Summary of forced degradation results

S. No	Stress condition	Time	%Assay of active substance	Mass balance (%assay + %degradation products)	Remarks
1	Acid degradation (1 N HCl)	1/2 hr	--	--	--
2	Alkali degradation (1N NaOH)	1/2 hr	--	--	--
3	H_2O_2 degradation (3%)	1/2 hr	98.43	98.50	Mild degradation formed
4	UV degradation	24 hr	99.65	99.86	No degradation products formed
5	Thermal degradation (60^0C)	1/2 hr	99.71	99.76	No degradation products formed

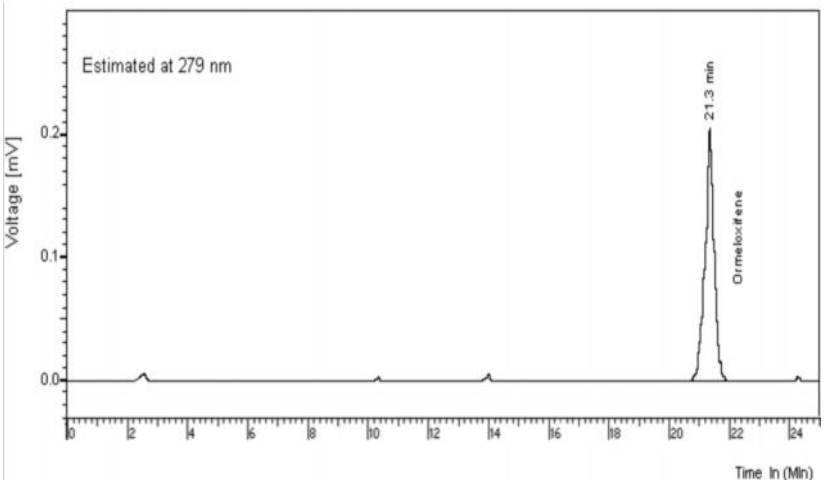

Figure: 2 The simple chromatogram of standard Ormeloxifene

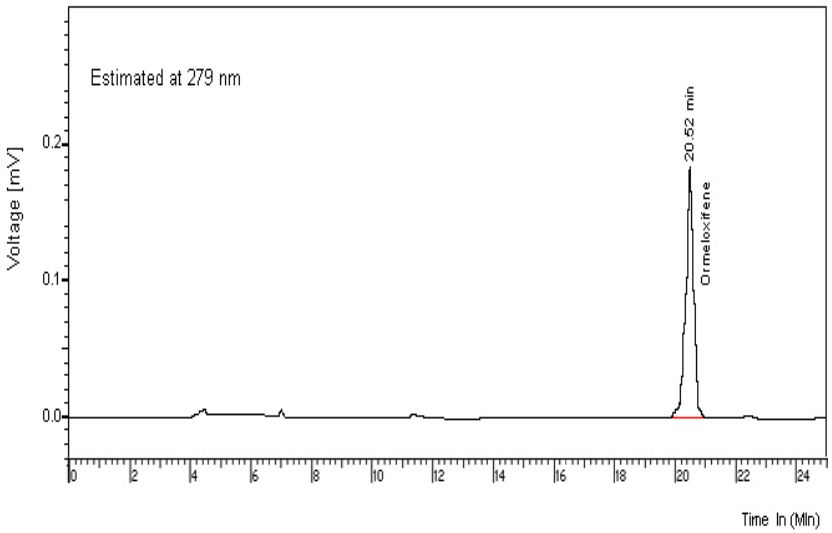

Figure: 3 The simple chromatogram of test Ormeloxifene

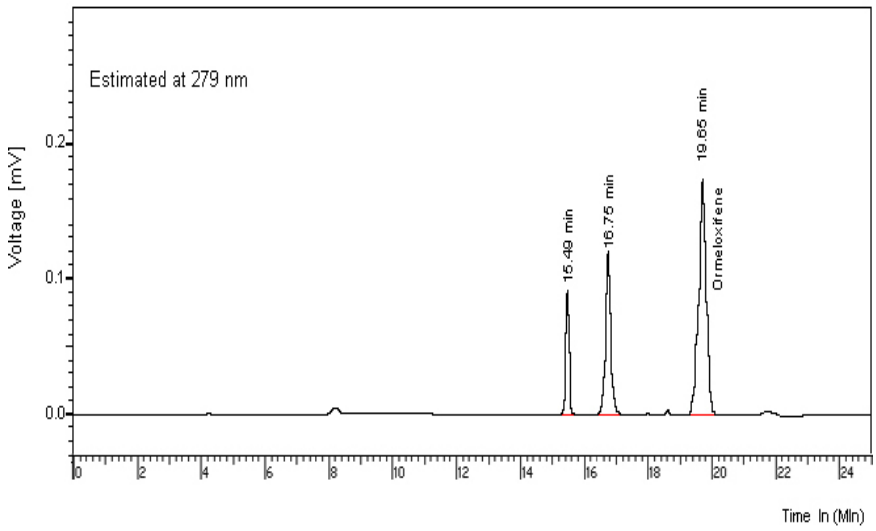

Figure: 4 The simple chromatogram of Hydrogen Peroxide degraded sample.

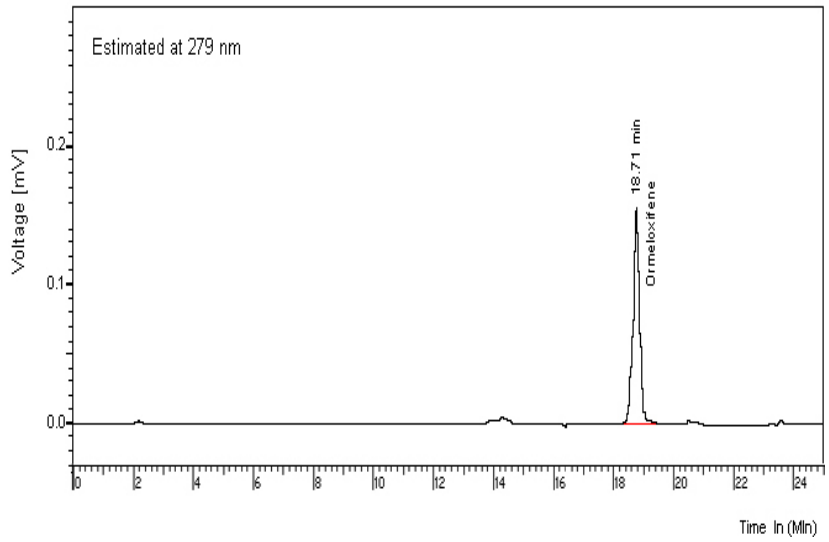

Figure: 5 The simple chromatogram of UV degraded sample.

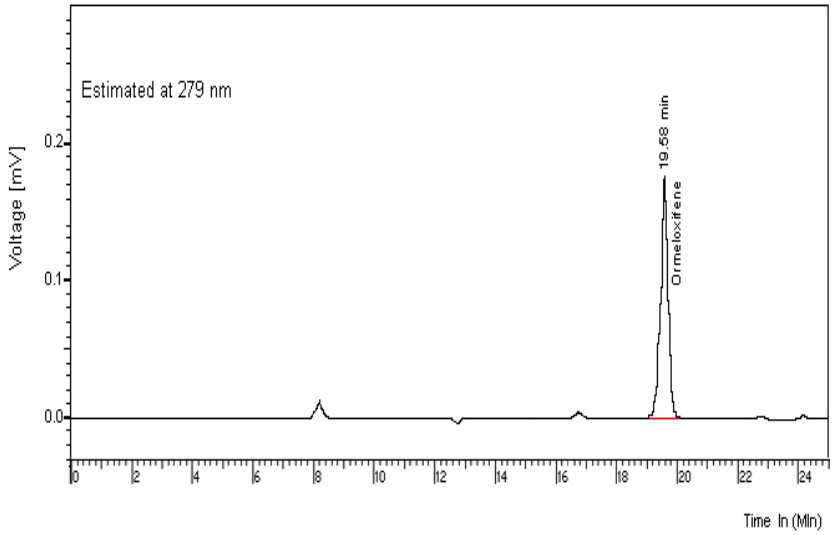

Figure: 6 The simple chromatogram of Thermal degraded sample.

RESULTS AND DISCUSSION

i. Method of development

The chromatographic conditions were optimized with a view to develop a stability- indicating assay method. Two different columns were tried as under chromatographic conditions namely, XTerra® RP18, 250 x 4.6 mm, 5 µ (water, Ireland) and Luna C_8 (Octylsilane), 250 x 4.6 mm, 5 µ (Phenomenax, USA). Luna C8 gave good peak shape but a lower retention. The chromatographic conditions finally comprised of a mobile-phase in the ratio of Methanol: Acetonitrile (40:60) at a flow rate of 1.5 mL / min using XTerra® RP18 column; 250 x 4.6 mm; 5 µ (G. L. Sciences, Japan) at 279 nm.

ii. Calibration curve

These results indicate that the response is linear over the range of 50, 100, 150, 200, 250 and 300ng/ml of Ormeloxifene with coefficient of regression, R^2, value as 0.9986 shown in **table: 1**. The value of correlation coefficient, slope and intercept were 0.9986, 194.5, and 1073.1 respectively.

Validation of the method

i. Precision

The %RSD for repeatability of sample preparation is 0.711%. This shows that precision of the method is satisfactory as % relative standard deviation is not more than ± 2.0%. **Table: 2** represent the precision of method.

ii. Ruggedness and robustness of the method

Method robustness and ruggedness was determined by analyzing same sample at normal operating conditions and also by changing some operating analytical

conditions such as column make, mobile phase composition, flow rate, instrument and analyst. The deliberate aforementioned changes in parameters alter the result of Ormeloxifene 0.28% to method precision study, which is not a significant change. The robustness and ruggedness of the method is established as the % deviation from mean assay value obtain from precision study is less than ±2.0%. **Table: 3** represents the ruggedness and robustness of the method.

iii. Accuracy

The accuracy of the method was established by recovery studies. Results indicate that the individual recovery of Ormeloxifene ranges from 100.96 % to 102.42 % with mean recovery of 101.438 % and % relative standard deviation of 0.54%. The recovery of Ormeloxifene by proposed method is satisfactory as % relative standard deviation is not more than ± 2.0% and mean recovery between 98.0 - 103.0%. **Table: 4** represents the accuracy of method.

iv. Analysis of the marketed formulation

The drug content was found to be 99.83% with a % RSD of 0.71%. It was noted that no degradation of Ormeloxifene had occurred in the marketed formulation that were analysed by this method. The low RSD value indicated the suitability of this method for routine analysis of Ormeloxifene in pharmaceutical dosage form.

v. Stability- indicating property

The chromatogram of no stress treatment of control and sample showed no additional peaks **(Figure: 2 & 3).** The retention time (RT) of standard and sample were 21.3 min and 20.52 min respectively. The chromatogram of acid degraded and alkali degraded sample showed no additional peaks. The chromatogram of hydrogen peroxide degraded sample showed additional peaks at RT of 15.49, 16.75 and 19.65 min respectively **(Figure: 4).** The chromatogram of UV degraded sample showed additional peak at RT of 18.71 min. **(Figure: 5).** The chromatogram of thermal degraded sample showed additional peak at RT of 19.58 min **(Figure: 6).** Rest of the peaks, if any, were from its blank or placebo in each of these specified conditions. In each forced degradation samples where additional peaks were observed, the response of the drug was changing from the initial control sample. This indicates that the drug is not susceptible to acid-base hydrolysis degradation but susceptible to hydrogen peroxide degradation, UV degradation and thermal degradation.

CONCLUSION

The developed HPLC technique is precise, specific, accurate and stability indicating. Statistical analysis proves that the method is reproducible and selective for the analysis of Ormeloxifene in pharmaceutical dosage form. The method can be used to determine the purity of the drug available from various sources. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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