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INNOVATIVE METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF ORMELOXIFENE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The present method describes the development of a validated RP-HPLC for determination of ormeloxifene hydrochloride in presence of its degradation products or other pharmaceutical excipients. The drug substance was subjected to stress conditions of acid (2N HCl), alkali (2N NaOH), oxidative (30%H_2O_2), neutral, thermal and photolytic studies. Seperation was carried out on Agilent XDB C18 column,(250×4.6mm I.D, Particle size 5µm) by using mobile phase consisting of a mixture of buffer: acetonitrile: methanol (20:35:45,v/v/v) with the flow rate of 1.0mL/min. UV detection was performed at 245nm and retention time was found to be 5.48min. The method was validated for linearity, accuracy and precision. Linearity for ormeloxifene was in the range of 30-90µg/mL with correlation coefficient values 0.9999. The mean recoveries were found in the range of 99.73-100.84% and the %RSD was found to be less than 2% indicated, that the method is more accurate and precise. The Limit of Detection and Limit of Quantification were 0.11µg/ml and 0.32ì g/ml respectively, which indicate sensitivity of the method. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by proposed HPLC method.

Key Words: *Ormeloxifene; RP-HPLC.*

INTRODUCTION

Ormeloxifene hydrochloride (Fig.1), chemically known as trans-7-methoxy-2,2-dimethyl-3-phenyl-4-[4-(2-pyrrolidino-ethoxy)phenyl]chroman hydrochloride[1] is one of the selective estrogen receptor modulator(SERMs) pertains to III generation SERM, antagonizes the effect of estrogen on uterine and breast tissue and stimulates its effect on vagina, bone, cardiovascular and central nervous system. Thus, it is especially beneficial in perimenopausal women as it has no uterine stimulation, prevents bone loss, does not increase the risk of breast cancer, lowers cholesterol level and maintains cognitive function of the brain. It has the additional advantage of reducing premenstrual symptoms, mastalgia and dysmenorrhea. When ormeloxifene was used as a contraceptive, its beneficial effect on menorrhagia and endometriosis was observed, which led to controlled trials for the management of menorrhagia after its approval for this indication[2].

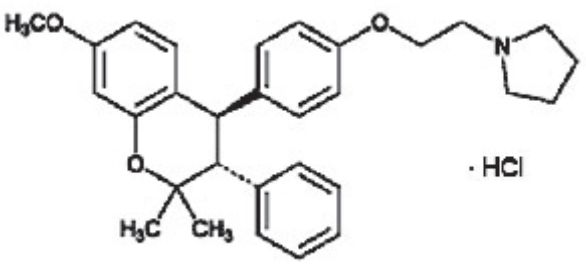

Fig.1: *Chemical structure of ormeloxifene hydrochloride*

Stability can be defined as the capacity of a drug substance or product to remain within established specifications and to maintain its identity, strength, quality, and purity throughout expiration dating period. Stability test of an active pharmaceutical ingredient or drug product can provide evidence on the drug quality and if it can be influenced by a variety of environmental factors such as temperature and humidity. The drug product stability guideline Q1A (R2) issued by the International Conference on Harmonization (ICH)[3] suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products.

There are some studies reported for determination of ormeloxifene by LC-MS/MS in rat plasma[4] & Pharmacokinetic activity on rats[5]. Praveen Kumar M, *et al.* have published HPLC method in formulations[6] the results were not correlated with text matter. Hence we made an attempt to develop simple, accurate and more reliable stability-indicating HPLC method for estimation of ormeloxifene in bulk and pharmaceutical dosage forms.

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MATERIALS AND REAGENTS

Ormeloxifene hydrochloride bulk drug provided from Spectrum Pharma Research solutions, Hyderabad and tablet were purchased from local market under the brand name SEVISTA manufactured by Torrent Pharmaceuticals Ltd. HPLC grade water from Rankem, India. HPLC grade acetonitrile, 30%H_2O_2 and triethylamine from SD Fine-Chem Limited, Mumbai, India. HPLC grade methanol and hydrophilic 0.45µm Polyvinylidene fluoride filters procured from Merck Millipore, Mumbai, India.

Chromatographic Conditions and Equipments

HPLC (Waters, with Empower 2 software) with PDA Detector, dry air oven (Cintex, Mumbai, India), and ultrasonic bath sonicator were used. All chromatographic experiments were performed in the isocratic mode. Separation was achieved on Agilent XDB C18 (250 × 4.6 mm I.D.; Particle size 5µm) column as stationary phase by using mixture of buffer: acetonitrile: methanol (20:35:45 v/v/v) as mobile phase. Other parameters such as run time 8 minutes, 1.0 mL/min as flow rate, injection volume of 50.0µL and column temperature of 30°C were finalized during development. Ormeloxifene was detected at 245 nm. Methanol was used as diluent. The stress degraded samples were analyzed by using a PDA detector covering the range of 200–400 nm.

Standard Solution Preparation

A standard stock solution of ormeloxifene was prepared by dissolving 10mg of drug in methanol in order to make concentration of 1mg/mL.

Sample Solution Preparation

Accurately weighed 20 tablets were powdered and equivalent amount of 60mg of ormeloxifene was transferred to a 100mL volumetric flask. About 70.0mL of diluent was added to this and sonicated in an ultrasonic bath for 10 minutes with intermittent shaking. Finally the volume was made up to the mark with diluent. The above solution was filtered through 0.45µm filter.

RESULTS AND DISCUSSION

Method Development and Optimization

Prime objective of an RP-HPLC method development for determination of ormeloxifene in pharmaceutical dosage form was that the method should be able to determine assay of drug and should be precise, accurate, reproducible, specific, and stability indicating. All degradation products from stress conditions should be well separated. Method should be simple so that it can be useful in analytical research and quality control laboratory for routine use.

Mobile Phase and Chromatographic Conditions Optimization

Method development was tried with Phenomenex Luna C18 (150 × 4.6 mm I.D.; Particle size 5 µm) column, as stationary phase and buffer (0.01M Potassium dihydrogen orthophosphate p^H 4.2 adjusted by orthophosphoric acid) : acetonitrile, (60:40 v/v) as mobile phase at a flow rate 1.0 ml/ min, No Peak was found under this condition. Further trials were carried out with different mobile phase compositions by keeping rest of the chromatographic conditions were unchanged. Good separation with asymmetry of the peak was obtained by using buffer: acetonitrile: methanol (20:35:45v/v/v). Finally, well resolved peak with a good symmetry was obtained at 5.42 min by replacing the column with Agilent XDB C18 (250 × 4.6mm, 5µm) under same mobile phase composition (Figure 2). The amount of drug present in the marketed formulation was calculated and the % purity was found to be 99.45%. The results were incorporated in Table 1.

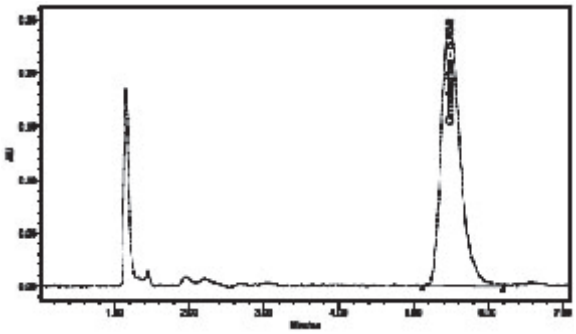

Fig. 2: *Standard chromatogram of ormeloxifene*

Table 1: *Summary of assay results for Ormeloxifene*

Formulation used	Label claim	Amount found*	%Assay*
Ormeloxifene	60mg	59.68mg*	99.45* ± 0.55

**Average of six determinations*

Validation of method

After successful development of LC method, it was subjected to method validation as per ICH guideline[7]. Analytical method validation was carried out by means of linearity, precision, accuracy, robustness, system suitability and forced degradation studies.

Linearity

Different aliquots of ormeloxifene standard stock solutions were prepared and injected into chromatographic system. The linearity of the method was demonstrated over the concentration range of 30 to 90µg / ml of the target concentration. Calibration curve of ormeloxifene was constructed by plotting peak area vs. concentration with a correlation coefficient of 0.9999 (Figure 3).

Precision

The precision of the method was demonstrated by system precision and method precision. In the system precision, standard solution of ormeloxifene was injected six times and the % RSD of peak area was found to be

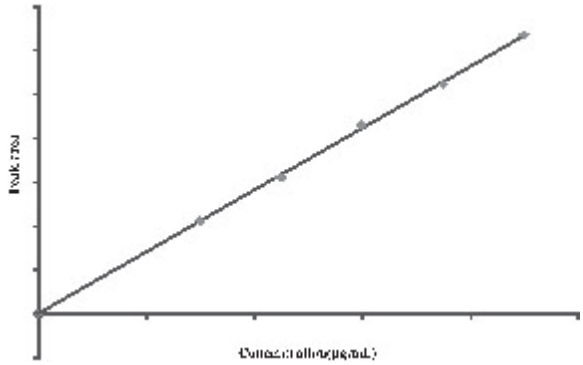

Fig. 3: *Calibration graph of ormeloxifene at 245nm.*

less than 1.5. In the method precision, the pre-analyzed sample solution was injected in triplicate within a day and on three different days and the %RSD was found to be less than 2, which indicates that the method is more precise.

Accuracy

Accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the pre-analyzed sample solution at 3 different concentrations i.e 80%, 100% and 120%. The resulting solutions were analyzed in triplicate at each level as per the ICH guidelines. The % recovery was given in Table 2. The mean recoveries of Ormeloxifene were found to be in the range of 99.73-100.84%.

Table 2: *Accuracy Results of ormeloxifene*

Spiked Level	Spiked Concentration (µg/m L)	Obtained Concentration (µg/m L)	% Recovery	% Mean Recovery
80% Ormeloxifene	60+48	108.01	100.02	99.94
	60+48	107.81	99.83	
	60+48	107.77	99.79	
100% Ormeloxifene	60+60	121.03	100.86	100.84
	60+60	120.25	100.21	
	60+60	121.74	101.45	
120% Ormeloxifene	60+72	133.05	100.80	99.73
	60+72	131.45	99.59	
	60+72	131.76	98.82	

LOD and LOQ

The LOD and LOQ were determined based on the standard deviation of response of the calibration curve. The residual standard deviation of the regression line and slope of the calibration curve were used to calculate the LOD and LOQ. The values were found to be 0.11µg/mL and 0.32µg/mL, respectively.

Robustness

As defined by the ICH, the robustness of an analytical procedure describes its capability to remain unaffected by small and deliberate variations in method parameters. The robustness as a measure of method capacity to remain unaffected by small but deliberate changes in chromatographic conditions was studied by testing influence of small changes in flow rate (1.0 ±0.1ml/min), change in column oven temperature (25°C±5°C), and change in mobile phase composition with ±0.2 %. There is no significant changes in the results, thus the method is more robust.

System suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (t_R), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the standard solution at a concentration of 60µg/mL. The results were incorporated in Table. 3.

Table 3: *Validation Parameters of ormeloxifene*

Parameters	Ormeloxifene
Retention Time (min)	*5.482*
Peak asymmetric Factor	*1.16*
No. of theortical Plates	*2689*
%RSD of peak area	*0.98*
Linearity range (µg/mL)	*30-90*
Correlation co-efficient	*0.9999*
LOD(µg/mL)	*0.11*
LOQ(µg/mL)	*0.32*
Accuracy (%)	*99.73-100.84*
Precision (%RSD)	
System precison	*0.7*
Method precison	*0.9*

Forced Degradation Studies.

Forced degradation studies under acidic, alkaline, oxidative, thermal, neutral and photolytic conditions were performed by heating and refluxing using a heating mantle with temperature control, which was carried out in a stability chamber equipped with light sources as defined under option 2 of the ICH guideline Q1B. Sample solutions were prepared separately at concentration of 60ìg/ml in 2N HCl, 2N NaOH & 30% H_2O_2, heated at 60°c for 2hrs and sonicated for 30min. In addition, neutral degradation was studied by preparing a sample solution with H_2O and refluxed for 7 hrs at 60°c, thermal degradation was studied by placing powder sample on petri plate and kept in hot air oven for 5hrs at 100°c and photolytic degradation was studied by keeping powder sample under UV light for one day, further both samples were diluted to get 60µg/ml with diluent. Different aliquots of the solutions were injected into the HPLC system and the resulting chromatograms were recorded (Figure 4 to 9). In above all the conditions, the drug was degraded and the % of degradation was reported in Table. 4.

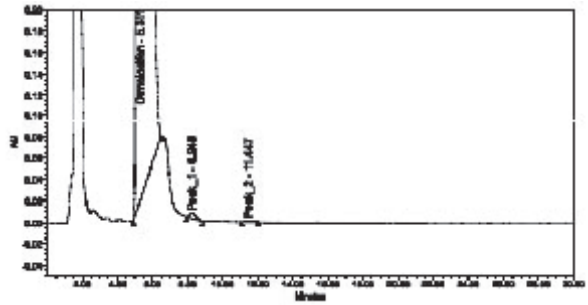

Fig. 4: *Typical Chromatogram of Acid Stressed Sample*

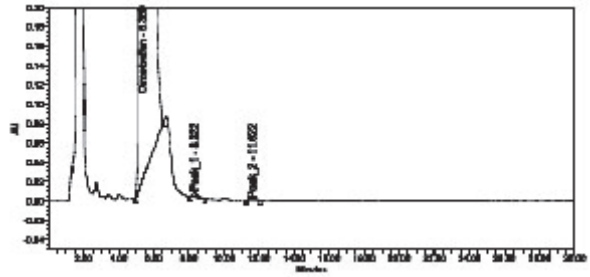

Fig. 5: *Typical Chromatogram of Alkali Stressed Sample*

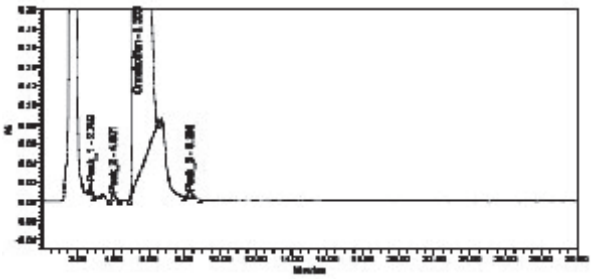

Fig. 6: *Typical Chromatogram of 30% H_2O_2 Stressed Sample*

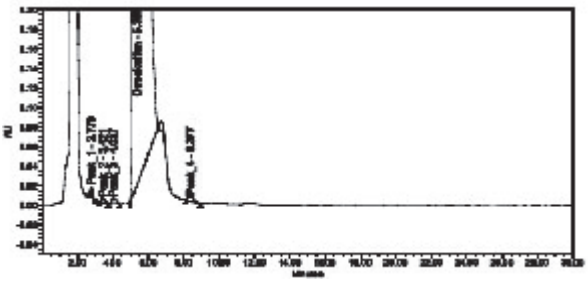

Fig. 7: *Chromatogram of Thermal Stressed sample*

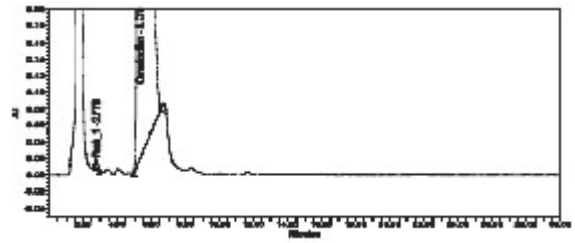

Fig. 8: *Typical Chromatogram of Neutral Stressed Sample*

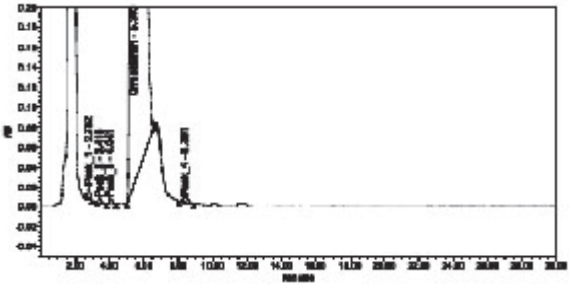

Fig. 9: *Typical Chromatogram of Photolytic Stressed Sample*

Table 4: *Forced Degradation studies of Ormeloxifene*

S.NO	Stressed Condition	t_R in min	Peak area	% Purity	% of degradation
1.	2N HCl	5.311	3938102	90.54	9.50
2.	2 N NaOH	5.359	3987659	91.64	8.36
3.	30 % H_2O_2	5.353	3863919	88.80	11.20
4.	Thermal	5.389	3921492	90.12	9.88
5.	Neutral	5.370	4187153	92.42	7.58
6.	Photolytic	5.380	4024800	96.22	3.78

CONCLUSION

The proposed method was used successfully to determine ormeloxifene in raw material and tablets even in presence of degradation products. The results of validation studies showed that the Stability indicating LC method is accurate, significant linearity and precision. The stability tests results showed that ormeloxifene is significantly unstable and the degraded products were separated from the main peak. Thus the proposed HPLC method is useful for routine quality control analysis of ormeloxifene in bulk and its formulations.

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