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Method Development and Validation of RP-HPLC Method for the Estimation of Ormeloxifene

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Abstract:

A new simple, specific, accurate, precise RP-HPLC method has been developed for the estimation of Ormeloxifene. The chromatographic separation for Ormeloxifene was achieved with mobile phase containing methanol :ACN(70:30 v/v), agilent C18 column (4.6 x150 mm) 5 µ at room temperature and UV detection at 274nm.The compounds were eluted in the isocratic mode at a flow rate of 1ml/min. The retention time of Ormeloxifene was found to be 2.497min. The method was validated according to ICH guideline for linearity, specificity, precision, accuracy, LOD, LOQ and robustness in accordance with ICH guidelines.

1. Introduction

Ormeloxifene hydrochloride (also called as centchroman) (Figure 1), chemically known as trans - 7- methoxy - 2, 2 - dimethyl – 3 – phenyl – 4 - [4 (2-pyrrolidino-ethoxy)phenyl]chroman hydrochloride is one of the selective estrogen receptor modulator (SERMs) (Suneetha A et al., 2014). It is best known as a non-hormonal, non-steroidal oral contraceptive which is taken once per week (Annu M et al., 2009). Ormeloxifene is primarily used as a contraceptive, but it may also be effective for dysfunctional uterine bleeding and advanced breast cancer (Dhananjay BS et al., 2013).

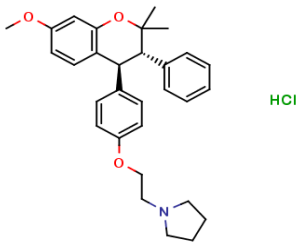

Figure 1: Structure of Ormeloxifene hydrochloride

Literature reveals that works were carried out for determination of Ormeloxifene by LC-MS/MS in rat plasma (SheelendraPratap Singh et al.,2008), pharmacokinetic activity on rats (Paliwal JK et al., 1996), HPLC methods in formulations (Praveen Kumar M et al., 2011), stability indicating HPLC method in bulk and pharmaceutical dosage forms (A. Suneetha et al., 2014), there are no analytical methods reported for the estimation Ormeloxifene by RP-HPLC method. Hence, it was felt that, there is a need of new analytical method development for the estimation of Ormeloxifene in pharmaceutical dosage form.

2. Materials and methods

The API of Ormeloxifene was received from KP labs Hyderabad.

2.1 Chemicals and reagents used

All the chemicals and reagents were supplied by Merck Ltd., India; Qualigens Fine Chemicals Ltd., Mumbai, India.

2.2 Instruments used

Method development was carried out using HPLC-auto sampler-UV detector (Separation module 2695, UV detector 2487).

2.3 Selection of mobile phase

- Methanol :ACN(70:30 v/v)

- Reasons: To decrease the retention and improve separation. Good Response, Area, Tailing factor, Resolution.

2.4 Selection of wavelength

10 mg of Ormeloxifene was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Ormeloxifene. The isobestic point was taken as detection wavelength. Ormeloxifene showed absorbance's maxima at 274nm. The spectrums are shown in Figure 2.

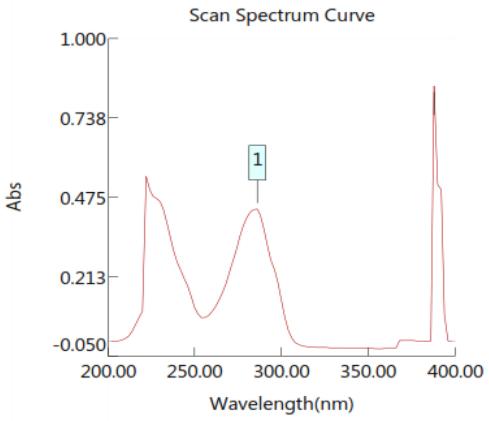

Figure 2: Spectrum showing overlapping spectrum of Ormeloxifene

2.5 Preparation of the Ormeloxifene standard and sample solution

2.5.1 Sample solution preparation:

10 mg of Ormeloxifene tablet powder was accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

2.5.2 Standard solution preparation

10 mg Ormeloxifene working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

2.6 System suitability

- Tailing factor for the peaks due to Ormeloxifene in standard solution should not be more than 1.5.
- Theoretical plates for the Ormeloxifene peaks in standard solution should not be less than 2000.

2.7 Validation Parameters

The method was validated according to ICH guideline for linearity, specificity, precision, accuracy, LOD, LOQ and robustness (ICH guidelines 1996).

3. Results and discussion

3.1 Optimized chromatographic conditions for simultaneous estimations of Ormeloxifene by RP-HPLC method

Column	: Agilent (5µm, 4.6x150mm)
Column temperature	: Ambient
Wavelength	: 274 nm
Mobile phase ratio	: Methanol:ACN(70:30% v/v)
Flow rate	: 1.0 ml/min
Auto sampler temperature	: Ambient
Injection volume	: 10µl
Run time	: 7.0 minutes

3.2 Assay calculation for Ormeloxifene

The assay study was performed for the Ormeloxifene. Each three injections of sample and standard were injected into chromatographic system.

The retention time of Ormeloxifene was found to be 2.497mins. The system suitability parameters for Ormeloxifene such as theoretical plates and tailing factor were found to be 4187.6, 1.5. The % purity Ormeloxifene in pharmaceutical dosage form was found to be 98.94%.

3.3 Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank. It was found that there was no interference of impurities in retention time of analytical peak.

3.4 Linearity

The linearity study was performed for the concentration of 15-75 ppm ormeloxifene. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The results are tabulated in Table 1.The correlation coefficient was found to be 0.999 (NLT 0.999).

3.5 Accuracy

The accuracy study was performed for 50%, 100% and 150% for Ormeloxifene. Each level was injected in triplicate into chromatographic system. The a rea of each level was used for calculation of % recovery. Results are tabulated in Table 2. The % recovery was found to be 99.95% (NLT 98% and NMT 102%)

3.6 Precision

- Repeatability
- Intermediate Precision

3.6.1 Repeatability

The precision study was performed for five injections of Ormeloxifene. Each standard injection was injected into chromatographic system.

The area of each standard injection was used for calculation of % RSD. The method precision study was performed, %RSD of ormeloxifene was found to be 0.24 (NMT 2). Results are tabulated in Table 3.

3.6.2 Intermediate precision/Ruggedness

The intermediate precision study was performed for five injections of Ormeloxifene. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of % RSD. Results are tabulated in Table 4.

The intermediate precision was performed for %RSD of Ormeloxifene was found to be 0.15 (NMT 2).

3.7 Detection limit

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$\text{LOD} = 3.3 \text{ X } \frac{\sigma}{S}$$

Where

σ - Standard deviation (SD)

S - Slope

The LOD for ormeloxifene was found to be 3.04.

3.8 Quantitation limit

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$\text{LOQ} = 10 \text{ X } \frac{\sigma}{S}$$

Where

σ - Standard deviation

S - Slope

The LOQ was performed for ormeloxifene was found to be 10.14.

3.9 Robustness

The robustness was performed for the flow rate variations from 0.8ml/min to 1.2ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Ormeloxifene. The method is robust only in less flow condition and the method is robust even by change in the mobile phase ±5%.

The results are summarized in table 7 on evaluation of the results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min.The method is robust only in less flow condition.

On evaluation of the results, it can be concluded that the variation in ±5% organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the mobile phase ±5%.

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Table 1: Linearity results for Ormelixifene

S.No	Linearity Level	Concentration	Area
1	I	15 ppm	244840
2	II	30 ppm	491451
3	III	45 ppm	677620
4	IV	60 ppm	873311
5	V	75 ppm	1148958
Correlation Coefficient			0.999

Table 2: Showing accuracy results for Ormeloxifene

%Concentration (at specification level)	Average Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	728287	5	4.96	99.91%	99.95%
100%	1378202	10	9.98	99.18%	
150%	2115480	15	15.02	99.60%	

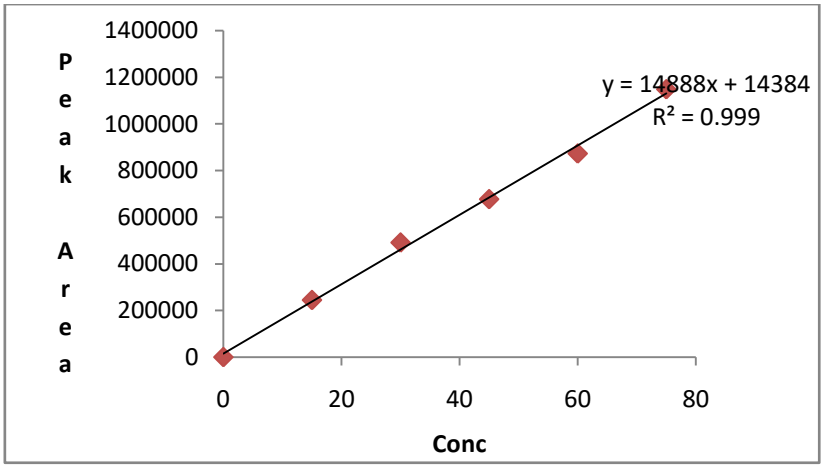

Figure 3: Showing calibration graph for ormeloxifene

Table 3: Repeatability results for Ormeloxifene

S.No	Name	RT	Area	Height (µV)
1	Ormeloxifene	2.423	693877	117760
2	Ormeloxifene	2.424	696531	117366
3	Ormeloxifene	2.424	693977	117612
4	Ormeloxifene	2.424	695278	117573
5	Ormeloxifene	2.423	697676	117829
Mean			695468	
Std. Dev.			1642.7	
% RSD			0.24	

Table 4: Showing results for intermediate precision of Ormeloxifene

S.No	Name	RT	`Area	`Height(µV)
1	Ormeloxifene	2.423	693078	117646
2	Ormeloxifene	2.424	693338	117177
3	Ormeloxifene	2.424	695080	117535
4	Ormeloxifene	2.424	694843	117534
5	Ormeloxifene	2.423	695336	117665
Mean			694335	
Std. Dev.			1047.5	
% RSD			0.15	

Table 5: Showing results for limit of detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg/ml)
Ormeloxifene	1642	14888	0.36

Table 6: Showing results for limit of quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg/ml)
Ormeloxifene	1642	14888	1.10

Table 7: Showing system suitability results for Ormeloxifene

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	SP Tailing
1	0.8	4187	1.5
2	1	4512	1.4
3	1.2	4084	1.4

Table 8: Showing system suitability results for Ormeloxifene

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	4194	1.5
2	*Actual	4524	1.5
3	5 % more	3097	1.4

4. Conclusion

The developed RP-HPLC method is simple and economical. In proposed method good resolution was obtained and the method was validated according to ICH guidelines. Hence, it can be applied for routine analysis of formulation.

Conflict of interest

None declared

5. References

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