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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF ULIPRISTAL ACETATE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, novel, precise and accurate stability indicating RP-HPLC method was developed and validated for the estimation of Ulipristal Acetate in pharmaceutical dosage form. A Phenoxneome C18 (150 mm x 4.6 mm, 5 µm) column was used as stationary phase with mobile phase consisting of 0.1% ortho phosphoric acid and acetonitrile in the ratio of 50:50 v/v (pH was adjusted to 4.0 with triethyl amine). The flow rate was maintained at 1.0 mL/min and effluents were monitored at 223 nm. The retention time was 1.895 min. The linearity of the method was observed in the concentration range of 20-100 µg/mL with correlation coefficient of 0.999. The method developed was validated for linearity, precision, accuracy, system suitability and forced degradation studies like acidic, alkaline, oxidative and neutral stress conditions were performed as per ICH guidelines. The results obtained in the study were within the acceptable limits and hence this method can be used for the estimation of Ulipristal Acetate in pharmaceutical dosage form.

INTRODUCTION

Ulipristal Acetate (Figure 1) is the selective progesterone receptor modulator (SPRM) for the treatment of uterine fibroids and also used as medication for emergency birth control [1]. Chemically it is [(8S,11R,13S,14S,17R)-17-acetyl-11-[4-(dimethylamino)phenyl]-13-methyl-3-oxo-1,2,6,7,8,11,12,14,15,16-decahydrocyclopenta [a]phenanthren-17-yl] acetate. Ulipristal Acetate prevents progesterone from binding to the receptor, leading to blockage of gene transcription inhibiting synthesis of proteins necessary to begin and maintain pregnancy and also acts by inhibiting the ovulation. [2-4]

Literature survey revealed that few HPLC methods [5-6] were reported for the estimation of Ulipristal Acetate in pharmaceutical formulations. Hence a new, sensitive and efficient HPLC method was developed and validated as per ICH guidelines [7-8] for the estimation of Ulipristal Acetate in bulk and pharmaceutical dosage form.

MATERIALS AND METHODS

Instrumentation

To develop a high pressure liquid chromatographic method for quantitative estimation of Ulipristal Acetate using Agilent Technologies 1260 infinity binary HPLC instrument on a Phenoxneome C18 (150 mm x 4.6 mm, 5 µm) analytical column was used. The instrument is equipped with a pump, sampler and PDA detector. A 20 µL rheodyne injector port was used for injecting the samples. Data was analyzed by using EZ Chrome Open Lab software.

Chemicals and solvents

The reference sample of Ulipristal Acetate was obtained from Shree Icon Pharmaceutical Laboratories, Vijayawada, India. Commercially available Ulipristal Acetate tablets claimed to contain 5 mg of Ulipristal Acetate was purchased from local market. Methanol (HPLC grade), acetonitrile (HPLC grade), ortho phosphoric acid (AR grade) and triethyl amine (AR grade) were purchased from Merck (India) Ltd, Mumbai, India.

Chromatographic conditions

A mixture of 0.1% ortho phosphoric acid: acetonitrile (50:50 v/v, pH 4.0 was adjusted with triethyl amine) was found to be the most suitable mobile phase for ideal chromatographic separation of Ulipristal Acetate. The solvent mixture was filtered and sonicated before use for 5 minutes. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 20 µL and the column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 223 nm. The run time was set at 5 min.

Preparation of mobile phase and diluent

500 mL of the 0.1% ortho phosphoric acid (pH adjusted to 4.0 with triethyl amine) buffer was mixed with 500 mL of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The water and acetonirile phase was used as diluent.

Preparation of standard solution

About 10 mg of Ulipristal Acetate is accurately weighed and transferred into a 10 mL (1000 µg/mL) clean dry volumetric flask containing mobile phase. The solution was sonicated for 5 min and the drug was dissolved completely. The volume was made up to the mark with a further quantity of the mobile phase to get a stock concentration of Ulipristal Acetate. Further pipette 1.0 mL of the above stock solution into a 10 mL volumetric flask (100 µg/mL) and the volume was made up to the mark with the mobile phase.

Preparation of sample solution

20 tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 10 mg of Ulipristal Acetate is transferred into a 10 mL (1000 µg/mL) clean dry volumetric flask containing mobile phase. The solution was filtered and sonicated for 5 min. The volume was made up to the mark with a further quantity of the mobile phase to get a stock concentration of Ulipristal Acetate. Further pipette 0.6 mL of the above stock solution into a 10 mL volumetric flask (60 µg/mL) and the volume was made up to the mark with the mobile phase.

METHOD VALIDATION

Linearity

Linearity was performed by preparing standard solution of Ulipristal Acetate at different concentration levels i.e., 20-100 µg/mL. The absorbance was measured at 223 nm. Each measurement was carried out in triplicate. Linearity was proven by regression analysis by the least square method.

Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. The intermediate precision of the method was confirmed by intra-day and inter-day analysis. The concentration used for the precision studies is 60 µg/mL. To study the intra-day and inter-day precision, the analysis of drugs was repeated for six times in the same day and in different days. Six replicate mixed standard solution of Ulipristal Acetate was measured with the same concentration and the %RSD was calculated.

Accuracy

The accuracy of the method was assessed by recovery study of Ulipristal Acetate in the dosage form at three concentration levels. A known amount of standard drug was added to the fixed amount of pre-analyzed drug sample solution. Percent recovery was calculated by comparing the peak area before and after the addition of the standard drug. The standard addition method was performed at three concentration levels in triplicate at 80%, 100% and 120%.

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution using the developed HPLC method.

Ruggedness

The ruggedness of the method was determined by carrying out the experiment on different instruments by different operators using different columns of similar types. Samples of Ulipristal Acetate at 60 µg/mL concentration were analyzed by different analysts.

Robustness

To demonstrate the robustness of the method, the solution was prepared as per test method and injected at different variable conditions like changes in flow rate, wavelength, mobile phase composition and pH.

STABILITY STUDIES

Degradation studies were performed and the degraded samples were injected.

Control sample

20 tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 10 mg of Ulipristal Acetate is transferred into a 10 mL (1000 µg/mL) clean dry volumetric flask containing mobile phase. The solution was filtered and sonicated for 5 min. The volume was made up to the mark with a further quantity of the mobile phase to get a stock concentration of Ulipristal Acetate. Further pipette 0.6 mL of the above stock solution into a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase.

Acid degradation studies

To 1 mL of stock solution of Ulipristal Acetate, 1 mL of 2N hydrochloric acid was added and refluxed for 30 mins at 60^{0}C. The resultant solution was diluted to obtain 60 µg/mL solution and 20 µL solution were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation studies

To 1 mL of stock solution of Ulipristal Acetate, 1 mL of 2N sodium hydroxide was added and refluxed for 30 mins at 60^{0}C. The resultant solution was diluted to obtain 60 µg/mL solution and 20 µL solution were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative degradation studies

To 1 mL of stock solution of Ulipristal Acetate, 1 mL of 20% hydrogen peroxide was added. The solution were kept for 30 mins at 60^{0}C. The resultant solution was diluted to obtain 60 µg/mL solution and 20 µL solution were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral degradation studies

Stress testing under neutral conditions was studied by refluxing the standard Ulipristal Acetate solution in water for 6 hrs at a temperature of 60ºC. The resultant solution was diluted to obtain 60 µg/mL solution and 20 µL solution were injected into the system and the chromatograms were recorded to assess the stability of sample.

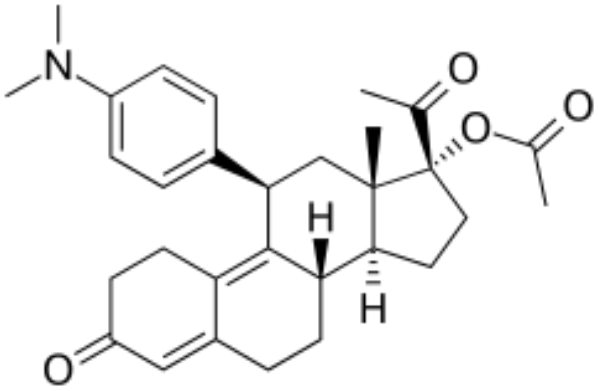

Fig. 1: Chemical structure of Ulipristal Acetate

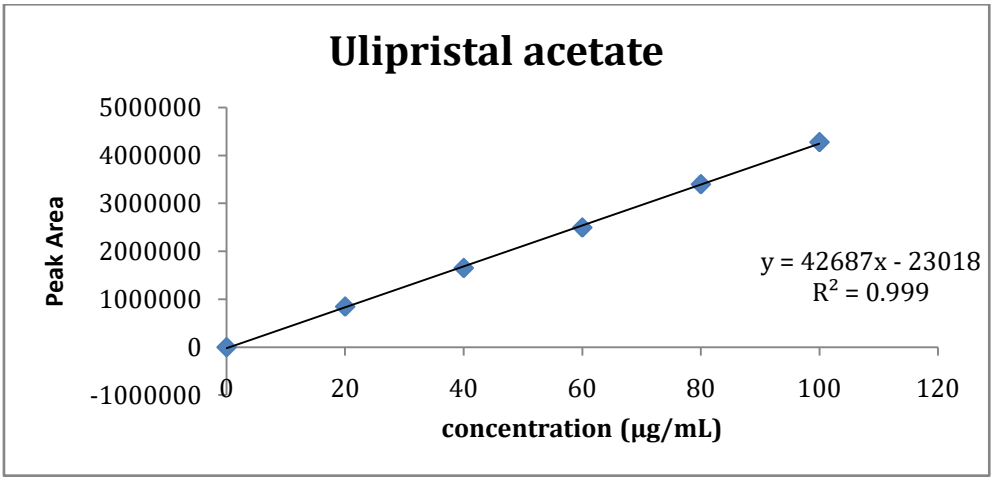

Fig. 2: Linearity curve of Ulipristal Acetate

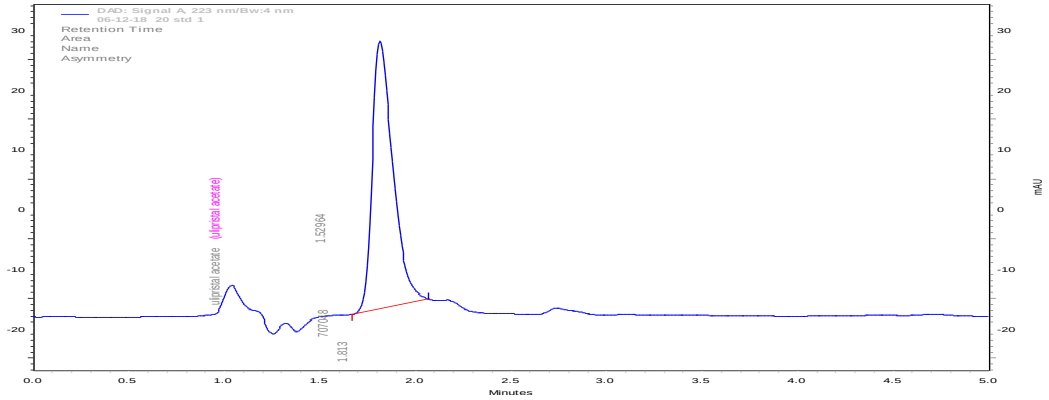

Fig. 3: Typical chromatogram of Ulipristal Acetate

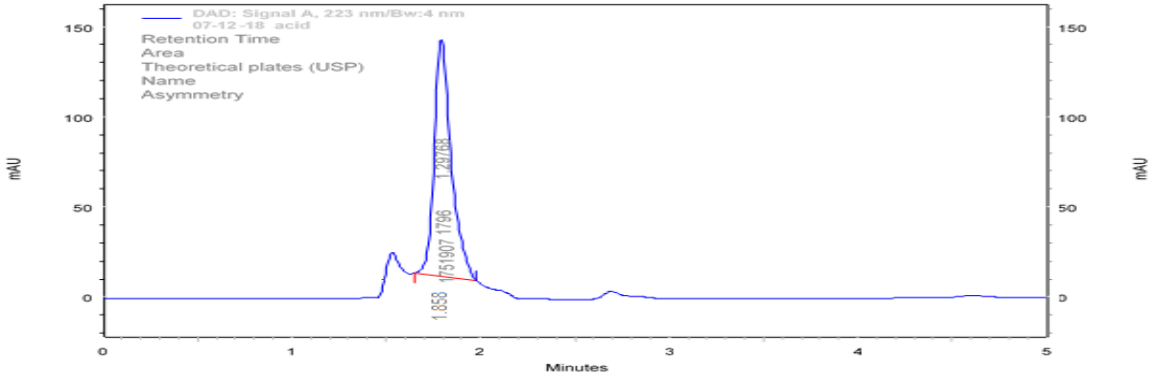

Fig. 4: Chromatogram of Ulipristal Acetate in acid degradation

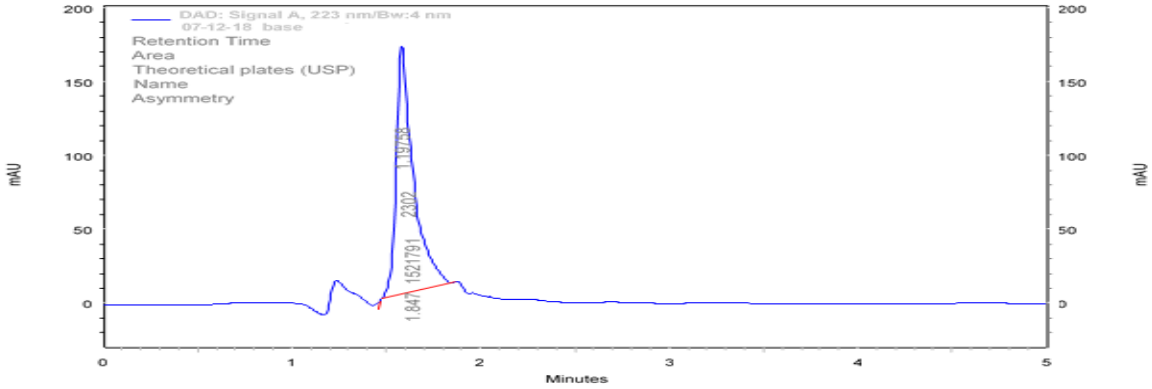

Fig. 5: Chromatogram of Ulipristal Acetate in alkali degradation

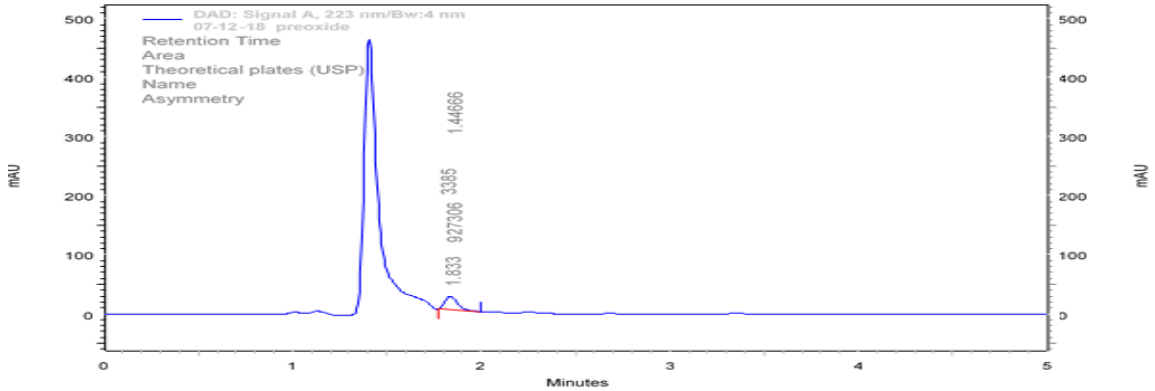

Fig. 6: Chromatogram of Ulipristal Acetate in oxidative degradation

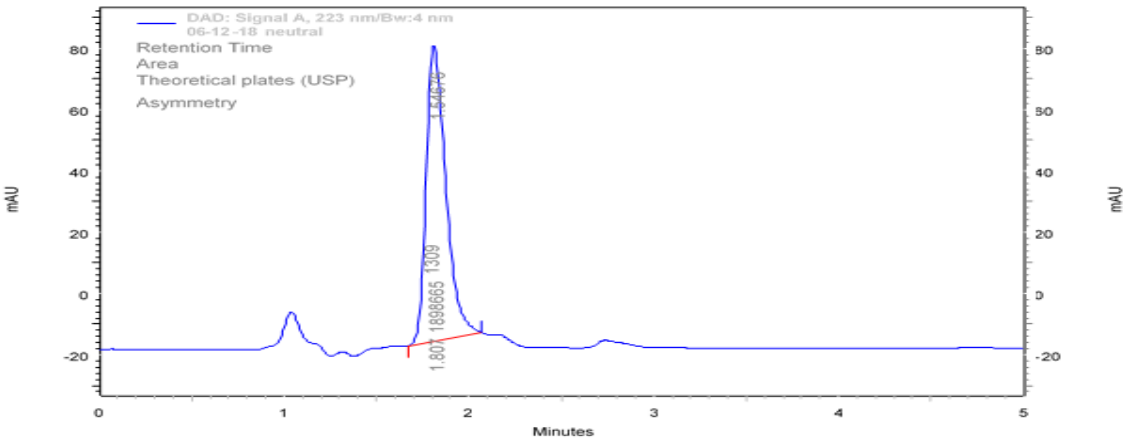

Fig. 7: Chromatogram of Ulipristal Acetate in neutral degradation

Table 1: Optimized chromatographic conditions of Ulipristal Acetate

Parameter	Condition
Mobile phase	Ortho phosporic acid:Acetonitrile (50:50 v/v)
pH	4.0
Diluent	Water:Acetonitrilee
Column	Phenoxneome C18 (150 mm × 4.6 mm, 5 µm)
Column temperature	Ambient
Wave length	223 nm
Injection volume	20 µL
Flow rate	1.0 mL/min
Run time	5 min
Retention time	1.895 min

Table 2: Linearity results of Ulipristal Acetate

S. No.	Concentration (µg/mL)	Peak area
1	0	0
2	20	84667
3	40	16510
4	60	24954
5	80	33986
6	100	42760
Slope		42687
Intercept		23018
Regression Equation (y)		y=42687x-23018
Correlation Coefficient		0.999

Table 3: Precision results for Ulipristal Acetate

S. No.	Intra-day Time (Hours)	Peak area	Inter-day Time (Days)	Peak area
1	0	2425428	Day1 (n=3)	2221391
2	3	2468027		
3	6	2430414	Day 2 (n=3)	2185709
4	9	2424927		
5	12	2396338	Day 3 (n=3)	2219279
6	15	2397420		

Mean	2423759	Mean	2208793
SD	26282.12	SD	20019
%RSD	1.084	%RSD	0.906

Table 4: Recovery results of Ulipristal Acetate

Recovery/Spike level at about [%]	Amount of Drug added (µg/mL)	Amount of Drug found (µg/mL)	% Recovery
80	40	39.26	98.15
100	60	60.87	101.45
120	100	99.87	99.87

Table 5: System suitability parameters of Ulipristal Acetate

Parameter	Results
Linearity range (µg/mL)	20-100
Correlation coefficient	0.999
Theoretical plates (N)	2300
Tailing factor	1.5
LOD (µg/mL)	0.064
LOQ (µg/mL)	0.190

Table 6: Forced degradation study results of Ulipristal Acetate

Stress conditions	%Assay	%Degradation
Control	100	-
Acid	87.60	12.40
Alkali	76.10	23.90
Oxidative	46.37	53.63
Neutral	94.94	05.06

RESULTS AND DISCUSSION

In the present work, a simple, novel, precise and accurate stability indicating RP-HPLC method has been optimized, developed and validated for the determination of Ulipristal Acetate in pharmaceutical formulations by using Phenoxneome C18 (150 mm x 4.6 mm, 5µm) in isocratic mode with mobile phase composed of mixture of 0.1% ortho phosphoric acid and acetonitrile in the ratio of 50:50 v/v (pH 4.0 was adjusted with triethyl amine) resulted the chromatographic peak obtained was in good shape, better resolved and almost free from tailing. The flow rate was 1.0 mL/min and the drug component was measured with PDA detector at 223 nm. The results of optimized HPLC conditions were shown in Table 1.

The method was linear in the range of 20 to 100 µg/mL for Ulipristal Acetate with correlation coefficient of 0.999. The linearity results were shown in Table 2 and the linearity curve was shown in Figure 2. The %RSD for the intra-day and inter-day precision for Ulipristal Acetate was found to be 1.084 and 0.906, which indicates the method is precise. The results of precision studies were shown in Table 3. The %recoveries of Ulipristal Acetate were found in the range of 98.15-101.45% and the %mean recovery was found to be 99.82%, which indicates the method is accurate. The results of recovery studies were shown in Table 4.

The retention time of Ulipristal Acetate was 1.895 min, cuts down on overall time of sample analysis and the method was more cost effective as it utilizes very less quantity of mobile phase. The number of theoretical plates was 2300 and tailing factor was 1.5 for Ulipristal Acetate, which indicates efficient performance of the column. Typical chromatogram of drug Ulipristal Acetate was shown in Figure 3. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in the formulation did not interfere with the estimation of the drug Ulipristal Acetate by the proposed HPLC method.

Selectivity of the method was demonstrated by the absence of any interfering peaks at the retention time of the drug. The limit of detection and limit of quantitation for Ulipristal Acetate were found to be 0.064 µg/mL and 0.190 µg/mL, which indicate the sensitivity of the method. A system suitability test was performed to evaluate the chromatographic

parameters and the summary of system suitability parameters were shown in Table 5. Validated method was applied for the determination of Ulipristal Acetate in commercial formulations.

Stability studies of Ulipristal Acetate under different stress conditions indicated the following degradation behavior. In acidic degradation, the degradation product was appeared at retention time of 1.858 min and the %degradation is 12.40%. In alkali degradation, the degradation product was appeared at retention time of 1.847 min and the %degradation is 23.90%. In oxidative degradation, the degradation product was appeared at retention time of 1.833 min and the %degradation is 53.63%. In neutral degradation, the degradation product was appeared at retention time of 1.809 min and the %degradation is 5.06%. The results of stability studies were shown in Table 6. The typical chromatograms of degradation behavior of Ulipristal Acetate in different stress conditions are shown in Figure 4 to Figure 7.

CONCLUSION

The present study represents the development of a stability indicating RP-HPLC method for determination of Ulipristal Acetate by following the recommendations of ICH guidelines. The proposed method showed acceptable accuracy, precision, selectivity and wide linear concentration range. The results of analysis proved that the method is suitable for the determination of Ulipristal Acetate in bulk and tablet dosage form without any interference from the degradation products and it is recommended for routine quality control analysis of the Ulipristal Acetate in pharmaceutical formulation.

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