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

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Abstract:
A simple and selective LC method is described for the determination of Ulipristal in tablet dosage forms. Chromatographic separation was achieved on a c18 column using mobile phase consisting of a mixture of Phosphate buffer(KH2PO4) pH4.0: Acetonitrile (30:70v/v/v),with detection of 223 nm. Linearity was observed in the range 60-140 µg /ml for Ulipristal (r2 =0.999) for drugs estimated by the proposed methods was in good agreement with the label claim. From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Ulipristal was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

Keywords: *Ulipristal, RP-HPLC, Development, Validation*

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INTRODUCTION:

Ulipristal is a selective progesterone receptor modulator used for the purposes of emergency contraception (Ella) and for the treatment of uterine fibroids (Fibristal). It is a derivative of 19-norprogesterone and has both antagonistic and partial agonist activity at the progesterone receptor. It also binds to glucocorticoid receptor, however compared to mifepristone (a progesterone receptor antagonist), ulipristal is more tolerable and has lower glucocorticoid activity and better binding affinity. The exact mechanism of action of ulipristal has been heavily debated. On one hand, the majority of official prescribing information labels, monographs, and prior research studies for ulipristal indicated as an emergency contraceptive suggest that its primary mechanism of action revolves around inhibiting or delaying ovulation by suppressing surges in LH that result in the postponement of follicular rupture IUPAC name is 14-acetyl-17-[4-(dimethylamino)phenyl]-14-hydroxy-15-methyltetracyclo[8.7.0.0^{2,7}.0^{11,15}]heptadec a-1,6-dien-5-one. Molecular formula $C_{28}H_{35}NO_3$. Molecular Weight is 433.5.

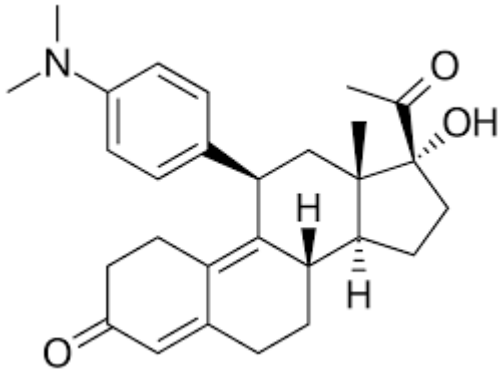

Figure 1: Structure of Ulipristal

Literature survey reveals that the Ulipristal in both single and simultaneous with other drugs can be estimated by RPHPLC in pharmaceutical dosage forms [5-7] The objective of this study is to develop a simple, fast, economical, selective, accurate, precise and sensitive RP-HPLC method for the determination of Ulipristal in bulk and its pharmaceutical dosage forms suitable for routine quality control analysis. This work makes an attempt to develop a new sensitive and accurate RP-HPLC method for estimation of UPA in bulk and pharmaceutical dosage form and to validate the developed method in accordance with International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines.[8]

MATERIALS AND METHODS:

Chemicals and Reagents: Ulipristal is gift samples obtained from Chandra labs, Hyderabad. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 254 nm with column Zodiac ODS, RP-18,250×4.6mm ID, 5µm Particle size, dimensions at Ambient temperature. The optimized mobile phase consists of Phosphate buffer(KH2PO4): Acetonitrile(30:70). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation of buffer:

The buffer solution was prepared by dissolving accurately weighed 0.900 g of anhydrous disodium hydrogen phosphate and 1.298 g of citric acid monohydrate in sufficient water to produce 1000 ml. The pH was adjusted to 3.0 using phosphoric acid and sonicated to dissolve.

Preparation of mobile phase:

The Mobile Phase was prepared by mixing HPLC grade acetonitrile (ACN) was mixed with the buffer in a ratio of 30: 70 v/v. It was then sonicated for about 30 min and filtered through 0.45-micron membrane filter which was used for analysis of Ulipristal.

Diluant Preparation:

Mobile phase is used as Diluant.

Standard sample

Standard stock solution of ULIPRISTAL (microgram/ml) were prepared by dissolving 100 mg of ULIPRISTALwas dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5min and dilute to 100 ml with mobile phase. Further dilutions are prepared in 5 replicates of 100µg/ml of ULIPRISTAL was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Tablet sample

20 tablets (each tablet contains 10mg of ULIPRISTAL) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of ULIPRISTAL (µg/ml) were

prepared by dissolving weight equivalent to 100mg of ULIPRISTALand dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 100µg/ml of ULIPRISTAL was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Procedure:

20µL of the standard, sample are injected into the chromatographic system and the areas for peaks are measured and the %Assay are calculated by using the formulae.

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 8 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Zodiac ODS, RP-18,250×4.6mm ID, 5µm Particle size, the mobile phase of composition phosphate buffer (pH 3.0) : Acetonitrile in the ratio of 30:70 (% v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Ulipristal in tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity: Ulipristal working standard solutions were prepared across the range of the analytical method with a minimum of 5 concentrations that are within the specified range 60-140 µg/ml for 5 replicating injections were taken and calculated the %RSD. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 80%, 100%,120%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Ulipristal and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies: The system precision of the test method was performed by injecting 6 replicate determination of standard preparation injections were injected and the % RSD was calculated. The %RSD for the area of six replicate injections was found. The results are shown in table 5.

Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different Analyst by using different make column of same dimensions. The results are shown in table 6.

Robustness: Robustness of assay method was carried out with variation of flow rate. Standard preparation was prepared and performed analysis as per test method and evaluated the system suitability parameters. As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method. The results are shown in table 7.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 8.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

Forced degradation studies

The forced degradation study is considered a vital analytical aspect of the drug development program for small molecules. Forced degradation, commonly known as stress testing, The ICH definition of stress testing for the drug product is “studies undertaken to assess the effect to severe conditions on the drug product. Such studies include photo stability testing and specific testing on certain products like metered dose inhalers, creams, emulsions etc. As per FDA guideline “Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods”. The results are shown in

table 9.

RESULTS AND DISCUSSION:

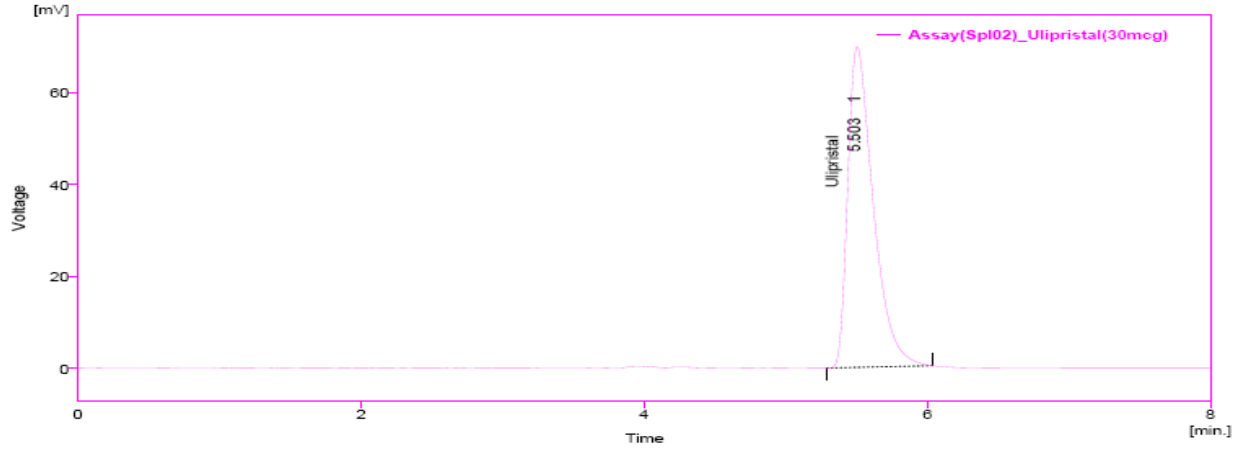

Figure 2: Standard chromatogram

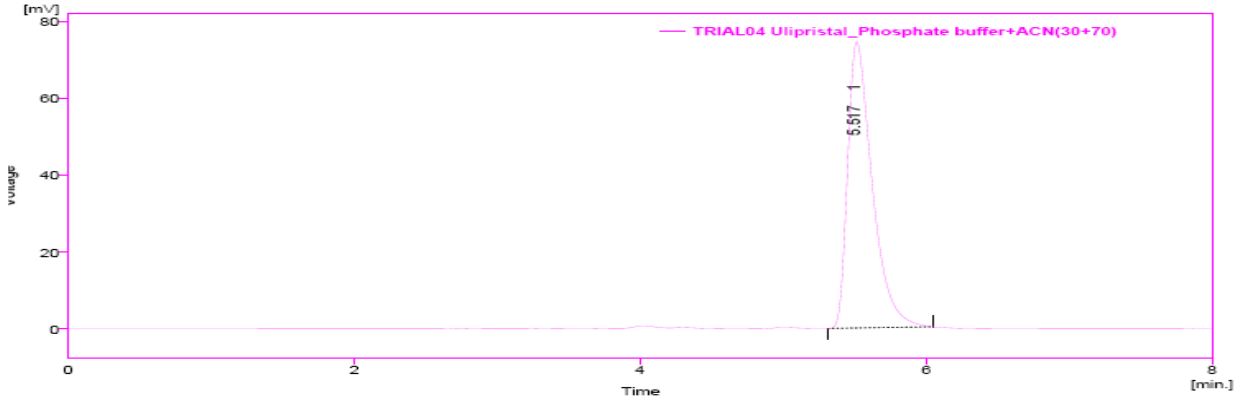

Figure 3: Sample chromatogram

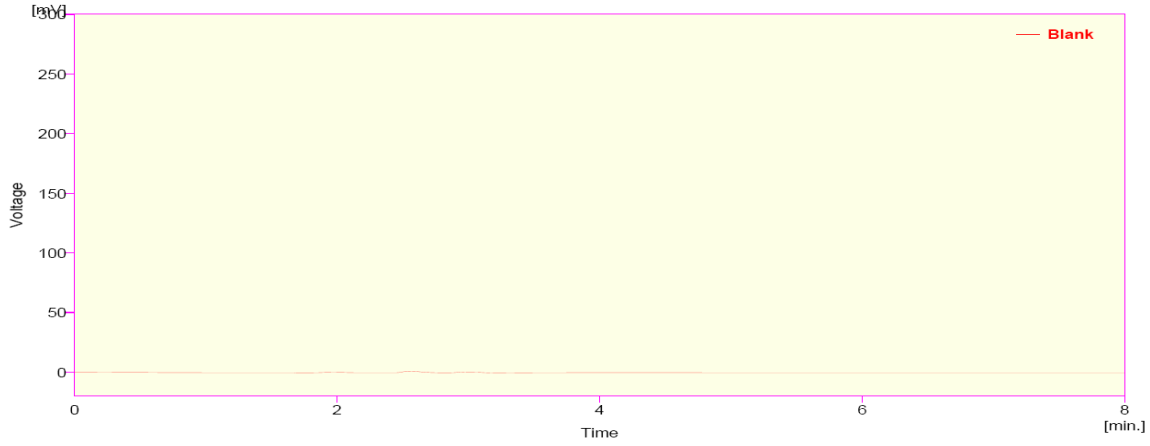

Figure 4: Blank chromatogram

Table 1: System suitability parameters

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	5.50	870.251	4489	1.890
2	5.49	875.623	4485	1.890
3	5.48	873.529	4427	1.896
4	5.50	874.123	4434	1.876
5	5.49	873.263	4434	1.890
6	5.49	874.103	4419	1.894
Mean	2.2182	875.236	-	-
SD	0.0361	21.537	-	-
%RSD	**1.63**	**1.05**	-	-

Table 2: Assay results for Ulipristal

ULIPRISTAL		
	Standard Area	**Sample Area**
Injection-1	873.254	874.316
Injection-2	875.134	871.445
Injection-3	875.177	874.919
Injection-4	411.533	875.134
Injection-5	868.506	873.263
Average Area	877.791	877.386
Tablet average weight	80.2 mg	
Standard weight	100.1 mg	
Sample weight	800.094 mg	
Label amount	10 mg	
std. purity	99.25	
Amount found in mg	9.96 mg	
Assay(%purity)	99.62 %	

Table 3: Linearity results of Ulipristal

S.No.	Conc.(µg/ml)	Area
1	60	443.806
2	80	631.638
3	100	809.924
4	120	1001.338
5	140	1208.208

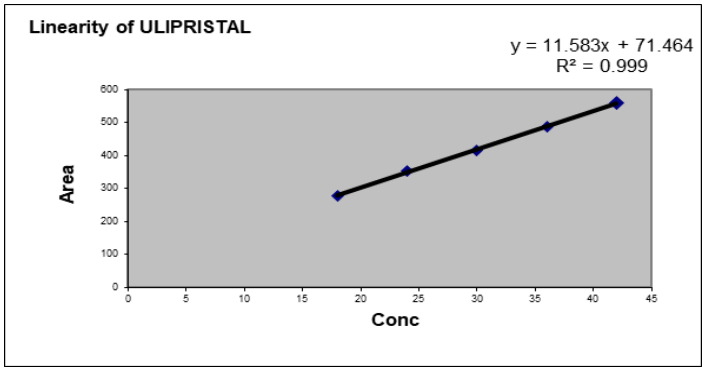

Figure 5: Linearity graph for Ulipristal

Table 4: Showing accuracy results for Ulipristal

Recovery level	Accuracy ULIPRISTAL					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
80%	100	**806.587**	804.784	101.93	101.93	100.29%
	100	**807.258**				
	100	**800.506**				
100%	120	**977.637**	974.915	120.60	100.50	
	120	**983.452**				
	120	**963.655**				
120%	140	**1185.399**	1185.984	142.13	101.52	
	140	**1193.566**				
	140	**1177.583**				

Table 5: Precision results for Ulipristal

ULIPRISTAL		
S.No.	Rt	Area
1	5.55	869.97
2	5.55	887.077
3	5.537	886.968
4	5.517	889.723
5	5.480	870.553
6	5.520	877.263
avg	5.5257	880.259
stdev	0.0265	8.833
%RSD	0.48	1.00

Table 6. Ruggedness results of Ulipristal

ULIPRISTAL	**%Assay**
Analyst 01	99.73
Analysts 02	99.80
%RSD	0.05%

Robustness results

Table 7: Flow variation and Wavelength results for Ulipristal

Parameter	ULIPRISTAL	
	Retention time(min)	Tailing factor
Flow Rate 0.8 ml/min	9.631	1.854
Wavelength 297nm	5.493	1.850

Table 8: LOD, LOQ of Ulipristal

Drug	LOD	LOQ
Ulipristal	2.18	6.59

Table 9: Forced degradation studies of Ulipristal

Name of the Degradation	Condition	Peak Purity	Peak Purity Value	%Assay
Thermal Degradation	60°C/7Days	PASS	+	98.05
PhotolyticDegradation	1.2mill/LUX Hours	PASS	+	98.06
Acid Degradation	5mL of 3N HCl/4Hrs at	PASS	+	99.96
Base Degradation	5mL of 3N NaOH	PASS	+	99.94
Peroxide Degradation	5mL of 10% H_2O_2/4Hrs at Bench top	PASS	+	99.03

CONCLUSION:

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Ulipristal was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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