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Novel Method for Chromatographic Determination of Ulipristal Acetate in Pure Form and Formulation by Using RP-HPLC

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Abstract

A method for the sensitive and exact determination of active compound Ulipristal Acetate in the bulk and marketed dosage form was developed as well as validated in this study. Ulipristal Acetate was measured in both bulk and pharmaceutical dose form. As the mobile phase, a 60:40 (v/v%) combination of acetonitrile and methanol was employed. Analyses were carried out with the help of a Symmetry C18, ODS, Reverse Phase column, with flow rates of one millilitre per minute, injection volumes of 20µl, run times of six minutes, and detection wavelengths of 275 nm. In order to verify reproducibility (within a day in triplicate) and moderate accuracy, six injections were performed. The findings were represented as percent relative standard deviation within and between days (% RSD). Analyte concentrations ranging from 20µg/ml to 50µg/ml were used to test the method's linearity. Precision investigations produced % RSD findings that were less than 0.78%, which was considered acceptable. The findings of HPLC indicates the 0.999 correlation coefficient (R2) value between the technique and the data. When used with appropriate values, the technique was also shown to be accurate and resilient. As a result, Ulipristal Acetate demonstrated the detection limit of 0.08 µg/mL and quantitation limits of 0.24 µg/mL. Using this approach, ulipristal acetate may be routinely determined in the bulk and marketed drugs, according to the study results.

Keywords: Ulipristal Acetate, Acetonitrile, Methanol, LOD, LOQ, RP-HPLC.

1. Introduction

A particular progesterone receptor modulator, Ulipristal (Figure 1) is used to treat uterine fibroids and as a crisis contraceptive measure [1,2]. As a subordinate of 19-norprogesterone, it exhibits both antagonistic and agonistic activity at the progesterone receptor [3,4]. A progesterone receptor antagonist, Ulipristal is also linked to glucocorticoid receptors but is more permeable than Mifepristone and has a lower rate of progesterone receptor movement as well as a better restraint proclivity compared to Mifepristone [5,6]. A better adequacy and response profile compared to the standard use of ulipristal have led to its recommendation as a first-line therapy for crisis contraception [7,8].

Diverse writers reported physical and chemical characteristics, and ulipristal acetate was subjected to various analytical procedures, both alone and in combination with other medications [9,10]. There are spectrophotometric approaches for estimating ulipristal-derived acetic acid, and there is an RP-HPLC strategy for estimating it. We developed a straightforward, exact, and precise

scientific technique for estimating ulipristal-acetic acid derivation and expanded it for their assurance in detailing, based on the requirement for a reasonable RP-HPLC strategy for routine examination of ulipristal-acetic acid derivation in definitions. An essential step in validating a strategy's capabilities is its encirclement and archiving [11,12].

In addition, the method's usefulness in determining the drug's composition in a business strategy was demonstrated. The approach was approved in accordance with USP and ICH rules for testing dynamic fixing [13,14]. Limits such as framework reasonableness and linearity were accepted along with specifics such as explicitness, roughness, and power. The approach was also approved in terms of the discovery breaking point and the measurement cutoff point. This approach aims to analyse a segment using a set of criteria. According to the researchers, this approach was shown to be suitable for the examination of pharmaceutical measuring structures. For this reason, it was decided to create a novel analytical technique for the simultaneous quantification of ulipristal acetate using reverse-phase HPLC [15].

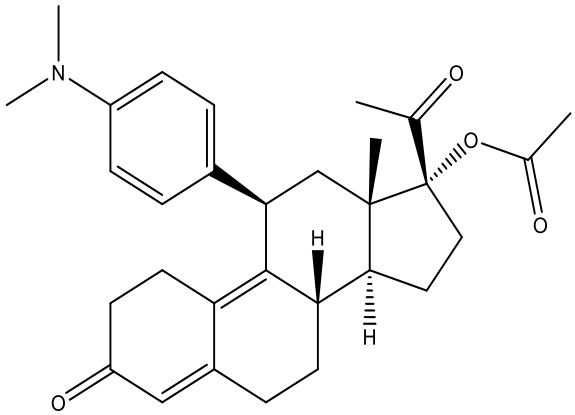

Figure 1: Chemical Structure of Ulipristal Acetate

2. Materials and Methods

2.1. Materials

For the current study, active constituents Ulipristal Acetate was acquired as a gift sample from Shree Icon Pharm. Ltd in Vijayawada, India. Further, Ulipristal Acetate tablets (ellaOne) was purchased from the local market, that claimed to contain 30 mg of Ulipristal Acetate. Merck India Ltd. in Mumbai provided the methanol and acetonitrile for this study. The HPLC system employed was HPLC (Waters). An UV-Visible detector and a pump are included in the device. Each of the samples was introduced into the system via a 20 µL injector port. Empower 2 software was employed to analyse the data.

2.2 Methods

2.2.1. Mobile Phase Preparation

The mobile phase (100mL) was made at three different mixture, solution A (Methanol: Acetonitrile), solution B (Acetonitrile: Water) and solution C (Methanol: Water) for the purpose of optimization (Table 1). The whole process was done under flow rate at 1.0mL/min. Mean time the injection volume was set at 20µL for every injection and U.V detection done at 275nm. The intense peak was observed using mixture of acetonitrile: water (60:40) (Table 2).

Table 1: Illustration of process efficiency

Column types	Mobile system	Flow Rate	Wave length	Assertion	Outcomes
Symmetry C18, ODS, Reverse Phase	Methanol: Acetonitrile = 40:60	1.0ml/min	275nm	Very Low response	Method rejected
	Methanol: Acetonitrile= 55:45	1.0ml/min	275nm	Low response	Method rejected
	Acetonitrile: Water = 50:50	1.0ml/min	275nm	Tailing peaks	Method rejected
	Methanol: Water = 70:30	1.0ml/min	275nm	Resolution was not good	Method rejected
	Acetonitrile: Water = 70:30	1.0ml/min	275nm	Tailing peak	Method rejected
	Acetonitrile: Water= 60:40	1.0ml/min	275nm	Nice peak	Method accepted

Table 2: Optimization of Ulipristal Acetate chromatography conditions

Variable	Requirement
Mobile phase for study	Acetonitrile: Methanol (60:40 v/v)
Column	Symmetry ODS RP C18,5□m, 15mm x 4.6mm i.d.
Detection Wavelength	275nm
Column Temperature	Ambient
Run time	6 min.
Temperature of Auto sampler	Ambient
Flow rate	1.0 ml/ min.

Diluent	Mobile Phase
Volume of Injection	20µl
Elution type	Isocratic
Retention time	2.570 minutes

2.2.1.1 Standard stock solution preparation

In a 10mL volumetric flask containing mobile phase, added 10 mg of ulipristal acetate to create a standard stock solution. Following a 10-minute sonication session, the medication was entirely dissolved and the mobile phase was added to bring it back up to the required volume.

2.2.1.2 Standard working solution preparation

A standard working solution was prepared by diluting 0.5mL of the stock solution with 10 ml mobile phase (50µg/mL) in volumetric flask.

2.2.2 Selection of wavelength

For the detection wavelength, 200-400nm UV spectrum scans of the ulipristal acetate solution were used. At 230 nm, the absorption was at its highest.

2.2.3 Chromatographic conditions

Isocratic elution on a Symmetry* C18 column (250 mm x 4.6 mm, 5m) at a wavelength of 275 nm was used for the chromatographic separation at room temperature (25 °C). In the study of Ulipristal Acetate chromatographic segregation, it was discovered that the mobile phase having Acetonitrile and Water [ACN: Water; 60:40 (v/v)] was the greatest effective solution. Prior usage, the mobile phase was filtered and further it was sonicated for 10 minutes to remove any impurities. It was pushed through the column at a rate of one millilitre per minute for a total of six minutes. For each run, the injection volume was 20 µL. It was necessary to equilibrate the column prior to injecting the drug solution. The compound was discovered at a wavelength of 230 nm.

2.3 Method validation

RP-HPLC technique was verified for specificity, linearity, sensitivity, precision and accuracy as well as stability. Analytical techniques were validated in accordance with the principles set out by the International Conference on Harmonization (ICH).

2.3.1 Accuracy

2.3.1.1 Recovery study

It was necessary to conduct a recovery test with ulipristal acetate in order to determine how was accurate the proposed method. The methodology precision being tested by measuring recoveries of ulipristal acetate using the standard addition approach. The results were promising. In order to pre-quantify the sample solution (10 µg/ml), three different ulipristal acetate standard solutions (80%, 100%, and 120%) being mixed to the stock solution [16]. The obtained results were represented in (Table 3).

Table 3: Precision results for Ulipristal Acetate

Number of injections	**R_t (Minutes)**	**Tailing Factor**	**Peak Area (AUC)**
Replicate – 1	2.792	1.01	186125
Replicate – 2	2.795	1.02	186651
Replicate – 3	2.792	1.13	184858
Replicate – 4	2.793	1.04	183813
Replicate – 5	2.794	1.29	187216
Replicate – 6	2.792	1.05	187611
Average			**186045.7**
Standard Deviation			**1455.199**
% RSD			**0.782175**

2.3.2 Precision

2.3.2.1 Repeatability

Analysing of several homogenous samples, the precision of the analytical technique was evaluated. The peak area and peak symmetry characteristics were used to establish reproducibility to check the repeatability and intermediate precision. Each experiment consisted of six injections, with the findings presented as a % RSD [17].

2.3.3 Intermediate Precision

2.3.3.1 Intra-assay & inter-assay

With respect to ulipristal acetate, the intra day and inter day variations of the suggested technique revealed high average assay findings and further lower standard deviation as well as percent RSD values (% RSD 2 percent) in a day as well as between days.

2.3.4 Linearity & Range

Five working standard solutions (0-50 µg/mL) were introduced into the HPLC system on three separate occasions. In order to evaluate the instrument response was directly proportional to analyte concentration, a calibration curve (X-axis) and average peak area (Y-axis) were created. Linear regression analysis was used to determine the regression equation and the co-relation coefficient [18].

2.3.5 Estimation of LOD and LOQ

The proposed HPLC technique was used to determine the limit of detection (LOD) and limit of quantitation (LOQ) by injecting progressively lower quantities of the standard solution [19].

2.3.6 Robustness

Aspects of the test procedure included preparing the solution according to the test method and injecting it into the system at a variety of settings, comprising varying the flow rate, wavelength, and mobile phase compositions.

2.3.7 System Suitability Parameter

In many analytical methods, system suitability assessment is a key step in the process. It is assumed that equipment, electronic components, analytical procedures and samples to be examined are part of a single system that may be assessed.

2.3.8 Estimation of Ulipristal acetate in marketed drugs

The mean mass of twenty pharmaceutical dosage forms was determined using the I.P. technique, which was applied to all of the samples. It is necessary to weigh out 25 mg of Ulipristal Acetate, which is then put to a 25mL clean, dry volumetric flask that has been filled with mobile phase. As a result of the filtering and sonication for 15 minutes, the solution was ready for use. In order to get Ulipristal Acetate as a stock concentration, a further amount of mobile phase was added. Ten millilitres of the previous solution were then dilute with mobile phase to get 100 millilitres. The membrane filter was used to filter the solution and sonicated to remove gases before it was analysed. Five replicates of the prepared solution were injected into the HPLC apparatus, and the results were noted. As a second injection of the reference solution, the peak regions were also recorded using HPLC.

2.3.8.1.Assay

$$Assay\ \% = \frac{T * W * DT * P}{S * DS * TS * 100} * Avg.Wt$$

Whereas,

T = Using test preparation, measurement of the peak area of a medication

S = Peak area of drug with standard

W = Measurement of Standard weight (mg)

TS = Measurement of Sample weight (mg)

DS = Measurement of dilution of Standard solution

DT = Measurement of dilution of Sample solution

P = Standard solution percentage purity

2.3.9. Stability studies

As a result of varied stress situations, the rate and degree of degradation of the API (ulipristal acetate) was observed. When compared to real-time or long-term stability testing, such sort of accelerated stability test helps in predicting the outcome of the drug that is anticipated to emerge following long-term storage in a very short period of time (as opposed to the latter). Heat degradation, photolytic degradation, and oxidative degradation were also examined in combination to acid and basic hydrolysis.

3. RESULTS AND DISCUSSION

An isocratic mode was used in the present work with a Symmetry ODS RP C18 column in isocratic mode and a mobile phase consisting of Acetonitrile and Methanol in the ratio of 60:40 v/v to design and verify a simple, new, accurate, and precise RP-HPLC technique for the measurement of Ulipristal Acetate in marketed drugs. The flow rate was one millilitre per minute, and the drug component was analysed using an ultraviolet/visible detector at 230 nm. Optimal HPLC settings yielded the following results, as indicated in Tables 1 and 2. The findings of the precision of studies were shown in (Table-3) of this report. We discovered that Ulipristal Acetate could be recovered with an accuracy of 98% to 100.89%, with an average recovery efficiency of 99.7% in our experiments. The recovery studies outcomes of the sample are demonstrated in Table 4. The intra-day and inter-day accuracy of Ulipristal Acetate was found to be 0.886% RSD and 0.776% RSD, respectively (Table 5), indicating that the method is dependable. The approach was predictable in the concentration range of 10 to 50 µg/mL, Ulipristal Acetate, with a R2 value of 0.999. The linearity data is presented in Table 6, and the linearity curve is depicted in(Figure-2). Ulipristal Acetate had detection limit of 0.08 µg/mL and quantitation limits of 0.24 µg/mL, indicating that it had detection and quantitation limitations.

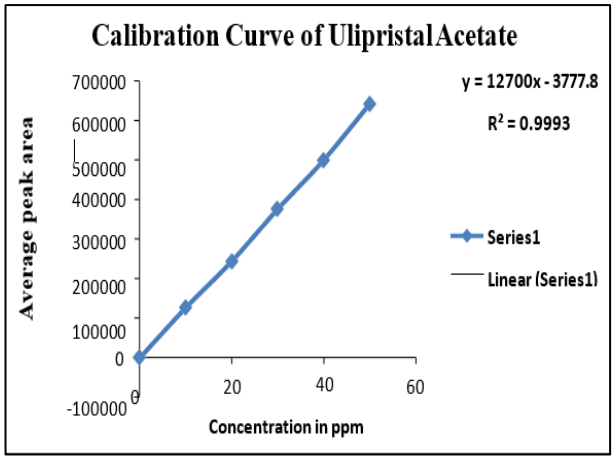

Figure 2: Pictorial Linearity Curve of Ulipristal Acetate

An increase in the optimal rate of flow of ±0.1 ml/min was done to test for robustness, resulting in a decrease in the percent RSD from (0.61 to 0.48%) for each determined increase in the flow rate, as measured by the method's percent RSD. Whereas, a 4 °C temperature shift resulted in a 0.63% to 0.72% change in RSD, respectively. On the other hand, a change of ±3 nm in the method's optimal detection wavelength (230 nm) was made, which resulted in an increase in percent RSD from (0.93 to 0.95%) and an increase in percent RSD from (0.93 to 0.95%) (Table 7). In order to assess chromatographic parameters, a system suitability test was conducted, and the results are summarised in Table 8. Ulipristal Acetate was determined using a validated technique. The estimation of Ulipristal acetate (ellaOne) in marketed formulation was found to be 29.58 □0.687 mean, and % assay observed at 99.79 □ 0.247 (Table 9).

The retention time was observed at 2.57 minutes for Ulipristal Acetate reduces overall sample analysis time. The technique is more cost-effective due to its use of a little amount of mobile phase. According to Ulipristal Acetate's the number of theoretical plates was 4214, tailing factor of 1.29, the column performed efficiently (Table 10). The chromatogram of Ulipristal Acetate is illustrated in Figure 3. The chromatogram of the formulation showed no interfering peaks during the run period, suggesting that the

excipients employed in the formulation did not interfere with the suggested HPLC method's measurement of Ulipristal Acetate. The selectivity of the approach was confirmed by the absence of other conflicting peak throughout the drug's retention duration.

Ulipristal Acetate degraded in the various manner, according to the results of stability studies conducted under various stress situations. During acid hydrolysis, the deterioration component first emerged at a retention time of 2.59 min, and the total amount of degradation products was 18.64 mg. The appearance of the degradation product at 2.59 min retention time and the number of degraded products is 16.63 were observed in basic hydrolysis. Thermal degradation produced a degradation product with a retention time of 2.63 minutes and a deteriorated product with a concentration of 1.08. Photolytic degradation produces degradation products that appear with a retention time of 2.57 minutes and have a degradation product concentration of 3.67. In the case of hydrogen peroxide degradation, the degradation product occurred after 2.56 minutes of retention time, and the degraded product has a pH of 10.59. Tables 11 and 12 show the findings of stability investigations and forced deterioration.

Table 4: Ulipristal Acetate recovery findings

ID of sample	**Concentration (µg/ml)**		**R_t**	**PeakArea of sample**	**% Pure drug Recovery**	**Statistical Analysis**
	Amount Added	**Amount present**				
S1: 80 %	8	8.036155	2.560	156421	100.1601	Mean= 99.3073%
S2: 80 %	8	8.087732	2.563	155412	99.49803	S.D. = 0.96244
S3: 80 %	8	7.989834	2.561	153531	98.26378	% R.S.D.= 0.96915%
S4: 100 %	10	10.0029	2.273	192210	98.91496	Mean= 99.67104%
S5: 100 %	10	10.06592	2.572	193421	99.55066	S.D. = 0.8229
S6: 100 %	10	10.16476	2.590	195320	100.5475	% R.S.D.= 0.82561%
S7: 120 %	12	12.04989	2.597	231541	99.6343	Mean= 100.2838%
S8: 120 %	12	12.13228	2.602	233124	100.3268	S.D. = 0.629055
S9: 120 %	12	12.19932	2.631	234412	100.8902	%RSD = 0.6272

Table 5: Intra-assay and inter-assay findings are reported

Concentration of API drug (µg/ml)	**Observed drug(µg/ml)**			
	Intra-Day		**Inter-Day**	
	Mean value(n=6)	**% RSD**	**Mean value (n=6)**	**% RSD**
8	6.95	0.69	8.02	0.94
10	9.06	0.95	9.59	0.72
12	11.78	0.99	11.09	0.67

Table 6: Linearity Results of drugs

Concentration(µg/ml)	**Mean AUC (n=6)**
0	0
10	126465
20	243214
30	374782
40	498192
50	639624

Table 7: The outcome of the Robustness test technique

Parameter	**% RSD**
0.9 ml/min flow rate	0.48
1.1 ml/min flow rate	0.61
System Temperature (23^0C)	0.72
System Temperature (27^0C)	0.63
Detection at 230 nm	0.93
Detection at 234 nm	0.95

Table 8: System Suitability Parameters Information

Parameters	**Limit used**	**Outcomes**
Test Resolution	Rs > 2	7.86
Test Asymmetry	T ≤ 2	ulipristal acetate=0.26
Theoretical plate	N > 2000	ulipristal acetate=4265
Tailing Factor	T<2	ulipristal acetate=1.29

Table 9: Recovery Data for estimation of Ulipristal acetate with marketed formulation (ellaOne)

UlipristalAcetate (Brand name)	**Drug (mg)**	**Mean (±SD) amount (mg) using (n=6)**	**%Assay (± SD)**
ellaOne (30mg)	30mg	29.58 ± 0.687	99.79 ± 0.247

Table 10: Chromatogram results of Ulipristal acetate

Name of drug	**R_t**	**Peak Area of drug**	**Tailing Factor of drug**	**Plate Count**
Ulipristal acetate	2.570	197762	1.29	4214

Table 11: Data of stability study of ulipristal acetate by HPLC

Parameters	**Rt**	**Peak Area of drug**	**Tailing Factor of drug**	**Plate Count**
Acid hydrolysis	2.590	160899	1.35	4754
Basic hydrolysis	2.590	164874	1.36	4654
Thermal degradation	2.631	195626	1.34	4365
Photolytic degradation	2.572	190504	1.32	4587
Oxidation with (3%) H_2O_2	2.560	176819	1.30	4635

Table 12: Data of Ulipristal Acetate API has been subjected to forced degradation tests

Different Stress condition	**Duration (hr)**	**Active component assay**	**Degraded products assay**	**%Mass Balance**
0.1 M HCl Acid Hydrolysis	24	81.36	18.64	100.0
0.I M NaOH Basic Hydrolysis	24	83.37	16.63	100.0
Thermal Degradation at 50 ^{0}C	24	98.92	1.08	100.0
UV (254nm)	24	96.33	3.67	100.0
3 % Hydrogen peroxide	24	89.41	10.59	100.0

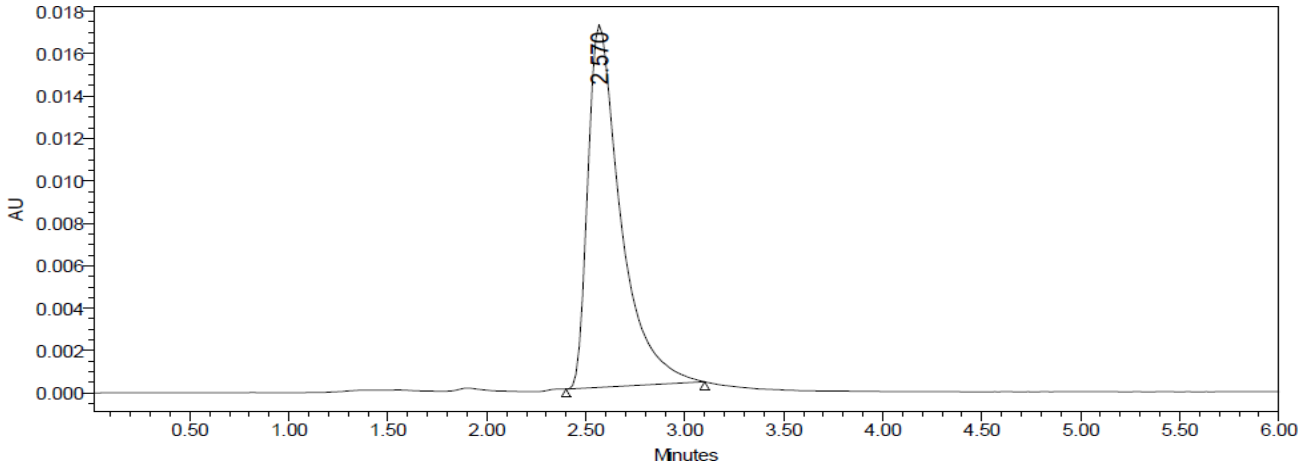

Fig 3: Pictorial Chromatogram for Ulipristal Acetate

4. Conclusion

Follow-up to ICH guidelines led to the creation of a Ulipristal Acetate RP-HPLC technique with stability indicators. The selectivity, precision, accuracy, and linear concentration range of the suggested technique were satisfactory. According to the results of the analyses, this method is adequate for evaluating Ulipristal Acetate in bulk and marketed tablets without interfering with the determination of deterioration compounds. It is recommended that Ulipristal Acetate be tested on a frequent basis for quality control purposes in pharmaceutical preparations.

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Conflict of interest

None

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