A novel stability indicating RP-HPLC Method for the estimation of ulipristal - A Selective Progesterone Receptor Modulator

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The present research article described the novel HPLC technique that was developed for the estimation of ulipristal in an API and tablet formulation. Symmetry, C18, 250mm x 4.6mm i.d., 5 μm Particle size, column with 0.4 ml/min flow rate, was used for the chromatographic separation. Phosphate buffer, pH 4.6 and methanol as a mobile phase (20:80) with a flow rate of 1 ml/min and eluent at 304 nm was monitored for the mobile phase. In compliance with ICH guidelines, the method was validated. Ulipristal was eluted in this technique with a retention time of 1.516 minutes. The limit of detection was 0.075 μg/ml and the limit of quantification was found 0.25 μg/mL. The calibration curve plot was found linear over the concentration ranges 5-150 μg/mL, with the regression coefficient (R²) of 0.990. The % assay of the marketed dosage form was found at 97.40 %, which is within the acceptance level. The present method of ulipristal force degradation study was performed as per the guidelines. The results of the force degradation study show the highest 11.39% degradation in an acidic stressed condition, in comparison to alkaline, peroxide, thermal and photolytic stressed conditions. The experiential evidence of all the study results revealed the suitability of the quantification of ulipristal in API and tablet formulation routinely.

INTRODUCTION

Ulipristal, which is used as an emergency contraceptive, is a selective progesterone receptor modulator. As a contraceptive drug, a progestin and a modulator of progesterone receptors, it has a role. It is a steroid of 3-oxo-Delta (4), a steroid ester; an acetate ester; a steroid of 20-oxo, and a tertiary amino compound (Marci et al., 2014) (Figure 1).

Ulipristal binds to the progesterone receptor (PR), thus inhibiting gene expression mediated by PR and intervening in the reproductive system with progesterone activity. This agent can also suppress the growth of uterine leiomyomatosis as a result (Puchar et al., 2015). Ulipristal is also used in the treatment of uterine fibroids, also known as myomas, which are non-cancerous womb growths (uterus). Sometimes they can cause heavy or painful cycles, swelling of the tummy and urinary problems (Rabe et al., 2013; Lyseng-Williamson and Croxtall, 2012). Ulipristal is considered an important molecule for the treatment of the above-mentioned conditions and not included in any pharmacopoeia. Therefore the determination of ulipristal with a suitable analytical method is important.

The literature review on the available HPLC method for ulipristal reveals that there is an existence of two reported methods. The reported methods have their own limitations as follows, In one reported
method (Shi et al., 2014), the reported retention time of 3.04 minute with a narrow linearity range of 20-50 μg/mL and used acetonitrile (80%) as a part of the mobile phase, which is generally considered as a costly solvent. In another reported method (Rao et al., 2019) also utilized costly acetonitrile and orthophosphoric acid as a mobile phase. Whereas they also utilized acetonitrile and water as a diluent. Overall this method can be considered as cumbersome. Therefore keeping all facts in mind the efforts were taken to eradicate all the above disadvantages from the reported methods and to develop a reliable, fast method for the estimation of ulipristal using HPLC technique and validate the method as per the requirements of ICH guidelines (Mondal et al., 2014; Harikrishnan et al., 2020).

In this article, the development of a novel method has been represented with several validation parameters and can be utilized for the routine estimation of ulipristal in bulk as well in the marketed dosage form.

MATERIALS AND METHODS

Chemicals and Reagents

The present working standards Ulipristal (99.98%) was procured from the Akumentis Healthcare Ltd, Thane, Maharashtra, India. The tablets (Fibristal tablet, manufactured by Akumentis Healthcare Ltd) were purchased from the local market of Hyderabad, India. All required chemicals and reagents were purchased from Finer chemical Ltd, Fisher Scientific and Merck.

Instrumentation Conditions

The ulipristal was analysed using High-performance liquid chromatography (HPLC) Acquity Waters, PDA detector with software empower 2, equipped with an auto sampler and PDA detector. The analytical column Symmetry, C18, 250mm x 4.6mm. i.d., 5mm Particle size, with the flow rate 0.4 ml/min (isocratic) was utilised. The analytical balance 0.1mg sensitivity (Af_coset ER-200A), pH meter (Adwa – AD 1020).

Standard ulipristal preparation for the analysis

50 mg of ulipristal standard was transferred into 50 ml volumetric flask, dissolved & makeup to volume with the mobile phase. Further dilution was done by transferring 0.2 ml of the above solution into a 10ml volumetric flask and makeup to volume with mobile phase to achieve the final concentrations of 20 μg/mL.

Preparation of 0.05M Phosphate buffer Solution

About 6.8043 grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000 ml beaker, dissolved and diluted to 1000ml with HPLC water. The pH was adjusted to 4.6 with orthophosphoric acid.

Preparation of Mobile Phase

The mobile phase, which is utilised for the analysis of ulipristal, is a mixture of phosphate buffer (pH 4.6 with orthophosphoric acid) and methanol in the volume ratio of 20:80. 800 ml (80%) of Methanol and 200 ml (20%) of the phosphate buffer was well combined and degasified for 15 minutes in an ultrasonic water bath. The solution was filtered under vacuum filtration through a 0.45 μm filter.

Assay of ulipristal marketed formulation

Twenty Fibristal tablets (each tablet 5mg ulipristal) from Akumentis Healthcare Ltd, Thane, Maharashtra, India, were measured correctly and powdered finely in a glass mortar. A 100 ml standard flask was transferred to a weight equal to 100 mg ulipristal. For a duration of 10 min, 10 ml of methanol was added and gently swirled. The clear solution was then filtered and transferred through a Whatmann No 1 filter paper into the flask, and up to 100 ml of diluent (Methanol: phosphate buffer; 80:20) volume was formed. There was a concentration of 1mg/ml in the resulting solution. To obtain the final concentration of 100 μg/ml of ulipristal, precisely pipette 1 ml of the above solution into a 100 ml regular flask and make it up to a volume of diluent. Further pipette out 1ml from the 100μg/ml solution and transferred into 10 ml standard flask and the volume was made up to the mark using a diluent to achieve the concentration of 10μg/ml of ulipristal. The prepared latest solution was injected into the chromatographic system. The chromatogram was recorded.

Method validation

System suitability

It was conducted (Mondal et al., 2013) to legitimize whether the analytical framework is operating correctly during the method production of ulipristal. The injection of six replicates of the regular solution of ulipristal was performed. The percent RSD of a set of optimized parameters was measured, such as peak area, theoretical plates, retention time and asymmetric factor.

Specificity

Using 20 mg of a placebo, which is equal to one ulipristal tablet dissolved in 100 ml of the mobile phase. The interference of the placebo test of the ulipristal sample solution was conducted. Then the solution (placebo) was handled as a normal solution. To determine the potential interfering peaks,
the solution was injected into the chromatographic device.

**Accuracy**

Recovery analysis was performed (Mondal et al., 2019) at different levels (80 %, 100 % and 120 %) of pure ulipristal to justify the accuracy of the established method. In order to achieve the different quantities, different amounts of standard ulipristal were incorporated to a set concentration in the ulipristal tablet sample solution. This study was performed three times, and the percentage of recovery and the percentage of mean recovery was calculated. The percent recovery should be between 98.0 per cent to 102.0 per cent for each point.

**Precision**

This study of the present developed method of ulipristal was studied by determining the ulipristal sample solution. It was evaluated by analyzing the six ulipristal sample solutions in triplicate (n=6). Intraday and inter-day precision were resolute by analysing six times in three different concentrations of ulipristal, i.e 20 µg/ml, 40 µg/ml and 60 µg/ml.

The chromatograms were recorded. The peak area and the retention time of the ulipristal was determined and the relative standard deviation (RSD) was calculated. The percent RSD should not be more than 2 percent for the region of six standard injections performance.

**Linearity**

An aliquot from this solution was diluted with mobile phase in five different concentrations to 5-150 µg/ml of ulipristal to execute the standard linearity solution of ulipristal as stated earlier. The calibration curve was plotted for the ulipristal was subjected to regression analysis. In the defined range, the relationship between the concentration and peak area should be linear and the coefficient of correlation should not be less than 0.99.

**Limit of detection (LOD)**

Ulipristal solution with a final concentration of 0.075 µg/ml was prepared from the stock solution for this analysis. The prepared solution was filtered and injected into the chromatographic device. The signal to noise (S/N ratio) value for the LOD solution must be 3. The LOD solution of ulipristal was prepared and injected three times and the area measured for all three HPLC injections was measured. The percent RSD for the six replicate injections region was found to be within limits defined.

**Limit of quantitation (LOQ)**

This study involved the preparation of 0.25 µg/ml of ulipristal concentration that was prepared from the series of dilution from the standard ulipristal solution. The prepared sample was transferred into the chromatographic device and injected. The signal to noise (S/N ratio) value for the LOQ solution must be 10. The LOQ solutions were prepared for three injections and calculated the area for all three HPLC injections. The percent RSD for the six replicate injections region was found to be within limits defined.

**Robustness**

This feature is used to determine the ability of the developed method to remain unaffected by small, deliberate adjustments in the method’s parameters and provides a measure of its reliability during regular use. The flow rate, the detection wavelength, and the composition of the organic solvent were deliberately modified from the normal chromatographic conditions by injecting the ulipristal working standard solution into the HPLC system.

**Force degradation study of ulipristal**

Stress testing was carried out in the environmental test chamber (Acamus Technologies, India) at 60°C and 75 percent relative humidity (Shobharani et al., 2014), as prescribed by ICH stress conditions such as acidic, oxidative, alkaline, photolytic and thermal stresses.

**Acid hydrolysis**

Forced degradation in acidic media was achieved by adding 1 ml of 1 M HCl to 1 ml of ulipristal stock solution and the mixture was heated for approximately 2 hours at 80°C and the solution was neutralized by adding 1 M NaOH and held sideways for 24 hours and injected.

**Alkaline hydrolysis**

Forced degradation of basic media was achieved by adding 1 ml 1 M NaOH to 1 mL of stock solution and the mixture is heated for about 2 hours at 80°C and the solution is neutralized by including 1 M HCl and held sideways for 24 hours. It is injected with the prepared solution and chromatograms are recorded.

**Oxidative Degradation**

In a clean and dry 100 ml volumetric flask, 10 mg of pure ulipristal was accurately weighed. To make it soluble, 30 ml of 3% H2O2 and a little methanol were added to it and then held as such for 24 hrs in darkness.

A final volume of up to 100 ml was produced. Using water to prepare 10 µg/ml of solution and finally dilute it to 10 mL. The above sample was injected against a mobile phase blank in the HPLC method and a chromatogram was obtained.
Photolytic degradation

In a clean and dry Petri dish, about 10 mg of pure ulipristal was taken. It was held without interruption in a UV cabinet at a wavelength of 254 nm for 24 hours. 1 mg of the UV-exposed drug was accurately weighed and transferred to a clean and dry 10 ml volumetric flask. The UV light exposed analyte was first dissolved in methanol and made up to the mobile phase mark and eventually diluted to 10 mL to prepare a solution of 10 μg/ml. This solution was injected into the HPLC device against a mobile phase blank and obtained a chromatogram.

Thermal degradation

10 mg of pure analyte was correctly measured and transferred to a clean and dry round bottom flask. There was 30 ml of HPLC water applied to it. It was then refluxed uninterruptedly in a water bath at 60 °C for 6 hrs. The mixture of the medication and water was allowed to cool to room temperature after the reflux was over. Up to 100 ml of the final volume was produced and finally diluted to 10 mL to prepare 10 μg/ml of solution. A blank solution was inserted into the HPLC device against it.

RESULTS AND DISCUSSION

Method development

In the API and tablet formulation of ulipristal, different HPLC chromatographic conditions were hoped to obtain an optimized method for ulipristal estimation. During the original trials, many parameters such as column type, mobile phase composition, mobile phase pH and diluents were ranged. In order to achieve the desired composition of the mobile process for system optimization, different proportions of solvents, the buffer, have been tested. Finally, with the mobile phase phosphate buffer, pH 4.6 and methanol as a mobile phase with a volume ratio of 20:80 with 1 mL/min, flow rate. Ulipristal was eluted with high-quality peak shape and low retention time. The retention time of 1.516 minutes was observed with the detection at 304 nm for the ulipristal. The developed method was validated in accordance with ICH guidelines. Figure 2 displays an optimized chromatogram.

Method validation

The developed, optimized condition was utilized for the study of validation parameters for ulipristal. The assay result of the ulipristal marketed tablet dosage form of ulipristal shows the percent purity of 97.40%, the obtained chromatogram was depicted in Figure 3 and the result was shown in Table 1.

A percentage assay results in the marketed ulipristal tablet, suggesting the appropriateness of the present developed method for the analysis of ulipristal in the tablet dosage form.

The results of the specificity study, it was observed that no peaks of excipients were detected at the retention time of the ulipristal and supported the method’s specificity. A system suitability study was performed to confirm the satisfactory operation of the equipment used for analytical measurements. The percentage RSD of several criteria have been
Table 1: Assay of ulipristal marketed formulations

| Ulipristal marketed formulation | Labeled claimed | Amount obtained* | Percentage purity of ulipristal* |
|---------------------------------|-----------------|------------------|----------------------------------|
| Fibristal tablets (each tablet 5mg ulipristal) from Akumentis Health-care Ltd, Thane. | 5 mg | 4.87 mg | 97.40% |

*average of three replicates

Table 2: Summarized results of the validation parameters

| Parameters                        | Ulipristal |
|-----------------------------------|------------|
| LOD mg/ml                         | 0.075      |
| LOQ mg/ml                         | 0.25       |
| Linearity range (mg/ml)           | 5-150      |
| Regression co-efficient           | 0.990      |
| % *Mean recovery (accuracy)       | 98.84      |
| Intraday precision** (% RSD)      | 0.47       |
| Inter-day precision** (% RSD)     | 0.63       |
| % RSD of tailing factor* (robustness study) | 0.43 |

*Average of three replicates.  ** Average of six replicates
considered such as peak area, tailing factors, theoretical plates and retention time, and found 0.08, 0.51, 3.80 and 1.33. The results of accuracy as a mean % recovery was found 98.84, and the % RSD was not more than 2 % as shown in Table 2.

The percent recovery was found as a result of accuracy within the appropriate range, i.e. within 95-105 percent as shown in the outcome, supporting the accuracy of the established process. The % RSD of the intra and inter-day precision study was found 0.47 and 0.63. The results of the precision study were also revealed in the table of validation parameters and the results of the precision analysis showed that the new methodology was found to be specific.

The linearity study was performed in the range of concentrations 5-150 \( \mu \text{g/ml} \), and the correlation coefficient was obtained is 0.990 for the ulipristal. The linearity overlay chromatogram, as shown in Figure 4. The obtained linear range indicated the wide determination region of the ulipristal with the required precision in the linearity analysis of the developed method, and the correlation coefficient for the ulipristal was found to be 0.990, indicating its defined linearity.

Robustness study of the developed ulipristal HPLC method was carried out by changing the parameters from the optimized chromatographic conditions such as changes in mobile phase composition (±2%), detection wavelength (±2 nm), changes in flow rate (±0.1ml/min). The % RSD of the tailing factor, which was considered as a parameter was established at 0.43 as shown in the validation parameters table. The % RSD of the tailing factor was found less than 2, which authorize the robustness of the developed method since there was no significant changes were observed on the deliberate changes in the optimized parameters.

The sensitivity of the method developed has been demonstrated by the detection limit and the quantitation limit. Concentrations of detection and quantitation found for ulipristal at a very low limit. The detection and quantitation limit of ulipristal were found to 0.075 \( \mu \text{g/ml} \) and 0.25 \( \mu \text{g/ml} \), respectively. Degradation studies of ulipristal were performed under the influence of several stressed conditions such as acid, alkali, oxidation, thermal, photolytic conditions.

Degradation was found in all stressed condition except photolytic influence. The acidic stressed condition shows the degradation of 11.39%; alkaline stress condition shows 9.48 %, peroxide condition shows 6.22%. The thermal degradation shows 1.28 % of degradation respectively. Table 3 revealed the detailed results and Figure 5 showed the chromatograms.

Results of the degradation analysis of ulipristal showed that stressed conditions of acid, alkaline and peroxide contribute to further degradation compared to other stressed conditions. Thermal degradation results in few degradations, while no degradation has been identified in photolytic conditions. The chromatograms of the ulipristal were found very particular in all stressed circumstances.

**CONCLUSION**

Focused on the experiential evidence of the present established ulipristal method, authors are strongly declaring the method’s novelty over the very few methods available. The new HPLC approach is ‘rapid’ since within 1.516 minutes, it dramatically reduced the total analysis time, which considers the lowest analysis time needed for ulipristal analysis. The present method considered “stability indicating” because under stressed conditions, not as much degradation was observed and excellent separation of ulipristal among the other degraded peaks was also observed. According to the acceptance criteria of ICH Q2B guidelines, the results of the validation parameters were noted and found. Therefore, the present method developed can be used for the routine analytical and quality control assay of ulipristal in bulk as well as tablet dosage form as a novel, accurate, validated method of ulipristal.

| Sample Name (degradation types) | Mean Area* | % Assay | % degradation |
|-------------------------------|------------|---------|---------------|
| Acid                          | 100351     | 88.61   | 11.39         |
| Base                          | 103654     | 90.50   | 9.48          |
| Peroxide                      | 105451     | 93.78   | 6.22          |
| Photolytic                    | 132089     | 99.46   | 0.64          |
| Thermal                       | 120145     | 98.72   | 1.28          |

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**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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