ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

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# Ulipristal acetate determination using MBTH

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*Article History:*

Received on: 08.07.2019
Revised on: 15.10.2019
Accepted on: 21.10.2019

*Keywords:*

Ulipristal acetate,
MBTH,
Oxidative coupling,
Method development,
Validation

ABSTRACT

A simple visible spectrophotometric method is proposed for the determination of ulipristal acetate present in bulk and tablet formulation. The currently proposed method is established based on MBTH oxidation by ferric ions to form an active coupling species (electrophile), followed by its coupling with the ulipristal in acidic medium to form high intensified green colored chromophore having $\lambda$max at 609 nm. Validated the method as per the current guidelines of ICH. Beer's law was obeyed in the concentration range of 6.25 – 37.50 $\mu$g mL$^{-1}$ with a high regression coefficient (r > 0.999). Reproducibility, accuracy, and precision of the method are evident from the low values of R.S.D. This method can be used in quality control laboratories for routine analysis of ulipristal acetate in bulk drug and pharmaceutical dosage forms.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i4.1646

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## INTRODUCTION

Ulipristal acetate (UPA) delays the ovulation process approximately for 5 days and hence is useful to prevent inadvertent pregnancy. Tissue selective mixed progesterone agonist is exerted by it. It also exerts antagonist effects in endometrial tissue and myometrial (Attardi *et al.*, 2004). The P4 activity in target tissues is blocked due to its selective progesterone receptor modulating activity. Its oral bioavailability is good. Fibroids management is possible by the administration of a single oral dosage per day due to its good half-life (Pohl *et al.*, 2015). Its initial development was done by NICHD, USA, and later stage by HRA Pharma (Attardi *et al.*, 2004; Gainer and Ulmann, 2003). Its development was originally aimed at gynecological applications. CDB/VA-2914 is its other name. With the trade name Ella, UPA got FDA's approval in 2010 to use it as an emergency contraceptive (Fine *et al.*, 2010). Esmya® was the trade name product from Gedeon Richter (UK) Ltd. Approval was granted for it in EU for alternating treatment of uterine fibroids symptoms (European Medicines Agency, 2016; Garnock-Jones and Duggan, 2017). It has a steroidal structure (Figure 1). It has free solubility nature in solvents like $CHCl_3$, $CH_3OH$, and $CH_3CN$, but in water has sparingly soluble nature (Prajapati, 2015). Different methods were proposed for the determination of UPA by using UV (Prajapati, 2015), HPLC-gradient (Béni *et al.*, 2014), HPLC-isocratic (Gong and Zhu, 2015) and LC-MS/MS (Pappula *et al.*, 2017; Nandakumar *et al.*, 2017). But no visible spectrophotometric method was reported. Hence, a method is proposed using MBTH as a coupling agent and then validated for its applicability in routine analysis.

## MATERIALS AND METHODS

### Preparation of reagents

### Preparation of standard drug solution

The standard drug of ulipristal acetate (50 mg) was weighed accurately and transferred to a 100 ml volumetric flask. It was dissolved properly and diluted up to the mark with methanol to obtain a final concentration of 500 $\mu$g/ml (stock solution).

### Preparation of reagents

1 M HCl was used to prepare ferric chloride (3% w/v) solution. Distilled water and methanol were used respectively to prepared MBTH (0.5% w/v) and ulipristal solutions (standard stock and working).

### Instrumentation

Analytical grade chemicals were used throughout the study, and solutions were prepared using distilled water. A double beam spectrophotometer (Shimadzu UV-1700) was used along with Shimadzu UV-Probe 2.10 software. Standard quartz cuvettes were used for analysis.

## RESULTS AND DISCUSSION

### Absorption Spectrum of Coloured Complex

Oxidation (Gorumutchu *et al.*, 2019b; Gorumutchu and Nadh, 2018; Gorumutchu and Ratnakaram, 2019a) and ion-pair formation (Gorumutchu *et al.*, 2018, 2019a; Gorumutchu and Ratnakaram, 2019b) reactions are the preferred in the visible spectrophotometric estimation of drugs. MBTH involved oxidative coupling is used in the present method to form a chromophore. A characteristic absorption maximum was observed at 609 nm for the developed chromophore in the determination of ulipristal by visible spectrophotometry (Figure 2).

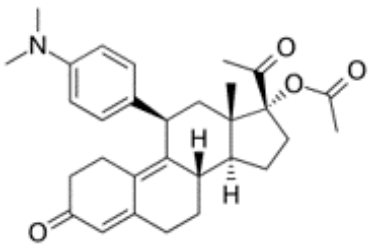

**Figure 1: Chemical structure of ulipristal Acetate**

### Reaction Conditions and their Optimization

The univariate approach was followed to optimize the parameters one-by-one in which one parameter conditions are varied while maintaining the other conditions at constant. Optimized the reaction conditions to form a colored solution with the highest absorbance. Researchers carried out quantitative estimation based on oxidative coupling nature of MBTH in acidic (Ramachandra and Naidu, 2017; Hadi and Mouayed, 2017; Reddy *et al.*, 2016; Kumar *et al.*, 2015; Varsha *et al.*, 2015; Sudhir *et al.*, 2013, 2019), neutral (Sastry and Rao, 1989) and basic media (Pospíšilová *et al.*, 1998, 1990). But, complicated reactions were reported in an alkaline medium like precipitation of by-products and promoted oxidation of electrophile (II) (Tharpa *et al.*, 2010). Taking into consideration of these, reactions were carried out in acidic medium by many researchers (Pospíšilová *et al.*, 1998) and hence adopted in the present case. The addition of water-

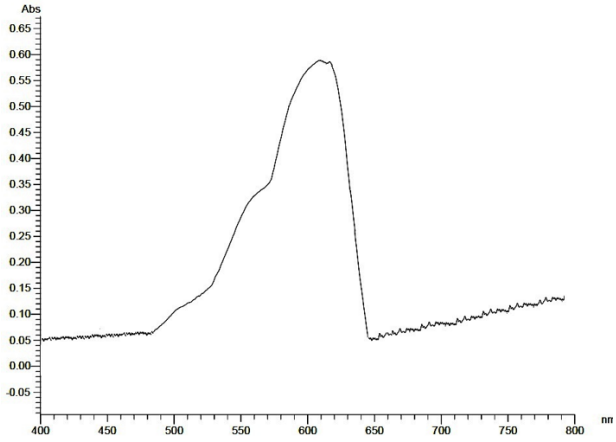


**Figure 2: Visible spectrum of Ulipristal-MBTH complex**

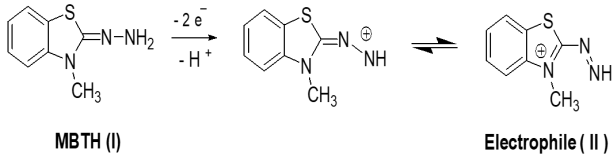


**Figure 3: Formation of electrophile ($E^+$)**

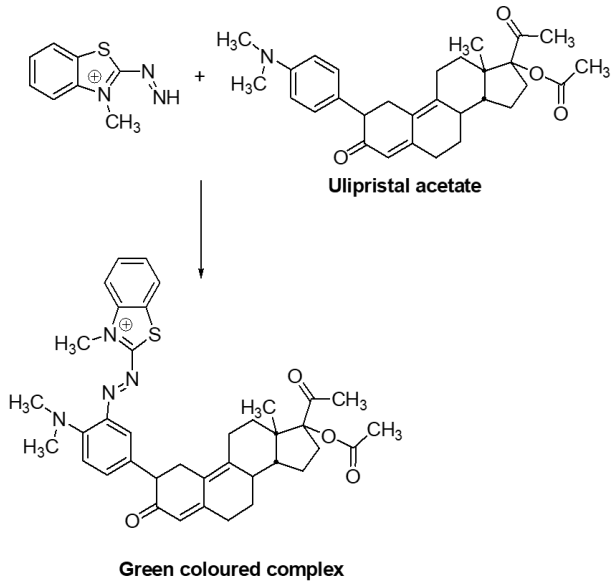


**Figure 4: Coloured complex formation between ulipristal and electrophile ($E^+$)**

**Table 1: Calibration curve values**

| Concentration ($\mu$g mL$^{-1}$) | Absorbance* |
|---|---|
| 6.25 | 0.1587 |
| 12.50 | 0.3024 |
| 18.75 | 0.4522 |
| 25.00 | 0.6012 |
| 31.25 | 0.7584 |
| 37.50 | 0.9044 |

* Average of three determinations

**Table 2: Key parameters of method development & validation**

| S. No. | Parameter | Observation |
|---|---|---|
| | Optical characteristics | |
| 1. | Apparent molar absorptivity (l mol$^{-1}$ cm$^{-1}$) | 1.2 $\times 10^4$ |
| 2. | Sandell's sensitivity ($\mu$g cm$^{-2}$A$^{-1}$) | 0.041 |
| | Regression analysis | |
| 1. | Slope | 0.024 |
| 2. | Intercept | 0.005 |
| 3. | Regression coefficient (r) | 0.9999 |
| | Validation parameters | |
| 1. | $\lambda$ max (nm) | 609 |
| 2. | Beer's Law Limit (Linearity, $\mu$g mL$^{-1}$) | 6.25 – 37.50 |
| 3. | Limit of detection ($\mu$g mL$^{-1}$) | 0.10 |
| 4. | Limit of quantitation ($\mu$g mL$^{-1}$) | 0.33 |
| 5 | Minimum stability period (hours) | 4 |

**Table 3: Recovery of Ulipristal Acetate**

| Level of recovery (%) | Amount of drug recovered ($\mu$g mL$^{-1}$) (Practical) | Statistical evaluation | | % Recovery = Practical x 100/ Theoretical |
|---|---|---|---|---|
| 50 | 18.74 | Mean | 18.74 | 99.95 |
| | 18.73 | SD | 0.012 | 99.89 |
| | 18.76 | %RSD | 0.067 | 100.05 |
| 100 | 25.01 | Mean | 24.99 | 100.04 |
| | 24.99 | SD | 0.012 | 99.96 |
| | 24.98 | %RSD | 0.050 | 99.92 |
| 150 | 31.23 | Mean | 31.24 | 99.94 |
| | 31.26 | SD | 0.012 | 100.03 |
| | 31.24 | %RSD | 0.040 | 99.97 |

1. Nominal concentration used (a): 12.50 $\mu$g mL$^{-1}$
2. Amount of drug added (b): 6.25,12.50 and 18.75 $\mu$g mL$^{-1}$ respectively for 50%, 100% and 150% recovery levels
3. Theoretical amount: Total amount of drug (a + b) = 18.75, 25.00, 31.25 $\mu$g mL$^{-1}$respectively for 50%, 100% and 150% recovery levels

**Table 4: Precision studies**

| Concentration of Drug ($\mu$g mL$^{-1}$) | Concentration* | | | |
|---|---|---|---|---|
| | Intraday (Mean ± SD) ($\mu$g mL$^{-1}$) | % RSD | Inter-day (Mean ± SD) ($\mu$g mL$^{-1}$) | % RSD |
| 6.25 | 6.251±0.0012 | 0.019 | 6.252±0.0022 | 0.035 |
| 18.75 | 18.752±0.005 | 0.027 | 18.758±0.032 | 0.171 |
| 37.50 | 37.504±0.008 | 0.021 | 37.511±0.029 | 0.077 |

* Average of six determinations

**Table 5: Method ruggedness**

| Test Concentration of Drug ($\mu$g mL$^{-1}$) | Concentration* Analyst change | |
|---|---|---|
| | Mean ± SD ($\mu$g mL$^{-1}$) | % RSD |
| 6.25 | 6.251±0.001 | 0.016 |
| 18.75 | 18.752±0.041 | 0.219 |
| 37.50 | 37.501±0.036 | 0.096 |

* Average of six determinations

**Table 6: Assay of Pharmaceutical Formulation**

| Formulation | Labeled amount (mg) | Amount found* (mg) | % Drug Recovered | %RSD |
|---|---|---|---|---|
| Esmya® | 5 | 5.0314±0.0005 | 100.63 | 0.010 |

* Average of three determinations

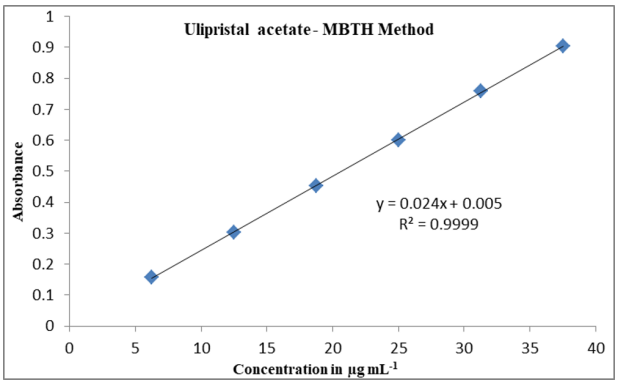


**Figure 5: Calibration graph of UA-MBTH chromophore**

miscible organic solvents (minimum of 30% v/v) was suggested to improve the solubility and stability of products (Pospíšilová *et al.*, 1990). However, no such requirement was found in the present case. Water was the diluting solvent, and the ambient temperature was maintained. The best suitable oxidant was ferric chloride. The optimized procedure is as follows. 3 mL $FeCl_3$ solution was added to all the flasks comprising an aliquot of standard working solution of ulipristal (100 $\mu$g mL$^{-1}$). Then 3 mL of MBTH solution was added. Intermittent stirring was done for 15 min. Then made up to the mark using distilled water in a 10 mL volumetric flask.

**Chromophore Formation and Chemistry**

MBTH is one of the popular analytical reagent for the determination of a spectrum of organic compounds, ozone, enzymes etc (Siah *et al.*, 2017; Hadi and Mouayed, 2017; Anthon and Barrett, 2002; Setti *et al.*, 1998; Furnival *et al.*, 1983; Wychen *et al.*, 2017). In addition, it is a widely used oxidative coupling agent to determine phenolic / nitrogen-containing organic compounds or pharmaceutical drugs, holding those functional groups in structures (Tharpa *et al.*, 2010). Further literature shows that spectrophotometric determination of many pharmaceutical compounds bearing amine group is carried out using MBTH as a chromogenic agent (Giri *et al.*, 2019b,a; Kumar *et al.*, 2013, 2014) in presence of an oxidant.

In this study, an electrophilic intermediate (II) is formed in the acid medium by the oxidation of MBTH (I) using ferric ions. It involves two electrons loss and one deprotonation (Figure 3). So

formed electrophile (II) acts as a good active coupling agent (El-Yazbi *et al.*, 1993). Hence, a chromophore is formed by its electrophilic substitution on ulipristal. A green-colored oxidative-coupling product with high intensity was obtained by electrophilic substitution of Electrophile II on the most nucleophilic site of ulipristal acetate. Limiting the logarithmic method (Alarfaj *et al.*, 2009) helped to confirm the mono substitution of the electrophile on ulipristal with 1:1 stoichiometry (ulipristal: MBTH). Then the topic of discussion is a site of substitution on ulipristal. A carbon atom with high electron density is attacked by the electrophile. Based on the literature, it is clear that the best choice of coupling spot is a para position to –OH / $-NH_2$ group on an aromatic ring. However, if the para position is occupied, the next choice of electrophile substitution is o-position, having less steric hindrance (Sastry and Rao, 1989). Hence, the formation of chromophore takes place, as shown in Figure 4. As the para position is occupied, the substitution of electrophile takes place at the ortho position of ulipristal to form the chromophore

### Validation of Method

### Linearity and range

The calibration curve was found to be linear (Figure 5) for the noted optical density values of different concentrations (Table 1). The correlation coefficient for the linear regression equation (y = 0.024x + 0.005) was greater than 0.9999 (Figure 5), and hence, the linearity of the proposed analytical method was tested. Table 2 represents different optical and regression parameters.

### Accuracy

% recovery values are in the range of 99.89 – 100.05 (Table 3). Accuracy of the method is evident from small values of S.D. as well as %RSD.

### Precision

%RSD results of inter-day and intraday precision were observed in the range 0.019 - 0.027 and 0.035 – 0.171, respectively (Table 4), indicating the satisfactory precision of the method.

### Ruggedness

Confirmed the method ruggedness as the difference between two analyst values is insignificant (Table 5).

### Limits of d etection and quantification

Based on slope and SD values, LOD and LOQ values were determined (Giri *et al.*, 2019b; Sethi, 2001; ICH Guidelines, 2015) and, found to be 0.10 and 0.33 $\mu$g $mL^{-1}$ respectively.

### Analysis of Pharmaceutical Formulations

Spectrophotometry is the most opted method for analysis in developing countries (Kumar *et al.*, 2013; Sudhir *et al.*, 2013; Kumar *et al.*, 2014). API recovery values from the tablet formulation (Esmya®) are very good in the present study (Table 6). Hence, this method can be used for routine analysis.

## CONCLUSION

As the para position of ulipristal is occupied, the substitution of electrophile takes place at the ortho position to form the chromophore. This is a simple and straightforward method and can be used as an alternative to expensive methods.

## ACKNOWLEDGEMENT

Authors are thankful to Acharya Nagarjuna University and GITAM-Bengaluru Campus for providing facilities to carry out the present research work.

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