**ISSN 0974-3618 (Print)**
**0974-360X (Online)**

**www.rjptonline.org**

***RESEARCH ARTICLE***

# Extractive Spectrophotometric Determination of Ulipristal Acetate using Naphthol Blue Black

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## ABSTRACT:

Ulipristal acetate is used to treat uterine fibroids and for emergency birth control. The present study is a first report on development of a visible spectrophotometric method for determination of Ulipristal acetate present in bulk and tablet formulation. The method involves the sequential addition of HCl (0.1 N) and Napthol Blue Black solution to Ulipristal acetate. Cation formed on tertiary amine group of Ulipristal acetate attracts anion of naphthol blue black (an acid dye) to develop a coloured ion-association complex. From the aqueous phase, the chromophore is extractable into chloroform, which exhibits $\lambda_{max}$ at 640 nm. As per the existing guidelines of ICH, various parameters of the method were tested for validation. Regression analysis ($r > 0.999$) shows that the plotted calibration curve exhibits good linearity in the studied range of concentration (2.50 – 15.00 μg $mL^{-1}$). The % recovery values falls in 99.80 – 100.72 range. %RSD results of both precision studies were observed in the range 0.007 – 0.560, indicating the satisfactory precision of the method. Low values of R.S.D. (< 1 %) were observed indicating that the proposed method is reproducible, accurate and precise. The proposed method can be used in quality control laboratories for routine analysis of Ulipristal acetate (bulk drug and pharmaceutical dosage forms) without requirement of expensive instruments.

**KEYWORDS:** Ulipristal acetate, naphthol blue black, Ion-association complex, Method development, Validation.

## INTRODUCTION:

Ulipristal acetate (UPA) is also known as CDB/VA-2914. It prevent sunintentional pregnancy by adjourning ovulation for about5 days. It exerts tissue selective mixed progesterone agonist and antagonist effects in myometrial and endometrial tissue[1]. It is a selective progesterone receptor modulator that blocks the activity of P4 in target tissues. It has good oral bioavailability and a half-life allowing one single oral administration per day for the management of fibroids[2]. It was initially developed by the National Institutes of Child Health and Human Development (NICHD) and then by HRA pharma[1,3].

UPA was originally developed for gynecological applications. FDA's approval was granted for UPA in August 2010 in US for use as an emergency contraceptive with trade name Ella[4]. Gedeon Richter (UK) Ltd produces with the trade name Esmya®. It was also approved in the EU for the intermittent treatment of symptoms of uterine fibroids[5-6]. Chemically it is [17α-acetoxy-11β-(4-N,N-dimethyl aminophenyl)-19-norpregna-4,9-diene-3,20-dione] (Fig. 1) and having a steroidal structure. It is a white amorphous powder, sparingly soluble in water and freely soluble in methanol, acetonitrile and chloroform[7].

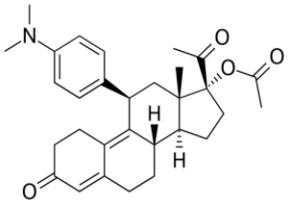


**Fig. 1: Chemical Structure of Ulipristal acetate**

Received on 18.11.2018 Modified on 07.12.2018
Accepted on 26.12.2018 
*Research J. Pharm. and Tech. 2019; 12(3): 1347-1352.*
**DOI: 10.5958/0974-360X.2019.00226.9**

A thorough literature survey shows that LC-MS/MS[8-9], HPLC-gradient[10], HPLC-isocratic[11] and UV[7] methods were proposed to determine of ulipristal. Therefore, the current study reports development and validation of a flexible extractive spectrophotometric method for the determination of ulipristal in bulk drug and tablet dosage formulations.

## MATERIALS AND METHODS:

Techomp (UV 2310) double beam UV-Visible Spectrophotometer with HITACHI software version 2.0 was used to measure the absorbance. Quartz cuvetts (10 mm path length) were used for the analysis. Digital pH meter (Elico LI-120) and balance (Shimadzu AUX-220) were used to weigh the samples and to measure pH respectively. Spectroscopic measurements were conducted at room temperature (25 ± 5°C). All chemicals used in the present study were AR grade. In the entire process, used water was double distilled.

### Preparation of reagents:

### Preparation of standard drug solution:

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

### Preparation of naphthol blue black (NBB):

200 mg of NBB was accurately weighed and dissolved in 100 mL of distilled water. Then impurities in NBB solution were removed by washing the dye solution with equal volume of chloroform and used the separated dye solution.

## RESULTS AND DISCUSSIONS:

An extractive-spectrophotometric method was used for the determination of Ulipristal acetate, an organic base. Exploitation of such reactions of naphthol blue black was a basis for estimation of different drugs containing amine group(s)[12-18]. The proposed method is centered on the reaction between organic base (Ulipristal acetate) and with an acidic dye (naphthol blue black) to produce an ion-association complex which can be extracted into organic solvent (chloroform)[19]. Then spectrophotometrically determined the separated chromophore.

### Absorption Spectrum of Coloured Complex:

A characteristic absorption maximum was observed at 640 nm for the developed chormophore in determination of Ulipristal acetate by visible spectrophotometry (Fig. 2).

### Optimization of Reactions Conditions:

To establish a rapid and quantitative development of a highly stable and sensitive ion-association complex, it is necessary to ascertain the optimum conditions.

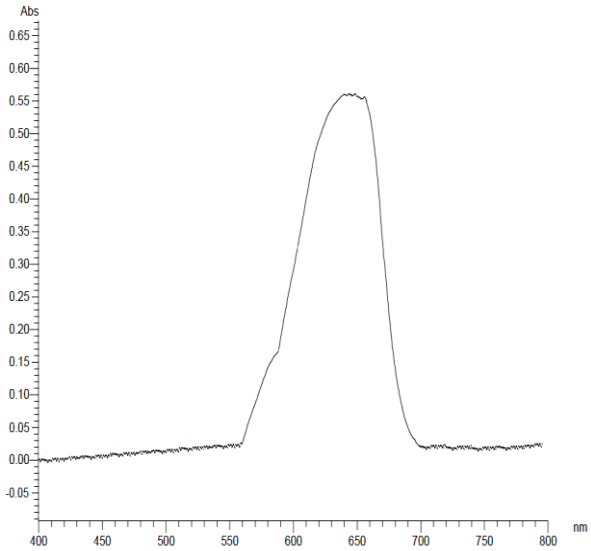
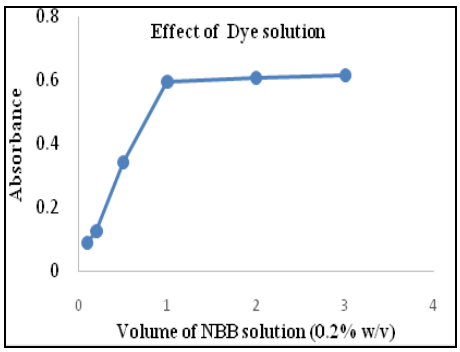


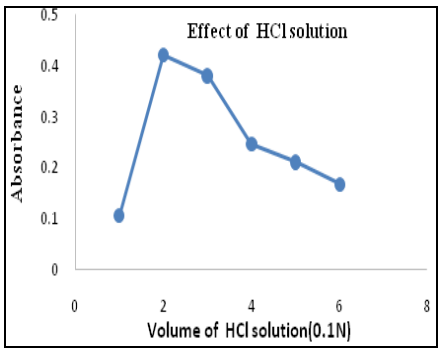

**Fig. 2. Visible spectrum of UPA-NBB ion-association complex**

Reaction conditions affecting the development, sensitivity and stability of colored product are volume/concentration of solutions (NBB, acid) and extracting solvent, time for the formation and stability of coloured product, and temperature. Variation of reaction conditions was carried out to optimize. Colour species absorbance was measured in establishment of optimum values by changing the conditions of one parameter at a time and by maintaining fixed conditions for others.

Maximum absorption values were found with one ml of NBB solution (Fig 3.a). Absorbance increased up to 2 ml acid concentration and then decreased (Fig 3.a). Though acidic condition is necessary to create cationic ionic form of ulipristal, higher concentration of acid may result in suppression of acid dye hydrolysis which may hamper the formation of ionic pair. Different time intervals (1 to 10 min) were chosen to study the optimum time required for the formation at room temperature. Absorbance values show that maximum intensity of colour is achieved almost immediately and is stable almost upto two hours. Hence, a minimum period of two minutes was maintained as contact time between the reagents. The effect of sequence of addition of reagents on the formation of chromogen was studied. The observed absorbance values indicate that the sequence "ulipristal + acid + naphthol blue black" can be considered for addition of reactants and reagents. Effect of temperature on coloured complex stability was inspected at various temperatures and found that the absorbance values are reproducible in the range 20 to 35°C. But coloured solution was found to be unstable beyond that temperature. Hence, all the studies were carried out at room temperature. Chloroform was found to be the best suitable solvent for extraction of chromophore from aqueous solution (Fig 4).




a




b.

**Fig. 3. Optimization of volumes of (a) NBB and (b) HCl solution**

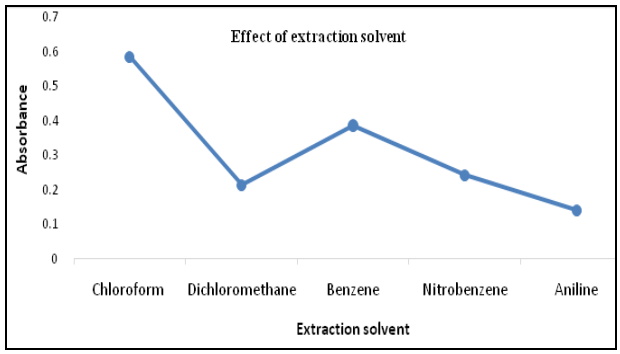



**Fig. 4. Optimization of solvent**

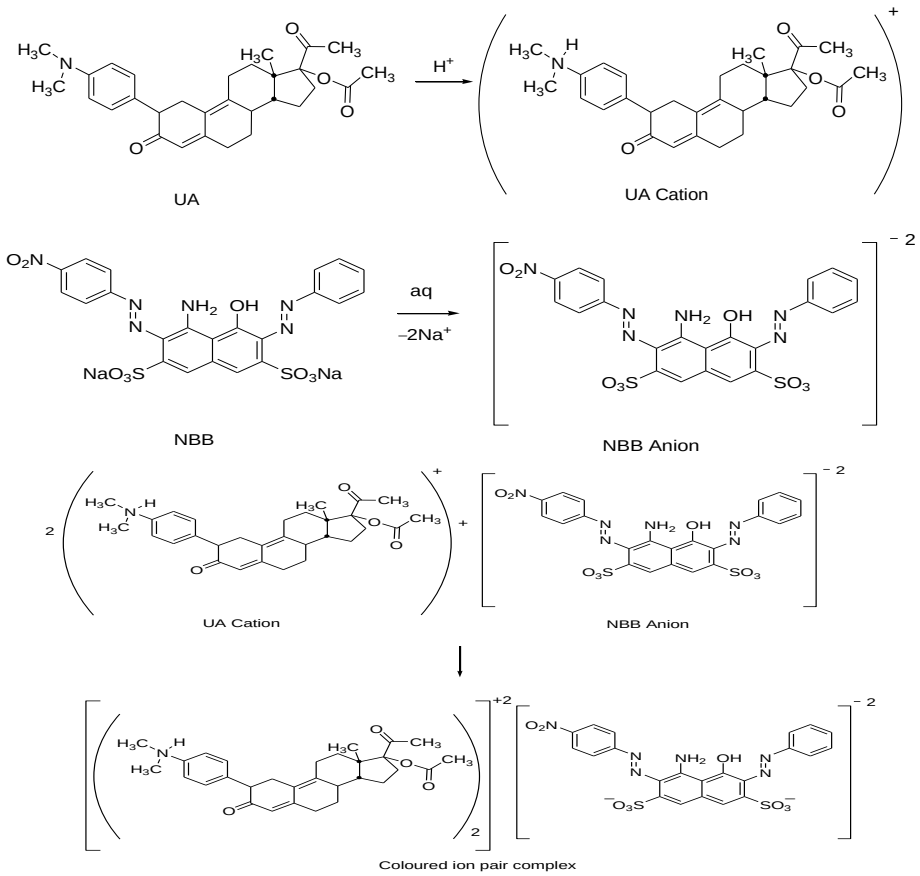



**Fig. 5. Reaction of Ulipristal acetate with naphthol blue black**

**Optimized method procedure:**

Aliquots of standard ulipristal solution were taken into a series of 100 mL separating funnels. 2 mL of HCl solution (0.1 N) and 1 mL of naphthol blue black were added successively. The total volume of the aqueous phase in each separating funnel was adjusted to 15 mL with distill water. To each separating funnel, 10 ml of Chloroform was added and the contents were shaken for 2 min. The two phases were allowed to separate. The separated chloroform layer was dried over anhydrous sodium sulfate. The absorbance of the blue colored ion-pair complex was measured at 640 nm against the reagent blank within the stability period.

**Chromophore Formation and Chemistry:**

The chemistry of the proposed analytical method is based on functional groups present in basic ulipristal acetate and acidic Naphthol blue black (NBB). A tertiary amine group is present in ulipristal acetate and considered as a good target. It forms a cation in acidic medium by protonation on the nitrogen of amine. Naphthol blue black (NBB) is an acid dye and two sulphonate groups present on it undergo hydrolysis in aqueous medium to give a dibasic anion. The oppositely charged ulipristal acetate cations (two in number) and NBB anion (one in number) are held together by electrostatic attraction between them to form 2:1 ion-association complex which acts as a single entity. The ion-association complex is coloured and is quantitatively extractable from aqueous to organic phase (chloroform) (**Fig. 5**). Based on the number of amine groups present on those pharmaceutical drug molecules, previous researchers reported the involvement of either 1:1 complex [12-16] or 2:1 complex [17-18].

**Validation of Method:**

**Linearity and range:**

Chromophore was developed for a sequence of ulipristal standard solutions (2.50 – 15.00 µg $mL^{-1}$) under the optimized experimental conditions. Each point on the calibration curve represents the average of three independent measurements of absorbance (Table 1). Least squares method was used to derive the regression equation for the measured absorbance values. Obtained a linear Beers law plot with a good correlation value (> 0.999) indicating a linear relationship between absorbance and concentration (Fig. 6). The corresponding linear regression equation is y = 0.0558x + 0.0077. Hence, the linearity of the proposed analytical method was tested. Table 2 represents different optical and regression parameters.

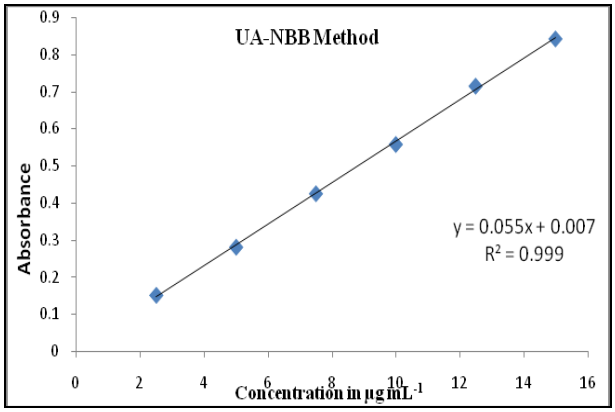



**Fig. 6. Calibration graph of Ulipristal acetate**

**Table 1. Calibration curve values**

| Concentration (µg $mL^{-1}$) | Absorbance* |
|---|---|
| 2.50 | 0.1524 |
| 5.00 | 0.2818 |
| 7.50 | 0.4258 |
| 10.00 | 0.5485 |
| 12.50 | 0.7154 |
| 15.00 | 0.8425 |
| * Average of three determinations | |

**Table 2.Key Parameters of Present Study**

| S. No. | Parameter | Observation |
|---|---|---|
| **Optical characteristics** | | |
| 1. | Apparent molar absorptivity (l $mol^{-1}$ $cm^{-1}$) | $2.71\times10^4$ |
| 2. | Sandell's sensitivity (µg $cm^{-2}A^{-1}$) | 0.0175 |
| **Regression analysis** | | |
| 1. | Slope | **0.0558** |
| 2. | Intercept | **0.0077** |
| 3. | Regression coefficient (*r*) | 0.9994 |
| **Validation parameters** | | |
| 1. | $\lambda_{max}$ | 640 nm |
| 2. | Beer's Law Limit (Linearity, µg $mL^{-1}$) | 2.50 – 15.00 |
| 3. | Limit of detection (µg $mL^{-1}$) | 0.12 |
| 4. | Limit of quantitation (µg $mL^{-1}$) | 0.58 |
| 5 | Stability period | 2 hours |

**Table 3. Recovery of Ulipristal Acetate**

| Level of recovery (%) | Amount of drug recovered (µg $mL^{-1}$) (Practical) | Statistical evaluation | | % Recovery |
|---|---|---|---|---|
| 50 | 7.55 | Mean | 7.55 | 100.67 |
| | 7.54 | SD | 0.008 | 100.53 |
| | 7.56 | %RSD | 0.108 | 100.80 |
| 100 | 9.98 | Mean | 9.99 | 99.80 |
| | 10.01 | SD | 0.012 | 100.10 |
| | 9.99 | %RSD | 0.124 | 99.90 |
| 150 | 12.58 | Mean | 12.58 | 100.64 |
| | 12.57 | SD | 0.008 | 100.56 |
| | 12.59 | %RSD | 0.065 | 100.72 |

% Recovery = Practical x 100/ Theoretical  Nominal concentration used (a): 5.00µg $mL^{-1}$

Amount of drug added (b): 2.5, 5 and 7.5 µg $mL^{-1}$ respectively for 50%, 100% and 150% recovery levels

Theoretical amount: Total amount of drug (a + b) = 7.50, 10.00, 12.50 µg $mL^{-1}$ respectively for 50%, 100% and 150% recovery levels

**Accuracy:**
Recovery studies confirmed the proposed method accuracy using standard additions method's. This was accomplished by the addition of various quantities (50% to 150%) of Ulipristal acetate bulk sample to fixed quantify (5.00µg $mL^{-1}$) in order to maintain the total amount of drug (theoretical) concentration within the linearity range. Table 3 shows that the % recovery values falls in 99.80–100.80 range (Table 3). Current method can be considered to be of highly accurate due to small values of %RSD as well as S.D.

**Precision:**
Inter-day and intraday precision were studied by selecting different three concentrations of Ulipristal acetate in the above selected range for linearity (2.50 – 15.00µg $mL^{-1}$). Analysis of each concentration (of six independent series) was carryout out on consecutive days (six in numbers) as well as on the same day (Table 4). %RSD results of both precision studies were observed in the range 0.080 - 0.139 and 0.120 – 0.560 respectively, indicating the satisfactory precision of the method.

**Table 4. Intraday and inter-day precision readings of the proposed method**

| Concentration of Drug (µg $mL^{-1}$) | Concentration* | | | |
|---|---|---|---|---|
| | Intraday (Mean ± SD) (µg $mL^{-1}$) | % RSD | Inter-day (Mean ± SD) (µg $mL^{-1}$) | % RSD |
| 2.50 | 2.501±0.002 | 0.080 | 2.500±0.014 | 0.560 |
| 10.00 | 10.054±0.014 | 0.139 | 10.021±0.026 | 0.259 |
| 15.00 | 15.027±0.018 | 0.120 | 15.012±0.018 | 0.120 |

***** Average of six determinations

**Ruggedness:**
Assay of different amounts of Ulipristal acetate (2.50, 10.00 and 15.00µg $mL^{-1}$) was carried out by two different analysts on different days under the above given method optimized conditions in order to appraise the ruggedness of the current developed method. Lack of significant difference in the values produced by different analysts indicates the evidence for reproducible results (Table 5). Hence, ruggedness of this method is confirmed.

**Table 5. Ruggedness data of Ulipristal acetateby two analysts at different days**

| Test Concentration of Drug (µg $mL^{-1}$) | Concentration* | |
|---|---|---|
| | Mean ± SD (µg $mL^{-1}$) | % RSD |
| 2.50 | 2.504±0.008 | 0.319 |
| 10.00 | 10.021±0.002 | 0.020 |
| 15.00 | 15.003±0.001 | 0.007 |

***** Average of six determinations

**Limits of detection and quantification:**
As per the ICH guidelines (2005), LOD and LOQ were calculated to determine the sensitivity of the proposed method using formulae (3.3×σ/S) and (10×σ/S) respectively [20-21], where S (calibration curve slope) and σ (S.D. of the response). The corresponding calculated values for Ulipristal acetate determination are given below.

LOD = 0.12µg $mL^{-1}$ and
LOQ = 0.40µg $mL^{-1}$

**Analysis of Pharmaceutical Formulations:**
Sonicated the tablet powder equivalent to 50 mg of tablet formulation (10 Esmya®tablets) with chloroform (3×25 mL). Then filtered the solution using 0.45 µm nylon membrane filter and cumulative chloroform extract was made upto 100 mL using the same solvent. Evaporated the solvent from this chloroform extract (20 mL) and then dissolved in minimum quantity of dilute HCl (0.1 N). Later diluted to 50 mL with distilled water. Further diluted with distilled water to obtain a standard solution of 100 µg/mL. To determine the amount of Ulipristal acetate present in the tablet formulations, the above suggested method was used (Table6) because the recovery values of the API is good. It indicates the non-interference to the above method from common excipients. In developing countries, the most opted analytical technique is spectrophotometry to carry out the routine analysis in QC laboratories of industries [22-26]. Hence, the above method which comprises naphthol blue black as a complexing agent can be applied to determine the quantity of Ulipristal acetate present in pure and tablet formulations.

**Table 6. Estimation of Ulipristal acetate in formulation**

| Tablet Formulation | Labeled Amount (g) | Amount found*(g) | % Drug Recovered | % RSD |
|---|---|---|---|---|
| Esmya® | 5 | 5.0008±0.0002 | 100.02 | 0.004 |

* Average of three determinations

## CONCLUSIONS:

The proposed method is the first visible spectrophotometric estimation of Ulipristal acetate in bulk drug form and tablet formulation. Formation of extractive ion-pair complex by the acid dye (Naphthol Blue Black) was used to develop a spectrophotometric method to quantify Ulipristal acetate. It exhibited reasonable precision and accuracy. For all the validation parameters, %RSD values were very small (< 1) and it indicates the validity of method. Moreover, it does not require costly and highly sophisticated instruments like HPLC and LCMS/MS.

Non-interference of excipients is evident from the good agreement between API recovered from tablet formulations and respective label claims. Hence, this simple method could be used for routine analysis in quality control laboratories.

**List of symbols and Abbreviations:**
S: Calibration curve slope
σ: Standard deviation of the response
R.S.D.: Relative Standard Deviation
HPLC: High Performance Liquid Chromatography
GC: Gas Chromatography
NBB: naphthol blue black
LOD: Limit of quantification
LOQ: Limit of quantification

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