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VALIDATED, VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF MIFEPRISTONE USING Cr (VI) AS AN ANALYTICAL REAGENT

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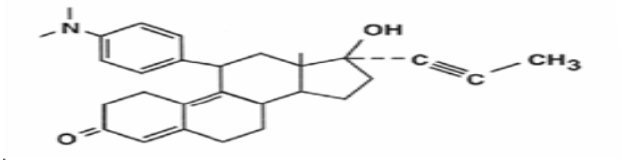
ABSTRACT

The present research article reports a validated visible spectrophotometric method for the estimation of mifepristone in commercial samples (tablets). The proposed method is based on the colour reaction between Cr(VI) and mifepristone in the pH range 1.0-5.0 forming a yellow coloured complex. This colour reaction is exploited for the development of a visible spectrophotometric procedure for the determination of mifepristone. The absorption spectrum of the complex shows a maximum absorbance at 430nm. pH 2.0 is selected for analytical studies. The absorbance of the complex varied linearly with the amount of mifepristone. The straight line relation between absorbance and amount of mifepristone obeys the equation A = 0.0080C- 0.0001. The linear plot indicates that Beer's law is obeyed in the range 10.0-100.0 µg/ml of mifepristone. The molar absorptivity and sandell's sensitivity are 3.558×10^3 l mol^{-1} cm^{-1} and 0.1207 µg cm^{-2} respectively. The standard deviation of the method for ten determinations of 30 µg/ml mifepristone is 0.0026. The correlation coefficient (γ) of the experimental data of the calibration plot is 0.9997. The proposed spectrophotometric method was validated according to ICH specifications such as, linearity, accuracy, precision, LOD, LOQ and ruggedness were studied. The method was applied for the assay of mifepristone in dosage forms.

Key Words:- Mifepristone, Cr(VI), Visible Spectrophotometry, Method validation.

INTRODUCTION

Mifepristone is a substituted 19-nor steroid compound chemically designated as 11β-[*p*-(Dimethylamino) phenyl]-17β-hydroxy-17-(1-propynyl) estra-4,9-dien-3-one. Its empirical formula is $C_{29}H_{35}NO_2$. Its structural formula is



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The compound is a yellow powder with a molecular weight of 429.6 and a melting point of 191-196°C. It is very soluble in methanol, chloroform and acetone and poorly soluble in water, hexane and isopropyl ether.

Mifepristone a clinically approved progesterone receptor antagonist effectively terminates pregnancy and offers therapeutic promise for endometriosis uterine fibroids and breast cancer. The clinical usefulness of mifepristone is potentially compromised due to over glucocorticoid receptor antagonism.

In early 1980s researchers at the pharmaceutical company Rochelle Velat (Paris, France) published the initial paper describing the antiprogestin mifepristone. Synthesis of this compound created a new field of interest

in reproductive endocrinology and made possible new approaches in the study of reproductive physiology. Use of hormones antogonists for medical and scientific purpose has increased since the introduction of these synthetic progesterone anti-hormone compounds. Since mifepristone is the first compound developed in the category of progesterone antogonists it has become the bench mark by which all other anti-progesterone are evaluated. Mifepristone has been used in a variety of species as a competitive inhibitor of progesterone, the principal hormone necessary for maintenance of pregnancy, there by terminating pregnancy.

The primary action of progesterone is to initiate and maintain pregnancy. During pregnancy, progesterone inhibits myometrial contractility and maintains the uterus in a quiescent state. Additional actions of progesterone include its facilitation of the LH surge and transformation of endometrium from a proliferative to a secretory state. Together with estradiol, progesterone also maintains endometrial integrity. It is the decrease of these steroids at the end of the luteal phase, which is responsible for menstrual bleeding.

Mifepristone pharmacokinetics is characterized by rapid absorption, a long half-life of 20-30 hours and high micromolar serum concentrations following injection of doses of >100mg of the drug. Because its metabolites, which still retain considerable affinity toward human progesterone and glucocorticoied receptors has the same biological actions as mifepristone itself, many recent clinical studies on pregnancy termination and emergency contraception have focused on the decrease of the dose of mifepristone form 200 – 600 mg to 2 – 100 mg.

Mifepristone also directly promotes uterine contractions. It also increases the myometrial response and sensitivity to exogenous prostaglandins, which further enhances uterine contractions. In addition, mifepristone induces the release of prostaglandins by decidual cells and pomotes an accumulation of prostaglandins by inhibiting their breakdown. Another important action of mifepristone is to dilate and soften the uterine cervix. Although mifepristone is predominantly a progesterone antagonist, under certain circumstances such as in estrogen-treated post-menopausal women, it may function as a progesterone agonist.

Mifepristone is determined voltametrically using DNA – modified carbon paste electrode by Kai Gu *et al.,* (Gu K *et al.,* 2000). A simple sensitive and validated HPLC method is developed for mifepristone determination in wild canid serum (Stith C, Delwar Hussain M, 2003) and Zhiyon Guo *et al*., developed a highly sensitive HPLC method for mifepristone determination in human plasma (Guo Z *et al*., 2006). A high performance liquid chromatographic method for the determination of mifepristone in human plasma is developed using norethisterone as an internal standard (Guo Z *et al*., 2007). Simultanous determination of mifepristone and monodimethyal miferpristone in human plasma by liquid chromatography –tandem mass spectrometry method[5] is reported (Cheng T *et al*., 2009). Zhiyong *et al*., reported a HPLC-UV method for the simultaneous determination of rivanol and mifepristone in human plasma with solid phase extraction (Zhiyong G *et al*., 2007). Devadusu *et al*., (2012) reported absorbance ratio method for the determination of mifepristone.

The above survey of literature shows no report of a direct visible spectrophotometric method for mifepristone. In continuation of our work on development of simple visible spectrophotometric methods for the assay drugs in pharmaceutical formulations, now we report simple visible spectrophotmetric procedure validated as per ICH guidelines for the determination of mifepristone (Reddy KNR *et al*., 2014).

MATERIALS AND METHODS

All chemicals and solvents used were of analytical reagent grade.

Solutions

Chromium (VI) solution

Requisite quantity of $K_2Cr_2O_7$ (GPR Merck) is dissolved in double distilled water and made up to the mark in a 100 ml volumetric flask to get 1.0 x 10^{-2} M stock solution of chromium (VI). The resulting solution is standardized (Marczenko Z, 1976).

Mifepristone Solution

100 mg of mifepristone is dissolved in ethanol made up to mark into a 100 ml volumetric flask. This solution is suitably diluted to get the required concentrations

Buffer solutions

Buffer solutions are prepared by adopting the standard procedures reported in the literature (Perrin DD and Dempsy B, 1978). The solutions employed for the preparation are given below.

pH	Constituents
0.5 – 3.0	1 M Sodium acetate + 1 M Hydrochloric acid
3.0 – 6.0	0.2 M Sodium acetate + 0.2 M Acetic acid
7.0	1.0 M Sodium acetate + 0.2 M Acetic acid
8.0 – 12.0	2.0 M Ammonia + 2.0 M ammonium chloride

Instruments employed

a) **UV-Visible recording spectrophotometer (UV – 160A)**

Shimadzo Corporation Spectrophotometric Instrument Plant, Analytical Instruments Division, Kyoto, Japan developed a versatile and indigenous microprocessor based UV-Visible recording spectrophotometer (UV-160A).

b) **ELICO digital pH meter**

ELICO digital pH meter manufactured by M/s ELICO Private Limited, Hyderabad, India is used for measuring the pH of buffer solutions. The instrument has a temperature compensate arrangement. The reproducibility of measurements is within ± 0.01 pH.

Procedure

A known number of tablets are weighed and ground to a fine powder. A portion of the powder containing 100 mg of the active component is accurately weighed into a 100 ml calibrated flask, 60ml of distilled water are added and shaken thoroughly for about 20 minutes to extract the drug. The contents are diluted to the mark, mixed well and filtered using quantitative filter paper to remove the insoluble residue. The filtrate is diluted to get required concentration of drug.

Absorption spectrum

The absorption spectra of the Cr(VI) solution and mifepristone solution in buffer solution of pH 2.0 and that of the experimental solution containing solutions of the Cr(VI) mifepristone and the buffer (pH 2.0) against the buffer blank are recorded in the wavelength range 300-650nm. The spectra are presented in fig.1

The spectra presented in fig.1 show that the complex has an absorption maximum at 430 nm. Neither Cr(VI) nor mifepristone have significant absorbance at 430 nm. Hence, analytical studies are made at 430 nm.

Assay of mifepristone

The present method for the determination mifepristone is applied for its determination in a pharmaceutical sample. A known aliquot of pharmaceutical sample solution of mifepristone is added to a 10ml volumetric flask containing 5 ml of buffer solution of pH 2.0 and 1ml of Cr(VI) [5 x 10^{-3}M] solution. The contents are made upto the mark with distilled water. The absorbance is measured at 430 nm against the Cr(VI) blank after heating the experimental solution to 60^0C for 30 minutes and cooling it to room temperature. The amount of mifepristone is computed from the predetermined calibration plot at 430 nm.

Effect of time on the absorbance of the experimental solution

The effect of time on the colour intensity of the experimental solution containing buffer solution of pH 2.0 ,Cr(VI) and mifepristone solution was studied by measuring its absorbance at 430nm at different time intervals. The studies revels that at room temperature the experimental solution attains maximum absorbance only after 25 hours of mixing the constituent solutions. Hence, the effect of increase in temperature on the absorbance of experimental solution is studied.

Effect of temperature on the absorbance of experimental solution

To determine at which temperature the experimental solution quickly attains maximum absorbance, experimental solution absorbance was measured at different temperatures. The results are presented in Table -1**.**The results in Table -1 indicate that the absorbance attains maximum value at 60^0 C. The absorbance is, therefore, measured after heating the experimental solution to 60^0C for 30 minutes and cooling it to room temperature

Effect of excipients

Various amounts of excipients that are generally associated with mifepristone in its pharmaceutical formulations are added to a fixed amount of mifepristone (10µg/ml) solution and the absorbance measurements are made under optimal conditions, The concentration (µg/ml) at which various excipients do not cause an error of more than ± 4% in absorbance of the complex solution is taken as the tolerance limit. The results are summarized in Table -2.

The data in Table- 2 reveal that various excipients that are associated with mifepristone in pharmaceutical formulations do not interfere when present even in large quantities in the determination of mifepristone making the method highly selective.

RESULTS AND DISCUSSION

Mifepristone reacts with Cr(VI) in the pH range 1.0-5.0 forming a yellow coloured complex solution. The absorption spectrum of the yellow colored Cr(VI) – Mifepristone complex shows (Fig-1) an absorption maximum at 430 nm. At this wavelength either Cr(VI) or mifepristone have no absorbance. The colour intensity of the complex is found to be maximum at pH 1.5-2.5 Hence studies were carried at pH 2.0, where the interference due to excipients or diverse ions is negligible. The color intensity attains a maximum after 30 minutes of mixing of various components at 60^OC. There after the color of the

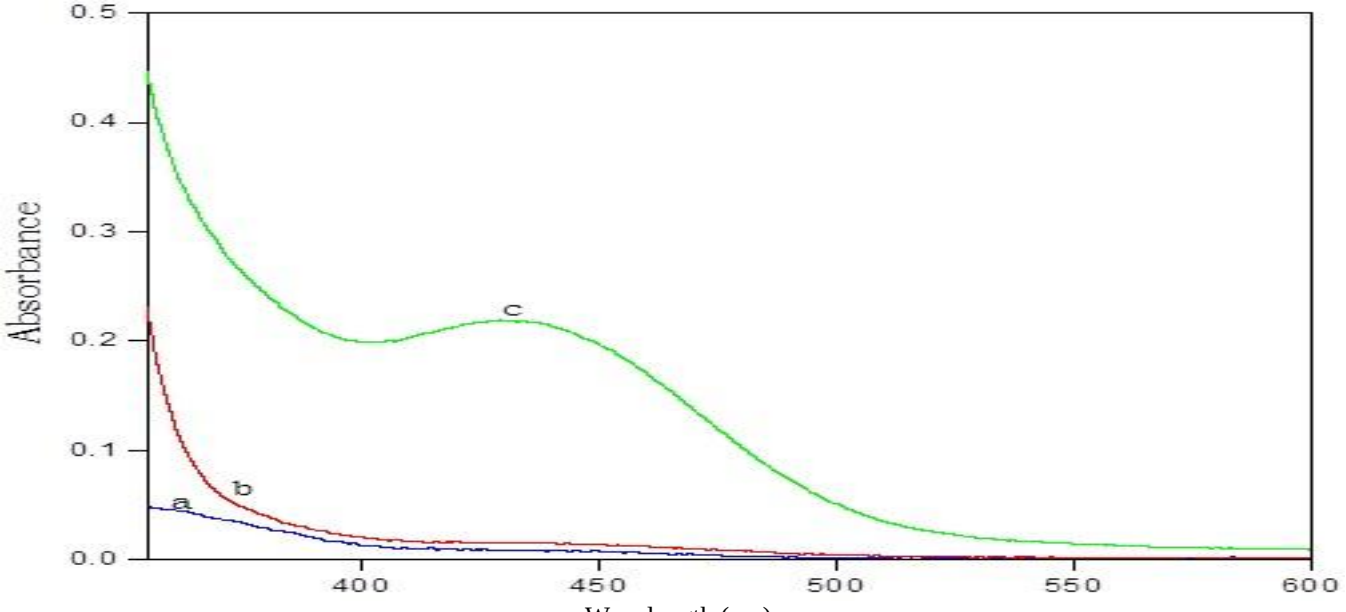
complex remains stable for more than 20 hours. The order of mixing of various components of the reaction mixture (buffer, Cr(VI) solution and mifepristone solution) did not have any effect on the maximum absorbance of the experimental solution. Further a study of the influence of surfactants on the absorbance of the complex showed that none of the surfactants studied (TritionX-100, SDS,CPC etc) had any effect on the maximum absorbance of the complex solution The absorbance varied linearly with the concentration of mifepristone. Beer's law is obeyed in the range 10.0-100.0 µg/ml of mifepristone. The straight line plot conforms to the equation A = 0.0080 C - 0.0001. Optical characteristics and regression data are presented in Table-3.The method was applied successfully for the determination of mifepristone in pharmaceutical tablets.

The data are presented in Table-4.

Method Validation and Statistical Analysis

The developed method was validated as per official specifications of ICH[11]. The validation parameters were found to be accurate and precise. Statistical results are expressed in terms of, mean ± SD, %RSD and student t-test values are calculated with the aid of Excel-2007. Dfferences were considered significant at the 95% confidence interval. Repeatability of the method was verified by intra day and inter day precision studies (Table-5). Accuracy of the method was studied by recovery studies and the results are presented in Table-6, Ruggedness studies were carried out by changing the analyst and the results are given in Table-7.

Fig 1. Absorption spectra of a) Cr (VI) vs. buffer blank b) MPT vs. buffer blank; c) Cr(VI) – MPT vs. buffer blank



[Cr(VI)] = 5.0 x 10^{-4}M; [MPT] = 6.0 x 10^{-5}M

Table 1. Effect of temperature on the absorbance of the Experimental solution

[mifepristone] = 8 x 10^{-5}M pH = 2.0

[chromium(VI)] = 5 x 10^{-4}M λ = 430 nm

Temperature(^{0}C)	Absorbance
40	0.130
50	0.250
60	0.365
65	0.367
70	0.368

Table 2. Tolerance limit of excipients

Amount of MPT = 30.0 µg/ml pH = 2.0

Excipient	Tolerance limit (µg/ml)
Fructose	2019
Glucose	1448
Sucrose	2200
Lactose	2735
Gelatin	2910
Starch	2287
Sodium Alginate	2128
Boric Acid	3039
Magnesium stearate	2534

Table 3. Optical and regression data of the Proposed method for mifepristone

Parameter	Mifepristone
λ_{max} (nm)	430
Beer's law limits (µg/ml)	10.0 – 100.0
Limits of detection (µg/ml)	3.0114
Limits of quantization (µg/ml)	9.0342
Molar absorptivity ($l.mo1^{-1}cm^{-1}$)	3.558×10^3
Sandell's Sensitivity ($\mu g/cm^2$)	0.1207
Regression equation (y= a + b x)	
Slope (b)	0.0080
Intercept (a)	-0.0001
Correlation coefficient (γ)	0.9997
Standard deviation (Sd)	0.0026

Table 4. Intra- and Inter- day precision studies of mifepristone (n=3, p=0.05)

Con(µg/ml)	Mean absorbance ±SD		%RSD		t-value
	Day-1	Day-2	Day-1	Day-2	
30	0.246±0.002	0.245±0.001	1.02	1.03	0.651
40	0.331±0.001	0.333±0.003	0.46	0.92	0.445
50	0.416±0.002	0.414±0.002	0.48	0.61	0.512

Table 5. Assay of mifepristone in pharmaceutical formulation

Sample (Manufacturer – Formulation)	Label Claim(mg)	Amount found (mg)	Error (%)
BRAND-I(MIFEGEST-Zydus Cadila health care Ltd -Tablet)	200.0	201.9	0.95
BRAND-II (MIFEPRIN-Sunpharma Industries Ltd-Tablet	200.0	202.3	1.15

***Average of Seven determination**

Table 6. Recovery studies for mifepristone in tablets

Tablet	Amount of Sample(µg/ml)	Amount of Drug added(µg/ml)	Amount Recovered(µg/ml)	% of Recovery ± SD
Brand—I (MIFEGEST-Zydus Cadila health care Ltd -Tablet)	20	20	40.18	100.45±0.002
	20	30	48.92	97.84±0.002
	20	40	58.96	98.26±0.001
Brand-II (MIFEPRIN-Sunpharma Industries Ltd-Tablet)	30	20	50.18	100.36±0.002
	30	30	60.19	100.37±0.002
	30	40	70.48	100.68±0.002

Table 7. Ruggedness studies for the mifepristone in tablets

Tablet	Analyst- I			Analyst- II	
	Label Claim(mg)	Amount found*(mg)	(%)Recovery +SD	Amount found *(mg)	(%)Recovery +SD
BRAND-I	200.0	200.25	100.12+0.001	199.45	99.72 + 0.001
BRAND- II	200.0	199.50	99.75+0.001		

*****Average of Seven determination

CONCLUSION

The proposed method for the determination of mifepristone is a simple, highly selective, visible spectrophotometric procedure. The method is not only, precise and sensitive but also is within the reach of an ordinary clinical laboratory. The linearity parameters and the corresponding regression data indicate excellent linear relationship (γ =0.9997). A survey of literature did not show no report of a simple, sensitive, selective direct visible spectrophotometric procedure for the assay of mifepristone in pharmaceutical formulations. Other methods reported for its determination either use costly and sophisticated instrumentation that require expertise in operation or suffer from interference of various excipients.

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