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Quantitative Determination of Mifepristone in Pharmaceutical Samples By, Visible Spectrophotometric Method Using Ce (IV) As an Analytical Reagent

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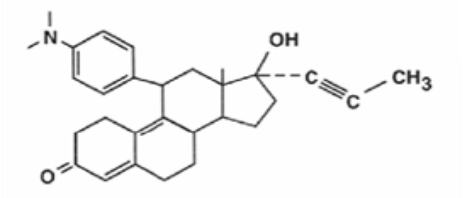
ABSTRACT

A simple, sensitive and accurate visible spectrophotometric method has been developed for the assay of mifepristone. The method is based on the colour reaction between Ce (IV) and mifepristone in the pH range 1.0-5.0.The golden yellow coloured complex is exploited for the development of a visible spectrophotometric procedure for the determination of mifepristone. The absorption spectrum of the complex shows maximum absorbance at 430nm at pH 2.0. Beer's law is obeyed in the range 1.5-30.0 µg/ml of mifepristone. The molar absorptivity and sandell's sensitivity are 1.714×10^4 l mol^{-1} cm^{-1} and 0.0251µg cm^{-2} respectively. The standard deviation of the method for ten determinations of 10 µg/ml mifepristone is 0.0013. The correlation coefficient (γ) of the experimental data of the calibration plot is 0.9999. The proposed spectrophotometric method was validated according to ICH specifications. The validation parameters such as, linearity, accuracy, precision, LOD, LOQ and ruggedness were studied. The method for the quantitative assay of mifepristone was successfully applied for its assay in pharmaceutical formulations.

Keywords: Mifepristone, Ce (IV), 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



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.

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Mifepristone is determined voltametrically using DNA – modified carbon paste electrode by Kai Gu *et al*. [1] A simple sensitive and validated HPLC method is developed for mifepristone determination in wild canid serum [2] and Zhiyon Guo *et al*., developed a highly sensitive HPLC method for mifepristone determination in human plasma. [3] A high performance liquid chromatographic method [4] for the determination of mifepristone in human plasma is developed using norethisterone as an internal standard. Simultaneous monodimethyal
mifepristone in human plasma by liquid chromatography – tandem mass spectrometry method [5] is reported. Zhiyong *et al.*, reported a HPLC-UV method for the simultaneous determination of rivanol and mifepristone in human plasma with solid phase extraction. [6] Devadusu [7] *et al*., reported absorbance ratio method for the determination of mifepristone. Spectrophotometric methods [8-9] for the quantitative estimation of drugs in pharmaceutical formulation are easier and inexpensive.

The above survey of literature shows no report of a direct visible spectrophotometric method for the assay of mifepristone. In continuation of our work on development of simple visible spectrophotometric methods [10] for the assay drugs in pharmaceutical formulations, we now report a simple visible spectrophotometric procedure validated as per ICH guidelines for the determination of mifepristone.

MATERIALS AND METHODS

All chemicals and solvents used were of analytical reagent grade.

Preparation of stock solutions

Cerium (IV) solution

Stock solution of ammonium ceric nitrate (S.D. Fine Chemicals) (1.0×10^{-2}M) is prepared by dissolving requisite quantity in double distilled water and standardized spectrophotometrically. [11]

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. [12]

Instruments employed

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

Shimadzu 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.

Assay of mifepristone

A known aliquot of the standard solution of mifepristone is added to a 10 ml volumetric flask containing 5 ml of buffer solution of pH 2.0 and 1ml of Ce (IV) [5×10^{-3}M] solution. The contents are made up to the mark and its absorbance is measured at 430nm. The amount of mifepristone is estimated from a predetermined calibration plot between absorbance and the amount of mifepristone.

Assay of mifepristone in pharmaceutical samples

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 Ce (IV) [5×10^{-3}M] solution. The contents are made up to the mark with distilled water. The absorbance is measured at 430 nm against the Ce (IV) blank after heating the experimental solution to 60°C 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 temperature on the absorbance of experimental solution

The studies on the effect of time on the colour intensity of the experimental solution revealed that at room temperature it attains maximum absorbance only after 20 hours of mixing the constituent solutions. Hence, the effect of temperature on the absorbance of experimental solution was studied. 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°C. Hence, the absorbance is measured after heating the experimental solution to 60°C 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 even in large quantities in the determination of mifepristone making the method highly selective.

Table 1: Effect of temperature on the absorbance of the experimental solution

Temperature(°C)	Absorbance
40	0.115
50	0.235
60	0.355
65	0.355
70	0.354

[mifepristone] = 2.0×10^{-5}M pH = 2.0
[cerium (IV)] = 5×10^{-4}M λ = 430 nm

Table 2: Tolerance limit of excipients

Excipient	Tolerance limit (µg/ml)
Fructose	1923
Glucose	1379
Sucrose	2095
Lactose	2605
Gelatin	2771
Starch	2178
Sodium Alginate	2027
Boric Acid	2894
Magnesium stearate	2413

Amount of MPT = 10.0µg/ml pH = 2.0

Table 3: Optical and regression data of the proposed method for mifepristone

Parameter	Mifepristone
λ_{max} (nm)	430
Beer's law limits (µg/ml)	1.5 - 30.0
Limits of detection (µg/ml)	0.5567
Limits of quantization (µg/ml)	1.4010
Molar absorptivity ($l.mol^{-1}cm^{-1}$)	1.714×10^4
Sandell's Sensitivity (µg/cm^2)	0.0251
Regression equation (y= a + b x)	
Slope (b)	0.0375
Intercept (a)	0.0013
Correlation coefficient (γ)	0.9999
Standard deviation (Sd)	0.0017

RESULTS AND DISCUSSION

Mifepristone reacts with Ce (IV) in the pH range 1.0-5.0 forming a golden yellow coloured complex solution. The absorption spectrum (Fig. 1) of the golden yellow colored Ce (IV) – Mifepristone complex shows an absorption maximum at 430 nm. At this wavelength either Ce (IV) or mifepristone have no absorbance. The colour intensity of the complex is found to be maximum in the pH range 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 maximum value after 30 minutes of mixing of various components at 60°C. 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, Ce (IV) solution and mifepristone solution) did not have any effect on the maximum colour intensity. Further a study of the influence of surfactants on the absorbance of the complex showed that none of the surfactants studied (Trition X-100, SDS, and CPC etc) had any effect on the maximum colour intensity of the complex.

The absorbance varied linearly with the concentration of mifepristone. Beer's law is obeyed in the range 1.5-30.0 µg/ml of mifepristone. The straight line plot obeyes the relation A = 0.0375 C + 0.0013. 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.

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	
15	0.587±0.001	0.584±0.001	0.26	0.28	0.05
20	0.783±0.002	0.782±0.001	0.32	0.20	0.47
30	1.173±0.002	1.174±0.001	0.21	0.34	0.691

Table 5: Assay of mifepristone in pharmaceutical formulation

Sample (Manufacturer-Formulation)	Label Claim (mg)	Amount found *(mg)	Error (%)
BRAND-I (MIFEPRIN- Sun Pharmaceutical Industries Ltd -Tablet)	200.0	201.98	0.99
BRAND-II (MTPILL-CIPLA 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 (MTPILL-CIPLA Ltd-Tablet)	25	20	45.18	100.40±0.001
	25	30	55.48	100.82±0.001
	25	40	64.60	99.38±0.002
Brand-II (Mifeprin- Sun pharmaceutical Ltd- Tablet)	30	20	49.20	98.40±0.002
	30	30	60.19	100.37±0.001
	30	40	70.23	100.32±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**	198.8	99.4±0.001	199.45	99.72±0.001
BRAND-II	**200.0**	200.35	100.17±0.001	200.56	100.28±0.002

***Average of Seven determination

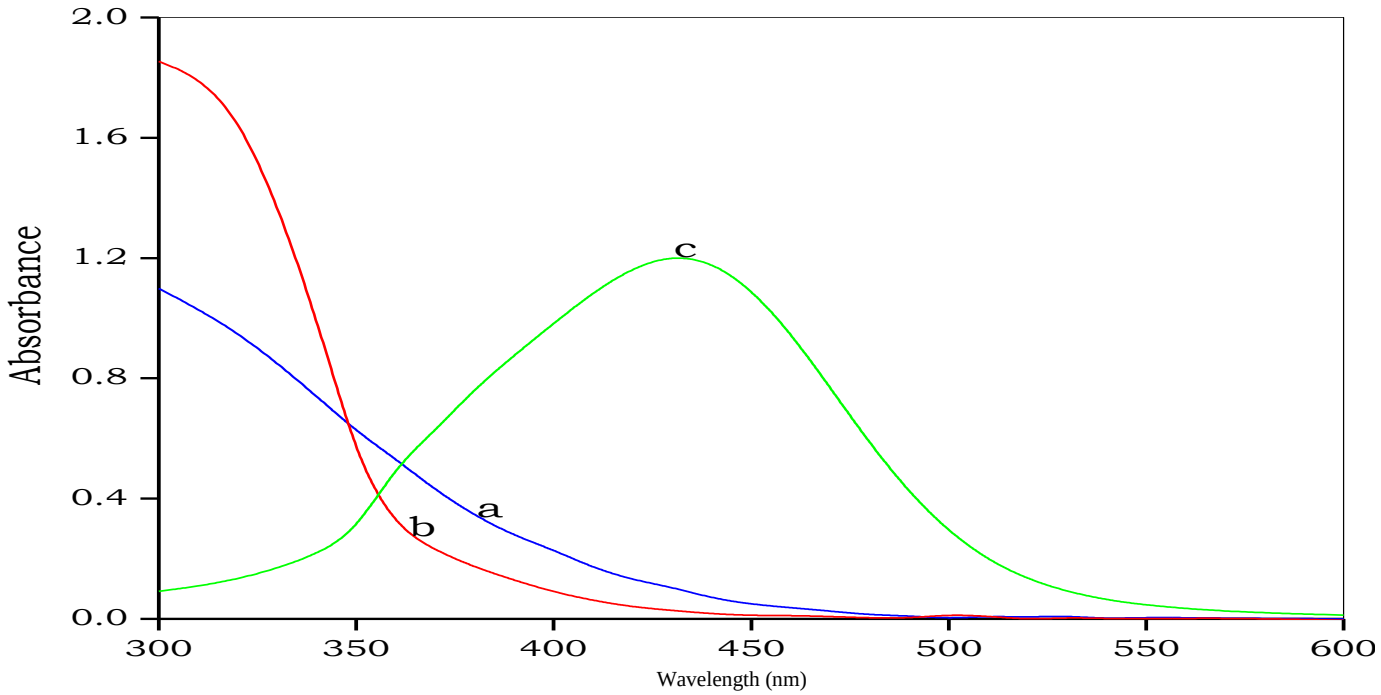

Fig. 1: Absorption spectra of a) Ce (IV) vs. buffer blank b) MPT vs. buffer blank; c) Ce (IV) – MPT vs. buffer blank [Ce (IV)] = 5.0 × 10^{-3}M; [MPT] = 5.0 × 10^{-4}M

Method Validation and Statistical Analysis

The developed method was validated as per official specifications of ICH. [13] 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. Differences were considered significant at the 95% confidence interval. Repeatability of the method was verified by intraday 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. The proposed method for the assay of mifepristone is a simple, highly selective, and sensitive visible spectrophotometric procedure. The method is not only, precise but also is within the reach of an ordinary clinical laboratory. A survey of literature did not show any report of a simple, sensitive, selective direct visible spectrophotometric procedure for the assay of mifepristone in pharmaceutical formulations. Other methods reported in the literature for its determination either use costly and sophisticated instrumentation or suffer from interference of various excipients.

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