Int. J. Drug Res. Tech. 2014, Vol. 4 (2), 14-20 ISSN 2277 - 1506

International Journal of Drug Research and Technology

Available online at http://www.ijdrt.com

Original Research Paper

METHOD DEVELOPMENT AND VALIDATION OF A VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE ASSAY OF MIFEPRISTONE IN PHARMACEUTICIAL FORMULATIONS USING GOLD (III)

K. Nagabhushana Reddy, A. Giri , B. Saritha, B. Vijayabhaskara Reddy and **T. Sreenivasulu Reddy***

Department of Chemistry, S. K. University, Anantapur, Andhra Pradesh, India

ABSTRACT

Gold (III) reacts with mifepristone in the pH range 1.5-3.5 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 o f the complex shows a maximum at 430 nm. pH 2.5 is selected for analytical studies. A ten fold excess of Au (III) is sufficient to produce maximum absorbance. The composition of the complex is 1.1 [Au (III): MPT]. The absorbance of the complex varied linearly with the amount of mifepristone. The straight line relation between absorbance and amount of mifepristone is A = 0.0242C+ 0.0003. The linear plot shows that Beer's law is obeyed in the range 2.0-45.0 µg/ml of mifepristone. The molar absorptivity and sandell's sensitivity are 1.040×10^4 l mol^{-1} cm^{-1} and 0.0413 µg cm^{-2} respectively. The standard deviation of the method for ten determinations of 15 µg/ml mifepristone is 0.0014. 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. The validation parameters such as, linearity, accuracy, precision, LOD, LOQ and ruggedness were studied. The proposed method for the quantitative assay of mifepristone was successfully applied for its assay in pharmaceutical formulations. The method is accurate, precise, highly sensitive and selective, for the assay of mifepristone.

Keywords: Mifepristone, Au (III), Visible spectrophotometry, Method validation.

INTRODUCTION

Mifepristone is a substituted 19-nor steroid compound chemically designated as11β-[p-(Dimethyl amino) phenyl]-17β-hydroxy-17-(1-propynyl) estra-4,9-dien-3-one. Its empirical formula is $C_{29}H_{35}NO_2$.

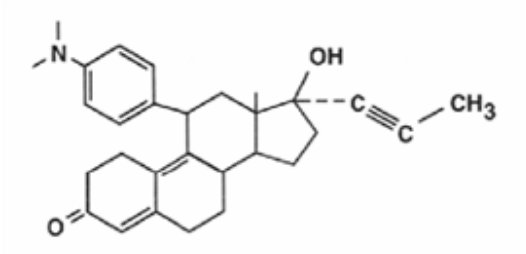

Figure 1: Structural of Mifepristone

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 >100 mg of the drug. Because its metabolites, which still retain considerable affinity toward human progesterone and glucocorticoid 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*.[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 determination of mifepristone and monodimethyal miferpristone 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] 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[7] for the assay drugs in pharmaceutical formulations, now we report simple visible spectrophotmetric procedure validated as per ICH guidelines for the determination of mifepristone.

MATERIALS AND METHODS

All chemicals and solvents used were of analytical reagent grade.

Solutions

Gold (III) Solution

1gm of chloroauric acid (Johnson Mathews, materials technology, U. K.) is dissolved in distilled water after adding few drops dilute HCl. The solution is made upto the mark in 100 ml volumetric flask. The gold content of the solution is determined by rhodamine B method.[8] The working solutions are prepared by diluting the stock solution

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.[9] 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

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).

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, 60 ml 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 gold (III) solution and mifepristone solution in buffer solution of pH 2.5 and that of the experimental solution containing solutions of the gold(III), mifepristone and the buffer (pH 2.5) against the buffer blank are recorded in the wavelength range 300-650nm The spectra are presented in figure1. The spectra in figure1 show that the complex has an absorption maximum at 430 nm. Both gold (III) and mifepristone have negligible absorbance at 430 nm. Hence, analytical studies were 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 10 ml volumetric flask containing 5 ml of buffer solution of pH 2.5 and 1ml of gold (III) [5 x 10^{-3}M] solution. The contents are made up to the mark with distilled water. The absorbance of the resulting solution is measured at 430 nm against the gold (III) blank after 30 minutes of mixing the various components. 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 2.5, gold (III) and mifepristone solution was studied by measuring its absorbance at 430 nm at different time intervals and the data are given table1.The data in table1 show that the absorbance of the complex solution attains a maximum value after 30 minutes of mixing the various component solutions. There after it is observed that the absorbance remains constant for more than 20 hours.

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 (15 µg/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.

RESULTS AND DISCUSSION

Mifepristone reacts with Au (III) in the pH range 1.0-5.0 forming a yellow coloured complex solution. The absorption spectrum of the yellow colored Au (III)-Mifepristone complex shows (figure-1) an absorption maximum at 430 nm. At this wavelength either Au (III) or mifepristone have no absorbance. The colour intensity of the complex is is found to be maximum at pH 1.5-3.5 Hence studies were carried at pH 2.5, 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 28 ^{O}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, Au (III) 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 (TritionX-100, SDS, CPC etc.) had any effect on the maximum colour intensity of the complex. A ten fold molar excess of Au (III) is sufficient to produce maximum absorbance. The absorbance varied linearly with the concentration of mifepristone. Beer's law is obeyed in the range 2.0-45.0 µg/ml of mifepristone. The straight line plot obeyed the equation A = 0.0242 C + 0.0003. Optical characteristics and regression data are presented in table 3.The method was applied successfully for the assay of mifepristone in pharmaceutical formulation (tablets).The data are presented in table 4.

Method Validation and Statistical Analysis

The developed method was validated as per official specifications of ICH[10]. 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 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.

CONCLUSION

The proposed method for the determination of mifepristone is a simple, rapid, 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 or suffer from interference of various excipients.

ACKNOWLEDGEMENTS

The authors thank the department of Chemistry of S.K.University, Anantapur-515003, for providing the necessary facilities.

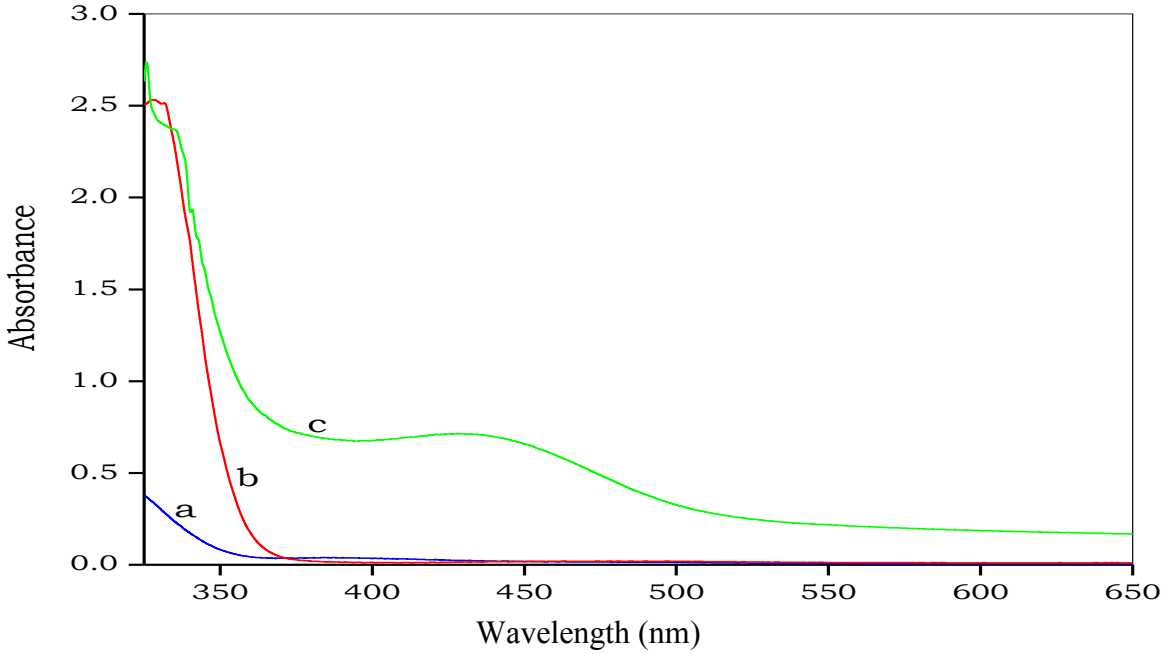

Figure 2: Absorption spectra of **a**) Au (III) vs buffer blank **b**) MPT vs buffer blank; **c**) Au (III)-MPT vs buffer blank
[Au (III)] = 5.0 x 10^{-3}M; MPT= 1.0 x 10^{-5}M

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

Time (minutes)	Absorbance
5	0.103
10	0.206
20	0.322
30	0.395
45	0.396
60	0.397
90	0.396
120	0.394

[Mifepristone] = 4 x 10^{-5}M pH = 2.5
[Gold (III)] = 5 x 10^{-4}M λ = 430 nm

Table 2: Tolerance limit of excipients

Excipients	Tolerance limit (µg/ml)
Fructose	1831
Glucose	1313
Sucrose	1995
Lactose	2481
Gelatin	2639
Starch	2074
Sodium Alginate	1930
Boric Acid	2756
Magnesium stearate	2298

Amount of MPT = 15 µg/ml pH = 2.5

Table 3: Optical and regression characteristics of the Proposed method for mifepristone

Parameter	Mifepristone
λ_{max} (nm)	430
Beer's law limits (µg/ml)	2.0 – 45.0
Limits of detection (µg/ml)	0.1883
Limits of quantization (µg/ml)	0.5650
Molar absorptivity ($l.mol^{-1}cm^{-1}$)	1.040×10^4
Sandell's Sensitivity ($\mu g/cm^2$)	0.0413
Regression equation (y= a + b x)	
Slope (b)	0.0242
Intercept (a)	0.0003
Correlation coefficient (γ)	0.9997
Standard deviation (Sd)	0.0014

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

Conc. (µg/ml)	Mean absorbance ± SD		% RSD		Calculated value of t
	DAY-1	DAY-2	DAY-1	DAY-2	
20	0.492±0.001	0. 491±0.001	0.31	0.20	0.189
30	0.739±0.002	0.737±0.001	0.34	0.14	0.209
40	0.985±0.003	0.982±0.001	0.36	0.16	0.246

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	198.5	0.75
BRAND-II (MIFEGEST Zydus Cadila Health care Ltd.-Tablet)	200.0	197.8	-1.10

*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 of drug Recovered (µg/ml)	%Recovery ±SD
BRAND-I (MIFEPRIN- Sun Pharmaceutical Industries Ltd -Tablet)	20	20	39.00	97.50 ±0.00203
	20	30	49.20	98.4±0.001
	20	40	61.15	101.95+0.001
BRAND-II (MIFEGEST Zydus Cadila Health care -Ltd.-Tablet)	30	20	51.12	102.24 ±0.001
	30	30	60.10	100.16±0.002
	30	40	70.30	100.40±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	201.20	100.60±0.001	198.5	99.25 ± 0.001
BRAND- II	200.0	197.8	98.9±0.001		

*Average of Seven determination

REFERENCES

1. Gu, K; Zhu, J; Zhu, Y; Xu, J and Chen, HY (2000), "Voltammetric determination of mifepristone at a DNA-modified carbon paste electrode", ***Fresenius Journal of Analytical Chemistry***, 368 (8),832-835.
2. Stith, C Delwar and Hussain, M (2003), "Determination of mifepristone levels in wild canid serum using liquid chromatography", ***Journal of Chromatography B***,794,(1), 9-15.
3. Guo, Z; Chu, C; Gengxin, Yin; Mingli, He; Keqin, Fu and Jianli, Wu (2006), "A highly sensitive HPLC method for mifepristone determination in human plasma", ***Journal of Chromatography B***, 832 (2), 181-184.
4. Guo, Z; Sui, Wang; Danyi, Wei and Jinxia, Zhai (2007), "Development of a high-performance liquid chromatographic method for the determination of mifepristone in human plasma using norethisterone as an internal standard: application to pharmacokinetics",***Contraception***, 76, (3), 228-232,
5. Cheng, T; Hui-chang, Bi; Guo-ping, Z; Xiao, C; Zhi-ying, H and Min, H (2009), "Simultaneous determination of mifepristone and monodemethyl-mifepristone in human plasma by liquid chromatography-tandem mass spectrometry method using levonorgestrel as an internal standard: application to a pharmacokinetic study", ***Biomedical Chromatography***, 23(1), 71-80.
6. Zhiyong, G; Danyi, W; Gengxin, Y; Sui, W; Shasha, Z; Yun, C and Jinxia, Z (2007), "Simultaneous determination of rivanol and mifepristone in human plasma by a HPLC-UV method with solid-phase extraction", ***Journal of Chromatography B***, 856 (1-2), 312-317
7. Reddy, KNR; Giri, A; Sarita, B and Reddy, TS (2014), "Method Development and Validation for the Quantitative Estimation of Tizanidine Using Pd(II)", ***Int. J. Pharmaceutical Analysis***, 39(1), 1199-1204
8. Marczenko, Z (1976), "***Spectrophotometric Determination of Elements***", 1st Ed., Ellis Horwood, John Wiley & Sons Inc., New York, 351.
9. Perrin, DD and Dempsy, B (1978), "***Buffers for pH and Metal Ion Control***", Chapman and Hall, London,128.
10. ICH Guideline (2005), "***Q2(R1) Validation of Analytical Procedures: Text and Methodology***", London.

Correspondence Author:
T. Sreenivasulu Reddy
Department of Chemistry, S. K. University, Anantapur, Andhra Pradesh, India
Email: tsrreddychem@gmail.com

Cite This Article: K, Nagabhushana Reddy; A, Giri; B, Saritha; B, Vijayabhaskara Reddy and T, Sreenivasulu Reddy (2014), "Method development and validation of a visible spectrophotometric method for the assay of Mifepristone in pharmaceuticial formulations using gold (III)", ***International Journal of Drug Research and Technology***, Vol. 4 (2), 14-20.

INTERNATIONAL JOURNAL OF DRUG RESEARCH AND TECHNOLOGY