Research Article

Development and Validation of Stability Indicating HPTLC Method for Estimation of Mifepristone

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ABSTRACT

Objective: To develop and validate stability indicating HPTLC method for determination of Mifepristone. **Methods:** The chromatographic development of the drug was carried out on aluminum plates pre-coated with silica gel 60 F_{254} as stationary phase using Toluene: Ethyl Acetate (7:3 v/v) as mobile phase. Detection wavelength chosen was 302 nm. **Result:** The retention factor (R_f) for Mifepristone was found to be 0.47 ± 0.02. The drug was subjected to stress testing as per ICH Q1A(R2).There was no interference of any degradant at Rf of Mifepristone. The developed method was successfully validated according to ICH Q2R1 guidelines. The calibration curve was found to be linear over a range of 100 - 500 ng/ band. The accuracy of the method is indicated by good recovery in the range of 100.35 - 102.97% with less than 2% of RSD. The limit of detection and limit of quantification were found to be 4.73 and 14.35 ng/band respectively. **Conclusion:** A new simple, accurate, precise and specific stability- indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for determination of Mifepristone in bulk and pharmaceutical dosage form without any interference from the excipients. The proposed method can be used for routine determination of Mifepristone as it offers several advantages such as rapid, cost effective, simple mobile phase and sample preparation steps.

Keywords: Forced degradation, high performance thin layer chromatography (HPTLC), mifepristone, stability-indicating method, validation

Received 4 March 2015 Received in revised form 6 April 2015 Accepted 8 April 2015

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INTRODUCTION

Mifepristone is a synthetic, steroidal antiprogestogen and antiglucocorticoid drug. It 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. It is a yellow powder with a molecular weight of 429.6. It is very soluble in methanol, chloroform and acetone and poorly soluble in water, hexane and isopropyl ether [1, 2]. Mifepristone terminates early pregnancy by blocking the activity of progesterone at progesterone receptors. Mifepristone is also sometimes used to treat tumors of the brain, endometriosis (growth of uterus tissue outside the uterus), or fibroids (noncancerous tumors in the uterus) [3].

The literature survey revealed few methods like UV-spectroscopy [4], colorimetric [5-7] and HPLC for the determination of Mifepristone [8-14].

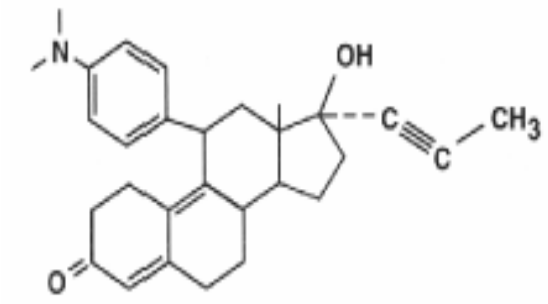

Figure 1: Chemical structure of mifepristone

The above survey of literature shows no report of stability indicating HPTLC method for estimation of mifepristone. The aim of the study was to develop a simple, accurate, precise and specific stability indicating HPTLC method for the determination of

Mifepristone. The present work involve stress degradation as per ICH Q1A(R2) and Q1B [15,16]. The proposed method was validated for linearity, accuracy, precision, robustness, LOD and LOQ according to ICH guidelines [17].

MATERIALS AND METHODS

Chemicals and Reagents

Mifepristone was provided as a gift sample by Cipla pharmaceutical Ltd., Mumbai. Ethyl Acetate, Methanol and Toluene of AR grade purchased from Merck Pvt. Ltd. Mumbai, India. Hydrochloric acid, hydrogen peroxide and sodium hydroxide were purchased from LOBA CHEMIE PVT. LTD. Mumbai.

Selection of detection wavelength

The standard solution of Mifepristone in methanol was scanned over the range of 200 – 400 nm by using UV-Visible spectrophotometer. Wavelength 302 nm was selected for analysis where Mifepristone showed higher absorbance (**Fig. 2**).

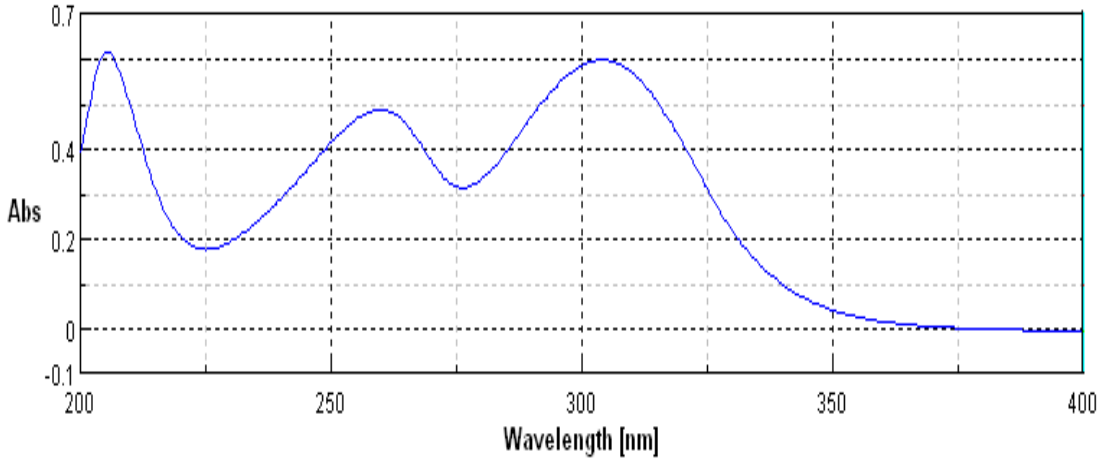

Figure 2: UV spectrum of mifepristone (10 µg/ml)

Instrumentations and Chromatographic conditions

Chromatographic separation of drug was performed on Aluminum plates pre-coated with silica gel 60 F_{254}, (10 cm × 10 cm with 250 µm layer thickness). Samples were applied on the plate as a band of 4 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). The mobile phase was composed of Toluene:Ethyl acetate(7:3v/v). CAMAG twin trough glass chamber10 cm × 10 cm was used for linear ascending development of TLC plate under 15 min saturation conditions and 10 mL of mobile phase was used per run, migration distance was 90 mm. Densitometric scanning was performed using Camag TLC scanner 3 in the range of 400-200 nm, operated by winCATS software, slit dimensions were 3.00 x 0.45 mm and Deuterium lamp was used as a radiation source.

Preparation of Standard stock solution

A standard stock solution of Mifepristone was prepared by dissolving 5 mg of drug in 10 ml of methanol to get concentration of 500µg/ml. From the standard stock solution, working standard solution was prepared to contain 50µg/ml of Mifepristone.

Preparation of sample solution

Twenty tablets of Mifepristone were weighed and powdered. A mass of powder equivalent to 5 mg of Mifepristone was accurately weighed and transferred to 10 ml volumetric flask and was diluted with methanol, sonicated for 10 min and the volume made to 10 ml (500 µg/ml). Solution was filtered and further dilutions were made to get the final concentration of 50 µg/ml of which 6 µl volumes was applied on plate. The procedure was repeated six times.

Stress degradation study of bulk drug

Stress degradation studies were carried under condition of acid/ base/ neutral hydrolysis, oxidation, dry heat and photolysis. For each study, samples were prepared as follows

1. The blank subjected to stress in the same manner as the drug solution.

2. Working standard solution of Mifepristone subjected to stress condition.

Dry heat and photolytic degradation were carried out in solid state. 6µL of the resultant solution was then applied at TLC plate and densitogram was developed.

Stress test conditions like strength of reagent and exposure time were optimized to get 10-30% degradation. The optimized conditions are as follows:

Degradation under alkali catalyzed hydrolytic condition

To 1 mL of 500 µg.mL^{-1}solution of Mifepristone, 1mL of 1 N NaOH was added. The volume was made upto 10 mL with methanol. The above solution was kept for 4 hrs at room temperature in dark place.

Degradation under acid catalyzed hydrolytic condition

To 1 mL of 500 µg.mL^{-1} solution of Mifepristone, 1 mL of 0.01N HCl was added. The volume was made upto 10 mL with methanol. The above solution was kept for 15 mins at room temperature in dark place.

Degradation under neutral hydrolytic condition

To 1 mL of 500 µg.mL^{-1}solution of Mifepristone, 1mL of distilled water was added. The volume was made upto 10 mL with methanol. The above solution was kept for 4 hrs at room temperature in dark place.

Degradation under oxidative condition

To 1 mL of 500 µg.mL^{-1}solution of Mifepristone, 1 mL of 6% H_2O_2 was added. The volume was made upto 10 mL with methanol. The above solution was kept for 24 hrs at room temperature in dark place.

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (80^0 C) for a period of 6 hours. Sample was withdrawn, dissolved in methanol and diluted to get 50µg mL^{-1}.

Photo-degradation studies

The photo degradation study of the drug was performed by exposing the drug to UV light providing illumination of NLT 200 watt hr/m^2 and separately to cool white fluorescent light of NLT 1.2 million Lux-Hr. After exposure 5 mg of drug was accurately weighed and transferred to 10 mL of volumetric flask; the volume was made up with methanol to obtain 500 µg.mL^{-1} solution. Further dilutions made to get 50 µg mL^{-1}.

Multiple wavelength scanning was done for each stress condition to locate the peak of degradation product; but no peak of degradation product was obtained even upon spotting ten times higher concentration. The drug peak area reduced under stress conditions.

Method Validation

The method was validated according to the ICH Q2 (R1) guidelines for the following parameters.

Specificity

The specificity of the method was ascertained by peak purity profiling studies in winCATS software. It involves comparison of UV spectra at peak start, middle and end. The peak purity values were found to be more than 0.998, indicating the noninterference of any other peak of degradation product or impurity.

Linearity and Range

From the standard stock solution (500µg/ml) of Mifepristone, solution was prepared containing 50µg/ml of Mifepristone. This solution was used for spotting. Six replicates per concentration were spotted. The linearity was determined by analyzing five concentrations over the concentration range of 100-500 ng/band for Mifepristone. The peak areas were plotted against the corresponding concentrations to obtain the calibration graph. The LOD and LOQ were calculated based on the equation:

$LOD = 3.3 \times \sigma / S$ and $LOQ = 10 \times \sigma / S$.

Where, σ is standard deviation of the lowest response of linearity equation and S is slope of the calibration curve of the analyte.

Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 80, 100 and 120%. Basic concentration of sample chosen was 200 ng/band. % recovery was determined from linearity equation.

Precision

The precision of the method was demonstrated by Intra-day and Inter-day variation studies. In the Intra-day studies 3 replicates of 3 concentrations were analyzed on the same day, for the inter day variation studies, 3 replicates of 3 concentrations

were analyzed on 3 consecutive days and % RSD was calculated.

Robustness

Robustness of the method was determined by introducing small deliberate changes in mobile phase ratio, chamber saturation time, time from spotting to development and development to scanning. For all changes in conditions, the samples were analyzed in triplicate and the effects on the peak area and rf value was noted. It was found that results did not vary by more than 2%.

RESULTS AND DISCUSSION

Optimization of mobile phase

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 302 nm. The stationary phase used for study of Mifepristone was Aluminum plates pre-coated with silica gel 60 F_{254}, (10 cm × 10 cm with 250 µm layer thickness. Different ratios of mobile phase constituents were studied, mobile phase with Toluene: Ethyl Acetate in the ratio of 7:3 v/v was chosen due to good symmetrical peak. Retention Factor was 0.47 ± 0.03. The analytical method was found linear over the range of 100-500ng/band.

Forced degradation studies

Forced degradation studies were conducted to evaluate the stability and specificity of the method. No degradation product of Mifepristone was observed when the drug was subjected to acidic, basic and neutral treatment, exposure to UV light, heat and to oxidation conditions. The drug peaks obtained from all the stressed samples were found to be homogenous and pure. The drug was found to be photo labile. Hence the method is found to be specific. The results are given in (**Table 1**).

Table 1: Summary of stress degradation of mifepristone

Stress degradation conditions	Percent recovery (%)	Percent degraded (%)	Peak purity data	
			r(s,m)	r(m,e)
Initial	100	-	0.9992	0.999
Base (1 N NaOH, kept for 4 hrs)	84.04	15.96	0.9993	0.9998
Acid (0.01 N HCl, Kept for 15 min)	79.09	20.91	0.9992	0.9992
H_2O_2 6% v/v (kept for 24 hrs)	75	25	0.9998	0.9997
Neutral (kept for 4 hrs)	83.51	16.49	0.9997	0.9994
Heat dry (80^0C, 6 hrs)	86.53	13.47	0.9998	0.9997
Photo stability (UV, 200 watt hrs/square meter)	69.12	30.88	0.9992	0.9990
Florescence (1.2 million Lux. Hrs)	35.77	64.23	0.9998	0.9994

Linearity and Range

The linear calibration range was found to be 100 to 500 ng/band. The calibration curve obtained by the least square regression analysis between average peak area and concentration showed linear relationship with a correlation coefficient of 0.997, the equation of the calibration curve found for Mifepristone was y = 22.391x - 342.54 (**Fig. 3**).

Accuracy and Precision

Recovery of standard drug was found to be 100.35 - 102.97 % with less than 2% of RSD values, indicating that the proposed method was accurate (**Table 2**). (**Table 3**) shows the precision study results. The RSD values for intraday and interday-precision were not more than 2 %, indicating the repeatability and reproducibility of the method.

Limit of detection and quantification (LOD and LOQ)

The LOD (Limit of Detection) and LOQ (Limit of quantitation) were estimated from the standard deviation of the lowest response and the slope of the calibration curve. LOD and LOQ were found to be4.73 ng/ band and 14.35 ng/band respectively.

Robustness

The %RSD values of all robustness parameters were examined and found to be within the limit of 2%, showed that the proposed method was robust (**Table 4**).

Solution Stability

Freshly prepared solution was kept in a freeze (cool condition) after use. UV absorbance of freeze solution was compared with absorbance of fresh solution. It was observed that drug solution have stability of 5 days.

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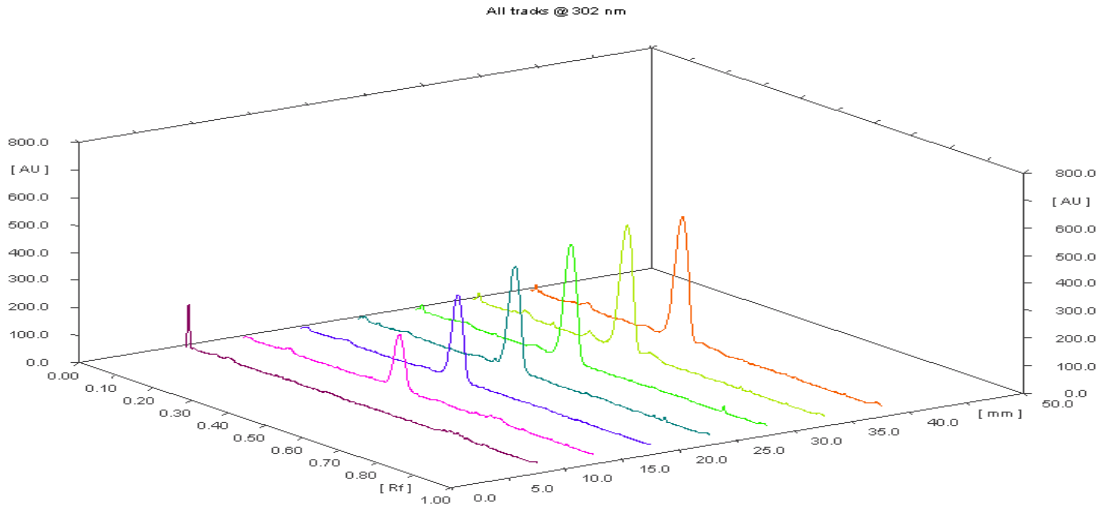

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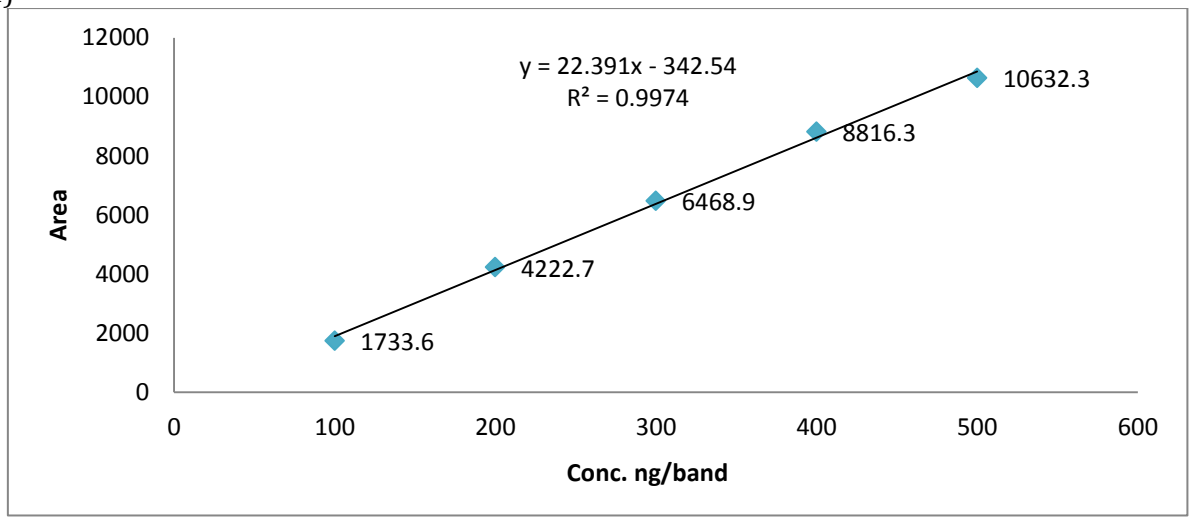

Figure 3: I) Densitogram of linearity of mifepristone (100-500 ng/band), II) Calibration curve for mifepristone

Table 2: Accuracy of the proposed method

Level (%)	Theoretical Conc. (ng/band)	Avg. of Area	Recovered Conc. (ng/band)	% Recovery
80	360	7746.7	361.27	100.35
100	400	8725.8	404.99	101.24
120	440	9802.3	453.07	102.97

Table 3: Precision study

Concentration (ng/band)	Mean area*	SD	% RSD
Inter day			
300	6401.3	56.58	0.88
400	8387.85	86.33	1.02
500	10312.33	80.579	1.78
Intra day			
300	6503.56	56.78	0.87
400	8830.067	47.22	0.53
500	10661.37	80.579	0.54

* Mean of three replicates

Table 4: Results of robustness study

Sr. No.	Parameter	Robust condition	% RSD
1.	Saturation time (15min) ± 2 min.	13min	0.16
		17min	0.41
2.	Mobile phase composition Toluene: Ethyl Acetate (7:3 v/v) ±0.2 toluene	Toluene: Ethyl Acetate (6.8: 3.2 v/v)	0.67
		Toluene: Ethyl Acetate (7.3: 2.7 v/v)	0.36
3	Time from spotting to development (immediate)	After 30min.	0.43
		After 1hr	0.54
4	Time from development to scanning (immediate)	After 30min.	1.45
		After 1hr	1.78

Table 5: Summary of validation study

Sr. No.	Validation parameters	Results
1.	Specificity	Specific
2.	Linearity Range	$y = 22.391x - 342.54$ ($R^2 = 0.9974$) 100- 500ng/band
3.	Accuracy	% Recovery
	80 %	100.35
	100 %	101.24
	120 %	102.97
4.	Precision	(% RSD)
	A) Interday precision	1.22
	B) Intraday precision	0.64
5.	Limit of Detection	4.73 ng/band
6.	Limit of Quantitation	14.35 ng/band
7.	Robustness	Robust

CONCLUSION

The developed method was found to be simple, sensitive, selective, cost-effective and time saving for analysis of Mifepristone in tablet without any interference from the excipients. The results indicated the suitability of the method to study stability of Mifepristone under various forced degradation conditions as prescribed by ICH Q1A(R2) guidelines.

ACKNOWLEDGEMENT

The authors would like to thank to Cipla Pharmaceuticals Ltd., Mumbai for providing working standard of Mifepristone and also to the Principal and Management, AISSMS College of Pharmacy, Pune for providing required facilities for research work.

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