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Analytical Method Development and Validation for Estimation of Mifepristone in Pure and Pharmaceutical Dosage Form

Sheetal Bastia [a], Vaibhav Gawade [b*], Vitthal Chopade [c], Rahul Jagtap [b] and Vishal Modi [d]

[a] Department of Pharmaceutical Quality Assurance, SSR College of Pharmacy, Affiliated to Savitribai Phule Pune University, Silvassa, D&NH-396230, India.
[b] Department of Pharmaceutical Chemistry, Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune-411018, India.
[c] Department of Pharmaceutical Chemistry, PES Modern college of pharmacy, Yamuna Nagar Nigdi, Pune-44, India.
[d] Department of Pharmaceutical Chemistry, SSR College of Pharmacy, Affiliated to Savitribai Phule Pune University, Silvassa, D&NH- 396230, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Mifepristone structurally belongs to the class of anti-progesterone steroids, which are used as an oral contraceptive. The reverse phase HPLC method was designed in a simplified and rapid way for the estimation of Mifepristone in bulk as well as tablets. The method was established using a Kromasil C_{18} column of dimensions of 250mm×4.6mm and a particle size of 5m.The used mobile phase was Acetonitrile: Water (70:30, v/v). The pump was pumped at 1 ml/min at a temperature of about 30 ± 2 °C and the eluted analyte was identified at 305 nm. Mifepristone eluted with a mean retention time of 6.27 minutes. The intended method was validated as per ICH (International Council for Harmonisation) guidelines, indicating a high degree of specificity, system suitability, accuracy, precision, and robustness. The LOD (Limit of detection) was found to be 0.7238 ppm and the limit of measurement was 0.9562 ppm. The method linearity was found to be between 1-6µg/ml, with an R^2 of 0.9923. In accuracy studies, the percent recovery was found to be between 99.39% - 100.50%. The method was discovered to be precise as the values of the percent RSD

**Corresponding author: E-mail: gawadevaibhav6060@gmail.com;*

were found to be less than 2.0% for both intraday and interday. The method was discovered to be reliable and robust. Mifepristone in marketed pharmaceutical tablet dosage form was effectively quantified using the established Reverse Phase HPLC method.

Keywords: Limit of detection; anti-progesterone; reverse phase; robustness; ICH guideline.

ABBREVIATIONS

MIF : Mifepristone
ACETONITRILE : Acetonitrile,
ICH :International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

1. INTRODUCTION

Unintended pregnancies are those that are unplanned or unwanted during conception. It is one of the most troubling public health complications and a severe reproductive health issue, which includes accidental pregnancy [1]. The World Health Organization (WHO) states that approximately 70,000 maternal deaths are due to the complications of unintended pregnancy, which further results in legal unsafe abortion, and that about 585,000 women die each year due to the complications of unintended pregnancy that result in child birth [2]. Mifepristone is a 19-nor steroid with an effective and competitive antagonist of glucocorticoids and progesterone to give anti-progesterone activity [3]. It has major special effects on ovulation [4]. If given precisely in between the follicular phases, it leads to delayed follicular maturation and ovulation as compared to normal. If this drug is given continuously, then ovulation is prevented [5]. It inhibits the growth of secretory endometrium, which leads to the production of menstruation [6].Mifepristone has anti-glucocorticoid and anti-androgenic properties when combined with glucocorticoid and androgen receptors [7-9].The use of mifepristone in the first trimester of pregnancy further leads to abortion. It is also used as a post-coital contraceptive [8-11]. The mechanism of action of Mifepristone is presented in:

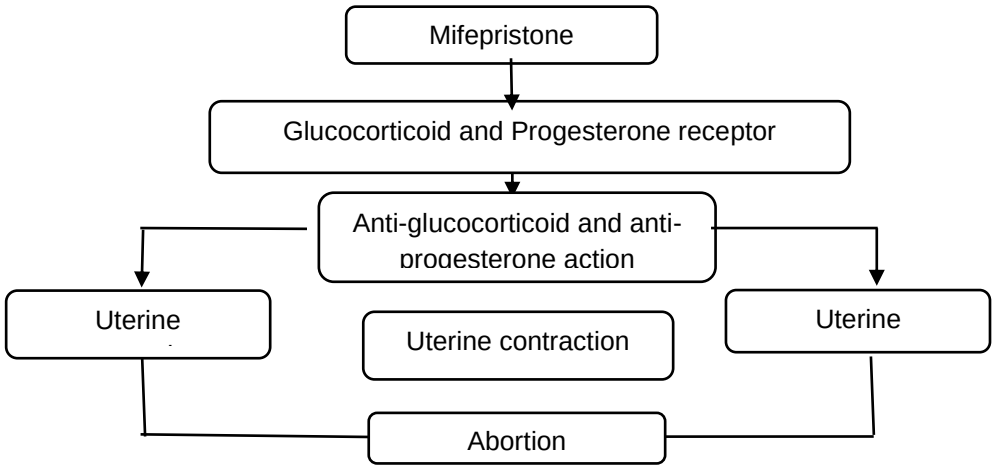

Fig. 1. Mechanism of mifepristone

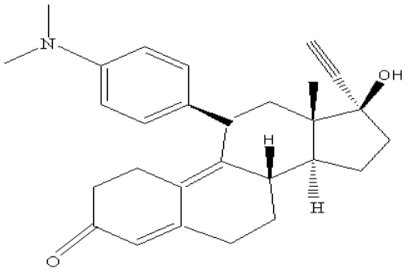

Fig. 2. Structure of mifepristone

An exhaustive search reveals that no analytical techniques like UV, HPLC, and stability indicating methods for the determination of Mifepristone as an individual have been reported [12-14]. There was no method reported to quantify Mifepristone by HPLC. However, a fresh method has been created for estimating Mifepristone that is accurate, specific, precise, and repeatable [14-16].

2 EXPERIMENTAL

2.1 Methods

A standard drug of Mifepristone (purity of 99% w/w) was obtained. .Both HPLC grade Methanol and HPLC grade Water were purchased in Mumbai, India by Fisher Scientific India Pvt. Ltd. and Rankem, Haryana, India. Termipil kit tablets manufactured by Alkem Labs contain 200 mg of Mifepristone as per label claim and were acquired from a pharmacy shop.

2.2 Chromatographic Conditions and HPLC Instrumentation

HPLC studies were carried out using the Jasco HPLC system 4000 series, consisting of a quaternary pump (PU-4180-LPG), a degasser, an auto-sampler (AS-4050) and a UV detector. All the chromatograms obtained were evaluated using Chrom-NAV version 2.0 software. The used mobile phase was Acetonitrile: Water (70:30, v/v). Flow rate of 1 ml/min, which was fine-tuned by trial and error. At a distance of 305 mm, detection was made.

3 METHODS

3.1 Preparation of Solutions

3.1.1 Standard stock solution preparation.

The Mifepristone standard stock solution was prepared by properly weighing 5 mg of medication and transferring it to a 50-ml flask with a modest amount of mobile phase. i.e., Acetonitrile: Water(70:30,v/v) to dissolve the drug was then sonicated for 10 minutes, and the remaining mobile phase was added to make the final volume up to 50 ml to obtain a stock solution containing 100 ppm of Mifepristone.

3.1.2 Sample solution preparation

To prepare the sample solution, weigh 5mg of equivalent Mifepristone tablet powder into a 50ml flask and add a tiny amount of mobile phase into the volumetric flask. (Acetonitrile: Water (70:30), v/v), it was then sonicated for about 10 minutes and the remaining mobile phase was added to make the final volume up to 50 ml to obtain a stock solution containing 100 ppm of Mifepristone.

3.2 Chart 1. Chromatographic Conditions

Criteria	Condition used
Stationary Phase	Kromasil C-18 (250 mm × 4.6mm,5µm)
Mobile Phase	Acetonitrile: Water (70:30, v/v).
Flow	1 ml /min
Detection	305 nm
Run time	10 minute

3.3 Method Validation

Specificity, method suitability, precision, LOD, LOQ, linearity, accuracy, and robustness were used to validate the optimised method according to ICH guidelines.

3.4 Specificity

Specificity was evaluated by analysing the standard (100ppm) and test (100ppm) and comparing the spectra, and the presence of interference was checked.

3.5 Limit of Detection (LOD) & Limit of Quantification

The limit of detection and the limit of quantification Mifepristone for the proposed method were estimated using the standard deviation method. A calibration curve was prepared in the detection and quantitation range (1-6 ppm).

$$LOD = 3.3\times\sigma/S$$

$$LOQ = 10\times\sigma/S$$

S = Slope of the Calibration curve.
σ= Std deviation of y-intercepts of calibration line.

3.6 Range and Linearity

By injecting 6 different concentrations of standard Mifepristone solutions in the range of 1–6 ppm, the linearity of the new technique was determined. Six solutions of different

concentrations were prepared from a 100 ppm standard stock solution by pipetting out 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ml of stock solution were added to a 10 ml volumetric flask, with the remaining volume made up with mobile phase. The resulting solutions were injected in triplicates into HPLC under optimised chromatographic conditions and the area was measured. An average area for each concentration was calculated. A calibration curve was plotted and interpreted, and R^2 was determined.

3.7 Accuracy

The percentage recovery was used to assess the accuracy of the developed approach. Percentage Recovery was calculated at three levels (50%, 100%, and 150%) at three different concentrations of solutions (i.e.1.5 ppm, 3 ppm, and 4.5 ppm) in triplicates to evaluate the accuracy. 3 different concentrations (i.e. 1.5 ppm, 3 ppm, and 4.5 ppm) were prepared from 100 ppm of standard stock solution and one concentration (i.e. 1 ppm) from 100 ppm of sample solution. The resulting solutions were injected in triplicates to HPLC under optimised chromatographic conditions. A known amount of sample solution (1 ppm) was spiked into three different concentrations of standard solutions (i.e., 1.5 ppm, 3 ppm, and 4.5 ppm). These solutions were injected in triplicates into the HPLC for analysis. Percentage recovery at each level was calculated using a formula:

$$\% \text{ Recovery} = \frac{\text{Measured Value}}{\text{True value}} \text{x } 100$$

3.8 Precision

The intraday and inter-day precision of the devised approach were investigated. Sample solutions of Mifepristone in three concentration ranges (i.e., 1.5 ppm, 3 ppm, and 4.5 ppm) were prepared and injected in triplicates into HPLC for analysis. At each concentration level, the peak area was measured and the percent RSD was calculated. Similarly, the intraday and inter-day precision studies were done.

Intraday precision: in intraday precision, analysis was conducted twice at various times on the same day.

Interday precision: In interday precision, analysis was conducted for two consecutive days.

3.9 Robustness

The method's robustness was assessed by fluctuating method parameters such as change wavelength (303nm, 307nm), flow rate change (0.9 ml/min, 1 ml/min), and mobile phase change composition to (Acetonitrile: Water) (69:31) and (71:29). The robustness was assessed by analysing standard solutions of 3ppm (n= 6) and sample solutions of 3ppm (n= 2) of Mifepristone, and the% RSD was determined.

4. ANALYSIS OF MIFEPRISTONE TABLETS

To analyse the tablet mixture, 5 mg of Mifepristone tablet powder was carefully weighed in a 50-ml volumetric flask, the mobile phase was added, and the flask was sonicated for 15 minutes. The mobile phase was used to dilute the solutions to a volume of 50 ml, shake the solution, and filter to obtain the stock solution containing 100 ppm of Mifepristone. The solution was runned in HPLC in triplicate. The% w/w of Mifepristone in each tablet was calculated using the formula:

$$\%\frac{\text{w}}{\text{w}} = \frac{\text{Area of sample}}{\text{Area of standard}} \text{x} \frac{\text{Weight of standard}}{\text{Dilution factor of standard}} \text{x} \frac{\text{Dilution factor of sample}}{\text{Weight of sample}} \text{x Purity of std}$$

5 RESULTS AND DISCUSSIONS

5.1 Method Development

During method development, various mobile phases were tried at different flow rates and their effects on retention time, capacity factor, area, peak symmetry, and numbers of theoretical plates were evaluated in HPLC to get the optimum resolution. To assess the amount of Mifepristone, a simple, specific, selective, and accurate reverse phase HPLC technique was designed and validated according to ICH guidelines in this work. Acetonitrile and water in different quantities were tested, and a ratio of Acetonitrile: Water (70:30) was carefully chosen as a suitable combination which gave accepted resolution and system suitability. The chromatogram of the developed method of standard Mifepristone is shown in Fig. 3.

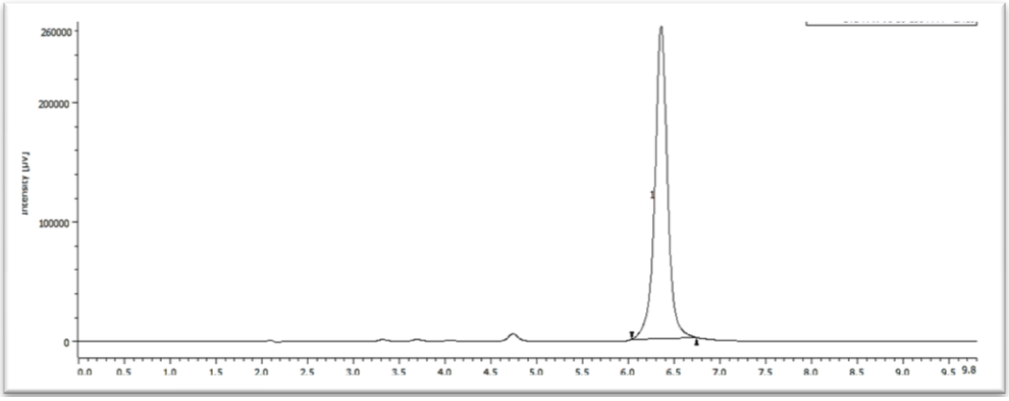

Fig. 3. Standard mifepristone chromatogram

5.2 Specificity

It was found that there was no interference in tablets from the mobile phase and excipient. The chromatogram of the blank solution, sample solution, and standard solution is shown in Figs. 4,5,6.

5.3 System Suitability Test

The number of theoretical plates was found to be more than 100,000.The tailing factor was less than 2.0. Hence, the developed method is suitable for estimation of Mifepristone as shown in Tables (1 and 2).

5.4 LOD and LOQ

The suggested method's LOD and LOQ were found to be 0.7238 and 0.9562, respectively, at concentrations ranging from 1-6 ppm, as shown in Table 3. The developed method is sensitive enough to detect and quantify the drug at a ppm level.

5.5 Linearity

The method was linear over concentration, ranging between 1-6 ppm, as shown in Table 4. The R^2 was 0.9923 and the regression equation Y = 32780 x + 2571.8. The R^2 was greater than 0.99, so the method was stated to be linear and is shown in Fig. 7.

5.6 Accuracy

The result of the established method was discovered to be accurate, as the result of the percent recovery was within the range. The percentage recovery was found to be between 99.39% - 100.50% as shown in Table 5 and 6.

5.7 Precision

The result was demonstrated by intraday and intraday precision at three concentration levels: 1.5 ppm, 3 ppm, and 4.5 ppm. The values of% RSD obtained at each level of both intraday and intraday precision were less than 2, as shown in Tables 7, 8, and 9,10. As a result, the proposed approach was discovered to be precise.

5.8 Robustness

The method was stated to be robust as the result of%RSD was less than 2. The% w/w was found between 98% and 102% for both unaltered conditions as shown in Table 11 and altered conditions as shown in Tables 12, 13, 14.

Mifepristone is currently used as a contraceptive. From a literature review, it was found that there is no reported Revere Phase HPLC method for estimation of Mifepristone. This research work describes the development and validation of an analytical method for the analysis of Mifepristone. Kromasil C18 column of dimensions 250mm×4.6mm particle size 5 µm and Acetonitrile: Water(70:30, v/v) as the mobile phase at wavelength 305nm.Following the ICH guidelines, the devised approach was validated. With a concentration range of 1-6 ppm and a regression coefficient (R^2) of 0.9923, the desired approach was found to be linear. The method for determination of Mifepristone using HPLC met the acceptance criteria with respect to selectivity, system suitability, precision, accuracy, linearity, and robustness over a theoretical concentration range of 1-6 ppm. Mifepristone analysis could be performed using the designed and validated method.

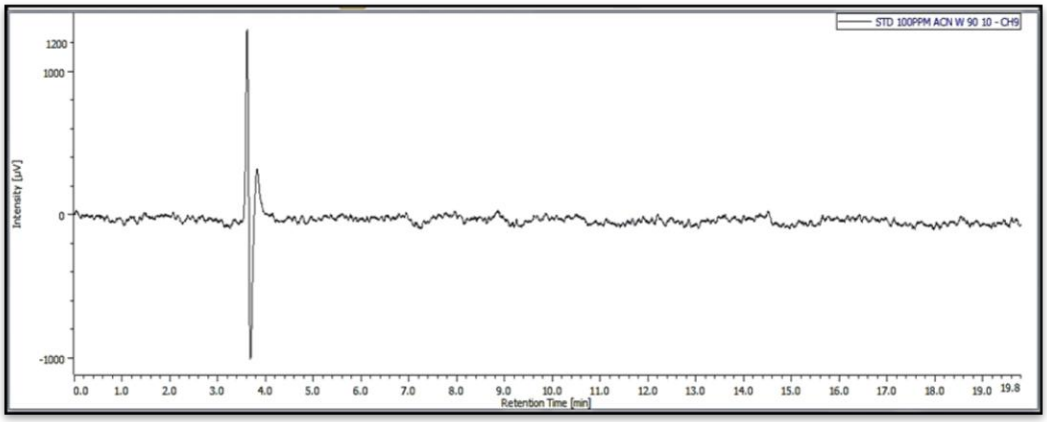

Fig. 4. Blank Chromatogram

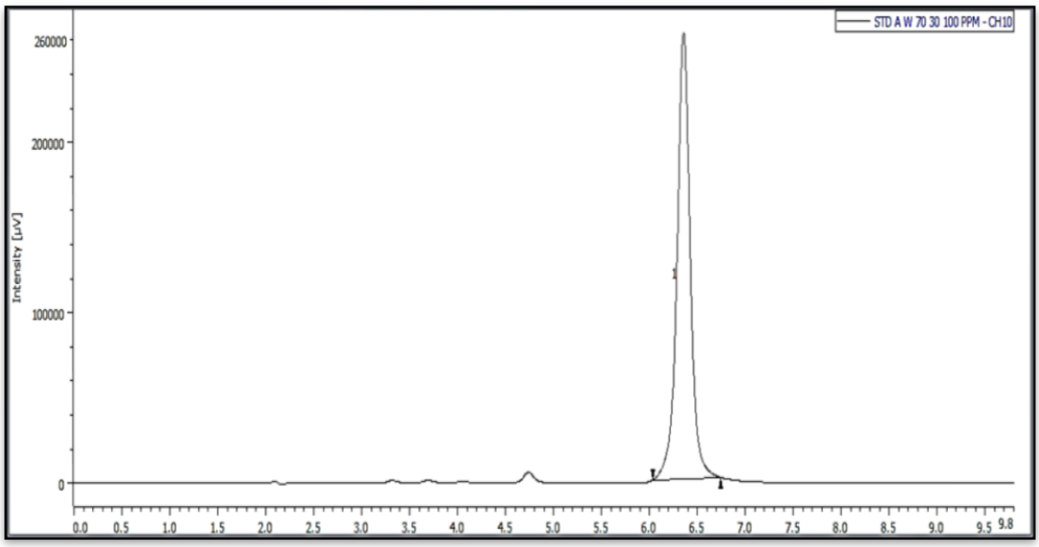

Fig. 5. Standard chromatogram

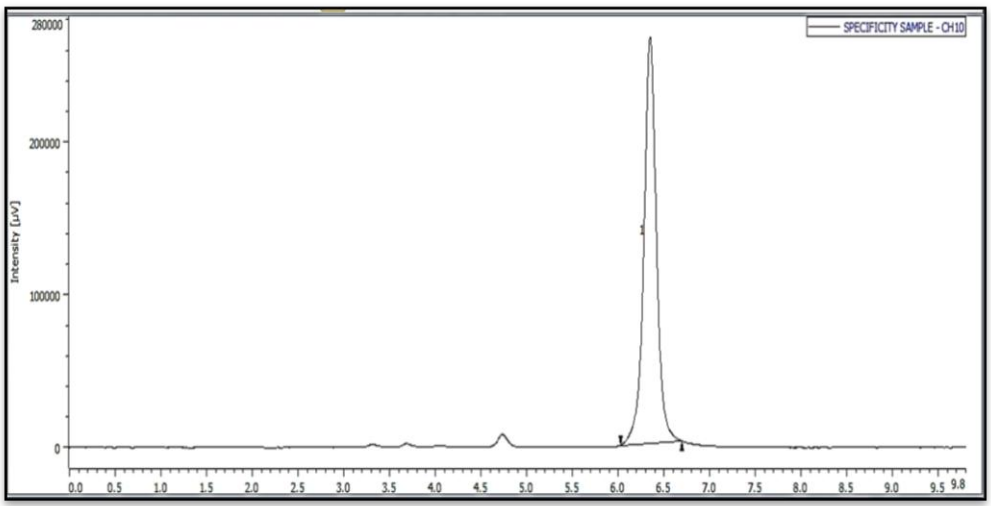

Fig. 6. Chromatogram of test.

Table 1. Mifepristone standard solution system suitability results

tR	Area	NTP	Height	Symmetric factor	%RSD
6.27	2446023	5114	123999	1.400	
6.277	2421958	4985	119117	1.428	
6.279	2448511	5144	124869	1.403	
6.280	2454430	5046	122274	1.418	
6.277	2441044	5253	127562	1.424	
6.277	2430670	6310	154563	1.318	0.4949

Table 2. Results of system suitability for sample solution of Mifepristone

tR	Area	NTP	Height	Symmetric factor	%RSD
6.277	2617420	9542	238574	1.157	
6.277	2592888	11295	267178	1.065	0.6658

Table 3. Results of LOD and LOQ

Sr. No.	Parameters	Results
1.	Calibration curve for detection and quantification range	1-6 ppm
2.	Y-Intercept	2571.8
3.	Slope	32780
4.	LOD	0.2589
5.	LOQ	0.7845

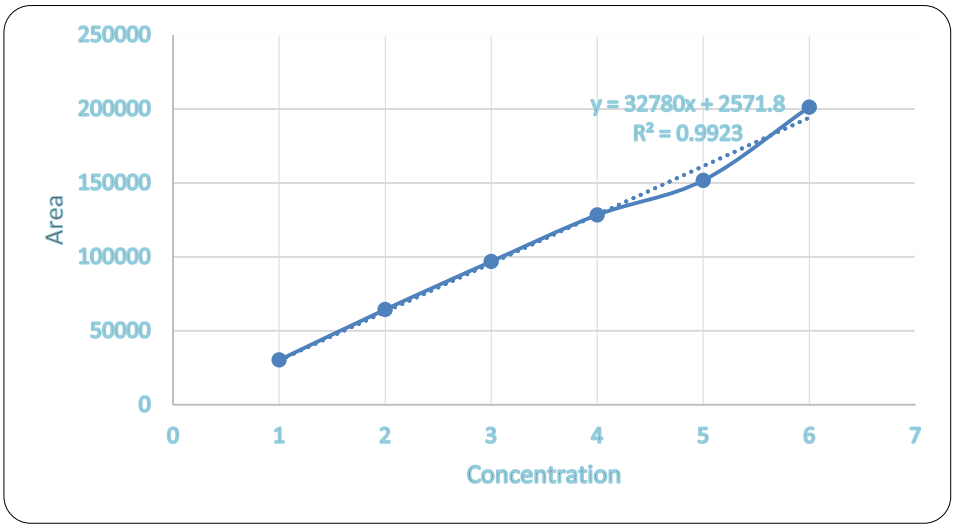

Fig.7.Calibration curve of mifepristone

Table 4. Results of linearity study for mifepristone

Conc. (ppm)	Area 1	Area2	Area3	Average area
1	33017	30253	27861	30377
2	69709	65930	57717	64452
3	100184	90565	99890	96879
4	12832	128288	128422	128346
5	15242	153924	148718	151689
6	200198	203318	200082	201199
Regression equation	Y = 32780 x + 2571.8			
R^2	0.9923			

Table 5. Results of accuracy of developed HPLC method at three different levels (50%, 100%, 150%)

Levels	Conc. (ppm)	Replicate	Area
(Test) 0	1	1	27144
		2	22039
		3	22439
(Std) 50	1.5	1	43851
		2	46340
		3	45694
(Std) 100	3	1	74426
		2	75760
		3	75383
(Std) 150	4.5	1	104831
		2	106036
		3	106598
(spike) 50	1.5+1	1	71412
		2	68535
		3	68037
(spike) 100	3+1	1	101240
		2	97769
		3	99405
(spike) 150	4.5+1	1	129932
		2	127887
		3	129367

Table 6. Results of% recovery of mifepristone by developed HPLC method

% Levels	Replicate	% Recovery	Mean% recovery ±SD	% RSD
50%	1	100.95		
	2	100.33	100.35% ± 0.5853	0.5832
	3	99.78		
100%	1	99.55		
	2	99.96	100.50% ± 0.2084	0.2089
	3	102		
150%	1	98.02		
	2	99.82	99.39% ± 1.185	1.1922
	3	100.30		

Table 7. Shows the results of the developed HPLC method's intraday precision

Conc. (ppm)	Area 1	Area 2	Area 3	Mean	% RSD
1.5	39200	39268	39194	39220.6	0.1
3	72962	72859	71346	72389	1.25
4.5	112515	112288	113994	112932.3	0.82

Table 8. Result of intraday precision of developed HPLC method

Conc. (ppm)	Area 1	Area 2	Area3	Mean	% RSD
1.5	36055	36977	36531	36521	1.26
3	66861	67539	67050	67150	0.52
4.5	110650	112449	111663	111587.3	0.8

Table 9. Result of interday precision of developed HPLC method

Conc. (ppm)	Area 1	Area 2	Area 3	Mean	% RSD
1.5	36025	35764	36036	35941.67	0.4283
3	70227	71012	69331	70190	1.1983
4.5	112470	114682	111733	112961.7	1.3586

Table 10. Result of interday precision of developed HPLC method

Conc. (ppm)	Area 1	Area 2	Area 3	Mean	% RSD
1.5	35079	34987	35631	35232.33	0.9885
3	67849	66643	67619	67370.33	0.9504
4.5	112599	113459	114371	113476.3	0.7808

Table 11. Results of robustness study (unaltered)

Conc. (ppm)		t_R	Area
		6.367	64813
		6.347	66123
Std	3 ppm	6.357	65727
		6.357	64892
		6.353	63196
		6.360	66022
Test	3 ppm	6.370	65005
		6.373	65577
SD = 944.0522 Mean = 65169.38 %RSD = 1.44			

Table 12. Results of effect of change in flow rate on proposed HPLC method for mifepristone

Flow rate of 0.9 ml/min				Flow rate of 1.1 ml/min			
Conc. (ppm)		t_R	Area	Conc. (ppm)		t_R	Area
Std	3 ppm	7.090	69477	Std	3 ppm	6.383	62422
		7.097	70574			6.377	61904
		7.097	70038			6.377	61433
		7,097	70737			6.383	61817
		7.097	69047			6.323	61820
		7.093	70830			6.387	62320
Test	3 ppm	7.093	76550	Test	3 ppm	6.420	64359
		7.093	75236			6.380	63897
SD = 635.3607 Mean = 70186.13 % RSD = 0.9053				SD = 1060.22 Mean = 62496.5 % RSD = 1.69			

Table 13. Results of effect of change in wavelength on proposed HPLC method for mifepristone

303 nm				307 nm			
Conc. (ppm)		t_R	Area	Conc. (ppm))		t_R	Area
Std	3 ppm	6.367	64514	Std	3 ppm	6.367	65464
		6.347	67514			6.350	65478
		6.357	66763			6.357	65470
		6.357	65642			6.357	63816

303 nm				307 nm			
Conc. (ppm)		**t_R**	**Area**	**Conc. (ppm))**		**t_R**	**Area**
		6.353	64151			6.353	64423
		6.360	66018			6.360	63655
Test	3 ppm	6.370	65176	Test	3 ppm	6.370	65924
		6.370	6330			6.373	67244
SD = 1115.621				SD = 1184.798			
Mean = 65638.5				Mean = 65184.25			
% RSD = 1.69				% RSD = 1.81			

Table 14. Result of analysis of tablet by HPLC

Drug	Conc. (ppm)	Area	% RSD	% Assay
Mifepristone	100	2617420 2592888 2632670	1.4582	102%

6. CONCLUSIONS

In accordance with ICH guidelines, all validity parameters for the methods developed were studied. All limits have been shown to be exact, specific, targeted, reliable, and reproductive. The procedure should also be used in pure and pharmaceutical dosing for the regular study of mifepristone.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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