Original Research Article

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

ABSTRACT:

Mifepristone is structurally belongs to the class of anti-progesterone steroids, which used as an oral contraception. Reverse phase HPLC method was designed in simplified rapid way for the estimation of Mifepristone in bulk as well as tablets.The method established using KromasilC_{18} column of dimensions 250mm×4.6mm particle size 5µm.The used mobile phase was Acetonitrile:Water(70:30,v/v).Pumped at1 ml/min and temperature of about 30 ± 2 °C and the eluted analyte were identified at 305 nm. Mifepristone eluted with mean retention time of 6.27 minutes also the intended method was validated as per ICH(International Council for Harmonisation) guideline in with indicating high degree of specificity, system suitability, accuracy, precision, robustness. The LOD (Limit of detection) was found to be 0.7238 ppm and Limit of measurement was 0.9562 ppm. The methods linearity was found between 1-6µg/ml, with an R^2 of 0.9923. In accuracy studies, % recovery was found to be between 99.39% - 100.50 %. The method was discovered to be precised as the values of % RSD were found to be less than 2.0 % for both intraday and interday. The method was discovered to be reliable & 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.

1. INTRODUCTION:

Unintended pregnancies are the one which are unplanned or unwanted during conception. It is one of the most troubling public health complications and a sever reproductive health issue which includes accidental pregnancy[1].The World Health Organization (WHO) states that approximately about 70,000 maternal death is due to the complication of unintended pregnancy which further result edinil legal unsafe abortion and about 585000 women die each year due to the complication of un intended pregnancy but result in the 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 its major special effects on ovulation[4]. If given precisely in between the follicular phase, it leads to delayed follicular maturation & ovulation as compared to normal. If this drug is given continuously then ovulation is prevented [5]. It damages the growth of secretory endometriumand that further result in the production of menses[6]. Mifepristone when further combines to a glucocorticoid receptor and androgen receptors give anti-glucocorticoid and anti-androgenic action[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

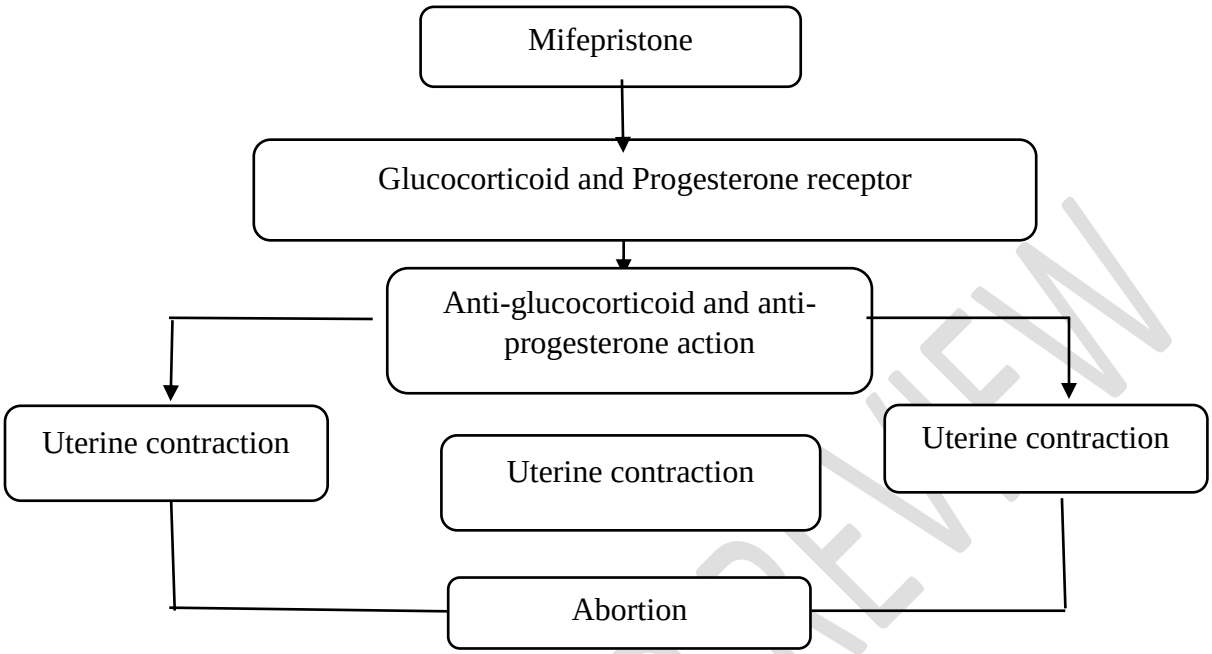


Fig.1.Mechanism of Mifepristone

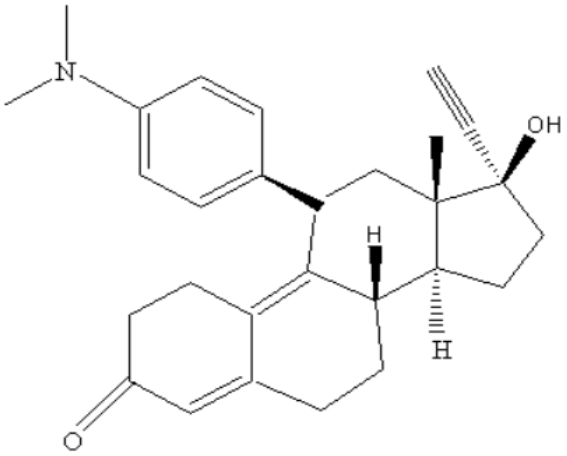


Fig.2.Structure of Mifepristone

Exhaustive search reveals that no any analytical techniques like UV, HPLC, and Stability indicating method for determination of Mifepristone as 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

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

2.2 HPLC Instrumentation and chromatographic condition:

HPLC studies was carried using Jasco HPLC system 4000 series consisting of quaternary pump(PU-4180-LPG), degasser, auto-sampler (AS-4050) and UV detector. All the chromatogram obtained was evaluated using Chrom-NAV version2.0 software. The used mobile phase was Acetonitrile: Water (70:30,v/v) Flow rate of 1ml/min, which was fine-tuned by trial and error. At a distance of 305mm, detection was made,

3 METHODS

3.1 Preparation of solutions

3.1.1 Standard stock solution preparation.

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

3.4 Specificity

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

3.5 Limit of detection (LOD) & Limit of quantification:

The Limit of detection &Limit of quantification Mifepristone of the proposed method was estimated using standard deviation method. Calibration curve were prepared in the detection and quantitation range (1-6ppm)

$$\mathbf{LOD = 3.3\times\sigma/S}$$

$$\mathbf{LOQ = 10\times\sigma/S}$$

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 1-6ppm,linearity of new technique was determined. Six solutions of different concentration were prepared from 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 was added to a 10ml volumetric flask, with the remaining volume made up with mobile phase. The resulting solutions were injected in triplicates to HPLC under optimized chromatographic conditions and area was measured. Average area for each concentration was calculated. 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 solutions (i.e.1.5 ppm,3ppm and 4.5ppm) in triplicates to evaluate the accuracy. 3 different concentrations (i.e. 1.5 ppm,3ppm and 4.5ppm) 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 optimized chromatographic conditions, known amount of sample solution (1 ppm) was spiked in three different concentration of standard solutions (i.e. 1.5 ppm,3ppm and 4.5ppm). These solutions were injected in triplicates in HPLC for analyses. Percentage recovery at each level was calculated using 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 in HPLC for analyses. At each concentration level the peak area was measured and % RSD was calculated. Similarly, the intraday and inter-day precision studies were done.

Intraday precision: In intraday precision, analysis was conducted twice at various time 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 aschangein wavelength(303nm,307nm), flow rate change(0.9ml/min,1,1ml/min), mobile phase change composition to (Acetonitrile:Water) (69:31)and (71:29).The robustness was assessedby analyzing standard solution of 3ppm (n=6) and sample solution of 3ppm(n=2) of Mifepristone and% RSD was determined.

4. Analysis of Mifepristonetablets

To analyse the tablet mixture, 5 mg of Mifepristone tablet powder was carefully weighed in a 50 ml volumetric flask, 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 filtered to obtain the stock solution containing 100 ppm of Mifepristone. The solution was runned in HPLC in triplicate. The % w/w of Mifepristone in tablet was calculated using formula:

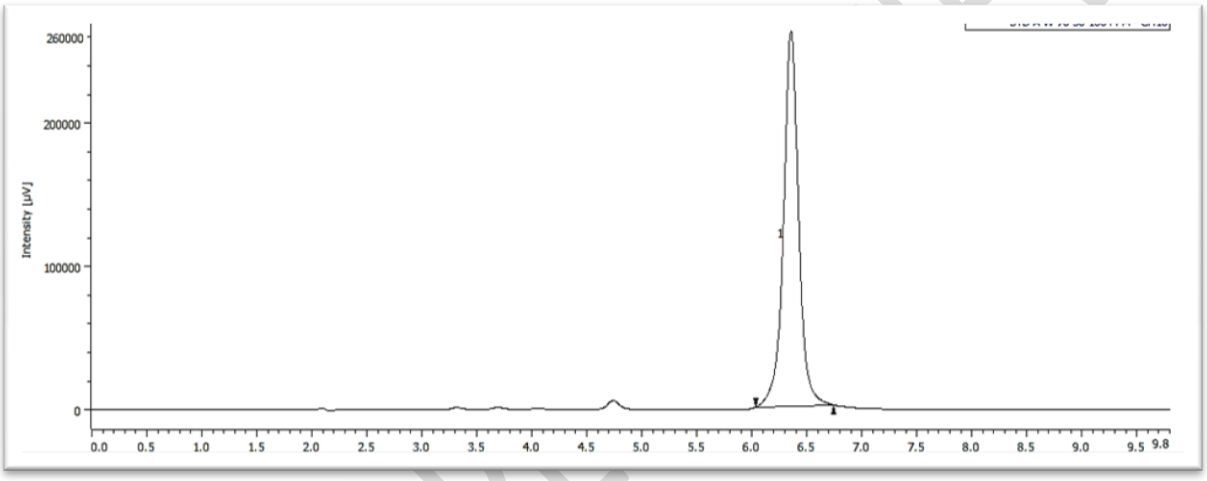
$$\%\frac{\text{w}}{\text{w}} = \frac{\text{Area of sample}}{\text{Area of standard}} \text{x} \frac{\text{Weight of standard}}{\text{Dilution factor of standard}} \text{x} \frac{\text{Dilution factor of sample}}{\text{Weight of sample}} \text{x Purity of std}$$

5 RESULTS & DISCUSSIONS

5.1 Method development

During method development, various mobile phase was tried at different flow rate and theireffects on retention time, capacity factor, area, peak symmetry and number of theoretical plateswere evaluated in HPLC to get 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 & 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 developed method of standard Mifepristone is shown in Figure3.

Fig.3. Standard Mifepristone chromatogram



5.2 Specificity

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

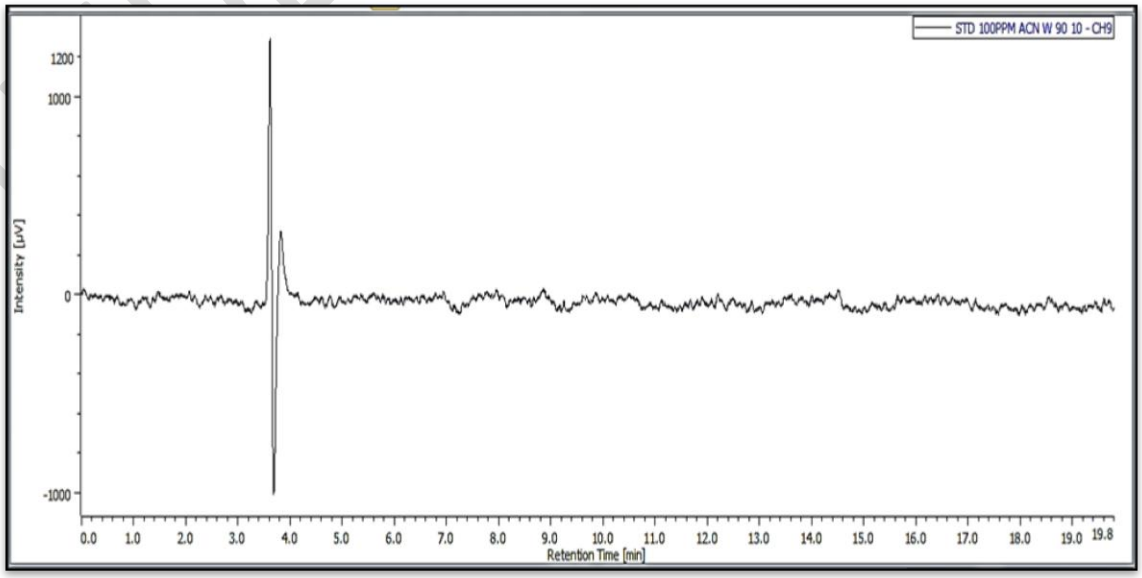


Fig. 4.Blank Chromatogram

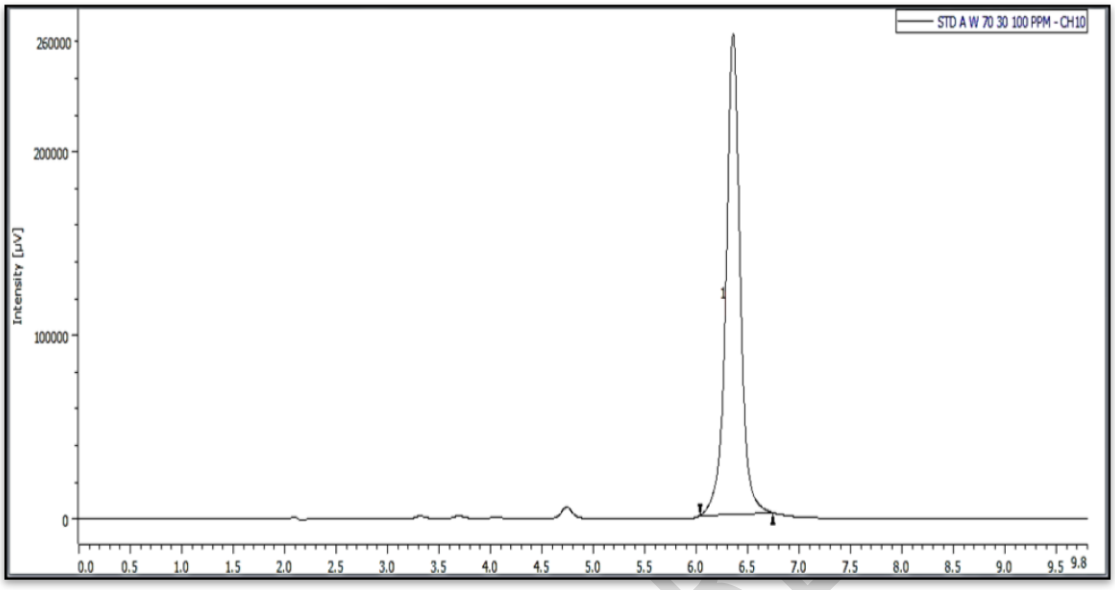


Fig.5.Standard chromatogram

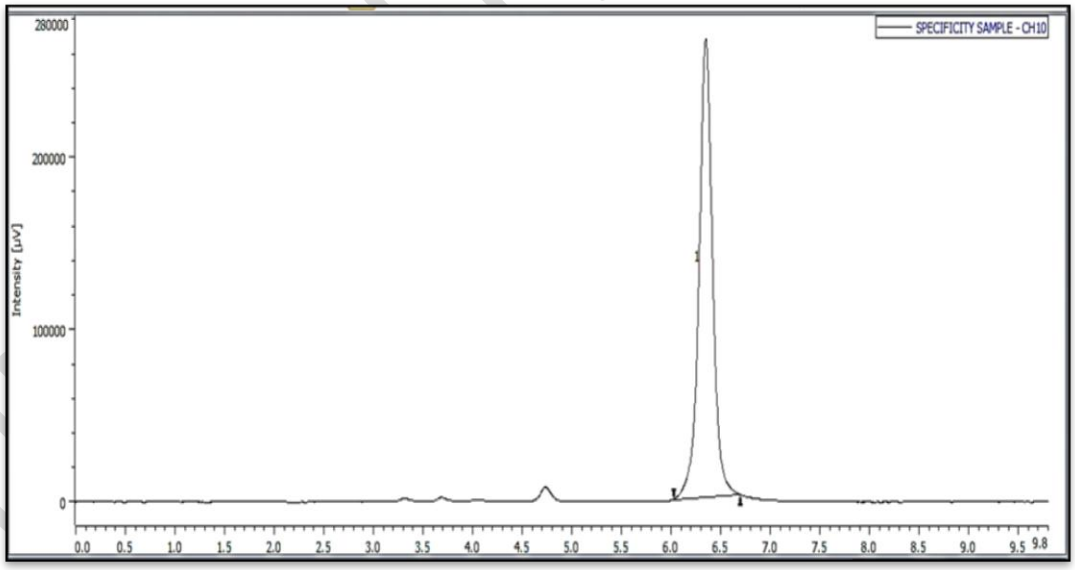


Fig.6.Chromatogram of test

5.3 System suitability test

Number of theoretical plate was found to be more than10000.The tailing factor was less than2.0. Hence the developed method is suitable for estimation of Mifepristone as shown in (Table1 & 2)

Table 1. Mifepristone standard solution system suitability results

tR	Area	NTP	Height	Symmetric factor	%RSD
6.277	2446023	5114	123999	1.400	0.4949
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	

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	0.6658
6.277	2592888	11295	267178	1.065	

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.As the developed method can detect and quantify the drug upto ppm level, thus sensitive.

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

5.5 Linearity

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

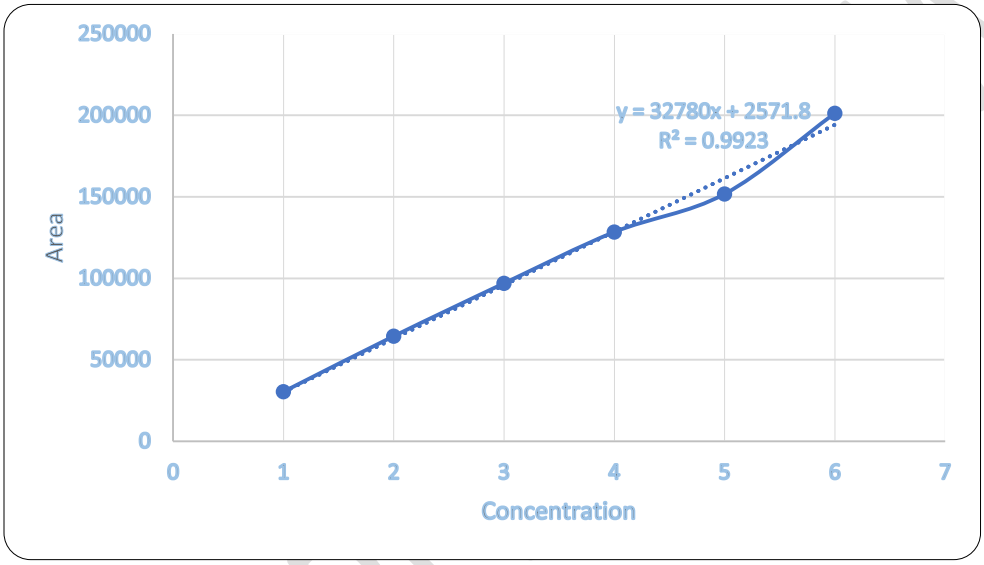


Fig.7.Calibration curve of Mifepristone

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

5.6 Accuracy

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

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	100.35% ± 0.5853	0.5832
	2	100.33		
	3	99.78		
100%	1	99.55	100.50% ± 0.2084	0.2089
	2	99.96		
	3	102		
150%	1	98.02	99.39% ± 1.185	1.1922
	2	99.82		
	3	100.30		

5.7 Precision

The result was demonstrated by intraday andintraday precision at three concentration levels1.5ppm,3ppm and4.5ppm.The values of % RSD obtained at each level of both intraday and intraday precision was less than 2 as shown in Table 7,8,9,10.As a result, the proposed approach was discovered to be precise.

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

5.8 Robustness:

The method was stated robust, as the result of %RSD was less than 2. The % w/w was fond between 98%-102% for both unaltered as shown in Table11 and altered conditions as shown in Table12,13,14.

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				**SD = 1060.22**			
Mean = 70186.13				**Mean = 62496.5**			
% RSD = 0.9053				**% 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
		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	1.4582	102 %
		2592888		
		2632670		

Mifepristone is currently used as a contraceptive. From literature review it was found that there is no reported Revere Phase HPLC method for estimation of Mifepristone. This research work describes development & validation of analytical method for analysis of Mifepristone. Kromasil C_{18} 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.

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.

ABBREVIATIONS

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

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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