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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF MIFEPRISTONE AND MISOPROSTOL IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, economic, sensitive, precise, efficient and reproducible RP-HPLC method was developed and validated for the quantitative simultaneous estimation of Mifepristone and Misoprostol in their pharmaceutical dosage form. The proposed method utilizes Symmetry-C_8 (150×4.6mm, 5µm) column and the separation was achieved by using a mixture of 0.02M Potassium Di Hydrogen Orthophosphate+0.03M Di Potassium Hydrogen Orthophosphate pH was adjusted to 3.5 using H_3PO_4:Acetonitrile(60:40) as the mobile phase at a flow rate of 1.0mL/min and column temperature was maintained at 30°C. Quantitation was achieved with UV detection at 251nm. Retention time of Mifepristone and Misoprostol were found to be 5.547min and 2.971min respectively. The method was validated for specificity, linearity, precision, accuracy and robustness. Method is specific as no interference from the blank and excipients were observed at the Retention time of any of the active ingredients. The linear regression analysis data for the calibration plots showed a good linear relationship over the concentration range of 200-1200µg/mL for Mifepristone and 0.2-1.20µg/mL for Misoprostol respectively. The Limit of Detection values for Mifepristone and Misoprostol were found to be 0.54µg/ml and 0.69µg/ml respectively. The Limit of Quantitation values for Mifepristone and Misoprostol were found to be 1.65µg/ml and 2.1µg/ml respectively. Percentage RSD of the Mifepristone and Misoprostol were found to be 0.16 and 0.212 respectively. Percentage Recovery was found to be 99.48% to 99.69% for Mifepristone and 99.70% to 99.99% for Misoprostol respectively. Statistical analysis showed that the method is repeatable and selective for the estimation of Mifepristone and Misoprostol bulk and in their pharmaceutical dosage form.

Keywords *– Mifepristone, Misoprostol, RP-HPLC, Validation.*

1. INTRODUCTION

The combination of mifepristone and misoprostol causes expulsion of the products of conception through decidual necrosis, myometrial contractions, and cervical softening. The efficacy and tolerability of Mifepristone in combination with Misoprostol for termination of early pregnancy (up to 49 days of amenorrhea) are established.

Mifepristone,*11-(4-Dimethylamino-phenyl)-17-hydroxy-13-methyl-17-prop-1-ynyl-1,2,6,7,8,11,12,13,14,15,16,17-dodecahydro-cyclopenta[a]phenanthren-3-one,* is a progestational and glucocorticoid hormone antagonist[1-2]. The chemical structure of mifepristone was shown in Fig.1. Its inhibition of progesterone induces bleeding during the luteal phase and in early pregnancy by releasing

endogenous prostaglandins from the endometrial or decidua. As a glucocorticoid receptor antagonist, the drug is used to treat hypercortisolism in patients with no pituitary cushing syndrome.

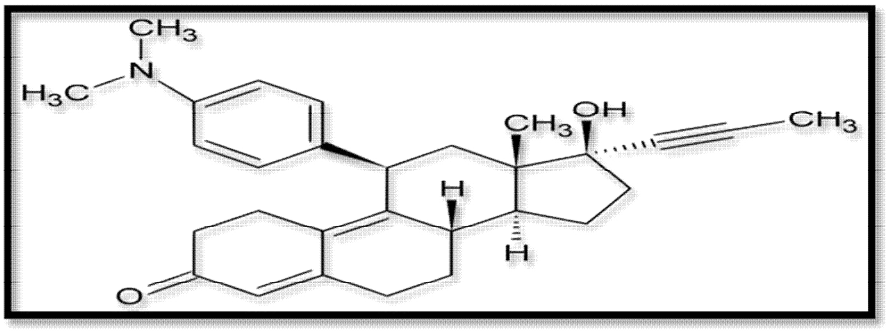


Fig.1: Chemical structure

The anti-progestational activity of mifepristone results from competitive interaction with progesterone at progesterone-receptor sites. Based on studies with various oral doses in several animal species, the compound inhibits the activity of endogenous or exogenous progesterone which results in the termination of pregnancy.

Literature survey reveals that Spectrophotometric and HPLC methods have been reported for their individual analysis and also along with other combinations in bulk drug and pharmaceutical formulations. The methods reported for mifepristone are development and in vitro/in vivo evaluation of a silastic intra vaginal ring for mifepristone delivery by HPLC method[4], simultaneous determination of mifepristone and monodemethyl-mifepristone in human plasma by liquid chromatography–tandem mass spectrometry method[5], development of a high-performance liquid chromatographic method for the determination of mifepristone in human plasma using norethisterone[6], HPLC method for the determination of ng mifepristone in human plasma[7], determination of mifepristone levels in wild canid serum using liquid chromatography[8].

Misoprostol, *Methyl 7-((1R, 2R, 3R)-3-hydroxy-2-((S, E)-4-hydroxy-4-methyloct-1-enyl)-5-oxocyclopentyl) heptanoate* is a synthetic analogue of natural prostaglandin E1[9]. It produces a dose-related inhibition of gastric acid and pepsin secretion, enhances mucosal resistance to injury. It is an effective anti-ulcer agent and also has oxytocic properties. The chemical structure of Misoprostol was shown in figure-2. This compound belongs to the class of organic compounds known as prostaglandins and related compounds. These are unsaturated carboxylic acids consisting of a 20 carbon skeleton that also contains a five member ring, and are biosynthesized from the fatty acid arachidonic acid.

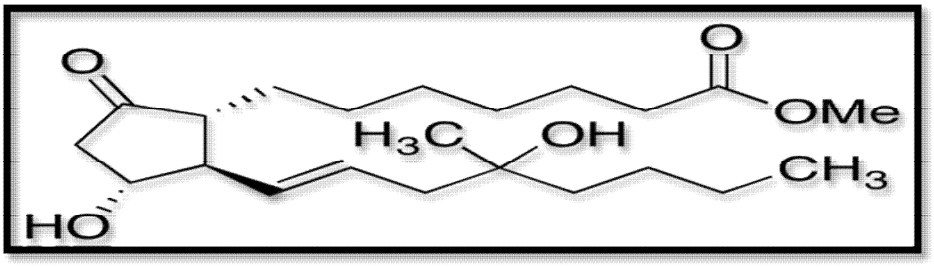


Fig.2: Chemical structure

Misoprostol seems to inhibit gastric acid secretion by a direct action on the parietal cells through binding to the prostaglandin receptor. The activity of this receptor is mediated by G proteins which normally activate adenylate cyclase. Misoprostol has also been shown to

increase the amplitude and frequency of uterine contractions during pregnancy via selective binding to the EP-2/EP-3 prostanoid receptors.

Literature survey reveals that Spectrophotometric and HPLC methods have been reported for their individual analysis and also along with other combinations in bulk drug and pharmaceutical formulations. The methods reported for misoprostol are validated HPLC method for misoprostol and combination with diclofenac sodium in bulk drug and formulation[10], development and validation of highly sensitive method for determination of misoprostol free acid in human plasma by liquid chromatography-electrospray ionization tandem mass spectrometry[11], development and validation of an *in vitro* dissolution method with HPLC analysis for misoprostol[12].

Literature survey reveals that a simultaneous estimation method was not reported for the combination of Mifepristone and Misoprostol drugs. So an attempt was made to develop and validate an economic, rapid reverse-phase high performance liquid chromatographic method for the estimation of Mifepristone and Misoprostol in bulk and pharmaceutical dosage form. The method was validated and found to be accurate, precise, efficient and reproducible.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Mifepristone and Misoprostol were obtained from Danco labs and Novel labs respectively. Methanol (HPLC grade), Acetonitrile (HPLC grade), water (HPLC grade) and 0.02M Potassium Di Hydrogen Orthophosphate, 0.03M Di Potassium Hydrogen Orthophosphate were obtained from Merck chemicals. Distilled water was prepared using a Milli-Q system (Millipore). Nylon syringe filters (0.45 µm) were from Millipore.

2.2 Equipment

Chromatographic separation was achieved using HPLC System (Waters Alliance 2695 Separation Module) containing UV detector. The output signal was monitored and processed using Empower Software®. The analytical balance used was from Sartorius, Model-BSA224SCW. UV spectrophotometer used was from Shimadzu, UV-3000.

2.3 Selection of UV Wavelength

10ppm solution of each Mifepristone and Misoprostol was prepared separately in methanol. UV scan of the above solutions were carried out over a wavelength range of 200–400 nm by using the Shimadzu UV spectrophotometer, Model- UV-3000. The detection wavelength was set at 251 nm because all the components exhibited higher responses. An overlaid UV absorption spectrum is shown in Fig.3.

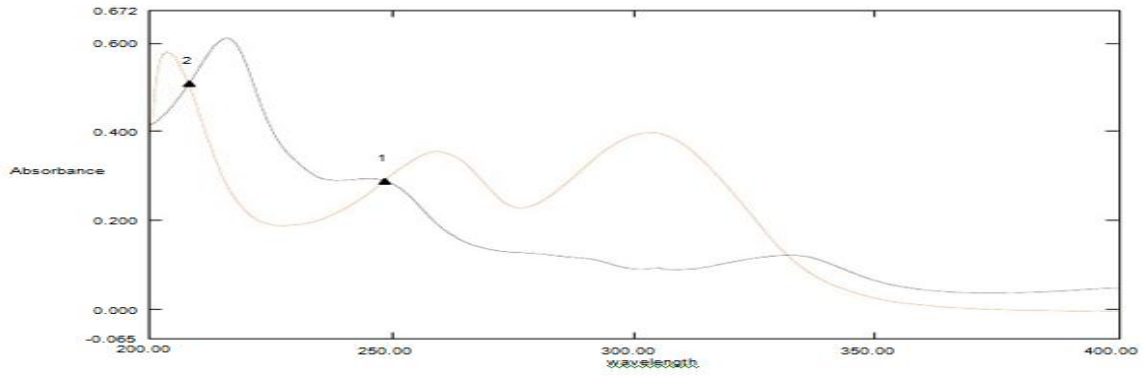


Fig.3: Isobestic point of Mifepristone and Misoprostol

2.4 HPLC Analytical Conditions

The proposed method utilizes Symmetry-C_8 (150×4.6mm, 5µm) column was used as the stationary phase and the separation was achieved by using a mixture of 0.02M Potassium Di Hydrogen Orthophosphate+0.03M Di Potassium Hydrogen Orthophosphate, pH was adjusted to 3.5 using H_3PO_4: Acetonitrile (60:40) as the mobile phase at a flow rate of 1.0mL/min and column temperature was maintained at 30°C. The detector was set at the wavelength of 251nm. Injection volume was 20µl. Sample and standard preparation was done in a solvent mixture of methanol and water in the ratio of 50:50v/v.

2.5 Preparation of standard solution

Weighed accurately 0.2 mg of Misoprostol working standard and 200 mg of Mifepristone working standard into 25 ml volumetric flask. 10 ml of diluent was added, sonicated for 10 minutes and made up to volume with the diluent. 1 ml of standard stock solution was transferred into 10 ml volumetric flask and made up to volume with diluent.

2.6 Preparation of Sample solution

Preweighed sample of 20 tablets was finely powdered and sample quantitatively equivalent to 0.2 mg of Misopristol and 200 mg of Mifepristone was weighed accurately and transferred into 25 ml volumetric flask. 10 ml of diluent was added, shake well, sonicated for 10 minutes and dilute to volume with diluent. Filter the solution. Dilute 1 ml of filtrate to 10 ml with diluent.

2.7 Method validation

The developed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines[13], Validation of Analytical Procedures[14-15]: Q2(R1), for the parameters like system suitability, linearity and range, precision (repeatability), intermediate precision (ruggedness), specificity, accuracy and robustness.

2.8 System suitability

The system suitability studies can be defined as tests to ensure that the method can generate results of accuracy and precision. The system suitability studies were carried out as specified in USP. The parameters like number of theoretical plates (N), Tailing factor, Resolution (Rs) and relative standard deviation (RSD) of peak height or peak area or repetitive injections were studied. The system suitability test performed according to USP36.14. The standard solution was injected six times and results were recorded to find the adequate peak separation (resolution), percentage relative standard deviation for area, retention time, symmetry factor and theoretical plates.

2.9 Precision

The precision of an analytical method expresses the closeness of agreement (degree of scatter) between the series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Method precision was evaluated by injecting six different sample preparation. Different analyst from the same laboratory evaluated the intermediate precision of the method. The assay of these samples was determined. Precision and intermediate precision of the method was evaluated by calculating the %RSD.

2.10 Linearity

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. The linearity of detector response was determined by preparing a series of solution of the working standards (mixture of all active ingredients) over the range of 80% to 120% of targeted concentration. These solutions were injected into the chromatographic system and response area was recorded.

2.11 Limit of Detection

The detection of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantiated under stated experimental conditions. Limit of detection values were found to be 0.69µg/ml and 0.54µg/ml respectively.

2.12 Limit of Quantification

The quantification limit of an individual analytical procedure is defined as the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. Limit of quantification values of Misoprostol and mifepristone were found to be 2.1µg/ml and 1.65µg/ml respectively.

2.13 Accuracy

To study the accuracy of the method recovery experiments were carried out. The accuracy of the test method was determined by preparing recovery samples (spiking method) at the level of 80%, 100% and 120% of targeted concentration. The recovery samples were prepared in triplicate at each level. The contents were determined from the respective chromatograms. The samples at different levels were chromatographed and the percentage recovery for the amount added was calculated.

2.14 Robustness

Small, deliberate changes in temperature and flow rate were made to the chromatographic condition. A study was performed to determine the effect of variation in the temperature and flow rate. Standard solution prepared as per the test method and was injected into the HPLC system at 35°C and 45°C temperature. Flow rate change was done by varying flow rate at 0.8 ml/min and 1.2 ml/min.

3. RESULTS AND DISCUSSION

System suitability parameters proved that the proposed method suits for the simultaneous estimation of Mifepristone and Misoprostol. Chromatogram for Mifepristone and Misoprostol was found satisfactory on Symmetry-C_8 (150×4.6mm, 5µm). Drug peaks were found symmetrical as observed from asymmetry factor. Resolution of the proposed method was satisfactory. The optimized chromatographic conditions were shown in Table-1. Representative chromatograms were shown in Fig.5 and 6. System suitability parameters were given in Table-2. UV detection was set at 251nm.The data of precision were given in Table-3 and 4. The percentage RSD value was less than 2%. Sensitivity of the method was good and also linearity was observed over a wide concentration range of 200-1200µg/mL for Mifepristone and 0.2-1.20µg/mL for Misoprostol respectively. The correlation coefficients for individual analytes were found to be within the limits for Misoprostol r^2 =0.9995 and for mifepristone r^2 =0.9996. The linearity data were given in Table-5 and Fig.4. Accuracy of the method was determined by recovery with spiked concentration of pure drug at three levels for Mifepristone and Misoprostol. Recovery of drug was well within the acceptance limits of 98.0-102.0%. %RSD obtained from the precision results was less than 2.0%. So the developed method was said to be accurate and precise. Variation in temperature and flow rate, it was observed that there were

no marked changes obtained in the chromatograms, which demonstrated that the method developed is robust. Resolution, symmetry factor and theoretical plate limits for flow rate variation and temperature variation are within the acceptance criteria, which show that the method exhibit good system suitability under given set of conditions.

Table 1: Optimized chromatographic conditions and system suitability parameters for proposed HPLC method for Mifepristone and Misoprostol

Parameter	**Chromatographic conditions**
Instrument	WATERS 2695-High performance liquid chromatography
Column	SYMMETRY-C_8 Column (150×4.6mm,5µm).
Detector	WATERS 2498 UV detector
Diluent	Water:Methanol(50:50%v/v)
Mobile phase	0.02M Potassium Di Hydrogen Orthophosphate+0.03M Di Potassium Hydrogen Orthophosphate pH was adjusted to 3.5 using H_3PO_4:Acetonitrile(60:40)
Flow rate	1.0ml/min
Detector wavelength	251nm
Run time	10min
Retention time	2.971(Misoprostol) 5.547(Mifepristone)
Injection volume	20µl
Mode of separation	Isocratic mode
Column temperature	30°C

Table 2: Results of System suitability

Parameters	**Misoprostol**	**Mifepristone**
Retention time	2.971	5.547
Plate number	3606.16	7367.19
Tailing factor	1.09	1.01

Table 3: Results of System precision

Injection No.	Area of Misoprostol	Area of Mifepristone
1	299947	2594732
2	300688	2602820
3	300840	2602590
4	300104	2601038
5	301309	2608071
6	301550	2601832
MEAN	300740	2601847
Standard deviation	636.5	4278.20
%Relative standard deviation	0.212	0.16

Acceptance criteria: Percentage RSD should not be more than 2%.

Table 3: Results of Method Precision

Injection No.	Area of Misoprostol	Area of Mifepristone
1	300452	2595765
2	300348	2604833
3	301254	2601285
4	300332	2600845
5	302342	2602065
6	301462	2604185
MEAN	301032	2601496
Standard deviation	805.5	3224.99
%Relative standard deviation	0.268	0.12

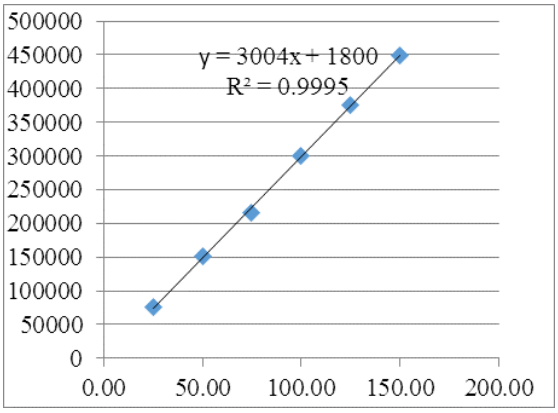
Acceptance criteria: %RSD should not be more than 2%.

Table 5: Results of linearity data

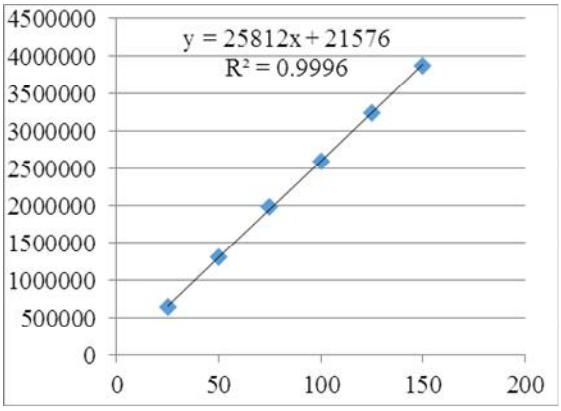
Linearity level	Peak Area of Misoprostol	Peak Area of Mifepristone
25%	75341	654657
50%	151139	1308618
75%	214990	1985539
100%	300364	2598655
125%	375130	3247711
150%	449690	3885729

Table 6: Limit of detection (LOD) and Limit of quantification (LOQ)

Drugs	Limit of detection (LOD)	Limit of quantification (LOQ)
Mifepristone	0.54µg/Ml	1.65µg/mL
Misoprostol	0.69µg/Ml	2.1µg/mL



Misoprostol



Mifepristone

Fig.4: calibration curves for Misoprostol and Mifepristone

Table 7: Results of accuracy data

Analyte	Concentration	Average Area	Amount added (mg)	Amount found (mg)	% Recovery
Misoprostol	80%	240984	0.16	0.15952	99.70%
	100%	301245	0.2	0.19998	99.99%
	120%	364145	0.24	0.2399	99.96%
Mifepristone	80%	2045245	160	159.168	99.48%
	100%	2596541	200	199.38	99.69%
	120%	3098815	240	238.896	99.54%

%Recovery = amount found/ amount added × 100

Acceptance criteria: The %Recovery should be100 ± 2%

Table 8: Results of Robustness data

Parameter	Misoprostol		Mifepristone	
	Retention time	Theoretical plates	Retention time	Theoretical plates
Flow rate-(0.8ml)	2.986	5353.03	5.998	11942.07
Flow rate-(1.2ml)	2.741	4230.99	5.090	10264.16
Column Temperature-(25°C)	2.973	4551.88	5.986	11309.80
Column Temperature-(35°C)	2.983	4748.38	5.876	10852.43

Acceptance criteria: Retention time of sample peaks should be comparable with proposed method.

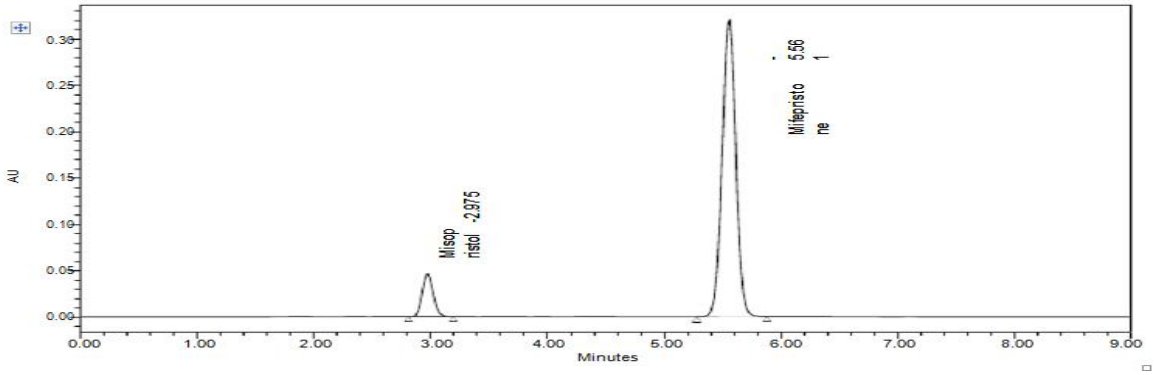


Fig.5: HPLC chromatogram of standard Misoprostol and Mifepristone

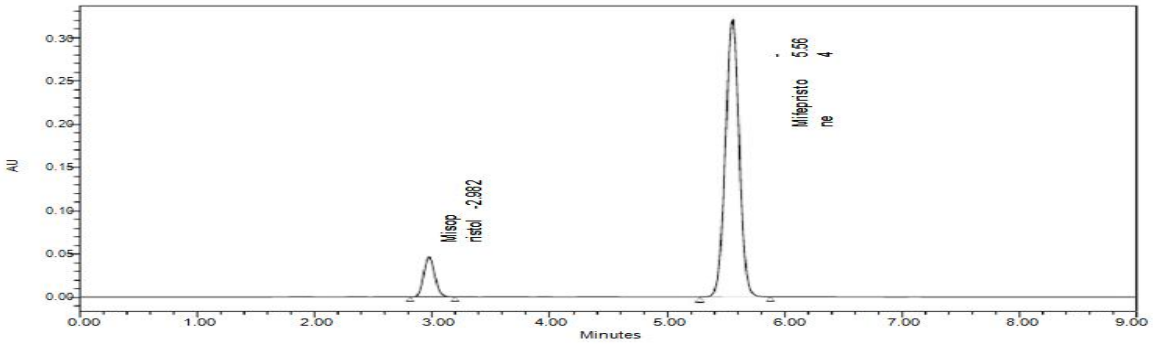


Fig.6: HPLC chromatogram of sample Misoprostol and Mifepristone

4. CONCLUSION

The RP-HPLC method developed for quantitative simultaneous estimation of Mifepristone and Misoprostol in bulk and pharmaceutical dosage form is simple, economic, sensitive, precise, efficient and reproducible and is suitable for its intended purpose. The method was validated as per ICH guidelines, showing satisfactory data for all the method validation parameters tested. Hence, the proposed method can be employed for assessing the quantitative determination of Mifepristone and Misoprostol in bulk and pharmaceutical dosage form.

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REFERENCES

1.http://www.drugbank.ca/drugs/DBO1609.

2.www.drugs.com/availability/generic-exjade.html.

3.U.S. Food and Drug Administration. Mifeprex (mifepristone) Information; [cited Jul 14]. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/label/2000/20687lbl.htm

4.X Duan, M Ning. "Development and in vitro/in vivo evaluation of a silastic intra vaginal ring for mifepristone delivery". Indian journal of pharmaceutical sciences. 2015, 77(3): 335-342.

5.Cheng Tang, Hui-chang Bi et al. "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. Jan 23 2009, 23(1): 71-80.

6.Guo Z, Wang S et al. Development of a high-performance liquid chromatographic method for the determination of mifepristone in human plasma using norethisterone as an internal standard: application to pharmacokinetic study. Contraception. Sep2007, 76(3): 228-32.

7.Geo Z, Chu C et al. An HPLC method for the determination of ng mifepristone in human plasma. Journal of Chromatography B. 2006, 832: 181–184.

8.Stith C, Hussain MD et al. "Determination of mifepristone levels in wild canid serum using liquid chromatography". Journal of Chromatography B. 2003, 794: 9-15.

9.Parfit K and Martindale. The complete drug reference; 35th edition; Vol-I, Pharmaceutical press, 2007, 1877.

10.Sunil R. Dhaneshwar, Vidhya K. Bhusari. "Validated HPLC Method for Simultaneous Quantitation of Diclofenac Sodium and Misoprostol in Bulk Drug and Formulation". Der Chemica Sinica Journal from Pelagia Research Library. 2010, 1(2): 110-118.

11.Vijaya Bharathi D, Jagadeesh B et al. Development and validation of highly sensitive method for determination of misoprostol free acid in human plasma by liquid chromatography-electrospray ionization tandem mass spectrometry: application to a clinical pharmacokinetic study. Sep 2011, 879(26): 2827-33.

12.Jigar Mehta, Kanhaiyalal Patidar, et al. Development and validation of an *in vitro* dissolution method with HPLC analysis for misoprostol in formulated dosage form 2010, 1: 198-201.

13.The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use, Validation of Analytical Procedure: Text and Methodology, ICH Q2 (R1), 2005, Geneva, Switzerland.

14.ICH guidelines, "Validation of Analytical Procedure: Methodology Q2B", I.C.H. Harmonized Tripartite Guidelines. 1996, 6-13.

15. ICH guidelines, "Validation of Analytical Procedures Q2A", ICH Harmonized Tripartite Guideline, Mar. 1995, 1-5.