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DEVELOPMENT AND VALIDATION OF SIMPLE AND VERY SENSITIVE HPLC METHOD WITH UV DETECTION FOR MISOPROSTOL- 0.1 MG TABLETS

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ABSTRACT

A selective HPLC method with ultraviolet detector was described for the determination of Misoprostol 0.1 mg tablets by invitro dissolution study. After a confined dissolution period the misoprostol present in the aliquots of dissolution media was derivatized by methanolic potassium hydroxide. The technique involves the conversion of misoprostol to its derivative which will show maximum absorbance at 285nm. The chromatographic separation was performed using reversed phase, Thermo MOS Hypersil, 150x4.6mm, 5µ column. The mobile phase was composed of 50 volumes of buffer and 50 volumes of methanol, the buffer consisting of 10 ml triethylamine in 1000 ml of water adjusted to pH 6.0 ± 0.05 with ortho-phosphoric acid, the detector used is a simple ultraviolet detector and the monitored wavelength is 285nm. The method is subjected for monitoring the parameters like specificity, accuracy, precision, linearity, ruggedness, robustness and solution stability. All the parameters are found to be at very appreciable level.

KEY WORDS: Misoprostol, Tablets, Dissolution method, U V Detection, High performance liquid chromatography.

1. INTRODUCTION

Misoprostol is a molecule having Molecular formulae $C_{22}H_{38}O_5$, Molecular weight 382.5, chemically it is mixture of methyl 7-[(1*RS*,2*RS*,3*RS*)-3-hyd8EPMy-2-[(1*E*,4*RS*)-4-hydroxy-4-

methyloct-1-enyl]-5-oxocyclopentyl] heptanoate and methyl 7-[(1*RS*,2*RS*,3*RS*)-3-hyd8EPMy-2-[(1*E*,4*SR*)-4-hyd8EPMy-4-methyloct-1-enyl]-5-oxocyclopentyl] heptanoate.

Misoprostol used in the therapy to prevent non-steroidal anti-inflammatory drug induced gastric ulcers, for treating missed miscarriage, in some cases used to induce labor. Postpartum hemorrhage is a leading cause of material morbidity and mortality. Active management of the third stage of labour, including use of uterotonic agent has been shown to reduce blood loss. Misoprostol (a prostaglandin E1 analogue) has been suggested for this purpose because it has strong uterotonic effects and can be given orally. It does not need refrigeration for storage. Misoprostol alone is used for terminating early pregnancies [1-8]. A growing body of evidence has now shown that Misoprostol can be used as a single agent to induce early abortion. Misoprostol is an orally active prostaglandin analogue which affects the uterine tone [9]. A large number of studies are available using Mifepristone followed by Misoprostol which results in abortion in more than 90% of women with pregnancies up to 7 weeks of gestation [10,11]. Misoprostol reduces serious gastrointestinal complications in patients with rheumatoid arthritis receiving Nonsteroidal anti-inflammatory drugs [12]. Some methods are available for dissolution of misoprostol, liquid chromatographic methods are reported for determination of Misoprostol by direct method where the injection volume is very high and the dose of is Misoprostol 0.2 mg in tablets [13]. There are methods where misoprostol is estimated with other drugs directly by high performance liquid chromatographic methods [14]. The drug is official in pharmacopoeias, but dissolution methods are not available [15-17].

There are no reports or literature for the determination of Misoprostol 0.1 mg tablets by invitro dissolution study.

2. MATERIALS AND METHODS

2.1 Materials

Misoprostol reference standard procured from United States of pharmacopoeia and Misoprostol 1% HPMC dispersion was procured from Chemvon biotechnology Co Ltd, Shanghai, China were used. The pharmaceutical formulation as tablets having label claim 0.1mg of misoprostol with other excipients was from Cipla limited, India. Methanol of HPLC grade was purchased from Merck. Potassium hydroxide Merck (AR grade) Ortho-phosphoric acid Merck, (AR grade) and triethylamine Merck, (AR grade) for mobile phase high pure HPLC grade water (Millipore, Bedford, MA, USA), for dissolution media Purified water were used in the present experimental study.

2.2 Chromatographic conditions

The mobile phase was prepared by mixing 500 ml of methanol with 500 ml of buffer, made by adding 10 ml of triethylamine in 1000 ml of water and adjusted to pH 6.0 ± 0.05 with ortho-phosphoric acid, and filtered through 0.45µ nylon filter. The chromatographic reversed phase separation was performed by using Thermo MOS Hypersil, 150x4.6mm, 5µ column. The HPLC system (Shimadzu 2010) set at a flow rate of 1.5 ml/min, column oven configured at 25ºC and ultraviolet detector configured at 285 nm were used.

The samples were injected using auto sampler chiller temperature configured at 20ºC. The approximate retention times for misoprostol and its derivative were 5 and 8 minutes respectively. The misoprostol was monitored at 203 nm and its derivative was monitored at 285 nm in the specificity study, the total run time was 15 minutes.

2.3 Reagents Required

Methanolic Potassium hydroxide: Accurately weighed and transferred 5grams of Potassium hydroxide in 100 ml volumetric flask and added 5ml of water, allowed to melt the pellets with the self generated heat and made up to volume with methanol.

Hydrochloric acid solution: Transferred 8.5 ml of concentrated hydrochloric acid in 100ml volumetric flask containing 50 ml of water, mix well and made up to the volume with water.

2.4 Standard stock solution

Weighed 100 mg of misoprostol dispersion (1% in HPMC) in 200 ml dry volumetric flask (Note: spread well to avoid heap formation, heap can results swelling of HPMC will create problem in releasing of misoprostol) and added 50 ml of water to disperse and sonicated for 10 min, made up to volume with water and mixed well.

20 ml of above stock transferred to 500ml volumetric flask and made up to volume.

2.5 Derivatization process

Transferred 20 ml of Blank water, standard and sample in different 25 ml volumetric flasks. To each flask added 2 ml of methanolic potassium hydroxide, allow it to stand for 1 hour at room temperature with intermittent shaking to undergo the process of derivatization and added 2ml of hydrochloric acid solution to neutralize. Mix well and made up to volume with water, filtered through 0.45µ nylon filter and injected.

2.6 Dissolution condition

The dissolution experiments were performed on Electrolab dissolution apparatus. The testing was optimized and performed in compliance with USP chapter-711 using the apparatus with Paddle (Type-II), configured to the speed of 50 revolutions per minute with dissolution media as purified water, volume of 500ml adjusted to temperature 37ºC ± 0.5, the dissolution optimum time point was considered as 30 minutes.

The formulation is tablet and hence paddles are selected, the minimum revolutions per minute are 50 selected for monitoring the stability changes in the formulation, water is weak buffer, based on the nature of the molecule as it is neutral hence water is selected as dissolution media and the dose is very less (0.1 mg maximum 0.2 mg) 500 ml is well sufficient to attain sink condition was selected.

2.7 Sample preparation

After completion of dissolution time allow to settle for 15 min and pipette out 25 ml in 50 ml test tubes. Transferred the 20 ml of above sample in different 25 ml volumetric flasks, to each flask 2 ml of methanolic potassium hydroxide was added, allow it to stand for 1 hour with intermittent shaking to undergo the process of derivatization and 2ml of hydrochloric acid solution was added to neutralize. Mix well and made up to volume with water, filtered through 0.45µ nylon filter and injected. Standard and sample concentrations were 0.0002 mg/mL

2.8 Validation parameters

The dissolution method was developed based on the identification test in USP and validated as per USP & ICH guidelines [18-21].

For the examination of specificity of the method, drug-free sample matrix was derivatized and tested. The sample was prepared according to the confined time points and derivatization procedures described in sections 2.5. Then, a chromatogram of a sample containing 8-epimisoprostol was compared with a chromatogram of the placebo and found to be no interference.

Linearity of the calibration curve was estimated for the peak area of derivative ranging from 0.0001 mg/ml to 0.0003 mg/ml.

Intra-day precision of the elaborated method was determined for 0.0002 mg/ml Misoprostol derivative concentrations, for six samples of each concentration. Inter-day precision was

estimated for the sample on the different day using the same concentration. The precision was expressed as CV. Accuracy was estimated for the concentration of 0.0001, 0.0002 and 0.0003 mg/ml of Misoprostol derivative concentrations of the method. The recovery obtained was between 98 to 102 %. The samples were extracted according to the procedure described in Section 2.5.

To demonstrate stability of the standard solution by comparing data of absolute difference in % assay at each interval with respect to initial value. The standard and sample solutions were stored at room temperature and analyzed at initial, about 6, 12 and 24 hours. The absolute differences between % assay values be within 2.0 % with respect to the initial value are considered as stable.

The system was allowed to stabilize for 2 hours. Standard solution and sample solution were prepared and kept at room temperature. Blank injected in once, as a system suitability standard solution was injected in six replicates. Samples injected in single and were analyzed as described in analytical methodology. From above experimental study, standard and sample solutions are found stable up to 24 hours at room temperature.

3.0 RESULTS AND DISCUSSION

3.1 Derivatization procedure and HPLC

The drug misoprostol was having low ultraviolet absorption, the response was very low and also the dose is also a limiting step for detection. Dissolution of such drugs can be estimated, but it demands for sensitive detectors like mass. Author was confined to estimate the drug on UV detector by using the technique of simple derivatization, the absorbance of the molecule was considerably increased by the derivatization process, the measurable wavelength shifted to 284nm. Where misoprostol it self having absorbance at 203nm. The derivatization agent used was very simple, stable, easily available and cost effective

3.2 Optimization of derivatization agent concentration

The derivatization reagent was selected (Reference of USP) potassium hydroxide; the reagent concentration was optimized by preparing different concentrations of potassium hydroxide 0.1, 0.2, 0.5, 1 and 2 molar and used for the study to get complete derivatization. From the above systematic study it was concluded that the 1N potassium hydroxide was sufficient for complete derivatization.

3.3 Optimization of derivatization time

The time required for the complete derivatization of the misoprostol was confined by systematic study of neutralization and injection of samples at different intervals like 10, 20, 30, 45, 60, 90, 120, and 180 minutes.

The time required for complete derivatization was found to be 60 minutes.

3.4 Optimization of derivatization volume

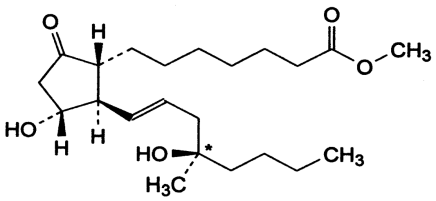
The derivative agent used in the study was 1N potassium hydroxide, the volume of the agent required for complete derivatization was optimized by using different volumes of reagent 0.1 ml 0.2 ml, 0.5ml, 1 ml, 2 ml, and 3ml in the reaction.

The 1.0 ml was found to be sufficient and hence 2.0 ml was used as the optimum reagent volume required for the derivatization.

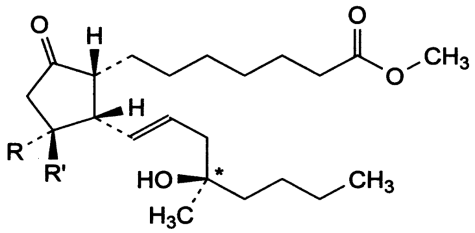
3.5 Optimization of derivatization temperature

Temperature is the most important factor can influence the rate of reaction. The experiment was conducted for optimization of temperature. Different temperature was used 20, 37 and 50ºC. The 50 ºC was found to be more effective, but author selected 37ºC to avoid volume changes with changing the temperature. Hence the experiment was performed at 37ºC.

Figure-1



its epimer at C* and their enantiomers



A.R=H,R^1=OH: mixture of methyl 7-[(1*RS*,2*SR*,3*SR*)-3-hyd8EPMy-2-[(1*E*,4*RS*)-4-hyd8EPMy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate and methyl 7-[(1*RS*,2*SR*,3*SR*)-3-hyd8EPMy-2-[(1*E*,4*SR*)-4-hyd8EPMy-4-methyloct-1-enyl]-5-oxocyclopentyl] heptanoate (8-epimisoprostol).

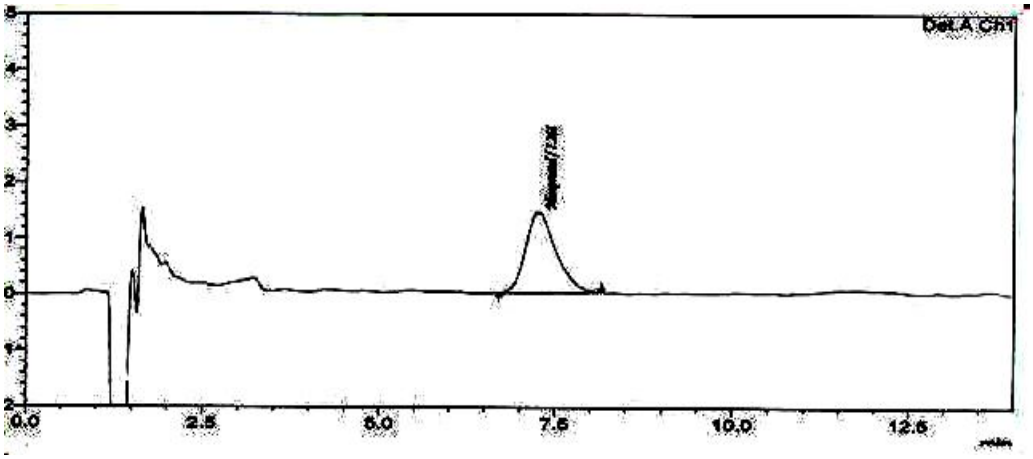


Fig-2: Standard (Misoprostol Derivative)

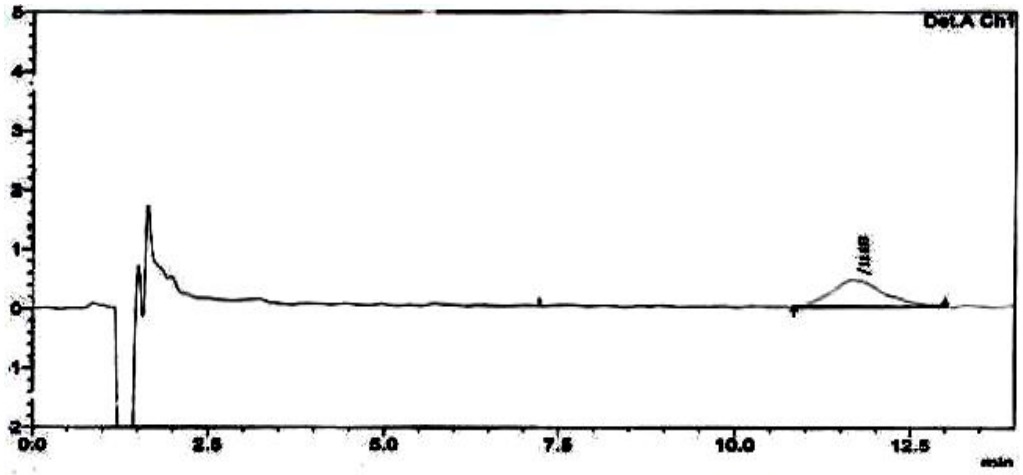


Fig-3: Placebo

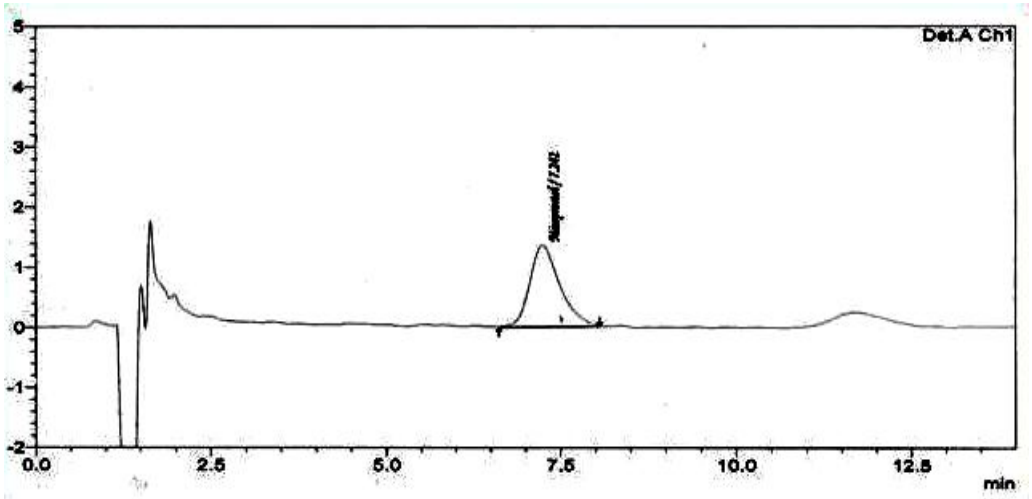
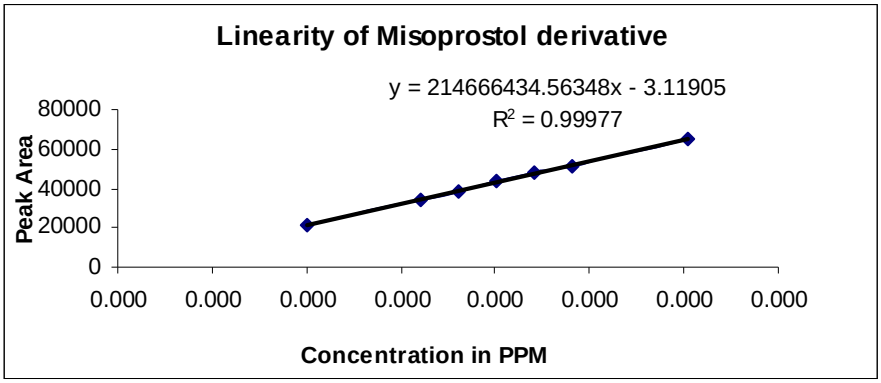


Fig- 4: Sample

Validation Tables:

1) **Linearity :**

Linearity level	Conc. in ppm	Experimental area (a)	Predicted area (y)	Residuals (b)
I	0.000101	21498	21577	-79.3
II	0.000161	34399	34526	-126.5
III	0.000181	38863	38842	21.4
IV	0.000201	43270	43158	112.3
V	0.000221	47877	47474	403.2
VI	0.000241	51582	51790	-207.9
VII	0.000302	64615	64738	-123.1
Correlation	0.9999			
Intercept (c)	-3.12			
Slope (m)	214666435			



Linearity plot

2) **Accuracy for Misoprostol derivative:**

Accuracy level	Mcg/ml added	Mcg/ml found	% Recovery
50% Spl-1	0.0001	0.0001	98.24
50% Spl-2	0.0001	0.0001	96.90
50% Spl-3	0.0001	0.0001	99.28
100% Spl-1	0.0002	0.0002	98.85
100% Spl-2	0.0002	0.0002	97.50
100% Spl-3	0.0002	0.0002	99.90
150% Spl-1	0.0003	0.0003	99.41

150% Spl-2	0.0003	0.0003	100.65
150% Spl-3	0.0003	0.0003	100.58
		Mean	99.03
		S.D	1.30
		%RSD	1.31

3) Precision:

a) System Precision

Injection	Response (Area) for Misoprostol Derivative
1	43162
2	42830
3	42641
4	42215
5	42637
6	42211
Mean	42616
SD	366.11
%RSD	0.86

b) Method precision

Injection	% Label Claim of Misoprostol Derivative
1	101.20
2	99.40
3	101.00
4	100.40
5	101.20
6	100.80
Mean	100.67
SD	0.69
%RSD	0.68

c) Intermediate precision

Injection	Day-1	Day-2
1	101.20	100.00
2	99.40	99.00
3	101.00	101.00
4	100.40	99.00
5	101.20	101.00
6	100.80	101.00
Mean	100.67	100.17
SD	0.69	0.98
%RSD	0.68	0.98
%RSD	0.85	

4) Solution stability

a) Stability of analytical standard solution for Misoprostol derivative

S. No.	Time	% Label claim of Misoprostol Derivative	%Correlation
1	Initial standard	99.8	-------
2	7 Hours standard	100.6	100.8
3	15 Hours standard	101.2	99.4
4	20 Hours standard	99.3	98.1
5	24 Hours standard	98.9	99.6

b) Stability of analytical standard solution for Misoprostol derivative

S. No.	Time	% Label claim of Misoprostol Derivative	%Correlation
1	Initial sample	98.5	------
2	7 Hours sample	98.6	100.1
3	15 Hours sample	99.6	101.1
4	20 Hours sample	100	101.5
5	24 Hours sample	99.8	101.2

4. CONCLUSION

A successful high performance liquid chromatographic method is developed for dissolution samples estimation, the special quality of the method is derivatization process. Misoprostol is molecule not only having very less UV absorbance, moreover the dose is very less, the monitoring wavelength is resides in weak UV region below 205nm and hence it is very difficult to develop accurate and precise methods, here the derivatization gives soluble stable product to estimate near 285nm at higher wavelengths base line noise is largely reduced, the step resulted viability to precision and accuracy of the method. The method was validated as per ICH guidelines and found the method is simple, specific, accurate, precise, linear, rugged and robust, the solution are stable more than a day.

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