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# Method Development and Validation of Mifepristone and Misoprostol by RP-HPLC

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**ABSTRACT —** A rapid, sensitive, and accurate RP-HPLC method was developed for the identification and quantification of Misoprostol and Mifepristone using a Waters HPLC system equipped with a PDA detector. Chromatographic separation was achieved on an Inertsil ODS-C18 column (250 × 4.6 mm, 5 µm). The mobile phase consisted of Acetonitrile and Water in a ratio of 85:15 (v/v), filtered and degassed prior to use. The flow rate was maintained at 1.0 mL/min and detection was performed at 305 nm.

**Key words:** Mifepristone, Misoprostol, RP-HPLC

## I. INTRODUCTION

Medabon is a brand name for a combination therapy containing two medicines, mifepristone and misoprostol, used for the medical termination of an early pregnancy. Mifepristone (6) is an anti-progestational steroid that works by blocking the effects of progesterone, a natural hormone essential for maintaining pregnancy. By inhibiting progesterone from binding to its receptors in the uterus, mifepristone causes the uterine lining (endometrium) to break down and the embryo to detach. It also causes the cervix to soften and dilate, preparing the uterus for expulsion of the pregnancy contents. Misoprostol (7-8) is a synthetic prostaglandin E1 analog. Taken 24 to 48 hours after mifepristone, misoprostol binds to specific receptors on uterine smooth muscle cells, causing powerful and coordinated contractions of the uterus. These contractions help to expel the detached embryo and other pregnancy tissues, effectively completing the medical abortion.

When used in sequence, the two drugs work synergistically to terminate the pregnancy. The regimen is typically used for medical termination of an intrauterine pregnancy up to 70 days of gestation.

## II.MATERIALS AND METHODS

**Preparation of Stock solution**: 100 mg of Mifepristone and 100 mg of Misoprostol API standards were accurately weighed and are transferred into two separate 100 ml volumetric flasks, dissolved in mobile phase, then sonicated for 20 minutes to obtain 1000µg/ml.

**Preparation of working standard solution**: From the above standard stock solution, 4 ml from each solution were transferred into 100ml volumetric flasks, made up to the volume with mobile phase to get 40µg/ml of Mifepristoneand Misoprostol.

## III. RESULTS AND DISCUSSION

**Method validation**: Validation parameters include specificity, linearity, range, accuracy, precision, limit of detection, limit of quantification, robustness and assay (1-5).

**Specificity**: Specificity is the ability to assessing equivocally the analyte in the presence of components which may be expected to be present. Typically, these components include impurities, degradants, matrix etc. Blank solution and standard solutions of Mifepristone(40µg/ml) and Misoprostol (40µg/ ml) were injected into the HPLC system. The peak purity data of Mifepristone and Misoprostol were compared. There should not be any interference at the retention time of the main peaks (9-14).

**Linearity:** Linearity for the drugs Mifepristone and Misoprostol was determined by preparing the standard solutions at six concentrations levels in the range of 20-70µg/ml for Mifepristone and 20-70µg/ml for Misoprostol from stock solution. The linearity charts of Mifepristone and Misoprostol were shown in figure no 2&3. The correlation coefficient was found to be 0.9995 and 0.9993 for Mifepristone and Misoprostol respectively. Linearity results were tabulated in table 2.

**Accuracy:** Accuracy was performed by spiking known amounts of standard solution to sample solution at three different concentrations levels (50%, 100%, 150%) and there by analysed for %RSD which should not be more than 2.0. The % recovery was calculated, and the results were reported in table no. 3 & 4.

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**Precision:** The precision of the analytical method was studied by injecting six replicates of standard containing 40µg/ml of Mifepristone and 40µg/ml of Misoprostol which were injected into HPLC system. The % RSD was calculated, and the results were reported in table no.5 & 6.

**Limit of Detection (LOD) and Limit of Quantification (LOQ):** The limit of detection was defined as the concentration which yields a signal - to – noise ratio 3:1 whereas the limit of quantification was calculated to be the lowest concentration that could be measured with signal - to – noise ratio10:1. LOD and LOQ were calculated from slope and standard deviation. The results were tabulated in table no. 7.

**Robustness:** The smallest deliberate changes in method like change in flow rate are made but there were no predictable changes in the results and are in the range as per ICH guidelines. Conditions like decrease in flow rate (0.8 ml/min), increase in flow rate (1.2 ml/min) were maintained and samples were injected in duplicate manner. System suitability parameters were not much affected, and all the parameters were passed. % RSD was found to be within the limits and results were tabulated in table no.8.

**Assay:** Assay was conducted on marketed formulation and mean % assay was found. The results were tabulated in table no. 9.

**Table1: Optimized Chromatographic conditions**

| **Parameters** | **Method** |
|---|---|
| Stationary Phase(column) | Inertsil -ODS $C_{18}$(250 x 4.6 mm, 5 µ) |
| Mobile Phase | Acetonitrile: Water (85:15) |
| Flow rate (ml/min) | 1.0 ml/min |
| Run time(minutes) | 8 min |
| Temperature in the column | Ambient |
| Volume of injection (µl) | 20 |
| Detection Wavelength | 305nm |
| Drug RT (min) | 3.476 min for Mifepristone and 5.281 for Misoprostol. |

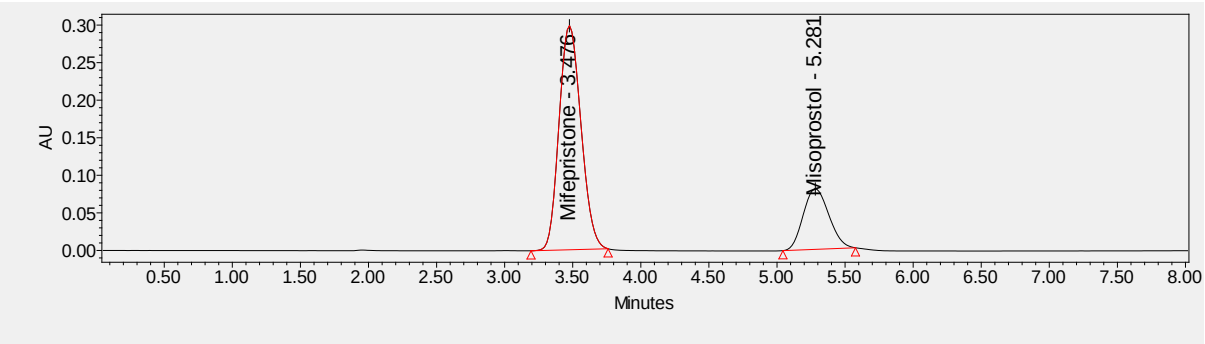


**Figure 1: Optimized chromatogram**

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**Table 2: Linearity data of Mifepristone and Misoprostol**

| **Mifepristone** | | **Misoprostol** | |
|---|---|---|---|
| Conc (µg/ml) | Peak area | Conc (µg/ml) | Peak area |
| 20 | 1437638 | 20 | 569634 |
| 30 | 2236342 | 30 | 826934 |
| 40 | 3080291 | 40 | 1095325 |
| 50 | 3822602 | 50 | 1363541 |
| 60 | 4592637 | 60 | 1597642 |
| 70 | 5291546 | 70 | 1863472 |

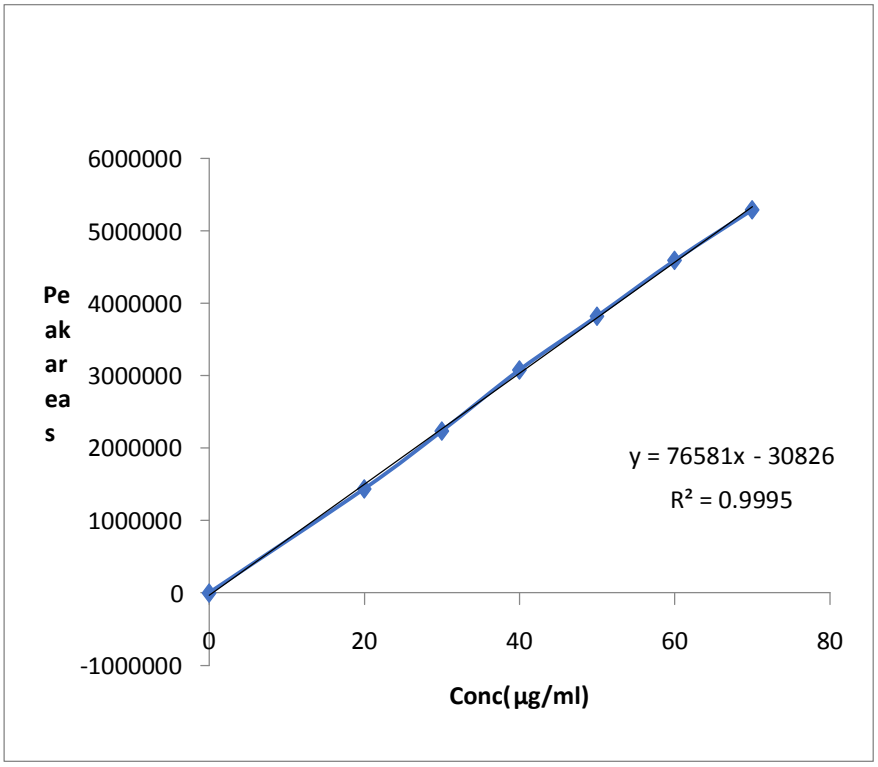


**Figure 2: Calibration Curve of Mifepristone**

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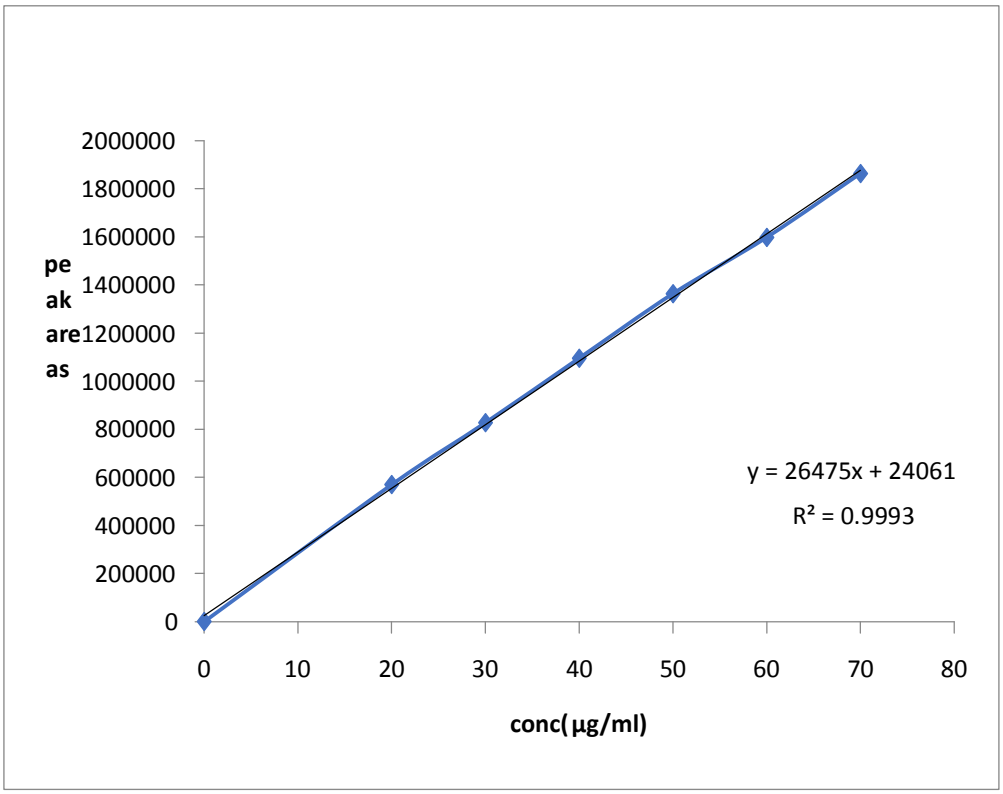


Figure 3: Calibration Curve of Misoprostol

**Table 3: Accuracy data of Mifepristone**

| Concentration % of spiked level | Amount added (ppm) | Amount found (ppm) | % Recover y | Statistical Analysis of % Recover y |
|---|---|---|---|---|
| 50% - 1 | 20 | 20.02 | 100.01 | 99.96 |
| 50% - 2 | 20 | 19.95 | 99.92 | |
| 50% - 3 | 20 | 19.86 | 99.90 | 0.654 |
| 100 % - 1 | 40 | 39.96 | 98.91 | 99.90 |
| 100 % - 2 | 40 | 39.93 | 99. 94 | |
| 100% - 3 | 40 | 39.96 | 99.12 | 0.768 |
| 150% - 1 | 60 | 59.99 | 99.98 | 100.08 |
| 150% - 2 | 60 | 60.06 | 100.05 | |
| 150% - 3 | 60 | 60.03 | 100.03 | 0.543 |

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**Table 4: Accuracy data of Misoprostol**

| Concentration % of spiked level | Amount added (ppm) | Amount found (ppm) | % Recover y | Statistical Analysis of % Recover y |
|---|---|---|---|---|
| 50% - 1 | 20 | 19.93 | 99.92 | 99.99 |
| 50% - 2 | 20 | 19.89 | 99.86 | |
| 50% - 3 | 20 | 20.03 | 100.01 | 0.964 |
| 100 % - 1 | 40 | 39.97 | 99.96 | 99.98 |
| 100 % - 2 | 40 | 40.08 | 100.06 | |
| 100% - 3 | 40 | 40.04 | 100.03 | 0.784 |
| 150% - 1 | 60 | 59.98 | 98.93 | 99.96 |
| 150% - 2 | 60 | 59.98 | 99.94 | |
| 150% - 3 | 60 | 59.94 | 99.93 | 0.876 |

**Table 5: System precision data of Mifepristone and Misoprostol**

| S. No | Peak areas of Mifepristone | Peak areas of Misoprostol |
|---|---|---|
| 1 | 3080564 | 1096324 |
| 2 | 3084567 | 1095462 |
| 3 | 3083475 | 1093478 |
| 4 | 3083756 | 1094375 |
| 5 | 3080457 | 1092357 |
| Mean | 3082564 | 1094399 |
| SD | 1917.175 | 1569.568 |
| % RSD | 0.062194 | 0.143418 |

**Table 6: Method precision data of Mifepristone and Misoprostol**

| S. No | Peak areas of Mifepristone | Peak areas of Misoprostol |
|---|---|---|
| 1 | 3080634 | 1093487 |
| 2 | 3080752 | 1091785 |
| 3 | 3080185 | 1093621 |
| 4 | 3080664 | 1095462 |
| 5 | 3080741 | 1093237 |
| Mean | 3080561 | 1093141 |
| SD | 226.4816 | 14930.42 |
| % RSD | 0.007352 | 0.136583 |

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**Table 7: LOD and LOQ data of Mifepristone and Misoprostol**

| Drug Name | LOD (µg/ml) | LOQ (µg/ml) |
|---|---|---|
| Mifepristone | 0.08 | 0.23 |
| Misoprostol | 0.073 | 0.22 |

**Table 8: Robustness data of Mifepristone and Misoprostol**

| S . No | Drug Name | Condition | Peak area | % RSD |
|---|---|---|---|---|
| 1 | Mifepristone | Decreased Flow rate of 0.8 ml/min | 2959258 | 0.871 |
| 2 | | Increased Flow rate of 1.2 ml/min | 3166072 | 0.125 |
| 3 | Misoprostol | Decreased Flow rate of 0.8 ml/min | 1086320 | 0.176 |
| 4 | | Increased Flow rate of 1.2 ml/min | 1097503 | 0.018 |

**Table 9: Assay data of Mifepristone and Misoprostol**

| S. No | Peak area of Mifepristone | % Assay | Peak area of Misoprostol | % Assay |
|---|---|---|---|---|
| 1 | 3080567 | | 1096478 | |
| 2 | 3084563 | | 1092345 | |
| 3 | 3080385 | | 1093289 | |
| 4 | 3080661 | | 1092168 | |
| 5 | 3080634 | | 1093385 | |
| 6 | 3082642 | 101.61 | 1093227 | 100.98 |

## IV. CONCLUSION

The developed RP-HPLC method was validated as per ICH guidelines. All the system suitability parameters were within the range as stated by ICH guidelines. Interference peaks were not observed in blank, standard and sample chromatogram. Hence simple, precise and accurate, sensitive, specific and robust method was developed and validated. This can be used in quality control department with respect to routine analysis.

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