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METHOD DEVELOPMENT, VALIDATION AND STABILITY INDICATING STUDY OF METHYLERGOMETRINE MALEATE USING RP-HPLC

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ABSTRACT

The aim of present study was to develop a simple, economical, precise and accurate RP-HPLC method along with its validation and to perform the stability indicating study for the estimation of methylergometrine maleate in formulation of injection. RP-HPLC method was developed by using Phenomenex Luna (c18 (2), 250 x 4.6 mm, 5 µ) and a combination of ammonium acetate: acetonitrile: in the ratio of 70:30 v/v as mobile phase, adjusted to pH 6.5 by glacial acetic acid. The developed method was validated by showing the results of various validation parameters which are within the limits. Linearity ranges were 12-28 µg/ml, correlation coefficient was 0.9979 and mean recovery was found to be 99.79%. Further, stability indicating study has been performed and it showed positive results for acid degradation, alkali degradation, oxidation and sun-light exposure. Interferences of impurity peak were evaluated and found to be within limit. The developed method was found to be simple, sensitive, accurate and precise for analysis of

methylergometrine maleate. The method can be adopted for analysis of drug in pharmaceutical dosage forms.

Keywords: Stability, HPLC, Injectable, Degradation

1. INTRODUCTION

Methylergometrine (MEM) is oxytocic drug indicated for the prevention and control of excessive bleeding following vaginal childbirth **[1, 2]**. It is chemically 9, 10-didehydro-N-[1-(hydroxymethyl)-propyl]-D-lysergamide. MEM acts directly on the smooth muscle of the uterus and increases the tone, and amplitude of rhythmic contractions through binding and the resultant antagonism of the dopamine D_1 receptor **[3]**. Thus, it induces a rapid tetanic uterotonic effect which shortens third stage of labour and reduces blood loss. Literature reveals many methods for the estimation of MEM that include HPLC with fluorescence, UV, LC-MS-MS, chromatographic and mass spectrometric methods **[4-6]**. Even metabolites of MEM in physiological specimens were estimated by capillary electrophoresis **[7]**. The bioavailability and pharmacokinetic of oral MEM in plasma was estimated by an automated pre-column: (OPS-2) coupled to liquid chromatography with photochemical degradation and electrochemical detection **[8-11]**. The objective of present study was to develop and validate a sensitive RP-HPLC based analytical method with a simple composition, low cost of mobile phase for determination of MEM and to evaluate stability indicating study in its parenteral dosage form.

Structure of MEM **(Figure 1)**:

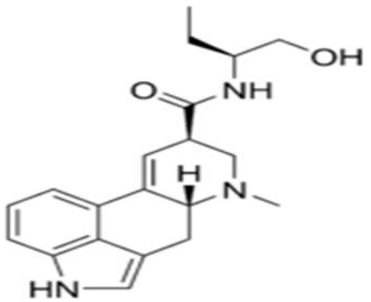

Figure 1: Structure of MEM

2. MATERIALS AND METHODS:

2.1 Apparatus

HPLC measurements were made on Shimadzu, (Class-VP Software), model 2489 series with UV detector and pneumatic pump.

2.1.2 Reagents and solutions

All chemicals and reagents were of analytical grade and solutions were prepared freshly with HPLC water. MEM sample was gifted by Mercury Lab, Vadodara, Gujarat.

2.1.3 Chromatographic condition

The analysis was performed by using HPLC instrument. The column used was Phenomenex Luna C 18 (2), 250 x 4.6 mm, 5 µ. The mobile phase consisted of buffer

(1.927 gm of ammonium acetate dissolved in 1000 ml water and pH was adjusted to 6.5) and acetonitrile. It was filtered through membrane filter (0.45µ), degassed and pumped from the solvent reservoir into the column in the ratio of 70:30(v/v). The flow rate of mobile phase was maintained at 1.0 ml/min and detection wavelength was set at 310.0 nm with a run time of 12 min. The volume of injection loop was 20µl. Prior to injection of the drug solution, the column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

2.1.4 Preparation of standard stock solution

Accurately weighed 49.6 mg of MEM was transferred into the 50 ml volumetric flask. 20 ml of mobile phase was added, sonicated to dissolve and volume was diluted up to the mark and mixed well.

2.1.5 Preparation of sample solution

2.5 ml of Formulation was taken in 25 ml volumetric flask. 10 ml of diluent was added to this solution. The mixture was sonicated to dissolve the drug and finally the volume was made up to 25 ml with HPLC grade water.

2.1.6 Calibration curve

Aliquots of standard stock solution (0.2 mg/ml) of MEM were taken into 10 ml volumetric flasks separately and were diluted up to the mark with mobile phase to obtain the final concentration of 12, 16, 20, 24 and 28 µg/ml of MEM. These solutions were injected into chromatographic system, chromatograms were obtained and peak area was determined for each concentration of drug solution. Prepared linearity plot of area Vs concentration and calculate the Y-intercept, Y-intercept bias (at 100% concentration level), slope of regression line, correlation co-efficient, R^2 value and % relative standard deviation of response factor.

2.1.7 Method Validation

The proposed method was validated in terms of linearity, range, repeatability, method precision, intermediate precision, accuracy, LOD, LOQ, robustness, ruggedness and system suitability. **2.1.8 Linearity and range**

The linearity of response for MEM was determined by preparing and injecting solutions with concentrations of 12 µg/ml to 28 µg/ml.

2.1.9 Repeatability

Intraday and interday repeatability of the proposed method was determined on the API.

2.1.10 (A) Intraday

The solutions of three different concentrations viz. 12 µg/ml, 20 µg/ml and 28 µg/ml were prepared and six replicates of each were injected into HPLC system on the

same day. The % relative standard deviation was calculated.

2.1.11 (B) Interday

Fresh solutions of three different concentrations viz. 12 µg/ml, 20 µg/ml and 28 µg/ml were prepared and six replicates of each were injected into HPLC system on 3 consecutive days. The % relative standard deviation was calculated.

2.1.12 Precision

2.1.12.1 Method Precision

Six sample solutions of 20 µg/ml were prepared from the formulation and analysed by the proposed method to check the method precision.

2.1.12.2 Intermediate precision (Interday)

Three fresh sample solutions, each of 20 µg/ml, were prepared from the injection formulation and analysed on 3 consecutive days to check intermediate precision.

2.1.13 Limit of Detection and Limit of Quantitation

Limit of Detection (LOD) and limit of Quantitation (LOQ) for MEM was determined by visual method. In this, subsequent dilutions of different concentrations of MEM were prepared and injected into HPLC system. The limit of Quantitation (LOQ) was determined as the minimum concentration at which the % RSD of three replicates were below 10.0 %. The limit of detection (LOD) was determined as the concentration at which the peak of MEM was detectable.

2.1.14 Accuracy

Accuracy (recovery) studies were performed by spiking MEM API in MEM injection at levels 80, 90,100,110 and 120 % of label claim.

2.1.15 Robustness

Robustness of the method was determined by small, deliberate changes in flow rate, mobile phase ratio and pH of mobile phase. Flow rate was changed to 1.0 ± 005 ml/min. The mobile phase ratio was changed to 30 ± 1% for acetonitrile. pH of mobile phase was changed to 6.5 ± 0.1.

2.1.16 Ruggedness

Method ruggedness was determined by analysing the sample at normal operating conditions and also by changing some of the operating analytical conditions such as column, instrument, analyst mobile phase composition and flow rate.

2.2 Stability indicating study [12]

2.2.1 Forced degradation studies of MEM

In order to establish stability-indicating nature of the method, pure MEM (API), and pharmaceutical formulation were subjected to various stress conditions to conduct forced degradation studies. Standard stock solution is used for forced degradation studies. Stress studies were carried out under the conditions of acid/base hydrolysis, oxidation, thermal,

UV light as mentioned in ICH Q1A (R2) guideline.

2.2.2 Preparation of acid induced degradation product

2.5 ml of injection sample was accurately pipette out and transferred into the 25 ml volumetric flask and sonicated to dissolve. 10 ml of diluent and 2 ml of HCL (2M) were added to it and this solution was heated at 80°C for 60 minutes. After cooling at room temperature pH of the solution was adjusted to 7 with 2 ml NaOH (2M). Then diluted up to the mark with diluent and mixed well.

2.2.3 Preparation of base induced degradation product

2.5 ml of injection sample accurately pipette out and transferred into the 25 ml volumetric flask and sonicated to dissolve. 10 ml of diluent and 2ml of NaOH (1M) was added to it and this solution was heated at 80°C for 60 minutes. After cooling at room temperature pH of the solution was adjusted to 7 with 2 ml of HCL (1M). Then diluted up to the mark with diluent and mixed well. This solution was injected to HPLC.

2.2.4 Preparation of H_2O_2 induced degradation product

2.5 ml of injection sample accurately pipette out and transferred into the 25 ml volumetric flask and sonicated to dissolve. 10 ml of diluent and 5 ml of 3% H_2O_2 was added to it and this solution was heated at 80°C for 60 minutes. After cooling at room temperature it was diluted up to the mark with diluent and mixed well. This solution was injected to HPLC.

2.2.5 Preparation of photo degradation product

1 ml of injection sample accurately pipette out which was previously exposed to sunlight for 30 minutes and transferred into the 10 ml volumetric flask. Afterwards, sufficient amount of diluent was added and solution was sonicated to dissolve it, then diluted up to the mark with diluent and mixed well. This solution was injected to HPLC.

3. RESULTS & DISCUSSION

High Performance Liquid Chromatographic method was developed for the determination of MEM in Formulation .The conditions were optimized to obtain an adequate separation and detection of the eluted compound. Here various trials were taken based on the available literature to optimise HPLC conditions for method development of MEM in Formulation. For the selection of buffer various trials were taken using sodium citrate, sodium phosphate and ammonium acetate along with acetonitrile in different ratios. Various optimisations of buffer and acetonitrile were done and finally ammonium acetate; acetonitrile in the ratio of 70: 30 was selected because of high resolution and better peak purity. Then trials were taken with

different HPLC columns like Phenomenex Luna C18 (2) (250×4.6 mm) 5µ and Cosmosil C18 (250x4.6mm) 5µ column. Out of these two, Phenomenex Luna C18 (2) (250×4.6 mm) 5µ was giving satisfactory results in terms of retention time, area, peak purity and theoretical plates. The λ max of the MEM was also found by scanning in UV spectrophotometer in the range of 200 nm to 350 nm in different solvents like methanol, water, acetonitrile etc, and found to be 310 nm. Typical chromatogram of MEM is depicted in figure 2. This value is same as reported in the literature. From this we can conclude that the drug MEM remains stable in different solvents and does not show any change in its peak properties. The proposed method was found to be linear in the concentration range of 12 to 28 µg/ml with correlation coefficient (r^2) 0.9979, slope 130600 and intercept 33977. The calibration curve is shown in figure 3. The method was validated in terms of accuracy, precision, repeatability, LOD, LOQ, ruggedness, system suitability and the results are recorded in **Table 1**. **[13, 14]**.

The results of interday and intraday precision (% RSD less than 2), method precision and intermediate precision **(Table 1)** indicate that the proposed method is precise enough for the analysis of drug. For specificity, no peaks are detected at the retention time of MEM. MEM peak and impurities peak in standard preparation and in assay preparation are spectrally pure **[15]**. The retention time of the peak obtained due to MEM in the assay preparations is correspond with that obtained from standard preparation. For accuracy, the % recovery at each level of MEM is between 99.0% to 100.0%. RSD for % recovery at each level is less than 2.00%.For precision, the % RSD of MEM was found to be less than 2%.

For Robustness, during small changes in the method, the % RSD of MEM was found to be less than 2 %. Limit of detection and limit of Quantitation were found to be 1.471 µg/ml and 4.4588 µg/ml respectively. Values of recovery ± SD was found to be greater than 98.0% indicated that the proposed method is accurate for the analysis of drug. Thus, a simple reverse phase liquid chromatographic method has been developed and subsequently stability indication and validation for the formulation was carried out **[16, 17]**. Here we used mobile phase consisting of ammonium acetate buffer having pH 6.5 adjusted with glacial acetic acid solution. The column was used Phenomenex Luna C18 (2) (250×4.6 mm) 5µ with flow rate 1 ml/min using UV detection at 310 nm. The retention time of MEM was found to be 6.123. For stability indication, drug is treated with 1% solutions of HCL, NaOH and 3% H_2O_2 at 80

ºC were done and results are depicted in **Table 2 and Figure 1, 2**. Here interference of impurity peak was evaluated. The RP-HPLC method for MEM in its parenteral dosage forms is established as per the ICH guidelines. MEM is very important in treatment of contraction of the uterus muscle in pregnancy and also used in the treatment of the migraine. On detailed literature survey, it was found that though MEM have been analyzed by many methods but in order to have the further good data it was analysed by RP-HPLC method. The peak purity data of standard solution, test solution and spiked sample solution proved the specificity of the proposed method. The results of precision, % recovery and all other validation parameters are within acceptance criteria. The results of stability indicating study in acid, alkali, oxidation and sun-light degradation was also within the limits. Chromatographic peak purity results indicated the absence of co-eluting peaks with the main peak of MEM, which demonstrated the specificity of assay method for estimation of MEM in presence of degradation products. The method was validated in terms of linearity, range, repeatability, precision, accuracy, LOD, LOQ, solution stability, specificity, ruggedness and system suitability.

Table 1: Summary of validation parameters for the proposed method

Parameters	Result
Linearity	12-28 µg/ml
Repeatability; Intraday precision	0.76%
Interday Precision	0.50%
Method Precision	100.04±0.27
Intermediate Precision	99.76±0.50
Accuracy; 80%	100.3%
90%	99.14%
100%	99.54%
110%	100.41%
120%	100.51%
Assay	100.04%
Limit of Detection	1.471µg/ml
Limit of Quantitation	4.4588µg/ml

Table 2: Results of the Different Stability Indicating Studies

Degradation study	Peak purity	Limit	Status
Acid degradation	0.9999	NLT 0.98	Pass
Alkali degradation	0.9957		Pass
Peroxide degradation	0.9819		Pass
Sun light exposure	0.9963		Pass

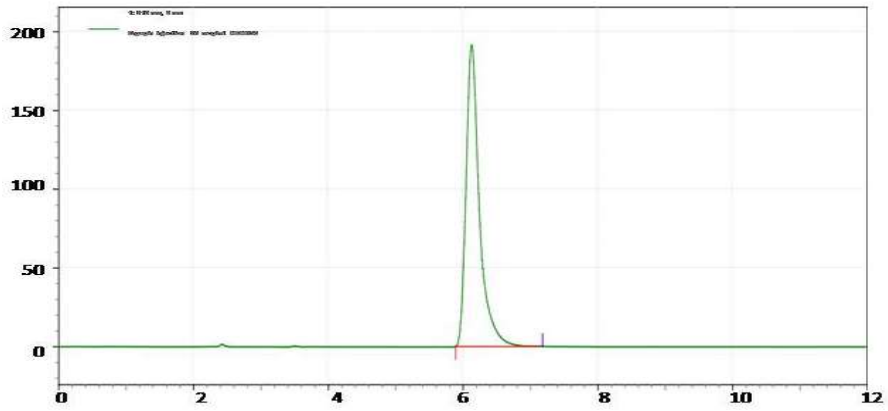

Figure 2: Development of Chromatogram for Methylergometrine

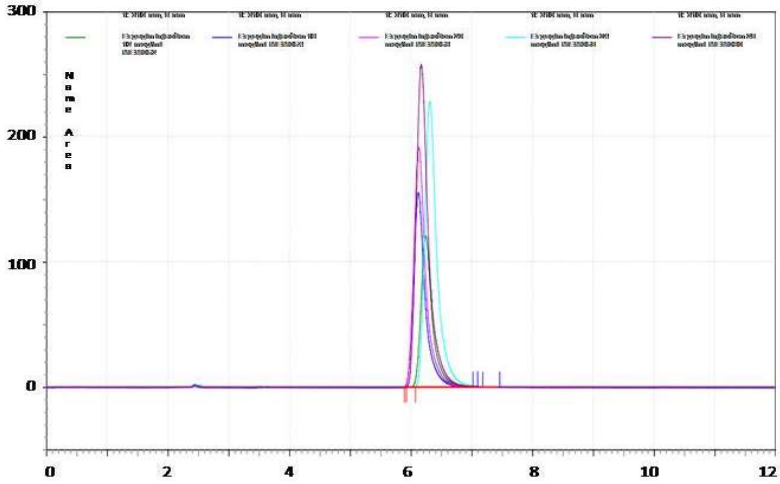

Figure 3: Linearity Overlay plot of Different Test Concentration

4. CONCLUSION

It may be concluded that developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, and robust. Hence it is expected to be used for the routine analysis of MEM and may reveal potential utility of the proposed method for quality control laboratories.

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