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METHOD DEVELOPMENT AND PARTIAL VALIDATION OF THE MAFENIDE ACETATE DRUG IN SEMISOLID DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, specific, accurate and precise RP HPLC method has been developed for the determination of Mafenide from Semisolid dosage form by reverse phase HPLC. C18 column (Xterra RP 18, 4.6 x250mm, 5.0µm). The sample was analyzed using Potassium phosphate mono basic buffer (pH 2.5± 0.05): Methanol (87:13) as a mobile phase at a flow rate of 1.0ml/min and detection at 267nm. The retention time for Mafenide was found to be 2.852 min. The stability assay was performed and was validated for accuracy, precision, linearity, specificity and sensitivity in accordance with ICH guidelines. Validation revealed the method is specific, rapid, precise, reliable, and reproducible. Calibration plots were linear over the 50%-150% of targeted concentration ranges for drug. The method can be used for estimation of Mafenide drug in semisolid dosage form.

Keywords: Method Development, Validation of Mafenide, Semisolid Dosage Form.

INTRODUCTION

Mafenide Acetate is a Benzene sulfonamide, 4-(amino methyl)-, monoacetate or Amino-*p*-toluene sulfonamide monoacetate. This medication is used alone or with other medications to help prevent and treat wound infections in patients with severe burns. Mafenide is a drug applied to the skin that belongs to a class of drugs known as sulfa antibiotics. It works by killing bacteria that may infect an open ound. Killing bacteria helps to promote wound healing and to decrease the risk of the bacteria spreading to surrounding skin or to the blood, thereby helping to prevent a serious blood infection (sepsis). Literature survey reveals that there is no method available in HPLC methods for estimation of Mafenide Acetate. Method has been reported for the estimation of mafenide Acetate in semisolid dosage form. Present work emphasizes on the stability testing of mafenide Acetate in semisolid dosage form by RP-HPLC (Anonymous 1 and 2; David G. Watson, 1999).

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EXPERIMENTAL

A Waters High performance liquid chromatography system, the purity determination performed on a stainless steel column 250mm long, 4.6mm internal diameter filled with Octadecylsilane chemically

bonded to porous silica particles of 5µm diameter reverse phase C18 column (Xterra RP 18, 4.6 x250mm,5.0µm particle size). Optimized chromatographic conditions are listed in Table 1.

MATERIALS AND CHEMICALS

Pure samples of USP Mafenide Acetate RS were obtained from merk for the estimation Mafenide Acetate in commercial formulations. HPLC grade Monobasic Potassium phosphate, Hexane sulphonic acid sodium salt , Phosphoric acid solution and Methanol were procured from institute and of Rankem ltd. High pure water prepared by using Millipore Milli Q plus purification system.

Preparation of Standard Solution

Weigh accurately and transfer about 25mg of USP Mafenide acetate Reference standard into a 25 mL volumetric flask. Add about 15 ml of Diluent and sonicate to dissolve. Dilute to volume with diluent. Mix well. (Mafenide Acetate standard concentration of about 1000µg/mL).

Preparation of Sample Solution

Constitute the cream as directed in the labeling. Transfer an accurately measured volume of the constituted cream, equivalent to 100mg of mafenide acetate, to a100ml volumetric flask, dilute it about 70 ml of diluents and mix well. Then mixed up the volume with diluents.(Mafenide Acetate Sample concentration of about 1000µg/mL).

Validation of the Method

The method was validated in terms of system precision, linearity, precision and specificity of the sample applications. The linearity of the method was investigated by serially diluting the stock solutions of mafenide and measured the absorbance at 267nm. Calibration curves where constructed by plotting the area against the concentration. Losartan Potassium shows the linearity in the concentration range from 50-150% of targeted concentration with correlation coefficient of 0.9998.Precision was found to be lower than 1%. Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by different analysts using similar operational and environmental conditions (Beckett AH and Stenlake JB, 1999; David Harvey, 2000; James W. Munson, 2001; Hobert H. Willard, 1986).

RESULTS AND DISCUSSION

To evaluate the linearity range of mafenide acetate, varying concentrations of standard solution s is diluted ranging from 50% to 150% of the target concentration (1000µg/ml) were injected into HPLC system. The linearity graph was plotted from 50% to 150%. A calibration curve was constructed for each sample by plotting the peak area obtained the concentration.

The linearity data for Mafenide acetate are presented as follows (fig-1).

Method Precision

Precision of the assay method was determined by injecting, in duplicate, six (6) individual samples of mafenide acetate. The samples were prepared as per the method. The results are tabulated in table 3.

Specificity

Blank, standard, mafenide Acetate for Topical solution sample, spiked sample, Individual Impurity were prepared and injected into the chromatographic system for identification and impurity interference with the mafenide peak (Douglas A. Skoog, 1979; Frank A Settle, 2004). The blank and Impurity interference are tabulated in table 4.

System Precision Ruggedness

The standard solutions prepared by analyst-1 and analyst -2 are injected in different HPLC systems, on different day, using a different column. The system suitability parameters calculated (Frank Settle, 2004; Kaur H, 2006) by analyst -2 can be compared with those of Analyst -1. They are tabulated in table 5.

Method Precision Ruggedness

The sample solutions prepared by Analyst-1 and Analyst-2 are injected in different HPLC systems, on a different day, using a different column. The assay values calculated (Fengshan Yu *et al*., 2009) by Analyst-2 can be compared with those of Analyst-1 are tabulated in table 6.

Solution Stability

The Mafenide acetate precision sample and standard solutions were prepared (Gary D. Christian, 2004; ICH Harmonised Tripartite Guidelines). Replicate injections of the standard solution were made at the following time intervals: initial, 24 hours. The values were compared to initial standard area and they are tabulated in table 7.

Sample solution were injected at 24 hours time interval, and %area difference was compared to the initial area generated by these samples and are tabulated in table 8.

From the above experimental data results and parameters it was concluded that the developed RP-HPLC method has the following advantages.

Ø The standard and sample preparation requires less time.
Ø No tedious extraction procedure was involved in the analytical process.
Ø Suitable for the analysis of raw materials. Run time required for recording chromatograms were less than 5 times.

Table 1. Optimized Chromatographic conditions

Parameter	**Optimized condition**
Instrument	Waters HPLC/Empower software/PDA detector
Column	(Xterra RP 18, 4.6 x250mm,5.0µm particle size)
Mobile phase*	Potassium phosphate mono basic buffer(pH 2.5± 0.05): Methanol(87:13)
Flow rate	1.0ml/min
Detection	267nm
Injection volume	20µl
Temperature	Ambient

*Filtered through a 0.45µ membrane filter (Millipore), degassed and sonicated

Table 2. System Suitability Parameters

Analytes	RT(N=5)	%RSD (N=5) Limit (NMT2.0)
Mafenide Acetate	2.8	0.27

Method Precision

Table 3. Method precision study of Mafenide acetate for topical solution

SNO	**% ASSAY**
1	99.47
2	99.35
3	99.59
4	99.41
5	99.28
6	99.82
MEAN	99.48
%RSD	0.19

Table 4. Blank and Impurity interference

Sample ID	**Interference**	**Retention time**
Blank	Nil	NA
Mafenide related Compound A	Nil	7.07
Sample name	**Purity angle**	**Purity threshold**
Spiked name	0.191	1.052

Table 5. System precision ruggedness study of Mafenide standard

	Analyst-1	**Analyst-2**
SNO	**PEAK AREA RESPONSE**	**PEAK AREA RESPONSE**
1	2938855	2891090
2	2945051	2884273
3	2950750	2890041
4	2956462	2891038
5	2958793	2891613
MEAN	2949982	2889611
%RSD	0.27	0.10

Table 6. Method precision ruggedness study of Mafenide acetate for topical solution

S.NO	Analyst-1	Analyst-2
	%Assay	%Assay
1	99.47	99.64
2	99.35	99.73
3	99.59	99.85
4	99.41	100.14
5	99.28	100.04
6	99.82	99.99
MEAN	99.48	99.90
%RSD	0.19	0.19

Table 7. Solution stability of Mafenide standard solution

Initial area	24 hours area	%area difference
2949982	2957378	0.25

Table 8. Solution stability of Mafenide acetate for topical sample solution

Initial area	24 hours area	% area difference
2900226	2918187	0.61

Fig 1. Linearity Curve of Mafenide Acetate

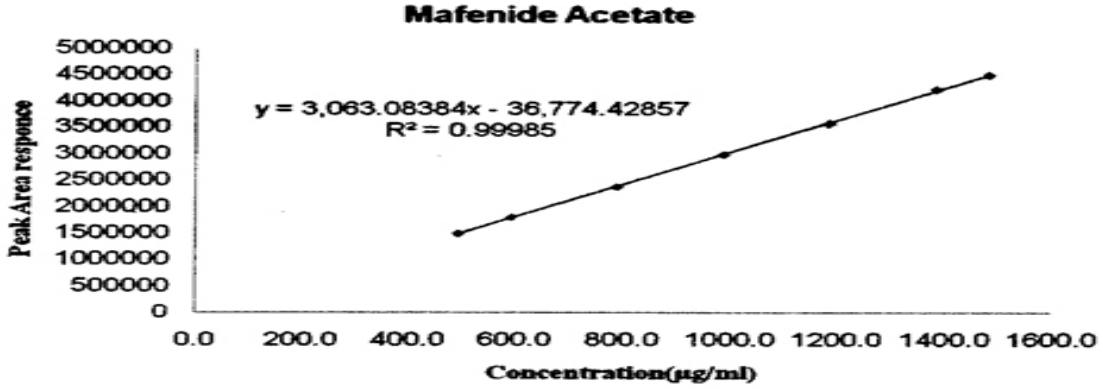


Fig 2. Typical chromatogram of mafenideacetate .

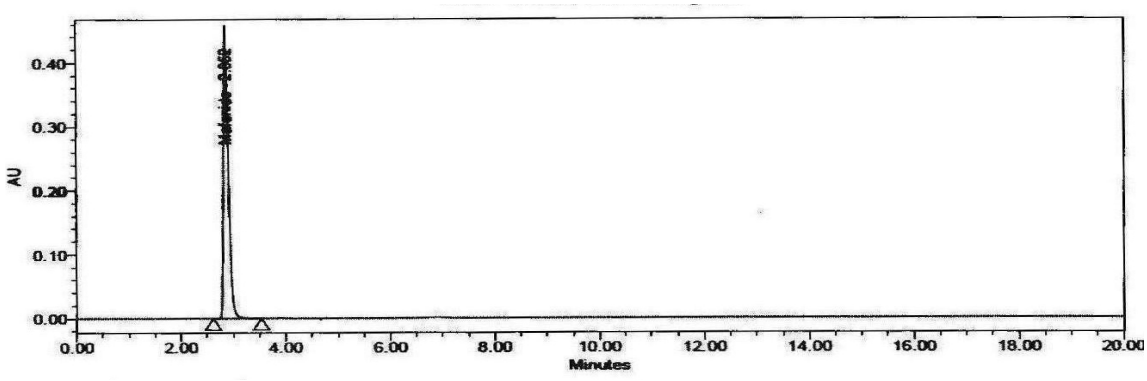
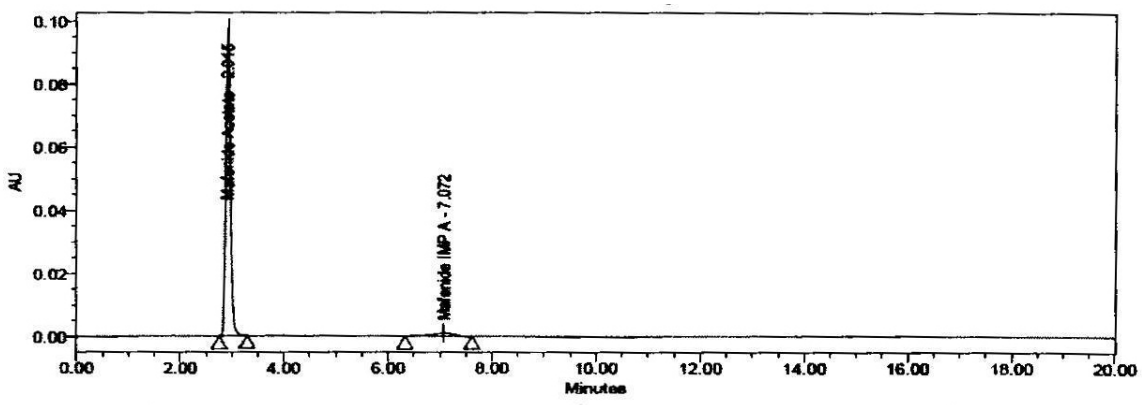


Fig 3. Typical chromatogram of spiked sample .



CONCLUSION

The proposed HPLC method validation for estimation of Mafenide acetate USP is carried out as per ICH and USP Guidelines. System suitability test id established and recorded, the method found to be specific for validation of estimation of Mafenide acetate USP. The method found to be linear in the specified range. Hence, this method stands validated and can be used for routine analysis.

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