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Research Article

Pharmaceutical analysis for Novel drug formulation

Analytical Procedure for determination and statistical validation of novel drug Mafenide acetate in pharmaceutical formulations

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Abstract: Requirement for a sophisticated analytical method using HPLC and HPTLC is in high demand to meet the needs of a small scale industry for analysis of drugs that are relatively expensive. Hence a simple method was proposed in the routine determination of Mafenide acetate in pharmaceutical formulations and bulk dosage forms that can be less expensive. An analytical method was developed for the estimation of Mafenide acetate drug substance by liquid chromatography. The chromatographic separation was achieved on phenyl column (Eclipse XDB-Phenyl 250*4.6, 5um) at ambient temperature. The separation was achieved employing a mobile phase consisting of 0.1 %v/v Trifluoroacetic acid in water: Methanol (10:90). The flow rate was 1.0 ml/ minute and ultraviolet detector at 245nm. The average retention time for Mafenide acetate was 3.3 minutes. The proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were checked and are found within the acceptable range. The assay methods were found to be linear ranging from 50-150 µg/ml for Mafenide acetate. The parameters considered for the procedure are related limit, selectivity, linearity, range, accuracy and precision are defined. Thesample solution leads to unequivocal, absolute identification of the analyte peak of interest apart from all other matrix components. The objective of our work is to form a basis for production procedure and control, which are designed to assure that the drug products have the identity, Quality, and purity. The results obtained could be treated as simple, sensitive and reproducible for determination of Mafenide acetate in pharmaceutical formulations.

Keywords: Mafenide Acetate, HPLC, EclipseXDB-Phenyl, Methanol and Validation

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1. INTRODUCTION

Pharmaceutical analysis plays a major role today, and it can be considered as an interdisciplinary subject. Pharmaceutical analysis derives its principles from various branches of science like Chemistry, Physics, Microbiology, Nuclear Science, Electronics, etc. Analytical methods development and validation[1,2,12] play important roles in the discovery, development, and manufacture of pharmaceuticals[1,3-5]. The current guidelines provided by good manufacturing practice (CGMP) and Food and Drug Administration (FDA) insist on methods that involve analysis with greater sensitivity and reproducibility[1]. Development of a method of analysis is usually based on prior art (or) existing literature, using the same (or) quite similar instrumentation. It is rare today that an HPLC-based method is developed that is not related in the same way (or) compared to existing, literature based approaches. –To meet the requirements HPLC (High performance liquid chromatography)[6-10] is the method of choice used by the pharmaceutical industries to assess and assay the intact drug and degradation products[11] . The appropriate selection and chromatographic conditions ensure that the HPLC method will have the desired specificity. UV spectroscopy is also a simple analytical tool widely used for determination and validation of drugs[11]. Hence for the assay of the selected drugs HPLC and UV spectroscopy has been choose for these proposed methods[1].The drug Mafenide acetate is used as a tropical cream either with other drugs or alone for treating wounds and the infected area of skin[2,12]. Specifically it is extensively used for severe skin burns. The developed chromatographic methods validation should be developed in such a way that it should be as per ICH [2,13] or US FDA guidelines for all the critical parameters. To access the precision and to evaluate the results of analysis the analyst must use statistical methods. These methods include confidence limit, regression analysis to establish calibration curves. In each analysis the critical response parameters must be optimized and recognized if possible. Pharmaceutical analytical techniques are applied mainly in two areas, quantitative analysis and qualitative analysis, although there are several other applications. The concept of analytical chemistry lies in the simple, precise and accurate measurements. These determinations require highly sophisticated instruments and methods like Mass Spectroscopy, Gas chromatography, HPTLC[14], HPLC[15-18], etc. RPHPLC[11, 19-21] method is sensitive, accurate, precise and desirable for routine estimation of drugs in formulations. Thereby it is advantageous than volumetric methods. Many HPLC methods were developed and validated for the quantitative determination of various marketed drugs. Analytical method development and validation places an important role in drug discovery and manufacture of pharmaceuticals. These methods are used to ensure the identity, purity, potency and performance of drug products. The majority of analytical development effort goes into validating a stability indicating method. So it is a quantitative analytical method based on the structure and chemical properties of each active ingredient of the drug formulation.[3] Most of the drugs can be analyzed by HPLC[15-18] method because of several advantages like rapidity, specificity, accuracy, precision, reproducibility, ease of automation and eliminates tedious extraction and isolation procedures. On the literature survey, it was found that most of the analytical methods available for the above mentioned drug are applicable for quantification in plasma samples[22], the most widely used method being liquid chromatography-mass chromatography[7]. So it is felt that there is a need to develop accurate, precise analytical methods for the estimation of the drug in solid dosage formulations[23].A comprehensive method was developed and validated using Mafenide acetate in bulk and pharmaceutical dosage forms. The method developed is simple, accurate and economical for analysis of Mafenide acetate.

2. MATERIALS AND METHODS

2.1 Mafenide Acetate

Mafenide[8-9] (INN; usually as mafenide acetate, trade name Sulfamylon) is a sulfonamide[24]-type medication used as an antibiotic. 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 wound[2]. 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).

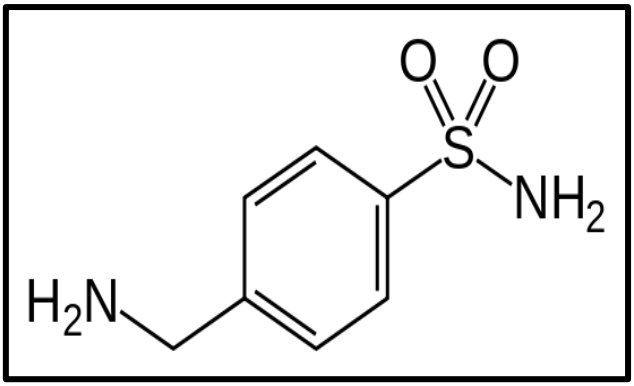


Fig 1: Mafenide Acetate

Fig 1: Structure of Mafenide Acetate with chemical name 4-(Amino methyl) benzene sulfonamide having molecular formula $C_7H_{10}N_2O_2S$, Molecular weight 186.233g/mol, PKa-10.27 and is soluble in methanol and water. The objective of the present work is to develop a HPLC method for the determination of Mafenide acetate in tablets and validation of the process, to be employed in routine analysis. Our project work was carried out by incorporating the Reverse phase High performance Liquid chromatography (HPLC)[15-18] and the developed method was validated according to ICH guidelines for its various parameters [28-29].

Table 1: Equipments and Models Used

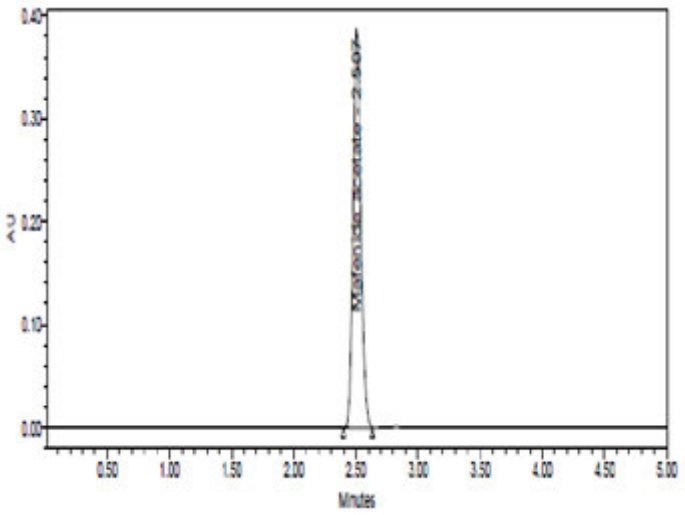
S.NO	Equipment's	Model	Company
1	Electronic Balance	SAB224CL	SCALETEC
2	UltraSonicator	SE60US	ENERTECH
4	Thermal oven	---------	YAMATO
5	pH Meter	PH-7000	SMIS
6	Filter Paper 0.45 microns	---------	MILLIPORE

Equipments required for the proposed analytical procedure.

Table 1:Explains the equipment used for the proposed analytical procedure. Electronic Balance SAB224CL , Made SCALETEC was used, Ultrasonicator model SE60US Made ENERTECH,Thermal oven Made: YAMATO, pH Meter with pH-7000 model and Made: SMIS,Filter Paper-0.45 microns of company MILLIPORE is used to perform the work

2.2 *Optimised Chromatographic Conditions*

Buffer: 0.1% Trifluoroacetic acid (Qualigens) in water Mobile Phase: Buffer: Methanol (Qualigens) (300:700), Column: Eclipse XDB- Phenyl 250*4.6mm, 5um, Flow Rate: 1.0ml/min Ambient Volume, 10ul Detector: 230nm Diluents: Water: Methanol (50:50).



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Mafenide acetate	2.507	1705712	100.00	7329	1.15

Fig 2: Chromatogram for the optimized method

In figure 2: RT was found to be good and the peak symmetry of drug was good. The theoretical plate count and tailing were within the limits and it is used for the validation of the method.

2.3 *Preparation Of Mobile Phase*

Transfer 500 ml of HPLC water into 1000 ml of beaker and add Trifluoroacetic acid. Transfer the above solution 300ml of Trifluoroacetic acid, 700ml of Methanol is used as a mobile phase. They are mixed and sonicated for 20min.

2.4 *Preparation Of Standard Solution*

A 25mg of pure Mafenide acetate was weighed and transferred to 25 ml of volumetric flask and dissolved in Water. The flask was shaken and volume was made up to mark with Water[13,14] to give a primary stock solution containing 1000 µg/ml. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark[12] with Water to give a solution containing 100 µg/ml of Mafenide acetate.

2.5 *Preparation Of Sample Solution*

A 25mg of Mafenide acetate sample was weighed and transferred to 25 ml of volumetric flask and dissolved in

Water. The flask was shaken and volume was made up to mark with water to give a primary stock solution containing 1000 µg/ml. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with water to give a solution containing 100 µg/ml of Mafenide acetate . Separately injected both the standard[15,16] (2 injections) and sample preparations (2 injections) into the chromatographic system and recorded the peak area responses.

3. RESULTS AND DISCUSSION

For any analytical procedure to be performed different parameters are taken into consideration that are suitable as per the guidelines given by ICH[2,13].Acceptance criteria for various parameters for validation such as specificity, RT, %RSD, linearity range, correlation coefficient, accuracy, robustness[30-31]are reflected in Table 3 and graphs 3.1 (Fig 3-Fig 9) .

3.1 Graphs/Chromatograms

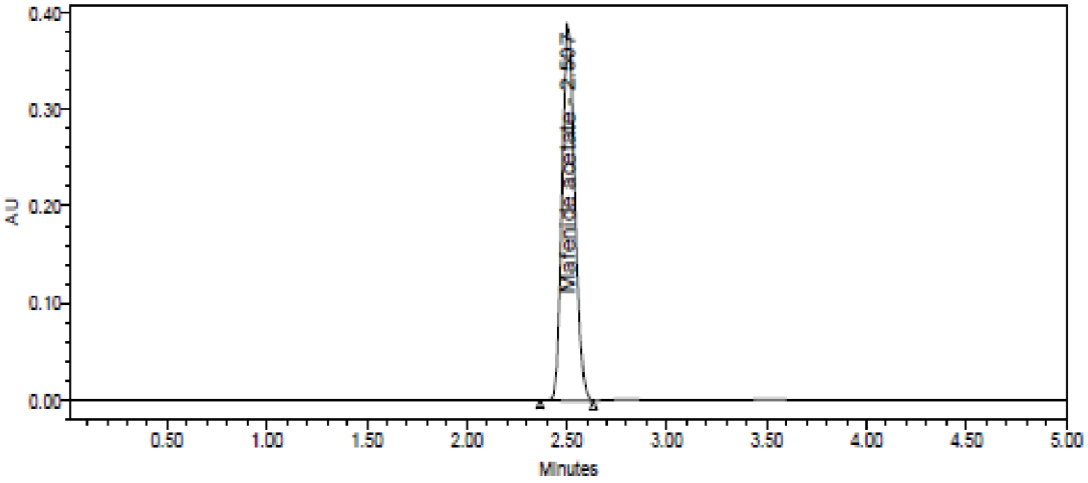


Fig 3: System Suitability Typical Chromatogram of Sample

Fig 3 explains the results of system suitability from the sample showed uniform retention time, theoretical plate count, tailing factor and resolution for the drug indicate a good system for analysis. The acceptance range is not less than 2000 and is acceptable as per the ICH regulations[30.]

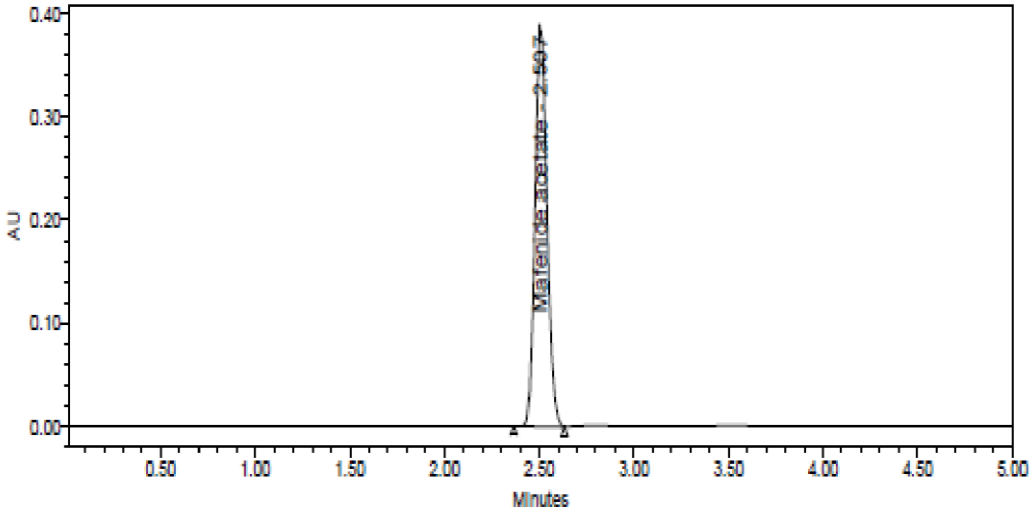


Fig 4: Specificity Chromatogram representing specificity of sample

Fig 4 explains that retention time for standard, sample and commercial product of Mafenide acetate are same. This proves that, recipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective[2 ,30.]

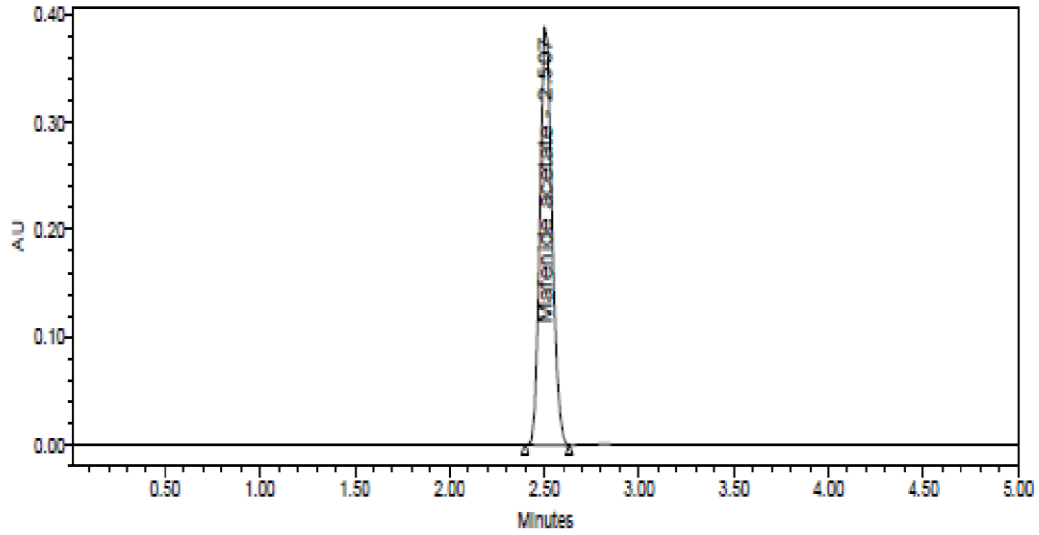


Fig 5: Method Precision Chromatogram for precision

Fig 5 explains that the percentage relative standard deviation (%RSD) was found to be less than 2% which proves that method is precise[30].

Table:2 Linearity Levels

S.NO	Level	Area
1.	50	896552
2.	75	1275273
3.	100	1689787
4.	125	2180494
5.	150	2569186
Correlation coefficient		0.9991

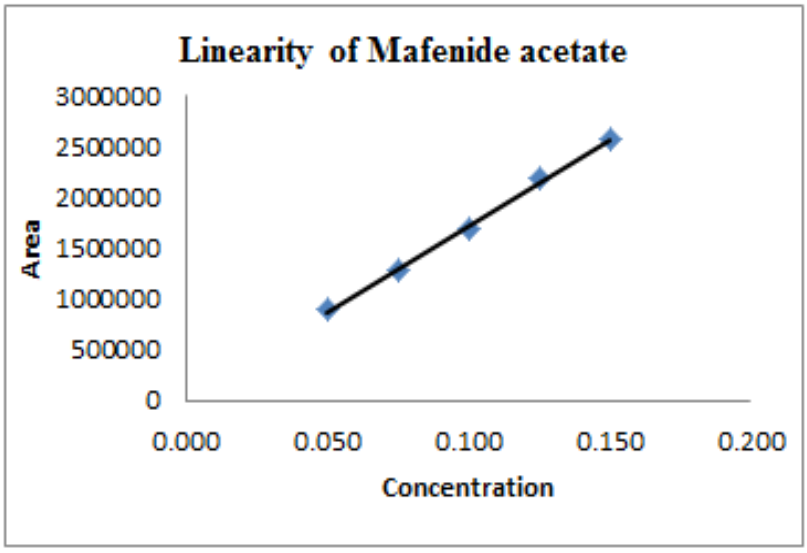


Fig 6: Linearity: Linearity plot of Mafenide acetate

Table 3 and Fig 6 explains the linear relationship between peak areas versus concentrations was observed for Mafenide acetate in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.9991 for Mafenide acetate which proves that the method is linear in the range of 50% to 150%.The linearity plot is also satisfying the norms as per the ICH guidelines [30].

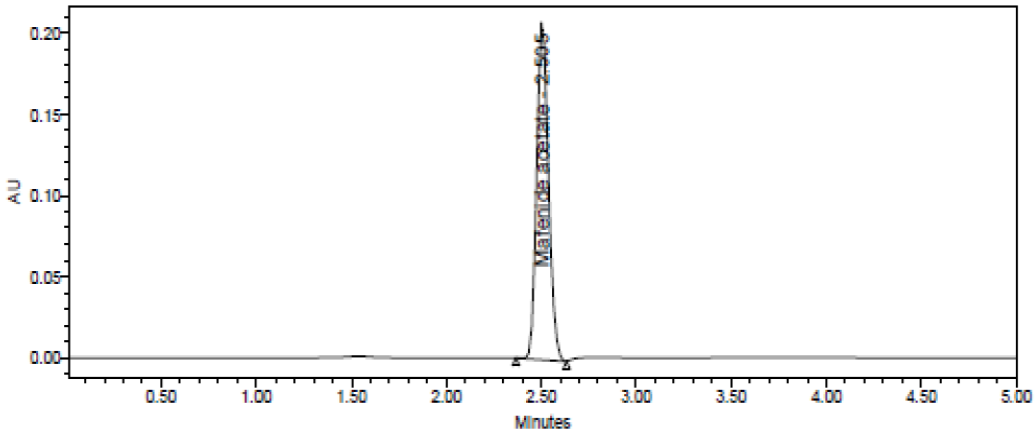


Fig 7: Accuracy: Typical chromatogram for Accuracy 50 %

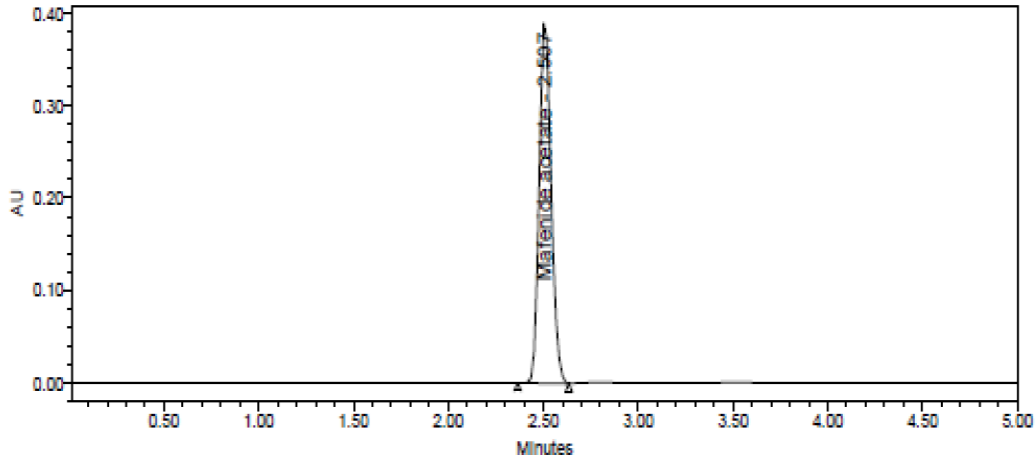


Fig 8: Typical chromatogram for Accuracy 100 %

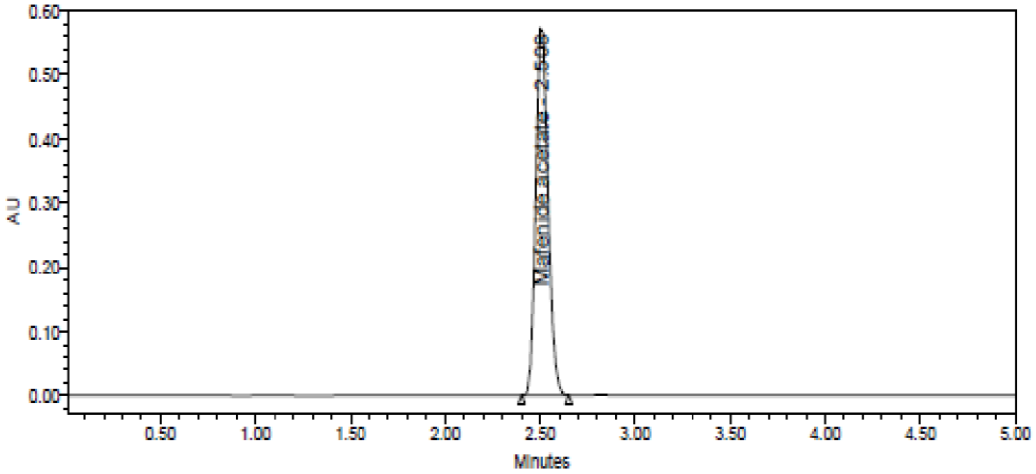


Fig 9: Typical chromatogram for Accuracy 150 %

Fig 7 at accuracy level 50%, Fig:8 at accuracy level 100% and Fig: 9 at accuracy level 150% shows that the method is highly accurate. A linear relationship between peak areas versus concentration was observed for Mefenide acetate in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.9991 for Mafenide acetate which prove that the method is linear in range of 50% to 150% and the results obtained were as par with IHC guidelines[30-31].

Table 3: Parameters and Results with Acceptance Criteria

S.No	Parameter	Result	Acceptance Criteria
1	System suitability Theoretical plates Asymmetry Retention time %RSD	 7325 1.15 2.507 0.28	 Not less than 2000 Not more than 2.0 Not more than 2.0
2	Specificity	Specific	Specific
3	Method precision(%RSD)	0.62	Not more than 2.0%
4	Linearity Range(mcg/ml) Correlation coefficient(r^2)	50.0-150.0 0.9991	Not less than 0.990
5	Accuracy (Mean % recovery) 50% 100% 150%	 99.2 99.7 99.6	 97 - 103%
6	Robustness	All the system suitability parameters are within the limits.	

The optical characteristics, precision and accuracy of the proposed method above mentioned table is about the parameters measured

In Table 3: Various parameters are taken into consideration as per the ICH[2,13]guidelines . System Suitability **:** Tailing factor for the peaks due to Mafenide acetate in standard solution should not be more than 2.0.Theoretical plates for the Mafenide acetate peaks in standard solution should not be less than 2000[2]. Asymmetry and RT should not be more than 2.0.The results obtained within the range. Specificity of standard **s**olution, sample, blank and placebo were prepared as per test procedure and injected into the HPLC system Chromatogram of standard and sample should be identical with near Retention time. A study to establish the interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure. The acceptance criteria for chromatogram of blank should not show any peak at the retention time of analyte peak. There is no interference due to blank at the retention time of analyte as shown in Fig:4. Hence the method is specific. A graph of standard versus the actual concentration in µg/ml and determined the coefficient of correlation and basis for 100% response. Linearity regression coefficient of average peak area response of replicate injections plotted against respective concentration should not be less than 0.999. The % y-intercept as obtained from the linearity data (without extrapolation through origin 0, 0) should be within ±2.0. As per the statistical evaluation. The injection reproducibility requirements are met if the %RSD [30-31] for peak areas is not more than 2.0 and for retention times is not more than 2.0[30]. The average percentage recovery was between 98-102% and Relative standard deviation of these recovery concentrations was less than 2%[2,13]..Results of accuracy study are presented in the above table. All the results indicate that the method is highly accurate**.** Results of variability were summarized in the above table. Percentage relative standard deviation (%RSD) was found to be less than 2% which proves that method is precise.The results of Robustness of the present method had shown that changes are not significant we can say that the method is Robust[30].

4. CONCLUSION

From the above experimental results it was concluded that, this newly developed method for the simultaneous estimation of Mafenide Acetate was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective. This study could be helpful to understand the work procedures and handling of SOP of UV Spectrophotometer, HPLC with calculating the statistical parameters and knowledge about the indicating method.. The proposed method can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories. Therefore, this validated method could be applied to demonstrate the ability of the different instrumental systems for control and sustained release tablets.

5. AUTHOR CONTRIBUTION STATEMENT

Dr. Choragudi S.F and Sharmila Thokala has designed and directed through the project; Navya Yanamandra K.S. and Chandrika kethineni performed the experiments; Sharmila Thokala has developed the literature framework and Navya Yanamandra analyzed the conducting of the experiment and acquired the results. Dr. Chorgudi S.F. and Chandrika Kethineni reverified the results and rectified the mistakes if any. NavyaYanamandra, under the guidance of Dr. Chorgudi S F scripted down this paper. Both authors read and approve the final version of the manuscript

6. CONFLICT OF INTEREST

Conflict of interest declared none.

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