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Development and validation of Polyhexa methylene guanidine HCL in its bulk and dosage form by RP- HPLC

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Abstract:
A simple, precision and accuracy HPLC method was developed the estimation of Polyhexa methylene guanidine analysis of formulation, consisting of an Methanol: water (60: 40 % v/v). The chromatographic condition was set at a Flow rate of 1 ml/min with the UV detector at 240 nm. The above method was optimized with a view to develop an assay method for Polyhexa methylene guanidine. Several mobile phase compositions were tried to resolve the peaks of Polyhexa methylene guanidine. The optimum mobile phase containing methanol: water (60: 40 % v/v) was selected because it was found ideal to resolve the analyte peaks of the drug. Quantification was achieved with UV detections at 240 nm based on peak area and absorbence. As per USP requirements system suitability studies were carried out and freshly prepared standard solutions of Polyhexa methylene guanidine.

Keywords: Polyhexa methylene guanidine, RP-HPLC, Method development, Validation.

INTRODUCTION:

Polyhexamethylene guanidine hydrochloride (PHMG) is an antibacterial polymer containing a guanidine group in its main chain. It dissolves in water easily and forms into colorless, odorless solution. This PHMG chemical can be used as a broad-spectrum and high-efficiency disinfectant. Polyhexamethylene guanidine hydrochloride is low toxic, steady, non-flammable, non-explosive, and non-corrosive to stainless steel, copper, carbon steel, wood, and plastic.

Because of its unique bactericidal mechanisms, almost all kinds of bacteria shall be killed efficiently and will not develop resistance action. PHMG disinfectant is a high molecular polymer that can easily be washed away. It is non-corrosive to the skin and can not be easily absorbed by human organs. Polyhexamethylene guanidine hydrochloride performs good biocompatibility. This chemical can be widely used in textile, animal husbandry, aquaculture, medical sterilization, and daily disinfectant.

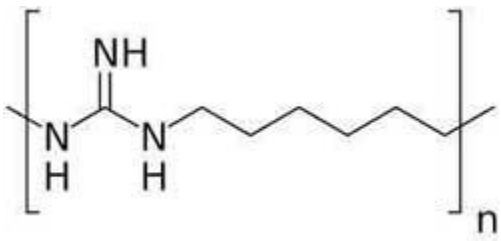

Figure 1: Structure of Polyhexa methylene guanidine

The literature survey revealed that There are very few methods reported in the literature for analysis of Polyhexa methylene guanidine alone or in combination with other drugs in the pure form and pharmaceuticals formulations by RP-HPLC.[3-7] In view of the need for a suitable, cost-effective RP-

HPLC method for routine analysis of Polyhexa methylene guanidine estimation of in pharmaceutical dosage form. Attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Polyhexa methylene guanidine. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the estimation of Polyhexa methylene guanidine in pharmaceutical dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Polyhexa methylene guanidine were Purchased from market. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 240 nm with column Symmetry C_{18} (4.6 X 150 mm; 5µm Waters), dimensions at Ambient temperature. The optimized mobile phase consists of methanol: water (60:40%v/v). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation Mobile phase:

Mix a mixture of above methanol (60%), 400 mL of HPLC water (40%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Standard Solution Preparation

Accurately weigh and transfer 10 mg of Polyhexa methylene guanidine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 368.0 mg of Polyhexa methylene guanidine sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of Polyhexa methylene guanidine of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Polyhexa methylene guanidine peaks and calculate the %Assay by using the formulae.

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 10 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Symmetry C_{18} (4.6 X 150 mm; 5µm Waters), the mobile phase of composition methanol: water (60:40%v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Polyhexa methylene guanidine in tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity: Polyhexa methylene guanidine working standard solutions were prepared across the range of the analytical method with a minimum of 5 concentrations that are within the specified range (10-50 µg/ml) low level (10 µg/ml) and higher level (50µg/ml) for 5 replicating injections were taken and calculated the %RSD. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%,150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Polyhexa methylene guanidine and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies: The system precision of the test method was performed by injecting 5 replicate determination of standard preparation injections were injected and the % RSD was calculated. The %RSD for the area of five replicate injections was found. The results are shown in table 5.

Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The results are shown in table 6.

Robustness: Robustness of assay method was carried out with variation of flow rate. Standard preparation was prepared and performed analysis as per test method and evaluated the system suitability parameters. As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method. The results are shown in table 7,8.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 9.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

RESULTS AND DISCUSSION

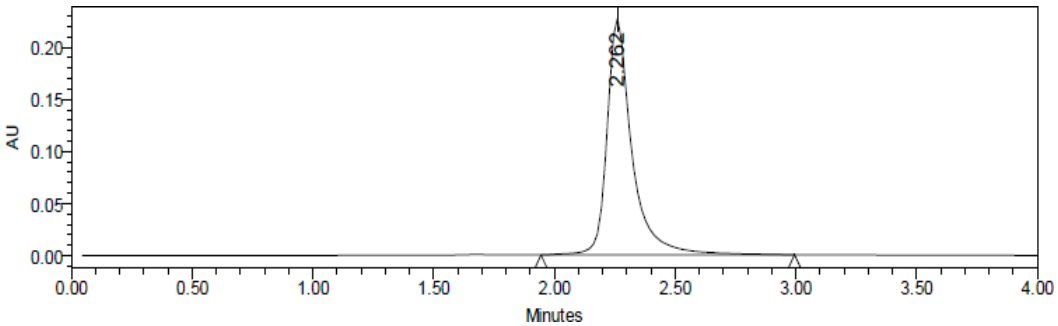

Figure 2: Standard chromatogram

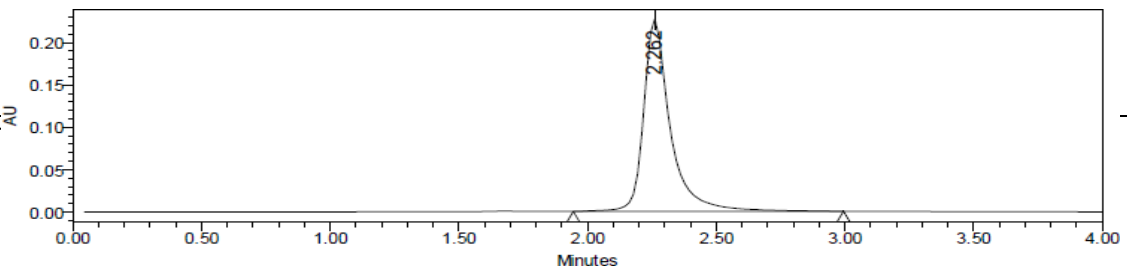

Figure 3: Sample chromatogram

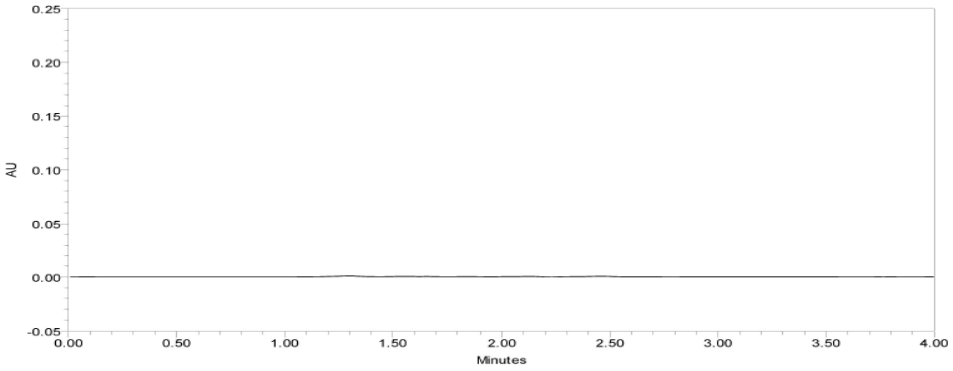

Figure 4: Blank chromatogram

Table 1: System suitability parameters

	Tailing factor	Theoretical Plates
Polyhexa methylene guanidine	1.5	2804.8

Table 2: Assay results for Polyhexa methylene guanidine

	Label Claim (mg)	% Assay
Polyhexa methylene guanidine	10	99.6

Table 3: Linearity results of Polyhexa methylene guanidine

S.No	Linearity Level	Concentration	Area
1	I	10ppm	682741
2	II	20ppm	1201305
3	III	30ppm	1627183
4	IV	40ppm	2180552
5	V	50ppm	2716958
Correlation Coefficient			0.999

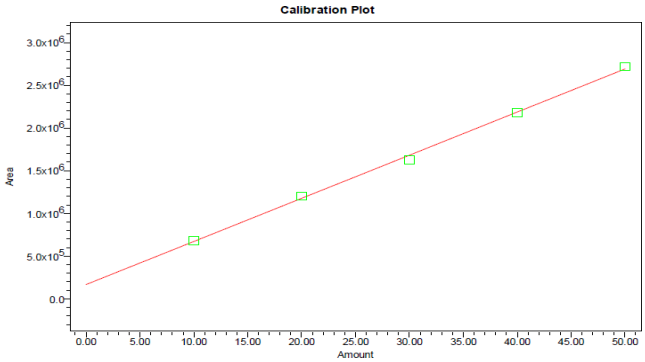

Figure 5: Linearity graph for Polyhexa methylene guanidine

Table 4: Showing accuracy results for Polyhexa methylene guanidine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	823686.2	5.0	5.0	100.1%	
100%	1634793	10	9.93	99.3%	
150%	2451939	15.0	14.9	99.3%	99.5%

Table 5: Precision results for Polyhexa methylene guanidine

Injection	Area
Injection-1	1631295
Injection-2	1630511
Injection-3	1636464
Injection-4	1628557
Injection-5	1635684
Average	1632502.2
Standard Deviation	3420.4
%RSD	0.2

Table 6. Ruggedness results of Polyhexa methylene guanidine

Injection	Area
Injection-1	1639701
Injection-2	1645897
Injection-3	1640705
Injection-4	1637036
Injection-5	1638609
Average	1640389.4
Standard Deviation	3365.9

%RSD	0.2

Robustness results

Table 7: Flow variation results for Polyhexa methylene guanidine

S.No	Flow Rate(ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	3353.0	1.5
2	1	2804.8	1.5
3	1.2	2384.0	1.4

Table 8: Change in organic composition results for Polyhexa methylene guanidine

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2396.0	1.3
2	*Actual	2804.8	1.5
3	10% more	2218.0	1.4

Table 9: LOD, LOQ of Polyhexa methylene guanidine

Drug	LOD	LOQ
Polyhexa methylene guanidine	2.273	2.268

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the estimation of Polyhexa methylene guanidine in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Polyhexa methylene guanidine in pure and its pharmaceutical dosage forms.

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