Faris Ameen, J. Global Trends Pharm Sci, 2021; 12 (2): 9437 - 9441

ISSN–2230-7346
Journal of Global Trends in Pharmaceutical Sciences

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE DETERMINATION OF AMINOGUANIDINE HCL

Faris Ameen A*[1], S Kathirvel[1], R. S. Chandan[2], R. Maruthi[2] and Vishnuraj. AR[1]

[1]Department of Pharmaceutical Analysis, National college of Pharmacy, KMCT Group of Institution, Manassery , Mukkam , Kozhikode, Kerala – 673602, India
[2]Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru - 570015, Karnataka, India.

*Corresponding Author E-mail: **farisameenponnad@gmail.com**

ARTICLE INFO	ABSTRACT
Key Words RP-HPLC method; Aminoguanidine HCl; ICH; HPLC with UV detector Access this article online Website: https://www.jgtps.com/ Quick Response Code:	Aminoguanidine is a single carbon compound used for the inhibition of formation of advanced glycation end products. A novel reversed-phase (RP) HPLC method for quantitative analysis of Aminoguanidine hydrochloride has been developed and validated in the bulk drug and tablet dosage form. HPLC Quantification was done using a RP C_{18} column, ortho-phosphoric acid buffer and methanol in the ratio of 90:10 v/v used as a mobile phase. Flow rate was maintained at 1mL/minute. The retention time (RT) of Aminoguanidine HCl was observed as 2.5 min at 220 nm by using UV detector. Linearity range was 50-90 µg/ml and coefficient of correlation was found to be 0.998. The proposed method has been validated according to ICH guidelines. The method was validated for system suitability, linearity, limits of detection and quantification, precision, selectivity, accuracy and ruggedness

INTRODUCTION

Aminoguanidine (AG) is the prototype of a therapeutic agent used to prevent advanced glycation end products (AGEs) from forming[1,2]. This interacts easily with alpha and beta-dicarbonyl compounds to prevent advanced glycation end products from being developed[3,4]. Aggregation of AGEs is a risk factor for the progression of diabetes and it's complications[5,6].AG also has other pharmacological actions, it inhibits nitric oxide synthase and semicarbazide-sensitive amine oxidase (SSAO). It is a highly reactive nucleophilic reagent with several biological molecules like pyridoxal phosphate and glucose[7,8]. It also decreased serum cholesterol, LDL and triglycerides in diabetic patients [5]. Aminoguanidine can prevent glycation as well as the browning reactions[9]. A study conducted on rats found that Aminoguanidine may prevent hypertrophy by inhibiting non-enzymatic glycation products, rather than by inhibiting Nitric oxide development in rats[10]. Aminoguanidine is a single-carbon compound, whose unequalled structure makes it effective of acting derivative as a guanidine, hydrazine, or formamide. The present method is a quick and precise RP-HPLC method for both bulk and tablet dosage analysis of Aminoguanidine HCl.

MATERIALS AND METHODS
Chemicals and reagents:

Aminoguanidine Hydrochloride standard were purchased from Tokyo Chemical Industry Co., Ltd, Tokyo, AminoPro tablets labelled to contain Aminoguanidine 75 mg, manufactured profound products were procured

9437

from market. All the chemicals used were HPLC grade, procured from Merck pharmaceuticals

Instrumentation and Chromatographic conditions

For the HPLC method development, Shimadzu LC-20AD HPLC system, with UV Detector SPD-20A was used, 10 μl of the sample was injected into Phenomenex luna C_{18} column (250 mm X 4.60 mm 5μ), ortho-phosphoric acid buffer and methanol in the ratio of 90:10 v/v are used as a mobile phase. Flow rate was maintained at 1mL/minute. The wavelength was set to 220 nm in UV detector. The temperature of the oven was 40 ℃. PHENEX PTFE0.02μm syringe sensor is used for filtration purposes.

Preparation of buffer for mobile phase:

Mixing 1.0 ml of ortho phosphoric acid in 1000 ml of Milli pore water and pH was adjusted with triethylamine for the preparation of mobile phase.

Preparation of standard stock solution:

For the HPLC method, 10 mg of Aminoguanidine Hydrochloride standard was weighted and makeup to 10 ml by using HPLC grade Methanol to obtain 1mg/ml concentration. From the solution, the standard stock solutions of 50, 60, 70, 80, and 90μg/ml were prepared.

Preparation of the sample solution

Weighed and finely powdered twenty tablets; a powder amount equal to 10 mg of Aminoguanidine Hydrochloride was weighed and makeup to 10 ml by using HPLC grade Methanol to obtain 1mg/ml concentration. From the above sample solution, pipette out 0.4ml and makeup to 10ml by using Methanol (HPLC grade) to get a 40μg/ml solution.

RESULT AND DISCUSSION

The simple, precise, and quick RP-HPLC technique for the determination of Aminoguanidine HCl has been developed. The method developed was validated as stated in ICH Q2(R1) guidelines. The RT of Aminoguanidine HCl was found to be 2.5min from the chromatogram. The coefficient of correlation was found to be 0.998.

System suitability

Standard stock of Aminoguanidine HCl was injected 6 times for testing system suitability

Linearity:

It is the ability to obtain experimental results equal to the specimen's analyte content. The calibration curve was obtained with five concentrations of the standard solution (50-90μg/ml). coefficient of correlation, slope and intercept was calculated from the graph

Precision

The method's repeatability has been checked using different concentrations of the drug 50, 70 and 100 μg/ml. The above solutions were prepared from stock solution and used to inject in interday and intraday for the evaluation of precision.

Accuracy   pH   was   adjusted towith triethyl

It's the closeness of the value obtained to the true sample value. The exactness of the approaches was calculated by the recovery percentage of the standard drug that was added to sample solutions. The study was carried out by adding 50%, 100%, 150% of standard drug to the sample solution.

### Limit of detection and limit of quantification

The limit of detection (LOD) was found to be 0.027849 µg/ml and The Limit of quantitation (LOQ) was found to be 0.084391 µg/ml

Robustness

It is a method's ability to stay unchanged when small differences in parameters are applied. The robustness of the system defined by small variations in the wavelength, flow rate, column temperature.

Ruggedness

It is a measure of the reproducibility of results by planned operating conditions from lab to lab and from changing analyst. The ruggedness of the developed method is validated.
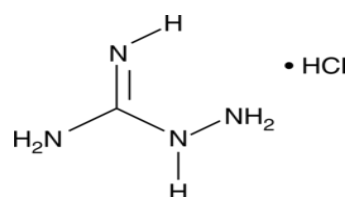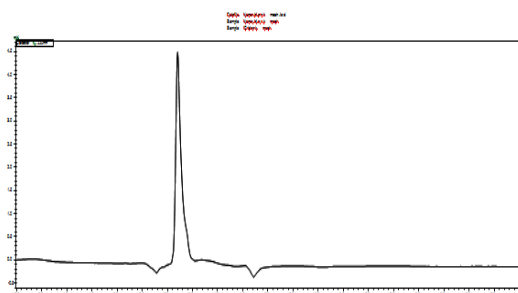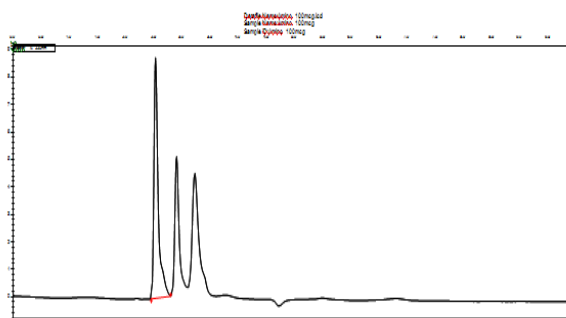
Figure 2: Blank chromatogram

Figure 3: Standard chromatogram of Aminoguanidine HCl at 100µg/ml showing RT at 2.5 min

Table 1: System suitability results

Parameters	Acceptance criteria	Results
Tailing factor	NMT 2.0	1.926
Theoretical plates	NLT 2000.0	8755

Table 2 : Calibration data of Aminoguanidine

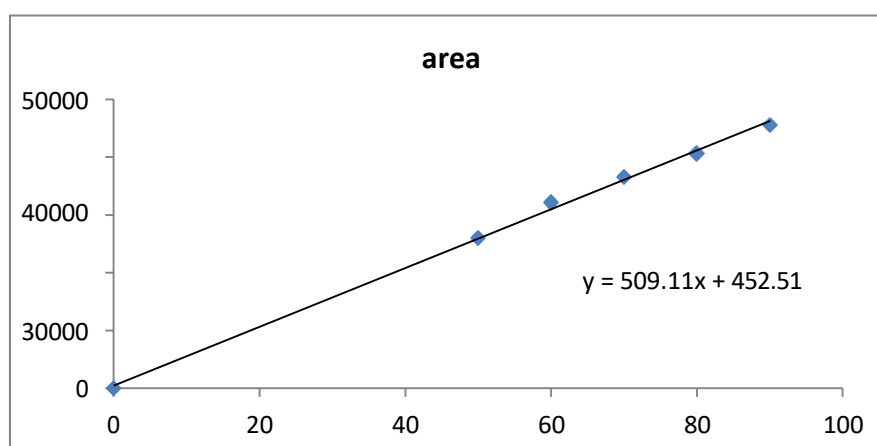| Sl.no	Concentration (µg/mL)	Peak Area
1	0	0
2	50	25996
3	60	32106
4	70	36550
5	80	40659
6	90	45591

Figure 4: Calibration curve for Aminoguanidine HCl

Table 2: Method precision intraday-interday studies

Method	Conc. Range (µg/mL)	% RSD (Intra-day N=6)	% RSD (inter-day N=6)
HPLC	50	0.79	1.40
	70	0.77	0.83
	90	0.82	0.67

Table 3 : Recovery studies of Aminoguanidine

	Level of recovery		
	50%	**100%**	**150%**
	60 µg/mL	**80 µg/mL**	**100 µg/mL**
Peak Area	31950	40773	52264
Mean % recovery	99.46	99.15	98.46

Table 4: Robustness data

Parameters	Change in units	Acceptance criteria	Results
Wavelength	220 ±3	%RSD ≤ 2	1.428
Flow rate	1ml/min ±0.1	%RSD ≤ 2	1.356
Column temperature	40˚c ±5˚c	%RSD ≤ 2	0.766

Table 5 : Ruggedness data

Concentration (µg/mL)	Trial 1 (Peak area)	Trial 2 (Peak area)	Mean (Peak area)	SD	%RSD
By changing the analyst					
0	0	0	0	0	0
50	25996	26346	26171	247.4874	0.945655
60	32106	32276	32191	120.2082	0.373422
70	36550	36986	36768	308.2986	0.838497
80	40659	40312	40485.5	245.3661	0.606059
90	45591	45408	45499.5	129.4005	0.2844
By changing the instrument					
0	0	0	0	0	0
50	26058	26683	26370.5	441.9417	1.675894
60	32138	32920	32529	552.9575	1.699891
70	36595	37361	36978	541.6438	1.464773
80	40676	41146	40911	332.3402	0.812349
90	46279	47314	46796.5	731.8555	1.563911

CONCLUSION

The developed RP-HPLC has been validated for system suitability, linearity, precision, Accuracy, LOD & LOQ, robustness and ruggedness in compliance with the ICH guidelines. It was inferred from the above finding that the developed method was reliable, accurate and unique for the detection of Aminoguanidine HCl.

Acknowledgement

The authors express their sincere thanks to the Principal, JSS College of Pharmacy, Mysuru and JSS Academy of Higher Education & Research, Mysuru for providing the necessary facilities to carry out the research work.

Conflicts of interest:

The author confirms no conflicts of interest.

REFERENCES:

1. M. Brownlee, H. Vlassara, A. Kooney, P. Ulrich, A. Cerami, Science 232 (1986) 1629–1632

2. Thornalley, P. (2003). Use of aminoguanidine (Pimagedine) to prevent the formation of advanced glycation endproducts. *Archives of Biochemistry and Biophysics*, 419(1), pp.31-40

3. N. Ahmed, O.K. Argirov, H.S. Minhas, C.A. Cordeiro, P.J. Thornalley, Biochem. J. 364 (2002) 1–14.

4. N. Ahmed, P.J. Thornalley, Biochem. J. 364 (2002) 15–24.

5. Soulis T, Cooper ME, Vranes D, Bucala R, Jerums G. Effects of aminoguanidine in preventing experimental diabetic nephropathy are related to the duration of treatment. Kidney international. 1996 Aug 1;50(2):627-34.

6. Joly, G.A., Ayres, M., Chelly, F., et al. Effects of NG-methyl-L-arginine, NG-nitro-L-arginine, and Aminoguanidine on constitutive and inducible nitric oxide synthase in rat aorta. Biochemical and Biophysical Research Communications 199, 147-154 (1994)

7. Misko, T.P., Moore, W.M., Kasten, T.P., et al. Selective inhibition of the inducible nitric oxide synthase by Aminoguanidine. European Journal of Pharmacology 233, 119-125 (1993)

8. R. Bucala, Z. Makita, G. Vega, S. Grundy, T. Koschinsky, A. Cerami, H. Vlassara, Proc. Natl. Acad. Sci. USA 91 (1994)9441–9445

9. Lewis B, Harding J. The effects of aminoguanidine on the glycation (non-enzymic glycosylation) of lens proteins. Experimental Eye Research. 1990;50(5):463-467

10. Stadler K, Jenei V, Somogyi A, Jakus J. Beneficial effects of aminoguanidine on the cardiovascular system of diabetic rats. Diabetes/Metabolism Research and Rev 2018.