

Ion chromatography

Assay of guanidine in pharmaceutical formulations

Authors

Sachin Patil and Jeff Rohrer

Thermo Fisher Scientific, Sunnyvale, CA

Keywords

Dionex IonPac CS20 column,
suppressed conductivity detection,
RFIC, Dionex CDRS 600, HPIC,
Dionex EGC 500

Goal

To develop an IC method for the determination of guanidine in pharmaceutical formulations using an RFIC system with suppressed conductivity detection

Introduction

Guanidine hydrochloride is a prescription drug used for the treatment of muscle weakness and easy fatigue caused by a rare disease known as the myasthenic syndrome of Eaton-Lambert.¹ Lambert-Eaton myasthenic syndrome is an antibody-mediated autoimmune disease that is caused by serum auto-antibodies. It leads to muscle weakness and autonomic dysfunction.² Guanidine has also been used for the treatment of botulism.³ Guanidine is limited in therapeutic use because of harmful side effects that accompany its administration—bone marrow suppression and renal failure.⁴

Currently used methods to determine guanidine include gas chromatography (GC) and high-performance liquid chromatography (HPLC) methods. These methods require derivatization before sample analysis. One GC method uses derivatization with glyoxal.⁵ Derivatization using isovaleroylacetone (IVA) and ethyl chloroformate (ECF) has also been used for a GC determination of guanidine.⁶ An HPLC method has been described for the determination of guanidine salts that requires derivatization with acetyl acetone.⁷ Another HPLC method uses fluorescence detection and requires reaction with 9,10-phenanthrenequinone before detection.⁸

The goal of this work is to design an ion chromatography (IC) method that uses a Thermo Scientific™ Dionex™ IonPac™ CS20 cation-exchange column, electrolytically

generated MSA eluent, and suppressed conductivity detection to determine guanidine in pharmaceutical formulations. IC offers a significant improvement over existing guanidine assays because it does not require derivatization of guanidine prior to analysis. The Dionex IonPac CS20 column provides a large separation window between monovalent and divalent cations. This allows most amines to elute after the alkali metals but before the alkaline earth metals, thus moving the amines away from matrix ions that would normally make quantitation difficult. Its selectivity makes the Dionex IonPac CS20 column particularly useful for determining a variety of alkyl and alkanol amines.

The eluent used in this separation is generated using a Thermo Scientific™ Dionex™ EGC 500 MSA Eluent Generator Cartridge and purified online using a Thermo Scientific™ Dionex™ CR-CTC 500 Continuously Regenerated Cation Trap Column (CR-CTC 500). The Thermo Scientific™ Dionex™ CDRS 600 (2 mm) Cation Electrolytically Regenerated Suppressor produces the regenerant ions necessary for eluent suppression and allows continuous operation with minimum maintenance. The RFIC system requires only deionized (DI) water as the carrier, significantly simplifying system operation and improving analytical reproducibility. The method also requires no time-, resource-, or money-consuming analyte derivatization.

The method proposed in this application note was validated following the guidelines outlined in USP General Chapter <1225>, Validation of Compendial Procedures¹¹ to meet the requirements of a validated guanidine assay.

Experimental

Equipment and consumables:

- A Thermo Scientific™ Dionex™ ICS-5000+ Reagent-Free Ion Chromatography system* including
 - DP Dual Pump with degas option
 - DC detector compartment with single temperature zone
- Thermo Scientific™ Dionex™ AS-AP autosampler (P/N 074926) with cooling tray option (recommended)
- Dionex EGC 500 MSA Eluent Generator Cartridge (P/N 075779)
- Dionex CR-CTC 500 Continuously Regenerated Cation Trap Column (P/N 075551)
- 2.5 µL sample loop
- 10 mL polystyrene autosampler vials, with caps (P/N 074228)
- Sterile assembled micro-centrifuge tubes with screw cap, 1.5 mL (Sarstedt P/N 72.692.005)

- Thermo Scientific™ Titan™ 3, 0.45 µm syringe filter units, nylon membrane, 30 mm diameter (P/N 44526-NN)

* Equivalent or improved results can be achieved using the Thermo Scientific™ Dionex™ ICS-6000 system.

Parameter	Setting
Columns	Dionex IonPac CS20 2 × 250 mm analytical column (P/N 302606) Dionex IonPac CG20 guard column 2 × 50 mm (P/N 302607)
Eluent flow rate	0.3 mL/min
Column temperature	40 °C
Run time	8 min
Injection volume	2.5 µL (Full loop)
Back pressure	~3,000 psi (1 psi = 6.89476 kpa)
Eluent	50 mM MSA
Eluent source	Dionex EGC 500 MSA
Detection	Suppressed Conductivity Detection using Dionex CDRS 600, 2 mm suppressor (P/N 088670)
Suppressor current	44 mA
Compartment temperature	25 °C

Method conditions

Reagents and chemicals

- Guanidine hydrochloride (Sigma P/N G4505)
- Mannitol (Sigma, P/N M9546)
- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better

Preparation of solutions and reagents

Guanidine stock solution 1000 mg/L

Accurately weigh 161 mg of guanidine hydrochloride and dissolve in DI water in a 125 mL polypropylene bottle. Adjust the weight to 100 g with DI water. Prepare a 100 mg/L secondary stock solution by 10-fold dilution of the primary stock solution.

Calibration standards

On the day of the analysis, dilute the secondary stock solution to prepare calibration standards with concentrations of 15, 10, 7.5, 5.0, 2.5, 1.5, 0.625, 0.31, and 0.2 mg/L of guanidine. For example, for the 10 mg/L standard, perform a 1:10 dilution of the 100 mg/L standard by adding 10 g of 100 mg/L standard to a 125 mL polypropylene bottle. Add DI water to a final weight of 100 g, cap, and store the bottle at 4 °C until needed.

Simulated matrix sample for accuracy study

A simulated matrix sample was prepared by dissolving two tablets of an over-the-counter paracetamol tablet formulation into 50 mL of DI water. The suspension was filtered through a 0.45 µm nylon syringe filter and this solution was used for the spike recovery experiment.

Robustness study

Following the guidelines of USP Physical Tests, <621> Chromatography,¹⁰ evaluate the robustness of this method by examining the retention time (RT), peak asymmetry, and resolution after imposing small variations ($\pm 10\%$) in procedural parameters (e.g., flow rate, eluent gradient concentration, column temperature). Inject a standard containing 2 mg/L guanidine. Apply the same procedure to another column set. Test the following variations:

- Flow rate at 0.27 mL/min, 0.3 mL/min, 0.33 mL/min
- Column temperature at 36 °C, 40 °C, and 44 °C
- MSA eluent initial concentrations at 45 mM, 50 mM, 55 mM

Note - The underlined parameters belong to the main method and the other two are the tested variations.

Results and discussion

Separation

Separation of guanidine was achieved using a Dionex IonPac CS20, 2 × 250 mm column under isocratic elution conditions. Figure 1 shows separation of a 2 mg/L guanidine solution. To achieve fast separation without interference from the nearest cations, a high eluent concentration (50 mM) was required throughout the run. Magnesium elutes well after the guanidine peak. The total method run time is 8 min, which is sufficiently long to ensure that cations eluting after magnesium are removed prior to the next injection. It also accounts for the increase in retention time encountered during some of the conditions of the robustness study.

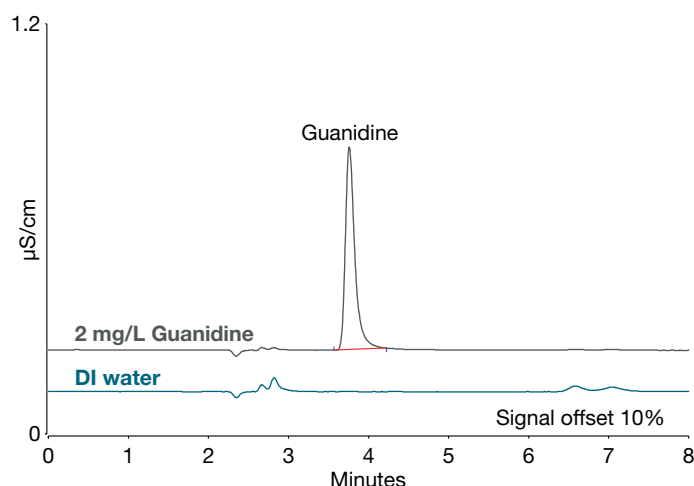


Figure 1. Separation of guanidine on a Dionex IonPac CS20 column

Method linearity and precision

The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the USP General Chapter <1225> guidelines recommend a minimum of five concentrations to establish linearity in an assay.⁹ For a drug substance or finished product, the minimum specified range is 80 to 120% of the test concentration. Method linearity was studied using guanidine standards at ten concentration levels ranging from 0.1 to 10 mg/L. The measured coefficient of determination value for a linear fit was 0.9999.

Assay precision was evaluated by injecting three replicates at three guanidine concentration levels, 0.2, 1, and 5 mg/L, and expressed as the RSDs of retention time and peak area from the series of measurements. The RT RSDs were $\leq 0.44\%$, and the peak area RSDs were $\leq 2.11\%$ (Table 1).

Table 1. Guanidine retention time and peak area precision (n=3)

	Guanidine conc. (mg/L)		
	0.25	0.34	1.74
RT RSD	0.34	0.44	0.27
Peak area RSD	1.74	2.11	1.2

Method sensitivity

Though method sensitivity is not important when assaying guanidine as a major component of a formulation, it is important if guanidine is measured as a related substance or as the analyte in a limit test. Method sensitivity was determined by analyzing guanidine standards and adjusting concentrations until S/N ratios of ~ 3 (LOD) and ~ 10 (LOQ) were obtained. To determine the LODs and LOQs, the baseline noise was first determined by measuring the peak-to-peak noise in a representative 1 min segment of the baseline where no peaks elute but close to the peaks of interest. The signal was determined from the average peak height of three injections of guanidine. The LOD and LOQ for guanidine were 0.0045 mg/L and 0.0125 mg/L, respectively (Table 2). Figure 2 shows chromatograms obtained using 0.0045 and 0.0125 mg/L injections of guanidine.

Table 2. Guanidine method sensitivity (n=5)

LOD (mg/L)	LOQ (mg/L)
0.0045	0.0125

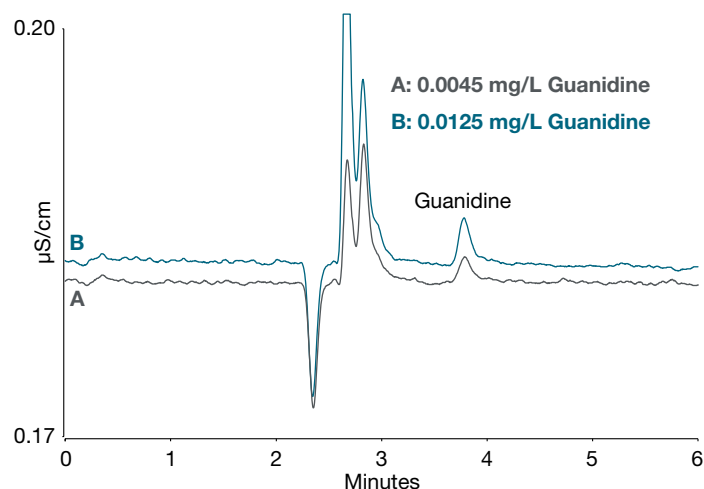


Figure 2. Guanidine sensitivity on a Dionex IonPac CS20 column

Table 3. Spike recovery experiment (n=3)

Analyte	Recovery (%)		
	1 mg/L spike	2.5 mg/L spike	5 mg/L spike
Guanidine	100	103	102

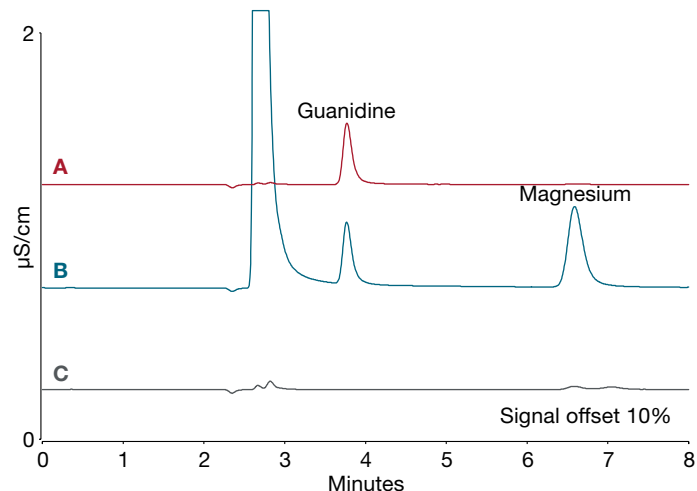


Figure 3. Analysis of (A) 1 mg/L guanidine standard, (B) simulated matrix sample spiked with 1 mg/L guanidine, and (C) DI water

Method accuracy

In the absence of a commercial guanidine formulation, a simulated matrix sample was prepared using an over-the-counter tablet formulation as described in the preparation of reagents and solutions section. Accuracy studies were conducted by spiking 125 mg each of guanidine and mannitol into the simulated matrix sample. Mannitol was spiked as it was found to be present in the guanidine tablet formulation¹¹ but missing in the tablet formulation used here. Guanidine was spiked at three different levels as shown in Table 3. The spiked solutions were diluted with DI water to bring the spike levels to 1, 2.5, and 5 mg/L. Figure 3 shows the chromatogram obtained for the solution spiked with 1 mg/L guanidine. All three levels yielded good guanidine recoveries indicating good method accuracy.

Method robustness

Method robustness was studied by introducing $\pm 10\%$ changes to method conditions and monitoring changes to key chromatographical parameters—retention time, asymmetry, and resolution (to magnesium). Robustness studies were performed on two different columns. The peak asymmetry was measured using the USP formula.¹⁰ A standard (10 mg/L guanidine) was injected three times (n=3) at each chromatographic condition. As shown in Tables 4 and 5, only eluent concentration has a significant effect on guanidine chromatography.

Table 4. Robustness studies on column 1 (n=3)

Condition	Difference (%)				
	Retention time		Peak asymmetry		Resolution
	Guanidine	Magnesium	Guanidine	Magnesium	Guanidine
-10% Flow rate	10.6	9.92	2.81	1.81	-0.26
+10% Flow rate	-9.06	-9.31	-1.30	-0.16	0.22
-10% Eluent	4.45	15.3	3.90	6.74	15.1
+10% Eluent	-4.23	-12.9	2.16	-4.28	-15.9
-10% Column temp.	2.05	0.19	3.03	0.16	-3.69
+10% Column temp.	-2.01	-0.15	2.16	1.48	3.36

Table 5. Robustness studies on column 2 (n=3)

Condition	Difference (%)				
	Retention time		Peak asymmetry		Resolution
	Guanidine	Magnesium	Guanidine	Magnesium	Guanidine
-10% Flow rate	11.6	12.30	4.29	3.06	-0.27
+10% Flow rate	-8.91	-8.57	-4.06	-3.40	0.97
-10% Eluent	4.48	15.5	2.71	6.96	15.51
+10% Eluent	-3.85	-12.1	-0.90	-5.77	-16.0
-10% Column temp.	2.27	0.88	2.03	-1.02	-3.58
+10% Column temp.	-1.61	0.74	1.13	1.53	3.54

Conclusions

This study describes an IC-based assay for the determination of guanidine in a simulated pharmaceutical formulation. Guanidine was separated on a cation-exchange column and detected by suppressed conductivity in 8 min. This method allows the concentration of guanidine to be determined in an automated way, circumventing the need to perform pre-analysis sample derivatization. This assay for guanidine was validated to meet the analytical performance characteristics outlined in USP General Chapter <1225>, Validation of Compendial Procedures, and was shown to measure accurately the content of guanidine in a simulated pharmaceutical formulation. This assay offers a simple, accurate, and robust measurement approach to determine guanidine. It should be applicable to other pharmaceutical formulations.

References:

1. Keogh M.; Sedehizadeh, S; Maddison, P. Treatment for Lambert-Eaton myasthenic syndrome. *Cochrane Database Syst. Rev.* **2011**, <https://doi.org/10.1002/14651858.CD003279.pub3>
2. Verschuuren, J.J.; Wirtz, P.W.; Titulaer, M.J.; Willems, L.N.; van Gerven, J. Available treatment options for the management of Lambert-Eaton myasthenic syndrome. *Expert. Opin. Pharmacother.* **2006**, *7*(10), 1323–1336.
3. Constantinos, C. A. and Koupparis, M. A. Automated flow injection spectrophotometric non-aqueous pseudotitrations of amines and their hydrohalide salts. *Analyst*, **1988**, *113*, 755.
4. Blumhardt, L.D.; Joeke, A. M.; Marshall, J.; Philalithis, P. E. Guanidine treatment and impaired renal function in the Eaton-Lambert syndrome. *Br. Med. J.* **1977**, *1*(6066), 946–947.
5. Majidano, S.A.; Khuhawar, M.Y. Gas Chromatographic Determination of Guanidino Compounds in Uremic Patients Using Glyoxal as Derivatizing Reagent. *J. Chromatogr. Sci.* **2012**, *50*(5), 380–386.
6. Zounr, R. A. et al. Improved Gas Chromatographic Determination of Guanidino Compounds Using Isovaleroylacetone and Ethyl Chloroformate as Derivatizing Reagents. *Anal. Sci.* **2016**, *32*(2), 141–146.
7. Waldemar Palaitis, W.; Curran, J. R. HPLC Assay for Guanidine Salts Based on Pre-Column Derivatization with Acetylacetone. *J. Chromatogr. Sci.* **1984**, *22*(3), 99–103.
8. Yamamoto, Y. et al. Quantitative Analysis of Methylguanidine and Guanidine in Physiologic Fluids by High-performance Liquid Chromatography-fluorescence Detection Method. *J. of Chromatogr.* **1979**, *162*(1), 23–29.
9. Validation of Compendial Methods, General Chapter <1225>. U.S. Pharmacopeia/ National Formulary: Rockville, MD, USP36-NF31; p 983.
10. Physical Tests, <621> Chromatography. U.S. Pharmacopeia/ National Formulary (USP): Rockville, MD, USP36-NF21; p 268.
11. Guanidine hydrochloride tablet. [Online] https://www.merck.com/product/usa/pi_circulars/g/guanidine_hydrochloride/guanidine_pi.pdf

Learn more at thermofisher.com/ic