Original Article

Stability of Diluted Adenosine Solutions in Polyolefin Infusion Bags

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Abstract

Background: Intravenous or intracoronary adenosine is used in the cardiac catheterization lab to achieve maximal coronary blood flow and determine fractional flow reserve.

Objective: To determine the stability of adenosine 10 and 50 µg/mL in either 0.9% sodium chloride injection or 5% dextrose injection in polyolefin infusion bags stored at 2 temperatures, refrigeration (2°C–8°C) or controlled room temperature (20°C–25°C).

Methods: Adenosine 10 µg/mL and 50 µg/mL solutions were prepared in 50 mL polyolefin infusion bags containing 0.9% sodium chloride injection or 5% dextrose injection and stored at controlled room temperature or under refrigeration. Each combination of concentration, diluent, and storage was prepared in triplicate. Samples were assayed using stability-indicating, reversed-phase high-performance liquid chromatography immediately at time 0 and at 24 hours, 48 hours, 7 days, and 14 days. Stability was defined as retaining 90% to 110% of the initial adenosine concentration. The samples were also visually inspected against a light background for clarity, color, and the presence of particulate matter.

Results: After 14 days, all samples retained 99% to 101% of the initial adenosine concentration. No considerable change in pH or visual appearance was noted. The stability data indicated no significant loss of drug due to chemical degradation or physical interactions during storage.

Conclusion: Adenosine solutions of 10 and 50 µg/mL were stable for at least 14 days in 50 mL polyolefin infusion bags of 0.9% sodium chloride injection or 5% dextrose injection stored at controlled room temperature and refrigerated conditions.

Key Words—adenosine, concentration, fractional flow reserve, stability

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Adenosine, an endogenous nucleoside, is used clinically to convert paroxysmal supraventricular tachycardia (PSVT) to sinus rhythm by slowing the conduction time through the AV node and by interrupting the reentry pathway.¹ In vivo, it is synthesized in the myocardium and is released in response to increased metabolic oxygen demands and ischemia. Adenosine administration results in coronary vasodilation and increased blood flow.² Its pharmacological properties allow for its use in the measurement of fractional flow reserve (FFR) in patients with multivessel coronary artery disease who

are undergoing percutaneous coronary intervention (PCI).^{3,4} FFR indicates stenosis severity by determining the ratio of the maximal blood flow in a diseased vessel to the maximum blood flow in the same vessel without stenosis. The calculation of FFR allows clinicians to accurately identify specific lesions most likely to induce myocardial ischemia.²

Currently, administrations of intravenous and intracoronary adenosine are used in a cardiac catheterization setting to achieve maximal coronary blood flow and determine FFR. Because adenosine requires continuous infusion administration and is used in

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an emergency setting, stability information would be beneficial for cost saving and accelerated medication delivery time. Two previous stability studies of diluted solutions of adenosine have demonstrated stability with the following concentrations: 50, 100, 200, and 750 µg/mL.^{5,6} Because no information is available on the stability of adenosine solutions at lower concentrations, the purpose of this study was to determine the stability of adenosine 10 µg/mL and 50 µg/mL solutions in 50 mL polyolefin infusion bags containing 0.9% sodium chloride injection or 5% dextrose injection at controlled room temperature (20°C-25 °C) or under refrigeration (2°C-8°C).

METHODS

Reverse-Phase High-Performance Liquid Chromatography

A Shimadzu model LC-2010A HT HPLC instrument (HPLC 2010A with LCSolutions; Shimadzu Scientific Instruments, Marlborough, MA) with a C18, 4.6 x 150 mm, 3 µm, 100 Å column (Luna C18 column [3 µm particle size, 4.6 mm x 150 mm]; Phenomenex, Inc, USA) and a guard cartridge system (SecurityGuard Cartridge C18 [3 mm x 4 mm]; Phenomenex, Inc, USA) with a C18, 4 x 3 mm cartridge maintained at 40°C was used for this study. The instrument contained a degasser, autosampler, and UV detector set at 259 nm. A stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was created and used. Data collection and processing were done using Shimadzu LC Solution software. The mobile phase consisted of methanol (Methanol HPLC grade; Fisher Scientific, Fair Lawn, NJ, lot 115982) and distilled water (7:93 vol/vol) with 0.1% trifluoroacetic acid (trifluoroacetic acid, 99%; ACROS Organics, NJ, lot B0517715). Adenosine was eluted at a flow rate of 0.5 mL/min after an injection of

10 µL. Under these conditions, adenosine eluted with an average retention time of 7 minutes.

Five standard solutions of adenosine, with concentrations of 1, 5, 10, 30, and 50 µg/mL, were prepared from 100 µg/mL stock solution with distilled water and used to establish linearity $r^2 > 0.999$ of the detector response. The 100 µg/mL adenosine stock solution was prepared using 10 mg of adenosine powder (adenosine, 99+%; ACROS Organics, NJ, lot A0263503) in 100 mL volumetric, using distilled water to make up the volume. These standards were passed through 0.45 µm, 13-mm diameter disposable nylon syringe filters (13-mm syringe filter [0.45 µm, nylon, non-sterile]; Fisherbrand, Ireland, lot R9PN60120) and assayed by RP-HPLC in triplicate on 3 separate days. Six sets of filtered standards were frozen in HPLC vials for use at each time point in the stability study after linearity was established following the first day's successful runs. Intraday and interday variability were determined and are depicted in Tables 1 and 2, respectively.

A forced degradation study was performed to verify that the assay was stability-indicating, with the ability to resolve degradation products from the parent compound. A 100 µg/mL adenosine stock solution was again used. From this stock solution, four 10 µg/mL solutions of 10 mL each were prepared using distilled water as the diluents and treated under separate conditions. Four samples were prepared as follows: sample 1 was adjusted to pH 2 with 1 M hydrochloric acid and incubated at 60°C, sample 2 was adjusted to pH 12 with 1 M sodium hydroxide and incubated at 60°C, sample 3 was spiked to a concentration of 3% hydrogen peroxide and incubated at 60°C, and sample 4 was spiked to a concentration of 3% hydrogen peroxide and placed in direct sunlight. RP-HPLC analysis was performed at time 0 and at

Table 1. Intraday standard results of adenosine with concentrations of 1, 5, 10, 30, and 50 µg/mL

Concentration (µg/mL)	Peak area			Average	SD	%CV
	Run #1	Run #2	Run #3			
0	0	0	0	0	-	-
1	63,068	63,114	62,911	63,031	106	0.17
5	313,430	315,180	316,161	314,924	1,383	0.44
10	632,797	635,095	634,614	634,169	1,212	0.19
30	1,964,048	1,966,455	1,967,198	1,965,900	1,647	0.08
50	3,285,579	3,294,835	3,295,877	3,292,097	5,669	0.17

Note: %CV = coefficient of variation.

Table 2. Interday standard results of adenosine with concentrations of 1, 5, 10, 30, and 50 µg/mL

Concentration (µg/mL)	Peak area			Interday average	Interday SD	Interday %CV
	May 29, 2012 average	May 31, 2012 average	June 2, 2012 average			
0	0	0	0	0	–	–
1	63,392	63,031	62,325	62,916	542.75	0.86
5	315,309	314,924	312,673	314,302	1,423.45	0.45
10	635,441	634,169	632,286	633,965	1,587.31	0.25
30	1,966,374	1,965,900	1,966,368	1,966,214	271.56	0.01
50	3,297,544	3,292,097	3,291,768	3,293,803	3,244.06	0.10

Note: %CV = coefficient of variation.

every 24 hours until at least 10% degradation had occurred, which occurred by the 72-hour time point. All degradation products were well separated from the parent drug with retention times shorter than 6 minutes.

Stability Study

The stability of adenosine 10 µg/mL and 50 µg/mL in 0.9% sodium chloride injection or 5% dextrose injection in 50 mL polyolefin infusion bags stored at controlled room temperature (20°C-25°C) and refrigeration (2°C-8°C) was tested as follows.

The average overfill volume of three 50 mL polyolefin infusion bags of 0.9% sodium chloride injection (0.9% sodium chloride injection in PAB container [copolymer of ethylene and propylene]; B.Braun Medical Inc., Irvine, CA, lot J2D929) and 5% dextrose injection (5% dextrose injection in PAB

container [copolymer of ethylene and propylene]; B.Braun Medical Inc., Irvine, CA, lot J2C904) was determined for the lots and expirations used for this stability study. The average overfill of the 50 mL IV bag of 0.9% sodium chloride injection and 5% dextrose injection was 7.3 mL and 6.3 mL, respectively. For the 2 strengths being studied, the volume of overfill was withdrawn along with the volume of adenosine 3 mg/mL solution (adenosine injection 3 mg/mL, USP; APP Pharm, Schaumburg, IL, lot 6003500) to be injected. Adenosine 10 µg/mL and 50 µg/mL were prepared in triplicate in either 0.9% sodium chloride or 5% dextrose and stored at either controlled room temperature (20°C-25°C) or refrigeration (2°C-8°C), yielding a total of 24 prepared samples. In addition to this, each diluent was also stored under controlled room temperature or refrigeration without adenosine added, 4 more samples

Table 3. Stability of adenosine 10 and 50 µg/mL in either 0.9% sodium chloride injection or 5% dextrose injection in polyolefin infusion bags stored at refrigeration (2°C-8°C) or at controlled room temperature (20°C-25°C)

Concentration (µg/mL)	Diluent (50 mL IV bag)	Storage (°C)	Actual initial adenosine concentration (mcg/mL)	% Initial concentration remaining			
				24 hours	48 hours	7 days	14 days
10	NS	20-25	10.33 ± 0.41	100.4 ± 0.1	101.0 ± 0.0	100.7 ± 0.3	100.1 ± 0.1
		2-8	10.05 ± 0.39	100.4 ± 0.1	100.8 ± 0.2	100.8 ± 0.2	100.1 ± 0.1
	D5W	20-25	10.03 ± 0.32	100.4 ± 0.1	100.4 ± 0.1	100.0 ± 0.6	99.2 ± 0.1
		2-8	10.43 ± 0.20	100.0 ± 0.2	100.1 ± 0.1	99.8 ± 0.1	99.7 ± 0.1
50	NS	20-25	48.97 ± 1.07	100.1 ± 0.0	100.1 ± 0.8	100.1 ± 0.8	100.1 ± 0.1
		2-8	48.45 ± 0.59	100.2 ± 0.1	100.2 ± 0.0	100.2 ± 0.0	100.0 ± 0.0
	D5W	20-25	50.09 ± 1.37	100.1 ± 0.0	100.2 ± 0.1	99.7 ± 0.1	99.7 ± 0.2
		2-8	50.68 ± 1.96	100.0 ± 0.1	100.0 ± 0.1	99.8 ± 0.2	99.8 ± 0.1

Note: NS = 0.9% sodium chloride injection; D5W = 5% dextrose injection.

Figure 1. Calibration curve.

total, to serve as controls should unexpected results arise. All preparations were made using aseptic techniques in a class II biological safety cabinet (ESCO Airstream Class II Biological Safety Cabinet; ESCO Technologies, Hatboro, PA). At each stability time point, the samples were visually inspected for clarity, color, and presence of particulate matter; 2 mL was aseptically withdrawn for pH measurements and 1 mL for RP-HPLC analysis.

The initial concentration of adenosine in solution was determined using the regression equation from the calibration curve, Concentration ($\mu\text{g/mL}$) = 65964/(Peak Area), from the time 0 analyses as depicted in

Figure 1. Using the peak areas, percent initial concentration remaining was determined using the average of the triplicate $\pm$ SD. Both initial concentration and percent initial concentration are depicted in Table 3. For this study, stability was defined as 90% to 110% of the time 0 concentration with no significant changes in visual appearance or pH.

pH Measurements

For each sampling time, the pH meter (*SevenEasy* pH meter; Mettler-Toledo Inc., Columbus, OH) was calibrated with pH 4 (buffer solution pH 4.00; Fisher Scientific, Fair Lawn, NJ, lot 085812) and pH 7

Table 4. Stability study of pH measurements of adenosine 10 and 50 $\mu\text{g/mL}$ in either 0.9% sodium chloride injection or 5% dextrose injection in polyolefin infusion bags stored at refrigeration (2°C - 8°C) or at controlled room temperature (20°C - 25°C)

Concentration ($\mu\text{g/mL}$)	Diluent (50 mL IV bag)	Storage ($^\circ\text{C}$)	pH (Mean $\pm$ SD)				
			0 hours	24 hours	48 hours	7 days	14 days
10	NS	20-25	4.94 $\pm$ 0.21	5.08 $\pm$ 0.08	4.95 $\pm$ 0.06	4.97 $\pm$ 0.02	5.02 $\pm$ 0.05
		2-8	4.77 $\pm$ 0.03	4.98 $\pm$ 0.01	4.90 $\pm$ 0.02	4.96 $\pm$ 0.02	4.95 $\pm$ 0.02
	D5W	20-25	4.43 $\pm$ 0.03	4.45 $\pm$ 0.02	4.43 $\pm$ 0.01	4.43 $\pm$ 0.00	4.48 $\pm$ 0.03
		2-8	4.43 $\pm$ 0.01	4.45 $\pm$ 0.03	4.42 $\pm$ 0.01	4.44 $\pm$ 0.02	4.50 $\pm$ 0.02
50	NS	20-25	4.93 $\pm$ 0.01	5.06 $\pm$ 0.04	5.00 $\pm$ 0.02	5.07 $\pm$ 0.02	5.08 $\pm$ 0.02
		2-8	4.88 $\pm$ 0.02	5.04 $\pm$ 0.03	4.97 $\pm$ 0.01	5.05 $\pm$ 0.03	5.09 $\pm$ 0.01
	D5W	20-25	4.55 $\pm$ 0.01	4.56 $\pm$ 0.01	4.54 $\pm$ 0.00	4.55 $\pm$ 0.01	4.60 $\pm$ 0.01
		2-8	4.52 $\pm$ 0.03	4.57 $\pm$ 0.01	4.53 $\pm$ 0.01	4.55 $\pm$ 0.01	4.61 $\pm$ 0.01

Note: NS = 0.9% sodium chloride injection; D5W = 5% dextrose injection.

(buffer solution pH 7.00; Fisher Scientific, Fair Lawn, NJ, lot 084727) buffer solutions, yielding a slope of 97% or greater at 25°C. The average pH values for each triplicate $\pm$ SD were determined and are depicted in **Table 4**. For this study, stability was defined as a pH measurement consistent with the package labels of each component used, specifically 3.5 to 7.5.^{1,7,8}

RESULTS AND DISCUSSION

All solutions were visually clear, colorless, and free of particulate matter at all time points. The initial pH values of the samples were consistent with the acceptable pH ranges specified in the package insert, that is, pH 4.5 to 7.0 for 9% sodium chloride injection and pH 3.5 to 6.5 for 5% dextrose injection. There was no considerable change in pH observed throughout the study as shown in **Table 4**. All samples retained greater than 90% of the initial concentration at all time points, suggesting little to no loss of adenosine due to chemical degradation or physical adsorption to polyolefin infusion bag packaging as shown in **Table 3**.

In future studies, it would be beneficial to perform this study with the use of polyvinyl chloride (PVC) bags, which are less expensive and more commonly seen in the hospital setting, in order to obtain a complete range of stability data. Although PVC IV bags were unavailable at the time of this study, the use of polyolefin infusion bags provides unique stability data. In recent years, there has been a shift for many hospitals to use DEHP-free IV bags, as the plasticizer has been shown to have effects on the development of the male reproductive system and production of normal sperm in young laboratory animals.⁹ Polyolefin infusion bags consist of a polymer of ethylene and propylene and are latex-free, PVC-free, and DEHP-free. The plastic container is developed for parenteral medication and is commonly referred to as *PAB*

infusion bags.^{7,8} The copolymer contains no plasticizers and exhibits no leachability, which can be considered an advantage when preparing a low-concentration adenosine formulation.

CONCLUSION

Adenosine solutions of 10 and 50 μ g/mL concentrations in 50 mL polyolefin infusion bags of 0.9% sodium chloride injection and 5% dextrose injection were stable when stored in the refrigerator or at controlled room temperature for a period of up to 14 days.

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