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Stability of Diluted Adenosine Solutions in Polyolefin Infusion Bags

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Stability of Diluted Adenosine Solutions in Polyolefin Infusion Bags

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.

Disciplines

Pharmacy and Pharmaceutical Sciences

Comments

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TITLE:

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 $\mu\text{g}/\text{mL}$ in either 0.9% sodium chloride injection or 5% dextrose injection in polyolefin infusion bags stored at two temperatures, refrigeration (2 - 8°C) or at controlled room temperature (20 - 25°C).

METHODS

Adenosine 10 $\mu\text{g}/\text{mL}$ and 50 $\mu\text{g}/\text{mL}$ 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 (20 - 25 °C) or under refrigeration (2 - 8 °C). Each combination of concentration, diluent, and storage was prepared in triplicate. Samples were assayed using a stability-indicating, reversed-phase high performance liquid chromatography immediately at time zero and at 24 hours, 48 hours, 7 days, and 14 days. Stability was defined as retaining 90% - 110% of the initial adenosine concentration. The samples were also visually inspected against a light back ground for clarity, color, and the presence of particulate matter.

RESULTS

After 14 days, all samples retained 99 - 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 $\mu\text{g}/\text{mL}$ 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, stability, concentration, fractional flow reserve

INTRODUCTION:

Adenosine, an endogenous nucleoside, is clinically used 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 allows 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. Since adenosine requires continuous infusion administration and is used in 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 $\mu\text{g}/\text{mL}$.^{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 $\mu\text{g}/\text{mL}$ and 50 $\mu\text{g}/\text{mL}$ solutions in 50 mL polyolefin infusion bags containing 0.9% sodium chloride injection or 5% dextrose injection at controlled room temperature (20 - 25 °C) or under refrigeration (2 - 8 °C).

METHODS:

RP-HPLC:

A Shimadzu model LC-2010A HT HPLC instrument^a with a C18, 4.6x150 mm, 3 μ m, 100 A column^b and a guard cartridge system^c with a C18, 4x3 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 was done using Shimadzu LC Solution software. The mobile phase consisted of methanol^d and distilled water (7:93 v/v) with 0.1% trifluoroacetic acid^e. Adenosine was eluted at a flow rate of 0.5 mL/min after an injection of 10 μ L. 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 μ g/mL, were prepared from 100 μ g/mL stock solution with distilled water and used to establish linearity $r^2 > 0.999$ of the detector response. The 100 μ g/mL adenosine stock solution was prepared using 10 mg of adenosine powder^f in 100 mL volumetric, using distilled water to make up the volume. These standards were passed through 0.45 μ m, 13 mm diameter disposable nylon syringe filters^g and assayed by RP-HPLC in triplicate on three 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. Intra-day and inter-day variability was determined and is depicted in Tables 1 and 2, respectively.

TABLE 1.

Intraday standard results of adenosine with concentrations of 1, 5, 10, 30, and 50 μ g/mL

TABLE 2.

Interday standard results of adenosine with concentrations of 1, 5, 10, 30, and 50 $\mu\text{g/mL}$

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 $\mu\text{g/mL}$ adenosine stock solution was again used. From this stock solution, four 10 $\mu\text{g/mL}$ 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 one was adjusted to pH 2 with 1 M hydrochloric acid and incubated at 60 °C, sample two was adjusted to pH 12 with 1 M sodium hydroxide and incubated at 60 °C, sample three was spiked to a concentration of 3% hydrogen peroxide and incubated at 60 °C, and sample four was spiked to a concentration of 3% hydrogen peroxide and placed in direct sunlight. RP-HPLC analysis was performed at time zero and at 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-min.

STABILITY STUDY:

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

The average overfill volume of three 50 mL polyolefin infusion bags of 0.9% sodium chloride injection^h and 5% dextrose injectionⁱ 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

two strengths being studied, the volume of overflow was withdrawn along with the volume of adenosine 3 mg/mL solution^j 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 - 25 °C) or refrigeration (2 - 8 °C), yielding a total of 24 prepared samples. In addition to this, each diluent was also stored under controlled room temperature (20 - 25 °C) or refrigeration (2 - 8 °C) without adenosine added, four more samples total, to serve as controls should unexpected results arise. All preparations were made using aseptic techniques in a class II biological safety cabinet^k. 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, $Concentration (\mu g/mL) = 65964/(Peak Area)$, from the time zero analyses as depicted in Figure 1. Using the peak areas, % initial concentration remaining was determined using the average of the triplicate \pm standard deviation. Both initial concentration and percent initial concentration are depicted in Table 3. For this study, stability was defined as 90% - 110% of the time zero concentration with no significant changes in visual appearance or pH.

FIGURE 1.

Calibration Curve

TABLE 3.

Stability 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 two temperatures, refrigeration (2 - 8 $^{\circ}\text{C}$) or at controlled room temperature (20 - 25 $^{\circ}\text{C}$).

pH MEASUREMENTS:

For each sampling time, the pH meter^l was calibrated with pH 4^m and pH 7ⁿ buffer solutions, yielding a slope $\geq 97\%$ at 25 $^{\circ}\text{C}$. The average pH values for each triplicate \pm standard deviation 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}

TABLE 4.

Stability Study 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 two temperatures, refrigeration (2 - 8 $^{\circ}\text{C}$) or at controlled room temperature (20 - 25 $^{\circ}\text{C}$).

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, i.e. pH 4.5 – 7.0 for 9% sodium chloride injection and pH 3.5 – 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 bags packaging as shown in Table 3.

In future studies, it would be beneficial to perform this study with the use of 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 polyvinyl chloride (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 µg/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.

FOOTNOTES:

^aHPLC 2010A with LCSolutions. Shimadzu Scientific Instruments, Marlborough, MA.

^bLuna C18 column (3 µm particle size, 4.6 mm x 150 mm). Phenomenex, Inc, USA.

^cSecurityGuard Cartridge C18 (3 mm x 4 mm). Phenomenex, Inc, USA.

^dMethanol HPLC Grade. Fisher Scientific, Fair Lawn, NJ, lot 115982.

^eTrifluoroacetic acid, 99%. ACROS Organics, NJ, lot B0517715.

^fAdenosine, 99+%. ACROS Organics, NJ, lot A0263503.

^g13 mm Syringe Filter (0.45 µm, Nylon, Non-Sterile). Fisherbrand, Ireland, lot R9PN60120.

^h0.9% sodium chloride injection in PAB® container (copolymer of ethylene and propylene).

B.Braun Medical Inc., Irvine, CA, lot J2D929.

ⁱ5% dextrose injection in PAB® container (copolymer of ethylene and propylene). B.Braun

Medical Inc., Irvine, CA, lot J2C904.

^jAdenosine injection 3 mg/ml, USP. APP Pharm, Schaumburg, IL, lot 6003500.

^kESCO Airstream Class II Biological Safety Cabinet. ESCO Technologies, Hatboro, PA.

^lSevenEasy pH meter. Mettler-Toledo Inc., Columbus, OH.

^mBuffer solution pH 4.00. Fisher Scientific, Fair Lawn, NJ, lot 085812.

ⁿBuffer solution pH 7.00. Fisher Scientific, Fair Lawn, NJ, lot 084727.

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TABLE 1.
Intraday standard results of adenosine with concentrations of 1, 5, 10, 30, and 50 $\mu\text{g/mL}$

Concentration (mcg/mL)	Peak Area				SD	%CV
	Run #1	Run #2	Run #3	Average		
0	0	0	0	0	-	-
1	63068	63114	62911	63031	106	0.17
5	313430	315180	316161	314924	1383	0.44
10	632797	635095	634614	634169	1212	0.19
30	1964048	1966455	1967198	1965900	1647	0.08
50	3285579	3294835	3295877	3292097	5669	0.17

TABLE 2.
Interday standard results of adenosine with concentrations of 1, 5, 10, 30, and 50 $\mu\text{g/mL}$

Concentration (mcg/mL)	Peak Area				Interday SD	Interday %CV
	2012/05/29 Average	2012/05/31 Average	2012/06/02 Average	Interday Average		
0	0	0	0	0	-	-
1	63392	63031	62325	62916	542.75	0.86
5	315309	314924	312673	314302	1423.45	0.45
10	635441	634169	632286	633965	1587.31	0.25
30	1966374	1965900	1966368	1966214	271.56	0.01
50	3297544	3292097	3291768	3293803	3244.06	0.10

TABLE 3.

Stability 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 two temperatures, refrigeration (2 - 8°C) or at controlled room temperature (20 - 25°C).

Concentration (mcg/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	25	10.33 \pm 0.41	100.4 \pm 0.1	101.0 \pm 0.0	100.7 \pm 0.3	100.1 \pm 0.1
		5	10.05 \pm 0.39	100.4 \pm 0.1	100.8 \pm 0.2	100.8 \pm 0.2	100.1 \pm 0.1
	D5W	25	10.03 \pm 0.32	100.4 \pm 0.1	100.4 \pm 0.1	100.0 \pm 0.6	99.2 \pm 0.1
		5	10.43 \pm 0.20	100.0 \pm 0.2	100.1 \pm 0.1	99.8 \pm 0.1	99.7 \pm 0.1
50	NS	25	48.97 \pm 1.07	100.1 \pm 0.0	100.1 \pm 0.8	100.1 \pm 0.8	100.1 \pm 0.1
		5	48.45 \pm 0.59	100.2 \pm 0.1	100.2 \pm 0.0	100.2 \pm 0.0	100.0 \pm 0.0
	D5W	25	50.09 \pm 1.37	100.1 \pm 0.0	100.2 \pm 0.1	99.7 \pm 0.1	99.7 \pm 0.2
		5	50.68 \pm 1.96	100.0 \pm 0.1	100.0 \pm 0.1	99.8 \pm 0.2	99.8 \pm 0.1

TABLE 4.

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

Concentration (mcg/mL)	Diluent (50 mL IV bag)	Storage (°C)	pH (Mean \pm SD)				
			0 hours	24 hours	48 hours	7 days	14 days
10	NS	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
		5	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	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
		5	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	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
		5	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	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
		5	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