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Stability of diluted adenosine solutions in polyvinyl chloride infusion bags

Melissa Kaltenbach
St. John Fisher College

David Hutchinson
St. John Fisher College, dhutchinson@sjfc.edu

Jessica E. Bollinger
Cleveland Clinic

Fang Zhao
St. John Fisher College, fzhao@sjfc.edu

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Stability of diluted adenosine solutions in polyvinyl chloride infusion bags

Abstract

Purpose The stability of diluted adenosine solutions in polyvinyl chloride infusion bags was studied.

Methods Adenosine 50-, 100-, and 220-μg/mL solutions were prepared in 50-mL polyvinyl chloride (PVC) infusion bags containing 0.9% sodium chloride injection or 5% dextrose injection and stored at room temperature (23–25 °C) or under refrigeration (2–8 °C). Each sample of every combination of concentration, diluent, and storage temperature was prepared in triplicate, yielding 36 samples. The samples were assayed using a stability-indicating, reverse-phase high-performance liquid chromatographic method immediately after preparation (time zero) and at 24 hours, 48 hours, 7 days, and 14 days. pH was measured at time zero, 48 hours, 7 days, and 14 days. Time zero concentrations were calculated from the equation produced from a calibration curve of standards ranging from 10 to 500 μg/mL. Samples were also visually inspected against a light background for clarity, color, and the presence of crystalline particulate matter. Stability was defined as retaining at least 90% of the initial adenosine concentration.

Results After 14 days, all samples retained greater than 98% of the initial adenosine concentration, with no evidence of adsorption, visible precipitation, or considerable change in pH, suggesting minimal to no loss of product due to degradation or adsorption.

Conclusion Adenosine 50-, 100-, and 220-μg/mL solutions in 50-mL PVC infusion bags containing 0.9% sodium chloride injection or 5% dextrose injection stored at room temperature and refrigerated conditions were stable for at least 14 days.

Disciplines

Pharmacy and Pharmaceutical Sciences

Comments

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Stability of diluted adenosine solutions in IV bags stored at two different temperatures

Abstract

Purpose: The objective of this study was to determine the stability of adenosine at concentrations of 50, 100, and 220 µg/ml in 50 ml IV bags of either 0.9% sodium chloride injection or 5% dextrose injection over the period of 14 days stored in the refrigerator (2-8°C) or at room temperature (23-25°C) using high performance liquid chromatography (HPLC).

Methods: Using aseptic techniques, adenosine solutions were prepared at concentrations of 50, 100, and 220 µg/ml in 50 ml IV bags of 0.9% sodium chloride injection and 5% dextrose injection and stored at room temperature or refrigerated. Each sample of every combination of concentration, diluent, and storage temperature was made in triplicate for a total of 36 samples. The samples were visually inspected for microbial growth and assayed using high-performance liquid chromatography at time zero as well as 24 hours, 48 hours, 7 days, and 14 days. Measurements of pH were taken at time zero, 48 hours, 7 days, and 14 days. Time zero concentrations were calculated from the equation produced from a calibration curve of standards ranging from 10 µg/ml to 500 µg/ml. Stability was then defined as retaining ≥ 90% of the time zero concentration.

Results: After 14 days, all sample preparations contained greater than 90% of the initial (time zero) adenosine concentration with no evidence of adsorption, visible growth, or considerable change in pH.

Conclusion: Adenosine solutions of 50, 100, and 220 µg/ml concentrations in 50 ml IV bags of 0.9% sodium chloride injection and 5% dextrose injection were stable when stored in the refrigerator or at room temperature for a period of at least 14 days.

Background

Adenosine is an endogenous nucleoside present in all cells of the body. Clinically, it is used to convert paroxysmal supraventricular tachycardia to sinus rhythm. This is accomplished by the slowing of conduction time through the A-V node and by interrupting the reentry pathways through the A-V node.[1] In recent years, there has been interest in the use of adenosine to restore hepatic artery flow (HAF) postoperative liver transplantation. Currently, animal data has shown that restoring the hepatic artery buffer response results in improved survival.[2] In this study, 9 female pigs received continuous infusions of adenosine at 0.7 µg/kg/minute until the HAF was greater than 250 ml/minute.

Currently, an ongoing pilot study aims to demonstrate that adenosine infused into the hepatic artery of adult liver transplant recipients will produce an increase in HAF. Low doses of adenosine ranging from 0.7-5.0 mcg/kg/minute are infused via a 16G central venous catheter line into a branch of the hepatic artery (normally ligated during liver transplant). To maintain patency of the artery with sufficient flow from the catheter and simultaneously achieve low doses of adenosine, low concentrations are necessary for infusion into the catheter for up to 48 hours post-transplantation. There is little data on the stability of diluted solutions of adenosine. One study reports adenosine diluted to 750 µg/ml with 0.9% sodium chloride injection, 5% dextrose injection, lactated Ringer's injection, or 5% dextrose and lactated Ringer's injection as being stable at 25, 5, and -15°C for at least 14 days.[3] Because no information is available on the stability of adenosine solutions at lower concentrations, this study was designed to determine the stability of adenosine solutions of 50, 100, and 220 µg/ml prepared in 50 ml polyvinyl chloride (PVC) IV bags of 9% sodium chloride injection and 5% dextrose injection at room temperature (23-25°C) and refrigerated (2-8°C) for 14 days.

Methods

RP-HPLC

A stability indicating reverse phase-high performance liquid chromatography (RP-HPLC) method using a Shimadzu model LC-2010A HT HPLC instrument[a] with a C18 3.5 µm, 4.6 x 150 mm column[b] maintained at 40 °C was employed in this stability study. The Shimadzu instrument contained a degasser, autosampler, and UV detector within. The UV detector was set at 259 nm, the wavelength at which adenosine is absorbed absorbs UV light.[3] Shimadzu LC Solution software was used for data collection and processing. The mobile phase consisted of methanol[c] and distilled water (5:95 v/v) with 0.1% trifluoroacetic acid[d]. Injections of 1 µl samples of adenosine eluted at a rate of 0.5 ml/min with an average retention time of 6 minutes.

Assay Validation

In accordance with guidelines provided by the American Journal of Hospital Pharmacy, a stability-indicating assay was used.[4] A forced degradation study was conducted to show the ability of the assay to separate the degradation products from the parent drug. A stock solution was prepared by placing 10 mg of adenosine powder[e] in a 10 ml volumetric, with distilled water making up the remaining balance, and then stirred for 45 minutes to ensure complete dissolution. From the stock solution, four 100 µg/ml solutions, 10 ml each were prepared with distilled water as the diluents. Using a previously reported procedure, the samples were prepared as follows[3]: Sample 1 was adjusted to pH 2 with 1*M* hydrochloric acid and incubated at 60 °C for 95 hours. Sample 2 was adjusted to pH 12 with 1*M* sodium hydroxide and incubated at 60 °C for 95 hours. Sample 3 was spiked with 3% hydrogen peroxide (final concentration) and incubated at 60 °C for 95 hours. Sample 4 was spiked with 3% hydrogen peroxide and placed in a window sill in

direct sunlight for 95 hours. RP-HPLC analysis was performed at time zero and at 95 hours for each sample.

Standards of adenosine at 10, 50, 100, 300, and 500 µg/ml with distilled water as the diluents were prepared from a stock solution of 25 mg/ml. These standards were assayed by RP-HPLC in triplicate on three consecutive days. Table 1 shows the intra-day variability of the assay. The inter-day results were calculated from the intra-day data and are shown in Table 2.

At time zero, another set of standards were prepared using the same concentrations and method as the standards above. Using linear regression, a calibration curve was constructed of the peak area of adenosine against adenosine concentration. The curve was linear over the range of concentrations ($r^2 = 0.999$). See Table 3 for the complete data.

Stability Study

Before preparing the samples, the contents of three 50 ml bags of 9% sodium chloride injection[f] of the same lot and three 50 ml bags of 5% dextrose injection[g] of the same lot were emptied and measured to determine the overfill volume. The average overfill volume for each of the diluents was 7 ml. For each concentration being studied (50, 100, and 220 µg/ml), the volume of the adenosine solution to be injected was withdrawn from each bag along with the 7 ml overfill. Adenosine[h] 50, 100, and 220 µg/ml samples were prepared in triplicate in either 9% sodium chloride injection or 5% dextrose injection and stored at room temperature (23-25 °C) or refrigerated (2-8 °C) for a total of 36 samples. All preparations were done using aseptic techniques in a class II biological safety cabinet[i].

Visual inspection and RP-HPLC was performed at time zero, 24 hours, 48 hours, 7 days, and 14 days. When the bags were removed from their controlled-temperature environments, a sample was collected and inspected against a light background to inspect for clarity, color and

the presence of crystalline particulate matter. At time zero, 48 hours, 7 days, and 14 days, 2 ml of each sample was withdrawn for pH measurements. On each day that pH was measured, the pH meter[j] was calibrated with pH 4[k] and pH 7[l] buffer solutions yielding a slope ≥ 97% at 25.0 °C. The average of the pH values of each triplicate ± the standard deviation was calculated. For RP-HPLC analysis, 1 ml was withdrawn from each sample. The equation y = 6450.1x – 21984 from the calibration curve was used to calculate the initial concentration as well as the percent of the initial concentration remaining using the average of the triplicate ± the standard deviation. Stability for this study was defined as ≥ 90% of the initial concentration remaining.

Results and Discussion

At the start of the study, each sample was visually inspected with no precipitation found. Time zero concentrations and pH measurements were also recorded. At the end of the 14 day study, all samples remained clear and free of precipitation. All samples retained greater than 98% of the initial concentration at all time points, suggesting minimal if any loss of product due to degradation or adsorption (Table 4).

According to the manufacturers, the pH of adenosine 3 mg/ml solution[h] is 5.5 – 7.5, 0.9% sodium chloride injection[f] is 4.5 – 7.0, and 5% dextrose injection[g] is 3.2 – 6.5. Due to the small sample volume (2 ml) used and the wide range of values, the pH measurements were inconsistent and variable (Table 5). However, the pH of the samples with 0.9% sodium chloride were consistently higher (pH 6.24 – 8.07) than the pH of the samples with 5% dextrose (pH 5.80 – 6.84). Another set of samples were prepared in the same manner to verify the accuracy of the time zero pH measurements. These samples also showed variability but remained in the same pH range.

Conclusion

Adenosine 50, 100, and 220 µg/ml solutions in 50 ml IV bags of 0.9% sodium chloride injection and 5% dextrose injection stored at room temperature and refrigerated conditions were stable for at least 14 days.

Footnotes

[a]HPLC 2010A with LCSolutions. Shimadzu Scientific Instruments, Marlborough, MA.

[b]Symmetry C18 column (3.5-µm particle size, 4.6 mm x 150 nm). Waters Corp., Milford, MA.

[c]Methanol HPLC Grade. Fisher Scientific, Fair Lawn, NJ, lot 096609.

[d]Trifluoroacetic acid, 99%. ACROS Organics, NJ, lot B0514618.

[e]Adenosine, 99+%. ACROS Organics, NJ, lot A0263503

[f]0.9% sodium chloride injection. Hospira, Inc., Lake Forest, IL, lot 74-025-JT

[g]5% dextrose injection. Hospira, Inc., Lake Forest, IL, lot 69-249-JT.

[h]Adenosine injection 6 mg/ml (3 mg/ml). Ben Venue Labs, Inc., Bedford, OH, lot 1360824.

[i]ESCO Airstream Class II Biological Safety Cabinet. ESCO Technologies, Hatboro, PA.

[j]SevenEasy pH meter. Mettler-Toledo Inc., Columbus, OH.

[k]Buffer solution pH 4.00. Fisher Scientific, Fair Lawn, NJ, lot 085812.

[l]Buffer solution pH 7.00. Fisher Scientific, Fair Lawn, NJ, lot 084727.

References

1. Adenosine Injection USP package insert. Bedford, OH: Ben Venue Laboratories; 2006 Jan.
2. Kelly DM, Zhu x, Shiba H et al. Adenosine restores the hepatic artery buffer response and improves survival in a porcine model of small-for-size syndrome. *Liver Transpl*. 2009; 15:1448-57.
3. Ketkar VA, Kolling WM, Nardviriyakul N et al. Stability of undiluted and diluted adenosine at three temperatures in syringes and bags. *Am J Health-Syst Pharm*. 1998; 55:466-70.
4. Trissel LA. Avoiding common flaws in stability and compatibility studies of injectable drugs. *Am J Hosp Pharm*. 1983; 40:1159-60.

Table 1. Intra-day Variability Standard Results for Adenosine at 10, 50, 100, 300 and 500 µg/mL

Day 1 Standards

Conc. µg/ml[a]	Area A	Area B	Area C	Ave. Area[b]	Intra-day Std.[c]	Intra-day % CV[d]
10	61549	61378	61863	61597	246	0.40
50	302566	306729	306342	305212	2300	0.75
100	609603	614814	607911	610776	3598	0.59
300	1817457	1813071	1810339	1813622	3591	0.20
500	3025820	3018918	3020640	3021793	3592	0.12

Day 2 Standards

Conc. µg/ml[a]	Area A	Area B	Area C	Ave. Area[b]	Intra-day Std.[c]	Intra-day % CV[d]
10	61393	60999	61801	61398	401	0.65
50	305829	302480	302857	303722	1834	0.60
100	616212	614914	614943	615356	741	0.12
300	1825585	1805919	1802873	1811459	12328	0.68
500	3005765	3023395	2998304	3009155	12884	0.43

Day 3 Standards

Conc. µg/ml[a]	Area A	Area B	Area C	Ave. Area[b]	Intra-day Std.[c]	Intra-day % CV[d]
10	61784	61776	61085	61548	401	0.65
50	302942	305830	327739	312170	13560	4.34
100	610699	611201	611829	611243	566	0.09
300	1807224	1808661	1798488	1804791	5506	0.31
500	2999500	3005103	3000509	3001704	2987	0.10

[a]Nominal concentration.

[b]Average of areas A, B, and C.

[c]Intra-day standard deviation.

[d]Intra-day % coefficient of variation.

Table 4. Stability of Adenosine 50 µg/mL, 100 µg/mL and 220 µg/mL in 0.9% Sodium Infection and 5% Dextrose Injection Stored in Polyvinyl Chloride Bags

Average Storage Temperature (°C)[a]	Diluent[b]	Actual Initial Drug Concentration (µg/ml)[c]	% Initial Concentration Remaining			
			24 hr	48 hr	7 days	14 days
Nominal Concentration: 50 µg/ml						
5	NS	53.55 ± 0.41	99.4±0.3	99.5±0.7	99.7±0.5	98.6±0.7
25	NS	53.15 ± 0.28	100.5±0.5	100.4±07	100.8±0.9	100.3±0.3
5	D5W	52.41 ± 0.26	100.6±0.7	99.9±0.2	99.7±0.6	99.4±0.3
25	D5W	52.04 ± 0.76	100.6±1.0	99.9±0.5	100.1±0.1	100.8±0.3
Nominal Concentration: 100 µg/ml						
5	NS	107.39 ± 2.32	99.5±0.3	99.9±0.6	99.7±1.0	99.5±0.5
25	NS	108.15 ± 1.17	99.7±0.1	99.7±0.2	99.6±0.3	100.5±0.8
5	D5W	109.14 ± 5.00	100.5±0.8	99.5±0.9	99.6±1.0	100.1±1.3
25	D5W	111.31 ± 2.18	99.7±0.5	99.2±0.4	99.4±0.6	99.6±0.3
Nominal Concentration: 220 µg/ml						
5	NS	224.15 ± 2.76	101.1±0.6	99.7±1.0	99.9±1.3	99.2±1.1
25	NS	232.63 ± 7.05	98.3±1.7	99.7±1.7	98.3±1.9	98.7±1.7
5	D5W	231.28 ± 4.24	101.5±0.7	99.5±0.1	99.4±0.6	99.4±1.2

25	D5W	234.44 ± 1.37	99.4±0.8	99.0±0.4	99.7±0.8	99.8±0.2

[a]Refrigerated temperature range 2 - 8°C. Room temperature range 23 - 25°C.

[b]NS = 0.9% sodium chloride injection, 50 ml PVC IV bag.

D5W = 5% dextrose injection, 50 ml PVC IV bag.

[c]Mean ± S.D., n = 3.

Table 5. pH measurements of adenosine 50 µg/mL, 100 µg/mL and 220 µg/mL in 0.9% Sodium Infection and 5% Dextrose Injection Stored in Polyvinyl Chloride Bags

Nominal Concentration (µg/ml)	Average Storage Temperature (°C)[a]	Diluent[b]	pH Measurement (Mean ± SD, n = 3)			
			Time Zero	48 Hr	7 Days	14 Days
50	5	NS	6.43 ± 0.19	7.58±0.27	7.71±0.13	7.34±0.84
50	25	NS	6.59 ± 0.08	8.00±0.06	8.00±0.19	6.83±0.26
50	5	D5W	6.31 ± 0.28	6.86±0.07	6.77±0.13	6.48±0.32
50	25	D5W	5.80 ± 0.74	6.80±0.03	6.67±0.11	6.41±0.26
100	5	NS	6.24 ± 0.12	7.57±0.39	7.87±0.18	6.81±0.14
100	25	NS	6.38 ± 0.17	7.88±0.14	8.07±0.04	6.78±0.03
100	5	D5W	6.20 ± 0.34	6.78±0.07	6.32±0.59	6.24±0.28
100	25	D5W	6.62 ± 0.08	6.71±0.04	6.64±0.09	6.16±0.59
220	5	NS	7.31 ± 0.83	7.82±0.07	7.87±0.09	6.68±0.14
220	25	NS	7.89 ± 0.02	7.95±0.02	7.83±0.11	6.70±0.17
220	5	D5W	6.84 ± 0.06	6.84±0.03	6.69±0.02	6.18±0.29
220	25	D5W	6.77 ± 0.06	6.76±0.11	6.70±0.04	6.45±0.23

[a]Refrigerated temperature range 2 - 8°C. Room temperature range 23 - 25°C.

[b]NS = 0.9% sodium chloride injection, 50 ml PVC IV bag.

D5W = 5% dextrose injection, 50 ml PVC IV bag.