Stability Of Azacitidine Solutions In Sterile Water For Injection

Ivan Tyono[1], Shirley Law[2], Lauren F. Charbonneau[1], and Scott E. Walker[2,3]

[1]Odette Cancer Centre Department of Pharmacy, [2]Sunnybrook Health Sciences Centre Department of Pharmacy, and the [3]Faculty of Pharmacy, University of Toronto, Toronto, Ontario.

INTRODUCTION

Azacitidine is currently indicated for the treatment of intermediate-2 & high-risk Myelodysplastic Syndrome (MDS) and Acute Myeloid Leukemia (AML) with 20-30% blasts with multi lineage dysplasia for patients unable to tolerate high dose chemotherapy.

The original VIDAZA® product monograph indicated that following reconstitution the product must be refrigerated immediately, and may be held under refrigerated conditions (2°C - 8°C) for up to 8 hours. After removal from refrigerated conditions, the suspension may be allowed to equilibrate to room temperature for up to 30 minutes prior to administration.

A 2012 CJHP publication identified that reconstitution with cold sterile water for injection (SWFI) is an important step in extending the before use date.

Extending the before use date would reduce wastage of many drugs. NAPRA guidelines state that the Beyond Use Date must not exceed the earliest of the dates established by the following two criteria:

- Expiration date based on chemical and physical stability according to reference texts
- Storage time related to risk of microbial contamination

The introduction of a generic version of azacitidine (Dr.Reddy's) in 2017 raised questions of the stability of the generic formulation and the validity of extending stability from one brand to another.

OBJECTIVES

The objective of this study was to evaluate the stability of reconstituted 25 mg/mL and 10 mg/mL azacitidine in the original manufacturer's vial and in 5mL polypropylene syringes at -20^{0}C, 4^{0}C and 25^{0}C.

Interested in a copy of this poster or other Sunnybrook Posters?

Scan the QR code or Go to http://metrodis.org/SB_PPC2015.html

and download the poster from this site.

NONE of the authors of this poster have any personal or financial relationships with any commercial entities that may have a direct or indirect interest in the subject matter of this presentation.

This stability study and the azacitidine used in this study was supplied by DrReddy's Laboratory through an Unrestricted Educational Grant.

METHODS

Liquid Chromatographic Method & Validation

The liquid chromatographic system, consisting of a Waters Nova Pak column and a potassium phosphate mobile run at 1 mL/min with UV detection, separated azacitidine from degradation products.(Figure 1) Accuracy and reproducibility of standard curves and Quality Control Samples was tested over 5 days and inter and intra-day errors tabulated.

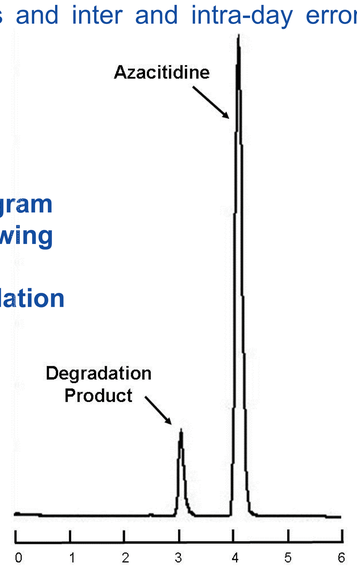

Figure 1. Typical chromatogram of azacitidine showing separation of the azacitidine degradation product from azacitidine.

Stability Study: Vials at 25°C.

On study day 0, 6 vials were reconstituted with SWFI as per manufacturer's instruction and 6 were prepared with COLD (4^{0}C) SWFI to prepare concentrations of 10 mg/mL and 25 mg/mL. All vials were stored at room temperature (25^{0}C). Immediately following reconstitution and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 24hr the concentration of azacitidine was determined.

Stability Study: Vials and Syringes at 4^{0}C.

On study day 0, 8 vials were reconstituted with COLD (4^{0}C) SWFI to prepare vials and syringes with concentrations of 10 mg/mL and 25 mg/mL. All vials and syringes were refrigerated (4^{0}C) and sampled at 8, 24, 32, 72 and 96hr. The concentration of azacitidine was determined in duplicate.

Stability Study: Vials and Syringes at -20^{0}C.

On study day 0, 8 vials were reconstituted with COLD (4^{0}C) SWFI to prepare vials and syringes with concentrations of 10 mg/mL and 25 mg/mL. All vials and syringes were stored in the freezer at -20^{0}C. Immediately following reconstitution and on days 1, 3, 7, 10, 14, 17 and 21 the azacitidine concentration was determined in duplicate.

Data Reduction and Statistical Analysis

Analysis of variance was used to test differences in degradation rate between various temperature–diluent–concentration combinations. The 5% level was used as the a priori cut-off for significance. The concentration of a solution on a particular day was considered "acceptable" or "within acceptable limits" if it was greater than 90% of the initial concentration (as determined on day 0) and the amount found on that day, with 95% confidence.

RESULTS

Assay Validation

The absolute deviation from the known concentration from quality control samples on any day averaged 2.1%. The error of replicate analysis within a day averaged less than 0.29% for the standards. Inter-day variation, as measured by the observed standard deviation of regression (Sy.x) for percent remaining, averaged 0.88% across all temperature, concentration and container combinations.

Assay validation demonstrated that the method was accurate and reproducible and stability indicating, separating azacitidine from its degradation products.

Stability of Azacitidine at -20°C.

During the 21-day study period, there was no apparent loss of the azacitidine concentration of solutions stored in glass vials or polypropylene syringes (Table 1).

Stability of Azacitidine at 4°C.

During the 4-day refrigerated study period, approximately 30% of the azacitidine was lost in both syringes and the manufacturer's glass vials. (Table 2) Degradation products were observed in samples.

Stability of Azacitidine at 25°C.

During the 24 hour (1440 minute) room temperature study, there was more than 10% loss within 3 hours (Table 3) when reconstitution occurred with room temperature SWFI. When reconstitution occurred with cold SWFI, 10% loss occurred after 4hours. Degradation products were observed in samples.

Analysis of variance revealed differences in percent remaining due to time but not concentration or container.

The data was also analyzed by linear regression, and T-90 was calculated for each concentration-container combination. When the 95% confidence limits for the degradation rates were used to estimate T-90, a beyond-use date of 262 minutes was observed at 25C with cold reconstitution, 26.92 hours or longer was observed at 4C and more than 100 days at -20C.

Table 1. Concentrations and T-90 at -20^{0}C

	Original Glass vial -20 C 25mg/mL	Original Glass vial -20 C 10mg/mL	BD Syringe -20 C 25mg/mL	BD Syringe -20 C 10mg/mL
Observed Initial Concentration (mg/mL)	25.00	9.94	24.96	9.95
Study Day				
0	100.00	100.00	100.00	100.00
1	99.20	99.04	99.09	99.10
3	99.40	99.26	99.83	99.84
7	99.59	99.28	100.05	99.35
10	100.20	99.65	100.18	99.58
14	98.98	98.31	99.86	99.91
17	99.11	98.58	99.98	100.86
21	99.65	99.22	99.26	99.21
Rate of Change in Concentration (%/day) Slope	-0.0119	-0.0359	0.00	0.01
Standard Deviation of Regression (Sy.x)	0.454	0.500	0.424	0.596
Time to Achieve 90% of Initial (T-90) Days	841.80	278.40	3755.60	-669.81
Shortest T-90 (95% CI) in Days	151.02	104.43	187.58	177.44

Table 2. Concentrations and T-90 at 4^{0}C

	Original Glass vial 4C 25mg/mL	Original Glass vial 4C 10mg/mL	BD Syringe 4C 25mg/mL	BD Syringe 4C 10mg/mL
Expected Concentration				
Observed Initial Concentration (mg/mL)	25.01	9.93	24.98	9.96
Hours				
0	100.00	100.00	100.00	100.00
8	99.60	99.79	99.59	99.39
24	93.09	94.27	93.32	94.16
32	89.88	90.83	89.49	90.83
72	75.53	76.78	75.98	76.93
96	70.76	70.88	71.16	70.88
Rate of Change in Concentration (%/hour) Slope	-0.3259	-0.3232	-0.321	-0.320
Standard Deviation of Regression (Sy.x)	1.385	1.152	1.396	0.999
Time to Achieve 90% of Initial (T-90) Hours	30.69	30.94	31.20	31.21
Shortest T-90 (95% CI) in Hours	26.92	27.69	27.28	28.30

Table 3. Concentrations and T-90 at 25^{0}C

	Original Glass vial 25^{0}C[1,3] 25^{0}C[1]	Original Glass vial 25^{0}C[1,3] 25^{0}C[1]	Original Glass vial 25^{0}C[2,4] 4^{0}C[2]	Original Glass vial 25^{0}C[2,4] 4^{0}C[2]
Reconstitution Temperature of SWFI[1,2]				
Nominal concentration (mg/mL)	25mg/mL	10mg/mL	25mg/mL	10mg/mL
Observed Initial Concentration (mg/mL)	25.08 mg/mL	9.96 mg/mL	24.81mg/mL	10.01 mg/mL
Study Minute				
0	100.00	100.00	100.00	100.00
60	94.27	94.81	98.34	98.53
120	91.20	91.80	96.00	96.25
180	88.44	90.14	92.96	93.09
240	85.48	86.74	90.11	91.53
300	83.70	85.15	88.03	88.55
360	81.62	82.81	86.02	86.83
420	78.85	80.35	85.04	84.94
480	77.22	78.13	82.47	82.85
540	75.42	75.64	80.87	81.54
600	73.59	74.38	79.19	79.25
1440	55.36	56.56	62.60	63.00
Rate of Change in Concentration (%/minute) Slope	-0.0488	-0.0452	-0.0355	-0.0353
Standard Deviation of Regression (Sy.x)	1.291	1.090	0.738	0.531
Time to Achieve 90% of Initial (T-90) Minutes	204.87	221.19	281.54	283.35
Shortest T-90 (95% CI) in Minutes	168.74	185.07	261.97	268.81

1. Azacitidine reconstituted with room temperature sterile water for injection.
2. Azacitidine reconstituted with cold (4^{0}C) sterile water for injection.
3. To improve fit and prediction of T-90, regression analysis considered only data from 0-360 minutes, maintaining the correlation coefficient above 0.98 and the intercept greater than 98%.
4. To improve fit and prediction of T-90, regression analysis considered only data from 0-600 minutes, maintaining the correlation coefficient above 0.98 and the intercept greater than 98%.

CONCLUSION

There appears to be no difference in the stability of the Dr.Reddy's and Celgene formulations.

Reconstitution with cold SWFI is an important step in extending the before use date. We recommend that immediately following reconstitution *with cold* SWFI, vials and syringes be stored at 4°C. The maximum period of storage at 4°C is 8 hours. If a vial is unused after 8 hours or at the end of the day, we recommend the vial be placed in the freezer at -20°C. The maximum period of storage at -20°C is 4 days. At any time prior to the 4th day the vial can be removed from the freezer, thawed, syringes prepared and the solution administered to patients. If the time between removal from the freezer and administration to the patient does not exceed 2 hours (30 minutes to thaw and 1.5 hours to allow delivery and administration to the patient) more than 90% of the initial concentration will remain with 95% confidence.

If these storage conditions / limitations are used, wastage can be reduced (possibly eliminated) providing significant cost savings.