Physical Compatibility and Stability of Ascorbic acid Injection in Polyvinyl Chloride Minibags at 4ºC and Room Temperature (25ºC).

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INTRODUCTION

Ascorbic acid is currently being evaluated for efficacy in sepsis where a dose of 50 mg/kg will be administered intravenously every 6 hours.

Published data demonstrate the stability of ascorbic acid (AA) 30-mg/mL and 50-mg/mL solutions for 96 hours. However, If a 50 mg/kg dose is diluted in 50 mL, a 50 kg patient would receive 2.5 g, corresponding to an estimated actual concentration of 37.0 or 40.0 mg/mL (considering overfill and drug volume using the Sandoz or Mylan formulations, respectively), while a 130 kg patient would receive 6.5 g dose, corresponding to a concentration of 77.8 mg/mL or 92.2 mg/mL (including overfill and drug volume) for the available formulations manufactured by Sandoz [250 mg/mL] and Mylan [500 mg/mL], respectively

Therefore, available data provides stability for patients that weigh less than 64 kg (Mylan) or 72 kg (Sandoz) and for only up to 96 hours, covering potentially less than half of our patients.

OBJECTIVES

To evaluate the physical compatibility and chemical stability of ascorbic acid injection diluted in either 50mL of NS or D5W in PVC minibags during storage over 14 days at 25ºC and at 4ºC, protected from light (PFL).

METHODS

Liquid Chromatographic Method

The liquid chromatographic system consisted of a mixture of 5% methanol and 95% 0.05M phosphoric acid. This was pumped through a 15 cm x 4.6 mm 5 µm reverse-phase SB-CN column (Agilent Zorbax SB-CN) at 1.0 mL/min. The column effluent was monitored at 246 nm, the UV maxima of ascorbic acid (see Figure 1).

Assay Validation

The method was validated to ensure reproducibility, accuracy and assay specificity. Accuracy and reproducibility of standard curves and samples was tested over 5 days prior to the study. Inter and intra-day errors of reproducibility were assessed by the coefficients of variation and the standard deviation of regression.

Stability Study: Vials and Syringes at 4ºC and 25ºC.

On study day 0, 37mg/mL and 78mg/mL concentrations (Sandoz) and 40mg/mL and 92mg/mL concentrations (Mylan) of AA were prepared and PFL. 3 units of each container were stored at room temperature and 3 were stored in the refrigerator. Concentration and physical inspection were completed on study days 0, 0.33, 1, 1.33, 2, 3, 4, 7, 10 and 14.

Data Reduction and Statistical Analysis

Chemical stability was based on the intersection of the lower limit of the 95% confidence interval of the observed degradation rate and the time to achieve 90% of the initial concentration (T-90).

Analysis of variance was used to test differences in degradation rate between the different storage temperatures, concentrations and manufacturers. The 5% level was used as the *a priori* cut-off for significance.

RESULTS

Assay Validation

The analytical method measured the concentration of ascorbic acid specifically. Although degradation products were not observed in chromatograms as the concentration of ascorbic acid declined (Figure 1), specificity was confirmed with the following results. Chromatograms from all degraded samples were inspected for the appearance of additional peaks and the ascorbic acid peak was compared between samples for changes in concentration, retention time, and peak shape (electronic overlay and numerical calculation of tailing). UV spectral purity (200-798 nm, 6 nm bandwidth, deuterium lamp: UV6000, Thermo Separation Products, Fremont, CA) of the ascorbic acid from a sodium hypochlorite degraded sample (13.77% remaining), was compared to the spectrum of the authentic un-degraded sample of ascorbic acid in water taken at time zero. UV spectra similarity was calculated as 99.97%. Figure 1.

During Assay Validation and the study period, ascorbic acid was measured accurately (average absolute deviation from known: 2.12%) and reproducibly (intra-day CV(%): 0.24%). (inter-day CV(%): 1.67%). During the study period, the standard deviation of regression (Sy.x), a measure of inter-day variation, ranged from 0.33 – 0.81% -Tables1 and 2.

Concentration Results

During the study period all solutions retained more than 97.7% of the initial concentration at 4ºC, and at 25ºC more than 88% remained after 14 days, with both products, and PFL .

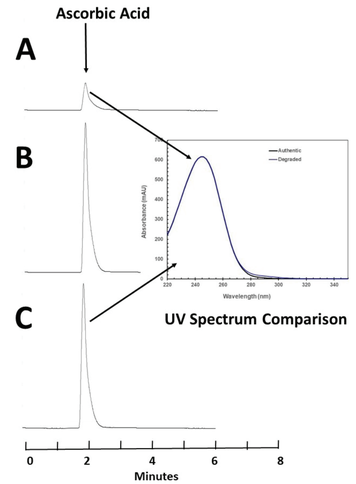
Analysis of Variance demonstrated a significant effect of study days (time) and temperature on the percent remaining ($p<0.001$) but solution ($p>0.519$), concentration ($p>0.677$) and manufacturer ($p>0.808$) had no significant effect.

CONCLUSIONS

Ascorbic acid concentrations between 36.6 and 91.4 mg/mL, diluted in either NS or D5W and stored in PVC minibags are physically and chemically stable for at least 14 days at 4ºC and 10 days at room temperature (25ºC), with protection from light.

Establishing a Beyond Use Date should be based on current NAPRA/USP-797 Guidelines and consideration of the contamination rate in your institution.

Figure 1. Representative Chromatograms



Chromatogram A represents a 80 mg/mL solution of ascorbic acid after the addition of sodium hypochlorite, which resulted in degradation of ascorbic acid , leaving 13.8% of the initial concentration. No degradation products are observed. To confirm specificity, the 'purity' of the ascorbic acid peak, the UV spectrum was compared to the UV spectra observed in a fresh un-degraded AA peak (over-layed spectra, - similarity 99.97%). Chromatogram C represented a 77 mg/mL solution in NS on study day zero. Chromatogram B represents the same 77 mg/mL solution in NS after 10 days storage at room temperature. The observed percent remaining was 93.36%

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Table 1. Percent Remaining on Each Study Day With Calculation of the Time to Achieve 90% Remaining (T-90) with 95% Confidence. Manufacturer: Sandoz

Solution	NS	NS	NS	NS	D5W	D5W	D5W	D5W
Temperature	4C	RT	4C	RT	4C	RT	4C	RT
Nominal concentration (mg/mL)	37 mg/mL	37 mg/mL	78 mg/mL	78 mg/mL	37 mg/mL	37 mg/mL	78 mg/mL	78 mg/mL
Study Day \ Actual concentration (mg/mL)	36.64	36.71	77.42	77.21	36.85	36.87	77.21	77.21
0	100.00±0.49	100.00±0.81	100.00±0.24	100.00±0.13	100.00±0.32	100.00±0.13	100.00±0.11	100.00±0.18
0.33	99.66±0.31	99.66±0.52	99.27±0.06	99.06±0.24	99.30±0.15	99.39±0.11	98.62±0.37	99.11±0.16
1	99.23±0.09	99.20±0.46	99.68±0.24	99.42±0.13	98.92±0.24	99.20±0.20	99.58±0.40	99.49±0.38
1.33	98.56±0.45	98.76±0.57	99.26±0.87	99.10±0.18	98.79±0.54	98.35±0.25	99.54±0.27	99.58±0.33
2	98.77±0.38	98.11±0.44	98.89±0.64	98.64±0.54	98.60±1.30	100.14±1.02	99.00±0.61	98.92±0.14
3	98.62±0.15	97.47±0.82	98.74±0.27	97.80±0.54	99.16±0.83	97.83±0.10	99.03±0.23	97.85±0.22
4	98.74±0.11	96.36±0.80	97.72±0.62	96.47±0.36	98.85±0.62	96.72±0.28	99.06±0.03	97.11±0.14
7	98.44±0.26	95.20±0.83	97.74±0.25	94.82±0.47	97.98±0.85	95.18±0.27	98.83±0.32	95.22±0.19
10	99.09±0.31	93.18±0.74	98.36±0.04	93.08±1.08	99.11±1.17	92.91±0.71	99.39±0.16	92.58±1.01
14	98.91±1.01	88.45±0.81	98.56±0.06	90.11±0.20	99.32±1.15	88.02±0.28	98.94±0.17	90.76±0.45
Rate of Change of Concentration (%/day – Slope)	-0.0368	-0.7625	-0.0965	-0.6929	-0.0146	-0.8068	-0.0265	-0.6837
Intercept	99.16	99.89	99.23	99.81	99.06	100.22	99.31	99.98
Correlation (r)	-0.3408	-0.9905	-0.5912	-0.9954	-0.1288	-0.9800	-0.2968	-0.9931
Standard Deviation of Regression (Sy.x)	0.501	0.524	0.650	0.330	0.556	0.808	0.421	0.398
Confidence Interval for slope	0.0828	0.0865	0.1073	0.0546	0.0919	0.1335	0.0696	0.0658
Fastest Slope 95% Confidence (%/day)	-0.1196	-0.8490	-0.2039	-0.7475	-0.1065	-0.9403	-0.0961	-0.7495
Upper Limit95% Confidence	0.0460	-0.6760	0.0108	-0.6383	0.0772	-0.6732	0.0431	-0.6180
Shortest T-90 (95% CI) days	**83.63**	**11.78**	**49.05**	**13.38**	**93.92**	**10.64**	**104.01**	**13.34**

Table 2. Percent Remaining on Each Study Day With Calculation of the Time to Achieve 90% Remaining (T-90) with 95% Confidence. Manufacturer: Mylan

Solution	NS	NS	NS	NS	D5W	D5W	D5W	D5W
Temperature	4C	RT	4C	RT	4C	RT	4C	RT
Nominal concentration (mg/mL)	40	40	92	92	40	40	92	92
Study Day\ Actual concentration (mg/mL)	39.45	39.48	91.25	91.23	39.30	39.54	91.38	91.26
0	100.00±0.71	100.00±0.46	100.00±0.20	100.00±0.22	100.00±0.24	100.00±0.57	100.00±0.46	100.00±0.42
0.33	100.02±0.07	99.99±0.20	99.90±0.27	99.73±0.18	99.95±0.05	100.00±0.23	100.13±0.42	99.33±0.72
1	99.07±0.15	99.26±0.09	99.13±0.19	98.89±0.12	99.27±0.14	99.21±0.36	99.34±0.42	99.79±0.59
1.33	98.82±0.47	98.99±0.15	98.98±0.29	99.03±0.11	99.28±0.34	99.19±0.44	99.08±0.65	99.71±0.32
2	98.94±0.36	99.08±0.29	99.08±0.36	99.12±0.14	99.50±0.29	99.07±0.61	99.24±0.83	99.82±0.38
3	98.20±0.35	97.57±0.52	98.95±0.19	97.54±0.50	99.04±0.13	97.70±0.16	99.44±0.69	97.81±0.38
4	99.06±0.63	96.24±0.75	98.62±0.16	96.37±1.00	99.05±0.65	96.17±0.74	99.16±0.94	96.16±0.17
7	98.77±0.69	94.70±0.32	98.08±0.56	95.22±0.35	98.82±0.58	94.35±0.57	99.17±0.74	95.00±0.53
10	99.49±0.72	92.66±0.42	98.77±0.26	93.18±0.42	99.54±0.68	92.67±0.74	99.86±0.70	92.26±0.98
14	99.09±0.78	88.39±0.77	98.02±0.39	90.08±0.57	98.99±0.54	88.18±1.26	98.87±0.54	89.39±0.73
Rate of Change of Concentration (%/day – Slope)	-0.0198	-0.8110	-0.1106	-0.6932	-0.0430	-0.8288	-0.0359	-0.7850
Intercept	99.23	100.15	99.42	99.87	99.53	100.19	99.58	100.28
Correlation (r)	-0.1644	-0.9945	-0.7909	-0.9936	-0.4995	-0.9940	-0.3938	-0.9871
Standard Deviation of Regression (Sy.x)	0.585	0.421	0.422	0.390	0.368	0.449	0.414	0.629
Confidence Interval for slope	0.0966	0.0695	0.0698	0.0644	0.0608	0.0741	0.0684	0.1038
Fastest Slope 95% Confidence (%/day)	-0.1164	-0.8805	-0.1804	-0.7576	-0.1039	-0.9029	-0.1043	-0.8889
Upper Limit95% Confidence	0.0769	-0.7415	-0.0408	-0.6289	0.0178	-0.7546	0.0324	-0.6812
Shortest T-90 (95% CI) days	**85.91**	**11.36**	**55.42**	**13.20**	**96.27**	**11.08**	**95.90**	**11.25**