Stability of 2.5mg/mL Indocyanine Green (ICG) Solutions Stored in Syringes at 25ºC, 4ºC, -20ºC and -67ºC.

William Perks[1], Shirley Law[1], Romina Marchesano[1], Scott E Walker[1,2].

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

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INTRODUCTION

The Indocyanine Green (ICG) prescribing information indicates that ICG must be used within 6 hours. There is no indication of the storage temperature.

Although there are a number of previously published ICG stability studies, they are focused on lower concentrations and / or the stability in human plasma.

Due to increasing use of ICG, waste and recent backorders, chemical stability data was sought to preserve inventory and provide extended stability data.

OBJECTIVES

To evaluate the chemical stability of ICG prepared in polypropylene (PP) syringes at concentrations of 2.5mg/mL, reconstituted with Sterile Water for Injection (SWFI) and stored at 25ºC, 4ºC and in the freezer at -20ºC and -67ºC.

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METHODS

Liquid Chromatographic Method

The liquid chromatographic system consisted of a mixture of 40% acetonitrile and 60% 0.05 mol/L phosphoric acid with 0.01 M sodium lauryl sulfate. This was pumped through a 15 cm x 4.6 mm reverse-phase SB-CN column (Agilent Zorbax SB-CN) at 1.0 mL/min. The column effluent was monitored at 770 nm.

Assay Validation

The analytical method was validated to ensure reproducibility, accuracy and assay specificity. The system was shown to be capable of separating indocyanine green from its degradation products (Figure 1). Accuracy and reproducibility of standard curves was tested over 5 days. 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, ICG solutions of 2.5mg/mL were prepared in 5mL PP syringes, reconstituted with SWFI. Three syringes were stored at 25ºC, 4ºC, -20ºC or -67ºC. ICG concentrations were determined 8 or more times over each study period using a validated stability indicating analytical method.

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. The 5% level was used as the a priori cut-off for significance.

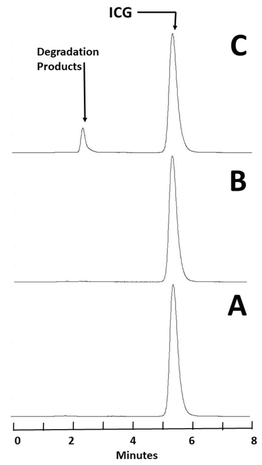

Figure 1.
Chromatogram A represents a solution of 2.5mg/mL solution of ICG in SWFI on study day zero. Chromatogram B was observed after 28 days storage of a 2.5mg/mL solution at -20ºC. Degradation products are not visually evident and 100% remains. Chromatogram C was observed after 48 hours storage of a 2500 mcg/mL solution at 25ºC. A degradation product is observed at 2.2 minutes. 86% of the initial ICG concentration was observed to remain.

RESULTS

Assay Validation

The analytical method separated degradation products from ICG (Figure 1) and measured the concentration specifically, accurately (average: 2.23%) and reproducibly (intra-day CV(%): 1.74% ; or standard deviation of regression (Sy.x): 0.15 – 1.03% -Tables 1 - 3).

Concentration Results

At room temperature (Table 1), 2.5mg/mL solutions retained more than 90% of the initial concentration for about 35 hours. There was no apparent or significant difference in ICG stability due to the source of SWFI (Table 1. Akorn vs. Baxter vs. Hospira: p = 0.0791). The calculated T-90, with 95% confidence, was 34.1 hours.

During storage at 4ºC (Table 2), 2.5mg/mL solutions retained more than 90% of the initial concentration for approximately 41-hr. Reconstitution with cold SWFI (4ºC), reduced the rate of ICG degradation, (p =0.0119) (data not shown). The calculated T-90, with 95% confidence, was 41.74 hr.

During storage at either -20ºC or -67ºC (Table 3), more than 98% was retained for 28 days. The calculated T-90, with 95% confidence, exceeded the 28-day study period for syringes stored in the freezer at either -20ºC or -67ºC.

Table 1. Percent Remaining on Storage at 25ºC, testing the Manufacturer source of SWFI

Source of SWFI used for Reconstitution[1]	Akorn	Baxter	Hospira
Observed Initial Concentration (mg/mL)	2.51	2.51	2.50
Time (hours)			
0	100.00±0.02	100.00±0.09	100.00±0.08
0.5	99.59±0.03	99.54±0.10	99.79±0.44
1	99.48±0.03	99.42±0.11	99.38±0.09
1.5	98.87±0.06	98.78±0.13	98.78±0.11
2	98.39±0.14	98.42±0.16	98.32±0.19
3	98.19±0.14	98.19±0.08	98.21±0.09
4	97.79±0.04	97.65±0.08	97.56±0.21
6	97.73±0.04	97.64±0.11	97.69±0.04
24	94.93±0.26	95.06±0.21	95.71±0.68
30	92.79±0.21	92.79±0.33	92.86±0.06
48	86.62±0.06	86.31±0.10	86.20±0.17
Degradation Rate (Rate of Change of Concentration [%/hr]	-0.2461	-0.2489	-0.2469
Intercept	99.45	99.42	99.47
Correlation Coefficient	-0.9839	-0.9804	-0.9705
Standard Deviation of Regression (Sy.x)	0.751	0.841	1.031
Time to Achieve 90% of initial (T-90) Hours	40.63	40.18	40.50
Confidence Interval for slope	0.0337	0.0377	0.0463
Fastest Rate of Change in Concentration (%/hour) 95%CI	-0.2124	-0.2112	-0.2007
Shortest T-90 (95% CI) Hours	35.74	34.89	34.11

Table 2. Percent Remaining during Storage at 4ºC, testing the Effect of SWFI Reconstitution Temperature.

Observed Initial Concentration (mg/mL)	2.50
Time (Hours)	
0	100.00±0.07
8	98.93±0.12
24	94.42±0.09
32	93.58±0.10
72	82.89±0.37
96	77.56±0.31
120	69.82±0.06
Degradation Rate (Rate of Change of Concentration [%/hr] - Slope	-0.240
Intercept	100.49
Correlation Coefficient	-0.999
Standard Deviation of Regression (Sy.x)	0.540
Time to Achieve 90% of initial (T-90) HOURS	41.74
Confidence Interval for slope	0.01781
Fastest Rate of Change in Concentration (%/hour) 95% Confidence	-0.2571
Shortest T-90 (95% CI) Hours	41.74

Table 3. Percent Remaining During Storage at -20ºC and -67ºC.

Observed Initial Concentration (mg/mL)	2.50	2.50
Study Day / Storage Temperature	-20ºC	-67ºC
0	100.00±0.06	100.00±0.01
1	99.83±0.02	99.97±0.05
3	99.90±0.03	100.73±1.25
7	99.77±0.14	99.83±0.05
14	99.82±0.05	100.07±0.16
17	100.00±0.12	99.99±0.28
21	100.04±0.19	100.05±0.15
28	100.42±0.20	100.44±0.15
Rate of Change in Concentration (%/Day) Slope	0.014	0.004
Intercept	99.81	100.09
Correlation Coefficient I	0.715	0.135
Standard Deviation of Regression Sy.x	0.156	0.317
Time to Achieve 90% of Initial (T-90) Days	694.99	2,558.59
Confidence Interval for slope	0.01405	0.02865
Upper Limit95% Confidence	0.0284	0.0326
Shortest T-90 (95% CI) - days	351.58	307.11

CONCLUSIONS

This study demonstrated that the source of SWFI does not significantly affect the stability of ICG.

This study also that demonstrated that 2.5mg/mL solutions of ICG may be stored frozen at -20ºC or -67ºC for up to 28 days, but at 25ºC or 4ºC, solutions should be stored for only 34-41 hours, respectively.

Since syringes must be stored for some period of time at 4ºC or room temperature, Syringes stored in the freezer for up 28-days can be withdrawn from the freezer, allowed to thaw, but should be used within 24-hours of withdrawal from the freezer. Under these conditions more than 92.5% of the initial ICG concentration will remain at 24 hours.