UNEXPECTED RESULTS FOR SAMPLE COLLECTION AND HANDLING STABILITY ASSESSMENT FOR SUMATRIPTAN IN HUMAN PLASMA

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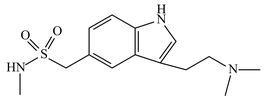
INTRODUCTION

Determination of sample collection and handling stability (SCHS) is a requirement for validation of bioanalytical methods. Stability of sumatriptan in human whole blood for 120 minutes at ambient temperature did not meet pre-defined acceptance criteria (+/- 15% of control). As sumatriptan was stable in plasma for 23 hours at ambient temperature, degradation of the compound in whole blood was not expected. It was then hypothesized that partitioning of sumatriptan between plasma and red blood cells was not immediate and was impacting the results of the early time points. Evaluation of the particulate layer from ambient temperature samples were tested as well as further evaluation of the compound in whole blood at varying conditions (ice-water bath and 37°C).

Figure 1. Sumatriptan Structure



METHOD

- Whole blood was drawn fresh in-house and fortified with sumatriptan at 0.150 and 100 ng/mL. Assuming plasma accounted for approximately 50% of the whole blood and all of the spiked sumatriptan would migrate to the plasma layer, the target plasma concentrations of the low and high concentration samples were 0.300 and 200 ng/mL respectively.
- After preparation, the samples were incubated in an ice-water bath, at ambient temperature, and at 37°C for multiple time points between 0 and 120 minutes.
- The 0 minute time point served as a “control” for comparison of the other time points. This was necessary since the target concentrations were approximations as opposed to true theoretical concentrations.
- At each time point, samples were centrifuged, and the plasma layer was immediately frozen at -20°C.
- For samples incubated at ambient temperatures for 30 minutes, the red cell fraction was also stored at -20°C for testing.
- The collected plasma samples were analyzed using a validated method for the quantitation of sumatriptan in human plasma:
  - After slight acidification, samples were loaded onto an HLB solid-phase extraction plate.
  - Samples were washed with a low percentage organic:aqueous solution before elution with a high percentage organic:aqueous solution.
  - Eluted samples were directly injected onto an SCX analytical column with a high percentage organic:acidic aqueous mobile phase.
  - An ESI source detected positive ions in the MRM mode using an AB SCIEX API 4000™.
- Red blood cells were analyzed with the same chromatographic and instrument conditions after a protein crash of the cellular material.

Table 1. SCHS at Ambient Temperature 0 vs. 120 minutes

	SCHS A 0.300 ng/mL		DF = 20 SCHS D 200 ng/mL	
	0 minutes	120 minutes	0 minutes	120 minutes
	0.168	0.120	120	81.4
	0.170	0.117	121	80.8
	0.178	0.109	124	81.5
	0.170	0.116	118	80.5
	0.186	0.122	120	80.6
	0.175	0.115	118	80.1
Mean	0.175	0.117	120	80.8
% CV	3.9	3.9	1.9	0.7
% of Control		66.9		67.3
n	6	6	6	6

Table 2. Total Sumatriptan in Plasma and Red Blood Cell Fraction After 30 minutes at Ambient Temperature

	SCHS A 0.300 ng/mL		DF = 20 SCHS D 200 ng/mL	
	Plasma	Red Cell Fraction	Plasma	Red Cell Fraction
	0.129	0.178	91.8	120
	0.138	0.181	89.1	119
	0.126	0.180	89.7	123
	0.126	0.185	88.2	119
	0.126		90.2	
	0.132		92.5	
Mean	0.130	0.181	90.3	120
% CV	3.7	1.6	1.8	1.7
Total Concentration		0.311		210
% of Theoretical		103.7		105.2
n	6	4	6	4

RESULTS OVERVIEW

- The initial comparison for 0 to 120 minute samples held at ambient temperature failed acceptance criteria. (Table 1)
- Additional comparisons of multiple time points between 0 and 60 minutes at ambient temperature showed a steady concentration decline to approximately 60% of control occurred between 0 and 30 minutes. There was negligible difference between concentrations of the 30 and 60 minute samples. (Figure 2)
- Summation of the concentrations discovered in the plasma and red blood cell fractions at the 30 minute time point at ambient temperature were equivalent to the amount of sumatriptan spiked into the whole blood. (Table 2)
- Comparisons of time points between 0 and 60 minutes in an ice-water bath demonstrated the same pattern, but at a slower rate. Changing light conditions had no effect on results. (Figure 3)
- Samples at multiple time points between 0 and 60 minutes collected from three separate donors at 37°C showed a steep drop in concentration between 0 and 10 minutes to approximately 70% of control with no further significant reduction between 10 and 60 minutes. (Figure 4)

Figure 2. SCHS at Ambient Temperature as a Percentage of Control

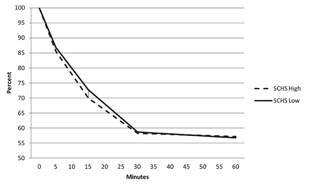


Figure 3. SCHS Temperature/Light Comparison

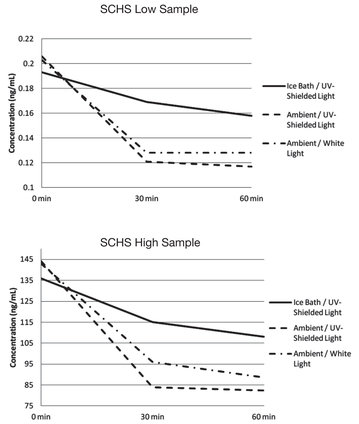
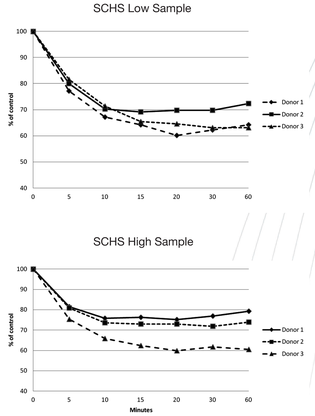


Figure 4. SCHS at 37°C as a Percent of Control (Multiple Donors)



CONCLUSION

The temperature/time dependent concentration decline is generally indicative of a stability issue. However, since sumatriptan was shown to be stable in plasma, further investigation was warranted to determine if there was truly a stability issue in whole blood. Summation of results from the plasma and red cell fraction showed no loss of sumatriptan which proved it was in fact stable in whole blood. Since the drop in concentration reached an end point, it was determined that equilibrium was achievable and continuing loss would not likely occur. Therefore, the original hypothesis was supported. Disequilibrium in the partitioning between the plasma and red blood cell fractions, an artifact caused by the preparation of fortified samples for testing, should be considered as a potential cause of apparent analyte instability in whole blood.