Stability of Ethanol Certified Standards Evaluation

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Approved: 5/2/19 KMM

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Purpose:

The purpose of this study was to evaluate the stability of ethanol certified standards over a six month period of time. Currently the Florida Department of Law Enforcement utilizes certified reference standards provided by Cerilliant: a SIGMA-ALDRICH company. These standards are used to calibrate and verify the proper identification and quantitation of ethanol for forensic headspace gas chromatography analysis. These certified reference standards are accompanied by a Certificate of Analysis which lists the manufacturer's recommendation for use and storage. Although the certificates list an expiration date, they do clarify that this expiration is separate from shelf-life and the individual user must determine shelf-life for their purposes.

Experimentation:

The Toxicology section at the Florida Department of Law Enforcement utilizes several different concentrations of certified reference standards for the calibration and verification of ethanol. The standards used in this study consisted of eight concentrations: 0.020, 0.025, 0.050, 0.080, 0.100, 0.200, 0.300, and 0.400g%. These represent the concentrations used in the Orlando Regional Operations Center and spans a range of concentrations typically reported for this type of analysis. Two separate ampules were tested for each concentration (Sets A and B). All of the standards were purchased from Cerilliant and certified to these specified concentrations. The ampules are said to contain 1.2mL of solution. Aliquots consisting of 100µL at each concentration level were sampled and tested after a specified number of days: 0, 7, 14, 21, 28, 38, 42, 52, 63, and 185 days. After initial opening (Day 0), the standards were transferred to a new, clean, single-use glass vial and sealed with a screw-top lid. This is consistent with how ethanol standards are currently stored at the laboratory. The standards were all stored under refrigeration while not in use.

Several steps were taken to standardize the testing method performed. All aliquots were taken by the same analyst utilizing the same pipette dilutor (OROC PIP-15). The internal standard solution (14-367) was kept consistent over all runs. Additionally, the standards were also run utilizing the same analytical method (AA-4r3). A calibration curve was established on each day of analysis using a freshly opened set of calibrators and controls.

Sets A and B were from the following vials:

Concentration g%	A - STD#	B - STD#	Cerilliant Lot #
0.020	686AO	686AP	FN08101401
0.025	807BI	807BJ	FN06221502
0.050	915K	915L	FN04271601
0.080	945K	945L	FN04171701
0.100	911K	911L	FN08101601
0.200	913K	913L	FN03301601
0.300	879AO	879AP	FN02121601
0.400	914U	914V	FN05131606

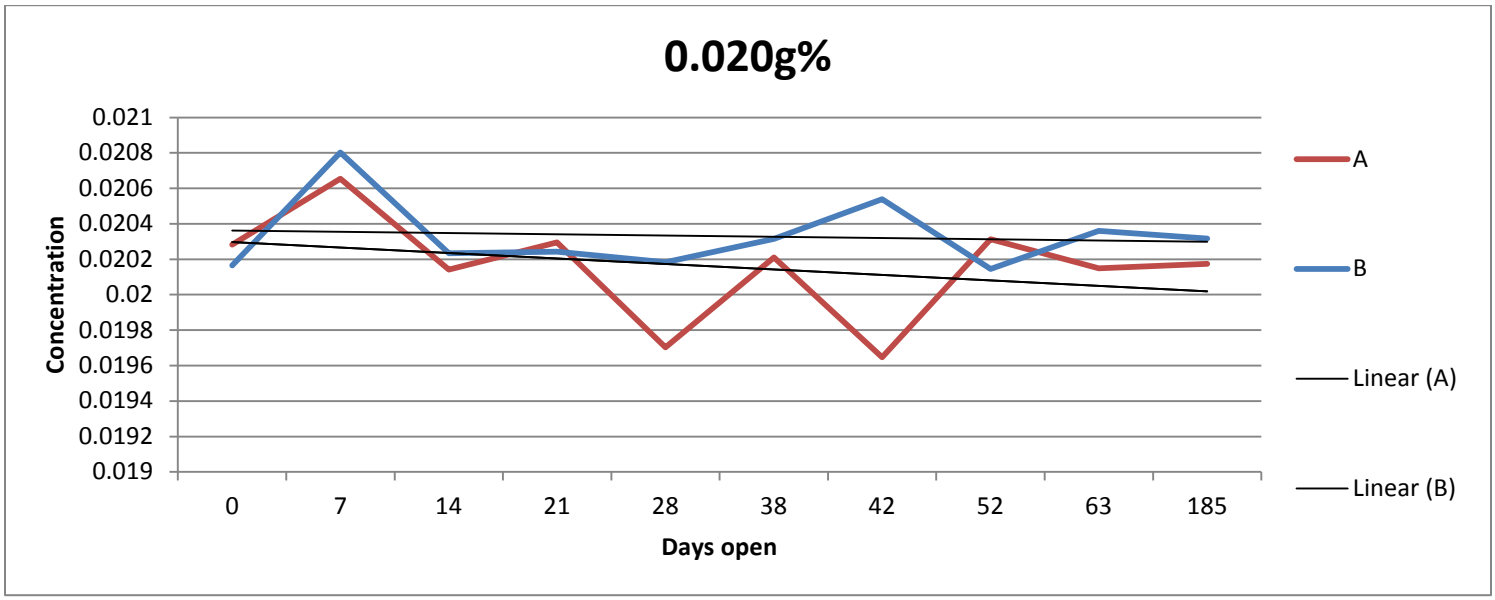
Results:

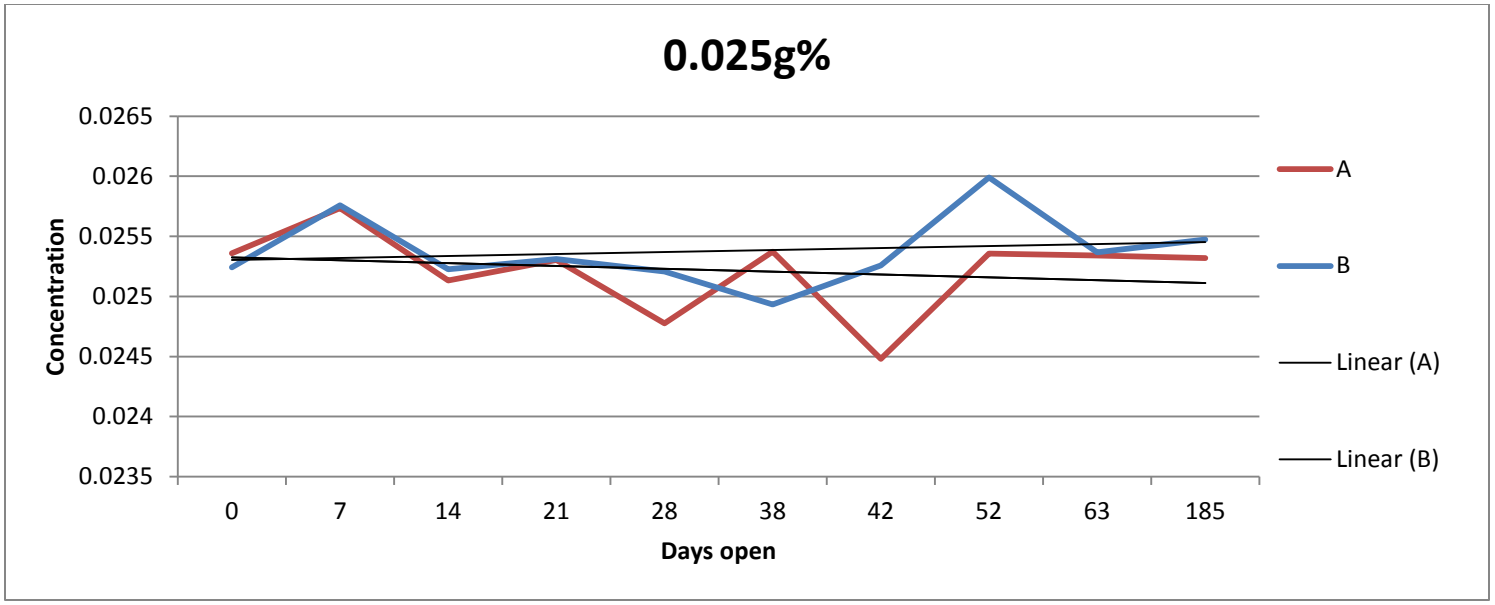
Each of the eight levels was analyzed in duplicate: Set A and Set B. The below shows a summary of the results of all data collected from Day 0 to Day 185 consisting of ten separate analysis.

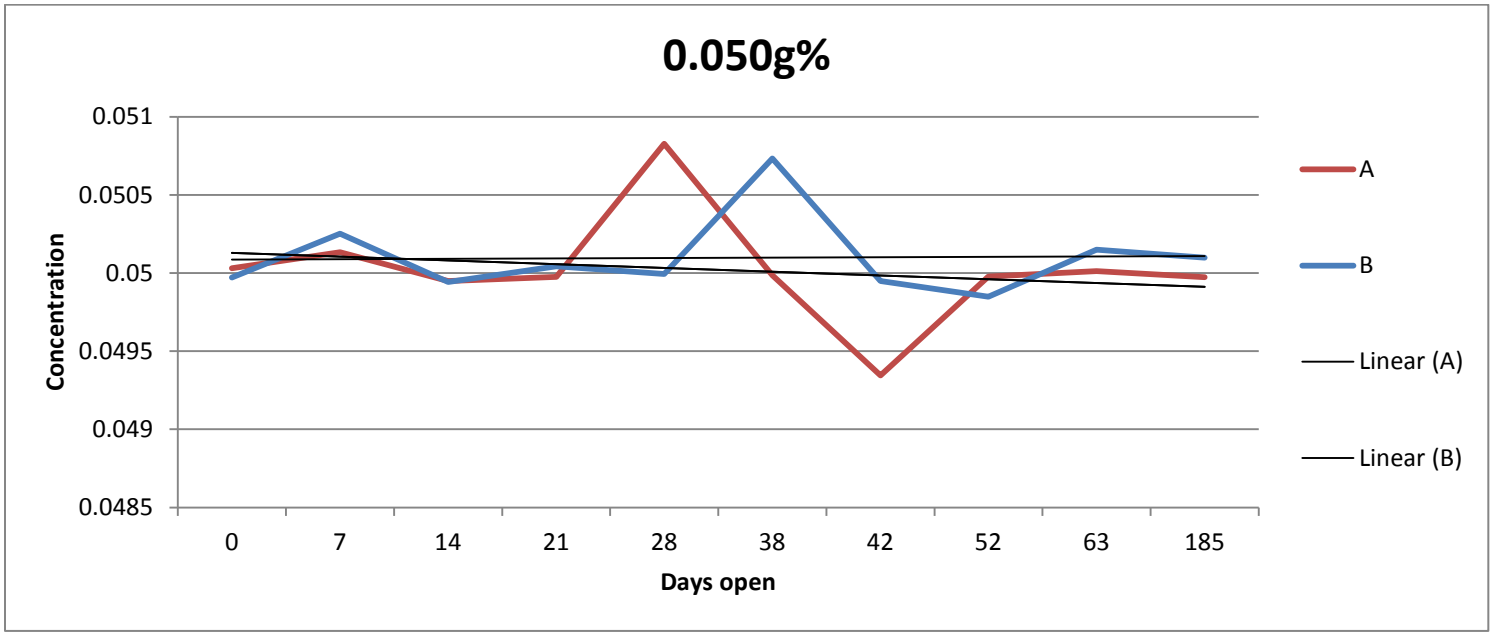
Set A								
	0.020g%	0.025g%	0.050g%	0.080g%	0.100g%	0.200g%	0.300g%	0.400g%
Mean	0.020157	0.025218	0.050021	0.079742	0.100012	0.199670	0.300665	0.401601
Std Dev	0.000279	0.000333	0.000337	0.000322	0.000590	0.000404	0.000589	0.003914
Min	0.019647	0.024480	0.049344	0.078921	0.099642	0.198993	0.299195	0.399996
Max	0.020654	0.025735	0.050828	0.080027	0.101763	0.200324	0.301516	0.413272

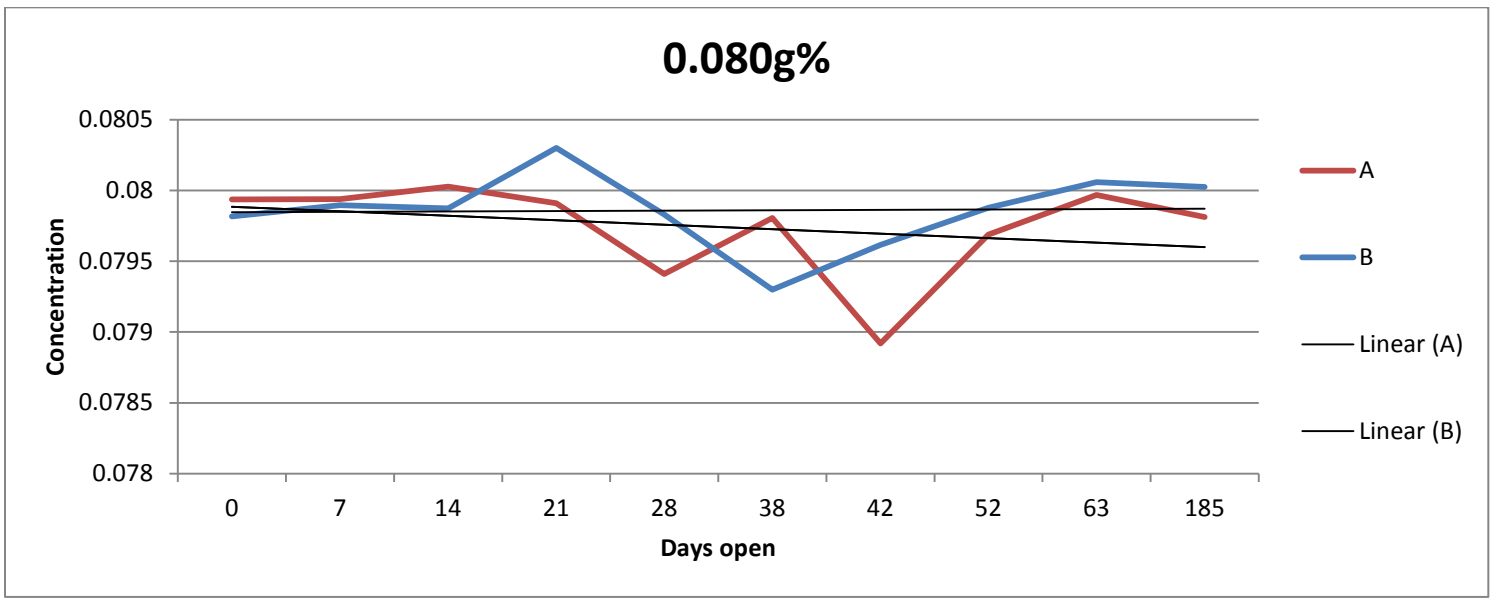
Set B								
	0.020g%	0.025g%	0.050g%	0.080g%	0.100g%	0.200g%	0.300g%	0.400g%
Mean	0.020330	0.025377	0.050098	0.079859	0.099793	0.199808	0.300673	0.400585
Std Dev	0.000192	0.000286	0.000239	0.000252	0.000256	0.000574	0.001202	0.000778
Min	0.020145	0.024933	0.049849	0.079300	0.099227	0.198529	0.298466	0.399865
Max	0.020802	0.025990	0.050733	0.080301	0.100189	0.200525	0.302754	0.402377

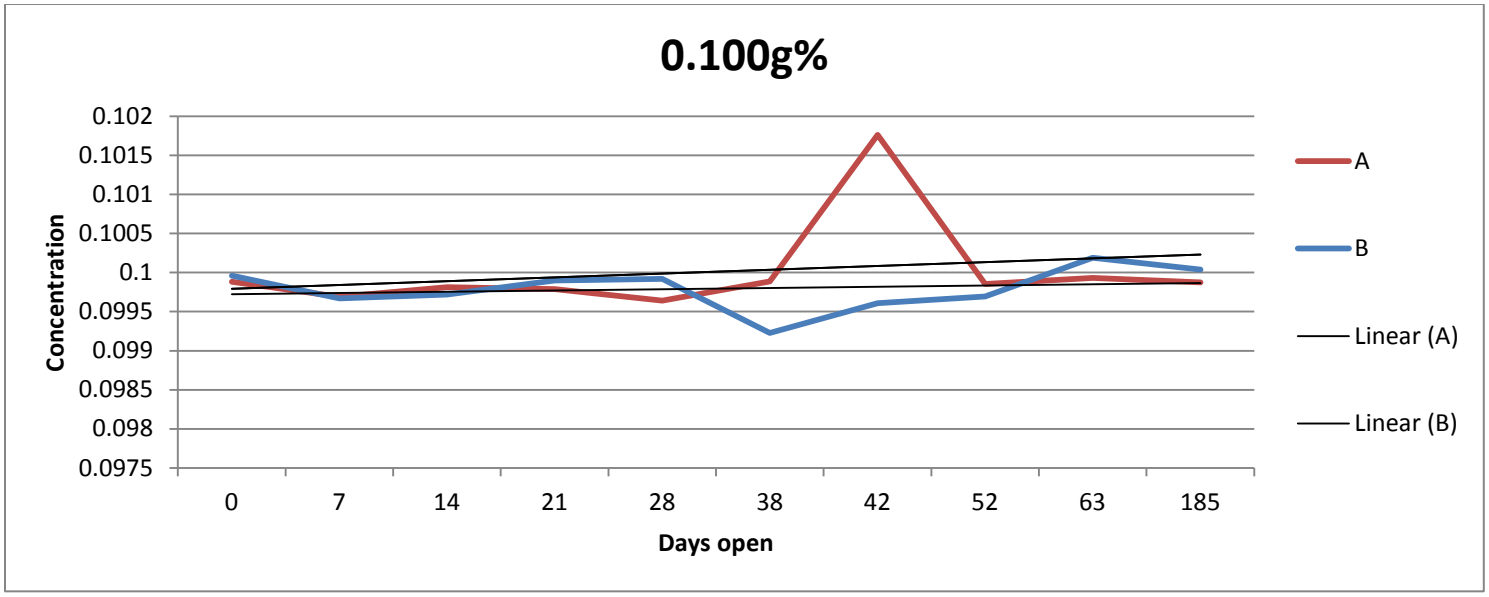
In order to determine if a trend existed over the time tested, a linear least squares regression was calculated for each data set. Below are graphs showing the result of each concentration measurement over days open for each concentration.

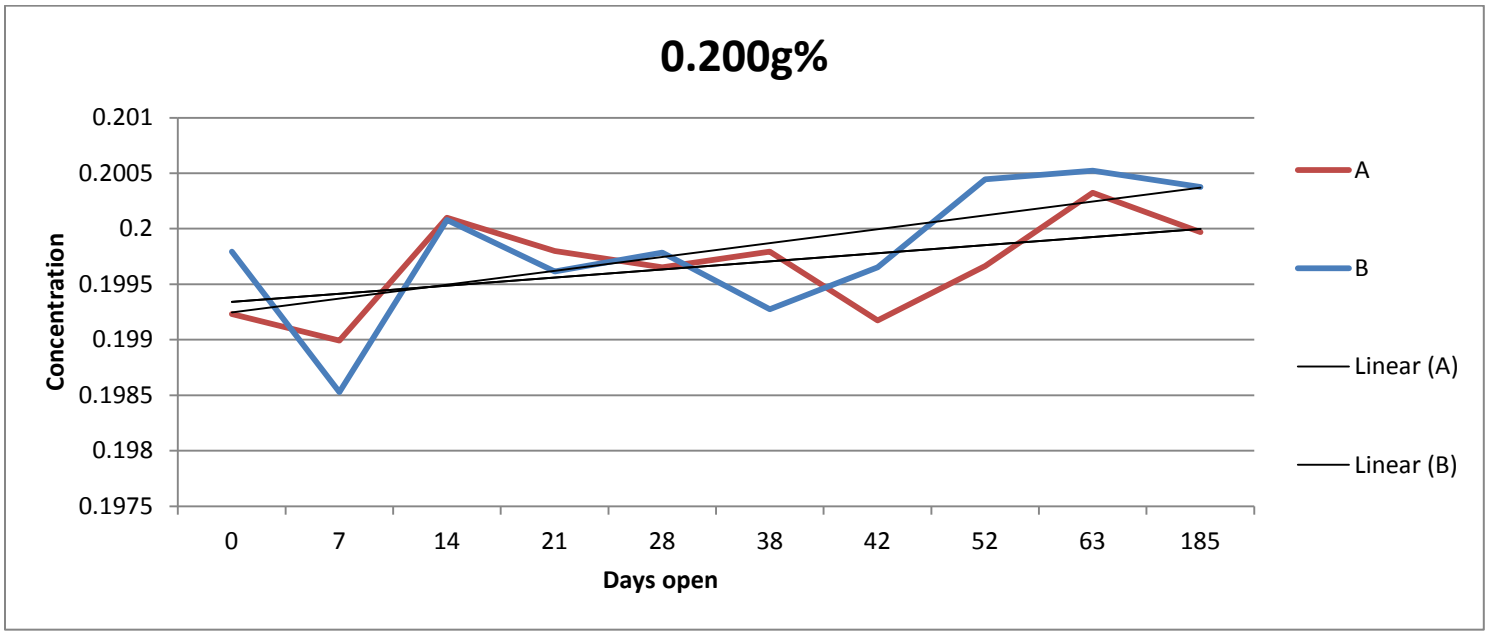


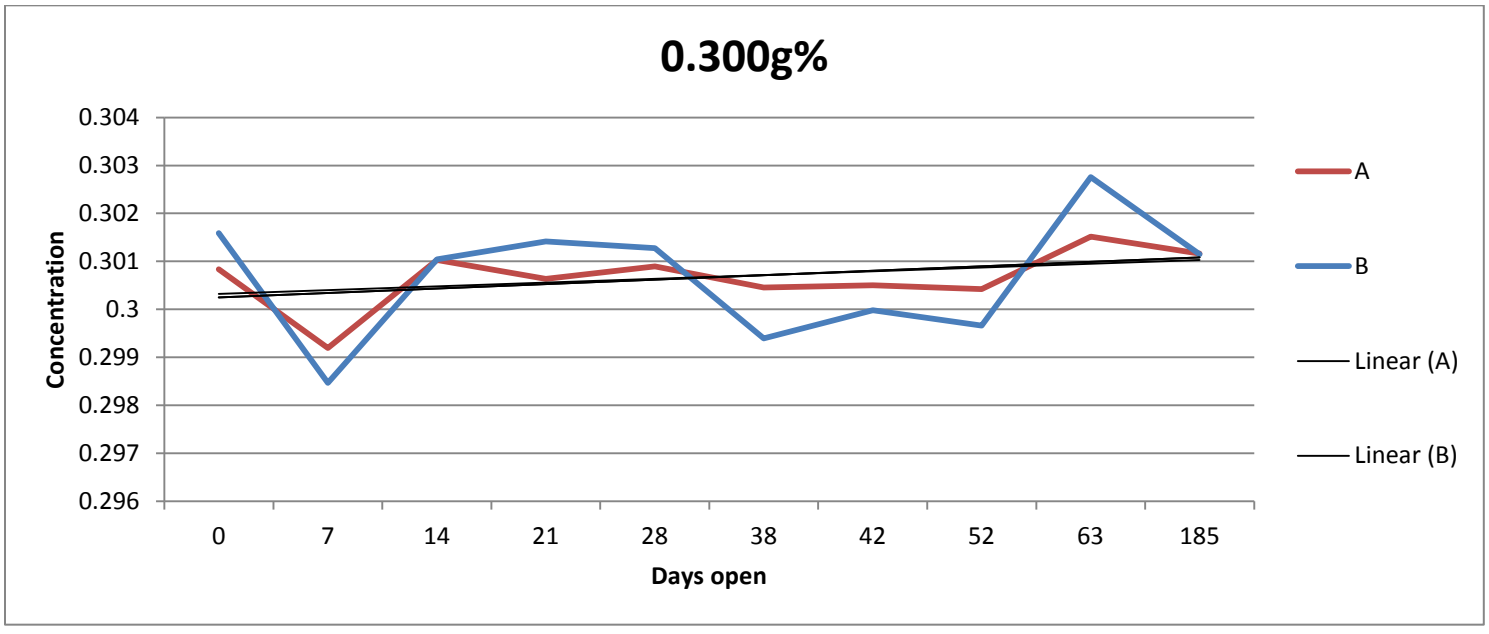

0.025g%
0.0265
0.026
0.0255
0.025
0.0245
0.024
0.0235
Concentration
0
7
14
21
28
38
42
52
63
185
Days open
A
B
Linear (A)
Linear (B)

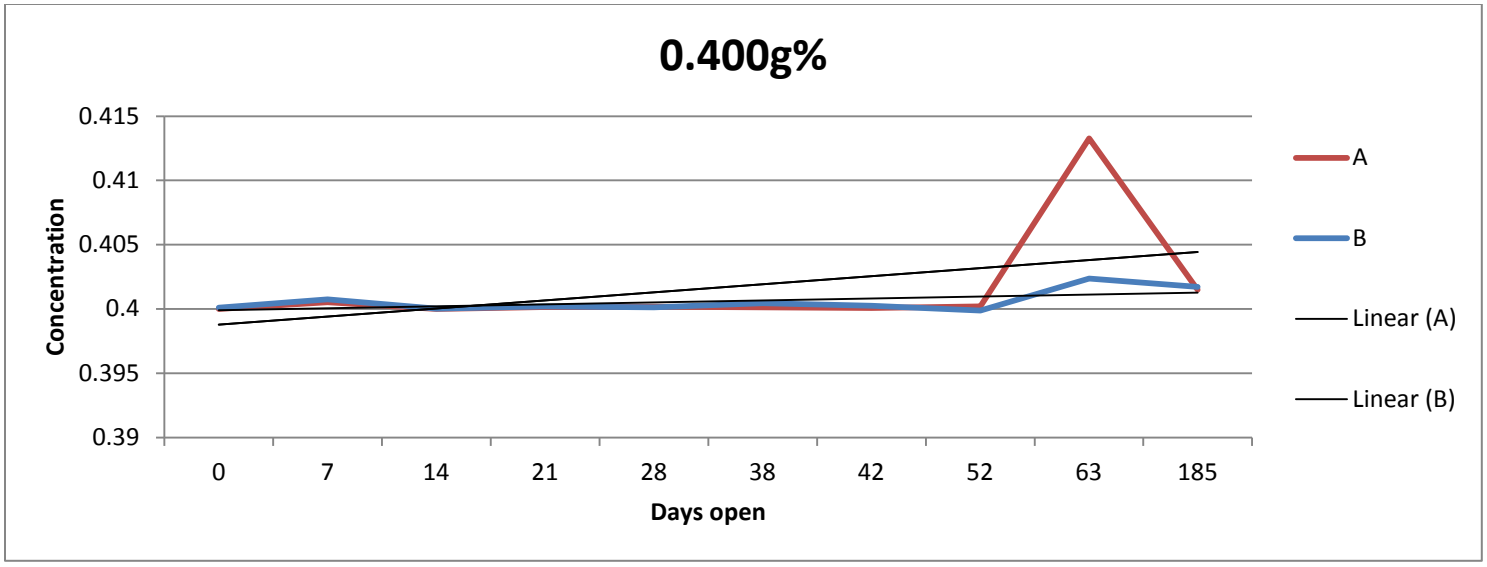

0.050g%
0.051
0.0505
0.05
0.0495
0.049
0.0485
Concentration
0
7
14
21
28
38
42
52
63
185
Days open
A
B
Linear (A)
Linear (B)


0.080g%
0.0805
0.08
0.0795
0.079
0.0785
0.078
Concentration
0
7
14
21
28
38
42
52
63
185
Days open
A
B
Linear (A)
Linear (B)


0.100g%
0.102
0.1015
0.101
0.1005
0.1
0.0995
0.099
0.0985
0.098
0.0975
Concentration
0
7
14
21
28
38
42
52
63
185
Days open
A
B
Linear (A)
Linear (B)


0.200g%
0.201
0.2005
0.2
0.1995
0.199
0.1985
0.198
0.1975
Concentration
0
7
14
21
28
38
42
52
63
185
Days open
A
B
Linear (A)
Linear (B)


0.300g%
0.304
0.303
0.302
0.301
0.3
0.299
0.298
0.297
0.296
Concentration
0
7
14
21
28
38
42
52
63
185
Days open
A
B
Linear (A)
Linear (B)



Below is a table of the calculated slopes of the linear least squares regression line for each of the concentration levels.

Vial	Slope
0.020 - A	-0.000000595
0.020 - B	-0.000000234
0.025 - A	0.000000019
0.025 - B	0.000000865
0.050 - A	-0.000000794
0.050 - B	0.000000119
0.080 - A	-0.000000318
0.080 - B	0.000000845
0.100 - A	0.000000396
0.100 - B	0.000001571
0.200 - A	0.000003308
0.200 - B	0.000005808
0.300 - A	0.000004700
0.300 - B	0.000004380
0.400 - A	0.000016711
0.400 - B	0.000009326

Conclusions:

Each of the eight evaluated levels was analyzed in duplicate represented by Sets A and B. Regardless of the duration of time (days) open, all resulting concentrations of previously opened standards obtained from analysis with freshly prepared calibration curves were consistent with the certified value of the standard within ±0.005g% or ±5%, whichever was larger. All calibration curves generated with freshly prepared standards had r^2 values of 0.99 or greater.

A linear least squares regression line was calculated for each vial tested over the course of 185 days. The slope of each line was then evaluated to determine if a relationship existed between the concentration of the standards and time (days) open. The standard error of each slope was calculated. Utilizing a significance level of 0.05 (95% confidence) and calculated P-values, the slopes of each line was not found to be significantly different from zero. Thus no relationship between concentration and time was demonstrated.

The Cerilliant ethanol certified reference standards have shown to maintain stability for at least 185 days at the Florida Department of Law Enforcement once opened. It is recommended that long term storage of previously open ethanol certified reference materials utilize clean, new, screw-top single use vials and remain under refrigeration while not in use.

Reference:

McClave, James T and Sincich, Terry (2009). *Statistics, Eleventh Edition*. Upper Saddle River, New Jersey: Prentice Hall.