

Failed Proficiency Result for 11-Nor-9-Carboxy-THC

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Background

In February of 2023, our laboratory received an unacceptable result for Delta-9-THC-COOH, with a SDI of +2.5 (acceptability limits ± 2 SDI). The quality management department mandated an outlier investigation be performed to elucidate root cause and clinical impact.

The drug test implemented quantifies 74 analytes. Test is split into two analyses due to different gradient methods, which also allows to segregate analytes by ESI polarity.

Method and Material

Calibrators are prepared in-house using certified reference material (Cerilliant, Round Rock, TX) spiked into commercially procured drug free urine (UTAK, Valencia, CA) with added potassium fluoride (4 mg/mL). 76 total standards are spiked into urine solvent to prepare 100 mL of stock calibrator solution. Stock serves as top level calibrant, and 6 lower levels are prepared from dilutions of stock.

Calibrants are aliquoted into 2 mL polypropylene tubes, and stored long term at -80°C. A new set of calibrators are pulled from freezer at the start of work week (typically Monday), and then kept in 4°C refrigerated temperature during interim, until they are discarded at end of work week. Calibrants have an expiry of 1 year at -80°C.

Assay preparation is performed in 96 well MCX SPE plate. 50 μ L of calibrant, QC, or clinical sample and 100 μ L of IS solution (isotope-labeled standards in 0.5 M NH₄Ac buffer and β -glucuronidase) are added to wells, and then incubated at 70°C for two hours. Following incubation, solid phase extraction is performed. Collected elutions are diluted in 2% acetonitrile/1% formic acid in water. Analysis is performed on Waters Acquity UPLC coupled to Waters Xevo TQ-S micro triple quad mass spectrometer, utilizing negative electrospray ionization and selective reaction monitoring. Compounds are separated on a 1.7 μ m 100 mm C18 column by gradient reverse phase chromatography with A and B mobile phase compositions of 0.005% formic acid in H₂O and 0.01% formic acid in acetonitrile, respectively.

Quality control material consists of 3 levels. For THC-COOH, the low, middle, and high QC have means of 48, 374, and 926 ng/mL, respectively.

Results & Discussion

Proficiency sample contained 5 total analytes, and only delta-9-THC-COOH yielded an unacceptable result (Table 1). Investigation showed a decreasing daily slope value for the week the PT sample was analyzed, and that if the sample had been reprocessed using calibrations from earlier days, the results would have been acceptable (Table 2). Retrospective study showed this declining trend when slopes were averaged over a two-month span (Table 3, N=37). The other five analytes included in the negative ESI mode (all barbiturates) do not demonstrate this decline.

Calibrator use protocol was changed so that sets would be discarded after use on Wednesday, and a new set would be put into use on Thursday. This set would then be discarded on Friday. Slopes were monitored for two months after this change was implemented. Declining trend was ameliorated, as shown in Table 4.

Review of QC during same time frames did not yield expected improvement. As shown in Charts 4 and 5, QC performance appeared largely unchanged.

Conclusion

Though the QC performance does not support our hypothesis, the THC-COOH slope values definitively did show a decline relative the other 5 negative ESI analytes, and this decline was corrected when the protocol was changed. Our laboratory continues to troubleshoot this challenging analyte. We are re-evaluating our calibrator preparation protocol. Literature (doi: 10.3390/metabo12090801) suggests THC-COOH adsorptive loss in urine matrix, and recommends use of glass for containers. Consideration of divesting the large panel for smaller class specific analyses has also been discussed.

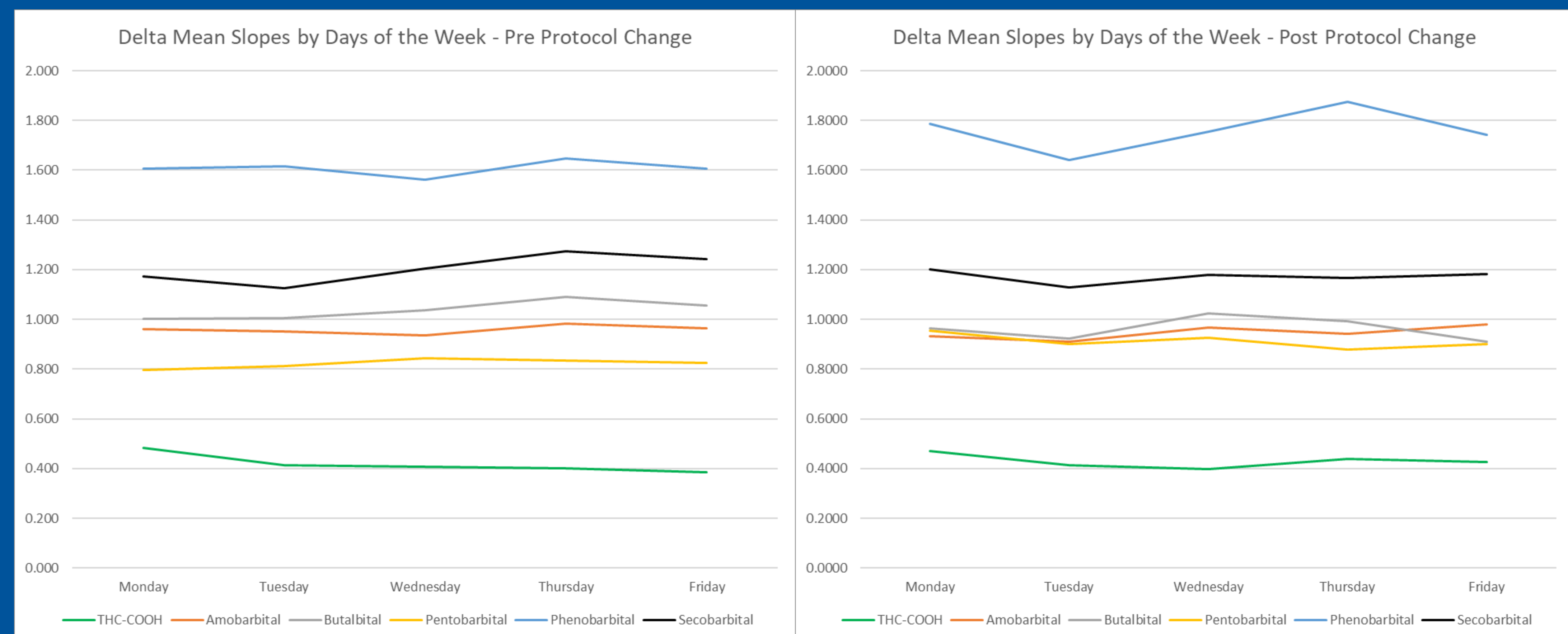
Declining slope trend in 11-Nor-9-Carboxy-THC corrected by change in calibrator protocol.

Analyte	SDI	%CV
Delta-9-THC-COOH	+2.5	26%
Butalbital	-0.9	19%
Fentanyl	+0.3	19%
Norfentanyl	-0.7	18%
Gabapentin	+0.3	23%

Table 1. List of analytes included in the PT sample, of which THC-COOH failed. Other analytes do not show bias as observed for THC-COOH result.

Day of Calibration	Result (ng/mL)	THC-COOH Cal Slope	R ²	Peer Mean	Peer SD	SDI
Monday	195	0.608	0.991	174.14	44.81	0.5
Tuesday	217	0.544	0.989			1.0
Wednesday	260	0.454	0.974			1.9
Thursday	286	0.412	0.970			2.5
Friday	298	0.395	0.979			2.8

Table 2. Table shows submission result for evaluation (in red), as well as the result when processed using calibrations from different days of the week. Table also shows slope of THC-COOH calibration and correlation coefficient throughout the week. Also shown is the SDI for each day's result based on the peer mean and standard deviation.



Analyte	Monday	Tuesday	Wednesday	Thursday	Friday
THC	0.4818	0.4137	0.4078	0.3997	0.3852
Amobarbital	0.9601	0.9519	0.9363	0.9841	0.9655
Butalbital	1.0025	1.0056	1.0361	1.0896	1.0565
Pentobarbital	0.7965	0.8129	0.8446	0.8354	0.8236
Phenobarbital	1.6052	1.6163	1.5623	1.6475	1.6077
Secobarbital	1.1742	1.1263	1.2039	1.2728	1.2412

Table 3. Table showing mean slopes by day of the week for the 6 analytes in ESI negative analysis from one of our instruments, named Delta, over a span of two months (N=37). Data in table is plotted in Chart 1 above.

Analyte	Monday	Tuesday	Wednesday	Thursday	Friday
THC	0.4690	0.4137	0.3989	0.4395	0.4270
Amobarbital	0.9322	0.9093	0.9661	0.9426	0.9784
Butalbital	0.9627	0.9230	1.0238	0.9911	0.9094
Pentobarbital	0.9534	0.9005	0.9272	0.8793	0.9011
Phenobarbital	1.7869	1.6397	1.7538	1.8745	1.7415
Secobarbital	1.2005	1.1279	1.1785	1.1671	1.1834

Table 4. Table showing mean slopes for 6 ESI negative analytes after calibrator protocol change was implemented. Slopes are from same instrument as Table 3, and were obtained over a period of two months (N=45). Data in table plotted in Chart 2 above.

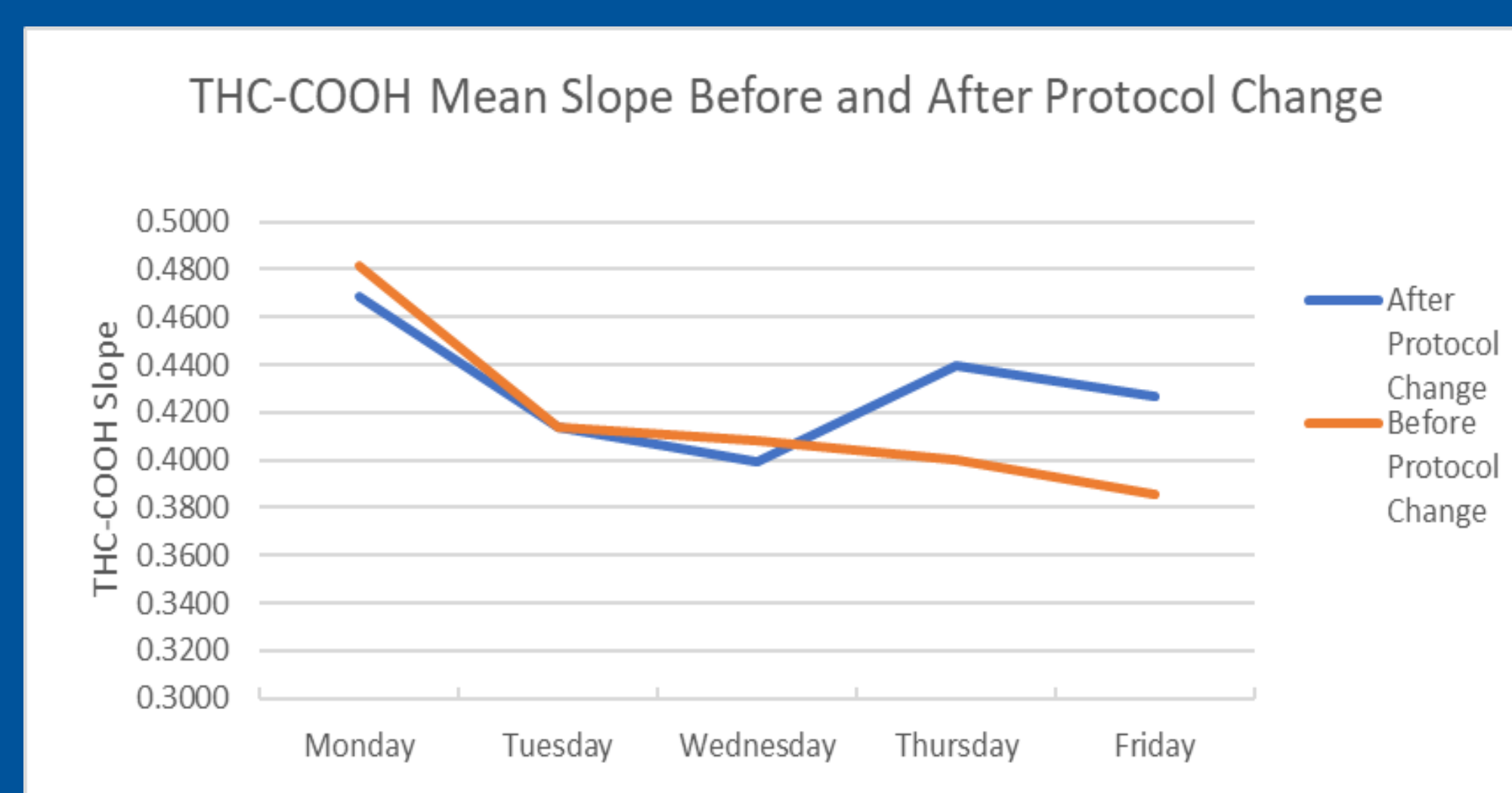


Chart 3. Mean slopes for THC-COOH before and after protocol change in same chart. Correction on Thursday is apparent.

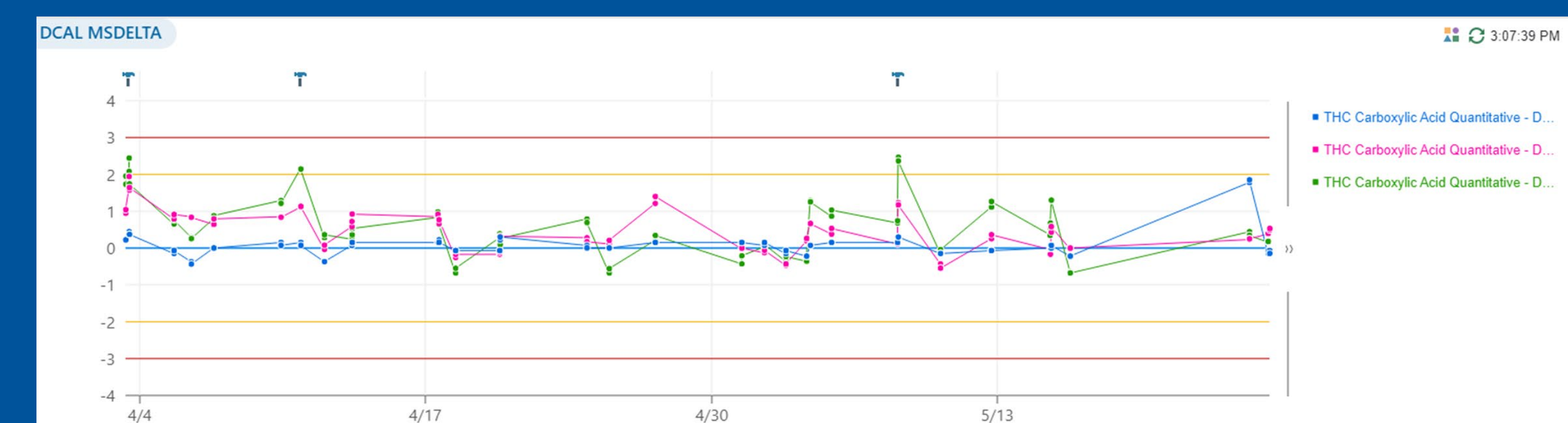


Chart 4. Levey-Jennings chart showing QC performance of 3 quality control materials (Low, Mid, High) during time frame prior to protocol change implementation.

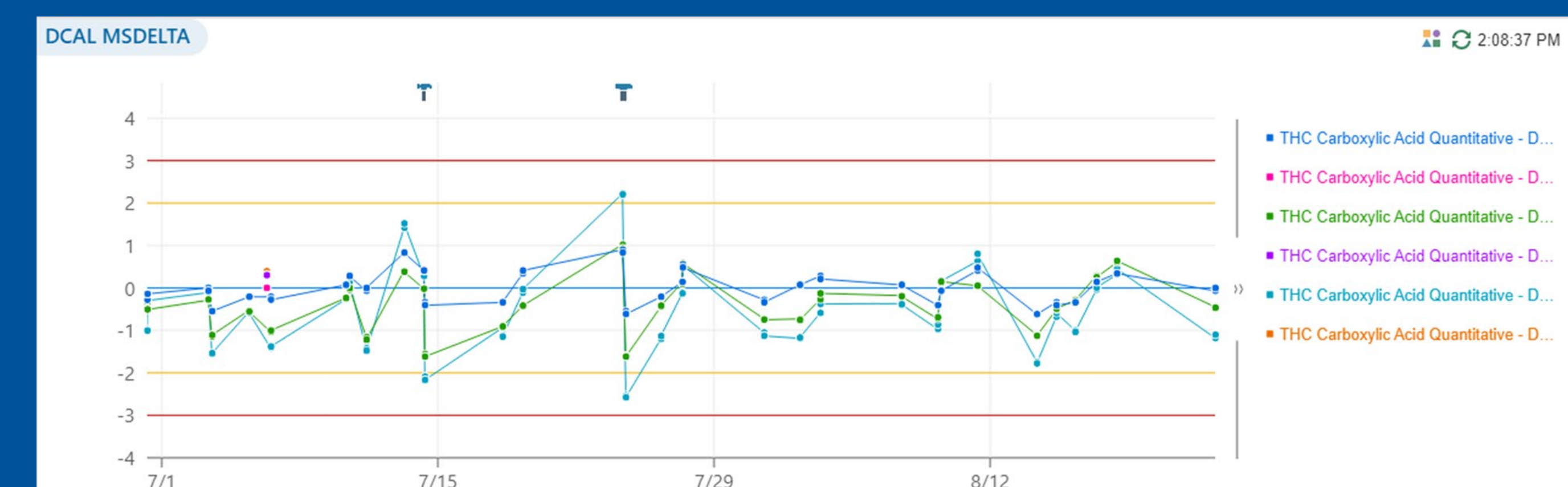


Chart 5. Levey-Jennings chart showing QC performance of 3 quality control materials during time frame after change to calibrator protocol.