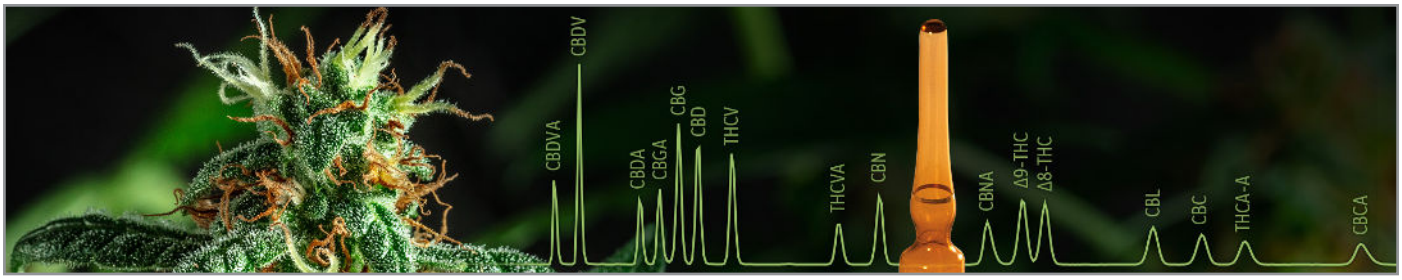


Stability Study of Mixed Neutral and Acidic Cannabinoid Standards

By Melinda Urich

Abstract

This study examines the stability of working solutions prepared by combining neutral and acidic cannabinoid standards. Stability was assessed for 16 cannabinoids across 30 days for solutions prepared in commonly used diluents and stored under typical lab conditions.

Related Products

- *Raptor ARC-18 2.7 µm, 150 mm x 4.6 mm (cat.# 9314A65)*
- *Cannabinoids Acids 7 Standard (cat.# 34144)*
- *Cannabinoids Neutrals 9 Standard (cat.# 34132)*

Introduction

To ensure accurate reporting, it is essential that the stability of cannabinoid standards be studied. For example, if improperly manufactured or stored, tetrahydrocannabinolic acid (THCA) will convert to its methoxy counterpart, tetrahydrocannabinol (THC), giving invalid results for both analytes. To extend stability and simplify the preparation of working standards, Restek has formulated two certified reference materials (CRMs) comprised of 16 commonly analyzed cannabinoids. These standards are formulated in two high-concentration (1000 µg/mL) solutions: a cannabinoids acids 7 mix (cat.# 34144) and a cannabinoids neutrals 9 mix (cat.# 34132). Using these two CRMs instead of 16 single-compound standards saves labs time and money, and minimizes preparation errors. More important, because the acids and neutrals are formulated separately in appropriate diluents instead of as a single solution, long-term stability can be increased by preventing decarboxylation of the acids.

Stability studies on sealed ampuls of both the cannabinoid acids and neutrals CRMs have been performed by Restek under accelerated conditions for standard stability for up to four weeks. However, to assess the stability of working standards, the current study was conducted by combining the cannabinoid CRMs in commonly used diluents and storing the mixed solutions under typical lab conditions.

Experimental

The components of each cannabinoid CRM are given below. To measure the stability of a combined solution of Restek's cannabinoids acids 7 standard (cat.# 34144) and cannabinoids neutrals 9 standard (cat.# 34132), duration, temperature, and diluent parameters (Figure 1) were selected to represent use in a typical production lab. Peak response (area) data was then collected and compared. Data was normalized to Day 0 results, and the acceptance criterion was defined as ±5% of the original response on Day 0.

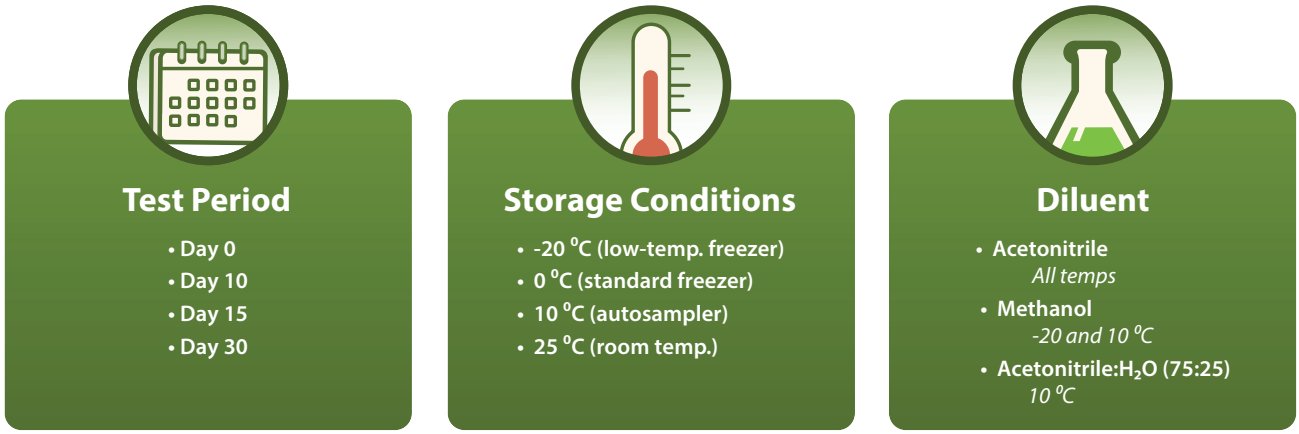
Cannabinoids Acids 7 Standard (cat.# 34144)
1000 µg/mL, acetonitrile with 1% DIPEA and 0.05% ascorbic acid

- Cannabichromenic acid (CBCA) (185505-15-1)
- Cannabidiolic acid (CBDA) (1244-58-2)
- Cannabidivarinic acid (CBDVA) (31932-13-5)
- Cannabigerolic acid (CBGA) (25555-57-1)
- Cannabinolic acid (CBNA) (2808-39-1)
- Tetrahydrocannabinolic acid (THCA-A) (23978-85-0)
- Tetrahydrocannabivarinic acid (THCVA) (39986-26-0)

Cannabinoids Neutrals 9 Standard (cat.# 34132)
1000 µg/mL in purge-and-trap methanol

- Cannabichromene (CBC) (20675-51-8)
- Cannabicyclol (CBL) (21366-63-2)
- Cannabidiol (CBD) (13956-29-1)
- Cannabidivarin (CBDV) (24274-48-4)
- Cannabigerol (CBG) (25654-31-3)
- Cannabinol (CBN) (521-35-7)
- d8-Tetrahydrocannabinol (d8-THC) (5957-75-5)
- d9-Tetrahydrocannabinol (d9-THC) (1972-08-3)
- Tetrahydrocannabivarin (THCV) (31262-37-0)

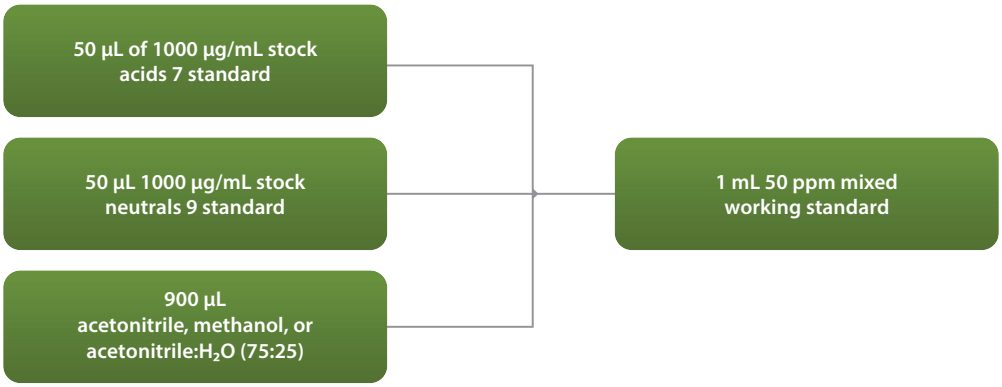
Figure 1: Experimental Parameters for Testing the Stability of Prepared Cannabinoid Standards (50 ppm Mixtures of Acids and Neutrals)



Standard Solution Preparation

Prior to dilution, storage and handling of both cannabinoid CRMs followed their certificates of analysis (CoA), including the recommended storage condition of -20 °C and sonication prior to use. Using aliquots from the same ampuls for each CRM, mixed standards were prepared at 50 ppm in each diluent to a final volume of 1 mL following the procedure in Figure 2. Each working standard was prepared in a 2 mL amber vial, capped, and then stored under one of the test conditions described in Figure 1.

Figure 2: Sample Preparation of 50 ppm Working Standards (Combined Cannabinoid Acids and Neutrals CRMs)



Analytical Conditions

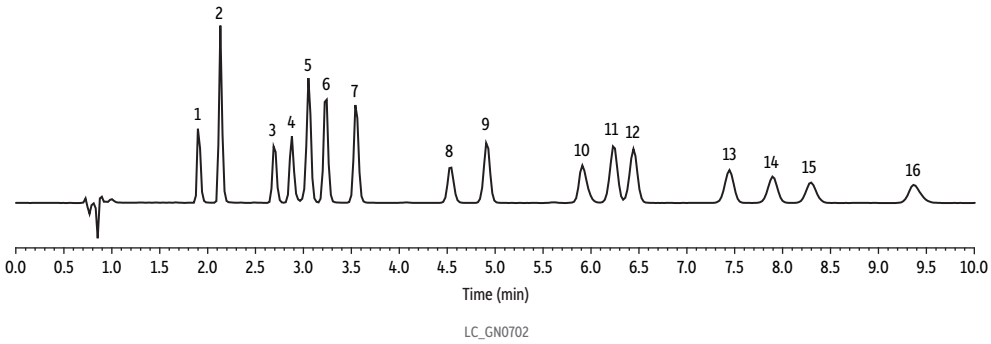
Analytes were monitored using the following chromatographic conditions. On each test day, the prepared working standards were removed from their respective storage conditions, analyzed, recapped, and then returned to their storage conditions after analysis.

Column: Raptor ARC-18 2.7 µm 150 mm x 4.6 mm (cat. # 9314A65)
Injection volume: 5 µL
Mobile phase A: Water, 5 mM ammonium formate, 0.1% formic acid
Mobile phase B: Acetonitrile, 0.1% formic acid
Flow rate: 1.5 mL/min
Instrument: Shimadzu Nexera X2
Detector: Shimadzu DAD @ 228 nm
Temperature: 30 °C
Gradient: Isocratic, 75% B
Run time: 11 minutes

Results and Discussion

The example chromatogram in Figure 3 shows good peak shape and separation for all 16 neutral and acidic cannabinoids on a Raptor ARC-18 column. Each working standard was injected in triplicate, and the area values for each cannabinoid were averaged and normalized to the average Day 0 area values to produce the relative response results presented in Tables I and II and Figures 4-10. Overall, the data indicate that the cannabinoids in the mixed acids and neutrals working standards were stable across most diluent and storage condition combinations. Responses for all analytes were within the ±5% criteria, except for CBDV, CBG, and CBD in room temperature storage (25 °C) on day 30. In addition, it was observed that the acetonitrile:water (75:25) diluent data showed an increasing response, which suggests evaporation of the diluent.

Figure 3: Representative Chromatogram for a 50-ppm Mixed Standard Blended from Acidic and Neutral Cannabinoid CRMs (Room Temperature, Day 30).



Peaks	t_R (min)	Peaks	t_R (min)
1. Cannabidivarinic acid (CBDVA)	1.91	9. Cannabinol (CBN)	4.93
2. Cannabidivarin (CBDV)	2.15	10. Cannabinolic acid (CBNA)	5.91
3. Cannabidiolic acid (CBDA)	2.69	11. Δ9-Tetrahydrocannabinol (Δ9-THC)	6.23
4. Cannabigerolic acid (CBGA)	2.90	12. Δ8- Tetrahydrocannabinol (Δ8-THC)	6.45
5. Cannabigerol (CBG)	3.05	13. Cannabicyclol (CBL)	7.45
6. Cannabidiol (CBD)	3.21	14. Cannabichromene (CBC)	7.90
7. Tetrahydrocannabivarin (THCV)	3.58	15. δ-9-Tetrahydrocannabinolic acid-A (THCA-A)	8.30
8. Tetrahydrocannabivarinic acid (THCVA)	4.52	16. Cannabichromenic acid (CBCA)	9.37

Column: Raptor ARC-18 (cat.# 9314A65); Dimensions: 150 mm x 4.6 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Temp.: 30 °C; **Standard/Sample:** Cannabinoids acids 7 standard, 1000 µg/mL, acetonitrile with 1% DIPEA and 0.05% ascorbic acid (cat.# 34144); Cannabinoids neutrals 9 standard, 1000 µg/mL, P&T methanol (cat.# 34132); Diluent: Acetonitrile; Conc.: 50 ppm; Inj. Vol.: 5 µL; **Mobile Phase:** ; A: Water, 5 mM ammonium formate, 0.1% formic acid; B: Acetonitrile, 0.1% formic acid; Gradient (%B): 0.00 min (75%); 11.00 min (75%); **Flow:** 1.5 mL/min; **Detector:** Shimadzu DAD @ 228 nm**; Instrument:** Shimadzu Nexera X2**; Sample Preparation:** To prepare the working standards, 50 µL of the cannabinoids acids 7 standard (cat.# 34144); 50 µL of the cannabinoids neutrals 9 standard (cat.# 34132); and 900 µL of acetonitrile were aliquoted into 2 mL, screw-thread vials (cat.# 21143), capped with short-cap, screw vial closures (cat.# 24498), and stored at room temperature for 30 days.

Table I: Stability of Neutral Cannabinoids in Mixed Working Standards Stored Under Test Conditions (% Response Relative to Day 0). Green Indicates Result Passes the ±5% Acceptance Criteria, Yellow Indicates a Borderline Result, Red Indicates a Failing Result.

Storage Temperature	- 20 °C						0 °C		
Diluent	Acetonitrile			Methanol			Acetonitrile		
Day	10	15	30	10	15	30	10	15	30
Cannabidivarin	103	101	102	98	99	98	102	102	104
Cannabigerol	103	102	103	99	98	98	102	103	103
Cannabidiol	103	102	102	98	98	98	102	102	103
Tetrahydrocannabivarin	103	102	102	98	99	98	104	102	103
Cannabinol	103	102	102	98	98	97	103	102	103
Δ9-Tetrahydrocannabinol	104	103	102	98	98	97	101	101	101
Δ8-Tetrahydrocannabinol	103	101	102	98	99	98	103	102	104
Cannabicyclol	104	103	102	99	99	97	102	102	103
Cannabichromene	104	102	102	98	98	97	102	102	103

Storage Temperature	10 °C									25 °C		
Diluent	Acetonitrile			Methanol			Acetonitrile:Water			Acetonitrile		
Day	10	15	30	10	15	30	10	15	30	10	15	30
Cannabidivarin	99	100	100	98	98	97	102	102	102	101	98	88
Cannabigerol	99	102	101	98	99	99	102	103	103	99	95	87
Cannabidiol	99	101	101	97	98	98	102	103	103	102	99	93
Tetrahydrocannabivarin	99	101	101	97	98	98	102	103	104	103	102	103
Cannabinol	99	102	102	96	98	98	103	103	104	103	102	104
Δ9-Tetrahydrocannabinol	99	102	102	96	98	98	102	103	104	103	102	103
Δ8-Tetrahydrocannabinol	99	101	101	97	98	98	103	103	104	104	102	105
Cannabicyclol	99	102	102	96	98	98	102	103	104	104	103	104
Cannabichromene	99	102	102	96	98	98	102	103	104	104	103	105

Table II: Stability of Acidic Cannabinoids in Mixed Working Standards Stored Under Test Conditions (% Response Relative to Day 0). Green Indicates Result Passes the ±5% Acceptance Criteria, Yellow Indicates a Borderline Result, Red Indicates a Failing Result

Storage Temperature	- 20 °C						0 °C		
Diluent	Acetonitrile			Methanol			Acetonitrile		
Day	10	15	30	10	15	30	10	15	30
Cannabidivarinic acid	102	100	100	98	99	97	100	100	101
Cannabidiolic acid	102	100	100	98	98	97	101	101	101
Cannabigerolic acid	102	100	100	98	97	97	100	100	101
Tetrahydrocannabivarinic acid	102	100	101	98	98	97	100	100	101
Cannabinolic acid	101	101	101	98	98	97	100	101	101
Tetrahydrocannabinolic acid	102	104	100	98	98	97	100	100	101
Cannabichromenic acid	102	102	101	98	98	97	101	101	101

Storage Temperature	10 °C									25 °C		
Diluent	Acetonitrile			Methanol			Acetonitrile:Water			Acetonitrile		
Day	10	15	30	10	15	30	10	15	30	10	15	30
Cannabidivarinic acid	97	99	99	96	98	97	99	100	101	101	99	95
Cannabidiolic acid	98	100	100	96	98	97	101	102	102	101	99	96
Cannabigerolic acid	98	99	99	96	97	97	101	102	103	101	98	95
Tetrahydrocannabivarinic acid	98	99	100	96	97	98	100	101	102	101	99	99
Cannabinolic acid	99	100	101	96	97	98	100	102	103	102	101	104
Tetrahydrocannabinolic acid	99	100	100	96	98	97	100	101	102	101	99	100
Cannabichromenic acid	100	101	101	97	98	98	101	102	103	103	101	103

Figure 4: Percent Response Results Relative to Day 0 for 16 Cannabinoids at 50 ppm in a Mixed Standard Prepared in Acetonitrile, Stored at -20 °C, and Analyzed on Days 0, 10, 15, and 30

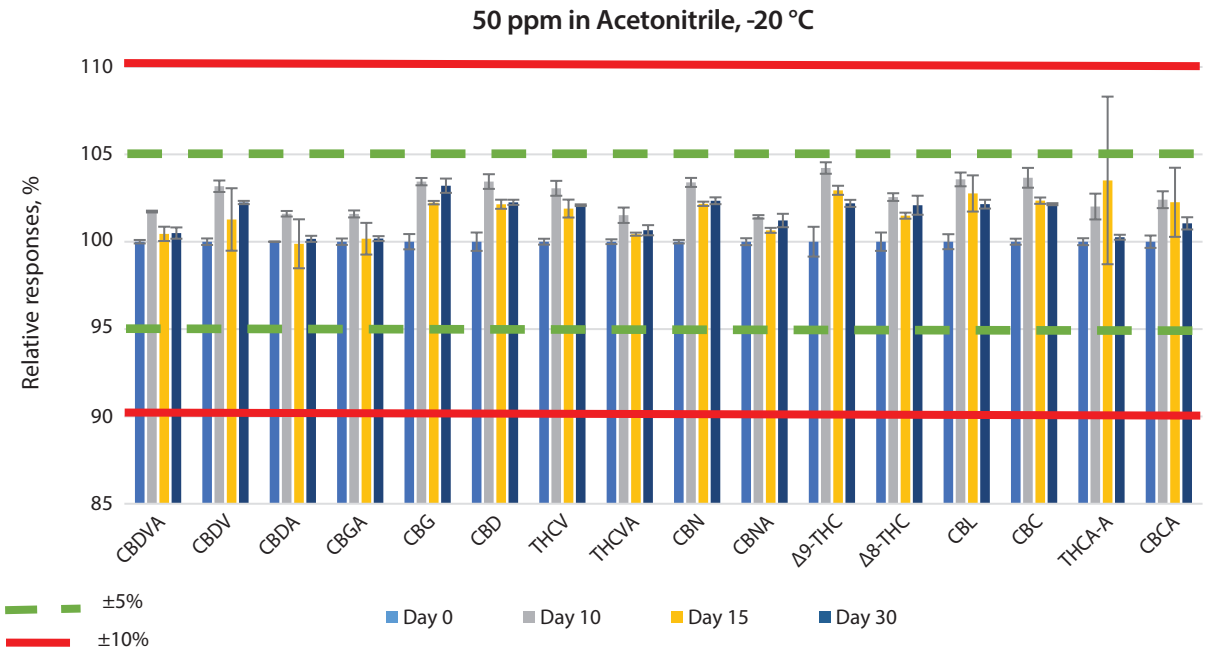


Figure 5: Percent Response Results Relative to Day 0 for 16 Cannabinoids at 50 ppm in a Mixed Standard Prepared in Acetonitrile, Stored at 0 °C, and Analyzed on Days 0, 10, 15, and 30

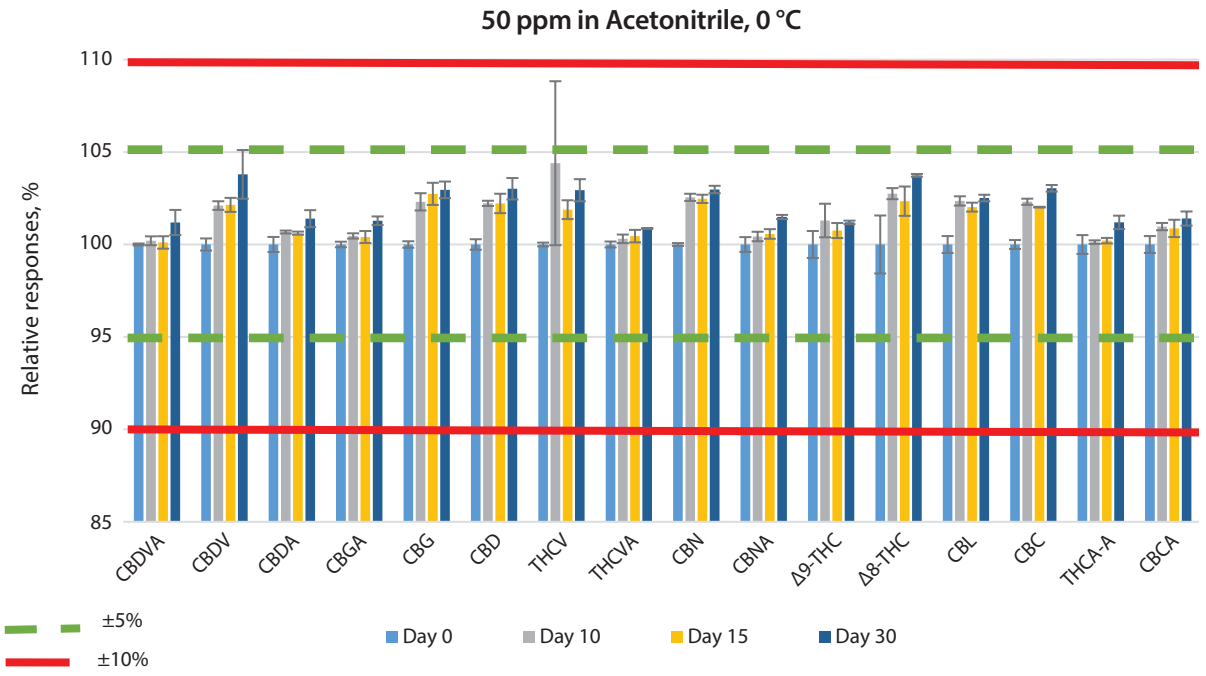


Figure 6: Percent Response Results Relative to Day 0 for 16 Cannabinoids at 50 ppm in a Mixed Standard Prepared in Acetonitrile, Stored at 10 °C, and Analyzed on Days 0, 10, 15, and 30

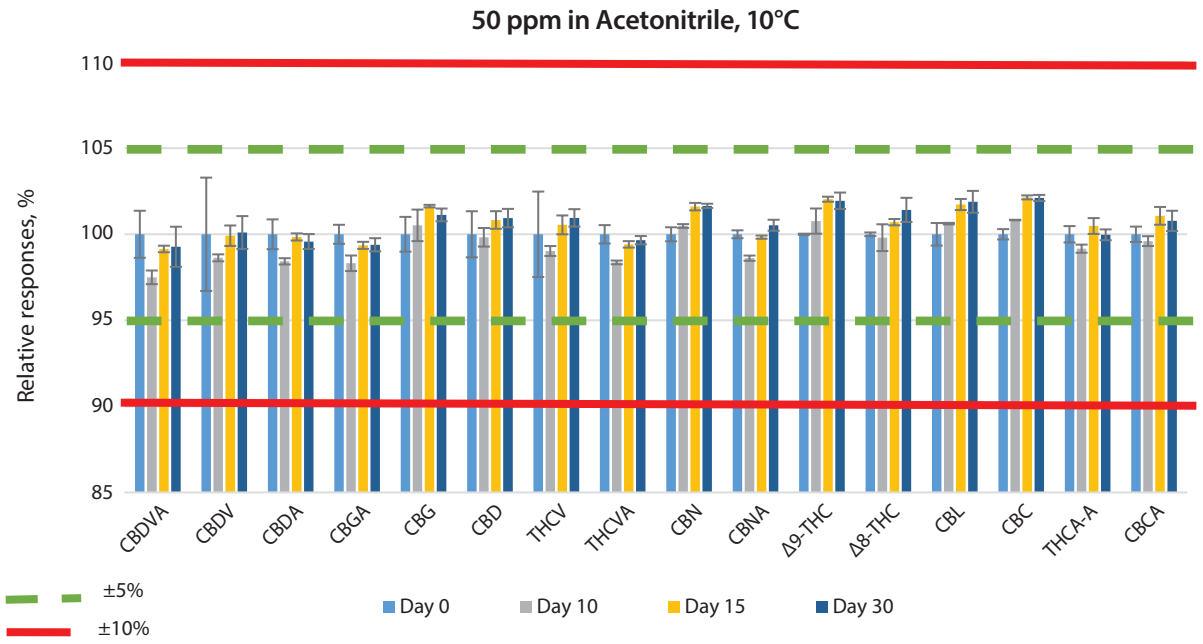


Figure 7: Percent Response Results Relative to Day 0 for 16 Cannabinoids at 50 ppm in a Mixed Standard Prepared in Acetonitrile, Stored at 25 °C, and Analyzed on Days 0, 10, 15, and 30

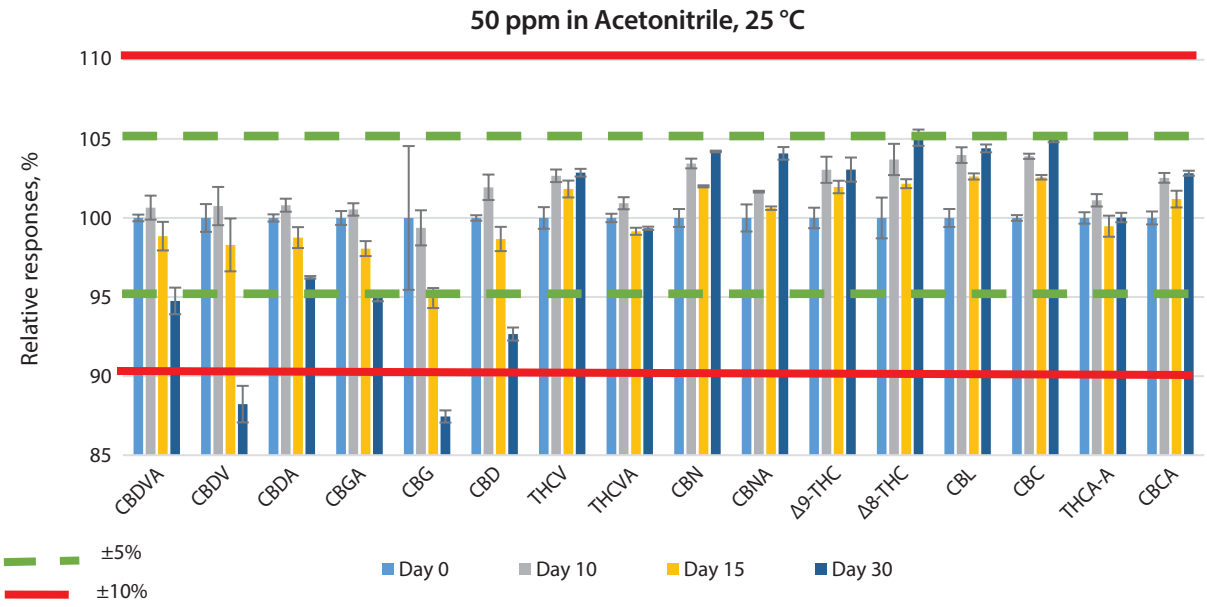


Figure 8: Percent Response Results Relative to Day 0 for 16 Cannabinoids at 50 ppm in a Mixed Standard Prepared in Methanol, Stored at 10 °C, and Analyzed on Days 0, 10, 15, and 30

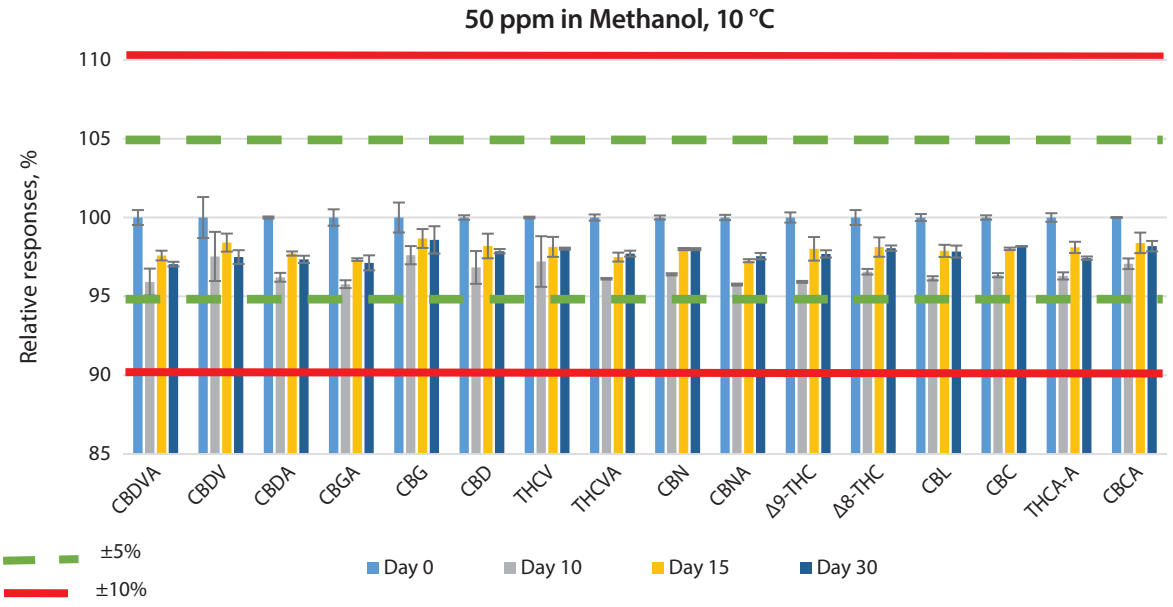


Figure 9: Percent Response Results Relative to Day 0 for 16 Cannabinoids at 50 ppm in a Mixed Standard Prepared in Methanol, Stored at -20 °C, and Analyzed on Days 0, 10, 15, and 30

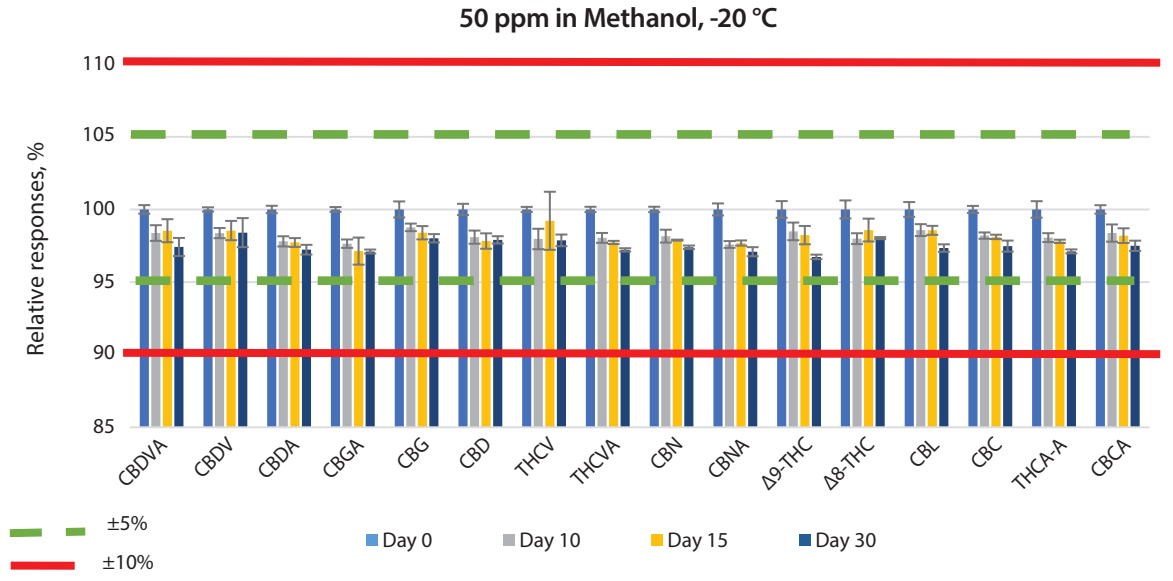
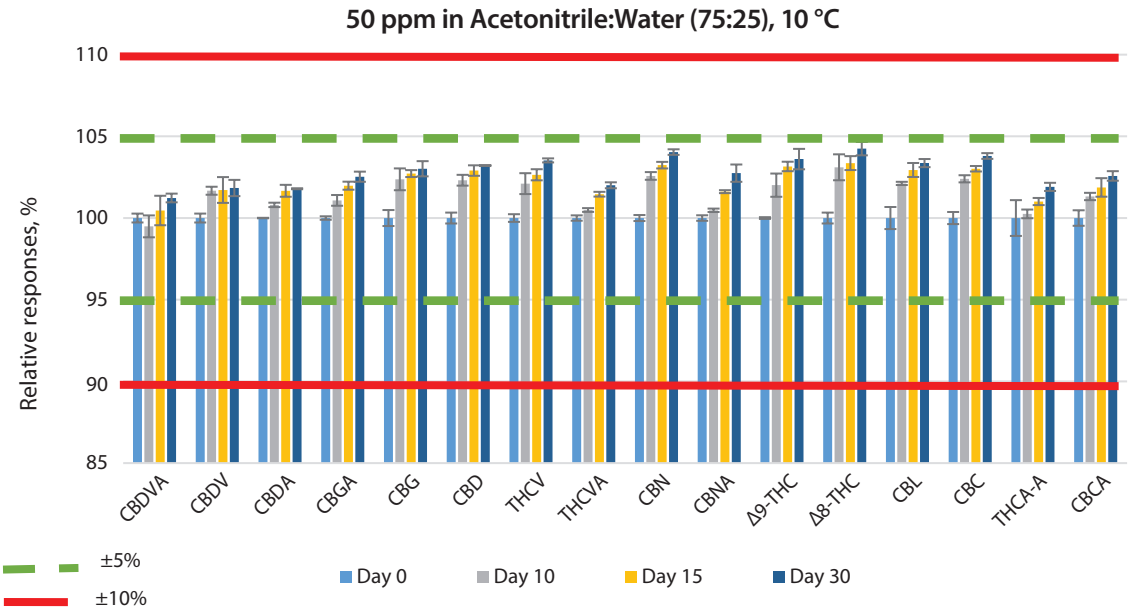


Figure 10: Percent Response Results Relative to Day 0 for 16 Cannabinoids at 50 ppm in a Mixed Standard Prepared in Acetonitrile:Water (75:25); Stored at 10 °C; and Analyzed on Days 0, 10, 15, and 30



Conclusion

A certificate of analysis for a CRM gives the expiration date of a properly stored, unopened ampul. Once opened, the solutions should be transferred to an appropriate vial and stored under recommended conditions between use. Like all analytes, cannabinoid stability is affected by the environmental and handling conditions to which the CRM and working standards are exposed. While Restek has completed stability studies on both sealed CRM ampuls and mixed working standard containing both acidic and neutral cannabinoids, it is strongly advised that labs conduct their own stability studies and implement SOPs to address the use and handling of CRMs and the preparation of working standards. During the current study, the cannabinoids were generally stable across the test conditions; however, for the most accurate results we recommend, especially when combining multicomponent ampuls, that standards be prepared fresh daily to prevent degradation. In addition, having technicians who prepare samples also prepare their own QC samples and calibration curves is a best practice because it can reduce variation. More information about Restek reference standard manufacturing and testing can be found at https://www.restek.com/articles/restek-reference-standards.

Raptor ARC-18

- Ideal for high-throughput LC-MS/MS applications with minimal sample preparation.
- Well-balanced retention profile for better detection and integration of large, multiclass analyte lists.
- Sterically protected to endure low-pH mobile phases without sacrificing retention or peak quality.
- Part of Restek's Raptor LC column line featuring 1.8, 2.7, and 5 µm SPP core-shell silica.

Catalog No.	Product Name	Units
9314A65	Raptor ARC-18, 2.7 µm, 150 mm x 4.6 mm	ea.

Cannabinoids Acids 7

Cannabichromenic Acid (CBCA) (185505-15-1)
Cannabidiolic Acid (CBDA) (1244-58-2)
Cannabidivarinic Acid (CBDVA) (31932-13-5)
Cannabigerolic Acid (CBGA) (25555-57-1)
Cannabinolic Acid (CBNA) (2808-39-1)
Tetrahydrocannabinolic Acid (THCA-A) (23978-85-0)
Tetrahydrocannabivarinic Acid (THCVA) (39986-26-0)

Catalog No.	Concentration	Solvent	Volume	Units
34144	1000 µg/mL	Acetonitrile with 1% DIPEA and 0.05% Ascorbic Acid	1 mL/ampul	ea.

Cannabinoids Neutrals 9

Cannabichromene (CBC) (20675-51-8)
Cannabicyclol (CBL) (21366-63-2)
Cannabidiol (CBD) (13956-29-1)
Cannabidivarin (CBDV) (24274-48-4)
Cannabigerol (CBG) (25654-31-3)
Cannabinol (CBN) (521-35-7)
d8-Tetrahydrocannabinol (d8-THC) (5957-75-5)
d9-Tetrahydrocannabinol (d9-THC) (1972-08-3)
Tetrahydrocannabivarin (THCV) (31262-37-0)

Catalog No.	Concentration	Solvent	Volume	Units
34132	1000 µg/mL	P&T Methanol	1 mL/ampul	ea.

RESTEK
Pure Chromatography

Questions? Contact us or your local Restek representative (www.restek.com/contact-us).

www.restek.com

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