

Measuring the Stability of Neutral and Acidic Cannabinoid Certified Reference Material for Instrument Calibration

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Introduction

Validated methods for cannabis potency testing often assess many characteristics, including, but not limited to, accuracy, precision, and linearity. One essential starting point to begin validation is procurement of analytical reference standards. Abiding by strict environmental controls and stringent stability testing, Restek's ISO-accredited lab developed two certified reference standards to assist with validation and calibration. Sixteen commonly analyzed cannabinoids of interest were selected to develop two high concentration (1000 µg/mL) reference materials, an acids 7 mix and neutrals 9 mix. Offering the 16 component mix over two ampoules, compared to 16 single ampoules, allows users flexibility, as well as time and cost savings by minimizing errors when preparing calibration curves and QCs.

Background

The selection and separation of these cannabinoids offers long term storage stability, due to key additives. The stability of the cannabinoids are crucial for quantitation of key compounds such as THC and THCA. If improperly manufactured or stored, THCA will convert to THC giving invalid results for both analytes.

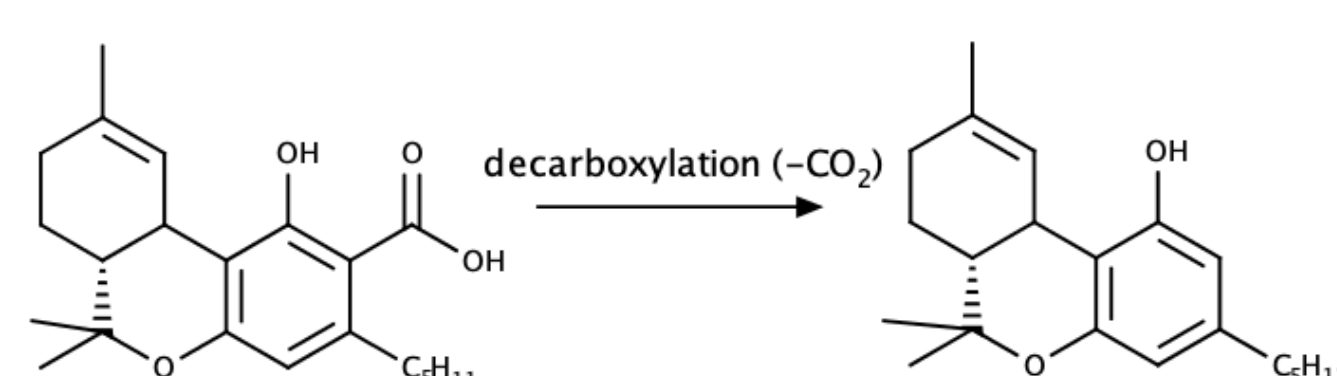


Figure 1: Shows THCA conversion to THC

To assess the stability of these two mixes once mixed, a study was conducted assessing the stability of the combined acids 7 and neutrals 9 mixes. Component break down for each mix is shown in Table I and II.

Compound Name	Synonym	CAS #	Compound Name	Synonym	CAS #
Cannabichromenic Acid	CBCA	185505-15-1	Cannabichromene	CBC	20675-51-8
Cannabidiolic Acid	CBDA	1244-58-2	Cannabicyclol	CBL	21366-63-2
Cannabidivarinic Acid	CBDVA	31932-13-5	Cannabidiol	CBD	13956-29-1
Cannabigerolic Acid	CBGA	25555-57-1	Cannabidivarin	CBDV	24274-48-4
Cannabinolic Acid	CBNA	2808-39-1	Cannabigerol	CBG	25654-31-3
Tetrahydrocannabinolic Acid	THCA-A	23978-85-0	Cannabinol	CBN	521-35-7
Tetrahydrocannabivarinic Acid	THCVA	39986-26-0	Δ8-Tetrahydrocannabinol	Δ8-THC	5957-75-5
			Δ9-Tetrahydrocannabinol	Δ9-THC	1972-08-3
			Tetrahydrocannabivarin	THCV	31262-37-0

Table I: Cannabinoid Acids 7 Standard Components (Cat.# 34144)

Table II: Cannabinoid Neutrals 9 Standard Components (Cat.# 34132)

Experimental Design

Test Period

Days: 0, 10, 15 and 30

Storage Conditions

-20, 0, 10 and 25 °C

- 20 °C – representative low temp freezer conditions
- 0 °C – representative of standard freezer conditions
- 10 °C – representative of auto sampler conditions
- 25 °C – representative of room temperature conditions

Diluent

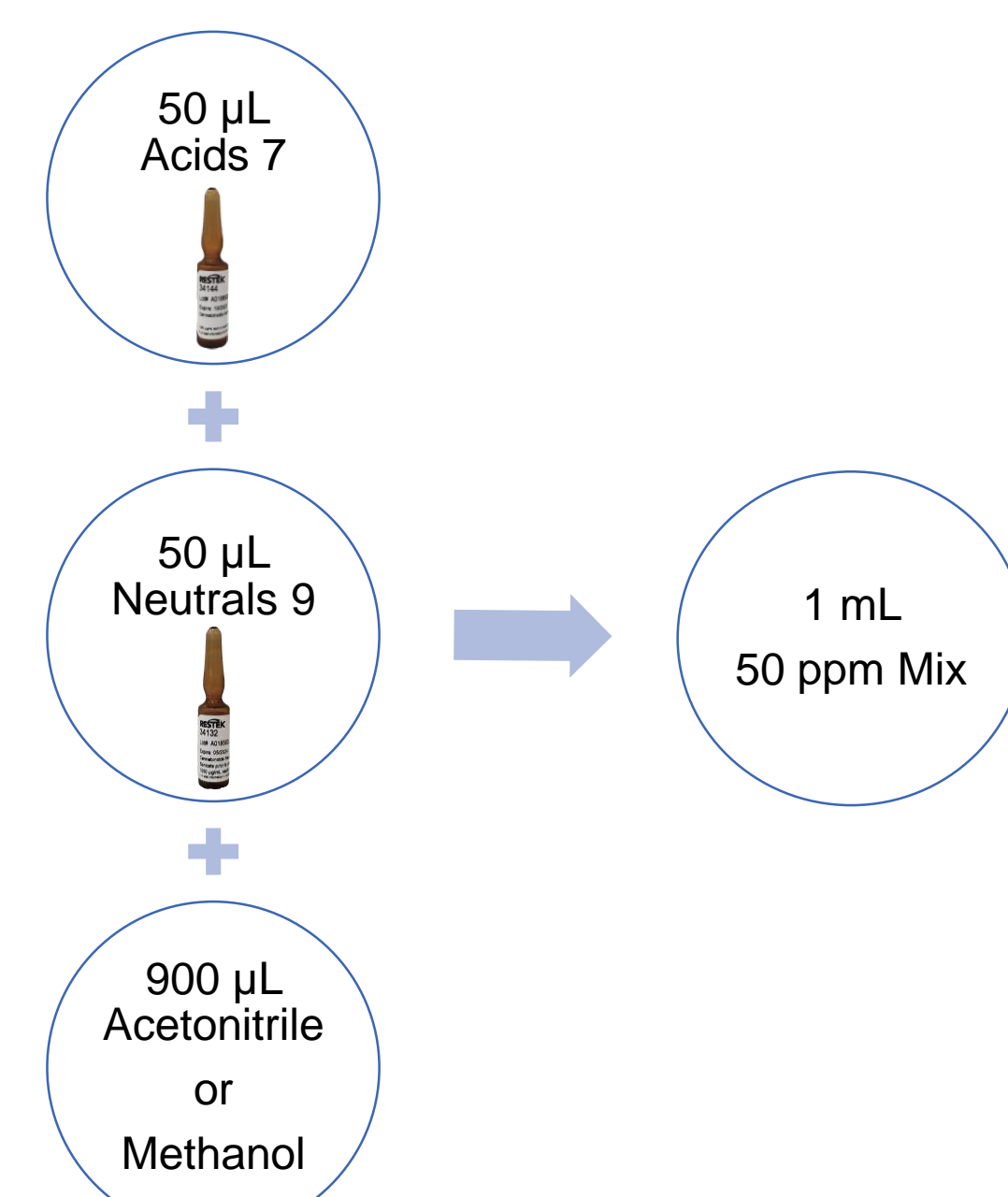
Methanol and Acetonitrile

- Methanol – -20 and 10 °C
- Acetonitrile – -20, 0, 10 and 25 °C

Assessed conditions using mix of combined acids 7 and neutrals 9 reference standards.

Acceptance Criteria: ±5 % of original response at "Day 0"

Sample Preparation



Each prepared vial was stored at respective conditions outlined. Caps were replaced after each analysis



Analytical Method

Column:	Raptor ARC-18 2.7 µm 150 mm x 4.6 mm (Cat # 9314A62)	
Standards:	Acids 7 (Cat # 34144) Neutrals 9 (Cat # 34132)	
Diluent:	Acetonitrile	
Inj. Vol.	5 µL	
MP A:	Water, 5 mM ammonium formate, 0.1% formic acid	
MP B:	Acetonitrile, 0.1% formic acid	
Flow:	1.5 mL/min	
Detector	UV-vis @ 228 nm	
Temp:	30 °C	
Gradient:	Isocratic 25:75 MPA:MPB	
	Time (min)	(%) B
	0.00	75
	11.00	75

Table III: Method conditions for analysis of stability

Results

Storage Temperature	- 20°C			0°C			10°C			25°C								
	Acetonitrile	Methanol	Acetonitrile	Acetonitrile	Methanol	Acetonitrile	Acetonitrile	Methanol	Acetonitrile	Acetonitrile	Methanol	Acetonitrile						
Day	10	15	30	10	15	30	10	15	30	10	15	30	10	15	30			
Cannabidivarin	103	101	102	98	99	98	102	102	104	99	100	100	98	98	97	101	98	88
Cannabigerol	103	102	103	99	98	98	102	103	103	99	102	101	98	99	99	99	95	87
Cannabidiol	103	102	102	98	98	98	102	102	103	99	101	101	97	98	98	102	99	93
Tetrahydrocannabivarin	103	102	102	98	99	98	104	102	103	99	101	101	97	98	98	103	102	103
Cannabinol	103	102	102	98	98	97	103	102	103	99	102	102	96	98	98	103	102	104
Δ9-Tetrahydrocannabinol	104	103	102	98	98	97	101	101	101	99	102	102	96	98	98	103	102	103
Δ8-Tetrahydrocannabinol	103	101	102	98	99	98	103	102	104	99	101	101	97	98	98	104	102	105
Cannabicyclol	104	103	102	99	99	97	102	102	103	99	102	102	96	98	98	104	103	104
Cannabichromene	104	102	102	98	98	97	102	102	103	99	102	102	96	98	98	104	103	105

Table IV: Results of Neutrals 9 compounds at specified conditions, % response compared to normalized "day 0" response. Red indicates outside of specified criteria, yellow indicates on border of ±5% criteria, green indicates passes criteria.

Storage Temperature	- 20°C			0°C			10°C			25°C								
	Acetonitrile	Methanol	Acetonitrile	Acetonitrile	Methanol	Acetonitrile	Acetonitrile	Methanol	Acetonitrile	Acetonitrile	Methanol	Acetonitrile						
Day	10	15	30	10	15	30	10	15	30	10	15	30	10	15	30			
Cannabidivarinic Acid	102	100	100	98	99	97	100	100	101	97	99	99	96	98	97	101	99	95
Cannabidiolic Acid	102	100	100	98	98	97	101	101	101	98	100	100	96	98	97	101	99	96
Cannabigerolic Acid	102	100	100	98	97	97	100	100	101	98	99	99	96	97	97	101	98	95
Tetrahydrocannabivarinic Acid	102	100	101	98	98	97	100	100	101	98	99	100	96	97	98	101	99	99
Cannabinolic Acid	101	101	101	98	98	97	100	101	101	99	100	101	96	97	98	102	101	104
Tetrahydrocannabinolic Acid	102	104	100	98	98	97	100	100	101	99	100	100	96	98	97	101	99	100
Cannabichromenic Acid	102	102	101	98	98	97	101	101	101	100	101	101	97	98	98	103	101	103

Table V: Results of Acids 7 compounds at specified conditions, % response compared to normalized "day 0" response. Yellow indicates on border of ±5% criteria, green indicates passes criteria.

Results (cont.)

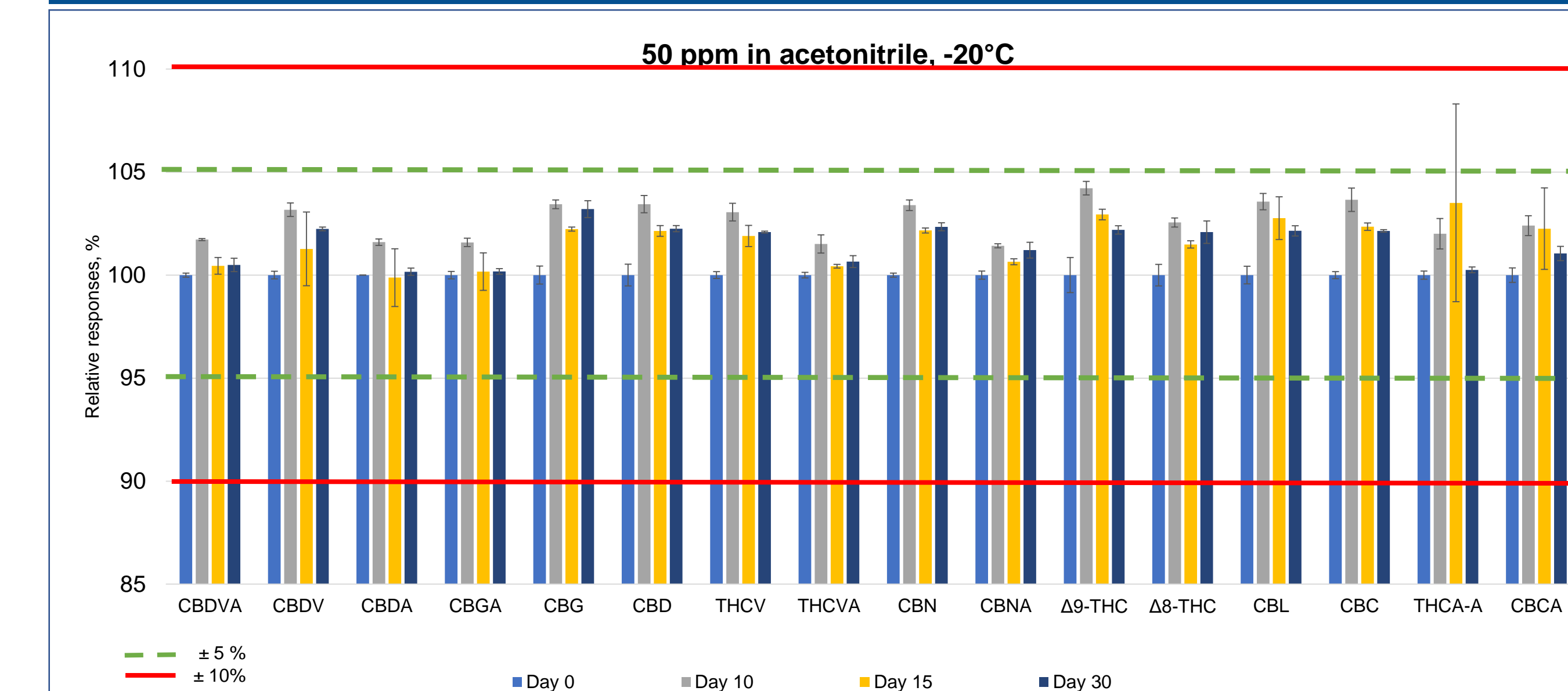


Figure I: Relative %response results of 50 ppm mix for day 0, 10, 15 and 30

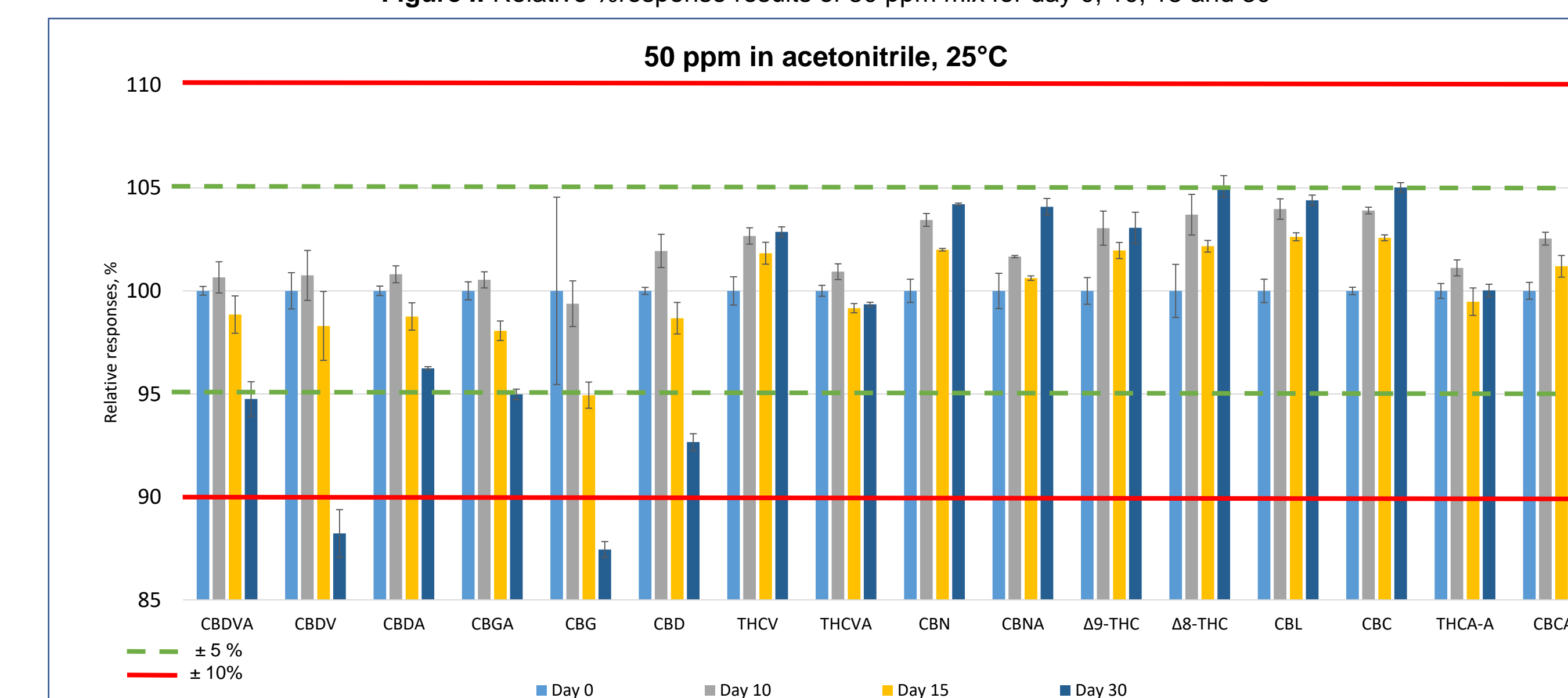
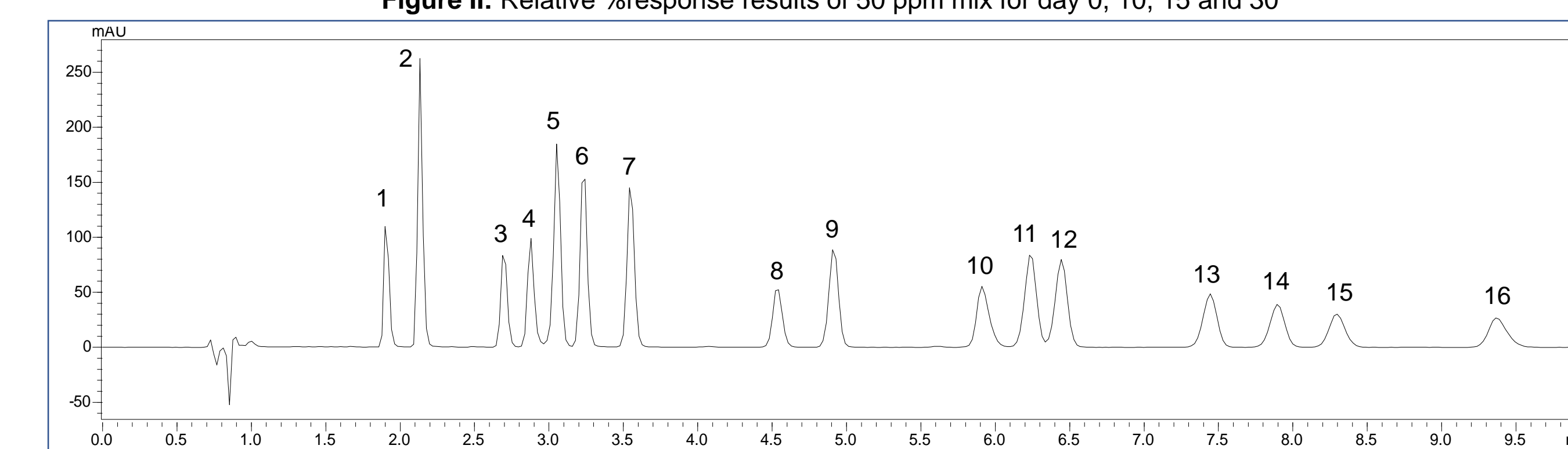


Figure II: Relative %response results of 50 ppm mix for day 0, 10, 15 and 30



Peak #	Compound	Peak #	Compound
1	Cannabidivarinic acid (CBDVA)	9	Cannabinol (CBN)
2	Cannabidivarin (CBDV)	10	Cannabinolic acid (CBNA)
3	Cannabidiolic acid (CBDA)	11	Δ9-Tetrahydrocannabinol (Δ9-THC)
4	Cannabigerolic acid (CBGA)	12	Δ8-Tetrahydrocannabinol (Δ8-THC)
5	Cannabigerol (CBG)	13	Cannabicyclol (CBL)
6	Cannabidiol (CBD)	14	Cannabichromene (CBC)
7	Tetrahydrocannabivarin (THCV)	15	Tetrahydrocannabinolic acid A (THCA-A)
8	Tetrahydrocannabivarinic acid (THCVA)	16	Cannabichromenic acid (CBCA)

Figure III: Representative chromatogram for mix Acids 7 and Neutrals 9 reference standard

Conclusions and Recommendations

During this study, it was observed, vials of mixed reference material stored at the designated conditions all behaved similar to each other, with the exception of room temperature storage at 30 days.

A CoA for certified reference material indicates expiration of a properly stored, unopened ampoule. Once opened, the solutions should be transferred to the appropriate vial and stored under recommended conditions between use. The stability of each product is subject to environmental and handling conditions by which the product is exposed. When combining multi-component ampoules, it is recommended to prepare calibrations standards daily to prevent degradation and improve accuracy of results. It is strongly advised labs conduct their own stability studies and implement SOPs to address use and handling of certified reference materials.