

Analyte Stability Issues during Sample Preparation: Recommendations for the Best Practices

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Outline

Background

Recommendations for the Best Practices

Examples: Case I and Case II

Conclusion

Background

Ensuring analyte stability under various environmental conditions is critical for creation of an accurate and precise quantitative method.

Known environmental conditions causing instability can be intentionally avoided during method development.

It's very important to comprehensively understand the compound stability before developing a quantitative method.

Recommendations for the Best Practices

Assess the stability of analytes under a variety of environmental conditions.

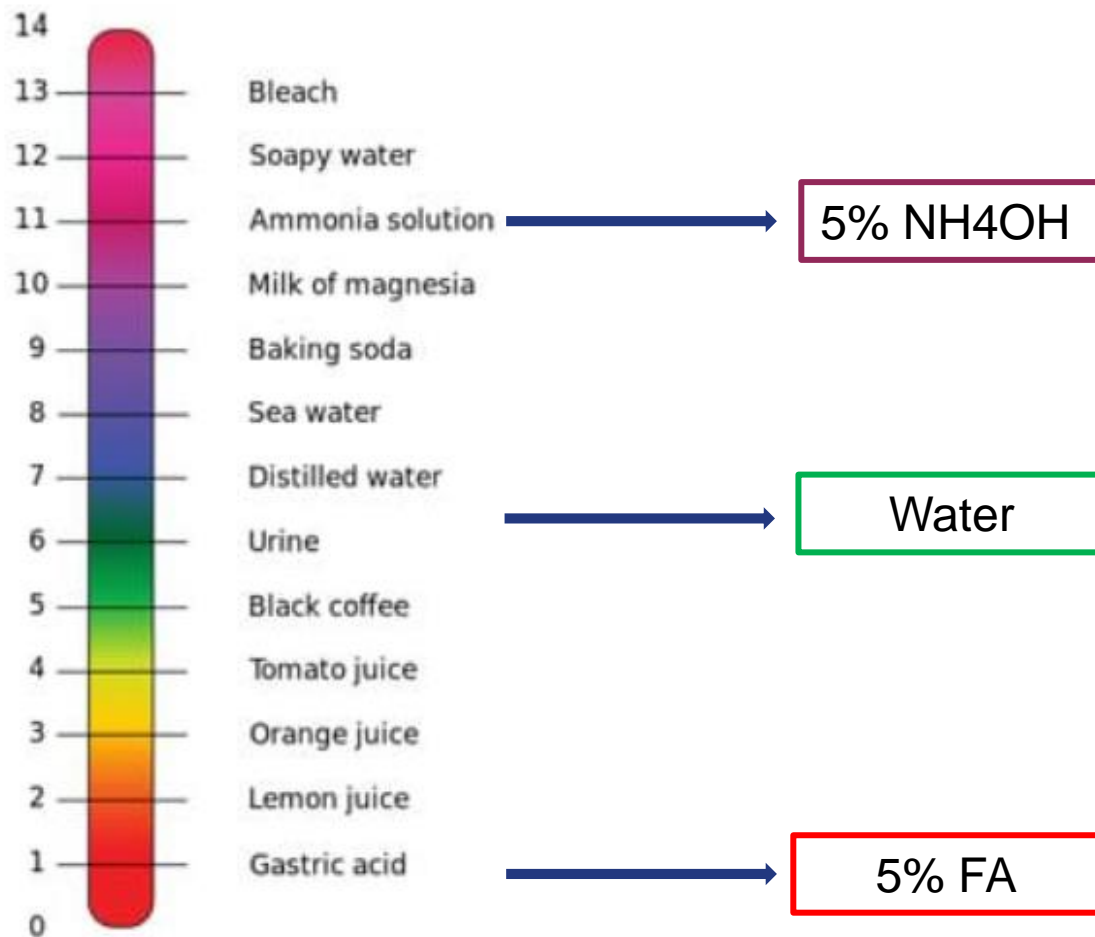
Three main environmental conditions are pH, light exposure and temperature.

The assessment should use extreme conditions.

Apply appropriate pH, light and temperature conditions during sample preparation to prevent instability issue.

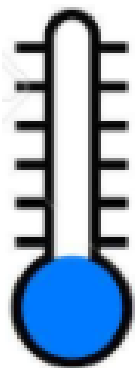
Recommendations for the Best Practices

Different pH Levels



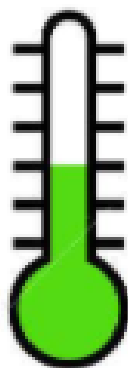
Recommendations for the Best Practices

Different Temperatures



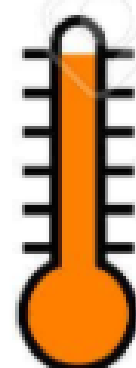
cold

Ice-Water



optimal

RT



heat

60 °C

Recommendations for the Best Practices

Different Light Exposures



Light Protection



Light Exposure

Recommendations for the Best Practices

Stability Experiment Design

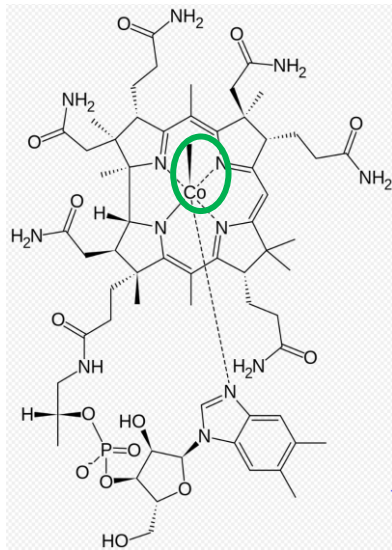
Sample	Replicates	Conditions
Solution-Acidic	3	Light Protection, RT (Control)
Solution-Acidic	3	Elevated Light, RT
Solution-Acidic	3	Light Protection, 60 C
Solution-Neutral	3	Light Protection, RT (Control)
Solution-Neutral	3	Elevated Light, RT
Solution-Neutral	3	Light Protection, 60 C
Solution-Basic	3	Light Protection, RT (Control)
Solution-Basic	3	Elevated Light, RT
Solution-Basic	3	Light Protection, 60 C

Examples: Case I---Vitamin B₁₂ Stability Study

USP and AOAC have compendia method only for B₁₂ raw material, while finished goods needs sensitive and fast method.

Vitamin B₁₂ supplements are typically derived from two sources: Methylcobalamin (mB12) and Cyanocobalamin (cB12).

mB12 is a better source, but its product has stability issues.

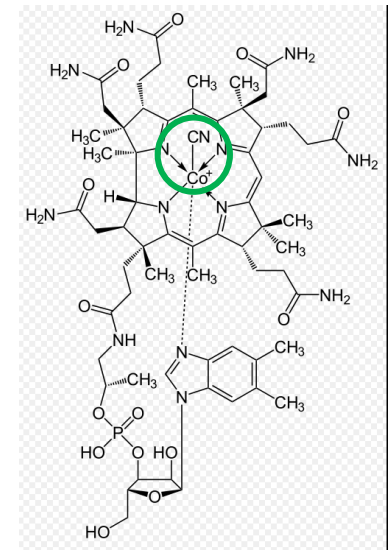


mB₁₂

mB₁₂ improves bioavailability

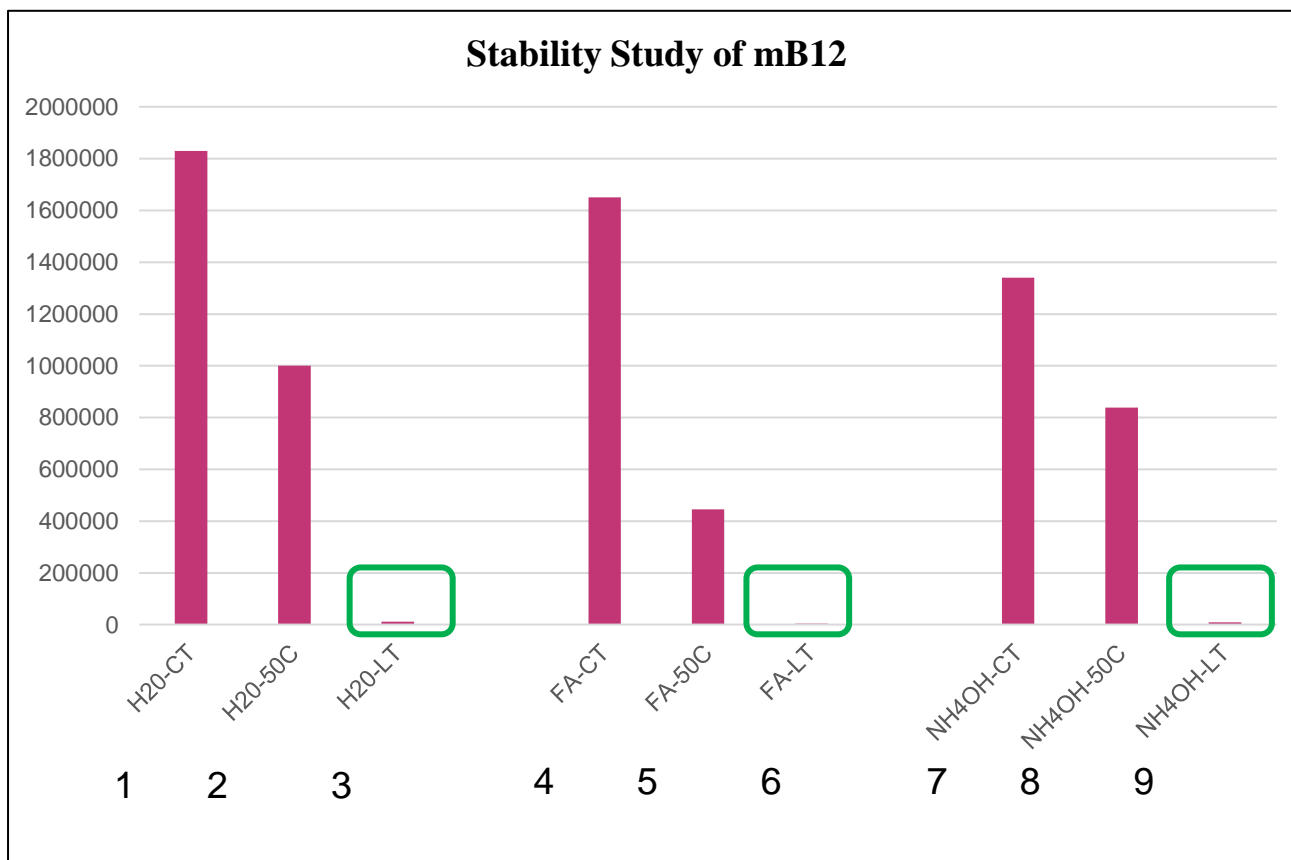
mB₁₂ has better retention in tissues

mB₁₂ contains no toxic cyanide



cB₁₂

Examples: Case I---Vitamin B₁₂ Stability Study

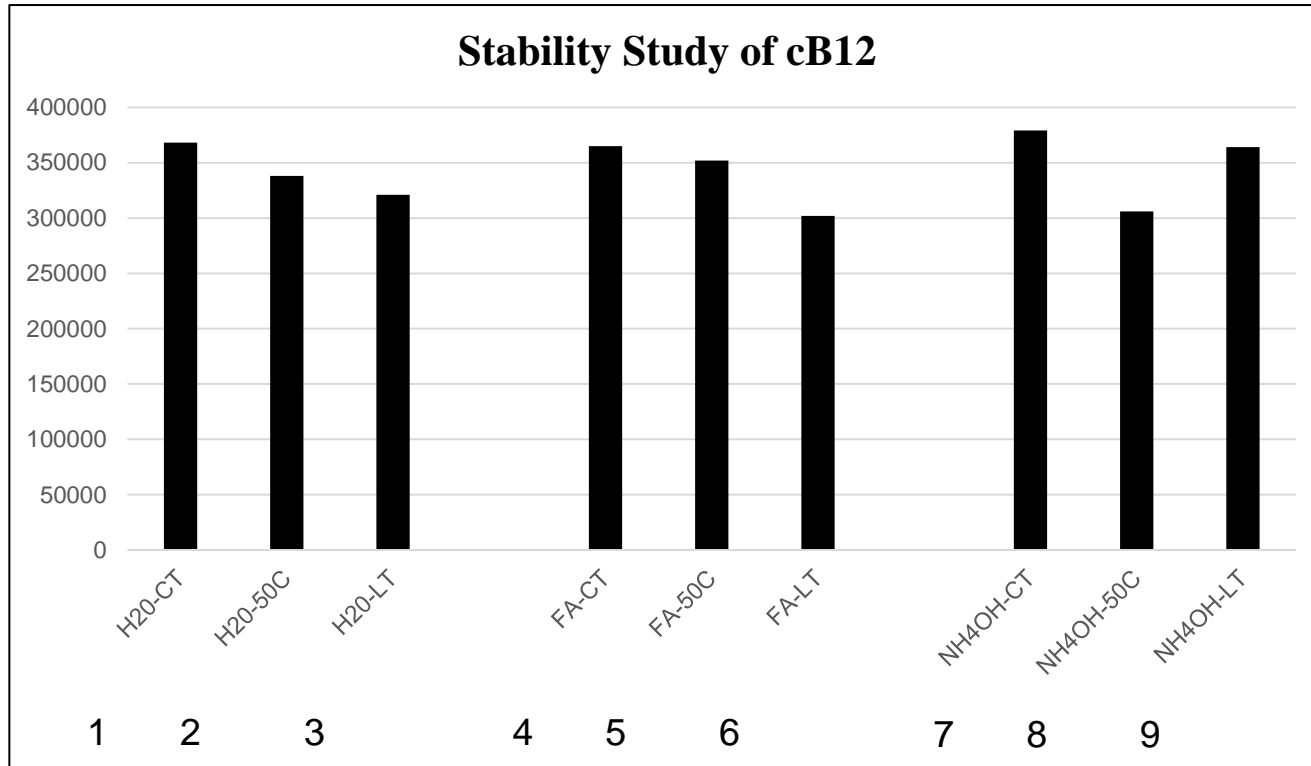


Light sensitive

Temperature sensitive

pH sensitive

Examples: Case I---*Vitamin B₁₂ Stability Study*



Light sensitive

Temperature sensitive

pH sensitive

Examples: Case I---*Vitamin B₁₂ Stability Study*

1. Sample is stored in black bag.
2. Sample is prepared in amber container under yellow light.
3. Sample is extracted in neutral solvent.
4. Sample is sonicated in ice-water and stored at 1-8 °C.



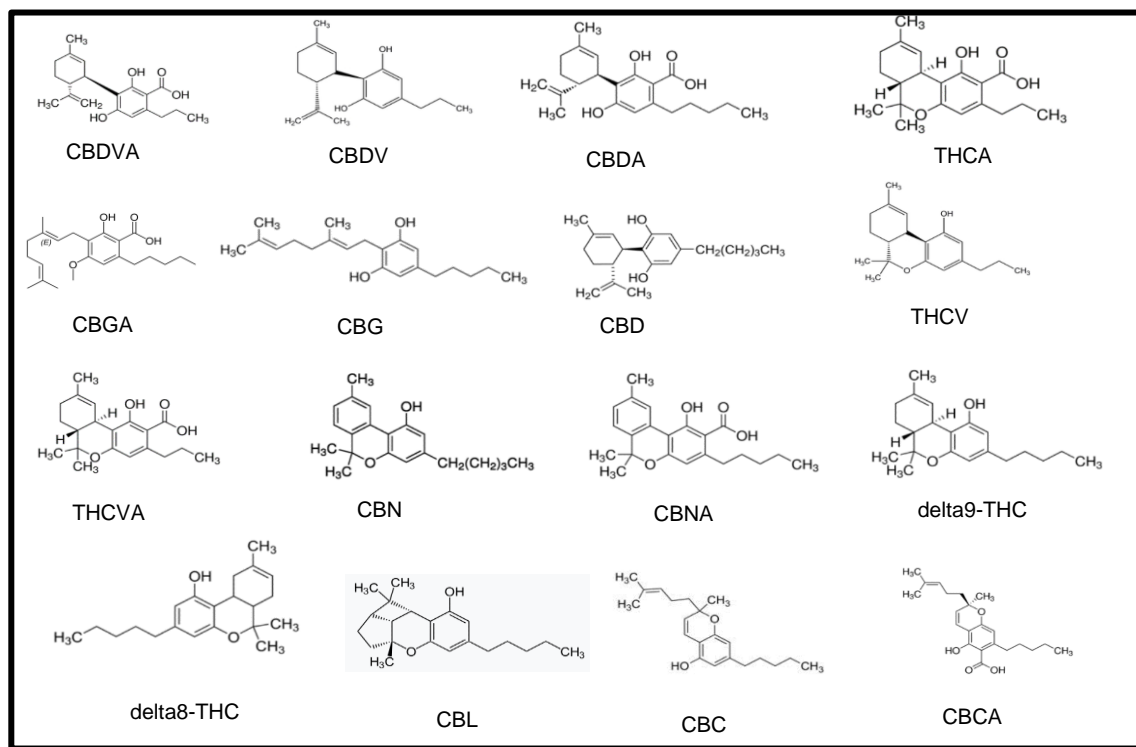
Examples: Case II---*CBD Stability Study*

In 2018, AOAC has published a method to quantify 10 cannabinoids (CBDs) with the range of 0.500-10.0 µg/mL in cannabis.

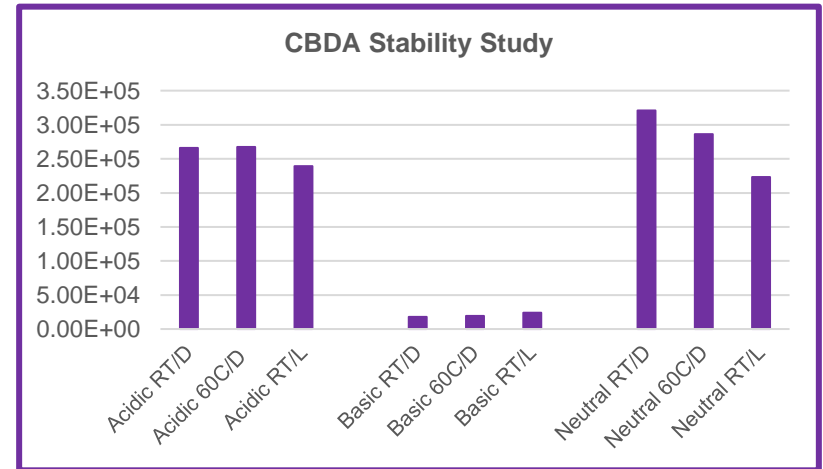
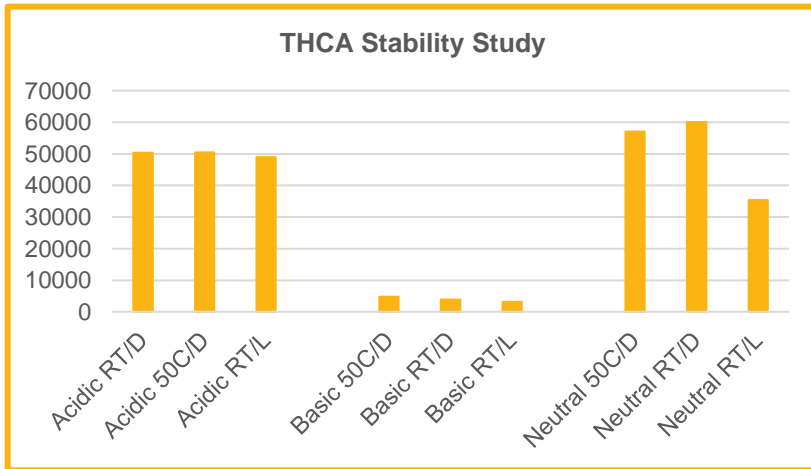
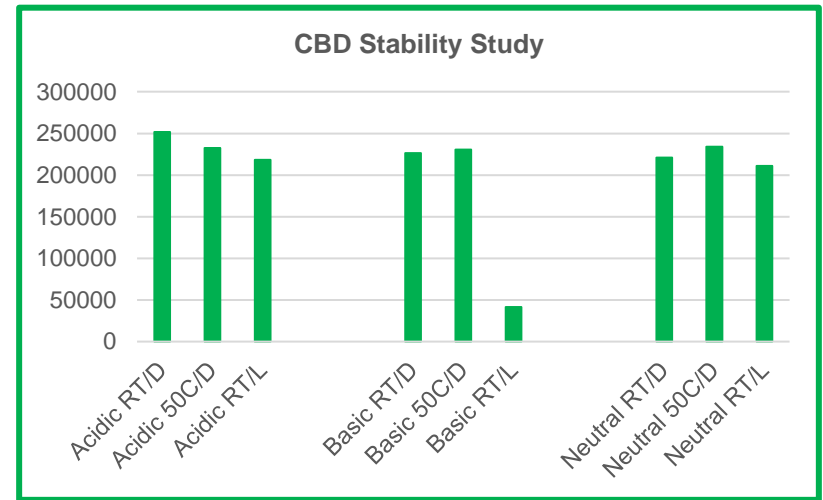
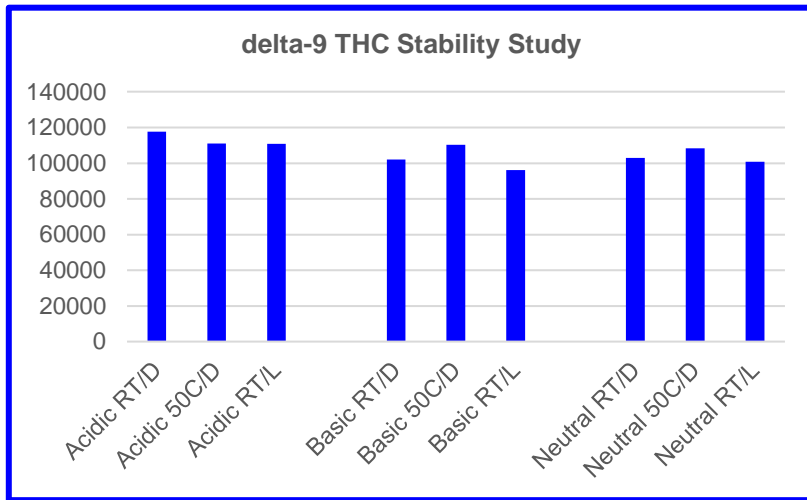
The standard solutions are expensive, but only have 3 days stability indicated in AOAC method, .

It's very important to understand the stability information for these cannabinoids.

Analyte and IS	Abbreviation
Cannabidivarinic acid	CBDVA
Cannabidivarin	CBDV
Cannabidiolic acid	CBDA
Tetrahydrocannabinolic Acid	THCA
Cannabigerolic acid	CBGA
Cannabigerol	CBG
Cannabidiol	CBD
Tetrahydrocannabivarin	THCV
Tetrahydrocannabivarinic acid	THCVA
Cannabinol	CBN
Cannabinolic Acid	CBNA
Delta-9-Tetrahydrocannabinol	delta9-THC
Delta-8-Tetrahydrocannabinol	delta8-THC
Cannabicyclol	CBL
Cannabichromene	CBC
Cannabichromenic Acid	CBCA
Cannabidiol-D ₃ (IS)	CBD-d3
Cannabinol-D ₃ (IS)	CBN-d3
Delta-9-Tetrahydrocannabinol-D3 (IS)	delta9-THC-d3



Examples: Case II---*CBD Stability Study*



Examples: Case II---*CBD Stability Study*

Sample preparation is in amber container under yellow light.

The optimized solvent is acidic.

Sample is sonicated at room temperature for 15 mins.

Concentration	Storage Temperature	Storage Container	Stability (days)
Standard (50.0 ug/mL)	-20 °C	Amber/Glass	149
Standard (20-2000 ng/mL)	1 °C-8 °C	Amber/Glass	4
Extract 1 (20-2000 ng/mL)	1 °C-8 °C	Amber/Glass	9
Extract 2 (20-2000 ng/mL)	1 °C-8 °C	Amber/Glass	4

Conclusions

With the best practices the analyte stability could be comprehensively evaluated.

The information of analyte stability can provide the optimized storage conditions for original sample and extracted sample.

The information of analyte stability can provide the optimized sample preparation conditions.

The quantitative method is more accurate and precise with comprehensively understanding the analyte stability



Questions?

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