WELCOME

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Evaluation of Cannabinoids Reference Standards

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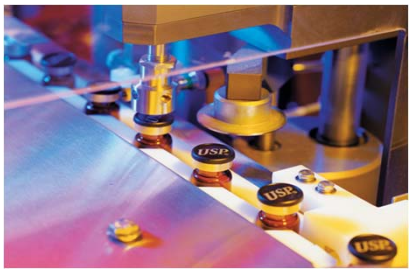
2

USP Reference Standards Development

Reference Standards Evaluation

USP Reference Standards

- **Highly characterized specimens of**
  - Drug substances
  - Excipients
  - Impurities
  - Biologics
  - Food Ingredients
  - Dietary Supplements
  - Compendial Reagents
  - Performance Test Tablets



- **Rigorously tested within USP Labs, Industry, and Government Labs**
- **Intended for use in Compendial Methods**
- **Users are responsible for determining the suitability of use for non-USP compendial use**

4

USP Reference Standards History

- **USP Reference Standards History**
  - USP X–1926: First mention of future availability
  - USP XI–1936: First list of USP Reference Standards (6 standards)
  - USP has provided Reference Standards since 1936
  - Over 80 years of history and experience
- **Less Than 200 in 1965 to More Than 3900 in 2020**
- **Several 100s are at various stages of development**

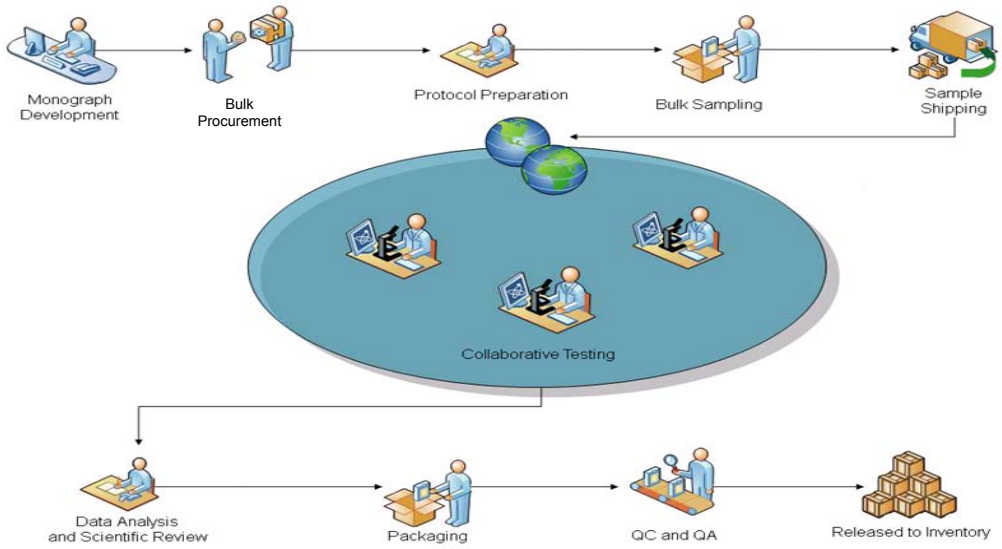
5

Types of USP Reference Standards

- **Quantitative**
  - Assay - Generally > 99.5 % HPLC Purity
  - Impurities - Generally > 98 % HPLC Purity
- **Qualitative**
  - ID, Resolution, peak identification
- **Special category**
  - Melting point, particle size, dissolution verification tablets
- **Non USP Compendial Use**
  - Not required for use in compedial methods
  - Service to industry

6

Reference Standards Development Overview



7

Reference Standard Testing Methods

- **Test Methods Typically Employed**
  - Appearance
  - IR, Raman, UV/Vis
  - NMR (qNMR)
  - MS
  - Elemental Analysis (CHN)
  - Thermal (TGA, DSC)
  - Melting point/range
  - DVS
  - XRPD
  - Chromatography (HPLC, GC, TLC)
  - Water (KF)
  - Residual Solvents (GC-HS)
  - Volatiles (LOD)
  - ROI / Sulfated Ash
  - ICP
  - Titration
  - Other techniques as needed

8

Summary

- USP Reference Standards are thoroughly characterized materials for use in Compendial Methods
- Developed for Quantitative, Qualitative, Special Category and Non-USP Compendial Use
- Processes based on Quality Systems (Manuals, SOPs) and Quality Assurance oversight
- Undergo collaborative testing to determine the suitability as Reference Standards
- Reference Standards undergo Periodic Suitability testing for continued use

9

Cannabis Reference Standards

Cannabis Inflorescence for Medical Purposes – USP Considerations for Quality Attributes, Journal of Natural Products, ***2020****, 83,* 1334-1351.

Analysis methods are provided in the *Supplemental Information*.

- Types of cannabis inflorescence can be identified by macro- and micro-scopic characteristics in their *native* form.
- HPTLC, HPLC and GC methods are provided for identifying *processed* cannabis profile and quantifying its constituents. USP provide 5 RSs to support these analysis procedures, 3 are for quantitative assay standard and 2 for qualitative peak ID uses.

10

Cannabis Reference Standards

#1651621 Delta-9-Tetrahydrocannabinol (Δ^9-THC, or Dronabinol)

#1089149 Cannabidiol (CBD)

#1089161 Cannabidiol Solution

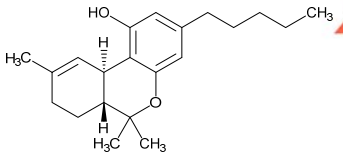
#1089172 Cannabinoid Acids Mixture

#1089183 Cannabinoids Mixture

- Internal standard can be use in place of USP standards for quantification purpose (using conversion factor or RRF). See detail description in publication and supplemental information.
- 2 Mixtures RSs are recommended by *Cannabis Expert Panel* which covers major constituents of interests.

11

1651621 Delta-9-Tetrahydrocannabinol (Δ^9-THC)



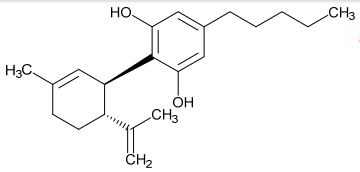
- Current Lot: R045H0
- Monograph uses:

Monograph	Compendium	ID	Assay	Related Compounds	Dissolution	Uniformity of Dosage Units
Dronabinol	USP 42/NF 37	IR, TLC	HPLC	HPLC	--	--
Dronabinol Capsules	USP 42/NF 37	HPLC	HPLC	--	HPLC	HPLC

- ID methods: IR(197F), NMR(1D & 2D), MS, TLC, HPLC
- Assigned value: 0.995 mg/mL in Methanol (Assay against previous lot)

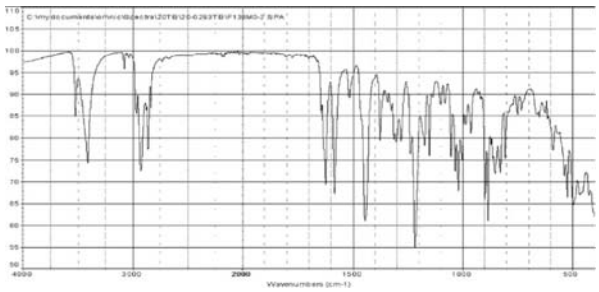
12

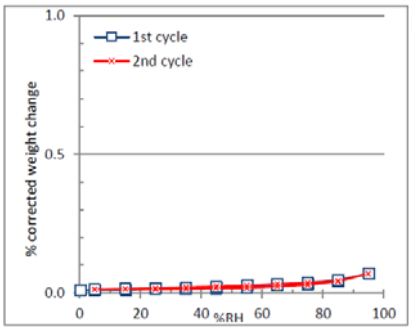
1089149 Cannabidiol (CBD)

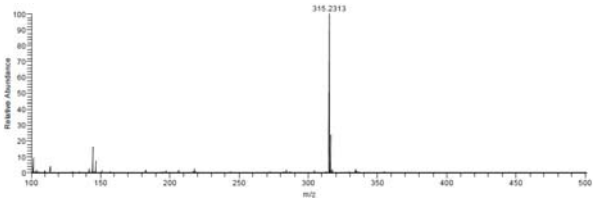


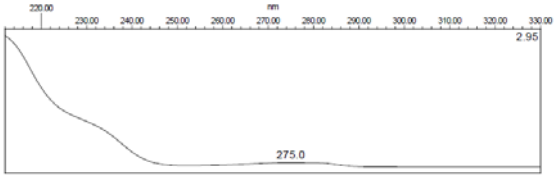
- Current Lot: F138M0
- Developed as a service in response to recent industry interest
- Quantitative and Qualitative uses (assay, peak ID, resolution probe) in HPLC and GC
- ID methods: IR(197A), NMR(1D & 2D), MS, EA, HPLC
- Assigned value: 1.00 mg/mg (by Mass balance from KF, RS, ROI, Chromatography impurities)
- Additional test results: UV (from PDA), Vapor sorption (minimally affected by change of %RH)

1089149 Cannabidiol (CBD)









Elemental Analysis (Collaborators [A])	*Anal. Calcd.* for $C_{21}H_{30}O_2$: C = 80.21%, H = 9.62% Found: C = 80.05%, H = 9.58% (as is basis)

14

1089149 Cannabidiol (CBD)

Assigned value by Mass balance calculation

Analyte/Test *Test Reference*	Reported As (units)	Collaborator Test Result				
		[A]	[A]	[A2]	[D]	Average
Water, (KF) <921>	%w/w	0.01	0.03	0.01	--	0.02
Residual Solvents, (RS) <467>	%w/w	NR	NR	NR	--	0.00
Residue on Ignition, (ROI) <281>	%w/w	0.03	0.02	0.00	--	0.02
Chromatographic impurities, (T) *Journal of Natural Products*	%TDA	0.00	--	0.02	0.00	0.01

=[(100.0 - ∑%TDA) ÷ 100] × [(100.0 – ∑%w/w) ÷ 100]
=[(100.0 - T) ÷ 100] × [(100.0 – (KF + RS + ROI)) ÷ 100]
=[(100.0 – 0.01) ÷ 100] × [(100.0 – (0.02 + 0.00 + 0.02)) ÷ 100] = 0.9999 x 0.9996
= 0.9995

1.00 mg of cannabidiol ($C_{21}H_{30}O_2$) per mg of material on the as is basis

NR: non-reportable amount of isooctane was found and use 0.00 for calculation purpose.

15

1089161 Cannabidiol Solution

- Current Lot: F136F0
- Developed as a service in response to recent industry interest.
- Quantitative and Quantitative uses (assay, peak ID, resolution probe) in HPLC and GC
- ID methods: HPLC, LC-MS (MS^2)
- Assigned value: 1.02 mg/mg in Methanol (Assay against #1089149 CBD)
- Additional test results: UV (from PDA)

16

1089183 Cannabinoids Mixture

- Current Lot: F136D0
- Developed as a service in response to recent industry interest
- Qualitative uses (peak ID, resolution probe) in HPTLC, HPLC, GC.
- ID methods: HPLC, LC-MS (compared with commercial available standards)
- Content: following components in 1 mL of Methanol
  - 0.075 mg Delta-9-Tetrahydrocannabinol (Δ^9-THC)
  - 0.025 mg Delta-8-Tetrahydrocannabinol (Δ^8-THC)
  - 0.050 mg Cannabidiol (CBD)
  - 0.025 mg Cannabinol (CBN)
  - 0.025 mg Cannabichromene (CBC)
  - 0.025 mg Cannabigerol (CBG)
  - 0.025 mg Tetrahydrocannabivarin (THCV)
  - 0.025 mg Cannabidivarin (CBDV)
  - ✓Concentrations provided for information only!
- Additional test results: UV (from PDA)

17

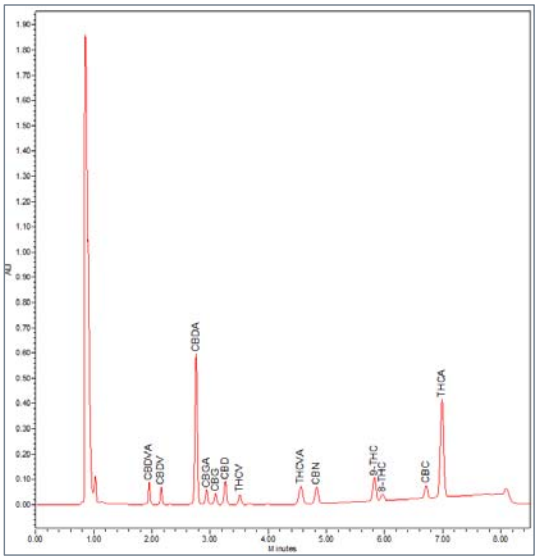
1089172 Cannabinoid Acids Mixture

- Current Lot: F136E0
- Developed as a service in response to recent industry interest.
- Qualitative uses (peak ID, resolution probe) in HPTLC, HPLC, GC
- ID methods: HPLC, LC-MS (compared with commercial available standards)
- Content: following components in 1 mL mixture of acetonitrile (38%) and triethylamine (62%) with 0.15 mg of Ascorbic acid as stabilizer:
  - 0.25 mg Tetrahydrocannabinolic Acid (THCA)
  - 0.25 mg Cannabidiolic Acid (CBDA)
  - 0.050 mg Tetrahydrocannabivarinic Acid (THCVA)
  - 0.025 mg Cannabidivarinic Acid (CBDVA)
  - 0.025 mg Cannabigerolic Acid (CBGA)
  - ✓ Concentrations provided for information only!

18

1089183 Cannabinoids Mixture

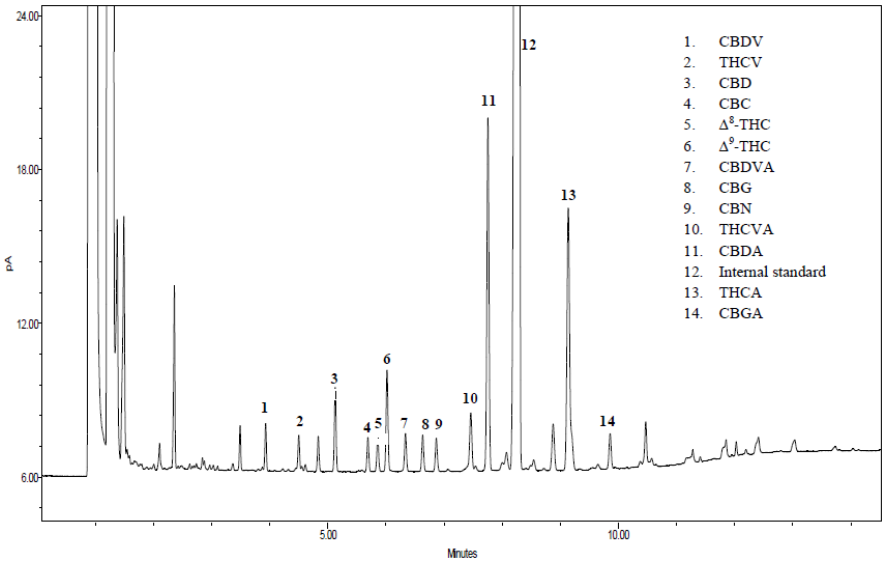
HPLC Typical Chromatogram (Standard solution C)



19

1089183 Cannabinoids Mixture

GC Typical Chromatogram (Standard solution C)



20

Stability of Cannabinoid Acids Mixture

- Supplier indicated Cannabinoid acids solution were not stable even at low temperature.
- Early in-house studies on THCA also confirmed this observation.
- Selection of stabilizer was proposed which including the use of Butylated hydroxyanisole (BHA), Ascorbic acid, Triethylamine (TEA) and combinations.
- The preliminary results showed both Ascorbic acid and TEA significantly improved the stability of THCA.
- Second stage of testing was performed with a combination of TEA and Ascorbic acid since ascorbate anion is the active species of the anti-oxidant property.

Abbreviated stability study of Acids Mixture

- A short term preliminary test (3 and 10 days):
  - THCA (250 µg/mL), THCVA (25 µg/mL), CBDA (250 µg/mL), CBDVA (25 µg/mL), CBGA (25 µg/mL), Ascorbic Acid and Triethylamine (TEA) in Acetonitrile.
  - Prepared in-house and packaged under Argon.
  - Samples stored @ 25° & 60%RH *vs*. -70° freezer (as standard)
- 10 days results shows stability trend of 2~5 >1 > 4 > 3 based on HPLC test

	1	2	3	4	5
Ascorbic Acid (µg/mL)	0	0	150	150	150
TEA (µg/mL)	250	350	0	300	400
Sum of acids* (mM)	1.62	1.62	2.47	2.47	2.47
Excess amount of TEA (mM)	0.86	1.84	--	0.5	1.49

* Include ascorbic acid (a vinylogous carboxylic acid)

Formulation of Cannabinoid Acids Mixture

- Based on the results, amount of TEA is proposed to be about 2 moles *equiv*. in excess of acids. The formulation became a mixture of acetonitrile (38%) and triethylamine (62%), by *v/v*, with 0.15 mg of Ascorbic acid as stabilizer.
- Need to be neutralized with Formic acid before use.
- Long term stability study in progress

QUESTIONS?

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Thank You!

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