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Application Note

Cannabis Potency Testing:
Method Development and Cost Considerations

Overview of key method optimization parameters and a featured low-cost methanol method.

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Introduction

Cannabinoid testing is conducted for a variety of reasons, but always to identify and quantitate various cannabinoids in a source tissue or sample, such as tinctures, essential oils, or foods. This application note discusses common parameters of potency testing that can be problematic to method robustness, and presents a method that addresses these while offering cost-savings over most HPLC methods currently employed in testing laboratories.

Method Requirements

Most current cannabinoid analysis methods for routine testing are by reversed-phase chromatography(RPC) with UV detection, and as such require chromatographic separation of the analytes. As the number of analytes needed to be identified and quantified increases, greater selectivity is required of a method. This often involves fine tuning of method parameters that can include column chemistry, pH, buffer strength, temperature, and composition of organic modifier.

Mobile Phase pH

At least two parameters that can be problematic are pH and temperature. Fundamental good practice for RPC, dictates that the pH of a mobile phase should be well-removed from the pKa of the analytes. This is because if the pH is too close to the analyte pKa, there can be large shifts in the retention of the analyte with only a small change in pH. If the mobile phase isn't buffered well, then there can be very significant local pH shifts within the migrating analyte band. Therefore mobile phase pH should be at least 1 pH unit removed from the closest analyte pKa. In the case of cannabinoids, the analyte pKas are generally between pH 3.5 and 4.

Unfortunately, this is also the approximate pH of dilute formic acid/ammonium formate mobile phases commonly employed for cannabinoid analysis. This is however, ironic, for "good" reason: that is, it is at this pH that critical pair resolutions are observed, despite efforts at method development to keep the pH substantially lower or higher, in order to better control the state of ionization of the analytes. Nevertheless it remains, that in terms of good RPC practice for development of robust methods, this is clearly not ideal.

Column Temperature

It's not uncommon to see optimized methods that indicate temperatures of 30 °C or less. There's nothing inherently problematic with that if the column oven has an integrated cooling unit. Many HPLC column ovens, especially lower-cost units, are configured only with a heating element. Therefore any attempts to control temperature at or near room temperature are problematic. When temperature control at or near such values are critical for resolution, users will often be frustrated with too much retention variation if the ovens aren't equipped with a cooling unit and thus cannot maintain a constant temperature.

Organic Modifier

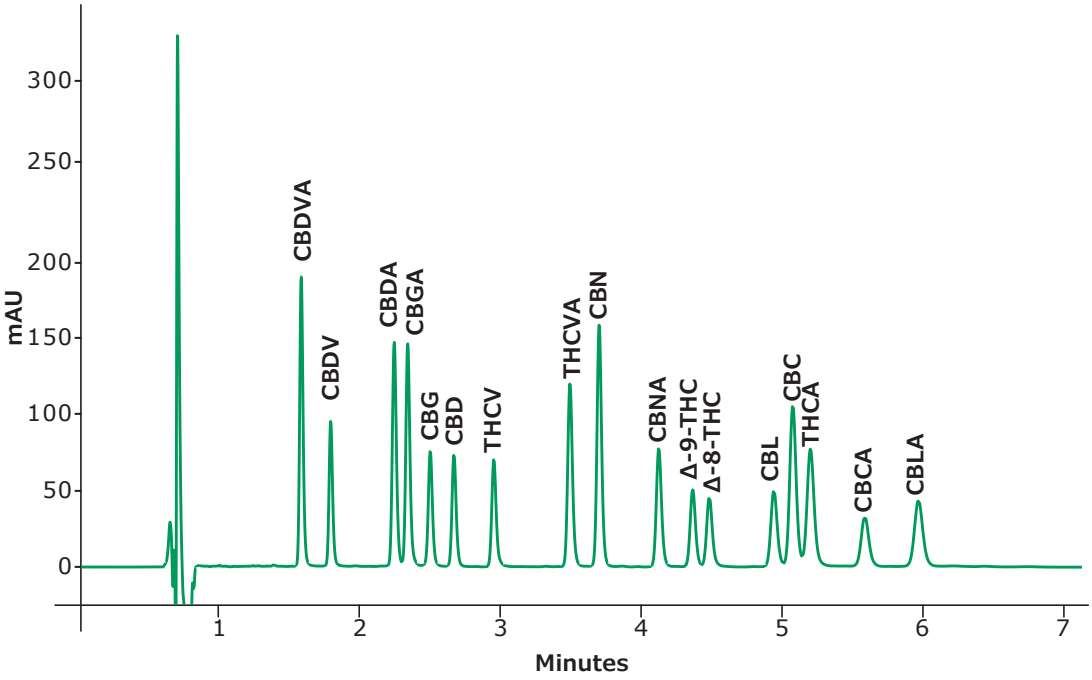
Most often, solutions for RPC resolution of multiple cannabinoids employ acetonitrile as the organic modifier. This is simply because it has permitted quicker and easier solutions for resolution of all sample components. However, the one downfall with acetonitrile, compared to the other common RPC modifier methanol, is cost. Acetonitrile can cost 3 times as much or more.

Case Study – Low Cost Methanol Method

Herein we present an alternate method for analysis of up to 17 cannabinoids that has the following attributes:

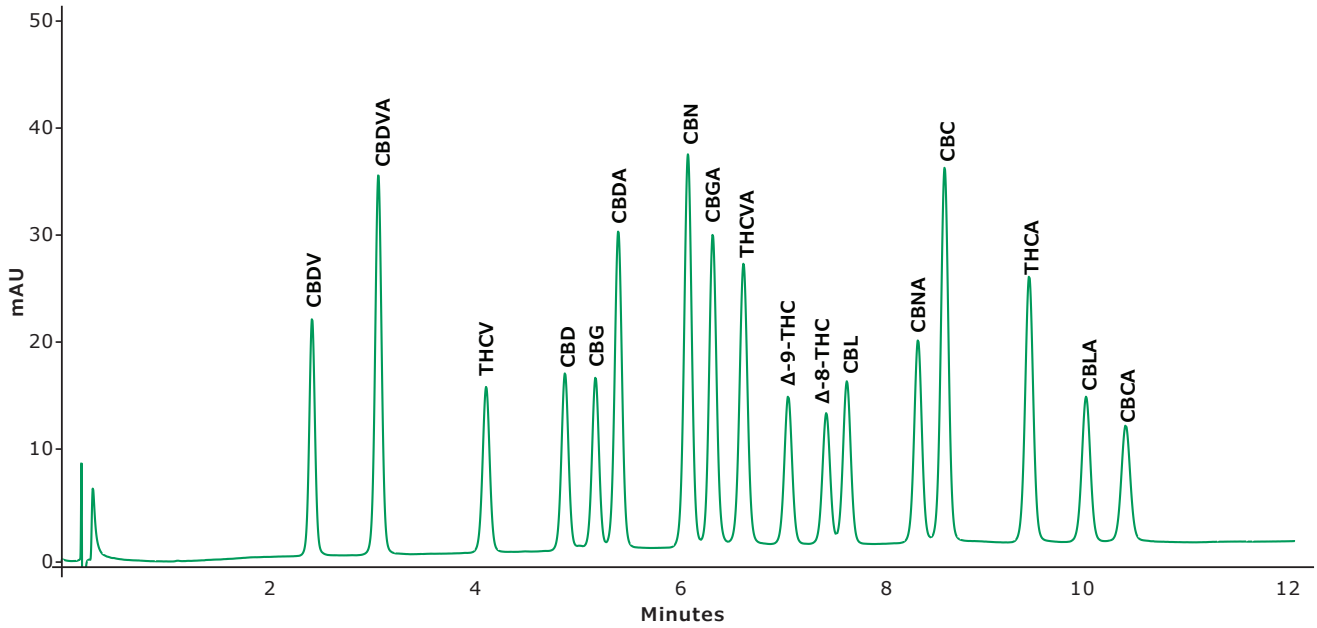
- Mobile phase pH is adequately removed from the analyte pKas (the pH of mobile phase A is ~2.6).
- A higher column temperature is used that can be readily controlled by lower-cost heating-only column ovens
- The organic modifier is methanol, thus potentially lowering costs considerably
- The associated backpressures are such that the method doesn't require use of a UHPLC.

Figure 1. Chromatogram of a typical acetonitrile-based gradient method for potency testing.



Method Conditions	
Column:	Ascentis Express C18, 2.7 µm, 150 x 3 mm (**53816-U**)
Mobile Phase A:	5 mM Ammonium Formate + 0.1% Formic acid in water
Mobile Phase B:	0.1% Formic acid in acetonitrile
Flow:	0.4 mL/min
Gradient:	75% B to 90% B in 2 min, hold at 90% B 5 min
Injection:	3 µL, 25 µg/mL
Column Temp.:	25 °C
Detection:	UV, 228 nm
Max Pressure:	530 bar (7690 psi)

Figure 2. Chromatogram of low-cost methanol-based gradient method for potency testing.



Method Conditions	
Column:	Ascentis Express C8, 2.7 µm, 50 x 2.1 mm (**53831-U**)
Mobile Phase A:	0.1% Formic acid in water
Mobile Phase B:	0.06% Formic acid in methanol
Flow:	0.6 mL/min
Gradient:	60% to 75% B in 8 min; hold at 75% B for 4 min
Injection:	0.5 µL, 25 mg/L
Column Temp.:	45 °C
Detection:	UV, 228 nm
Max Pressure:	270 bar (3920 psi).

Table 1. Example of Calculations for Solvent Consumption and Cost Per Injection

Method	Organic	mL	¢/mL	¢
Gradient	ACN	6.14	10.25	62.94
Gradient MeOH	MeOH	5.31	3.58	19.01

Discussion

In **Figure 1** you see a common method based on use of acetonitrile as the organic modifier. **Figure 2** is the alternate methanol-based method. While the runtime of the latter is increased 70%, it's still a very reasonable length, under 12 min. Even with the added length, the cost calculations (**Table 1**) of the volume of organic solvent consumed is about 3-fold less. As long as the throughput is sufficient, this represents a cost-savings that can be readily realized. For either method, further gains in resolution can be attained by using the smaller particle 2 µm Ascentis Express columns.

Featured and Related Products

	Product Description	Cat. No.
	HPLC Columns	
	Ascentis® Express C8 column, 2.7 µm, 50 x 2.1 mm I.D.	**53831-U**
	Ascentis Express C18, 2.7 µm, 150 x 3 mm I.D.	**53816-U**
	Ascentis® Express C8, 2.7 µm guard cartridge 5 mm × 2.1 mm, pkg of 3 ea	**53509-U**
	Ascentis® Express C18, 2.7 µm guard cartridge 5 mm × 3 mm, pkg of 3 ea	**53504-U**
	Ascentis® Express Guard Cartridge Holder	**53500-U**
	Accessories	
	Certified Vial Kit, Low Adsorption (LA), 2 mL, pk of 100 volume 2 mL, amber glass vial (with marking spot), natural PTFE/silicone septa, thread 9 mm	**29653-U**
	Certified Reference Materials	
	Cannabinoid Mixture (Acids) 6 Component including CBCA, CBDVA, CBDA, CBGA, THCVA, and THCA-A in 1% DIPEA and 0.05% Ascorbic acid in Acetonitrile, each analyte at 500µg/mL, certified reference material, ampule of 1mL, Cerilliant®	**C-218**
	Cannabinoid Mixture (Neutrals) 8 Component including CBG, Cannabinol, CBD, CBDV, (-)-delta8-THC, (-)-delta9-THC, and THCV in Acetonitrile, each analyte at 500µg/mL, certified reference material, ampule of 1mL, Cerilliant®	**C-219**
	Cannabidivarinic Acid (CBDVA) solution 1.0 mg/mL in acetonitrile, certified reference material, ampule of 1 mL, Cerilliant®	**C-152**
	1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**C-140**
	Cannabidiolic acid (CBDA), 1.0 mg/mL in acetonitrile, ampule of 1 mL, certified reference material, Cerilliant®	**C-144**
	Cannabigerolic acid (CBGA), 1.0 mg/mL in acetonitrile, ampule of 1 mL, certified reference material, Cerilliant®	**C-142**
	Cannabigerol (CBG), 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**C-141**
	Cannabidiol solution, 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**C-045**
	Tetrahydrocannabivarin (THCV), 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**T-094**
	Tetrahydrocannabivarinic acid (THCVA), 1.0 mg/mL in acetonitrile, certified reference material, ampule of 1 mL, Cerilliant®	**T-111**
	Cannabinol (CBN), 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**C-046**
	Cannabinolic acid (CBNA), 1.0 mg/mL in acetonitrile, certified reference material, ampule of 1 mL, Cerilliant®	**C-153**
	Δ9-tetrahydrocannabinol (Δ9-THC), 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**T-005**
	Δ8-tetrahydrocannabinol (Δ8-THC), 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**T-032**
	Cannabicyclol (CBL), 1.0 mg/mL in acetonitrile, certified reference material, ampule of 1 mL, Cerilliant®	**C-154**
	Cannabichromene (CBC), 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**C-143**
	Δ9-tetrahydrocannabinolic acid (THCA), 1.0 mg/mL in acetonitrile, ampule of 1 mL, certified reference material, Cerilliant®	**T-093**
	Cannabichromenic acid (CBCA), 1.0 mg/mL in acetonitrile, certified reference material, ampule of 1 mL, Cerilliant®	**C-150**
	Cannabicyclolic acid (CBLA), 0.5 mg/mL in acetonitrile, certified reference material, ampule of 1 mL, Cerilliant®	**C-171**
	Water, Solvents and Chemicals	
	Methanol, UHPLC grade, Sigma Aldrich	**900688**
	Formic acid 98% - 100%, for LC-MS, LiChropur™	**5330020050**
	Acetonitrile with 0.1 % (v/v) formic acid for UHPLC, Sigma Aldrich	**900686**
	Ammonium formate ≥99.0%, for LC-MS, LiChropur™	**70221**
	Ultrapure water from Milli-Q® system or bottled water	**Milli-Q® IQ 7005 or 101262**

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