

APPLICATIONS

12 Cannabinoids for Potency Testing in Two Methods with Alternate Elution Orders by LC-UV

Scott Krepich and Jeff Layne
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA



Scott Krepich
Senior Field Application
Scientist

Scott enjoys surfing and eating. He is crazy about chromatography, because his mom is really into CSI and thinks that is what he does.

Introduction

While legalization of medical and recreational marijuana is proliferating through more and more states, potency testing needs are expanding as the amounts of each particular cannabinoid in a sample will impact its indication, evident by the emergence of purified, isolated cannabinoids for custom formulation.

Here we demonstrate 2 separate methods capable of quantifying 12 major cannabinoids of interest with different elution orders using methanol as the organic mobile phase in one and acetonitrile in the other. Methods with different elution orders can be advantageous when testing samples with different levels and ratios of each cannabinoids, where a large amount of one will resolve better if eluted after a neighboring compound in smaller levels.



LC Method Parameters

Methanol Mobile Phase Method

Column: Kinetex[®] 2.6 µm C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4462-AN
Mobile Phase: A: Water with 0.1% Formic acid (**other acids would work as well)
 B: Methanol with 0.1% Formic acid (**other acids would work as well)
 **For the cannabinoids – we do not know how the different acidic modifiers might affect the retention behavior of matrix peaks
Gradient:

Time (min)	B (%)
0	60
10	85

Flow Rate: 0.5 mL/min
Back Pressure: 240 Bar
Temperature: 50 °C
Detection: UV @ 230 nm

Acetonitrile Mobile Phase Method

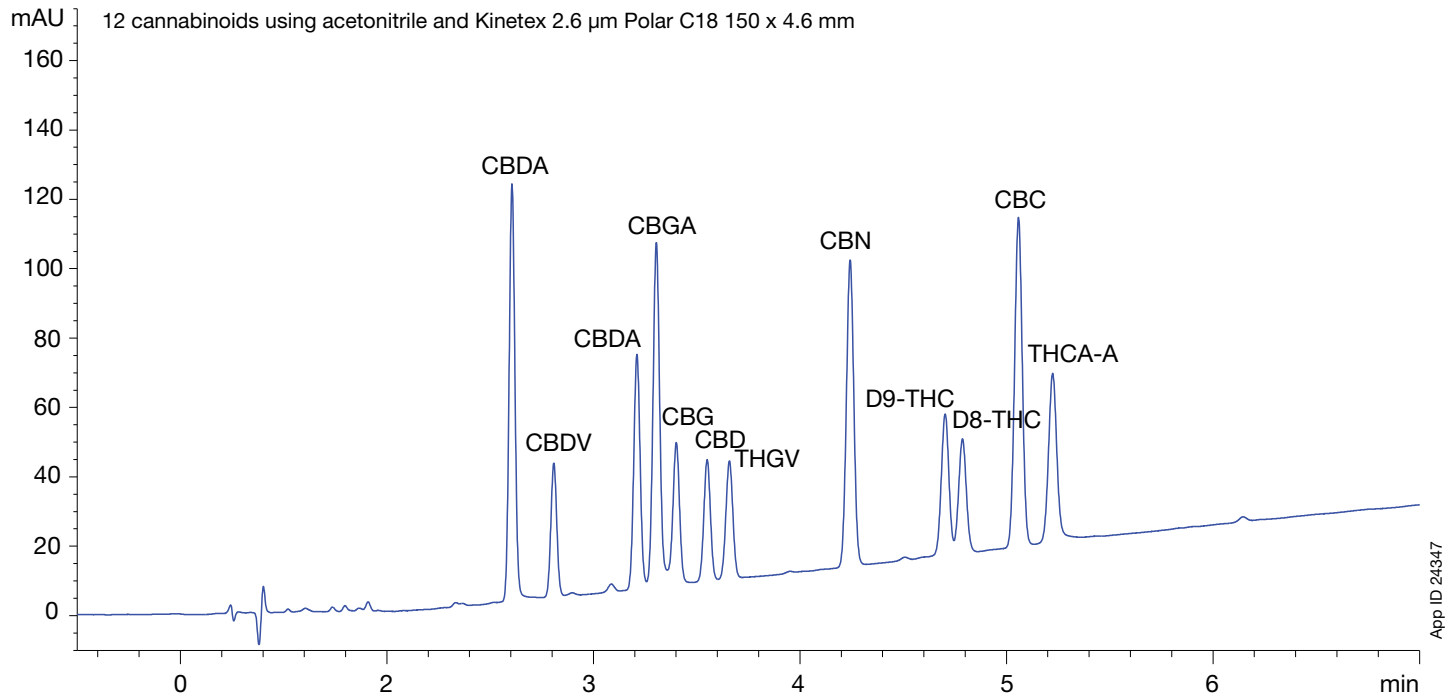
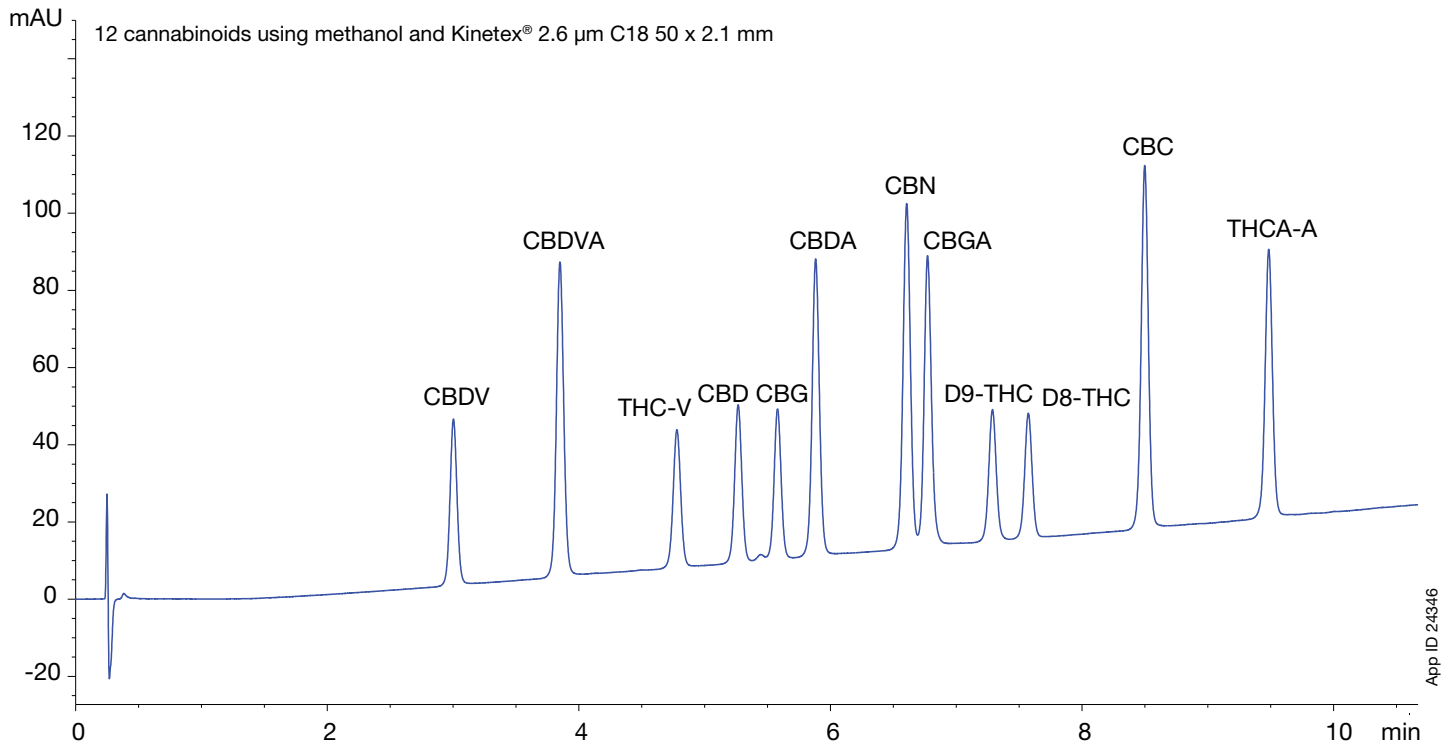
Column: Kinetex 2.6 µm Polar C18
Dimensions: 150 x 4.6 mm
Part No.: 00F-4759-E0
Mobile Phase: A: Water with 0.1% TFA (**0.1% FA, 0.1% H₃PO₄ would work as well)
 B: Acetonitrile with 0.1% TFA (**0.1% FA, 0.1% H₃PO₄ would work as well)
 **For the cannabinoids – we do not know how the different acidic modifiers might affect the retention behavior of matrix peaks
Gradient:

Time (min)	B (%)
0	75
7	100

Flow Rate: 1 mL/min
Back Pressure: 150 Bar
Temperature: 55 °C
Detection: UV @ 230 nm



Figure 1. Representative chromatograms



Results and Discussion

The primary method of 12 cannabinoids in this technical note was focused on the separation using acetonitrile as the strong mobile phase B solvent. Elutropic strength, gradient slope, acid modifier, column temperature, and different C18 column chemistries were among the primary parameters that were explored. Elutropic strength and gradient slope were among the most apparent influencers, with acid modifier, column temperature, flow rate and C18 column chemistries being minor, but potentially significant factors.

Applying these factors directly using methanol as the strong mobile phase B solvent was unsuccessful at first, and it was only until the breakthrough of developing a counter-intuitive method on a shorter column that successful separation of all 12 cannabinoids was revealed. While it's unclear exactly why, it may be that backpressure is a factor that influences separation, and since aqueous-methanol mixtures are much more viscous than aqueous-acetonitrile mixtures, perhaps the shorter column gave better separation with methanol due to backpressure. Some atypical Van Deemter behavior has been reported for these cannabinoids, perhaps due to their hydrophobic and oily nature, disrupting mass transfer in reversed phase conditions. If backpressure is a direct influencer on selectivity, particle size can be an additional parameter to explore beyond instrument platform generalities.

In comparing the final two methods, we can see that the method using methanol on a shorter column is actually a bit longer in runtime, with the last peak eluting at about 9.5 min, compared to about 5.5 min when using acetonitrile on the longer column.

The methanol method may be preferred due to the improved resolution between the peaks, with the critical pair being CBN and CBGA, which have a resolution value to 1.55 under these conditions and on this system (an Agilent 1100 Binary system). Depending upon the system used, better results can be obtained.

Conclusion

While some of the chromatographic parameters influencing cannabinoid selectivity may be contrary to traditional logic, the ability to make dramatic and refined changes can be useful in anticipation of a diverse range of sample matrices containing different interferences and levels of each cannabinoid. Here we've detailed two such solutions with orthogonal selectivity, that also yield opportunities for further exploration on other column chemistries, such as aromatic phenyl phases.

Table 1. Comparison of elution order and retention times when using the acetonitrile method and the methanol method.

	Elution Order MeOH	Elution Order AcCN
CBDV	1	2
CBDVA	2	1
THC-V	3	7
CBD	4	6
CBG	5	5
CBDA	6	3
CBN	7	8
CBGA	8	4
D9-THC	9	9
D8-THC	10	10
CDC	11	11
THCA-A	12	12



APPLICATIONS

Kinetex[®] Core-Shell LC Ordering Information

5 µm Minibore Columns (mm)					SecurityGuard [™] ULTRA Cartridges [‡]
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	00A-4601-AN	00B-4601-AN	00D-4601-AN	00F-4601-AN	AJO-8782 for 2.1 mm ID

5 µm MidBore [™] Columns (mm)				SecurityGuard [™] ULTRA Cartridges [‡]
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
C18	00B-4601-YO	00D-4601-YO	00F-4601-YO	AJO-8775 for 3.0 mm ID

5 µm Analytical Columns (mm)					SecurityGuard [™] ULTRA Cartridges [‡]
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
C18	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJO-8768 for 4.6 mm ID

Australia
t: +61 (0)2-9428-6444
f: +61 (0)2-9428-6445
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
f: +43 (0)1-319-1300
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
f: +1 (310) 328-7768
info@phenomenex.com

China
t: +86 400-606-8099
f: +86 (0)22 2532-1033
phen@agela.com

Denmark
t: +45 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
f: +33 (0)1 30 09 21 11
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
f: +49 (0)6021-58830-11
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
f: +91 (0)40-3012 2411
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
f: +39 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
f: +64 (0)9-4780952
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: +1 (800) 541-HPLC
f: +1 (310) 328-7768
info@phenomenex.com

Spain
t: +34 91-413-8613
f: +34 91-413-2290
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
f: +44 (0)1625-501796
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

**All other countries
Corporate Office USA** 
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

2.6 µm Minibore Columns (mm)						SecurityGuard [™] ULTRA Cartridges [‡]
Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	3/pk
Polar C18	00A-4759-AN	00B-4759-AN	—	00D-4759-AN	00F-4759-AN	AJO-9532
C18	00A-4462-AN	00B-4462-AN	00C-4462-AN	00D-4462-AN	00F-4462-AN	AJO-8782 for 2.1 mm ID

2.6 µm MidBore [™] Columns (mm)						SecurityGuard [™] ULTRA Cartridges [‡]
Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	3/pk
Polar C18	—	00B-4759-YO	—	00D-4759-YO	00F-4759-YO	AJO-9531
C18	00A-4462-YO	00B-4462-YO	00C-4462-YO	00D-4462-YO	00F-4462-YO	AJO-8775 for 3.0 mm ID

2.6 µm Analytical Columns (mm)						SecurityGuard [™] ULTRA Cartridges [‡]
Phases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	3/pk
Polar C18	—	00B-4759-E0	—	00D-4759-E0	00F-4759-E0	AJO-9530
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJO-8768 for 4.6 mm ID

1.7 µm Minibore Columns (mm)					SecurityGuard [™] ULTRA Cartridges [‡]
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	00A-4475-AN	00B-4475-AN	00D-4475-AN	00F-4475-AN	AJO-8782 for 2.1 mm ID

1.7 µm MidBore Columns (mm)				SecurityGuard [™] ULTRA Cartridges [‡]
Phases	30 x 3.0	50 x 3.0	100 x 3.0	3/pk
C18	—	00B-4475-YO	00D-4475-YO	AJO-8775 for 3.0 mm ID

1.3 µm Minibore Columns (mm)		
Phases	30 x 2.1	50 x 2.1
C18	00A-4515-AN	00B-4515-AN

guarantee

If Kinetex analytical columns do not provide at least an equivalent separation as compared to a competing column of the same particle size, similar phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

Terms and Conditions
Subject to Phenomenex Standard Terms and Conditions which may be viewed at www.phenomenex.com/TermsAndConditions.

Trademarks
Kinetex is a registered trademark and SecurityGuard and MidBore are trademarks of Phenomenex.
© 2017 Phenomenex, Inc. All rights reserved.

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com