APPLICATIONS

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Outside of the lab, Laura enjoys spoiling her dog Maggie and subjecting her husband to novel methods of torture, such as endless playlists of sad songs and long walks on the beach to catch Pokémon.

Clean-up and Analysis of 12 Cannabinoids in Whole Blood by LC-MS/MS Using Phree™ Phospholipid Removal Products and a Luna® Omega Polar C18 LC Column

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Overview

In addition to the psychoactive cannabinoid THC, there is growing interest in researching the potential medicinal benefits of the plant's other major cannabinoids which are non-psychoactive. This work presents a LC-MS/MS method for analysis of 12 cannabinoids and 2 metabolites from whole blood. Cannabinoids are very hydrophobic compounds, which elute late in reversed phase chromatography. Since phospholipids also elute in this region, matrix effects/ion suppression can be a concern. Through a simple sample preparation technique utilizing Phree Phospholipid Removal Products, removal of phospholipids, reduced matrix effects, and good recovery were achieved while avoiding the added inconvenience of a dry down step.

Materials

Reference standards and deuterated internal standards were purchased from Cerilliant® Corporation (Round Rock, TX). Additional reference standards were purchased from Restek® Corporation (Bellefonte, PA). Pooled Na_2EDTA human whole blood was purchased from BioreclamationIVT® (Westbury, NY). Ammonium formate and formic acid were purchased from Sigma-Aldrich® (St. Louis, MO). HPLC grade methanol and acetonitrile were purchased from Honeywell® (Morris Plains, NJ). Purified water was obtained using a Sartorius® arium® comfort II filtration system (Göttingen, Germany).

Experimental Conditions

Whole blood samples were prepared by protein precipitation with and without phospholipid removal. Protein precipitation samples were prepared by slowly adding 1000 µL of chilled acetonitrile with internal standard to 250 µL of whole blood while vortexing, followed by 10 minutes of centrifugation at 13,500 rpm. The supernatant was then analyzed and compared with phospholipid removal samples. Analyte recovery was tested at 100 ng/ mL as the ratio of the mean peak area of pre-extraction spiked whole blood samples divided by the mean peak area of post-extraction spiked whole blood samples (N=3 for both sets).

Phospholipid Removal Protocol

Cartridge:	Phree Phospholipid Removal, 1 mL Tube
Part No.:	8B-S133-TAK
Add:	1000 µL of chilled acetonitrile slowly with 20 µL of internal standard to 250 µL of whole blood while vortexing
Centrifuge:	Sample for 10 minutes at 13,500 rpm
Transfer:	Supernatant and add 15 µL concentrated Formic acid
Load:	Onto Phree Cartridge
Apply:	Vacuum and collect eluate for analysis

LC Method Conditions

Column:	Luna Omega 3 µm Polar C18
Dimensions:	50 x 2.1 mm
Part No.:	00B-4760-AN
Mobile Phase:	A: 10 mM Ammonium Formate in Water B: Methanol
Gradient:	**Time (min) % B** 0 50 5 95 7 95 7.1 50 10 50
Flow Rate:	0.4 mL/min
Injection Volume:	10 µL
Col. Temperature:	40 °C
System Backpressure:	~ 150 bar
Instrument:	Agilent® 1260 HPLC + SCIEX Triple Quad™ MS/MS

MS Method Conditions

Cannabinoids MRM Parameters:

Q1	Q3 (Quant)	Q3 (Qual)	ID	DP	EP	CE (Quant)	CE (Qual)	CXP
285.2	216.9	150.9	Cannabidivarin (CBDV)	-100	-10	-30	-24	-13
285.2	217	257	Tetrahydrocannabivarin (THCV)	-111	-10	-31	-33	-13
309.2	279.1	222.1	Cannabinol (CBN)	-120	-10	-40	-55	-13
313.2	191	203	Cannabichromene (CBC)	-110	-10	-27	-32	-13
313.2	179	245.1	Cannabidiol (CBD)	-106	-10	-26	-30	-13
313.2	245.1	191.1	delta-8-THC	-120	-10	-35	-35	-13
313.2	245.1	191.1	delta-9-THC	-130	-10	-35	-36	-13
315.2	191	136	Cannabigerol (CBG)	-119	-10	-31	-36	-13
329.2	311.1	217.1	Cannabidivarinic Acid (CBDVA)	-100	-10	-23	-34	-13
329.2	311.2	268.1	11-Hydroxy-THC (THC-OH)	-105	-10	-24	-36	-13
343.2	298.9	244.9	11-nor-9-carboxy-THC (THC-COOH)	-110	-10	-27	-25	-13
357.2	338.7	338.7	Cannabidiolic Acid (CBDA)	-100	-10	-27	-36	-13
357.2	312.5	312.5	Tetrahydrocannabinolic Acid (THCA-A)	-105	-10	-32	-41	-13
359.2	341.2	315.2	Cannabigerolic Acid (CBGA)	-110	-10	-26	-28	-13
316.2	248	NA	delta-9-THC-D3	-115	-10	-36	NA	-13
352.2	307.8	NA	THC-COOH-D9	-96	-10	-27	NA	-13

Phospholipids MRM Parameters:

Q1	Q3	ID	DP	EP	CE	CXP
526.5	184.4	LysoPC-1 d31	50	10	39	10
790.9	184.1	PC-1 d31	50	10	47	10
496.4	184.2	LysoPC-1	50	10	37	10
522.4	184.4	LysoPC-2	50	10	41	10
760.7	184.2	PC-1	50	10	47	10
786.8	184.2	PC-2	50	10	49	10
784	184	PC-4	50	10	49	10
184	184	PL (In-source)	200	10	7	10

MS Settings:

Curtain Gas (CUR)	25.0 psi
Collision Gas (CAD)	7
IonSpray Voltage (IS)	-4500 V
Temperature (TEM)	600 °C
Ion Source Gas 1 (Gas1)	50 psi
Ion Source Gas 2 (Gas2)	50 psi
Instrumentation	SCIEX Triple Quad™ 4500

Revision: 0
PHEN-RUO-00053

Figure 1.
Representative Chromatogram

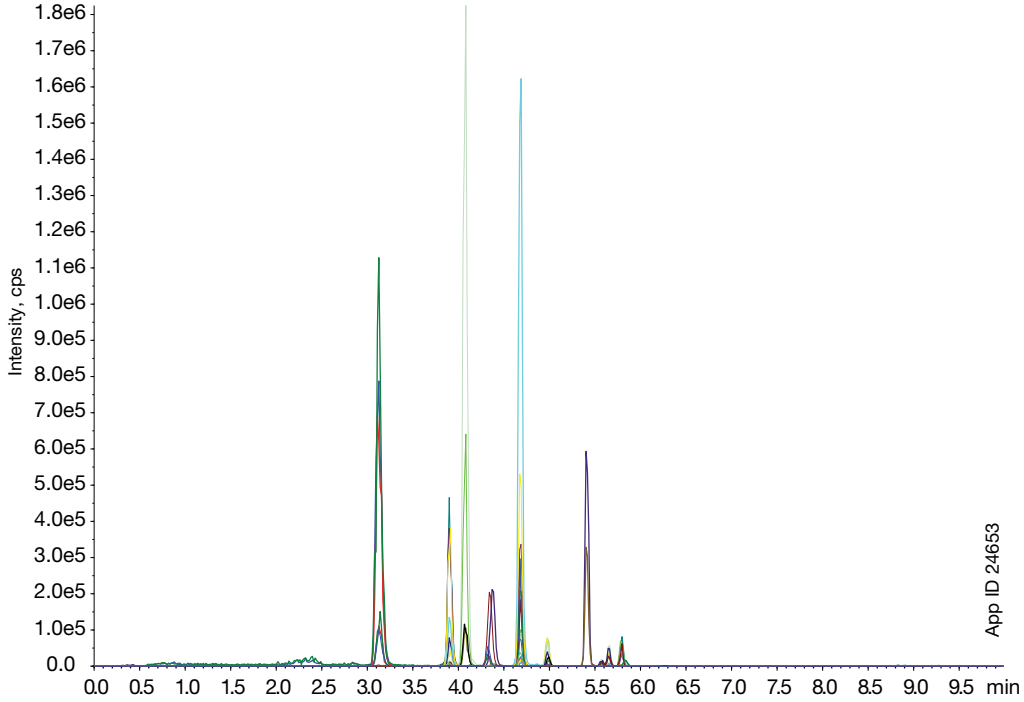
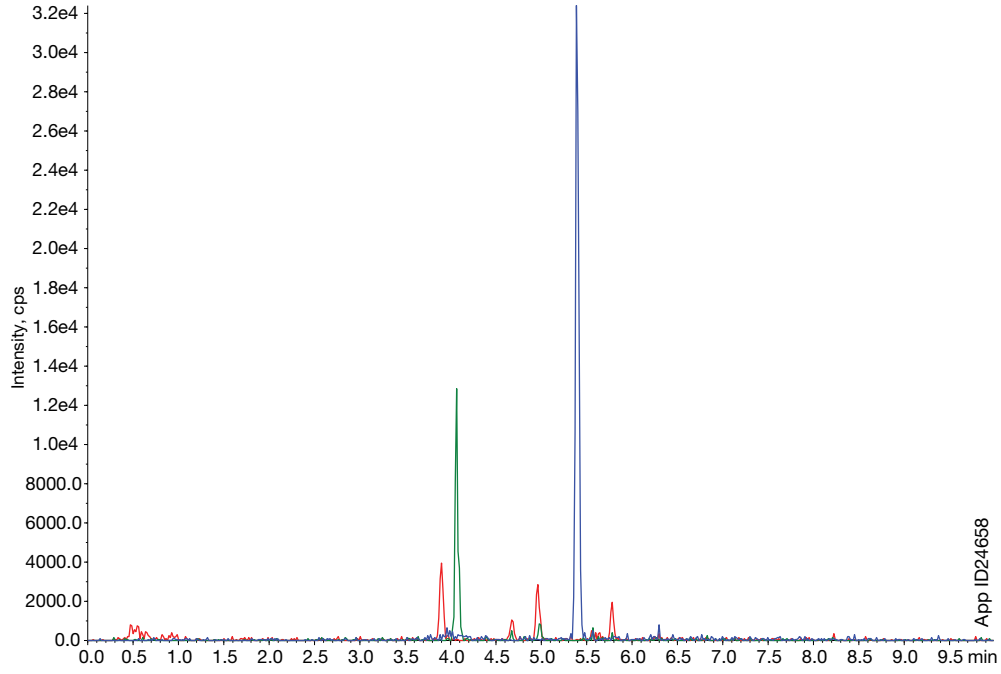


Figure 2.
Extracted chromatogram of CBG, CBD, and CBN, at 5 ng/mL



Resolution of Isobars

Figure 3.
Extracted chromatogram of CBDV, THCV

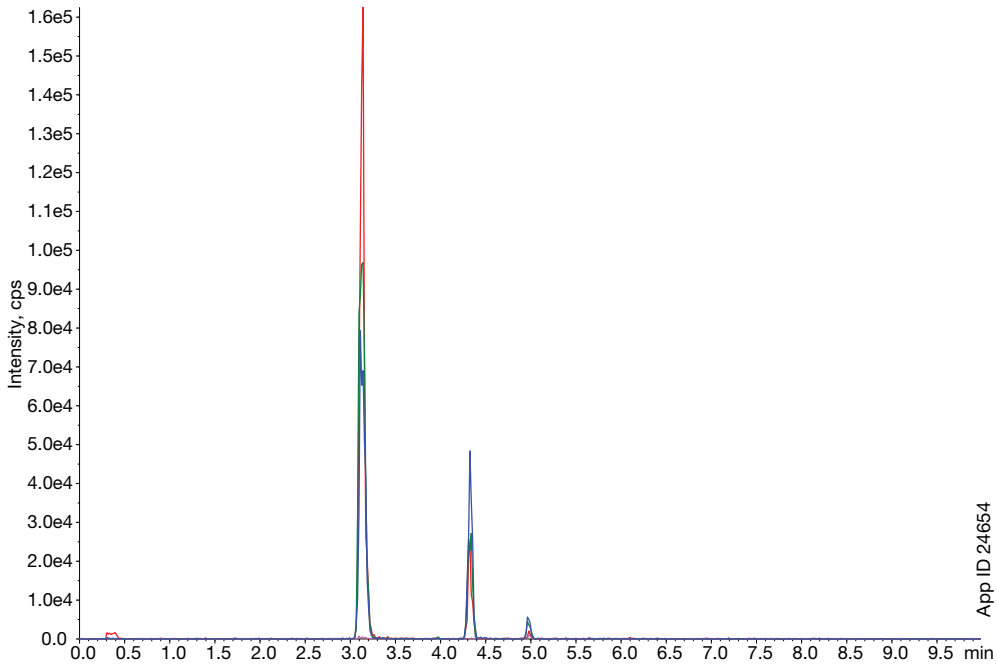


Figure 4.
Extracted chromatogram of CBD, delta-9-THC, delta-8-THC, CBC

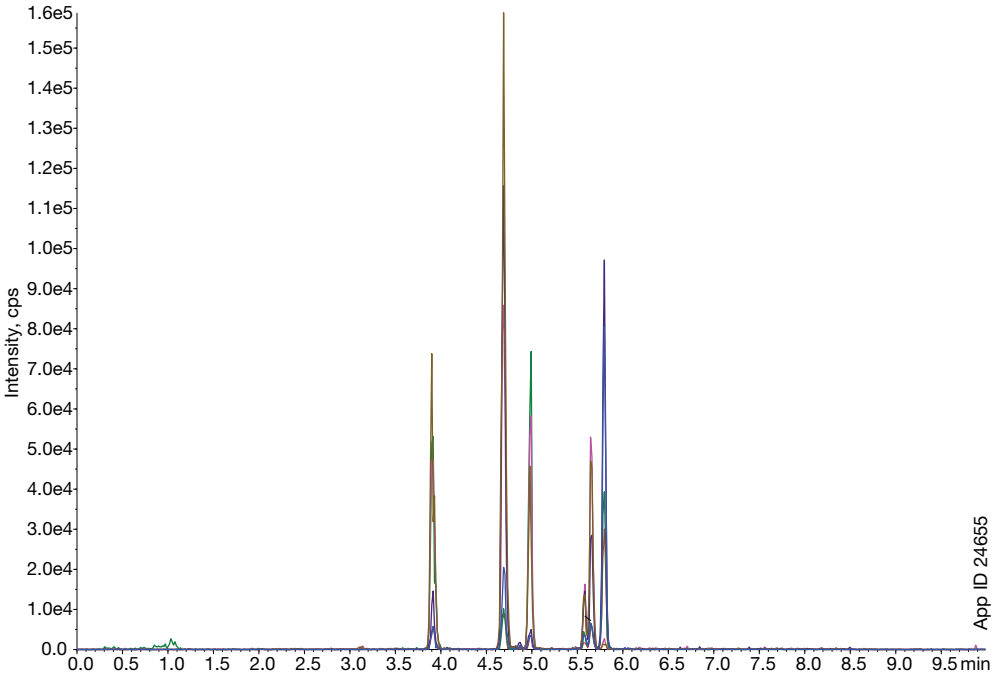


Figure 5.
Extracted chromatogram of CBDVA, THC-OH

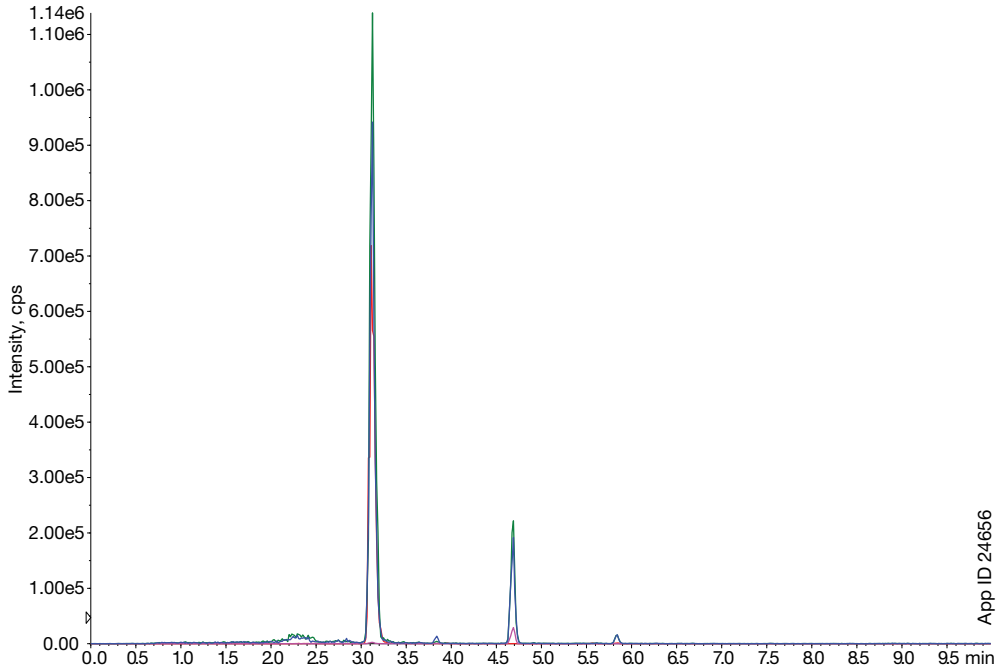


Figure 6.
Extracted chromatogram of CBDA, THCA-A

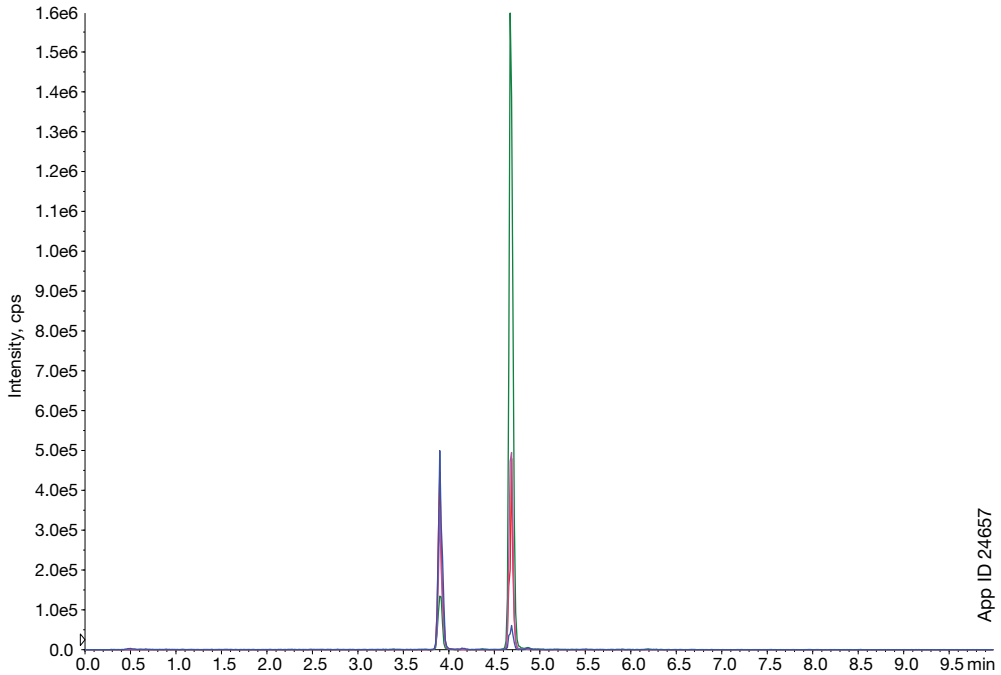


Figure 7.
Phospholipid Content Comparison

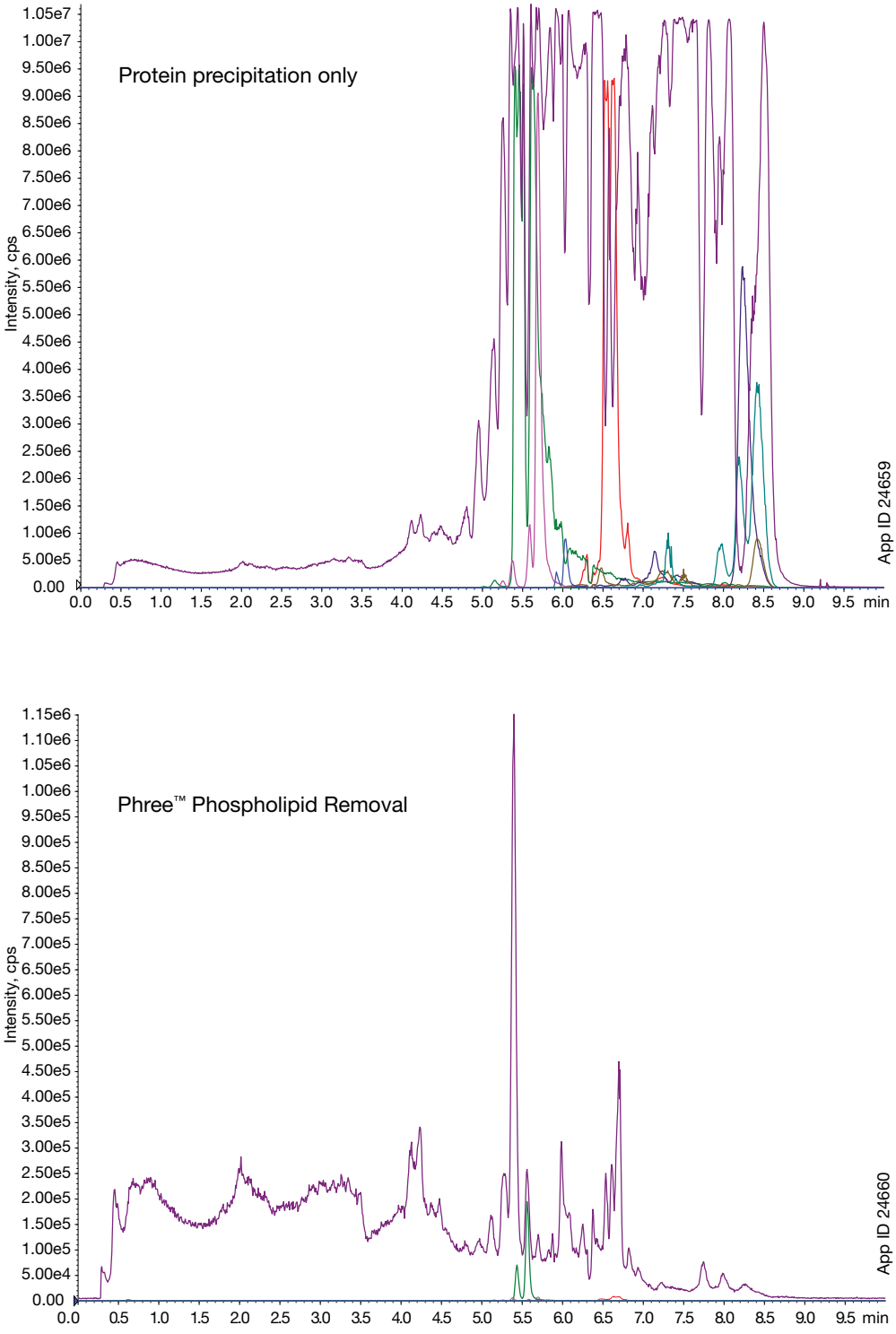


Table 1.
Effect of Phospholipids on Area Ratios

Analyte		Area Ratio for Extracted Sample vs. Neat Standard	
	Retention Time (min)	Protein Precipitation (%)	Phree™ Phospholipid Removal (%)
CBDVA	3.11	132	101
CBDA	3.91	109	98
CBGA	4.07	98	87
CBG	4.08	110	87
CBDV	4.34	100	89
THC-COOH	4.38	91	92
THCA-A	4.68	121	103
THC-OH	4.69	107	99
THCV	4.98	276	90
CBD	4.99	297	106
CBN	5.42	59	91
delta-9-THC	5.57	76	70
delta-8-THC	5.65	227	94
CBC	5.79	225	97

Results and Discussion

12 cannabinoids and 2 metabolites were analyzed in 10 minutes with clean-up using Phree Phospholipid Removal Products coupled with a Luna® Omega Polar C18 column, resolving 4 groups of isobaric species (**Figures 1-6**). Resolution between isobars CBDVA and THC-OH was increased by selecting a less acidic mobile phase A: aqueous 10 mM Ammonium formate rather than 0.1 % Formic acid. The retentive Polar C18 column chemistry produced good peak shape for these compounds in a mostly organic diluent, allowing for direct injection of the eluate and avoiding the need for a dry down step.

Analyte recovery for the extracted samples was comparable between protein precipitation and Phree Phospholipid Removal Products with a 1-13 % difference for all analytes except delta-9-THC, which had a 20 % difference.

A qualitative comparison of phospholipid content using several common MRM transitions shows that protein precipitation leaves behind a large amount of phospholipids which begin eluting around 5 minutes (**Figure 7**). The phospholipid removal cartridge removes the majority of these phospholipids and in addition, the quantitative effects of phospholipids . The analyte to internal standard (**Table 1**). Compared to neat standards, protein precipitation samples were 127- 197 % over the expected area ratios. Phree extracted samples matched the neat standards within ±10 %. The exception was delta-9-THC, which has its own deuterated analog as the internal standard.

Matrix effects for delta-9-THC were 32 % for the protein precipitation samples indicating significant ion suppression while matrix effects for the phospholipid removal sample were only 101% (100% being no matrix effects). By using the deuterated analog delta-9-THC-D3 as internal standard, the area ratios for both preparation methods were brought within ±30% of their expected values demonstrating some normalization of matrix effects. Utilizing delta-9-THC-D3 as an internal standard for CBN, which elutes close to delta-9-THC, only brought protein precipitation samples to 59% of the expected peak area ratio compared to 91% with phospholipids removed (100% being completely normalized). This demonstrates the importance of either removing ion suppressing matrix components through sample preparation or having deuterated analogs of analytes of interest. Since stable isotope-labeled analogs are not yet commercially available for most of the late-eluting compounds, ion suppression may not be accurately normalized by having just a few internal standards and removing phospholipids and reducing matrix effects will be important for accurate quantitation.

Conclusion

The work here demonstrates a LC-MS/MS method for analysis of 12 cannabinoids and 2 metabolites from whole blood. Adding phospholipid removal after protein precipitation significantly reduced matrix effects which is especially important when stable isotope-labeled analogs are not available for each compound. Future work will include determining LODs, comparing positive mode ESI sensitivity, and testing analyte recovery at additional concentrations.

References

1. **A Unified Sample Preparation Procedure for General Unknown Screening (GUS) of Compounds in Whole Blood Samples**

   MSACL 2015 EU: Small Molecules | Thursday 1:30 PM Poster #30
   Seyed Sadjadi[1], Roy Gerona[2], Anita Wen[2], William Zeng[2], Thomas Lin[2], Shahana Huq[1], Sean Orlowicz[1] Francisco
   [1] Phenomenex, Inc.
   [2] University of California, San Francisco

2. **Strategies for the Assessment of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC–MS/MS**

   B. K. Matuszewski,* M. L. M. L. Constanzer, and, C. M. Chavez-Eng Analytical Chemistry 2003 75 (13), 3019-3030 DOI: 10.1021/ac020361s

3. **Analysis of Cannabinoids and Their Metabolites in Human**

   Urine Binnian Wei, Lanqing Wang, and Benjamin C. Blount Analytical Chemistry 2015 87 (20), 10183 10187 DOI: 10.1021/acs.analchem.5b02603

Ordering Information

Luna® Omega LC Column

3 µm Minibore Columns (mm)					SecurityGuard™ Cartridges (mm)
Phases	**30 x 2.1**	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**4 x 2.0***
Polar C18	00A-4760-AN	00B-4760-AN	00D-4760-AN	00F-4760-AN	AJ0-7600
				for ID:	2.0-3.0 mm

3 µm MidBore™ Columns (mm)				SecurityGuard Cartridges (mm)
Phases	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**4 x 2.0***
Polar C18	00B-4760-Y0	00D-4760-Y0	00F-4760-Y0	AJ0-7600
			for ID:	2.0-3.0 mm

3 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 3.0***
Polar C18	00B-4760-E0	00D-4760-E0	00F-4760-E0	00G-4760-E0	AJ0-7601
				for ID:	3.2-8.0 mm

*SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

Phree™ Phospholipid Removal Products

Part No.	Description	Unit
8B-S133-TAK	Phree Phospholipid Removal 1 mL Tube	100/box
8E-S133-TGB	Phree Phospholipid Removal 96-Well Plates	2/box

Accessories

Collection Plates (deep well, polypropylene)		
AH0-7192	96-Well Collection Plate 350 µL/well	50/pk
AH0-7193	96-Well Collection Plate 1 mL/well	50/pk
AH0-7194	96-Well Collection Plate 2 mL/well	50/pk
AH0-8635	96-Well Collection Plate, 2 mL Square/Round-Conical	50/pk
AH0-8636	96-Well Collection Plate, 2 mL Round/Round, 8 mm	50/pk
AH0-7279	96-Well Collection Plate, 1 mL/well Round, 7 mm	50/pk
Sealing Mats		
AH0-8597	Sealing Mats, Pierceable, 96-Square Well, Silicone	50/pk
AH0-8598	Sealing Mats, Pre-Slit, 96-Square Well, Silicone	50/pk
AH0-8631	Sealing Mats, Pierceable, 96-Round Well 7 mm, Silicone	50/pk
AH0-8632	Sealing Mats, Pre-Slit, 96-Round Well 7 mm, Silicone	50/pk
AH0-8633	Sealing Mats, Pierceable, 96-Round Well 8 mm, Silicone	50/pk
AH0-8634	Sealing Mats, Pre-Slit, 96-Round Well 8 mm, Silicone	50/pk
AH0-7362	Sealing Tape Pad	10/pk
Vacuum Manifolds		
AH0-6023*	SPE 12-Position Vacuum Manifold Set, for tubes	ea
AH0-6024*	SPE 24-Position Vacuum Manifold Set, for tubes	ea
AH0-8950	Strata 96-Well Plate Manifold, Universal with Vacuum Gauge	ea

*Manifolds include: Vacuum-tight glass chamber, vacuum gauge assembly, polypropylene lid with gasket, male and female luers and yellow end plugs, stopcock valves, collection rack assemblies, polypropylene needles, lid support legs. Waste container included with 12-position manifold.

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

APPLICATIONS

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