Vitamin E Acetate: Effective in Sunscreens, But Deadly through Vaping

Guest Author: Scott Krepich, Food and Environmental Application Scientist

While cannabis legalization continues to expand, so has the proliferation of various cannabis related products and delivery modes. However, the regulatory landscape is still murky, and many, if not most, of cannabis and cannabis related products are distributed through the grey or black market. This can be particularly dangerous to the US and Europe, where consumers are accustomed to rigorous safety standards for food, dietary supplements, and cosmetics. This has resulted in some abandoning common sense when it comes to the myriad of diverse edibles, tinctures, topicals, vaginal suppositories, and vaping formulations that have flooded the cannabis consumer market.

Vaping formulations may be among the most dangerous, as our respiratory systems are more fragile than our digestive system, and social acceptance of vaping is vastly exceeding safety studies and testing. This sentiment rang true on September 12th, 2019, when the CDC reported hundreds of incidents of acute respiratory distress syndrome, possibly associated with vaping.

Synthetic vitamin E oil (Tocopheryl acetate), a common component of lotions and sunscreens, is being formulated into many vaping oils as a more cost-effective thickener and is now included as a hazardous component for testing, along with other additives, pesticides, and toxins.

Along with the regulatory issues, another major challenge in cannabis testing are the diverse matrices that may contribute their own unique challenges in an effective analytical testing method. That being said, examining the chemical and physical properties of both the analytes and sample matrices, and extrapolating from existing methodologies, considering the underlying chemistries, can be a fast and effective way to integrate new samples and analytes into an existing workflow.

In the case of tocopheryl acetate in vape oils, I might start with a normal phase LC-UV/Fluorescence method, like the AOAC OMA 2012.10 found in the below technical application “Vitamin A and E Analysis from Infant Formula”.

TN-1190

APPLICATIONS

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Allen Misa

Food Industry Marketing Manager

Allen Misa is a downhill mountain biker who spends his days and weekends either riding off a face of a mountain or bouncing his daughters on his lap.

Vitamin A and E Analysis From Infant Formula Using Luna® 3µm NH_2 per AOAC OMA 2012.10

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Introduction

Millions of parents globally rely on infant formula to provide valuable nutrition for their infant children. Infant formula is supplemented with important nutrients to aid in the healthy development of the infant. Two of the most important nutrients include Vitamins A and E. To increase shelf-life, Vitamin A is typically added as either in the palmitate or acetate form. Vitamin E is typically added unmodified and in the acetate form. As part of the Quality Assurance (QA) process, the FDA requires infant formula manufacturers to quantify Vitamin A and Vitamin E levels in the final product. These compounds are effectively analyzed using HPLC with both FLD and UV detection, preceded by a Liquid-Liquid Extraction (LLE) sample preparation step. Presented is an optimized HPLC method using Luna 3µm NH_2.

Materials and Methods

Reagents and Chemicals

Vitamin A palmitate—Reference standard, Sigma (St. Louis, MO) Cat. No. R3375.
Vitamin A acetate—Reference standard, Sigma Cat. No.46958.
dl-α-Tocopherol acetate—Reference standard, Sigma Cat.No. T3376.
dl-α-Tocopherol—Reference standard, Sigma Cat. No.95240.
tert-butyl methyl ether —Mobile phase, Sigma Cat. 306975-1L.
Hexane —Mobile phase, Burdick & Jackson Cat. AH212-4.

Experimental Conditions

Sample Preparation (Per AOAC OMA 2012.10)

Powder Samples:

- Transfer 25 g accurately weighed into a 250 mL volumetric flask. Dissolve using distilled water (approximately 40 °C) cool and make up to 250 mL with distilled water. Transfer 5 mL reconstituted sample to a 50 mL screw top centrifuge tube.

Ready-To-Feed Samples or Concentrated Liquid Products:

- Transfer 5.0 mL thoroughly agitated sample directly to a 50 mL screw top centrifuge tube. Liquid samples should be analyzed from a freshly opened container stored/refrigerated for no more than 48h, and never analyzed from a frozen sample.

Directly Following Either Powder or Ready-To-Feed Samples:

- Add 5 mL 2% papain solution into the 50 mL screw top centrifuge tube
- Mix to disperse each sample, cap, and place the tubes in a 37 ± 2 °C water bath for 20–25 min. Remove the samples from the bath and cool. Place in a freezer for approximately 5 min or refrigerate for approximately 20 min.
- Add approximately 20 mL acidified methanol to each sample tube and mix.
- Accurately pipet 10.0 mL iso-octane into each sample tube.
- Close tightly to avoid leakage and shake the tube for 10 min preferably with a mechanical shaker.
- Centrifuge for 10 min at 4000 rpm to obtain a clear isooctane layer. Remove enough iso-octane from the centrifuge tube to fill an injection vial. This extract is ready for LC analysis.

Note: Typically, a 50 µL injection volume is used for the standards and sample extracts, but this can be varied (20–100 µL) to suit sensitivity.

LC-UV/LC-FLD Conditions

Column:	Luna 3µm NH_2
Dimensions:	150 x 4.6 mm
Part No.:	00F-4377-E0
Mobile Phase:	A: Hexane B: Hexane with 50% MTBE
Gradient:	**Time (min) % B** 0 0 3 0 8 100 12 100
Flow Rate:	1.0 mL/min
Injection:	50 µL
Temperature:	Ambient
Detection UV:	325 nm
Detection FLD:	280/310 nm (emission/excitation)
Sample:	1. Vitamin A Palmitate isomer 2. Vitamin A Palmitate 3. Vitamin A Acetate isomer 4. Vitamin A Acetate 5. Vitamin E Acetate 6. Vitamin E

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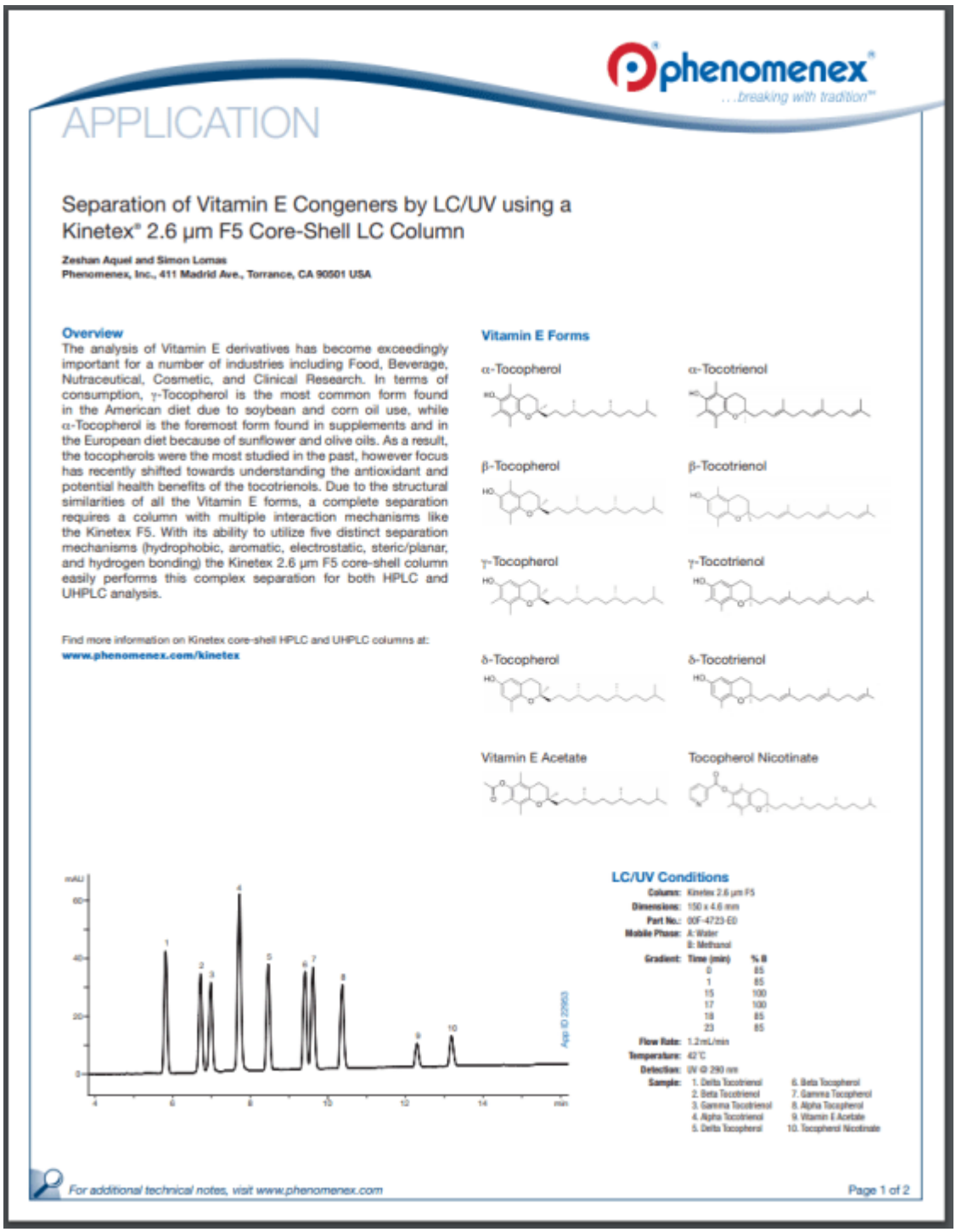
Or, since this is a hydrophobic, fat soluble vitamin, a high organic reversed phase LC-UV

method may also be effective, and could likely be integrated into an existing potency methodology, in anticipation of it eluting in a similar chromatographic region. See a similar analysis in the following technical applications “Separation of Vitamin E Congeners by LC/UV” and “16 Cannabinoids for Potency Testing by Practical LC-UV”.



APPLICATION

Separation of Vitamin E Congeners by LC/UV using a Kinetex® 2.6 µm F5 Core-Shell LC Column

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Overview

The analysis of Vitamin E derivatives has become exceedingly important for a number of industries including Food, Beverage, Nutraceutical, Cosmetic, and Clinical Research. In terms of consumption, γ-Tocopherol is the most common form found in the American diet due to soybean and corn oil use, while α-Tocopherol is the foremost form found in supplements and in the European diet because of sunflower and olive oils. As a result, the tocopherols were the most studied in the past, however focus has recently shifted towards understanding the antioxidant and potential health benefits of the tocotrienols. Due to the structural similarities of all the Vitamin E forms, a complete separation requires a column with multiple interaction mechanisms like the Kinetex F5. With its ability to utilize five distinct separation mechanisms (hydrophobic, aromatic, electrostatic, steric/planar, and hydrogen bonding) the Kinetex 2.6 µm F5 core-shell column easily performs this complex separation for both HPLC and UHPLC analysis.

Find more information on Kinetex core-shell HPLC and UHPLC columns at:
www.phenomenex.com/kinetex

Vitamin E Forms

α-Tocopherol
α-Tocotrienol
β-Tocopherol
β-Tocotrienol
γ-Tocopherol
γ-Tocotrienol
δ-Tocopherol
δ-Tocotrienol
Vitamin E Acetate
Tocopherol Nicotinate

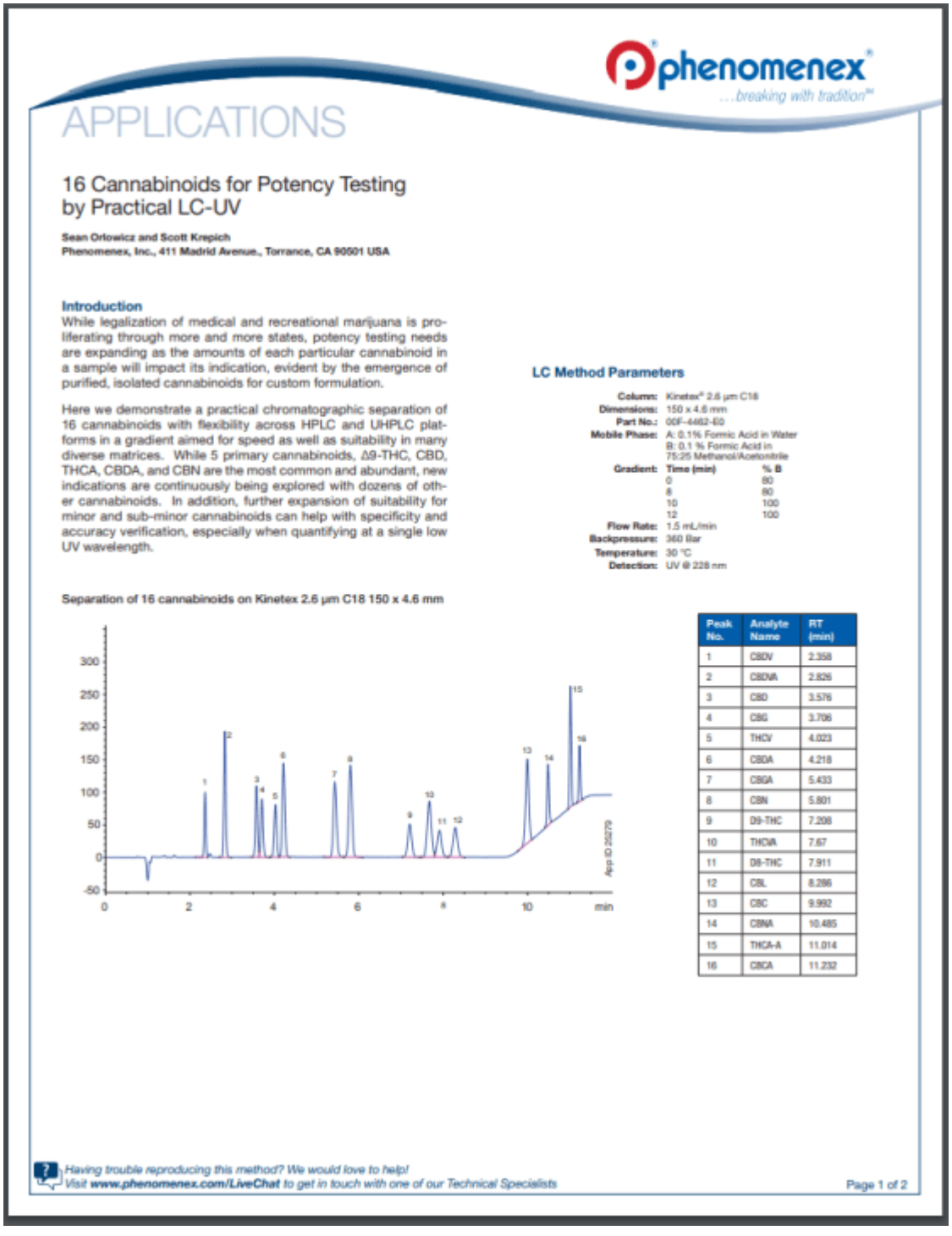
LC/UV Conditions

Column: Kinetex 2.6 µm F5
Dimensions: 150 x 4.6 mm
Part No.: 00F-4723-E0
Mobile Phase: A: Water
B: Methanol

Gradient:

Time (min)	% B
0	85
1	85
15	100
17	100
18	85
23	85

Flow Rate: 1.2mL/min
Temperature: 42°C
Detection: UV @ 290 nm
Sample:
1. Delta Tocotrienol
2. Beta Tocotrienol
3. Gamma Tocotrienol
4. Alpha Tocotrienol
5. Delta Tocopherol
6. Beta Tocopherol
7. Gamma Tocopherol
8. Alpha Tocopherol
9. Vitamin E Acetate
10. Tocopherol Nicotinate



APPLICATIONS

16 Cannabinoids for Potency Testing by Practical LC-UV

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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 a practical chromatographic separation of 16 cannabinoids with flexibility across HPLC and UHPLC platforms in a gradient aimed for speed as well as suitability in many diverse matrices. While 5 primary cannabinoids, Δ9-THC, CBD, THCA, CBDA, and CBN are the most common and abundant, new indications are continuously being explored with dozens of other cannabinoids. In addition, further expansion of suitability for minor and sub-minor cannabinoids can help with specificity and accuracy verification, especially when quantifying at a single low UV wavelength.

LC Method Parameters

Column: Kinetex® 2.6 µm C18
Dimensions: 150 x 4.6 mm
Part No.: 00F-4462-E0
Mobile Phase: A: 0.1% Formic Acid in Water
B: 0.1 % Formic Acid in 75:25 Methanol/Acetonitrile
Gradient:

Time (min)	% B
0	80
8	80
10	100
12	100

Flow Rate: 1.5 mL/min
Backpressure: 360 Bar
Temperature: 30 °C
Detection: UV @ 228 nm

Separation of 16 cannabinoids on Kinetex 2.6 µm C18 150 x 4.6 mm

Peak No.	Analyte Name	RT (min)
1	CBDV	2.358
2	CBDVA	2.826
3	CBD	3.576
4	CBG	3.706
5	THCV	4.023
6	CBDA	4.218
7	CBGA	5.433
8	CBN	5.801
9	D9-THC	7.208
10	THCVA	7.67
11	D8-THC	7.911
12	CBL	8.286
13	CBC	9.992
14	CBNA	10.485
15	THCA-A	11.014
16	CBCA	11.232

Having trouble reproducing this method? We would love to help!
Visit www.phenomenex.com/LiveChat to get in touch with one of our Technical Specialists

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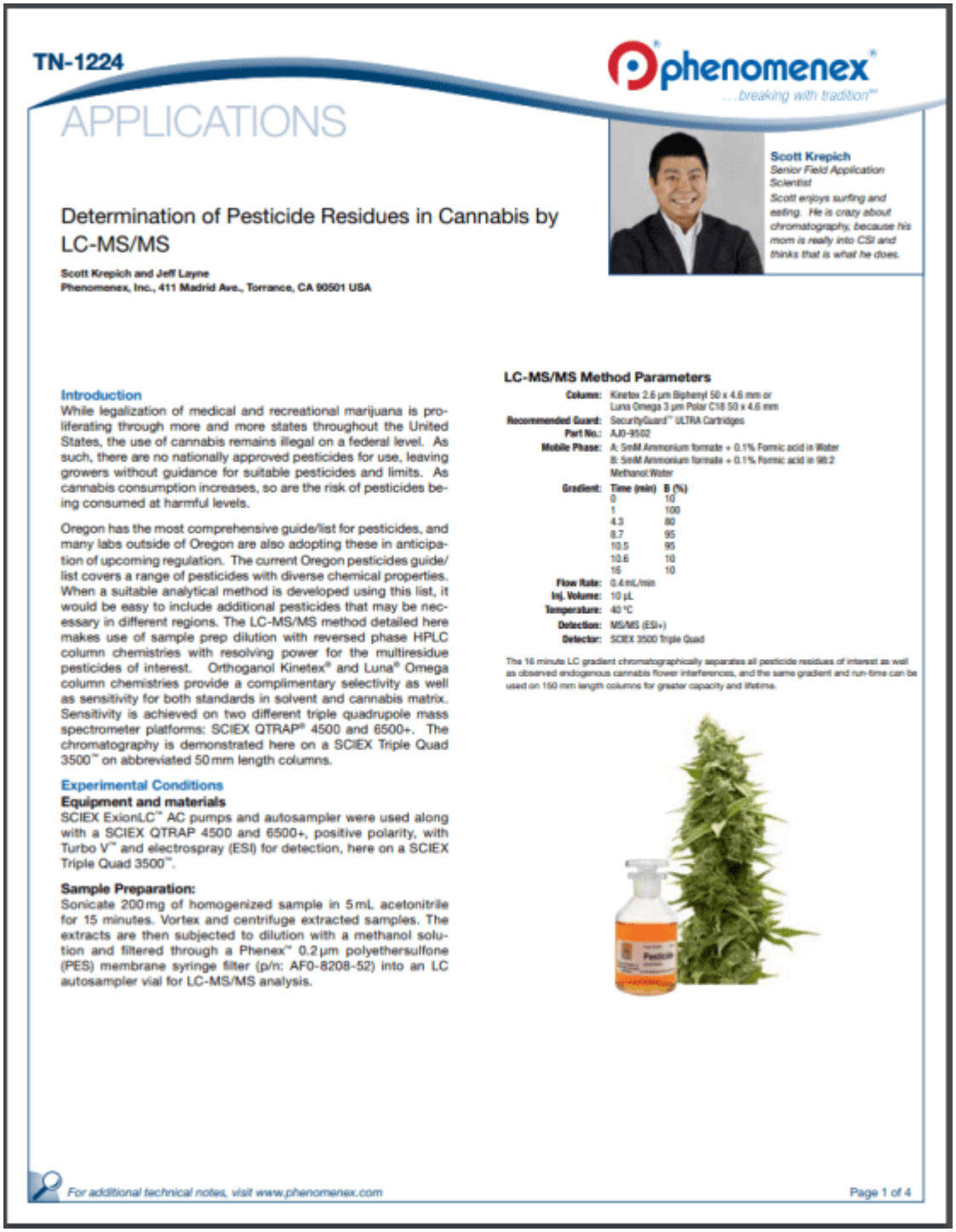
And lastly, especially if screening for trace amounts as a residue, it may be possible to

include it to an existing multi-residue pesticide LC-MS/MS workflow, similar to how some have effectively integrated some terpene and cannabinoid testing into their pesticide screens. For an example see “Determination of Pesticide Residues in Cannabis by LC-MS/MS”.



TN-1224

APPLICATIONS

Determination of Pesticide Residues in Cannabis by LC-MS/MS

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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 throughout the United States, the use of cannabis remains illegal on a federal level. As such, there are no nationally approved pesticides for use, leaving growers without guidance for suitable pesticides and limits. As cannabis consumption increases, so are the risk of pesticides being consumed at harmful levels.

Oregon has the most comprehensive guide/list for pesticides, and many labs outside of Oregon are also adopting these in anticipation of upcoming regulation. The current Oregon pesticides guide/list covers a range of pesticides with diverse chemical properties. When a suitable analytical method is developed using this list, it would be easy to include additional pesticides that may be necessary in different regions. The LC-MS/MS method detailed here makes use of sample prep dilution with reversed phase HPLC column chemistries with resolving power for the multiresidue pesticides of interest. Orthoganol Kinetex® and Luna® Omega column chemistries provide a complimentary selectivity as well as sensitivity for both standards in solvent and cannabis matrix. Sensitivity is achieved on two different triple quadrupole mass spectrometer platforms: SCIEX QTRAP® 4500 and 6500+. The chromatography is demonstrated here on a SCIEX Triple Quad 3500™ on abbreviated 50 mm length columns.

Experimental Conditions

Equipment and materials

SCIEX ExionLC™ AC pumps and autosampler were used along with a SCIEX QTRAP 4500 and 6500+, positive polarity, with Turbo V™ and electrospray (ESI) for detection, here on a SCIEX Triple Quad 3500™.

Sample Preparation:

Sonicate 200 mg of homogenized sample in 5 mL acetonitrile for 15 minutes. Vortex and centrifuge extracted samples. The extracts are then subjected to dilution with a methanol solution and filtered through a Phenex™ 0.2 µm polyethersulfone (PES) membrane syringe filter (p/n: AF0-8208-52) into an LC autosampler vial for LC-MS/MS analysis.

LC-MS/MS Method Parameters

Column:	Kinetex 2.6 µm Biphenyl 50 x 4.6 mm or Luna Omega 3 µm Polar C18 50 x 4.6 mm
Recommended Guard:	SecurityGuard™ ULTRA Cartridges
Part No.:	AJ0-9502
Mobile Phase:	A: 5mM Ammonium formate + 0.1% Formic acid in Water B: 5mM Ammonium formate + 0.1% Formic acid in 98:2 Methanol:Water
Gradient:	Time (min) B (%) 0 10 1 100 4.3 80 8.7 95 10.5 95 10.6 10 16 10
Flow Rate:	0.4 mL/min
Inj. Volume:	10 µL
Temperature:	40 °C
Detection:	MS/MS (ESI+)
Detector:	SCIEX 3500 Triple Quad

The 16 minute LC gradient chromatographically separates all pesticide residues of interest as well as observed endogenous cannabis flower interferences, and the same gradient and run-time can be used on 150 mm length columns for greater capacity and lifetime.

For more information or help with building your own method development of Vitamin E

acetate, reach out to our Technical Experts via Live Chat 24/7 around the world at www.phenomenex.com/chat.

If you found this article helpful or interesting, then you will also want to check out the following articles:

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