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Application News

No. HPLC-030

High Performance Liquid Chromatography

Tocopherol (Vitamin E) Analysis in Vaping E-Liquid by UHPLC-PDA

■ Introduction

Vaping illnesses have been a growing concern for the CDC with over 2,000 known cases and 39 confirmed deaths so far (CDC – November 2019). It is unclear what is causing the illnesses, but vitamin E acetate has been identified as a possible contributor. Vitamin E acetate has been found in the bronchoalveolar lavage (BAL) fluid for all currently tested samples by the CDC (CDC – November 2019).

Two methods have been developed using photodiode array detection (PDA) to separate three isomers of tocopherol (vitamin E): alpha-tocopherol acetate, alpha-tocopherol, and gamma-tocopherol. First is a 10-minute method to separate e-liquid matrix and nicotine from the cannabinoids and vitamin E isomers. Secondly, a quick 5-minute method was also developed with full baseline resolution for identification and quantitative analysis of the three Vitamin E isomers but without resolution between matrix, nicotine and the cannabinoids.

The new Nexera XR UHPLC system with the photodiode array (PDA) was used for method development. The Nexera XR allows for fast separation with low back pressure when coupled with a superficially porous column resulting in high efficiency. The PDA allows for simultaneous monitoring of three channels: 290 nm for vitamin E isomers, 260 nm for nicotine, and 228 nm for cannabinoids.

■ Analytical Conditions

Table 1: Instrument Method Parameters (10-minute method)

Liquid Chromatography	Nexera XR with PDA (LC-40B XR, SIL-40C XR, SPD-M40, CTO-40S, SCL-40, DGU-405)
Mobile Phase A	Water, 0.1% Formic Acid
Mobile Phase B	1:1 Methanol/Acetonitrile
MP Composition	Gradient, 70-95% - 1.5 minutes, hold 6.5 minutes
Column	Shim-pack Velox C18, 1.8 µm, 3.0 x 100 mm Shim-pack Velox EXP Guard, 3.0 x 5 mm
Oven temperature	40°C
Flow rate	0.8 mL/min
Wavelengths Monitored	290 nm, 260 nm, 228 nm
Run Time	10 minutes

Table 2: Instrument Method Parameters (5-minute method)

Liquid Chromatography	Nexera XR with PDA (LC-40B XR, SIL-40C XR, SPD-M40, CTO-40S, SCL-40, DGU-405)
Mobile Phase A	Water, 0.1% Formic Acid
Mobile Phase B	1:1 Methanol/Acetonitrile
MP Composition	Isocratic, 5:95
Column	Shim-pack Velox C18, 1.8 µm, 3.0 x 100 mm Shim-pack Velox EXP Guard, 3.0 x 5 mm
Oven temperature	40°C
Flow rate	0.8 mL/min
Wavelengths Monitored	290 nm, 260 nm, 228 nm
Run Time	5 minutes

■ Standard Preparation

Three forms of tocopherol (vitamin E) were used for this application. A stock CRM solution of Gamma-tocopherol (gamma vitamin E) at 1.0 mg/mL was purchased from Sigma-Aldrich/Cerilliant. For alpha-tocopherol (alpha vitamin E) and alpha-tocopherol acetate (alpha vitamin E acetate), each were purchased as neat (100 mg) standards from Sigma-Aldrich. Vitamin E standards were prepared into 6 calibration levels 100, 50, 25, 10, 5, and 1 ppm in methanol.

The nicotine and cannabinoid mixture was prepared as a combined 50 ppm solution. This mixture was used as a co-injection standard with the vitamin E standards to demonstrate the separation of vitamin E analytes from the nicotine and cannabinoids. Co-injection is a user-friendly pre-treatment program that allows for an injection of different sample vials with a pre-selected standard concentration. The 3-component cannabinoid mixture (CBD, d9-THC, and CBN) was purchased from Restek and the nicotine 1.0 mg/mL standard was purchased from Sigma-Aldrich.

■ Sample Preparation

E-liquid extraction was performed with approximately 500 mg of sample, extracting into 5 mL of isopropyl alcohol, and mixing by vortex. An additional 5 mL of methanol was added to the extracted sample and further vortexed. Then 500 µL of this mixture was diluted into 500 µL of methanol (2x dilution). 200 µL of the final diluted sample was added to 0.2 µM PTFE filter vials for HPLC injection. Samples E and F followed a similar preparation but instead required a 5x dilution prior to filtering.

Table 3: Vaping E-liquid Samples

Sample	CBD or Nicotine	Label Claim	Flavor	Ingredients
A – E-liquid	Nicotine	3 mg/mL	Blue Raspberry	Unknown, VG (vegetable glycerin)
B – E-liquid	Nicotine	6 mg/mL	Really Berry	Vegetable glycerin, propylene glycol, flavors, nicotine
C – E-liquid	CBD	100 mg	Blue Raspberry-Dragon Fruit	Natural cannabidiol isolate, propylene glycol, food grade vegetable glycerin, natural and artificial flavorings
D – E-liquid	CBD	100 mg	Lava Flow	Organic hemp derived CBD. Kosher vegetable glycerin, MCT oil, flavor concentrates
E – E-liquid	CBD	250 mg	Lemon-lime	Full spectrum cannabidiol blend, natural lemon-lime extract, and coconut oil
F – Coconut Oil	None	None	None	MCT coconut oil 100%

■ Results and Discussion

Method Development

The 10-minute method uses gradient flow to allow for separation of e-liquid matrix, nicotine, cannabinoids, and the vitamin E isomers. Figure 1 shows chromatographic separation of the vitamin E isomers starting near 5.5 minutes (black), the cannabinoids near 2 minutes (blue), and nicotine near 0.5 minutes (pink). The e-liquid matrix also elutes near nicotine (see Figure 4).

Three different wavelengths were determined for the different components of the e-liquid based on the λ max from the respective absorbance spectra. Wavelength 290 nm is used for the vitamin E isomers, 228 nm for cannabinoids, and 260 nm for nicotine (Figure 1).

Figure 1 shows that the vitamin E isomers also absorb near 5.5 minutes in chromatograms B and C at the lower wavelengths. It is not recommended to use wavelengths 228 nm and 260 nm for vitamin E determination, because some of the sample e-liquids have unknown analytes that have a similar retention time as alpha-vitamin E and absorb at the lower wavelengths (Figure 4). These unknowns do not absorb at the higher wavelengths. From this, 290 nm is used for vitamin E determination to avoid false positive identification.

The 5-minute method was developed as an isocratic method that can be used for a quick screening method to determine if vitamin E is present in vaping e-liquid. The three vitamin E isomers are fully separated from the other sample components, but the e-liquid matrix, cannabinoids and/or nicotine are not fully resolved (Figure 2).

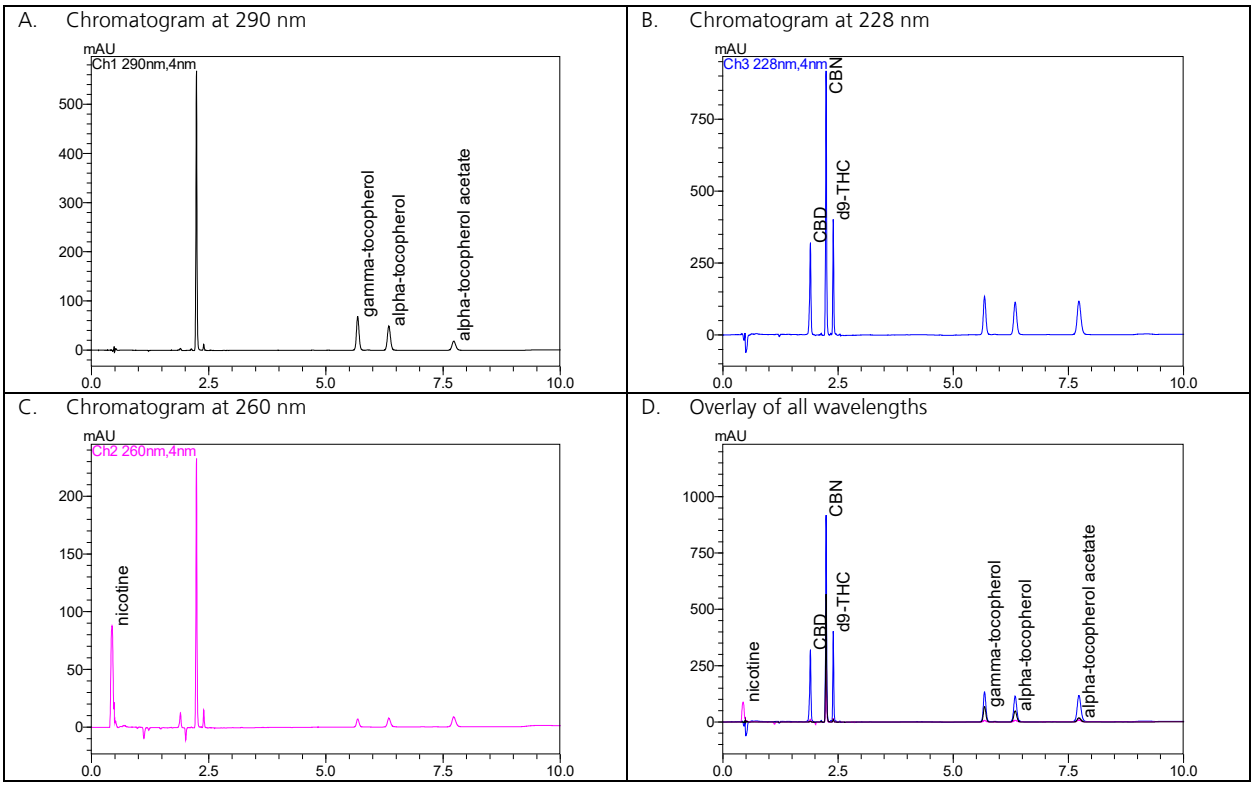

Figure 1: Vitamin E isomers at different wavelengths (10-minute method)

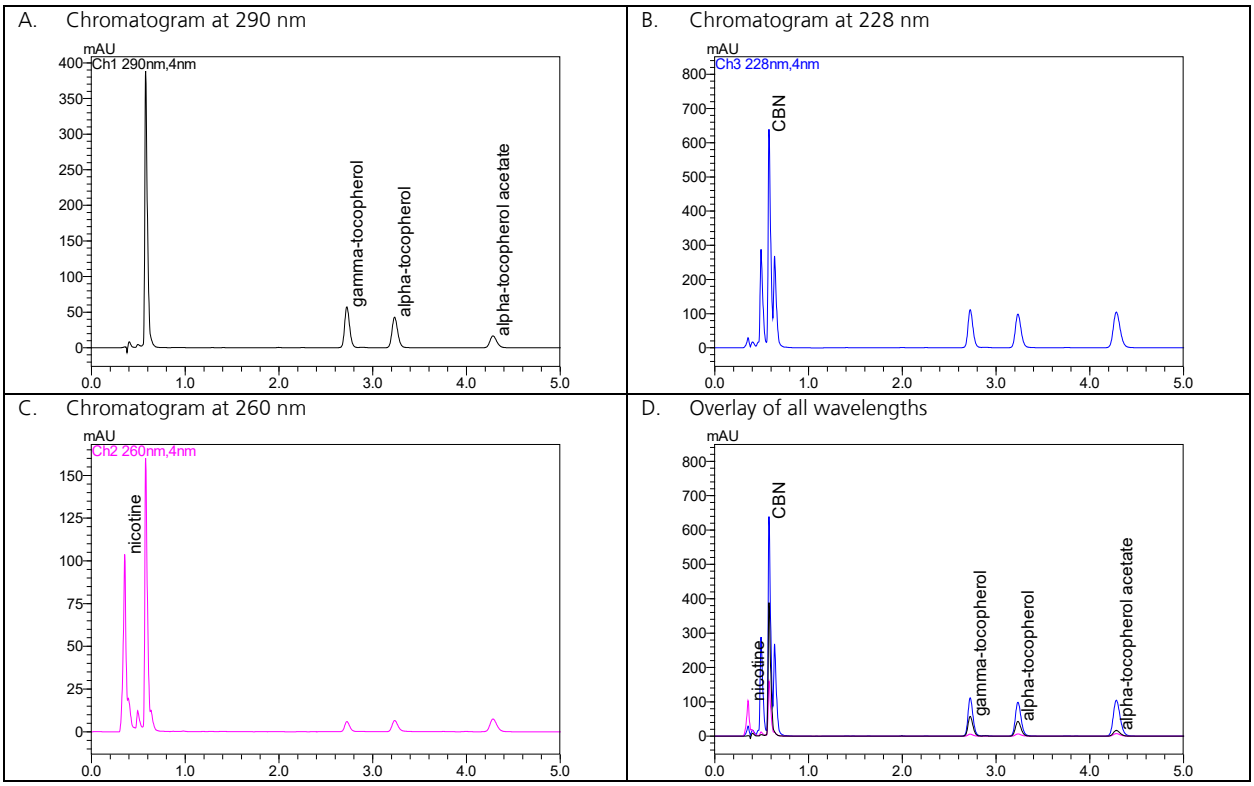

Figure 2: Vitamin E isomers at different wavelengths (5-minute method)

Calibration

Figure 3 shows the comparison of the vitamin E isomers at the varying concentrations with the calibration plot for both the 10- and 5-minute methods. The calibration curves of the six standards (100 – 1 ppm) of the three tocopherol isomers have an R^2 value of at least 0.999 (Table 4) for both the 10- and 5-minute methods. The accuracies of the calculated concentration are +/- 5%.

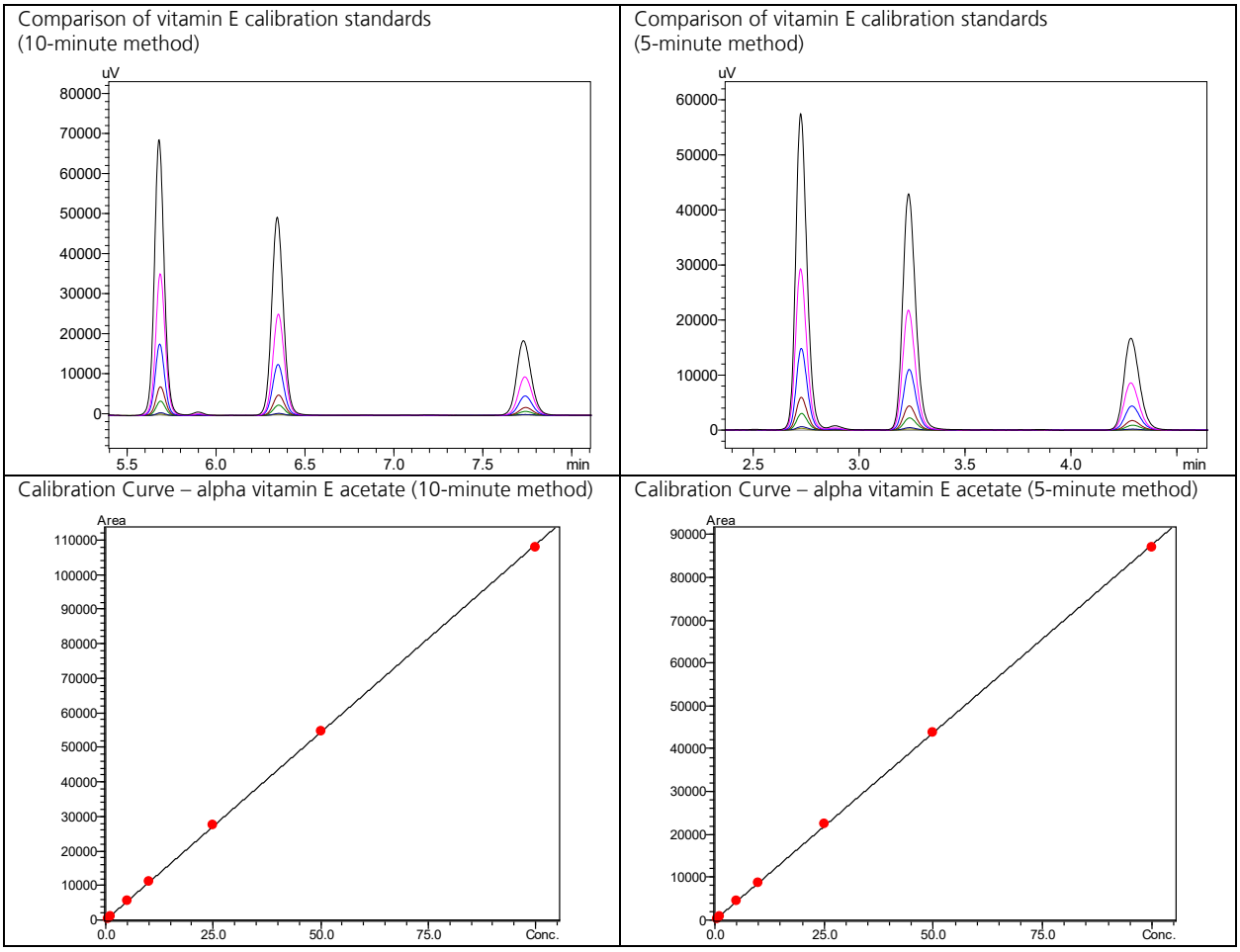

Figure 3: Calibration standard chromatograms and calibration curve of tocopherol isomers

Table 4: Calibration curve results of the vitamin E isomers (5-minute method)

10-minute method			
	gamma vitamin E	**alpha vitamin E**	**alpha vitamin E acetate**
Retention Time	5.682	6.364	7.727
R2	0.9997766	0.9998797	0.9999021
5-minute method			
	gamma vitamin E	**alpha vitamin E**	**alpha vitamin E acetate**
Retention Time	2.686	3.191	4.223
R2	0.9998746	0.9999525	0.9998544

■ Sample Results

A sample set of six e-liquids were acquired for analysis of vitamin E. These results can be seen in Figure 4 below. The three wavelength traces are overlaid (black – 290 nm, pink – 260 nm, and blue – 228 nm). Samples A and B show results of nicotine eluting near the sample matrix. These peaks are also eluting very early in the chromatography, indicating they are unretained on the column.

Samples C, D and E all result with a sample matrix peak and CBD cannabinoid identification. CBD, d9-THC, and CBN show retention on the column, but should not be used for quantitation as there could be other cannabinoids present not used in this standard mixture. These methods were not developed for quantitation of nicotine and cannabinoids but can be used qualitatively for product determination.

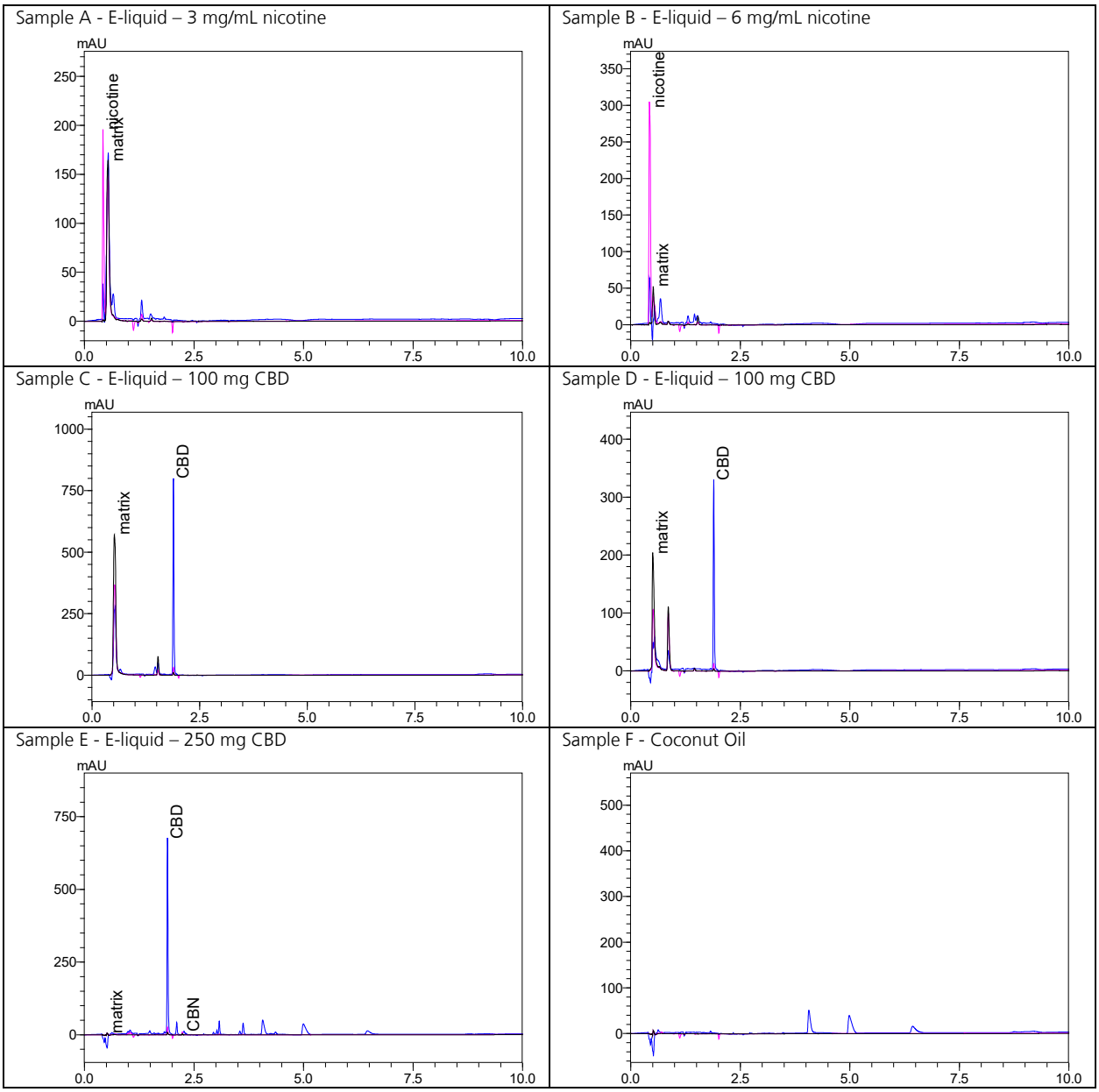

Figure 4: E-liquid Samples (nicotine – pink, cannabinoids – blue, vitamin E isomers – black)

Vitamin E was not detected in any of the samples using the method from this application. However, the e-liquid that used coconut oil (sample E) as a thickening agent did show unknown components that absorb at low wavelengths (near 228 nm). Coconut oil (sample F) was also analyzed under the same conditions, and the chromatogram overlay with e-liquid (sample E) can be seen below in Figure 5.

At 290 nm (Figure 5 – left), these unknown components are not seen, but at 228 nm (Figure 5 – right) there are several compounds found in both sample E and coconut oil. This demonstrates why it is not recommended to use 228 nm as the wavelength for vitamin E quantitation as false positives could be possible.

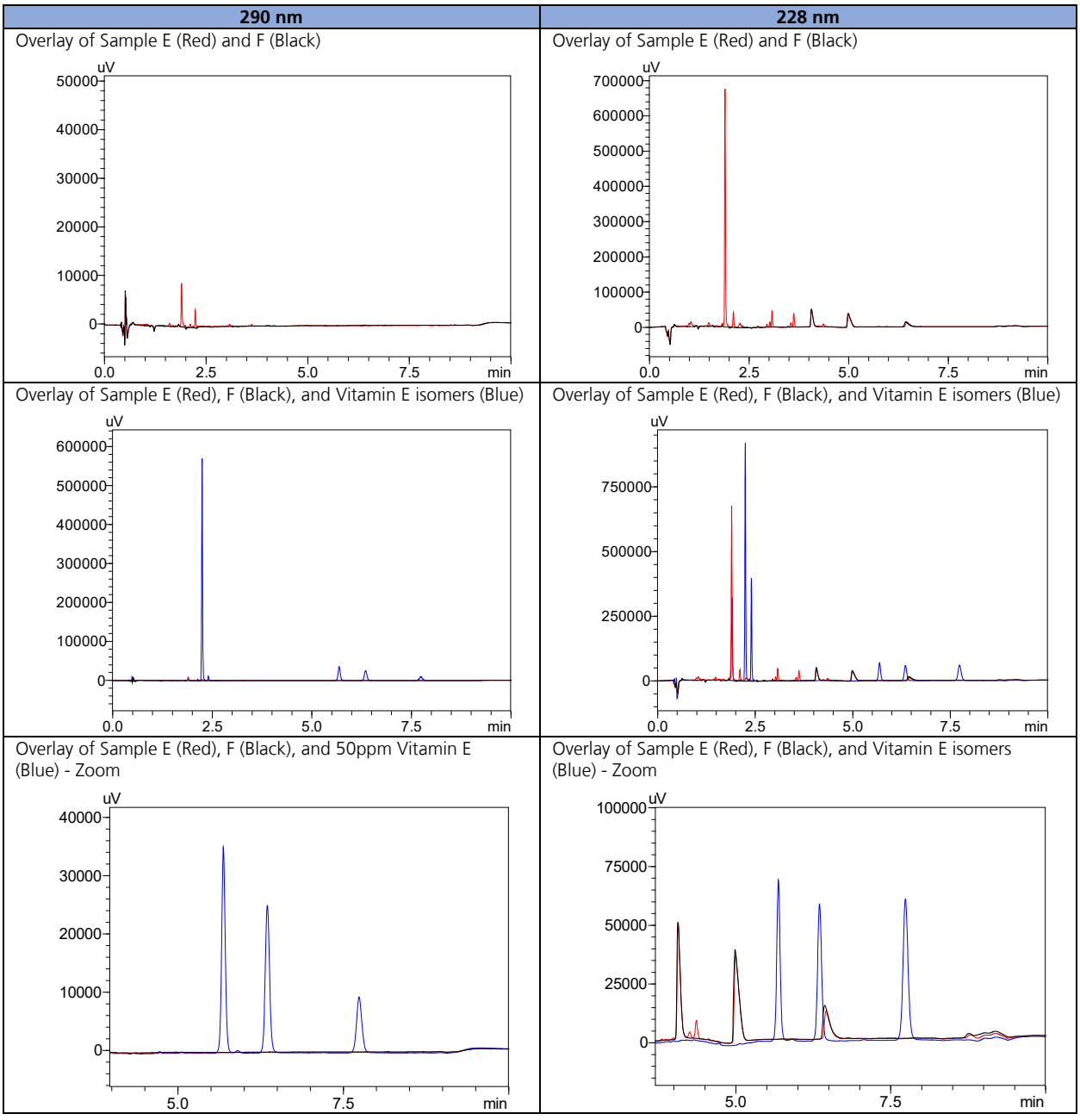

Figure 5: Overlay of Sample E. E-liquid 250 mg CBD with Sample F. Coconut Oil and 50 ppm vitamin E isomer mixture

■ Conclusion

The methods described in this application can be used as a screening tool for three vitamin E isomers in nicotine or cannabinoid e-liquid products. The quick 5-minute method will allow for vitamin E identification and quantitation, while the 10-minute method will also allow for determination between nicotine or cannabinoid-based products.

■ Consumables

- Shim-pack Velox C18, 1.8 µm, 3.0 x 100 mm (220-32008-02)
- Shim-pack Velox EXP Guard, 3.0 x 5 mm (220-32028-02)
- Restek Cannabinoid Standards – 3 components (Cat.# 34014) or Shimadzu 11-Component Cannabinoid Standard (220-91239-21)
- 0.2 µM PTFE Filter vials (220-91521-40)

Source

(2019) Outbreak of Lung Injury Associated with the Use of E-cigarette, or Vaping, Products. https://www.cdc.gov.

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