Analysis of Vitamin E and Vitamin E Acetate in Vape Oils

Triple Quadrupole Analysis of Vape Oils Produces High Quality Quantitative Results

Ritu Arora, Swetha Kaul[1]; Paul Winkler[2], Phil Taylor[3]
[1]Cannalysis, Santa Ana, CA, USA; [2]SCIEX, Framingham, USA, [3]SCIEX, Warrington, UK

Recently, a drastic increase in vaping related lung illnesses has been observed.[1] The cause of this unprecedented number of people gravely affected from vaping is of urgent concern to the CDC and FDA.[2] Vitamin E acetate, a compound used to thicken vaping liquids, has been implicated in the rise in the rate of observed lung illnesses.[3] While the link between lung illness and Vitamin E Acetate is not certain[1], there has been an increase in requests to have products tested for the presence of Vitamin E and Vitamin E Acetate.[4] LC-UV methods have historically been employed for these analytes, but the variability of the relevant matrices and the possibility of co-eluting interferences in a non-specific method demands that a more specific and reliable analytical approach be used to assure product safety. Increased specificity is a hallmark of Multiple Reaction Monitoring (MRM) analysis on a triple quadrupole mass spectrometer. The mass spectrometric approach using two MRM transitions for each analyte as well as an isotopically-labelled internal standard, ensures that the detected signal for the vitamin E and vitamin E acetate can indeed be attributed to the presence of these species and are not the artifact of complex matrix interferences.

This application note details a workflow for accurate and precise analysis of Vitamin E and Vitamin E Acetate in vape oils. The SCIEX Triple Quad™ 3500 LC-MS/MS system was leveraged to produce quantitative results which are of high quality, robust, and time efficient.

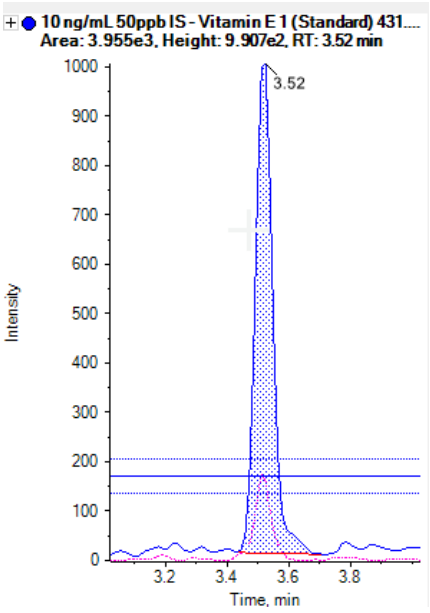

Figure 1. Vitamin E Acetate at 10ng/mL, showing acceptable ion ratio confirmation.

Key Advantages of the Vitamin E Method

- Quantitative method combines analyses for Vitamin E and Vitamin E Acetate
- MRM analysis for high specificity of detection
- Highly simplified sample preparation: “dilute-and-shoot”
- Fast 7-minute analysis
- Linear response for quantitation from 10ppb up to 500ppb with excellent precision (5%CV) for both analytes

Experimental

Sample Preparation: Samples were prepared for analysis by dissolving 500mg of sample in 40mL of methanol. The injection solvent contained Vitamin E d6 at a concentration of 50ppb. This was used as the internal standard for quantitation. The diluted sample was analyzed without any further processing.

Chromatography: Chromatographic separation was achieved using an Agilent Poroshell 120 EC-C18, 2.7µm, 100 X 4.6mm column with a solvent flow rate of 1.2 mL/min. The column oven was set to 50°C. 5 µL injection volume was used. The chromatographic gradient and mobile phases are outlined in Table 1.

Table 1. Gradient for Vitamin E Separation.

Time (min)	Mobile Phase B (%)
0.0	*95.0*
0.5	*100.0*
5.0	*100.0*
5.1	*95.0*
7.0	*95.0*

Mobile phase A: Water with with 5mM ammonium formate, 0.3% formic acid
Mobile phase B: Methanol with with 5mM ammonium formate, 0.3% formic acid

Mass Spectrometry: Analysis was performed on the SCIEX Triple Quad 3500 System with a Turbo V™ source using electrospray ionization (ESI) in the positive ion mode. Data were collected using the conditions shown in Table 2. Ion source and collision gas conditions were as follows: GS1 = 30, GS2 = 30, CUR = 35, CAD = 11, TEM = 300ºC.

Table 2. Compound-Specific Acquisition and Data Processing Parameters.

	Precursor	Fragment	DP (V)	CE (V)	RT (min)
Vitamin E 1	*431.1*	*165.1*	*121*	*37*	*3.53*
Vitamin E 2	*431.1*	*137.1*	*111*	*59*	*3.53*
Vitamin E Acetate 1	*473.2*	*207.1*	*176*	*25*	*4.38*
Vitamin E Acetate 2	*473.1*	*165.1*	*176*	*55*	*4.38*
Vitamin Ed6	*437.1*	*171.1*	*106*	*37*	*3.54*

Results and Discussion

Linearity, Precision and Sensitivity

Calibration curves for Vitamin E and Vitamin E Acetate were acquired from 10ppb to 500ppb. An example curve is shown in Figure 2. The top trace is the calibration curve for the primary MRM transition of Vitamin E and the bottom calibration is the primary MRM transition for Vitamin E Acetate. Both compounds exhibit excellent linearity over this range (r-value >0.98).

The calibration was run 5 consecutive times to demonstrate the precision and stability of the method. Very good reproducibility was obtained and is shown in Table 3. The percent CV for the 5 injections was 6% except for Vitamin E at 10ppb, which had a percent CV of 9%. The measured accuracy ranged from 83% to 118% and was generally within 10% of the expected value. These data demonstrate that highly reproducible analytical results are observed using this method. These values indicate that the accuracy expected to be obtained with this method will meet analytical requirements, based on the regulation in place for residues testing. Figure 3 shows the MRM group for Vitamin E Acetate for each of the five 10ppb injections. The ion ratios (ratio of primary MRM signal to secondary MRM signal) for each of the injections demonstrates that reliable ion ratios are consistently obtained even at low concentration.

Sample Results

Thirty-three vape oils from a wide variety of sources were analyzed using the method. Typical results from a subset of these samples are shown in Table 4. These representative results show that Vitamin E Acetate is detectable in all the products, while Vitamin E is not detected in any of the products. The data also demonstrate that there is no correlation between the concentration of Vitamin E and Vitamin E Acetate.

Table 4: Typical Values for Vitamin E and Vitamin E Acetate from Range of Vape Oils (Subset of 33 Samples Analyzed).

Sample	Vitamin E (ppb)	Vitamin E Acetate (ppb)
1	*<0*	*0.415*
2	*74.1*	*0.461*
3	*76.4*	*0.383*
4	*<0*	*0.532*
5	*<0*	*2.32*
6	*<0*	*2.02*
7	*106.7*	*0.594*
8	*72.7*	*0.524*

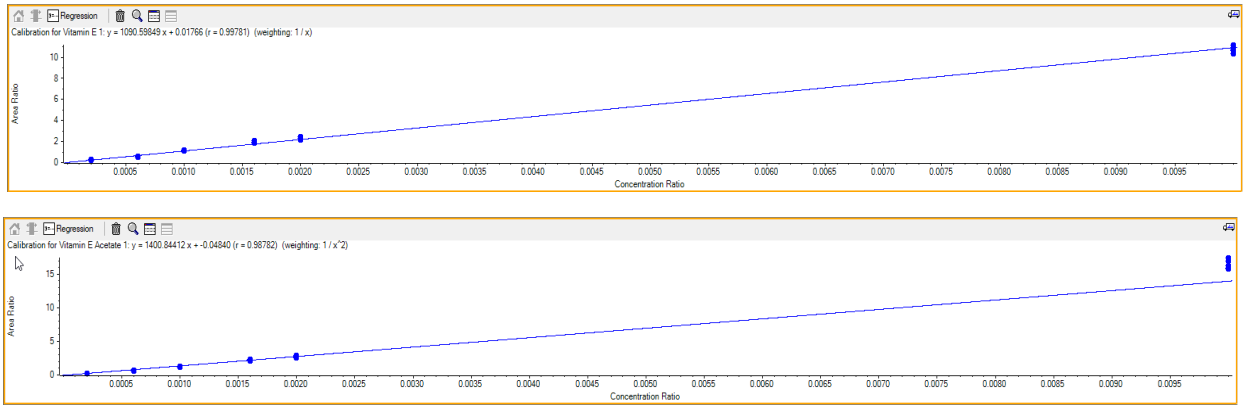

Figure 2: Example Calibration Curves. (Top) Calibration curve for Vitamin E from 10 to 500ppb. (Bottom) Calibration curve for Vitamin E Acetate from 10 to 500ppb.

Table 3. Precision and Accuracy for Five Consecutively Analyzed Calibration Curves.

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
1	Vitamin E 1	0.010	5 of 5	0.0	0.0	8.85	99.99
2	Vitamin E 1	0.030	5 of 5	0.0	0.0	5.22	85.57
3	Vitamin E 1	0.050	5 of 5	0.1	0.0	3.64	101.63
4	Vitamin E 1	0.080	5 of 5	0.1	0.0	4.15	109.59
5	Vitamin E 1	0.100	5 of 5	0.1	0.0	3.71	105.08
6	Vitamin E 1	0.500	5 of 5	0.5	0.0	2.67	98.15

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
1	Vitamin E Acetate 1	0.010	5 of 5	0.0	0.0	5.62	106.76
2	Vitamin E Acetate 1	0.030	5 of 5	0.0	0.0	5.54	83.03
3	Vitamin E Acetate 1	0.050	5 of 5	0.0	0.0	5.43	92.54
4	Vitamin E Acetate 1	0.080	5 of 5	0.1	0.0	2.90	101.75
5	Vitamin E Acetate 1	0.100	5 of 5	0.1	0.0	4.58	98.14
6	Vitamin E Acetate 1	0.500	5 of 5	0.6	0.0	4.40	117.78

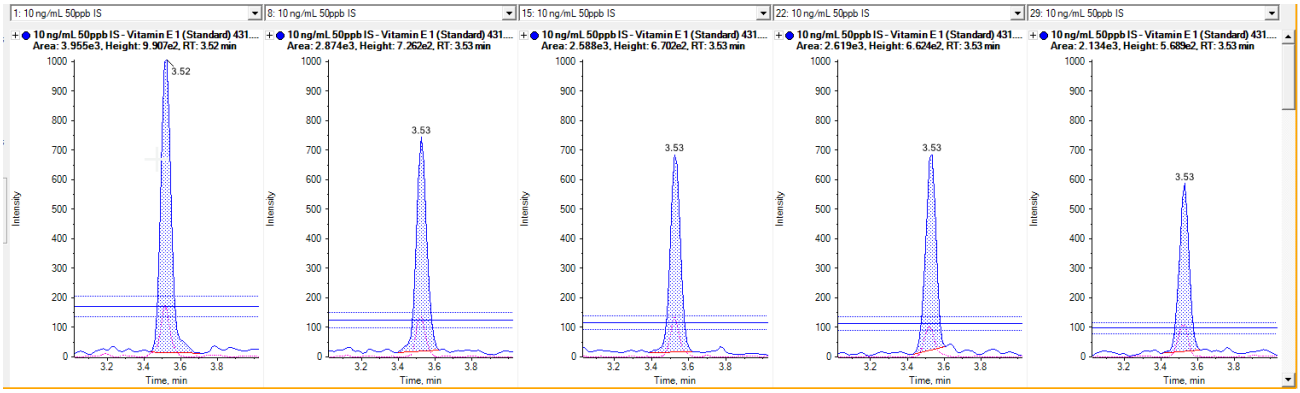

Figure 3: Example Chromatography. Peaks for the primary and secondary ions overlaid at 10ppb Vitamin E Acetate showing acceptable ion ratios for each injection.

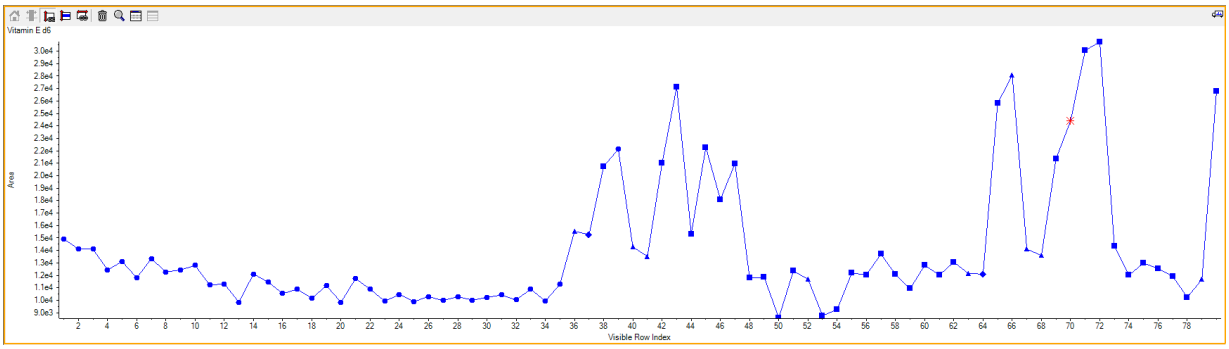

Figure 4: Peak Areas for the Internal Standard, Vitamin E d6. Circles represent calibration standards, Squares represent unknown samples, and triangles represent CCV standards.

The peak areas for the internal standard, Vitamin E d6 are shown in Figure 4. The plot shows area values for the injected samples of a single batch. The standards (represented by closed circles) run at the start of the batch show a very stable response during the analysis of the calibration solutions with an RSD of 12%. The IS areas for the samples (represented as squares) show elevated areas for some of the standards resulting in an RSD for the sequence of 37%. Elevated areas for the internal standard were not observed for all samples and appear to be related to those samples that had high concentrations of Vitamin E. The areas do, however, demonstrate the need for using an internal standard to achieve accurate quantitation.

The performance of the method was monitored during the run with the analysis of Continuing Calibration Verification standards (CCV). These samples were spiked with Vitamin E Acetate. The results for the method QC are shown in Table 5. The CCVs were stable during the sequence of injections with recoveries from 86 to 108%, which is within general acceptance criteria required for residue analysis methods. The CCVs were acquired with different concentrations throughout the sequence and further demonstrate that the method provides accurate quantitation across the calibration concentration range during sample analysis.

Conclusions

A method has been developed for the analysis of Vitamin E and Vitamin E Acetate that is suitable for the quantitative determination of these compounds in vaping oils from 0.01% to 0.5%. The method has been demonstrated to provide accurate and precise results during an extensive analysis of several actual samples of vaping oil.

Table 5: Continuing Calibration Verification Results

Sample Type	Spike Conc (ppm)	Calc Conc (ppm)	Accuracy (%)
CCV 1	*0.050*	*0.054*	*108*
CCV 2	*0.100*	*0.101*	*101*
CCV 3	*0.050*	*0.043*	*87*
CCV 4	*0.100*	*0.101*	*101*

References

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4. Swetha Kaul, Cannalysis Laboratories, Santa Ana, CA (September 26, 2019)

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
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