CUSTOMER APPLICATION NOTE 73327

Thermal decomposition of vitamin E acetate in a surrogate vaping environment

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Keywords: High-temperature headspace sampling, vaporizer, e-juice, ISQ 7000 GC-MS, TRACE 1300 GC, TriPlus 500 Headspace Autosampler

Goal

To demonstrate the use of high temperature headspace sampling as a surrogate vaporization environment and evaluate the formation of vitamin E acetate degradation products at temperatures specific to vaporization.

Introduction

"Products intended to be consumed by inhalation after high temperature vaporization" is a popular new category of consumer products that have evolved in a marketplace without appropriate regulatory oversight. This is not the fault of regulatory agencies, but rather the nature of the evolution from "products intended to be consumed by inhalation after combustion", such as cigarettes and joints, which have been determined by many regulatory agencies to be exceptionally dangerous, with the premature death of millions of people linked directly to smoking. This new product category was initially proposed as a safer alternative to and a means of aiding in the cessation of smoking. As the product category has evolved from a means of diverting users from behaviors considered detrimental into products that individuals are drawn to for their own means (for example, flavored e-liquids concentrates), the question regarding what ingredients or additives could be safely used must be considered.

The recent set of severe illnesses and 47 deaths[1] appeared as a cluster after a few years of widespread use of vaporizers. Many of these adverse health effects have been associated with vitamin E acetate (α-tocopheryl acetate; Figure 1), a product that is not toxic by ingestion and so would have never been identified as a problematic ingredient.[2] The use of vaporizers for the administration of vitamins, herbal supplements, botanicals, and even illegal recreational drugs, including LSD and fentanyl, has also been reported.[3,4] While such instances represent a minority of vaporizer use, they further highlight the importance of understanding the health effects of standard e-liquid components to improve the chances of finding causal relationships between health issues and atypical additives. Only by evaluating the decomposition products at the temperatures used in vaporizers can the risks associated with a given ingredient be properly characterized.

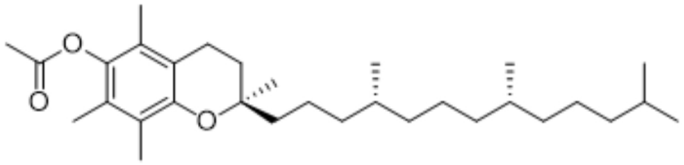

Figure 1. The chemical structure of vitamin E acetate (α-tocopheryl acetate)

Understanding the safety of vaporizers necessitates a complete picture of their chemistry, including the potential for thermal and oxidative breakdown of e-juice components within the vaporizer during routine use. Sampling degradation products directly from vaporizer output is a critical research objective that will contributed greatly to our understanding of vaporizers and their associated chemistry. However, as a matter of experimental design, these studies are limited as there exists a variety of confounding variables that may obscure attempts to characterize degradation products, the most notable being the temperature dependence the heating coils possess as a function of delivered voltage, coil age, cartridge volume, etc.[5,6]

This application note outlines an approach to understand the chemistry of vaporization using high-temperature headspace sampling coupled to gas chromatography-mass spectrometry (GC-MS). The precise temperature control afforded could improve consumer safety by providing evidence for tighter control of vaporization temperatures. Vitamin E acetate (Figure 1) is presented as an example of the utility of this approach, as recent CDC laboratory testing has detected this additive in bronchoalveolar lavage fluid samples obtained from e-cigarette product use–associated lung injury patients.[2]

Experimental

Analytical equipment

Gas chromatography separations were carried out on the Thermo Scientific™ TRACE™ 1300 Gas Chromatograph with the Thermo Scientific™ TriPlus™ 500 Headspace Autosampler, using a Thermo Scientific™ TraceGOLD TG-35MS 30 m × 0.25 mm i.d. × 0.25 µm capillary GC column (P/N 26094-1420). The MS analysis was performed on a Thermo Scientific™ ISQ™ 7000 Single Quadrupole GC-MS system (Figure 2).

Figure 2. TriPlus 500 Headspace Autosampler, TRACE 1300 Gas Chromatograph, and ISQ 7000 Single Quadrupole GC-MS

Consumables

GC parts

- Ferrule, Vespel, for Agilent™ Capillary Nut, 0.1–0.25 mm, Pack of 10 (P/N 290VA191)
- Headspace Vial, 20 mL, Crimp Top, Clear, Round Bottom, set of 125 (P/N 20-CV)
- Crimp Cap, 20 mm, Magnetic, Red Silicone/Natural PTFE, high temperature (300 °C), set of 500 (P/N 20-MCB-ST3HT)
- Electronic Handheld Crimper for 20 mm Crimp Caps, GEN 4 1/EA (P/N 60180-ECR20)
- GC column, TraceGOLD TG-35MS capillary (30 m × 0.25 mm × 0.25 µm) (P/N 26094-1420)

Kits

- Capillary Tool Kit for Thermo Scientific GCs (P/N 60180-784)
- GC Installation Kit (P/N 60180-888)
- GLD Pro Leak Detector (P/N 66002-001)
- GC/GCMS PTV Essentials Kit for liquid injection (P/N 26096-1420PTV-BNDL)
- GC/GCMS-SSL Essentials Kit for liquid injection (P/N 26096-1420SSL-BNDL)

Sample preparation and analysis

For each test, 1–2 mg of vitamin E acetate analytical standard (Sigma-Aldrich) was accurately weighed into a 20 mL headspace vial and sealed with high temperature 3.2 mm PTFE septa. Samples were incubated for 5 min at temperatures ranging from 180 to 300 °C and were subsequently analyzed using the parameters outlined in Tables 1 and 2. Comparative analysis was done using a 5 min incubation in the TriPlus 500 Headspace Autosampler oven at 180–300 °C (30 °C steps), followed by analysis as per Tables 1 and 2. The GC-MS transfer line and EI source were both set at 300 °C.

Table 1. GC temperature gradient using a TraceGOLD TG-35MS capillary GC column (30 m × 0.25 mm × 0.25 µm) and He (99.999%) carrier gas at 1.5 mL/min

Time (min)	Temp. (°C)	Rate (°C/min)	Hold (min)
2.0	35.0	-	10.0
11.5	130.0	10.0	-
19.0	290.0	30.0	2.2
25.0	320.0	30.0	5.0

Table 2. TriPlus 500 Headspace Autosampler method parameters (1000 µL sample loop). The inlet temperature was set to 125 °C with a 100:1 split ratio.

Parameter	Value
Incubation temp. (°C)	180–300
Incubation time (min)	5.0
Vial shaking	Fast
Pressurization mode	Pressure
Vial pressure (kPa)	100.0
Loop/sample path temp. (°C)*	200–300
Loop pressure (kPa)	50.0
Loop equilibration (min)	0.20
Injection mode	Standard
Injection time (min)	0.50
Inlet temp. (°C)	140.0
Purge flow (mL/min)	2.0

*The sample path temperature was 20 °C higher than the incubation temperature, except for 300 °C, which had equivalent loop and incubation temperatures.

Full scan MS spectra (29–500 *m/z*) were interrogated using the NIST Mass Spectral Search Program for the NIST/EPA/NIH EI and NIST Tandem Mass Spectral Library (version 2.3, 2017). In some instances, analytical standards were used to confirm NIST hits (Table 3). All plausible degradants were compared against suitable blanks (Figure 3) to ensure they were specific to vitamin E acetate.

Chromatography software

The Thermo Scientific™ Chromeleon™ Chromatography Data System was used for data acquisition and analysis.

Table 3. Thermal and oxidative degradation products of vitamin E acetate identified at 300 °C using high-temperature headspace sampling with GC-MS detection and spectral library matching

RT (min)	Putative ID	SI	RSI
1.35	formic acid*	933	950
1.42	acetone*	897	911
1.64	isobutyraldehyde	927	927
1.65	acetic acid*	869	909
1.72	methacrolein	890	908
1.93	2-butanone*	868	874
2.43	isovaleraldehyde	829	829
2.48	3-methyl-2-butanone	889	889
2.65	propanoic acid	846	881
2.71	acrylic acid	838	892
2.76	2,2-dimethylTHF	815	832
2.90	2-pentanone	872	899
2.95	4-penten-2-one	831	896
3.07	acetol	913	914
3.13	2,3-pentanedione	874	895
3.43	isobutyric acid	860	873
4.06	3-penten-2-one	914	916
4.27	2,6-dimethyl-1-heptene	920	925
4.56	2-hexanone*	714	809
4.98	isovaleric acid	838	855
5.06	3-methyl-2-butenal	908	936
5.66	pyruvic acid methyl ester	739	812
6.01	2,2-dimethyl-3(2H)-furanone	899	916
6.38	2-methylcyclopentyl acetate	742	835
7.40	2-methyl-6-heptanone	905	906
7.86	6-methyl-6-hepten-2-one	873	886
8.21	2,5-hexanedione	837	842
8.71	5,5-dimethyl-2(5H)-furanone	807	860
8.99	3-methyl-2,5-furandione	853	888
9.18	alpha-methyl-gamma-butyrolactone	764	802
9.31	pyroterebic acid	878	892
9.64	3,4-dimethyl-2-hexanone	756	823
9.74	3,6-heptanedione	839	928
9.97	levulinic acid	774	849
10.19	4-methyl-6-hepten-4-olide	821	920
10.43	3,4-dimethyl-2,5-furandione	886	909
11.56	5-methyl-1-undecene	787	822
12.06	2,7-octanedione	769	877
13.21	hexahydropseudoionone	902	902
15.73	3-formyl-4-hydroxy-2,5,6-trimethylphenyl acetate	930	948
17.41	acetic acid, 5,7,8-trimethyl-6-coumarinyl ester	882	901

* RT-matched to analytical standards

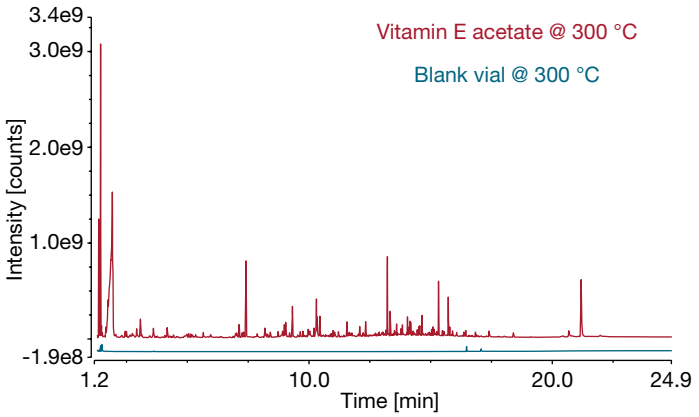

Figure 3. Blank vial results compared against a vial containing vitamin E acetate. Data are signal off-set by 3% but are otherwise scaled to the same y-axis. Both vials were incubated for 5 min at 300 °C, with the data demonstrating that the observed thermo-oxidative degradants are directly related to vitamin E acetate.

Results and discussion

As a proof-of-concept experiment, the decomposition of vitamin E acetate was observed at temperatures consistent with e-cigarettes and vaporizers by incubating vitamin E acetate in an oven at temperatures from 180 to 300 °C (contained in a sealed headspace vial). After confirming that vitamin E acetate and a variety of thermal and oxidative degradation byproducts were observed by incubating in the oven, the same experiment was repeated with the TriPlus 500 Headspace Autosampler used for incubating the samples. The data obtained from the TriPlus 500 Headspace Autosampler (Figure 4) were identical to that observed for the off-line oven reactions (data not shown).

Evaluation of the total ion chromatograms (TICs) for the different temperatures demonstrated significant thermal and oxidative decomposition products starting as low as 240 °C (Figure 4). The NIST database was searched to provide direction for the identification of the decomposition observed products (Table 3). Many of the compound identifications appear to involve oxidation of the aliphatic side-chain of vitamin E acetate, with a variety of aldehydes and ketones registering as plausible hits in the NIST database.

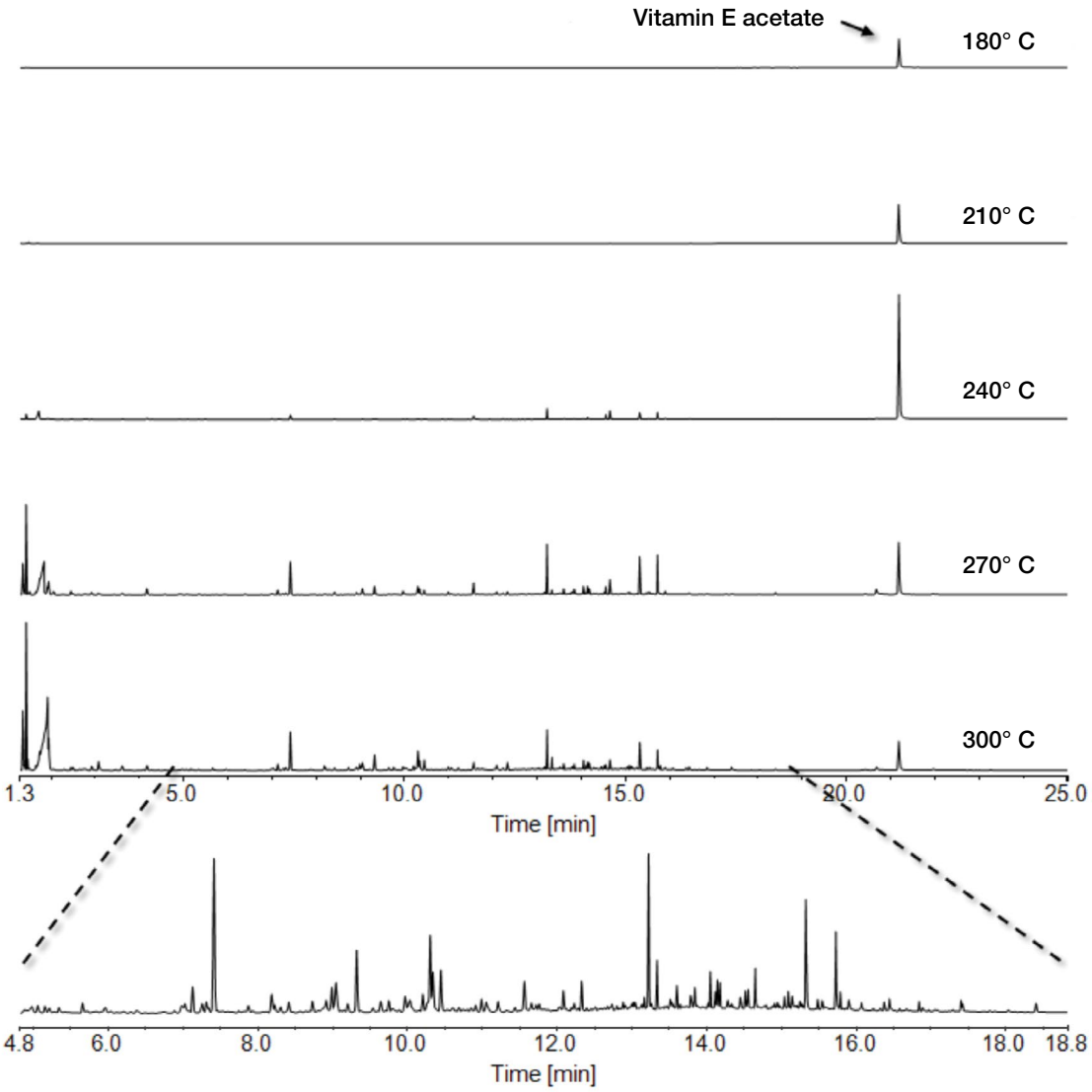

Figure 4. Total ion chromatograms (TICs) of potential thermal and oxidative degradation products of vitamin E acetate (21.2 min) as a function of incubation temperature (5 min at indicated temperatures) using high-temperature headspace sampling with GC-MS detection. Absolute TIC responses were normalized to the amount of vitamin E acetate in each vial and all chromatograms share the same signal axis.

In addition to the identifications made using the NIST database, several analytical standards were analyzed to confirm the NIST identifications of formic acid, acetone, acetic acid, 2-butanone, and 2-hexanone (Table 3). The concentrations of these confirmed degradation products were assessed semi-quantitatively and were determined to be less than the limits specified under residual solvents limits enforced by Health Canada. However, these limits are based on ingestion, not inhalation; the pharmacology of inhalation is significantly different than ingestion and this must be considered when evaluating the risks associated with vaporizers. Moreover, ingestion limits do not consider routine use of vaporization devices and how that could impact consumer health. This clearly points to the need for more detailed characterizations of the thermal/oxidative decomposition products of e-juice components and how they relate to consumer exposure during routine vaporizer use.

Lastly, it is worth noting that the incubations reported herein were performed with air in the headspace. It is likely that a different degradation profile would be obtained if samples were incubated in an inert atmosphere—such an approach would help with the identification of those degradants that were produced thermally versus those that were thermo-oxidative decomposition products.

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Conclusion

One of the fundamental criteria for developing an analytical methodology or standard is to determine if the testing protocol is “fit for purpose”. Relying on ingestion-based toxicity metrics to determine the safety of products “intended to be consumed by inhalation after high temperature vaporization” does not fit the criteria of being “fit for purpose”. It is equally obvious that products that are heated to temperatures from 150 to 450 °C (or higher) need to be evaluated based on what they would deliver to the user at these temperatures. The preliminary data presented here highlights the utility of high-temperature headspace sampling for the evaluation of products intended for inhalation after high-temperature vaporization. This surrogate vaporization environment enables tightly controlled temperature and time domain experiments that remove the variability associated with sampling directly from vaporization products. Detailed investigations into the decomposition of other e-juice/vaporization additives and/or ingredients are currently underway using the workflow outlined here. It is anticipated that approaches like this will be utilized by producers of e-juice and similar products, as well as by regulatory agencies, to inform legislative decisions regarding the safety of vaporization products and their constituents.

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