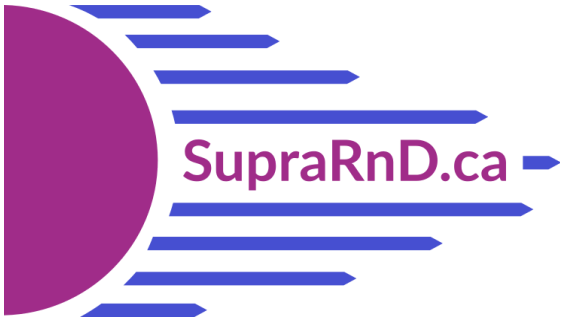
Vaporization Potential and

Effective Residual Solvent Analysis Report

Vitamin E Acetate, Squalane and Squalene

November 10, 2020

Prepared by



Supra Research and Development

Supra Research and Development Inc.
106-2293 Leckie Rd, Kelowna B.C. V1X 6Y5
http://suprarnd.ca/ | inquire@suprarnd.ca | 1-855-437-8772

Table of Contents

Supra Research and Development Inc.
106-2293 Leckie Rd, Kelowna B.C. V1X 6Y5
http://suprarnd.ca/ | inquire@suprarnd.ca | 1-855-437-8772

1.0 Introduction

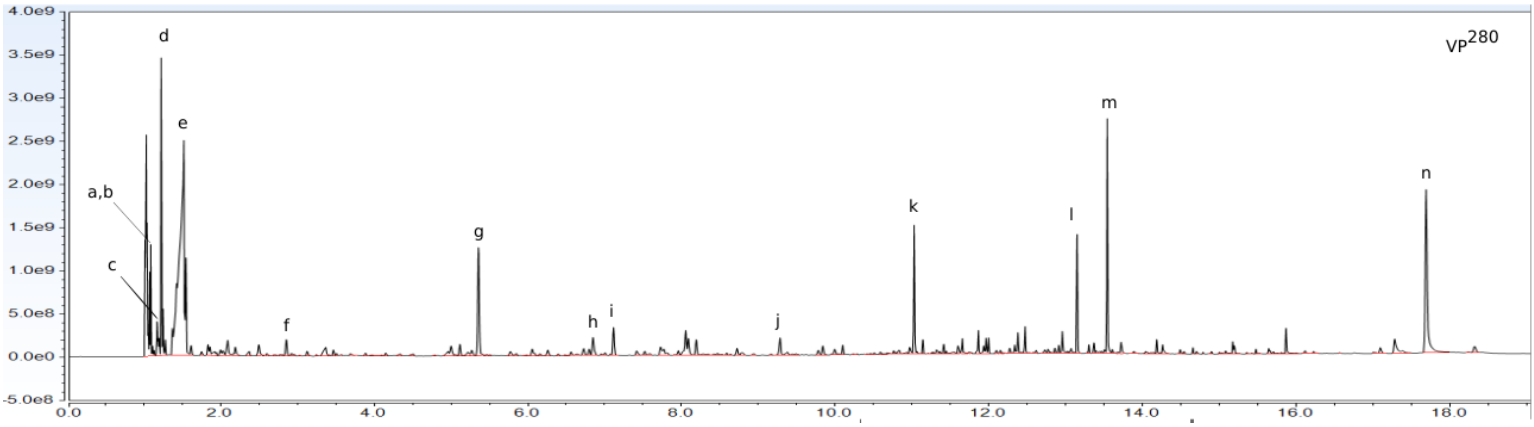
Consumer products that are intended to be consumed by inhalation after high temperature vaporization are a relatively new category of products that require a unique approach to determine the relative risks associated with consumer use. The most significant variable is that at elevated temperatures ingredients can rearrange, react and/or thermally degrade to create new chemical structures that can have fundamentally different chemical properties with different pharmacological consequences of use. This chemical change is dependent not only on the vaporization temperature but also on the composition of the material being vaporized. In some cases, compounds such as Vitamin E acetate which are Generally Regarded as Safe (“**GRAS”**) when introduced to a consumer at room temperature by ingestion may decompose to produce a complex mixture of chemical agents with significant toxicities at high temperatures. Furthermore, the lack of standardization for devices used to generate vapors after high temperature vaporization means that the temperature used is often unknown. Some of the compounds generated at elevated temperatures are themselves reactive and can further react, rearrange or decompose to alternate structures. This type of possible chemical behavior greatly complicated traditional chemical analysis as quantitation standards would also decompose at the temperatures in question. The sampling of vapors produced by devices is a potential approach to determine exposure risk for consumers of devices, however, the diversity of devices used makes determination of the correct devices to use for such studies a significant challenge. Regardless of the challenges, it is critically important to develop approaches to evaluate ingredients that could be used in products that are intended to be consumed by Inhalation after high temperature vaporization so that those materials that have a high likelihood of exposing the consumer to dangerous chemical agents are not used as ingredients. This work will highlight such an approach and apply it to the examination of 3 different potential ingredients, Vitamin E Acetate, Squalane and Squalene.

2.0 Vaporization Potential

Supra Research and Development (“**SUPRA**”) has developed an approach to determine the profile of the diverse range of thermally generated compounds generated by ingredients that are intended to be used in vaporizers. Rather than try and develop a standardized device for producing vapors, we use an analytical instrument that can heat a sample in a controlled manner and then collect and analyse the byproducts. The instrumentation we are using is called Headspace - Gas Chromatography Mass Spectrometry. In this approach a small quantity of sample is accurately heated in hermetically sealed glass vials to a series of well defined temperatures. At each temperature, a sample of the gas phase vapour, also called the “HeadSpace”, is collected and analysed. This analysis involves separation of individual chemical components in a Gas Chromatograph followed by detection in a Mass Spectrometer. The Mass Spectrometer allows for both identification of individual components as well as relative quantitation. The

information can be graphically displayed as a chromatogram where individual compounds are displayed as 'peaks'. A sample chromatogram is presented in Figure 1 below;

Figure 1: Vaporization Potential Chromatogram of Vitamin E Acetate collected at 280ºC



The Chromatograph shows the range of thermal degradation vaporization byproducts that are generated at a given temperature. We have defined this profile of products that can be produced at a given temperature as the Vaporization Potential ("**VP**"). This profile is temperature dependent and so to further define the profile we use the nomenclature **VP^{xyz}** where the number "xyz" is the temperature that the profile was gathered, for example **VP^{280}** is the Vaporization Potential profile collected at 280ºC.

The **VP** profiles are representative of the gas phase above a vaporized sample and thus the profile of chemical agents that would be delivered to the consumer when the user draws in this vapor when using a heated device. This information is critical to understanding the potential pharmacological consequences of inhaling the chemical profile generated at a specific temperature from a specific composition from a vaporized sample. However, at the current time there are no established regulatory limits to the quantity of chemical agents a user can safely be exposed to when using a vaporized product. The development of these types of regulatory standards and the universal acceptance of such standards would require a lengthy and potential contentious legal and scientific based process. Although, we fundamentally agree that this type of process has significant merit, there is also merit in finding an alternate approach that could identify additives, such as Vitamin E Acetate, that have been clearly linked to adverse health events, specifically the **EVALI** hospitalizations and deaths observed in late 2019 and 2020. **EVALI** is the name given by the US Centers for Disease Control and Prevention ("**CDC**") to the dangerous, newly identified lung disease linked to vaping. The name **EVALI** is an acronym that stands for e-cigarette or vaping product use-associated lung injury.

In order to develop an approach for screening ingredients and mixtures intended to be used in vaporization devices for their potential to produce dangerous chemical agents, we have developed an alternate approach we refer to as Equivalent Residual Solvent Analysis ("**ERSA**").

3.0 Equivalent Residual Solvent Analysis

Most finished consumer products intended for human consumption which could include exposure to solvents as extraction agents or chemical cleaning agents are required to be tested for Residual Solvents. This Residual Solvent Analysis is a well established approach and section 467 of the US Pharmacopeia ("**USP<467>**") outlines limits for a variety of potential residual solvents. These limits are universally accepted as levels that consumer products should not exceed in order to be safe. We have observed that many of the chemical agents observed when collecting VP data are in fact included on the residual solvent list. Given this we developed a testing protocol where we place a test sample in an hermetically sealed glass headspace vial, then heat this to a defined test temperature, say 240ºC, hold it for 5 minutes, then cool it to room temperature and then analysed this material using a validated Residual Solvent Analysis method. The validated Residual Solvent Analysis method we employ is also a Headspace-GCMS method, however, in this case the vial is only heated to 95ºC and an external calibration curve is used to quantify the observed residual solvents generated from the heated incubation step. We refer to this approach as Equivalent Residual Solvent Analysis ("**ERSA**"). If the residual solvent analysis indicates that a sample would fail, then we conclude that the material should not be used in any product intended for inhalation that heats the material at a temperature above the temperature at which it failed.

Even though the stated approach will work at any temperature, we have found that as the temperature approaches 300ºC almost all materials we have examined fail and for practical considerations we have selected a temperature of 280ºC as the highest test point in this study. Furthermore, we also consider 240ºC to be the highest temperature that any vaporization device should be set as, as above this the concentrations of problematic thermal degradation products increase drastically. Given that, we typically recommend that a **VP^{240}** be the test temperature for routine screening and the **ERSA** analysis at 240ºC be used as the definitive pass fail test criteria. We have also observed that 180ºC is a temperature where Cannabinoids, typical Terpenes and Nicotine and related chemical compounds are effectively vaporized with little or no thermal degradation. Although we have observed a few problematic compounds begin to thermally degrade at temperatures as low as 210ºC, most do not begin to degrade until the temperature exceeds 220ºC. With this in mind we can imagine a public health message that strongly discourages any vaporization above 420ºF or 215.6ºC.

4.0 Summary of results for Vitamin E Acetate, Squalane and Squalene

In this report 3 ingredients have been examined: Vitamin E Acetate, Squalane and Squalene. Each of these have "failed" the **ERSA** assessment at 240ºC.

4.1 Vitamin E Acetate

The chemical structure of Vitamin E Acetate is presented below. This compound was a known additive in e-juice and Cannabis concentrates associated with many of the **EVALI** hospitalizations and deaths observed in late 2019 and 2020. It has been suggested that this compound is responsible for many of the adverse health effects in the **EVALI** event.

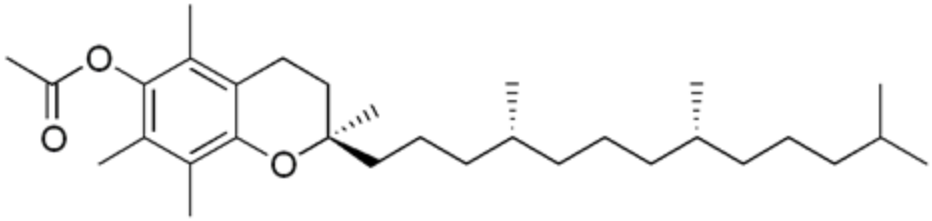


Figure 2: Chemical structure of Vitamin E Acetate

The VP profiles at a series of temperatures for Vitamin E Acetate is presented in Figure A.1 of Appendix A. The most dominant Oxidation products are Acetic acid and Formic acid and these are observed at sufficient quantities to have the compound fail the **ERSA** screening approach at 240ºC. This data is presented in Table A.2 presented in Appendix A.

4.2 Squalane

The chemical structure of squalene is presented in Figure 3 below. This is a possible ingredient that could be used in vaporization devices.

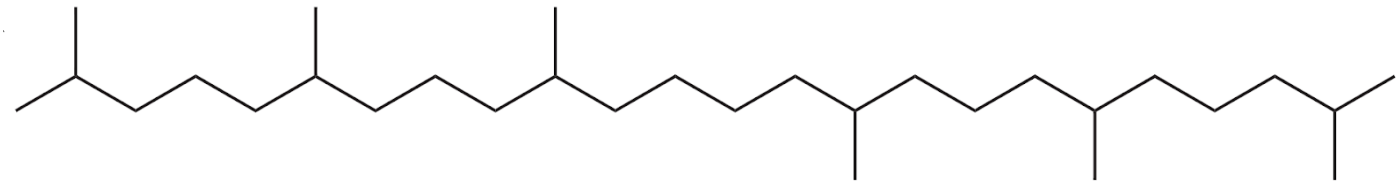


Figure 3: Chemical structure of squalane

The VP profiles at a series of temperatures for this compound is presented in Figure B.1 of Appendix B. The most dominant Oxidation products are Acetone, Methanol and Acetic acid and these are produced at sufficient quantities to have the compound fail the **ERSA** analysis at 240ºC. This **ERSA** data is presented in

Table B.2 presented in Appendix B. There are a diverse number of thermal degradation and oxidation products produced by squalane and based on this and the very high concentration of Acetone, Methanol and Acetic Acid we speculate that this additive would produce more diverse and adverse health effects as Vitamin E Acetate does.

4.3 Squalene

The chemical structure of squalene is presented in Figure 4 below. This is also a possible ingredient that could be used in vaporization devices.

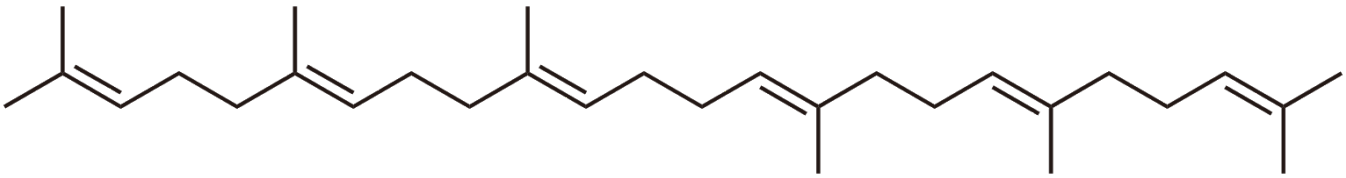


Figure 4: Chemical structure of squalene

The VP profiles at a series of temperatures for this compound is presented in Figure C.1 of Appendix C. There are a large number of Oxidation products generated including Acetone, Methanol, Acetic acid and Formic Acid that are produced at sufficient quantities to have the compound fail the **ERSA** analysis at 240ºC. This **ERSA** data is presented in Table C.2 presented in Appendix C. The diverse number of thermal degradation and oxidation products produced by squalene is of significant concern, especially, because this degradation begins at much lower temperature, 180ºC, than observed for other ingredients that we have studied previously. It is speculated that Squalene would produce more adverse health effects as Vitamin E Acetate does and that these adverse effects could begin at much lower vaporization temperatures.

5.0 Conclusion

The three compounds that we have examined in this report, Vitamin E Acetate, Squalane and Squalene each have failed the **ERSA** assessment protocol we have defined at 240ºC. Vitamin E Acetate has been identified as a problematic ingredient associated with **EVALI** hospitalizations and deaths. The data presented here suggests that Squalane and Squalene thermally degrade in a manner that produces higher levels of chemical agents than we observed for Vitamin E Acetate. From this, we speculate that these compounds could be more problematic than Vitamin E Acetate. However, it should be noted that these are speculations based on assumptions and this opinion is provided for discussion purposes only and is not intended to be a definitive statement on the safety of a given product or ingredient.

Supra Research and Development Inc.
106-2293 Leckie Rd, Kelowna B.C. V1X 6Y5
http://suprarnd.ca/ | inquire@suprarnd.ca | 1-855-437-8772

Appendix A: Sample Results for Vitamin E Acetate

Client ID: n.a.

Supra Details: α-Tocopheryl acetate (Vitamin E acetate) (Sigma-Aldrich PN#R1030 Lot#LRAC1696)

Batch ID: 201022_VP-RS-quant-Oregon

Submission Date: 2020 October 15

Reporting Date: 2020 November 12

Analysis Date: 2020 October 22

Analyst: RJH / SRS

Authorized By: Ryan Hayward

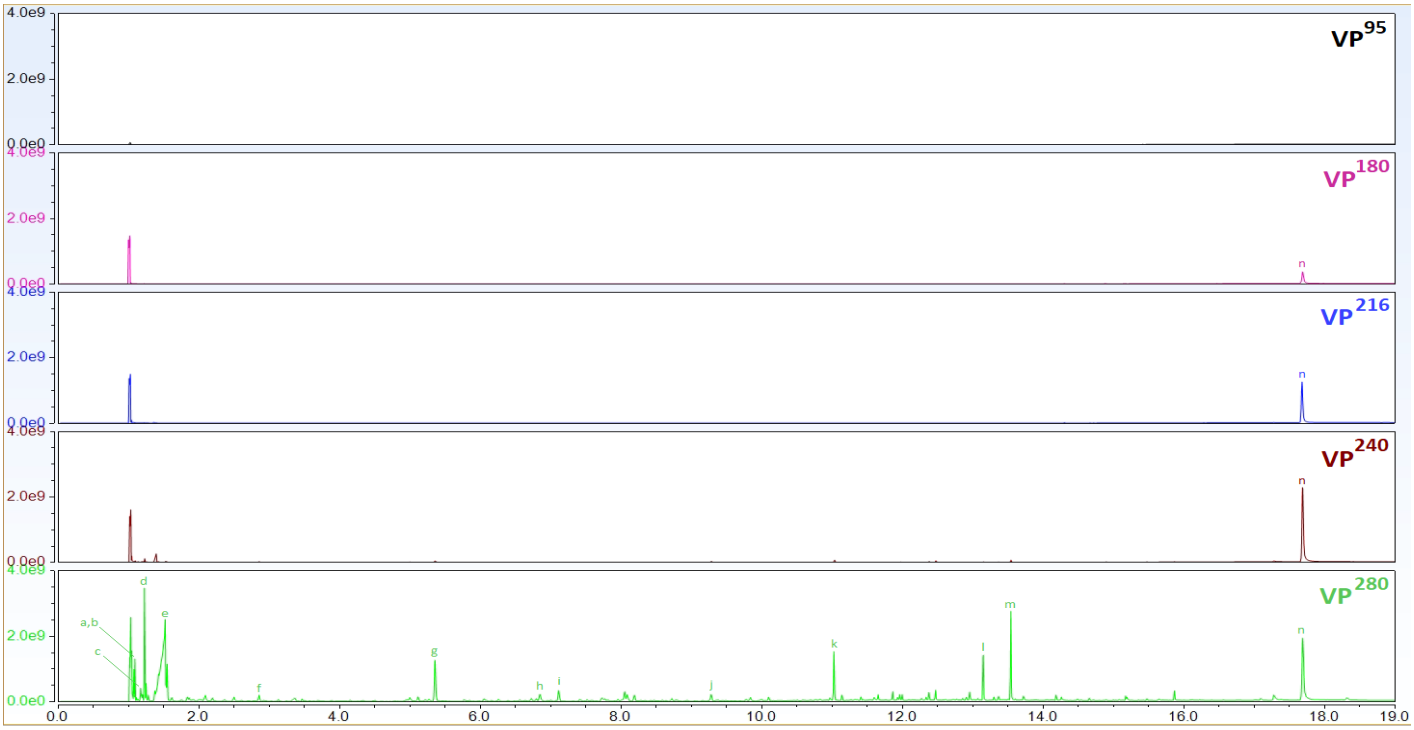
Job Function: Laboratory Manager

Date Authorized: 2020 November 10

Signature: 

Supra Research and Development Inc.
106-2293 Leckie Rd, Kelowna B.C. V1X 6Y5
http://suprarnd.ca/ | inquire@suprarnd.ca | 1-855-437-8772

Figure A.1: Vaporization Potential Chromatograms For Vitamin E Acetate



Total Ion Chromatograms (TICs) of VP^{95}, VP^{180}, VP^{216}, VP^{240} and VP^{280} of vitamin E acetate. The chromatograms are scaled to the same *y*-axes.

Table A.1: Identified peaks for Vitamin E Acetate (qualitative profile)

Compound	Retention time (min)	Chromatogram label
methanol	1.07	a
acetaldehyde*	1.09	b
oxalic acid*	1.17	c
acetone	1.23	d
formic acid	1.50	e
hexanal*	2.86	f
6-methyl-2-heptanone*	5.36	g
2-nonanone*	6.85	h
4-methyl-3-pentenoic acid*	7.13	i
4,8-dimethylnonanol*	9.28	j
6,10-dimethyl-2-undecanone*	11.04	k
6,10,14-trimethyl-2-pentadecanone*	13.15	l
3-formyl-4-hydroxy-2,5,6-trimethylphenyl acetate*	13.54	m
vitamin E acetate	17.69	n

List of identified compounds in thermally-treated samples (see Figure 1 for labelled chromatograms). Compounds marked with an asterisk (*) were identified using NIST library matching (>800 SI and RSI). All other compounds were identified using analytical standards

Supra Research and Development Inc.
106-2293 Leckie Rd, Kelowna B.C. V1X 6Y5
http://suprarnd.ca/ | inquire@suprarnd.ca | 1-855-437-8772

Table A.2: Equivalent Residual Solvent Analysis at 240ºC Vitamin E Acetate

	USP limit	VP^{240}
2-Butanone	5000	< 1000
2-Propanol	5000	nd
Acetone	5000	< 1000
Acetonitrile	410	nd
Benzene	2	nd
Cyclohexane	3880	nd
Ethanol	5000	< 1000
Ethyl formate	5000	nd
Hexane	290	nd
Isobutanol	5000	< 1000
Isopropyl acetate	5000	< 1000
Methanol	3000	< 600
Methylcyclohexane	1180	nd
n-Pentane	5000	< 1000
Acetic acid*	5000	> 10000
Formic acid*	5000	> 10000

Table 2 Description: Quantitated concentrations (parts-per-million [ppm] relative to original sample mass [Table 3]) of degradation products identified for each sample treatment at 240 ºC. Values were calculated using a full evaporation technique (FET) headspace method calibrated with residual solvent standards. Calibration ranges were 0.2x to 2x each analyte's USP limit. Results outside the calibration range are reported as greater than (>) or less than (<) the respective upper or lower limits of calibration. A semi-quantitative calibration was performed for formic acid and acetic acid. These compounds have been marked with an asterisk (*) and their results should be treated as estimates. Shaded values indicate failures.

Table A.3: Experimental details Vitamin E Acetate

After accurate weighing (Table 3), all samples were incubated in gas-tight headspace vials fitted with PTFE-lined silicone septa for temperatures ranging from 95 - 280 °C (*n* = 1/temperature). All incubations were performed for five minutes and included a blank vial alongside client formulations.

	Vaporization Potential ($VP^{°C}$)				
	VP^{95}	VP^{180}	VP^{216}	VP^{240}	VP^{280}
Vitamin E acetate (g)	0.0104	0.0098	0.0111	0.0111	0.0105

Masses of materials used for each temperature treatment. Samples were incubated at their designated temperature for five minutes to achieve an equilibrated headspace, from which 1 mL was sampled for analysis. Sampling was performed directly from the incubated vial to reflect delivery of volatiles into the headspace at respective temperatures.

Supra Research and Development Inc.
106-2293 Leckie Rd, Kelowna B.C. V1X 6Y5
http://suprarnd.ca/ | inquire@suprarnd.ca | 1-855-437-8772

Appendix B: Sample Results for Squalane

Client ID: n.a.

Sample Details: Squalane (Sigma-Aldrich PN#PMR1417 Lot#LRAC4099)

Batch ID: 201022_VP-RS-quant-Oregon

Submission Date: 2020 October 15

Reporting Date: 2020 November 12

Analysis Date: 2020 October 22

Analyst: RJH / SRS

Authorized By: Ryan Hayward

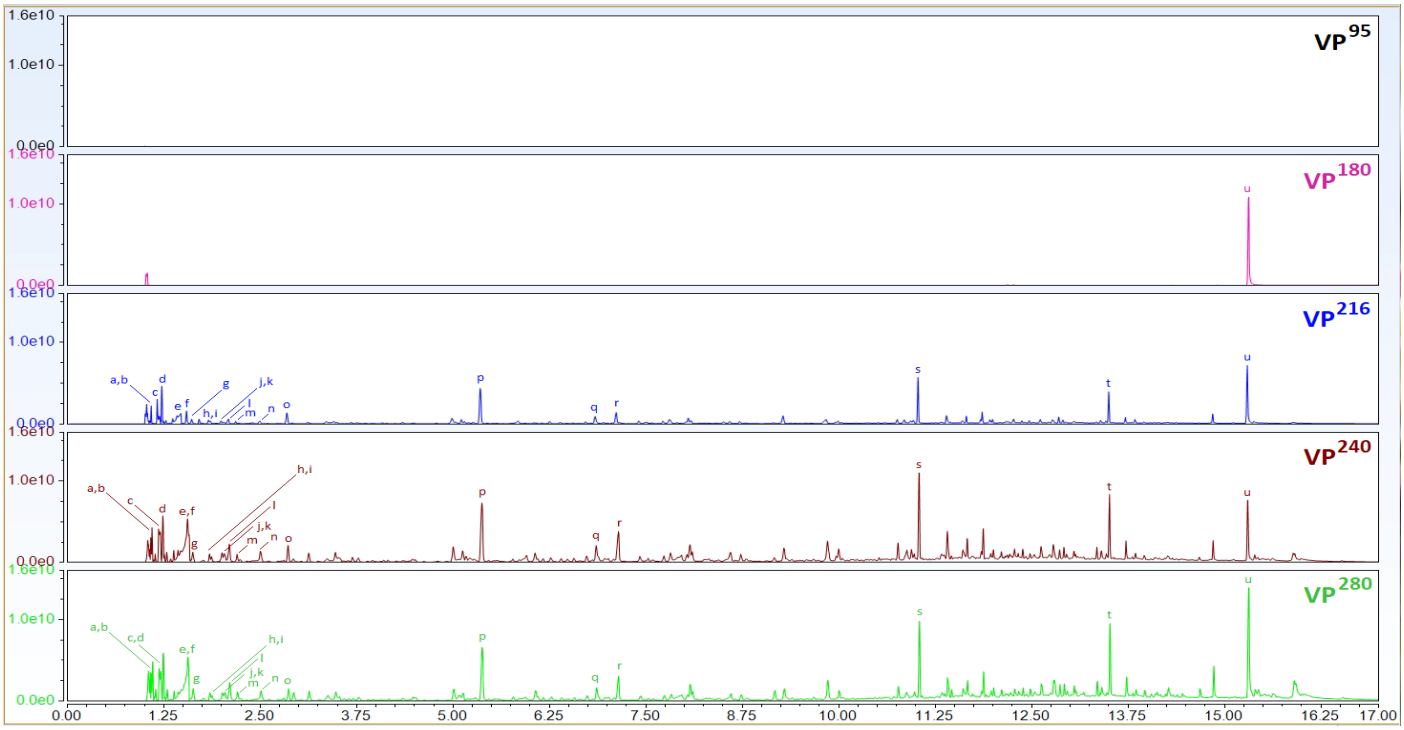
Job Function: Laboratory Manager

Date Authorized: 2020 November 10

Signature: 

Supra Research and Development Inc.
106-2293 Leckie Rd, Kelowna B.C. V1X 6Y5
http://suprarnd.ca/ | inquire@suprarnd.ca | 1-855-437-8772

Figure B.1: Vaporization Potential Chromatograms For Squalane



Total Ion Chromatograms (TICs) of VP^{95}, VP^{180}, VP^{216}, VP^{240} and VP^{280} of squalane. See Table 1 for peak labels. The chromatograms are scaled to the same *y*-axes.

Table B.1: Identified peaks for Squalane (qualitative profile)

Compound	Retention time (min)	Chromatogram label
methanol	1.07	a
acetaldehyde*	1.09	b
oxalic acid*	1.17	c
acetone	1.23	d
acetic acid	1.50	e
2-butanone	1.55	f
4-methyl-3-pentenal*	1.62	g
3-methylbutanal*	1.85	h
3-methyl-2-butanone*	1.87	i
2-methylheptane*	2.00	j
2,2-dimethyltethrahydrofuran	2.04	k
2-pentanone*	2.10	l
acetol*	2.19	m
2-hexanone*	2.50	n
hexanal*	2.86	o
6-methyl-2-heptanone*	5.36	p
2-nonanone*	6.85	q
4-methyl-3-pentenoic acid*	7.14	r
6,10-dimethyl-2-undecanone*	11.04	s
2-nonadecanone*	13.52	t
squalane	15.31	u

Supra Research and Development Inc.
106-2293 Leckie Rd, Kelowna B.C. V1X 6Y5
http://suprarnd.ca/ | inquire@suprarnd.ca | 1-855-437-8772

List of identified compounds in thermally-treated samples (see Figure B.1 for labelled chromatograms). Compounds marked with an asterisk (*) were putatively identified using NIST library matching (>800 SI and RSI). All other compounds were identified using analytical standards.

Table B.2: Equivalent Residual Solvent Analysis at 240ºC Squalane

	USP limit	VP^{240}
2-Propanol	5000	nd
Acetone	5000	> 10000
Acetonitrile	410	< 82
Benzene	2	nd
Cyclohexane	3880	< 776
Ethanol	5000	< 1000
Ethyl formate	5000	nd
Hexane	290	nd
Isobutanol	5000	nd
Isopropyl acetate	5000	nd
Methanol	3000	> 6000
Methylcyclohexane	1180	< 236
n-Pentane	5000	< 1000
Acetic acid*	5000	> 10000
Formic acid*	5000	< 1000

Quantitated concentrations (parts-per-million [ppm] relative to original sample mass [Table B.3]) of degradation products identified for each sample treatment at 240 ºC. Values were calculated using a full evaporation technique (FET) headspace method calibrated with residual solvent standards. Calibration ranges were 0.2x to 2x each analyte's USP limit. Results outside the calibration range are reported as greater than (>) or less than (<) the respective upper or lower limits of calibration. A semi-quantitative calibration was performed for formic acid and acetic acid. These compounds have been marked with an asterisk (*) and their results should be treated as estimates. Shaded values indicate failures.

Table B.3: Experimental details Squalane

After accurate weighing (Table B.3), all samples were incubated in gas-tight headspace vials fitted with PTFE-lined silicone septa for temperatures ranging from 180 - 300 °C (*n* = 1/temperature). All incubations were performed for five minutes and included a blank vial alongside client formulations.

	Vaporization Potential ($VP^{°C}$)				
	VP^{95}	VP^{180}	VP^{216}	VP^{240}	VP^{280}
Squalane (g)	0.0100	0.0094	0.0094	0.0103	0.0099

Masses of materials used for each temperature treatment. Samples were incubated at their designated temperature for five minutes to achieve an equilibrated headspace, from which 1 mL was sampled for analysis. Sampling was performed directly from the incubated vial to reflect delivery of volatiles into the headspace at respective temperatures.

Supra Research and Development Inc.
106-2293 Leckie Rd, Kelowna B.C. V1X 6Y5
http://suprarnd.ca/ | inquire@suprarnd.ca | 1-855-437-8772

Appendix C: Sample Results for Squalene

Client ID: n.a.

Supra Details: Squalene (Sigma-Aldrich PN#S3626 Lot#MKCJ2769)

Batch ID: 201022_VP-RS-quant-Oregon

Submission Date: 2020 October 15

Reporting Date: 2020 November 12

Analysis Date: 2020 October 22

Analyst: RJH / SRS

Authorized By: Ryan Hayward

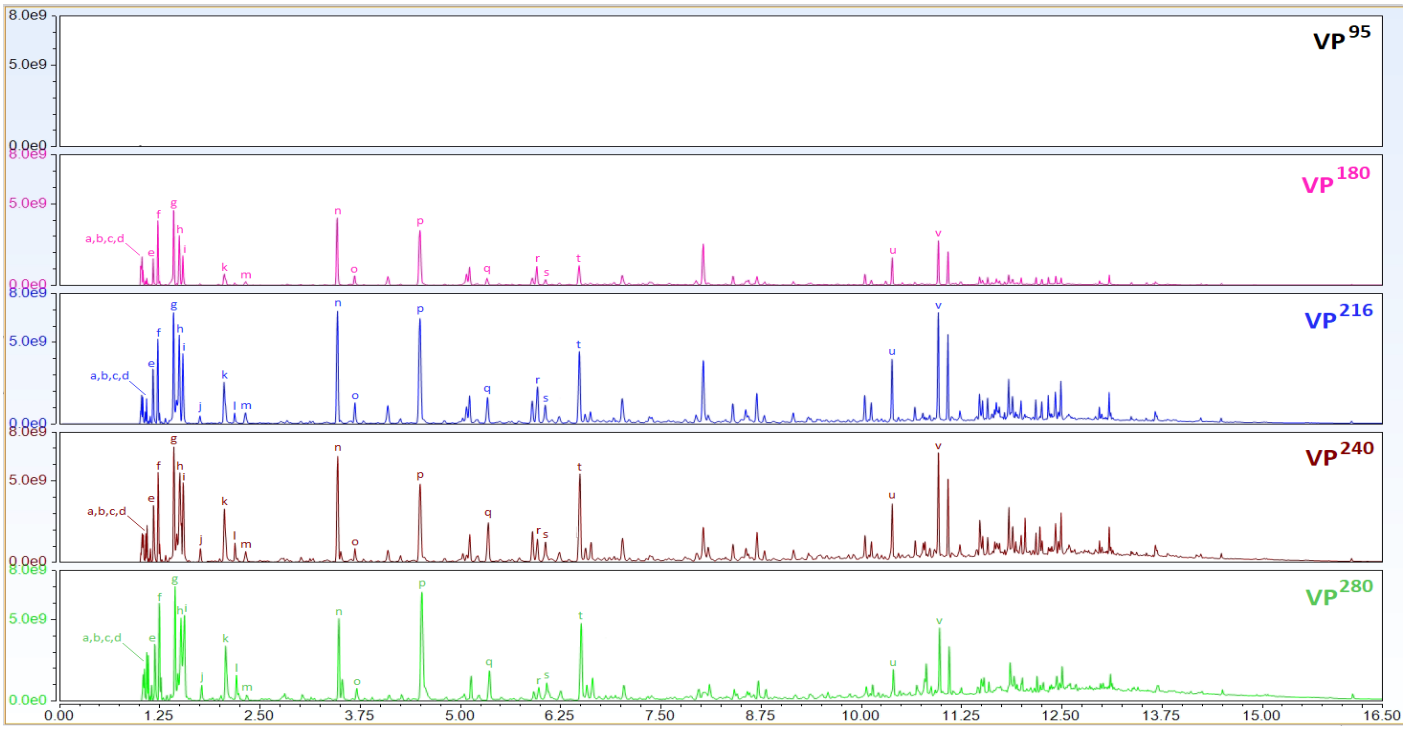
Job Function: Laboratory Manager

Date Authorized: 2020 November 10

Signature: 

Supra Research and Development Inc.
106-2293 Leckie Rd, Kelowna B.C. V1X 6Y5
http://suprarnd.ca/ | inquire@suprarnd.ca | 1-855-437-8772

Figure C.1: Vaporization Potential Chromatograms For Squalene



Total Ion Chromatograms (TICs) of VP^{95}, VP^{180}, VP^{216}, VP^{240} and VP^{280} of squalene. See Table 1 for peak labels. The chromatograms are scaled to the same *y*-axes.

Table C.1: Identified peaks for Squalene (qualitative profile)

Compound	Retention time (min)	Chromatogram label
methanol	1.07	a
acetaldehyde*	1.09	b
glyoxal*	1.10	c
ethanol	1.13	d
oxalic acid*	1.17	e
acetone	1.23	f
methacrolein*	1.42	g
2-methyl-3-buten-2-ol*	1.50	h
3-buten-2-one*	1.54	i
3-hydroxy-3-methyl-2-butanone*	1.76	j
3-ethyl-2,2-dimethyloxirane*	2.06	k
1-hydroxy-2-propanone*	2.18	l
1-ethyl-5-methylcyclopentene*	2.32	m
3-methyl-2-butenal*	3.46	n
4-hydroxy-2-butanone*	3.69	o
3-methylcyclopentyl acetate*	4.50	p
4,4,5-trimethyl-1,3-dioxan-5-ol*	5.36	q
2,3-dimethyl-3-buten-2-ol*	5.95	r
6-methyl-5-hepten-2-one*	6.06	s
1-(1-butenyloxy)pentane*	6.49	t
citral*	10.04	u
3,6-dimethyloctan-2-one*	10.96	v

Supra Research and Development Inc.
106-2293 Leckie Rd, Kelowna B.C. V1X 6Y5
http://suprarnd.ca/ | inquire@suprarnd.ca | 1-855-437-8772

List of identified compounds in thermally-treated samples (see Figure C.1 for labelled chromatograms). Compounds marked with an asterisk (*) were putatively identified using NIST library matching (>800 SI and RSI). All other compounds were identified using analytical standards.

Table C.2: Equivalent Residual Solvent Analysis at 240ºC Squalene

	USP limit	VP^{240}
2-Propanol	5000	< 1000
Acetone	5000	> 10000
Acetonitrile	410	nd
Benzene	2	0.4
Cyclohexane	3880	nd
Ethanol	5000	1382
Ethyl formate	5000	< 1000
Hexane	290	136
Isobutanol	5000	< 1000
Isopropyl acetate	5000	< 1000
Methanol	3000	> 6000
Methylcyclohexane	1180	< 236
n-Pentane	5000	< 1000
Acetic acid*	5000	> 10000
Formic acid*	5000	> 10000

Quantitated concentrations (parts-per-million [ppm] relative to original sample mass [Table 3]) of degradation products identified for each sample treatment at 240 ºC. Values were calculated using a full evaporation technique (FET) headspace method calibrated with residual solvent standards. Calibration ranges were 0.2x to 2x each analyte's USP limit. Results outside the calibration range are reported as greater than (>) or less than (<) the respective upper or lower limits of calibration. A semi-quantitative calibration was performed for formic acid and acetic acid. These compounds have been marked with an asterisk (*) and their results should be treated as estimates. Shaded values indicate failures.

Table C.3: Experimental details Squalene

After accurate weighing (Table 3), all samples were incubated in gas-tight headspace vials fitted with PTFE-lined silicone septa for temperatures ranging from 95 - 280 °C (*n* = 1/temperature). All incubations were performed for five minutes and included a blank vial alongside client formulations.

	Vaporization Potential ($VP^{°C}$)				
	VP^{95}	VP^{180}	VP^{216}	VP^{240}	VP^{280}
Squalene (g)	0.0096	0.0095	0.0102	0.0103	0.0111

Masses of materials used for each temperature treatment. Samples were incubated at their designated temperature for five minutes to achieve an equilibrated headspace, from which 1 mL was sampled for analysis. Sampling was performed directly from the incubated vial to reflect delivery of volatiles into the headspace at respective temperatures.

Supra Research and Development Inc.
106-2293 Leckie Rd, Kelowna B.C. V1X 6Y5
http://suprarnd.ca/ | inquire@suprarnd.ca | 1-855-437-8772

END OF REPORT

Results reported by Supra Research and Development are representative of the materials as provided by the client.

Results are provided for information only and are not intended to comment on the safety of a given product.

Supra Research and Development shall retain all reports in a secure manner to prevent unauthorized access. Supra reserves the right to charge for the reissuance of reports in some instances.

Supra Research and Development will retain samples for a minimum period of 45 days following the release of the report. After 90 days following the release of the report samples are subject to disposal.