



# ELECTRONIC CIGARETTES / E-CIGARETTE REFILL CONTAINER EMISSIONS REPORT

Analytical Summary Report in Support of the European Tobacco  
Product Directive (2014/40/EU) Article 20

## Product Details

<b>Product Form:</b>	E-Liquid (Refill Container)
<b>Brand:</b>	Black Aniseed
<b>Description:</b>	50/50 VG/PG
<b>Strength:</b>	18mg/ml
<b>Bottle Size:</b>	10ml
<b>SKU:</b>	NA
<b>Batch Tested:</b>	NS20288
<b>Client Job Ref:</b>	NA
<b>Client Name:</b>	Aquavape

**Approved By:** Lynne Hessel

**Approved Date:** 24/10/2016 16:37:39

**User ID:** LHessel

**Title:** Quality and Business Systems Manager

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**Client Name:** Aquavape  
**Description:** 50/50 VG/PG  
**Client Job Ref:** NA  
**Batch Number:** NS20288

**Brand:** Black Aniseed  
**SKU:** NA  
**Strength:** 18mg/ml  
**Bottle Size:** 10ml

## Emissions Data Summary

Component	Result	Units	CAS No	Description
Formaldehyde	4.4	µg/ten inhalations	50-00-0	Carbonyls in Emissions by HPLC-MS
Acetaldehyde	4.2	µg/ten inhalations	75-07-0	Carbonyls in Emissions by HPLC-MS
Propionaldehyde	Not Detected	µg/ten inhalations	123-38-6	Carbonyls in Emissions by HPLC-MS
Acrolein	Not Detected	µg/ten inhalations	107-02-8	Carbonyls in Emissions by HPLC-MS
Crotonaldehyde	Not Detected	µg/ten inhalations	123-73-9	Carbonyls in Emissions by HPLC-MS
Butraldehyde	Not Detected	µg/ten inhalations	123-72-8	Carbonyls in Emissions by HPLC-MS
Diacetyl (butane-2,3-dione)	Not Detected	µg/ten inhalations	431-03-08	Carbonyls in Emissions by HPLC-MS
Acetylpropionyl (2,3-pentanedione)	Not Detected	µg/ten inhalations	600-14-6	Carbonyls in Emissions by HPLC-MS
Aerosol Mass	51950.0	µg/ten inhalations	N/A	Carbonyls in Emissions by HPLC-MS

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**Client Name:** Aquavape**Description:** 50/50 VG/PG**Client Job Ref:** NA**Batch Number:** NS20288**Brand:** Black Aniseed**SKU:** NA**Strength:** 18mg/ml**Bottle Size:** 10ml

## ANALYTICAL METHOD

## PARAMETERS

The following tables detail the parameters utilised during the collection of the product emissions.

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<b>Client Job Ref:</b>	NA	<b>Strength:</b>	18mg/ml
<b>Batch Number:</b>	NS20288	<b>Bottle Size:</b>	10ml

## Analytical Method Parameters

Test Method	Carbonyls in Emissions by HPLC-MS
Inhalation Numbers Block 1	11 to 20
Device EC-ID (if available)	NA
Device Brand	Aquavape
Device Subtype Brand	Aquavape
Device available on the European Market	Yes
Device Power Setting	Fixed
Inhalation Volume (ml)	55
Inhalation Interval (Seconds)	30
Inhalation Time (Seconds)	3
Inhalation Profile	Square Wave
Inhalation Flow Rate (L/min)	1.10

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## ANALYTICAL DATA

The following tables detail all analytical data points generated during the emissions study including calculated mean values.

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**Client Job Ref:** NA  
**Batch Number:** NS20288

**Brand:** Black Aniseed  
**SKU:** NA  
**Strength:** 18mg/ml  
**Bottle Size:** 10ml

## Analytical Data

**Method:** Carbonyls in Emissions by HPLC-MS

Component	Value	Units	Reporting Threshold
Formaldehyde Block 1	4.4	µg/ten inhalations	1.0 µg/ten inhalations
<b>Formaldehyde Mean</b>	<b>4.4</b>	<b>µg/ten inhalations</b>	
Acetaldehyde Block 1	4.2	µg/ten inhalations	1.0 µg/ten inhalations
<b>Acetaldehyde Mean</b>	<b>4.2</b>	<b>µg/ten inhalations</b>	
Propionaldehyde Block 1	Not Detected	µg/ten inhalations	1.0 µg/ten inhalations
<b>Propionaldehyde Mean</b>	<b>Not Detected</b>	<b>µg/ten inhalations</b>	
Acrolein Block 1	Not Detected	µg/ten inhalations	1.0 µg/ten inhalations
<b>Acrolein Mean</b>	<b>Not Detected</b>	<b>µg/ten inhalations</b>	
Crotonaldehyde Block 1	Not Detected	µg/ten inhalations	1.0 µg/ten inhalations
<b>Crotonaldehyde Mean</b>	<b>Not Detected</b>	<b>µg/ten inhalations</b>	
Butyraldehyde Block 1	Not Detected	µg/ten inhalations	1.0 µg/ten inhalations
<b>Butyraldehyde Mean</b>	<b>Not Detected</b>	<b>µg/ten inhalations</b>	
Diacetyl (butane-2,3-dione) Block 1	Not Detected	µg/ten inhalations	1.0 µg/ten inhalations
<b>Diacetyl (butane-2,3-dione) Mean</b>	<b>Not Detected</b>	<b>µg/ten inhalations</b>	
Acetylpropionyl (2,3-pentanedione) Block 1	Not Detected	µg/ten inhalations	1.0 µg/ten inhalations
<b>Acetylpropionyl (2,3-pentanedione) Mean</b>	<b>Not Detected</b>	<b>µg/ten inhalations</b>	
<b>Mean Aerosol Mass</b>	<b>51950.0</b>	<b>µg/ten inhalations</b>	

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# ANALYTICAL METHOD SUMMARY

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## Method Title: Emissions - The Determination Of Carbonyl Compounds In Aerosol

### Overview

During the vapourisation process, the e-liquid is heated to temperatures often exceeding 300°C. These temperatures are sufficiently high to induce physical changes of e-liquids and chemical reactions between the constituents of e-liquids. Solvents contained in the nicotine formulation may undergo pyrolysis leading to formation of potentially toxic compounds.

Both glycerol and propylene glycol have been shown to thermally decompose at high temperatures generating low molecular weight carbonyl compounds with established toxic properties (e.g., formaldehyde, acetaldehyde and acrolein). The operating conditions of the e-cigarette device plays a pivotal role in determining the rate at which carbonyl compounds are produced during e-cigarette use. In addition, it is also known that the higher the propylene glycol content in the e-liquid, the greater the chance that higher levels of carbonyls will be detected in the vapour.

The method is designed to generate a known amount of aerosol under controlled sampling conditions from a specified e-liquid / e-cigarette combination, which is then captured in a derivatisation solution. (Derivatisation is a procedural technique that is required to modify the carbonyl compounds functionality in order to enable chromatographic separation and detection). The resulting liquid samples are stabilised and then analysed using high performance liquid chromatography with tandem mass spectrophotometric detection (HPLC-MS/MS).

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<b>Batch Number:</b>	NS20288	<b>Bottle Size:</b>	10ml

## Target Analytes

The following is a list of carbonyl compounds which may be produced from e-cigarettes during normal or reasonably foreseeable conditions.

Name	CAS N°	IARC Monograph Group*
<b>Formaldehyde</b>	50-00-0	Group 1
<b>Acetaldehyde</b>	75-07-0	Group 2B
<b>Propionaldehyde</b>	123-38-6	Group 3
<b>Acrolein</b>	107-02-8	Group 3
<b>Crotonaldehyde</b>	123-73-9	Group 3
<b>Butryaldehyde</b>	123-72-8	N/A
<b>Diacetyl</b>	431-03-8	N/A
<b>Acetylpropionyl</b>	600-14-6	N/A

\* IARC is the International Agency for Research on Cancer, an agency of the World Health Organization (WHO). IARC classifies carcinogens in five categories ranging from carcinogenic to humans (Group 1) to probably not carcinogenic to humans (Group 4). For more information please visit <http://monographs.iarc.fr/>

To date, there is no official guidance on appropriate toxicological safe levels of carbonyls permitted in e-cigarette vapour. To establish appropriate limits of detection / quantification (LOD / LOQ) levels for the analytical methodology, the following table outlines an interpretation of permitted daily occupational exposure limits as defined by the United States Department of Labor, Occupational Safety and Health Administration (OSHA) <[https://www.osha.gov/dts/chemicalsampling/toc.toc\\_chemsamp.html](https://www.osha.gov/dts/chemicalsampling/toc.toc_chemsamp.html)>. Where multiple limits are defined (e.g. by industry), the lower limit has been used in all cases. For the purposes of correlating exposure limits relative to daily e-cigarette usage, a maximum of 500 inhalations per day has been defined as 'normal or reasonably foreseeable conditions'. An 'alert limit' of 50% of the PEL (Permitted Exposure Limit) has been established as a guide.

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Name	Maximum PEL (mg)	Alert Limit 50% of PEL (µg/ day 500 inhalations)	Alert Limit 50% of PEL (µg/ 10 inhalations)	Reporting Threshold (µg/ 10 inhalations)
Formaldehyde	0.1	57.0	1.1	1.0
Acetaldehyde	261.3	130626.2	2612.5	1.0
Propionaldehyde	276	137824.1	2756.5	1.0
Acrolein	1.3	664.9	13.3	1.0
Crotonaldehyde	5.0	2494.0	49.9	1.0
Butryaldehyde	427.6	213793.5	4275.9	1.0
Diacetyl*	0.2	83.1	1.7	1.0
Acetylpropionyl*	11.9	5936.4	118.7	1.0

PEL = Permitted Exposure Limit

\* The detection of diacetyl and acetylpropionyl will be on a limit test basis only - i.e. methodology will confirm absence in the emissions below a specified level.

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## Methodology References

Method Reference	Reference Title
<b>CORESTA RECOMMENDED METHOD N° 81</b>	Routine Analytical Machine For E-Cigarette Aerosol Generation And Collection – Definitions And Standard Conditions (June 2015)
<b>ISO 7210:2013</b>	Routine Analytical Cigarette-Smoking Machine – Additional Test Methods For Machine Verification
<b>ISO 3308:2012</b>	Routine Analytical Cigarette-Smoking Machine – Definitions And Standard Conditions
<b>ISO 558:1980</b>	Conditioning And Testing – Standard Atmospheres - Definitions
<b>United States Department of Labor, Occupational Safety and Health Administration</b>	Chemical Sampling Information
<b>CORESTA RECOMMENDED METHOD N° 74</b>	Determination of Selected Carbonyls in Mainstream Cigarette Smoke By HPLC (July 2014)
<b>Nicotine &amp; Tobacco Research, Volume 17, Issue 2 Pg. 168-174</b>	Evaluation of electronic cigarette liquids and vapour for the presence of selected inhalation toxins. <i>Farsalinos et al</i>
<b>Nicotine &amp; Tobacco Research, Volume 16, Number 10 (October 2014) 1319-1326</b>	Carbonyl Compounds in Electronic Cigarette Vapors: Effects of Nicotine Solvent and Battery Output Voltage <i>Kosminder et al</i>
<b>Tob Control. 2014 March ; 23(2): 133-139</b>	Levels of selected carcinogens and toxicants in vapor from electronic cigarettes <i>Goniewicz et al</i>
<b>AFNOR XP D90-300-2</b>	Cigarettes électroniques et eliquides
<b>Journal of Regulatory Toxicology and Pharmacology 75 (2016) 58-65</b>	Effect Of Variable Power Levels On The Yield Of Total Aerosol Mass And Formation Of Aldehydes In e-Cigarette Aerosols <i>Gillman et al</i>
<b>CORESTA Task Force Technical Report</b>	2014 Electronic Cigarette Aerosol Parameters Study

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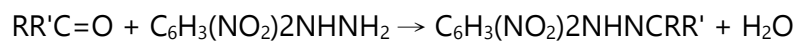
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## Method Principles

The materials (e-cigarette devices or e-liquid) under test will be operated under controlled environmental conditions using an automated vaping machine. A defined number of inhalations from each sample will be generated. The aerosol generated for each set of inhalations will be collected into an acidified derivatisation solution containing 2, 4-dinitrophenylhydrazine. This solution efficiently traps the aerosol and simultaneously converts the carbonyl compounds into a hydrazone species according to the following equation:



The conversion to a hydrazone form enables the original carbonyls to be detected at very low concentrations while improving the method selectivity. The acidified solution is subsequently neutralized to quench the reaction described above. Aliquots of the collected samples are then analysed by HPLC-MS/MS. To compensate for the highly variable matrix components which can cause significant modification of ionization in the mass spectrometer, isotopically stable deuterium labelled internal standards are used for the quantification of the carbonyl compounds.

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