



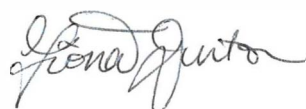
Test Report

Ready Mix Naturals e-liquid analysis

For the Attention of: Ready Mix

Study Reference	HAL23-043
Test Report Number	HAL23-043-0-1
Study Description	e-liquid HPHC analysis
Submitting Customer	Ready Mix
Customer Reference	Ready Mix Naturals
Date of Test Report	04-Sep-2023

Authorised on behalf of Element Materials Technology Analytical Services Ltd

 4th-Sept-2023

Laboratory Manager

Note:

- The results relate only to the items tested.
- Tests not covered under the scope of Element Manchester's ISO 17025 accreditation are identified with an asterisk (*).
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Element Sample ID	Customer Sample ID	Date Received
H85138	Nixodine / Vegetable Glycerine For Lab Testing / No Flavour - 100mg	24-Apr-2023
H85158	VG/Nixodine 6mg Solution without flavouring 30ml	24-Apr-2023

Glossary

Term	Definition
ACM	Aerosol Collected Mass
SD	Standard Deviation
%RSD	Relative Standard Deviation expressed as a percentage
LLD	Low-Liquid Detection
PG	Propylene Glycol
VG	Vegetable Glycerin
EG	Ethylene glycol
DEG	Diethylene glycol
LoQ/LLoQ	(Lower) Limit of Quantification as determined during method validation.
U	Uncertainty of measurement – expanded uncertainty with k=2 unless otherwise stated.
s	Seconds
mL	Millilitres
g	Grams
LC-MS	Liquid Chromatography-Mass Spectrometry
GC-MS	Gas Chromatography-Mass Spectrometry
GC-FID	Gas Chromatography-Flame Ionisation Detection
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry

Aerosol Generation Conditions

Aerosol samples were generated using a regime described in Table 1 and based upon those described in CRM 81 / ISO 20768.

Table 1

Instrument	Cerulean SM450e
Puff Volume	55 mL
Puff Duration	3 s
Puff Interval	30 s
Puff Block	Testing suite dependant
Vaping Angle	-45°
Puff Profile	Square Wave

CFPs and holders were weighed pre and post each puff block to enable ACM to be calculated.

Study Reference: HAL23-043

Test Method: TM_SOP_045* (e-liquid)

Major Components (Nicotine, Propylene Glycol, Glycerin and Menthol) in E-liquids and ENDS Emissions by GC-FID
Storage conditions: Ambient

The results of 3 replicates are described in Table 2.

Table 2

Element Sample ID	Test	Start Date	End Date	Nicotine	Mean	%RSD	PG	Mean	%RSD	VG	Mean	%RSD	Units
H85138	1	13-Jul-2023	17-Jul-2023	<0.08	n/a	n/a	<2	n/a	n/a	993	988	1.3%	mg/g
	2			<0.08						973			mg/g
	3			<0.08						998			mg/g
H85158	1	13-Jul-2023	17-Jul-2023	<0.08	n/a	n/a	<2	n/a	n/a	993	n/a	n/a	mg/g
	2			<0.08						>1,000*			mg/g
	3			<0.08						>1,000*			mg/g

*Greater than upper LOQ of method TM_SOP_045 for VG

Test Method: TM_SOP_045* (Aerosol Emissions)

Major Components (Nicotine, Propylene Glycol, Glycerin and Menthol) in E-liquids and ENDS Emissions by GC-FID
Storage conditions: Ambient

The results of 3 replicates are described in Table 3 - Table 4. Aerosol generated using Innokin Zlide Tank (0.48 Ohm Mesh Coil) and Evolv™ battery unit (power set at 12W)

Table 3:

Element Sample ID	Test	Start Date	End Date	Nicotine	Mean	%RSD	PG	Mean	%RSD	Units
H85138	1	21-Jul-2023	21-Jul-2023	<1.0	n/a	n/a	<25	n/a	n/a	µg/puff
	2			<1.0			<25			µg/puff
	3			<1.0			<25			µg/puff
H85158	1	21-Jul-2023	21-Jul-2023	<1.0	n/a	n/a	<25	n/a	n/a	µg/puff
	2			<1.0			<25			µg/puff
	3			<1.0			<25			µg/puff

Table 4:

Element Sample ID	Test	Start Date	End Date	VG	Mean	%RSD	Units	ACM	Mean	%RSD	Units
H85138	1	21-Jul-2023	21-Jul-2023	3199	3553	10.0%	µg/puff	0.0691	0.0737	7.11%	g
	2			3912			µg/puff	0.0810			g
	3			3547			µg/puff	0.0709			g
H85158	1	21-Jul-2023	21-Jul-2023	4337	4717	11.1%	µg/puff	0.0846	0.0897	5.52%	g
	2			5312			µg/puff	0.0964			g
	3			4502			µg/puff	0.0881			g

Test Method: TM_SOP_005 (Aerosol Emissions)

Mono-carbonyl analysis: Formaldehyde, acetaldehyde, butyraldehyde in e-liquids / aerosol emissions by LC-MS. Aerosol generated as described in Table 1. Aerosol generated using Innokin Zlide Tank (0.48 Ohm Mesh Coil) and Geekvape NOVA TC battery unit (power set at 12W)
Storage conditions: Ambient

The results of 3 replicates are described in Table 5 - Table 6

Note: Method deviation – d2-formaldehyde IS for calculated response of acetaldehyde (Risk Assessment (RA-034))

Table 5:

Element Sample ID	Test	Start Date	End Date	Formaldehyde	Mean	%RSD	Acetaldehyde	Mean	%RSD	Units
H85138	1	13-Jul-2023	14-Jul-2023	2.345	1.985	0.2	0.040	0.038	0.2	µg/puff
	2			1.460			0.030			µg/puff
	3			2.149			0.044			µg/puff
H85158	1	13-Jul-2023	14-Jul-2023	0.311	0.250	0.3	0.011	n/a	n/a	µg/puff
	2			0.152			<0.005			µg/puff
	3			0.286			0.006			µg/puff
U		-		23	-	-	27	-	-	%

Table 6:

Element Sample ID	Test	Start Date	End Date	Butyraldehyde	Mean	%RSD	ACM	Mean	%RSD	Units
H85138	1	13-Jul-2023	14-Jul-2023	<0.005	n/a	n/a	0.2326	0.215	0.1	g
	2			<0.005			0.1998			g
	3			<0.005			0.2138			g
H85158	1	13-Jul-2023	14-Jul-2023	<0.005	n/a	n/a	0.2526	0.353	0.4	g
	2			<0.005			0.5393			g
	3			<0.005			0.2675			g
U		-		10	-	-	-	-	-	%

Test Method: TM_SOP_050* & TM_SOP_001 (Aerosol Emissions)

Flavours and VOCs in e-liquids / aerosol emissions by GC-MS. Aerosol generated as described in Table 1.

Storage conditions: Ambient

The results of 3 replicates are described in Table 7 - Table 9. Aerosol generated using Innokin Zlide Tank (0.48 Ohm Mesh Coil) and Geekvape NOVA TC battery unit (power set at 12W).

Table 7:

Element Sample ID	Test	Start Date	End Date	2-3 Pentane-dione	Mean	%RSD	Acrylonitrile	Mean	%RSD	Benzene	Mean	%RSD	1-butanol	Mean	%RSD	Units
H85138	1	01-Aug-2023	01-Aug-2023	<0.10*	n/a	n/a	<0.10*	n/a	n/a	<0.10*	n/a	n/a	<0.10*	n/a	n/a	µg/puff
	2			<0.10*			<0.10*			<0.10*			<0.10*			µg/puff
	3			<0.10*			<0.10*			<0.10*			<0.10*			µg/puff
H85158	1	01-Aug-2023	01-Aug-2023	<0.10*	n/a	n/a	<0.10*	n/a	n/a	<0.10*	n/a	n/a	<0.10*	n/a	n/a	µg/puff
	2			<0.10*			<0.10*			<0.10*			<0.10*			µg/puff
	3			<0.10*			<0.10*			<0.10*			<0.10*			µg/puff

Table 8:

Element Sample ID	Test	Start Date	End Date	Diacetyl	Mean	%RSD	Isoamyl acetate	Mean	%RSD	Isobutyl acetate	Mean	%RSD	Methyl Acetate	Mean	%RSD	Units
H85138	1	01-Aug-2023	01-Aug-2023	<0.10*	n/a	n/a	<0.10*	n/a	n/a	<0.10*	n/a	n/a	<0.10*	n/a	n/a	µg/puff
	2			<0.10*			<0.10*			<0.10*			<0.10*			µg/puff
	3			<0.10*			<0.10*			<0.10*			<0.10*			µg/puff
H85158	1	01-Aug-2023	01-Aug-2023	<0.10*	n/a	n/a	<0.10*	n/a	n/a	<0.10*	n/a	n/a	<0.10*	n/a	n/a	µg/puff
	2			<0.10*			<0.10*			<0.10*			<0.10*			µg/puff
	3			<0.10*			<0.10*			<0.10*			<0.10*			µg/puff

Table 9:

Element Sample ID	Test	Start Date	End Date	Methyl acetate	Mean	%RSD	Toluene	Mean	%RSD	Units	ACM	Mean	%RSD	Units
H85138	1	01-Aug-2023	01-Aug-2023	<0.10*	n/a	n/a	<0.10*	n/a	n/a	µg/puff	0.2161*	n/a	n/a	g
	2			<0.10*			<0.10*			µg/puff	0.2162*			g
	3			<0.10*			<0.10*			µg/puff	0.2423*			g
H85158	1	01-Aug-2023	01-Aug-2023	<0.10*	n/a	n/a	<0.10*	n/a	n/a	µg/puff	0.2337*	n/a	n/a	g
	2			<0.10*			<0.10*			µg/puff	0.2351*			g
	3			<0.10*			<0.10*			µg/puff	0.1881*			g

Study Reference: HAL23-043

Test Method: TM_SOP_050* & TM_SOP_002 (Aerosol Emissions)

Flavours and VOCs in e-liquids / aerosol emissions by GC-MS. Aerosol generated as described in Table 1.

Storage conditions: Ambient

The results of 3 replicates are described in Table 10 - Table 11. Aerosol generated using Innokin Zlide Tank (0.48 Ohm Mesh Coil) and Geekvape NOVA TC battery unit (power set at 12W).

Table 10:

Element Sample ID	Test	Start Date	End Date	Acrolein	Mean	%RSD	Croton-Aldehyde	Mean	%RSD	Units	ACM	Mean	%RSD	Units
H85138	Rep 1	12-Jul-2023	12-Jul-2023	<0.10*	n/a	n/a	<0.50*	n/a	n/a	µg/puff	0.2648*	n/a	n/a	g
	Rep 2			<0.10*			<0.50*			µg/puff	0.1987*			g
	Rep 3			<0.10*			<0.50*			µg/puff	0.2239*			g
H85158	Rep 1	12-Jul-2023	12-Jul-2023	<0.10*	n/a	n/a	<0.50*	n/a	n/a	µg/puff	0.2121*	n/a	n/a	g
	Rep 2			<0.10*			<0.50*			µg/puff	0.2634*			g
	Rep 3			<0.10*			<0.50*			µg/puff	0.2798*			g

Table 11:

Element Sample ID	Test	Start Date	End Date	Furfural	Mean	%RSD	Propylene Oxide	Mean	%RSD	Units
H85138	Rep 1	12-Jul-2023	12-Jul-2023	0.76*	0.69*	13.61*	<0.50*	n/a	n/a	µg/puff
	Rep 2			0.58*			<0.50*			µg/puff
	Rep 3			0.71*			<0.50*			µg/puff
H85158	Rep 1	12-Jul-2023	12-Jul-2023	<0.50*	n/a	n/a	<0.50*	n/a	n/a	µg/puff
	Rep 2			<0.50*			<0.50*			µg/puff
	Rep 3			<0.50*			<0.50*			µg/puff

End of Test Report

Test Report Number: HAL23-043-0-1

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SMT LABS
SCIENTIFIC MATERIAL TESTING

6105 S. Ash Ave., Suite A-8, Tempe, AZ 85283; (480)-941-6598

Report No.: 23-08-0211.0002-RPT_1

Date: August 31, 2023

Customer: Ready Mix Naturals
1435 N. Highway 123
Bypass Sequin, TX 78155

Customer P.O.: Verbal

Sample: One Sample Bottle of 100 mg Nixodine Solution Product.

Objective: Characterize the Sample by Gas Chromatography-Mass Spectrometry (GC-MS) Analysis.

ISO 9001:2015
CERTIFIED COMPANY

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SUMMARY

1. Diluted samples of varying concentrations were made of the Nixodine sample for GC-MS survey analysis. Dichloromethane (DCM) and methanol were used to make diluted samples in concentrations. Dilutions were sonicated for 5 minutes, and analyzed by GC-MS.
2. The results of GC-MS analysis of the 10x methanol dilution sample are displayed in Table 1 with the base peak being underlined. Methanol proved to be a better solvent which enhanced dissolution and detected additional peaks in the sample when compared to a sample dissolved in dichloromethane.

Retention Time (min)	Component ID or Mass Spectrum (m/z)	Area-%
		10X Dilution
7.701	2-furanmethanol	0.093
13.51	Glycerin	92.05
14.83	29, 43, 59, 69, 87, 97, 113, <u>144</u>	0.03
15.46	Nixodine	6.97
15.98	Levoglucosan	0.75
16.33	29, 43, 60, <u>73</u> , 87, 98, 122	0.02
16.61	1,6-Anhydro-.beta.-D-glucofuranose	0.06
17.18	29, 43, 57, 74, <u>127</u> , 145, 187	0.03

Table 1. Nixodine Product Sample, GC-MS Analysis.

3. Two major components (> 1 area-%) were detected by GC-MS analysis: Glycerin and Nixodine.
4. If required, high purity reference standards can be obtained for mass spectral analysis. Reference materials will be required to quantify the components in the sample.
5. Based on the components detected, it may be best to use a Wax column for the glycerin due to the peak shape and tailing observed. Method development will be necessary to determine the appropriate conditions.
6. A Karl Fischer titration could be used to determine the water content of the sample.



MATERIAL ANALYSES

One sample bottle labelled Unchained Naturals Nixodine 100 mg product was provided for GC-MS analysis of its volatile components. The product sample, as received, is shown in Figure 1.



Figure 1. Nixodine Sample, As Received

The provided product sample was used to make four diluted test solutions at varying concentrations: 10x, 100x, and 1000x using DCM and 10x using methanol. All dilution samples were sonicated for five minutes before GC-MS analysis.



Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS is an analytical technique used for the separation and identification of volatile and semi-volatile organic compounds. The compounds are separated on the GC column and identified by matching their mass spectra to those of reference mass spectra contained in a digital library.

GC-MS analysis was performed using an Agilent 6890N Gas Chromatograph (GC) equipped with an Agilent 5973N Quadrupole Mass Selective Detector. A Restek Rtx-5MS column was used for the analysis. The GC oven was held at 40 °C for 6 min., then ramped 40 °C to 310 °C at 15 °C/min., and held at 310 °C for 10 min. A mass range of m/z 29-550 was scanned. Samples were run with and without a 4.0-minute solvent delay.

The total ion chromatogram (TIC) acquired from the 10x dissolved in dichloromethane sample is shown in Figure 2.

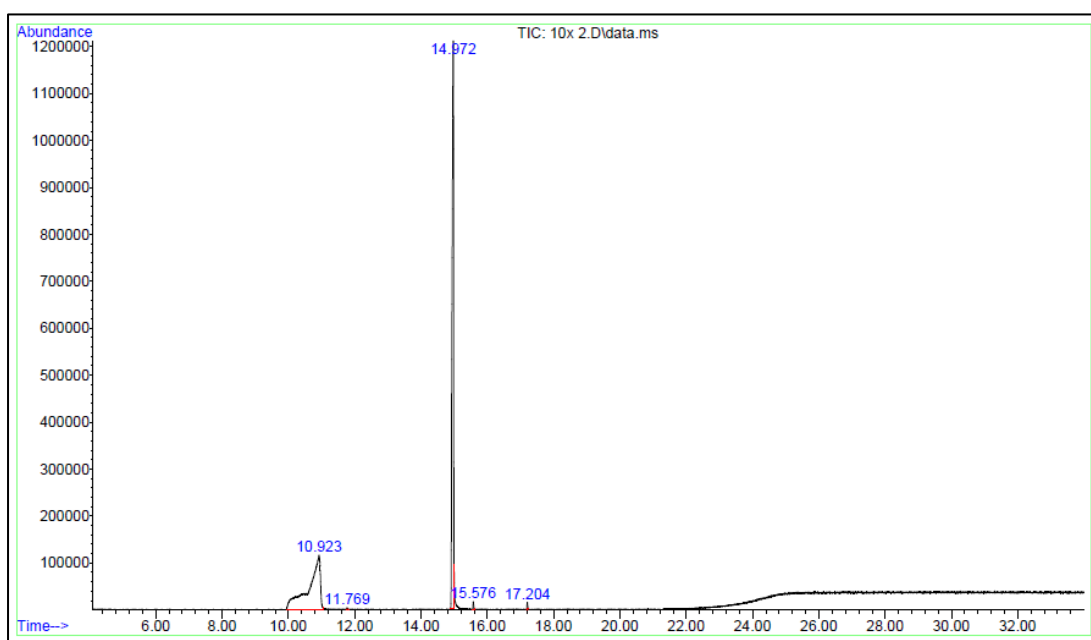


Figure 2. 10x Dilution Sample, TIC.

The GC-MS results of the 10x Dilution sample are shown in Table 2.



Retention Time (min)	Component ID or Mass Spectrum (m/z)	Area-%
		10X Dilution
10.92	Glycerin	42.53
11.77	Glycerin monoacetate	0.08
14.97	Nixodine	56.85
15.58	29, 43, 55, 61, 71, 78, 87, <u>99</u> , 117, 159	0.30
17.20	29, 43, 57, 67, 74, 84, 98, 115, <u>127</u> , 134, 145, 187	0.25

Table 2. Nixodine Product Sample, Dichloromethane, GC-MS Analysis.

All peaks were integrated; the resulting area counts were used to determine the relative area-percent of each detected component. The area-% is defined as the integrated area of an individual peak divided by total integrated area of all detected peaks x 100. As the detector responses are not corrected, these area-% values are relative and are not quantitative.

Two major components (> 1 area-%) were detected by GC-MS analysis: Glycerin and Nixodine.

Additional GC-MS testing was conducted using methanol as the solvent for further verification of constituent volatile molecules. The sample was prepared at a 1:10 concentration and the results of the GC-MS are shown in Table 3.

Retention Time (min)	Component ID or Mass Spectrum (m/z)	Area-%
		10X Dilution
7.701	2-furanmethanol	0.093
13.51	Glycerin	92.05
14.83	29, 43, 59, 69, 87, 97, 113, <u>144</u>	0.03
15.46	Nixodine	6.97
15.98	Levoglucosan	0.75
16.33	29, 43, 60, <u>73</u> , 87, 98, 122	0.02
16.61	1,6-Anhydro-.beta.-D-glucofuranose	0.06
17.18	29, 43, 57, 74, <u>127</u> , 145, 187	0.03

Table 3. Nixodine Product Sample, Methanol Dilution, GC-MS Analysis.

The total ion chromatogram (TIC) acquired from the 10x sample in Methanol is shown in Figure 3.



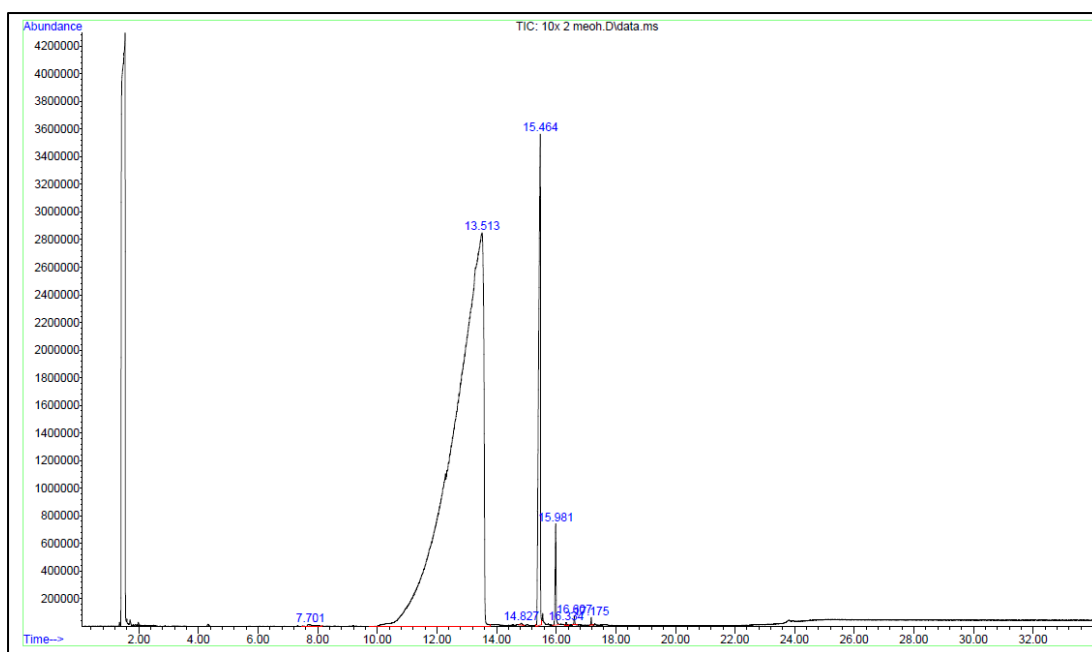


Figure 3. 10x Methanol Dilution Sample, TIC.

Methanol proved to be a better solvent which enhanced dissolution and detected additional peaks in the sample when compared to a sample dissolved in dichloromethane.

Please do not hesitate to contact us should you have any questions or concerns.

David De La Cruz
Director of Material Science Practice



SMT Labs Terms and Conditions

SMT Labs will retain samples for up to 30 days from the date of the final report. Samples will be discarded unless long-term storage arrangements have been made with the client.

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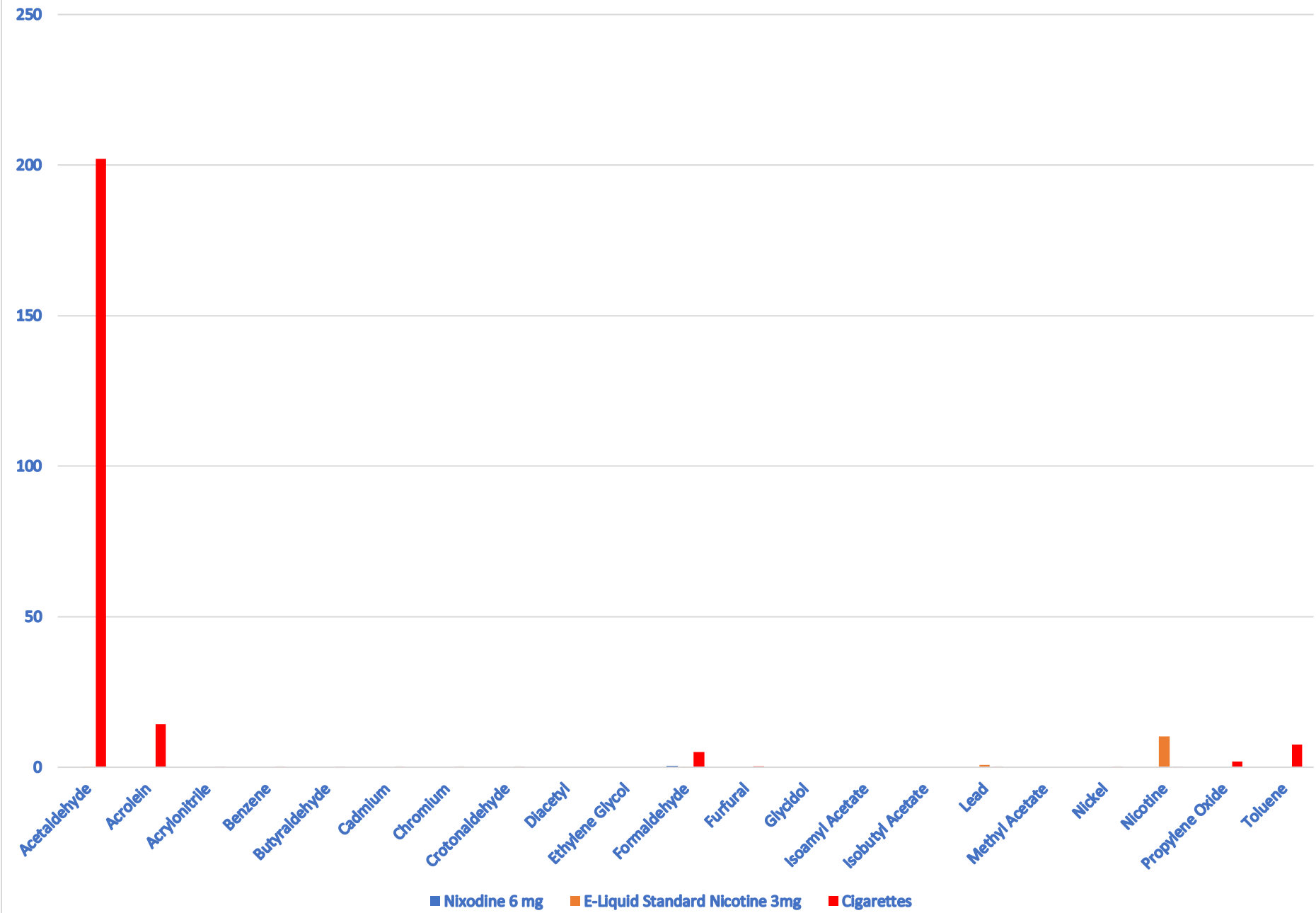
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Test data and conclusions pertain only to items analyzed. We accept no responsibility or liability for results due to non-representative test items, improper sampling, insufficient testing, or misinformation.

Should additional information become available, SMT labs reserves the right to supplement, amend or modify this report.



Hazardous Chemical Comparison



Constituent	Nixodine 6 mg	E-Liquid Standard Nicotine 3mg	Cigarettes
Acetaldehyde	N/A	0.029	202
Acrolein	N/A	N/A	14.4
Acrylonitrile	N/A	N/A	0.0509
Benzene	N/A	N/A	0.168
Butyraldehyde	N/A	N/A	0.14
Cadmium	N/A	N/A	0.172
Chromium	N/A	N/A	0.0429
Crotonaldehyde	N/A	N/A	0.0807
Diacetyl	N/A	N/A	N/A
Ethylene Glycol	N/A	N/A	N/A
Formaldehyde	0.25*	0.0842	4.96
Furfural	N/A	N/A	0.4
Glycidol	N/A	N/A	N/A
Isoamyl Acetate	N/A	N/A	N/A
Isobutyl Acetate	N/A	N/A	N/A
Lead	N/A	0.79	0.0926
Methyl Acetate	N/A	N/A	N/A
Nickel	N/A	N/A	0.0465
Nicotine	N/A	10.23	0.19
Propylene Oxide	N/A	N/A	2.01
Toluene	N/A	N/A	7.64

*** Testing produced dry wicking due to VG thickness; however, OSHA sets the limits for safe exposure, and even extreme conditions Nixodine falls well below this limit.**

0.75 ppm

The US Occupational Safety and Health Administration (OSHA) has established limits for the amount of formaldehyde that workers can be exposed to at their place of work. At present the limit is at 0.75 ppm on average over an 8-hour workday. Oct 24, 2022