CHEMNOVATIC Sp. z o.o. Sp. k.
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ANALYSIS REPORT NO 2023/07/0013/007_EN DATE: 31.07.2023

Particulars of the Client	Particulars of the sample/Description of the designation	
SUBSTITUTE SRL Sibiu, strandului, 4 Romania	Quantitative determination of aerosol components derived from e-liquids, according to agreed specification	
	Sample number	**Client`s designation**
	2023/07/0013/007	Lichid smokemania Traditional MTL 60vg 40pg 18mg

Date of receipt of samples: 21th July 2023
Date of completion of the analyses: 31th July 2023

The analyses have been conducted by:

CHEMNOVATIC Sp. z o.o. Sp. k.
Ludwika Spiessa 9
20-270 Lublin
VAT No PL946-264-59-31

Description of the delivered samples:

Transparent solutions of significant viscosity supplied in plastic containers.

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Results:

1. **Sample mass and puffs number for tobacco-specific nitrosamines, aldehydes and ketones determination.**

Sample signature	Subject of determination	Method identification**	The result of the analysis	Standard Deviation	Unit
2023/07/0013/007	Sample mass at 40 puffs. Vaping process to acetonitrile.	SL/2020/036 Ed. 3 of 05.05.2023, NA	0,3212	-	g

2. **Sample mass and puffs number for nicotine, propylene glycol, glycerin and volatile organic compounds determination.**

Sample signature	Subject of determination	Method identification**	The result of the analysis	Standard Deviation	Unit
2023/07/0013/007	Sample mass at 40 puffs. Vaping process to methanol.	SL/2020/036 Ed. 3 of 05.05.2023, NA	0,2396	-	g

3. **Sample mass and puffs number for heavy metals determination.**

Sample signature	Subject of determination	Method identification**	The result of the analysis	Standard Deviation	Unit
2023/07/0013/007	Sample mass at 40 puffs. Vaping process to water.	SL/2020/036 Ed. 3 of 05.05.2023, NA	0,1267	-	g

4. **Results of heavy metals determination.**

Sample signature	Subject of determination	Method identification**	The result of the analysis	Standard Deviation	Unit
2023/07/0013/007	Content of lead **Pb**	SL/2020/042 Ed. 1 of 03.09.2020, NA	<LOQ	-	µg/g
	Content of cadmium **Cd**		<LOQ	-	
	Content of arsenic **As**		<LOQ	-	
	Content of chrome **Cr**		<LOQ	-	
	Content of nickel **Ni**		<LOQ	-	
	Content of copper **Cu**		<LOQ	-	
	Content of aluminum **Al**		<LOQ	-	
	Content of tin **Sn**		<LOQ	-	
	Content of iron **Fe**		<LOQ	-	

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5. Results of volatile organic compounds determination.

Sample signature	Subject of determination	Method identification**	The result of the analysis	Uncertainty	Unit
2023/07/0013/007	Average content of **benzene**	SL/2020/037 Ed. 1 of 03.09.2020, NA	<LOQ	-	µg/g
	Average content of **xylenes**		<LOQ	-	
	Average content of **toluene**		<LOQ	-	
	Average content of **isoprene**		<LOQ	-	
	Average content of **1,3-butadiene**		<LOQ	-	
	Average content of **ethylene glycol**		<LOQ	-	
	Average content of **diethylene glycol**		<LOQ	-	

6. Results of aldehydes and ketones determination.

Sample signature	Subject of determination	Method identification**	The result of the analysis	Standard Deviation	Unit
2023/07/0013/007	Average content of **formaldehyde**	SL/2020/040 Ed. 1 of 03.09.2020, NA	11,53	1,15	µg/g
	Average content of **acetaldehyde**		<LOQ	-	
	Average content of **acrolein**		3,91	0,39	
	Average content of **crotonaldehyde**		<LOQ	-	
	Average content of **isovaleraldehyde**		<LOQ	-	
	Average content of **o,m,p-tolualdehyde**		<LOQ	-	
	Average content of **hexaldehyde**		<LOQ	-	
	Average content of **diacetyl**	SL/2020/037 Ed. 1 of 03.09.2020, NA	<LOQ	-	
	Average content of **acetyl propionyl**		<LOQ	-	

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7. Results of tobacco-specific nitrosamines determination.

Sample signature	Subject of determination	Method identification**	The result of the analysis	Standard Deviation	Unit
2023/07/0013/007	Average content of **NNK**	SL/2020/039 Ed. 2 of 22.10.2020, NA	<LOQ	-	µg/g
	Average content of **NNN**		<LOQ	-	

8. Results of nicotine, propylene glycol and glycerin determination after heating e-liquid.

Sample signature	Subject of determination	Method identification**	The result of the analysis	Uncertainty	Unit
2023/07/0013/007	Average content of **propylene glycol**	SL/2020/038 Ed. 3 of 03.09.2020, NA	167,2	18,4	mg/g***
	Average content of **glycerin**	SL/2020/038 Ed. 3 of 03.09.2020, NA	740,5	81,5	
	Average content of **nicotine**	SL/2020/038 Ed. 3 of 03.09.2020, A	12,0	1,3	
	Average number of **puffs**	NA	40	-	-
	Average nicotine **dose per puff**		0,07	0,01	mg/puff

** Determination method: A-accredited, NA-non-accredited, AS- accredited subcontractor, NAS – non-accredited subcontractor.

*** amount of nicotine [mg] per 1 g of vaped liquid; LOQ – limit of quantification.

Additional information:

I. Sampling conditions:

Sampling parameters:

- The air flow through the system was 1,1 L/min.
- The test consists of 3 sec. puff and 27 sec. relaxation time interval.
- Heater resistance: 1,5 Ω.
- The voltage applied to the heater: 3,7 V.
- The temperature of transfer line was set in the range of 80-100 °C.
- Used device: Volish.

II. Heavy metals determination method:

The aerosol was collected into the ultrapure water with nitric acid (trace analysis quality) in the absorber. The samples were analyzed directly on Agilent ICP-OES VDV 5100 System in the axial mode. The cyclon chamber and glass nebulizer was used. The RF Power was 1,20 kW and the plasma flow of argon was 12 L/min.

Table 1.The Limits of quantification of heavy metals.

Subject of designation	Unit	Limit of quantification
Content of lead **Pb**	µg/g	10,00
Content of cadmium **Cd**	µg/g	10,00
Content of arsenic **As**	µg/g	10,00
Content of chrome **Cr**	µg/g	10,00
Content of nickel **Ni**	µg/g	10,00
Content of copper **Cu**	µg/g	10,00
Content of aluminum **Al**	µg/g	10,00
Content of tin **Sn**	µg/g	10,00
Content of iron **Fe**	µg/g	10,00

III.Volatile Organic Compounds determination method:

The aerosol was collected to methanol in the absorber. Analysis of the standard solutions and the samples was performed with gas chromatography combined with mass spectrometry Shimadzu GCMS-QP2010 SE System. The quantitative analysis were performed in split injection mode by gradient temperature program and SIM detector mode. The Zebron WAX column was used with parameters: 30 m length; 0,25 I.D. mm and 0,25 µm of film thickness.

Table 2. The limits of quantification of volatile organic compounds.

Subject of designation	Unit	Limit of quantification
Content of **benzene**	µg/g	50,0
Content of **xylenes**	µg/g	50,0
Content of **toluene**	µg/g	50,0
Content of **isoprene**	µg/g	50,0
Content of **1,3-butadiene**	µg/g	50,0
Content of **ethylene glycol**	µg/g	250,0
Content of **diethylene glycol**	µg/g	50,0

IV.Aldehydes and ketones determination method:

The aerosol was collected to acetonitrile in the absorber. The analytes were derivatized in acetonitrile solution by 2,4-DNPH (dinitrophenylhydrazine in phosphoric acid). Analysis of the standard solutions and the samples was performed using ultraperformance liquid chromatography with diode-array detector coupled with tandem mass spectrometry UHPLC-PDA/MS/MS Shimadzu Nexera X2 8040. The Luna Omega column (1.6 um; C 18; 100 A LC Column 100x2.1 mm) was used for the determinations.

Table 3. The limits of quantification of aldehydes and ketones.

Subject of designation	Unit	Limit of quantification
Content of **formaldehyde**	µg/g	2,22
Content of **acetaldehyde**	µg/g	3,06
Content of **acrolein**	µg/g	3,70
Content of **crotonaldehyde**	µg/g	4,36
Content of **isovaleraldehyde**	µg/g	5,04
Content of **o,m,p- tolualdehyde**	µg/g	6,23
Content of **hexaldehyde**	µg/g	5,56
****Content of **diacetyl**	µg/g	50,0
****Content of **acetyl propionyl**	µg/g	25,0

****The aerosol was collected to methanol in the absorber. Analysis of the standard solutions and the samples was performed with gas chromatography combined with mass spectrometry Shimadzu GCMS-QP2010 SE System. The quantitative analysis were performed in split injection mode by gradient temperature program and SCAN and SIM detector mode. The Zebron WAX column was used with parameters: 30 m length; 0,25 I.D. mm and 0,25 µm of film thickness.

V.Tobacco-specific nitrosamines determination method:

The aerosol was collected to acetonitrile into the absorber. Analysis of the standard solutions and the samples was performed using ultraperformance liquid chromatography with diode-array detector coupled with tandem mass spectrometry UHPLC-PDA/MS/MS Shimadzu Nexera X2 8040. The Luna Omega column (1.6 um; C 18; 100 A LC Column 100x2.1 mm) was used for the determinations.

Table 4. The limits of quantification of tobacco-specific nitrosamines.

Subject of designation	Unit	Limit of quantification
Content of **TSNA: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)**	µg/g	2,5
Content of **TSNA: N-nitrosonornicotine (NNN)**		2,5

VI.Nicotine, propylene glycol and glycerin determination method:

The aerosol was collected to methanol into the absorber. Analysis of the standard solutions and the samples was performed with gas chromatography combined with flame ionization detector Shimadzu GC2010 Plus System. The quantitative analysis were done in split injection mode by isothermal and gradient temperature program. The Zebron ZB-624 column was used with parameters: 30 m length; 0,32 I.D. mm and 1,8 µm of film

thickness.

Table 5. The limits of quantification of nicotine.

Subject of designation	Unit	Limit of quantification
Content of **nicotine in aerosol after heating**	mg/g	2,5
Average nicotine **dose per puff**	mg/puff	0,03125

END OF REPORT

The report may not be published, in whole or in part, without the written consent of

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The obtained result applies only to the tested (received) samples.

Approved by:	**Marcin Górecki** Manager of Legal Regulations Department