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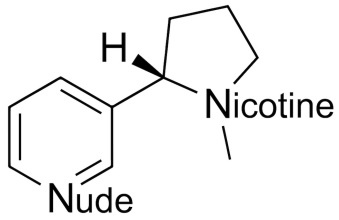

Standardized Puffing Regimen – Emissions Testing Protocol

The Following denotes the analytical procedure for the preparation of eLiquid samples for vaporization, collection, and analysis of the 21 analytes of interest for the EU Tobacco Product Directive application process. Samples will be puffed utilizing a smoking machine with a predetermined flow rate and sampling regimen per ISO 3308, 6585, 3402, and CORESTA methods 21 and 25. Following the standardized puffing regimen, samples will then be quantitated with GC/FID, HPLC/UV, or ICP/MS.

Inhalation Volume (mL) – 55
Inhalation Duration (seconds) – 4
Inhalation Interval (seconds) – 30
Inhalation Profile – Square wave
Puff count – 30 (triplicate of dosage – per 10 puffs)
Device Name – Kanger + ProTank
Device Setting – 25W 0.5ohm
Impinger or Cambridge Filter Holder (CFH)? – Impinger

An aliquot of the diluent is spiked with a known amount of internal standard. The aliquot is the capped, mixed, and injected into the analytical system. Analyte quantitation is confirmed by a 5-7 point calibration curve. Prior to the quantitation, the individual analytes were confirmed utilizing an attached mass spectrometer to the same gas chromatograph, confirming no less than 3 unique ion fragments of certified reference chemical.

Analysis by gas chromatography tandem flame ionization detection (GC/FID): Ethylene glycol, Diethylene glycol, nicotine, NNN, NNK, toluene, benzene, isoprene, 1,3-butadiene, diacetyl, acetyl propionyl.

Analysis by gas chromatography tandem mass spectrometry (HPLC/UV): Formaldehyde, Acetaldehyde, Acrolein, Crotonaldehyde

Analysis by inductively coupled plasma tandem mass spectrometry (ICP/MS): Cadmium**,** Calcium**,** Chromium**,** Copper**,** Lead**,** Nickel**,** Arsenic

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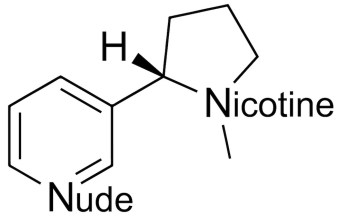

Standardized Puffing Regimen – Emissions Testing Protocol

The Following denotes the analytical procedure for the preparation of eLiquid samples for vaporization, collection, and analysis of the aldehyde content for the EU Tobacco Product Directive application process. Samples will be puffed utilizing a smoking machine with a predetermined flow rate and sampling regimen per ISO 3308, 6585, 3402, and CORESTA methods 21 and 25. Following the standardized puffing regimen, samples will then be quantitated with HPLC/UV.

Analyte – Acetaldehyde	Limit of Detection: 0.1ppm	At 3mg/mL	At 6mg/mL	At 12mg/mL
Brand Name:	**Flavor Name:**	**Concentration (PPM/10 inhalations)**		
Milkman	Milky O's	28.6	28.6	28.6

Analyte – Crotonaldehyde	Limit of Detection: 0.1ppm	At 3mg/mL	At 6mg/mL	At 12mg/mL
Brand Name:	**Flavor Name:**	**Concentration (PPM/10 inhalations)**		
Milkman	Milky O's	<0.1	<0.1	<0.1

Analyte – Formaldehyde	Limit of Detection: 0.1ppm	At 3mg/mL	At 6mg/mL	At 12mg/mL
Brand Name:	**Flavor Name:**	**Concentration (PPM/10 inhalations)**		
Milkman	Milky O's	3.08	3.08	3.08

Analyte – Acrolein	Limit of Detection: 0.1ppm	At 3mg/mL	At 6mg/mL	At 12mg/mL
Brand Name:	**Flavor Name:**	**Concentration (PPM/10 inhalations)**		
Milkman	Milky O's	<0.1	<0.1	<0.1

Operators for Emissions Protocol:	**Operators for Analytical Protocol:**	**Checker:**
M. Schwausch & D. Monaco	J. Polansky	J. Rubenstein
Validated By:		

Notes: Samples were received in good condition with factory seals intact. Chain of custody was properly maintained during the entirety of the sampling and receipt process. This document was drafted as a .pdf and contains two pages.

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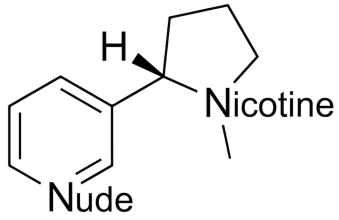

Standardized Puffing Regimen – Emissions Testing Protocol

The Following denotes the analytical procedure for the preparation of eLiquid samples for vaporization, collection, and analysis of the contaminating glycol content for the EU Tobacco Product Directive application process. Samples will be puffed utilizing a smoking machine with a predetermined flow rate and sampling regimen per ISO 3308, 6585, 3402, and CORESTA methods 21 and 25. Following the standardized puffing regimen, samples will then be quantitated with GC/FID.

Analyte – Ethylene Glycol	Limit of Detection: 1.0ppm	At 3mg/mL	At 6mg/mL	At 12mg/mL
Brand Name:	**Flavor Name:**	**Concentration (PPM)**		
Milkman	Milky O's	<1.0	<1.0	<1.0

Analyte – Diethylene Glycol	Limit of Detection: 1.0ppm	At 3mg/mL	At 6mg/mL	At 12mg/mL
Brand Name:	**Flavor Name:**	**Concentration (PPM)**		
Milkman	Milky O's	<1.0	<1.0	<1.0

Operators for Emissions Protocol:	**Operators for Analytical Protocol:**	**Checker:**
M. Schwausch & D. Monaco	J. Polansky	J. Rubenstein
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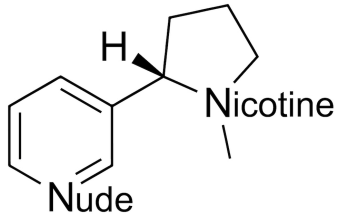

Standardized Puffing Regimen – Emissions Testing Protocol – Delivered Nicotine

The Following denotes the analytical procedure for the preparation of eLiquid samples for vaporization, collection, and analysis of the delivered nicotine content for the EU Tobacco Product Directive application process. Samples will be puffed utilizing a smoking machine with a predetermined flow rate and sampling regimen per ISO 3308, 6585, 3402, and CORESTA methods 21 and 25. Following the standardized puffing regimen, samples will then be quantitated with GC/FID.

		At 3mg/mL	At 6mg/mL	At 12mg/mL
Brand Name:	**Flavor Name:**	**Nicotine per dose (ug/10 inhalations) AKA (ug/dose)**		
Milkman	Milky O's	214.50	429.00	858.00

Operators for Emissions Protocol:	**Operators for Analytical Protocol:**	**Checker:**
M. Schwausch & D. Monaco	J. Rubenstein	J. Rubenstein
Validated By:		

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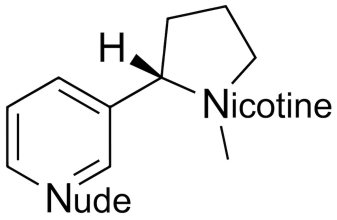

Standardized Puffing Regimen – Emissions Testing Protocol – Heavy Metals

The Following denotes the analytical procedure for the preparation of eLiquid samples for vaporization, collection, and analysis of the heavy metals of interest for the EU Tobacco Product Directive application process. Samples will be puffed utilizing a smoking machine with a predetermined flow rate and sampling regimen per ISO 3308, 6585, 3402, and CORESTA methods 21 and 25. Following the standardized puffing regimen, samples will then be quantitated with ICP/MS.

Sample Name:	**Milkman – Milky O's**		
Element	Sym	Limit of Detection (ppm)	ppm
Arsenic	As	0.25	<0.25
Cadmium	Cd	0.25	<0.25
Chromium	Cr	0.25	<0.25
Calcium	Ca	0.25	<0.25
Copper	Fe	0.25	<0.25
Lead	Pb	0.25	<0.25
Nickel	Ni	0.25	<0.25

Operators for Emissions Protocol:	**Operators for Analytical Protocol:**	**Checker:**
M. Schwausch & D. Monaco	J. Polansky	J. Rubenstein
Validated By:		

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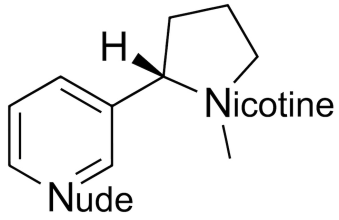

Standardized Puffing Regimen – Emissions Testing Protocol – Ketones

The Following denotes the analytical procedure for the preparation of eLiquid samples for vaporization, collection, and analysis of the ketone content for the EU Tobacco Product Directive application process. Samples will be puffed utilizing a smoking machine with a predetermined flow rate and sampling regimen per ISO 3308, 6585, 3402, and CORESTA methods 21 and 25. Following the standardized puffing regimen, samples will then be quantitated with GC/FID.

Analyte – 2,3-Butanedione (Diacetyl)	Limit of Detection: 0.25ppm	At 3mg/mL	At 6mg/mL	At 12mg/mL
Brand Name:	**Flavor Name:**	**Concentration (PPM)**		
Milkman	Milky O's	<0.25	<0.25	<0.25

Analyte – 2,3-Pentanedione (Acetyl Propionyl)	Limit of Detection: 0.25ppm	At 3mg/mL	At 6mg/mL	At 12mg/mL
Brand Name:	**Flavor Name:**	**Concentration (PPM)**		
Milkman	Milky O's	<0.25	<0.25	<0.25

Operators for Emissions Protocol:	**Operators for Analytical Protocol:**	**Checker:**
M. Schwausch, D. Monaco, & A. Setiomiguno	J. Polansky	J. Rubenstein
Validated By:		

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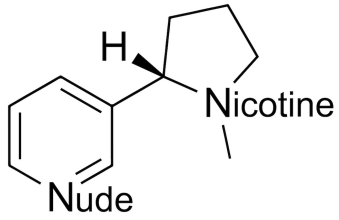

Standardized Puffing Regimen – Emissions Testing Protocol – TSNAs

The Following denotes the analytical procedure for the preparation of eLiquid samples for vaporization, collection, and analysis of the tobacco-specific nitrosamines (TSNA's) for the EU Tobacco Product Directive application process. Samples will be puffed utilizing a smoking machine with a predetermined flow rate and sampling regimen per ISO 3308, 6585, 3402, and CORESTA methods 21 and 25. Following the standardized puffing regimen, samples will then be quantitated with GC/FID.

Analyte – N-Nitrosonornicotine (NNN)	Limit of Detection: 0.25ppm	At 3mg/mL	At 6mg/mL	At 12mg/mL
Brand Name:	**Flavor Name:**	**Concentration (ppm)**		
Milkman	Milky O's	<0.25	<0.25	<0.25

Analyte – Nicotine-Derived Nitrosamine Ketone (NNK)	Limit of Detection: 0.25ppm	At 3mg/mL	At 6mg/mL	At 12mg/mL
Brand Name:	**Flavor Name:**	**Concentration (ppm)**		
Milkman	Milky O's	<0.25	<0.25	<0.25

Operators for Emissions Protocol:	**Operators for Analytical Protocol:**	**Checker:**
M. Schwausch & D. Monaco	J. Polansky	J. Rubenstein
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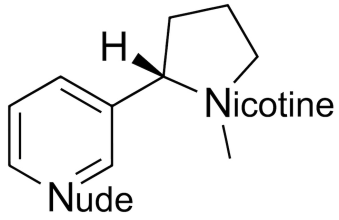

Standardized Puffing Regimen – Emissions Testing Protocol - Volatiles

The Following denotes the analytical procedure for the preparation of eLiquid samples for vaporization, collection, and analysis of the volatile content for the EU Tobacco Product Directive application process. Samples will be puffed utilizing a smoking machine with a predetermined flow rate and sampling regimen per ISO 3308, 6585, 3402, and CORESTA methods 21 and 25. Following the standardized puffing regimen, samples will then be quantitated with GC/FID.

Analyte – Benzene	Limit of Detection: 1.0ppm	At 3mg/mL	At 6mg/mL	At 12mg/mL
Brand Name:	**Flavor Name:**	**Concentration (PPM)**		
Milkman	Milky O's	<1.0	<1.0	<1.0

Analyte – 1,3-Butadiene	Limit of Detection: 1.0ppm	At 3mg/mL	At 6mg/mL	At 12mg/mL
Brand Name:	**Flavor Name:**	**Concentration (PPM)**		
Milkman	Milky O's	<1.0	<1.0	<1.0

Analyte – Isoprene	Limit of Detection: 1.0ppm	At 3mg/mL	At 6mg/mL	At 12mg/mL
Brand Name:	**Flavor Name:**	**Concentration (PPM)**		
Milkman	Milky O's	<1.0	<1.0	<1.0

Analyte – Toluene	Limit of Detection: 1.0ppm	At 3mg/mL	At 6mg/mL	At 12mg/mL
Brand Name:	**Flavor Name:**	**Concentration (PPM)**		
Milkman	Milky O's	<1.0	<1.0	<1.0

Operators for Emissions Protocol:	Operators for Analytical Protocol:	Checker:
M. Schwausch & D. Monaco	J. Polansky	J. Rubenstein
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