Non-targeted Analysis Using Gas Chromatography Mass Spectrometry for Evaluation of Chemical Composition for E-Vapor Products

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2020 CORESTA Online Congress

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BACKGROUND

PMTA ENDS Guidance*

"FDA also recommends that you **include a complete list of uniquely identified constituents**, including those listed below, as appropriate for your product, **and other toxic chemicals contained within the product or delivered by the product, such as a reaction product from leaching or aging and aerosol generated through the heating of the product**… "

"This information should include the **established shelf life of the product** and changes in pH and constituents **(including HPHCs and other toxic chemicals) over the lifespan of the product**... "

Goal:

Develop a GC/MS Non-Targeted Analysis (NTA) method to characterize the volatile and semi-volatile compounds present in the aerosol from ENDS products

* Taken from US Dept HHS, 2019, Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems (ENDS)

NON-TARGETED ANALYSIS BY GC/MS

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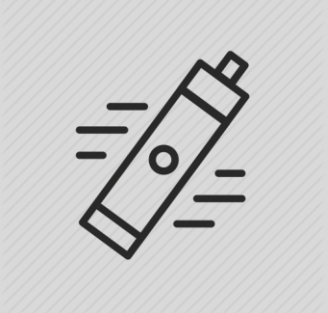
Sample Analysis

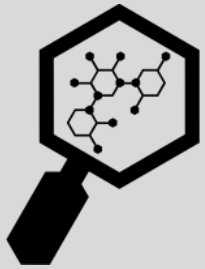


- Aerosol Collection
- Sample Preparation
- Instrumentation

Data Processing

- MS Deconvolution
- Compound Identification
- MassHunter Unknowns*

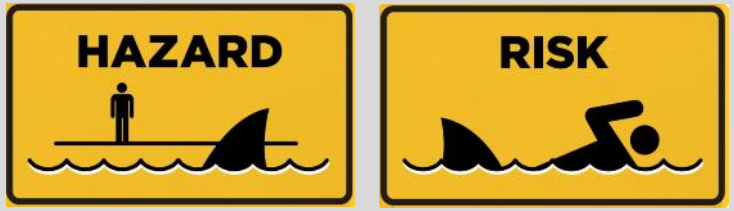
Unknown Identification



- Custom Library
- High Resolution Mass Spectrometry

Custom Reporting

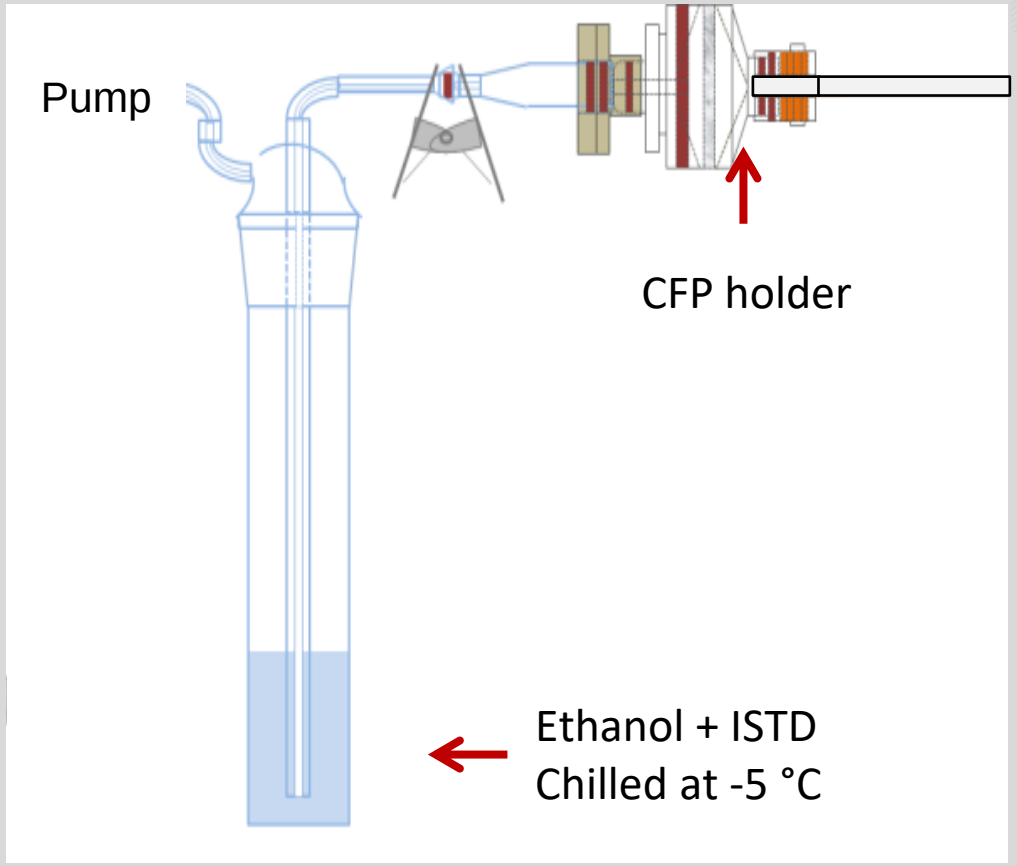
Risk Assessment



PMTA

* MassHunter software is licensed and distributed by Agilent Technologies

AEROSOL COLLECTION AND SAMPLE PREPARATION



Aerosol Collection

- Linear smoking machine (Borgwaldt LX20)
- Intense puffing regime: 55 cc, 5 s, interval 30 s, square
- Target Aerosol Mass*: ~ 0.8 g
- Collected on a 55 mm Cambridge filter pad (CFP) with a trailing impinger containing 10 mL of extraction solvent
- Combine CFP and impinger contents and extract on rotator for 30 minutes.

E-Liquid Extraction

- Remove e-liquid from cartridge (~0.8 g)
- Combine with 10 mL extraction solvent (Ethanol w/ IS)
- Extract on rotator for 30 minutes

*Device dependent parameter

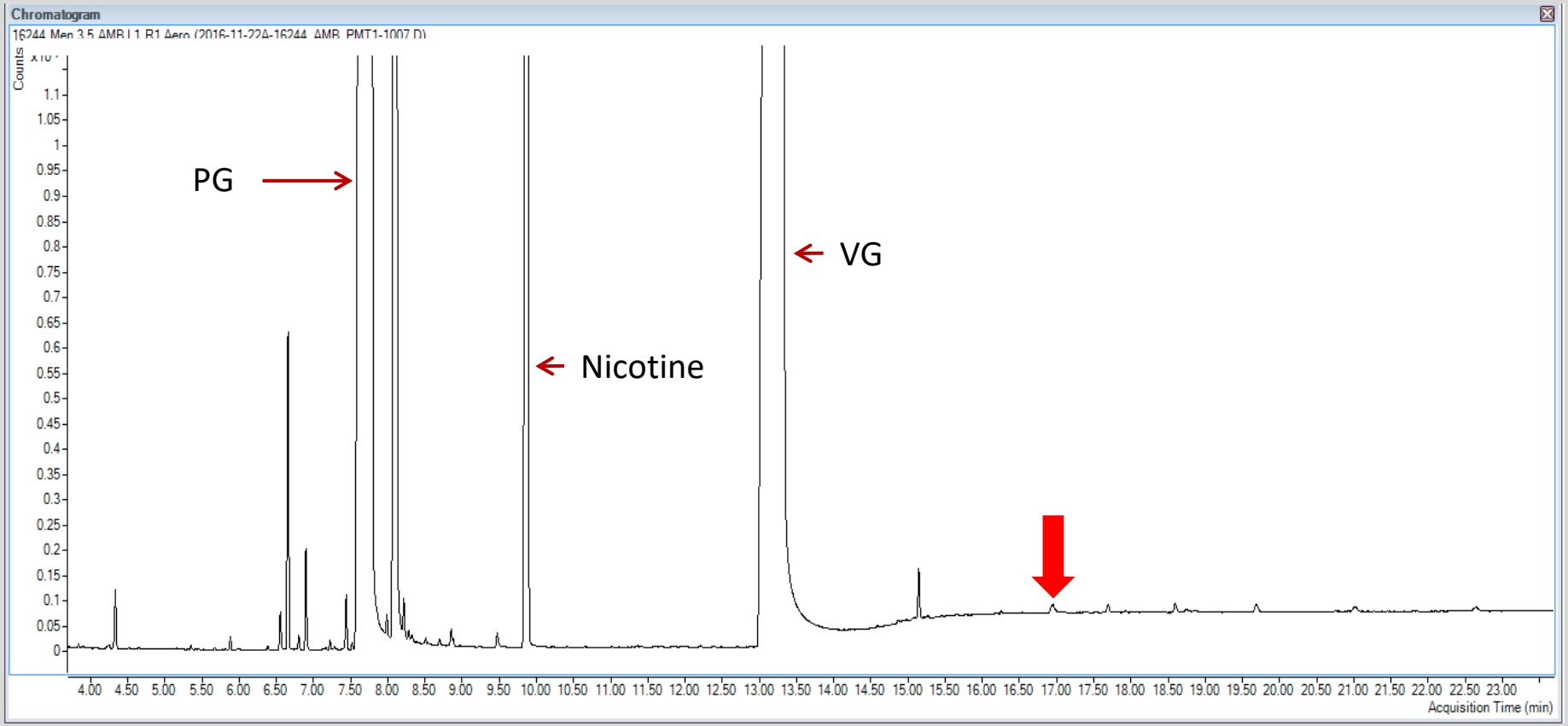
INSTRUMENTATION

Agilent Technologies GC/MS (unit mass) in Electron Ionization (EI) mode

Column: Restek Stabilwax (30 m x 0.25 mm x 0.25 µm)

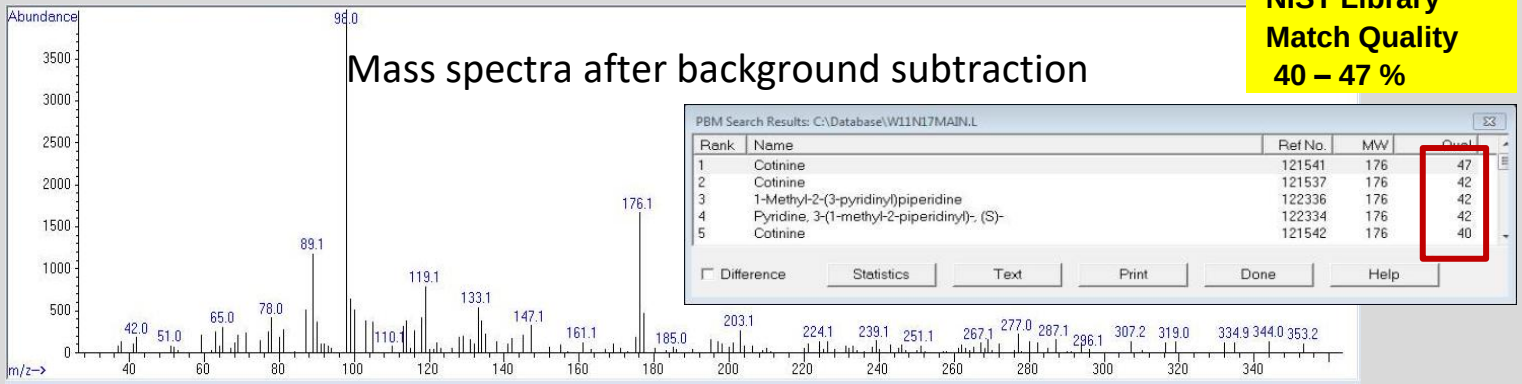
Run time: 25 minutes

EXAMPLE GC/MS CHROMATOGRAM AEROSOL SAMPLE



PG- Propylene glycol, VG- Vegetable glycerin

MS DECONVOLUTION AND COMPOUND IDENTIFICATION



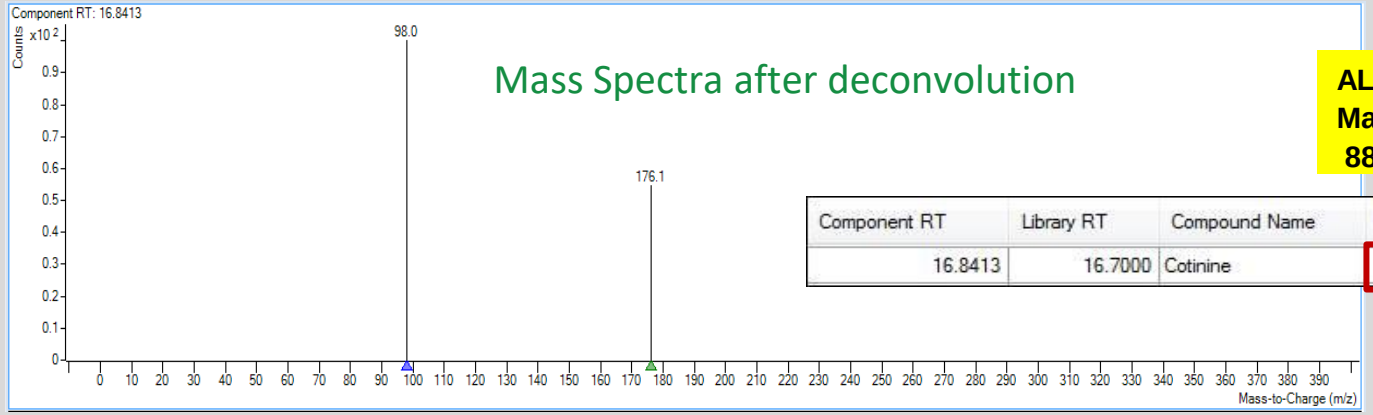
Mass spectra after background subtraction

NIST Library Match Quality 40 – 47 %

PBM Search Results: C:\Database\W11N17MAIN.L

Rank	Name	Ref No.	MW	Qual
1	Cotinine	121541	176	47
2	Cotinine	121537	176	42
3	1-Methyl-2-(3-pyridinyl)piperidine	122336	176	42
4	Pyridine, 3-(1-methyl-2-piperidinyl)-, (S)-	122334	176	42
5	Cotinine	121542	176	40

Difference | Statistics | Text | Print | Done | Help



Mass Spectra after deconvolution

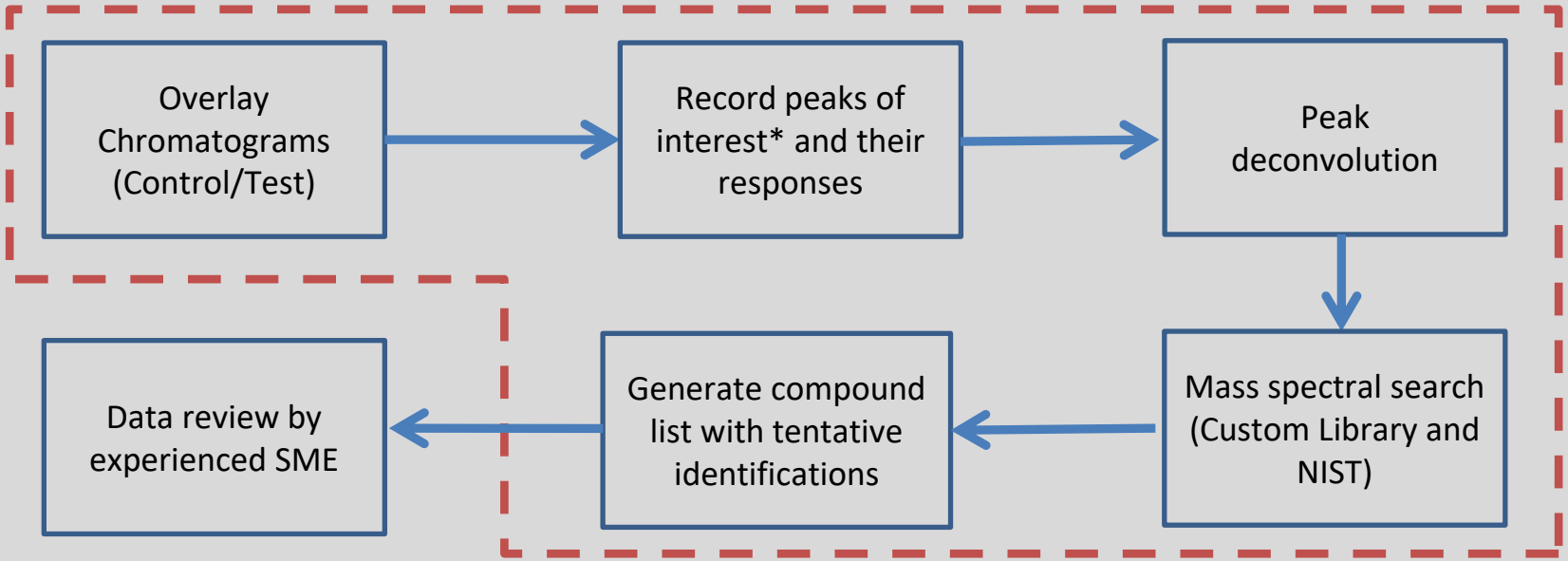
ALCS Library Match Quality 88 %

Component RT	Library RT	Compound Name	Match Factor
16.8413	16.7000	Cotinine	88.0

Mass spectral deconvolution: Important tool for improved compound identification

DATA PROCESSING

Automated with MassHunter Unknowns

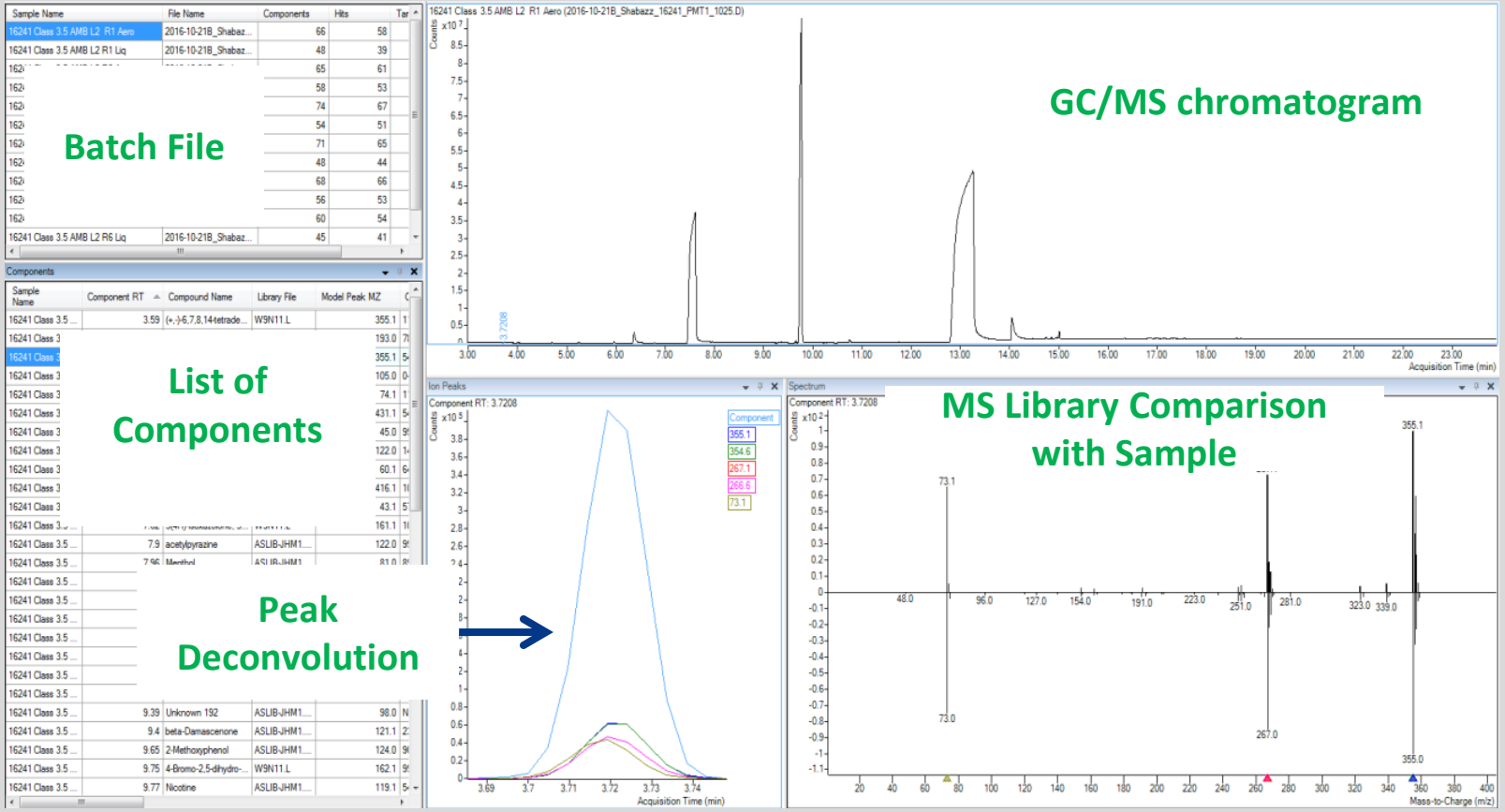


Example: 1 lot with 6 replicates (aerosol and liquid)

- Manual process: **~6 hours**
- Automated process: **~1-2 hours**

* Identify peaks that are new or increasing in concentration compared to control (fresh formulation)

MASSHUNTER UNKNOWNS ANALYSIS SOFTWARE



METHOD VALIDATION

Method Requirements:

- Semi-quantitative method for the chemical characterization of ENDS (aerosol and liquids)
- Provide identification of compounds that are new or are present at higher concentrations compared to a control
- Capable of detecting compounds as low as 1 ppm

Model Compounds
Hydroxyacetone
Piperonal
2,3,5-trimethylpyrazine
Menthone
(E)-Beta-damascone
Cinnamic acid methyl ester
Myosmine
Cotinine

Model Matrices[1]	
F1	50/50/15 – PG/VG/Water[2] + 2.5 % NBW
F2	50/50/0 – PG/VG/Water[2] + 2.5 % NBW
F3	50/50/15 - PG/VG/Water[2] + 0 % NBW
F4	80/20/15 – PG/VG/Water[2] + 0 % NBW
F5	20/80/15 – PG/VG/Water[2] + 0 % NBW
	Commercial matrices - 2

1 - NBW = Nicotine by weight
2 - PG/VG ratios are for the remaining percent after addition of water and nicotine

METHOD VALIDATION: ACCURACY

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Analyte Concentration (PPM) = Analyte Response × MRF*

	Hydroxyacetone	2,3,5-trimethylpyrazine	Menthone	(E)-Beta-Damascone	Cinnamic acid methyl ester	Myosmine	Piperonal	Cotinine
2 PPM								
F1	87 %	98 %	48 %	67 %	102 %	93 %	106 %	97 %
F2	116 %	103 %	50 %	83 %	107 %	90 %	116 %	100 %
F3	81 %	103 %	52 %	84 %	110 %	84 %	120 %	193 %**
F4	69 %	99 %	51 %	78 %	107 %	79 %	111 %	99 %
F5	93 %	104 %	50 %	80 %	105 %	79 %	116 %	99 %
5 PPM								
F1	66 %	108 %	52 %	83 %	111 %	94 %	119 %	108 %
F2	62%	101 %	49 %	75 %	99 %	81 %	111 %	96 %
F3	63 %	104 %	50 %	79 %	107 %	81 %	116 %	99 %
F4	52 %	97 %	48 %	75 %	102 %	74 %	104 %	97 %
F5	87 %	108 %	51 %	79 %	109 %	77 %	114 %	102 %
10 PPM								
F1	44 %	100 %	47 %	75 %	103 %	84 %	110 %	98 %
F2	49 %	102 %	50 %	77 %	106 %	81 %	113 %	99 %
F3	50 %	100 %	48 %	74 %	100 %	74 %	109 %	94 %
F4	43 %	96 %	48 %	72 %	98 %	72 %	105 %	94 %
F5	68 %	104 %	50%	78 %	107 %	78 %	109 %	94 %

Accuracy ranges from approximately 0.5x to 2x of the target concentration

* Manual Response Factor = IS Area/IS Concentration (g/mL) X Sample weight (g)/Extraction volume (mL)

** Higher value was a result of different mass spec ion selected by the software for quantitation

METHOD VALIDATION: LIMIT OF DETECTION (LOD)

Purpose: Determine the lowest level that an analyte could be detected and accurately identified

Minimum requirements:

- A signal to noise ratio > 8:1 with a library match factor score > 55.
- The ability to correctly identify compounds more than 50 % of the time

Analyzed six (6) replicate injections or fortified e-liquid samples prepared at concentrations ranging from 0.5 ppm to 5.0 ppm.

Fortified Matrix Concentration (PPM)	0.5	**0.7**	1.0	2.0	5.0
Number of compounds/8, with confirmed identifications	3	**4**	6	7	8
% Correct	38 %	**50 %**	75 %	88 %	100 %

- A threshold or “cut-off” value of 0.5 ppm determined using criteria in the U.S. FDA – Guidelines for the Validation of Chemical Methods for the FDA FVM Program*

The method LOD was determined to be 0.7 ppm, with a threshold value of 0.5 ppm

*Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 2nd edition, April 2015, pages 16-17, section 3.4

METHOD VALIDATION: SENSITIVITY

Purpose: Determine the sensitivity of the method to detect a change in analyte concentrations compared to a control formulation

Statistical approach: $$P(|\bar{x}_t - \bar{x}_c| > k\sigma_m) = P\left(\frac{|\bar{x}_t - \bar{x}_c|}{\sqrt{2}\sigma_m} > \frac{k}{\sqrt{2}}\right) \Rightarrow k = \sqrt{2}\, t \cong 6\sigma_m$$

Example	Hydroxyacetone	2,3,5-trimethylpyrazine	Menthone	(E)-Beta-Damascone	Cinnamic acid methyl ester	Myosmine	Piperonal	Cotinine
Day 1 Mean	3.10	5.45	2.60	4.32	5.55	7.63	6.24	9.45
Day 2 Mean	2.49	4.74	2.37	ND	5.04	7.38	5.91	8.11
Day 3 Mean	3.27	5.08	2.44	4.17	5.00	8.15	6.19	9.20
Grand Mean (x_c)	2.95	5.09	2.47	4.24	5.19	7.72	6.11	8.92
S.D.	0.41	0.35	0.12	0.10	0.31	0.39	0.18	0.71
Mean+6xSD (x_t)	5.42	7.21	3.17	4.85	7.04	10.09	7.19	13.20
Fold Increase	1.84	1.42	1.28	1.14	1.35	1.31	1.18	1.48

Fold Increase = (Mean + 6 × S.D.) / Mean

1.4 fold increase was detectable based on the overall average from matrices and analytes

METHOD VALIDATION: SELECTIVITY

Purpose: Evaluate the ability of the method to identify extraneous peaks and detect peaks that were ≥ 1.4 fold increase compared to a control formulation

- Fresh and aged e-liquid samples were evaluated
- All compounds detected were identified using internal custom library
- Determine number of peaks ≥ 1.4 fold compared to the fresh formulation

Sample	Correct/Total	Percent (%) of Compounds Identified Correctly	≥ 1.4 Fold Increase
Tobacco Flavor e-Liquid	44/47	91.6	1
Menthol Flavor e-Liquid	108/119	90.8	3
Tobacco Flavor Aerosol	53/55	96.4	3
Menthol Flavor Aerosol	110/118	93.2	3
Overall Average	N/A	93.0	1.8

> 90 % of compounds were correctly identified base on our internal custom library with a match factor score > 85 and a 1.4 fold increase for analytes was detected

METHOD VALIDATION: SUMMARY OF CRITICAL PARAMETERS

Validation Parameter	Established Criteria
Precision & Accuracy (n = 6, 3 days)	- %RSD ≤ 8.5 for all matrices/concentration levels[1] - Estimated concentrations[2] range - 0.5x to 2x the target value
LOD	- 0.7 ppm with a threshold value of 0.5 ppm
Selectivity	- Compounds that increase by 1.4 fold can be detected by this method - > 90% compounds were correctly identified[3]

1 - Data not included in presentation
2 - Calculated on the basis of manual response factor of internal standard
3 - Using a custom library with a match factor score > 85

Validation results demonstrate that method is fit for purpose

SUMMARY

- A semi-quantitative method for non-targeted analysis (NTA) by GC/MS was developed using MassHunter Unknowns software for automated data processing
- MassHunter Unknowns software automates and significantly reduces data processing time and the ALCS custom internal mass spectral library improves consistency of peak identification
- The NTA method was fully validated based on requirements listed in "*Guidelines for the Validation of Chemical Methods for the FDA FVM Program*"
- The GC/MS Non-targeted analysis method is suitable for chemical characterization of e-vapor aerosol and liquid formulations

THANK YOU

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