Chemical Characterization of E-Vapor Products using Non-targeted Analysis by Gas Chromatography Mass Spectrometry

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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 method to characterize the volatile and semi-volatile compounds present in the aerosol and e-liquid from ENDS products.

* Taken from the US Dept HHS (Food and Drug Administration), 2019, Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems (ENDS), Guidance for industry.
- (Text highlighted in blue above denotes added emphasis.)

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Non-Targeted Analysis by GC/MS

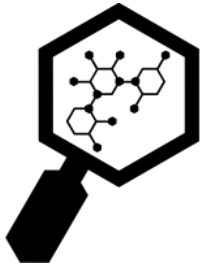
Sample Analysis

1) Aerosol Collection or e-Liquid Removal
2) Sample Preparation
3) Analysis by Instrumentation

Data Processing

1) MS Deconvolution
2) Compound Identification
3) MassHunter Unknowns Analysis*

Compound Identification



1) Custom Library
2) High Resolution Mass Spectrometry

Custom Reporting

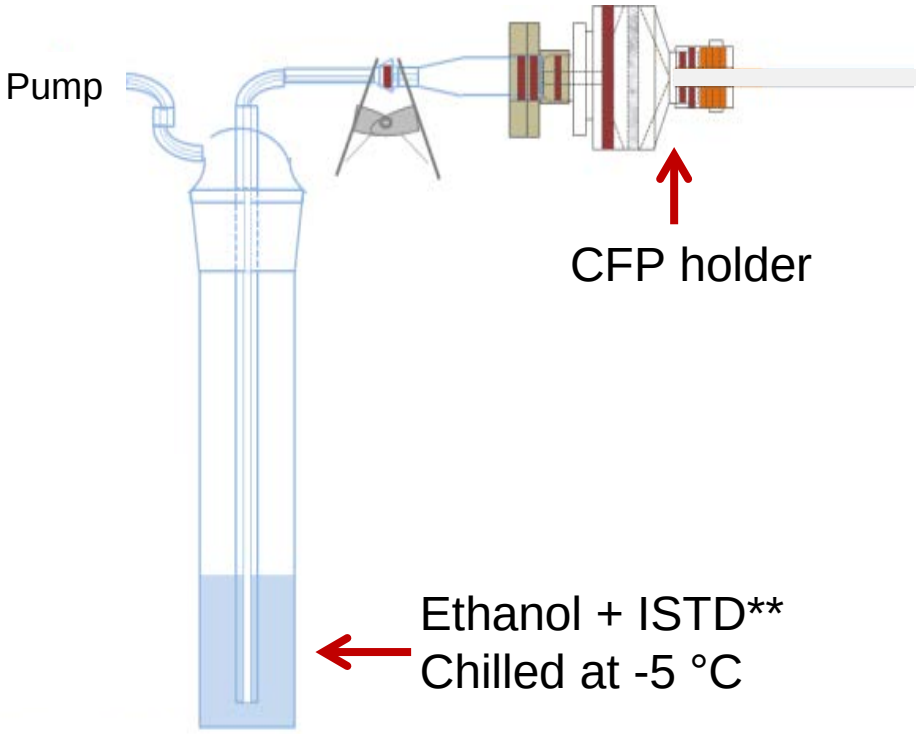
Risk Assessment

- Toxicological evaluation

PMTA**

* MassHunter Unknowns Analysis software is licensed and distributed by Agilent Technologies.
** PMTA – Pre-Market Tobacco Authorization

Aerosol Collection and Sample Preparation



Aerosol Collection

- **Linear smoking machine (Borgwaldt LX20)**
- **Intense puffing regime: 55 cc puff volume, 5 s puff duration, 30 s interval, square wave puff profile**
- **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 (Ethanol w/ ISTD).**
- **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/ ISTD)**
- **Extract on rotator for 30 minutes**

*Device dependent parameter
**Internal Standard (ISTD)

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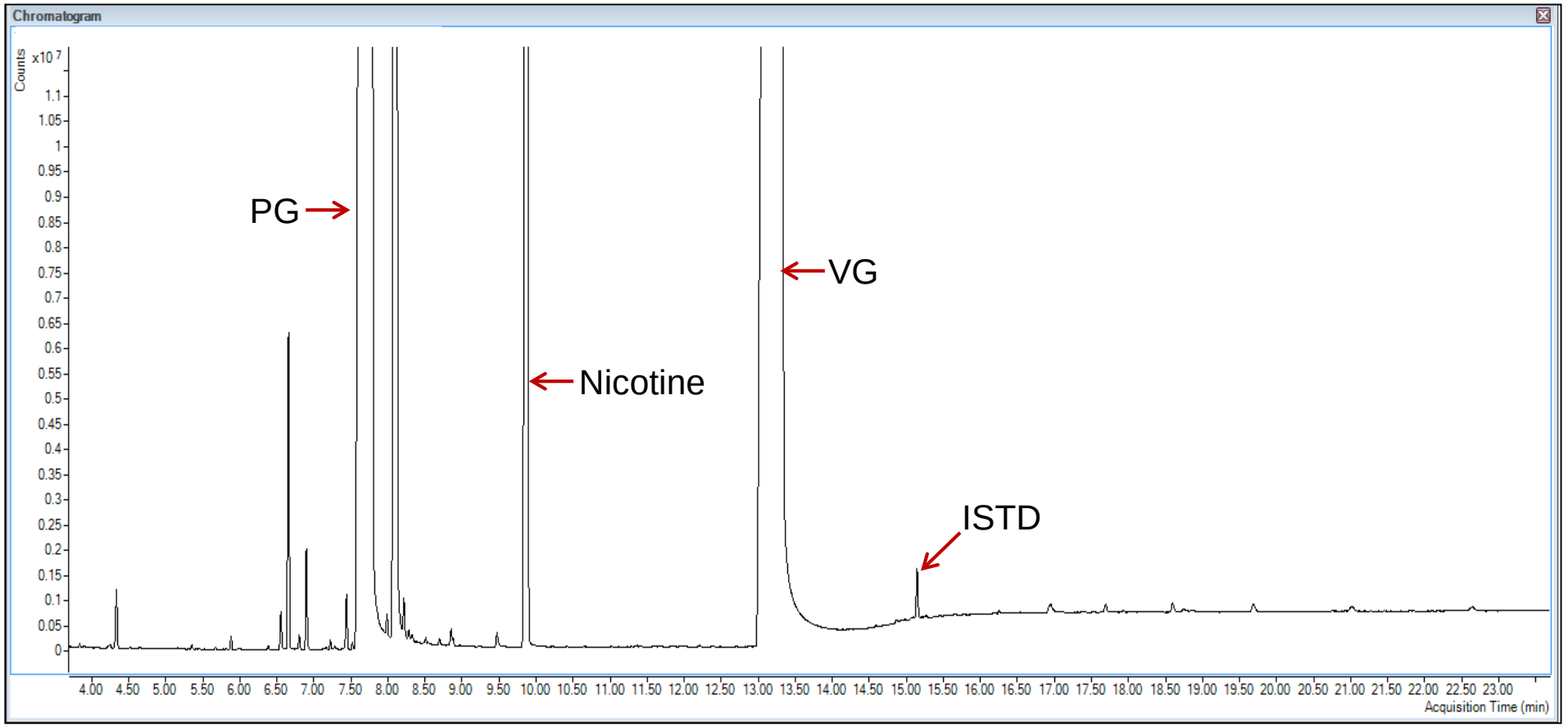
Instrumentation

Agilent Technologies GC/MS (single quad) in Electron Ionization (EI) mode

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

Run time: ~24 minutes

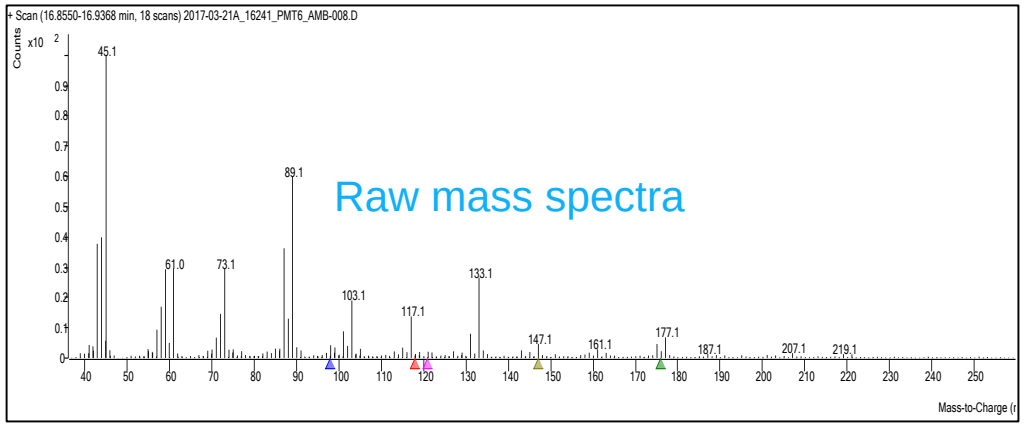
Example GC/MS Chromatogram Aerosol Sample

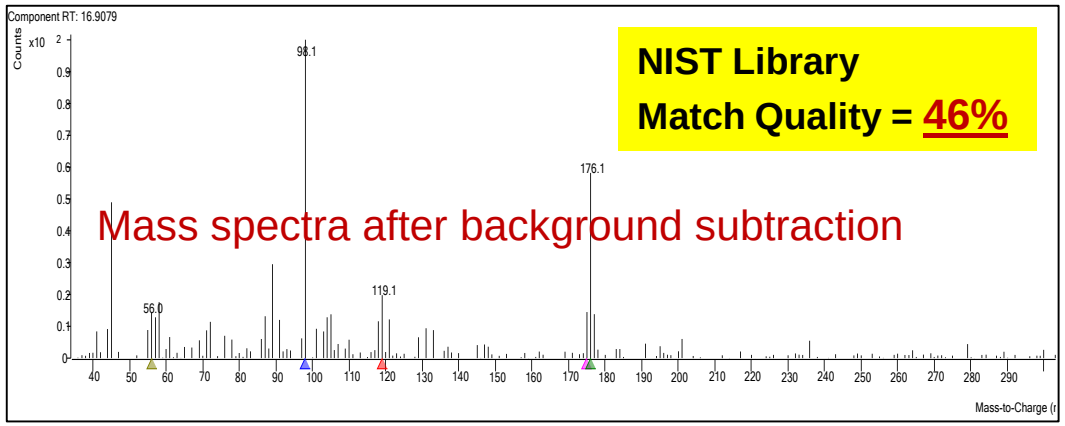


PG- Propylene glycol, VG- Vegetable glycerin, ISTD - Internal Standard (6-Methylcoumarin)

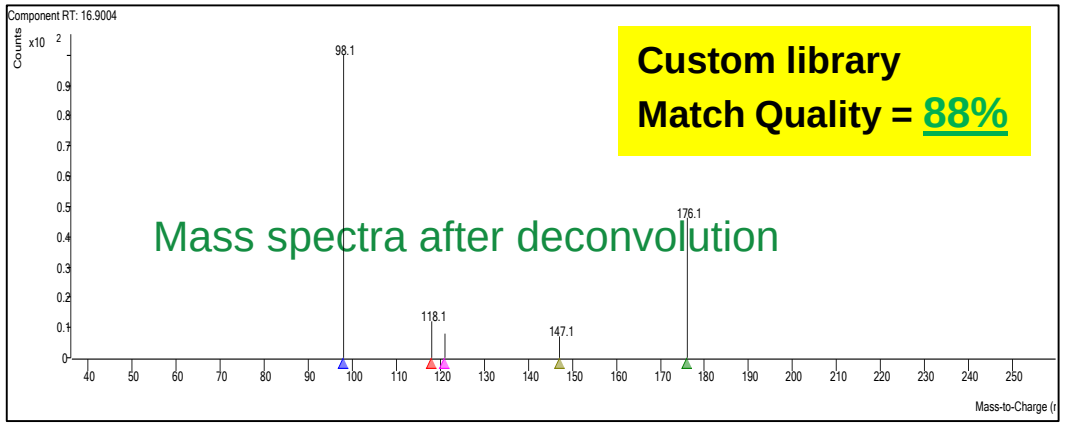
MassHunter Unknowns Deconvolution

<u>Deconvolution</u>: Extracts the "clean" spectra from background noise based on both retention time and peak shape.
Note: This is based on method parameters such as retention time window, base peak shape, and min/max number of ion peaks.





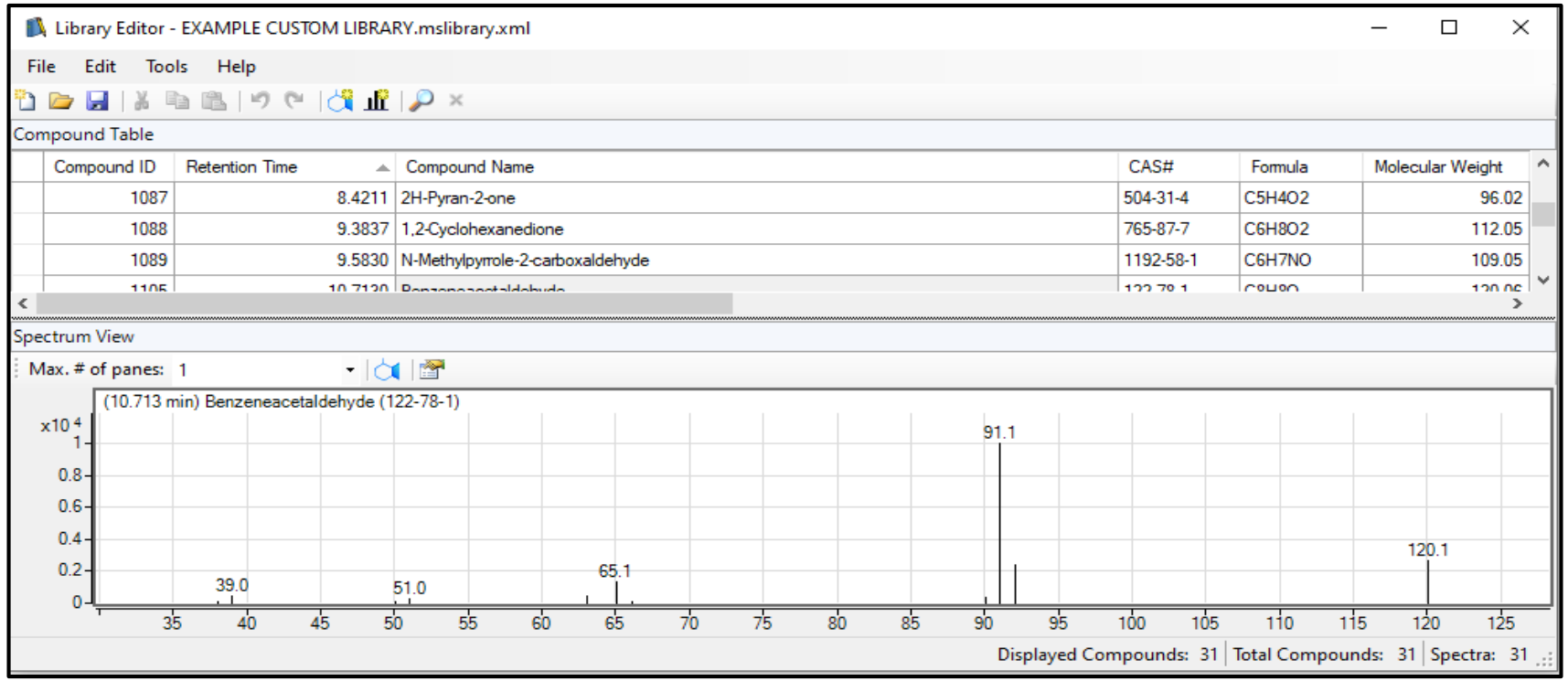
NIST Library
Match Quality = 46%



Custom library
Match Quality = 88%

MassHunter Library Editor Software

Agilent MassHunter Library Editor software is used to create custom libraries which are applied to data.

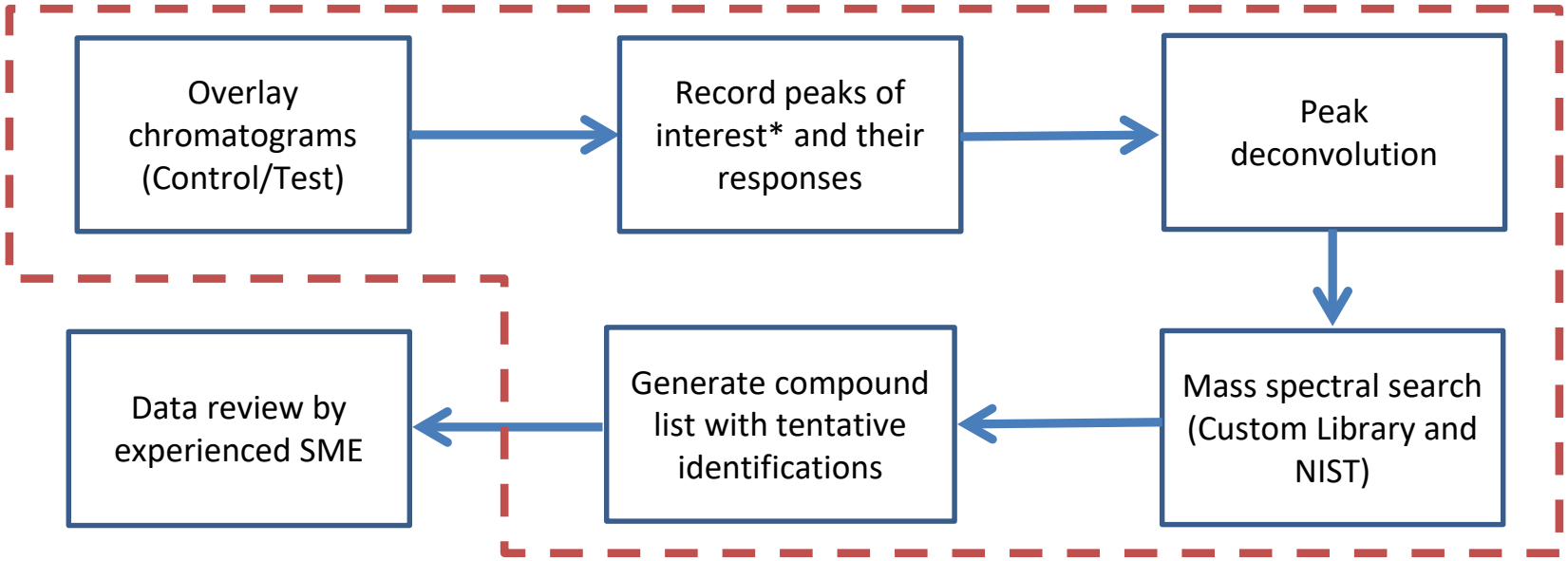


Custom library:

- Includes ~1100 mass spectral entries with compound RT, CAS#, formula, molecular weight.
- Includes > 600 mass spectral entries confirmed with reference standards.

Data Processing – Automation with MH Unknowns

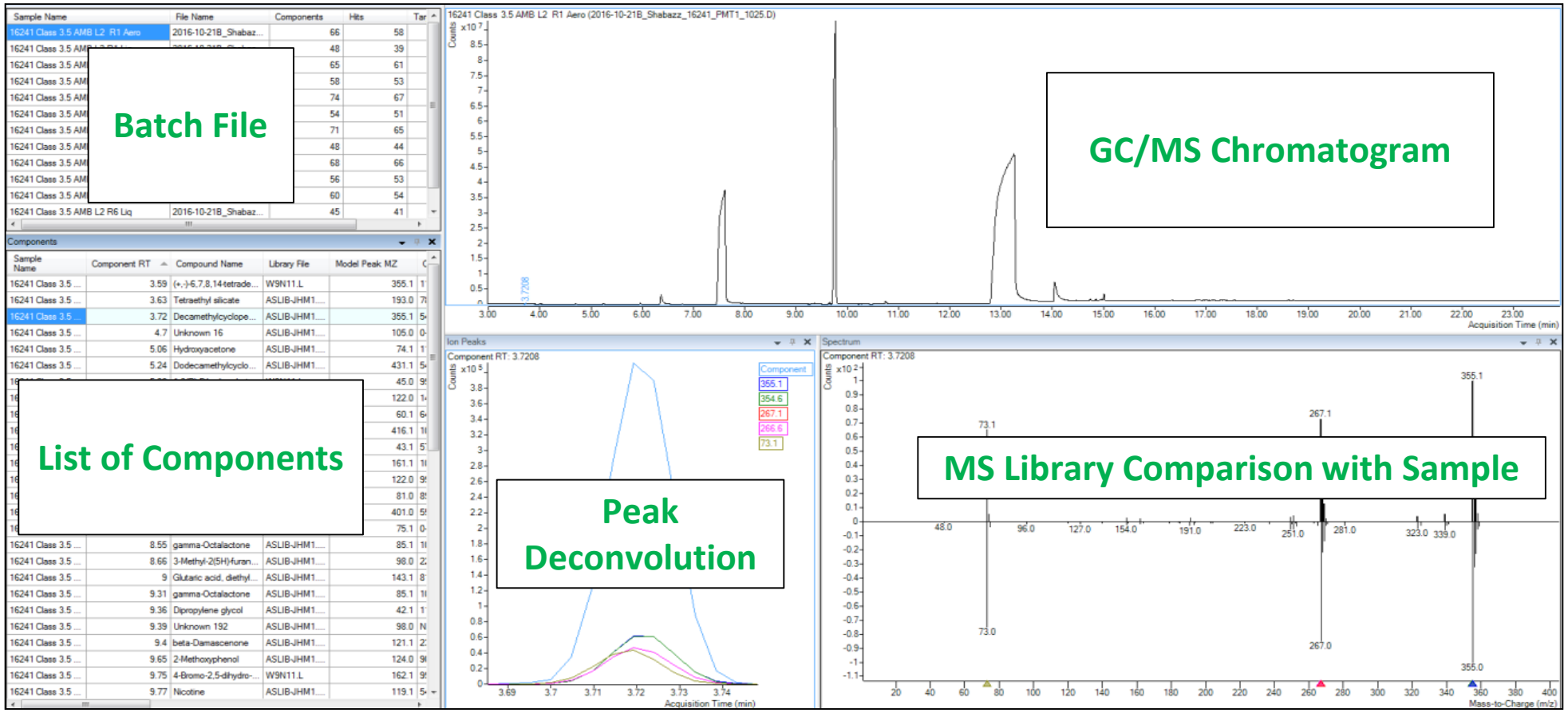
Automation with MassHunter Unknowns



- Efficiency of software for 1 lot with 6 sample replicates (aerosols and e-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 e-liquids)
- Provide identification of compounds that are new or are present at higher concentrations compared to a control
- Capable of detecting compounds ≥ 1 ppm

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

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

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

Method Validation: Accuracy

$$\% \text{ Accuracy} = \frac{\textit{Measured Amount (PPM)}}{\textit{Background Amount (PPM)+ Fortified Amount (PPM)}} \times 100\%$$

Concentration F1-F5 matrices	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 fortified concentration

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

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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 of 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 FDA FVM criteria*

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 Foods and Veterinary Medicine (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
Grand Mean + 6 x S.D. (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 = *Grand Mean* + $(6^{df} \times S.D.)$ / *Grand Mean* = *1.4*

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

Fold increase *df* = degrees of freedom

Method Validation: Selectivity

<u>Purpose</u>: 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 the custom library.
- Determine number of peaks in the aged samples ≥ 1.4-fold increase in comparison to the fresh formulation.

Sample	Correct/Total Number of peaks	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 based on custom library match factor scores > 85 and a 1.4-fold increase for analytes detected

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Method Validation: Summary of Critical Parameters

Validation results demonstrate that the method is fit for purpose

Validation Parameter	Established Criteria
Precision & Accuracy (n = 6, over 3 days)	- %RSD ≤ 8.5 for all matrices/concentration levels[1] - Estimated concentrations[2] ranges from 0.5x to 2x the target value
Limit of detection (LOD)	- 0.7 ppm with a threshold value of 0.5 ppm
Selectivity	- Compounds with1.4-fold increase 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

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Summary

- Developed and validated a semi-quantitative method for non-targeted analysis by GC/MS using MassHunter Unknowns Analysis software
- Developed data processing automation w/ custom library which significantly saves time
- Method is suitable for chemical characterization of e-vapor aerosol and e-liquid formulations
- Method is under ISO17025 scope of accreditation
- Method can be used for detecting chemical changes over time to determine the lifespan of e-vapor products

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THANK YOU

Questions?