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

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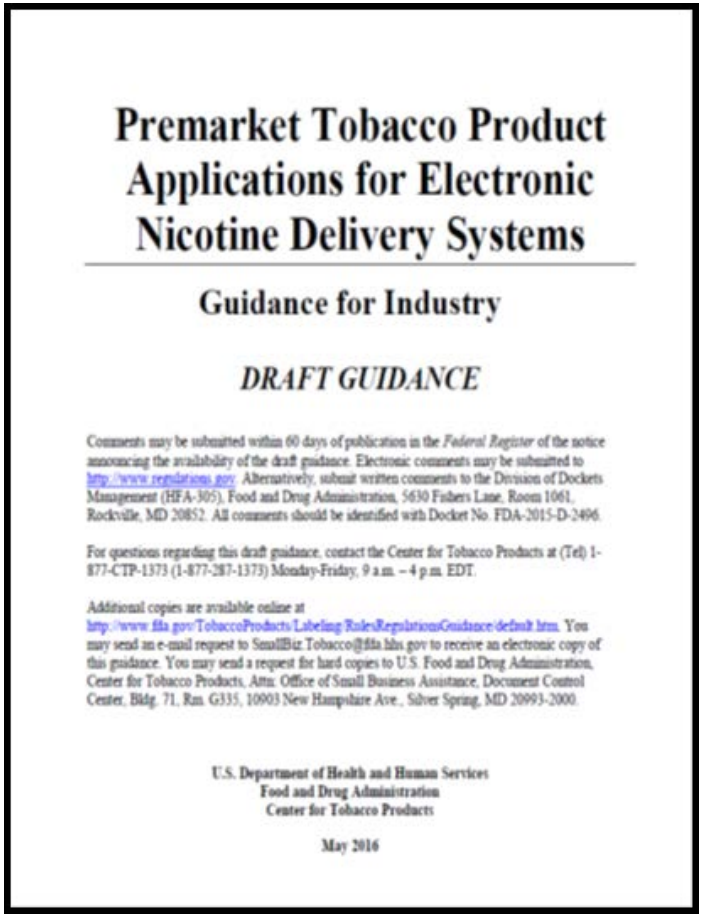
FDA draft guidance for PMTA for ENDS



Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems

Guidance for Industry

DRAFT GUIDANCE

Comments may be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Electronic comments may be submitted to http://www.regulations.gov. Alternatively, submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852. All comments should be identified with Docket No. FDA-2015-D-2496.

For questions regarding this draft guidance, contact the Center for Tobacco Products at (Tel) 1-877-CTP-1373 (1-877-287-1373) Monday-Friday, 9 a.m. – 4 p.m. EDT.

Additional copies are available online at http://www.fda.gov/TobaccoProducts/Labeling/RulesRegulationsGuidance/default.htm. You may send an e-mail request to SmallBiz.Tobacco@fda.hhs.gov to receive an electronic copy of this guidance. You may send a request for hard copies to U.S. Food and Drug Administration, Center for Tobacco Products, Attn: Office of Small Business Assistance, Document Control Center, Bldg. 71, Rm. G335, 10903 New Hampshire Ave., Silver Spring, MD 20993-2000.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Tobacco Products

May 2016

PMTA: Premarket Tobacco Product Application
ENDS: Electronic Nicotine Delivery System
HPHC: Harmful and potential harmful constituents

Section H – Scientific studies and analysis

"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... "

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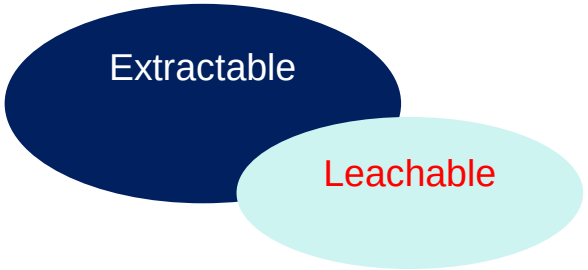
Text in Blue denotes added emphasis

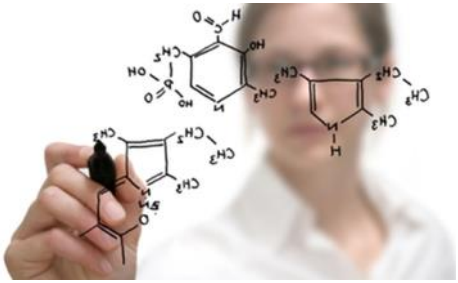
Approaches for chemical characterization

- Evaluation of device components

  - Extractable and Leachable



- Targeted analysis (Nicotine, Nicotine degradants, HPHCs, etc.)



- Non-Targeted Analysis (NTA)
  - GC/MS profiling (Volatile and semi volatile compounds)
  - LC/MS profiling (Non volatile compounds)

- Data obtained supports risk assessment

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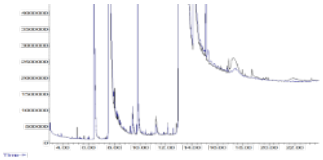
Non-targeted analysis by GC/MS



GC/MS Profile

Risk Assessment

Sample Aerosol/Liquid

Data Analysis

MassHunter Software

Compound Identification/ Confirmation

Custom Library

HRMS confirmation

Reporting

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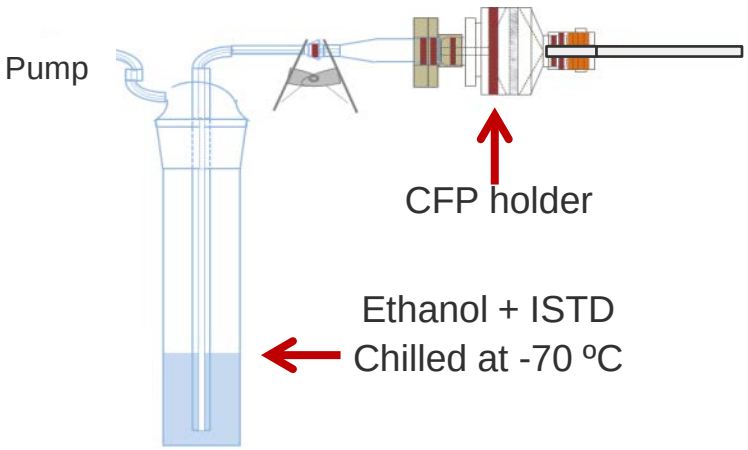
HRMS: High Resolution Mass Spectroscopy

Generation of samples : Aerosol and liquid



Analyze Sample Extract

GC-MS

- **<u>Aerosol sample collection</u>**

- Linear smoking machine (Borgwaldt LX20)

- Puffing regime: 55 cc, 5 s, interval 30 s, square wave, # of puffs - 140

- Collected on a 55 mm Cambridge filter pad (CFP) with a trailing impinger with 10 mL extraction solvent

- **<u>Liquid sample analysis</u>**

- Liquid from cartridge (~0.8 g) + 10 mL Extraction solvent

- Extract on rotator for 30 minutes

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ISTD: Internal standard

GC/MS profiling method

- **Instrumentation setup**

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

- Column: Restek Stabilwax (30m x 0.25mm x 0.25µm) with 5 meter Integra guard

- Run time: 24 min

Agilent GC-MS

- **Method applications**

- Analysis of aged samples

- Stability assessment

- Stress testing

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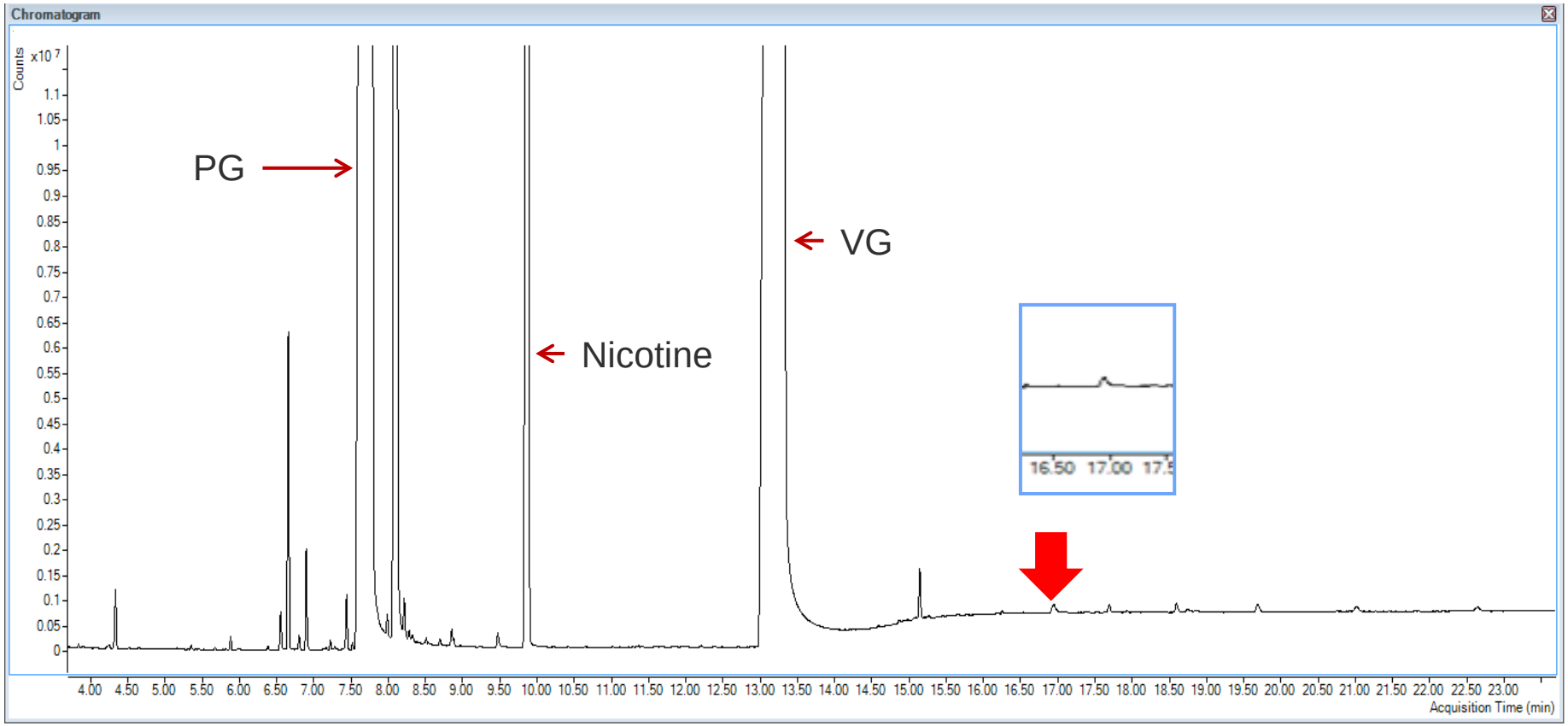
GC/MS chromatogram: Aerosol of commercial e-vapor product



PG- Propylene glycol, VG- Vegetable glycerin

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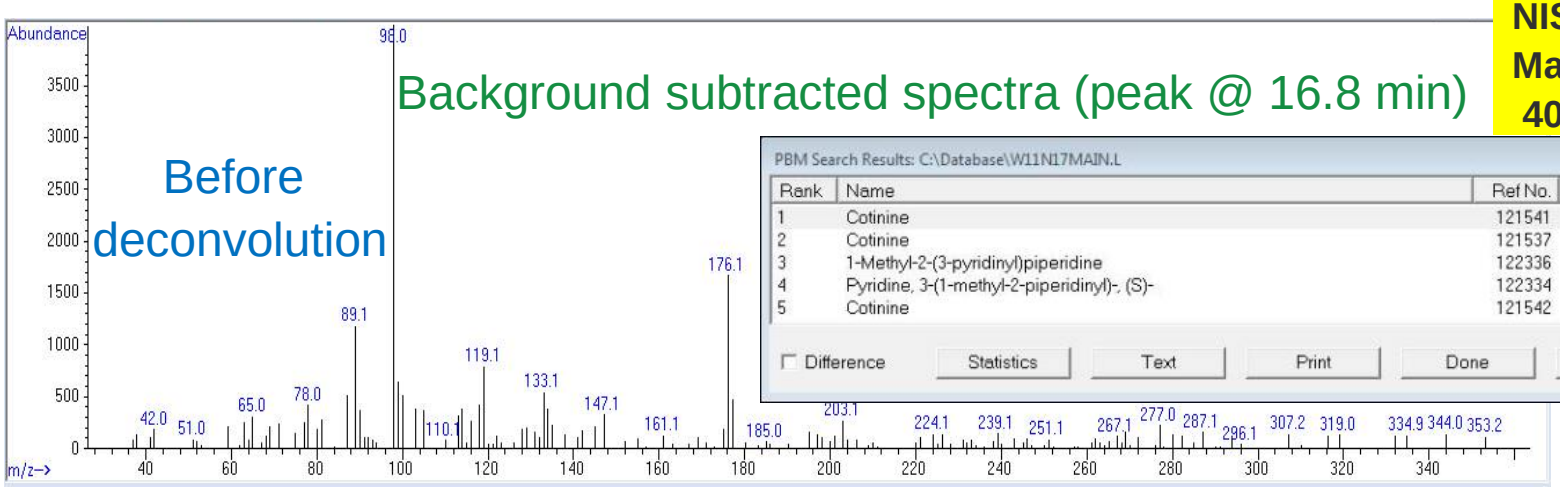
MS deconvolution and library search

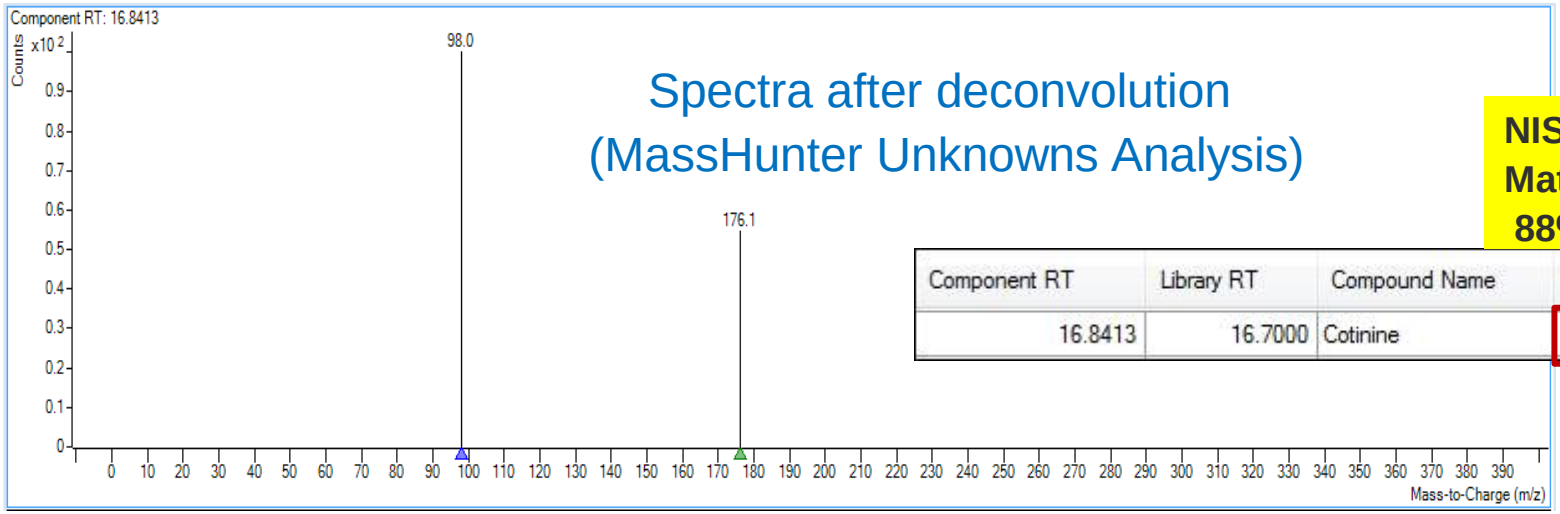


NIST library Match Quality 40-47%

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



NIST 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 identification from Library

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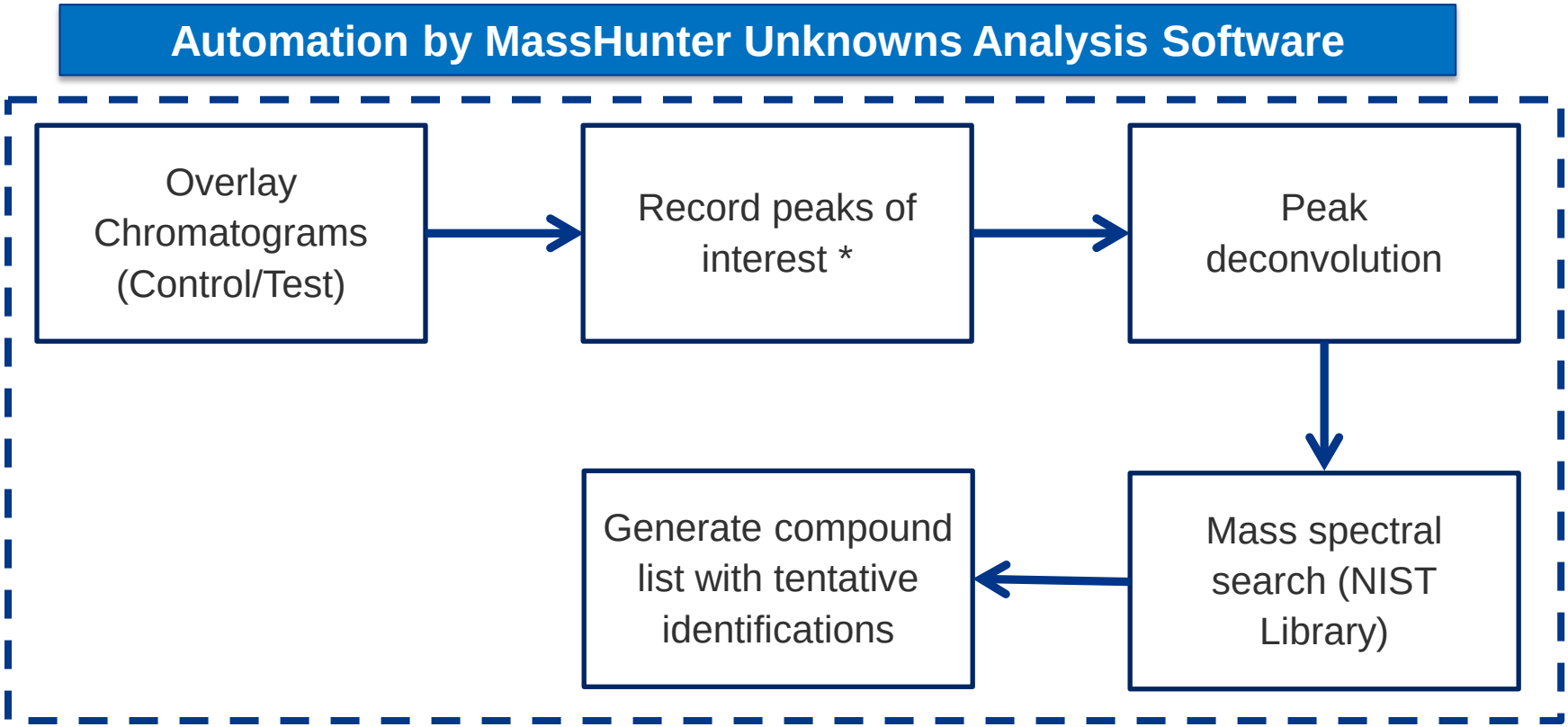
GC/MS profiling data analysis



Example : Aerosol and liquid analysis (6 replicates each)

- # of total peaks ~10-100 per analysis
- **Manual process: ~ 6 hours**
- **Automated process: ~ 2 hours**

* Peaks that are new or increasing in concentration compared to control

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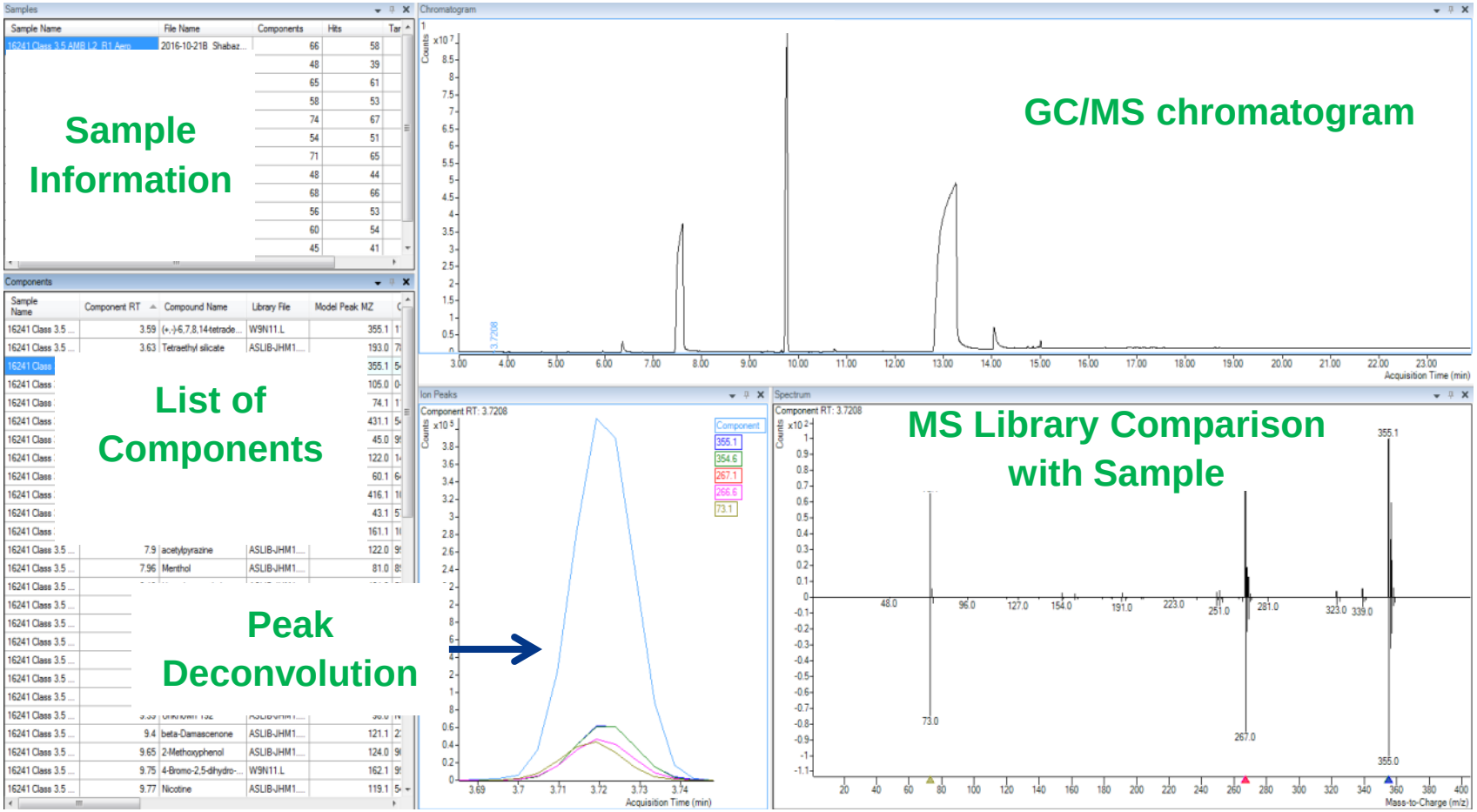
Data processing by MassHunter Unknowns Analysis



Component list is generated from automatic spectral deconvolution and library search

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Other tools to support GC/MS data processing

- AMDIS used for deconvolution and ChemStation as secondary verification tools

- Compounds are confirmed using unit mass and HRMS with reference standard when available

- Established an in-house custom mass spectral library including information:
  - Mass spectra
  - Retention time
  - Compound name
  - Formula
  - CAS number

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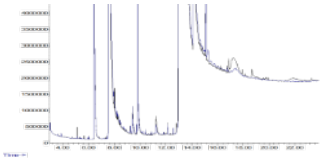
Non-targeted analysis by GC/MS



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GC/MS profiling method validation

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GC/MS profiling method validation

- Validation compounds were selected by classification (e.g., flavors, degradation products, etc.) and retention time
- Matrices representing wide range of PG/VG concentrations were included
- All matrices were fortified using the validation compounds

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

Unfortified e-liquid matrices[1]
50/50/15 – PG/VG/H_2O + 2.5% NBW
50/50/0 – PG/VG/H_2O + 2.5% NBW
50/50/15 – PG/VG/H_2O + 0% NBW
80/20/15 – PG/VG/H_2O + 0% NBW
20/80/15 – PG/VG/H_2O + 0% NBW
Flavored e-liquids (n=2)

[1] NBW = Nicotine by weight

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GC/MS profiling method validation

Validation Parameter	Elements Evaluated
Calibration	NA
Accuracy	✓
Instrument precision	✓
Repeatability and Intermediate precision	✓
Reproducibility	NA
Selectivity	✓
LOD	✓
LOQ	NA
Robustness	✓
Stability	✓
System suitability	✓

NA: Not Applicable

Follows FDA recommendations for validation of Chemical method*

* *Guidelines for the Validation of Chemical Methods for the FDA FVM Program,* 2nd edition, April 2015

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Critical method validation results

Validation Parameter	Established Criteria
Precision & Accuracy (n=6, 3 days)	- %RSD ≤ 8.5 for all matrices/concentration levels - Estimated concentrations* range - 0.5 to 2 times the actual value
Selectivity	- >99% compounds were identified with library match quality of 85 or higher - Compounds that increase by 1.4 fold can be detected by this method
LOD	- 0.7 ppm

* Calculated on the basis of manual response factor of internal standard

Validation results demonstrate that method is fit for purpose

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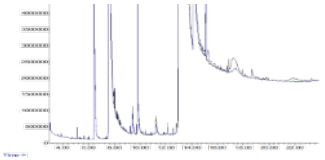
Non-targeted analysis by GC/MS



GC/MS Profile

Risk Assessment

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HRMS confirmation

Reporting

Validated Method

HRMS: High Resolution Mass Spectroscopy

HRMS – Orbitrap for identification of unknowns

- Resolving power up to 100,000 for small molecule identification
- Offers high resolution and sensitivity in full scan mode
- High mass accuracy and isotope fingerprint (e.g., ^{15}N and ^{13}C) can be used to confirm formulas
- Ion source - Electron ionization (EI) and Chemical ionization (CI)

ThermoFisher Q-Exactive GC-Orbitrap

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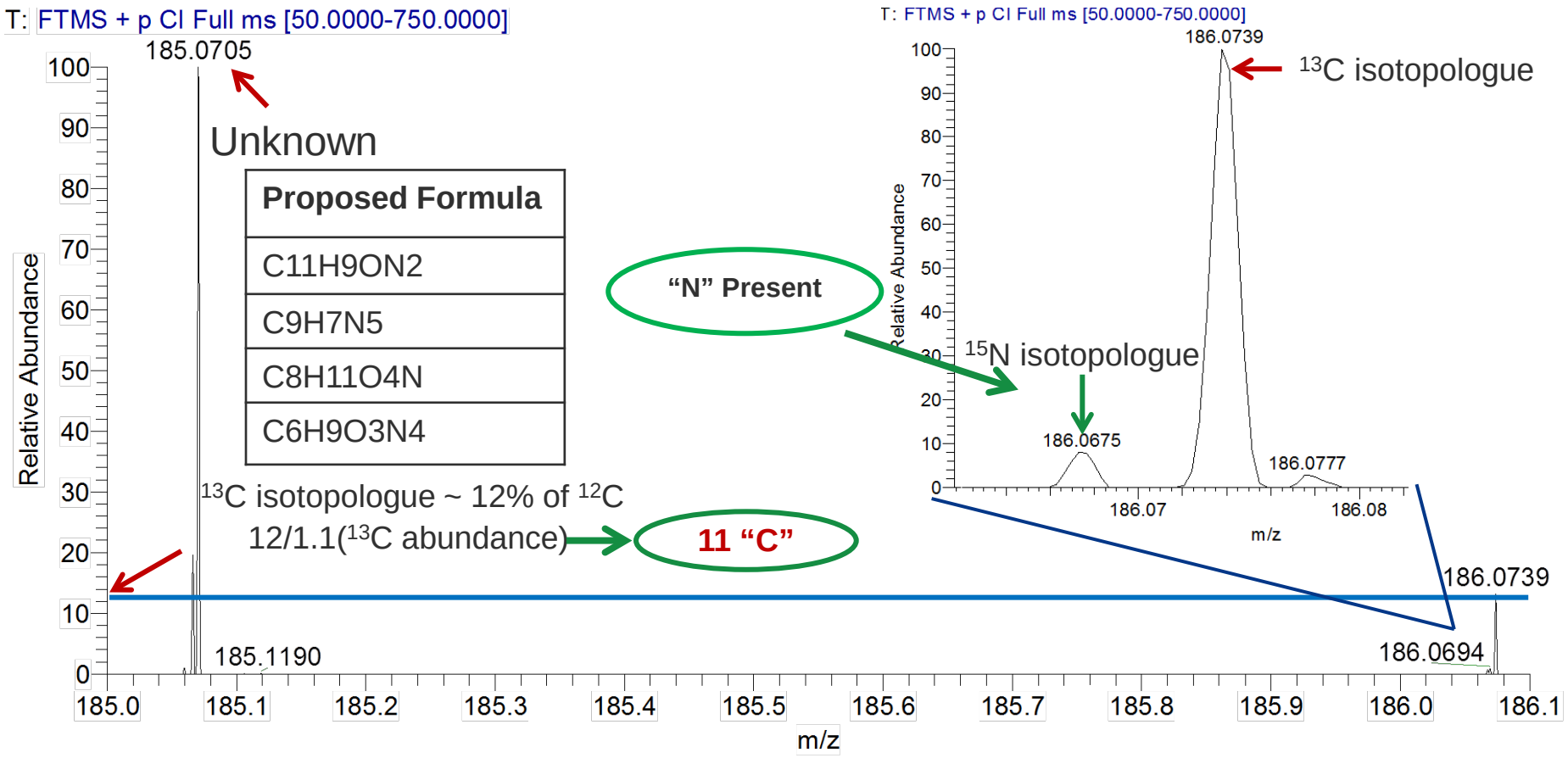
Example: HRMS identification of an unknown



CI spectra to confirm the molecular ion and formula for the unknown compound

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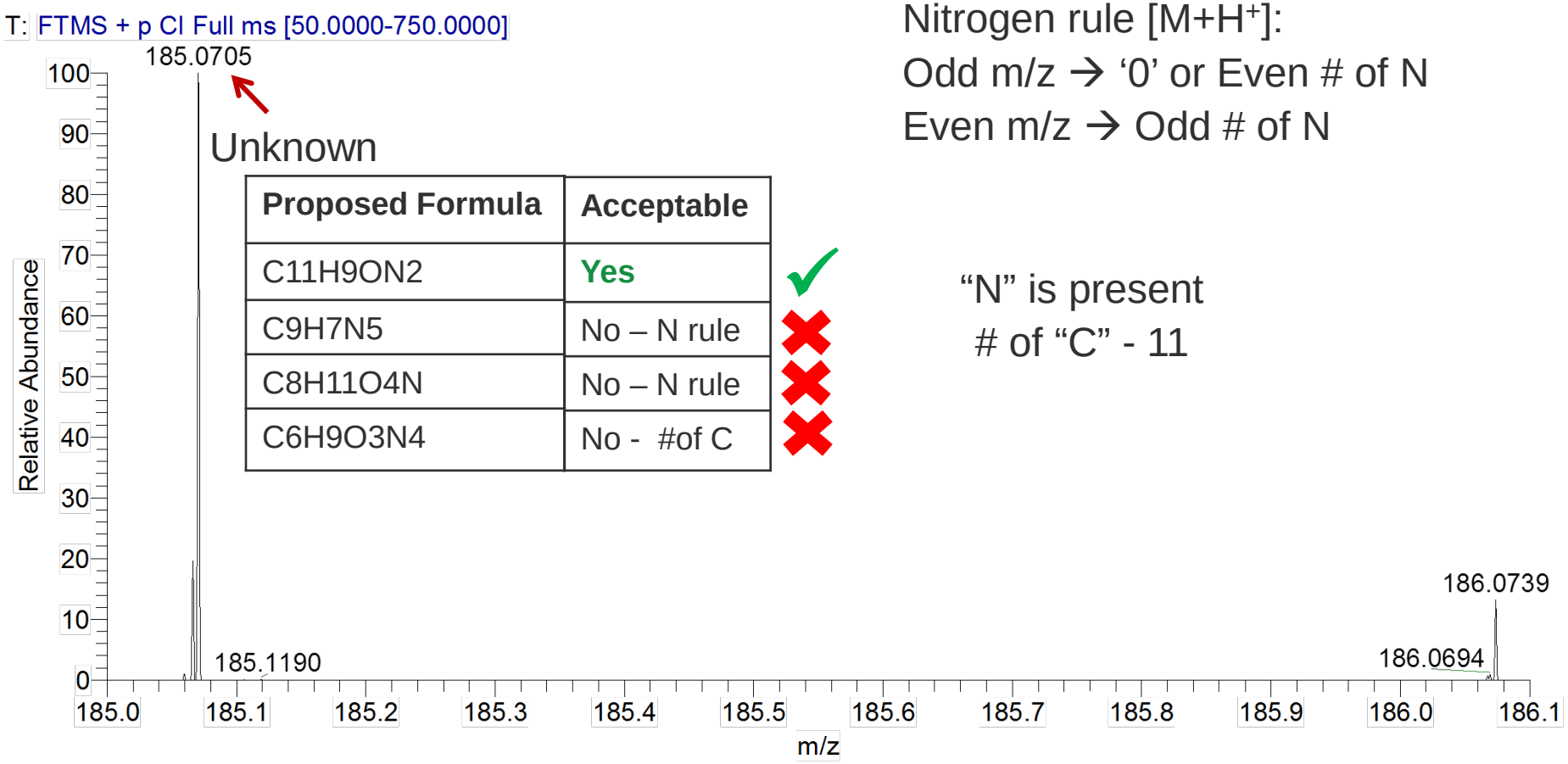
Example: HRMS identification of an unknown



Proposed Formula	Acceptable
C11H9ON2	Yes
C9H7N5	No – N rule
C8H11O4N	No – N rule
C6H9O3N4	No - #of C

Nitrogen rule [$M+H^+$]:

Odd m/z → '0' or Even # of N

Even m/z → Odd # of N

"N" is present

of "C" - 11

Confirmation of molecular formula based on exact mass and isotopologues

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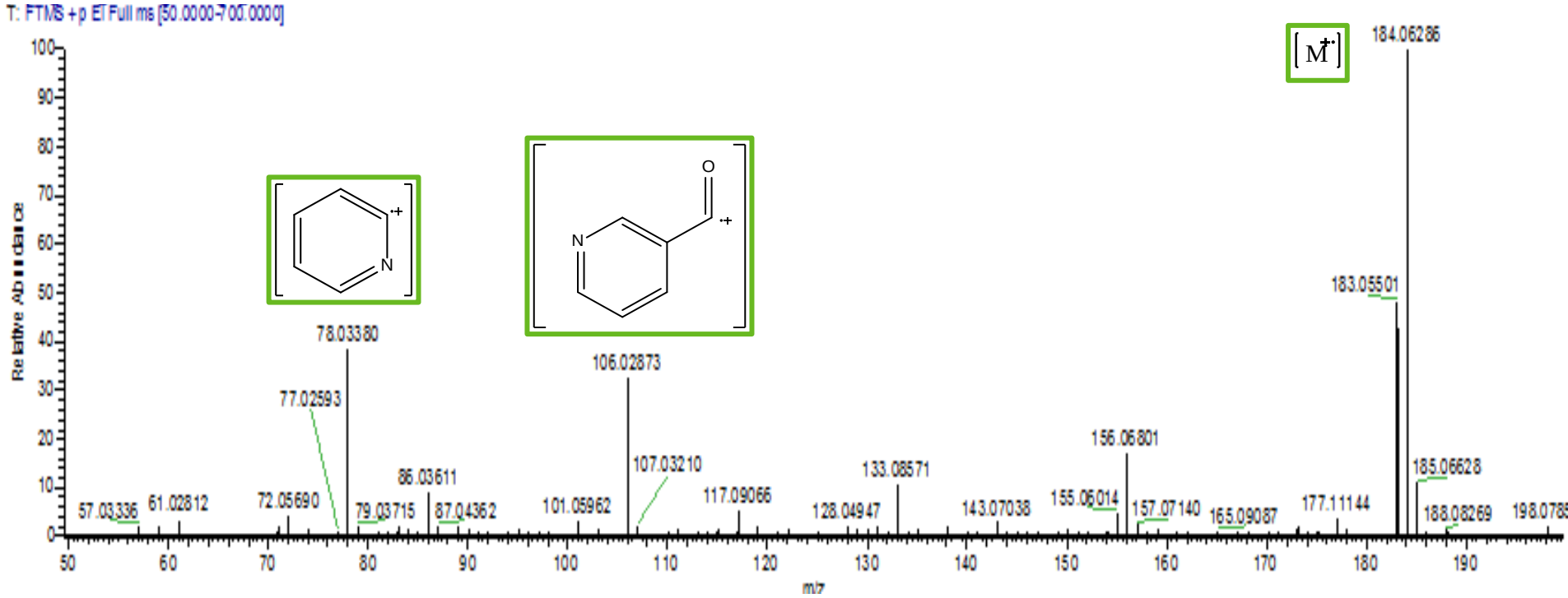
Proposed structures and confirmation

- Fragmentation patterns for EI and CI spectra were evaluated to propose structure



EI – Spectral fragmentation were assigned

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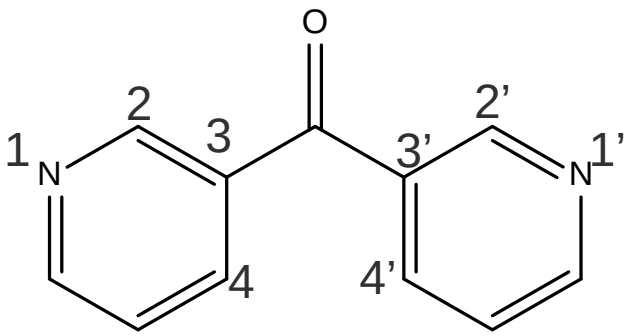
Proposed structures and confirmation

- Fragmentation patterns for EI and CI spectra were evaluated to propose structure
- Multiple structures are possible
- Available isomers were ordered from commercial sources



(3,3')

Other possible isomers:
(3,4'), (2,2'), **(2,3')**, **(4,4')**, (2,4')

Isomer std.	3,4'	2,3'	3,3'	4,4'
GC/MS Retention time (min)	16.25	16.35	16.63	16.85

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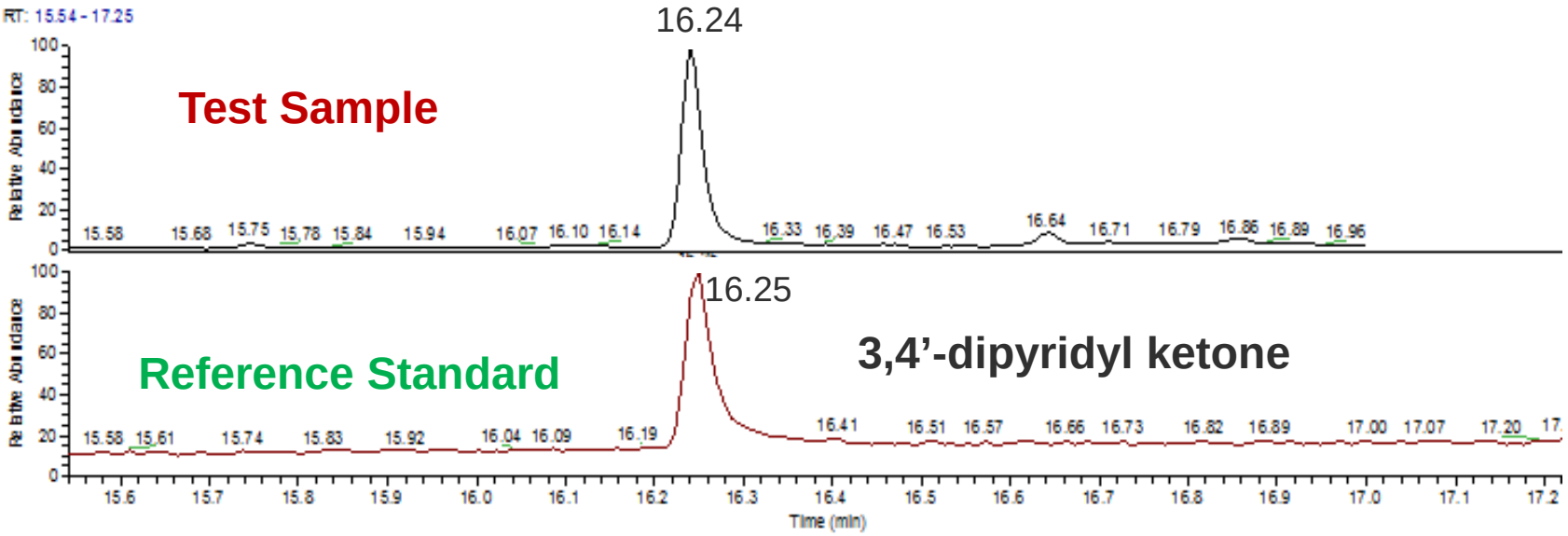
Confirmation by reference standard

Total ion chromatogram (GC/HRMS)



Identity was confirmed by reference standard match with Mass spectra & Retention time

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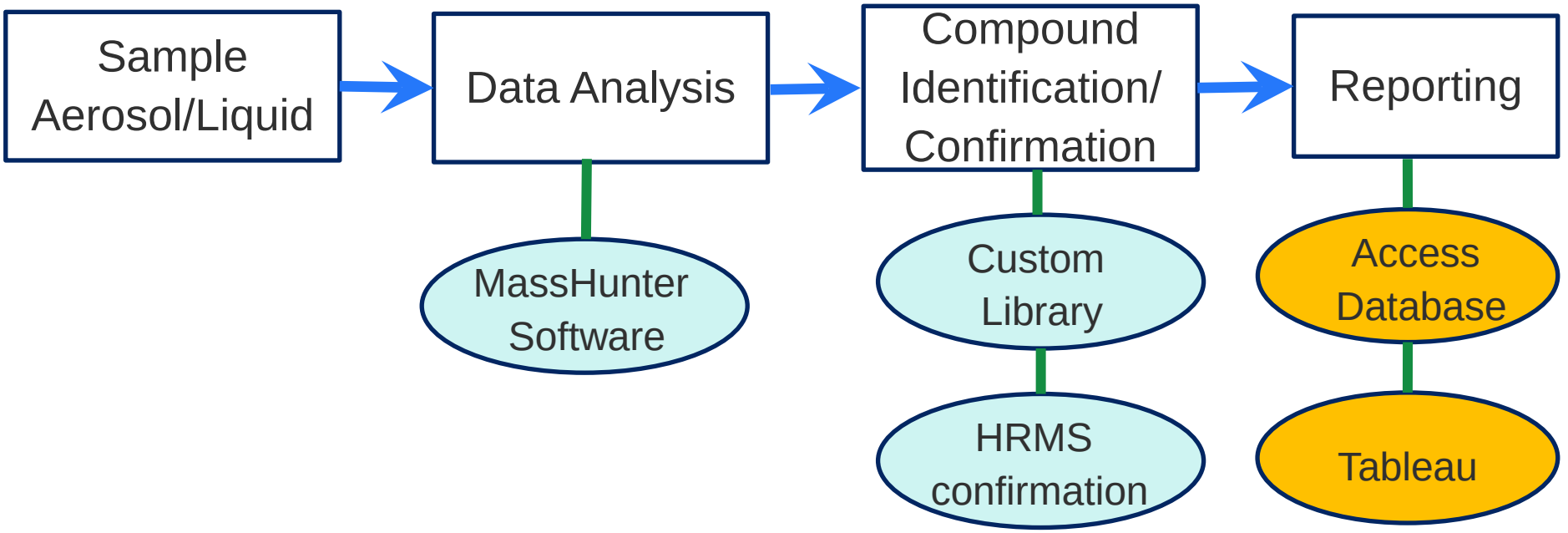
Non-targeted analysis for chemical characterization



✓ Validated Method

✓ Compound Confirmation

Data Storage & Visualization

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HRMS: High Resolution Mass Spectroscopy

Conclusion

- Automated workflows were developed for data analysis and reporting
- High resolution accurate mass spectrometry was used for compound identification and confirmation
- Non-targeted GC/MS analysis approach is applicable for chemical characterization of e-vapor aerosol and liquids formulations

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