Compound Identification Process for GC-MS Non-Targeted Analysis of JUUL Aerosol using a Custom Mass Spectral Library

Juul Labs Science

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Introduction

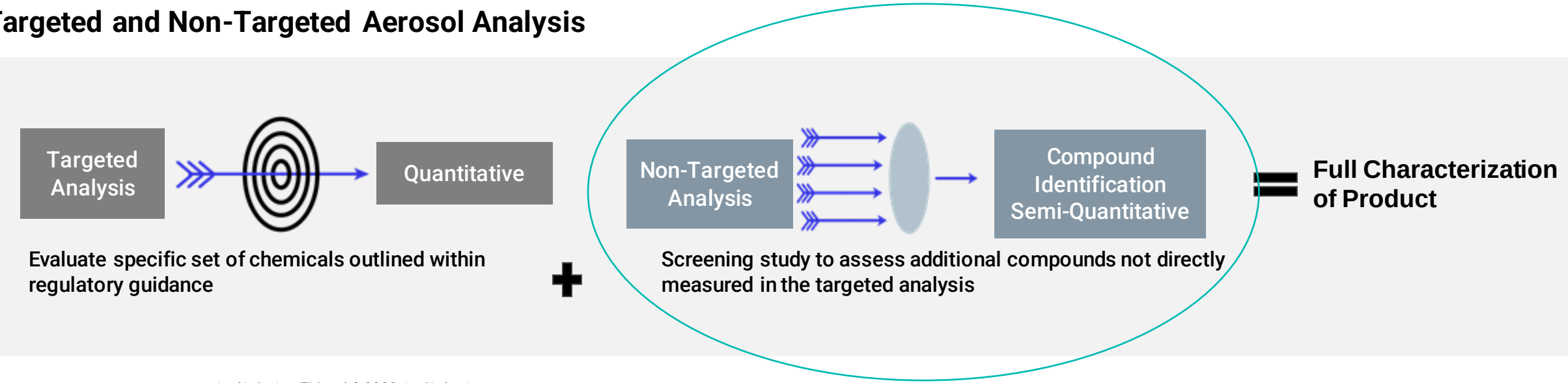
PMTA guidance for ENDS recommends:

"a list of uniquely identified constituents or chemicals, including … other toxic chemicals contained within the product or delivered by the product to be provided"

Objective:

- Full Chemical characterization of the ENDS aerosol
- Determination of aerosol constituent emission levels
- Risk assessment

Targeted and Non-Targeted Aerosol Analysis



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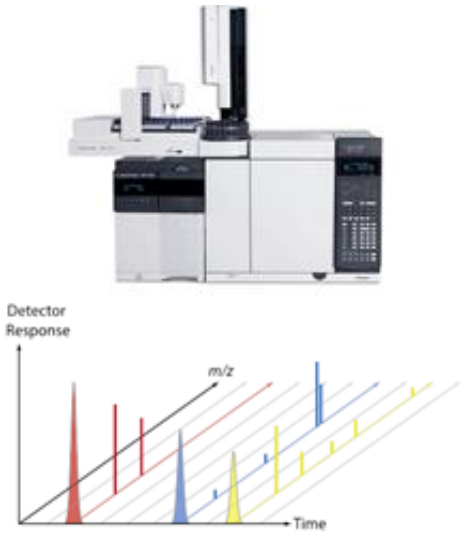
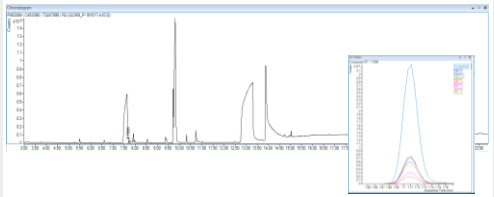
Non-Targeted Analysis of ENDS by GC-MS

Sample Collection

Data Acquisition

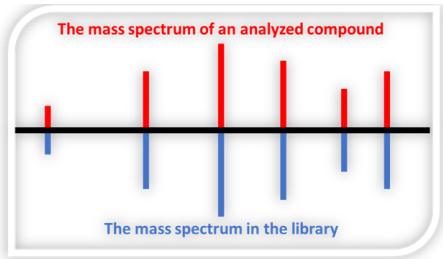


Data Processing



Peak detection
Deconvolution
(compound extraction)
Estimated concentration

Compound Identification



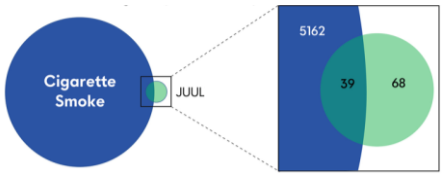
Mass spectral databases and libraries

NIST Custom Library

Interpretation

Compound categorization and rationalization

Comparison to cigarette smoke



Risk Assessment

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Non-Targeted Analysis: Compound Identification

- Many published NTA studies rely heavily on NIST and/or other pre-established vendor libraries for compound identification

- There are several limitations in solely relying on pre-established vendor databases:
  - Need for a match factor threshold (“good” match/identification ≥80)
  - Poor match factor due to matrix effects
  - Not all e-vapor related compounds are included
  - Inconsistent compound identification between samples
  - The NIST database mostly consists of non-deconvoluted mass spectra

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Mass Spectral Libraries – Comparison of Match Factor Threshold

Mass Spectral Database

267,376 compounds

Metabolites, drugs, pesticides, surfactants, etc.

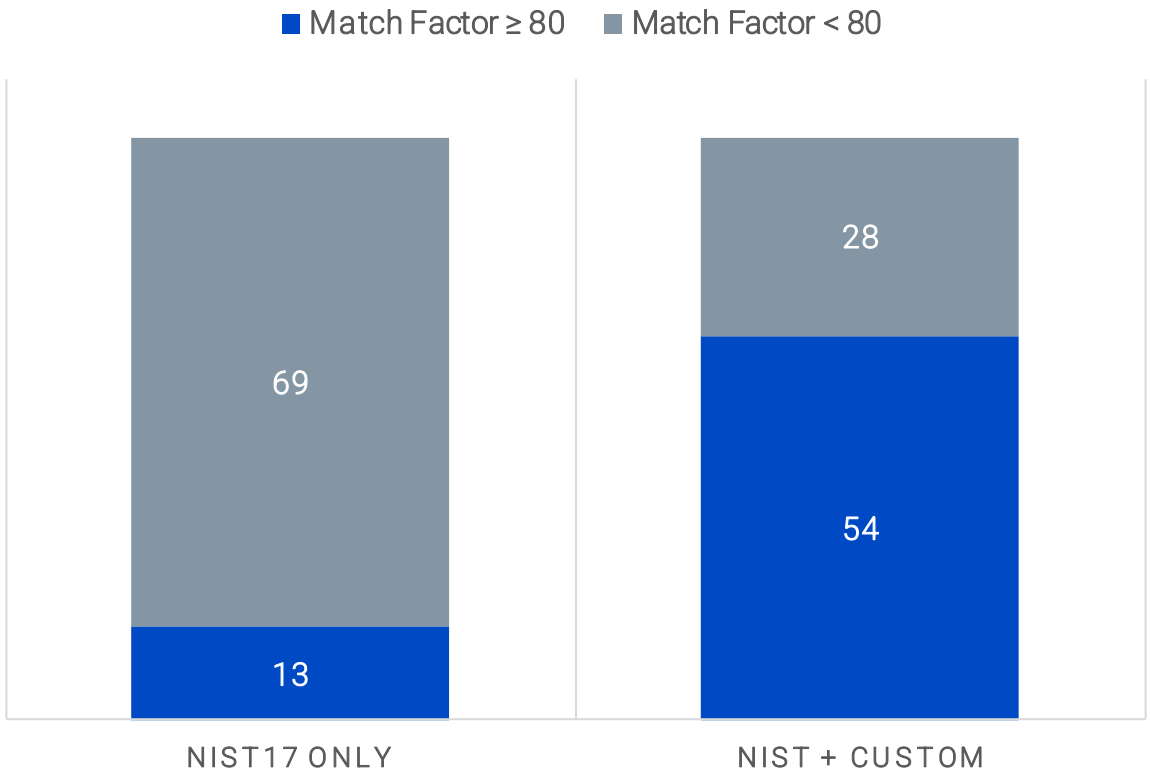
Custom mass spectral library

Reference standard (matrix matched)

Tentative identifications

Previously observed unknown compounds

JUUL Golden Tobacco 5.0% Aerosol NTA Constituents*



*Primary constituents (propylene glycol, glycerol, nicotine, and benzoic acid) are not included in reporting

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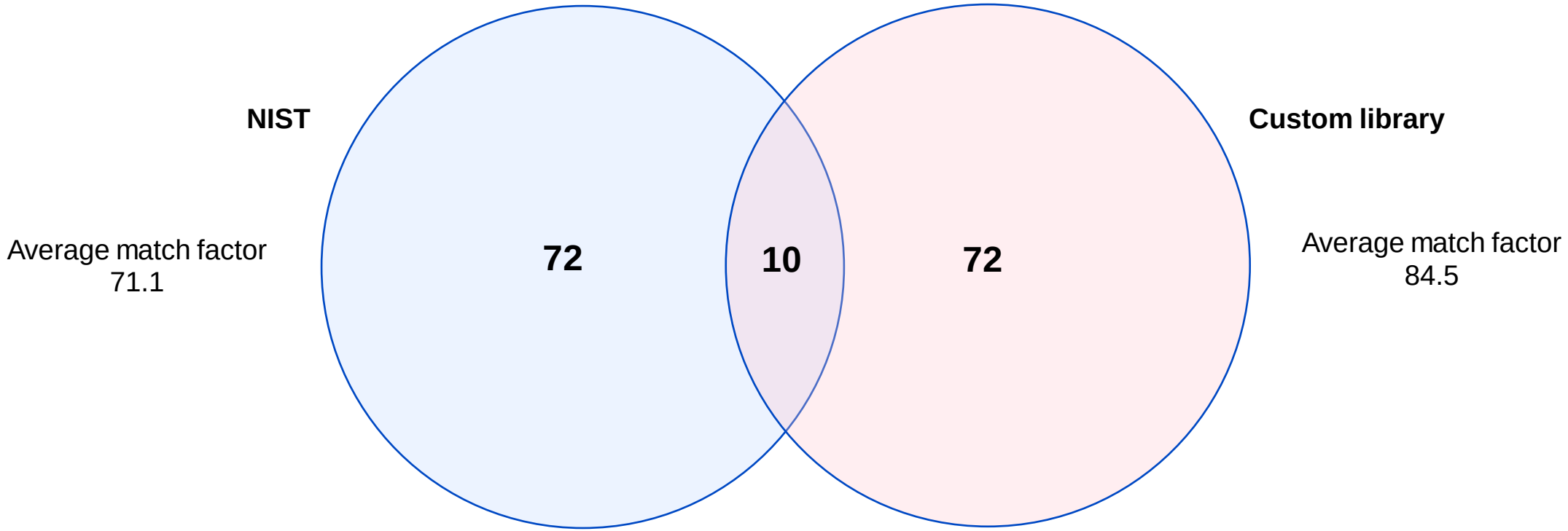
Identification Comparison

Sample set: Golden Tobacco 5% aerosol

NIST identifications were compared to custom library search results

10 out of 82 (~12%) accurate identifications by NIST (compounds present at relatively high concentrations)



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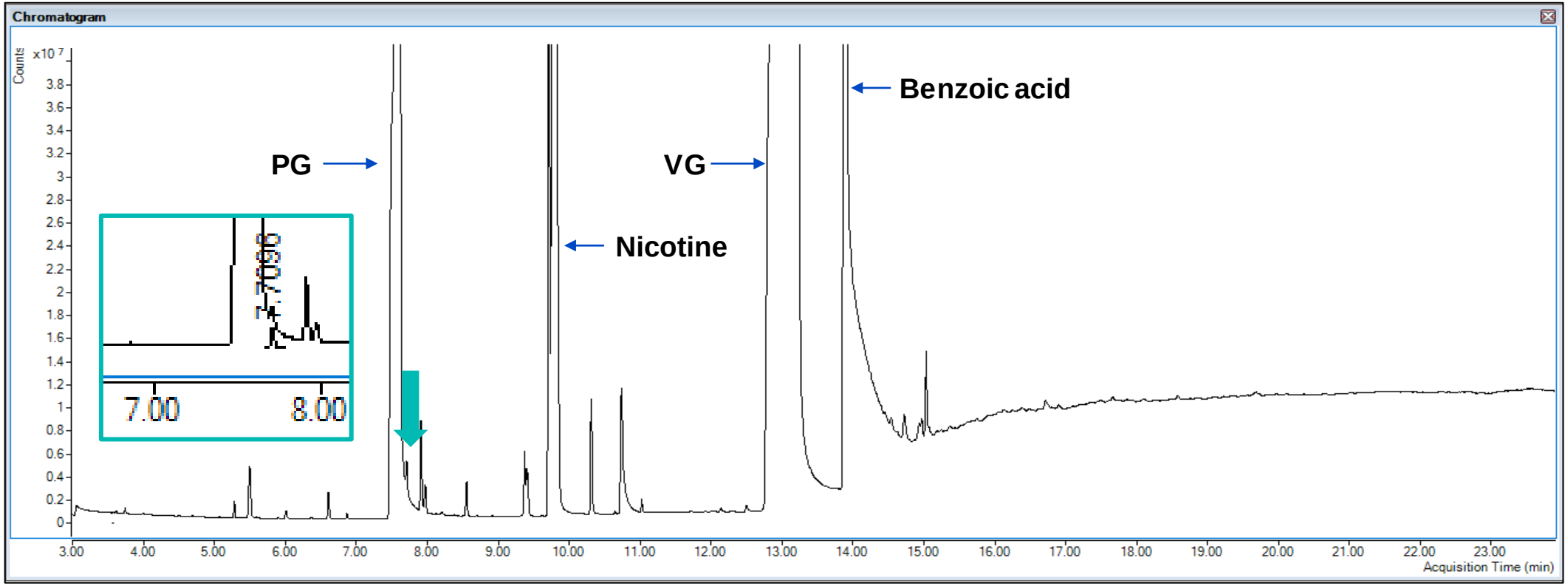
Impact of Matrix Effects on Compound Identification



PG – Propylene glycol; VG – Vegetable glycerin

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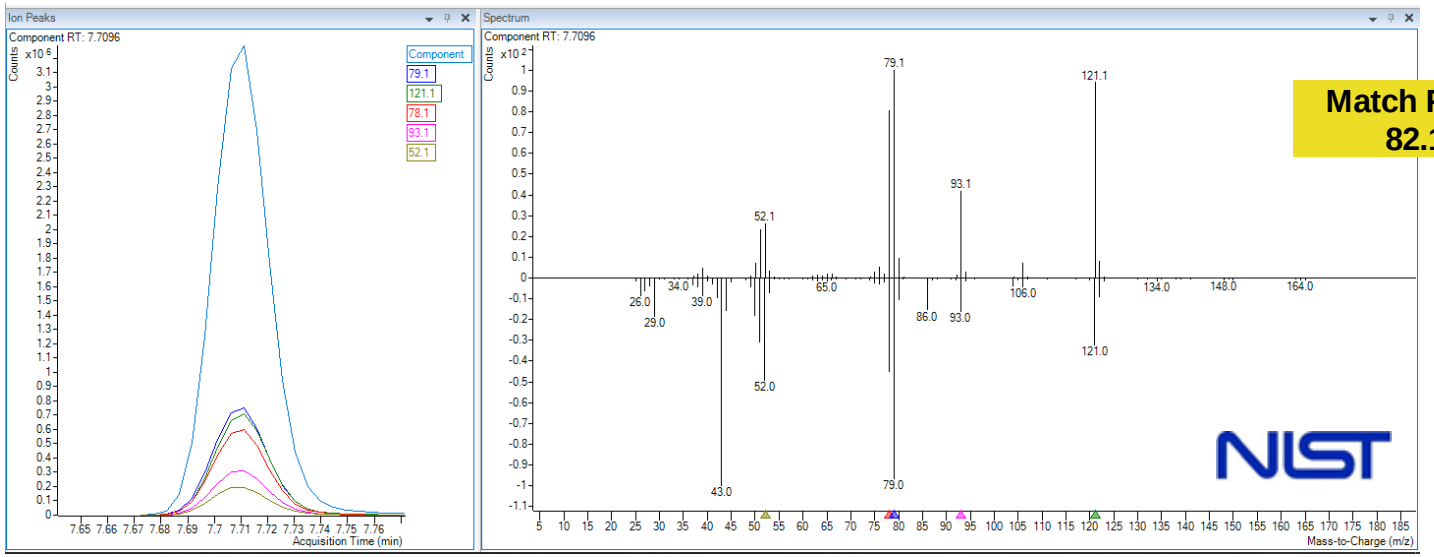
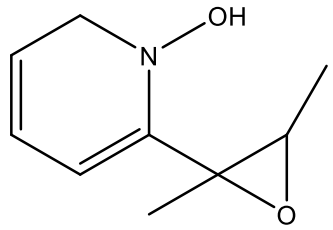
Impact of Matrix Effects on Compound Identification



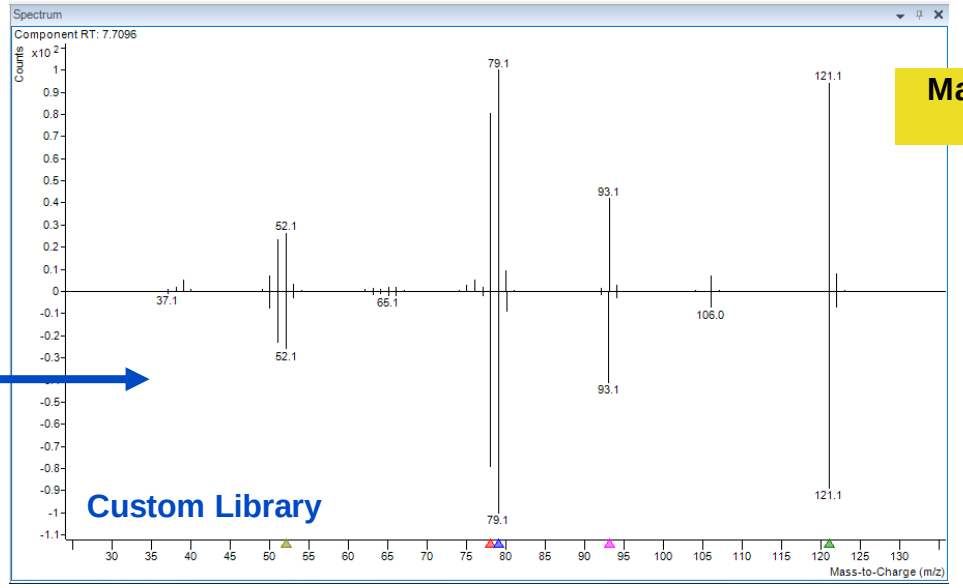
Match Factor 82.15

"Good" match score, but incorrect identification

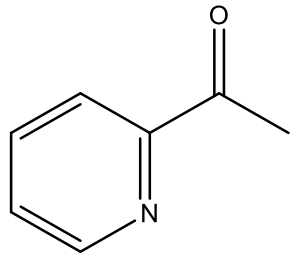


2-(2,3-Dimethyl-oxiran-2-yl)-pyridin-1-ol

Matrix matched reference standard



Match Factor 92.65



Component RT = 7.70 min
Library RT = 7.68 min

Ethanone, 1-(2-pyridinyl)-

Confirmed by mass spectral match and retention time match

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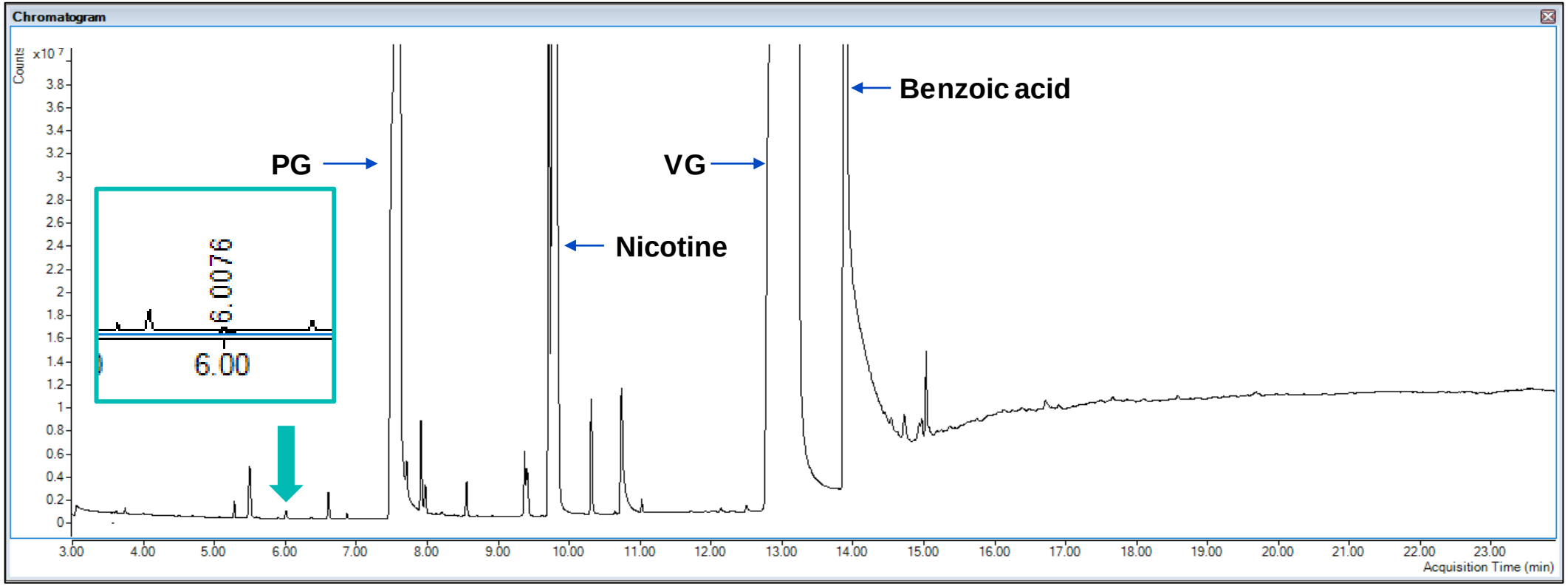
Improved Match Factor



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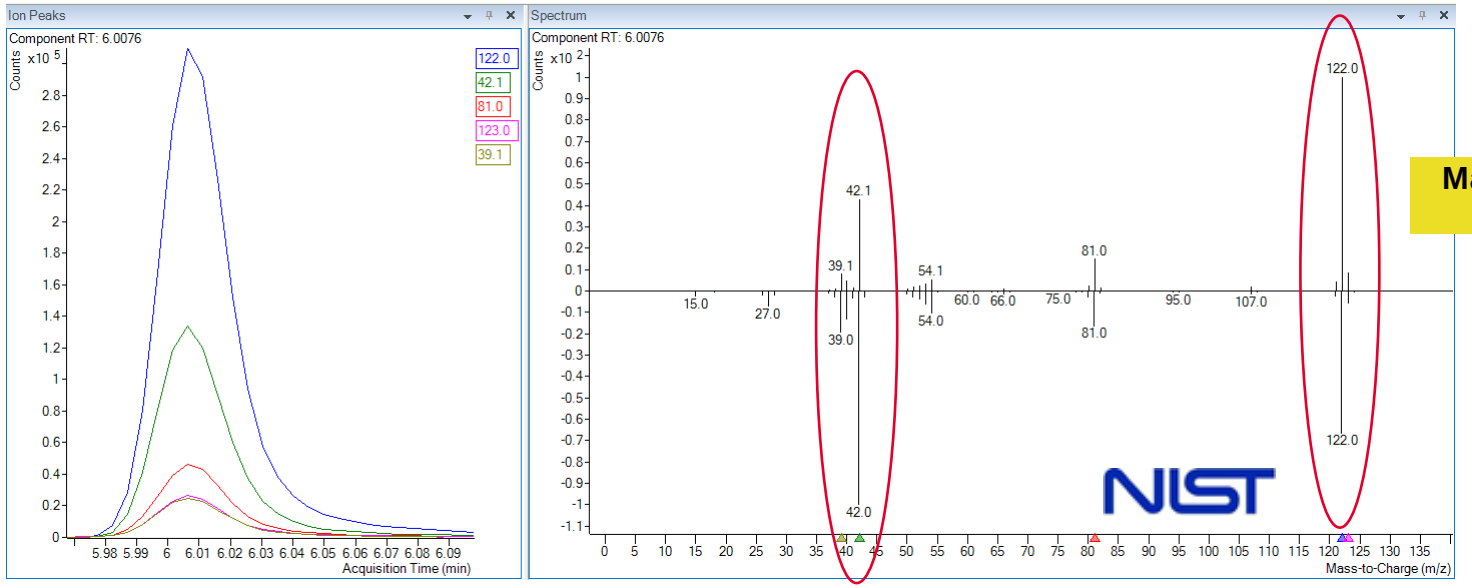
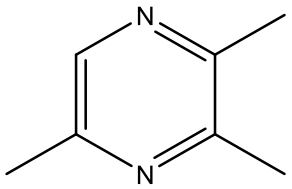
Improved Match Factor



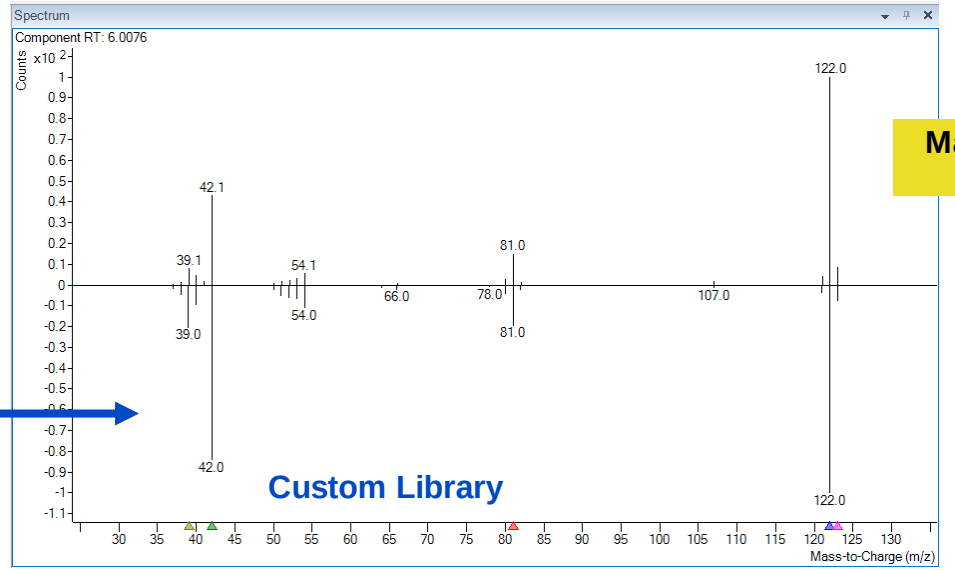
Match Factor 72.64



Correct identification

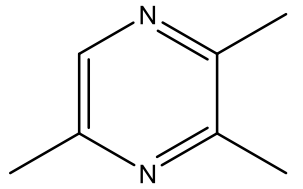
Differences in ion ratios

Matrix matched reference standard



Match Factor 96.87

Component RT = 6.00 min
Library RT = 6.00 min



Trimethylpyrazine

Confirmed by mass spectral match and retention time match

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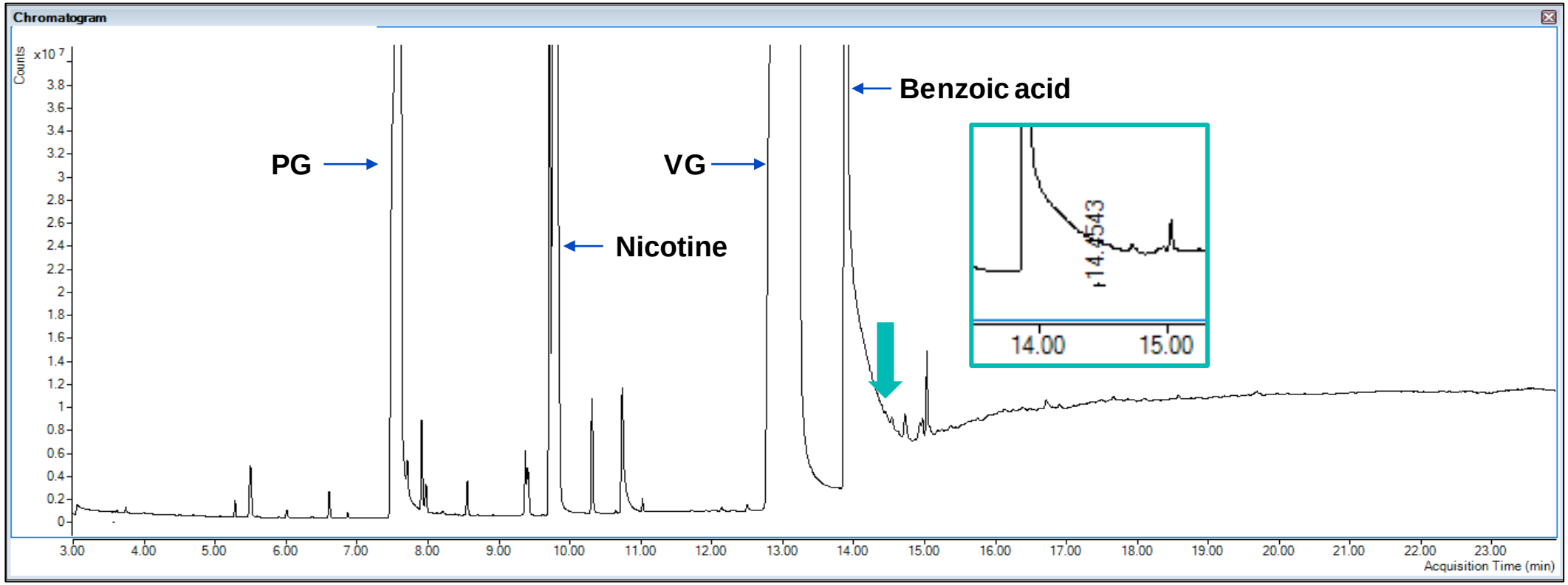
Absence in NIST Database



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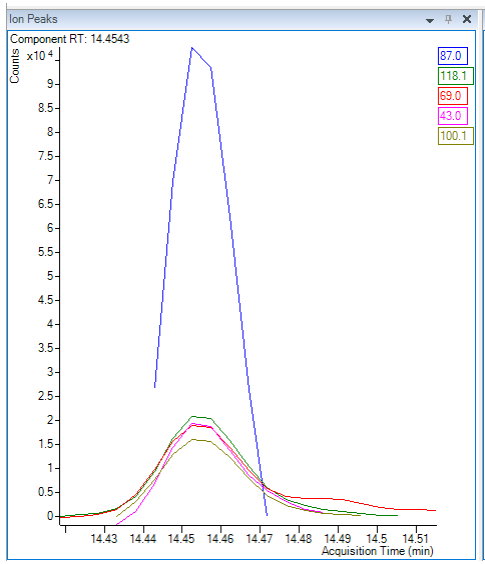
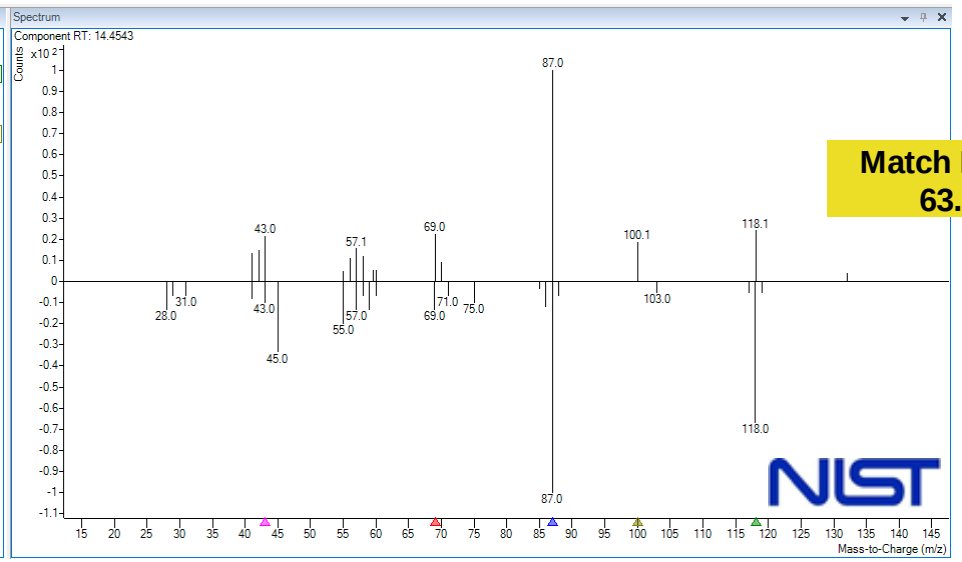
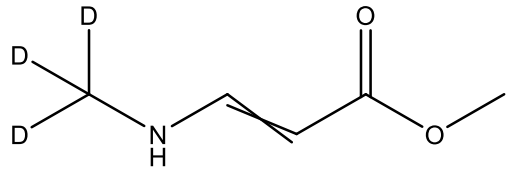


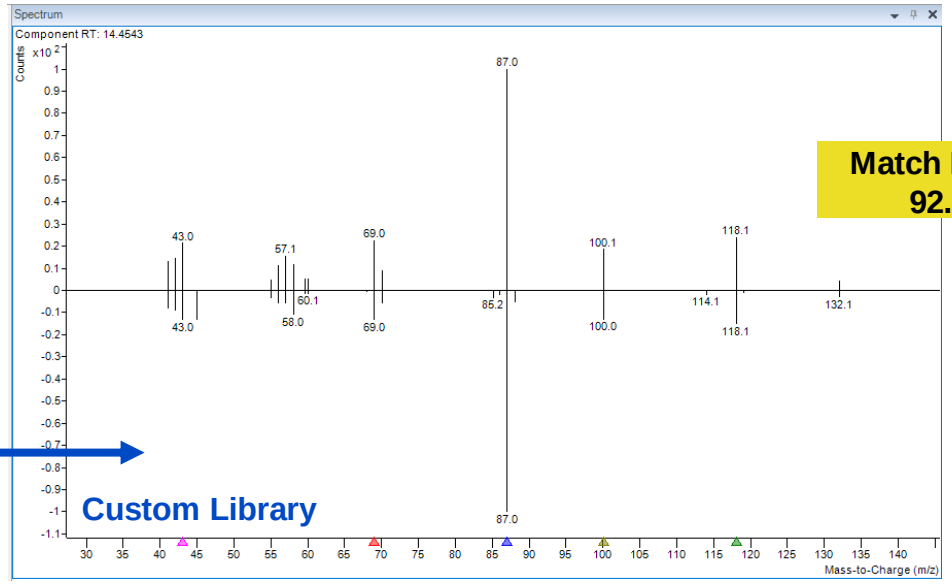


Match Factor 63.46

Incorrect identification due to absence of compound mass spectrum.



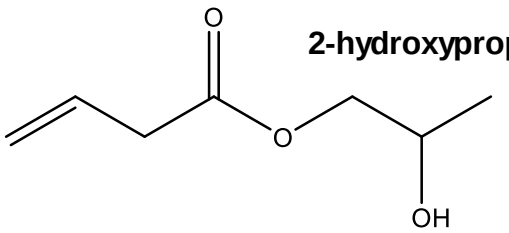
Initially labeled as an unknown compound



Match Factor 92.10

Component RT = 14.45 min
Library RT = 14.50 min

Custom synthesized reference standard



2-hydroxypropyl but-3-enoate

Butyrolactone + PG reaction product

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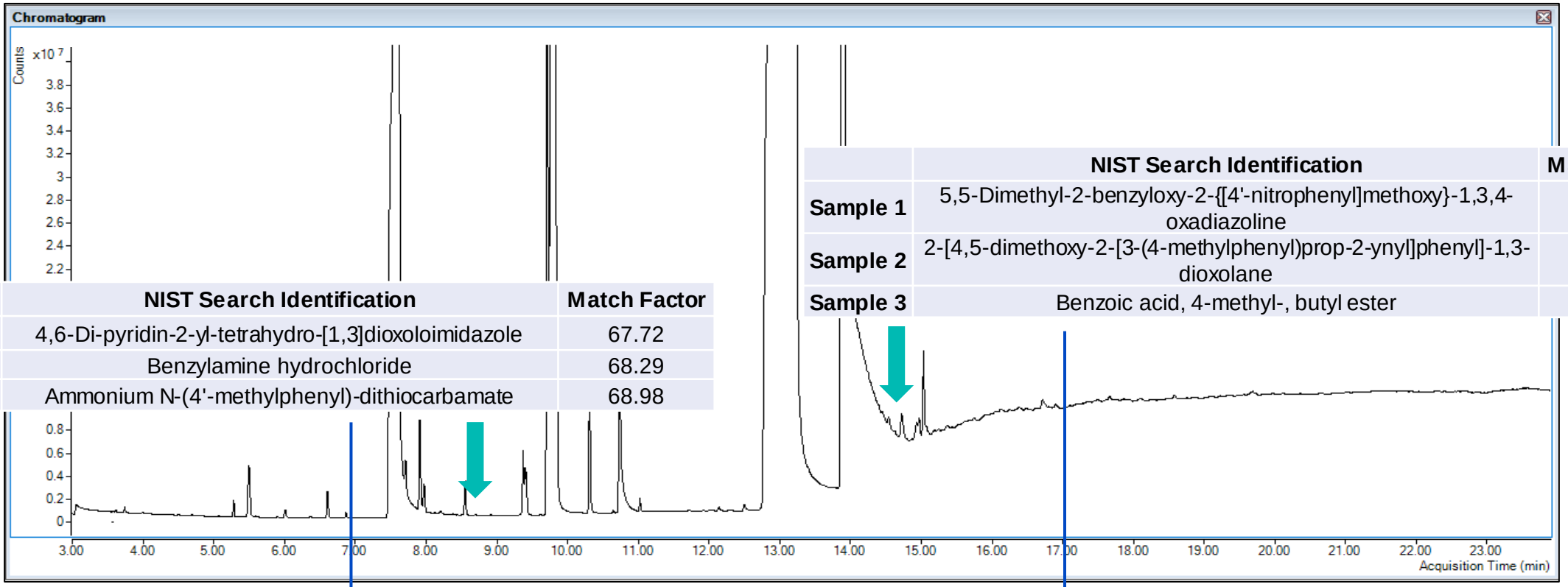
Inconsistent Compound Identification



	NIST Search Identification	Match Factor
Sample 1	4,6-Di-pyridin-2-yl-tetrahydro-[1,3]dioxoloimidazole	67.72
Sample 2	Benzylamine hydrochloride	68.29
Sample 3	Ammonium N-(4'-methylphenyl)-dithiocarbamate	68.98

	NIST Search Identification	Match Factor
Sample 1	5,5-Dimethyl-2-benzyloxy-2-{[4'-nitrophenyl]methoxy}-1,3,4-oxadiazoline	74.42
Sample 2	2-[4,5-dimethoxy-2-[3-(4-methylphenyl)prop-2-ynyl]phenyl]-1,3-dioxolane	76.42
Sample 3	Benzoic acid, 4-methyl-, butyl ester	68.07

Custom Library search

Nicotinealdehyde RRT 0.577 **Identification Confirmed**

Custom Library search

Unknown (RRT 0.972)

Relative Retention Time (RRT) = compound RT / internal standard RT

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Summary

- Limitations using NIST database only search for chemical identification of ENDS aerosol:
  - < 20 % of the detected compounds had an acceptable tentative identification (match factor ≥ 80)
  - Peaks co-eluting with primary constituents exhibited poor match due to matrix effects
  - Inconsistent identifications between samples

- A comparison of the compound identifications when performing a search including and excluding the custom library combined with the NIST17 database for JUUL aerosol resulted in < 20% common identifications.

- The use of a custom library greatly increased acceptable tentative identifications by consistent detection and peak tracking based on relative retention time (RRT).

- The use of a custom mass spectral library is a valuable addition to the non-targeted assessment approach.

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