FINGERPRINTING OF E-LIQUIDS BY THE DETERMINATION OF UNTARGETED COMPOUNDS USING TWO MODERN ANALYTICAL APPROACHES

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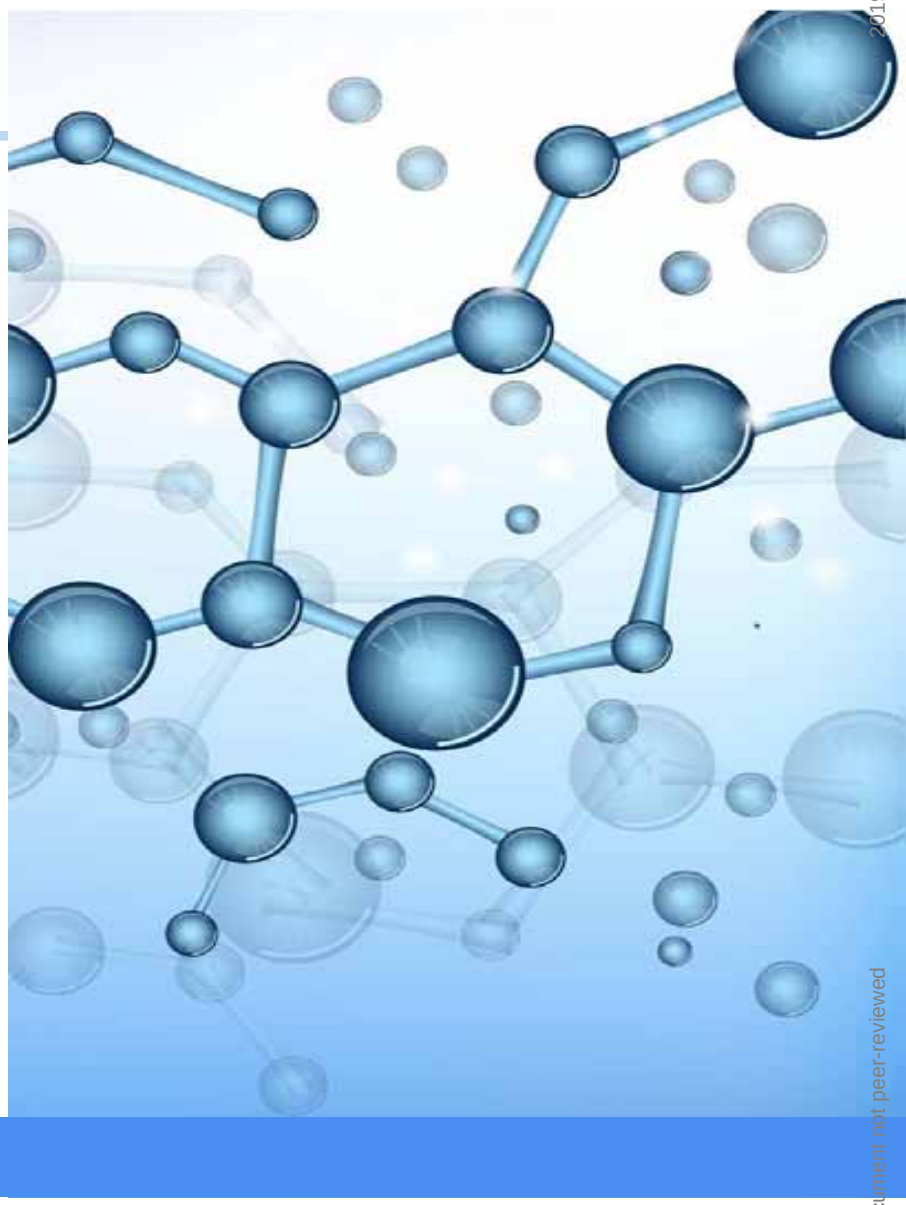


Presentation Outline

- Background Information
- Analysis Techniques
  - HS-SMPE GC-MS
  - LC-MS QToF
- Project Design
- Analytical Results
  - Interpretation of Data
- Summary
  - Additional uses

Background – Why is Untargeted Analysis Important?

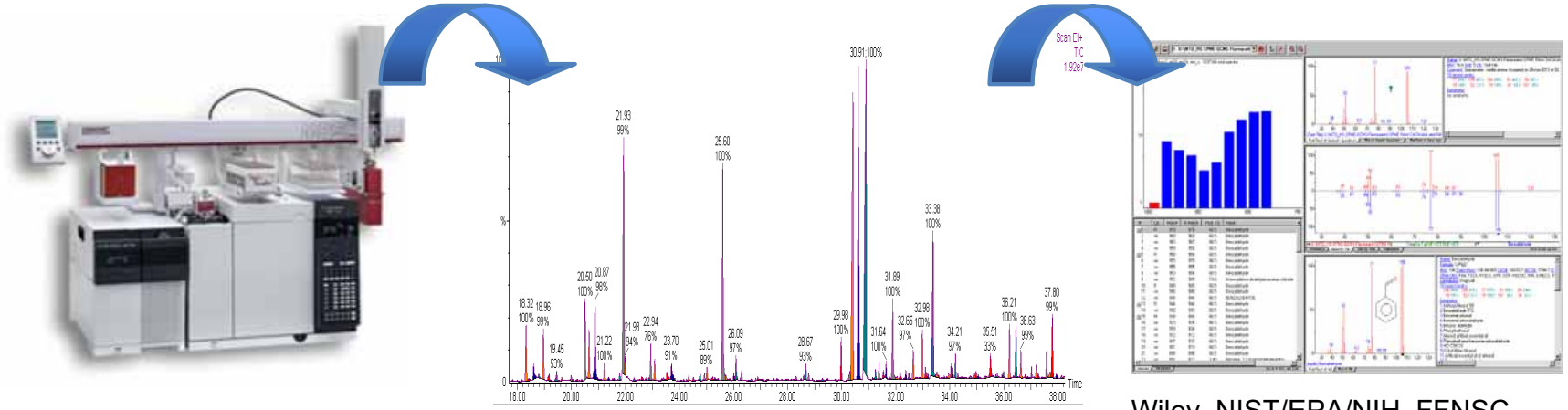
- To monitor potential changes to a product that could occur during storage or between lot preparations
  - Storage conditions
  - Heat
  - Air
  - Light

- Isomerization
- Oxidation
- Dehydrogenation
- Polymerization
- Thermal rearrangements
- Leachables

QUALITY and SAFETY

Headspace - Solid Phase Microextraction - GCMS

HS-SPME-GCMS **Chromatographic Profiles** **ID – Spectral Confirmation**



Wiley, NIST/EPA/NIH, FFNSC

- *67th TSRC (2013) Williamsburg, VA, USA - Profiling of Various Tobacco Products*
- *68th TSRC (2014) Charlottesville, VA, USA - Determination of Potential Flavor Additives in Tobacco Products*
- *2014 CORESTA CONGRESS, Québec City, Canada - Determination of Pyrazine Flavourants in Electronic Cigarette Cartridges and Liquids*

Headspace - Solid Phase Microextraction - GCMS

0.1 g of e-liquid (0.05 g for menthol) in 10 ml vial

20 µl of ISTD and 2 ml of 3.0 M KCl (aq)

ISTD: d6-benzene, d5-acetophenone, d5-benzophenone

Equilibrate at room temp for 2 hours

HS-SPME-GCMS analysis

Instrument Parameters	
SPME Fiber	Supelco 50/30 µm Stableflex DVB/CAR/PDMS
GC Column	DB WAX 30 m L × 0.25 mm ID × 0.50 µm
Incubation	50 °C for 5 min, 250 rpm interval agitation, 20 sec agitation and 2 sec rest
Extraction	50 °C for 20 min
Desorption	260 °C for 5 min in GC inlet
Injector conditions	260°C held for 60 min, splitless
Oven program	40°C for 5 min. Ramped at 4°C/min to 180°C for 0 min, Ramped at 8°C/min to 240°C for 4.5 min, and Ramped at 10°C/min to 260°C for 6 min. Total run time = 60 min
MS Mode	Full scan (35-400 m/z)

Headspace - Solid Phase Microextraction - GCMS

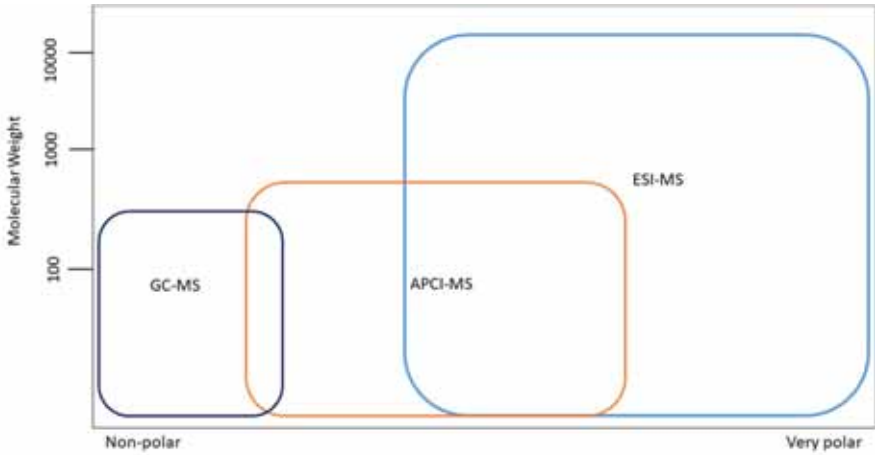
Advantages

- Simple, solvent free sample preparation
- High sensitivity and throughput
  - Low background / Good reproducibility
- Fully automated
- Useful for a wide range of volatile or semi-volatile compounds
- Fast screening method
  - Compound identification
  - Compound semi-quantification

Limitations

- Competition for active sites
- Molecular weight range
- Polarity

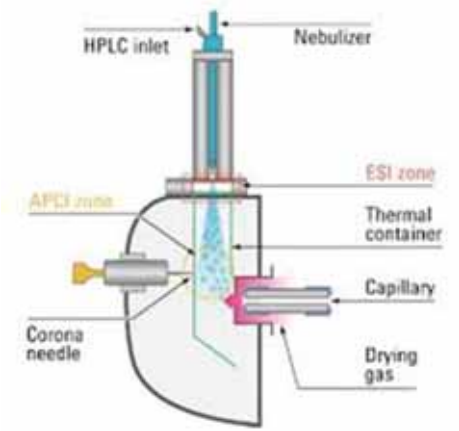
Potential Missing Information!



LC -Time-of-flight Mass Spectrometry (LC-ToF-MS)

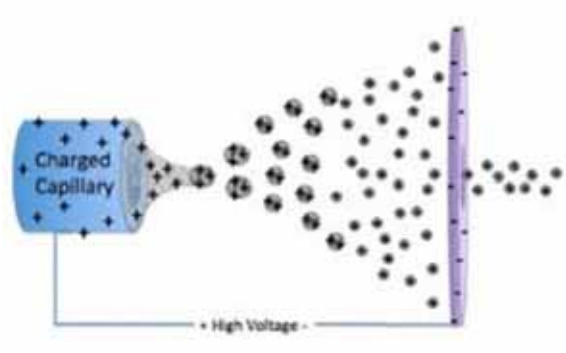
APCI-MS

- Alcohols; Aldehydes; Fatty acids; Amides; Halogenated Aliphatics



ESI-MS

- Acids and Bases; Conjugated Carbonyls; Nitrosamines; modified Sugars



LC -Time-of-flight Mass Spectrometry (LC-ToF-MS)

- Agilent 6545 Q-TOF LC/MS
- Agilent 1290 Infinity II HPLC system

- 0.5 - 2ppm accuracy
  - accurate to 4th decimal in ToF mode
- 12k - 20k mass resolution (FWHM)

- Molecular formula determination of ions based on mass defect, isotope patterns
- Structural determination based on additional CID-MS/MS experiments

LC -Time-of-flight Mass Spectrometry (LC-ToF-MS)

- HPLC conditions
  - reversed phase C18 column
  - 5mM ammonium acetate/methanol mobile phases
- Four MS modes
  - ESI positive and negative
  - APCI positive and negative
- Mass range of 70 - 750m/z

- 100mg of e-liquid
  - *isotopically labelled TSNA compounds acting as internal standards*
- 25mL in 5mM ammonium acetate

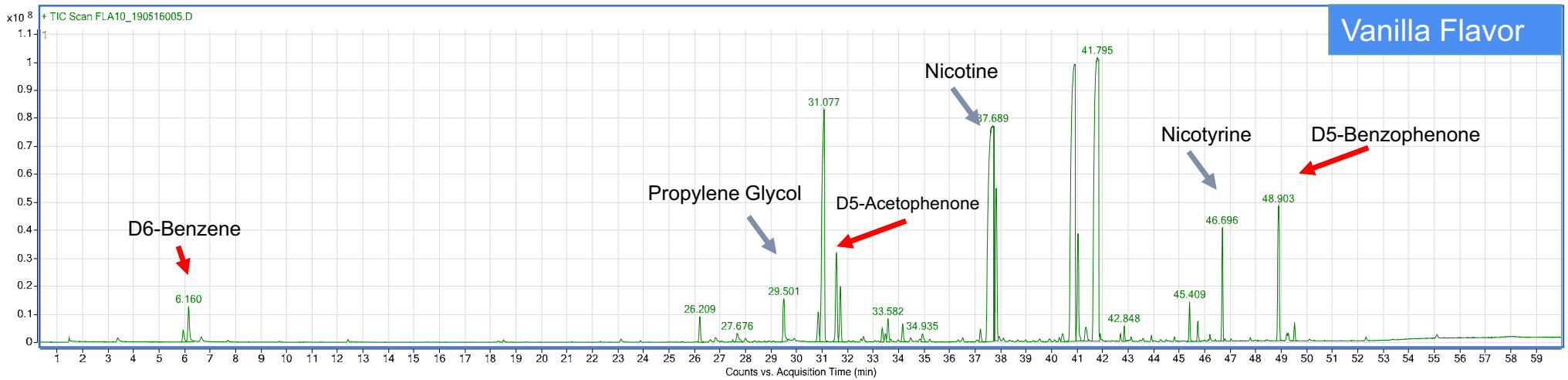
Best balance of low level detection capability while minimizing potential for detector/column saturation

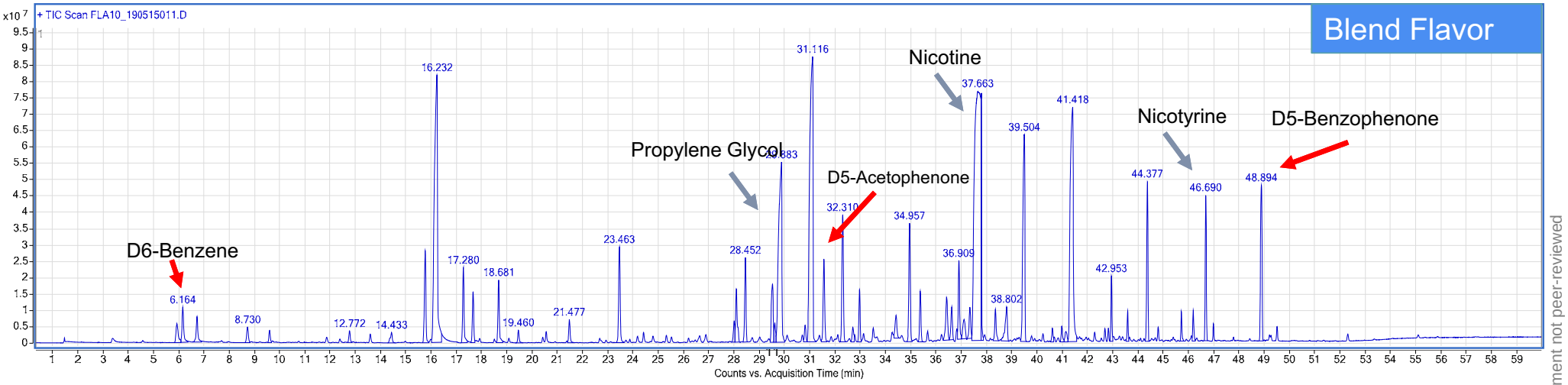
Experimental Design

- Six different e-liquids; Blend, Vanilla, Menthol, Tobacco, Cherry, Mocha
- Two Storage Conditions
  - Ambient (Room Temperature)
  - Accelerated (40 ºC, 75% RH)
- Three months storage time

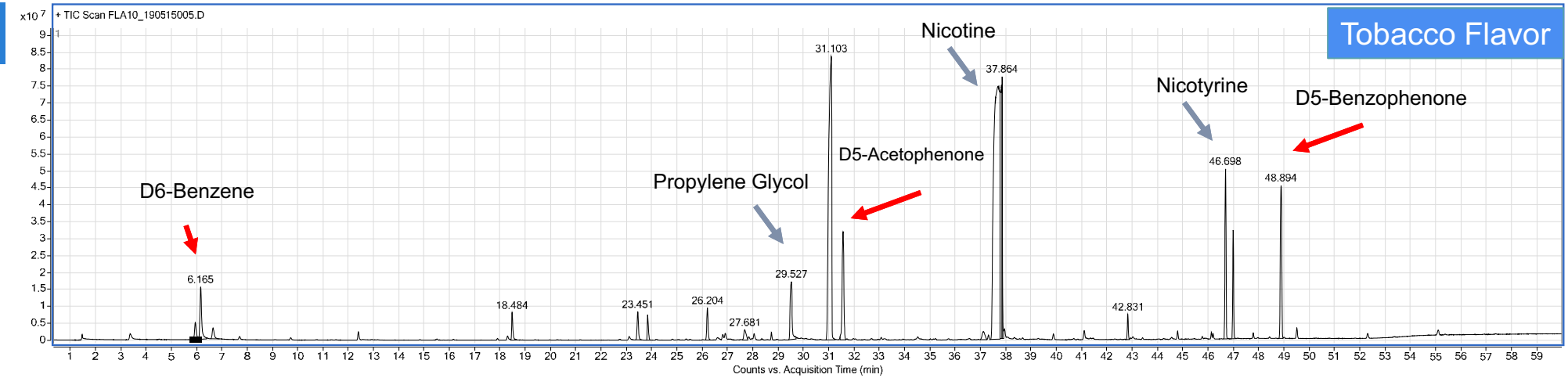
- HS-SMPE GC-MS
- LC-MS QToF

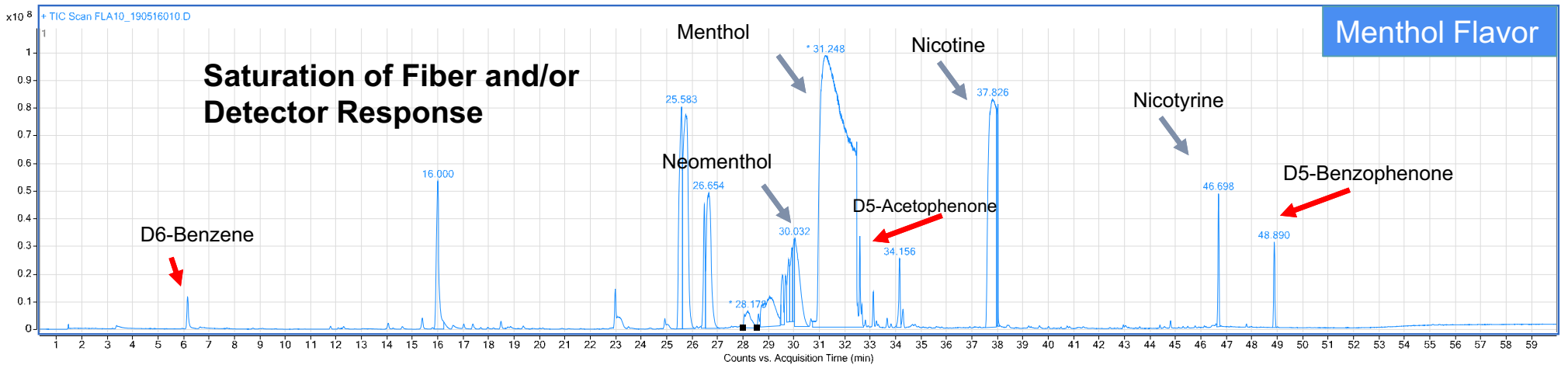
Results: HS-SMPE GC-MS (*Chromatography*)





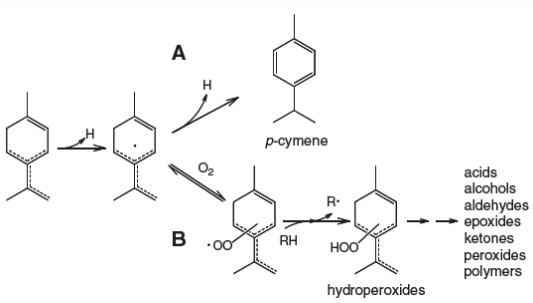
Results: HS-SMPE GC-MS (*Chromatography*)





Results: HS-SMPE GC-MS (123 *Identified Compounds*)

No.	Compound	Tobacco	Vanilla	Menthol	Blend
1	Acetone			✓	✓
2	Methyl Acetate	✓	✓	✓	✓
3	Ethyl Acetate				✓
4	Methyl Propionate				✓
5	Ethanol	✓	✓		✓
6	α-Pinene			✓	✓
7	α-Thujene			✓	
8	Ethyl Butyrate				✓
9	Butyl Acetate				✓
10	β-Pinene			✓	
11	Sabinene			✓	
12	Butyraldehyde Propylene Acetal (4-Methyl-2-propyl-1,3-dioxolane)				✓
13	β-myrcene			✓	✓
14	α-Phellandrene				✓
15	1-Butanol			✓	
16	α-Terpinene			✓	✓
17	Limonene	✓		✓	✓
18	Methyl Hexoate	✓	✓		
19	Limonene		✓		✓
20	Eucalyptol			✓	✓
21	Ethyl Hexanoate				✓
22	γ-Terpinene			✓	✓
23	β-Ocimene			✓	✓
24	3-Octanone			✓	
25	p-cymene			✓	✓
26	Terpinolene			✓	✓
27	Octanal		✓		
28	Amyl Isovalerate			✓	
29	Isoamyl Isovalerate (Apple Oil)				✓
30	(3E)-Hexenyl Acetate				✓



p-cymene often identified in aged essential oils

44 % decrease in T=3 months at room temperature

50 % decrease in T=3 months, at room temperature

Results: *continued*)

No.	Compound	Tobacco	Vanilla	Menthol	Blend
35	2,3-Dimethylpyrazine				✓
36	1-Hexanol			✓	
37	2-Ethyl-1-hexyl acetate				✓
38	3-Hexen-1-ol	✓			✓
39	Methyl Octanoate				✓
40	3-Octanol			✓	
53	Benzaldehyde	✓	✓		
54	Ethyl Chrysanthemate (Not Occurred in Nature, used for fragrance)				✓
55	Linalool			✓	✓
56	1-Octanol	✓	✓		✓
57	Linalyl Acetate				✓
58	Menthyl Acetate			✓	
59	α-Isophorone				✓
60	Neomenthol			✓	
87	Ethyl Phenacetate				✓
88	Nerol				✓
89	β-Damascenone				✓
90	Corylone (Cyclotene Hydrate, Wood Smoke)				✓
91	Ethyl Dodecanoate				✓
92	Geraniol				✓
93	Benzaldehyde Propylene Glycol Acetal	✓			✓
94	Geranyl acetone	✓	✓		
95	Benzyl Alcohol	✓	✓		
119	γ-Undecalactone		✓		✓
120	Menthalactone			✓	
121	Propenylguaethol	✓			✓
122	Heliotropine Propylene Glycol Acetal		✓		✓
123	Vanillin		✓		

26 % decrease in T=3 months – accelerated conditions, no changes in RT

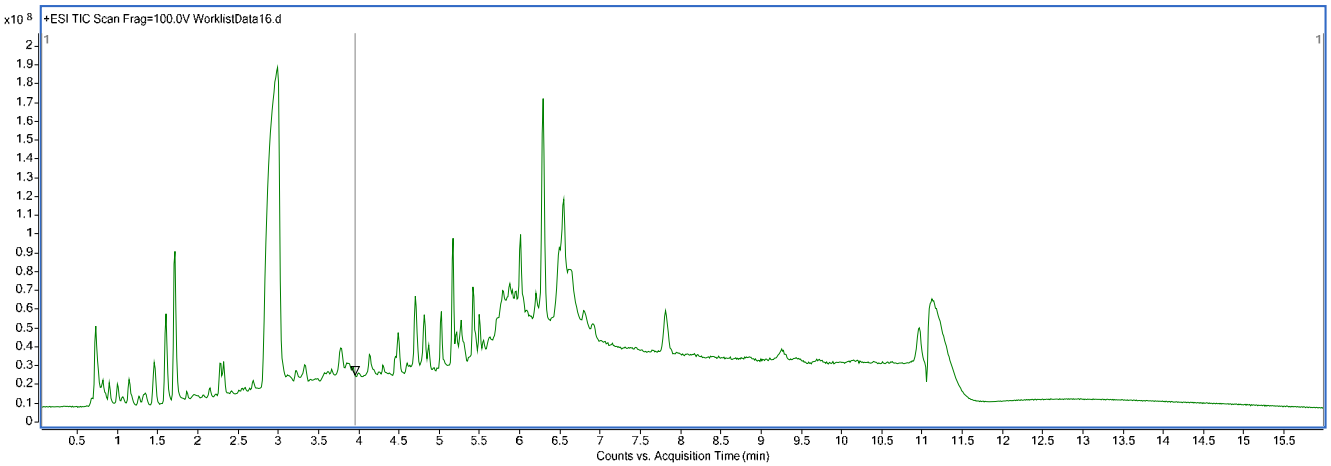
25 % decrease in T=3 months, at room temperature

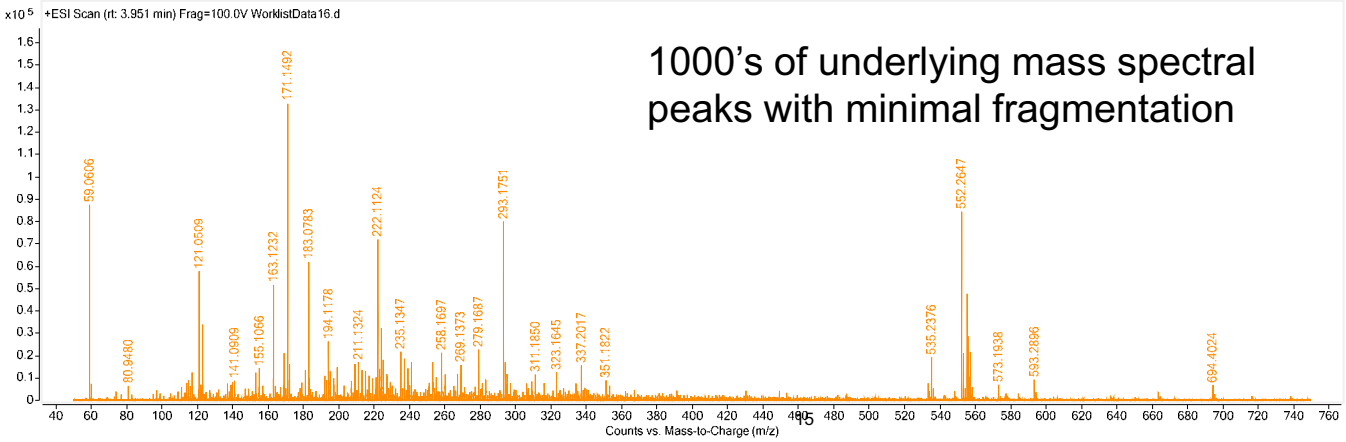
35 % decrease in T=3 months, at room temperature

25 % decrease in T=3 months – accelerated conditions

Results: LC-ToF-MS (Chromatography)

- “Unknown” Discovery

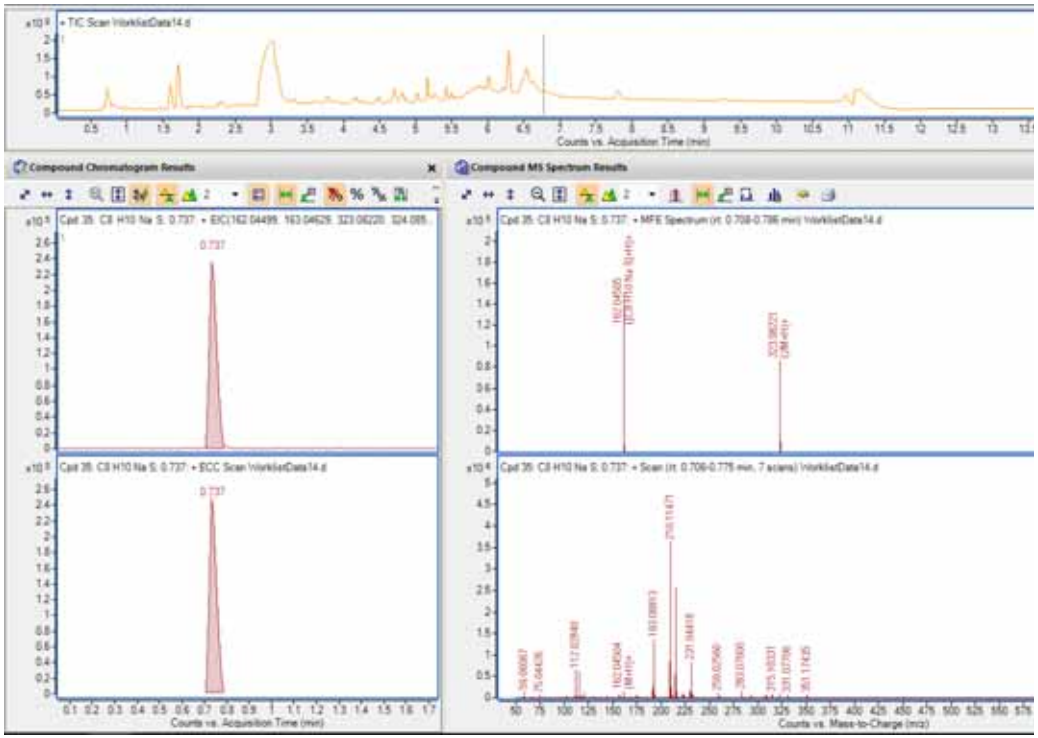




1000’s of underlying mass spectral peaks with minimal fragmentation

Results: LC-ToF-MS (Chromatographic Interpretation)

- Chromatogram / Spectrum Deconvolution / Molecular Feature Extraction (MFE)



- Algorithm searches spectrum for ions exhibiting “peak like” intensity changes and performs EIC
- Also searches for potential redundant data from isotope peaks/dimers/alternate adducts
- Data with a defined m/z and RT referred to as a **Molecular Feature**

Molecular Feature Acceptance Criteria

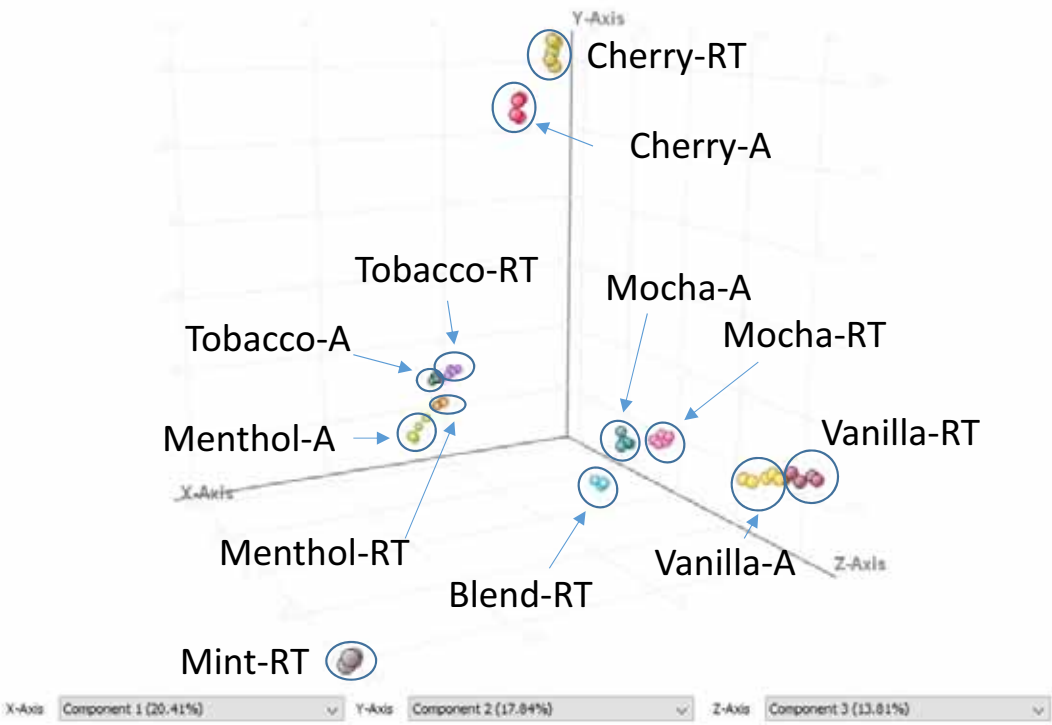
- RT range between 0.5-10 minutes
  - Retention variation below 0.2min
- Peak must occur in at least 60% of all replicates of sample grouping
- m/z variation must be within ±5.0ppm
  - Intensity of at least 3000 counts
  - S/N > 10
- Mass defect filter corresponding to 3 - 30 Carbon with 0 - 20 degrees unsaturation and N, O, S, Na, and Cl heteroatoms

Results: LC-ToF-MS (Principle Component Analysis)

- A data set with a large numbers of variables (i.e. MS features) is transformed, creating new variables (called principal components or PCs (e.g. PC1, PC2, etc.)) from them, using linear combinations of the original variables.
  - Each feature is given a “loading value” which gives a relative weight in which each feature contributed to the PC.
  - Data (features) which show little to no variance or high amounts of random variance are essentially ignored in the vector calculation;
- By plotting the loadings averages for each sample, a scores plot (most of the PCA data shown in this presentation) can be created which can be qualitatively analyzed to identify trends within the samples.

Results: LC-ToF-MS (ESI - Positive Mode) > 7200 unique features

RT = Room Temperature storage; A= 40°C/75% humidity



- Numerous features corresponding to formula typical of alkaloids (MW < 300) and nicotine degradation; cocoa and vanilla beans
- Many formula typical of highly conjugated aromatic aldehydes (e.g. benzaldehyde, vanillin etc.)
- Formula corresponding to amino acids, and amines also abundant
- Samples prepared in glass show additional non-plastic related features which suggest that some compounds may adhere to plastics

Each product has a unique “finger-print” of compounds of ~100-600 features.

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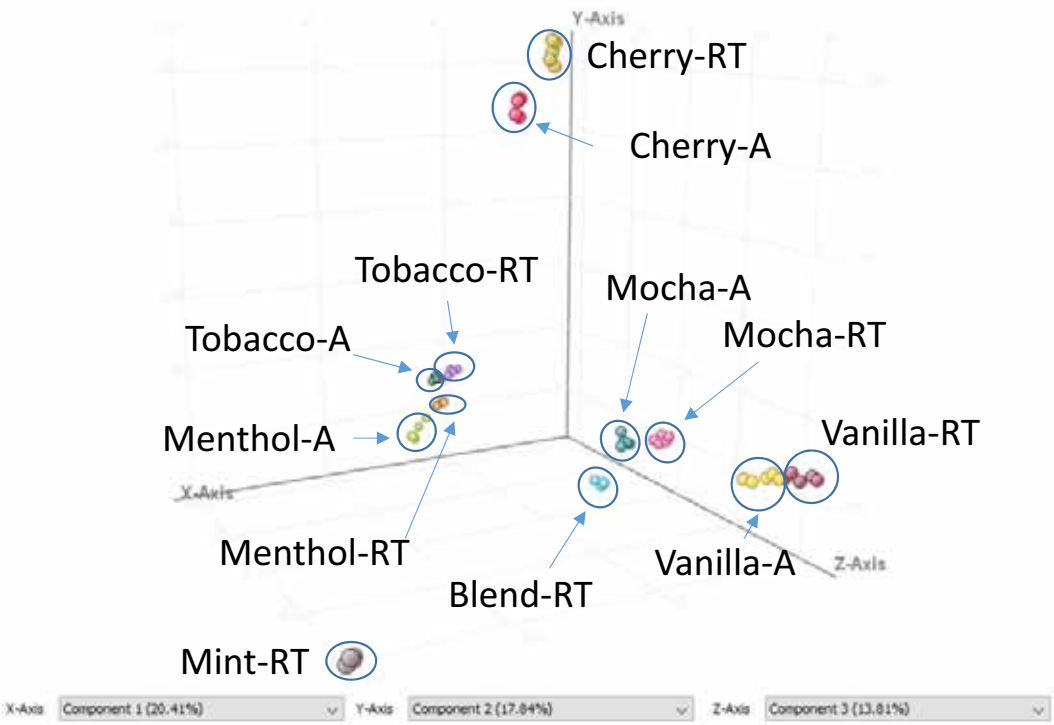


- Numerous features corresponding to formula typical of alkaloids (MW < 300) and nicotine degradation; cocoa and vanilla beans
- Many formula typical of highly conjugated aromatic aldehydes (e.g. benzaldehyde, vanillin etc.)
- Formula corresponding to amino acids, and amines also abundant
- Samples prepared in glass and plastic vials at 3 month mark show much less phthalates compared to initial experiment suggests methanol used in mobile phase likely source of contamination
- Samples prepared in glass show additional non-plastic related features which suggest that some compounds may adhere to plastics

Each product has a unique “finger-print” of compounds of ~100-600 features.

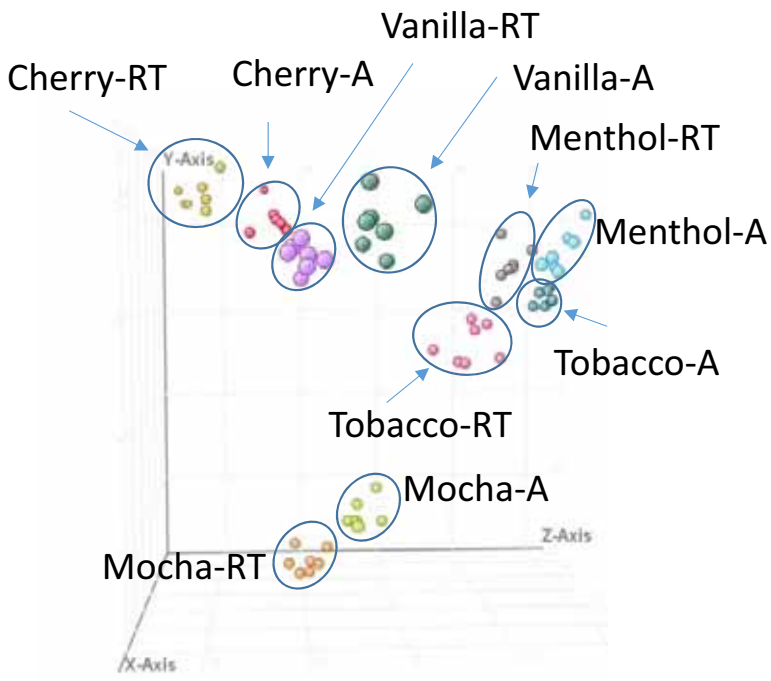
Results: LC-ToF-MS (ESI - Positive Mode)

Comparison of storage conditions

- Cotinine and other known nicotine degradation products significantly higher in samples stored under accelerated conditions
- Many other features can only be identified by chemical formula-MSMS data required for structural elucidation

	Vanilla	Cherry	Mocha	Tobacco	Menthol
# features elevated in accelerated samples	191 (~3.1% total)	17 (~0.5%)	344 (~7.9%)	165 (~3.7%)	460 (~16.2)
# features elevated in RT samples	63 (~1.0%)	14 (~0.5%)	70 (~1.6%)	147 (~3.3%)	153 (~5.4%)
Total detected features	6237	3635	4378	4481	2843

Results: PCA scores plot - Storage (ESI - Negative Mode)



Over 1700 unique features detected

- Many organic acid/fatty acid like chemical formulas detected
- Oxidized sugar (acids) and reduced sugar (alcohols) isomers detected - possible additive or break-down products of sweeteners

PC2 (Z-Axis) qualitatively predicts Room temperature-Accelerated differences

Results: LC-ToF-MS (ESI - Negative Mode)

Comparison of storage conditions

	Vanilla	Cherry	Mocha	Tobacco	Menthol
# features elevated in accelerated samples	64 (~ 6.4% of total)	94 (~12 %)	29 (~ 1.9%)	69 (~ 10%)	102 (~ 15%)
# features elevated in RT samples	45 (~ 4.8%)	45 (~ 5.9%)	20 (~ 1.3%)	4 (~0.06 %)	75 (~ 11%)
Total detected features	1003	758	1495	667	688

***Note**: Considerably fewer features than EI (+)*

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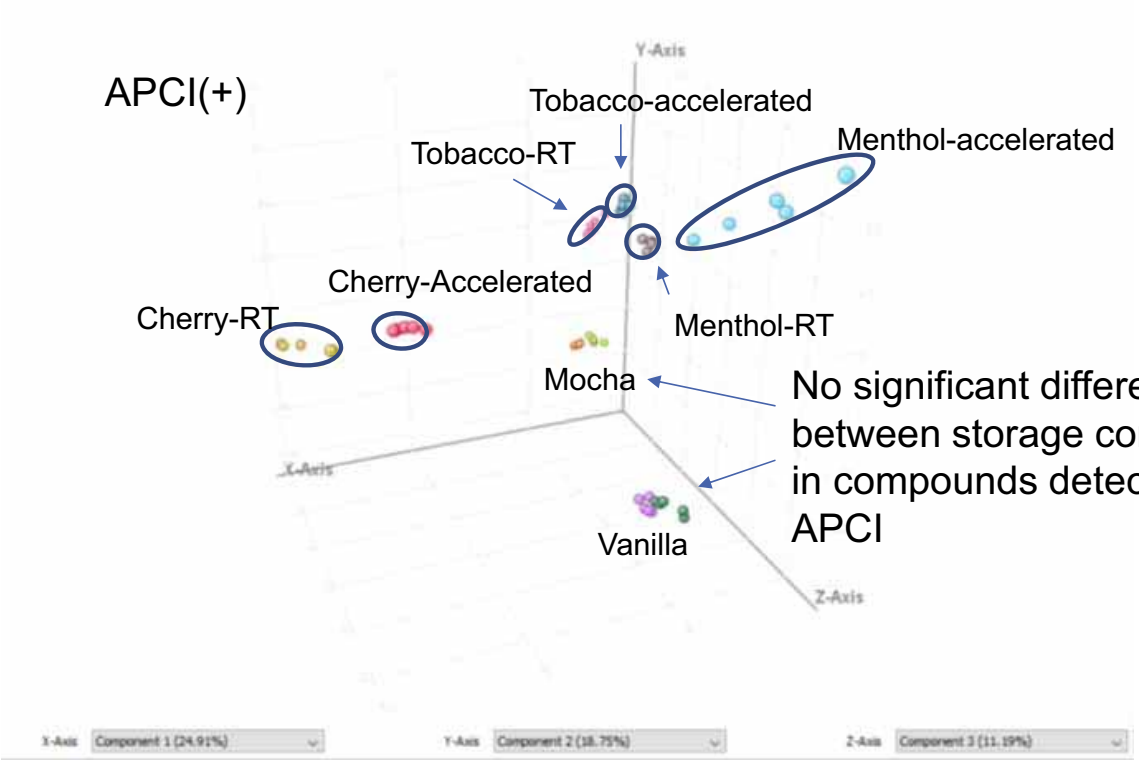
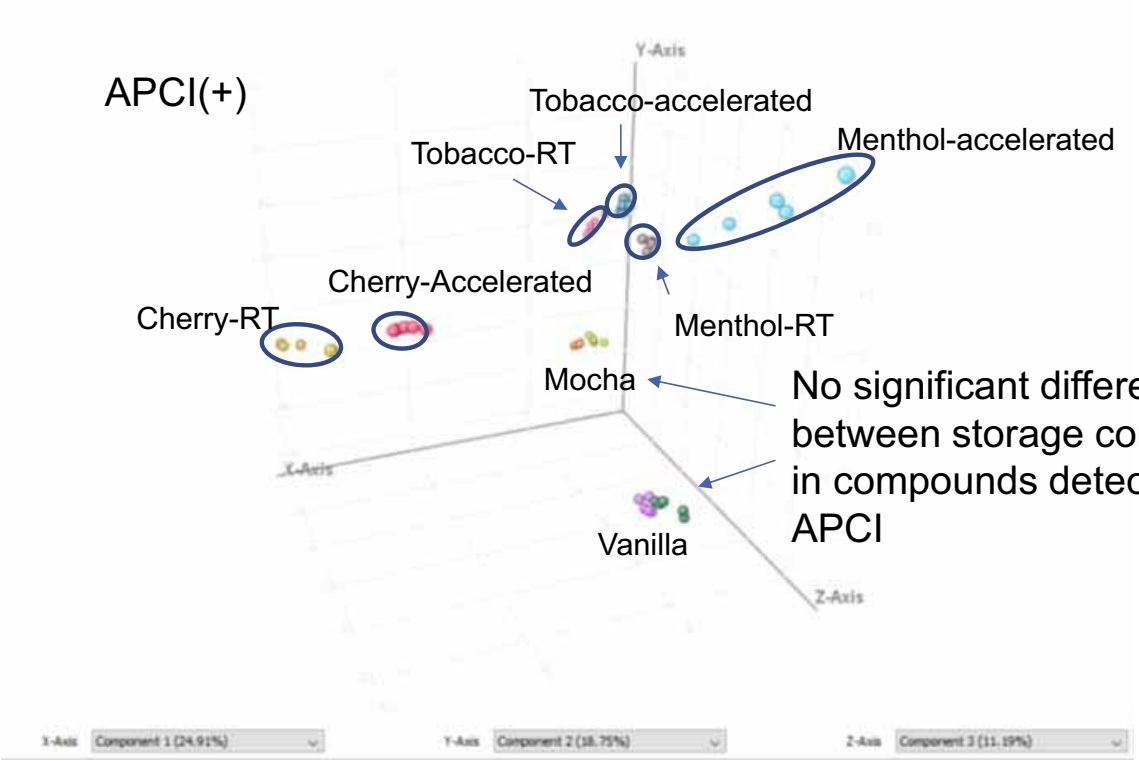
Results: Atmospheric Pressure Chemical Ionization (APCI)

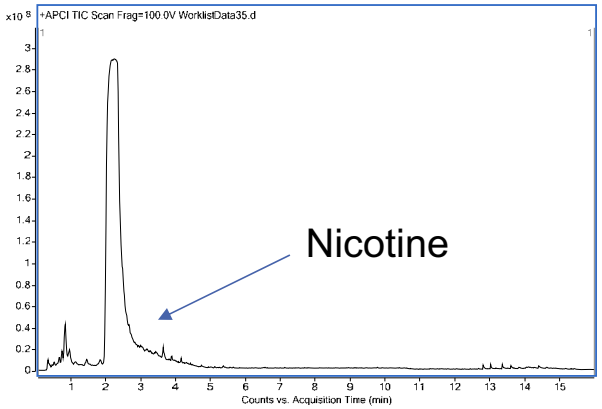


- ~ 50% redundant with ESI (+)
- ~90% redundant with ESI (-)

~650 unique features APCI (+); ~100 APCI (-)

Results: Atmospheric Pressure Chemical Ionization (APCI)



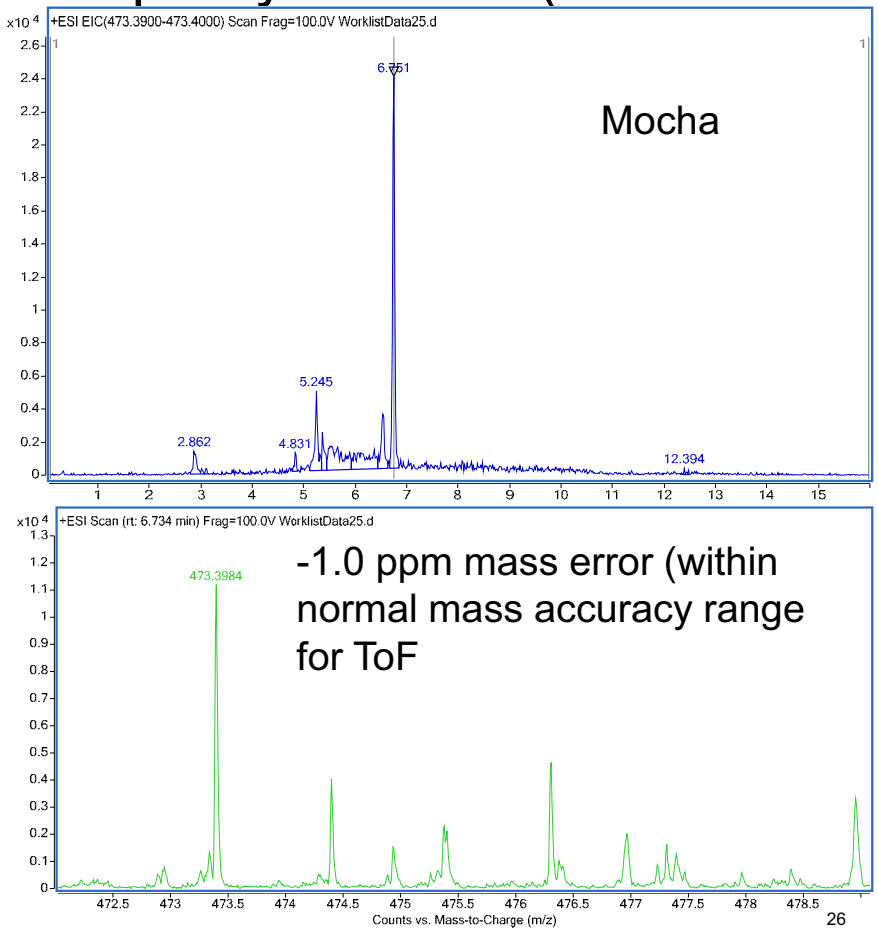


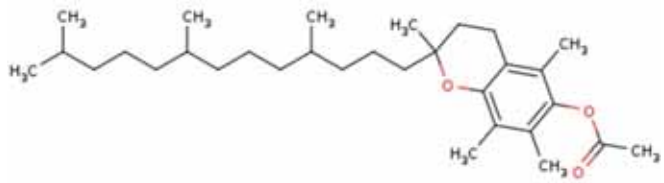
- ~ 50% redundant with ESI (+)
- ~90% redundant with ESI (-)

~650 unique features APCI (+); ~100 APCI (-)

LC-ToF-MS – Retrospective Evaluation of Data

- Tocopheryl acetate (vitamin E acetate)



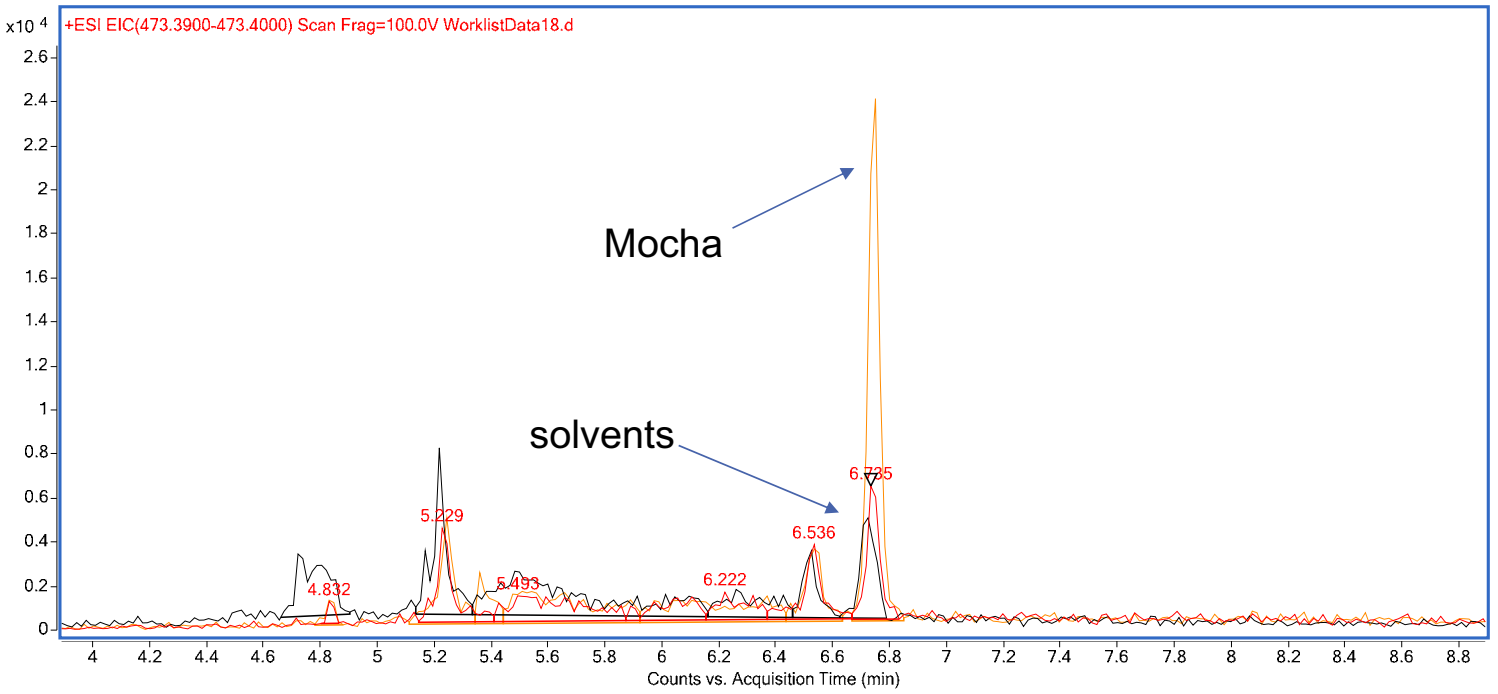


$[C_{31}H_{52}O_3+H]^+$=473.3989 amu

Hit from Metlin Scripps metabolomic database

LC-ToF-MS – Retrospective Evaluation of Data

- Tocopheryl acetate (vitamin E acetate)
- Overlay: Mocha and 2 solvents - one before - one after



Summary

- HS-SMPE GC-MS has been demonstrated as a useful tool for the identification (and semi-quantification) of a wide range of volatile or semi-volatile compounds present in e-liquids
  - Can be used to monitor changes in products between lot preparations or changes that may occur during storage
  - Methodology is limited to low polarity and lower molecular weight compounds
- LC-MS QToF has been demonstrated as a useful tool for untargeted analysis
- Considerably more compounds (features) are discovered in comparison to HS-SMPE GC-MS

Summary

- The number of compounds (features) identified is dependent on ionization mode
  - ESI - Positive Mode > 7200 unique features
  - ESI - Negative Mode > 1700 unique features
  - APCI - Positive Mode ~ 650 unique features
  - APCI - Negative Mode ~ 100 unique features
- Positive identification (i.e. structural determination) requires additional CID-MS/MS experiments.
- Data collected by LC-MS QToF can be used in “retrospective” investigations for the presence of possible targeted compounds

Acknowledgements

- Kenneth Chalcraft
- Paulina Biernacka and David Li

- Wendy Wagstaff

THANK YOU !

Results: *continued*)

No.	Compound	Tobacco	Vanilla	Menthol	Blend
31	Hexanal Propylene Glycol Acetal-1				✓
32	3-Octyl Acetate			✓	
33	Ethyl Heptoate (grape oil)				✓
34	Hexanal Propylene Glycol Acetal-2				✓
35	2,3-Dimethylpyrazine				✓
36	1-Hexanol			✓	
37	2-Ethyl-1-hexyl acetate				✓
38	3-Hexen-1-ol	✓			✓
39	Methyl Octanoate				✓
40	3-Octanol			✓	
41	Nonanal		✓		
42	2,3,5-Trimethylpyrazine	✓			✓
43	β-Isophorone				✓
44	Ethyl Octanoate				✓
45	4-Methyl Anisole				✓
46	3-Ethyl-2,5-Dimethylpyrazine	✓			
47	1-Octen-3-ol			✓	
48	1-Heptanol		✓		
49	3-Ethyl-2,5-Dimethylpyrazine	✓			
50	Menthone	✓	✓	✓	✓
51	2-Ethyl-1-Hexanol	✓	✓		
52	Isomenthone			✓	✓
53	Benzaldehyde	✓	✓		
54	Ethyl Chrysanthemate (Not Occurred in Nature, used for fragrance)				✓
55	Linalool			✓	✓
56	1-Octanol	✓	✓		✓
57	Linalyl Acetate				✓
58	Menthyl Acetate			✓	
59	α-Isophorone				✓
60	Neomenthol			✓	

26 % decrease in T=3 months – accelerated conditions, no changes in RT

25 % decrease in T=3 months, at room temperature

Results: *continued*)

No.	Compound	Tobacco	Vanilla	Menthol	Blend
61	2-Acetylpyridine				✓
62	β-Famesene Isomer		✓		
63	γ-Phenylpropyl Acetate				✓
64	2-Acetylpyrazine				✓
65	Menthol	✓		✓	✓
66	(E)-β-Farnesene		✓		
67	Diethyl Butanedioate				✓
68	Italicene isomer		✓		
69	α-Farnesene isomer		✓		
70	Menthol Isomer			✓	
71	δ-Terpineol			✓	✓
72	4-Oxoisophorone				✓
73	α-Methylbenzyl acetate				✓
74	α-terpineol			✓	✓
75	Sabinol (Natural substance, found in pepermint, not for flavor use)			✓	
76	β-Bisabolene		✓		
77	cis-γ-Bisabolene		✓		
78	Germacrene D			✓	
79	(E,E)-α-Farnesene		✓		
80	Piperitone			✓	
81	Carvone			✓	
82	Geranyl acetate				✓
83	1-Decanol	✓			
84	Bisabolene Isomer		✓		
85	α,α-Dimethylphenethyl Acetate				✓
86	α-curcumene		✓		
87	Ethyl Phenacetate				✓
88	Nerol				✓
89	β-Damascenone				✓
90	Corylone (Cyclotene Hydrate, Wood Smoke)				✓
91	Ethyl Dodecanoate				✓
92	Geraniol				✓
93	Benzaldehyde Propylene Glycol Acetal	✓			✓
94	Geranyl acetone	✓	✓		
95	Benzyl Alcohol	✓	✓		

35 % decrease in T=3 months, at room temperature

HS-SMPE GC-MS Results: *continued*)

No.	Compound	Tobacco	Vanilla	Menthol	Blend
96	α-Isomethyl Ionone				✓
97	β-Ionone				✓
98	Jasmone			✓	
99	1-Dodecanol	✓	✓		
100	(6E)-3,7,11-trimethyldodeca-6,10-dien-3-ol		✓		
101	Megastigmadienone Isomer				✓
102	Caryophyllene Oxide			✓	
103	(E)-Nerolidol		✓		
104	β-Methyl Ionone				✓
105	Isoamyl Phenylacetate		✓		
106	Phenol	✓	✓		
107	Ethyl maltol				✓
108	(Z)-Nerolidol		✓		
109	Ledol		✓		
110	Methyl Methanthranilate				✓
111	Aliphatic Ester				✓
112	Cedrol	✓			
113	Spathulenol (Natural substance, not for flavor use)			✓	
114	Aliphatic Ester				✓
115	γ-decalactone		✓		
116	Ambroxide (Amberlyn or Ambrox)				✓
117	Thymol			✓	
118	Piperonal		✓		
119	γ-Undecalactone		✓		✓
120	Menthalactone			✓	
121	Propenylguaethol	✓			✓
122	Heliotropine Propylene Glycol Acetal		✓		✓
123	Vanillin		✓		

25 % decrease in T=3 months – accelerated conditions