

Poster Reprint

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# Qualitative and Quantitative Characterization of Whiskey with Accurate Mass Time of Flight LCMS

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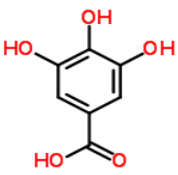
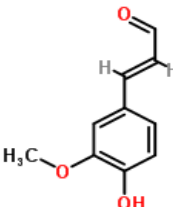
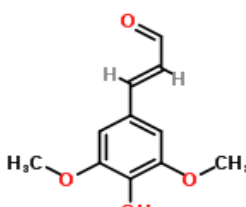
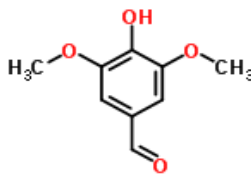
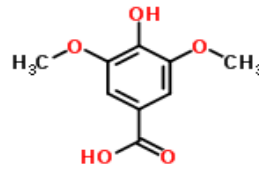
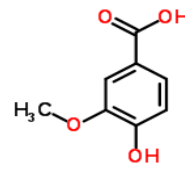
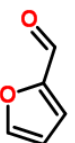
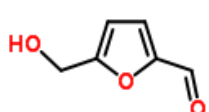
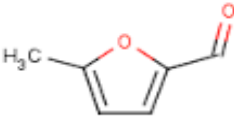
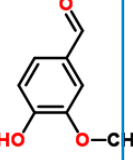
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## Introduction

Many types of spirits are aged in oak barrels for the absorbance of color and flavors. The barrels are reused, moving from whiskies to bourbons to sheries and tequila. Ten compounds must be present for a spirit to be considered barrel aged. Counterfeiters market non barrel aged spirits as barrel aged. In order to prosecute these counterfeiters, there must be proof that a product is fake. Congeners are minor compounds other than ethanol that occur naturally in distilling and fermenting process. This method adds another level of confirmation to the presence of the congeners. With the addition of fragment confirmation, we have now ways to prove the authenticity of spirits can be proven by the simultaneous confirmation of fragments, retention time, isotopic fidelity and spacing and accurate mass. Congeners are quantified with reference standards.

### Congeners

<b>Gallic Acid</b>  $C_7H_6O_5$ 170.0215	<b>Coniferaldehyde</b>  $C_{10}H_{10}O_3$ 178.0630	<b>Sinapaldehyde</b>  $C_{11}H_{12}O_4$ 208.0736	
<b>Syringic Acid</b>  $C_9H_{10}O_4$ 182.0579	<b>Syringaldehyde</b>  $C_9H_{10}O_5$ 198.0528	<b>Vanillic Acid</b>  $C_8H_8O_4$ 168.0423	
<b>Furfural</b>  $C_5H_4O_2$ 96.0211	<b>5-HMF</b>  $C_6H_6O_3$ 126.0317	<b>5-Methylfurfural</b>  $C_6H_6O_2$ 110.0368	<b>Vanillin</b>  $C_8H_8O_3$ 152.0473

## Experimental

One reference standard containing 10 nonvolatile congeners was run via LCMS TOF. A four-level calibration curve was created and run in triplicate. Seven samples of commercial whiskies were purchased and in quintuplicate for both quantitative and qualitative analysis. Known samples of non-aged spirits and long barrel aged spirits were analyzed. The multi-congener standard was included in this data set.

This data set was imported into Agilent Mass Profiler Professional software. The spirits were profiled by their mass characteristics. The experiment was repeated using all ion methodology. The all-ion method yielded LCMSMS-like spectral confirmation of the congeners in the spirits.

The samples were again rerun using all ions methodology with high and low fragmentor voltages. That data were analyzed against a Congener Agilent MassHunter Personal Compound Database and Library (PCDL) Manager for verification.



### TOF MS Parameters

G6230B	Value
Ion Mode	Dual ESI,
+ Mass Range	30-950 m/z
Scan Rate	4 Hz
Reference Masses	121.0509, 922.0098
Drying gas flow	9 L/min
Drying gas temp	300 °C
Capillary Voltage	4000 V
Fragmentor	145 V
All Ions Fragmentor EXP	100 V, 170 V, 270 V
Skimmer	65 V
Octapole RF peak	750

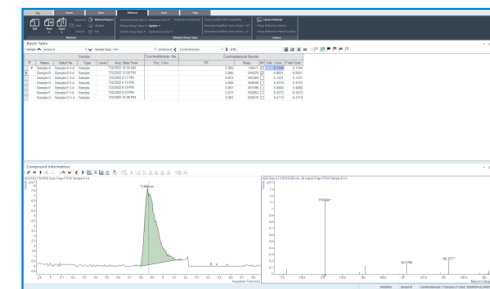
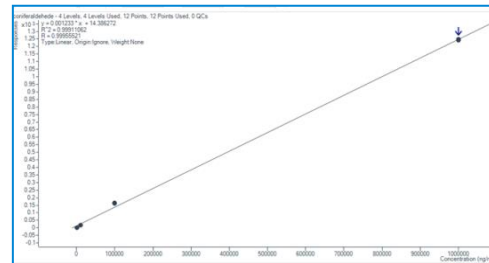
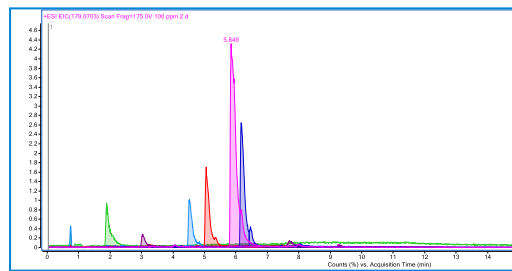
### LC Parameters

Prime UHPLC	Value
Column Temp	35 C
Injection volume	30-950 m/z
DAD Lambda	280
Flow Rate	0.5 ml/min
Mobile Phase A	0.1% Formic in H <sub>2</sub> O; Initial condition 95 %
Mobile Phase B	0.1% Formic Methanol; Initial condition 5%
Time 12 minutes	% A 5 [0.1% Formic in H <sub>2</sub> O] % B 95 0.1% Formic Methanol
Run Times	Stop time = 15 minutes Post time = % minutes
Column	ZORBAX RRHT StableBond CN, 3.0 x 100 mm, 1.8 μm, 600 bar, 80Å,

## Results and Discussion

### Quantitative Results

Extracted ions were used to create calibration curves from 1 ppm to 1000 ppm. 4 levels at 1, 10, 100, 1000 ppm.

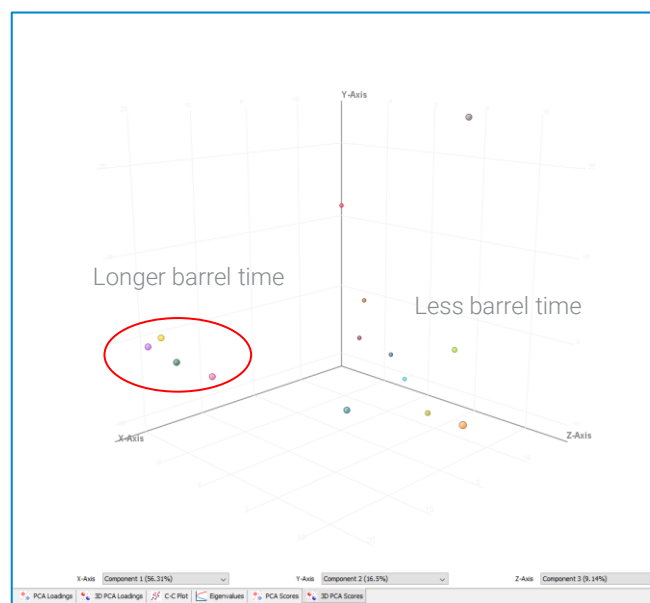
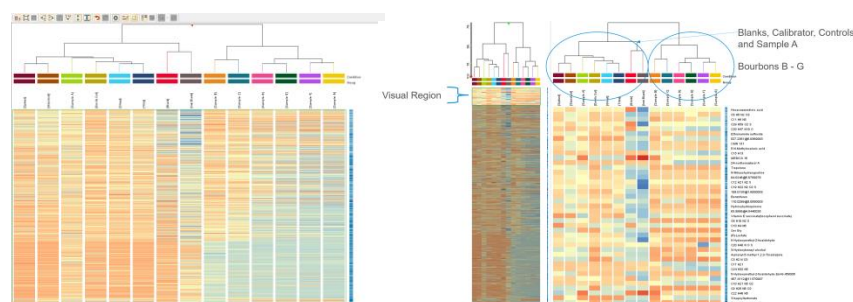


Congeners	R <sup>2</sup>	m/z
Vanillic Acid	0.999	169.0495
Syringic Acid	0.999	199.0601
Vanillin	0.999	153.0546
Syringaldehyde	0.999	183.0652
Coniferaldehyde	0.999	179.0703
Sinapaldehyde	0.999	209.0808
5 - methylfuran	0.999	111.0441
Gallic Acid	0.999	170.0275
Furfural	0.999	96.0225
5 - Hydroxymethylfuraldehyde (HMF)	0.999	126.0307

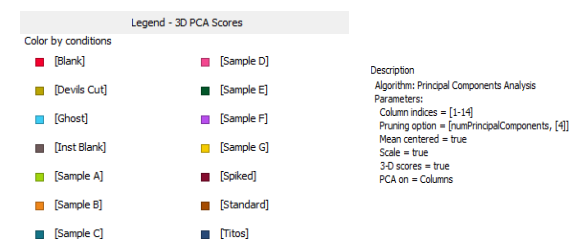
Sample	Compound	Concentration (ppm)	Sample	Compound	Concentration (ppm)
A	Vanillin	2.394	F	Vanillin	3.89
	5 HMF	1.609		Vanillic Acid	40.95
B	Vanillin	1.26	Syringic Acid	4.37	
	Vanillic Acid	45.21	Syringaldehyde	13.46	
	Syringic Acid	2.34	Sinapaldehyde	6.98	
	Syringaldehyde	10.59	Gallic Acid	12.57	
	Sinapaldehyde	29.99	5 HMF	0.96	
	Gallic Acid	12.37	G	Vanillin	0.9
5 HMF	2.02	Vanillic Acid		44.46	
C	Vanillin	0.95	Syringic Acid	1.2	
	Vanillic Acid	45.41	Syringaldehyde	8.01	
	Syringic Acid	1.603	Sinapaldehyde	20.25	
	Syringaldehyde	7.74	Gallic Acid	8.23	
	5 HMF	0.69	5 HMF	7.83	
D	Vanillic Acid	46.47	E	Vanillin	0.92
	Syringic Acid	0.38 ppm		Vanillic Acid	45.33
	Syringaldehyde	5.24		Syringic Acid	1.05
	Sinapaldehyde	0.47		Sinapaldehyde	10.44
	5 HMF	0.58		5 HMF	0.63
			Gallic Acid	7.55	
			Syringaldehyde	7.31	

### Data Analysis 3D PCA

Analysis via LC-TOF Calibrators, controls (Bourbons), blanks, samples N=5 for each, Total dataset = 97 files  
 Feature Finding Agilent Profinder  
 Data Analysis: 97 raw data files, "loose" parameters, non-recursive  
 Statistical Analysis Agilent Mass Profiler Professional (MPP)

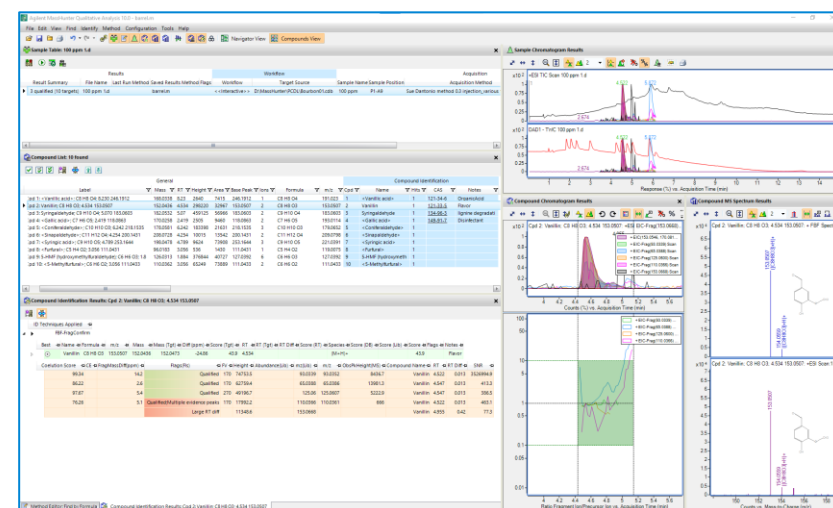
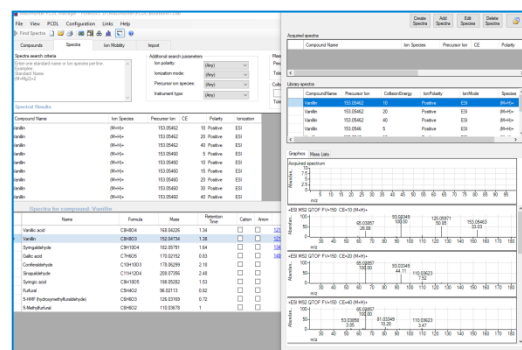


The workflow allowed characterization where all spirit samples clustered properly. Duplicate samples paired correctly. All analysis was done blindly and tacitly validating the MPP tools



### Data base for Qualitative Evaluation

Name	Formula	Mass	Retention Time	Clarity	Acid	CAS	ChemSpider	PubChem
Vanillic acid	C <sub>11</sub> H <sub>10</sub> O <sub>4</sub>	186.04296	1.34	<input type="checkbox"/>	<input type="checkbox"/>	322-34-5	1330	44Hydroxy-3-methoxybenzoic acid
Vanillin	C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>	152.04746	1.38	<input type="checkbox"/>	<input type="checkbox"/>	322-33-3	33864-54-8	4-Hydroxy-3-methoxybenzaldehyde
Sinapaldehyde	C <sub>11</sub> H <sub>10</sub> O <sub>4</sub>	186.04296	1.64	<input type="checkbox"/>	<input type="checkbox"/>	334-96-3	3332	4-Hydroxy-3,5-dimethoxybenzaldehyde
Gallic acid	C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>	170.02152	0.83	<input type="checkbox"/>	<input type="checkbox"/>	148-91-7	361	3,4,5-Trihydroxybenzoic acid
Coniferaldehyde	C <sub>13</sub> H <sub>12</sub> O <sub>3</sub>	176.06299	2.18	<input type="checkbox"/>	<input type="checkbox"/>			
Sinapaldehyde	C <sub>11</sub> H <sub>10</sub> O <sub>4</sub>	186.04296	2.48	<input type="checkbox"/>	<input type="checkbox"/>			
Syringic acid	C <sub>11</sub> H <sub>10</sub> O <sub>6</sub>	186.05292	1.53	<input type="checkbox"/>	<input type="checkbox"/>			
Furfural	C <sub>5</sub> H <sub>4</sub> O <sub>2</sub>	96.02113	0.82	<input type="checkbox"/>	<input type="checkbox"/>			
5-HMF Pyridoxymethylfuraldehyde	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.03159	0.72	<input type="checkbox"/>	<input type="checkbox"/>			
5-Methylfuran	C <sub>5</sub> H <sub>6</sub> O	98.06716	1	<input type="checkbox"/>	<input type="checkbox"/>			



A barrel aging PCDL has been created previously from all ions data. This data base has been used at sites in industry for qualification and of barrel aged spirits. The 100 ppm Congener standard was used to verify method performance. All Congeners were found with ion confirmation.

Results for 100 ppm standard

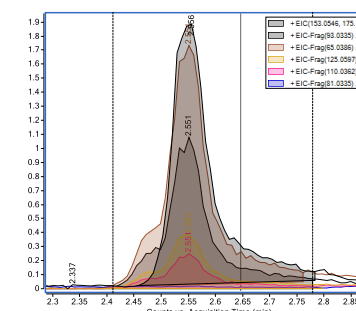


## Qualitative analysis with All Ions Methodology

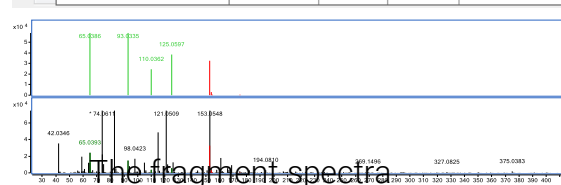
The known barrel aged Bourbon with all 10 congeners was run and analyzed with the Congener PCDL.

Label	Formula	m/z	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score (Tgt)	Flags (Tgt)
Cpd 1: <Furfural>	C5 H4 O2	97.0286	96.0213	96.0211	2.14	87.59	Qualified
Cpd 2: <5-Methylfuran>	C6 H6 O2	111.044	110.0367	110.0368	-0.4	99.95	Qualified
Cpd 3: <5-HMF>	C6 H6 O3	127.0389	126.0316	126.0317	-0.64	99.81	Qualified
Cpd 4: Vanillin	C8 H8 O3	153.0546	152.0473	152.0473	-0.01	97.41	Qualified
Cpd 5: <Vanillic Acid>	C8 H8 O4	169.0495	168.0423	168.0423	0.18	98.09	Qualified
Cpd 6: Gallic Acid	C7 H6 O5	171.0287	170.0214	170.0215	-0.83	99.57	Qualified
Cpd 7: <Coniferaldehyde>	C10 H10 O3	179.0702	178.0629	178.063	-0.53	99.38	Qualified
Cpd 8: <Syringic Acid>	C9 H10 O4	183.0651	182.0578	182.0579	-0.5	99.32	Qualified
Cpd 9: Syringaldehyde	C9 H10 O5	221.0421	198.0528	198.0528	-0.17	93.33	Qualified
Cpd 10: Sinapaldehyde	C11 H12 O4	209.0807	208.0734	208.0736	-0.97	99.35	Qualified

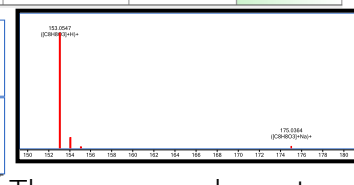
ID	Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (Tgt)	RT Diff	Score (RT)	Species	Flags	Notes
93.0326	Vanillin	C8 H8 O3	153.0547	152.0474	152.0473	-0.38	99.72	2.581	2.585	-0.004	100	(M+)(-M+)		



Overlay of EICs of fragment ions

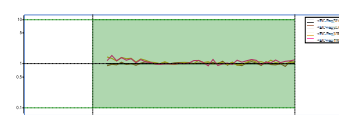


The fragment spectra

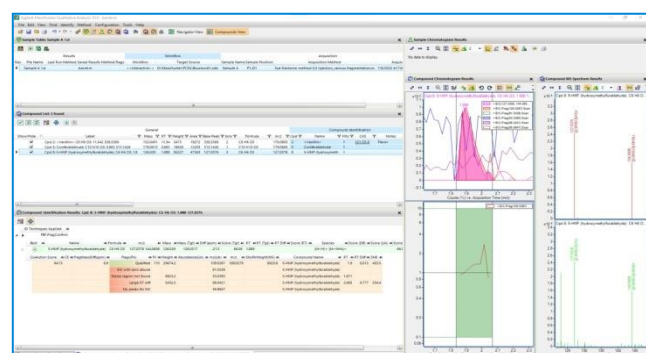


The compound spectra

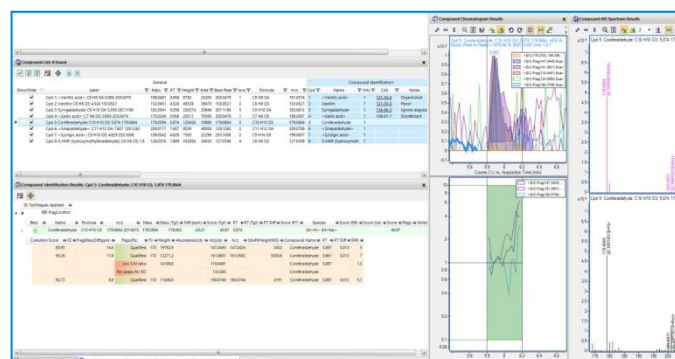
Each of the 10 compounds in the mix was qualified by retention time, accurate mass, fragment matching, Isotopic spacing and pattern. When a compound was qualified, an overlay of fragment retention times is created along with a Coelution plot.



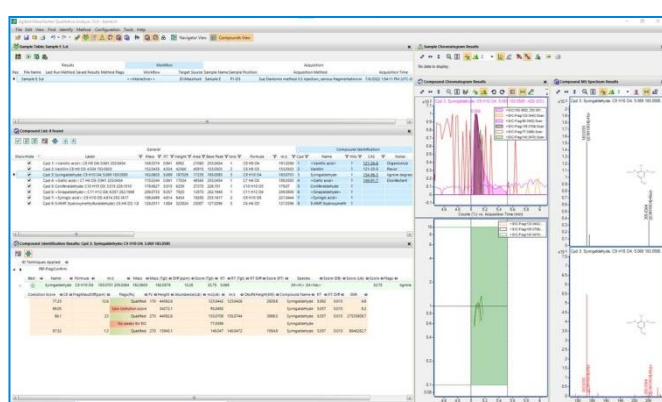
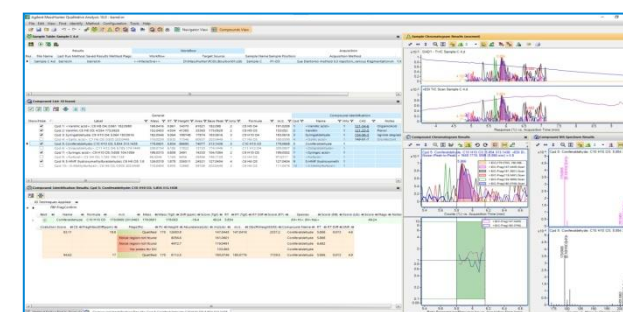
Coelution plot of fragment spectra retention times



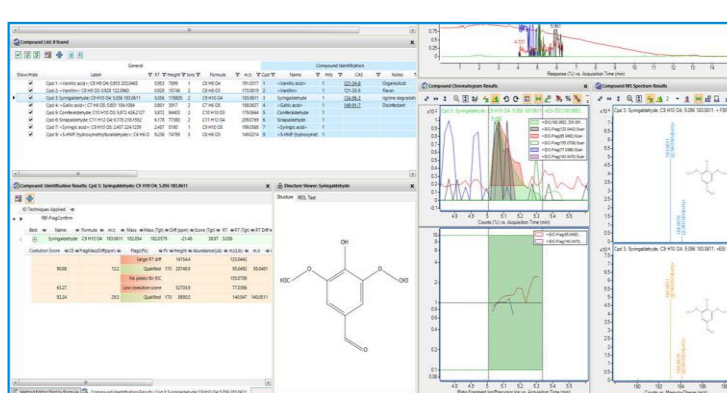
Sample A shows only 3 Congeners Matching a vodka sample and non aged bourbon.



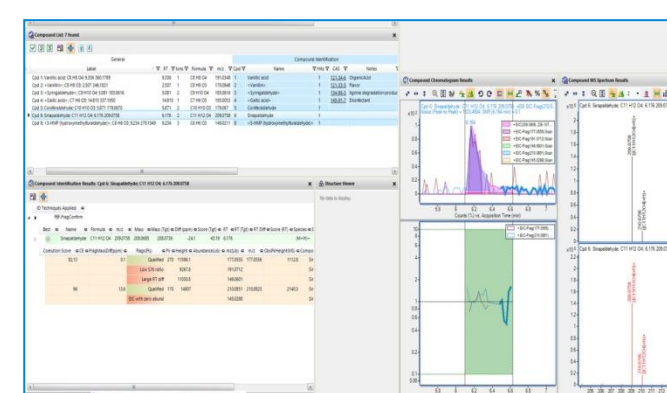
Sample B, C and D appear to be very similar. The congeners are found in low concentrations.



Sample E exhibits a golden color with an aroma. Definite barrel aging is concluded.



Sample F, dark brown with rich aroma, shows definite presence of congeners. Sample F is a barrel aged whiskey



Sample G, darkest brown with rich aroma, shows definite presence of congeners and is a barrel aged whiskey

## Conclusions

We are able to use the time of flight for both qualitative and quantitative work. With all ion methodology, we see the ability to have MS/MS like spectral confirmation.

<https://www.agilent.com/en/promotions/asms>

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