ASMS 2017
Poster number:
MP - 273

Allergens in Perfume by GC/Q-TOF with a Low Energy EI Source

Matthew Curtis; Jennifer Sanderson; Tim Peck
Agilent Technologies, Santa Clara, CA

Introduction

The number of suspected flavor and fragrance allergens found in consumer products is driving the need to develop improved analytical methods that can be used to quantitate regulated compounds as well as screen for new and/or unreported compounds. While targeted allergens can be identified by library search, new compound identification, especially in complex matrices, can prove to be challenging. Employing a high-resolution quadrupole time of flight mass spectrometer with a new EI source capable of low energy ionization for this analysis expands the amount of unknown compounds that can be identified by molecular ion, with good resolving power to separate it from the matrix and accurate mass assignment for confidence in identification.

Presented here is a workflow that can be employed in screening, confirming and quantitating known allergens and identifying new and unknown compounds that may be allergens or have other impacts on biological systems. The creation of a user-created compound database and library for increased confidence of the identification of target analytes.

Figure 1: Agilent 7250 GC/Q-TOF

Experimental

Sample Preparation

A fragrance allergen standards kit was purchased from Restek (33105). This contained 31 components separated into three solutions by their functional groups. This solution was diluted from 400 mg L^{-1} to 1 mg L^{-1} to create a 9-point calibration curve. 1,4-dibromobenzene and 4,4'-dibromobiphenyl were used as internal standards.

11 commercially available perfumes and colognes were diluted 10:1 with MTBE prior to analysis.

Instrumentation

The new Agilent 7250 GC/Q-TOF (Figure 1) equipped with the 7890B GC was used for this analysis. A 0.5 microliter injection was made into a multi-mode inlet at 280°C, with a 200:1 split flow into the Agilent UI low-pressure drop liner with glass-wool. Separation was performed on a DB-17MS UI 20 m x 180 µm x 0.18 µm with a He carrier gas flow of 1.6 mL/min. The International Fragrance Association has an analytical procedure for the analysis of allergens; this method was utilized for the work presented here[1].

The Q-TOF system was equipped with a new high efficiency EI source heated at 280°C for 70eV and 250°C for low-eV. Operated in full scan mode, spectral data was collected at a mass range of 40-700 m/z with an acquisition rate of 5Hz. Ionization using the new Low Energy-EI source was operated at the standard electron energy (70eV) and at lower electron energy optimized for this method (12.5eV). Data analysis was performed using Agilent MassHunter Software.

Workflow/Post Data Analysis

-Build Personal Compound Database and Library (PCDL) for both ionization energies

-Use the PCDL to quickly screen the commercial perfumes for target analytes.

-Use the PCDL to create a quantitation batch of the standard compounds

-Analyze and quantitate commercial fragrances for target allergens

-Use the quantitation batch, and the NIST14 library to identify components not included in the PCDL

-Use 12.5eV to confirm high m/z ions to aid in the identification of the molecular ion

Results and Discussion

Building a Unique Screening Library with Agilent PCDL Manager

Accurate mass libraries are easily created from a MassHunter datafile. MassHunter Qualitative Analysis is used to locate the analyte, extract the spectrum and add an identification for the compound (Figure 2). The Molecular Formula Generation algorithm confirms the molecular ion and fragment ions using accurate mass, isotope ratios, and isotope spacing.

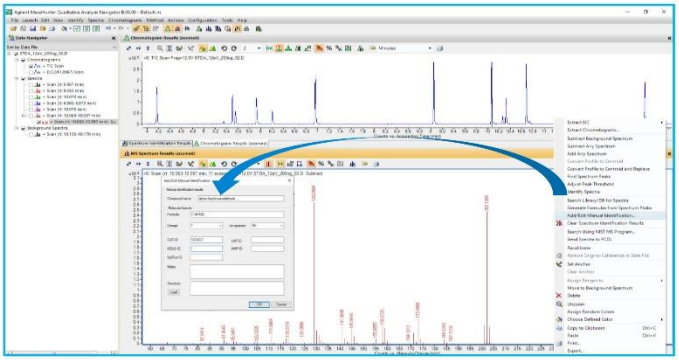

Figure 2: MassHunter Qualitative Analysis Manual Identification

After reviewing the identification, a right-click on the spectrum performs the transfer of all the compound information to the PCDL (Figure 3).

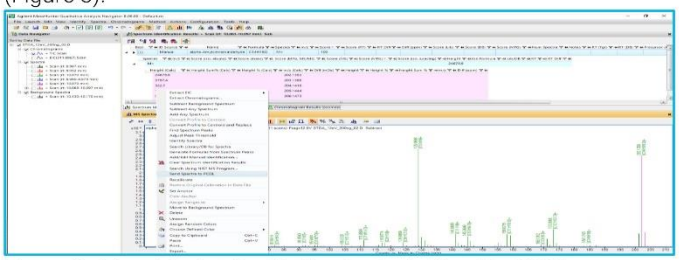

Figure 3: MassHunter Qualitative Analysis Send to PCDL

PCDL Manager includes the compound name, retention time, CAS #, molecular formula, structure, spectrum, and more (Figure 4). The PCDL can be used for screening targets or as a specific library to search prior to a general library (NIST).

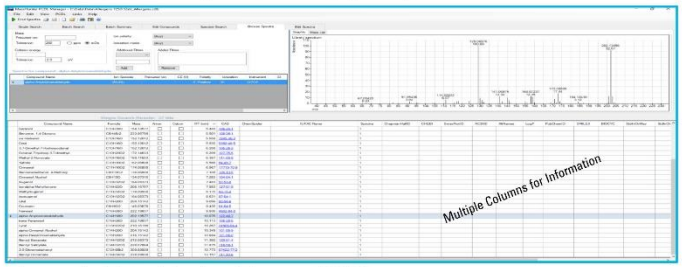

Figure 4: MassHunter Allergen PCDL

Utility of a User-Created PCDL

PCDL uses the accurate mass, isotope ratios, fragment ions, coelution graphs, and RT to confirm the identification of the target.

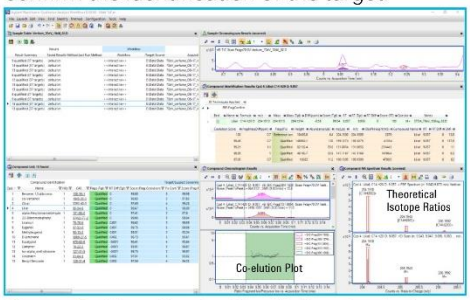

Figure 5: MassHunter Qualitative Analysis Screening

Deconvolution followed by a library search can also be performed in MassHunter Qualitative Analysis. Even when a unit mass library is used, the exact mass is calculated from the molecular formula and compared to the spectrum. This provides additional confirmation (Figure 6).

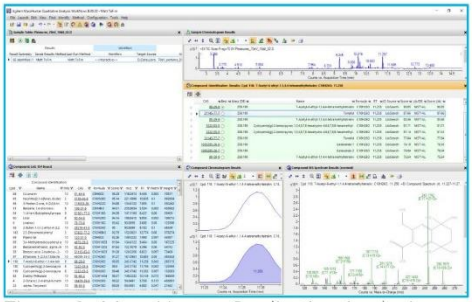

Figure 6: MassHunter Qualitative Analysis Unknown Identification (NIST14)

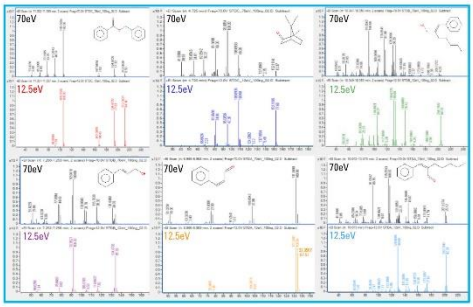

Figure 7: Comparison of 70eV and 12.5 eV of several allergens

Results and Discussion

Table 1: Quantitation of Target Analytes from 7 of the commercial products (10x due to dilution).

	Sample 1				Sample 2			Sample 3			Sample 4			Sample 5			Sample 6			Sample 7		
Compound Name	RT (min)	MZ	Mass Accuracy (mmu)	Final Conc. (µg/L)	MZ	Mass Accuracy (mmu)	Final Conc. (µg/L)	MZ	Mass Accuracy (mmu)	Final Conc. (µg/L)	MZ	Mass Accuracy (mmu)	Final Conc. (µg/L)	MZ	Mass Accuracy (mmu)	Final Conc. (µg/L)	MZ	Mass Accuracy (mmu)	Final Conc. (µg/L)	MZ	Mass Accuracy (mmu)	Final Conc. (µg/L)
D-Limonene	3.013	94.0777	0.1	20.1	94.0776	-0.1	1830.6	94.0776	0	50.4	94.0787	1	16.6	94.0775	-0.1	55.8	94.0778	0.2	26.4	94.0775	-0.1	592.4
Eucalypto2l	3.18				154.1357	0.6	11.6	154.1358	0.7	42.0	154.1351	0	77.2	154.1354	0.3	163.2	154.135	-0.1	10.3			
Camphor	4.8							152.12	0.5	43.2	152.1192	-0.3	103.6	152.1195	0	65.9						
Estragole	5.316	148.0888	0.6	19.2				148.0881	-0.1	19.1	148.0881	-0.1	23.4									
methyl 2-nonynoate	6.36	100.0517	-0.2	30.6																		
Safrole	6.57	161.0685	1	19.4	162.0675	0	18.6	162.0676	0.1	21.0	162.0677	0.2	19.8	162.0679	0.4	21.0				162.0673	-0.2	19.3
iso-alpha-methylionone	7.952	150.104	0.2	1103.1				150.1041	0.3	881.5	150.1037	0	3252.6	150.1039	0.1	1526.8	150.1039	0.1	873.4	150.1037	0	5624.5
Coumarin	9.434	146.0363	0.2	3010.0	146.0367	0.6	20.3	146.0363	0.2	141.3	146.0361	0	272.4	146.0363	0.2	74.3	146.0361	0	152.3	146.0358	-0.3	19.0
Benzyl Benzoate	11.353	212.0833	0.1	222.7	212.0832	0	412.5	212.084	0.8	22.2	212.0829	-0.3	58.0	212.0836	0.3	102.2	212.0832	-0.1	134.5	212.0827	-0.6	20.7
Benzoic acid, 2-hydroxy-, phenylmethyl ester	11.877	91.0544	0.2	127.7	91.0541	0	8269.4	91.054	-0.1	20.7	91.0552	1.1	20.5	91.0542	0.1	37.1	91.0543	0.2	95.0	91.054	-0.1	3213.5
Benzyl cinnamate	13.158	192.0936	0.3	16.7	192.0936	0.2	21.6							192.0923	-1.1	15.2						
methyl 2-octynoate	5.261																					
Phenylacetaldehyde	4.191				92.0621	0.1	22.7															
Citral	5.94	123.1148	-1.9	31.1				123.1172	0.5	37.6	123.1162	-0.5	109.1	123.1169	0.3	333.8	123.1175	0.8	38.2	123.1162	-0.5	166.6
Octanal, 7-hydroxy-3,7-dimethyl-	6.207	96.0934	0.2	517.8	96.0933	0	255.9	96.0936	0.3	62.5	96.0919	-1.3	70.2	96.0935	0.3	58.0	96.0933	0.1	2177.1	96.0924	-0.8	706.0
Cinnamal	6.974										132.0565	-0.3	18.5	132.0573	0.6	19.7						
Lilial	9.057				189.1273		4831.6	189.1275	0.3	310.3	189.1272	-0.1	591.4	189.1275	0.2	431.0	189.1276	0.3	17.8	189.1273	0	5085.2
alpha-Amylcinnamaldehyde	10.071	202.1351	0.1	79.6	202.1341	-0.8	18.8	202.1362	1.2	16.3				202.1352	0.3	20.9				202.1343	-0.7	17.5
Lyral	10.325				136.0882	0.1	241.4	136.0882	0.2	267.9	136.0882	0.1	465.9							136.0885	0.4	18.2
alpha-Hexylcinnamaldehyde	10.685	216.1531	2.6	18.5	216.1505	0	3427.5	216.1474	-3.1	39.4							216.1507	0.2	1105.2	216.15	-0.5	24.3
Linalool	3.684	93.0699	-0.1	2623.3	93.0699	-0.1	1662.6	93.0698	-0.2	593.4	93.0698	-0.2	1171.7	93.0699	-0.1	879.7	93.0698	-0.2	502.9	93.0698	-0.2	138.0
Benzyl alcohol	3.987	108.0569	-0.3	184.0	108.0568	-0.4	36.4	108.0571	-0.1	30.1	108.0567	-0.4	63.5	108.0566	-0.6	19.6	108.0567	-0.5	104.4	108.057	-0.2	47.4
Citronellol	4.965	81.0699	0	305.8	81.0698	-0.1	668.3				81.0698	-0.1	555.8				81.0699	0	1679.4	81.0698	-0.1	2078.3
Geraniol	5.442	69.0699	-0.1	454.2	69.07	0	2251.0				69.0699	-0.1	321.1	69.0699	-0.1	41.2	69.0699	-0.1	1073.9	69.0697	-0.3	1806.7
Benzenemethanol, 4-methoxy-	7.028																					
Cinnamyl Alcohol	7.251	134.0726	0	369.1							134.0719	-0.7	19.1	134.0726	0.1	19.9						
Eugenol	7.405	164.0834	0.1	529.1							164.0827	-0.6	17.8	164.0832	-0.1	2371.9	164.0832	-0.1	113.1	164.084	0.7	17.9
Methyleugenol	8.176	178.0989	0	21.6							178.1009	2	18.4	178.0986	-0.3	24.7	178.0992	0.3	22.8	178.0971	-1.8	18.3
Isoeugenol	8.626				164.0831	-0.1	25.0							164.0828	-0.5	24.1	164.084	0.7	19.4	164.0837	0.5	19.7
Farenol	10.118	136.1248	0.1	78.7	136.1206	-4.1	754.9	136.1229	-1.9	653.9	136.1241	-0.7	759.7							136.1231	-1.7	1233.1
alpha-amylcinnamic alcohol	10.405	130.0734	-4.2	23.1				130.0775	-0.2	22.9												

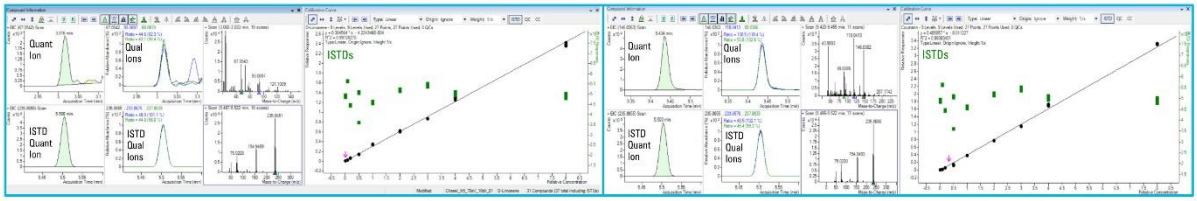

Figure 8: Calibration curve and qualifier ratios for D-Limonene (left) and Courmarin (right).

Unknowns Analysis with Accurate Mass and High Resolution

To identify additional components beyond the target list, MassHunter Unknowns Analysis was used to verify the target hits while separating other hits from the NIST14 library.

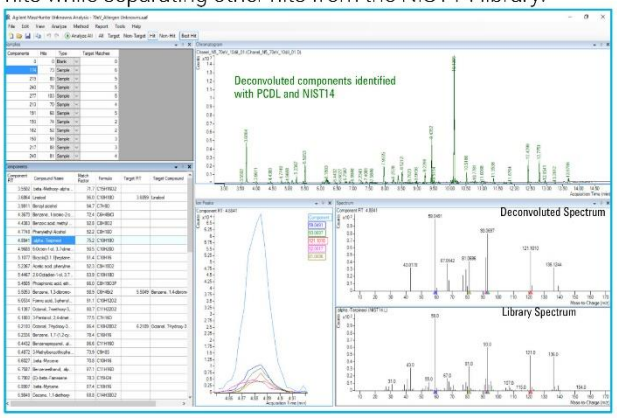

Figure 9: Unknowns Analysis for Known-Unknown identification

Conclusions

The Agilent 7250 GC/Q-TOF successfully analyzed allergens in fragrance products

- High mass accuracy, accurate isotope ratios provided high confidence of identification
- An accurate mass library was created for target quantitation and screening of allergens
- Low energy EI provided confidence in the identification of the molecular ion cluster and possible structural identification
- 31 allergen components were quantitated in 11 commercially available perfumes

References

[1]GC/MS Quantitation of Potential Fragrance Allergens in Fragrance Compounds. *International Fragrance Association*. V3, 2007