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Comprehensive chemical characterization of e-cigarette liquids using high-resolution Orbitrap GC-MS

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Keywords

Accurate mass, chemical components, electronic cigarettes, e-liquids, gas, GC, chromatography, high resolution accurate mass, HRAM, Orbitrap mass spectrometry, solid phase micro extraction, SPME, SPME Arrow

Goal

To demonstrate the utility of the Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS mass spectrometer for confident characterization of chemical content of electronic cigarette liquids.

Introduction

Electronic cigarettes were introduced in 2007 as alternative to conventional tobacco products, and their use has significantly increased worldwide. Despite their growing popularity, little is known about the potential impact of e-cigarettes on human health. This is especially important with regards to the presence of flavoring compounds, solvents, additives, and other components intentionally or unintentionally added with unclear long-term effects.[1]

In 2012, the U.S. Food and Drugs Administration (FDA), established a list of 93 “harmful and potentially harmful constituents” (HPHCs) in cigarette smoke, cigarette filler, and smokeless tobacco products.[2] Under section 904(a)(3) draft guidance of the Federal Food, Drug and Cosmetic Act (the FD&C Act), a representative subset of 20 HPHCs to be reported by tobacco product manufacturers for combustible products only are detailed.[3] Additionally under section 910 draft guidance of the FD&C Act, 29 HPHCs have been outlined in the Premarket Tobacco Products Applications (PMTA) guidance for Electronic Nicotine Delivery Systems (ENDS).[4]

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In May 2016 the Tobacco Products Directive (TPD) 2014/14/EU[5] introduced new rules for nicotine-containing electronic cigarettes and refill containers (Article 20), in order to protect human health and to meet the obligations of the European Union under the WHO Framework Convention on Tobacco Control.[6] In the UK the majority of the provisions under article 20 are implemented by the Medicines and Healthcare products Regulatory Agency (MHRA).[7] Other EU member states have transposed the EU TPD into their own national laws and assigned competent bodies to oversee.

Current analytical technologies used for the qualitative and quantitative assessment of electronic cigarette liquids (e-liquids) are liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS), but both techniques can have limitations with regards to mass accuracy, sensitivity, and linear dynamic range. GC-MS triple quadrupole and GC-FID would typically only be used for quantification of known compounds in e-liquids. Whereas a high resolution accurate mass (HRAM) approach can achieve confident targeted and non-targeted compound identifications.

There are several analytical challenges associated with the analysis of e-cigarette liquids using GC or GC-MS. To have good coverage of the chemical content, a GC or GC-MS platform that can sensitively and selectively detect chemical constituents, taking into account the variety and complexity of possible matrices, must be used. GC coupled to high resolution mass spectrometry is one of the most appropriate as it offers both the required sensitivity and selectivity. In particular, GC-Orbitrap MS with sub-ppm mass accuracy, versatility for sample introduction, and combined with unique software algorithms for automated deconvolution and extensive spectral libraries, make it a powerful solution for both qualitative and quantitative assessments of e-liquids, all while operating in full scan acquisition mode.

Although liquid injections are commonly used in GC-MS workflows for this analysis, an alternative is solid phase micro extraction (SPME),[8] which is a solvent-free technique that combines sample extraction with concentration in a single step. It consists of a fused-silica fiber coated with an organic phase that acts by extracting and concentrating the analytes present using selective adsorptive/absorptive processes.

The fiber can be exposed to the headspace or via direct immersion in the sample. The Thermo Scientific™ SPME Arrow addresses some of the limitations of SPME with improved fiber design and geometry, superior sensitivity, improved extraction efficiency, and higher mechanical robustness.

This work aims to demonstrate the applicability of SPME Arrow in combination with GC-Orbitrap technology for qualitative targeted and non-targeted analysis of chemical components of e-liquids. For confident confirmation of compounds identified, softer ionization modes (chemical ionization, CI) were employed, in addition to classical electron ionization (EI).

Experimental

Preparation of samples

Ten e-liquid samples were purchased locally and included both flavored and flavorless samples (Table 1). Two shortfill samples (c and i), supplied at 0 mg/mL specified nicotine level, were also analyzed. Shortfills are e-liquids that can be purchased in bottles larger than the regulated limit of 10 mL, into which the user can add a nicotine shot prior to use. They are not regulated under TPD within the UK as they contain 0% nicotine upon purchase.

Table 1. E-liquid samples used in the analysis, both flavored and flavorless samples, with declared nicotine levels of 0, 6 or 12 mg/mL

	Description	Bottle volume (mL)	Declared nicotine concentration (mg/mL)
a	Flavorless	10	0
b	Flavored (branded)	10	0
c	Flavored (branded)	50	0
d	Flavored (vanilla)	10	0
e	Flavored (mint)	10	0
f	Flavored (branded)	10	6
g	Flavorless	10	6
h	Flavored (lemon)	10	12
i	Flavored (strawberry)	50	0
j	Flavored (lemon)	10	0

For target and non-targeted qualitative analysis of e-liquids using SPME Arrow sample introduction: 100 µL of each e-liquid sample was first diluted to 10 mL with water (HPLC grade), mixed, then further diluted 50 µL to 1 mL with water (HPLC grade) in a 20 mL headspace vial (P/N 20-CV) with crimp cap (P/N 20-MCBC-ST3) ready for SPME Arrow analysis.

Sample blanks were also prepared taking 1 mL of water (HPLC grade) in a 20 mL headspace vial. In addition, all samples and blanks contained an internal standard (8-hydroxyquinoline) to a final concentration of 10 µg/mL.

Instrument and method setup

A Thermo Scientific™ Exactive™ GC Orbitrap™ mass spectrometer, coupled with a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph, configured with a Thermo Scientific™ TriPlus™ RSH™ SPME Arrow autosampler, and a Thermo Scientific™ Instant Connect split/splitless (SSL) injector with a SPME Arrow adaptor was used in all experiments.

Compound separation was achieved on a Thermo Scientific™ TG-WaxMS 30 m x 0.25 mm i.d. x 0.25 µm film capillary column (P/N 26088-1420).

The mass spectrometer was tuned, air leak checked, and calibrated in <1.5 min using FC43 (CAS 311-89-7) to achieve mass accuracy of <2 ppm. The system was operated using electron ionization (EI), as well as positive chemical ionization (PCI), and negative chemical ionization (NCI) modes using the fast, vent-free vacuum probe interlock tool. Data were acquired in full-scan and 60,000 mass resolution (full width at half maxima FWHM, measured at *m/z* 200). Additional details of the instrument parameters are shown in Tables 2–4, for the SPME Arrow analysis.

Table 2. TriPlus RSH autosampler conditions

Extraction parameters		
SPME Arrow fiber:	Thermo Scientific™ DVB / Carbon WR / PDMS (P/N 36SA11T1)	
Vial:	Fiber depth in vial (mm):	35
Incubation:	Temperature (°C): Time (min): Agitator speed (rpm):	60 10 500
Extraction:	Temperature (°C): Time (min): Stirring speed (rpm):	60 20 500
Fiber desorption:	Temperature (°C): Time (min): Fiber depth in injector (mm):	230 3.0 70
Fiber conditioning:	Temperature (°C): Time - pre desorb (min): Time - post desorb (min):	280 3.0 15

Table 3. GC and injector conditions

TRACE 1310 GC system parameters				
Liner:	SPME Arrow Liner 1.7 mm i.d. (P/N 453A0415-UI)			
Inlet temperature (°C):	230			
Carrier gas, (mL/min):	He, 1.2			
Inlet module and mode:	SSL, split mode			
Split ratio:	100:1			
Purge flow (mL/min):	5			
Column:	TG-WaxMS 30 m x 0.25 mm i.d. x 0.25 µm film capillary column (Thermo Scientific™ TraceGOLD™ GC Column) (P/N 26088-1420)			
Oven temperature program:	**RT (min)**	**Rate (°C/min)**	**Target temperature (°C)**	**Hold time (min)**
Initial	0	-	40	3.00
Final	3.00	13	250	6.00
Run time	25	-	-	-

Table 4. Mass spectrometer conditions

Ionization type:	EI	NCI	PCI
Transfer line (°C):	250		
Ion source (°C):	230	170	170
CI gas type:	n/a	Methane	Methane
CI gas flow (mL/min):	n/a	1.2	1.3
Electron energy (eV):	70		
Acquisition mode:	Full-scan		
Mass range (Da):	35–400	100–400	80–400
Mass resolution:	60,000 FWHM at *m/z* 200		

Data processing

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software. The TraceFinder single platform software integrates instrument control, method development functionality, and qualitative and quantitation data processing. TraceFinder also contains accurate mass spectral deconvolution and spectral matching functionality.

Results and discussion

E-liquids were analyzed qualitatively by targeting the subset of the US FDA list of HPHCs.[2,3] Moreover, an un-targeted approach was used to screen the samples for other potential toxic chemicals that may be present.

Target screening for known HPHC components in e-liquids

Where standards are not available, the Exactive GC Orbitrap, with high mass resolution, and excellent mass accuracy, provides the ability to qualitatively screen for known compounds, against a developed compound database (CDB) that contains the names, RTs, and exact masses of several EI fragment ions.

An e-liquid CDB was developed in-house (Figure 1), containing specific compounds of interest, including GC-amenable compounds from the representative subset of HPHCs detailed by the FDA.[3] The samples of interest were screened against this CDB, an example of the screening results is shown in Figure 2 for sample h (vanilla flavor).

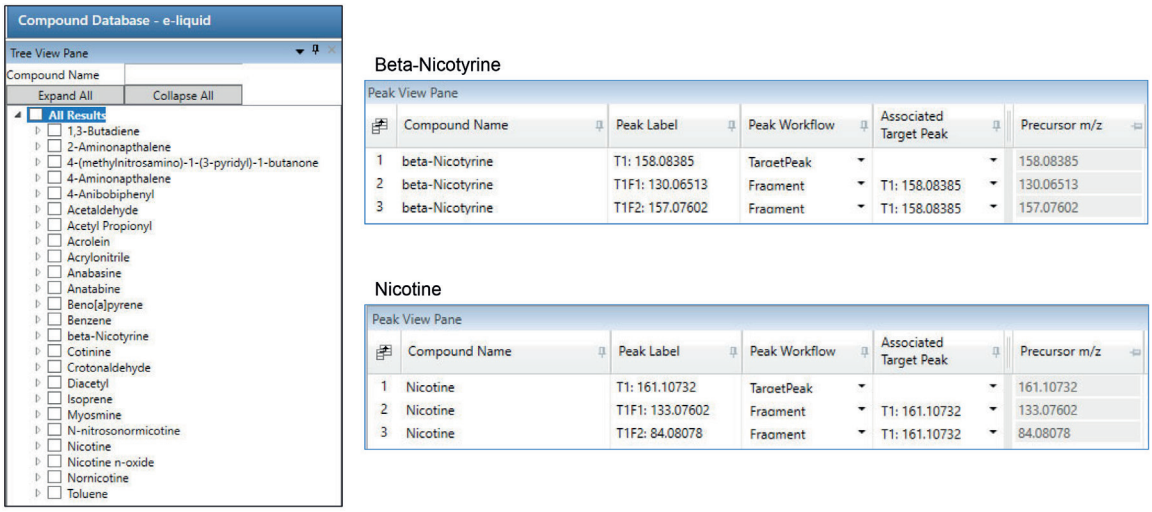

Figure 1. Compound database, with beta-nicotyrine and nicotine data expanded, illustrating the compound name, peak label, peak workflow, associated target peak, and the fragment *m/z*

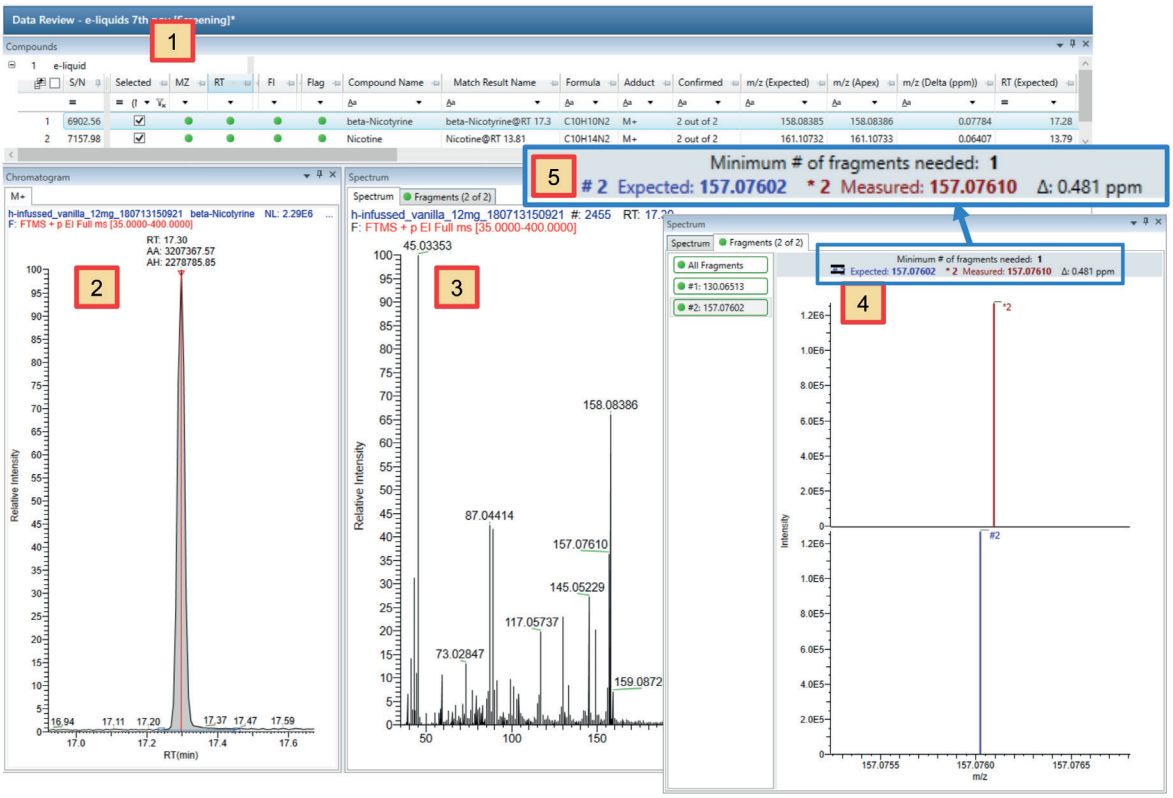

Figure 2. Target screening results for sample h (vanilla). Sections of the target screening results include [**1**] compounds matched in the sample, identified based on expected *m/z* and fragment ions (within ±5 ppm window), [**2**] Extracted ion chromatogram (XIC) for the selected compound, [**3**] component mass spectra, [**4**] fragment ion mass spectra observed (top), expected (bottom), ±5 ppm extracted window displayed, [**5**] for the selected fragment ion, ppm delta value for expected vs the measured *m/z*.

Non-targeted screening for unknown components in e-liquids

For non-targeted qualitative screening of e-liquids, full-scan data was first acquired using EI, followed by spectral deconvolution with library matching for putative compound identification.

For additional confidence in the identification of unknowns, a confirmation step using positive and negative chemical Ionization (PCI and NCI) is also mandatory. The workflow used for non-targeted screening is summarized in Figure 3.

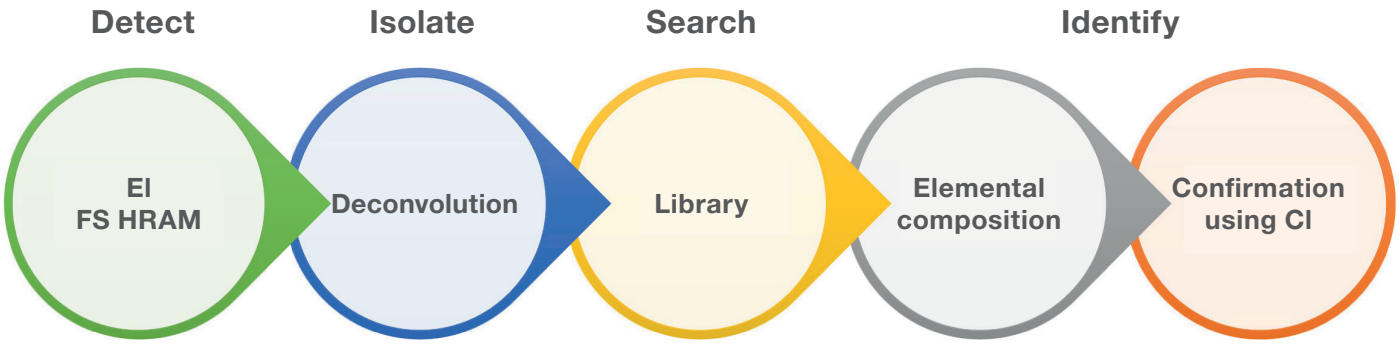

Figure 3. Workflow for the Exactive GC Orbitrap for non-targeted screening of e-liquids: full scan data acquired using EI full scan HRAM; spectral deconvolution with library search for putative compound identification; confirmation using chemical ionization (CI) data for added specificity and selectivity

Detect: Electron ionization, full scan

Full scan data (EI) was first acquired; example TICs are shown in Figure 4.

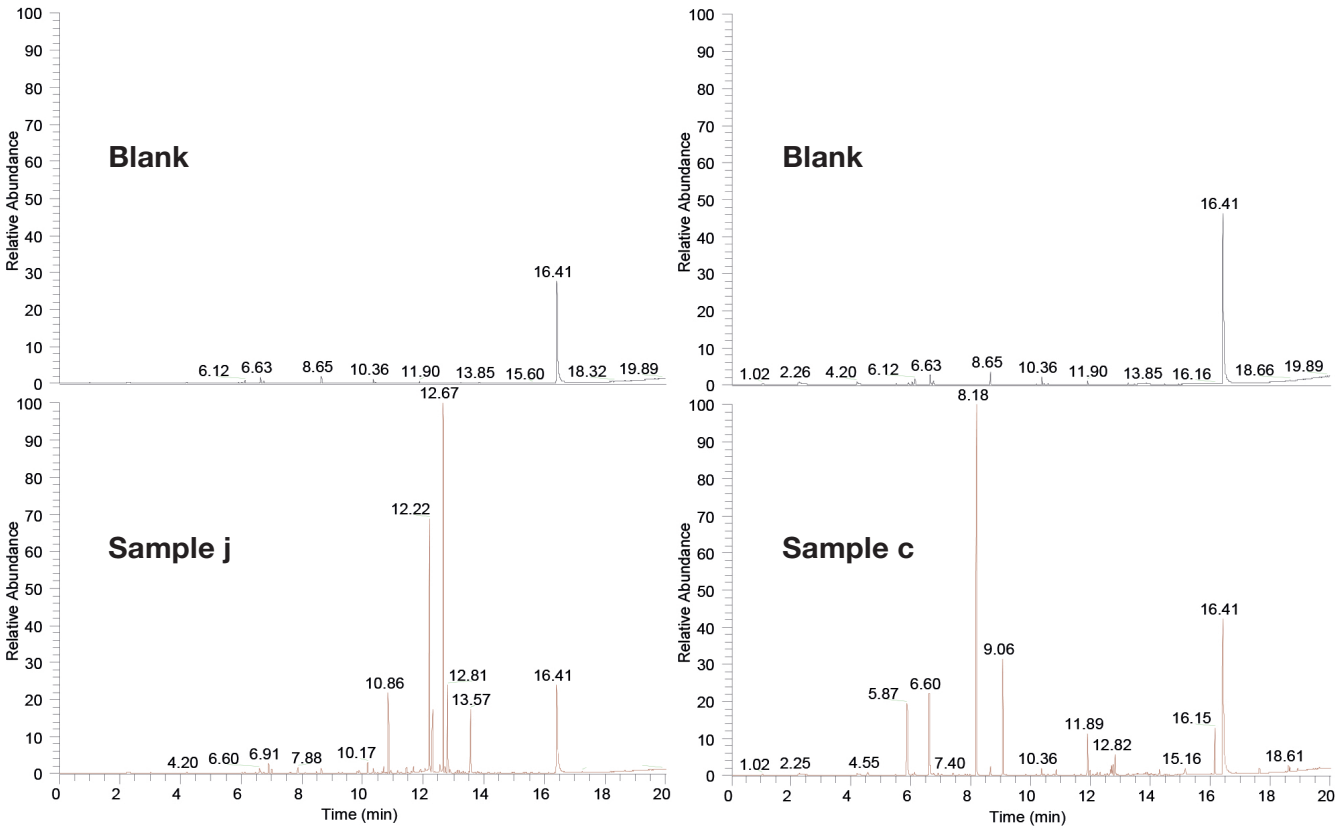

Figure 4. GC-MS total ion chromatograms (TICs normalized signal to the most intense sample) for EI full scan data obtained for e-liquid sample j (lemon flavor) (bottom left) and e-liquid shortfill sample c (branded flavor) (bottom right), and the associated sample blanks (top). Sample and blank relative abundance scales have been normalized for comparison. The peak at RT = 16.4 min is the internal standard (8-hydroxyquinline).

Isolate, search and identify: Deconvolution

Spectral deconvolution is available with TraceFinder software that is designed to automatically deconvolve chromatographic peaks into multiple components by aligning mass spectral peaks and performing a library search match on the deconvolved spectra.

An example of the deconvolution identification results achieved for sample j (lemon) is shown in Figure 5 for *p*-cymene. The main compounds detected in the analyzed e-liquids samples using the deconvolution plug-in are shown in Table 5.

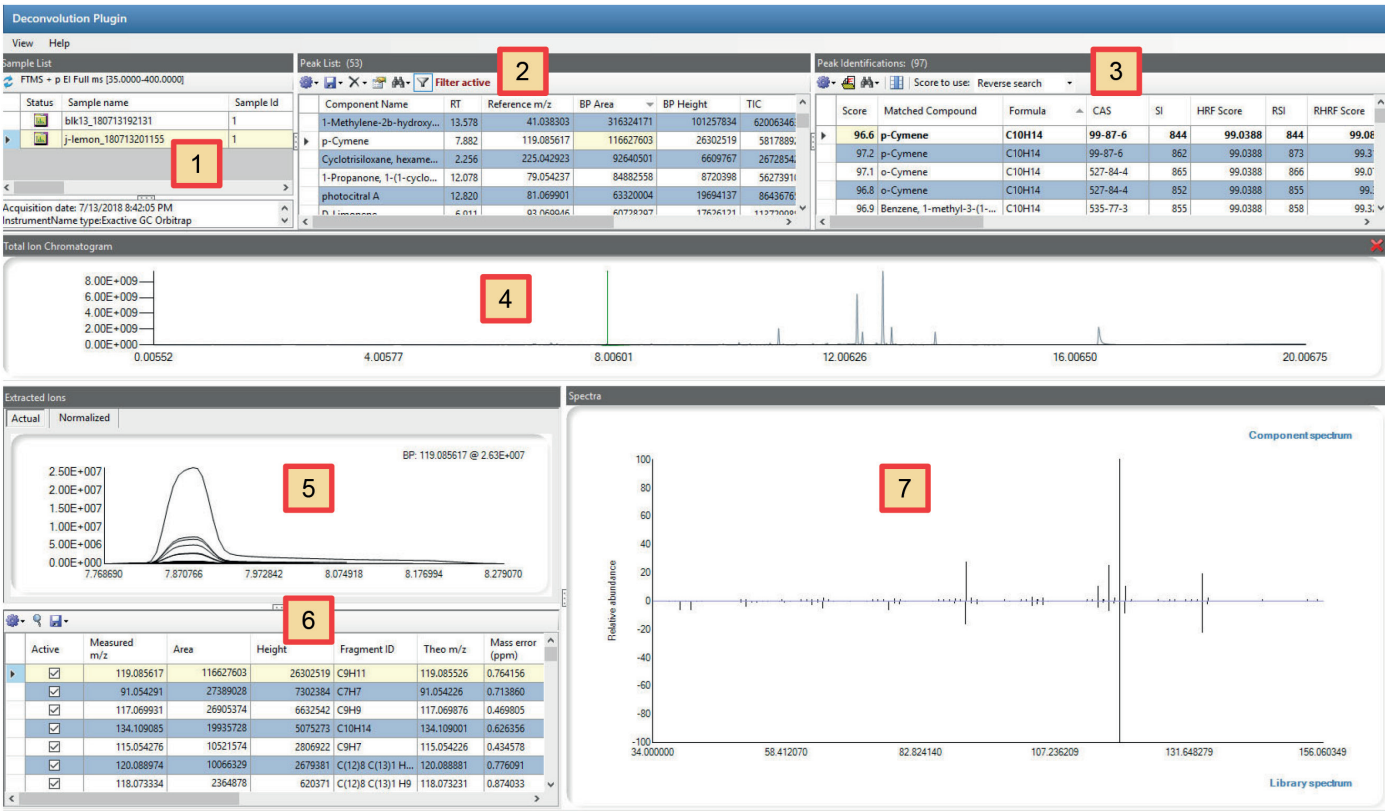

Figure 5. Deconvolution result browser for sample j (lemon), highlighting the identification of *p*-cymene. Sections of the deconvolution software include: [**1**] sample list; [**2**] deconvoluted peak list; [**3**] peak identification, highlighting library search result for the selected component in the peak list; [**4**] TIC; [**5**] overlay of the extracted ions of the deconvoluted component in the peak list; [**6**] list of annotated fragment ions from the deconvoluted EI spectrum and elemental composition based on elements in top hit; and [**7**] the deconvoluted component EI mass spectra (top) and the comparison to the library (bottom).

Table 5. The main compounds detected in the analyzed e-liquids, detailing the sample description, main compounds detected, the measured and theoretical *m/z* of the base peak, mass accuracy (ppm) for the base peak, the exact mass (M.W.) and CAS number for the detected compound, the RT, the identification scores for SI (search index score), HRF (High-Resolution Filtering score), and RSI (reverse index score)

Sample / description		Compounds detected	Formula	Base peak			Exact mass (*m/z*)	CAS No.	RT (min)	Identification scores		
				Measured (*m/z*)	Theoretical (*m/z*)	Mass accuracy (ppm)				SI	HRF	RSI
a	Flavorless	2,2,4-trimethyl-1, 3-Dioxolane	$C_6H_{12}O_2$	101.05974	101.05974	0.3	116.08373	1193-11-9	3.1	808	87	810
		1,3-Dioxolane, 2-ethyl-4-methyl-	$C_6H_{12}O_2$	87.04409	87.04406	0.4	116.08373	4359-46-0	4.3	838	85	841
b	Flavored (branded)	Butanoic acid, ethyl ester	$C_6H_{12}O_2$	43.05413	43.05423	2.2	116.08373	105-54	4.5	809	86	810
		Ethyl maltol	$C_7H_8O_3$	140.04663	140.04680	1.2	140.04734	4940-11-8	15.1	890	100	940
		Vanillin	$C_8H_8O_3$	151.03903	151.03897	0.0	152.04734	121-33-5	18.9	878	94	886
c	Flavored (branded)	n-Amyl isovalerate	$C_{10}H_{20}O_3$	70.07770	70.07770	0.0	172.14633	25415-62-7	8.2	829	86	859
		2-Pentyl acetate	$C_7H_{14}O_2$	43.01774	43.01784	2.4	130.09938	626-38-0	5.8	874	83	899
		Acetic acid, pentyl ester	$C_7H_{14}O_2$	43.01774	43.01784	2.4	130.09938	628-63-7	6.6	840	77	880
		2,3-Nonanedione	$C_9H_{16}O_2$	43.05412	43.05423	2.3	156.11503	6175-23-1	9.1	764	68	839
		Eugenol	$C_{10}H_{12}O_2$	164.08296	164.08318	1.3	164.08373	97-53-0	16.2	856	99	878
		Ethyl vanillin	$C_9H_{10}O_3$	137.02341	137.02332	0.6	166.06299	121-32-4	18.6	818	91	837
d	Flavored (vanilla)	Piperonal	$C_8H_6O_3$	149.02318	149.02332	1.0	150.03169	120-57-0	16.7	875	66	875
		Vanillin	$C_8H_8O_3$	151.03905	151.03897	0.5	152.04734	121-33-5	18.9	876	92	878
e	Flavored (mint)	(±)-Menthol	$C_{10}H_{20}O$	81.06996	81.06988	1.7	156.15142	15356-70-4	11.8	807	95	808
		D-menthone	$C_{10}H_{18}O$	139.11166	139.11174	0.6	154.13577	1196-31-2	10.1	785	93	843
		(±)-Menthol	$C_{10}H_{20}O$	81.06996	81.06988	1.1	156.15142	15356-70-4	11.4	823	93	824
		DL-menthone	$C_{10}H_{18}O$	112.08820	112.08827	0.6	154.13577	89-80-5	10.4	795	92	851
		Eucalyptol	$C_{10}H_{18}O$	93.07005	93.06988	1.9	154.13577	470-82-6	7.0	783	86	783
f	Flavored (branded)	Propyl pyruvate	$C_6H_{10}O_3$	43.05417	43.05423	1.4	130.06299	20279-43-0	12.7	761	95	904
		cis-Verbenol	$C_{10}H_{16}O$	79.05421	79.05423	0.2	152.12012	18881-04-4	12.2	728	84	728
		Butanoic acid, ethyl ester	$C_6H_{12}O_2$	43.05417	43.05423	1.4	116.08373	105-54	4.6	813	89	814
		Nicotine	$C_{10}H_{14}N_2$	84.08093	84.08078	1.9	162.11570	54-11-5	13.8	872	98	873
g	Flavorless	Nicotine	$C_{10}H_{14}N_2$	84.08093	84.08078	1.9	162.11570	54-11-5	13.8	872	98	873
h	Flavored (lemon)	Nicotine	$C_{10}H_{14}N_2$	84.08093	84.08078	1.9	162.11570	54-11-5	13.8	879	99	880
		Piperonal	$C_8H_6O_3$	149.02333	149.0233	0.1	150.03169	120-57-0	16.7	880	98	881
		Butanoic acid, ethyl ester	$C_6H_{12}O_2$	43.05412	43.05423	2.4	116.08373	105-54	4.6	882	91	882
i	Flavored (strawberry)	Cinnamic acid, methyl ester, (E)-	$C_{10}H_{10}O_2$	131.04919	131.04914	0.4	162.06808	1754-62-7	15.5	859	97	878
		γ-Decalactone	$C_{10}H_{18}O_2$	85.02843	85.02841	0.3	170.13068	706-14-9	16.0	801	95	807
		Butanoic acid, ethyl ester	$C_6H_{12}O_2$	43.05415	43.05423	1.7	116.08373	105-54	4.6	814	86	815
		Ethyl 2-methylbutanoate	$C_7H_{14}O_2$	102.06757	102.06753	0.4	130.09938	7452-79-1	4.8	786	89	809
j	Flavored (lemon)	Photocitral B	$C_{10}H_{16}O$	137.09610	137.09609	0.1	152.12012	6040-45-5	12.7	671	99	921
		cis-Verbenol	$C_{10}H_{16}O$	79.05424	79.05423	0.1	152.12012	1845-30-3	12.2	827	88	828
		cis-Geraniol	$C_{10}H_{18}O$	93.06995	93.06985	0.8	154.13577	106-25-2	13.6	742	79	743
		p-Cymene	$C_{10}H_{14}$	119.08562	119.08553	0.8	134.10955	99-87-6	7.9	862	100	873

Identify and confirm: Molecular ion confirmation using soft ionization

The spectral library match from the EI positive spectrum can be further confirmed by considering the chemical ionization (CI) data with added specificity and selectivity. Figure 6 shows TICs of an e-liquid (sample j, lemon flavor), analyzed using EI, PCI, and NCI.

Considering the peak at RT=12.2 min, the background subtracted mass spectra for mass spectra using EI and PCI are shown in Figure 7, and the NIST library search results from the EI-positive data are displayed in Figure 8, showing the NIST library search results, with the top match identified as *cis*-verbenol.

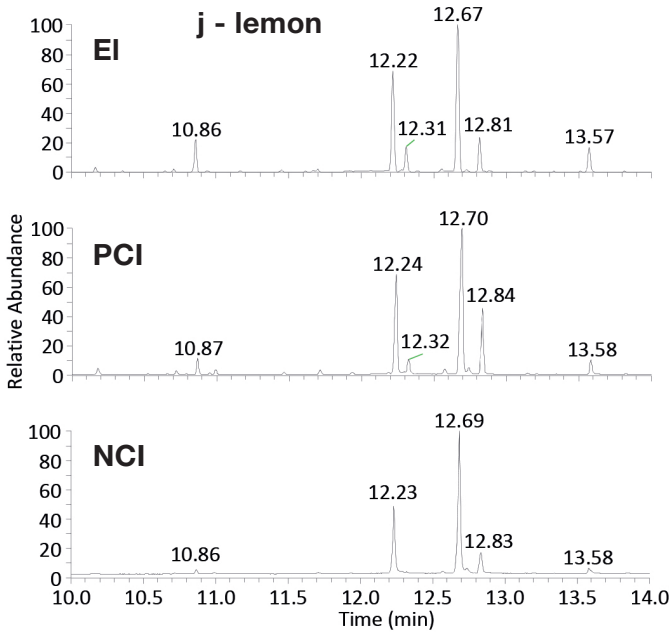

Figure 6. TIC for e-liquid sample j (lemon flavor), analyzed using EI, PCI, and NCI

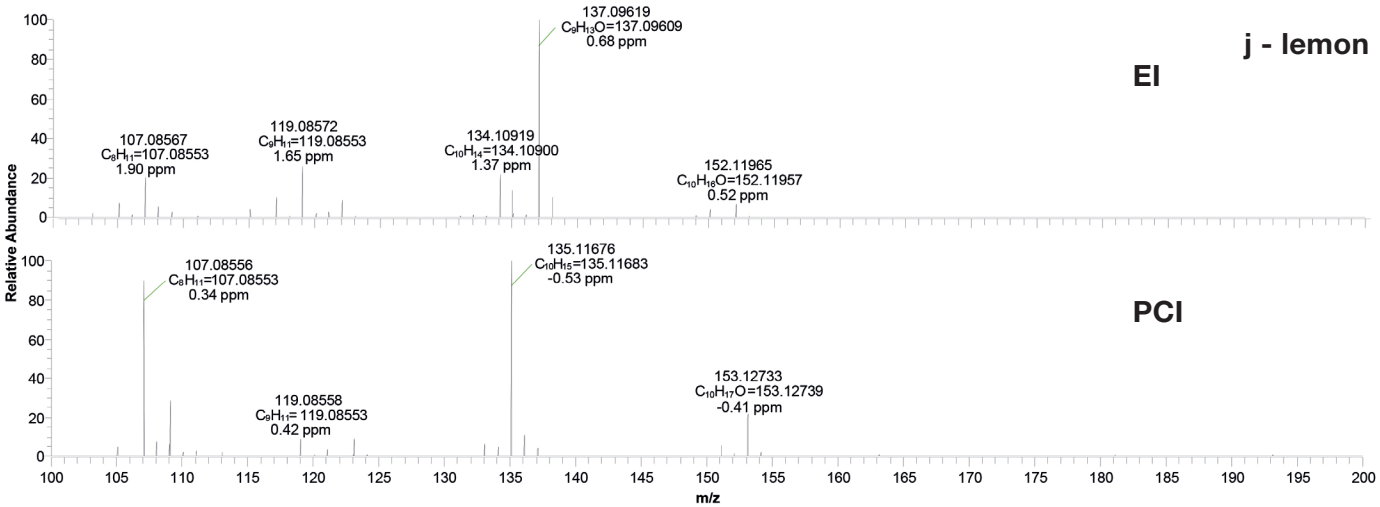

Figure 7. Mass spectra for peak at RT = 12.2 min (as displayed in Figure 6) in the e-liquid sample j, using EI and PCI. With annotation are the measured mass, the elemental composition, and the theoretical mass as well as the mass accuracy (ppm).

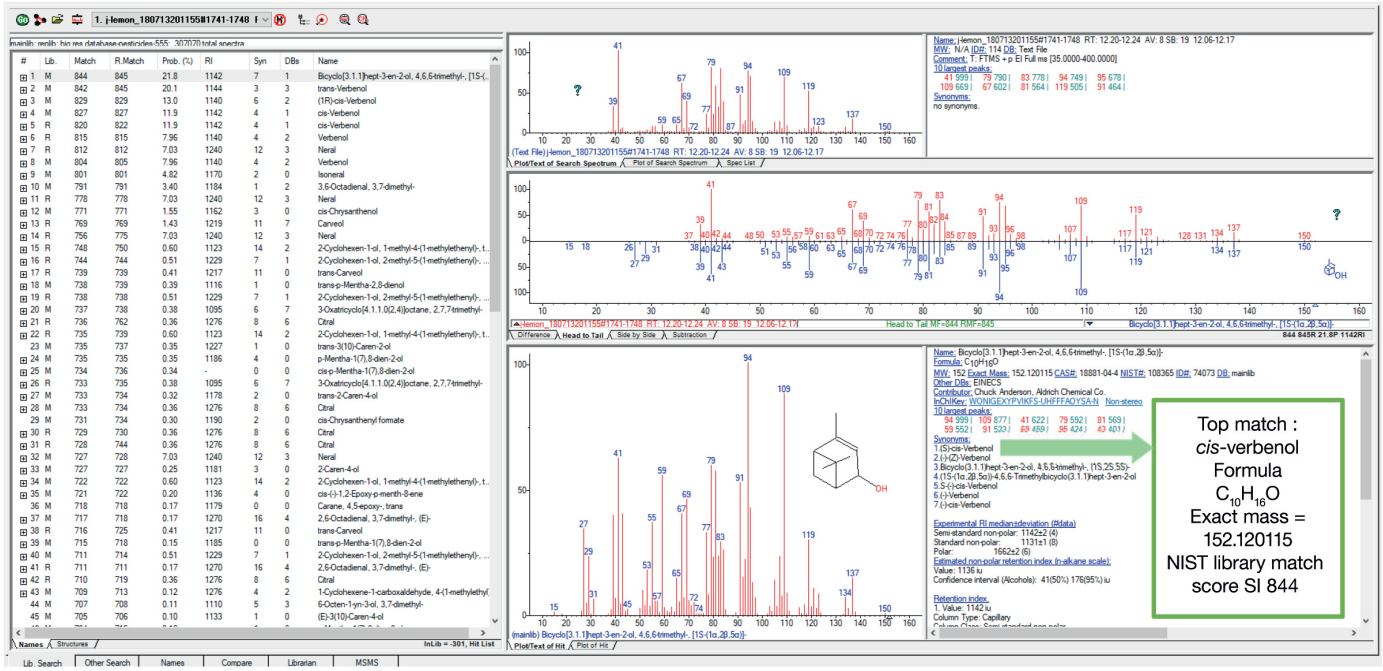

Figure 8. NIST library search results for peak at RT = 12.2 min, identified from the EI-positive data, with the top match identified as *cis*-verbenol

When PCI data is acquired using methane as the reagent gas, three main adducts are typically observed: $[M+H]^+$, $[M+C_2H_5]^+$, and $[M+C_3H_5]^+$. Confirmation of peak at RT = 12.2 min in the e-liquid sample j, as *cis*-verbenol is shown Figure 9, using PCI, where $[M+H]^+$, $[M+C_2H_5]^+$, and $[M+C_3H_5]^+$ are observed in the background subtracted mass spectra.

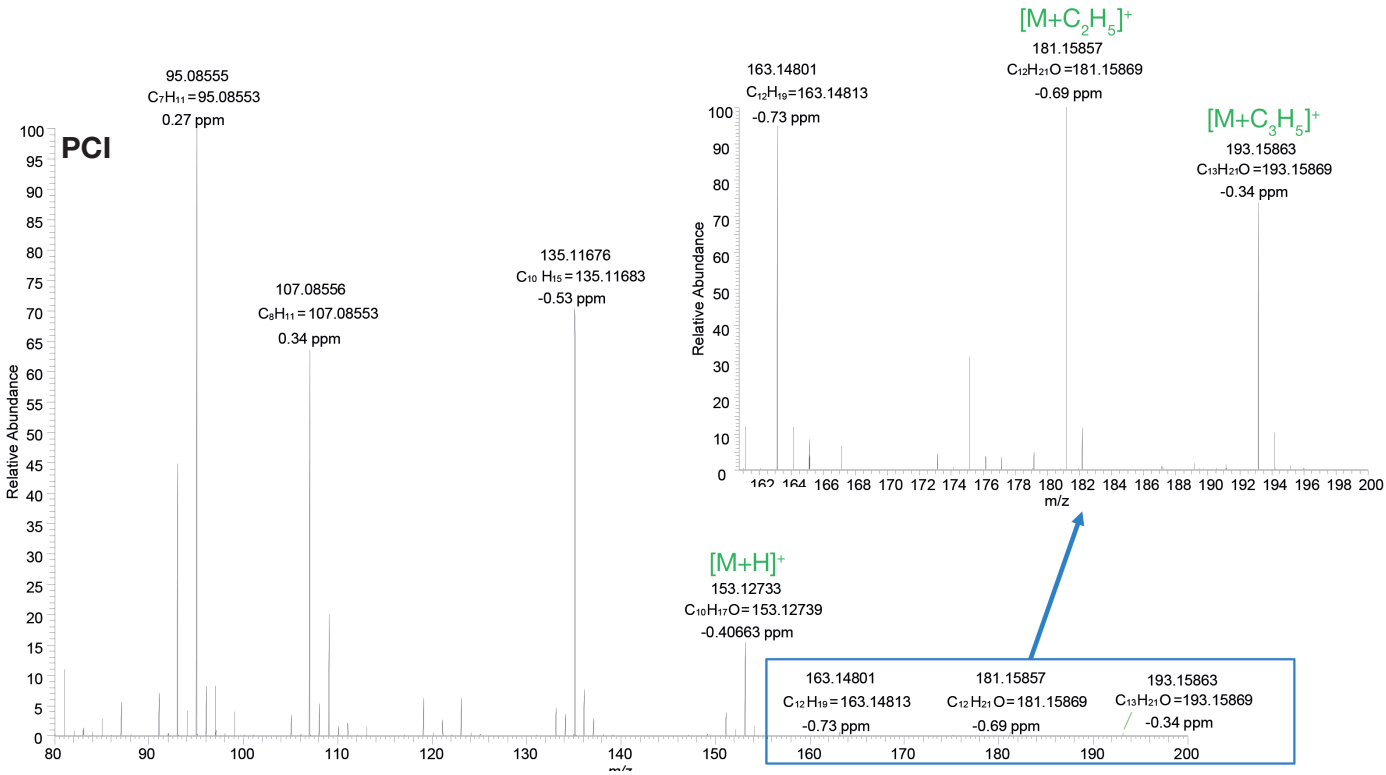

Figure 9. Compound confirmation for the peak at RT = 12.2 min (*cis*-verbenol), illustrating using PCI data. Highlighted are the presence of the protonated molecule $[M+H]^+$, $[M+C_2H_5]^+$, and $[M+C_3H_5]^+$. Annotated are the measured mass, the elemental composition, and the theoretical mass, as well as the mass accuracy (ppm).

Conclusions

- The results of this study demonstrate that using Orbitrap-based GC-MS technology, with unique intuitive software workflows for automated deconvolution and extensive spectral libraries, provides excellent solutions for the analysis of e-liquids.
- Efficient peak detection algorithms with spectral deconvolution and library searching, easily achievable in TraceFinder software, provide confident identification of components in the non-targeted screening of e-liquid samples.
- Additional confidence in compound identification is made in a timely manner (<5 min switchover from EI to CI without venting the system) using chemical ionization, with added specificity and selectivity. When using methane as the reagent gas, positive chemical ionization three main adducts are typical observed, and using the softer negative chemical ionization mode providing predominant product ion information.
- In the absence of chemical standards (often expensive or difficult to purchase) compound confirmation can be made using the in-house developed compound databases and taking advantage of the routine high resolving power (60k) and sub ppm mass accuracy that only the Exactive GC Orbitrap system offers.
- Simplified sample preparation of e-liquid samples using SPME Arrow utilizing the fully automated SPME Arrow workflow is available using the TriPlus RSH autosampler.

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