Qualitative and Quantitative Analysis of Electronic Cigarette Liquids using Gas Chromatography – Orbitrap Mass Spectrometry

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constituents" (HPHCs) in cigarette smoke, cigarette filler, and smokeless tobacco products.[2] Under section 904(a)(3) draft guidance of the Federal Food, Drug and Cosmetic Act (the FD&C Act), a representative subset of 20 HPHCs to be reported by tobacco product manufacturers for combustible products only are detailed.[3] Additionally under section 910 draft guidance of the FD&C Act, 29 HPHCs have been outlined in the Premarket Tobacco Products Applications (PMTA) guidance for Electronic Nicotine Delivery Systems (ENDS).[4]

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.

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). In addition, quantitative analysis of nicotine in e-liquids samples, was performed using split/splitless injection.

MATERIALS AND METHODS

Preparation of samples and standards

E-liquid samples were purchased locally and included both flavored and flavorless samples. Two shortfill samples, 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.

For target and non-targeted qualitative analysis of e-liquids using SPME Arrow sample introduction: each e-liquid sample was first diluted 100 µL 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 ready for SPME Arrow analysis. Sample blanks were also prepared taking 1 mL of water (HPLC grade) in a 20 mL headspace vial.

For the quantitative analysis of nicotine, e-liquid samples were diluted with acetonitrile (LC/MS grade), with internal standard addition prior to analysis. Calibration standards (ranging from 46 to 13,792 ng/mL nicotine) were prepared in acetonitrile, diluting from a certified e-liquid standard acquired from LGC (Teddington, UK).

Test Methods

An Exactive GC Orbitrap GC-MS mass spectrometer coupled with a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph, using a Thermo Scientific™ TriPlus™ RSH™ autosampler and a TG-WAXMS film capillary column were used in all experiments.

For the **qualitative targeted and non-targeted analysis of chemical components** of e-liquids SPME Arrow extraction conditions were optimized, considering fiber choice, incubation, extraction and fiber conditioning temperature and times. GC Orbitrap-MS methods, were developed using electron ionization (EI), and chemical ionization (CI). Additional details of instrument parameters are shown in Table 1.

For the **quantitative analysis of nicotine**, a GC Orbitrap-MS method was developed, using liquid split/splitless sample injection. Additional details of instrument parameters are shown in Table 2.

The mass spectrometer was tuned, air leak checked and calibrated prior to use. The mass spectrometer was tuned and calibrated in <1.5 min using FC43 (CAS 311-89-7) to achieve mass accuracy of <0.5 ppm.

Table 1. Instrument conditions used for qualitative targeted and non-targeted analysis of e-liquid chemical components [A] GC and injector conditions, [B] TriPlus RSH autosampler, and [C] Mass spectrometer conditions.

[A]

TRACE 1310 GC system parameters				
Liner:	1.8 mm ID (P/N: 453A0415)			
Inlet temperature (°C):	230			
Carrier gas, (mL/min):	He, 1.2			
Inlet module and mode:	SSL, split mode			
Split ratio:	100			
Purge flow (mL/min):	5			
Column:	TG-WAXMS 30 m x 0.25 mm I.D x 0.25 µm (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	-	-	-

[B]

Extraction Parameters	
SPME Arrow fiber:	DVB/ Carbon WR / PDMS SPME (P/N: 3656A111)
Vial:	Fiber Depth in Vial (mm): 35
Incubation:	Temperature (°C): 60
	Time (min): 10
	Agitator Speed (rpm): 500
Extraction:	Temperature (°C): 60
	Time (min): 20
	Stirring Speed (rpm): 500
Fiber desorption:	Temperature (°C): 230
	Time (min): 3.0
	Fiber Depth in Injector (mm): 70
Fiber conditioning:	Temperature (°C): 280
	Time - pre desorb (min): 3.0
	Time - post desorb (min): 15

[C]

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		

Table 2. Instruments conditions used for quantitative analysis of e-liquid [A] GC and injector conditions, and [B] mass spectrometer conditions.

[A]

TRACE 1310 GC system parameters				
Injection volume (µL):	1.0			
Liner:	SSL liner 4mm ID, 78.5 mm, Thermo Scientific™ (P/N: 453A1315)			
Inlet (°C):	260			
Carrier gas (mL/min):	He, 1.2			
Inlet module and mode:	SSL, split mode			
Split ratio:	10			
Purge flow (mL/min):	5.0			
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	-	-	-

[B]

Exactive GC Orbitrap mass spectrometer parameters	
Transfer line (°C):	250
Ionization type:	EI
Ion source (°C):	230
Electron energy (eV):	70
Acquisition Modes:	full-scan
Mass range (Da):	75-500
Mass resolution:	60,000 FWHM at m/z 200

Data Analysis

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 workflows. TraceFinder also contains accurate mass spectral deconvolution and spectral matching functionality.

RESULTS

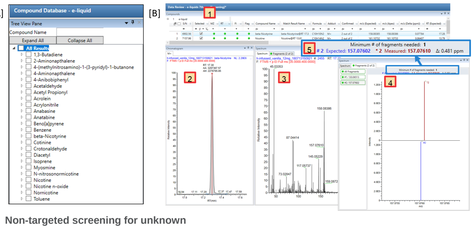
E-liquids were analyzed qualitatively by targeting the subset of the US FDA list of HPHCs.[2,3] Moreoever, an untargeted approach was used to screen the samples for other potential toxic chemicals that may be present.

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, 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 are shown in Figure 1, for a vanilla flavoured e-liquid sample.

Figure 1. [A] E-liquid compound database, [B] target screening results for a vanilla flavored e-liquid sample. 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

The typical workflow used for non-targeted screening is summarized in Figure 2. The full-scan data is first acquired using EI, followed by spectral deconvolution with library matching for putative compound identification. For additional confidence in the identification of components in e-liquids unknowns, a confirmation step using positive and negative chemical Ionization (PCI and NCI) is also mandatory.

Figure 2. 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

Figure 3. Deconvolution result browser for a lemon flavored e-liquid sample, highlighting the identification of *p*-cymene. Including: [**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).

Detect: Electron ionization, full scan

Full scan data (EI) was first acquired.

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.

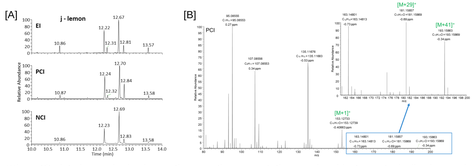
An example of the deconvolution identification results achieved for a lemon flavour e-liquid sample is shown in Figure 3 for *p*-cymene.

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 4[A] shows TICs of an lemon flavoured e-liquid, analyzed using EI, PCI, and NCI.

Considering the peak at RT=12.2 min, the background subtracted mass spectra using EI and PCI are shown in Figure [4B], with the top match in the NIST library search results, from the EI data identified as *cis*-verbenol.

Figure 4. [A] TIC for a lemon flavored e-liquid sample, analyzed using EI, PCI, and NCI, [B] mass spectra for peak at RT = 12.2 min using PCI (annotation are the measured mass, the elemental composition, and the theoretical mass as well as the mass accuracy (ppm)).

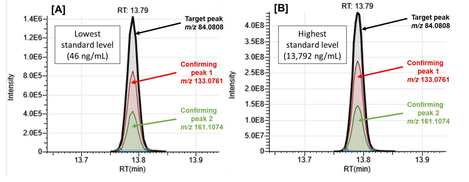


When PCI data is acquired using methane as the reagent, three main adducts are typically observed: $[M+H]^+$, $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$. Confirmation of peak at RT = 12.2 min in the lemon flavored e-liquid sample, when compared against the NIST library search result identified from the EI-positive data as *cis*-verbenol, using PCI where $[M+H]^+$, $[M+C_2H_5]^+$, and $[M+C_3H_5]^+$ are observed in the background subtracted mass spectra (see Figure 4[B]).

Quantitative analysis of e-liquids for nicotine

Using direct liquid injection, various analytical parameters including chromatographic resolution, instrument sensitivity, and linearity were accessed for the quantitative analysis of nicotine in e-liquid samples. The overlaid extracted ion chromatograms (EICs) achieved for target and confirming ions for nicotine over the calibration range are shown in Figure 5.

Figure 5. Overlaid EICs for target and confirming ions for [A] the lowest (46 ng/mL) and [B] the highest (13792 ng/mL) calibration standards in the developed quantification method.



Linearity was assessed using 11 calibration levels ranging from 46 to 13,792 ng/mL (equivalent to 0.046 to 13.79 mg/mL in the prepared e-liquid samples). Excellent linearity was demonstrated for nicotine, with an R^2 value of 0.9991, an average residual %RSD of 4.4. Mass accuracy of <1 ppm was obtained for all ions in the nicotine spectra (from the lowest to the highest standard).

Quantitative targeted analysis for nicotine in ten e-liquid samples, including flavored and flavorless, with declared nicotine levels of 0, 6, or 12 mg/mL, and two shortfill flavored e-liquid samples was performed. Replicate measurements for nicotine containing samples indicated excellent precision with %RSD < 3 achieved.

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, provide confident identification of components in the non-targeted screening of e-liquid samples.
- Additional confidence in compound identification using chemical ionization, with added specificity and selectivity. When using methane as the reagent gas for positive chemical ionization, three main adducts are typically observed, and using the softer negative chemical ionization mode can provide 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 offers.
- Simplified sample preparation of e-liquid samples using SPME Arrow, utilizing the fully automated SPME Arrow workflow available using the TriPlus RSH autosampler.
- In the targeted analysis of nicotine using liquid injection: linearity was demonstrated with R^2 = 0.9991 and residual values RSD% = 4.4%, over 11 calibration levels ranging from 46 to 13792 ng/mL (equivalent to 0.046 to 13.79 mg/mL in the prepared e-liquid samples); mass accuracy of <1 ppm was obtained for all ions in the nicotine spectra (from the lowest to the highest standard).
- Quantitative targeted analysis for nicotine in ten e-liquid samples, including flavored and flavorless, with claimed nicotine levels of 0, 6 or 12 mg/mL, and two shortfill flavored e-liquid samples. Replicate measurements for nicotine containing samples, indicated excellent precision with %RSD < 3 achieved.

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