Non-targeted Analysis using Gas Chromatography Mass Spectrometry to Evaluate Stability of E-vapor Products

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Abstract

In the newly regulated landscape of tobacco products there is an increasing need to fully characterize the chemical composition of aerosol in new products. Although aerosol from e-vapor products are considerably less complex than aerosols from Heat-Not-Burn (HNB) or mainstream smoke from cigarettes, there are still challenges that arise from the chemical composition of the matrix, flavors and other sensory technologies, and the potential for chemical interactions to occur during storage. Additional complexity is associated with efficiently collecting both volatile and semi-volatile compounds delivered in the aerosol from e-vapor products.

Aerosol samples were collected using a 55 mm Cambridge filter pad (CFP) with a trailing impinger containing 10 mL of ethanol chilled to -70 °C, to ensure both volatile and semi volatile compounds are captured. Samples were analyzed on an Agilent GC/MS system (7890B with 5977A) using a Restek Stabilwax® GC column (30 meter x 0.25mm ID x 0.25µm film) with an infused 5 meter integra guard column. Our workflow includes both Agilent MassHunter Unknowns Analysis and AMDIS (Automated Mass Spectral Deconvolution and Identification System) software for identification of extraneous peaks. We have show an automated workflow for data analysis that includes mass spectral deconvolution, peak detection, library searching and reporting. Identification of compounds not present in mass spectral libraries includes secondary analysis using high resolution mass spectrometry on a GC-OrbitrapTM with EI and CI ionization modes, allowing the identification of molecular formulas within 5 ppm of mass accuracy. Compound identification was confirmed through the use of reference standards.

Introduction

Gas Chromatography (GC) is a separation and quantitation method that has been used for the determination of nicotine and nicotine degradants in a variety of matrices. Mass spectrometry of ions generated by electron ionization (EI) has been a key technique for the identification of compound mainly due to the many fragment-rich spectra included in readily searchable libraries. These two orthogonal modes of analysis prove effective for estimating concentration as well as quantitating and identifying chemical components in a complex matrix. There are, however, cases where the above described approach is not able to identify a compound with confidence. In these instances high resolving power accurate mass spectrometry (HRAM) is used to gain further insights into the molecular formula and structure of the unknown chemical constituents. Mass measurements of less than 5 ppm mass accuracy are used to generate possible molecular formulas. However, there are likely many formulas which match the measured mass within the 5 ppm mass tolerance. In these cases chemical rules are applied to the mass spectrum, such as the nitrogen rule and rules associated with relative abundances and presets of fine isotope patterns in the A+1 and A+2 region of the mass spectrum to confirm molecular formula. Following unambiguous formula determination MS/MS experiments of the protonated parent give clues to the structure.

Experimental Method

Instruments

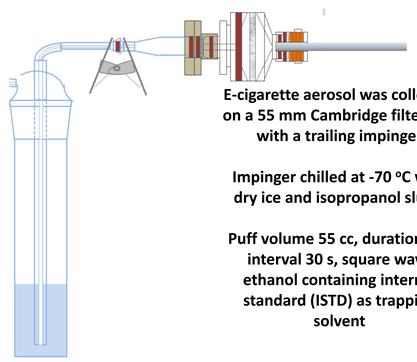
Samples were first analyzed on an Agilent GC/MS single quad system (7890B with 5977A) using a Restek Stabilwax® GC column (30 meter x 0.25mm ID x 0.25µm film) with an infused 5 meter integra guard column.

If needed samples were analyzed on a Thermo Scientific Orbitrap high resolving power MS, operated at 140,000 resolving power at m/z 200 in EI and CI modes.

Data Processing

Our workflow includes both Agilent MassHunter Unknowns Analysis and AMDIS (Automated Mass Spectral Deconvolution and Identification System) software for identification of extraneous peaks. Identification of compounds not present in mass spectral libraries includes secondary analysis HRAM spectrometry on a GC-Orbitrap with EI and CI modes, allowing for the identification of molecular formulas within 5 ppm mass accuracy. Compound identification was confirmed through the use of reference standards.

Aerosol Sample Collection

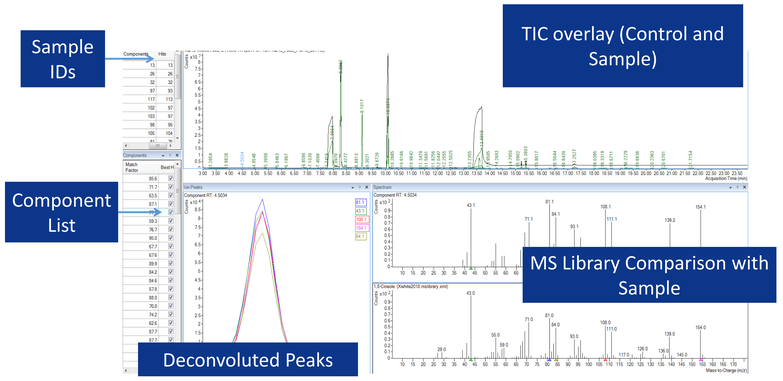


E-cigarette aerosol was collected on a 55 mm Cambridge filter pad with a trailing impinger

Impinger chilled at -70 °C with dry ice and isopropanol slurry

Puff volume 55 cc, duration 5 s, interval 30 s, square wave ethanol containing internal standard (ISTD) as trapping solvent

MassHunter Unknowns Automated Process

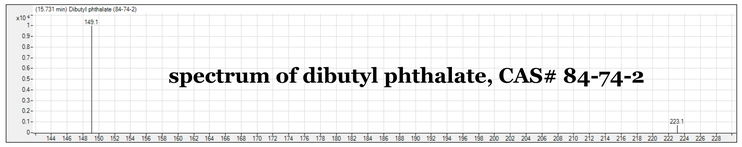


Component table is generated after automatic spectral deconvolution of the peaks and library search. ALCS custom In-house Mass Spectral Library was developed and used for data reprocessing and currently contains > 900 compounds. Identifications are confirmed by mass spectrum match factor, visual inspection of the peak/mass spectrum, and retention time.

Contaminant Identification - Custom Library to ID Compounds

An unknown peak was observed sporadically in aerosol samples and blanks. Upon investigation it was found that the O-rings used in the pad holders contain phthalates. While wiping the pad holders after aerosol collections it is possible for an analyst to contaminate the apparatus.

A certified Phthalate standard mix was analyzed and the ID was confirmed



In addition to unit resolution GC/MS analysis, all unknown confirmations are also being analyzed and verified using High Resolution GC Orbitrap MS.

Non-targeted Workflow

GC-MS analysis (EI)

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Automated data processing by MassHunter unknowns analysis

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Identification & Confirmation

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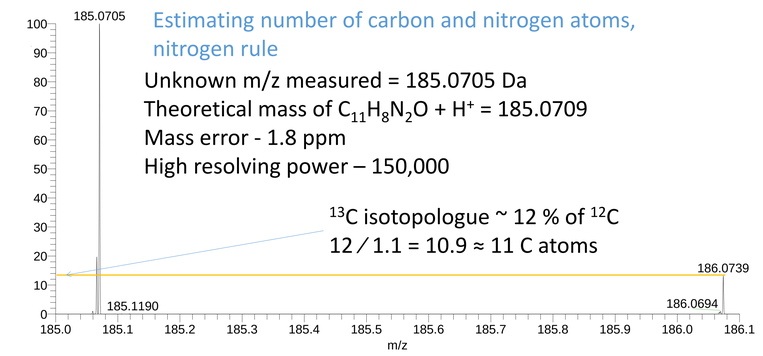
- GC-HRAM Orbitrap analysis (EI and CI)
- Confirmation by reference standard

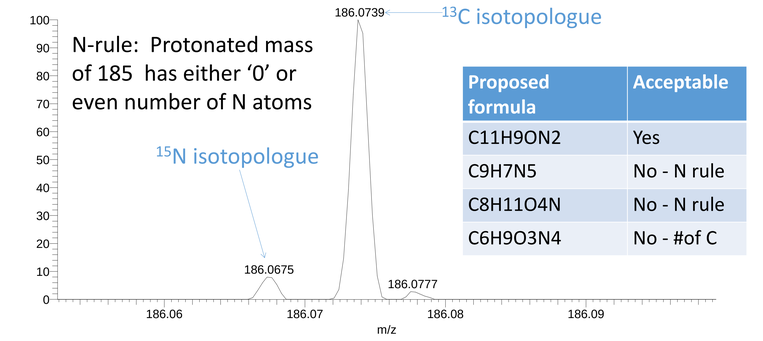
- Extraneous peaks (peaks not found in controls) are deconvoluted
- EI spectral search using NIST and internal custom library
- List of extraneous peaks are generated with identification and match quality
- Semi-quantification of compounds based on ISTD

- HRAM data dependent MS/MS Scan for extraneous peaks
- Fine isotope fingerprint of parent and fragments
- Molecular formula generation based on accurate mass
- TraceFinder or Compound Discoverer used for data analysis

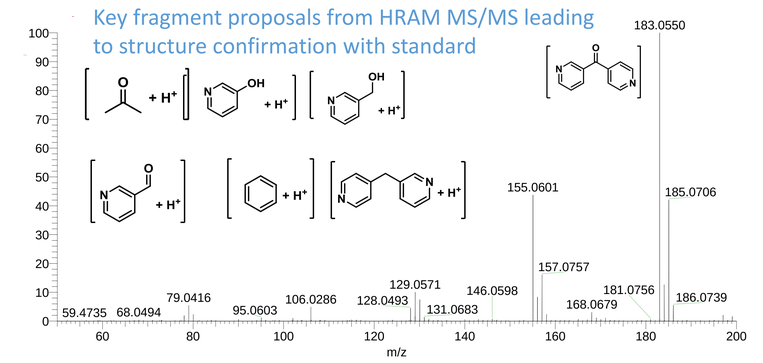
HRAM Identification

An unknown compound was observed and GC-MS single quad data was not sufficient for identification. Additional investigation was done using GC-Orbitrap HRAM spectrometry. Accurate mass and isotopologue information of ^{13}C and ^{15}N were used to obtain correct molecular formula for identification, followed by confirmation with a reference standard.





Proposed formula	Acceptable
C11H9ON2	Yes
C9H7N5	No - N rule
C8H11O4N	No - N rule
C6H9O3N4	No - #of C



Conclusions

An automated workflow was developed that deconvolutes peaks, searches the NIST and in-house custom mass spectral libraries and proposes identification and match quality.

When a compound has several high probability hits in the NIST spectral database or no high probability hit and/or analytical standards are not readily available, evaluating the mass spectrum using instruments with high resolving power becomes a necessary step to the elucidation of the compound identification.

Using reliable predictable parameters, such as exact mass and isotopologues high resolving power mass analysis provides more tools in the identification of unknown chemical compounds. Here we have shown the approach we use to profile e-cigarette aerosols and tools we use for molecular identification of unknowns.