

Transfer Rate Assessment for Eight Selected Flavors in e-Liquid Using GC-Q-TOF-MS and Mass Profiler Professional Software

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The aim of this investigation was to evaluate the performance of a refillable tank e-vapor system under normal usage conditions, with regards to the chemical composition of the e-liquid and the trapped aerosol samples. This study assessed the transfer rate of key flavor constituents from the e-liquid into the aerosol and monitored if any chemical changes occurred within the e-liquid over a period of 10 days.

A GC-Q-TOF-MS instrument was used to provide accurate mass measurement and high resolution information for compound identification. This non-targeted differential screening approach was used as a case study to better evaluate the deconvolution power of MassHunter 'Unknowns Analysis' software as well as the performance of the Mass Profiler Professional chemometric platform.

Materials & Methods

Aerosol Trapping and Sample Preparation

Aerosol generation was conducted using the CORESTA smoking regimen [1] over a period of 10 days. A total of 50 puffs were trapped using 4 devices in parallel (4 aerosol replicates). Each day, devices were used until the e-liquid were at the level of 0.6 mL, at which point e-liquid samples were taken for analysis. Tanks were then refilled with fresh e-liquid to the maximum fill level and the sampling/usage cycle repeated on the following day. The aerosol was collected using an Eagle Cold Trap, cooled with liquid nitrogen to -180°C (Figure 1a). Trapped aerosol and blank aerosol samples were solubilized using DCM/MeOH (80:20; 2 x 5 mL), containing internal standards and retention index markers.

E-liquid samples were weighed and diluted to 16.7 mg/mL with the same solvent used for the solubilization of the aerosol, which enabled a direct comparison of the e-liquid and aerosol composition. The aerosols were analyzed by GC-Q-TOF-MS in positive electron ionization acquisition mode (EI+) (Figure 1b).

GC-HR-MS Analysis

GC-HR-MS method was adapted from K. Lynam [2] to obtain a comprehensive analysis of the volatile and semi-volatile organic compounds, which covers compounds with Linear Retention Indices (LRI) below 1,900. Based upon two retention time prediction models (ACD/ChromGenius and RapidMiner [3]), this method can potentially cover 4,718 of the 9,845 molecules registered in an in-house flavors and tobacco-related compounds database [4].

The analysis was performed using an Agilent 7200A GC/Q-TOF. Analytical conditions are summarized in Table 1. MS raw files were processed using MassHunter Unknowns Analysis (Version B.07.01) and Mass Profiler Professional (MPP; Version 12.6.1, Agilent Technologies).

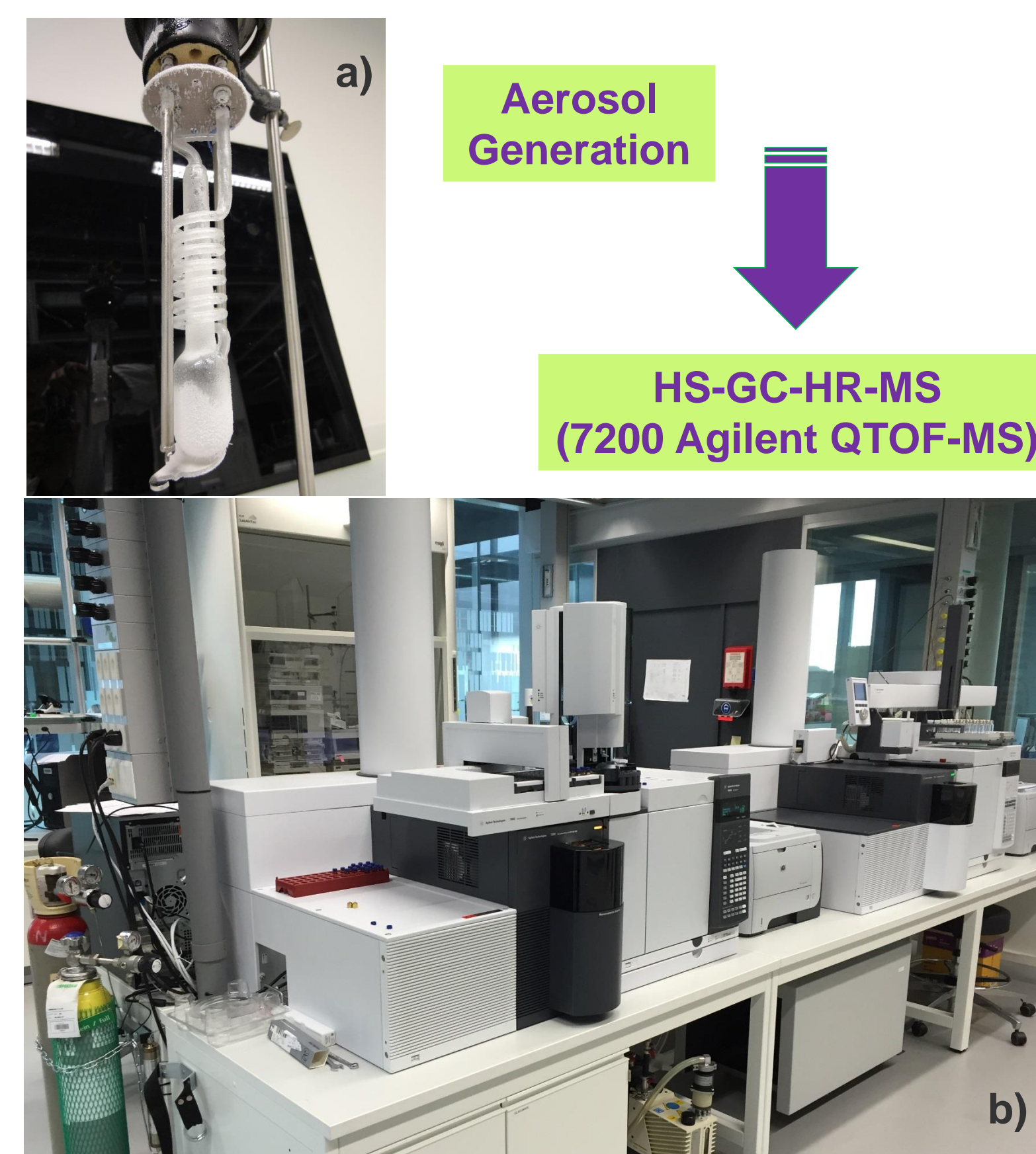


Table 1: Analytical conditions used to monitor volatile and semi-volatile constituents in e-liquid and e-aerosol samples using GC-HR-MS.

GC and MS Conditions	
Column	Agilent J&W DB-624UI (30 m, 0.25 μm , 1.4 μm)
Solvent delay	4.8 min
Injection Volume	1 μL
Multi Mode Inlet	220 $^{\circ}\text{C}$
Split ratio	5:1
Oven	35 $^{\circ}\text{C}$ for 2 min 35 $^{\circ}\text{C}$ to 250 $^{\circ}\text{C}$ ramp 10 $^{\circ}\text{C}/\text{min}$ 250 $^{\circ}\text{C}$ for 3 min
Transfer Line Temperature	260 $^{\circ}\text{C}$
Ionization mode	EI
Source Temperature	230 $^{\circ}\text{C}$
Quadrupole Temperature	150 $^{\circ}\text{C}$
Scan Range	m/z 22 to 500
Emission	35 μA
Ionization Energy	70 eV

Figure 1: Workflow for the analysis of aerosol samples generated with an Eagle Cold Trap (a) and analyzed by GC-HR-MS (b) to monitor volatile and semi-volatile compounds on a DB-624 GC column.

Results

Chromatographic Peak Deconvolution and Mass Spectrum Library Search

Data were deconvoluted using MassHunter Unknowns Analysis software (100 ppm mass extraction and retention time window of 25, 50, 75 and 100). The variable time window increased the number of components and improved the final matching score of the library. EI mass spectra of the deconvoluted peaks were compared to our high resolution mass spectral library matching spectra and experimental LRI values.

After subtracting the compounds present in the solvent, 31 compounds (consisting of key flavors, natural alkaloids and aerosol formers) were unambiguously confirmed with a Match Factor score above 40 and a Delta LRI of maximum ± 5 (Figure 2). Components were exported as .CEF format for further evaluation into Agilent MPP software.

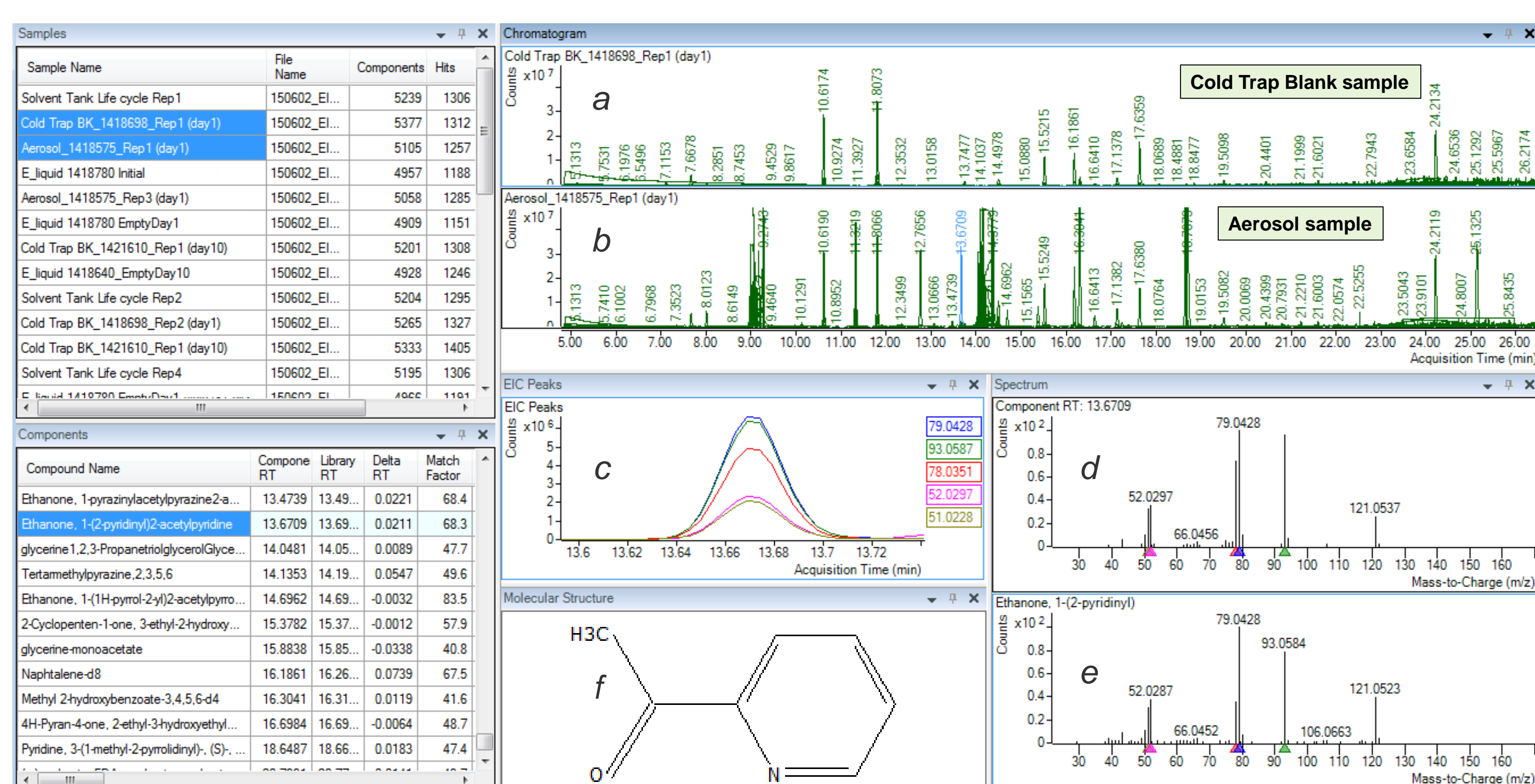


Figure 2: Chromatogram deconvolution and compound identification resulting from MassHunter Unknowns Analysis. Extracted components of blank aerosol (a) and an e-aerosol generated on day 1 (b), overlaid Extracted Ion Chromatograms (EICs) of a component (c), an accurate mass spectrum associated with the component (d) and in-house accurate mass library hit spectrum (e) associated with the molecular structure proposal (f).

Statistical Analysis with Mass Profiler Professional Software

Principal Component Analysis (PCA) was performed on 30 data files.

The clusters of the e-liquid samples (green) indicate a similar composition after 10 refills. All aerosol samples can be attributed to the same cluster (violet), however a slight difference can be observed between the 1st and 10th days.

In total 57,320 components were aligned, although the inconsistent extracted compounds were filtered out. A 1-way ANOVA interpretation (p value < 0.05 and a minimum 2-fold change) was used to determine if any chemical modifications occurred within 10 days.

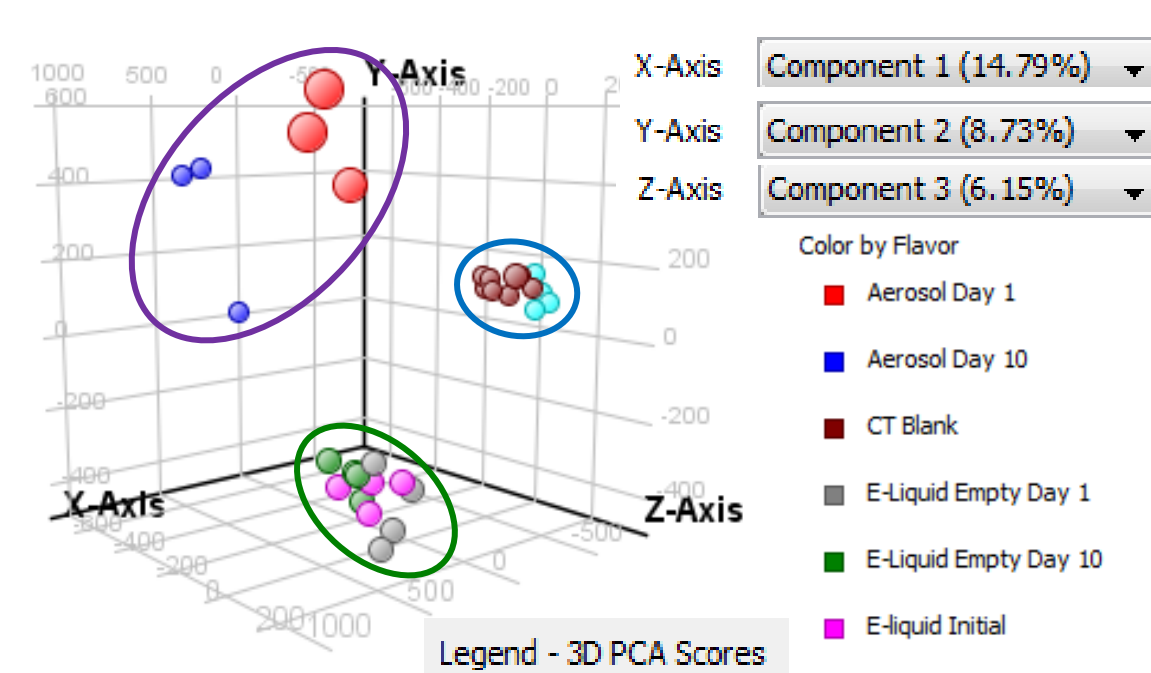


Figure 3: PCA plot of aerosol and e-liquid samples. The replicate of each sample group also cluster very closely. No injection was identified as an outlier.

Eight entities met the statistical analysis criteria. Masses were extracted (with ± 20 ppm) but none of these entities could be confirmed, highlighting problems associated with feature extraction, ions related to column bleed, noise and/or artifacts. Eight flavor compounds were investigated in order to assess transfer rates from e-liquid to aerosol (Figure 4). The concentration of these compounds were similar at each time point and their transfer rates were quantitative.

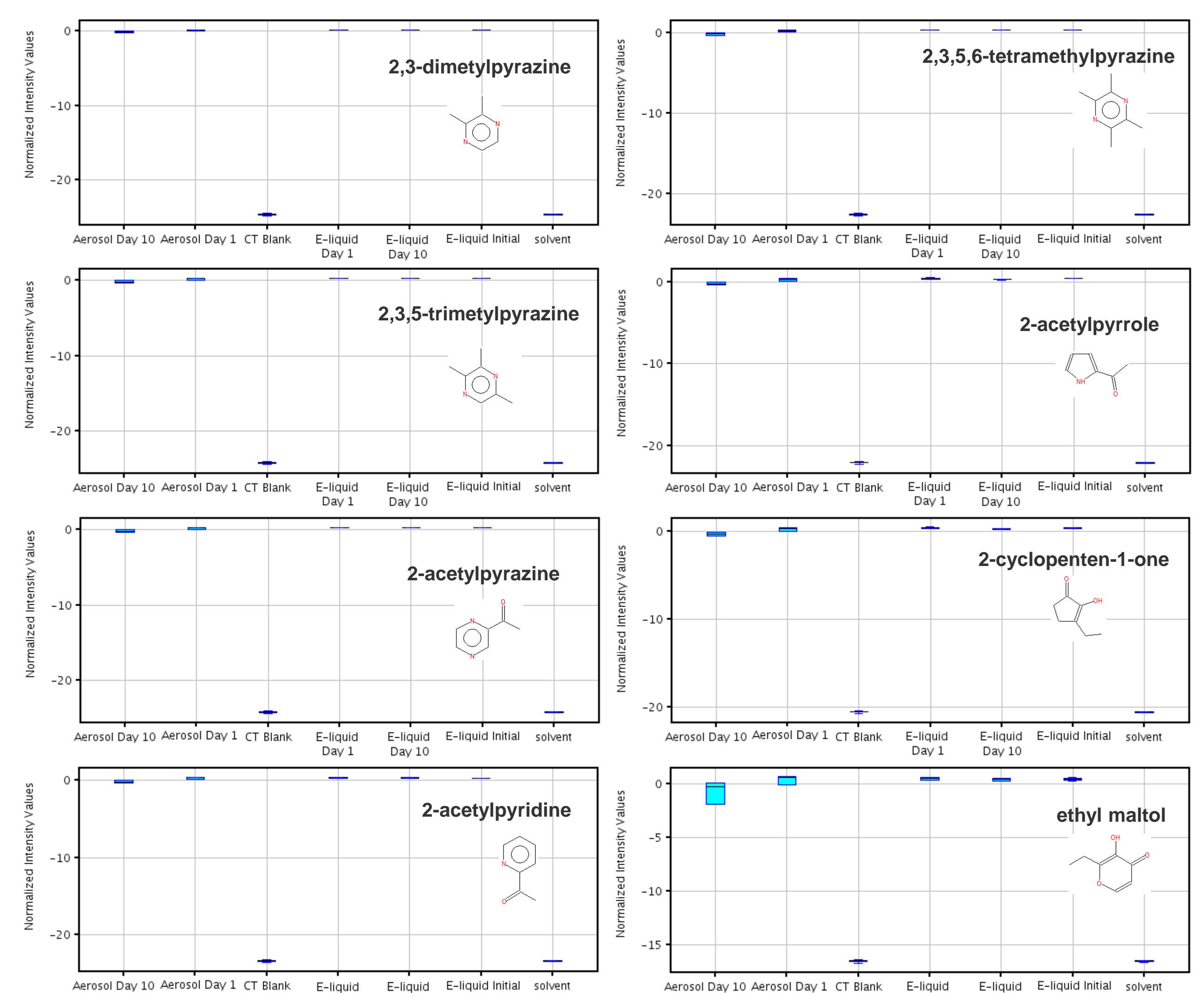


Figure 4: BoxWhisker Plot representation for the eight flavor constituents (normalized intensities).

Conclusion

- No significant difference was observed in e-liquid composition after 10 days
- No significant difference was observed between the aerosol generated on Day 1 and Day 10
- The transfer rate of the monitored flavor compounds was quantitative, although a slight decrease was observed for Day 10
- Results from MassHunter Unknowns Analysis software revealed a large set of compounds, however, some improvements (e.g., feature deconvolution) would facilitate the data evaluation
- The MPP workflow was demonstrated to be a powerful tool for statistical evaluation of large data sets

References

- http://www.coresta.org/Recommended_Methods/CRM_81.pdf
- <http://www.chem.agilent.com/Library/applications/5991-1136EN.pdf>
- "A Gas Chromatography High Resolution Time-of-Flight Mass Spectrometry Method to Characterize Complex Matrix of Aerosol Constituents" P. Guy *et al.*, IMSC 2014, Geneva (Switzerland)
- "Building an R&D chemical registration system" E. Martin *et al.* *J. Chemoinformatics* 2012, 4, 1, 1-14

