Identification of cooling agents in aerosols of an E-cigarette from unit mass resolution spectra enhanced to high mass accuracy

Serban Moldoveanu; Karen Kilby

R.J. Reynolds Tobacco Co., Winston-Salem, NC

Overview

The aerosols from a common commercial mentholated electronic cigarette (E-cigarette) were found to contain mainly glycerin, propylene glycol (PG), nicotine, and menthol. In addition to these compounds, five well defined chromatographic peaks were not possible to assign based on their mass spectra. The molecular formulas for the compounds generating these peaks were obtained with the help of a computer package that provides high mass accuracy from the data acquired on a conventional mass spectrometer of unit mass resolution (MassWorks 3.0.0.0, Cerno Bioscience LLC). The resulting molecular formulas were searched against a database of GRAS flavors (Flavor-Base 2004, Leffingwell & Associates). This allowed the identification in the aerosols of N-ethyl-*p*-menthane-3-carboxamide, menthone glycerol ketal (two isomers), L-menthyl lactate, and 2-sec-butylcyclohexanone. The spectra of these compounds were further confirmed using MassFrontier 7.0 software (Thermo Scientific).

Introduction

Electronic cigarettes (E-cigarettes) are battery-powered devices which simulate tobacco smoking. They use a heating element that vaporizes a liquid solution. The aerosols from a common commercial mentholated E-cigarette were analyzed, and some compounds had mass spectra not available in common mass spectral libraries. These compounds needed to be identified.

Method

1.The aerosols from the E-cigarette were collected on 44 mm Cambridge pads with a linear smoking machine (Cerulean SM 450). The pads were extracted with methanol.

2.The samples were analyzed on a 6890/5975 GC/MS (Agilent) equipped with a DB-WAXetr column 30m x 0.32mm with 0.5mm film.

3.Perfluorotributylamine (PFTBA) was automatically infused at the end of the chromatograms during data acquisition and used to generate correction factors for accurate mass. Mass spectra were collected as “raw scan” and with the threshold set at “zero” counts.

4.The spectra of unknowns were corrected to 0.01 Da mass precision using MassWorks package and an elemental composition was generated for unknowns.

5.The molecular formulas were searched against a GRAS flavor database (Flavor Base 2004) to identify the potential compounds

6.The spectrum of each compound was further evaluated using MassFrontier 7.0 software for validation.

Results

1. The total ion chromatogram for the aerosol sample is shown in Figure 1.

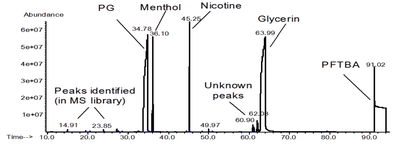


Figure 1. Total ion chromatogram of the sample, plus PFTBA.

The chromatogram shows the main peaks, and the unknown peaks.

2.Example of average area selected for generating the spectrum of one unknown peak is shown in Figure 2.

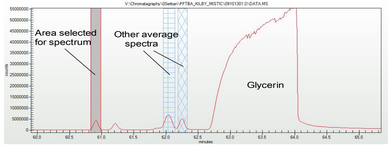


Figure 2. Window of the chromatogram showing the area selected for generating an average spectrum (from MassWorks 3.0.0.0.).

3.Average spectrum of the selected peak is shown in Figure 3.

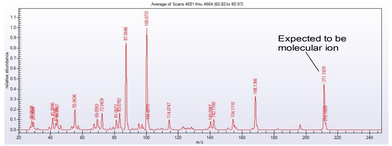


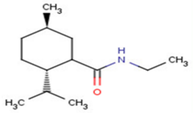
Figure 3. Average spectrum for the peak at 60.9 minutes (from MassWorks 3.0.0.0.).

4. The results of molecular formulas resulting from MassWorks are shown in Table 1.

Table 1. Molecular formulas from MassWorks for the peak eluting at 60.9 min.

Row	Formula	Mono Isotope	Mass Error (mDa)	Mass Error (ppm)	Spectral Accuracy
1	C13H25ON	211.1936	0.7144	3.3828	98.3374
2	C10H27O4	211.1909	-1.9656	-9.3073	96.8036
3	C9H27O3N2	211.2022	9.2677	43.8828	96.61
4	C6H29O6N	211.1995	6.5877	31.1927	93.7021
5	C5H27O6N2	211.1869	-5.9884	-28.3551	93.1122
6	C3H31O9	211.1968	3.9076	18.5025	90.683
7	C2H29O9N	211.1842	-8.6685	-41.0452	90.0863

5. Flavor-Base 2004 indicated that formula C13H25NO (first hit) corresponds to N-ethyl-*p*-menthane-3-carboxamide (CAS# 39711-79-0, FEMA 3455), which is a non-menthol compound producing a cooling sensation at 200 ppb.



6. The spectrum of peak eluting at 60.9 min is in agreement with the structure of N-ethyl-*p*-menthane-3-carboxamide. Various fragmentations predicted by MassFrontier 7.0. are shown in Figure 4.

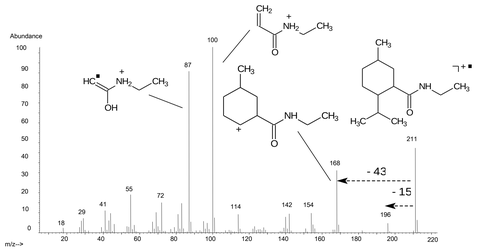
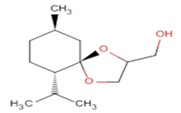
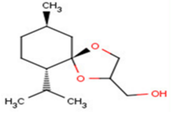


Figure 4. Mass spectrum assigned to N-ethyl-*p*-menthane-3-carboxamide, and fragmentations predicted with MassFrontier 7.0.

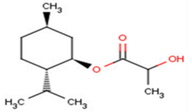
7. More molecules identified by this procedure:



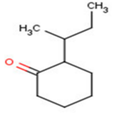
Menthone glycerol ketal (1)



Menthone glycerol ketal (2)



L-Menthyl lactate



2-Butan-2-ylcyclohexan-1-on

Menthone glycerol ketals (CAS# 67785-70-0, FEMA 3808 and CAS# 63187-91-7, FEMA 3807) provide trigeminal cooling similar to menthol, L-menthyl lactate (CAS# 59259-38-0, FEMA 3748) has no odor but provides lingering cooling menthol sensation, 2-butan-2-ylcyclohexan-1-one (CAS# 14765-30-1, FEMA 3261) has a strong minty taste.

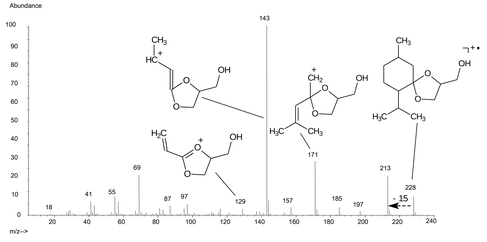


Figure 5. Mass spectrum assigned to menthone glycerol ketal, and fragmentations predicted with MassFrontier 7.0.

Conclusions

1. High mass accuracy can be generated from the data acquired on a conventional mass spectrometer of unit mass resolution (with MassWorks program from Cerno Bioscience LLC).

2.With high mass accuracy information, the correct molecular formulas can be generated for an unknown compound and corroborated with the information on the sample, the molecular structure can be determined.