1910 W. Saint Andrews Road ▪ Midland, MI 48640 ▪ USA
Phone: (989) 832-5555 ▪ Fax: (989) 832-5636 ▪ www.impactanalytical.com
A Michigan Molecular Institute Center of Excellence

Customer:	Cary Lee	**Report Number*:**	R11-0353
Company:	A Clean Cigarette	**Date Submitted:**	5/31/2011
Address:		**Report Date:**	6/14/2011
Databook #:	460 (ALP) 429 (LJK)	**Analyst(s):**	A. Payeur, L. Koski, M. Murphy
Samples:	S11-0923	**QC: (Initial/Date)**	KAR 6/15/2011

Analysis of E- Cigarette Fluid

Summary

Cary Lee of A Clean Cigarette submitted a sample of Normal High electronic cigarette fluid for analysis by gas chromatography-mass spectrometry (GC-MS) and inductively coupled plasma-optical emission spectroscopy (ICP-OES). The goal of this project was to quantitate the amount of nicotine, propylene glycol, and cotinine (if necessary) in the fluid, as well to determine the presence or absence of additional listed ingredients (ethanol, glycerol, acetylpyrazine, guaiacol, myosmine, vanillin). The sample was analyzed by ICP-OES to screen for unlisted inorganic materials such as lead and cadmium.

The Normal High electronic cigarette fluid (S11-0923) was found to contain 17.33 mg/mL nicotine by GC-MS and 95.5 % propylene glycol by GC-FID. Cotinine was not found in a high enough concentration to warrant quantitation. Glycerol, myosmine and vanillin were also detected by GC-MS.

The ICP-OES was utilized to analyze S11-0923 for elemental composition after the sample was microwave digested. Sulfur was detected at 2.05 µg/g of sample and Silicon was detected at 2.28 µg/g of sample. No other elements were detected above their individual limits of detection.

Experimental

Sample Identification

Impact Analytical ID	*Customer ID*
S11-0923	Normal High

GC-MS

The Normal High electronic cigarette fluid (S11-0923) was diluted 1:1 and 1:100 with methanol for analysis by gas chromatography mass spectrometry. The sample diluted 1:100 was used to quantitate nicotine in the sample. Nicotine standards were also prepared in methanol ranging in concentration from 0.10 to 0.25 mg/mL nicotine. A calibration curve was prepared from these standards.

All samples and standards were analyzed under the following chromatographic conditions. The gas chromatographic was operated using Impact Analytical SOP-MOL-013 as a guideline and peak identifications were made using the NIST 05 mass spectral database.

GC Method P11-034.M
Capillary Column: ZB-50MS, 30 m x 0.25 mm x 0.25 μm
Inlet: 250 °C
Carrier Gas (He) Flow: Constant Flow at 1 mL/min
Injection: 1 μL split injection, 50:1 split ratio
Temperature Program: 50 °C (2 min hold) increased at 10 °C/min to 320 °C (10 min hold)
Transfer Line: 300 °C
Detector: Agilent 5973 MSD at 230 °C, scan range m/z 50-700

GC-FID

The Normal High fluid (S11-0923) diluted 1:100 in methanol was also run by GC-FID under the following chromatographic conditions. Impact Analytical SOP-SEP-001 was used as a guideline for the GC-FID work.

GC Method P11-034.M
Capillary Column: ZB-50MS, 30 m x 0.25 mm x 0.25 μm
Inlet: 250 °C
Carrier Gas (He) Flow: Constant Flow at 1 mL/min
Injection: 1 μL split injection, 50:1 split ratio
Temperature Program: 50 °C (2 min hold) increased at 10 °C/min to 320 °C (10 min hold)
Detector: Flame ionization detector at 350 °C
H_2 flow: 30 mL/min
Air flow: 300 mL/min
Make up gas: 30 mL/min

ICP-OES

Sample was digested to eliminate organic interferents using the conditions outlined below:

Digestion Program Name:	Easy Oil
Number of Vessels:	4
Maximum Applied Energy:	800W
Maximum Temperature:	200˚C
Ramp Time:	20 minutes
Hold Time:	30 minutes
Cool Down Time:	30 minutes

Samples were analyzed by ICP-OES using the instrument conditions outlined below:

Instrument:	Thermo Scientific 6300 Series Duo
Method:	P11-0341 HW and P11-0341 LW
Energy Level:	1150 W
Nebulizer Gas Flow:	0.75 L/minute
Pump Rate:	25 rpm
Sample Aspiration Rate:	3.0 mL/minute

The method utilized scans through the emission spectrum for all elements detected by ICP-OES. Elements present emit emission lines which are then used to qualitatively identify elements. A semi-quantitative estimate of sample concentration is also provided for those elements.

Results and Discussion

GC-MS

An expanded GC-MS total ion chromatogram of the Normal High cigarette fluid (S11-0923) is shown in Figure 1. In this sample nicotine was identified at a retention time of approximately 13.668 minutes. The peaks eluting between 8 and 10 minutes were identified as propylene glycol, dipropylene glycol and similar isomers. Absolute quantitation of these compounds would require additional method development. However, semi-quantitation was performed and is provided below (see GC-FID results). The concentration of nicotine in the High Normal cigarette fluid (S11-0923) was calculated to be 17.33 mg/mL using the calibration curve shown in Figure 2.

Additional ingredients expected to be present in the High Normal cigarette fluid (S11-0923) include ethanol, glycerol, acetylpyrazine, guaiacol, myosmine, cotinine and vanillin. None of these ingredients were detected in the 1:100 dilution of the sample but some were detectable in the 1:1 dilution. Ethanol was not detected in either sample because the molecular weight is below the m/z 50 threshold set in the mass spectrometer settings. An expanded GC-MS total ion chromatogram of the High Normal cigarette fluid (S11-0923) 1:1 dilution is shown in Figure 3. In this chromatogram both glycerol and pyridine, or another similar analyte, are identified. Additional peaks present in this chromatogram were either related to propylene glycol, a fatty acid or were not identifiable.

Acetylpyrazine and guaiacol were not detected in this experiment. Expanded, extract ion chromatograms for myosmine, cotinine and vanillin are shown in Figures 4 through 6. Due to the low abundance of cotinine it was determined that quantitation was not necessary at this time.

GC-FID

The chromatogram obtained for Normal High cigarette fluid (S11-0923) 1:100 dilution in methanol by GC-FID is shown in Figure 7. The methanol solvent peak is at 2.394 minutes, nicotine is at 14.837 minutes, and earlier eluting peaks (around 10 minutes) are related to propylene glycol. Using FID area percent, it was determined that the Normal High cigarette fluid (S11-0923) is approximately 95.5 % propylene glycol and dipropylene glycol isomers.

ICP-OES

The digestion method utilized resulted in a clear, colorless sample without the presence of solids or suspensions. Sample size is determined by the microwave digesters which in turn determines the method detection limit. In order to minimize the method detection limit, the digestion was run on one aliquot and then a second aliquot added and digested a second time. If lower ethod detection limits are required in the future, this scheme could be expanded to increase the overall sample size.

Table I lists all of the elements that were investigated by this analysis and the resulting method detection limits. Only Sulfur and silicon were detected.

Table I. ICP-OES Detailed Results for Normal High Electronic Cigarette Fluid (S11-0923)

Atomic Number	Element	Analysis Results	Method Detection Limit		
47	Ag	Not Detected	<	1.138	µg/ g
13	Al	Not Detected	<	0.015	µg/ g
33	As	Not Detected	<	4.551	µg/ g
79	Au	Not Detected	<	1.365	µg/ g
5	B	Not Detected	<	1.138	µg/ g
56	Ba	Not Detected	<	1.138	µg/ g
4	Be	Not Detected	<	1.138	µg/ g
83	Bi	Not Detected	<	4.551	µg/ g
20	Ca	Not Detected	<	1.138	µg/ g
48	Cd	Not Detected	<	1.138	µg/ g
27	Co	Not Detected	<	1.138	µg/ g
24	Cr	Not Detected	<	0.910	µg/ g
29	Cu	Not Detected	<	1.138	µg/ g
26	Fe	Not Detected	<	1.138	µg/ g
32	Ge	Not Detected	<	22.753	µg/ g
72	Hf	Not Detected	<	9.101	µg/ g
80	Hg	Not Detected	<	2.730	µg/ g
53	I	Not Detected	<	227.531	µg/ g
49	In	Not Detected	<	40.956	µg/ g
77	Ir	Not Detected	<	9.101	µg/ g
19	K	Not Detected	<	1.138	µg/ g
3	Li	Not Detected	<	2.275	µg/ g
12	Mg	Not Detected	<	1.138	µg/ g
25	Mn	Not Detected	<	1.138	µg/ g

Atomic Number	Element	Analysis Results	Method Detection Limit		
42	Mo	Not Detected	<	1.138	µg/ g
11	Na	Not Detected	<	1.138	µg/ g
28	Ni	Not Detected	<	0.910	µg/ g
76	Os	Not Detected	<	11.377	µg/ g
15	P	Not Detected	<	0.130	µg/ g
82	Pb	Not Detected	<	2.275	µg/ g
46	Pd	Not Detected	<	4.551	µg/ g
78	Pt	Not Detected	<	45.506	µg/ g
45	Rh	Not Detected	<	11.377	µg/ g
16	**S**	**Detected**	**>**	**2.050**	**µg/ g**
51	Sb	Not Detected	<	4.551	µg/ g
21	Sc	Not Detected	<	1.138	µg/ g
34	Se	Not Detected	<	11.377	µg/ g
14	**Si**	**Detected**	**>**	**2.280**	**µg/ g**
50	Sn	Not Detected	<	1.138	µg/ g
38	Sr	Not Detected	<	1.138	µg/ g
73	Ta	Not Detected	<	20.478	µg/ g
52	Te	Not Detected	<	68.259	µg/ g
22	Ti	Not Detected	<	1.138	µg/ g
81	Tl	Not Detected	<	6.826	µg/ g
74	W	Not Detected	<	45.506	µg/ g
70	Yb	Not Detected	<	1.138	µg/ g
30	Zn	Not Detected	<	1.820	µg/ g
40	Zr	Not Detected	<	1.820	µg/ g

Qualitative Analysis conducted on a Thermo-Scientific iCAP 6300 Duo Inductively Coupled Plasma. The insturment method used was "Qualitative Analysis HW" and "Qualitative Analysis LW" with standard operating conditions.

Those elements ***shaded*** in the table above were found to be present in the sample analyzed. No attempt was made to quantitatively analyze this sample. Results presented for these elements are semi-quantitative estimates.

The **Method Detection Limit** indicated is the Instrument Detection Limit adjusted for sample preparation weights and volumes. Those elements NOT found may be present but at concentrations less then the instrument detection limit adjusted for the method weights and volumes.

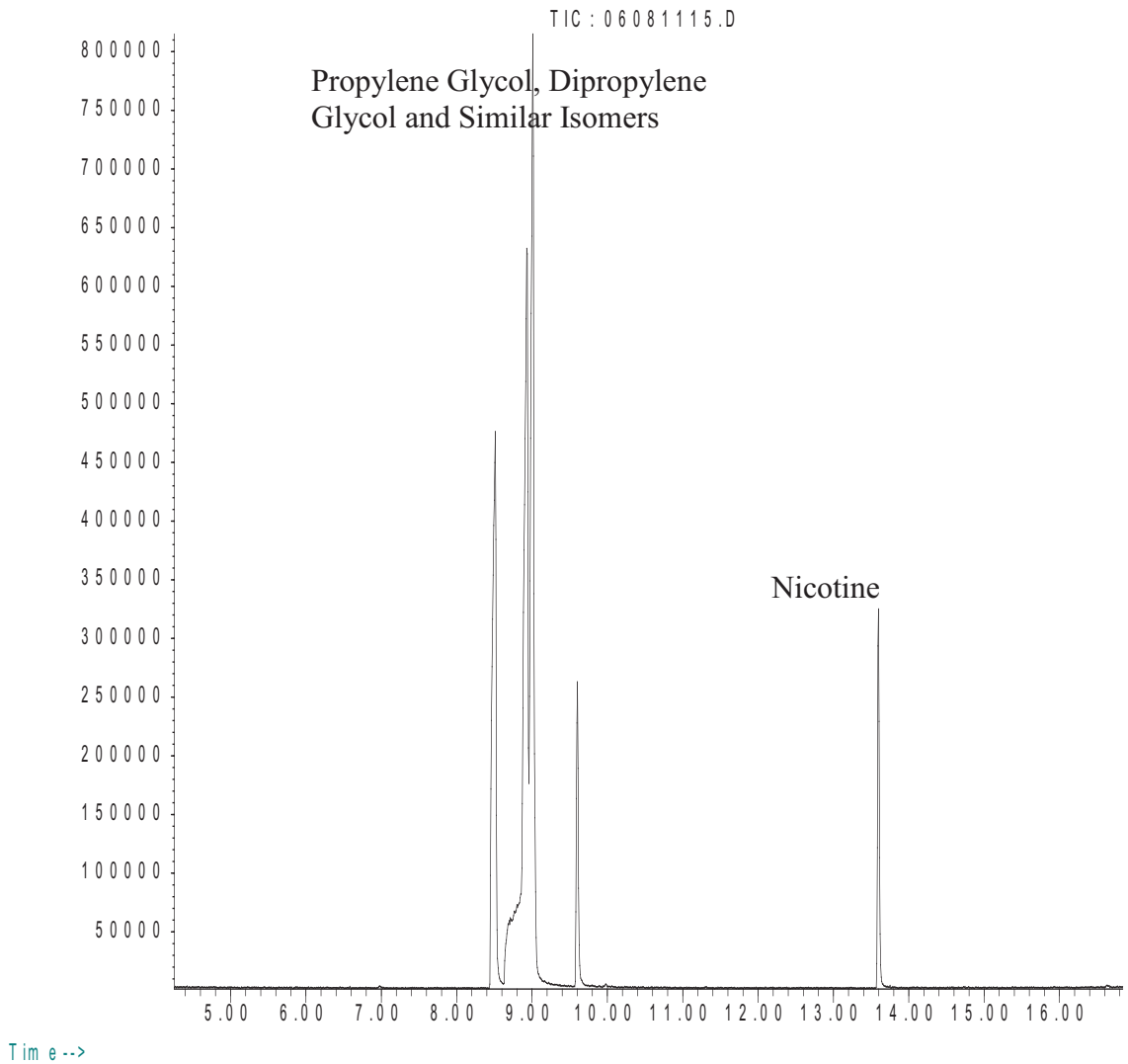

Figure 1. Expanded GC-MS total ion chromatogram for the Normal High cigarette fluid (S11-0923) diluted 1:100 with methanol.

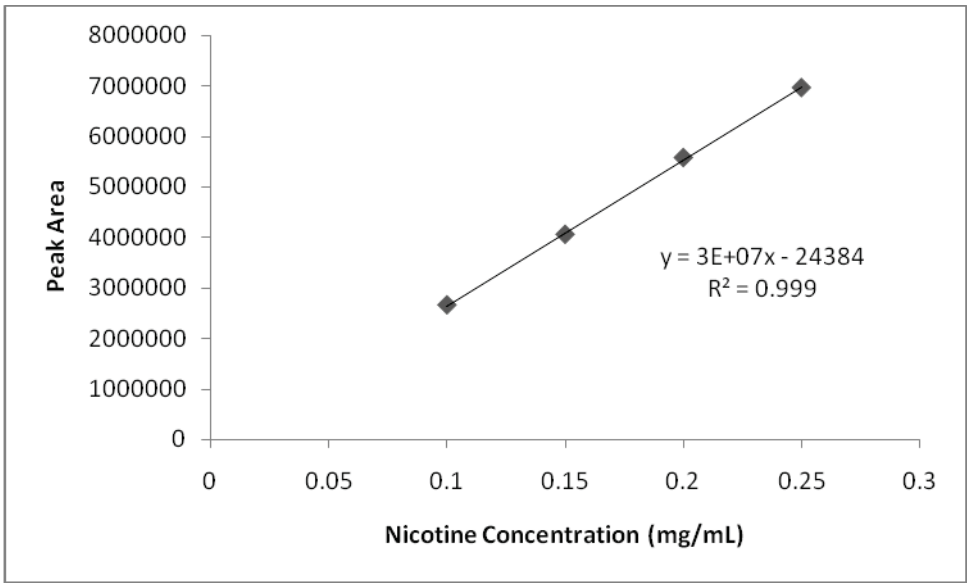

Figure 2. Calibration curve generated for nicotine standards preparred in methanol.

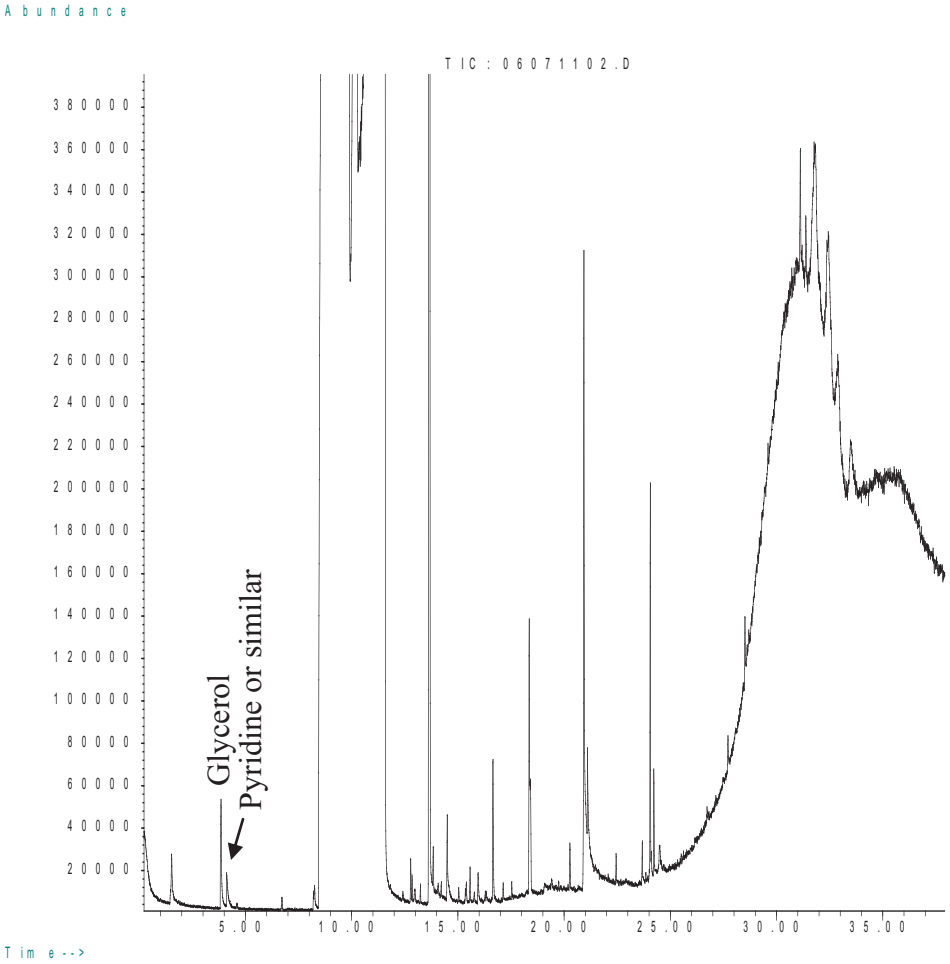

Figure 3. Expanded GC-MS total ion chromatogram of the High Normal cigarette fluid (S11-0923) 1:1 dilution showing the presence of glycerol and pyridine or a similar analyte.

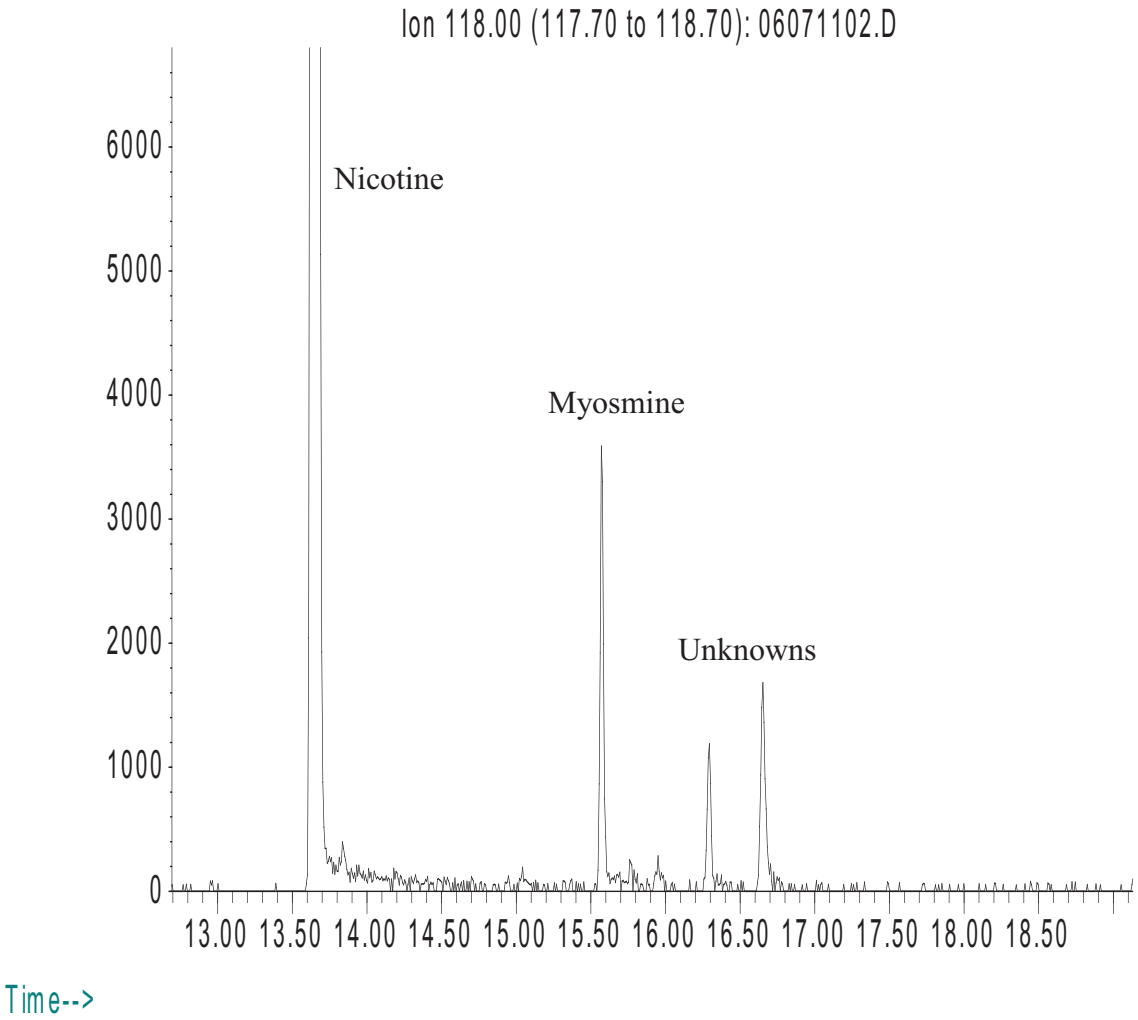

Figure 4. Expanded, extracted ion chromatogram of Normal High cigarette fluid (S11-0923) 1:1 dilution at m/z 118 showing detection of myosmine.

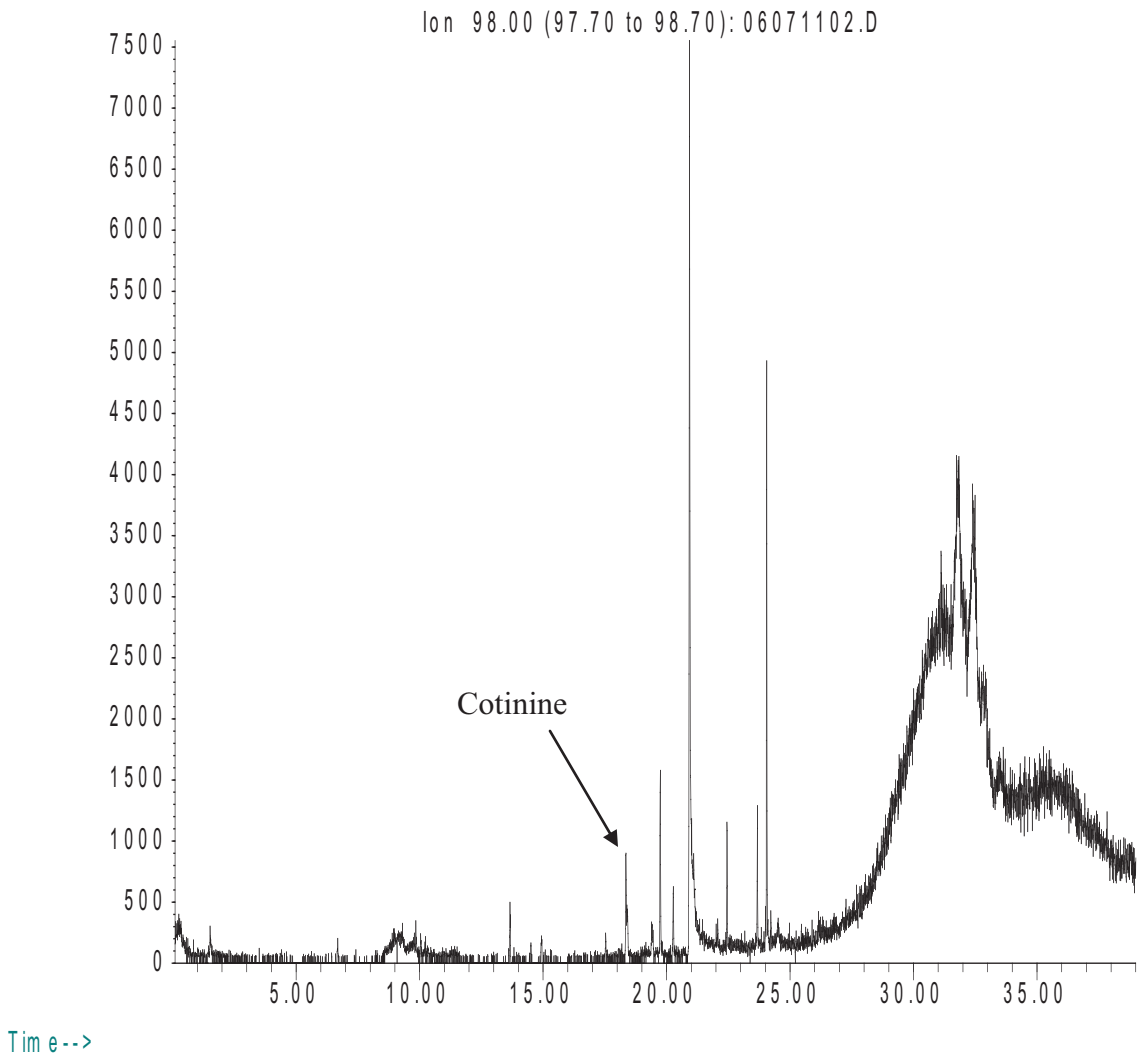

Figure 5. Expanded, extracted ion chromatogram, for High Normal cigarette fluid (S11-0923) 1:1 dilution showing the detection of cotinine at m/z 98. Other peaks present in the chromatogram were either unknowns or similar to long chains fatty acids.

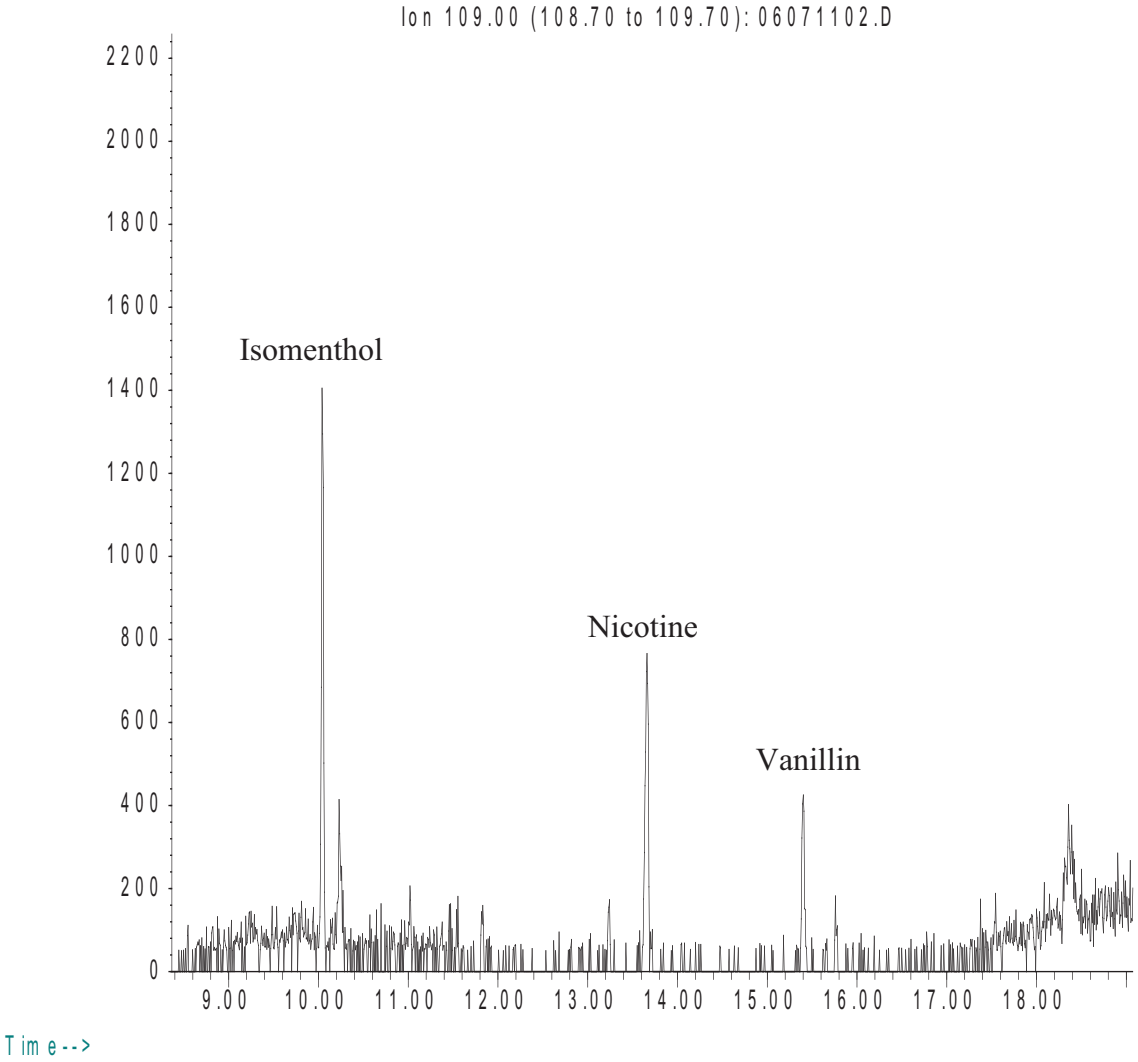

Figure 6. Expanded, extracted ion chromatogram, for High Normal cigarette fluid (S11-0923) 1:1 dilution showing the detection of isomenthol, nicotine and vanillin at m/z 109.

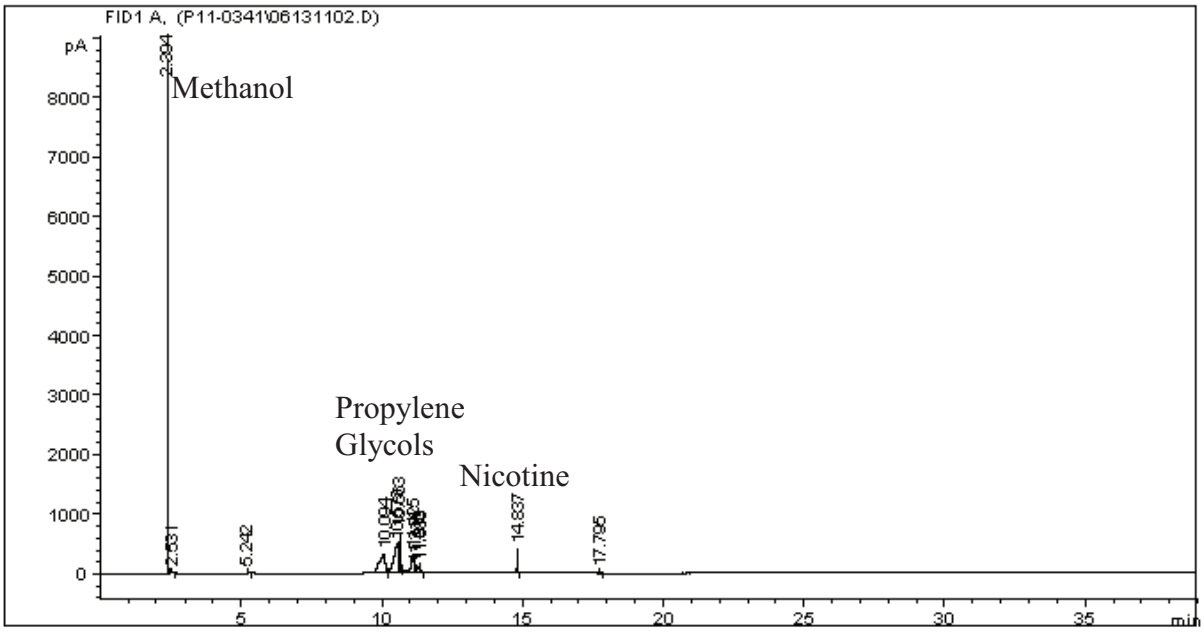

Figure 7. GC-FID chromatogram obtained for High Normal cigarette fluid (S11-0923) diluted 1:1 in methanol. The methanol solvent peak is at 2.394 minutes, nicotine is at 14.837 minutes and earlier eluting peaks (around 10 minutes) are related to propylene glycol.