MATERIALS CHARACTERIZATION REPORT

Report No.: 0706.04 **Date:** June 28, 2007

Customer:
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Customer P.O.: C.O.D.

Samples: One Crown7 Kit, Containing:

Crown7 Unit
2 Rechargeable Batteries
Charger
Two E-Cig Cartridges, opened (*for method development*)
One Pack of Five E-Cig Cartridges, Medium "11 mg", un-opened

Objectives: (i) Detect/Identify Semivolatiles and Volatiles in Extracts of the "As Received" E-Cig Cartridge and the Aerosol Produced by the Crown7 Device by Gas Chromatography-Mass Spectrometry (GC-MS)

(ii) Quantify Nicotine, Propylene Glycol, Glycerin and Water Observed by Gas Chromatography-Mass Spectrometry (GC-MS)

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ANALYZE
THE MATERIALS PROBLEM SOLVERS

SUMMARY

1. Solvent extracts of the filter+gel from the "as received" cartridge were found to contain the components listed in Summary Table I based on GC-MS data. Assignments were based on mass spectral data comparison. Components shown as m/z are listed ions with no suitable mass spectral match.

Summary Table I –
Component ID of "As Received" Cartridge (Filter+Gel)

Water	Phenoxyethyl isobutyrate
Ethanol	Hexadecanoic acid
Propylene glycol	m/z 45,56,59,63,89,91,104,131
Methyltartronic acid	m/z 45,56,59,63,89,91,104,131
Glycerin	m/z 45,56,59,74,89,118,132,145
Oxybis propanol (propylene glycol ether dimer)	m/z 45,56,104
Isopropyl methylpropanoate	m/z 45,56,59,89,91,114,131,135,145,163
Nicotine	m/z 45,56,89,91,114,131,145
Hydrazino imidazoline	m/z 43,71,128,141
Ethyl hydroxy 4H pyran one	m/z 43,45,59,71,89, 102,117,129

2. Major component contents of the "as received" cartridge are provided in Summary Table II. The quantified components sum accounts for 89.89 wt.-% of the contents of the analytical sample. The remaining ca. 10 % include the fibers, unidentified compounds listed as m/z in Summary Table I and possibly additional components. The nicotine concentration is 10.6 mg/cartridge (0.858 ± 0.02 wt.-% based on weight of cartridge plug, 1.2376 g, including fibers transferred to the extraction vessel).

Summary Table II –
Content of Major Components in an "As Received" Cartridge (Filter+Gel)

Component	Final Concentration (Wt.-%)
Nicotine	0.858 ± 0.02
Propylene Glycol	61.83 ± 1.0
Glycerin	18.80 ± 0.2
Water	8.402 ± 0.16

3. Both the interior and exterior of the interior threaded cap contained a visible layer of brown viscous liquid. Given the weight of the gel plug (about 1 g) and the suspicion that the extraneous material may differ from the gel plug, the amount of suspected gel remaining was not used in the experiments. After the filter plug was removed, the holder pieces were weighed, washed with isopropanol, dried and re-weighed. The mass losses were 0.128 grams (128 mg) to 0.151 grams (151 mg) for the two experiments. The amount of suspected external gel material is on the order of approximately 10% of the mass of the filter+gel plug used.

4. The five components listed in Summary Table III and their contents (in at least one of the "Aerosolization" experiments) are the only detectable components in chromatograms of the condensates by the analytical methodology. The collected material major component is water.

Summary Table III –
Concentration of Analytes
"Aerosolization" Experiments, Collected Condensates

Component	Final Concentration in Collected Condensate (Wt.-%) Experiment 2*	Final Concentration in Collected Condensate (Wt.-%) Experiment 3*
Ethanol	Detected, Not Quantified	13.80±0.77
Nicotine	<0.00042	1.46±0.042
Propylene Glycol	2.939 ± 0.05	3.507±0.090
Glycerin	<0.00012	Detected, Not Quantified
Water	Not Detectable by Method Used	66.19 ± 1.4

*Experiment 2, 38 mg of condensate; Experiment 3, 27 mg of condensate.

5. The "Aerosolization" experiments were performed to generate and collect the aerosol (atomized particles or a fine mist) in a vial. Mist evolution was actuated by inducing pressure differentials using a syringe piercing the collection vial which also held the device mouthpiece. The volume of total air displaced was 4.2 Liters and collected condensate amount ranged from 27-38 milligrams.

6. The "Aerosolization" Experiment results, by the methodology used, indicate a level of 0.39 milligram of nicotine and 3.7 milligrams of ethanol in the total collected condensate of Experiment 3. Variability(s) in the "aerosolization" experiment methodology may be determined with further experiments, if required.

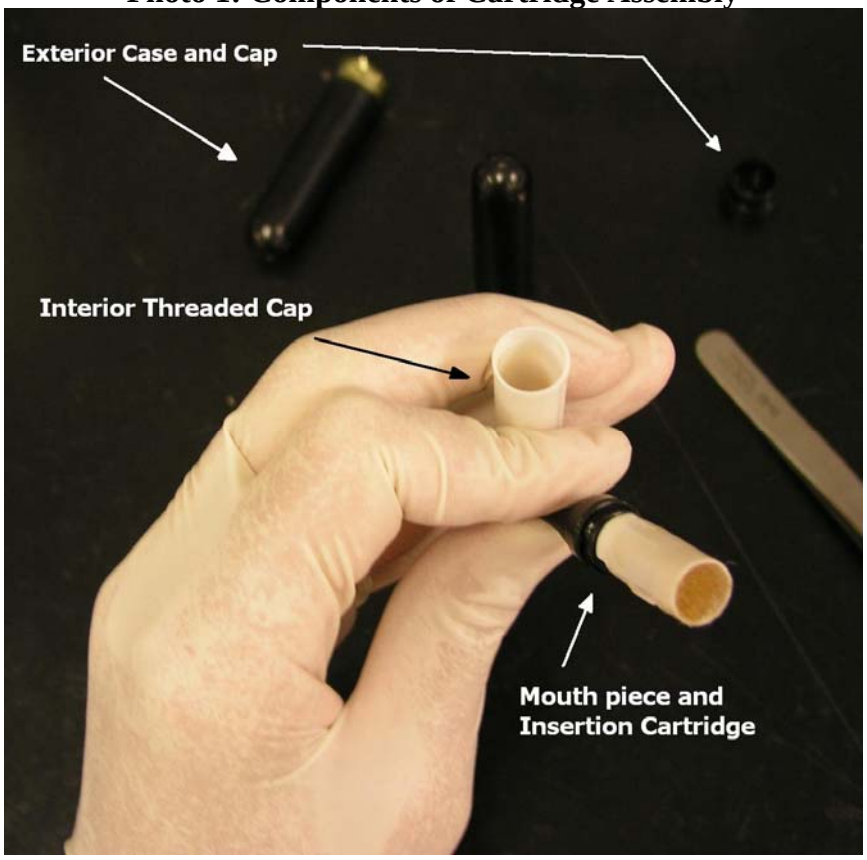
INTRODUCTION

The Crown7 Kit, described on the Title page, was received from R MacDonald on May 23rd. Five additional, unopened, cartridges (each E-Cig cartridge had "11mg" printed on the label) were received on June 5th. The objectives are described on the title page.

In order to evaluate the volatile and semi-volatile contents, two sampling techniques were used; one of the "as received" cartridge and one involving trapping the mist generated by the device or an "aerosolization" experiment.

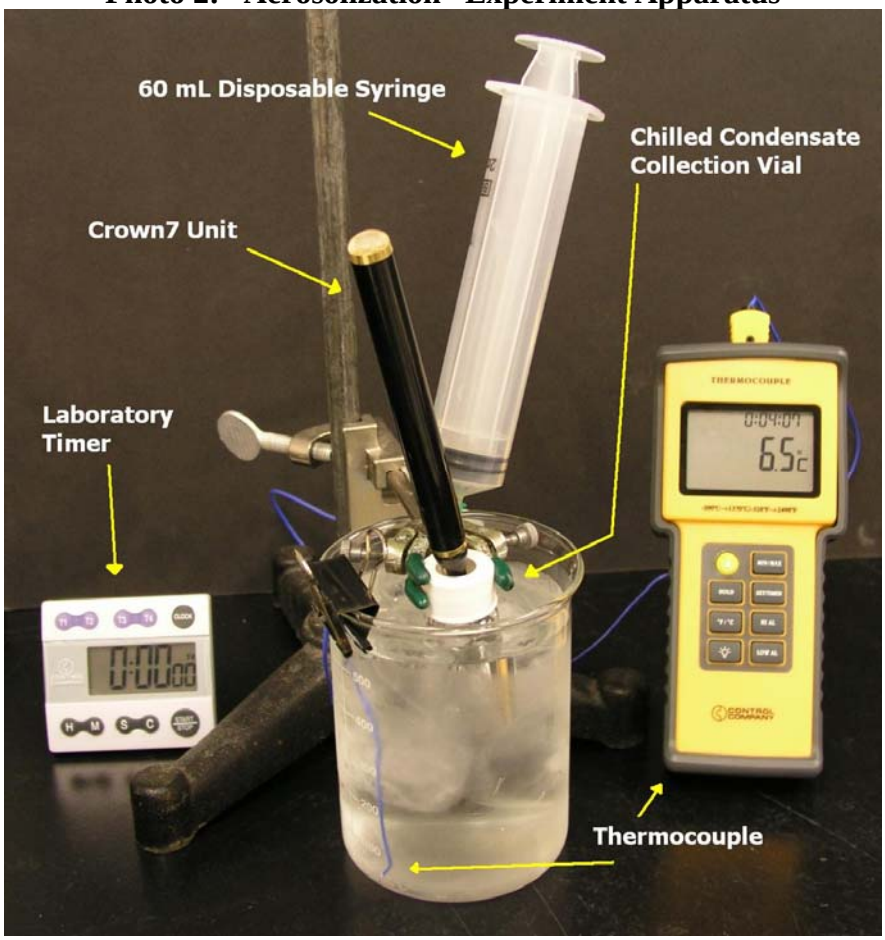
To determine the "as received" cartridge sample volatile and semi-volatile components, solvent extractions were performed directly on the removed fibrous plug ("gel plug") of material from the cartridge assembly. The solvent was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) techniques. Photo 1 illustrates the components of a cartridge assembly.

Photo 1: Components of Cartridge Assembly



To determine the volatile and semi-volatile components of the aerosol, "aerosolization" experiments were performed on new (unopened) cartridges using the Crown7 Kit device to generate and collect the aerosol (atomized particles or a fine mist) in a vial. Mist evolution was actuated by inducing pressure differentials using a syringe. Photo 2 illustrates the "aerosolization" experiment apparatus.

Photo 2: "Aerosolization" Experiment Apparatus



Once the Crown7 unit was loaded with an unopened cartridge, the mouthpiece was inserted through the Teflon coated septa (a small slice was made in the septum with a razor) of a 20 mL trace clean vial. A 60 mL disposable syringe with stainless steel needle was also inserted through the septa of the condensate collection vial (Photo 2). The condensate collection vial (e.g., the trace clean vial) was submerged in an ice water solution that remained at ca. 7 °C for experiment duration. A laboratory timer was started and the syringe was actuated from 0 mL to 20 mL (e.g., the volume of the condensate collection vial) ca. 7 times per minute. This process was continued for 30 min generating approx. 4.2 L of air passing through the apparatus. Some amount of visible atomized condensate was lost, likely, through the unit as the syringe was cycled from 20 mL to 0 mL (e.g., syringe down stroke). The loss of components due to this escape of condensate was minimized by the deliberately slow (ca. 7 cycles per minute) motion of the syringe. The experiment produced 38 mg of condensate in the chilled collection vial (Photo 2).

The experiment was repeated, utilizing an unopened cartridge, a new trace clean 20 mL vial and a new disposable syringe, to produce a second collection (27 mg) of condensate (aerosol). Each collected condensate was then diluted with solvent(s) for analysis by Gas Chromatography-Mass Spectrometry (GC-MS) techniques.

ANALYSIS

Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS is a valuable analytical technique for the separation and identification of volatile organic compounds. The compounds are separated on the GC column and identified by matching their mass spectra to those of reference mass spectra contained in the 125,000 spectra Wiley digital library (7^{th} Edition).

Instrumentation. GC-MS analysis was performed using a Varian CP-3800 Gas Chromatograph (GC) equipped with a Varian 1200L Quadrupole Mass Selective Detector and a CTC Analytics CombiPAL autosampling system. A Deactivated glass wool split inlet liner was used for chromatography under the following two sets of conditions.

Due to the varied composition of components in the extracts, two sets of analytical conditions were utilized for the analysis of the samples.

Instrument Conditions 1:

A Varian Factor Four 5ms capillary GC column (30 m x 0.25 mm with 0.25 μm film thickness) was utilized for analysis of the higher boiling components (e.g., nicotine, propylene glycol, glycerin, etc.) in conjunction with the following conditions:

Injector Temperature:	275 °C	Split Ratio:	20:1
Transfer Line Temperature:	280 °C	Injection Volume:	1.0 ul
He Flow Rate:	1.0 ml/min		
Column Oven:	40 °C hold 4 min, 40 °C to 280 °C at 20 °C/min		
MSD:	m/z 10-500 0.1 minute solvent delay		

Instrument Conditions 2:

A Phenomenex Zebron ZB-Wax capillary GC column (30 m x 0.25 mm with 0.25 μm film thickness) was utilized for analysis of lower boiling components (e.g., water, light solvents, etc) in conjunction with the following conditions:

Injector Temperature:	250 °C	Split Ratio:	20:1
Transfer Line Temperature:	280 °C	Injection Volume:	1.0 ul
He Flow Rate:	1.0 ml/min		
Column Oven:	40 °C hold 4 min, 40 °C to 225 °C at 20 °C/min, 225 °C hold 2 min		
MSD:	m/z 10-500 0.1 minute solvent delay		

The mass spectrometer was tuned prior to use.

Sample Preparation/Solvent Extraction of "Gel Plug". The exterior case and cap was removed from an unopened cartridge ("as received gel plug"). Next, the interior threaded cap was removed, exposing the insertion cartridge – attached to the black mouth piece. Utilizing clean forceps, the gel plug was removed from the insertion cartridge and added to a trace clean vial. It is worthy of noting that both the interior and exterior of the interior threaded cap contained a visible layer of brown viscous liquid. Given the weight of the gel plug (about 1 g, see Table I) and the suspicion that the material may differ from the gel plug, the amount of suspected gel remaining was not used in the experiments.

Due to the solubility differences of the anticipated range of components, a solvent mixture of acetonitrile and methylene chloride (ACN:DCM) in the proportion of 50:50 by weight was utilized for the extraction. The gel plug – saturated in a brownish fragrant liquid – was added to a 40 mL trace clean vial. Approximately 30 grams of ACN:DCM was added to the vial, which was agitated on a wrist shaker for 20 minutes.

The extraction procedure was repeated utilizing a second unopened cartridge ("gel plug"), with the exception of utilizing methanol for the extraction solvent.

After the filter plug was removed, the holder pieces were weighed, washed with isopropanol, dried and re-weighed. The mass loss was 0.128 grams (128 mg) for the ACN:DCM extract and 0.151 grams (151 mg) for the methanol extract. It is not certain if the substance may be attributed to the gel, hence the material was not used in any of the assay work. The amount of suspected external gel material is on the order of approximately 10% compared to the mass of the filter+gel plug (ca. 1.25 grams).

Table I
Weights of E-Cig Gel Plug

Identity	Weight, grams
E-Cig Gel Plug (ACN:DCM Extraction)	1.2376
E-Cig Gel Plug (Methanol Extraction)	1.2450

For comparison, the weights of the "aerosolization" experiment collected condensates are provided in Table II.

Table II
Weights of "Aerosolization" Experiment Condensates

Identity	Weight, grams
Experiment 2 (ACN:DCM Extraction)	0.0383
Experiment 3 (Methanol Extraction)	0.0273

Qualitative Analysis. Utilizing instrument conditions 1, undiluted portions of the ACN:DCM extracts from the as received gel plug and aerosolization experiment were analyzed. Prior to analysis, a portion of the ACN:DCM solvent system utilized for each extract was analyzed to ensure the purity of the solvent and system. The blank was observed to solely contain the expected components due to the solvents.

The stack plot comparison of the chromatogram of the "aerosolization" experiment 2 (top) and the gel plug extract (bottom) is displayed as Figure 1. It is worthy of noting that the condensate produced by the "aerosolization" experiment 2 was found to contain solely ethanol and propylene glycol, as noted in Figure 1. This differs significantly from the large number of components observed by the analysis of extract of the cartridge gel plug.

Table III contains the retention time and identity of each observed component from the ACN:DCM extraction of the gel plug. Components observed which are due to the solvent extraction system, (e.g., nitrogen, ACN, DCM, propanenitrile) are not listed in Table III. Components listed as m/z do not provide suitable mass spectra for correlation and are listed as unidentified with mass spectral data being provided.

Figure 1
GC-MS (Instrument Conditions 1, ACN:DCM)
Chromatograms of "Aerosolization" Experiment 2 and Gel Plug Extract

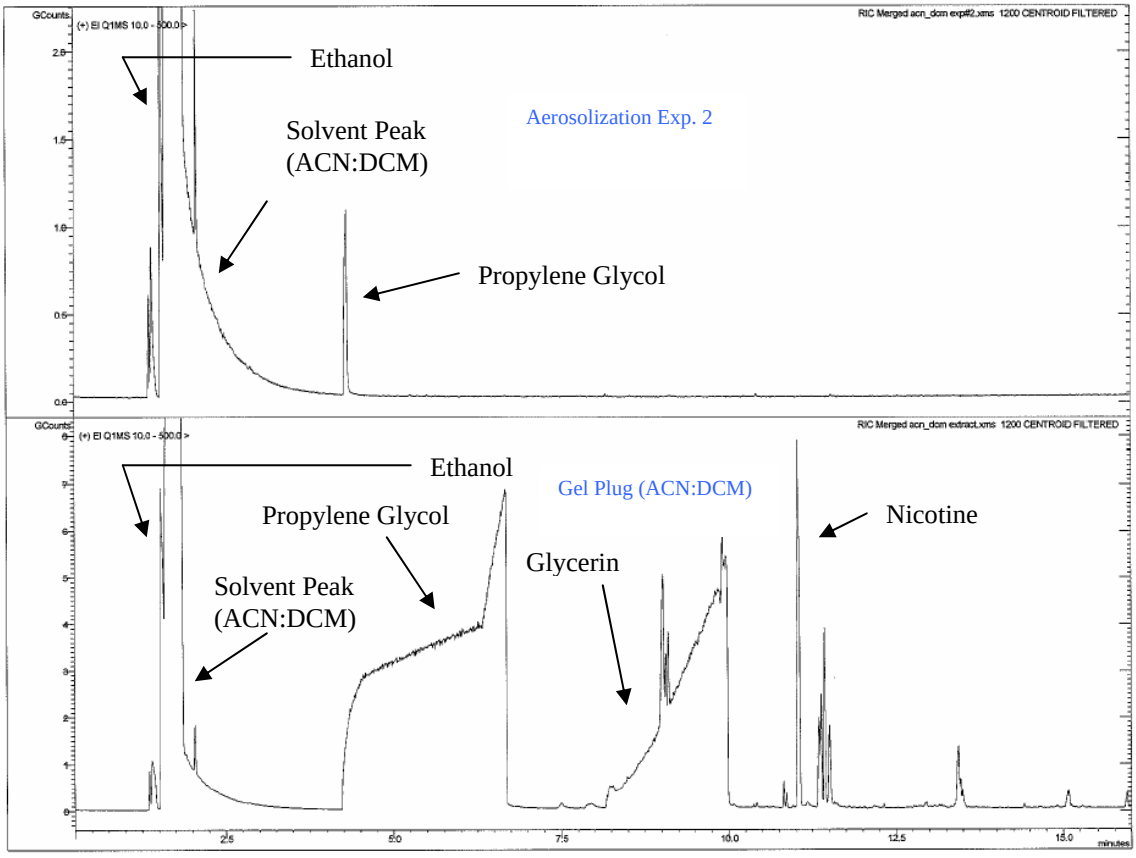
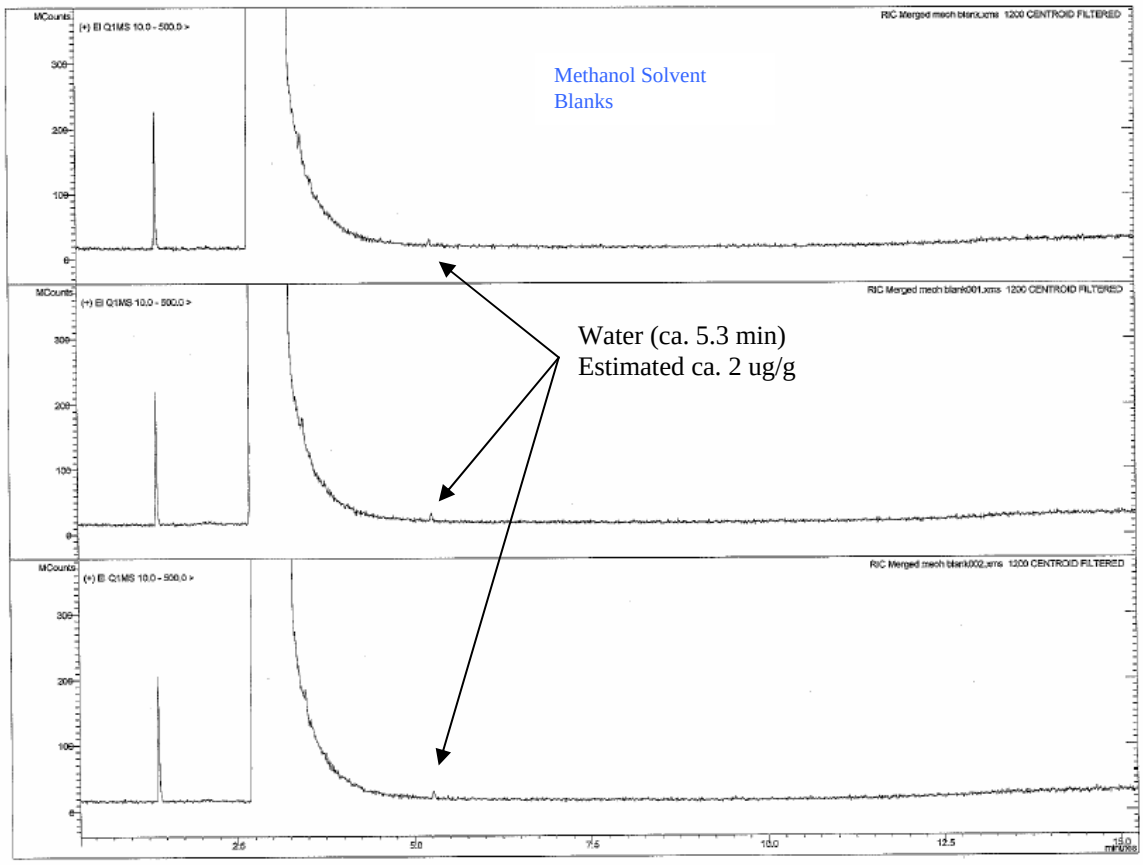


Table III
GC-MS (Instrument Conditions 1, ACN:DCM)
Mass Spectral Data Based Identifications of ACN:DCM Extract of Gel Plug

Retention Time (min)	Identity	Retention Time (min)	Identity
1.412	Water	11.350	m/z 45,56,59,63,89,91,104,131
1.541	Ethanol	11.380	m/z 45,56,59,63,89,91,104,131
6.674	Propylene glycol	11.432	Nicotine (artifact from saturation)
7.489	Methyltartronic acid	11.506	m/z 45,56,59,74,89,118,132,145
8.241	Glycerin	11.804	Hydrazino imidazoline
9.021	m/z 45,56,104	12.029	Phenoxyethyl isobutyrate
9.058	Oxybis propanol (propylene glycol ether dimer)		
9.103	Oxybis propanol (propylene glycol ether dimer)	13.435	m/z 45,56,59,89,91,114,131,135,145,163
9.909	Glycerin	14.414	Hexadecanoic acid
10.828	Isopropyl methylpropanoate	15.086	m/z 45,56,89,91,114,131,145,
11.037	Nicotine	15.936	m/z 43,71,128,141

Utilizing instrument conditions 2, portions of the "aerosolization" experiment 3 in methanol and the methanol extract of the gel plug were analyzed. Prior to analysis, a portion of the methanol utilized for each extract was analyzed to ensure the purity of the solvent and system. Figure 2 displays the chromatograms of the methanol blanks, injected in triplicate.

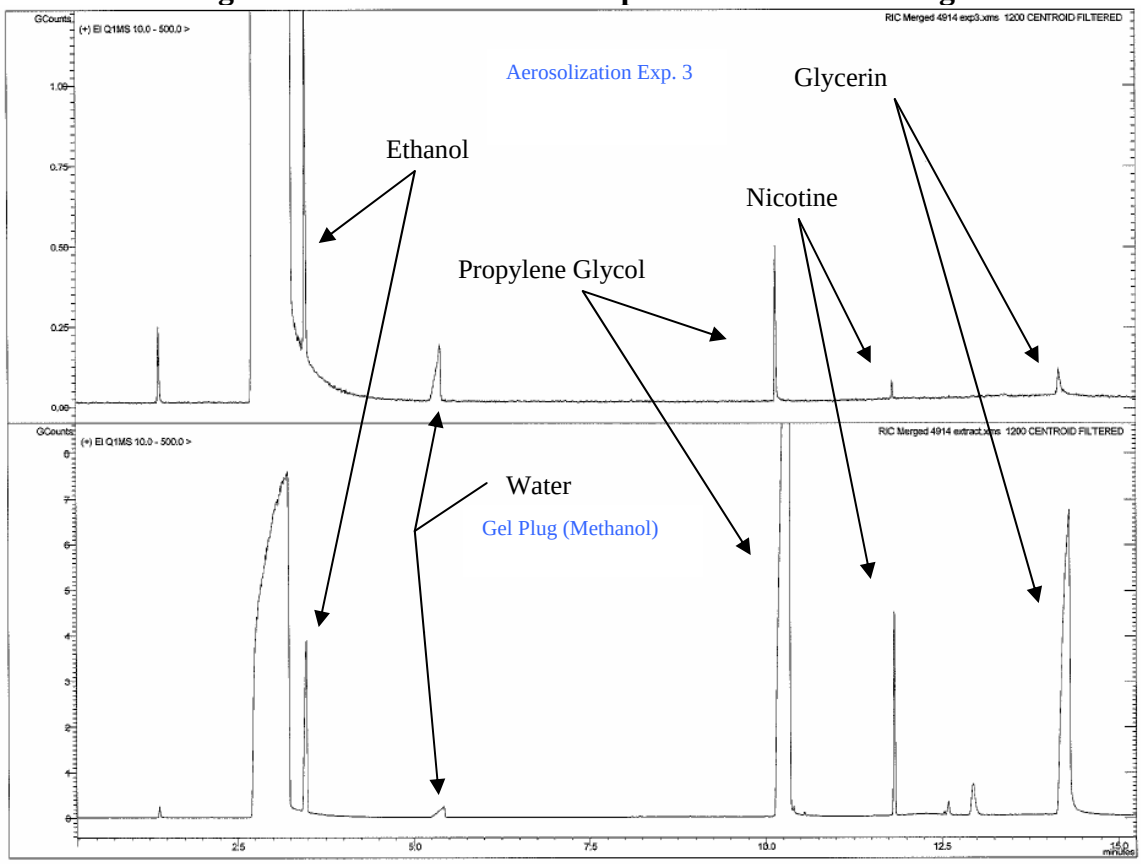
Figure 2
GC-MS (Instrument Conditions 2, Methanol)
Chromatograms of Methanol Solvent Blanks Prior to Sample Analysis



The blanks were observed to contain the expected components due to the solvent system (e.g., nitrogen, methanol). Additionally, trace amounts of water were observed in all blanks (ca. 5.3 min.). The concentration of water in the blanks was estimated to be ca. 2 ug/g, which is below the calibrated range for water and on the order of baseline noise.

The stack plot comparison of the chromatogram of the "aerosolization" experiment 3 extract (top) and the gel plug extract (bottom) is displayed as Figure 3.

Figure 3
GC-MS (Instrument Conditions 2, Methanol)
Chromatograms of "Aerosolization" Experiment 3 and Gel Plug Extract



As annotated in Figure 3, the chromatograms of the "aerosolization" extract and the gel plug extract were observed to contain similar major components. This similarity of major components between the two extracts was not observed with regard to the two extracts in the solvent system of ACN:DCM (Figure 1).

The reason for the differences is not known but possibilities may include:

Solubility differences of analytes in the two solvent systems used,

Differences in interactions of analytes in the analytical methodologies (e.g., different columns) used,

Variability(s) in the "aerosolization" experiment methodology.

The retention time and identity of all components observed for the "aerosolization" methanol extract and the gel plug methanol extract can be found in Tables IV – V. Components observed which are due to the solvent extraction system, (e.g., nitrogen and methanol) are not listed in Tables IV - V.

Table IV
Observed Components of Methanol Extract of Gel Plug

Retention Time (min)	Identity	Retention Time (min)	Identity
3.462	Ethanol	12.536	m/z 43,45,59,71,89, 102,117,129
5.424	Water	12.604	Ethyl hydroxy 4H pyran one
10.214	Propylene glycol	12.958	Oxybis propanol
11.836	Nicotine	14.304	Gylcerin

Table V
Observed Components of Methanol Extract of "Aerosolization" Experiment 3

Retention Time (min)	Identity	Retention Time (min)	Identity
3.467	Ethanol	11.808	Nicotine
5.352	Water	14.174	Glycerin
10.150	Propylene glycol		

Quantitative Analysis. Utilizing instrument conditions 1 and 2, as described previously, a multi level calibration was prepared and analyzed for each of the following components: nicotine, propylene glycol, glycerin, and water. The first three components (nicotine, propylene glycol and glycerin) contents were determined utilizing instrument conditions 1 and the ACN:DCM solvent system, while water content was determined utilizing instrument conditions 2, and methanol. After initial experiments were performed, ethanol was detected. Ethanol quantitation was only performed on "aerosolization" experiment 3 assay data.

Multi-level calibrations for each component were prepared and analyzed, followed by a calibration check standard. All calibration check standards were observed to fall within acceptable recovery ranges (e.g., ± 20% in a sample matrix). The trend lines generated by each calibration were observed to have reasonable r^2 value. The calibration for water was observed to achieve a better fit to the data when the trend line was not "forced" through zero. The formula utilized to calculate analyte concentration utilizing this model is $y = mx+b$, where "b" is the intercept. The intercept for the water calibration was determined to be 26301388.320. The slope and r^2 for the trend line determined for each calibrated component, is displayed in Table VI.

Table VI
Calibration Slope and r^2 values

Component	r^2 value	Slope
Nicotine	0.9975	21440891.935
Propylene Glycol	0.9994	8518059.669
Glycerin	0.9926	7615657.772
Water	0.9961	88110.536

Analytical data including final concentration for each calibrated component using instrument conditions 1 (ACN:DCM) are displayed in Table VII - VIII.

Table VII
Concentration of Analytes
For Gel Plug Extract (Instrument Conditions 1, ACN:DCM)

Component	Average Area ($\times 10^8$)	Dilution Factor	Concentration in Solution (ug/g)	Final Concentration (Wt.-%)
Nicotine	3.330	552.9	15.53	0.858 ± 0.02
Propylene Glycol	14.42	3653.	169.2	61.83 ± 1.0
Glycerin	3.918	3653.	51.45	18.80 ± 0.2
Water	8.876	83.40	1007.	8.402 ± 0.16

Table VIII
Concentration of Analytes
For "Aerosolization" Experiment 2 (Instrument Conditions 1, ACN:DCM)

Component	Average Area ($\times 10^7$)	Dilution Factor	Concentration in Solution (ug/g)	Final Concentration (Wt.-%)
Nicotine	n/d	n/a	n/d	<0.00042
Propylene Glycol	61.59	406.4	72.31	2.939 ± 0.05
Glycerin	n/d	n/a	n/d	<0.00012
Water	6.527	1496.	422.3	66.19 ± 1.4

An example of the calculation for the final concentration of nicotine in the gel plug extraction is provided below:

$$\frac{\text{Area of Analyte Peak}}{\text{Slope of Calibration Curve}} = \text{ppm analyte assayed in solution}$$

ppm analyte assayed in solution X dilution factor= ppm analyte in Sample

where dilution factor is

$$= \frac{\text{grams total}}{\text{g extracted sample}} \times \frac{\text{g total}}{\text{g extract}} \times \frac{\text{g total}}{\text{g extract diln1}} \times \frac{\text{g total}}{\text{g extract diln2}}.$$

$$= \frac{6.4661 \text{ g total}}{1.2376 \text{ g extracted sample}} \times \frac{0.5222 \text{ g total}}{0.0496 \text{ g extract}} \times \frac{2.0183 \text{ g total}}{0.2008 \text{ g ext diln1}} = 552.9$$

For Gel Plug Extract dilution injection#1:

$$\frac{337200000}{21440891.935} = 15.73 \text{ ppm nicotine assayed in solution}$$

$$15.73 \text{ ug/g (ppm) nicotine in solution} \times 552.9 \times \frac{1 \text{ g}}{1 \times 10^6 \text{ ug}} \times 100$$

= 0.870 Wt.-% nicotine in sample determined from injection1

The observed concentration of ethanol, nicotine and propylene glycol in the methanol extract of "Aerosolization" experiment 3 were determined utilizing multi-level calibrations for each analyte (instrument conditions 2). The trend lines generated by each calibration were observed to have reasonable r^2 value. The calibration for ethanol was observed to achieve a better fit to the data when the trend line was not "forced" through zero. The formula utilized to calculate analyte concentration utilizing this model is y = mx+b, where "b" is the intercept. The intercept for the water calibration was determined to be 21866415.671. The slope and r^2 for the trend line determined for each calibrated component, is displayed in Table IX.

Table IX
Calibration Slope and r^2 values

Component	r^2 value	Slope
Ethanol	0.9954	566157.808
Nicotine	0.9998	1814586.138
Propylene Glycol	0.9967	1608508.725

The average area counts, concentration (in solution), dilution factor utilized and final concentration for each calibrated component in each extract are displayed in Table X.

Table X
Concentration of Ethanol and Nicotine
For "Aerosolization" Experiment 3 Methanol Extract

Component	Average Area (x 10^8)	Dilution Factor	Concentration in Solution (ug/g)	Final Concentration (Wt.-%)
Ethanol	83.97	1258.	109.7	13.80 ± 0.77
Nicotine	2.722	97.73	150.0	1.46 ± 0.042
Propylene Glycol	5.772	97.73	358.8	3.507 ± 0.090

Spread sheets containing all the area counts, known concentrations of each calibration level, r^2 values, slopes, calibration check standard area counts and recoveries for each calibrated component can be found in the Appendix. All tune reports, chromatograms, and mass spectral correlations not included as figures can be found in the Appendix.

As questions arise during your review of this report, please do not hesitate to call.

ANALYZE Inc.

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