Characterization of Regal Cartridges for Electronic Cigarettes – Phase II

prepared for

inLife LLC
Irvine, CA

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12 November 2009

Client: inLife LLC
2569 McCabe Way
Irvine, CA 92614

Requestor: Tom Kiklas

Study: **Characterization of Regal Cartridges for Electronic Cigarettes – Phase II**

LIMS #: 20090304

SUMMARY
A liquid formulation used in electronic cigarettes was tested by gas chromatography mass spectrometry (GC-MS) to identify and determine relative concentrations of 4 Tobacco Specific Nitrosamines (TSNAs) and 8 Tobacco Specific Impurities (TSIs). The 4 nitrosamines were N-Nitrosoanabasine (NAB), N-Nitrosoanatabine (NAT), N-Nitrosonornicotine (NNN) and 4-(Methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK). The 8 Tobacco Specific Impurities were Nicotine 1 –oxide, Myosmine, Anatabine, 1-Methyl-3-nicotinoylpyrrolidine, Nornicotine, Anabasine, β-Nicotyrine and Cotinine. None of the TSNA compounds were found in this sample, but 2 of the 8 TSI's were found.

EXPERIMENTAL
One box of smoke cartridges along with an electronic cigarette was submitted for these experiments (Figure 1). The liquid product from the cartridge was characterized by GC-MS. The sample was designated as follows:

Table I: Sample Designations

LIMS #	Client ID	Description
20090304-01	inLIfe Regal Cartridges	Smoke Cartridges

For the Tobacco Specific Nitrosamines determination, individual Specificity standards were prepared in dichloromethane (DCM) at a concentration of 200ppm. Dilutions of these stock standards were prepared at 100, 50 and 20ppm to determine a Limit of Detection (LOD). The sample was prepared by disassembling an unopened e-cigarette cartridge, extracting the wick and isolating the liquid. 10µL of the liquid was dissolved in 1ml of DCM and mixed. This solution, along

with the specificity standards was characterized on a Shimadzu 2010S GC-MS instrument equipped with a ZB-5MSi (Phenomenex) high temperature amine capillary column. Observed peaks were identified based on spectral matches with commercial MS libraries and relative retention times (RRT) of the specificity standards.

The determination of Tobacco Specific Impurities (TSIs) closely resembled that of the TSNAs. Individual specificity standards for each of the eight TSIs were prepared in DCM at a concentration of 100ppm and diluted to 50 and 10ppm to determine their limit of detection. The sample was prepared identically to that in the TSNA experiment. The same equipment and column was utilized. Observed peaks were identified based on spectral matches to commercial MS libraries and RRT of the specificity standards.

The isolated liquid from the cartridge was also subjected to five different solvent extractions in order to substantiate the levels of TSNAs and TSIs in the sample. This was achieved by dissolving 10µL of liquid sample in 1ml of chloroform, methanol, acetonitrile, hexane and acetone. The resulting solutions were mixed and reduced to dryness under a stream of nitrogen. The resulting residue was reconstituted in 0.5ml DCM. These five solutions were injected onto the GC MS under the same conditions as the TSNA, TSI specificity standards and liquid sample extracted in DCM.

RESULTS & DISCUSSION

GC-MS chromatograms of the TSNA specificity standards showed relative retention times of NNK at ~13.576min, NNN = 12.659min, NAT = 12.850min and NAB = 13.034min. See figures 2 -5 for chromatograms. An overlay of the 10,000ppm sample in DCM with the four 20ppm TSNA specificity chromatograms showed no peaks matched to the RRT of the TSNAs (Figure 6), with the exception of a peak at the same retention time as NNK. Although the RRT of this unknown peak matched NNK, this peak does not match the molecular mass or mass-charge ratio (m/z) of NNK. An overlay of the liquid sample extracted in chloroform, methanol, acetonitrile, hexane and acetone also did not match the RRT of the TSNA specificity standards. Therefore, the amount of TSNAs in the product was clearly less than 20ppm (LOD).

GC-MS chromatograms of the TSI specificity standards (10ppm) showed relative retention times of Nicotine 1 –oxide = 13.223min, Myosmine = 10.319min, Anatabine = 11.172min, 1-Methyl-3-nicotinoylpyrrolidine = 12.069min, Nornicotine = 10.259min, Anabasine = 10.957min, ß-Nicotyrine = 10.905min and Cotinine = 12.366min. An overlay of the chromatograms at the 10ppm concentration level

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showed good resolution between peaks. See Figures 7-14 for chromatograms of TSI specificity standards.

An overlay of the liquid sample in DCM with each of the eight 10ppm TSI standards showed no matching peaks. However, chromatograms from the liquid sample extracted in chloroform, acetonitrile and acetone show matching peaks to ß-Nicotyrine; and Anatabine in the acetone extracted sample (Figures 15-17). The calculated amount of ß-Nicotyrine in the chloroform extracted sample was ~9ppm, 55ppm in acetonitrile and 53ppm in acetone. Therefore, the reportable level of ß-Nicotyrine in the sample was 55ppm, 14ppm for Anatabine and less than 10ppm for Nicotine 1 –oxide, Myosmine, 1-Methyl-3-nicotinoylpyrrolidine, Nornicotine, Anabasine and Cotinine.

The reportable levels of the TSNAs (N-Nitrosoanabasine (NAB), N-Nitrosoanatabine (NAT), N-Nitrosonornicotine (NNN) and 4-(Methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK) were less than 20ppm (LOD).

The analytical method used for this study focused on the determination of four TSNAs and eight TSIs in the liquid of an e-cigarette cartridge. Under the conditions employed in this study, the 4 TSNAs were not detected in this sample and were reported as less than the Limit of Detection. Two of the eight TSIs were observed. Table II summarizes these findings.

Table IIA: Tobacco Specific Nitrosamines (TSNA)

TSN	Concentration Detected (PPM)
N-Nitrosoanabasine (NAB)	< 20
N-Nitrosoanatabine (NAT)	<20
4-(Methylnitrosoamino)-1-(3-pyridineyl)-1-butanone (NNK)	<20
N-Nitrosonornicotine (NNN)	<20

Table IIB: Tobacco Specific Impurities (TSI)

TSI	Concentration Detected (PPM)
Nicotine 1-oxide	<10
Myosmine	<10
Anatabine	14
1-Methyl-3-nicotinoylpyrrolidine	<10
Nornicotine	<10
Anabasine	<10
ß-Nicotyrine	55
Cotinine	<10

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Figure 1: Regal Liquid Cartridge with Electronic Cigarette

Figure 2: GC MS TIC Chromatogram of N-Nitrosoanabasine (NAB) 20ppm

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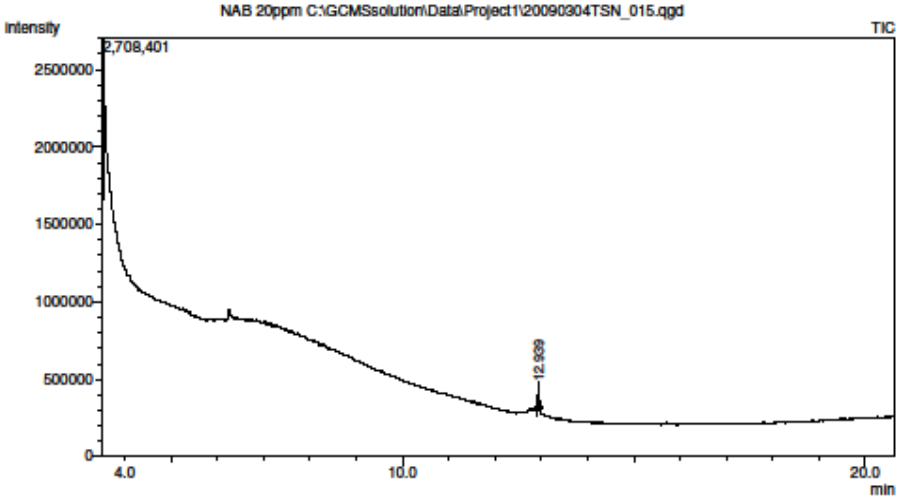
Sample Information

Analyzed by	: Admin
Analyzed	: 10/29/2009 3:19:57 PM
Sample Name	: NAB 20ppm
Sample ID	: NAB 20ppm
Tray	: Tray2
Vial #	: 9
Inj. Volume	: 1
Data File	: C:\GCMSsolution\Data\Project1\20090304TSN_015.qgd
Method File	: C:\GCMSsolution\Data\Project1\ZB-5MSi_8270D.qgm
Tuning File	: C:\GCMSsolution\System\Tune1\QP2010S.qgt

NAB 20ppm C:\GCMSsolution\Data\Project1\20090304TSN_015.qgd



Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Base m/z	Name
1	12.939	275022	100.00	170020	100.00	105.05	N-Nitrosoanabasine
		275022	100.00	170020	100.00		

C:\GCMSsolution\Data\Project1\20090304TSN_015.qgd 1 / 3

Figure 3: GC MS TIC Chromatogram of N-Nitrosonornicotine (NNN) 20ppm

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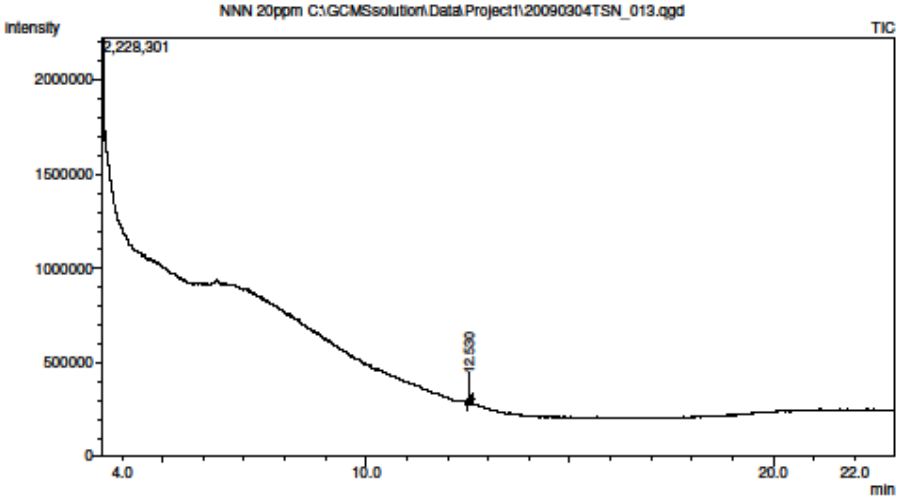
Sample Information

Analyzed by	: Admin
Analyzed	: 10/29/2009 2:14:31 PM
Sample Name	: NNN 20ppm
Sample ID	: NNN 20ppm
Tray	: Tray2
Vial #	: 7
Inj. Volume	: 1
Data File	: C:\GCMSsolution\Data\Project1\20090304TSN_013.qgd
Method File	: C:\GCMSsolution\Data\Project1\ZB-5MSi_8270D.qgm
Tuning File	: C:\GCMSsolution\System\Tune1\QP2010S.qgt

NNN 20ppm C:\GCMSsolution\Data\Project1\20090304TSN_013.qgd



Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Base m/z	Name
1	12.530	291023	100.00	150841	100.00	105.00	N-Nitrosonornicotine
		291023	100.00	150841	100.00		

C:\GCMSsolution\Data\Project1\20090304TSN_013.qgd 1 / 3

Figure 4: GC MS TIC Chromatogram of N-Nitrosoanatabine (NAT) 20ppm

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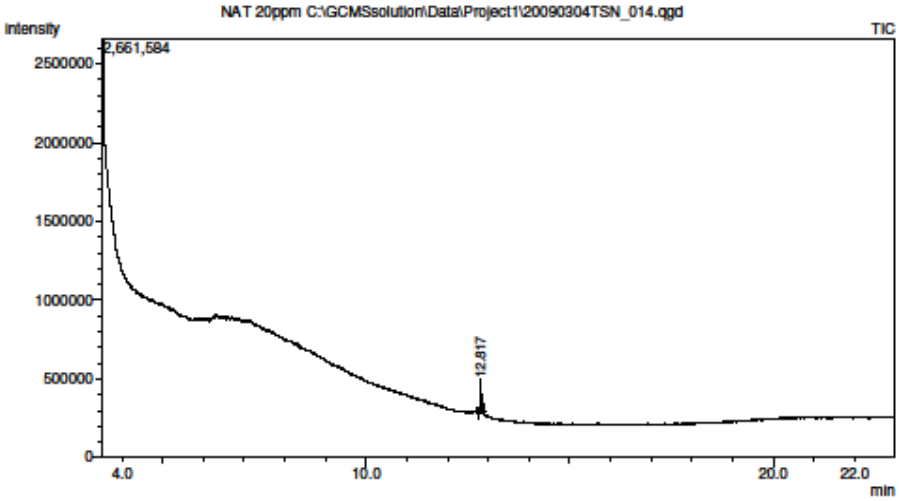
Sample Information

Analyzed by	: Admin
Analyzed	: 10/29/2009 2:47:16 PM
Sample Name	: NAT 20ppm
Sample ID	: NAT 20ppm
Tray	: Tray2
Vial #	: 8
Inj. Volume	: 1
Data File	: C:\GCMSsolution\Data\Project1\20090304TSN_014.qgd
Method File	: C:\GCMSsolution\Data\Project1\ZB-5MSi_8270D.qgm
Tuning File	: C:\GCMSsolution\System\Tune1\QP2010S.qgt

NAT 20ppm C:\GCMSsolution\Data\Project1\20090304TSN_014.qgd



Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Base m/z	Name
1	12.817	313240	100.00	202802	100.00	130.05	N-Nitrosoanatabine
		313240	100.00	202802	100.00		

C:\GCMSsolution\Data\Project1\20090304TSN_014.qgd 1 / 3

Figure 5: GC MS TIC Chromatogram of 4-(Methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK) 20ppm

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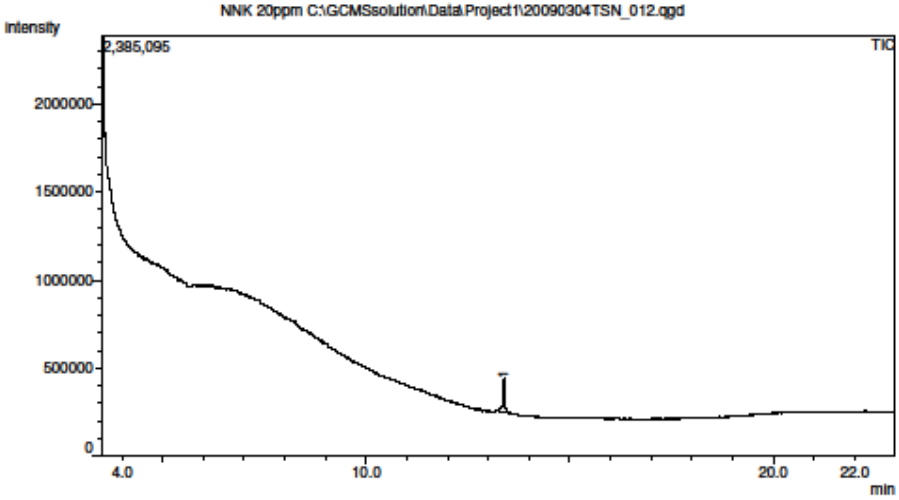
Sample Information

Analyzed by	: Admin
Analyzed	: 10/29/2009 1:41:44 PM
Sample Name	: NNK 20ppm
Sample ID	: NNK 20ppm
Tray	: Tray2
Vial #	: 6
Inj. Volume	: 1
Data File	: C:\GCMSsolution\Data\Project1\20090304TSN_012.qgd
Method File	: C:\GCMSsolution\Data\Project1\ZB-5MSi_8270D.qgm
Tuning File	: C:\GCMSsolution\System\Tune1\QP2010S.qgt

NNK 20ppm C:\GCMSsolution\Data\Project1\20090304TSN_012.qgd



Quantitative Result Table

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Base m/z	Name
1	13.385	597548	100.00	191867	100.00	78.05	4-(N-Nitroso-N-methylamino)-1-(3-pyridyl)-1-butanone
		597548	100.00	191867	100.00		

C:\GCMSsolution\Data\Project1\20090304TSN_012.qgd 1 / 3

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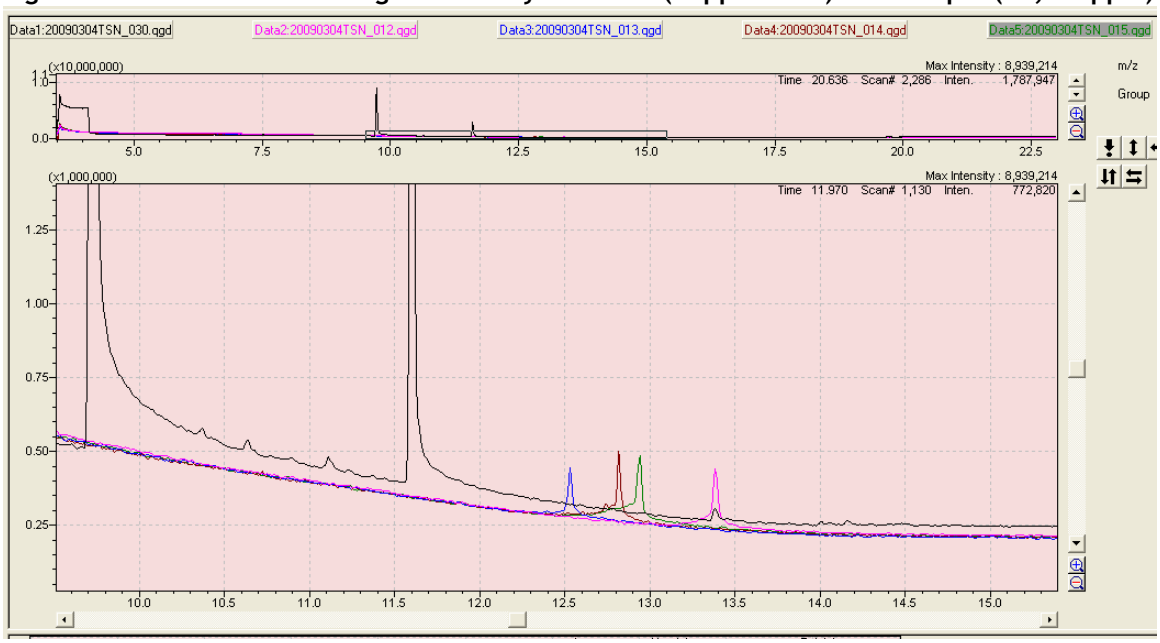
Figure 6: GC MS TIC Chromatogram overlay of TSNAs (20ppm each) and Sample (10,000ppm)



Black (20090304TSN_030) = Sample (10,000ppm) in DCM
Blue (20090304TSN_013) = NNN (10ppm)
Red (20090304TSN_014) = NAT (10ppm)
Green (20090304TSN_015) = NAB (10ppm)
Pink (20090304TSN_012) = NNK (10ppm)

Figure 7: GC MS TIC Chromatogram of Nicotine 1-Oxide 10ppm

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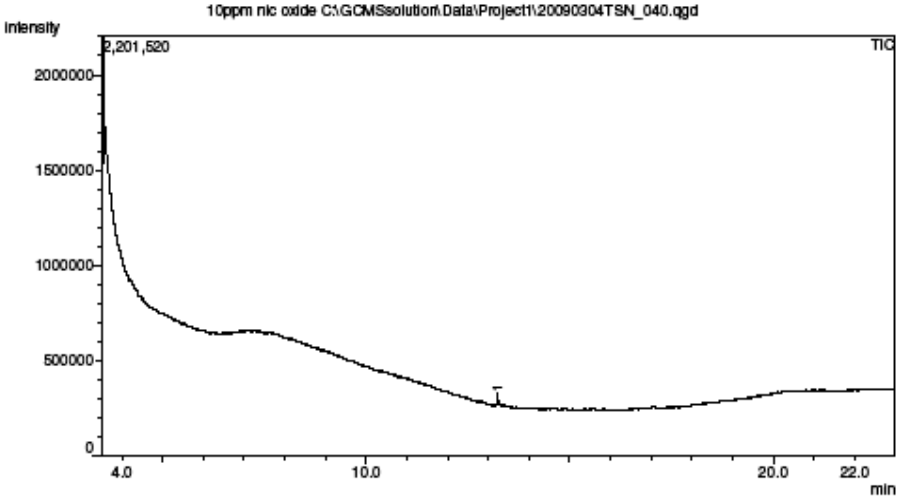
Sample Information

Analyzed by	: Admin
Analyzed	: 10/30/2009 4:33:42 PM
Sample Name	: 10ppm nic oxide
Sample ID	: 10ppm nic oxide
Tray	: Tray2
Vial #	: 29
Inj. Volume	: 1
Data File	: C:\GCMSsolution\Data\Project1\20090304TSN_040.qgd
Method File	: C:\GCMSsolution\Data\Project1\ZB-5MSi_8270D.qgm
Tuning File	: C:\GCMSsolution\System\Tune1\QP2010S.qgt

10ppm nic oxide C:\GCMSsolution\Data\Project1\20090304TSN_040.qgd



Quantitative Result Table

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Base m/z	Name
1	13.223	110912	100.00	63008	100.00	84.00	Nicotine-N-oxide
		110912	100.00	63008	100.00		

C:\GCMSsolution\Data\Project1\20090304TSN_040.qgd 1 / 3

Figure 8: GC MS TIC Chromatogram of Myosmine 10ppm

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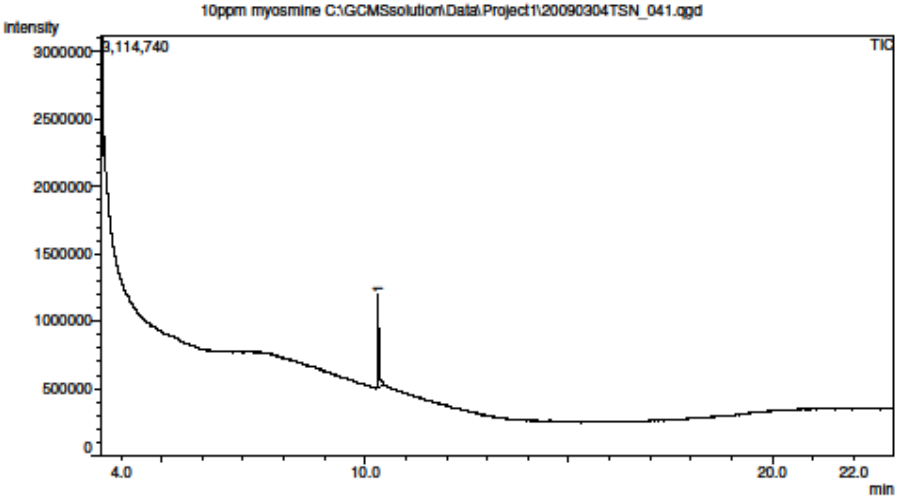
Sample Information

Analyzed by	: Admin
Analyzed	: 10/30/2009 5:06:07 PM
Sample Name	: 10ppm myosmine
Sample ID	: 10ppm myosmine
Tray	: Tray2
Vial #	: 30
Inj. Volume	: 1
Data File	: C:\GCMSsolution\Data\Project1\20090304TSN_041.qgd
Method File	: C:\GCMSsolution\Data\Project1\ZB-5MSi_8270D.qgm
Tuning File	: C:\GCMSsolution\System\Tune1\QP2010S.qgt

10ppm myosmine C:\GCMSsolution\Data\Project1\20090304TSN_041.qgd



Quantitative Result Table

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Base m/z	Name
1	10.319	1490515	100.00	697706	100.00	118.00	Myosmine
		1490515	100.00	697706	100.00		

C:\GCMSsolution\Data\Project1\20090304TSN_041.qgd 1 / 3

Figure 9: GC MS TIC Chromatogram of Anatabine 10ppm

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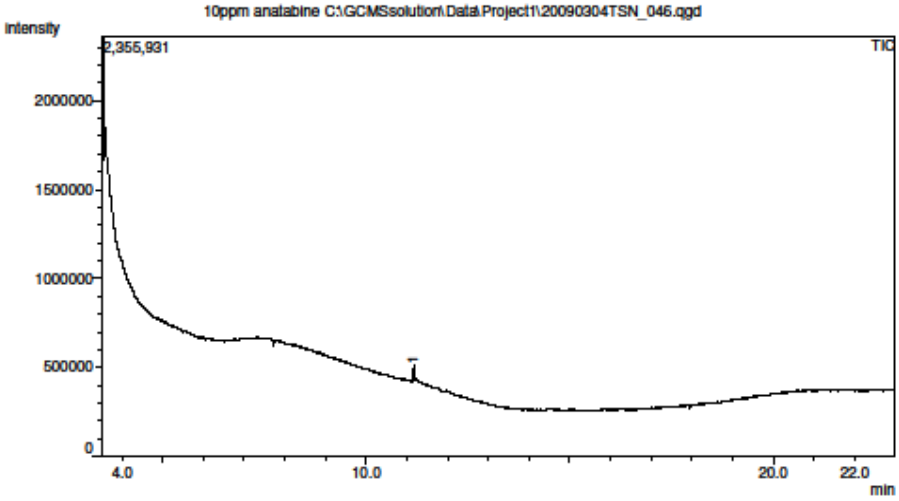
Sample Information

Analyzed by	: Admin
Analyzed	: 10/30/2009 7:47:40 PM
Sample Name	: 10ppm anatabine
Sample ID	: 10ppm anatabine
Tray	: Tray2
Vial #	: 35
Inj. Volume	: 1
Data File	: C:\GCMSsolution\Data\Project1\20090304TSN_046.qgd
Method File	: C:\GCMSsolution\Data\Project1\ZB-5MSi_8270D.qgm
Tuning File	: C:\GCMSsolution\System\Tune1\QP2010S.qgt

10ppm anatabine C:\GCMSsolution\Data\Project1\20090304TSN_046.qgd



Quantitative Result Table

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Base m/z	Name
1	11.172	195971	100.00	92443	100.00	251.80	Anatabine
		195971	100.00	92443	100.00		

C:\GCMSsolution\Data\Project1\20090304TSN_046.qgd 1 / 3

Figure 10: GC MS TIC Chromatogram of 1-Methyl-3-nicotinoylpyrrolidine (MNP) 10ppm

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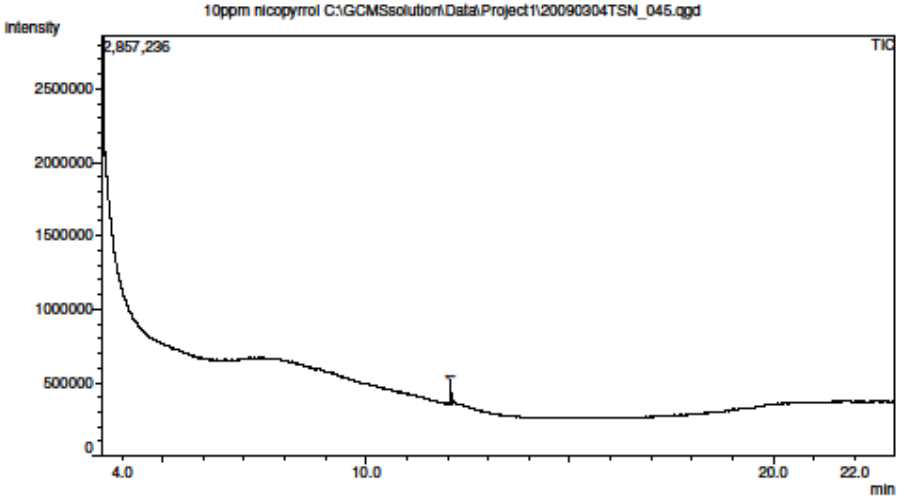
Sample Information

Analyzed by	: Admin
Analyzed	: 10/30/2009 7:15:27 PM
Sample Name	: 10ppm nicopyrrol
Sample ID	: 10ppm nicopyrrol
Tray	: Tray2
Vial #	: 34
Inj. Volume	: 1
Data File	: C:\GCMSsolution\Data\Project1\20090304TSN_045.qgd
Method File	: C:\GCMSsolution\Data\Project1\ZB-5MSi_8270D.qgm
Tuning File	: C:\GCMSsolution\System\Tune1\QP2010S.qgt

10ppm nicopyrrol C:\GCMSsolution\Data\Project1\20090304TSN_045.qgd



Quantitative Result Table

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Base m/z	Name
1	12.069	374319	100.00	159686	100.00	57.05	1-methyl-3-nicotinoylpyrrolidine (MNP)
		374319	100.00	159686	100.00		

C:\GCMSsolution\Data\Project1\20090304TSN_045.qgd 1 / 3

Figure 11: GC MS TIC Chromatogram of Nornicotine 10ppm

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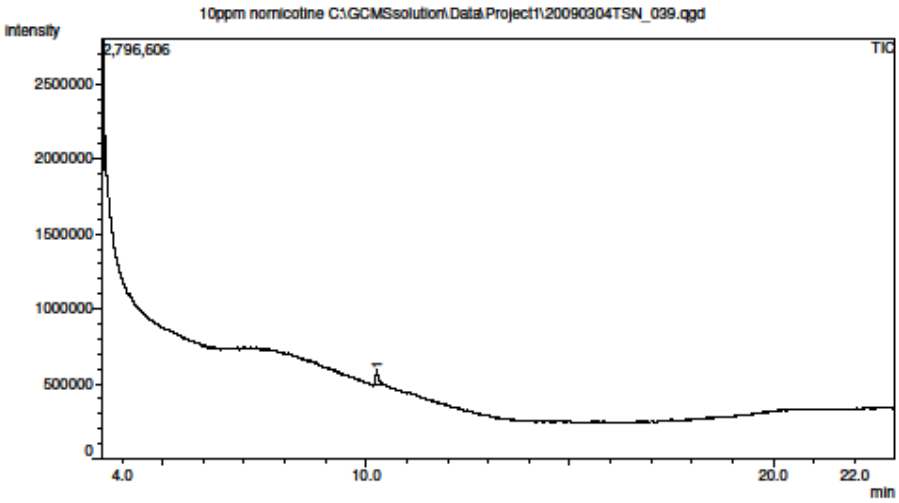
Sample Information

Analyzed by	: Admin
Analyzed	: 10/30/2009 4:01:18 PM
Sample Name	: 10ppm nornicotine
Sample ID	: 10ppm nornicotine
Tray	: Tray2
Vial #	: 28
Inj. Volume	: 1
Data File	: C:\GCMSsolution\Data\Project1\20090304TSN_039.qgd
Method File	: C:\GCMSsolution\Data\Project1\ZB-5MSi_8270D.qgm
Tuning File	: C:\GCMSsolution\System\Tune1\QP2010S.qgt



Quantitative Result Table

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Base m/z	Name
1	10.259	453033	100.00	107630	100.00	118.00	Nornicotine
		453033	100.00	107630	100.00		

C:\GCMSsolution\Data\Project1\20090304TSN_039.qgd 1 / 3

Figure 12: GC MS TIC Chromatogram of Anabasine 10ppm

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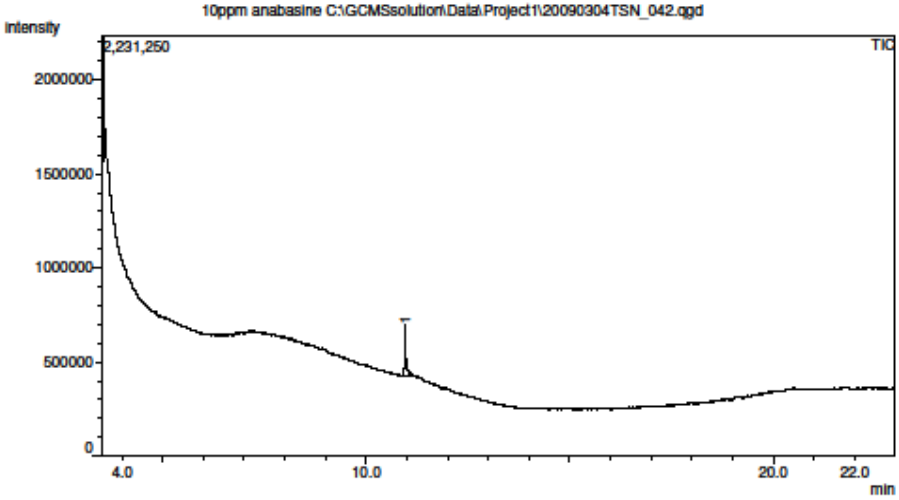
Sample Information

Analyzed by	: Admin
Analyzed	: 10/30/2009 5:38:26 PM
Sample Name	: 10ppm anabasine
Sample ID	: 10ppm anabasine
Tray	: Tray2
Vial #	: 31
Inj. Volume	: 1
Data File	: C:\GCMSsolution\Data\Project1\20090304TSN_042.qgd
Method File	: C:\GCMSsolution\Data\Project1\ZB-5MSi_8270D.qgm
Tuning File	: C:\GCMSsolution\System\Tune1\QP2010S.qgt

10ppm anabasine C:\GCMSsolution\Data\Project1\20090304TSN_042.qgd



Quantitative Result Table

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Base m/z	Name
1	10.957	770252	100.00	274551	100.00	84.00	Pyridine, 3-(2-piperidinyl)-, (S)- (Anabasine)
		770252	100.00	274551	100.00		

C:\GCMSsolution\Data\Project1\20090304TSN_042.qgd 1 / 3

Figure 13: GC MS TIC Chromatogram of ß-Nicotyrine 10ppm

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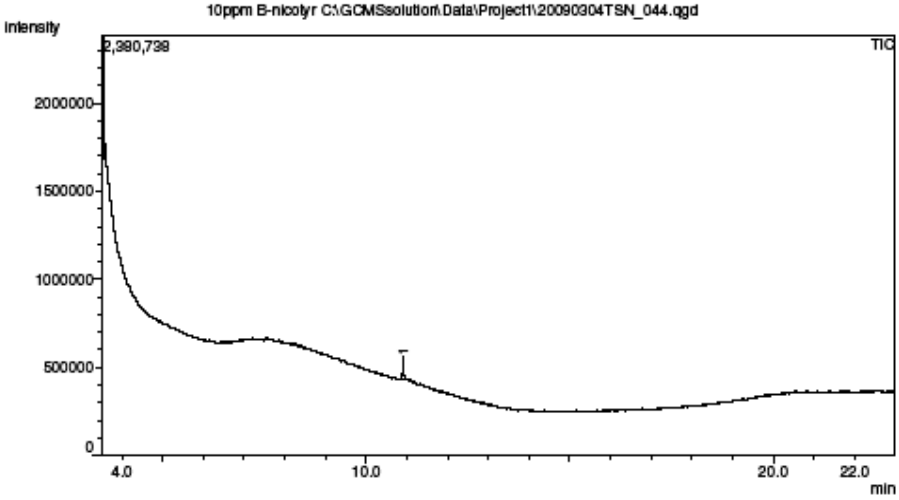
Sample Information

Analyzed by	: Admin
Analyzed	: 10/30/2009 6:43:10 PM
Sample Name	: 10ppm B-nicotyr
Sample ID	: 10ppm B-nicotyr
Tray	: Tray2
Vial #	: 33
Inj. Volume	: 1
Data File	: C:\GCMSsolution\Data\Project1\20090304TSN_044.qgd
Method File	: C:\GCMSsolution\Data\Project1\ZB-5MSi_8270D.qgm
Tuning File	: C:\GCMSsolution\System\Tune1\QP2010S.qgt

10ppm B-nicotyr C:\GCMSsolution\Data\Project1\20090304TSN_044.qgd



Quantitative Result Table

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Base m/z	Name
1	10.905	300464	100.00	135642	100.00	158.00	B-NICOTYRINE
		300464	100.00	135642	100.00		

C:\GCMSsolution\Data\Project1\20090304TSN_044.qgd 1 / 3

Figure 14: GC MS TIC Chromatogram of Cotinine 10ppm

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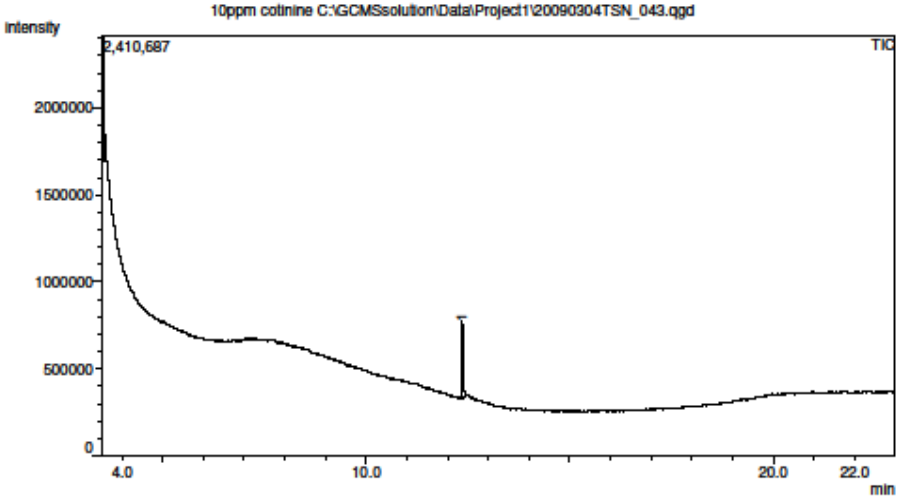
Sample Information

Analyzed by	: Admin
Analyzed	: 10/30/2009 6:10:46 PM
Sample Name	: 10ppm cotinine
Sample ID	: 10ppm cotinine
Tray	: Tray2
Vial #	: 32
Inj. Volume	: 1
Data File	: C:\GCMSsolution\Data\Project1\20090304TSN_043.qgd
Method File	: C:\GCMSsolution\Data\Project1\ZB-5MSi_8270D.qgm
Tuning File	: C:\GCMSsolution\System\Tune1\QP2010S.qgt

10ppm cotinine C:\GCMSsolution\Data\Project1\20090304TSN_043.qgd



Quantitative Result Table

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Base m/z	Name
1	12.366	831796	100.00	439455	100.00	98.00	2-Pyrrolidinone, 1-methyl-5-(3-pyridinyl)-, (S)- (CAS) Cotinine
		831796	100.00	439455	100.00		

C:\GCMSsolution\Data\Project1\20090304TSN_043.qgd 1 / 3

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Figure 15: GC MS TIC Chromatogram overlay of Acetone extracted sample(black), ß-nicotyrine (pink) and Anatabine (blue) (10ppm each)

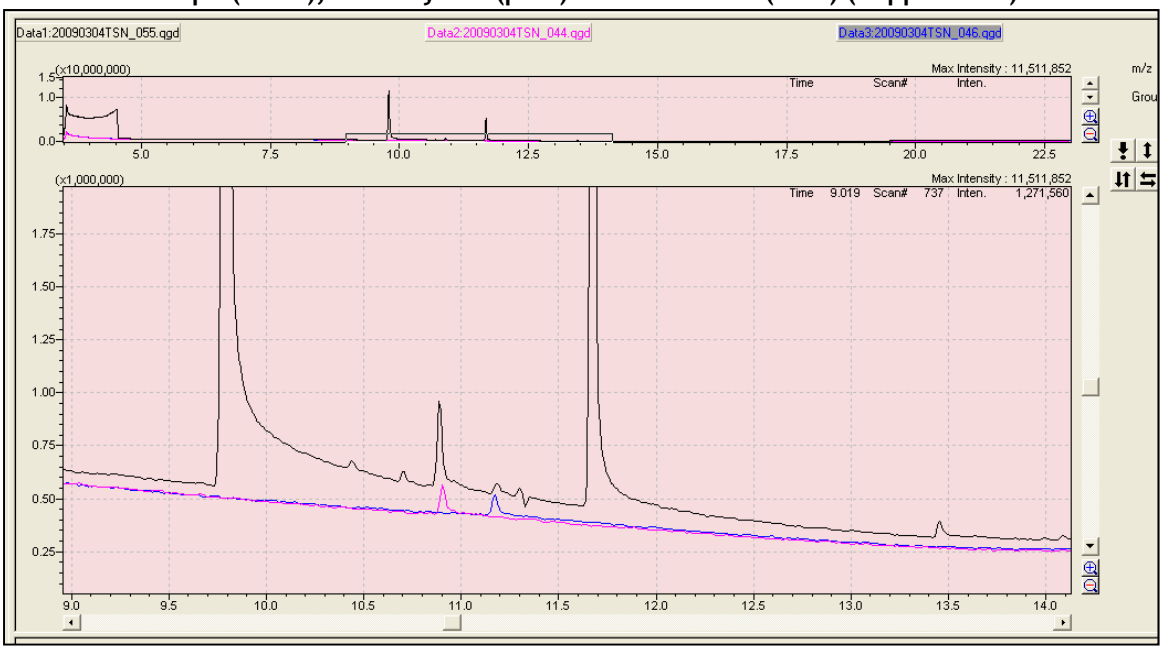
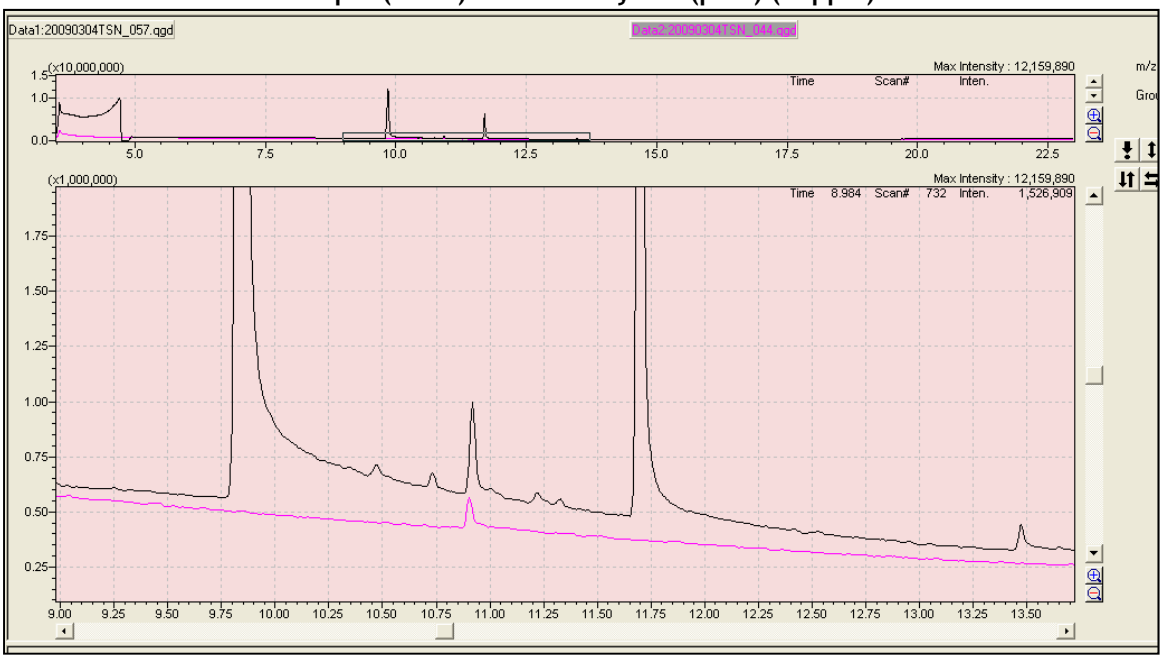


Figure 16: GC MS TIC Chromatogram overlay of Acetonitrile extracted sample (black) and ß-nicotyrine (pink) (10ppm)



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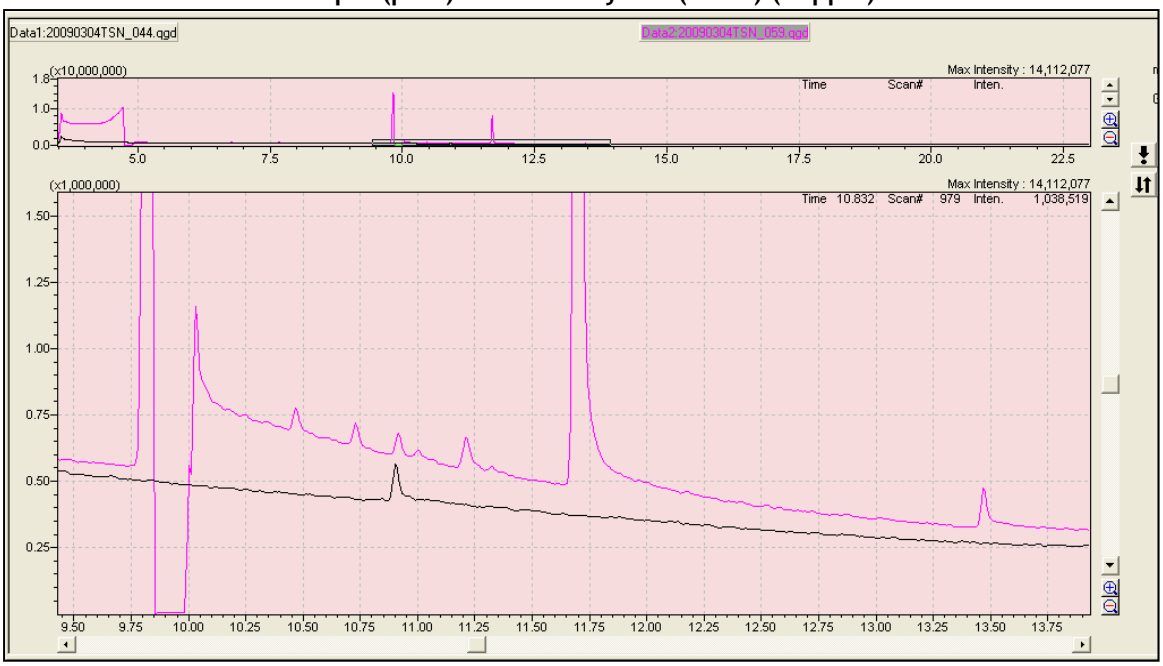
Figure 17: GC MS TIC Chromatogram overlay of Chloroform extracted sample (pink) and ß-nicotyrine (black) (10ppm)