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Case Study

Food Contact: Leachables in Tea

Released by:

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Job Number: Tea Case Study

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October 26, 2017

Customer
Contact
Address

Phone
Email

Dear Customer,

Please find enclosed the test results for your samples described as:

1. 100% pure black tea, Single Serving Cup
2. 100% pure black tea, Loose Tea Leaves
3. 100% pure black tea, Tea Bag

The following tests were performed:

1. Quadrupole Time-of-Flight Gas Chromatography Mass Spectrometry (QTOF GCMS)
2. Quadrupole Time of Flight Liquid Chromatography Mass Spectrometry (QTOF-LCMS)

Objective

The goal of this analysis was to compare leachables from the tea leaves with different brewing methods.

Summary of Results

Three Black Tea extracts were prepared via different brewing methods (labeled as *Single Serving Cup*, *Tea Bag*, and *Loose Tea Leaves*), and analyzed by QTOF-GCMS and QTOF-LCMS. Compounds detected in all three extracts were generally consistent, most of which are common natural compounds in tea leaves, such as caffeine, theophylline, and gallic acid. In addition, the *Single Serving Cup* was found to have trace levels of compounds consistent with cyclohexanone and 2-Hydroxy-iso-butyrophenone. A compound consistent with 2,4,6-tri-tert-butyl-phenol (antioxidant) was only detected in samples *Tea Bag* and *Loose Tea Leaves*.

Individual Test Results

A summary of the individual test results is provided below. All accompanying data, including spectra, has been included in the data section of this report.

Next Steps

The compounds unique to the *Single Serving Cup, Tea Bag* and *Loose Tea Leaves* samples can be quantitated by GCMS.

Sample Preparation

The brewing apparatus was first brewed with no *Single Serving Cup* installed five times in order to rinse the machine. Then 6 oz of hot water was collected from the brewing apparatus and used as a control blank. Three different formats for the tea (*Single Serving Cup, Tea Bag* or *Loose Team Leaves*) were compared. The same brand and type of tea was examined for all sample formats.

For sample *Single Serving Cup,* the Black Tea in a Single Serving Cup was brewed with 6 oz of hot water using the brewing apparatus, and the extract was collected.

For sample *Tea Bag*, the Black Tea in a tea bag was placed in a 250 mL glass container, and 6 oz of hot water from the brewing apparatus was added, and the tea bag was removed after 4 minutes.

For sample *Loose Tea Leaves*, the Black Tea in a tea bag were taken out from the tea bag, and the tea leaves were placed in a 250 mL glass container followed by adding 6 oz of hot water from the brewing apparatus. After 4 minutes, the tea extracted was collected by vacuum filtration using a paper filter.

QTOF GCMS

GCMS analysis was performed in electron impact modes. The spectra collected using electron impact (EI) ionization can be compared to the NIST mass spectral database for identification. In addition fragments can be identified using the accurate mass data collected. This ionization mode is high energy and generally causes a large amount of analyte fragmentation. In many cases the EI mass spectra collected only contain fragment ions making definitive unknown identification impossible for compounds not present in the mass spectral database. Chemical ionization (CI) provides less energy and causes significantly less fragmentation. The CI data collected can, in most cases, be used to determine the molecular formula for a particular compound using the molecular formula generation (MFG) algorithm.

Sample Preparation

An aliquot of each sample extract and the control blank was extracted with DCM at 1:1 ratio, and the DCM extracts were collected and analyzed by QTOF-GCMS without further preparation.

Results

Compounds detected in all three samples were generally consistent. The major compound detected in all three tea extracts was consistent with caffeine. Several trace level compounds consistent with cyclohexanone and 2-Hydroxy-iso-butyrophenone were only detected in sample *Single Serving Cup*, while a compound consistent with 2,4,6-tri-tert-butyl-phenol (antioxidant) was only detected in samples *Tea Bag* and *Loose Tea Leaves*.

The GCMS results are summarized in **Table 1**. **Figure 1** and **Figure 2** show overlays of chromatograms of the samples and a control blank.

Table 1
GCMS Results

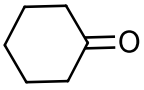
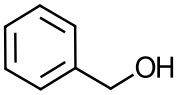
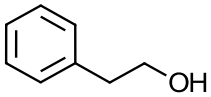
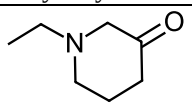
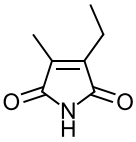
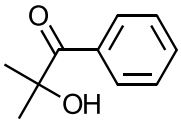
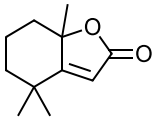
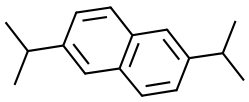
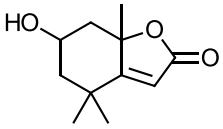
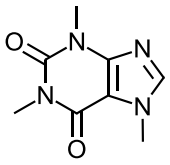
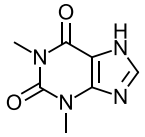
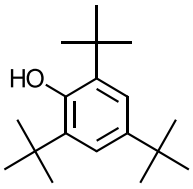
RT	Possible Identification	CAS	Samples		
			Single Serving Cup	*Tea Bag*	*Loose Tea Leaves*
7.986	 Cyclohexanone	108-94-1	X	N.D.	N.D.
10.260	 Benzyl alcohol	100-51-6	X	X	X
11.312	 Phenylethyl alcohol	60-12-8	X	X	X
11.527	 3-Piperidinone, 1-ethyl-	43152-93-8	X	X	X
12.583	 1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	20189-42-8	X	X	X
12.808	Unknown with fragment of m/z 111 (C_6H_9NO)	--	X	X	X
13.187	 2-Hydroxy-iso-butyrophenone	7473-98-5	X	N.D.	N.D.
15.554	 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	17092-92-1	X	X	X
16.435	Unknown with fragment of m/z 119	--	X	X	X

Table 1 GCMS Results					
RT	Possible Identification	CAS	Samples		
			Single Serving Cup	*Tea Bag*	*Loose Tea Leaves*
	(C_9H_{11}), and m/z 121 (C_9H_{13})				
16.616	 2,6-Diisopropylnaphthalene	24157-81-1	X	N.D.	X
16.660	2,6-Diisopropylnaphthalene isomer	--	X	X	X
16.771	Unknown with fragment of m/z 99 ($C_6H_{11}O$)	--	X	N.D.	N.D.
16.952	2,6-Diisopropylnaphthalene isomer	--	X	N.D.	X
16.982	2,6-Diisopropylnaphthalene isomer	--	X	N.D.	X
17.012	2,6-Diisopropylnaphthalene isomer	--	X	N.D.	X
17.334	 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	73410-02-3	X	X	X
17.934	 Caffeine	58-08-2	X	X	X
18.025	 Theophylline	58-55-9	X	X	X
18.238	 Phenol, 2,4,6-tri-tert-butyl-	732-26-3	N.D.	X	X
X – Detected; N.D. – Not Detected					

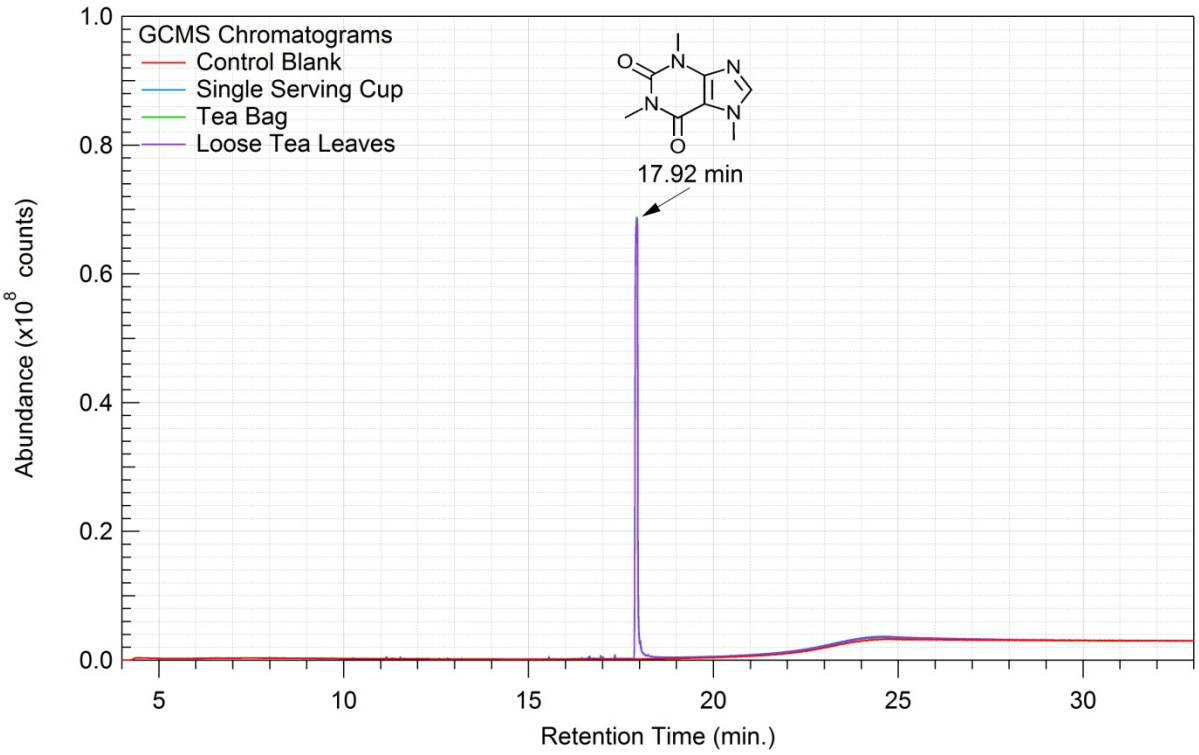


Figure 1- Overlay of GCMS chromatograms of the samples and a control blank.

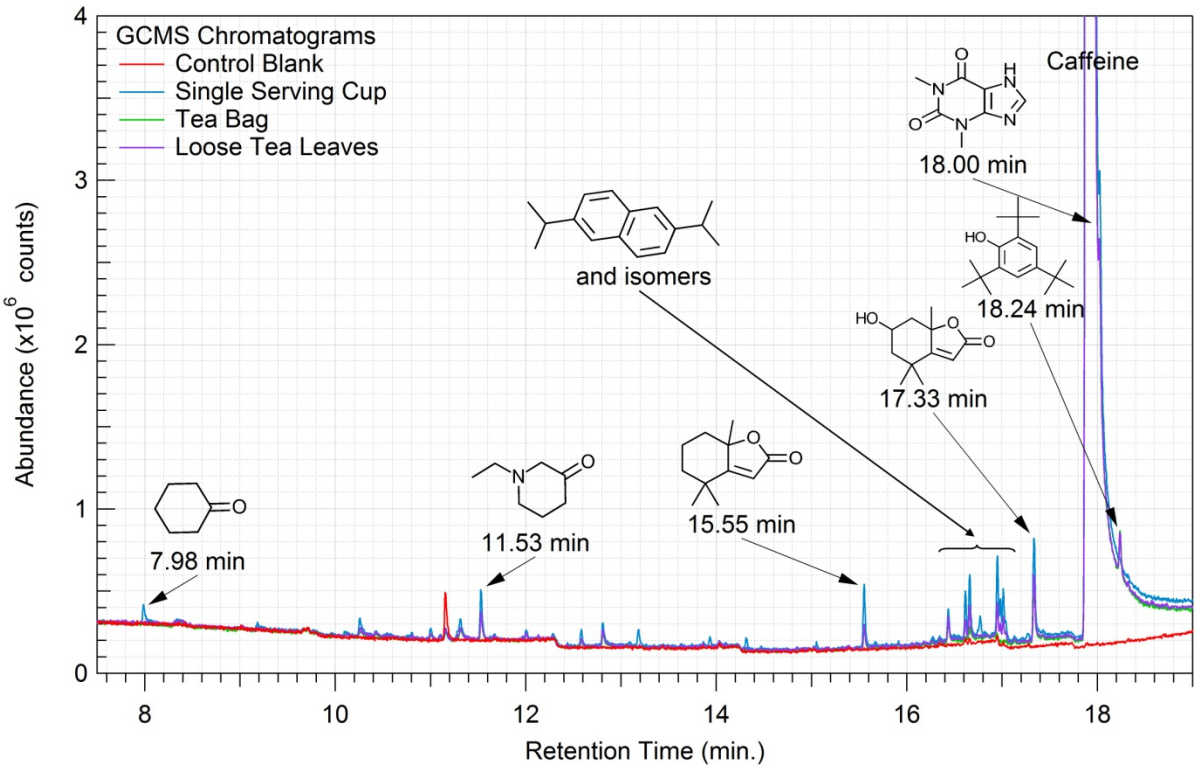


Figure 2- Overlay of GCMS chromatograms of the samples and a control blank, expanded.

QTOF LCMS

Background: QTOF-LCMS combines high mass accuracy time of flight mass spectroscopy with the power of a liquid chromatography separation to provide detailed information about the elemental composition of unknowns.

The presence of an additional quadrupole mass spectrometer (Q) provides the added capability to perform fragmentation experiments. This increases the confidence of unknown identification. It is preferable that a standard of the suspected unknown be analyzed under identical conditions as the sample. If the fragmentation patterns, high accuracy mass data, isotope patterns and LC retention times match for the unknown and standard then there is a very high probability that the identification is correct. It is possible to gain significant information about the structure of an unknown, even in cases in which standards are not available by using the molecular formula generation (MFG) algorithms contained in the Mass Hunter qualitative software.

LCMS requires that the molecule of interest be ionized. Thus, data is typically plotted in positive and negative modes indicating the charge on the ions. Ion formation is accomplished through the formation of a molecular adduct using a charge carrying species. Typical charge carriers in positive ion mode include H^+, Na^+, K^+, NH_4^+ etc. Thus the observed mass is typically the mass of the compound plus the mass of the charge carrier.

The nature of the mobile phase and the ionization conditions determine the ions formed. In negative ion, the loss of hydrogen is generally observed which results in the loss of one mass unit (1.0078 amu). Other transformations are also possible including dehydration, dimer formation, etc.

A number of plots are used to aid in interpreting QTOF-LCMS data. This includes Base Peak Chromatograms (BPC), Extracted Ion Chromatograms (EIC), Extracted Compound Chromatogram (ECC), Mass spectra (MS) and Product Ion Spectra (MSMS). A BPC is formed by plotting the most intense ion at a given retention time. This spectrum is particularly useful for identifying the retention time of unknowns. EICs are formed by plotting a single mass at all retention times. This could be considered a plot of peak intensity (~compound concentration) for a single compound (and its isomers) versus retention time. ECC's are the sum of all the ions determined to be related to a single compound.

MS spectra plot the observed masses and their intensities at a single retention time. MS/MS spectra show the fragmentation pattern for a single compound. Mass Spectra plot the mass to charge ratio (m/z) and not the mass of the compound.

All structures indicated represent best estimates based on the data observed. In most cases the MS/MS fragmentation spectra have been consulted briefly to aid in identification of possible structures.

Sample Preparation

The sample extracts and the control blank were diluted 10 times with water prior to the injection.

Results

Major compounds detected in all three samples were general consistent, most of which are common natural compounds in tea leaves, such as caffeine, theophylline, and gallic acid. No compounds consistent with common polymer degradants or polymer additives were detected in the samples.

Table 2 provides a summary of the LCMS results for the samples. **Figure 3** and **Figure 4** provide overlays of the base peak chromatograms (BPCs) obtained in positive and negative ionization modes, respectively.

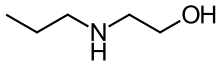
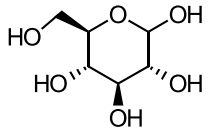
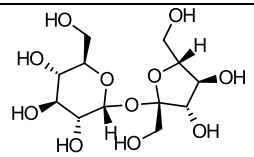
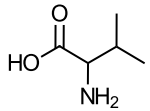
Table 2. Summary of LCMS Results										
RT	*Positive m/z*	*Negative m/z*	Mass	Best Match	Score	Diff.	Possible ID	*Single Serving Cup*	*Tea Bag*	*Loose Tea Leaves*
0.253		128.9595	129.967	$C_2 H_3 K O_4$	76.23	-1.11	Potassium salt	X	N.D.	X
0.254	104.1068		103.0995	$C_5 H_{13} N O$	99.27	1.64	 2-(Propylamino)ethanol	X	X	N.D.
0.273	381.0801		358.0904	$C_{15} H_{18} O_{10}$	85.28	-1.25	Natural compound in tea leaves	X	X	N.D.
0.275	193.0700 210.0966 215.0535 231.0261	191.0571 237.0608	192.0643	$C_7 H_{12} O_6$	95.36	-4.67	Natural compound in tea leaves	X	X	X
0.275		215.033	180.0639	$C_6 H_{12} O_6$	94.92	-2.99	 Glucose	X	X	N.D.
0.275		387.1148	342.1164	$C_{12} H_{22} O_{11}$	98.54	-0.56	 Sucrose	X	X	N.D.
0.276	118.0864 140.0684 156.0424		117.0791	$C_5 H_{11} N O_2$	90.04	-0.85	 Valine	N.D.	N.D.	X
0.306		311.0981 347.0742 357.1037	312.1054	$C_{11} H_{20} O_{10}$	97.97	0.71	Natural compound in tea leaves	X	X	X
0.343	175.1078 197.0895 213.0640	173.0934	174.1005	$C_7 H_{14} N_2 O_3$	89.09	-0.35	Amino acid, Natural compound in tea leaves	X	X	X
0.352	337.1577 359.1391 375.1131		336.1505	$C_{13} H_{24} N_2 O_8$	79.02	8.31	Natural compound in tea leaves	X	X	X

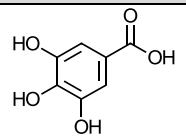
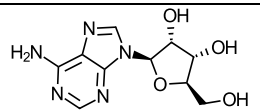
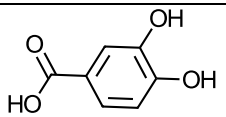
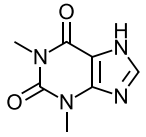
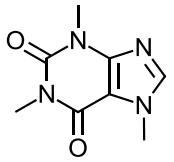
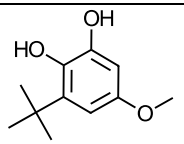
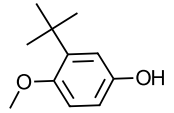
Table 2. Summary of LCMS Results										
RT	*Positive m/z*	*Negative m/z*	Mass	Best Match	Score	Diff.	Possible ID	*Single Serving Cup*	*Tea Bag*	*Loose Tea Leaves*
0.426		169.0145 283.0072	170.0217	$C_7 H_6 O_5$	89.11	-1.17	 Gallic acid	X	X	X
0.533	146.0810 168.0627 184.0372		145.0735	$C_6 H_{11} N O_3$	92.83	2.6	Amino acid, Natural compound in tea leaves	N.D.	N.D.	X
0.542	268.1041 290.0900		267.0968	$C_{10} H_{13} N_5 O_4$	99.89	-0.25	 Adenosine	N.D.	X	X
0.559	132.1015 154.0834 170.0591		131.0942	$C_6 H_{13} N O_2$	93.89	3.27	Natural compound in tea leaves	X	X	X
0.588	130.0861 152.0680 168.0410		129.0788	$C_6 H_{11} N O_2$	89.6	1.36	Natural compound in tea leaves	X	X	X
0.588	166.0860 188.0681		165.0786	$C_9 H_{11} N O_2$	87.45	2.06	Natural compound in tea leaves	X	X	N.D.
0.683		153.0193 267.0119	154.0266	$C_7 H_6 O_4$	89.8	0.11	 Protocatechuic acid	N.D.	N.D.	X
0.700	181.0722 203.0538 219.0255		180.065	$C_7 H_8 N_4 O_2$	86.96	-1.61	 Theophylline	X	X	X
1.876	365.1216		342.1323	$C_{16} H_{22} O_8$	97.37	-2.48	Natural compound in tea leaves	X	X	N.D.

Table 2. Summary of LCMS Results										
RT	*Positive m/z*	*Negative m/z*	Mass	Best Match	Score	Diff.	Possible ID	*Single Serving Cup*	*Tea Bag*	*Loose Tea Leaves*
1.984	195.0892 217.0696		194.0818	$C_8\ H_{10}\ N_4\ O_2$	81.61	-7.29	 Caffeine	X	X	X
2.026		337.0932 451.0870	338.1004	$C_{16}\ H_{18}\ O_8$	98.27	-0.72	Natural compound in tea leaves	X	X	X
2.545	401.158		378.1689	$C_{20}\ H_{26}\ O_7$	91.93	-2.73	Natural compound in tea leaves	X	X	X
2.545		609.1458 723.1348	610.1529	$C_{27}\ H_{30}\ O_{16}$	95	0.73	Natural compound in tea leaves	X	X	N.D.
2.581	465.1026 487.0843 503.0578	463.0878 577.0803	464.0945	$C_{21}\ H_{20}\ O_{12}$	81.23	2.14	Natural compound in tea leaves	X	X	X
2.590	197.1173 214.1447 219.0992 235.0738		196.1101	$C_{11}\ H_{16}\ O_3$	89.58	-0.57	 3-tert-butyl-4,5-dihydroxyanisole	X	X	N.D.
2.747	595.1674 617.1474 633.1296	593.1503 707.1416	594.1582	$C_{27}\ H_{30}\ O_{15}$	99.71	0.54	Natural compound in tea leaves	X	X	N.D.
2.784	449.1077 471.0897 487.0758	447.0927 561.0847	448.0992	$C_{21}\ H_{20}\ O_{11}$	93.19	3.12	Natural compound in tea leaves	X	X	N.D.
3.403	181.1224 198.1501 203.1023		180.1151	$C_{11}\ H_{16}\ O_2$	88.18	-0.54	 Butylated hydroxyanisole	X	N.D.	N.D.
4.013	263.1279 285.1098 301.0855		262.1207	$C_{15}\ H_{18}\ O_4$	98.41	-0.81	Natural compound in tea leaves	N.D.	X	X

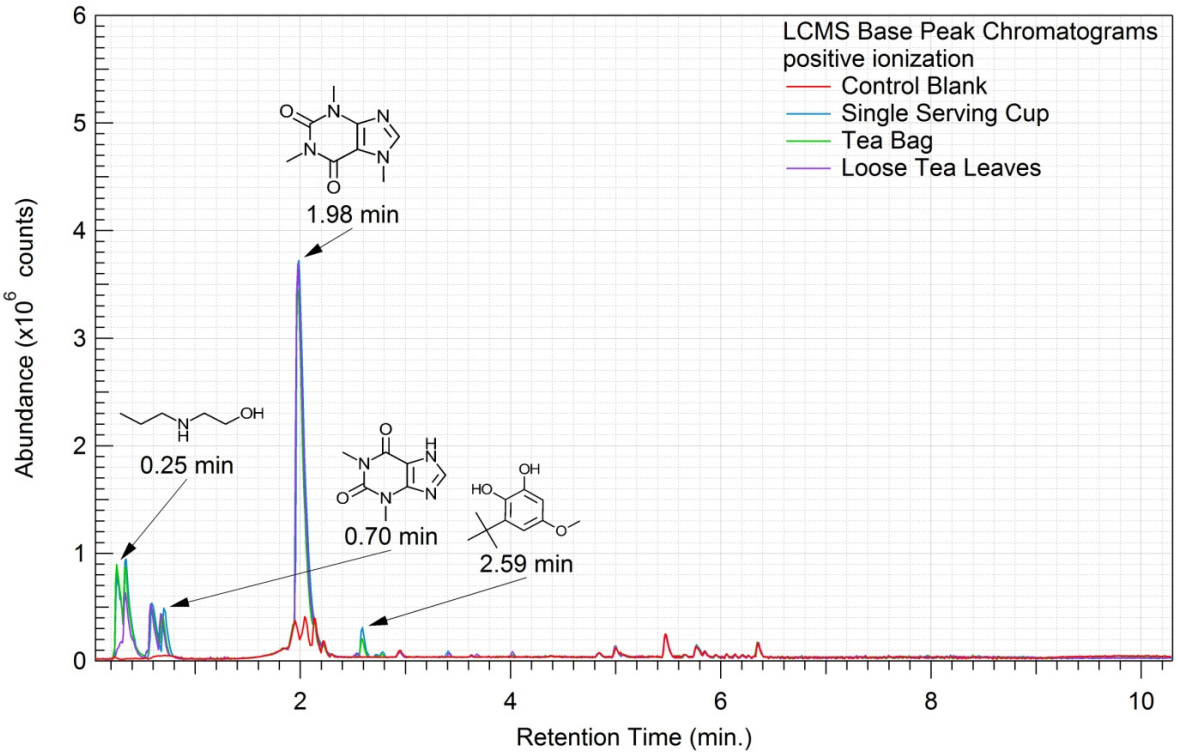


Figure 3- Overlay of LCMS base peak chromatograms, positive ionization.

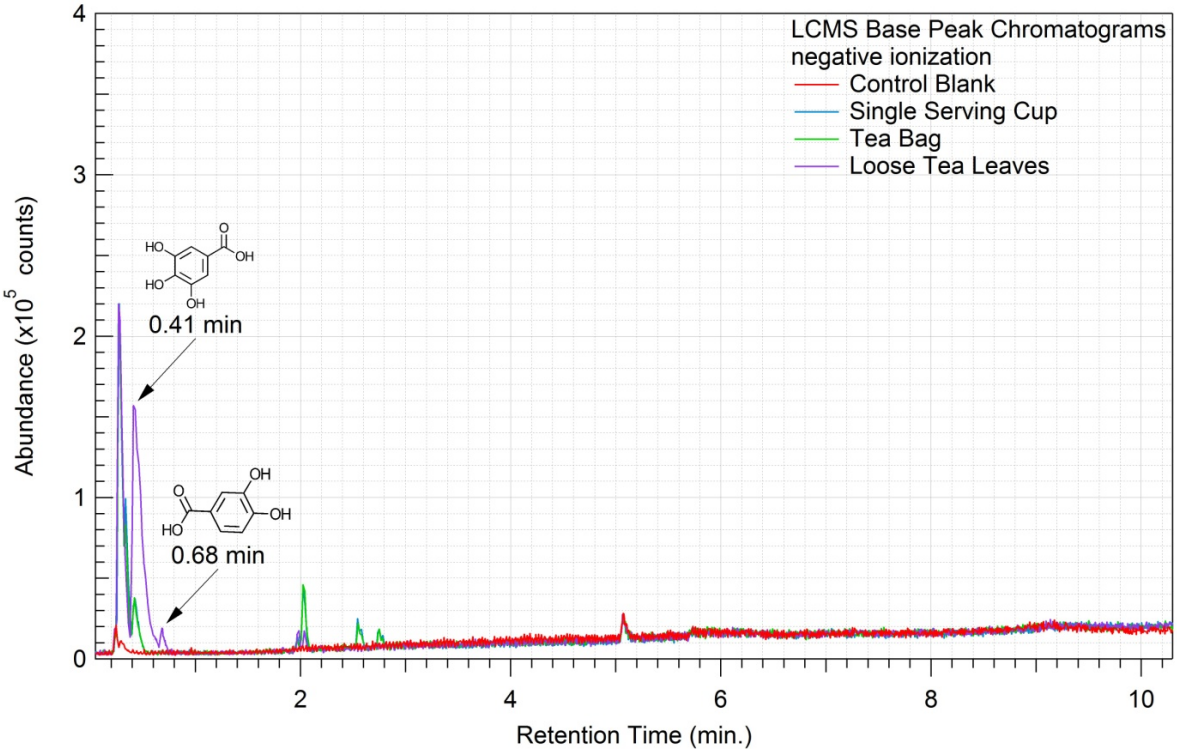


Figure 4 - Overlay of LCMS base peak chromatograms, negative ionization.

Analysis Conditions

This section of a Jordi report provides information on the methods used including instrument type, temperatures, solvents, sample preparation, etc. The specific conditions have been removed for this case study.

Closing Comments

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Jordi Labs specializes in polymer testing and has 30 years experience doing complete polymer deformulations. We are one of the few labs in the country specialized in this type of testing. We will work closely with you to help explain your test results and solve your problem. We appreciate your business and are looking forward to speaking with you concerning these results.

Sincerely,

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