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Analysis of Phenolic Antioxidants in Navy Mobility Fuels by Gas Chromatography-Mass Spectrometry

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14. ABSTRACT This study was undertaken to address the need for an improved analytical method to detect and quantify hindered phenolic antioxidant additives in Navy mobility fuels that overcomes the limitations of currently available methods. It was demonstrated that hindered phenols in fuels can be accurately quantified using capillary gas chromatography–mass spectrometry with selected ion monitoring (GC-MS-SIM) of mass fragments unique to each analyte. Using this approach, two methods were developed for the analysis of antioxidants in fuels: (1) A single column GC-MS-SIM method that, due to co-elution of fuel constituents, is only suitable for quantifying tri-t-butylphenol, and (2) a two column heart-cutting method that overcomes the problem of co-eluting fuel components, but requires modification of the instrument. The heart-cutting method was developed as a practical method for the routine determination of each of the five hindered phenolic antioxidants in any type of fuel, down to 0.05 ppm (MQL) with minimal interference from fuel. This offers a significant advantage over the traditional HPLC-ECD methods, which are more labor intensive and not capable of separating each of the individual phenolic antioxidants.														
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ABBREVIATIONS

AO	Antioxidant
ASTM	American Society for Testing and Materials
DTBP	2,6-di-t-butyl phenol
DTBMP	2,6-di-t-butyl-4-methyl phenol
ECD	Electrochemical detector
GC-MS	Gas chromatography with mass selective detection
HT	Hydrotreated
HPLC	High Performance Liquid Chromatography
In	Inhibitor
LC	Liquid Chromatography
MDL	Method detection limit
MQL	Method quantitation limit
NRL	Naval Research Laboratory
PPM	parts per million (milligrams per liter)
PSI	pounds per square inch
ROOH	hydroperoxide
RT	GC retention time
SIM	Selected Ion Monitoring
SRM	Selected Reaction Monitoring
SNR	signal-to-noise ratio
TBP	2-t-butyl phenol
TIC	Total Ion Chromatogram
TTBP	2,4,6-tri-t-butyl phenol
ULSD	Ultra-low sulfur diesel

1.0 OBJECTIVE

The objective of this study was to develop a practical and improved alternative to current methods available to quantify hindered phenolic antioxidants in fuels. This study focused on the development of a GC-MS-SIM and a quasi-two-dimensional GC-MS-SIM heart cutting method to quantify the constituents of approved antioxidant additives in jet and diesel fuels. Antioxidants are often used to meet military storage stability requirements and are required in hydrotreated and alternative fuels. It is often necessary to measure the antioxidant content of a fuel when there is question about specification compliance, or to determine if the addition an antioxidant additive package is necessary to ensure stability during long-term storage. The ability to monitor changes in concentrations of individual hindered phenolic antioxidants in fuel over time would also be a valuable laboratory tool. The only methods currently available for measuring antioxidant content in fuels are based on HPLC with electrochemical detection (HPLC-ECD). Those methods cannot discriminate between the different phenolic antioxidants that comprise the additive packages. HPLC-ECD analyses require specialized equipment, are difficult to conduct and are time consuming.

In this work, two fuel independent methods were successfully developed for the determination of the hindered phenolic antioxidants that are approved for use in Navy diesel fuels.

1. A single column GC-MS-SIM method which does not require specialized instrumentation ensuring analysis on standard GC-MS equipment. Co-eluting fuel constituents can interfere with the analytes of interest.
2. A quasi-two-dimensional GC-MS-SIM method employing a Deans switch to heart-cut targeted analytes onto a more polar secondary column for improved selectivity and elimination of interfering fuel constituents.

2.0 BACKGROUND

2.1 Fuel Autoxidation. Fuel stability is defined as the resistance of a fuel to undergo chemical processes that lead to either undesirable changes in properties or the formation of insoluble reaction products such as gums and sediments. Two types of stability, low-temperature storage and high-temperature thermal oxidation, are of concern. It is widely accepted that free-radical autoxidation is the predominant chemical mechanism that affects fuel stability during storage and use. A generalized mechanism for hydrocarbon autoxidation is shown in Scheme 1. Autoxidative degradation involves chemical changes that lead to oxidation of fuel molecules to form a variety of oxygenated species, often resulting in the accumulation of hydroperoxides (ROOH), as shown in equation 4. Hydroperoxides have been known to attack elastomers in the fuel system, particularly in the fuel control lines. Hydroperoxides can also play a key role in the degradation processes of aviation fuels by initiating a variety of free-radical reactions. Heteroatomic (i.e.,

nitrogen, sulfur, and oxygen) bearing species are particularly subject to involvement in these types of reactions.

Initiation:



Propagation:



Chain Branching:



Termination:



Scheme 1. Generalized hydrocarbon free-radical autoxidation mechanism.

Antioxidant additives have been developed and are widely used to inhibit this free-radical process by donating a hydrogen radical, which serves to terminate the radical chains, as shown in Scheme 2. These antioxidants are generally phenolic and are often hindered at the 2- and 5-positions to sterically stabilize the resulting phenoxy radical. The antioxidant 2,5-di-*t*-butyl-4-methylphenol (BHT) has been successfully used as an antioxidant in a wide variety of materials, where the electron donating character of the 4-methyl group further stabilizes the phenoxy radical. However, electron delocalization can occur to produce the 4-methyl radical, which can further react in accordance with equations 11 – 13. It has been shown¹ that two 2,5-di-*t*-butylphenol-4-methyl radicals can react with oxygen to form an oxygen bridged adduct (eq. 14) which has been reported² to be stable up to temperatures between 60°C and 150°C (eq. 15) .

Since hydrotreatment tends to remove naturally occurring phenols and thiols that can act to inhibit autoxidation, alternative fuels produced by hydrorefining tend to more readily undergo autoxidation and can be unstable without the aid of antioxidants. Previous examinations of

additive behavior on diesel fuel storage stability at elevated temperatures have produced mixed results. At 100 °C and 100 psig of oxygen, reductions in insolubles and hydroperoxides were reported by Nixon³ in the presence of two different hindered phenols, whereas these same antioxidants were ineffective in ASTM D4625⁴ long-term storage tests at atmospheric pressure and 43°C.

Inhibition:



Reactions with oxygen:



Disproportionation:



Scheme 2. Free-radical inhibition by hydrogen atom donation (In = inhibitor).

The effectiveness of phenolic antioxidants is related to the structure and concentration of the additive as well as the age and composition of the fuel. In addition, the nature of the test employed to assess thermal stability has often influenced the behavior of the antioxidants. While phenolic antioxidants have been used with varying degrees of success, evidence suggests that they are more effective^{5,6} when added at the refinery, or shortly thereafter.

2.2 Determination of Antioxidants in Fuels

Two analytical approaches, high performance liquid chromatography (HPLC) and GC have been used for the separation of phenolic AO compounds from a fuel sample matrix. Work done by Diehl et al.⁷ involved the use of (liquid chromatography) LC-MS with atmospheric pressure chemical ionization or electrospray to observe phenols and alcohols in gasoline and diesel fuels. Fuels were treated with ferrocene carboxylic acid chloride to derivatize phenolic compounds to

improve the ionization efficiency. Phenolic compounds were isolated from the fuel by several passes through silica gel columns. While Diehl et al. were able to resolve several types of the alcohols and phenolic compounds by LC-MS, the limited separation efficiency of LC prevented the separation of isomers. In SIM mode, the limit of detection was roughly 5 ppb for the investigated phenolic compounds, while in SRM mode 5x to 10x improvements were made in detection limit due to the improved selectivity from the decrease in duty cycle. One advantage to this technique is the ability to detect high molecular weight phenolic compounds. However, the tedious extraction procedure does not make this an ideal method for regular screening of AO in fuels. Additionally, common AO fuel packages consist of compounds with molecular weights below 300 m/z, making analysis by GC practical.

Bernbei et al.⁸ used a combined approach using HPLC with electrochemical detection (ECD) and GC-MS in SIM mode to look at specific AO's in fuels, namely 2,6-di-tert-butylmethylphenol, 2,4-dimethyl-6-tert-butylphenol, and 2,6-di-tert-butylphenol. The goal of this study was to reduce the amount of sample pre-treatment. HPLC-ECD requires no sample pre-treatment before analysis with a more rapid analysis time and resolution above 2.5 between the three analytes. The GC-MS method also shows greater resolution with a slightly longer analysis time (14 min). Each of these three compounds was easily isolated from the jet fuel matrix using the selectivity of the detectors and the resolution of the separation technique. This method maintained linearity over a wide calibration range from 5-100 ppm of AO. However, for the study of trace concentrations of AO in fuel, improvements to the MDL would be necessary. Additionally, HPLC-ECD lacks the required sensitivity to 2,6-di-tert-butylphenol (DTBP) and 2,4-dimethyl-6-tert-butylphenol (DMTBP).

GC methods are more commonly used for the analysis of phenolic compounds in fuel, due to the volatile nature of fuel components and the high separation efficiency of GC. Using a liquid-liquid extraction to separate the polar compounds from a jet fuel, Shin et al.⁹ were able to detect DTBP and DMTBP, and then analyze the extracted material by GC-MS. The Shin method improved the MDL to below 0.01 ppm. However, the liquid-liquid extraction in this method is not only labor intensive, but it also increases the inherent error in the measurement since it is dependent on accurately removing all the extraction solvent. Shin later improved the method by replacing the liquid-liquid extraction with solid phase extraction. The solid phase extraction is a less cumbersome extraction technique but had lower recovery efficiency when compared to the liquid-liquid extraction, and resulted in increasing the MDL to 0.5 ppm.

Heart-Cutting GC. When separating and quantifying analytes from a complicated sample matrix like fuel, it is necessary to minimize interfering compounds that mask the analytes response in order to obtain a more accurate and reproducible measurement. Jet and diesel fuels are complicated mixtures of a wide variety of hydrocarbons. The discrimination of these phenolic analytes in fuel will be difficult with a single GC column, due to co-elution of fuel constituents. Further separation on the basis of polarity can be obtained by the use of a second column with differing chemical selectivity. Simply connecting two columns in series will not

achieve the desired results when the second column becomes saturated. The technique of heart-cutting prevents the secondary column from being overwhelmed with material from the sample, and provides improved separation of a selected portion of the eluent from the first column through implementation of different column chemistry. Instrumentally, this is accomplished through the use of a pneumatically operated column flow switching device, known as a Deans switch. Heart-cutting was used in this study to attain separation of the phenolic analytes from co-eluting fuel constituents.

3.0 EXPERIMENTAL

Initially, a single column GC-MS-SIM method was developed but co-eluting fuel constituents limited the detection of all AO compounds except TTBP. This led to the development of a two-column method employing a Deans switch (Agilent Technologies). Both methods are reported here. In both instances, an Agilent (Santa Clara, CA) 7980/5975C GC-MS system was used. The ion source was operated in the electron ionization mode (70 eV, 300°C) using Agilent high-temperature filaments. Transfer line temperature was maintained at 250°C. The inlet used a Restek (Bellefonte, PA) thermolite 11mm septa with either an Agilent ultra-inert low pressure drop liner with deactivated glass wool or a Restek low pressure drop liner with deactivated wool. Injections were made using an Agilent 7693 autosampler with an Agilent 10 L gold standard syringe. Needle washes (n=3) of both dichloromethane (Fischer Scientific, ACS reagent grade) and heptane (Sigma-Aldrich, ACS reagent grade) were standard after each injection. Target analytes TBP (Aldrich, 99%), DTBP (Aldrich, 99%), DTBMP (Fischer Scientific, HPLC grade) and TTBMP (Aldrich, 98%) and fuel simulant dodecane (Sigma Aldrich, 99+%) were used as received. The antioxidant additive package AO-37 was obtained from Innospec and used as received.

Calibration Standards. Calibration standards containing 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.20, 0.1, and 0.05 ppm of each compound were prepared by serial dilution of a stock calibration mixture containing each of the AO compounds at 100 ppm in an additive-free F-76. All samples were injected at room temperature. It was found that better results are obtained when an additive-free fuel that most closely matches the sample matrix is used to prepare the standards.

3.1 Single Column Method

Neat one microliter samples were injected using a 100:1 split ratio with an injector temperature of 260 °C. The initial oven temperature was 170 °C which was held for 5 minutes, then ramped at 10 °C min⁻¹ to 230 °C. To ensure that any residual materials are baked off of the column, the oven was held at 300°C for 3 min after the oven program had completed. The column was an

Agilent DB-1MS, 60 m x 250 μm x 0.25 μm using helium as the carrier gas with a flow rate of 1.2 mL min^{-1} . The MS was run in SIM mode with the target ions listed in Table 1.

Table 1. Retention time and target ions for each compound, used in determining the SIM parameters (P = parent ion). Note that these timings are instrument dependent.

Compound	Acronym	Target Ions, m/z
2-tert-butylphenol	TBP	135P, 107, 150
2,6-di-tert-butylphenol	DTBP	191P, 163, 206
2,6-di-tert-butyl-4-methylphenol	DTBMP	205P, 177, 220
2,4,6-tri-tert-butylphenol	TTBP	247P, 248, 262
2,6-di-tert-butyl-4-sec-butyl-phenol	DTBSBP	233P, 247, 262

3.2 Heart-Cutting Method

The Agilent Technologies Deans Switch Calculator was used to determine the appropriate method settings for running the GC under the designated conditions. Within this calculator, the correct columns, column lengths and column diameters are entered in the appropriate boxes in order for the accurate values to be calculated for use in the method. In accordance with recommendations in the Agilent Deans Switch guide, these methods were developed using constant pressure mode. The selected column conditions, as well as starting temperature and starting flow values, were used to determine the inlet and pressure control module (PCM) pressures for the method (the PCM pressure is controlled by the Auxiliary EPC on Agilent 7890A GC systems).

Valve Timing. The Deans switch valve timing was determined by directing all primary column effluent to the flame ionization detector (FID). The retention time windows for each compound shown in Table 2 were thus obtained from the analyte retention times in heptane or methylene chloride. Typically, a window of 0.1 seconds both before and after each retention time was sufficient. It is important to note that, while similar, the retention time windows for any particular instrument can vary somewhat due to variations in column length or stationary phase condition. Thus, it is important that these timings be verified on the particular instrument used before proceeding with the analysis.

Operating Conditions. The FID heater was set to 300°C with a hydrogen flow of 30 mL min^{-1} . The air flow was set to 400 mL min^{-1} and the makeup gas flow (He) to 25 mL min^{-1} . Neat samples were injected using a split flow of 200 mL min^{-1} with split ratio of 100:1. The inlet was set to 285°C, the pressure set to 21.72 psi and septum purge flow to 3 mL min^{-1} . The MS transfer line temperature was maintained at 250°C. There are three columns used in this method, i.e., the

two columns used for the separation and a restrictor that couples the Deans Switch to the primary detector. The restrictor tubing length and diameter must match the values determined by the Agilent Calculator, which in this instance was 0.28 μm x 100 μm . Both of the other columns used for this method were 15 m x 250 μm x 0.25 μm , where the primary nonpolar column was a Restek Rxi-1ms and the secondary polar column was a Restek Rxi-17Sil MS. The pressure for column 1 was set to 21.72 psi. The pressure for columns 2 and 3 was set to 14.54 psi. Both columns were run at constant pressure.

Table 2. Event times, position of valves and set points for the Deans switch valve for each of the target antioxidant compounds. These retention time windows were determined using the FID chromatogram of the analytes in neat heptane.

Analyte	Time (min)	Event Type	Position	Setpoint
2-tert-butylphenol	7.9	Valve	Valve 1	On
	8.1	Valve	Valve 1	Off
2,6-di-tert-butylphenol	11.2	Valve	Valve 1	On
	11.6	Valve	Valve 1	Off
2,6-di-tert-butyl-4-methylphenol	13.0	Valve	Valve 1	On
	13.5	Valve	Valve 1	Off
2,4,6-tri-tert-butylphenol	15.4	Valve	Valve 1	On
	15.6	Valve	Valve 1	Off
2,6-di-tert-butyl-4-sec-butyl-phenol	16.1	Valve	Valve 1	On
	16.4	Valve	Valve 1	Off

The initial oven temperature was set to 60°C. The temperature was then increased at a rate of 20 °C/min to 100°C and held for 4 minutes. The temperature was then increased 10°C/min to 140°C and held for 2 minutes, followed by a final temperature ramp of 10°C/min ramp to 190°C/min with a post run at 300°C for 5 minutes. Equilibration time was 0.25 minutes and the columns used had a maximum operating temperature of 320°C. This analysis was performed in scan mode for the MS to allow the user to determine the appropriate times to switch between the various ions for selected ion monitoring (SIM) of each AO compound. The SIM ions used for each antioxidant compound were the same as listed in Table 1. The solvent delay was set to 6 minutes before MS acquisition started.

3.3 Calculations

Once the calibration curves are calculated and the data from the test samples are acquired, the quantitation of the antioxidants can be manually calculated with an MS Excel Spreadsheet, or in a more automated manner, using the Agilent MassHunter Quantitation version B.050.1 and Supplemental version B.06.00. Stepwise instructions are given for both methods in Appendix B,

and a detailed description of data importing and quantitation with MassHunter software is given in Appendix C.

4.0 RESULTS AND DISCUSSION

4.1 Single Column Method

An example total ion chromatogram of the calibration standards in dodecane is shown in Figure 1. The TBP elutes with the solvent tail of the dodecane fuel simulant, but none of the impurities in the dodecane interfere. The remaining three analytes were cleanly resolved. Four criteria were used to define a positive identification. First, the candidate peak must have a retention time within ± 0.20 minutes of the average retention time observed in the standards. Second, the target ions must co-maximize within ± 2 scans. Third, at least one secondary ion must be present with a signal-to-noise ratio (SNR) greater than 3. Lastly, at least one of the ion ratios of the primary to secondary ions must be within 20% relative to the calibration standards.

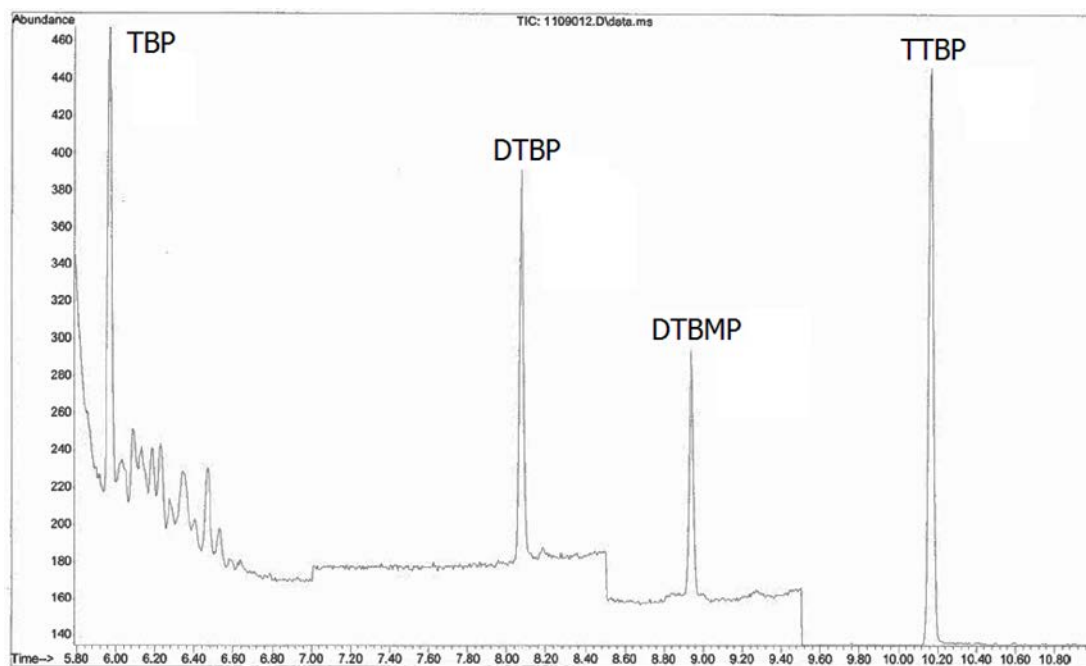


Figure 1. Total ion chromatogram for a 0.40 ppm standard of the Innospec AO-37 antioxidant package. Discontinuous breaks in the baseline mark the changes between different sets of SIM ions.

Method detection limits (MDL) and method quantitation limits (MQL) were determined by preparing seven spiked samples. The samples were prepared at a nominal concentration of 40 ppb and all analytes were detected with a SNR between 3 and 7 for the largest secondary ion. Method detection limits (MDL) and method quantitation limits (MQL) were determined at the 95% confidence limits and are reported in Table 3. These detection limits could be lowered if the hit criteria were changed to only require a signal-to-noise ratio $SNR > 3$ for the TIC as the secondary ions for multiply substituted phenol analytes are generally less than a third of the intensity of the primary ions. However this would significantly reduce the quality of the identification in a more complex matrix, such as a fuel.

The detection limits reported in Table 3 could likely be reduced by an additional two orders of magnitude by modifying the method to use a splitless injection. If this method is to be applicable to the entire expected concentration range, up to 24 ppm as specified by MIL-DTL-83133G, a calibration curve covering three orders of magnitude in concentration will be required. Maintaining linearity over such a wide concentration range is challenging on a GC-MS, and would likely require two separate calibration curves; one for concentrations greater than 1 ppm and one for concentrations less than 1 ppm. Alternatively, previous work not reported here has shown, a quadratic calibration curve could be generated that can cover the entire expected concentration range with an R^2 value greater than 0.999 and a mean relative prediction error of less than 15%.

Table 3. Analyte detection limits for the single column method.

Compound	MDL, ppb ($\mu\text{g/L}$)	MQL, ppb ($\mu\text{g/L}$)
2-tert-butylphenol	36.3	121
2,6-di-tert-butylphenol	39.3	131
2,6-di-tert-butyl-4-methylphenol	29.9	100
2,4,6-tri-tert-butylphenol	31.4	109

Analysis of AO-37. The Innospec additive package AO-37 was examined by the single column method to determine the composition and ensure that the major components of this commonly used additive were represented in the calibration standards. Analysis of a sample of dodecane containing 24 ppm (mg/L) of AO-37, revealed the composition as shown in Table 4.

Table 4. Concentrations of the targeted analytes found in a 24 ppm (mg/L) solution of the AO-37 antioxidant additive package in dodecane with the single column method.

Compound	Abbreviation	RT (min)	PPM (mg/L)
2,6-di-t-butylphenol	2,6-DTBP	9.68	18.0
2,4,6-tri-t-butylphenol	2,4,6-TTBP	11.78	4.2
2-t-butylphenol	2-TBP	7.22	0.9
2,4-di-t-butylphenol	2,4-DTBP	10.41	0.2
2,5-di-t-butylphenol	2,5-DTBP	11.02	0.2
4-t-butylphenol	4-TBP	8.77	0.2

4.2 Heart-cutting method

It quickly became evident when applying the single column GC-MS-SIM method to fuel samples, that co-eluting fuel constituents were interfering with the analyte ions of interest. This is a consequence of the fact that if there are too many ions entering the mass analyzer, the analyte signal will be suppressed. Figure 2 shows a TIC of a diesel fuel that contains the four phenolic oxidants in Table 3, from addition of the standard AO-37 additive package at 24 ppm, where it is clear that the phenolic antioxidants are being masked by fuel components. Only the tri-t-butylphenol (TTBP) was sufficiently unmasked by co-eluting fuel constituents to be quantifiable by this method. The single column method was successfully employed to measure TTBP depletion in hydrotreated Jet A fuels during ASTM D5304¹⁰ storage stability testing in an earlier NRL study¹¹. However, it is not necessarily valid to assume that all the components of the antioxidant package would be consumed at equal rates during thermal stress. Extrapolation from a single antioxidant would also not address comingling of different additive packages. Therefore, it was necessary to develop a method whereby all the antioxidants could be measured independently in fuel.

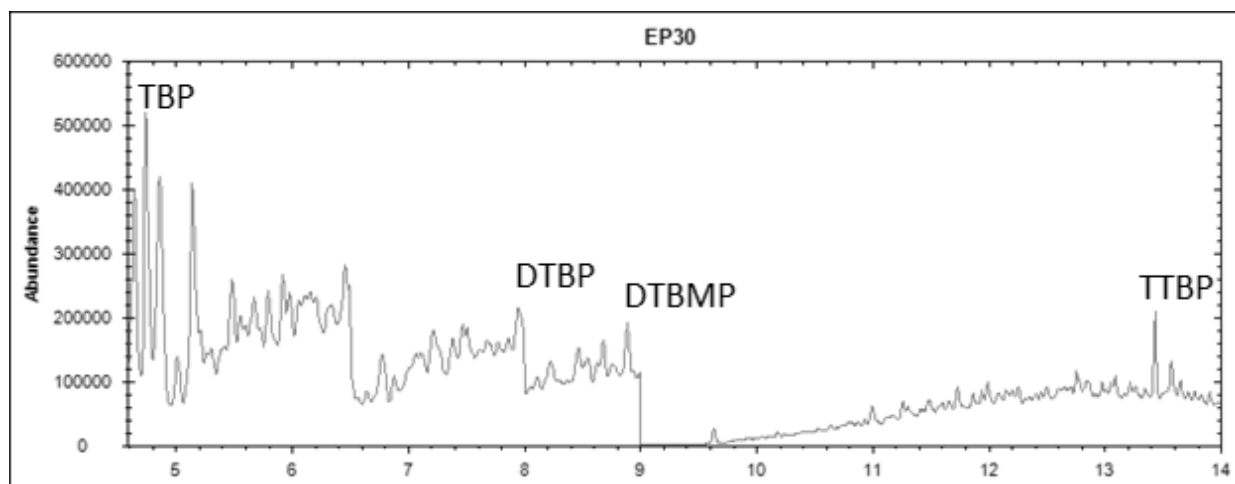


Figure 2. Total ion chromatogram from an F-76 diesel fuel containing 24 ppm of the AO-37 additive package, analyzed using single column GC-MS-SIM method.

Using the heart-cut method on the same fuel, one can easily detect all four AO present in the AO-37 sample in figure 3. Another commonly used antioxidant, 2,6-di-tert-butyl-4-sec-butylphenol (DTBSBP) was also included in the method.

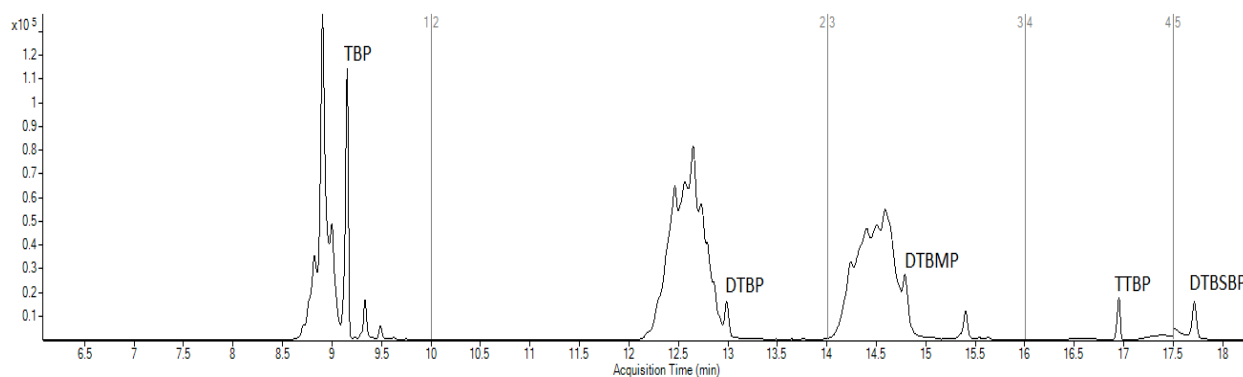


Figure 3. Total ion chromatogram from an ultra-low sulfur diesel fuel containing 6.25 ppm of the AO-37 additive package, analyzed using dual column Deans switch GC-MS-SIM method.

The MDL was determined with analyte concentrations as low as 10 ppb ($n=10$) in a calibration mixture diluted in heptane. The phenolic analytes TBP, DTBP, DTBMP and TTBP still maintained a SNR of greater than 10. MQL were measured by spiking a fuel sample with the appropriate amount of standard to give a more practical detection limit, with measured SNR of 7.

Table 5. The method detection and quantitation limits determined for the single column method.

Compound	MDL (ppb)	MQL (ppb)
2-tert-butylphenol	<10	97
2,6-di-tert-butylphenol	<10	190
2,6-di-tert-butyl-4-methylphenol	<10	395
2,4,6-tri-tert-butylphenol	<10	17
2,6-di-tert-butyl-4-sec-butylphenol	190	395

The linearity of this method is greater than the single column method over a larger concentration range prepared in heptane, maintaining an R^2 of 0.966 between 0.2 ppm and 50 ppm for TBP, DTBP, and TTBP. A range of 0.8 ppm to 50 ppm for DTBMP and DTBSBP achieved an R^2 of 0.942. Using methylene chloride as a sample solvent yielded better calibration curve reproducibility and linearity over the concentration range of 0.375 ppm to 50 ppm with R^2 at 0.99 for all five target analytes. It is likely that polarity mismatches between heptane and the more polar and aromatic phenolic analytes, resulted in sample preparation variabilities which would account for the improved linearity in methylene chloride. Figures 4 and 5 show the reproducibly over two days in heptane and methylene chloride calibration solvents, respectively.

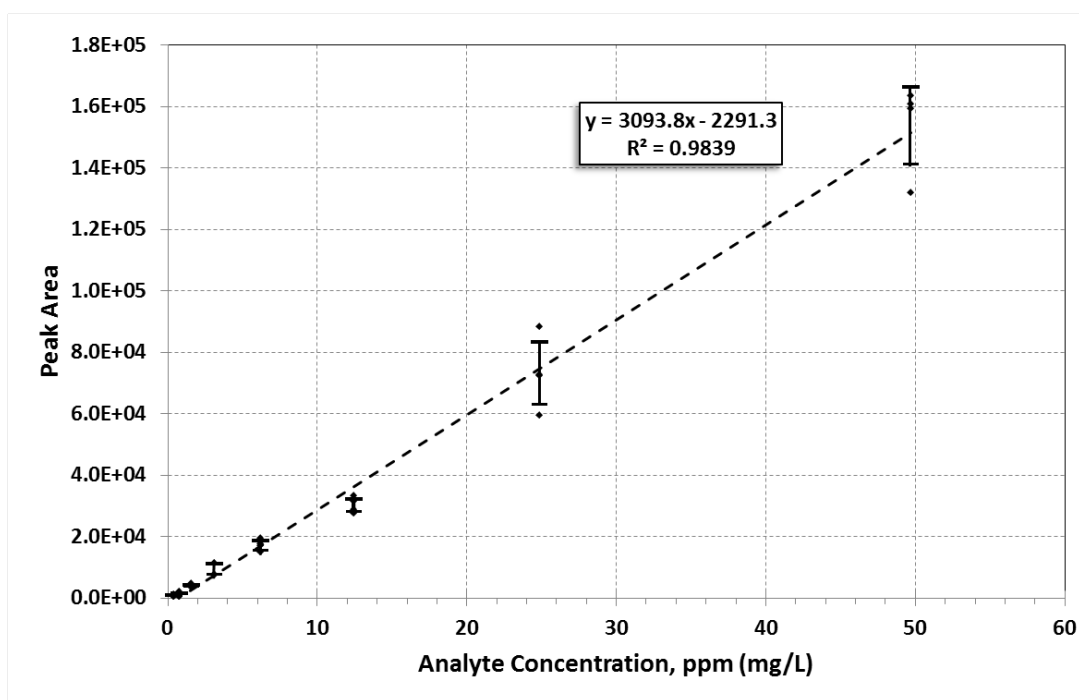


Figure 4. Two day calibration reproducibility from 0.388 ppm to 49.7 ppm TBP in heptane. Error bars represent $\pm$ a 95% confidence interval.

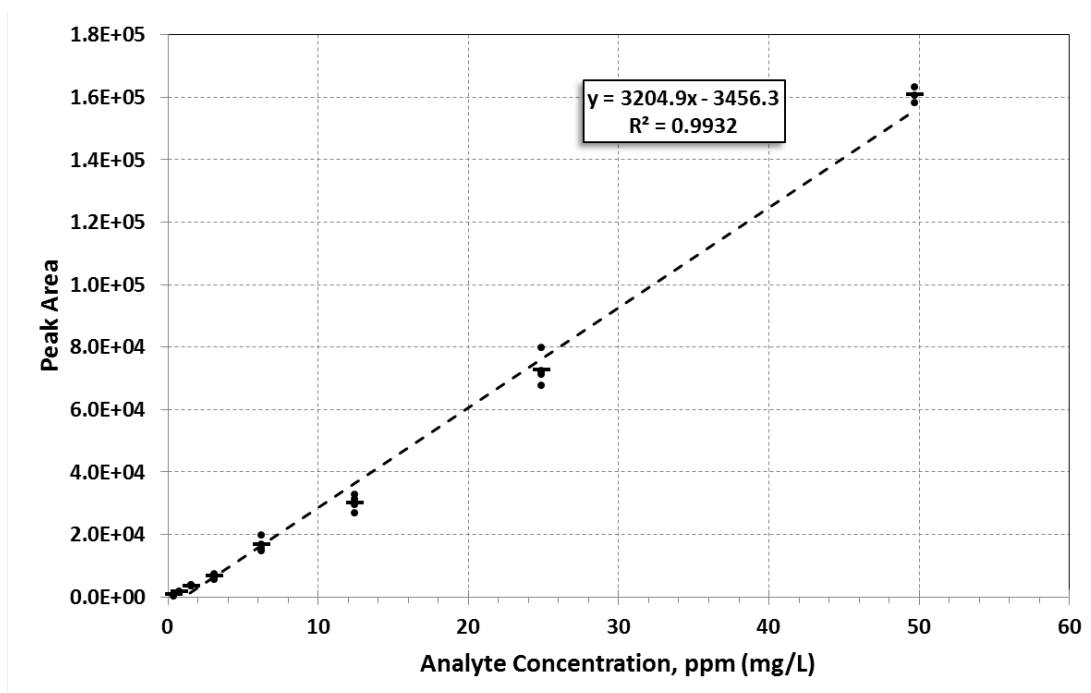


Figure 5. Two day calibration reproducibility from 0.388 ppm to 49.7 ppm TBP in dichloromethane. Error bars represent $\pm$ a 95% confidence interval.

Linearity was further improved for all analytes in the calibration standards from 0.09 ppm to 100 ppm when they were prepared in an AO free fuel. When standards were prepared in an AO free fuel, there was no detector saturation evident at high concentrations (greater than 50 ppm), whereas saturation was prominent with standards that used heptane instead of fuel. The calibration values in Table 6 and the curves shown graphically in Appendix A, figures A-1 through A-5, indicate that all analytes produced curves with an R^2 of 0.999 or greater. Using fuel as a solvent thus ensures a more accurate match of sample matrix between the calibration standards and the fuel samples.

Table 6. Calibration values obtained for the five analytes in an additive-free fuel.

Analyte	Slope (Peak area/PPM)	Linear Corr. (R^2)
2-tert-butylphenol	2883.6	0.9997
2,6-di-tert-butylphenol	4506.8	0.9998
2,6-di-tert-butyl-4-methylphenol	4504.7	0.9996
2,4,6-tri-tert-butylphenol	5581.4	0.9999
2,6-di-tert-butyl-4-sec-butylphenol	5731.8	0.9995

It should be noted that the calibration curves can vary with changes in the GC column and mass analyzer source. It is thus recommended that calibration be conducted at regular intervals, preferably just prior to analysis. The calibration curve linearity for the all five analytes should be close to what is shown here, before proceeding with the analysis.

5.0 CONCLUSIONS

It has been demonstrated that hindered phenols in fuels can be accurately quantified using GC-MS with selected ion monitoring of mass fragments unique to each analyte. Using this approach, two methods have been developed for the analysis of the AO components in fuel. The single column GC-MS-SIM method requires no modification of the GC-MS instrument, but is only suitable for quantifying one component, TTBP, in fuels, due to co-elution of fuel constituents. The heart-cutting method required installation of a Deans switch and a secondary column in the GC, with appropriate software control, but overcomes the problem of co-eluting fuel components. The heart-cutting method was thus developed as a practical method for the routine determination of each of the five hindered phenolic antioxidants in any type of fuel, down to 0.05 ppm (MQL) with minimal interference from fuel. This offers a significant advantage over the traditional HPLC-ECD methods which are more labor intensive and not capable of separating each of the individual phenolic antioxidants.

6.0 RECOMMENDATIONS FOR FURTHER STUDY

While the heart-cutting GC-MS-SIM method has been shown to be a significant improvement over existing methods to detect and quantify antioxidants in fuels, it does require modification of a standard GC-MS in order to conduct the analysis. An instrument that should be better suited for this analysis without modification is a GC-MS/MS triple quadrupole system operating in selected reaction monitoring (SRM) mode. While not as universally available as a standard GC-MS, it is anticipated that the GC-MS/MS would allow for greater detector selectivity so that only one column would be required for the analysis. The use of an internal standard such as 2,6 dimethylphenol, diphenylamine, or isotopes may also improve long term reproducibility between sample batches. It is thus recommended that the GC-MS/MS-SRM be investigated as an alternative to the heart-cutting GC-MS-SIM method, for those laboratories that have access to this instrumentation.

7.0 ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Navy Fuels & Lubes CFT, through the Naval Air Systems Command, Air 4.4.5 for providing funding support of this program.

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APPENDIX A: Calibration Curves for the Five Target Analytes

The following calibration curves were obtained for the five target analytes using concentrations ranging from 0.09 ppm to 100 ppm., measured in an additive-free fuel.

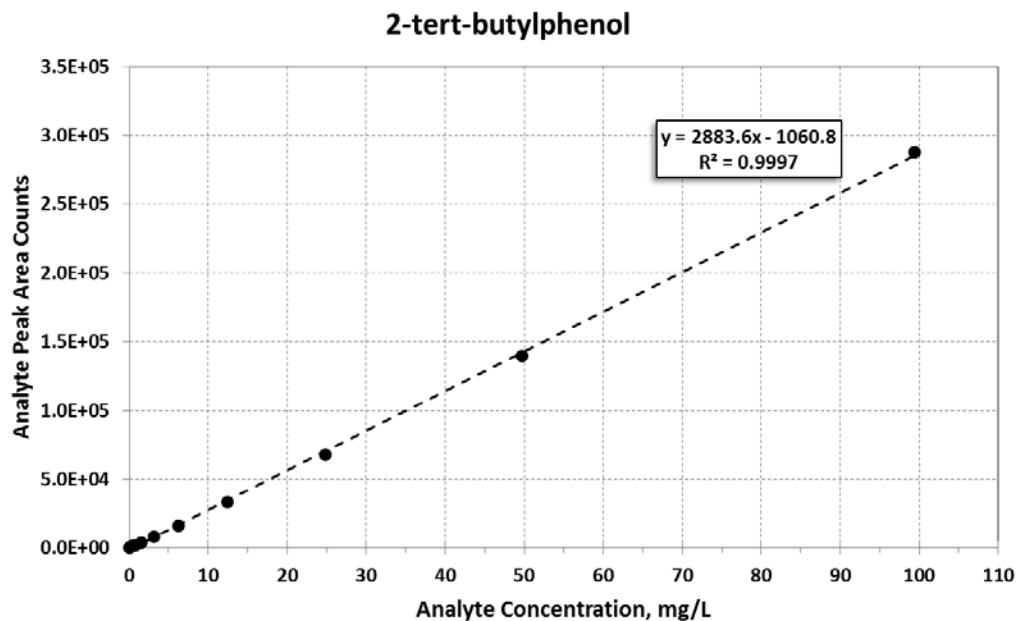


Figure A-1. Calibration curve obtained for TBP in an AO-free fuel.

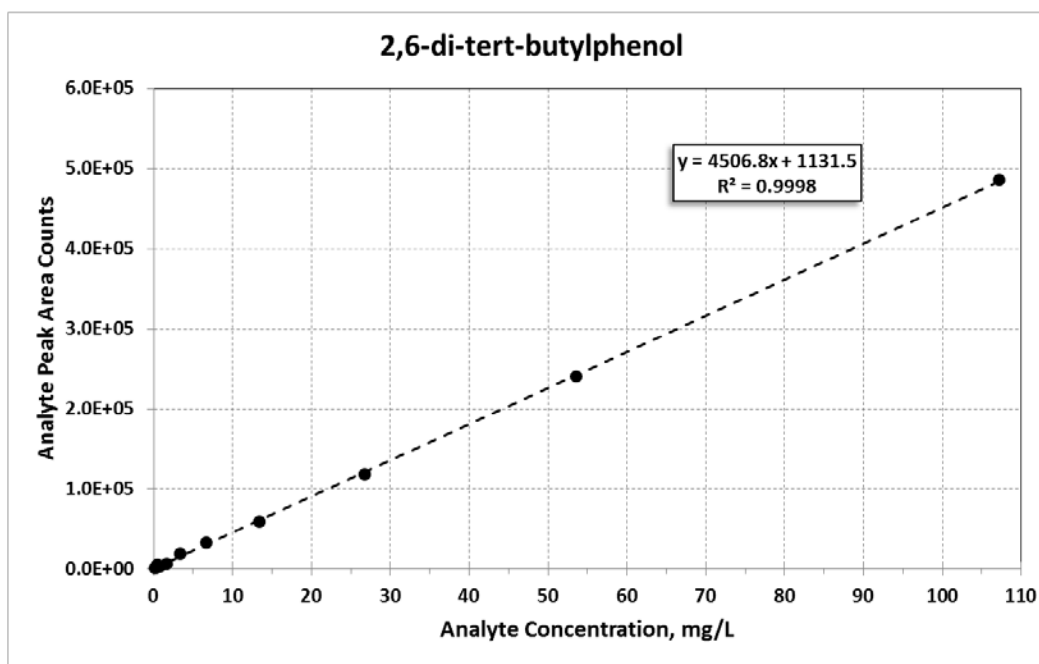


Figure A-2. Calibration curve obtained for DTBP in an AO-free fuel.

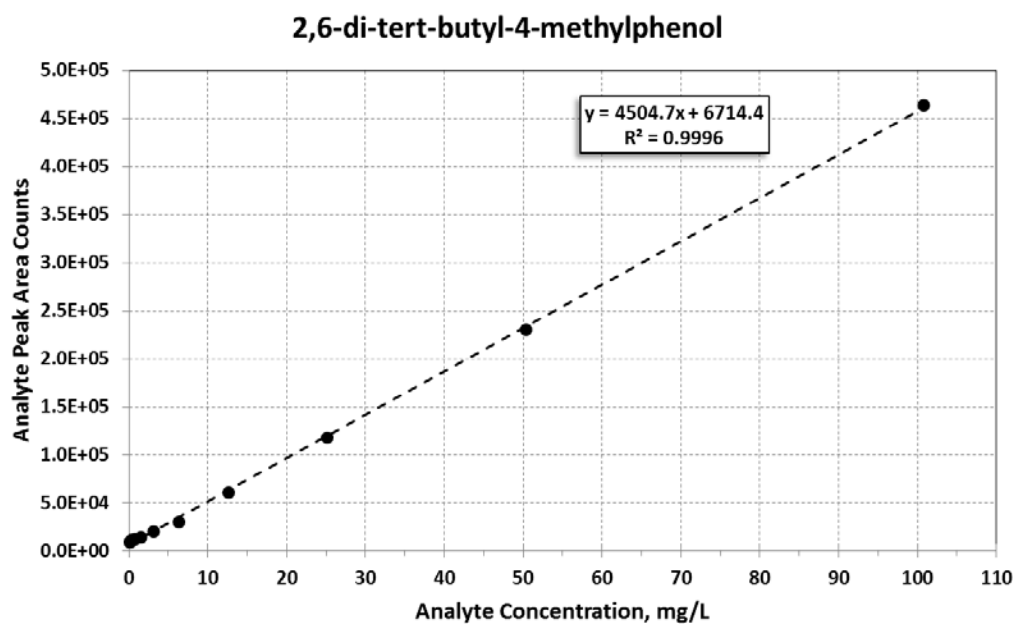


Figure A-3. Calibration curve obtained for DTBMP in an AO-free fuel.

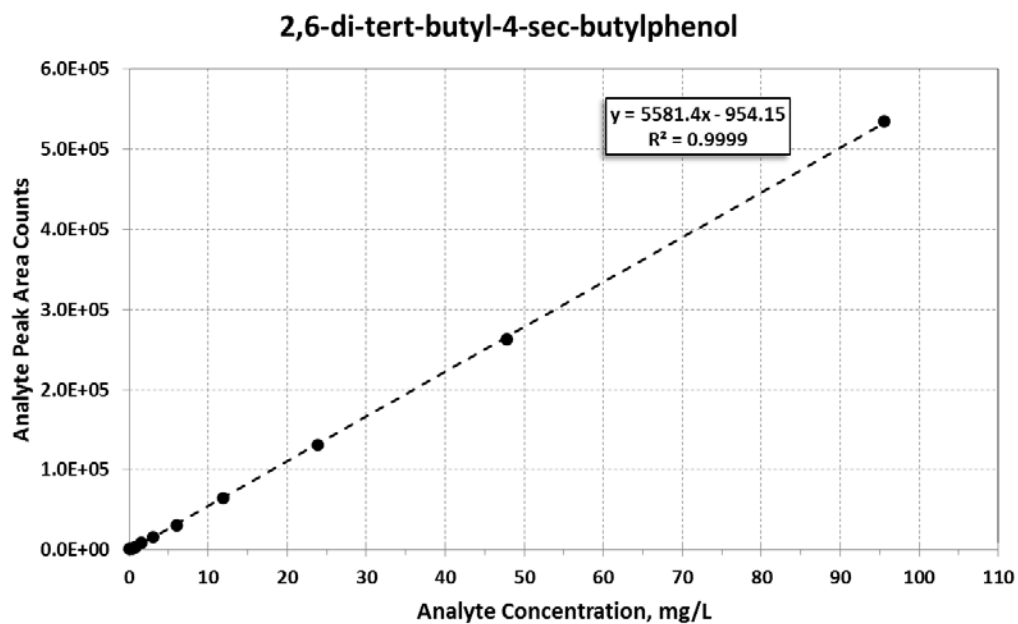


Figure A-4. Calibration curve obtained for DTBSBP in an AO-free fuel.

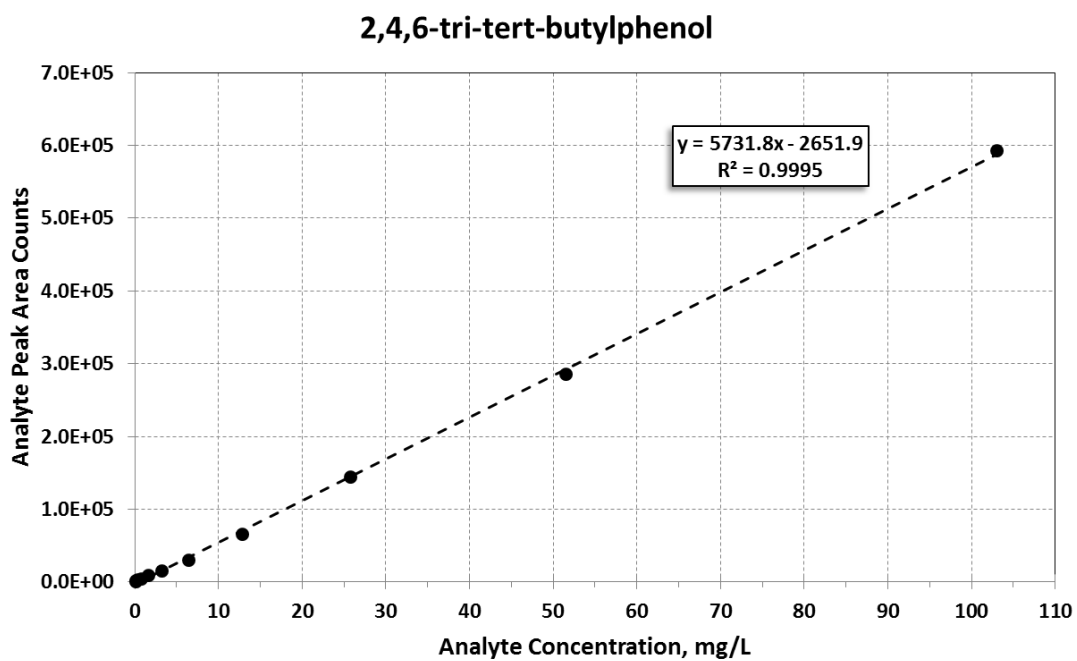


Figure A-5. Calibration curve obtained for TTBP in an AO-free fuel.

APPENDIX B: Manual Analysis Using a Spreadsheet and Automated Data Analysis with Agilent MassHunter Software

B.1 Manual Analysis Using a Spreadsheet

Calculating Calibration Curves

- Integrate all samples using the auto-integration program
- Use the MS Excel spreadsheet template, and enter the concentration and integration peak areas from the calibration standard analyses in the appropriate columns for each analyte, averaging each set of triplicate replicates.
- Create a third column for each analyte that calculates the square of the integration area.
- If it is not already present, customize quick access tool bar to add in “Analysis ToolPak” into the data tab.
- Click “Data Analysis” and open the regression calculator, calculate two regression ranges: a linear calibration curve from 0.03 to 3 ppm and a second order curve from 0.03 to 100 ppm
- Input Y range: select the cells containing the concentration values for that particular compound
- Input X range: select the area and area squared for the particular compound for the high concentration range and only the area for the low concentration range
- Output range: Select an empty location in the spreadsheet and hit “OK”
- Do this for all compounds and both calibration ranges

Analyzing Samples

- Integrate all samples using the same auto-integration program.
- Enter the integration values (peak areas) in the appropriate columns for each analyte in each sample.
- Below the integration values for each sample, the spreadsheet is set up to calculate analyte concentrations in ppm from the entered the peak areas.
- Use the values from the calibration curve (found under “Coefficients” in the output data) to calculate the concentrations from the integration areas for the fuel samples for each compound.
- Select the correct calibration range after calculating the concentration with one or both

- If sample concentration is below 3 ppm: $\text{concentration} = \text{Intercept} + X \text{ Variable } 1 * \text{area}$
- If above 3 ppm: $\text{concentration} = \text{Intercept} + X \text{ Variable } 1 * \text{area} + X \text{ Variable } 2 * (\text{area})^2$

B.2 Automated Data Analysis using Agilent MassHunter

Importing Data

- Transfer entire folder containing data of interest for analysis.
- Open GC MSD Translator by clicking the icon.
- Browse and select the folder containing the data of interest.
- Be sure to have “in-place translation” checked.
- Click “Start Translation” and the program will convert each file from the selected folder
- If there is an incomplete file due to a run not completing or being skipped for some other reason, there will be an error message stating that the file was outside the bounds of the array. If this occurs,
- Simply note which file(s) are incomplete. This can be distinguished by not having a check mark in the successful column.
- Close the translator.
- Open the folder the file is in and delete the file(s).
- Reopen translator and convert files again. Translating previously translated files does not cause a problem.
- Endure that all the data files have been translated. This can be double checked by seeing a check mark under the successful column within the Import MSD data files window.

Creating a New Batch

- Open MS Quantitation software
- All fields in view should be blank
- Create a new batch
- File > New Batch > Name the new batch > Open batch
- Add samples. File > Add samples > Select all the samples you would like to analyze including calibration curve samples

- All selected samples should be listed to the left side of the window.
- Making a Processing Method
- Highlight the highest concentration in the calibration curve in the list of samples.
- Method > Open > Open Method from Existing File > AO Processing Method 3 > Open
- The Method Editor window should automatically pop-up. Click exit.
- Another window will pop-up. Click “Apply Method to Batch”.
- Information should be added to the sample window and to the graphs.
- Method > Edit > Method Setup Tasks. Within the method setup tasks, the integration windows need to be checked.
- Click “Retention Time Setup”
- Confirm the retention time is correct for each compound. This will need to be updated as the GC column is replaced or cut.
- Save any changes: Method > Save As...
- Select the concentration setup on the left of the screen
- Tell MassHunter the calibration curve range
- Type in the actual concentration of each component in the Dil. High Conc. Column
- Type or select the dilution pattern.
- MassHunter will use the dilution pattern to create each level of calibration
- The number of points in the calibration curve will determine the number of levels in MassHunter. (i.e: 8 calibration curve points means 8 levels in MassHunter).
- Highlight the first compound at the top. For “Level Name Prefix” type ‘L’ and for “# of Levels” indicate the number of calibration points. (in our example this would be 8).
- Click “Create Levels”
- MassHunter should have created the levels and calculated the concentration at each level.
- Copy these levels and concentrations for each compound
- Right click on the compound levels were just added to
- Select “Copy Calibration Levels To...”
- Click “Select All” (or each specific sample for applying the levels to) and then okay.

- The copy should show the same calibration values from the first compound should be shown for each of the samples that you selected. This will be corrected when exiting the method editor.
- Exit the method editing and click “yes” to apply the method to the batch.
- Switch to single compound view
- Change the type from Sample to Cal for calibration standards
- Add the appropriate levels making sure it has the prefix
- Click “Analyze Batch”
- MassHunter should have processed the data but it is best to double check the results to ensure proper integration.
- View > Compounds-at-a-glance.... A new window should pop up with all the samples in the batch.
- To check the unknown samples: Layout > Samples\Targets\Auto Scale
- If samples have been integrated incorrectly, double click on the incorrectly integrated sample and make manual changes then exit.
- Check all samples for incorrectly integrated peaks.
- Close the compounds-at-a-glance window and reanalyze the batch.
- If there are red and/or blue highlights of samples in the batch table: These are alerts that are set up within the method to warn of issues with regards to the qualifier ratios which will be off depending on how your MS is operating. Use these alerts as a reminder to check and ensure MassHunter to be integrating the peaks correctly.

Optimizing the Calibration Curve

- Right click on the calibration curve
- Select “Curve fit assistant”
- Select a linear fit
- Right click on the plot
- Select “Accept Assistant Curve”
- Reanalyze the data
- Each compound should have its own separate calibration curve so be sure to check each curve individually to ensure everything seems reasonable

MassHunter Problems and Possible Solutions

- MassHunter tends to crash – be sure to save your data frequently!
- If MassHunter decides to not calculate concentration values for one component:
- Start over completely with a new batch
- Go to Analyze > Clear Calibration > Select All > OK then, Analyze Batch
- Go into the method editor and completely delete the compound from the retention time list. Before doing this, be sure to record the retention time, masses, qualifier ratios, time segment, etc. This information will be needed to re-add a new compound.

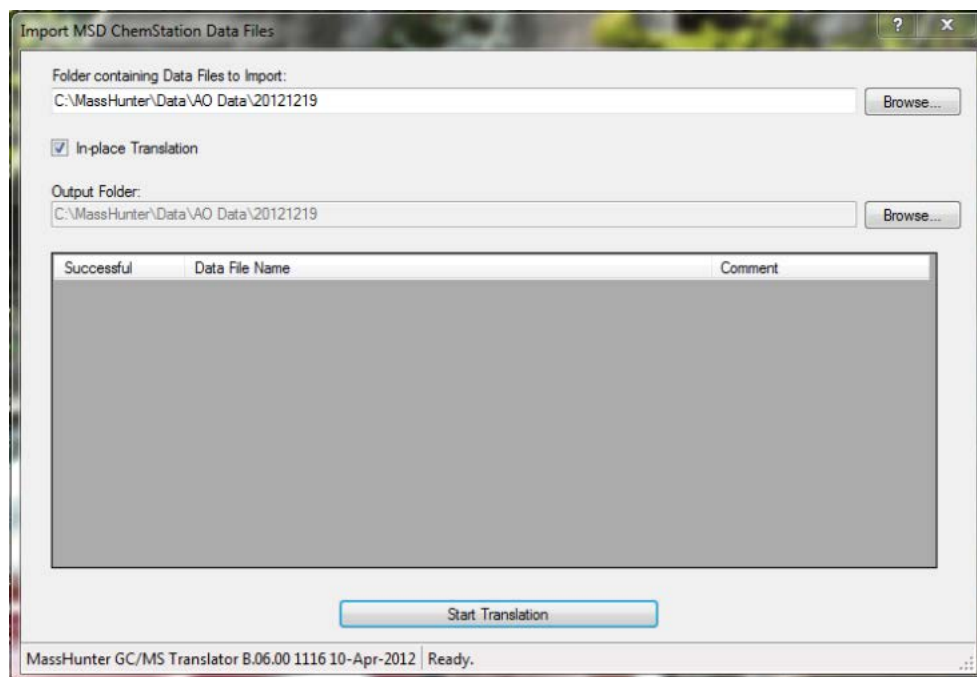
APPENDIX C: Walkthrough of MassHunter MSD Data Import and Analysis

This Appendix is an adjunct to the stepwise procedure outlined above in Appendix B. It is strongly recommended that the user be thoroughly familiar with the operation of the Agilent MassHunter software before attempting this analysis. This method requires, at a minimum, installation of the Agilent MassHunter MS Quantitation and the GC MSD Translator, found on the supplemental software disk. This walkthrough was prepared with Quantitation version B.050.1 and Supplemental version B.06.00. Currently, Windows 7 is the only supported operating system.

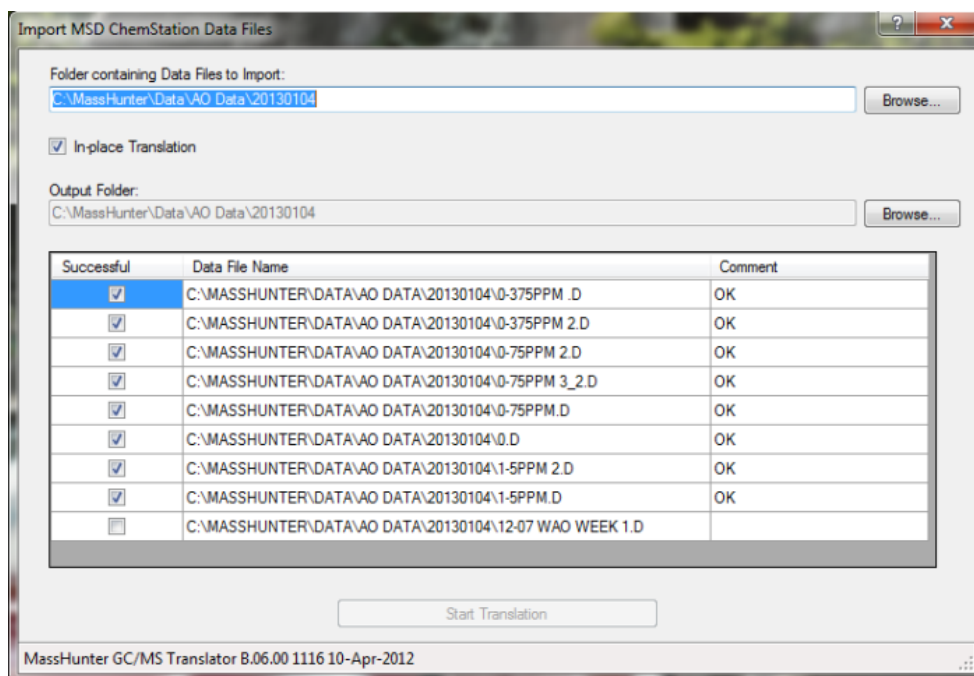
Importing Data

Transfer the entire Chemstation GC-MS data folder to the computer you will use to process the data. It is first necessary to convert the Chemstation data into a format that MassHunter can read.

Open the GC MSD Translator.

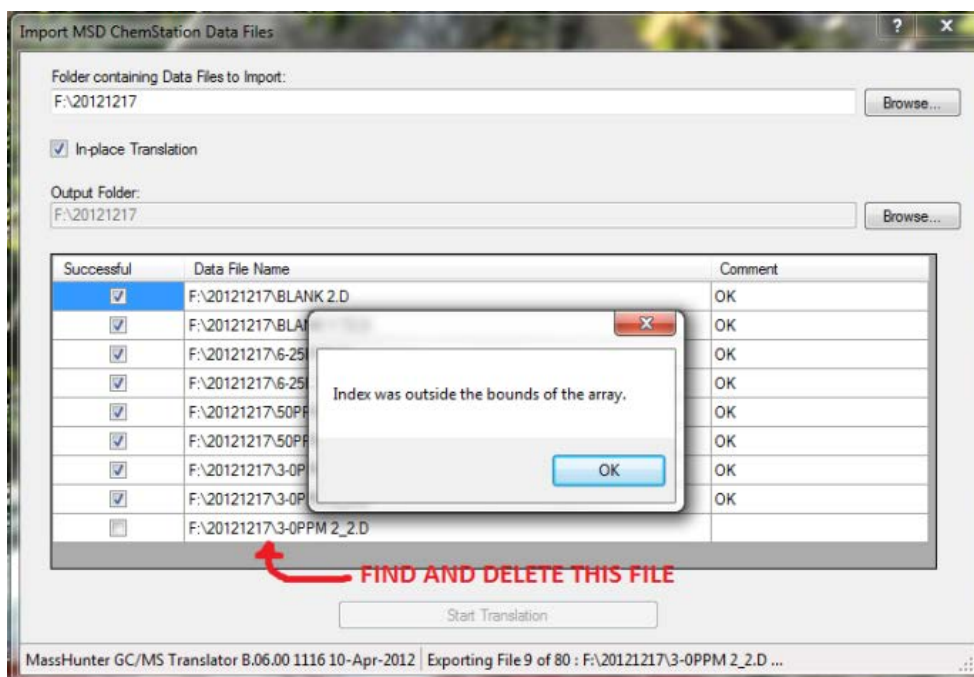


Find and select the folder containing the Chemstation data to be converted. Notice the check mark on the box labeled 'In-place Translation'. This indicates that the translator will add the necessary information to the Chemstation file. If it is unchecked, it will make a complete copy of the Chemstation data and add the MassHunter information into a new folder, i.e., the Output folder. In-place is more straightforward, so that will be the preferred mode. Click *Start translation* and the program will convert each file into MassHunter readable format.



Click the *Close* button when it appears, once the translation is complete.

Dealing with Incomplete Chemstation Files. If this dialog box appeared before all the files were translated, this indicates that a run was not completed or skipped because the translator was presented with an incomplete dataset.

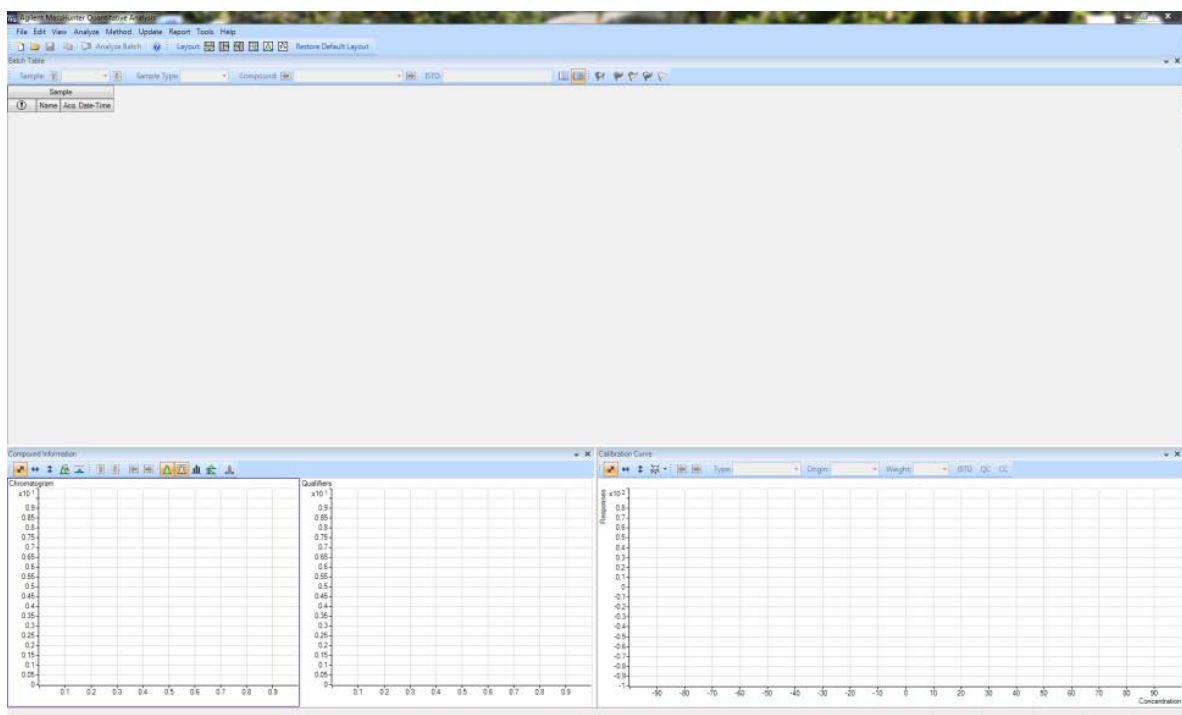


If this occurs, close the translator, find the incomplete file and delete it (the file name in the figure above is just an example). Then reopen the translator and initiate the file conversion once

again. If additional incomplete files are encountered, repeat the process of eliminating them until the translator successfully completes. Converting previously converted files does not cause a problem.

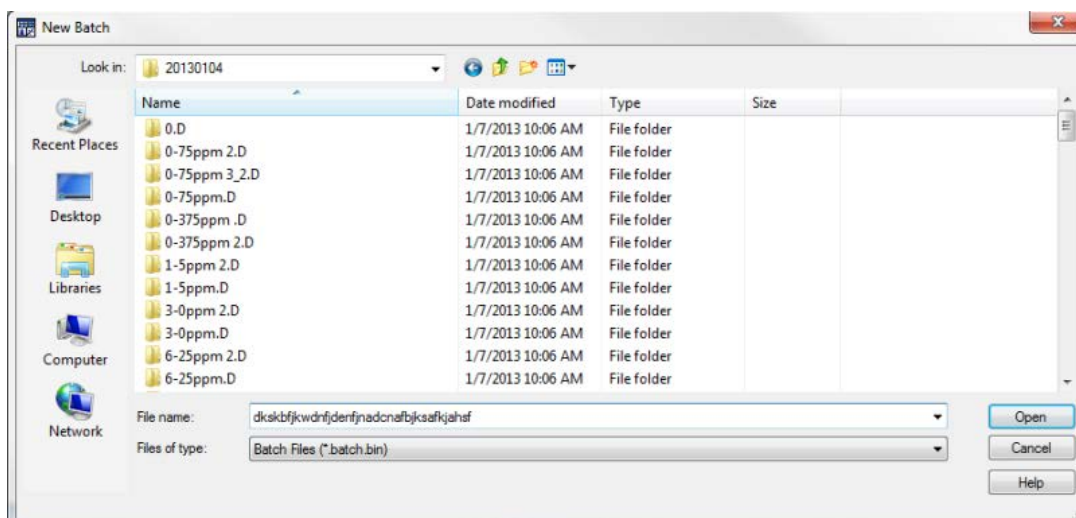
Opening and Using MassHunter

Since the object is to operate on MSD data, open then MS Quantitation software. You should see an empty set of panes, as shown below.

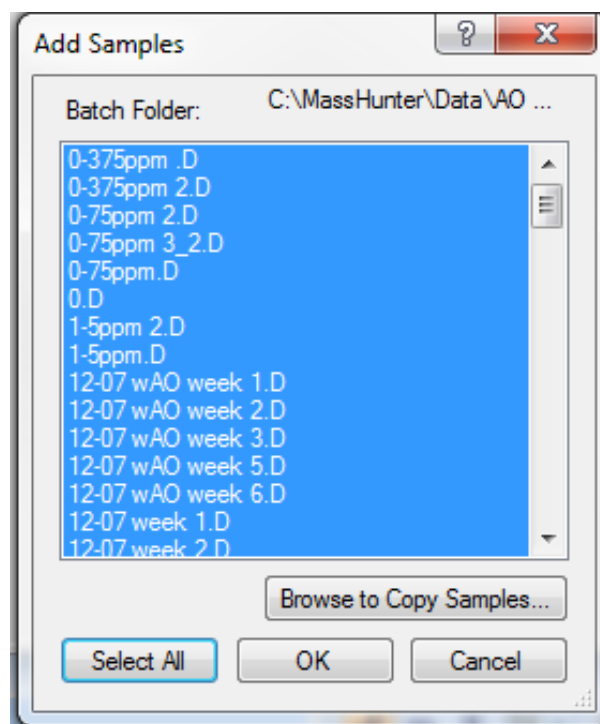


Create a Batch. The first step is to create a new *batch*. A batch contains all the samples you would like to analyze together and apply the same calibration to. A batch will independently hold all the processing method changes you apply to this particular batch of samples, and will not affect other batches.

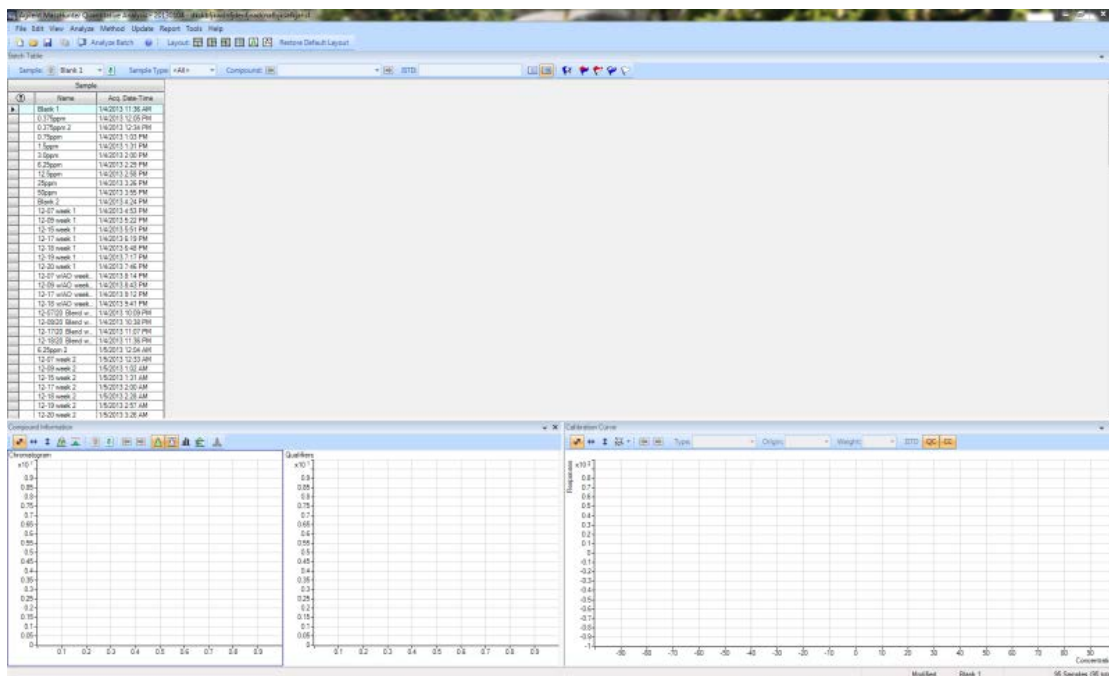
To create a new batch File click *New Batch...* and save it in any directory of choice, and give it a descriptive name. It is recommended that the batch is saved within the data folder to facilitate finding it later.



Adding samples to MassHunter. After the new batch has been created, the samples need to be added to MassHunter. This is done by clicking *File > Add Samples > Select All* (or highlight which samples you want), then click *OK*.

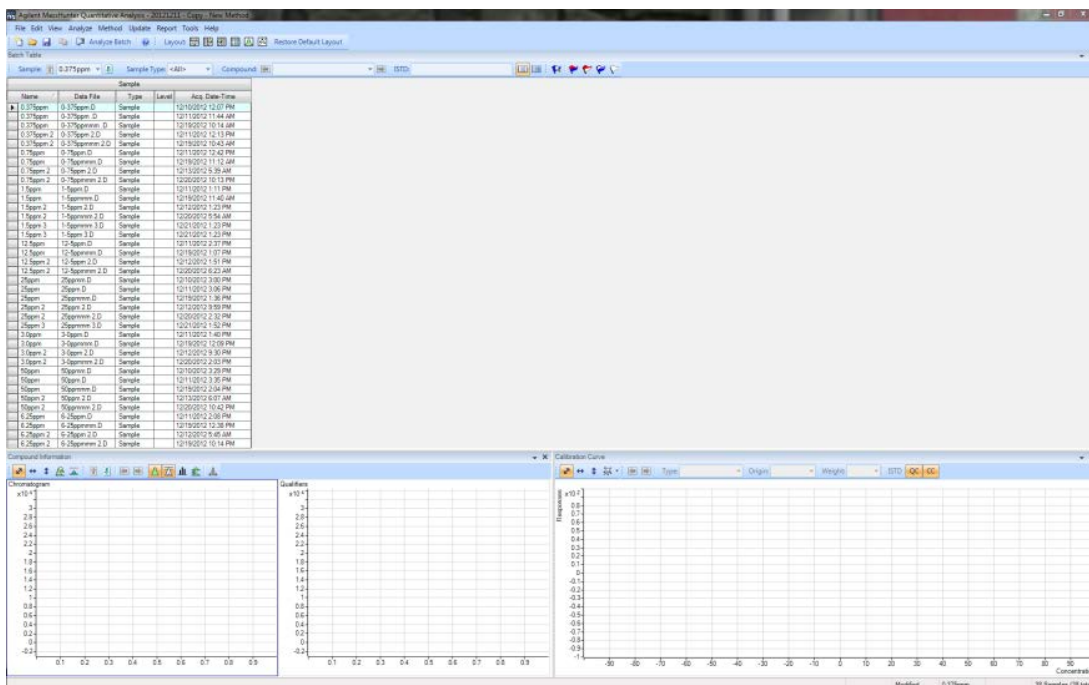


The figure above shows the samples that are in the same folder as the batch file. You can look around in other folders to add additional samples. Only converted samples will appear in this window.



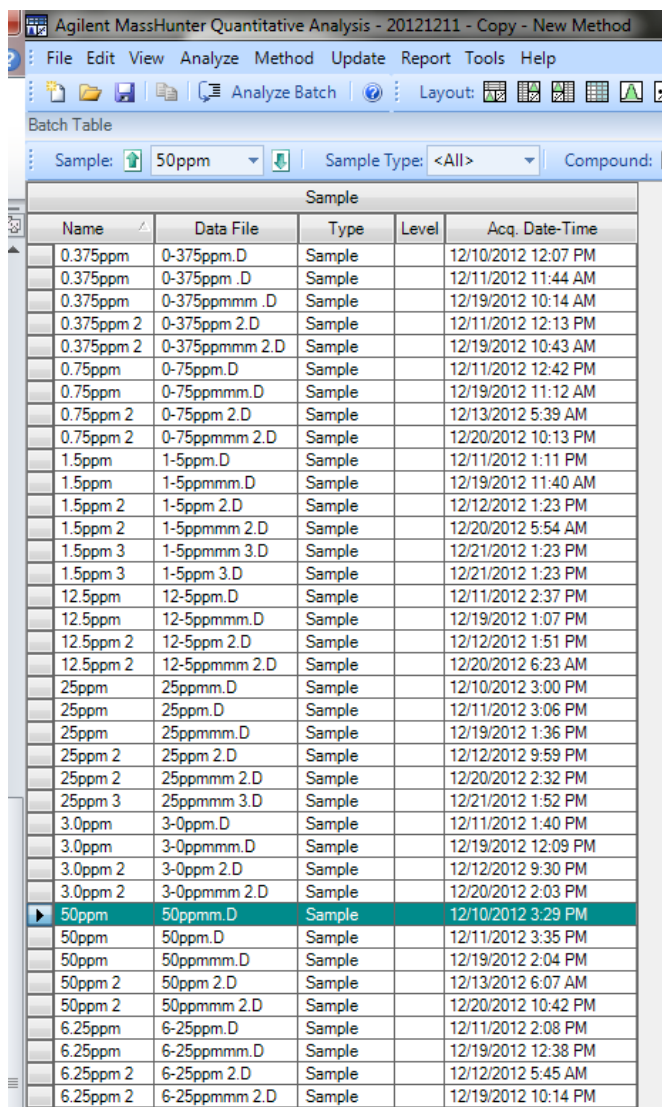
Generating a quantification method for single quadrupole SIM data in MassHunter Quantification MS.

It is now necessary to build a processing method that will create the calibration curve and integrate the analyte peaks. Start by opening MassHunter MS as shown previously, create a new batch and add your converted Chemstation sample files.



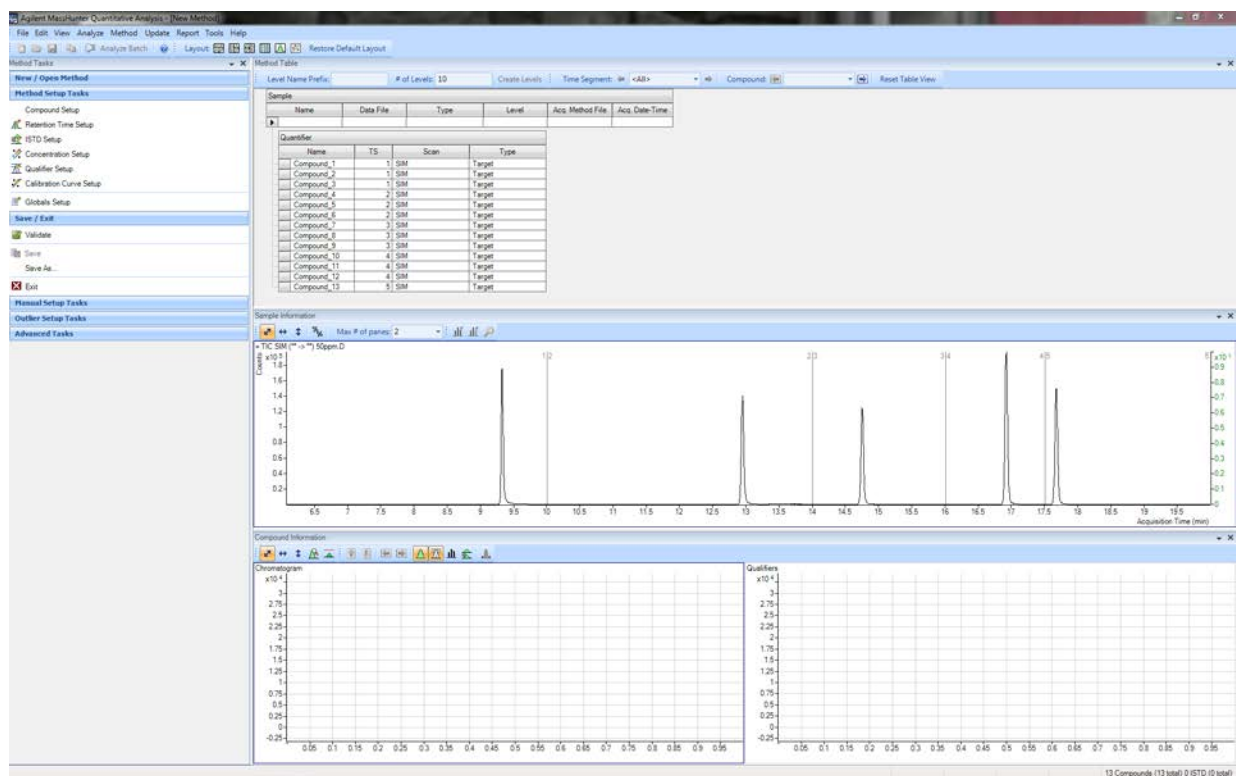
For this example, a series of calibration standards will be used to set up the method. If you have a processing method set up in Chemstation it is possible to convert the processing file using the method converter. However, this process does not work reliably in the version of the software used for this example.

Select the highest concentration standard, which has strong peak signals and facilitates setting up the method.



Sample				
Name	Data File	Type	Level	Acq. Date-Time
0.375ppm	0-375ppm.D	Sample		12/10/2012 12:07 PM
0.375ppm	0-375ppm.D	Sample		12/11/2012 11:44 AM
0.375ppm	0-375ppmm.D	Sample		12/19/2012 10:14 AM
0.375ppm 2	0-375ppm 2.D	Sample		12/11/2012 12:13 PM
0.375ppm 2	0-375ppmm 2.D	Sample		12/19/2012 10:43 AM
0.75ppm	0-75ppm.D	Sample		12/11/2012 12:42 PM
0.75ppm	0-75ppmm.D	Sample		12/19/2012 11:12 AM
0.75ppm 2	0-75ppm 2.D	Sample		12/13/2012 5:39 AM
0.75ppm 2	0-75ppmm 2.D	Sample		12/20/2012 10:13 PM
1.5ppm	1-5ppm.D	Sample		12/11/2012 1:11 PM
1.5ppm	1-5ppmm.D	Sample		12/19/2012 11:40 AM
1.5ppm 2	1-5ppm 2.D	Sample		12/12/2012 1:23 PM
1.5ppm 2	1-5ppmm 2.D	Sample		12/20/2012 5:54 AM
1.5ppm 3	1-5ppmm 3.D	Sample		12/21/2012 1:23 PM
1.5ppm 3	1-5ppm 3.D	Sample		12/21/2012 1:23 PM
12.5ppm	12-5ppm.D	Sample		12/11/2012 2:37 PM
12.5ppm	12-5ppmm.D	Sample		12/19/2012 1:07 PM
12.5ppm 2	12-5ppm 2.D	Sample		12/12/2012 1:51 PM
12.5ppm 2	12-5ppmm 2.D	Sample		12/20/2012 6:23 AM
25ppm	25ppm.D	Sample		12/10/2012 3:00 PM
25ppm	25ppm.D	Sample		12/11/2012 3:06 PM
25ppm	25ppmm.D	Sample		12/19/2012 1:36 PM
25ppm 2	25ppm 2.D	Sample		12/12/2012 9:59 PM
25ppm 2	25ppmm 2.D	Sample		12/20/2012 2:32 PM
25ppm 3	25ppmm 3.D	Sample		12/21/2012 1:52 PM
3.0ppm	3-0ppm.D	Sample		12/11/2012 1:40 PM
3.0ppm	3-0ppmm.D	Sample		12/19/2012 12:09 PM
3.0ppm 2	3-0ppm 2.D	Sample		12/12/2012 9:30 PM
3.0ppm 2	3-0ppmm 2.D	Sample		12/20/2012 2:03 PM
50ppm	50ppm.D	Sample		12/10/2012 3:29 PM
50ppm	50ppm.D	Sample		12/11/2012 3:35 PM
50ppm	50ppmm.D	Sample		12/19/2012 2:04 PM
50ppm 2	50ppm 2.D	Sample		12/13/2012 6:07 AM
50ppm 2	50ppmm 2.D	Sample		12/20/2012 10:42 PM
6.25ppm	6-25ppm.D	Sample		12/11/2012 2:08 PM
6.25ppm	6-25ppmm.D	Sample		12/19/2012 12:38 PM
6.25ppm 2	6-25ppm 2.D	Sample		12/12/2012 5:45 AM
6.25ppm 2	6-25ppmm 2.D	Sample		12/19/2012 10:14 PM

At the top of the screen, select *Method > New > New Method from Acquired SIM data...* , then select one of the high concentration standards > Open



The sample table now shows more compounds of interest, and the SIM TIC should be visible. Click on *Compound Setup* in the top of the left menu. The sample table should add a few extra columns as shown below.

Level Name Prefix: # of Levels: 10 Create Levels Time Segment: <All> Compound: [dropdown] Reset Table View							
Sample							
Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time		
Quantifier							
Name	TS	Scan	Type	MZ	RT	Ion Polarity	Criteria
Compound_1	1	SIM	Target	107.0	9.324	Positive	Close RT
Compound_2	1	SIM	Target	135.0	9.329	Positive	Close RT
Compound_3	1	SIM	Target	150.0	9.329	Positive	Close RT
Compound_4	2	SIM	Target	163.0	12.948	Positive	Close RT
Compound_5	2	SIM	Target	191.0	12.953	Positive	Close RT
Compound_6	2	SIM	Target	206.0	12.953	Positive	Close RT
Compound_7	3	SIM	Target	177.0	14.751	Positive	Close RT
Compound_8	3	SIM	Target	205.0	14.751	Positive	Close RT
Compound_9	3	SIM	Target	220.0	14.751	Positive	Close RT
Compound_10	4	SIM	Target	247.0	16.925	Positive	Close RT
Compound_11	4	SIM	Target	248.0	16.925	Positive	Close RT
Compound_12	4	SIM	Target	262.0	16.925	Positive	Close RT
Compound_13	5	SIM	Target	233.0	17.676	Positive	Close RT

All of the SIM traces are designated as compounds by default. Delete the extra SIM qualifiers and keep only the primary ions uses for quantitation. Take the time to name each compound as well.

Method Table

Level Name Prefix: # of Levels: 10 Create Levels Time Segment: <All> Compound: Reset Table View

Sample					
Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time
50ppm.D	50ppm.D				

Quantifier							
Name	TS	Scan	Type	MZ	RT	Ion Polarity	Criteria
TBP135	1	SIM	Target	135.0	9.329	Positive	Close RT
DTBP191	2	SIM	Target	191.0	12.953	Positive	Close RT
DTBMP205	3	SIM	Target	205.0	14.751	Positive	Close RT
TTBP247	4	SIM	Target	247.0	16.925	Positive	Close RT
DTBSBP233	5	SIM	Target	233.0	17.676	Positive	Close RT

Make sure to save your work each step of the way to prevent the loss of progress if MassHunter becomes unstable. Saving the method will also allow you to apply it more easily to other batches, otherwise the method will just be saved with the batch you are working on. Next, select *Retention Time Setup* from the upper left hand menu. Double check to make sure the time segments is correct (the column labeled TS), the scan type is SIM, and the sample type is Target.

The retention time should have been automatically determined by MassHunter but if adjustments need to be made, now is the time to make them. In the Left and Right RT Delta column, put in a reasonable time that will bracket the target analyte peak. MassHunter will NOT integrate outside of this window and will cut off peaks that drift outside. Make the integration window too large and MassHunter will integrate everything. Use the TIC at the bottom to determine a reasonable amount of time. In this example, both the left and right limits were set to 0.25 min. This can be adjusted later if it proves to be too narrow or too large. Select *Concentration Setup* from the upper left menu. Type in the units, the actual concentration of the most concentrated standard, and the dilution pattern used to make the curve. In this case, the highest concentration was around 50 ppm and 1:2 dilution patterns were used.

Method Table

Level Name Prefix: L # of Levels: 8 Create Levels Time Segment: <All> Compound: TBP135 Reset Table View

Sample					
Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time
50ppm.D	50ppm.D				

Quantifier						
Name	TS	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
TBP135	1	SIM	Target	49.7000	1:2	ng/ml
DTBP191	2	SIM	Target	53.6000	1:2	ng/ml
DTBMP205	3	SIM	Target	50.4000	1:2	ng/ml
TTBP247	4	SIM	Target	51.3000	1:2	ng/ml
DTBSBP233	5	SIM	Target	47.8000	1:2	ng/ml

In the next step, each calibration level is added to the method. Select TBP135 as shown above. At the top of the Method Table window, in the Level Name Prefix box type "L" or whatever prefix you want. In the # of Levels box type how many calibration standards you have (in this case 8). Click *Create Levels*.

Method Table

Level Name Prefix: L # of Levels: 8 Create Levels Time Segment: <All> Compound: TBP135 Reset Table View

Sample						
Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time	
50ppm.D	50ppm.D					

Quantifier						
Name	TS	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
TBP135	1	SIM	Target	49.7000	1:2	ng/ml

Calibration			
Level	Conc.	Response	Enable
L1	0.3883		☒
L2	0.7766		☒
L3	1.5531		☒
L4	3.1063		☒
L5	6.2125		☒
L6	12.4250		☒
L7	24.8500		☒
L8	49.7000		☒

In this example, all five analytes were mixed together, so the dilution pattern is the same and can be copied to the other compounds by either selecting each compound and using *Create Levels* or right click on TBP135 > *Copy Calibration Levels to* > *Select All* > *OK*. Now every compound should have eight calibration levels.

The next step is optional, but will provide a diagnostic tool for trouble shooting a sample. From the upper left menu select *Qualifier setup*. Qualifiers are tags that can be added that will work as an error trap to provide an alert of a problem and will be flagged in the batch window if they are out of specification. For this example, the remaining SIM ion ratios will be used as qualifiers to ensure proper identification of a compound. This information will not impact how MassHunter calculates concentration, but alerts of a potential problem. Right click on *TBP135* > *New Qualifier*.

Add another qualifier row as shown below.

Method Table

Level Name Prefix: L # of Levels: 8 Create Levels Time Segment: <All> Compound: TBP135 Reset Table View

Sample						
Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time	
50ppm.D	50ppm.D					

Quantifier						
Name	TS	Scan	Type	MZ	Uncertainty	Peak Filter Threshold
TBP135	1	SIM	Target	135.0	Relative	PeakAreaPctThresho...

Qualifier					
MZ	Rel. Resp.	Uncertainty	Area Sum	Qual. Min.	Qual. Max.
		20.0	☐		
		20.0	☐		

Quantifier						
Name	TS	Scan	Type	MZ	Uncertainty	Peak Filter Threshold
DTBP191	2	SIM	Target	191.0	Relative	PeakAreaPctThresho...
DTBMP205	3	SIM	Target	205.0	Relative	PeakAreaPctThresho...
TTBP247	4	SIM	Target	247.0	Relative	PeakAreaPctThresho...
DTBSBP233	5	SIM	Target	233.0	Relative	PeakAreaPctThresho...

Add the necessary information. For TBP, the two additional SIM ions were 107 and 150. The relative ratios compared to the main peak will most likely be slightly different due to slight differences between MS source cleanliness, and other operational conditions.

Method Table

Level Name Prefix: L # of Levels: 8 Create Levels Time Segment: <All> Compound: TBP135 Reset Table View

Sample	Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time
50ppm.D	50ppm.D					

Quantifier						
Name	TS	Scan	Type	MZ	Uncertainty	Peak Filter Threshold
TBP135	1	SIM	Target	135.0	Relative	PeakAreaPctThresho...

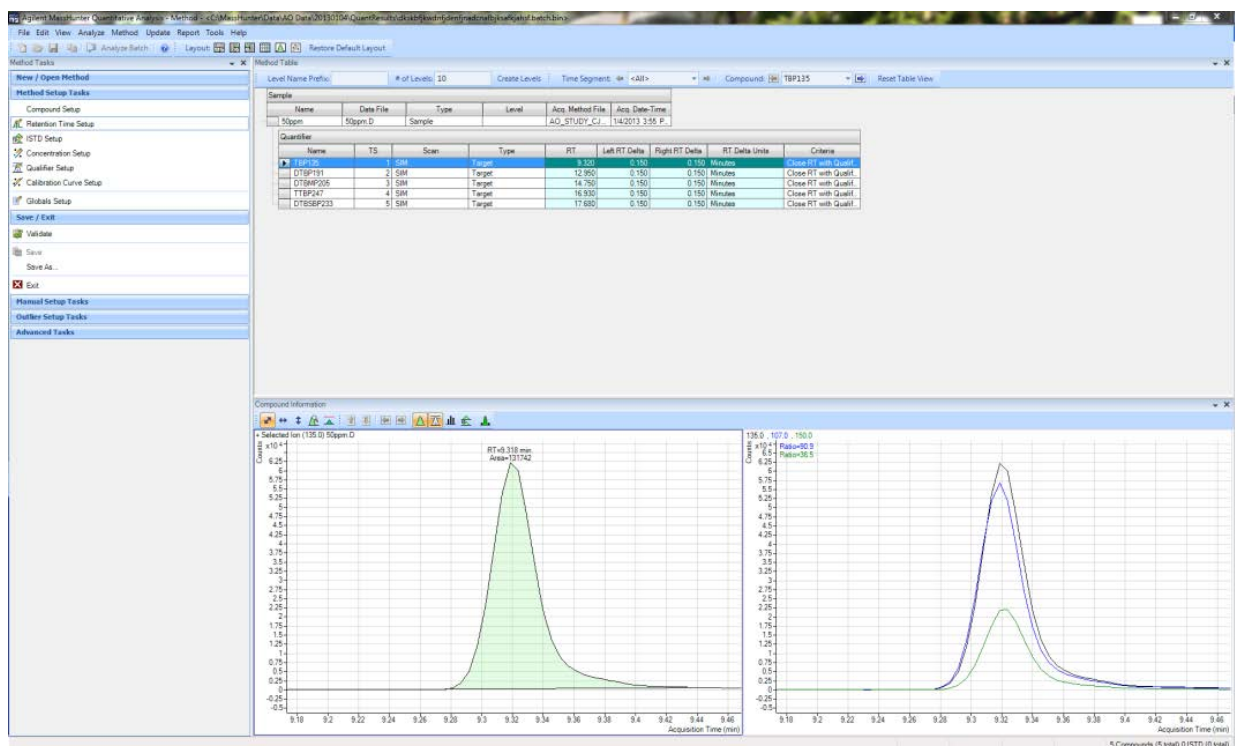
Qualifier					
MZ	Rel. Resp.	Uncertainty	Area Sum	Qual. Min.	Qual. Max.
107.0	80.0	20.0		64.00	96.00
150.0	50.0	20.0		40.00	60.00

Quantifier						
Name	TS	Scan	Type	MZ	Uncertainty	Peak Filter Threshold
DTBP191	2	SIM	Target	191.0	Relative	PeakAreaPctThresho...
DTBMP205	3	SIM	Target	205.0	Relative	PeakAreaPctThresho...
TTBP247	4	SIM	Target	247.0	Relative	PeakAreaPctThresho...
DTBSBP233	5	SIM	Target	233.0	Relative	PeakAreaPctThresho...

Repeat this process to add qualifiers for the other four analytes. The qualifier criteria can be broader if it is desired to receive alerts to only large deviations. Moreover, due to the complex nature of fuels, these ratios are rarely perfect.

This completes the method development, and minor fine tuning with retention time integration windows or qualifier ratios can always be customized at a later time to accommodate a particular application. Click *Exit* in the left menu and MassHunter will ask if you want the method to applied the batch, click *Yes* and continue with data processing.

Editing the method. Highlight the highest concentration of your calibration curve from the list of samples; this makes method editing easier as MassHunter only previews this selected data in the Method Editor. Then select: *Method > Open > Open Method from Existing File...* then Select, *AO Processing Method 3 > Open*. This will automatically open the Method Editor window. Click *exit* and a window will pop up. Click *apply method to batch*. This inputs the method and the graphs are no longer empty. Now return to the method editor by clicking *Method > Edit* (or press F10). On the left side of the screen a list of options will appear. Everything should be filled out with compound information but first, it is important to review the integration windows. Click on the *Retention Time Setup* to bring up a list of the five compounds of interest.



Confirm that the retention times are correct for each compound. This will need to be updated over time, as the GC column ages, is replaced or cut. The left and right delta determine how far MassHunter will look to find peaks, and these parameters can be changed if MassHunter starts integrating unwanted peaks or only partially includes wanted peaks. Once the changes are made save them in your own process method (*Method > Save As...*) so you do not have to change these parameters each time.

[illegible]

Level Name Prefix:		# of Levels: 10	Create Levels	Time Segment: <All>	Compound: TBP135	Reset Table View
Sample						
Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time	
50ppm	50ppm.D	Sample		AO_STUDY_CJ...	1/4/2013 3:55 P...	
Quantifier						
Name	TS	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
TBP135	1	SIM	Target	49.7000	1:2	ng/ml
DTBP191	2	SIM	Target	53.6000	1:2	ng/ml
DTBMP205	3	SIM	Target	50.4000	1:2	ng/ml
TTBP247	4	SIM	Target	51.5000	1:2	ng/ml
DTBSBP233	5	SIM	Target	47.8000	1:2	ng/ml

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Level Name Prefix:	# of Levels: 10	Create Levels	Time Segment: <All>	Compound: TBP135	Reset Table View
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Sample						
Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time	
50ppm	50ppm.D	Sample		AO_STUDY_CJ...	1/4/2013 3:55 P...	

Quantifier						
Name	TS	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
TBP135	1	SIM	Target	49.700	1:2	ng/ml
DTBP191	2	SIM	Target	53.600	1:2	ng/ml
DTBMP205	3	SIM	Target	50.400	1:2	ng/ml
TTBP247	4	SIM	Target	51.500	1:2	ng/ml
DTBSBP233	5	SIM	Target	47.800	1:2	ng/ml

MassHunter will use this dilution pattern to create each level of the calibration. Thus, serial dilution of a 50 ppm solution with a 1:2 dilution pattern, results in calibration points at 25, 12.5, 6.25 ppm, etc. For example, if it is desired to have 8 calibration points ranging from 50 ppm to 0.39 ppm, that would require eight dilution levels. Highlight the first compound and at the top, and use a prefix of 'L' and enter the number of levels as '8'. Then, click on *create levels*.

Level Name Prefix: L	# of Levels: 8	Create Levels	Time Segment: <All>	Compound: TBP135	Reset Table View
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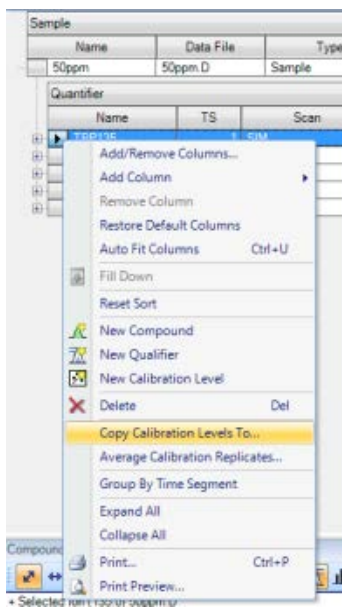
Sample						
Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time	
50ppm	50ppm.D	Sample		AO_STUDY_CJ...	1/4/2013 3:55 P...	

Quantifier						
Name	TS	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
TBP135	1	SIM	Target	49.7000	1:2	ng/ml

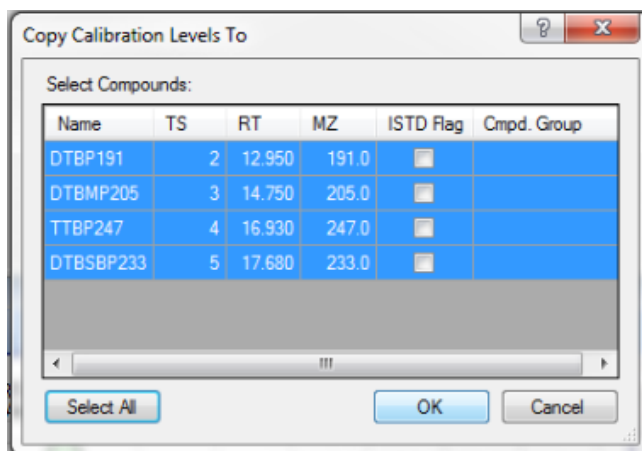
Calibration			
Level	Conc.	Response	Enable
L1	0.3883		☒
L2	0.7766		☒
L3	1.5531		☒
L4	3.1063		☒
L5	6.2125		☒
L6	12.4250		☒
L7	24.8500		☒
L8	49.7000		☒

Quantifier						
Name	TS	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
DTBP191	2	SIM	Target	53.6000	1:2	ng/ml
DTBMP205	3	SIM	Target	50.4000	1:2	ng/ml
TTBP247	4	SIM	Target	51.5000	1:2	ng/ml
DTBSBP233	5	SIM	Target	47.8000	1:2	ng/ml

This should result in the creation of eight levels with calculated concentrations at each level. Right click on the compound you just added levels too and select *Copy Calibration Levels To...*



Click *Select all* (or each sample you want to copy the levels too) and OK.



It should have copied the eight levels to each compound. MassHunter blindly copies the calibration values from the first compound, but it will correct itself automatically when the method editor is exited.

Sample						
Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time	
50ppm	50ppm.D	Sample		AO_STUDY_CJ...	1/4/2013 3:55 P...	

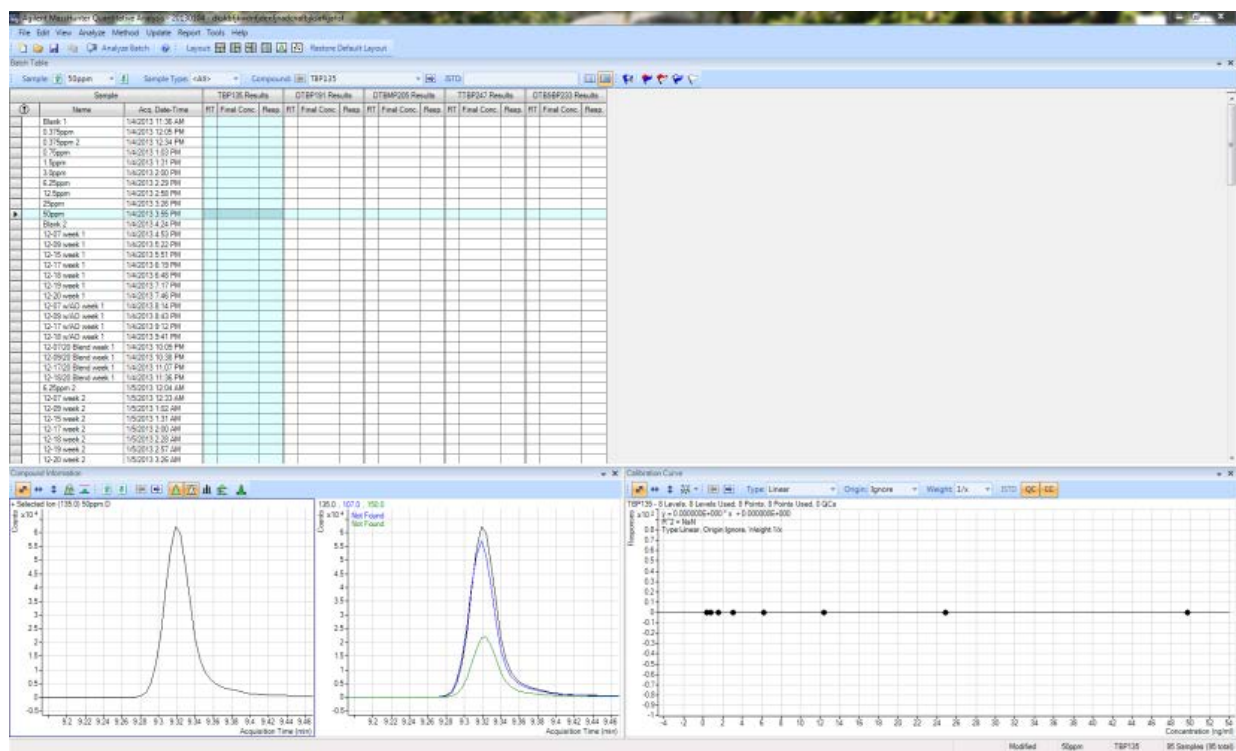
Quantifier						
Name	TS	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
TBP135	1	SIM	Target	49.7000	1:2	ng/ml
DTBP191	2	SIM	Target	53.6000	1:2	ng/ml

Calibration			
Level	Conc.	Response	Enable
L1	0.3883		☒
L2	0.7766		☒
L3	1.5531		☒
L4	3.1063		☒
L5	6.2125		☒
L6	12.4250		☒
L7	24.8500		☒
L8	49.7000		☒

Quantifier						
Name	TS	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
DTBMP205	3	SIM	Target	50.4000	1:2	ng/ml
TTBP247	4	SIM	Target	51.5000	1:2	ng/ml
DTBSBP233	5	SIM	Target	47.8000	1:2	ng/ml

If the dilution levels are the same, all that is necessary to update is the *Dil. High Conc.* every time you a new calibration stock solution is prepared. Save the processing method, so that it will not have to be necessary to repeat the level creation process each time, assuming you keep the same dilution pattern. It is not a problem if any particular calibration level is absent, since MassHunter will only add points to the calibration curve from the samples in the batch and if that particular concentration level was not included, it will be skipped and it will not show up in the generated calibration curve. This completes the method editing, so click *exit* then *yes* to apply the method to the batch.

Upon returning to the batch table after exiting the method editor, the screen should still look similar to the below figure.



Applying the calibration curve. This step informs MassHunter which samples are calibration points and at what levels those points correspond to. Switch to single compound view, and change the type from *Sample* to *Cal* for calibration standards and add the appropriate level MAKE SURE IT HAS THE PREFIX or MassHunter will not create a calibration curve. The Level has to be exactly how it was created in the method editor.

Batch Table

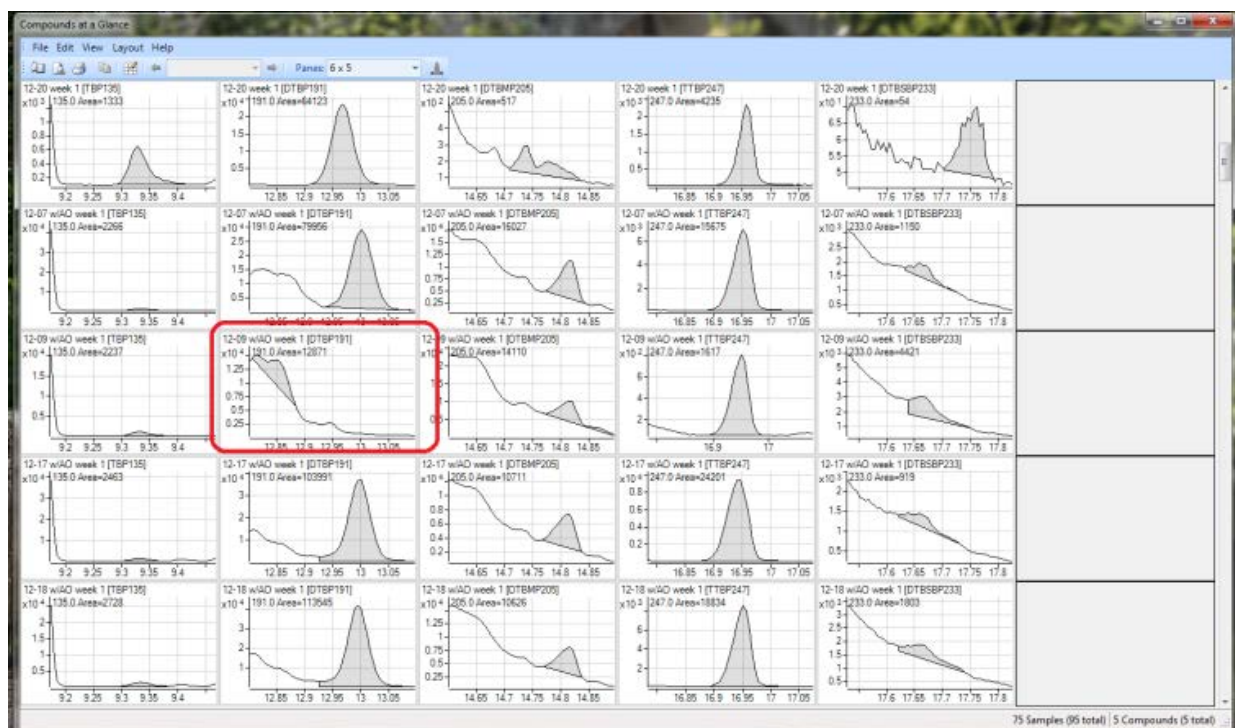
Sample: 6.25ppm Sample Type: <All> Compound: TBP135 ISTD:

Sample						TBP135...	TBP135 Results										Qualifier...	Qualifier...
?	▽	Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	S/N	Ratio	MI	Ratio	MI
		Blank 1	Blank 1.D	Blank		1/4/2013 11:36 AM												
		0.375ppm	0-375ppm.D	Cal	L1	1/4/2013 12:05 PM												
		0.375ppm 2	0-375ppm 2.D	Cal	L1	1/4/2013 12:34 PM												
		0.75ppm	0-75ppm.D	Cal	L2	1/4/2013 1:03 PM												
		1.5ppm	1-5ppm.D	Cal	L3	1/4/2013 1:31 PM												
		3.0ppm	3-0ppm.D	Cal	L4	1/4/2013 2:00 PM												
		6.25ppm	6-25ppm.D	Cal	L5	1/4/2013 2:29 PM												
		12.5ppm	12-5ppm.D	Cal	L6	1/4/2013 2:58 PM												
		25ppm	25ppm.D	Cal	L7	1/4/2013 3:26 PM												
		50ppm	50ppm.D	Cal	L8	1/4/2013 3:55 PM												
		Blank 2	Blank 2.D	Blank		1/4/2013 4:24 PM												
		12-07 week 1	12-07 week 1.D	Sample		1/4/2013 4:53 PM												
		12-09 week 1	12-09 week 1.D	Sample		1/4/2013 5:22 PM												
		12-15 week 1	12-15 week 1.D	Sample		1/4/2013 5:51 PM												
		12-17 week 1	12-17 week 1.D	Sample		1/4/2013 6:19 PM												
		12-18 week 1	12-18 week 1.D	Sample		1/4/2013 6:48 PM												
		12-19 week 1	12-19 week 1.D	Sample		1/4/2013 7:17 PM												
		12-20 week 1	12-20 week 1.D	Sample		1/4/2013 7:46 PM												
		12-07 w/AO week 1	12-07 w/AO week 1.D	Sample		1/4/2013 8:14 PM												
		12-09 w/AO week 1	12-09 w/AO week 1.D	Sample		1/4/2013 8:43 PM												
		12-17 w/AO week 1	12-17 w/AO week 1.D	Sample		1/4/2013 9:12 PM												
		12-18 w/AO week 1	12-18 w/AO week 1.D	Sample		1/4/2013 9:41 PM												
		12-07/20 Blend week 1	12-07-20 Blend week 1.D	Sample		1/4/2013 10:09 PM												
		12-09/20 Blend week 1	12-09-20 Blend week 1.D	Sample		1/4/2013 10:38 PM												
		12-17/20 Blend week 1	12-17-20 Blend week 1.D	Sample		1/4/2013 11:07 PM												
		12-18/20 Blend week 1	12-18-20 Blend week 1.D	Sample		1/4/2013 11:36 PM												
		6.25ppm 2	6-25ppm 2.D	Cal	L5	1/5/2013 12:04 AM												
		12-07 week 2	12-07 week 2.D	Sample		1/5/2013 12:33 AM												
		12-09 week 2	12-09 week 2.D	Sample		1/5/2013 1:02 AM												
		12-15 week 2	12-15 week 2.D	Sample		1/5/2013 1:31 AM												
		12-17 week 2	12-17 week 2.D	Sample		1/5/2013 2:00 AM												
		12-18 week 2	12-18 week 2.D	Sample		1/5/2013 2:28 AM												
		12-19 week 2	12-19 week 2.D	Sample		1/5/2013 2:57 AM												
		12-20 week 2	12-20 week 2.D	Sample		1/5/2013 3:26 AM												

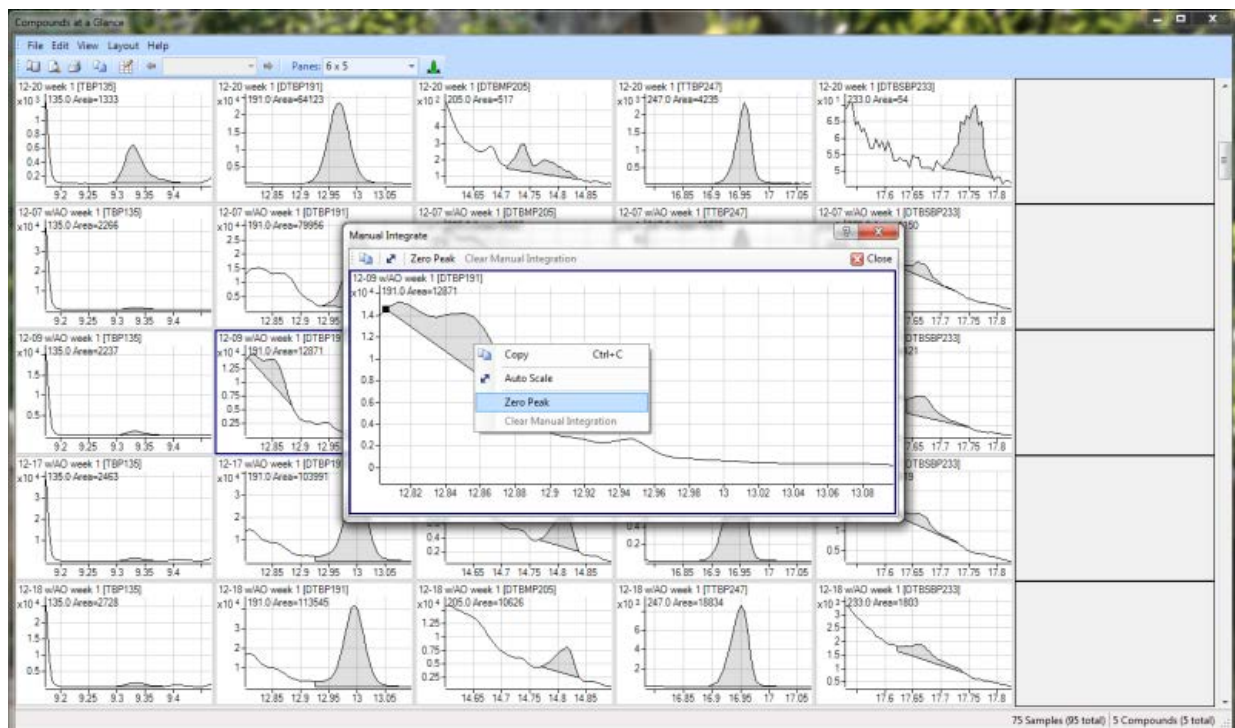
Single Compound View

Click on *Analyze batch* or F5 to display the processed data.

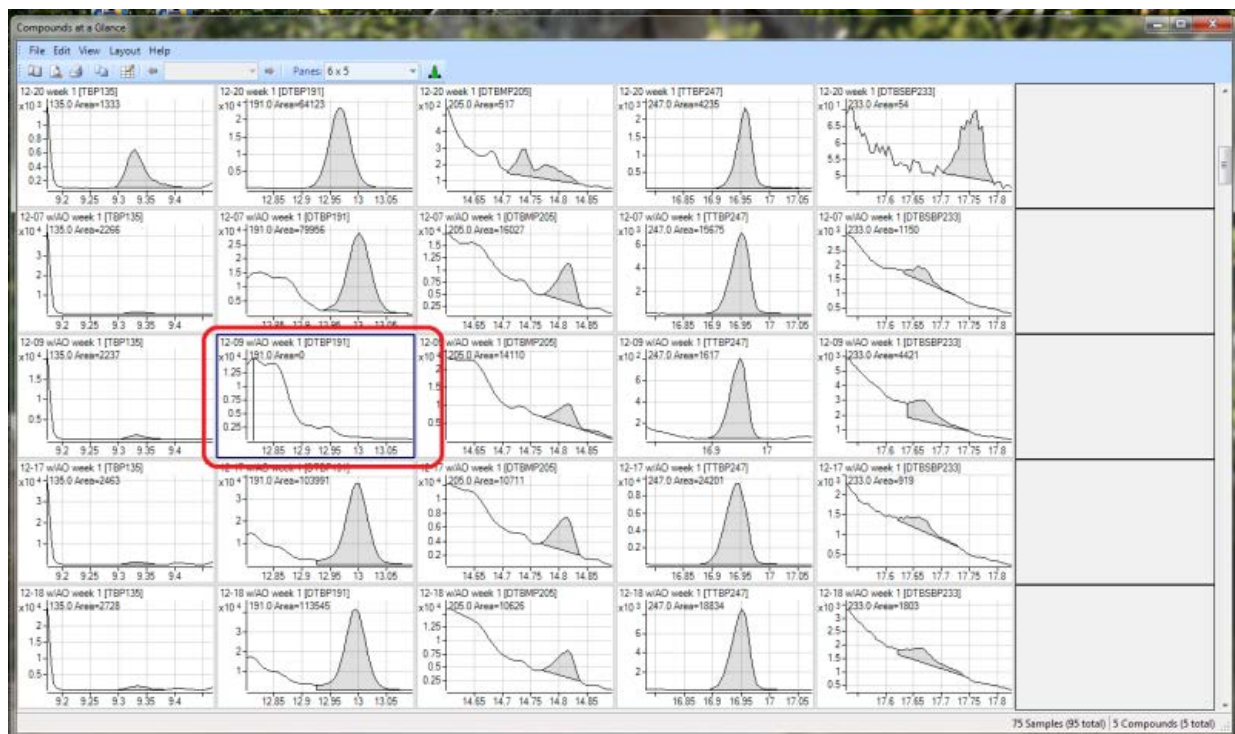
Now check to ensure that MassHunter has integrated the data appropriately. To do this quickly, go to *View > Compounds-at-a-glance...* and a new window will pop up with all the samples in the batch. To check just the unknown samples, click on *Layout > Samples\Targets\Auto Scale*.



The above figure highlights an instance where MassHunter had integrated something other than the target on a few samples. This can easily be corrected by double clicking on the suspect integration window, select *zero peak* to remove it, and exit. Check all samples, close the compounds-at-a-glance window and reanalyze the batch.

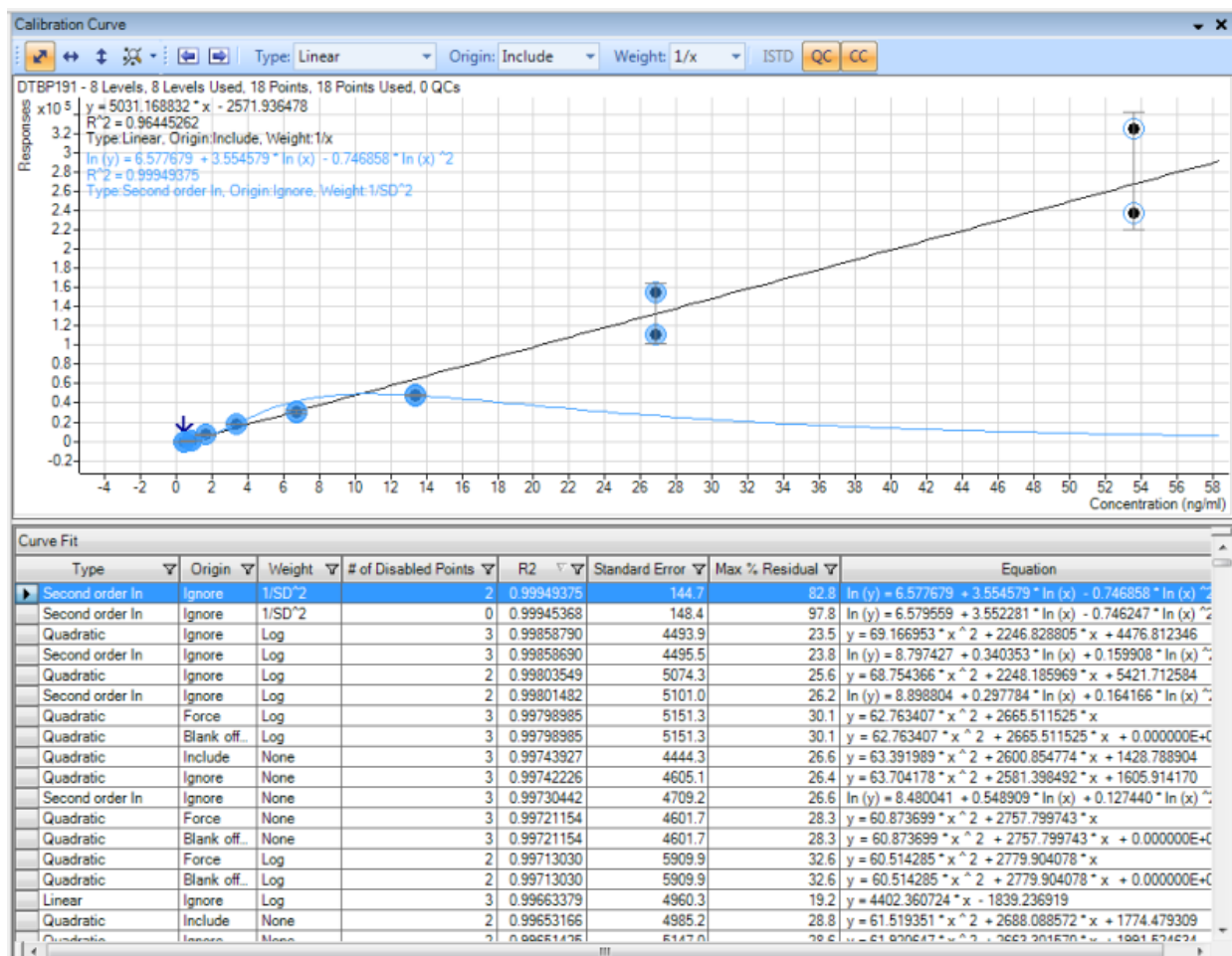


The below figure shows a misidentified peak that had been removed.



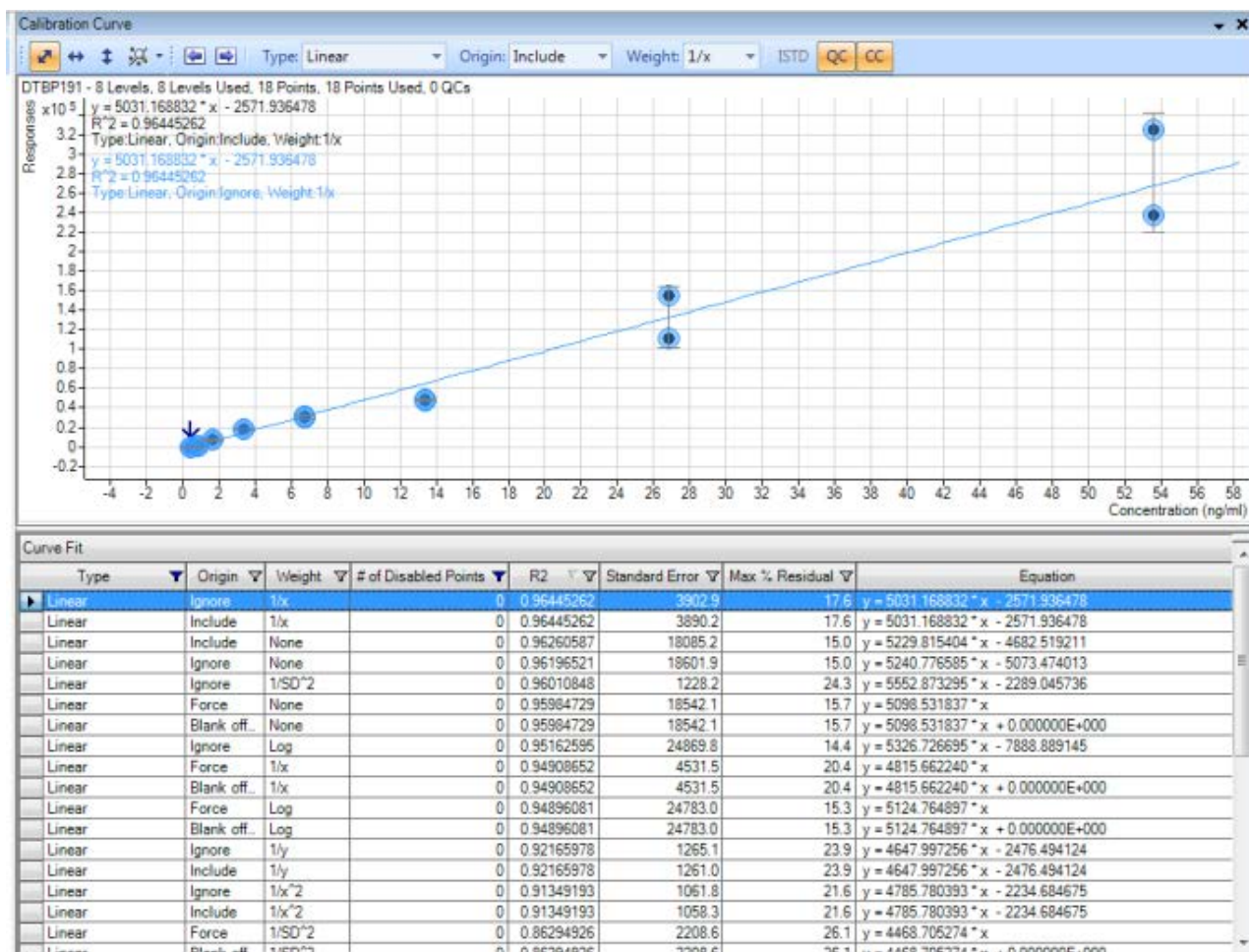
MassHunter may have red and blue highlights in the batch table. They are alerts that have been set up in the method to warn you of various issues. Some on the qualifier ratios will always be off depending on how your MS is operating. Regard alerts as more of a reminder to check and ensure that MassHunter is integrating correctly, or to make sure you are actually viewing the analyte of interest. It is not necessary to correct all the alerts, just use them as a quick check of the data for potential problems.

Checking calibration curve type. If the calibration data do not appear to be optimal, the type of curve, and its constraints might not be appropriate for the data. Right click on the calibration curve and select the curve fit assistant.

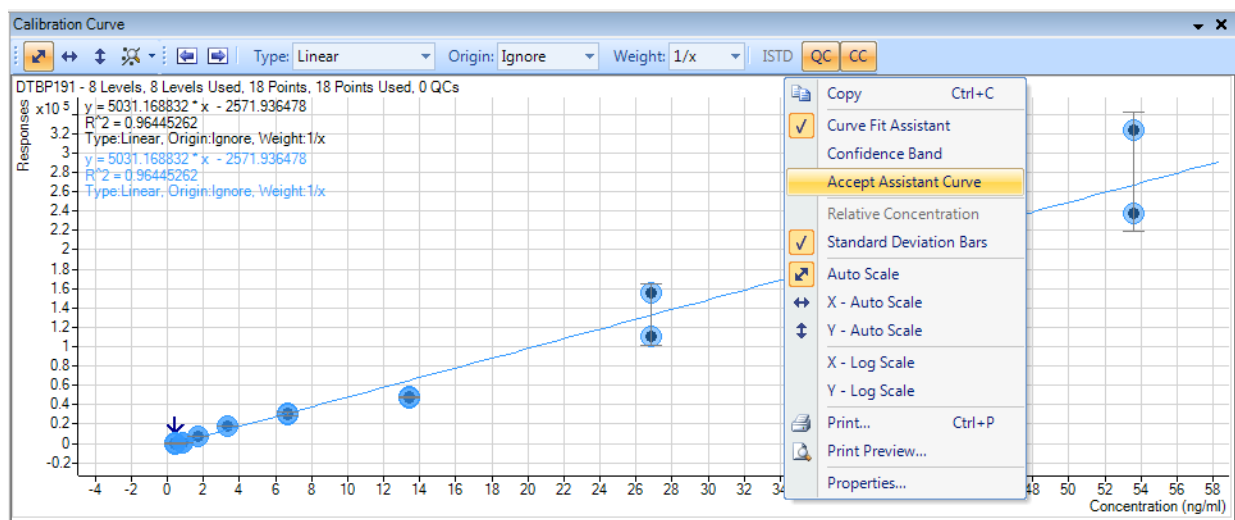


The curve fit assistant will present all available options for each curve. Do not simply pick the curve with the highest R^2 . As is shown in the figure above, the curve fit that produced the highest R^2 yielded a poor fit that did not represent all the data, and has discarded two data points. The below figure shows the linear fit that does not disable points. Each compound has its own

separate calibration curve, so it is necessary to repeat this procedure for each analyte calibration curve.



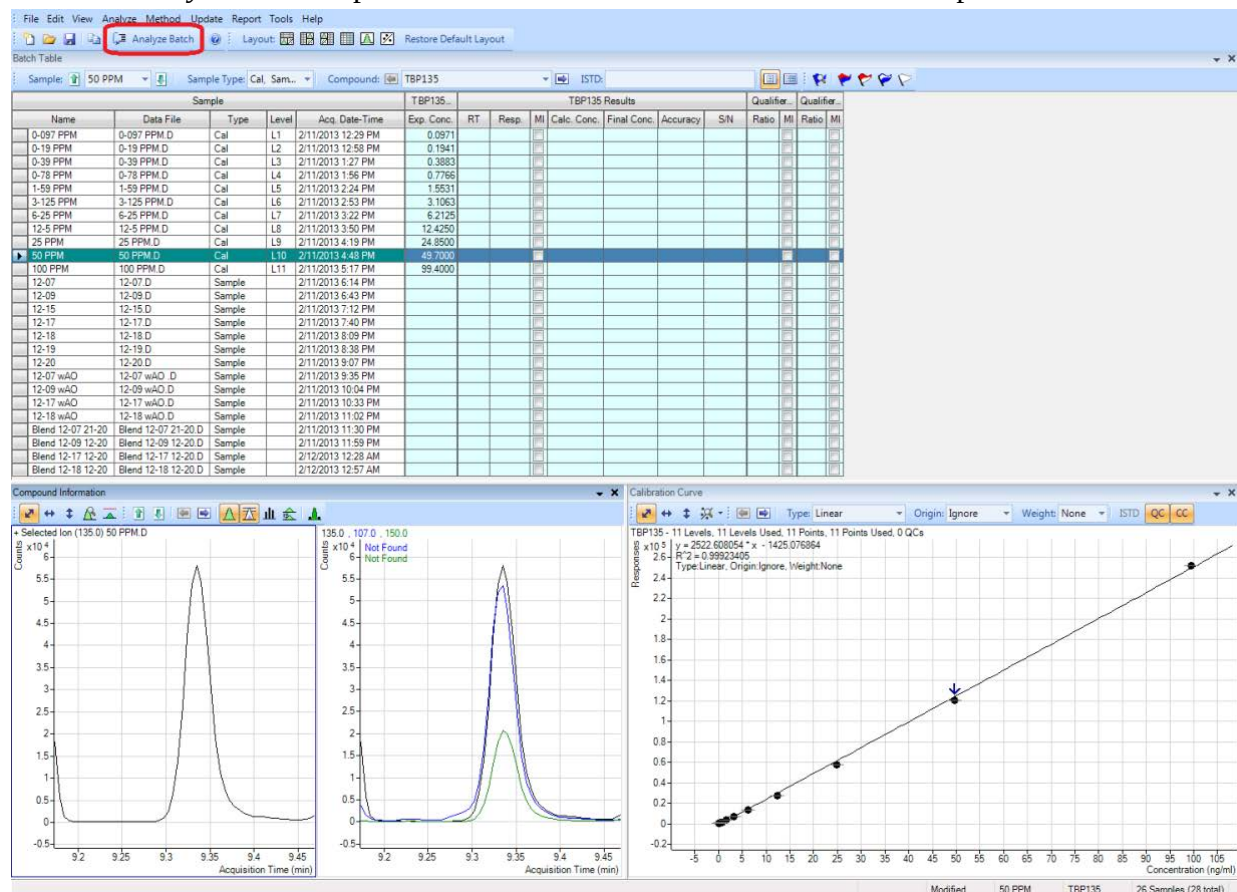
Right click on the plot and select *Accept Assistant Curve*



Reanalyze the data to ensure the best fit of the calibration curve. Note: it is recommended to save your data as MassHunter in case of a program crash.

Sample Analysis. Now that the calibration curve has been optimized, the sample data can be quantitated. (Note: the data set is different in this figure).

Click on *Analyze Batch* to process the results for both calibration and sample data.



The column “Final Conc.” lists the concentrations determined for each sample and standard.

File Edit View Analyze Method Update Report Tools Help

Analyze Batch Layout: Restore Default Layout

Batch Table

Sample: 0-78 PPM Sample Type: Cal, Sam... Compound: TBP135 ISTD:

Sample					TBP135 Results						
Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	Calc. Conc.	Final Conc.	Accuracy	S/N
0-097 PPM	0-097 PPM.D	Cal	L1	2/11/2013 12:29 PM	0.0971	9.297	22	0.150	0.1503	154.8	0.52
0-19 PPM	0-19 PPM.D	Cal	L2	2/11/2013 12:58 PM	0.1941	9.335	121	0.190	0.1907	98.2	2.64
0-39 PPM	0-39 PPM.D	Cal	L3	2/11/2013 1:27 PM	0.3883	9.329	677	0.417	0.4173	107.5	6.87
0-78 PPM	0-78 PPM.D	Cal	L4	2/11/2013 1:56 PM	0.7766	9.335	1260	0.655	0.6552	84.4	Infinity
1-59 PPM	1-59 PPM.D	Cal	L5	2/11/2013 2:24 PM	1.5531	9.335	2726	1.253	1.2534	80.7	Infinity
3-125 PPM	3-125 PPM.D	Cal	L6	2/11/2013 2:53 PM	3.1063	9.335	6726	2.885	2.8850	92.9	Infinity
6-25 PPM	6-25 PPM.D	Cal	L7	2/11/2013 3:22 PM	6.2125	9.335	13503	5.649	5.6496	90.9	451.62
12-5 PPM	12-5 PPM.D	Cal	L8	2/11/2013 3:50 PM	12.4250	9.334	27761	11.465	11.4657	92.3	157.83
25 PPM	25 PPM.D	Cal	L9	2/11/2013 4:19 PM	24.8500	9.335	57861	23.744	23.7443	95.6	415.59
50 PPM	50 PPM.D	Cal	L10	2/11/2013 4:48 PM	49.7000	9.335	120431	49.268	49.2686	99.1	Infinity
100 PPM	100 PPM.D	Cal	L11	2/11/2013 5:17 PM	99.4000	9.335	252205	103.023	103.0230	103.6	2641.13
12-07	12-07.D	Sample		2/11/2013 6:14 PM		9.297	0	0.141	0.1412		Infinity
12-09	12-09.D	Sample		2/11/2013 6:43 PM		9.281	0	0.141	0.1412		Infinity
12-15	12-15.D	Sample		2/11/2013 7:12 PM		9.308	0	0.141	0.1412		Infinity
12-17	12-17.D	Sample		2/11/2013 7:40 PM		9.335	0	0.141	0.1412		13.53
12-18	12-18.D	Sample		2/11/2013 8:09 PM		9.335	0	0.141	0.1412		10.60
12-19	12-19.D	Sample		2/11/2013 8:38 PM		9.335	0	0.141	0.1412		9.36
12-20	12-20.D	Sample		2/11/2013 9:07 PM		9.329	1269	0.659	0.6590		24.86
12-07 wAO	12-07 wAO.D	Sample		2/11/2013 9:35 PM		9.334	2215	1.044	1.0447		76.54
12-09 wAO	12-09 wAO.D	Sample		2/11/2013 10:04 PM		9.335	2604	1.203	1.2034		225.07
12-17 wAO	12-17 wAO.D	Sample		2/11/2013 10:33 PM		9.340	3132	1.418	1.4189		89.12
12-18 wAO	12-18 wAO.D	Sample		2/11/2013 11:02 PM		9.335	2747	1.261	1.2617		Infinity
Blend 12-07 21-20	Blend 12-07 21-20.D	Sample		2/11/2013 11:30 PM		9.335	661	0.410	0.4106		13.99
Blend 12-09 12-20	Blend 12-09 12-20.D	Sample		2/11/2013 11:59 PM		9.329	629	0.397	0.3978		13.43
Blend 12-17 12-20	Blend 12-17 12-20.D	Sample		2/12/2013 12:28 AM		9.335	630	0.398	0.3980		4.80
Blend 12-18 12-20	Blend 12-18 12-20.D	Sample		2/12/2013 12:57 AM		9.335	730	0.438	0.4389		10.13

This completes the analysis.