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Purpose

This project was developed to create a novel fire debris interpretation process that is more standardized, transparent, and objective. The specific goals and objectives were as follows:

Goal 1 – Develop and validate a data interpretation process for the identification of gasoline

Objective 1 – Determine and quantify significant identifying criteria for gasoline

Objective 2 – Based on the data, create a sufficiency graph with decision curves

Objective 3 – Detail an analysis method (similar to Analysis, Comparison, Evaluation, and Verification (ACE-V) Method) that facilitates documentation, independent verification and review

Goal 2 – Develop and validate a method for independent verification by a second examiner

Objective 4 – Using comparison software, develop protocols for transparent workflow

Objective 5 – Evaluate the process with mock samples containing complex matrices

Objective 6 – Evaluate the developed process in relation to current methodologies

Taken together, the goals and objectives of this study result in a quantitative sufficiency chart nested within an ACE-V workflow. This workflow includes the adaptation and utilization of a computerized documentation program that facilitates examiner evaluation and independent verification, ensuring reproducible data interpretation.

Although not typically mentioned in the chemistry disciplines of forensic science, subjectivity is at the forefront of discussions regarding the physical evidence disciplines. The current method of comparisons for Gas Chromatography-Mass Spectroscopy (GC-MS) fire debris data and the documentation process for final determinations are considered subjective and are based on the training and experience of the individual examiner. This can lead to inconsistencies in the quantity of information and sometimes the results that are provided from one analyst to another, particularly with complex samples. The lack of any standardized interpretation method for the analysis of GC-MS fire debris data indicated the need for the development and implementation of a novel objective methodology.

Prior to this research project, there was no validated guidance for favorable chromatographic pattern comparisons for the Total Ion Chromatograms (TIC) or Extracted Ion Profiles (EIPs) and documentation of the

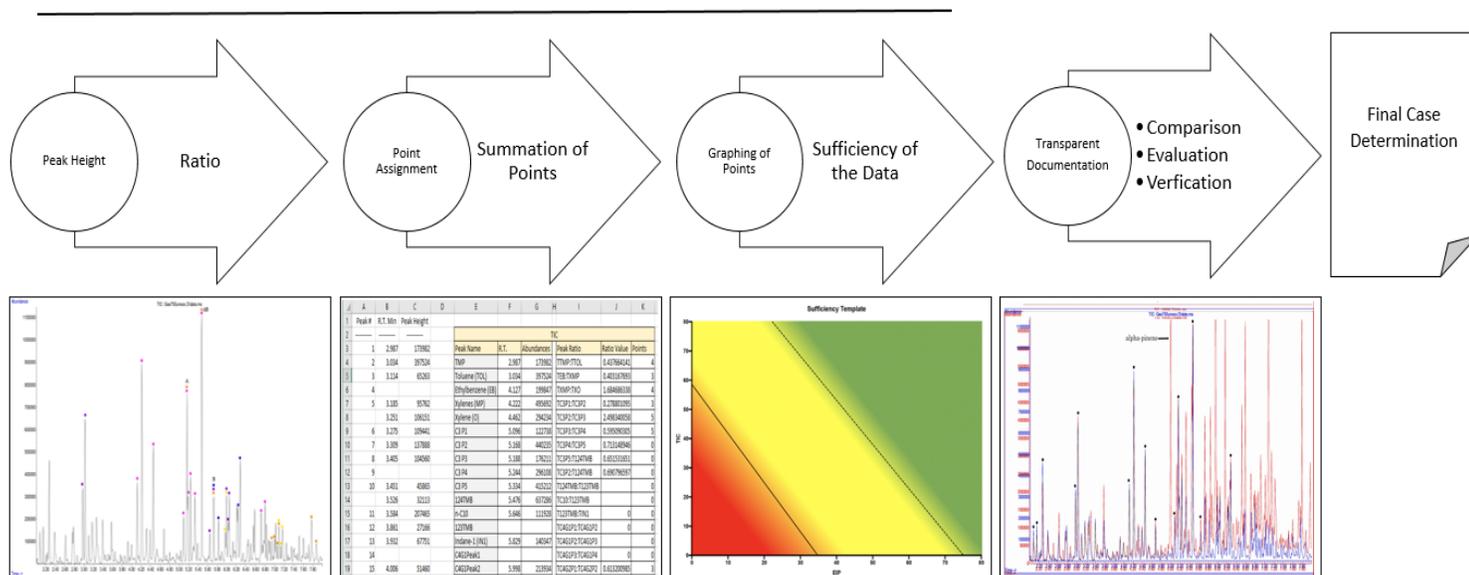
comparisons between the questioned and known samples did not have standardized requirements. On this basis, it was important to develop and validate new methods that have the potential to facilitate a transition from a subjective approach to a more objective, universally standardized approach.

Project Design

A methodology for establishing and measuring variables associated with quantity, quality, and confidence was optimized and applied to a control repository of gasoline and negative matrix samples. Key chromatographic features in the TIC and EIPs from the repository of control gasoline samples were evaluated and 61 chromatographic peak height ratios of interest were selected for the study. Statistical analysis was conducted to determine the variation observed for each of these ratios in the known gasoline samples and to determine the frequency of these key chromatographic features in the negative matrix samples. This information was evaluated to determine relative scores for each of these features. The scores were then used to create a validated sufficiency chart, which graphically displays the totality of data supporting a final determination. The results of this study were then integrated into an augmented ACE-V workflow, thereby ensuring usability, while maintaining consistent objectivity and transparency in fire debris examinations and comparisons. This is graphically summarized in Figure 1.

Figure 1 - Project Overview

Analysis



Establishing Quantitative Measures of Chromatographic Features in Gasoline and Negative Matrix Data

Gasoline is a mixture of many individual aromatic and aliphatic hydrocarbons, which can be observed in the TIC and EIP data. The chromatographic features of interest for this study (Table 1) include individual compounds that were selected from the TIC and EIPs because they are abundant, representative of the class of compound, and generally in a boiling range present at multiple evaporation levels.

Table 1 - Chromatographic Features of Interest

| Data Display | Ions used (m/z) | Number of Peaks Selected | Number of Peak Pair Ratios | Compounds of Interest |
|---------------------------|--------------------|--------------------------|----------------------------|--|
| TIC | All Tabulated | 34 | 21 | 2,3,4-trimethylpentane, toluene, ethylbenzene, xylenes (M,P,O), C3 alkylbenzenes, 1,2,4-trimethylbenzene, decane, 1,2,3-trimethylbenzene, indane, C4 alkylbenzenes, indanes and indenenes, C5 alkylbenzenes, naphthalene, dodecane, 1-methylnaphthalene, 2-methylnaphthalene |
| Alkane/isoalkane EIP | 57, 71, 85, 99 | 14 | 8 | Normal Alkanes within the medium boiling range (n-C10 to n-C12) and prominent branched alkanes that represent the isoparaffinic and distillate fraction with the n-C10 to n-C12 range |
| Aromatic/alkylbenzene EIP | 91, 105, 119, 133 | 25 | 15 | Toluene, ethylbenzene, xylenes (M,P,O), C3 alkylbenzenes, 1,2,4-trimethylbenzene, 1,2,3-trimethylbenzene, C4 alkylbenzenes, C5 alkylbenzenes |
| Indane EIP | 117, 118, 131, 132 | 11 | 6 | Indane and indene compounds that form the prominent peaks within the profile |
| Polynuclear Aromatic EIP | 128, 142, 156 | 3 | 1 | Naphthalene, 1-methylnaphthalene, 2-methylnaphthalene |

To establish the quantitative measures of the selected chromatographic features, a gasoline control and a negative matrix database were created. The gasoline control database was built with data from currently

marketed gasolines purchased from three geographic regions (Virginia, Florida, and Louisiana) and analyzed at different evaporation levels (Whole, 25%, 50%, 75%, and 90% Evaporated) by three different laboratory methods under their existing instrumental conditions (Table 2). The negative matrix database was built with data files from negative scene debris, lab burned substrate, and data files from the National Center for Forensic Science (NCFS) substrate database.

Table 2 - Gasoline Control Repository and Database

| GC-MS Method | | |
|--|--|---|
| Virginia Department of Forensic Science | Pinellas County Forensic Lab (Florida) | Louisiana State Police Department |
| Source Locations | | |
| 10 gasolines from different gas stations in Eastern Virginia | 10 gasolines from different gas stations around Pinellas County | 10 gasolines from different gas stations in Louisiana |
| Evaporation Levels | | |
| Each of the 10 samples, from the different gas stations, have a Whole, 25% Evaporated, 50% Evaporated, 75% Evaporated and 90% Evaporated aliquot, for a total of 50 aliquots | | |
| Summary | | |
| Sample Repository | 3 laboratory locations compiled samples from 30 different gas station locations at 5 levels of evaporation for a total of 150 gasoline samples | |
| Data Files | 150 gasoline samples analyzed on 3 different GC-MS methods for a total of 450 data files | |

The data points that form the respective databases were generated by collecting the peak heights of the key chromatographic features. The individual peak heights were then used in a ratio peak pair of closely eluting compounds within a given sample. After reviewing the cumulative peak pair ratio data, multiple comparisons were used to determine which peak pair ratios were statistically supported and, therefore, likely indicative of identifying criteria for gasoline. The goal of the comparisons of the peak pairs was to determine which peak pair ratios were statistically supported as being consistent in frequency and value in gasoline, while taking into account the source location, evaporation level and the instrumental analysis method (ANOVA rank). The frequency (Frequency rank) and variability (Wilcoxon rank) of the ratios of interest appearing in the negative matrix was also considered. The most statistically supported peak pairs were ranked and organized by color on the basis of their p-values for use in establishing the point values for the creation of the sufficiency graph. The color rank assignments are described in Table 3.

Table 3 - Description of the Color Rank Assignments.

| Color | Rank | Description |
|--------|---------------|---------------------------------|
| Purple | Highest Rank | ANOVA rank is a low number |
| | | Wilcoxon rank is a low number |
| | | Frequency rank is a low number |
| Pink | Middle Rank 1 | ANOVA rank is a low number |
| | | Wilcoxon rank is a low number |
| | | Frequency rank is a high number |
| Blue | Middle Rank 2 | ANOVA rank is a low number |
| | | Wilcoxon rank is a high number |
| | | Frequency rank is a low number |
| Green | Middle Rank 3 | ANOVA rank is a high number |
| | | Wilcoxon rank is a low number |
| | | Frequency rank is a low number |
| Yellow | Middle Rank 4 | ANOVA rank is a low number |
| | | Wilcoxon rank is a high number |
| | | Frequency rank is a high number |
| Orange | Middle Rank 5 | ANOVA rank is a high number |
| | | Wilcoxon rank is a low number |
| | | Frequency rank is a high number |
| Gray | Middle Rank 6 | ANOVA rank is a high number |
| | | Wilcoxon rank is a high number |
| | | Frequency rank is a low number |
| Red | Lowest Rank | ANOVA rank is a high number |
| | | Wilcoxon rank is a high number |
| | | Frequency rank is a high number |

Once established, the possible point values per color code that could be earned by each peak pair ratio were based on which of the three standard deviation ranges (± 1 , ± 2 , or ± 3) the peak pair ratio value falls within. The accumulated ratio points are then summed into two values which form the X and Y coordinate of the sufficiency graph; the X coordinate is the summation of the point values from the EIP ratios and the Y coordinate is the summation of the point values from the TIC ratios.

The proposed point system for the aforementioned ranked ratios was applied to sample data and plotted to generate the draft model of the sufficiency graph with quantitative placement of the decision lines. To further develop and validate the proposed quantitative model of the sufficiency graph, additional optimization steps were taken using a data-based approach and the reproducibility of the process was confirmed.

Augmentation and Application of the ACE-V Method

ACE-V is an acronym that represents a four-phase methodology for guiding examiners from pattern based disciplines through a systematic and structured approach to presenting the quality and quantity of data available.

The Analysis step of the ACE-V method consists of an objective and comprehensive observation of the quality and varying characteristics of the sample in question. The Comparison step of the ACE-V method focuses on the in-depth process of comparing the unknown sample to a reference gasoline sample, allowing confirmation and documentation of the shared characteristics. Based on the results of the Analysis step and the data-supported Comparison step, a conclusion is made and a supporting narrative is drafted during the Evaluation step of the ACE-V method. Lastly, the Verification step of the ACE-V method allows for the independent retesting of the conclusions that were made during the evaluation step. It is a form of peer review and serves to further confirm that the results are accurately and objectively represented by the initial examiner's conclusions. This original ACE-V methodology has been tailored to create a novel examination, documentation, and verification process for GC-MS fire debris data analysis.

This proposed adaptation of the ACE-V method (Appendix 1) provides guidance for how to process, compare, evaluate, and verify unknown samples in an objective and reproducible manner and incorporates software-based tools whose applications serve to enhance documentation transparency while standardizing the workflow.

Data Analysis

The quantitative evaluation of the gasoline control database was achieved by first performing a statistical comparison of the source, method, and evaporation level ratio data in a single three-way ANOVA model. Here the model considers the effect of one variable (source location, GC-MS method, evaporation) across the levels (three geographic regions, three laboratory GC-MS methods, and five evaporations) of the other variables simultaneously. That is, the effect on the model due to the method can be observed as the variation of different GC-MS method levels when source and evaporation levels are held constant. This model confirmed and controlled for the origin of variability within gasoline samples, revealing ratio pairs that were consistent and therefore most likely indicative of an identifying criteria for gasoline. For the interpretation of the ANOVA results, a Bonferroni correction was applied and produced a 0.00078 significance threshold for the

p-value. For the second evaluation, a frequency analysis was performed on the raw peak data for both the control gasoline data and the negative matrix data. This was used to quantitate the consistency of certain peaks being present in gasoline samples, but absent in negative matrix samples. The third evaluation utilized a Wilcoxon Ranked Sum Test to compare the patterns of the peak ratios within the negative matrix database (when both chromatographic features of a ratio were present) to the patterns of the peak ratios in the gasoline control database. This confirmed how variable or consistent the pattern was between the two datasets. For the interpretation of the Wilcoxon Ranked Sum Test results, a Bonferroni correction was applied and produced a 0.00106383 significance threshold for the p-value. Collectively, these evaluations were then used (as summarized in Table 4) to produce a statistically supported ranking for the acceptable variability of each peak height ratio among the selected key chromatographic peaks.

Table 4 – Description of Data Ranking for Establishing the Sufficiency Graph

| Database | Negative Matrix | Negative Matrix-Control Gasoline=Difference | Control Gasoline |
|------------------------|---|---|--|
| Dataset | Wilcoxon | Frequency | ANOVA |
| Description | Nonparametric comparison of the statistically significant difference between the gasoline control ratios and the negative matrix ratios | The raw frequency of peaks in gasoline samples, and in negative matrix samples | Parametric comparison of the statistically significant difference between the control ratios taking into account the complete model of variables |
| p-value | 0.00106383 | N/A | 0.00078 |
| Description of p-value | p-values that are >0.00106 show that the ratios between the negative and control database are not statistically significantly different; Consistent | No p-value, but rather the difference in frequency between the absence of the negative and control ratios as a percent. The higher the percent the bigger the difference in the absence of the ratio between the two databases, the smaller the percent the smaller the difference in the absence of the ratio. | p-values that are >0.00078 show that the ratios between the negative and control database are not statistically significantly different; Consistent |
| | p-values that are <0.00106 show that the ratios between the negative and control database are statistically significantly different; Variable | | p-values that are <0.00078 show that the ratios between the negative and control database are statistically significantly different; Variable |
| Ranking Convention | Ranked 1-47, on the basis of lowest p-value to highest p-value | Ranked 1-64, on the basis of high percentages to low percentages | Ranked 1-63, on the basis of highest p-value to lowest p-value |
| Ranking Description | Low numbers are more variable and high numbers are more consistent; variability is important here so low numbers are preferable. | Low numbers are those with the biggest difference in absence between the negative and control database. Low numbered ranks mean that the negative database ratio was most often absent, while in the control database the ratio was most often present; again low numbers are preferable. | Low numbers in the ranking are more consistent and high numbers in the ranking are more variable; consistency is important here so low numbers are preferable. |

Following the ranking of each ratio peak pair (organized by color), an earnable point value range was assigned. To generate the point value assignments, a peak ratio that was closer to the sample mean for a ratio’s aggregate control gasoline data received more points while those farther from the mean for a ratio’s aggregate control data received fewer points. That is, the points are earned by individual samples based on where their determined ratio value falls within a given standard deviation range (Figure 2); the ranges were generated based on the column means of the log transformed ratios for the aggregate of control samples. Each ratio peak pair is shown in Table 5 with the potential point values earned per color rank. The two lowest ranks (gray and red) are eliminated as they did not meet the statistical objectives.

Figure 2 - Point (Pts) Assignments Based on Standard Deviation (Std. Dev) and Distribution

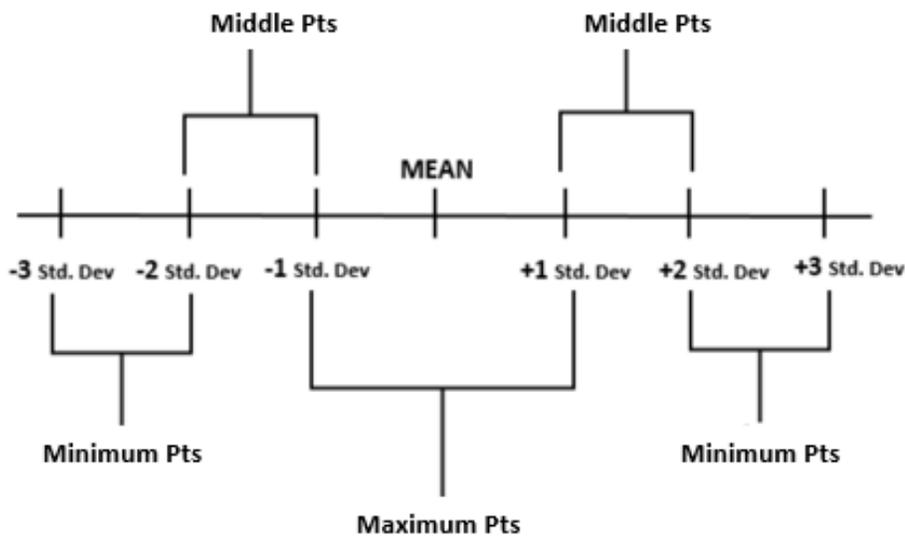


Table 5 - Color-Coded Final Rankings for All Peak Pair Ratios and Attainable Point Values

| Color Rank | Ratio Name | Maximum Points | Middle Points | Minimum Points |
|------------|-------------------|----------------|---------------|----------------|
| Purple | TTMP:TTOL | 6 | 5 | 4 |
| | TC4G1P2:TC4G1P3 | | | |
| | TC4G1P3:TC4G1P4 | | | |
| | TIN6:TIN7 | | | |
| | ALK4:ALK5 | | | |
| | ARC4G1P2:ARC4G1P3 | | | |
| | ARC5C4P5:ARC5P2 | | | |
| | ININ8:ININ9 | | | |
| | ININ9:ININ10 | | | |
| Pink | TEB:TXMP | 5 | 4 | 3 |
| | TXMP:TXO | | | |
| | TC3P1:TC3P2 | | | |
| | TC3P4:TC3P5 | | | |
| | TIN2:TIN3 | | | |
| | TIN4:TIN5 | | | |

| | | | | |
|--------|---|---|---|---|
| | ALK6:ALK8 ALKC11:ALKC12 ALK10:ALK11 ARC3P1:ARC3P2 ARC3P2:ARC3P3 ARC3P4:ARC3P5 ARC4G2P1:ARC4G2P2 ARC4G3P1:ARC4G3P2 ININ5:ININ6 | | | |
| Blue | TC4G2P1:TC4G2P2 ALK2:ALK3 ALK3:ALK4 ALK5:ALK7 ALK7:ALK9 | 4 | 3 | 2 |
| Green | TC10:T123TMB TC4G1P1:TC4G1P2 TIN5:TIN6 ARC4G1P3:ARC4G1P4 ARC5C4P1:ARC5C4P2 ININ4:ININ5 ININ10:ININ11 | 3 | 2 | 1 |
| Yellow | AR123TMB:ININ1 | 2 | 1 | 0 |
| Orange | TC3P2:TC3P3 TC3P3:TC3P4 TC3P5:T124TMB TC3P2:T124TMB T124TMB:T123TMB TC5P1:TC5P2 TC1PNA1:TC1PNA2 AREB:ARXMP ARXMP:ARXO ARC3P5:AR124TMB ARC3P2:AR124TMB AR124TMB:AR123TMB ARC4G1P1:ARC4G1P2 ININ6:ININ7 PNAC1PNA1:PNAC1PNA2 | 1 | 0 | 0 |
| Gray | T123TMB:TIN1 TC4G3P1:TC4G3P2 ALKC9:ALKDIS9 ALKISDS1:ALKISDS2 ALKISDS4:ALKC11 ARMX2:ARMX3 ARMX3:ARMX4 ARMX4:ARMX5 ARC3P5:ALKISO2 | 0 | 0 | 0 |
| Red | ALKC10:ALKDIS11 ARC3P3:ARC3P4 ININ2:ININ3 | 0 | 0 | 0 |

The proposed point system for the aforementioned ranked ratios (organized by color) was applied to the control gasoline data and plotted to generate the first draft model of the sufficiency graph. Quantitative placement of the decision lines was achieved using a Linear Discriminant Analysis. Optimization and validation to further develop the model of the sufficiency graph took place over several iterations and included several data sets.

The evaluations of the proposed processing method and validation of the sufficiency graph template were performed using additional databases (Table 6).

Table 6 - Database Summary for Sufficiency Graph Evaluation and Validation

| Database | Content | Purpose |
|----------|---------|---------|
|----------|---------|---------|

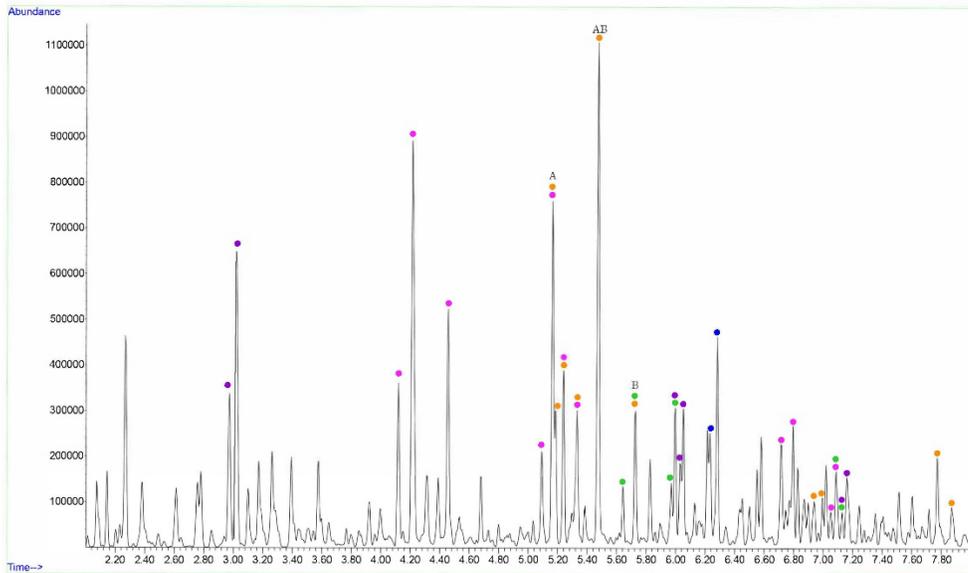
| | | |
|--|--|---|
| Other Reference Ignitable Liquids (ORIL) | Other classes of ignitable liquids and mixtures of ignitable liquid classes. | To test where non-gasoline ignitable liquids and mixtures of gasoline with non-gasoline ignitable liquids fall on the continuum of data based on the peaks selected and points assigned; to test points with the quantitative decision lines. |
| General Other Methods | 50% and 90% evaporated gasoline newer than 2016, analyzed using instrument methods currently in use at ten other forensic laboratories. | To test the resolution of other chromatographic methods using the proposed peak selection and point system process; subsequently, to determine/confirm the data point placement on the graph after LDA line placement. |
| Previously Determined Data Files | Case-like, non-laboratory created samples, with existing identifications of "Gasoline ID", "No ID", or "Other ID" processed by two examiners. | To test the efficacy of the proposed peak selection and point system process using samples likely to be seen in casework. |
| Reproducibility Testing | Three lab created "unknown" samples (1 negative, and 2 matrix plus gasoline), and three additional "unknown" samples (1 negative, 1 gasoline, and 1 matrix plus gasoline). | To test the reproducibility of the proposed method and determine potential inter-examiner variability, Virginia Department of Forensic Science Fire Debris Examiners were asked to analyze samples using the sufficiency method. Results were graphed pre- and post-training. |

The cumulative results of the databases were used to optimize and validate the sufficiency graph and contribute to its objectivity and usability.

Findings

The sufficiency method defines statistically supported identification criteria for the specific chromatographic features proposed, while also providing the relative statistical weight of each of these peak pair ratios with respect to one another. Further, it allows for enhanced documentation transparency with the inclusion of the graphical sufficiency output. The statistically supported identification criteria for the chromatographic features of interest was achieved by ranking the cumulative control and negative ratio data, and subsequently organizing the ranks by color; a point range was then assigned based on the color-associated rank. A visual summary of the colors used in the statistically supported ranking system for the ratios was generated for the TIC (Figure 3) and EIPs (Appendix 2) using the Adobe Elements software.

Figure 3 - Example TIC with Statistically Ranked Peak Ratios by Color

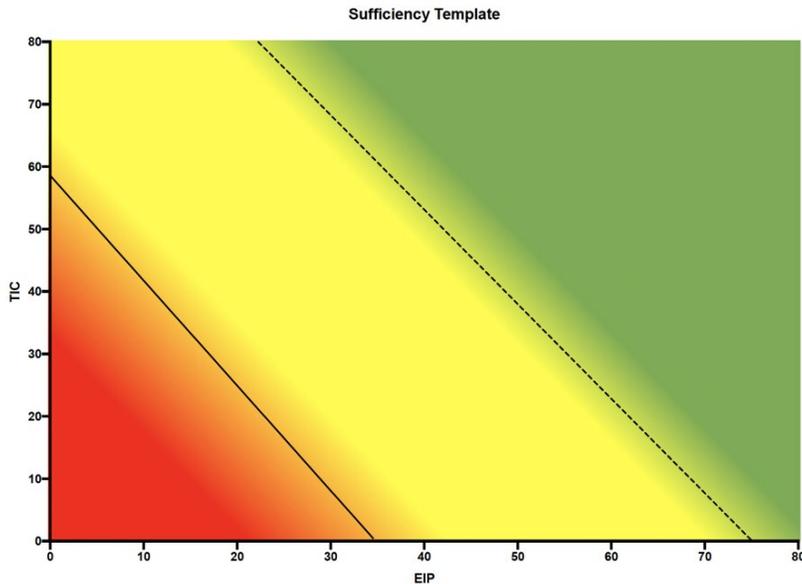


Referring to the figure, the colored dots above the key chromatographic peaks denote the statistically supported ranks of the ratios, which translates to the maximum potential points obtainable during the sufficiency evaluation. Purple denotes the highest rank, followed by Pink, Blue, Green, Yellow, and the lowest rank of Orange. Unless otherwise noted, moving from left to right, dots of the same color on two adjacent key chromatographic peaks form a given ratio. Dots labeled with a letter denote a ratio of two peaks that are not immediately adjacent.

The proposed point system for the aforementioned ranked ratios was applied to sample data and plotted to generate and validate the sufficiency graph with the decision lines (Figure 4). This graph was built and validated using a data-based approach to allow for an objective, reproducible visual representation of the quantity of data available to support the identification of gasoline in unknown samples. Although the basis of the finalized sufficiency template is a statistically supported and validated predictive model, it does not necessarily dictate a conclusion. Rather, the decision lines allow for the rapid identification of samples that have significant support for the identification of gasoline and those that do not have enough support to pursue gasoline. The sufficiency graph also informs the examiner, the reviewer, and potentially the trier of fact if a sample is complex with regard to the information available to support a gasoline identification. The finalized

sufficiency graph with the plotted unknown sample points is intended to become a part of the case record along with the documented decision made by the experienced examiner.

Figure 4 - Finalized Sufficiency Graph with Decision Lines



Implications for Criminal Justice Policy and Forensic Practices in the United States

The method developed and validated within this project has several positive implications for the field of forensic fire debris analysis and criminal justice practices in the United States. For gasoline, this study provides the examiners and trier of fact with an objective, statistically supported method to view and discuss the data available to support a conclusion. Within the novel and objective proposed method, the available data includes the ranking of chromatographic features of interest with respect to their statistical weight as well as the measure of acceptable pattern variation for each key chromatographic feature's ratio pair. During review, this data can be used to facilitate a transparent discussion between examiners; and, during a court hearing, it can be used to provide objective information to the trier of fact. These results fill a much needed void in the field of fire debris analysis in that it provides statistically validated, objective and transparent information to support a conclusion that expands on the current pattern comparison process.

The information generated within this research project has been disseminated using several different platforms. The materials were first presented at the 2020 American Academy of Forensic Sciences (AAFS) Meeting via an abstract and oral presentation. The material was also presented, with updates reflecting the progress of the project, at the Chemistry Plenary Session of the March 2020 Organization of Scientific Area Committees (OSAC) for Forensic Science, at a 2020 WebEx training for the New York Fire Debris Examiners Technical Working Group, and at the October 2020 ASTM International Workshop on Interlaboratory Studies. This study will be submitted for publication in the Journal of Forensic Chemistry as two journal articles. The first submission is in progress. The second submission and an abstract for the AAFS 2022 meeting are forthcoming.

Appendix

Appendix 1: Augmented ACE-V Method

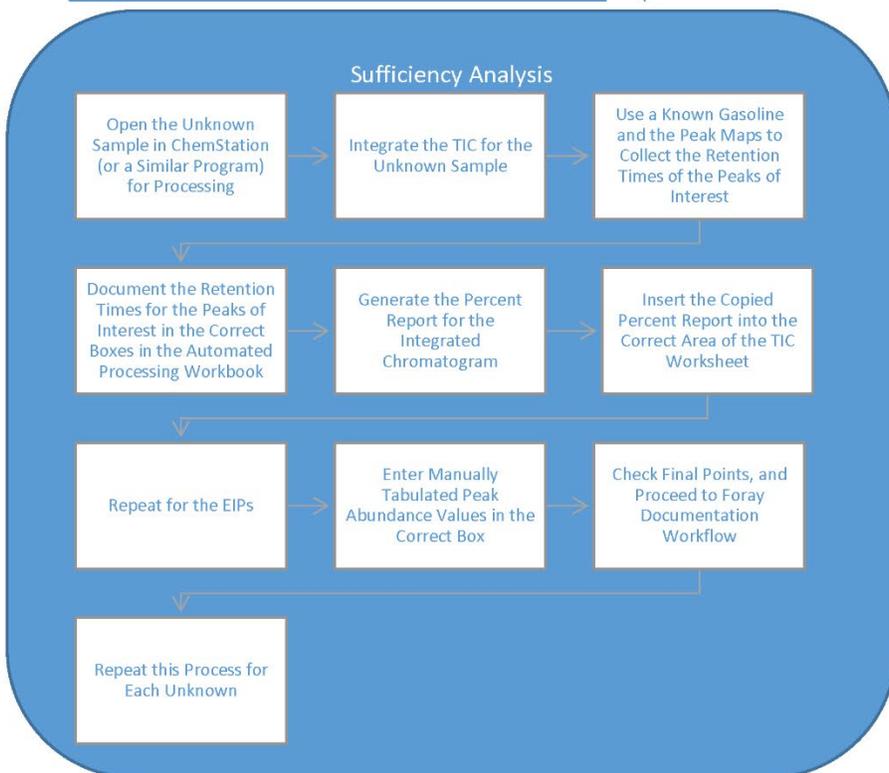
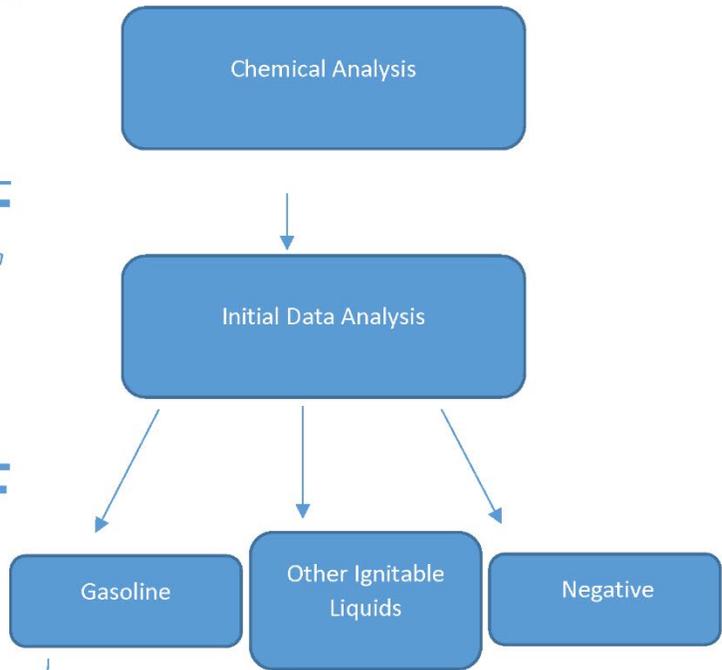
ANNOTATED FLOWCHART: ACE-V

ANALYSIS

Chemical Analysis: The Laboratory method for generating chromatograms for the unknown and reference compounds. The GC-MS method used should resolve the key diagnostic peaks.

Initial Data Analysis: The initial data interpretation to determine if chromatographic characteristics indicate that gasoline may be present. This step requires the use of a known reference based on the pattern present. Comprehensive guidance for this step is detailed further.

Preliminary Determination: The initial data analysis should suggest one of three outcomes: gasoline, other ignitable liquid, or negative. If gasoline is likely present, the Sufficiency Analysis should be pursued. Analysis processes for other ignitable liquids have not been developed. A negative determination can be made at two points, based on the analysis.



Sufficiency Analysis: The statistically supported process for determining the strength of the data to support an identification.

COMPARISON

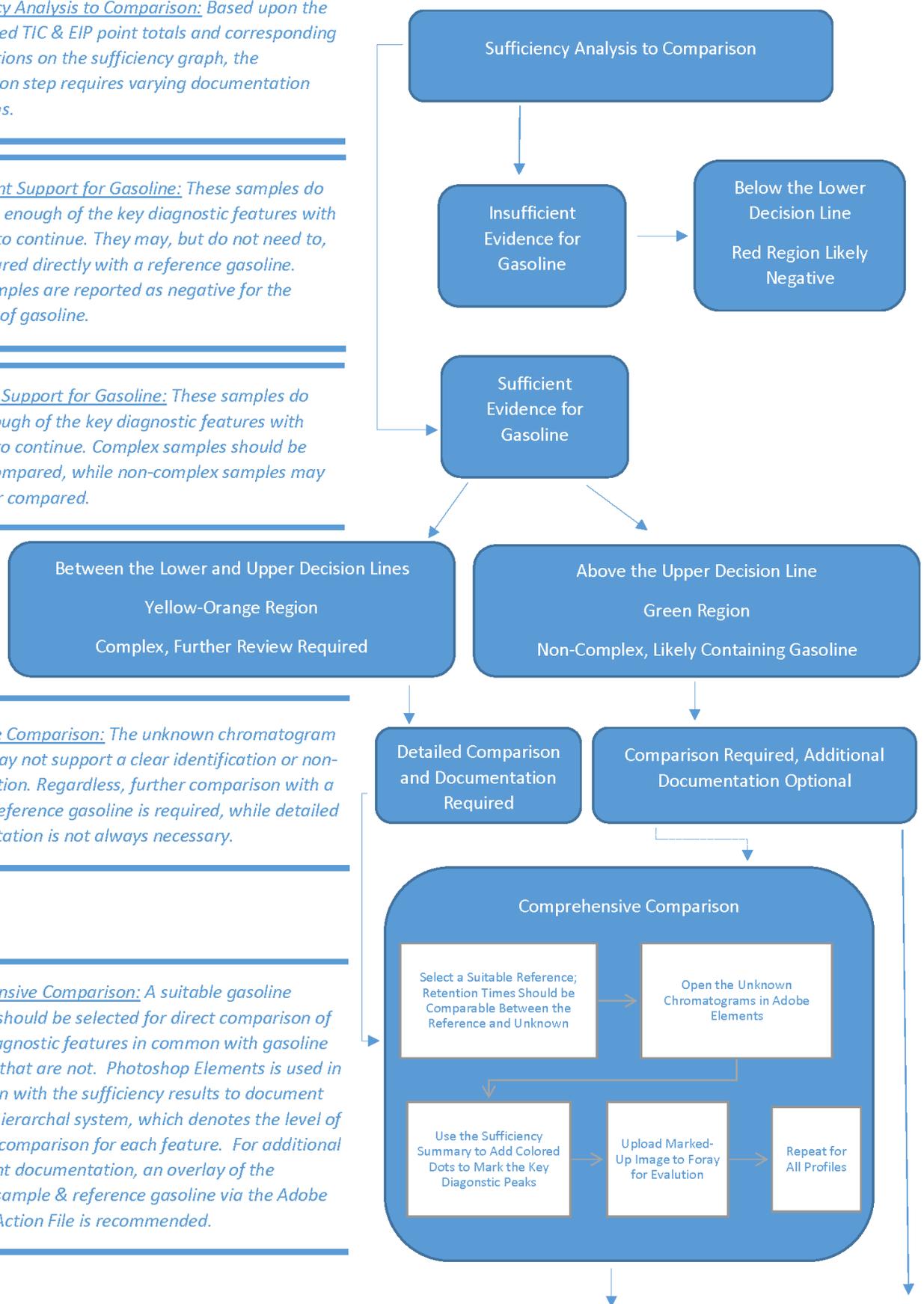
Sufficiency Analysis to Comparison: Based upon the determined TIC & EIP point totals and corresponding plot locations on the sufficiency graph, the comparison step requires varying documentation minimums.

Insufficient Support for Gasoline: These samples do not share enough of the key diagnostic features with gasoline to continue. They may, but do not need to, be compared directly with a reference gasoline. These samples are reported as negative for the presence of gasoline.

Sufficient Support for Gasoline: These samples do share enough of the key diagnostic features with gasoline to continue. Complex samples should be further compared, while non-complex samples may be further compared.

Reference Comparison: The unknown chromatogram may or may not support a clear identification or non-identification. Regardless, further comparison with a suitable reference gasoline is required, while detailed documentation is not always necessary.

Comprehensive Comparison: A suitable gasoline reference should be selected for direct comparison of the key diagnostic features in common with gasoline and those that are not. Photoshop Elements is used in conjunction with the sufficiency results to document the color hierarchal system, which denotes the level of statistical comparison for each feature. For additional transparent documentation, an overlay of the unknown sample & reference gasoline via the Adobe Elements Action File is recommended.



EVALUATION

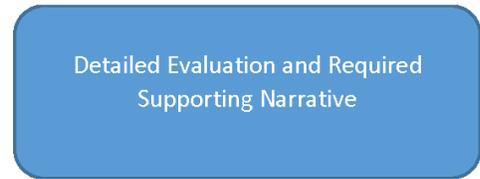
Evaluation: Depending on the outcome of the previous step, the conclusions may have enough support for a determination without a detailed evaluation. However, the complex region's detailed comparisons should always be critically evaluated at this step. All outcomes should have supporting narrative drafted.

Software facilitated documentation, including the results from the comparison step, should be completed via the Foray Adam System.

VERIFICATION

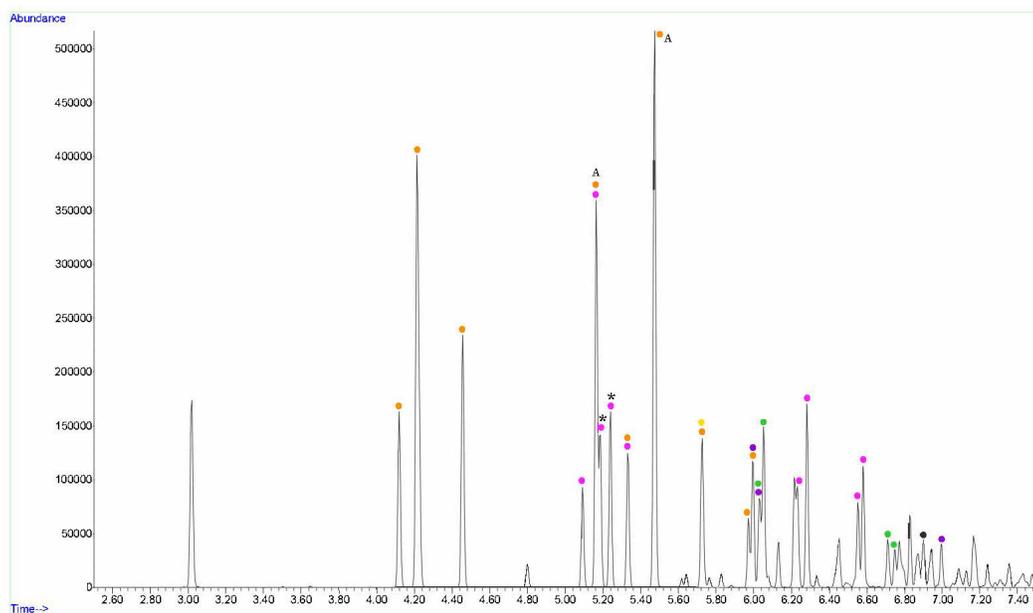
Verification: Recommended as a necessary step for all data that falls into the yellow, complex, region, but may not be necessary for the other non-complex data. This step involves a second experienced examiner evaluating the key diagnostic features documented during the comparison and the evaluation step to determine if there is agreement on the conclusion.

Software facilitated verification may be completed via the Foray Adam System.

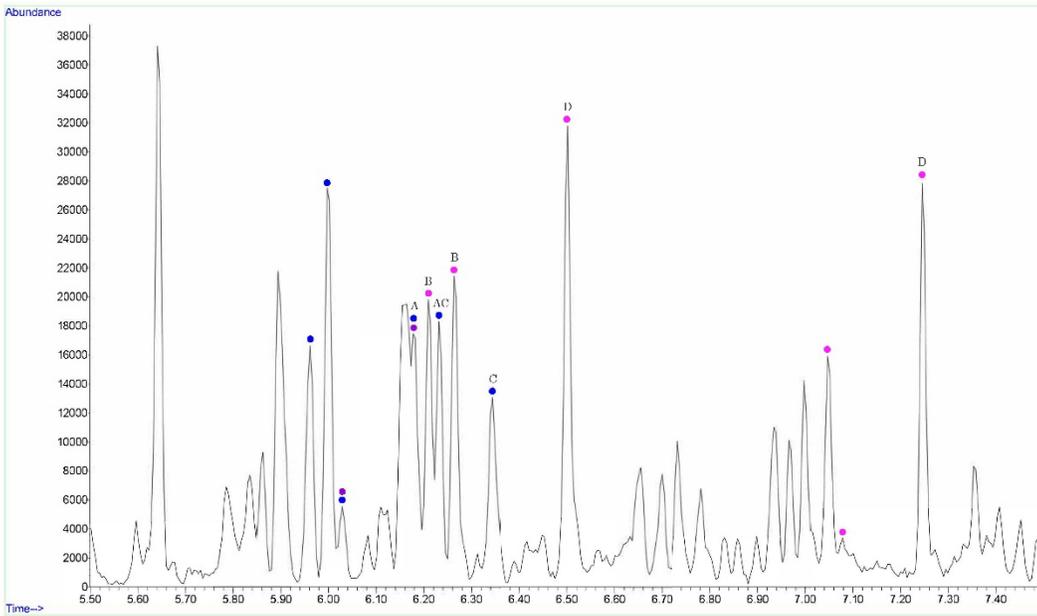


Appendix 2: EIPs with Statistically Ranked Peak Ratios by Color

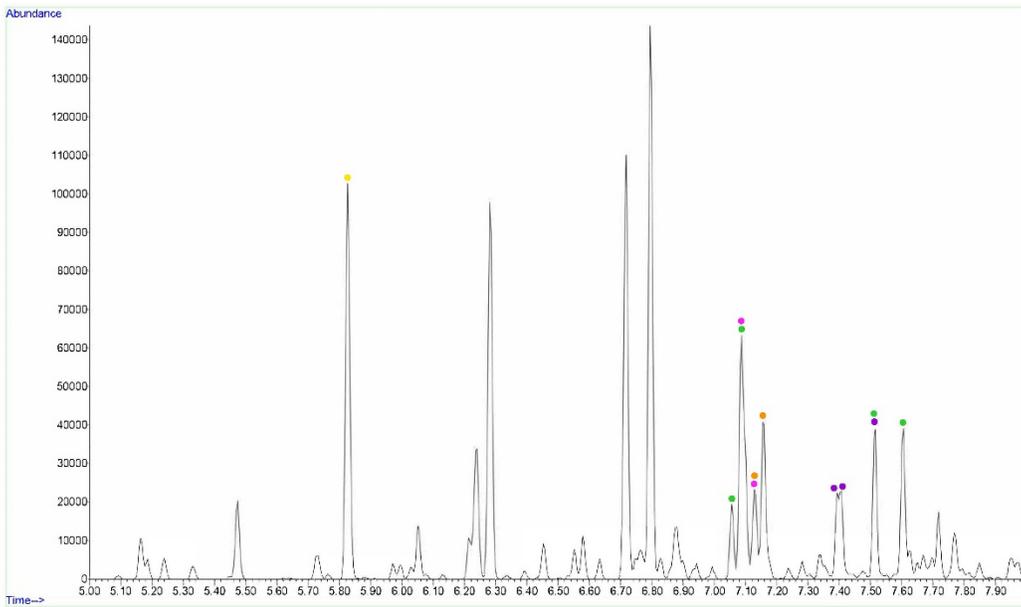
A. Aromatic Profile



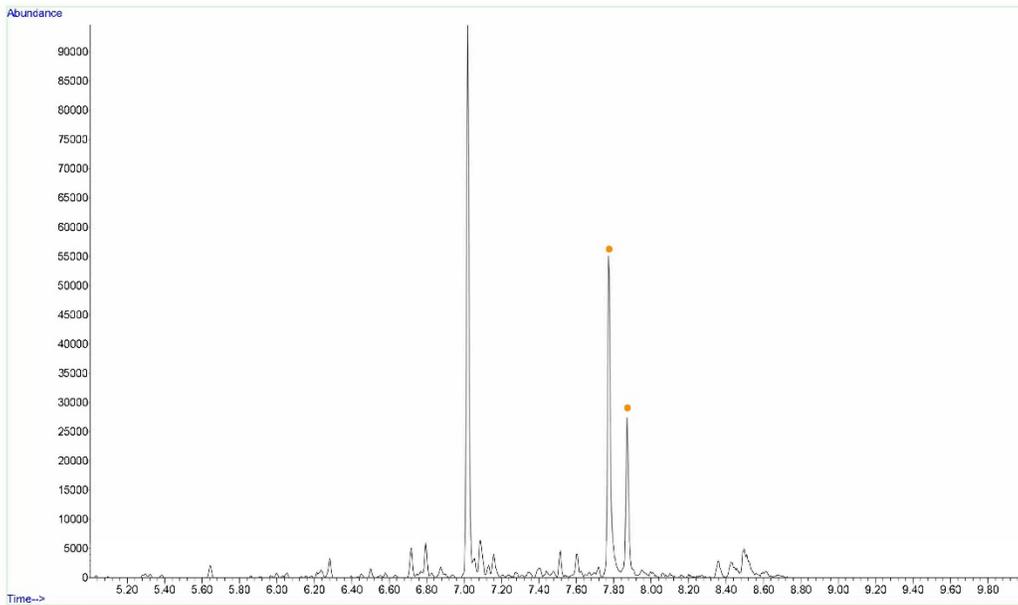
B. Alkane Profile



C. Indane Profile



D. Polynuclear Aromatic Profile



Appendix 2: The colored dots above the key chromatographic peaks denote the statistically supported ranks of the ratios, which subsequently translates to the maximum potential points obtainable during the sufficiency evaluation. As previously determined, Purple represents the highest rank, followed by Pink, Blue, Green, Yellow, and the lowest rank of Orange. Unless otherwise noted, moving from left to right, dots of the same color on two adjacent key diagnostic peaks form a given ratio. Dots labeled with a letter denote a ratio of two peaks that are not immediately adjacent. The asterisks denote two peaks that are adjacent to one another but do not form a significant ratio pair together; they are, however, included in other similarly ranked ratio pairs within the cluster of peaks. The green dots appear on two separate EIPs, but represent a single ratio pair.