Application Note

Forensic Toxicology

Agilent

Trusted Answers

# A Standard Workflow and Troubleshooting Process to Maintain and Troubleshoot the Flow Paths of the Agilent Intuvo GC and Conventional GCs


**Authors**

Samuel Fortener[1],
Kayci Gowins[2], and
Lynne Strainic[2]
Ohio Attorney General's
Bureau of Criminal
Investigations Laboratories
Bowling Green, Ohio[1]
London, Ohio[2]

Kirk Lokits and Robert Kubas
Agilent Technologies, Inc.



## Abstract

QC test mixes (probes) serve a dynamic purpose in ensuring the inertness, reproducibility, and functionality of a specific GC or GC/MS method.[1] The test mix chemistry of individual compounds to make up such a probe can vary widely depending on the column chemistry, detector selectivity, and method acquisition parameters. The QC test mixes discussed in this application note are commercially available through Agilent Technologies and are originally designed and optimized to probe the overall inertness and chromatographic condition of specific column dimensions and phases.[2,3] This work focuses on using existing column test mixtures to exercise and test the overall flow path of the GC and not solely the inertness and chromatographic condition of the GC column. GC methods were developed for four of the column test mixes with most run times under seven minutes. Specific areas of what constitutes a good QC test mix, what data (test mix) features to review, the steps to maintain data quality, and what to do when data quality decreases or fails QC guidelines, are discussed. Issues such as when to change the liner, gold seal, or guard chip, and when to perform column maintenance are examined.

## Introduction

As GC and GC/MS detection limits are continually pushed to lower and lower levels, the degree of surface activity within the flow path, from inlet to detector, becomes more critical. Agilent's introduction of inert inlet assemblies, gold seals, ion source bodies, inlet liners, columns, ferrules, and capillary flow technology splitters and column connectors has helped to reduce surface activity throughout the flow path.[4,5] Even though these individual components help to define a flow path of low surface activity, allowing for extended sample analysis and lower limits of detection, specific areas of the flow path will become more active than others based on sample matrix and concentration. Two of the most common questions asked by analysts are when to perform instrument maintenance and how to prioritize changing the components in the flow path.

As previously stated, areas of activity where target analytes interact can occur anywhere in the flow path, including the analytical column. Due to the multitude of problems attributed to active sites including loss of peak symmetry, peak height, peak area, resolution between peaks, and even loss of the peaks of interest (Figure 1), analysts may resort to addressing the problem by replacing whatever comes to mind, or using whatever consumable supplies are on hand. Although this approach might work sometimes and can allow for completion of a partially interrupted sequence or the analysis of a priority sample, the long-term desired effect of a stable, reproducible system for the next series of sample sequences may be short-lived or not happen at all, resulting in instrument downtime.

This work proposes a more logical and interactive approach to the workflow and troubleshooting processes involved in GC flow path troubleshooting and maintenance. Whether using conventional GC flow paths or the flow chip technology found in the Agilent Intuvo GC, the overall workflows are basically the same (Figure 2). Whenever troubleshooting a GC or GC/MS system it is a good idea to break the system into its individual functional groups (sample introduction, inlet, flow path, column, detector, data system) and focus on the functional group that makes the most sense and or is known for causing the problem at hand. As an example, upon injecting a sample into a GC or GC/MS system, no peaks are detected in the chromatogram but there are small extraneous peaks and noise on the baseline. Based on the process of breaking the GC or GC/MS into its various functional groups we have some of the possibilities listed in Table 1. If an analyst reviews the list of possibilities one-by-one to determine the efficacy of the probable cause, it could easily take up to half a day on/in the heating and cooling zones of a GC system and up to an entire day on GC/MS system heating and cooling zones. This would vent the MS manifold and pump the system back down to reach a stable high vacuum. If the analyst replaced each consumable component in each functional group, they could replace hundreds and possibly thousands of dollars' worth of parts. However, the longer you wait to perform maintenance on your GC or GC/MS system, the more time and consumables are involved in the repair and or maintenance process. A key to faster chromatographic troubleshooting is learning the art of reading the chromatogram. The chromatogram provides a wealth of knowledge and regardless of the application; the problems that plague a chromatogram are the same, so if one knows how to troubleshoot a chromatogram, this knowledge can be applied to any application. Specific things to look for in your chromatograms include comparing early and late eluting analytes to determine if they are exhibiting the same behavior, using historical data to compare the problematic chromatogram to an acceptable one using the software to overlay chromatograms to look for differences and differences over time, and keeping a logbook of maintenance, historical problems, or issues and what fixed or solved those issues.

Agilent column test mixes (probes) capitalize on this concept of reading the chromatogram by producing a standardized chromatogram on which to base your chromatographic troubleshooting observations. The various column test mixes consist of a mixture of components with varying chemical properties resulting in each component interacting in a specific manner with your column's stationary phase, thus generating a characteristic chromatogram for the column type it was designed for. Based on the chemistry of the column stationary phase, the probes can consist of n-alkanes and/or fatty acid methyl esters to measure retention time stability, alcohols for oxygen damage or exposed silanols, or acid and base components to differentiate acidic or basic column behavior (poor peak behavior and or peak tailing). A multitude of column test mixes/probes exist commercially and in-house and are optimized for the specific column stationary chemistries. Figures 3 through 5 illustrate a few of the more common column stationary phases and their respective column test probes. It should be noted that the acquisition parameters used to generate these chromatograms are not absolute or optimized for each component, but are generated based on overall component separation, peak shape, and minimal amount of runtime.

This study focuses on the concept of using the Agilent J&W DB-5ms column test probe in Figure 3 as a pre-emptive chromatographic test for not just the column status of a DB-5ms column but the entire flow path on two GC/MS Intuvo/5977B systems using real-world forensic drug chemistry samples. The Ohio Attorney General's Bureau of Criminal Investigations (BCI) Laboratories in London and Bowling Green Ohio developed the data acquisition, sample sequencing procedures, and chromatographic quality control (QC) criteria. The overall goal, in the shortest time possible, was to contaminate the flow path of two identical GC/MS systems, determine what areas of maintenance would need replacement prior to a guard chip failure, and continue running both systems until a new guard chip is required. Based on the actual number of case samples injected and the specific changes in chromatographic data quality generated over time, a logical workflow and early chromatographic indicators are developed to determine at what time which item(s) require maintenance to continue running the instrument.

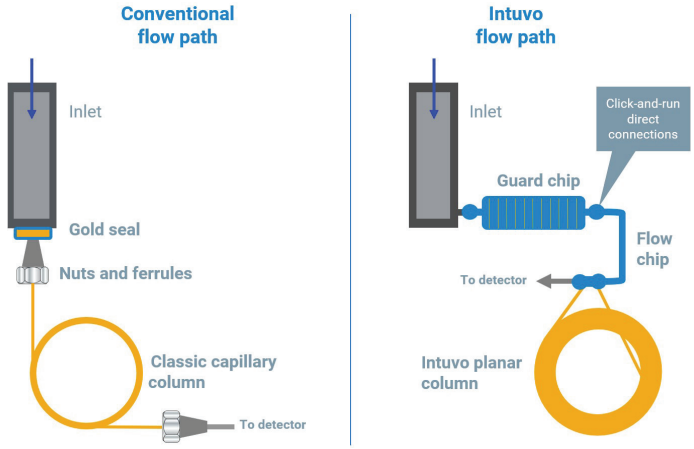



**Figure 2.** Comparison of conventional GC and the Agilent Intuvo GC flow paths.

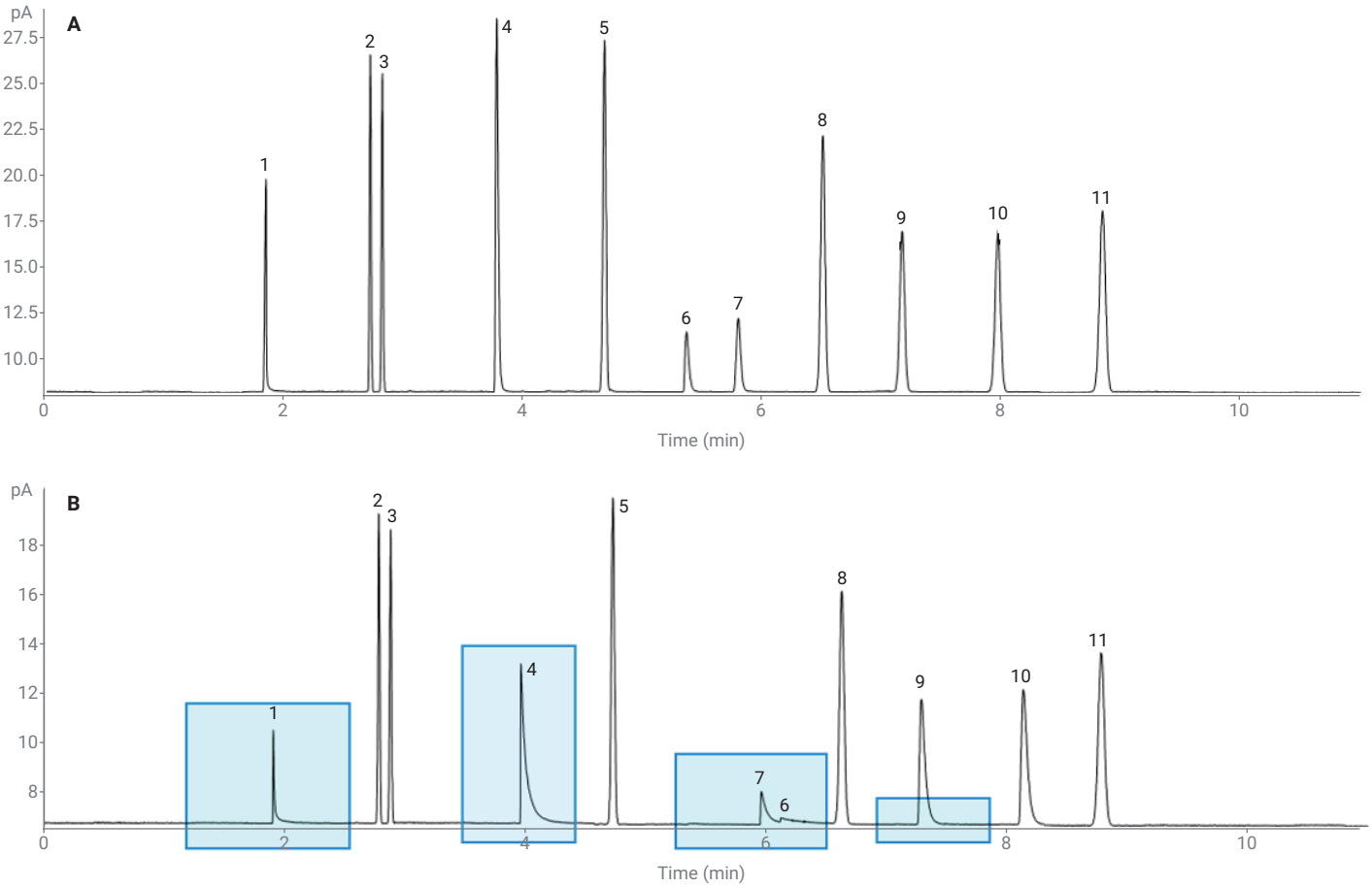



**Figure 1.** Illustrating acceptable chromatography and a chromatogram containing examples of undesirable analyte interactions.

**Table 1.** Example of a logical workflow process when troubleshooting "no peaks detected" on a GC or GC/MS system.

| Functional Group | What to Look for or Possible Cause |
|---|---|
| Sample Introduction<br>(Liquid Sampler, Headspace, PAL) | – Any LED error lights on<br>– Syringe plugged or plunger stuck<br>– Needle bent<br>– If the sample vial has been pierced |
| Inlet<br>(Split/Splitless, Multimode) | – Septum is cored<br>– Liner has active sites<br>– Gold seal has active sites |
| Intuvo Flow Path | Before the column:<br>– Guard chip has active sites<br>– Inlet chip has active sites<br>After the column:<br>– Detector chip has active sites<br>– MS tail chip has active sites |
| Column<br>(Packed, Megabore, Capillary) | – Installation depth incorrect<br>– Guard column has active sites<br>– Broken between inlet and detector<br>– Column has active sites |
| Detector<br>(FID, TCD, ECD, NPD, etc.) | – Electronics turned off<br>– Gases not on or set incorrectly<br>– Jet plugged |
| MS<br>(Single Quad, Triple Quad, Q-TOF) | – Column blocked in transferline<br>– Vacuum problem<br>– Filament burned out<br>– Wrong tune parameters<br>– Electronics/voltage issues |
| Data System | – Error messages on screen or logbook<br>– Wrong method loaded<br>– Lost communication to the instrument |

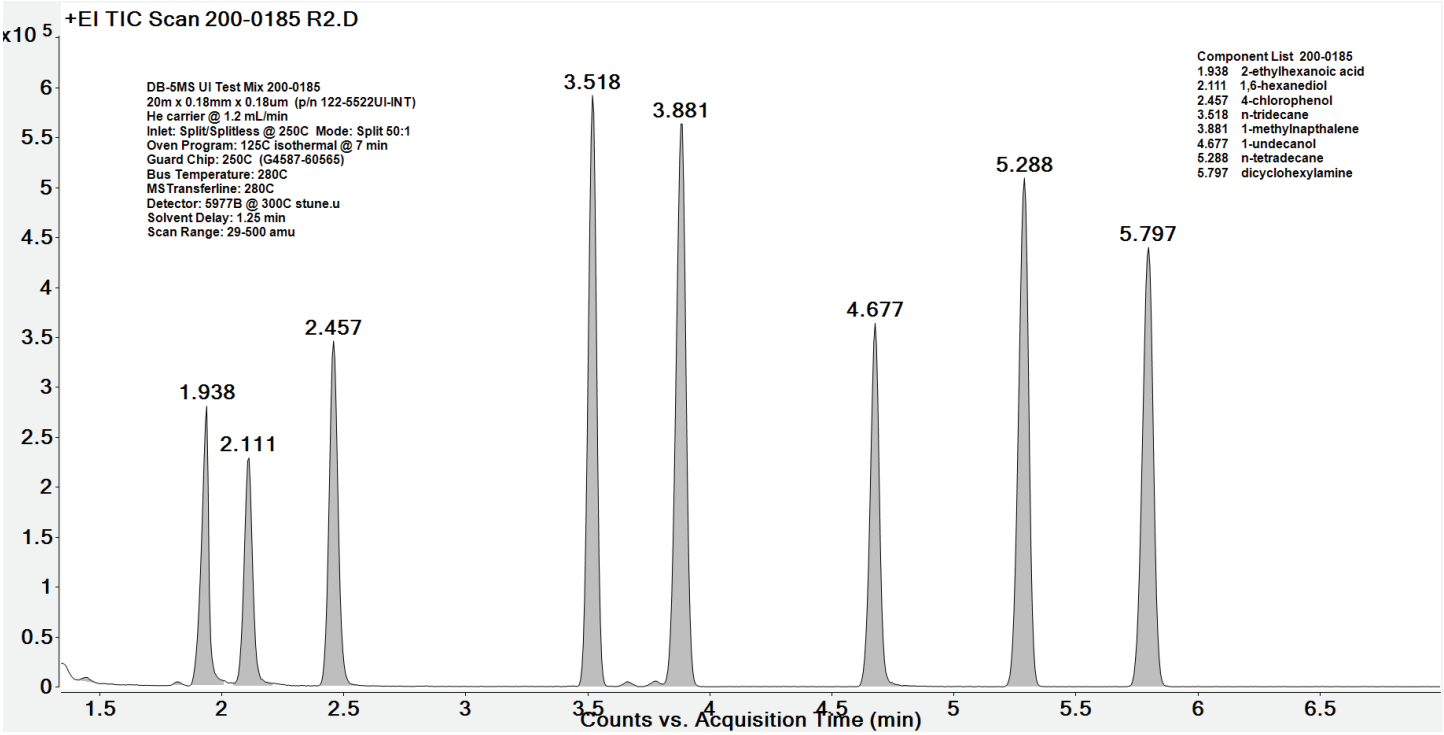



**Figure 3.** Acquisition parameters and component list for an Agilent Intuvo/5977B GC/MS system with an Agilent J&W DB-5ms UI stationary phase column.

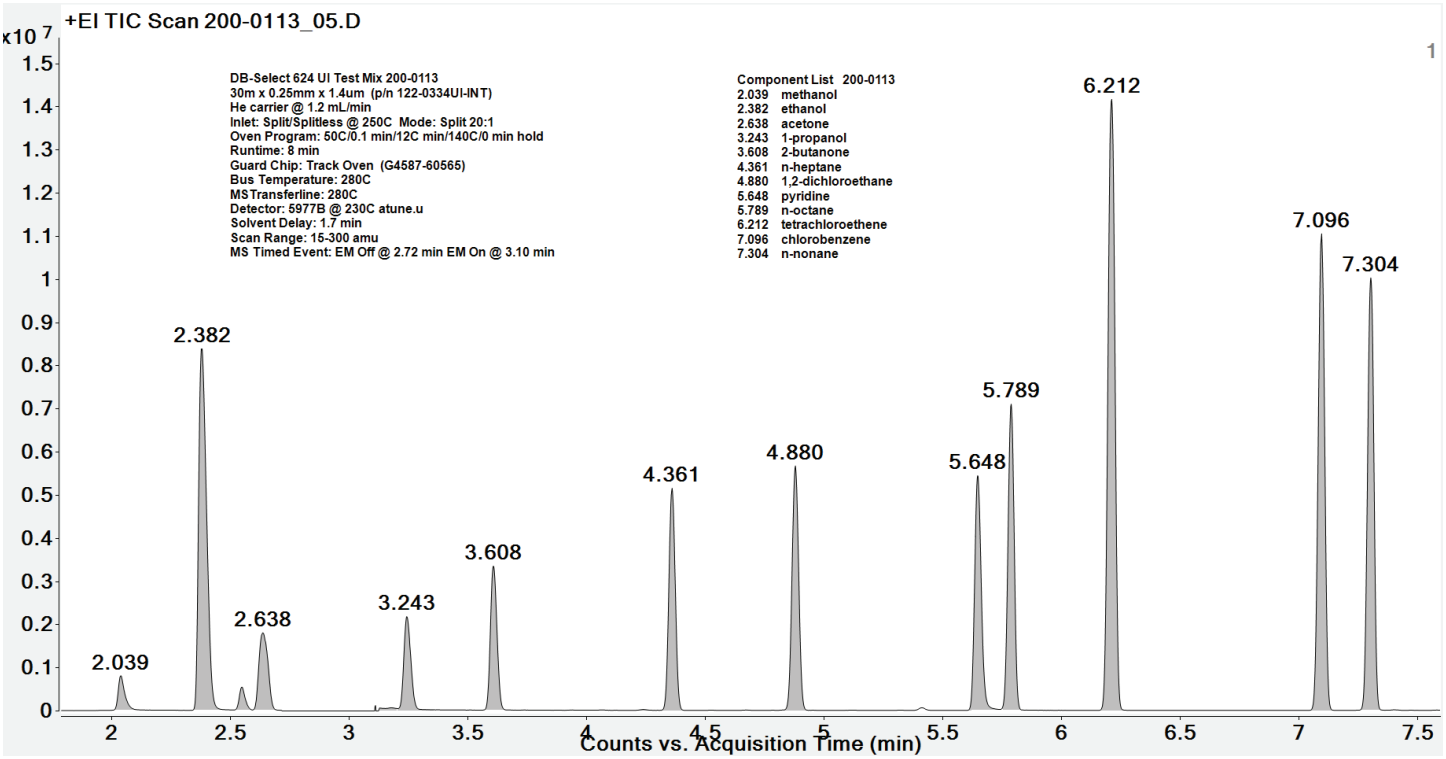



**Figure 4.** Acquisition parameters and component list for an Agilent Intuvo/5977B GC/MS system with an Agilent J&W DB-Select 624 UI stationary phase column.

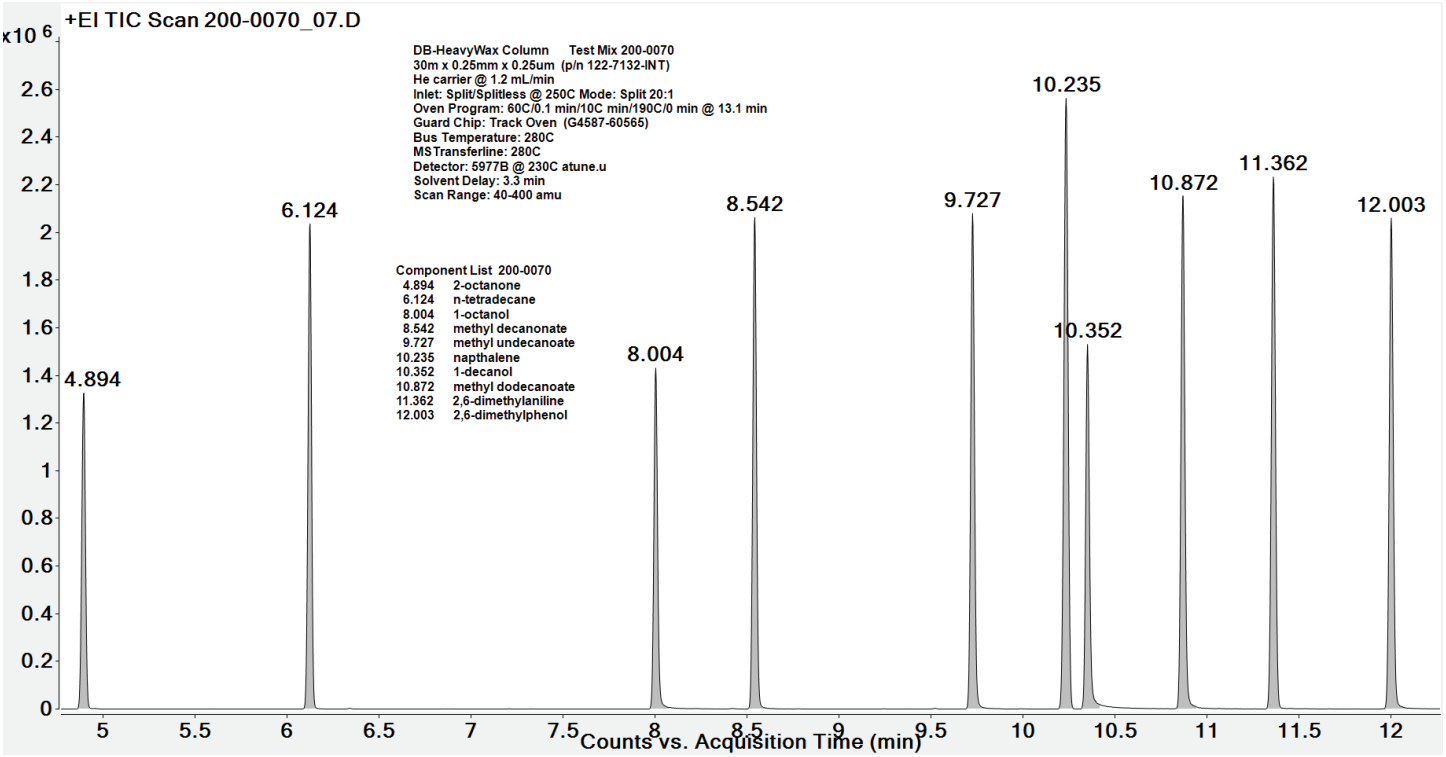



**Figure 5.** Acquisition parameters and component list for an Agilent Intuvo/5977B GC/MS system with an Agilent J&W DB-HeavyWax stationary phase column.

## Experimental

### Materials and supplies

- Agilent screw cap glass vials, 2 mL
- Agilent J&W DB-5ms column check mix 1 mL vial (part number 200-0185)
- Agilent J&W DB-5ms Ultra Inert GC column, 20 m, 0.18 mm, 0.18 µm (part number 122-5522UI_INT)
- Agilent liquid sampler tower and 150-vial sample tray
- Agilent Intuvo 9000 GC, inert split/splitless inlet with Electronic Pressure Controlled (EPC) Inlet
- Agilent 5977B MSD, inert EI source equipped
- Agilent MSD ChemStation Data Analysis software F.01.03
- Agilent MassHunter Acquisition software B.07.06
- Agilent MassHunter Qualitative Analysis software B.08.0
- Inlet liner, splitless, straight, deactivated, with glass wool
- Ultrahigh purity helium
- Methanol, hexane, chloroform, and ethyl acetate

### Sample and QC mix preparation

QC mixes are prepared using commercially available solid-dose and liquid drug reference standards prepared with HPLC grade chemical solvents. Class A volumetric glassware is used, when available. Solids are weighed and gravimetrically transferred to a 100 mL flask and if required by the method, gravimetric addition of liquid standard is performed. The mixture is then brought to volume with acetonitrile. The standard solution is then verified by instrumental analysis prior to use, by ensuring resolution, peak shape, and spectral fidelity requirements are met. The components and quantities listed below comprise the standard drug chemistry QC mix for the Ohio BCI laboratory.

**QC Mix 1**

- 50 mg caffeine
- 25 mg heroin
- 20 mg cocaine HCl
- 20 mg methaqualone
- 25 mg tetrahydrocannabinol (THC)*
- 50 mg alprazolam

**QC Mix 2**

- 100 mg hydromorphone
- 100 mg oxymorphone
- 100 mg buprenorphine

Samples were prepared by taking a representative piece of material from a larger amount and readying it for analysis by extraction using a variety of chemical solvents and reagents. Extraction techniques varied but most often included neat and basic extraction techniques. Methanol, acetonitrile, hexane, and chloroform were used for dry extractions and saturated sodium carbonate was used as the aqueous component of the basic extraction.

### Creation of the sample sequence table

Initial acquisition parameters for the DB-5ms column test probe mix were developed on newly installed Agilent Intuvo 5977B GC/MS systems with new septa and liners, and all data was generated on new DB-5ms UI 20 m, 0.18 mm, 0.18 µm capillary columns. Once acquisition parameters were optimized for the DB-5ms probe mix, an identical sequence protocol was developed to operate both GC/MS systems 24/7 to help minimize the time required to reach a guard chip failure. The sequence table begins with the laboratory's QC drug mix 1 containing caffeine (0.5 mg/mL), methaqualone (0.20 mg/mL), cocaine (0.20 mg/mL), heroin (0.25 mg/mL), THC (0.25 mg/mL), and alprazolam (0.50 mg/mL) (Figure 6). This is followed by the DB-5ms probe mix, and QC drug mix 2 consisting of hydromorphone, oxymorphone, and buprenorphine at 1mg/mL (Figure 7). QC drug mixes and casework samples were acquired under the laboratory's general drug screening method, and all casework samples were given non-descript sample number designations. Case samples encompassed a variety of sample matrices including but not limited to extracted tablets, powders, oils, hash liquids, mushrooms, and tars to name a few. After the injection of QC drug 2, 50 casework samples were run on other instruments throughout the week. The ensuing three injections consisted of the QC drug mixes and the DB-5ms test probe. Subsequent injections consisted of the same arrangement of casework samples, which allowed for a full sample tray to be maintained. Normally, solvent blanks are run between each sample and/or QC injections, however, to speed up the contamination process, blank solvent injections were eliminated from the sequence table. The sequence continued until one or more of the QC mixes or DB-5ms probe mix failed any of the chromatographic performance criteria. Upon a chromatographic failure, the system underwent maintenance and the sequence continued with the first three injections being the QC mixes and column probe. If all three mixes generated passing chromatographic criteria, the sequence was continued until another failure occurred, maintenance was performed, and the sequence restarted in the same manner until maintenance requires the replacement of the guard chip.

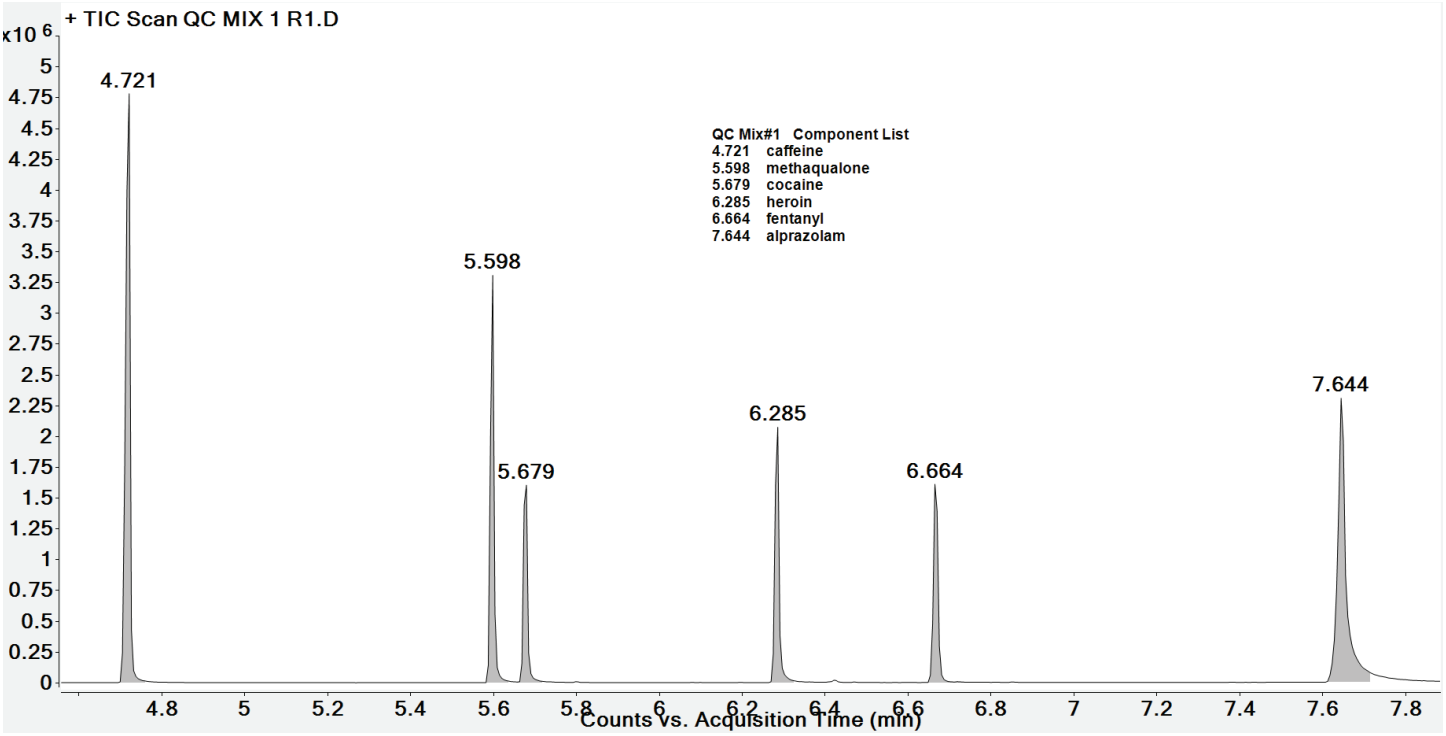



**Figure 6.** QC drug mix 1 containing caffeine, methaqualone, cocaine, heroin, THC, and alprazolam.

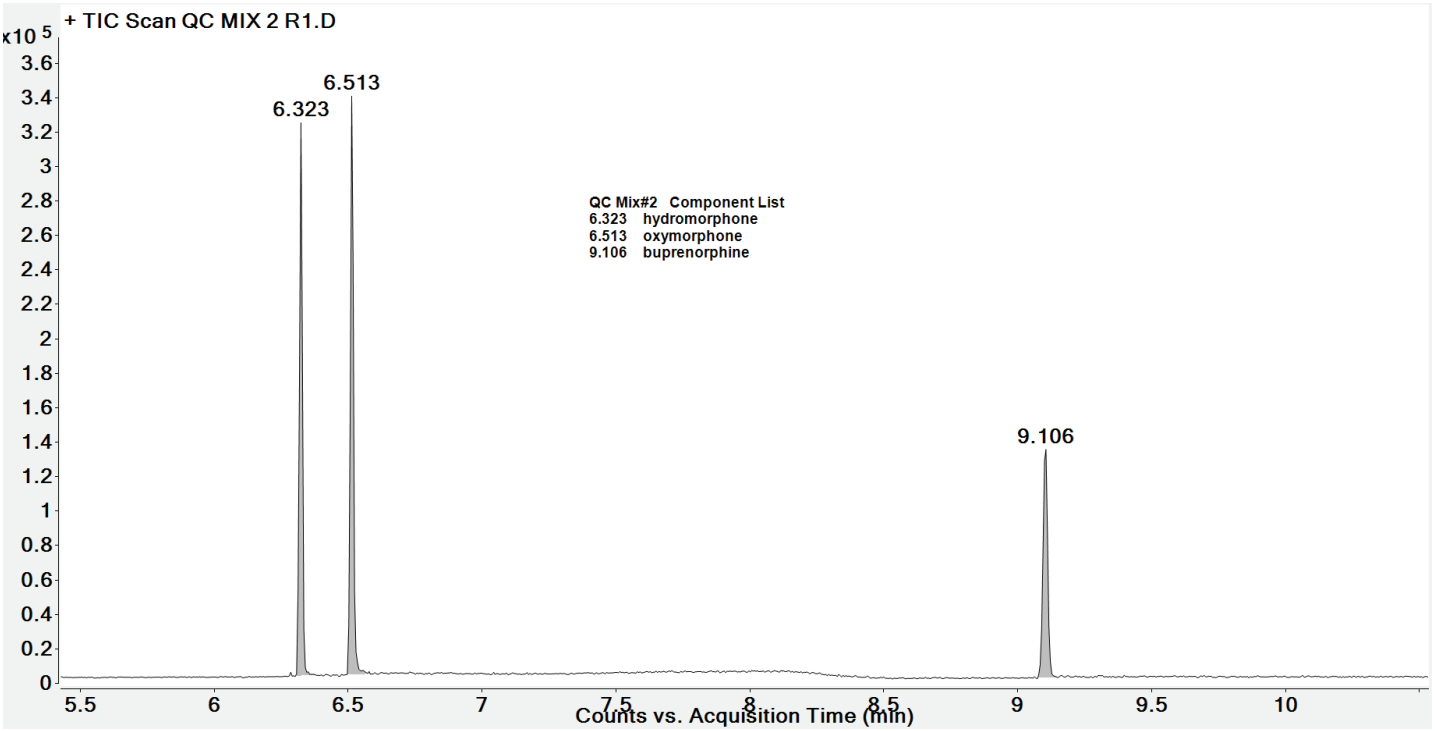
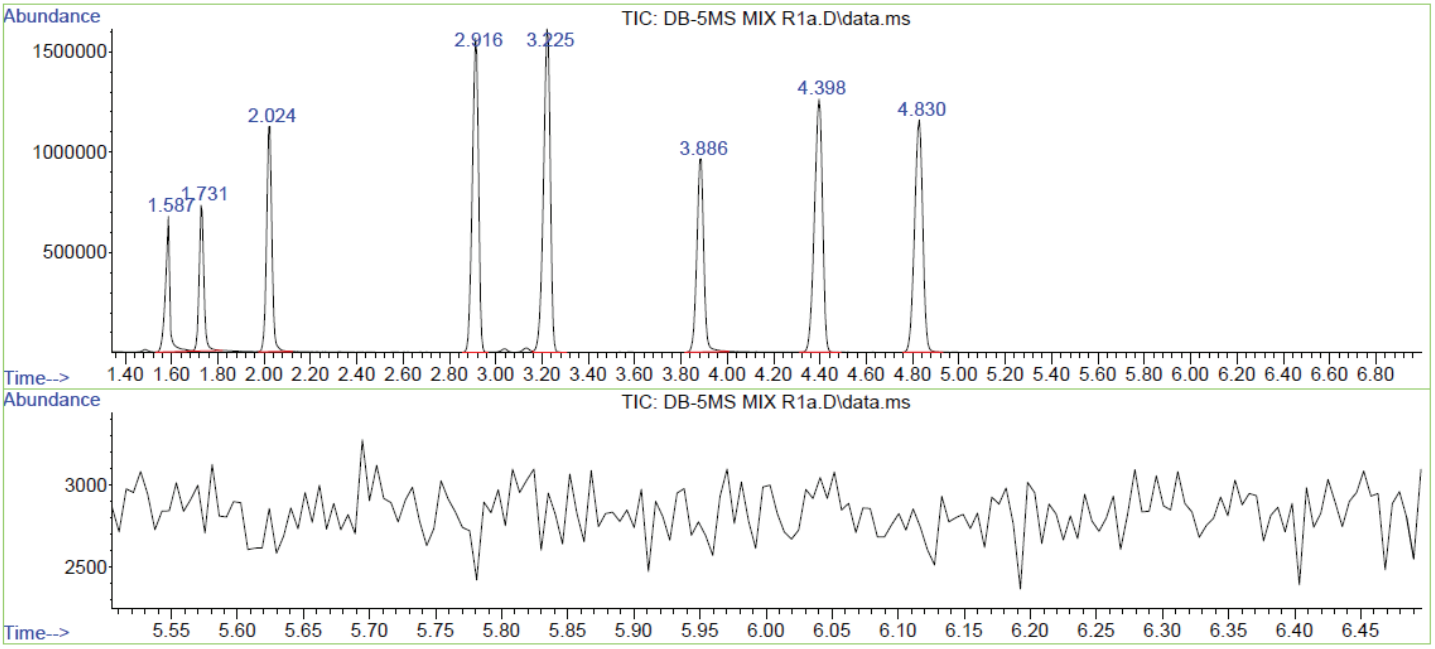



**Figure 7.** QC drug mix 2 containing hydromorphone, oxymorphone, and buprenorphine.

### Criteria for failure

There are numerous objective calculations and subjective (visual/pictorial) means within the ChemStation and MassHunter software and based on an analyst's experience, to measure chromatographic criteria, respectively. This study uses a combination of both MSD ChemStation Data Analysis software (Figure 8) and visual acuity to determine the degree of chromatographic failure. The ChemStation Performance Report is located under "Chromatogram" in the menu bar and near the end of the drop-down menu. Specific integrated parameters included retention time, peak area, peak width, resolution, and peak tailing (Table 2). Visual observations were based on missing peaks, extra peaks, increased peak tailing, peak symmetry, noisy baseline, and reduced response. Resolution between methaqualone and cocaine in QC Mix 1 was also a criterion considered in this work.




```
Noise characteristics:
  Noise start : 5.5  min.
  Noise end   : 6.5 min.
  Number of points: 184
  Maximum noise: 3277 abundances
  Minimum noise: 2366 abundances
  Peak-to-Peak noise: 911 abundances
  Root-mean-square noise: 153.586 abundances

The table below is based on an integrated chromatogram.
Tailing calculated at 10% height.
```

| # | RetTime | Area | BslPH | PWidth | Resol | Tailing | PtP S/N | RMS S/N | Plates |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.59 | 9022100 | 610505 | 0.025 | 0.00 | 0.77 | 670.15 | 3975.02 | 23001 |
| 2 | 1.73 | 10261831 | 723663 | 0.022 | 3.67 | 1.05 | 794.36 | 4711.79 | 35154 |
| 3 | 2.02 | 17739999 | 1133842 | 0.024 | 7.53 | 1.02 | 1244.61 | 7382.48 | 38760 |
| 4 | 2.92 | 29048070 | 1545549 | 0.030 | 19.54 | 0.89 | 1696.54 | 10063.11 | 53633 |
| 5 | 3.22 | 33061251 | 1602688 | 0.032 | 5.90 | 0.87 | 1759.26 | 10435.15 | 55620 |
| 6 | 3.89 | 19932608 | 965937 | 0.032 | 12.18 | 0.93 | 1060.30 | 6289.24 | 82215 |
| 7 | 4.40 | 30625176 | 1249347 | 0.039 | 8.54 | 0.87 | 1371.40 | 8134.54 | 71111 |
| 8 | 4.83 | 28331291 | 1148943 | 0.039 | 6.56 | 0.90 | 1261.19 | 7480.80 | 85685 |

**Figure 8.** ChemStation Performance Report from MSD ChemStation Data Analysis rev F.01.03.

**Table 2.** Integrated and visual chromatographic performance parameters.

| Parameter | Cutoff Valve |
|---|---|
| Reduced Peak Height | ≥ 50% |
| Reduced Peak Area | ≥ 10% |
| Peak Tailing | ≥ 1.5 |
| Resolution QC Mix 1 Methaqualone and Cocaine | ≤ 2% |
| Missing Peak(s) | Visual acuity |
| Extra Peak(s) | Visual acuity |
| Noisy Baseline | Visual acuity |
| Peak Symmetry | Visual acuity |
| Peak Width at ½ Height | TBD |

### Designing a logical maintenance and troubleshooting workflow

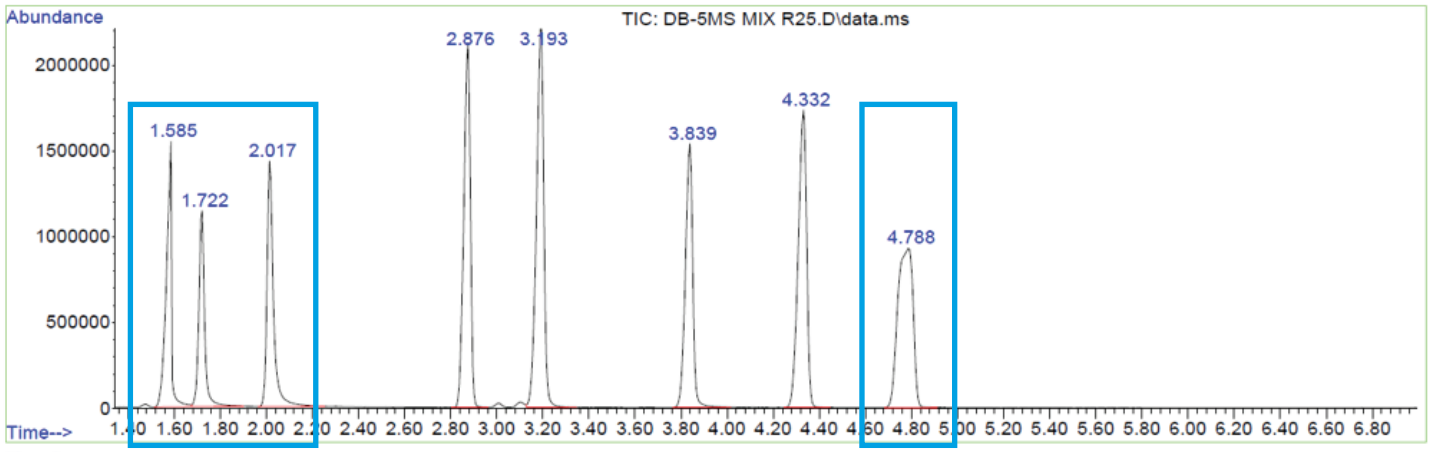
Due to the multidimensional nature of troubleshooting and maintaining analytical instrumentation, there exist a multitude of approaches an analyst can take based on their personal experience and knowledge of the instrumentation. That stated, the following is the approach taken based on the chromatographic evidence and focused within the project's boundaries. Septum replacement was automatically performed following 250 to 300 injections to minimize additional down time. Upon the occurrence of a chromatographic failure, as previously stated, the first step is based on what is in the sample's flow path, what makes the most sense, and what is least disruptive to the overall system. Since the septum is a non-issue, the next location that is easily accessible is the inlet liner, therefore, the liner is the first item replaced and the DB-5ms probe mix and QC mixes rerun. If that fixes the problem, the sequence is restarted until another failure occurs at which point the first step would be to replace the liner (based on the number and types of samples run) and recheck. If the liner does not fix the problem, the second step would be to replace the guard chip on an Intuvo system since it is the next item in the flow path. On a conventional GC or GC/MS system, the same logic applies, and if the liner replacement does not work in the first step, the next least intrusive approach would be to cut 2 to 3 inches off the head of the column and reinstall it into the inlet, making sure the column is at the appropriate depth in the inlet. If cutting the column does not resolve the issue, then replace the gold seal. In the case of the Intuvo, if replacing the guard chip does not resolve the poor chromatography, the next logical step in the flow path is the inlet chip. If replacing the gold seal in a conventional GC and replacing the inlet chip in an Intuvo does not correct the problem, then in both instances the column would be next based on flow path and degree of difficulty.

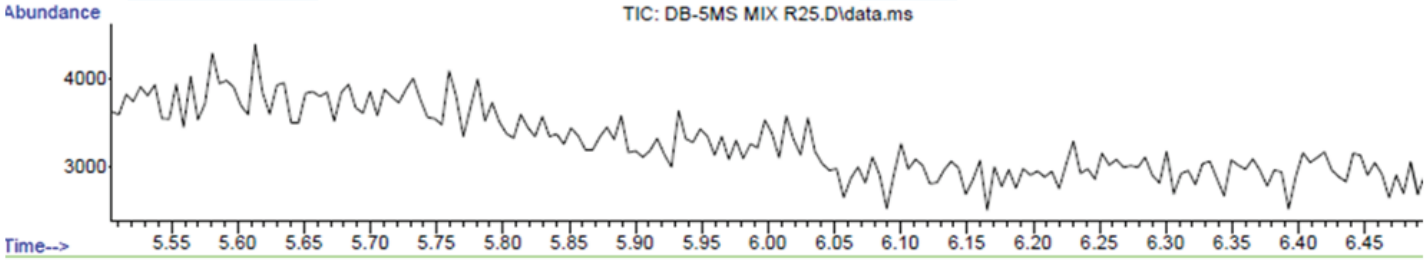
## Results and discussion

Both the Bowling Green and London BCI Laboratory sites followed the same sequence table of events as described previously, using casework samples, failure criteria, and maintenance workflow during this study. In the BCI Bowling Green Regional Laboratory, after approximately 1,450 casework samples were injected, the DB-5ms column check probe mix was injected and produced the ChemStation Performance Report depicted in Figure 9. The compound at 4.788 minutes is dicyclohexylamine, and it generated a much broader peak width response (0.063) compared to its peak width (0.039), illustrated in Figure 8 from earlier in the study. This is easily determined both visually and arithmetically through the peak width column in the performance reports. Dicyclohexylamine is a strong base (pKa = 10.4), which appears as a colorless liquid with a faint fishy odor and is used to make paints, varnishes, and detergents.[6] Peak tailing or lost response of a base indicates that a column, or in this instance, an area in the flow path, may be acidic; conversely, poor peak behavior of an acidic compound is indicative of possible basic active sites. Components with alcohol functional groups can interact with exposed silanols on the column or active sites within the flow path creating poor peak symmetry and peak tailing.[7] Based on the study's maintenance and troubleshooting workflow, the liner is the first consumable item to be replaced in the flow path. Once the liner is replaced, the peak shape and response of the dicyclohexylamine returns to its original character identified at the beginning of the sequence table. In Figure 10, note that the first three eluting compounds in from the DB-5ms probe mix, 2-ethylhexanoic acid, 1,6-hexanediol, and 4-chlorophenol, all have increased peak tailing listed in the table. Once the inlet liner was replaced, the individual peak shapes and tailing calculations improved and the sequence was able to continue. The BCI London Regional Laboratory began the DB-5ms probe mix evaluation study in a similar manner as demonstrated in the performance report found in Figure 11. The London Lab experienced a different series of liner changes compared to the Bowling Green Lab: the first inlet liner was replaced after approximately 300 injections, a second inlet liner was replaced after an additional 1,200 injections, and a third liner was replaced after an additional 400 injections; before, liner replacement did not cure the chromatographic failure and the guard chip had to be replaced to bring the system back to normal operation. There are multiple approaches to review the chromatographic data. Figure 12 displays overlaid chromatograms (using MassHunter Qualitative Analysis software) of the initial DB-5ms probe mix at the beginning of the evaluation (black) and the DB-5ms probe mix after ~1,500 injections (red).

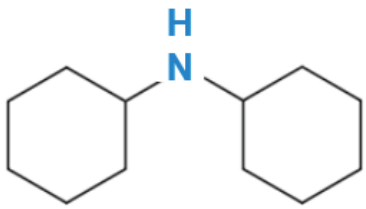
A cursory review of the overlay discloses an obvious reduction in component responses and a significant shift in retention times from the mid-to-late eluting components, but only slight variations in overall chromatographic peak shape. However, a closer view in Figure 13 exposes the considerable peak broadening of the 4-chlorophenol (red) when compared to the 4-chlorophenol at the beginning of the evaluation (black).

In this study, none of the QC drug mixes showed any depreciable differences as the number of injections progressed. Based on this observation, there may be alternative drug analytes that display a more reactive interaction with the flow path than those present in the current QC mixes, such as LSD and psilocybin, which have been observed for being notoriously difficult chromatographically.



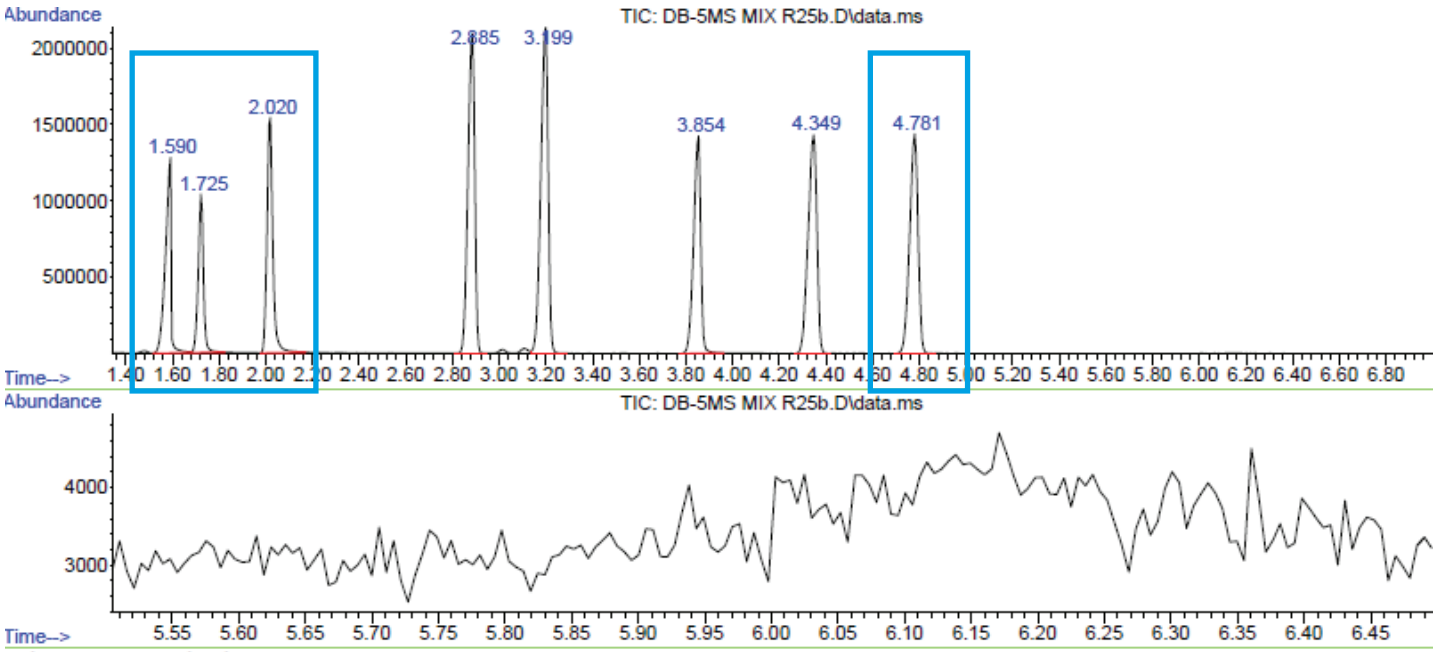

```
Noise characteristics:
  Noise start : 5.5  min.
  Noise end   : 6.5 min.
  Number of points: 184
  Maximum noise: 4389 abundances
  Minimum noise: 2512 abundances
  Peak-to-Peak noise: 1877 abundances
  Root-mean-square noise: 393.728 abundances

The table below is based on an integrated chromatogram.
Tailing calculated at 10% height.
```




Dicyclohexylamine

| | RetTime | Area | BslPH | PWidth | Resol | Tailing | PtP S/N | RMS S/N | Plates |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.58 | 24409064 | 1475822 | 0.024 | 0.00 | 0.67 | 786.27 | 3748.33 | 24391 |
| 2 | 1.72 | 19318744 | 1125410 | 0.026 | 3.24 | 1.12 | 599.58 | 2858.34 | 24350 |
| 3 | 2.02 | 27550307 | 1403187 | 0.029 | 6.30 | 1.19 | 747.57 | 3563.84 | 26164 |
| 4 | 2.88 | 42196061 | 2100343 | 0.031 | 16.67 | 0.88 | 1118.99 | 5334.50 | 46369 |
| 5 | 3.19 | 49020810 | 2213791 | 0.034 | 5.74 | 0.87 | 1179.43 | 5622.64 | 49535 |
| 6 | 3.84 | 33696730 | 1520432 | 0.035 | 11.14 | 0.83 | 810.03 | 3861.63 | 68009 |
| 7 | 4.33 | 44019516 | 1712343 | 0.040 | 7.81 | 0.84 | 912.28 | 4349.05 | 65424 |
| 8 | 4.79 | 42885532 | 927416 | 0.063 | 5.21 | 0.79 | 494.09 | 2355.47 | 31684 |

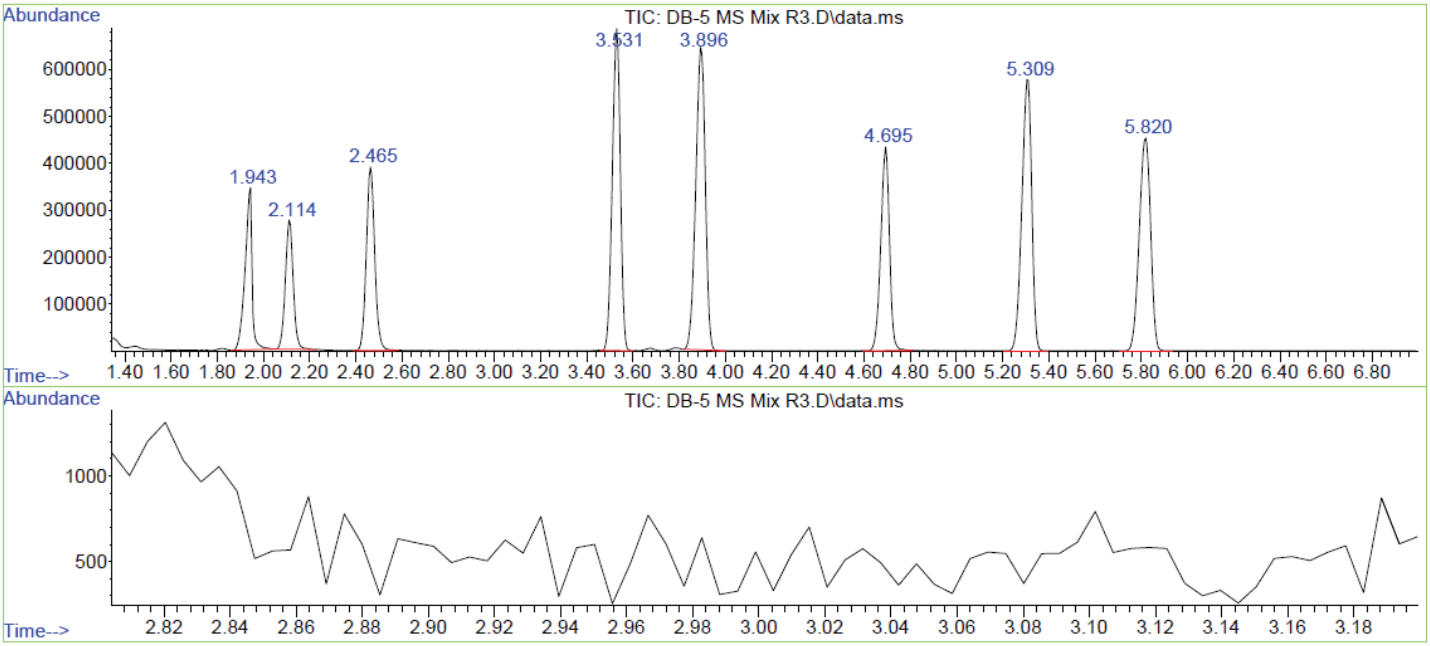
**Figure 9.** ChemStation Performance Report generated in the Bowling Green Lab after ~1,450 case samplers were injected.




```
Noise characteristics:
  Noise start : 5.5  min.
  Noise end   : 6.5 min.
  Number of points: 184
  Maximum noise: 4706 abundances
  Minimum noise: 2526 abundances
  Peak-to-Peak noise: 2180 abundances
  Root-mean-square noise: 465.32 abundances

The table below is based on an integrated chromatogram.
Tailing calculated at 10% height.
```

| | RetTime | Area | BslPH | PWidth | Resol | Tailing | PtP S/N | RMS S/N | Plates |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.59 | 21414131 | 1231596 | 0.026 | 0.00 | 0.59 | 564.95 | 2646.77 | 20930 |
| 2 | 1.72 | 15933279 | 1009801 | 0.024 | 3.19 | 0.87 | 463.21 | 2170.12 | 28376 |
| 3 | 2.02 | 26332900 | 1533976 | 0.026 | 6.97 | 1.12 | 703.66 | 3296.60 | 33944 |
| 4 | 2.89 | 42361417 | 2085170 | 0.032 | 17.61 | 0.84 | 956.50 | 4481.15 | 44481 |
| 5 | 3.20 | 46423965 | 2135618 | 0.034 | 5.58 | 0.79 | 979.64 | 4589.57 | 48668 |
| 6 | 3.85 | 29930442 | 1401626 | 0.033 | 11.47 | 0.80 | 642.95 | 3012.18 | 74572 |
| 7 | 4.35 | 37423794 | 1418229 | 0.040 | 7.93 | 0.84 | 650.56 | 3047.86 | 64184 |
| 8 | 4.78 | 36269384 | 1420462 | 0.040 | 6.39 | 0.89 | 651.59 | 3052.66 | 81106 |

**Figure 10.** ChemStation Performance Report generated in the Bowling Green Lab after the inlet liner was replaced.




Noise characteristics:
  Noise start : 2.8  min.
  Noise end   : 3.2 min.
  Number of points: 74
  Maximum noise: 1311 abundances
  Minimum noise: 255 abundances
  Peak-to-Peak noise: 1056 abundances
  Root-mean-square noise: 227.981 abundances

The table below is based on an integrated chromatogram.
Tailing calculated at 10% height.

| # | RetTime | Area | BslPH | PWidth | Resol | Tailing | PtP S/N | RMS S/N | Plates |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.94 | 7273708 | 337364 | 0.032 | 0.00 | 0.75 | 319.47 | 1479.79 | 20559 |
| 2 | 2.11 | 6562996 | 272803 | 0.037 | 2.92 | 0.99 | 258.34 | 1196.60 | 17911 |
| 3 | 2.47 | 9903062 | 387257 | 0.039 | 5.44 | 1.09 | 366.72 | 1698.64 | 22159 |
| 4 | 3.53 | 16577448 | 677896 | 0.039 | 16.16 | 0.95 | 641.95 | 2973.47 | 45769 |
| 5 | 3.90 | 18428872 | 642665 | 0.044 | 5.17 | 0.98 | 608.58 | 2818.94 | 42587 |
| 6 | 4.70 | 11288586 | 430354 | 0.041 | 10.99 | 0.94 | 407.53 | 1887.67 | 71317 |
| 7 | 5.31 | 17088577 | 579586 | 0.047 | 8.22 | 0.90 | 548.85 | 2542.25 | 71558 |
| 8 | 5.82 | 15487264 | 452765 | 0.055 | 5.93 | 0.93 | 428.76 | 1985.97 | 62297 |

**Figure 11.** ChemStation Performance Report generated in the London Lab at the beginning of the evaluation.

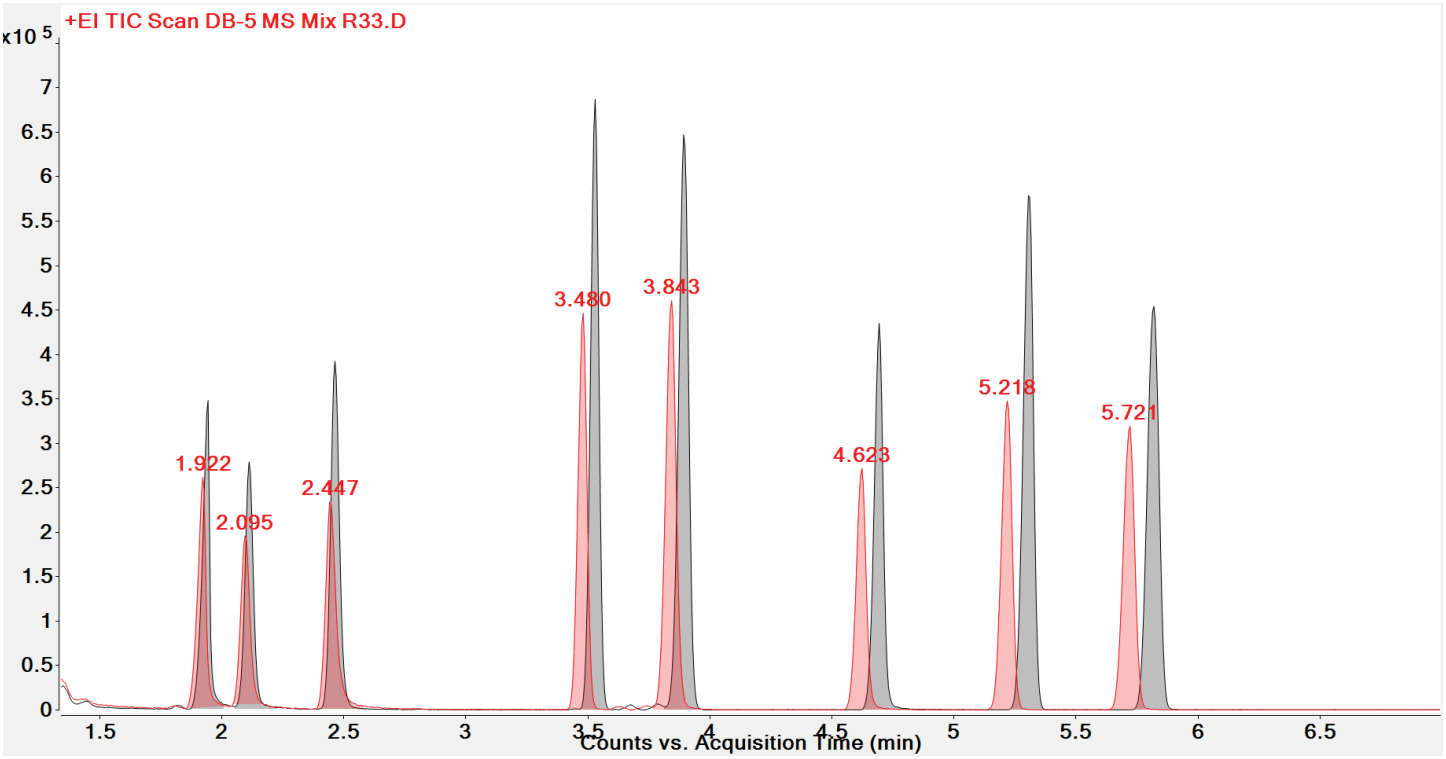



**Figure 12.** Total Ion Chromatogram (TIC) overlay of the DB-5ms column test probe mix produced at the beginning of the sequence table (black) and acquired again after ~1,500 injections (red).

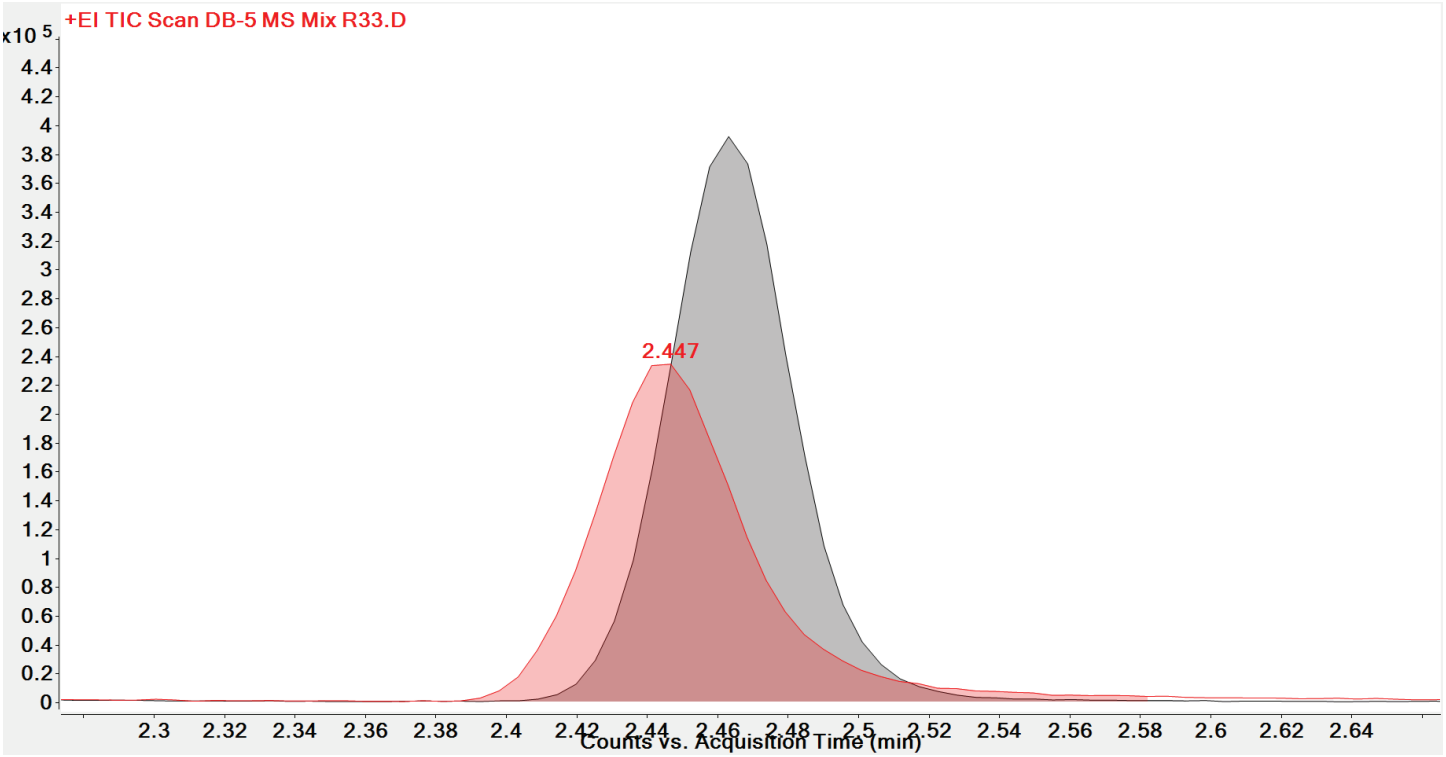



**Figure 13.** Zoomed-in view of 4-chlorophenol (black) at the beginning of the sequence and (red) after ~1,500 injections.

## Conclusion

Most laboratories have SOPs to verify the level of chromatographic functionality in their systems prior to sample analysis. However, many times those QC mixes do not probe or interact with the column stationary phase in a rigorous fashion and therefore do not produce pre-emptive chromatographic data indicative of a future chromatographic failure. Although column test probe mixes are produced to determine active sites and weaknesses within the column stationary phase surface, this study reveals they could help diagnose and determine when chromatographic integrity and performance is changing through the GC flow path. In theory, other column test mixes/probes based on their respective stationary phase chemistries should function similarly to the Agilent J&W DB-5ms probe mix used in this study, but would require additional testing. These workflow processes present a robust and selective method to determine the functionality of an overall chromatographic system. Applying these observations and resources along with the workflow processes depicted in this work should help to minimize instrument downtime and maximize instrumental performance and efficacy. There needs to be a conscientious thought process applied when defining which compounds of significance should go into your method's QC mixes to elevate the probing and exercising properties of the analytes in the overall chromatographic flow path.

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