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Introducing the Agilent InfinityLab LC Performance Workflow

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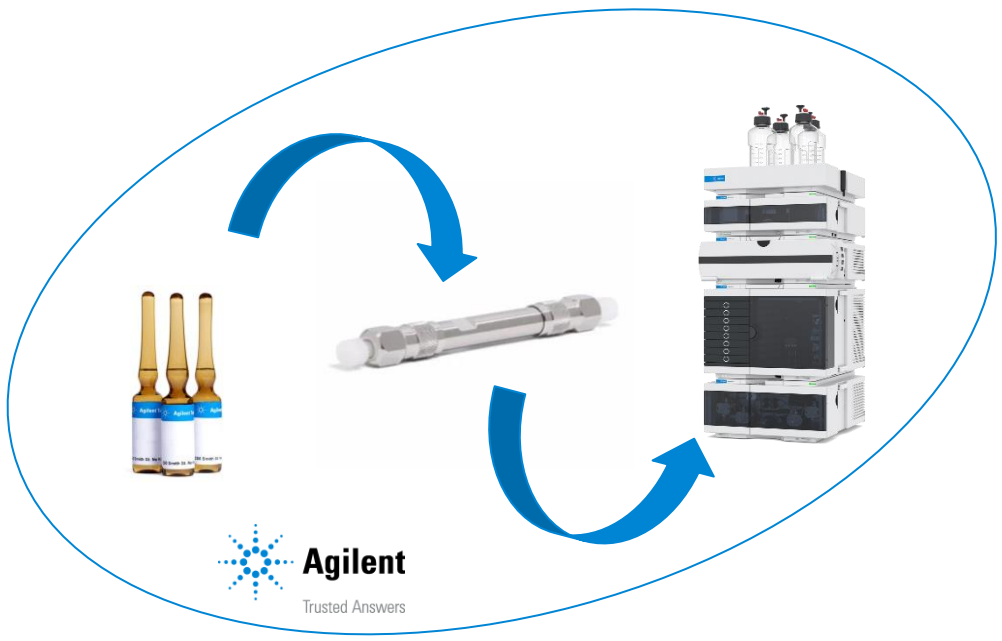
Introduction

When routine analysis starts to fail, it is crucial to have a rapid assessment of the failure is occurring where in the chromatographic detection system. This allows the user to quickly resolve the issue and get the instrument back online so that analysis can continue. While some failures are easily identifiable (such as a leak on the LC), other failures like detection, mobile phase delivery, and loss of chromatographic resolution can be more difficult to pinpoint. Failures can be identified by using a standard configuration of the LC system and a standard mixture specifically designed to probe the entire system, from injection to detection.



The Agilent InfinityLab LC workflow with the performance standard, column and LC stack with DAD.

The Agilent InfinityLab LC Performance Standard in combination with the InfinityLab LC Performance Check Column creates a powerful solution designed to probe LC systems that have various non-Mass Spectrometer detectors, such as UV, ELSD, and FLD. By running the performance workflow, the analysis of the compounds can produce a full system probe to check the system performance or troubleshoot the chromatographic system. This workflow is also flexible enough to use acetonitrile or methanol as the organic phase. Discussed here are the results that were produced from running the workflow on Agilent's InfinityLab II LC system using acetonitrile as the organic mobile phase.

Experimental

The LC method for the performance check is optimized for this column using LC grade acetonitrile (with 0.1% Formic Acid) and water. The parameters below also work with LC grade methanol (with 0.1% Formic Acid) and water.

1290 Infinity II LC Method with DAD detector	
Parameter	Value
Flow Rate	1 mL/min
Column Temp.	40 °C
Mobile Phase A	LC grade Water with 0.1% Formic Acid
Mobile Phase B1	LC grade acetonitrile with 0.1% Formic Acid
Inj. Volume	3 µL
%B Mobile Gradient	5% at 0 min
	95% at 3 min
	95% at 4.5 min
	5% at 5 min
Post Time	2 min
DAD Wavelength	265nm

Table 1: LC parameters used in the checkout method

Consumables Parts Required	Part Number
InfinityLab LC Performance Standard kit	5191-4547
InfinityLab LC Performance Check Column	699975-302C
InfinityLab Water for LC – 1x4L	5191-5120-001
InfinityLab Acetonitrile for LC – 1x4L	5191-5100-001
InfinityLab Methanol for LC – 1x4L	5191-5110-001
Formic acid	5191-4549
2 mL Amber vial, 100 pk	5190-4034
Blue preslit PFTE/silicone caps, 100 pk	5183-2076

Table 2: Consumable and orderable part numbers for the LC performance checkout workflow

Results and Discussion

The six analytes present in the InfinityLab LC Performance Standard are all easily detected using UV at 265 nm. Figure 1 shows the six analytes using acetonitrile with 0.1% formic acid as the organic mobile phase. Table 3 lists the analytes and their concentration in vial. This standard can be used as formulated without the need for any dilution.

Under the outlined method, caffeine elutes early in the gradient. Repeat injections of the standard will give sharp peaks, and no shifting in peak retention times when the system is operating normally. Should the peak start to split or shift retention time over an injection sequence, it could be indicative of a system leak or a compromise in the column, such as column bed compression. Another possible issue peak shifting can point to is the incomplete equilibrium of the mobile phases at the start of the gradient. The later eluting compounds will not show a significant difference; however, caffeine will have a large shift should this occur.

DEP and BZP act as a resolution pair during the gradient elution, with neither compound eluting near the aqueous or organic dominant gradient. The resolution $R_{DEP/BZP}$ is calculated by taking the difference in retention time and dividing it by the sum of the peak width at DEP and BZP act as a resolution pair during the gradient elution, with neither compound eluting near the aqueous or organic dominant gradient. The resolution $R_{DEP/BZP}$ is calculated by taking the difference in retention time and dividing it by the sum of the peak width at half height. Poor peak shapes and gradient changes will affect the resolution-established baseline resolution and can be an indicator of additional dwell or dead volumes in the system.

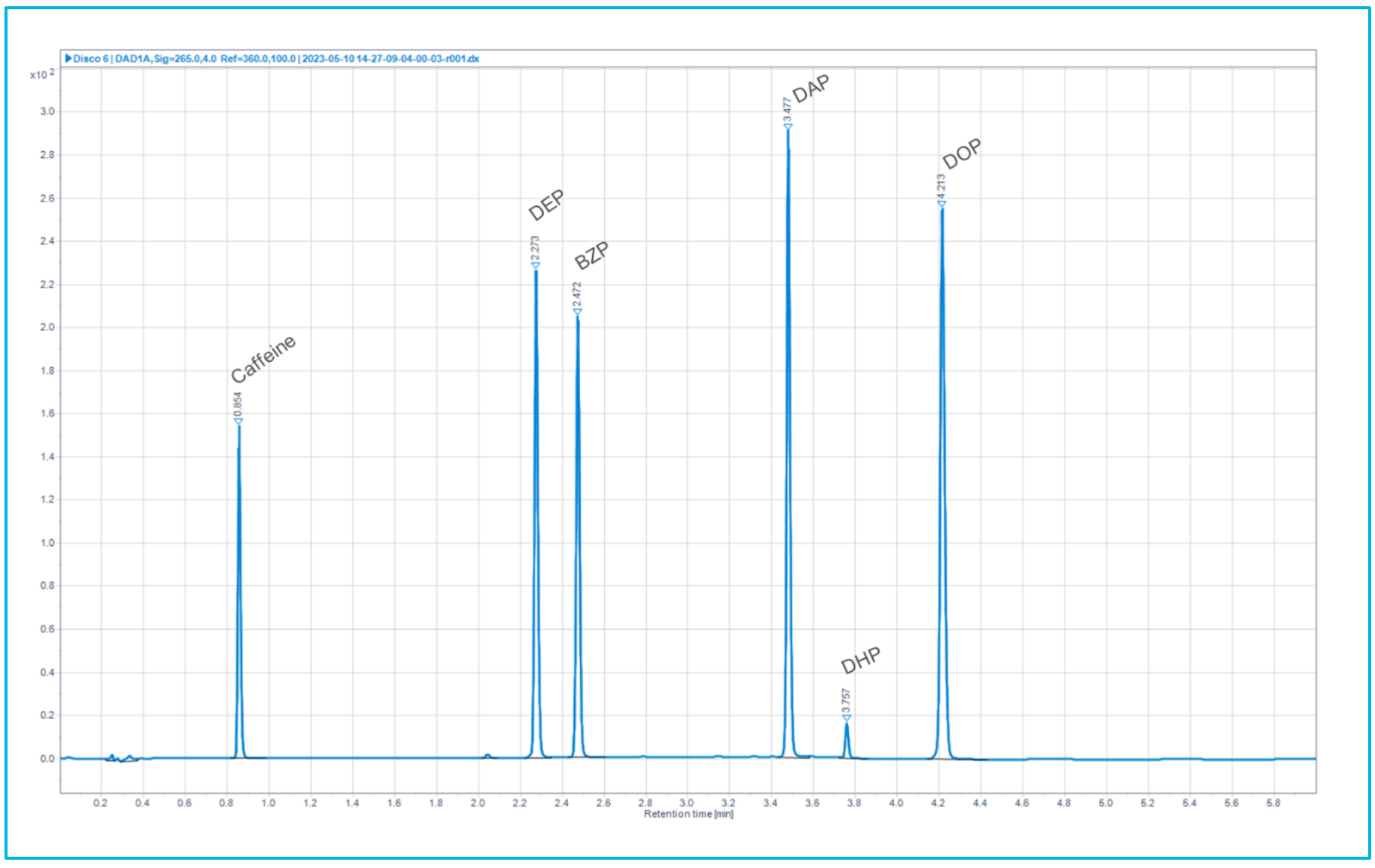


Figure 1: Chromatogram of the six analytes with UV detection using acetonitrile with 0.1% formic acid as the organic mobile phase.

Results and Discussion

Another useful pairing in this standard is DEP and DAP. The peak area ratio (PAR) for these compounds can establish the detector response at a specific wavelength. The conditions described yield a PAR of approximately 0.8 AU. A PAR that doubles this value could be indicative of loss of DAP at the injector.

Present at ~1.5% of the total chromatographic peak area, DHP can be clearly detected and quantitated. The ability to successfully integrate this peak demonstrates the detection ability for impurities at low levels. A raised baseline or poor peak shape could affect the ability of the detector to properly integrate this peak.

DOP is the most nonpolar analyte in this mix. It will only elute from a C18 column when 95 to 100% organic mobile phase elutes from the column, and when there is an isocratic hold at the end of the gradient program. DOP elution is consistent, whether methanol or acetonitrile are used as the organic mobile phase. Poor peak shapes and gradient changes will affect the resolution-established baseline resolution and can be an indicator of additional dwell or dead volumes in the system.

Analyte	Note/System Indicators	Affected System Component
Caffeine	A compound that elutes very early in the method will probe the LC pump for gradient delivery.	Pump
Benzophenone (BZP)	A critical pair with DEP.	Pump
Diethyl Phthalate (DEP)	Half of the elution pair that evaluates the resolving power of the chromatographic system.	Column
Diamyl Phthalate (DAP)	Paired with DEP, the resolution ratio between the two yields the gradient peak capacity. This peak area ratio (PAR) can also be used to evaluate loss at the injector.	Autosampler
Dihexyl Phthalate (DHP)	Spiked at low concentrations to monitor the ability to detect compounds at trace level (~ 1-2% of the total chromatogram).	Detector
Dioctyl Phthalate (DOP)	A late eluting compound used to ensure the gradient is appropriate for hydrophobic analytes, eluting at the end of the gradient.	Solvent composition

Table 3: The new performance check standard consists of six analytes designed to probe the entire LC system based on the method described. The six analytes are listed in Table 3.

Conclusions

When unexpected issues arise on your LC system, Agilent has delivered a robust workflow solution that can –

- Provide a complete solution with consumables and methodology required to evaluate if the LC instrument is performing properly
- Evaluate each module of the LC system to help quickly troubleshoot any potential issues and reduce downtime

To see the full body of work including an alternate organic mobile phase solvent and multiple detectors, scan the QR code below to access the application note.

References

1. 5994-6584EN Introducing the Agilent InfiniyLab LC Performance Standard. Agilent Technologies, Inc. July 28, 2023
2. Mutton, I; Boughtflower, B.; Taylor, N.; Brooke, D. The design and use of a simple System Suitability Test Mix for generic reverse phase high performance liquid chromatography–mass spectrometry systems and the implications for automated system monitoring using global software tracking. J. Chromatogr. A. 2011, 1218, 3711-3717.

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