White Paper

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# Introducing the Agilent InfinityLab LC Performance Standard


## Abstract

In this white paper, the development of a robust, stable mixture with six analytes that can be used to evaluate a reversed-phase HPLC is presented. The Agilent InfinityLab LC Performance Standard has been newly formulated to test the performance of an LC in standard configuration with various detectors including the ultraviolet detector (UV), fluorescence detector (FLD), and evaporative light scattering detector (ELSD).

# Introduction

When routine analysis starts to fail, it is crucial to have a rapid assessment of where in the chromatographic detection system the failure is occurring. 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 such as detection, mobile phase delivery, and loss of chromatographic resolution can be more difficult to pinpoint. Failure modes can be minimized with regular performance checks on the system. In either case, 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 Performance Standard is designed to probe LC systems that have various non-MS detectors such as UV, ELSD, and FLD. Any detector in line with UV can produce a full system probe to check the system performance or troubleshoot the chromatographic system.

# Experimental

## Materials

LC/MS-grade acetonitrile and methanol were used in this study, as they are the most common organic, reversed-phase LC solvents used in laboratories. LC/MS-grade 18 mΩ water was used as the aqueous phase. Each mobile phase was prepared with 0.1% formic acid, 99% purity (part number G2453-85060). The InfinityLab LC Performance Standard comes in 0.5 mL ampules, and does not require refrigeration or dilution. This allows for easier use, as the entire aliquot can be transferred to a 2 mL amber vial for analysis. Extra vials that come with the kit can be stored at room temperature. Table 1 lists the consumables and their part numbers for easy reference.

**Table 1.** List of Agilent consumables used for this method.

| Agilent Consumable | Part Number |
|---|---|
| InfinityLab LC Installation Standard Kit | 5191-4548 |
| InfinityLab LC Performance Standard Kit | 5191-4547 |
| InfinityLab LC Performance Column | 699975-302C |
| InfinityLab Ultrapure LC/MS Water | 5191-4498 |
| InfinityLab Ultrapure LC/MS Acetonitrile | 5191-4496 |
| InfinityLab Ultrapure LC/MS Methanol | 5191-4497 |
| Formic Acid | G2453-85060 |
| 2 mL Amber Vial, 100 pk | 5190-4034 |
| Blue Preslit PFTE/Silicone Caps, 100 pk | 5183-2076 |

## Instrumentation

An Agilent 1290 Infinity II LC with three different detectors (UV, ELSD, and FLD) was used to demonstrate the features of the InfinityLab LC Performance Standard. The UV detector is capable of a full-system performance check, as it can easily detect all six analytes present in the standard. The ELSD and FLD detectors can additionally be evaluated inline with the LC-UV to establish a baseline and monitor performance.

For each of the configurations demonstrated, the column used for LC performance testing is the Agilent InfinityLab Poroshell 120 EC-C18 3.0 × 50 mm, 2.7 µm LC column (part number 699975-302C). The LC method for the performance check is optimized for this column using both acetonitrile (0.1% formic acid) and methanol (0.1% formic acid). The conditions are listed in Table 2.

**Table 2.** Instrument method parameters used to analyze the Agilent InfinityLab LC Performance Checkout Standard.

| Parameter | Value |
|---|---|
| **Agilent 1290 Infinity II LC Method** | |
| Flow Rate | 1 mL/min |
| Column Temperature | 40 °C |
| Mobile Phase A | Ultrapure LC/MS-grade water with 0.1% formic acid |
| Mobile Phase B 1 | Ultrapure LC/MS-grade acetonitrile with 0.1% formic acid |
| Mobile Phase B 2 | Ultrapure LC/MS-grade methanol with 0.1% formic acid |
| Injection Volume | 3 µL |
| %B Mobile Gradient | 5% at 0 min<br>95% at 3 min<br>95% at 4.5 min<br>5% at 5 min |
| Post Time | 2 min |
| **Agilent 1290 Infinity II Detector Methods** | |
| **UV Detectors** | |
| Wavelength | 265 nm |
| **FLD** | |
| Excitation Wavelength | 230 nm |
| Emission Wavelength | 365 nm |
| **ELSD** | |
| Evaporation Temperature | 50 °C |
| Nebulizer Temperature | 50 °C |
| Gas Flow Rate | 1 SLM |
| Data Rate | 80 Hz |
| Smoothing | 30 sec |

## The InfinityLab LC Performance Standard

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.

**Table 3.** A list of the six analytes in the Agilent InfinityLab Performance Standard and their system indications.

| Analyte | Concentration (µg/mL) | Note/System Indicators |
|---|---|---|
| Caffeine | 20 | A compound that elutes very early in the method will probe the LC pump for gradient delivery. |
| Benzophenone (BZP) | 20 | A critical pair with DEP. |
| Diethyl Phthalate (DEP) | 350 | Half of the elution pair that evaluates the resolving power of the chromatographic system. |
| Diamyl Phthalate (DAP) | 600 | 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. |
| Dihexyl Phthalate (DHP) | 35 | Spiked at low concentrations to monitor the ability to detect compounds at trace level (~1 to 2% of the total chromatogram). |
| Dioctyl Phthalate (DOP) | 950 | A late-eluting compound used to ensure that the gradient is appropriate for hydrophobic analytes, eluting at the end of the gradient. |
| Methanol:Water 80:20 | – | Mix solvent that is nontoxic. |

## Results and discussion

### Detection using UV

The six analytes present in the InfinityLab 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.

Under the outlined method, caffeine elutes early on 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

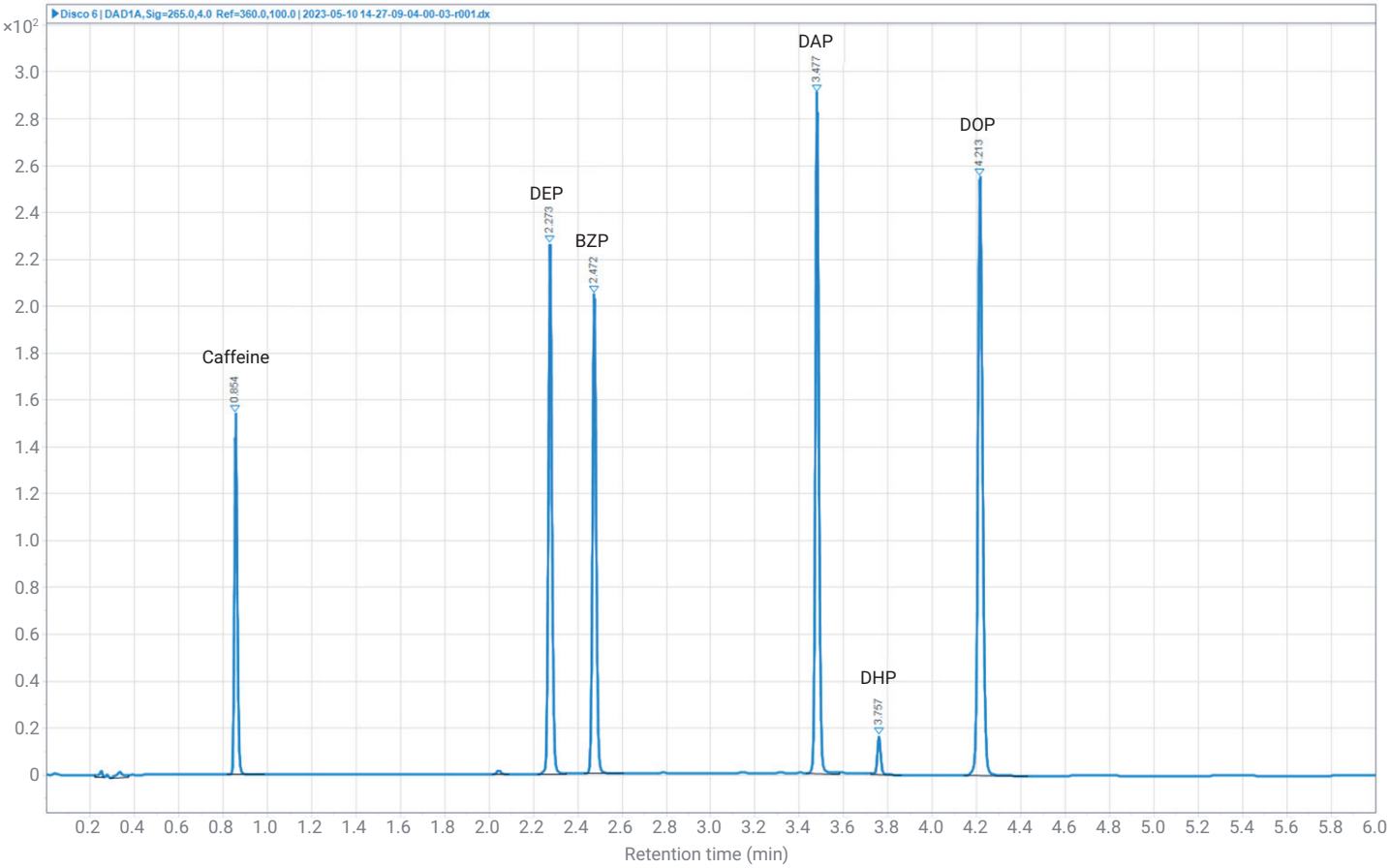


**Figure 1.** Chromatogram of the six analytes in the Agilent InfinityLab LC Performance Standard, analyzed using acetonitrile with 0.1% formic acid as the organic mobile phase.

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. This particular probe into early gradient delivery cannot be observed when using methanol with 0.1% formic acid as the organic mobile phase, as there is significantly more retention on the column, which can be seen in the chromatogram in Figure 2.

The performance column used in this study has a gradient peak capacity of 100, regardless of whether methanol or acetonitrile is used. The gradient peak capacity ($P_c$) is calculated using Equation 1, with $t_g$ representing the gradient time including the additional hold on the top of the gradient. The variable w represents the overall average of the peaks at half width for all six analytes.

$$P_c = 1 + \left(\frac{t_g}{w}\right)$$

**Equation 1.**

Because the gradient time is fixed, a change in the gradient peak capacity would be indicative of peak broadening, an indicator of column performance degradation.

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.

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.

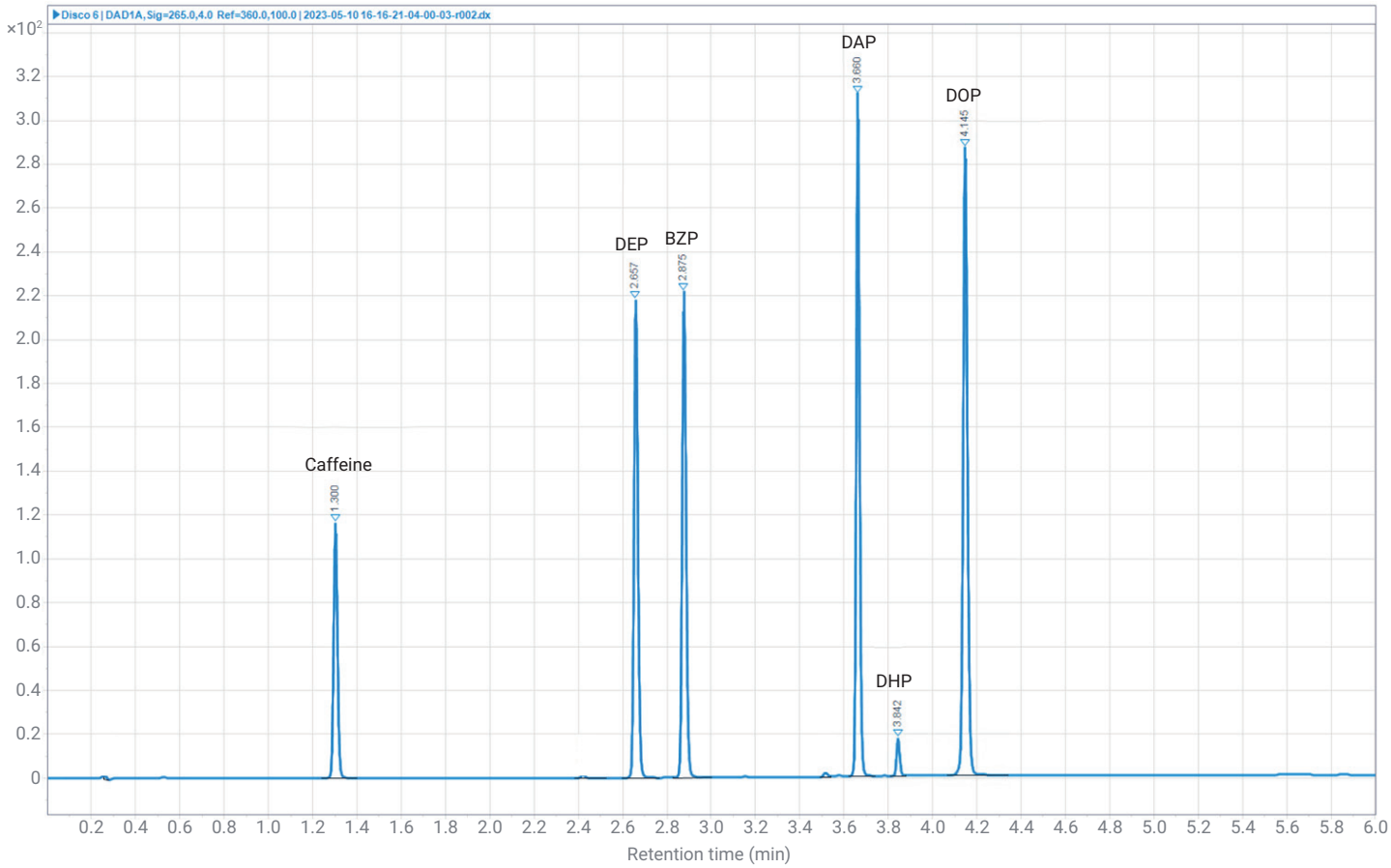


**Figure 2.** Chromatogram of the six analytes in the Agilent InfinityLab LC Performance Standard, analyzed using methanol with 0.1% formic acid as the organic mobile phase.

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.

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.

### Detection with FLD

Fluorescence detection works well with the phthalate compounds because they are easily detected. DHP can be detected with FLD, but is not quantitated at the level present in the standard. DEP, DAP, and DOP give a strong fluorescence response, and can therefore be used to establish a response on the FLD. In contrast, caffeine and BZP are not detected because they do not fluoresce. The chromatogram of these compounds is shown in Figure 3.

### Detection on ELSD

ELSD detection is best for large, nonvolatile analytes. DAP and DOP have a strong response on this detector. As shown in Figure 4, DHP can also be identified at trace levels. Caffeine, BZP, and DEP are too volatile to be detected by this method.

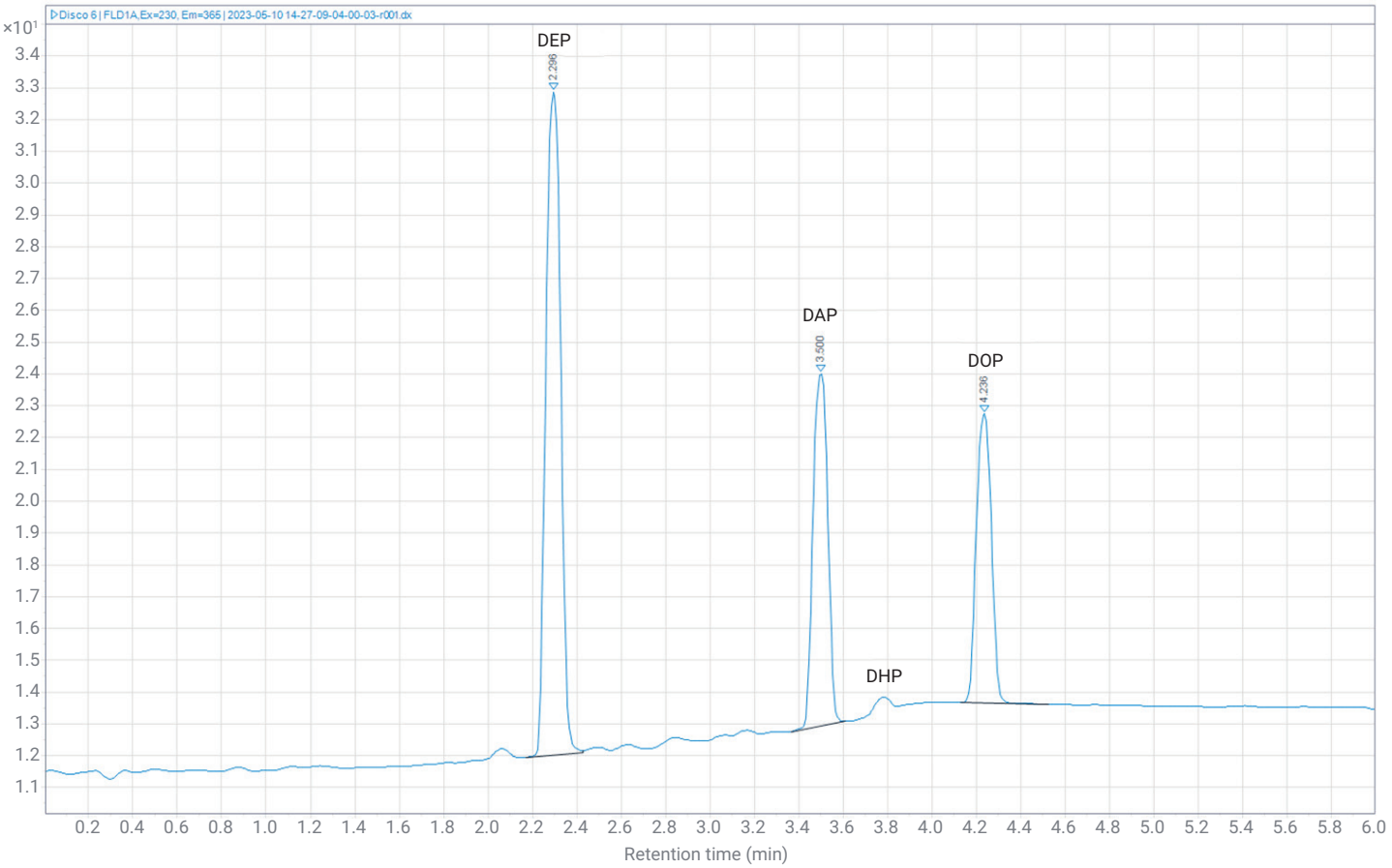


**Figure 3.** Chromatogram of three of the six analytes in the Agilent InfinityLab LC Performance Standard, analyzed using acetonitrile with 0.1% formic acid as the organic mobile phase.

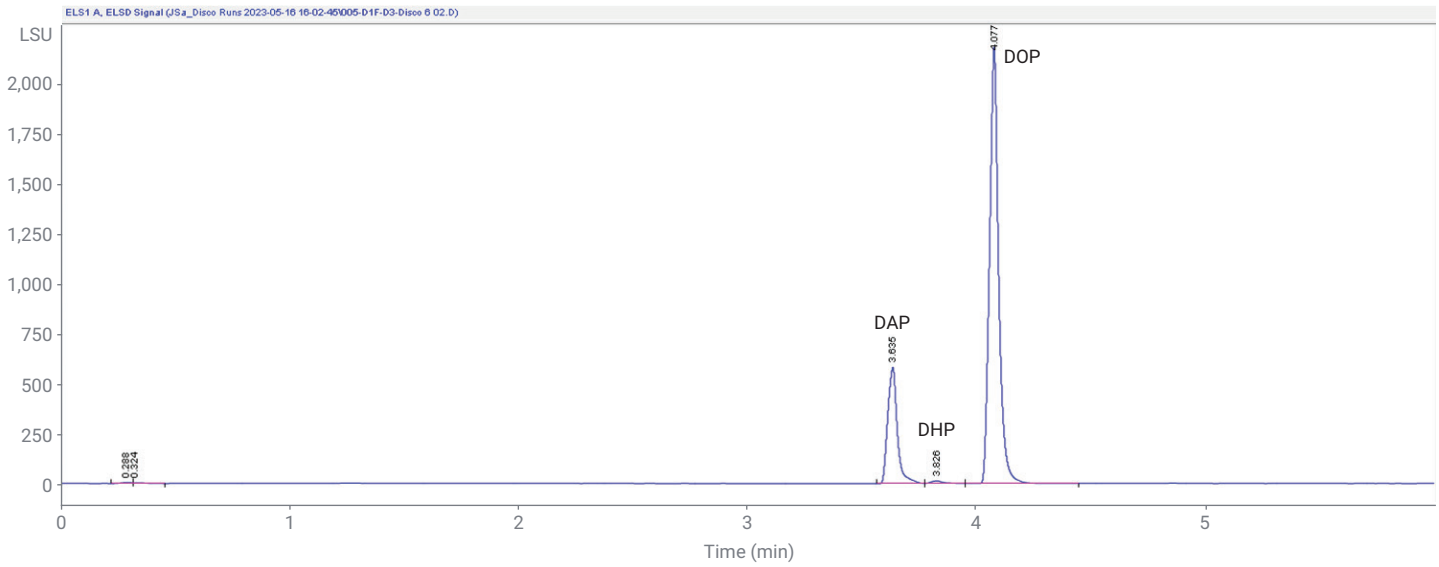


**Figure 4.** Chromatogram of the six analytes in the Agilent InfinityLab LC Performance Standard, analyzed using acetonitrile with 0.1% formic acid as the organic mobile phase.

## Conclusion

Agilent has developed a robust LC performance standard that can be used to evaluate the performance of an HPLC system with multiple detectors. This standard, when used with the performance column and prescribed methodology, will aid the user in assessing the system components and reducing the downtime of the instrument.

## Reference

1. Mutton, I. *et al.* 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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