Accurate Evaluation of the Finnigan Surveyor Autosampler Performance using a Deferred Standard

**Introduction**

The Deferred Standard (DS) concept was introduced more than 40 years ago with the purpose of monitoring the reliability of process chromatography. The DS consists of an injection of a pure compound during a chromatographic run. The DS injection is deferred from the sample injection. The delay between the sample injection and DS injection is under the user's control. This allows positioning the DS peak in any convenient location within the chromatogram. The DS can be thought of as a cross between an internal and external standard. The selection of a DS compound is easy because its elution time is controlled through the injection delay. Any compound compatible with the separation method can be used as long as it can be obtained as pure compound. A good choice may be one of the sample compounds, if available with sufficient purity.

The DS being a separate injection of a pure compound can be used to independently evaluate the reliability and reproducibility of a chromatographic system. Because the DS elutes during sample chromatographic runs, it allows validation of the current run and the analytical result.

In the case of autosampler evaluation, the DS allows for the isolation of the error contribution of the autosampler from the total system error. The DS is injected by a separate sampling system whose precision is known to be excellent (full loop injection) and reliable. The DS peak area is used to measure the precision of the HPLC system (pump, column, detector, data collection system) with the exclusion of the autosampler. Analyzing the results from the sample and the DS gives a direct measure of the autosampler reproducibility and guarantees that uncertainties from the rest of the HPLC system are not wrongly attributed to the autosampler operation.

**Autosampler Evaluation**

Various modes of autosampler operation are tested using the DS. These operational modes include full loop, partial loop, and variations of the loop-filling methods. The influence of temperature and other physical parameters are also investigated.

**Schematics**

A second sampling valve is inserted between the HPLC pump and the sampling valve. (Figure 1). The added valve is used to inject the DS. The complete setup includes, in addition to the standard equipment, a second sampling valve, a DS solution reservoir, and a peristaltic pump to fill the DS sampling loop (Figure 2). The operation of the peristaltic pump and the DS sampling valve are synchronized with the autosampler operation through the autosampler time functions.

- **Peristaltic Pump:** Ismatec™, 4 channel, 8 roller, 115/220V, Distributed by Cole-Parmer Instrument Co. Part #78017-10
- **Automated Sampling Valve:** Rheodyne EV700-100-SU Valve, 6 port, 2 position, Stainless Steel Valve, PEEK Rotor, Distributed by Sigma-Aldrich, Supelpro Part #53148-U
Procedure
For this experiment the peristaltic pump is turned on at the beginning of the run (time 0) with the DS sampling valve in the “Load” position. At 5.10 minutes the DS sampling valve is switched to the “Inject” position. The starting of the peristaltic pump and the DS injection time are a function of the application. The duration of the pumping should be long enough to ensure complete filling of the DS sampling loop. It takes 10 times the loop volume to have it filled completely to 99.9%. This provides for the best reproducibility. The DS should be injected at a time that ensures proper elution without interference from other peaks.

Results
The DS as a Diagnostic Tool
Figure 3 demonstrates the diagnostic capability of the DS method. The first important point is that the diagnostic is run during normal analytical operation of the instrument. A simple look at the DS peak area gives a quick indication of the performance level of the HPLC system.

The DS retention time provides more specific information about the pump performance or the possibility of leaks. DS peak shape analysis supplies detailed information about the column efficiency and may lead to early identification of performance degradation. Using the DS allows quantifying the performance of an HPLC system for each analytical run.

The same tests could be performed on one or several peaks of the sample but, because the sample is not necessarily consistent over time, the tests are less conclusive. In fact, combining analysis of peaks both from the sample and the DS may lead to a very powerful failure analysis of the chromatographic system.

A better diagnostic of the instrument leads to increased confidence in the results. Some redundancy in the analytical sequence may be eliminated without loss of confidence in the results, which leads to higher throughput, a significant contribution.
Figure 3. This illustrates the efficiency of a DS implementation. It is obvious from the very stable DS data that the HPLC system is working well. The relatively large variations of the sample peak can only be explained by a problem with the autosampler. Without the DS data, it would be difficult to identify the source of the problem.

Figure 4. This illustrates the efficiency of a DS implementation. It is obvious from the very stable DS data that the HPLC system is working well. The very stable sample data shows that the autosampler is working well.
Conclusion

The DS provides valuable information on the status of a chromatographic system. During the evaluation of an autosampler, this information allows for the separation of the sources of error due to the system itself from those contributed by the autosampler.

The DS is very important in all chromatographic applications. It significantly improves the confidence in the results without calling for redundant analysis, resulting in higher throughput.

References

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