

Innovative fraction collection with the Agilent 1100 Series purification platform

Application

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Abstract

Due to its high separation efficiency preparative HPLC is usually employed for compound purification in the pharmaceutical industry. In such purification processes, it is often overlooked that not only the development of an appropriate method accounts for a successful purification, but also the choice of right instrumentation. Only a perfectly designed instrument can ensure that the composition of a collected fraction is really in accordance with the corresponding section indicated in the chromatogram. As a consequence, when purifying compounds with such a system re-analyses would become

dispensable in most instances. In this Application Note we provide some technical insights into the innovative design of the Agilent 1100 Series purification platform. Fully automated delay volume calibration, low dispersion due to a low delay volume and real-time data processing contribute to a reliable system for high performance purification tasks.

Introduction

Ideally, the analyte composition that elutes as a fraction from the fraction collector needle tip corresponds in its composition exactly to the detector signal, that is, if an additional chromatogram would be recorded at the fraction collector needle tip, it would be identical to the chromatogram measured by a detector that is located after the column. However, in order to match this requirement the following effects that impact compound recovery and collection reliability have to be considered: delay volume, dispersion and system response. This note outlines how the innovative Agilent 1100 Series purification platform design takes into account the influence of these effects on the chromatographic results to provide reliable compound purification with high recoveries.



Delay volume calibration

The delay time is referred to as the time it takes for an analyte molecule to migrate from the detector cell to the fraction collector. In order to trigger start and stop of fraction collection precisely the delay time has to be determined. Later on, this delay time can easily be converted to the flow rate independent delay volume. Conventionally, the delay time is determined by injecting a dye and stopping the time until the dye appears at the fraction collector needle tip. Measuring the delay time in such a manner is not only laborious but also imprecise. Therefore, Agilent 1100 Series purification systems comprise an innovative delay volume calibration functionality¹. This patented² feature performs the measurement of the delay volume, fully automatically and precisely. The principle of the delay volume calibration is illustrated in figure 1.

In addition to the UV detector, a second detector, the so-called delay sensor, is integrated into the fraction collector. Whenever a delay calibrant is injected into the flow path both detectors record a signal. The time-delay between the two signals (minus the migration time between diverter valve and fraction delay sensor that is calculated internally) is the delay time. Depending on the flow rate used for the calibration procedure the exact delay volume is automatically calculated by the system and stored in the fraction collector memory. The precise delay time can now be calculated by the system for every flow rate. Re-calibration is not necessary.



Figure 1

Principle of Agilent's unique delay volume calibration

Dispersion

An often-overlooked phenomenon that impacts the compound distribution during migration from the detector to the fraction collector is dispersion. Dispersion equates with peak broadening and therefore impairs chromatographic resolution tremendously. According to the Aris-Taylor equation, band broadening is directly proportional to flow rate and tubing length, but proportional to the fourth power of the tubing inner diameter. This effect is impressively shown in figure 2. Consequently, connecting the detector with the fraction collector by tubing with an inappropriate i.d. will lead to bad purification results, such as a poor recovery or even a remixing of compounds. Therefore, a nonspecific fraction collector designed for a broad flow range might give acceptable results at high flow rates (column i.d. larger than 25 mm) but will sacrifice progressively recovery and purity when approaching low flow rates (column with i.d. smaller than 9 mm). Agilent therefore offers fraction collectors especially tuned for various purification scales. Each fraction collector type has been manufactured to provide optimal performance at dedicated flow rate ranges and column i.d. for highest compound purity and recovery. Additionally, it should be considered that each flow disturbance contributes to additional dispersion and therefore deteriorates chromatographic resolution. Sources for disturbances are columns, fittings, flow

cells, valves, etc. Their impact on purification results is in particular crucial at low flow rates. In order to keep the disturbance of the analyte composition on its way from the detector cell to the fraction collector needle tip as unchanged as possible, disturbance sources within the flow path should be avoided. A key part of every fraction collector and disturbance source is the diverter valve. The task of the diverter valve is to switch the flow from the column either to the waste or to the fraction collector needle tip. The impact on dispersion of such a valve type (solenoid valve, membrane type) conventionally built into many commercially available fraction collectors is displayed in figure 3. The considerable gain in peak broadening is clearly visible. Depending on flow rate and analyte concentration this effect tremendously impairs purification performance. In contrast, the Agilent diverter valve practically has no influence on the peak shape due to its innovative design (figure 4). Additionally, the Agilent diverter valve distinguishes from other commercially available valves by its robustness. Since it can be used at pressures up to 6 bar it guarantees leak-free and reliable operation under almost all conditions.



Figure 2

Impact of tubing i.d. on chromatographic resolution. Sample volume, tubing length and flow rate remained constant during the experiments



Figure 3

Impact of conventional diverter valve (solenoid valve, membrane type) on dispersion (flow rate 1 mL/min).



Figure 4

Impact of Agilent diverter valve (patented design) on dispersion (same conditions as in figure 3).

System-integrated intelligence

System-integrated intelligence not only allows users a tailored modular system set-up from the wide choice of Agilent 1100 Series modules but also accomplishes realtime data processing. Real-time data processing is of particular importance for instantaneous fraction collection and safe and reliable system operation even in the case of PC-power or network breakdown. In order to maintain such a flexible and safe system operation all Agilent 1100 Series modules communicate via a Controller Area Network (CAN). The outcome of this is fully PC-independent system operation. Fraction triggering still proceeds in real-time even if the communication between system and PC is disturbed by heavy network traffic, a busy CPU or or gets totally lost. Because of this fractions are collected precisely as indicated in the corresponding chromatogram. Besides CAN connection each Agilent 1100 Series module bears its own intelligence that starts becoming active as soon as the system receives a task from the PC. The PC just represents the interface between user and instrument. Its functionality is reduced to monitoring and evaluating experimental results.

Conclusion

Chromatographic purification systems have a crucial impact on purity and recovery of the target compounds. Generally re-analyses of the purified samples are performed in order to confirm purity of these compounds. Users' experiences have shown that the composition of the purified samples is usually different from what the corresponding chromatograms suggest. The eluent flow is susceptible to distrubances during its migration from the detector to the fraction collector that may tremendously impact the compositions of the collected fractions. In this Application Note we described some of the innovative features of the Agilent 1100 Series purification system, that ensure a nearly unperturbed analyte transport to the fraction device. Agilents' patented delay volume calibration guarantees a convenient and precise determination of the delay volume between detector and fraction collector, which is of significant importance for high purities and recoveries. Furthermore, we explain the sophisticated flow path design that keeps dispersion low and therefore contributes to high chromatographic resolution without remixing of previously separated peaks. Finally, Agilent's system-integrated intelligence principle provides real-time data processing for fast, reliable and precise fraction collection. Altogether, Agilent provides high performance purification systems for a broad application range and proven recoveries of nearly 100 %.

References

1.

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2.

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