

Refractive Index Detection and Isocratic Dial-a-Mix

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Key Words

Dial-a-Mix, RI Detection, Mixing, Refractive Index, Spin-Flow, Baseline Ripple

Goal

The performance of refractive index (RI) detection is strongly affected by even slight fluctuation of solvent composition. Therefore, instead of mixing solvents with a multi-channel pump, the mobile phase is commonly premixed. Premixing easily leads to variations caused by volume measuring and temperature changes. Also, premixing typically provides fixed amounts and an excess volume of mobile phase while mixing in the pump delivers exactly the amounts required. Next to preventing retention time shifts due to mobile phase variations, online mixing in the pump will save solvents and costs. The combination of the Thermo Scientific™ Dionex™ UltiMate™ 3000 Pumps with SmartFlow™ technology and high performance Thermo Scientific™ Spin-Flow™ mixing systems enables reliable on-line solvent mixing, even with sensitive RI detection. This can further help to save time and money during method development.



Figure 2. Selection of the Thermo Scientific SpinFlow mixer portfolio.



Figure 1. ERC RefractoMax 521 Refractive Index Detector.

Background

Refractive index detection is based on a unique measurement technique—the response on a compound's refractive index which is a universal bulk property of a substance. Most RI detectors used in liquid chromatography measure the difference between the refractive index of the analyte and the RI of the mobile phase and are referred to as differential refractive index detectors. RI detectors can be considered universal detectors. The universal response can be affected by several factors: temperature and pressure deviations as well as varying homogeneity of the mobile phase. Combining its components on-line may be critical if the mixing is not sufficient. Therefore, it may be necessary to use hand-mixed solvents to ensure a satisfying baseline noise. This can be very time-consuming and takes an increased amount of solvent, especially during method development.

The patented spin-flow mixing system ensures that on-line mixing (dial-a-mix) of solvents is an option for RI detection. The high efficiency of this technology keeps baseline noise low with both high- or low-pressure gradient pumps.

Solution

UltiMate 3000 Pumps are equipped with the patented Spin-Flow dual-step mixing system. The mixers ensure reliable mixing even with critical applications such as the on-line mixing of several solvents for RI detection. They enable dial-a-mix for fast and simple method development without the excessive consumption of solvents due to pre-mixing. Figure 3 shows a stacked chromatogram overlay of a typical RI application with the ERC™ RefractoMax 521 on a Thermo Scientific™ Hypersil GOLD™ Amino column. The chromatogram above was obtained with pre-mixed eluent, the baseline results from on-line mixed eluent. A mixture of acetonitrile and water is used, which is considered challenging for RI detection (Figure 3).

An increase of the baseline noise is observable due to solvent mixing, which has an impact on the signal to noise ratio (S/N). For routine carbohydrate analysis in foods or similar this does not impact assay results or robustness of the method. The decrease of the S/N is negligible when considering the height of the signals.

The used mixer provides a homogeneous solvent composition without extensive noise interfering with the separation. The difference of the baseline noise is barely visible. Especially the 1550 μL mixer size provides a smooth baseline that is sufficient for many applications.

Dial-a-mix is the ideal solution for method development in RI detection focused on best ease-of-use and minimum solvent consumption. The possible automation of various applications in one sequence is another advantage of this approach. This is possible as the reference cell of the RefractoMax 521 can be purged in an automated way between different applications. In combination with a switching valve, even two or more columns can be used.

Summary

UltiMate 3000 SmartFlow mixing technology is ideal for an efficient RI method development. Use our high performance mixers that are optimized for highly critical mixing requirements (1550 μL mixing volume) for optimum combination of reduced solvent dissipation and sufficiently low baseline noise. After the successful determination of the method and mobile phase conditions, switch to our medium- or small size mixer (400 μL to 35 μL mixing volume) and pre-mixed solvents to achieve lowest baseline noise and best limits-of-detection. Find an overview of our mixer portfolio, including related ordering information in Table 1.

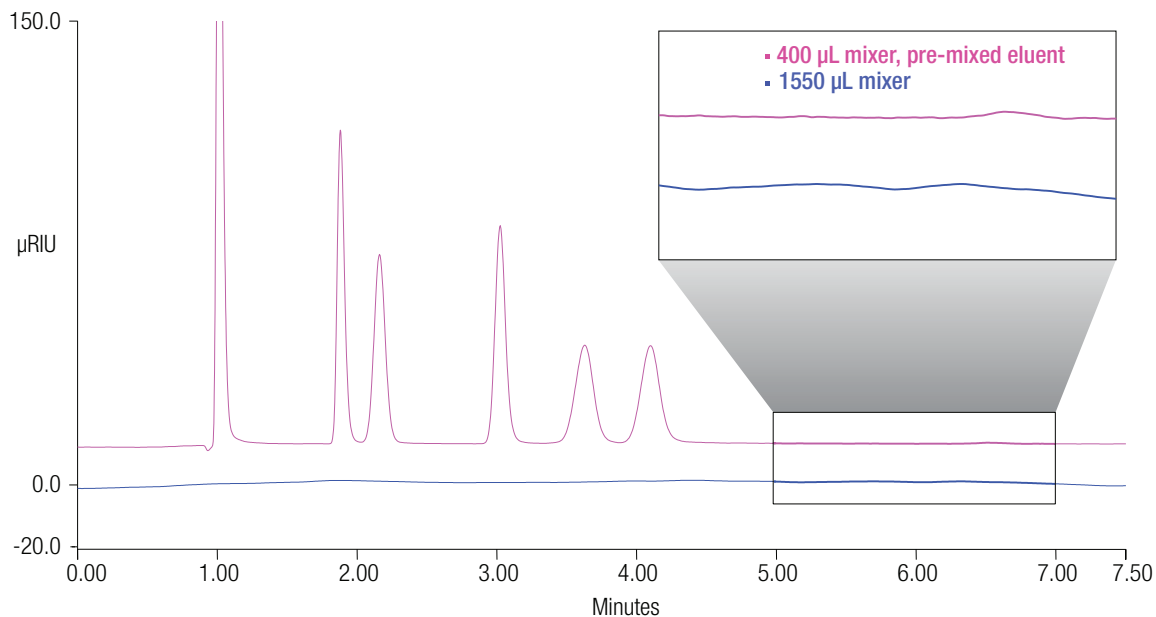


Figure 3. The stacked overlay of a chromatogram (premixed eluent) and a baseline obtained with on-line mixed eluents demonstrates that the right mixer enables the combination of on-line mixing and RI detection. This saves time and reduces the solvent consumption during method development.

Useful Links

For more information about the ERC RefractoMax 521 Refractive Index Detector and compatible UltiMate 3000 Basic Automated or Standard (SD) system setups including our mixer portfolio, visit www.thermoscientific.com. Find related ordering information below.

Description	Part Number
SpinFlow Mixers for the use with UltiMate 3000 Pumps	
Mixer for 35 µL mixing volume	
SD pumps*	6040.5000
RS pumps	6042.5000
Mixer for 100 µL mixing volume	
SD pumps*	6040.5100
RS pumps	6042.5100
Mixer for 200 µL mixing volume	6040.5110
Mixer for 400 µL mixing volume	6040.5310
Mixer for 800 µL mixing volume	6040.5750
Mixer for 1550 µL mixing volume	6040.5450
ERC RefractoMax 521 Refractive Index Detector	5060.0050

* except ISO-3100 SD pump

References

1. LC Troubleshooting: Avoiding Refractive Index Detector Problems, J.W. Dolan, LCGC Europe, December 2012
2. German patent DE102008037008, Mischvorrichtung für die Flüssigkeitschromatographie, April 2010

www.thermofisher.com/liquidchromatography

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