Application Note

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Purification of Reaction Mixtures Using Refractive Index Detection

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Abstract

Preparative high-performance liquid chromatography (HPLC) is the method of choice to isolate target compounds from complex liquid mixtures. If the target is not visible in a UV detector, alternative detection techniques must be used. Refractive index (RI) detection is a convenient and affordable choice for non-UV-active compounds. This application note demonstrates the purification of a reaction mixture using an Agilent 1290 Infinity II Preparative LC System with an RI detector in split flow.

Introduction

A crucial step in any organic synthesis is the separation and purification of the desired product from by-products, remaining starting materials and, if used, solvents and catalysts. In cases where physical and chemical properties, such as boiling point and solubility, are similar between the desired product and other species in the reaction mixture, purification by distillation, liquid/liquid extraction, or other means can be challenging. Preparative HPLC is the method of choice in these cases. This application note demonstrates the workflow for method development and purification of a reaction mixture. A reversed-phase (RP) HPLC purification method was developed by transferring and adapting an existing analytical scale method to the preparative scale. Purification was done on a 1290 Infinity II Preparative LC using RI detection in split mode, which enabled convenient monitoring of the non-UV-active compounds. The purification resulted in an increase in purity of the desired dioxolane product (Figure 1) from approximately 48 wt.% to 95 wt.% at a yield of 59%.

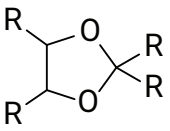

Figure 1. General chemical structure of the desired dioxolane product.

Experimental

Instrumentation

The 1290 Infinity II Preparative LC System used in this experiment comprised the following modules:

- Agilent 1290 Infinity II Preparative Binary Pump (G7161B) with 200 mL pump heads (option #206)
- Agilent 1290 Infinity II Preparative Open-Bed Sampler/Collector (G7158B)
- Agilent 1260 Infinity II Refractive Index Detector (G7162A)
- Agilent 1260 Infinity II Delay Coil Organizer (G9324A) with 1 mm inside diameter delay coils (option #210)

The flow path downstream of the column was divided between the delay coil organizer inlet and the RID using a stainless-steel T piece (part number 0100-1818). A capillary with dimensions of 0.17 × 500 mm connected the T piece with the RID, leading to a split ratio of approximately 50:1.

Column

- Analytical column: Agilent ZORBAX Eclipse XDB-C18, 4.6 × 150 mm, 5 µm (part number 993967-902)
- Preparative column: Agilent InfinityLab Pursuit XRs C18, 30 × 100 mm, 5 µm (part number INF6000100X300)

Software

Agilent OpenLab ChemStation for LC and LC/MS Systems, Rev. C.01.10 [287] or later versions

Sample

The sample is an acid-catalyzed, two-component reaction mixture containing approximately 45 wt.% of the desired dioxolane product and 55 wt.% of the two starting materials and side products, respectively.

Solvents

LC-grade acetonitrile (ACN) was purchased from VWR (Darmstadt, Germany). Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak). The mobile phase was premixed and degassed by a 15 minute treatment with a stream of helium.

Table 1. Chromatographic conditions of analytical and preparative runs.

Parameter	Value
Mobile Phase	15% ACN in water (v:v) – Premixed for method development – Mixed by pump during fractionation campaign
Flow Rate	40 mL/min
Injection Volume	750 µL
Sampler Method Preset	Preset 1: Polar Sample Matrix
Temperature	Ambient
RI Detection	25 °C Peak width > 0.025 min (0.5 s response time, 18.5 Hz) Signal polarity: positive (+)
Fraction Collection	Time based, 1.868 to 2.250 min

Results and discussion

Method scale-up and fractionation campaign

An existing and already optimized isocratic analytical scale separation method (Figure 2) was transferred to a preparative column by adjusting the injection volume and flow rate according to the increase in diameter. With the preparative-scale column being of a different type compared to the analytical-scale column, the separation was further optimized by adjusting the eluent composition and monitoring the resulting separation by RI detection in split mode. To ensure a smooth and undisturbed RI baseline, the eluent was premixed for all method development steps.

Since the product was required in the order of several hundred grams, the reaction mixture was injected undiluted onto the column and the injection volume was increased to the point where the degree of coelution with impurities was no longer tolerable. To assess the degree of coeluting impurities, a series of seven time slices was collected over the product peak (Figure 3) at different injection volumes and subsequently analyzed by GC-FID.

Figure 4 summarizes the GC-FID analyses. The two late-eluting hydrophobic impurities were removed completely. The starting materials, impurity I and impurity II, are present in all analyzed time slices. However, starting at slice 4, the sum of the peak areas of the product isomers accounted for > 97% of the total peak area excluding the solvent peaks. Since the amount of reaction mixture available was not the limiting factor, the amount of product collectible per injection was maximized while early-eluting portions of the peak that coeluted with starting materials, impurity I and II, were discarded. This led to a relatively low yield of 59% but maximized the amount of product collectible per injection. Therefore, in the final method, one time slice was collected per injection, comprising time slices 4 to 7 as shown in Figure 3.

To increase handling efficiency, the eluent was mixed by the pump instead of being premixed by hand for the fractionation campaign. While this leads to an increase in baseline noise in RI detection, it had no influence on the chromatographic separation and fraction collection.

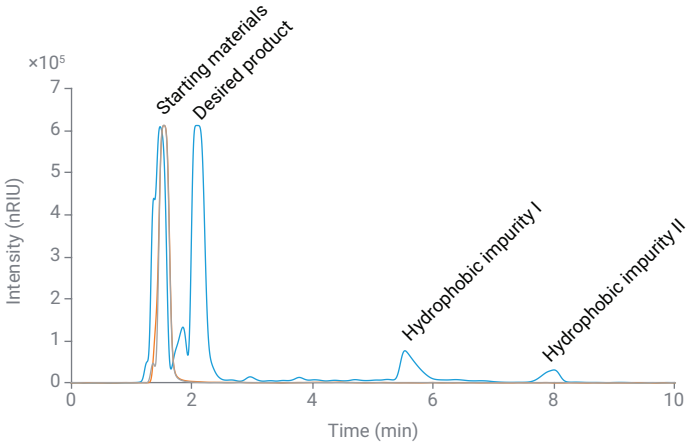

Figure 2. Overlay of reaction mixture (blue) and starting material (orange and grey) chromatograms.

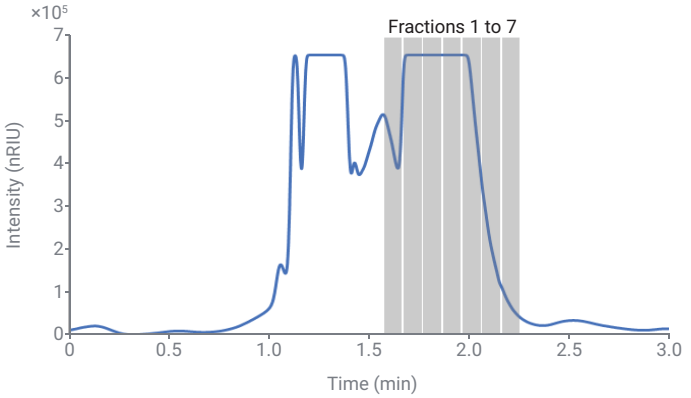

Figure 3. Preparative scale chromatogram of the reaction mixture with seven time slices to evaluate coeluting impurities by GC-FID.

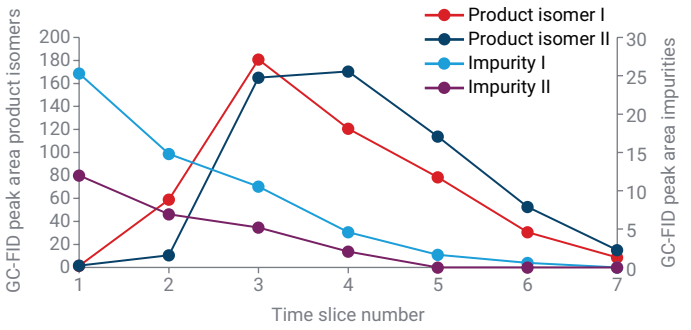

Figure 4. Relative time slice composition as determined by GC-FID.

Figure 5 shows a comparison of GC-FID chromatograms of a diluted reaction mixture sample and the collected eluent fraction containing the desired product. The selected fraction cut removed the hydrophobic impurities, while the starting materials, impurity I and impurity II, were greatly reduced. An enrichment of the desired product from approximately 45 peak area% to 95 peak area% relative to the starting materials and unwanted side products is observed. The eluent was subsequently removed by distillation and the desired product was obtained at 95 wt.% purity.

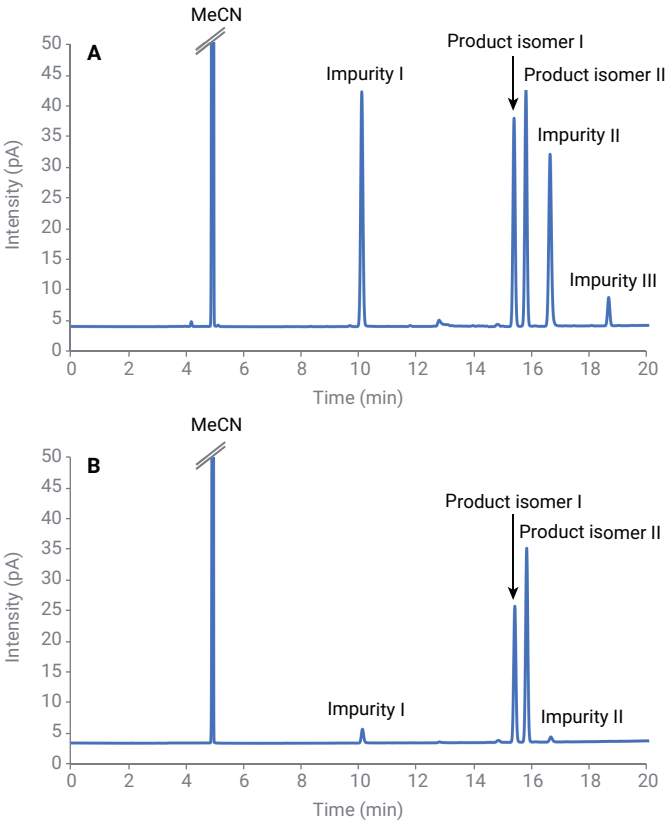

Figure 5. GC-FID chromatograms of the diluted reaction mixture (A) and the merged collected eluent fractions containing the desired product (B).

Conclusion

This application note demonstrates the purification of the desired dioxolane product from a reaction mixture by preparative high-performance liquid chromatography. Refractive index (RI) detection in split mode was used for method development and monitoring of the chromatographic performance during fractionation. This detection method is a convenient and affordable way of monitoring compounds that are not amenable to UV detection. The instrument setup enabled the purification of a dioxolane from a reaction mixture in the scale of several hundred grams from approximately 45 wt.% to 95 wt.% in a matter of weeks.

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