Technical Overview

Purify with more Confidence and Flexibility

InfinityLab LC purification solutions with column selection, solvent selection, and recovery collection

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Abstract

In preparative liquid chromatography, changes of the stationary phase or mobile phase are often required to enable successful separation and collection of the compounds of interest. This technical overview describes the purification of a sample under low and high pH conditions via two separate columns on a single system. Solvent and column selection are automated and conveniently stored within the method. Under high pH conditions, the sample was purified with high recovery (>99%); under low pH conditions, part of the sample was lost. Using a recovery collector, the flow was not diverted to waste but to a distinct recovery location, enabling salvage of the lost part of the sample.

Introduction

Preparative high-performance liquid chromatography (HPLC) is a common technique for the purification of small molecule mixtures. For many laboratories, it is not possible to dedicate one HPLC system to a certain method tailored to a specific sample. Often, operators must be flexible and run different methods on a single system, adjusting method and system parameters to the properties of the sample.

Some method parameters can be changed easily, for example, gradient steepness, starting conditions, flow rate, or injection volume. Switching to another solvent, a different modifier or pH, or selecting a column with alternate dimensions or selectivity, requires more manual interaction. To make method changes easier, faster, and more flexible, Agilent InfinityLab LC Purification Solutions offer solvent and column selection valves. This technical overview describes the separation of a preparative sample on two different columns under low and high pH conditions. Column and solvent selection are fully automated and conveniently integrated into the method. A recovery collector ensures that compounds that are missed by the fraction collector are stored in a separate bottle. These compounds of interest can be recovered in case of wrong choice of collection parameters.

Experimental

Instrumentation

The experiments described in this technical overview were conducted on an Agilent 1290 Infinity II Preparative LC/MSD System comprising the following modules:

- Agilent 1260 Infinity II Isocratic Pump (G7110B)
- Agilent 1290 Infinity II Preparative Binary Pump (G7161B) with 50 mL pump heads (option #202)
- Agilent 1290 Infinity II Preparative Open-Bed Sampler/Collector (G7158B) with 5 mL sample loop (option #241)
- Agilent 1290 Infinity Valve Drive (G1170A) with preparative 6-position/14-port valve head (G4734A)
- Agilent 1290 Infinity II MS Flow Modulator (G7170B)
- 2 × Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with 0.3 mm preparative flow cell (option #084)
- Agilent 1290 Infinity II Preparative Column Compartment (G7163B)
- Agilent 1260 Infinity II Valve-Based Fraction Collector (G7166A)
- Agilent LC/MSD XT (G6135B)

Columns

Two identical C18 columns, 30 × 150 mm, 5 µm with guard cartridges were used, one each assigned for low and high pH operation.

Software

Agilent OpenLab CDS ChemStation Edition for LC and LC/MS Systems, version C.01.10 [201]

Solvents

All solvents used were LC grade.

Method settings

Table 1. Chromatographic conditions of analytical and preparative runs.

Parameter	Value
Solvents	A1) 0.1% Formic acid in water A2) 10 mM Ammonium hydrogen carbonate in water, pH = 10 B1) 0.1% Formic acid in acetonitrile B2) Acetonitrile
Flow Rate	40 mL/min
Gradient	Time (min) %B 0 30 2.5 30 12.0 85 12.5 99
Stop Time	17.0 minutes
Injection Volume	1,000 µL
Sampler Settings	Preset for saturated nonpolar sample matrix Pre- and post-sample sandwich plugs: 500 µL Sandwich solvent: methanol:DMSO:water, 80:10:10, v:v:v
Column Selection Valve	Position 1: column A for low pH Position 2: column B for high pH
UV Detector 1 (Trigger)	Signal A: 280 ±70 nm, 4 nm slit width Peak width >0.025 minutes (10 Hz)
UV Detector 2 (Recovery)	Signal A: 280 ±70 nm, 4 nm slit width Peak width >0.025 minutes (10 Hz)
Flow Modulator	Split ratio 10,000:1 (M6) Turn on after 2.0 minutes
Fraction Collection	UV and MSD signals, AND condition UV: signal A, threshold and slope Threshold: 5 mAU Upslope: 2 mAU/s Downslope: 1 mAU/s

Table 2. MSD spray chamber and fraction collection settings.

Parameter	Value
Make Up Solvent	0.1% Formic acid in methanol:water (70:30)
Make Up Flow	0.5 mL/min
Ionization Source	Agilent Electrospray
Nebulizer Pressure	35 psig
Drying Gas Temperature	350 °C
Drying Gas Flow	12.0 L/min
Capillary Voltage	±3,000 V
Signals	1: negative scan *m/z* 100–1,000 2: positive scan *m/z* 100–1,000
Target Mass (*m/z*)	277.2; 222.1; 306.2
Ion Species	$[M-H]^-$, $[M+H]^+$
Trigger Settings	Signals 1 and 2 Threshold 50,000 cps Slope 5,000 cps/s

Results and discussion

A preparative sample composed of two neutral and one basic compound was separated with a linear gradient under different conditions. First, a method with acidic modifier in the mobile phase (A1, B1, see Table 1) was applied, using column A. Then, the same gradient was applied under basic conditions, using a different pair of eluents buffered at high pH (A2, B2, see Table 1). For the high-pH conditions, a separate column of the same type was used, allowing both columns to stay equilibrated to acidic and basic conditions, respectively. Column switching was employed using a 6-position/14-port column selection valve, which enabled linking the correct column to the low or high-pH eluents within the method.

Fraction collection was enabled in both methods, using the signals of the UV and the MS detector combined by a logical AND condition. The waste line of the fraction collector was connected to a second UV detector and a recovery collector, enabling detection and collection of peaks that were missed by the fraction collector.

Figure 1 shows a chromatogram overlay of the sample separated under low and high-pH conditions. The three sample components A, B, and C were separated and collected successfully in both cases. Compounds B and C elute at the same retention time under both low and high-pH conditions. These compounds neither have acidic nor basic moieties, and are not affected by changes of the pH in the mobile phase. Compound A was much less retained and showed strong peak tailing under low-pH conditions.

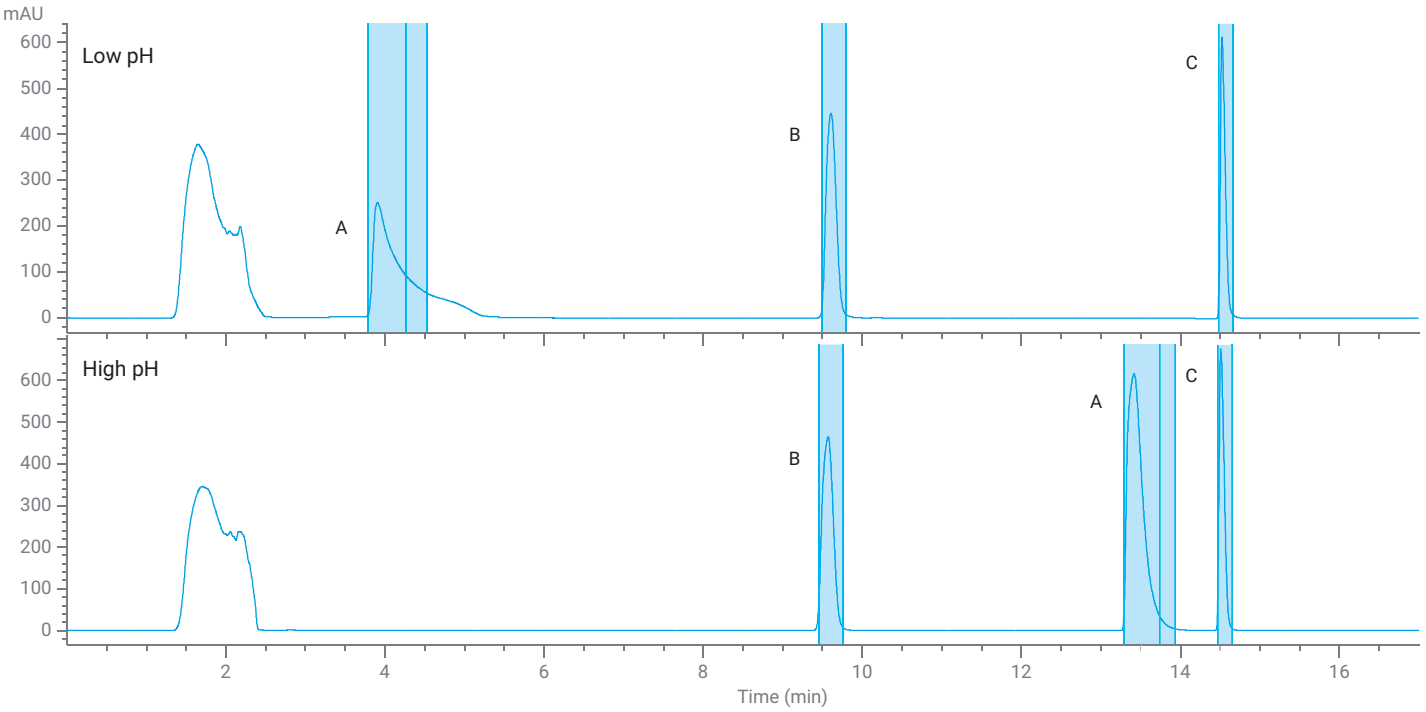

Figure 1. Chromatogram overlay (UV detector 1, 280 nm) of the sample separated under low (upper half) and high-pH conditions (lower half). Blue bars represent fraction collection events. Compound A is stronger retained and exhibits better peak shape under high-pH conditions.

This peak tailing is due to the basic properties of this compound. Under acidic conditions, the compound is protonated and positively charged, resulting in poor retention on the nonpolar C18 chains of the stationary phase. Peak shape improves under basic conditions, when compound A is not protonated and thus less polar.

Strong tailing of compound A under low-pH conditions also affects fraction collection. The method settings of the fraction collector contained a slope parameter, causing collection to stop once a certain downslope was reached. At low pH, this downslope parameter was fulfilled during the long tailing of the peak, before the signal fell to baseline again (see Figure 1, upper half). At high-pH conditions, with improved peak shape, the same slope parameter sustains collection until the signal has fallen to baseline again.

The consequences of this collection behavior become evident when looking at the signal of the second UV detector. Located in the waste line of the fraction collector, this second detector serves as verification of the fraction collection triggered by the first UV detector and the MSD. If a compound has been collected perfectly, no signal of that compound should be visible in the second UV detector. In fact, compounds B and C show a negligible peak area in UV detector 2, indicating correct fraction collection (see Figure 2). Compound A, however, produces a significant signal in UV detector 2 (peak A*, Figure 2). This signal confirms what is indicated by the fraction collection bands: the peak tail has not been collected and could have been lost.

With the recovery collector configured into the system, this part of the peak is collected together with the remaining eluent of the run. If compound A is precious, this recovery collection could be evaporated to dryness and re-injected to salvage the lost part of the first purification run. In a sequence of multiple samples, it is a default setting for the recovery collector to switch to a new location each sequence line. The operator can check the collected fractions and decide per run whether to keep or discard the recovery collection.

As shown in Figure 1, the peak shape of compound A improved significantly when the sample was analyzed under high-pH conditions. Fraction collection bands indicate that compound A has been collected properly. Comparing the signals of the two UV detectors confirms this observation: all compounds show only negligible signals in the recovery UV detector (data not shown).

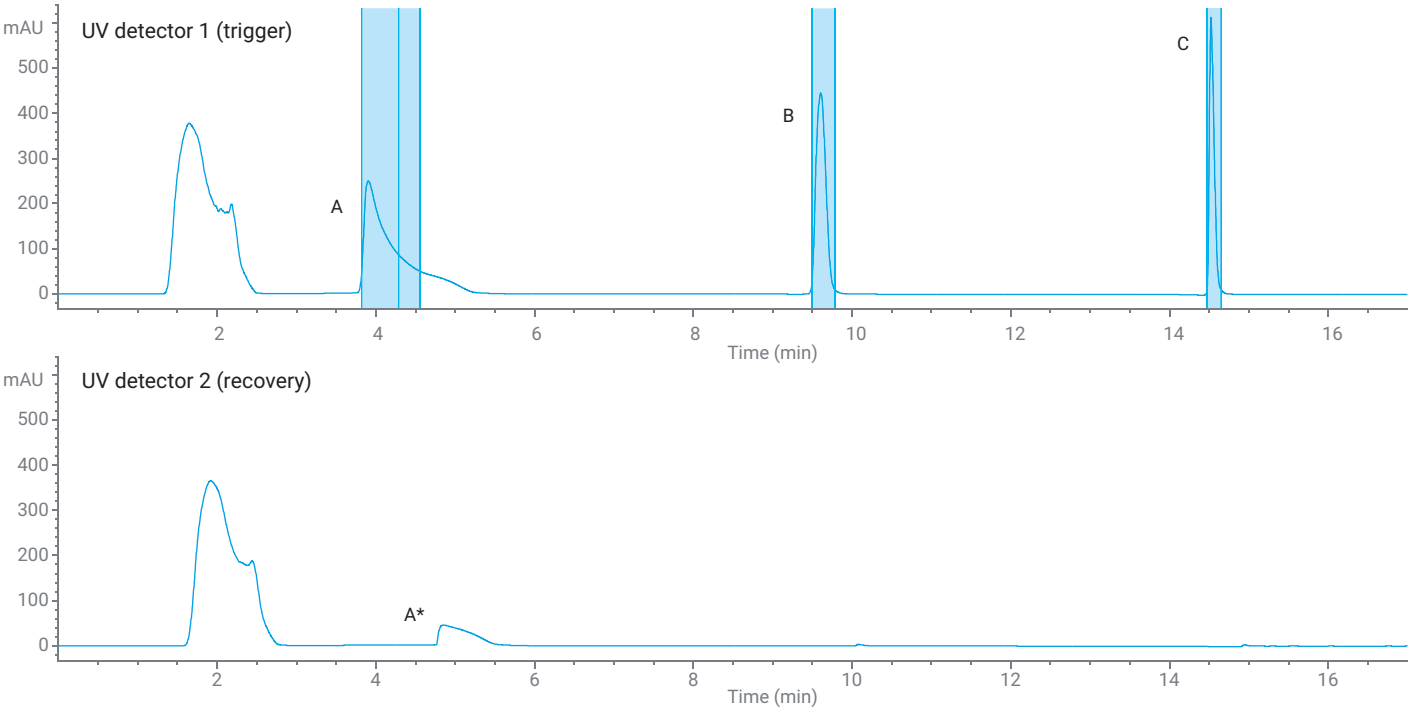

Figure 2. Chromatogram overlay (280 nm) of UV detector 1 (trigger) and 2 (recovery) of the separation at low pH condition. Blue bars represent fraction collection events. Compound A is only partly collected and appears with a significant signal in UV detector 2 (A*).

To evaluate fraction collection results of the low and high-pH methods, peak areas of all compounds detected by the two UV detectors were compared. The fraction recovery of each compound was then calculated as follows:

$$\text{recovery} = \frac{\text{area (UV1)} - \text{area (UV2)}}{\text{area (UV1)}} \times 100\%$$

Table 3 summarizes the peak areas and recovery calculations of all compounds for the low and high-pH separations. The recovery calculation shown here is just one of several ways to assess fraction recovery on a preparative LC system. This method does not consider injection inaccuracy and assumes identical path lengths of the flow cells in the two UV detectors. However, for a quick answer to the operator whether the compound of interest has been collected, this method provides an easy and reliable solution.

Table 3. Evaluation of peak areas under low and high-pH conditions, measured with the two UV detectors used for fraction and recovery collection, respectively. Only under high-pH conditions were all compounds collected with excellent recovery.

Method	Low pH			High pH		
Compound	A	B	C	A	B	C
Area UV Detector 1	6,722	3,919	2,645	8,579	4,533	3,020
Area UV Detector 2	1,207	22	28	40	19	12
Calculated Recovery	82.0%	99.4%	98.9%	99.5%	99.6%	99.6%

Conclusion

The Agilent 1290 Infinity II Preparative LC/MSD System with solvent and column selection provides a versatile tool for different purification tasks. Four solvents for binary gradients and up to six columns can easily be selected and stored in the method. Depending on the sample, the correct combination of solvent and column is only one click away. This technical overview demonstrates that a method executed at low and high-pH conditions, with the correct choice of mobile phase, is pivotal for successful fraction collection. Fraction recovery was above 99% with correct choice of pH, but as low as 82% with the wrong pH of the mobile phase. At the same time, InfinityLab LC Purification Solutions offer the possibility to cluster a fraction collector with a recovery collector. This combination adds more confidence to your purification tasks, since an uncollected compound can easily be recovered.

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