Application Note

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Efficient Purification of Crude Samples in Early-Stage Drug Discovery Using the Agilent 1290 Infinity II Preparative LC/MSD System

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Abstract

In early-stage drug discovery, high-purity compounds are needed for compound screening and lead identification. The Agilent 1290 Infinity II Preparative LC/MSD System, including the Agilent 1290 Infinity II Preparative Open-Bed Sampler/Collector, is ideally suited for purification of crude samples in early-stage drug discovery. The system offers mass-based fraction collection and precise delay calibration, which enables maximum purity and recovery of collected fractions and results in higher confidence in the quality of compounds studied during early stage drug discovery.

In this application note, paracetamol is purified from an example crude sample using the 1290 Infinity II Preparative LC/MSD System. Purity and recovery of the collected paracetamol are determined.

Introduction

In the early years of modern drug discovery, approaches were focused on compounds discovered through ethnobotanical knowledge or derived from natural products. Drug discovery nowadays has changed into a hypothesis-driven target-based approach, involving, among other techniques, high-throughput screening of compound libraries and rational drug design for identification of lead structures.[1] During screening of compounds and lead identification, high-purity compounds are needed to avoid errors and reduce cost.[2] Purification of crude reaction mixtures can be successfully accomplished by preparative-scale HPLC.[3]

The Agilent InfinityLab LC Purification Solutions[4] offer tailor-made LC purification solutions ranging from analytical to preparative scale for purification of multiple grams.

The Agilent 1290 Infinity II Preparative LC/MSD System, part of the InfinityLab LC Purification Solutions, enables high-throughput purification, delivering high-quality compounds, and is therefore ideally suited for purification of crude samples in early-stage drug discovery. The Agilent 1290 Infinity II Preparative Open-Bed Sampler/Collector combines sampler and fraction collector in one module and so optimizes use of lab bench space. It offers highest flexibility in terms of configuration of the sampler and fraction bed, injection from vials as well as microplates, and flexibility in terms of injection volume and fraction collection modes. The different fraction collection modes were described in previous Agilent technical overviews.[5–7] A built-in delay sensor in the 1290 Infinity II Preparative Open-Bed Sampler/Collector allows precise delay calibration. In combination with mass-based fraction collection, allowing highly specific and sensitive fraction triggering as offered by the 1290 Infinity II Preparative LC/MSD System, maximum purity and recovery of collected fractions are achieved. This results in higher confidence in the quality of compounds analyzed during early-stage drug discovery.

In this application note, exemplifying purification of a crude sample in early-stage drug discovery, the 1290 Infinity II Preparative LC/MSD System is used to purify paracetamol from a sample containing approximately 50% paracetamol, accompanied by its impurities. Purity and recovery of the collected paracetamol are determined.

Experimental

Equipment

The Agilent 1290 Infinity II Preparative LC/MSD System comprised the following modules:

- Agilent 1290 Infinity II Preparative Binary Pump (G7161B) with 200 mL/min pump heads (option #206)
- Agilent 1290 Infinity II Preparative Open-Bed Sampler/Collector (G7158B)
- Agilent 1290 Infinity II Preparative Column Compartment (G7163B)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with 0.3 mm preparative flow cell (option #084)
- Agilent 1290 Infinity II MS Flow Modulator (G7170B)
- Agilent 1260 Infinity II Isocratic Pump (G7110B)
- Agilent 1260 Infinity II Delay Coil Organizer (G9324A) with knitted delay coils for 15 to 40 mL/min (option #210)
- Agilent LC/MSD XT (G6135B)

The Agilent 1260 Infinity II LC System used for fraction reanalysis comprised the following modules:

- Agilent 1260 Infinity II Binary Pump (G7112B)
- Agilent 1260 Infinity II Multisampler (G7167A) with sample thermostat (option #101)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with standard flow cell (option #018)

Software

Agilent OpenLab CDS ChemStation Edition Rev. C.01.10 [239].

Columns

- Agilent Polaris C18-A, 21.2 × 150 mm, 5 µm (p/n A2000150X212)
- Agilent Polaris C18-A, 4.6 × 150 mm, 5 µm (p/n A2000150X046)

Chemicals

All solvents were LC grade. Acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Fresh ultrapure water was obtained from a Milli-Q Ultrapure Lab Water System equipped with a Millipak 0.22 µm membrane point-of-use cartridge (Millipore, Merck (Darmstadt, Germany)). Dimethyl sulfoxide (DMSO) and formic acid were purchased from Honeywell Riedel-de-Haen (Seelze, Germany) and VWR (Darmstadt, Germany), respectively. Paracetamol (acetaminophen) and its impurities were obtained from Sigma-Aldrich (Steinheim, Germany). The impurities obtained were:

A. (2-acetamidophenol)

D. (acetanilide)

E. (4'-hydroxyacetophenone)

F. (4-nitrophenol)

I. (2'-hydroxyacetophenone)

J. (4'-chloroacetanilide)

K. (4-aminophenol)

Standards

Individual stock solutions of paracetamol and its impurities A, D, E, F, I, J, and K were prepared at a concentration of approximately 100 mg/mL in DMSO. A standard mix representing a crude sample to be purified in early-stage drug discovery was prepared from the stock solutions. This crude sample had a concentration of approximately 100 mg/mL and contained 50% of the target compound paracetamol, as well as its impurities A, D, E, F, I, J, and K in different percentages.

For analytical-scale LC analysis, the crude sample was diluted 1:100 with acetonitrile/water (2/1; v/v). For determination of the purity and recovery of the purified target compound, the collected fractions were transferred to volumetric flasks and filled up to volume with acetonitrile/water (2/1; v/v). Calibration standards for determination of recovery were prepared in the concentration range of 10 to 1000 µg/mL by dilution of the paracetamol stock solution with acetonitrile/water (2/1; v/v).

Preparative-scale LC method

Parameter	Value	
Column	Agilent Polaris C18-A, 21.2 × 150 mm, 5 µm	
Solvent	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile	
Gradient	0.00 min – 3% B 0.70 min – 3% B 0.71 min – 13% B 2.38 min – 23% B 2.39 min – 100% B Stop time: 5 min Post time: 3 min	
Injection	Injection volume: 1000 µL, plug setting 3, pre- and post-sample sandwich plug: DMSO, post-sample plug: DMSO	
Flow rate	31.86 mL/min	
Temperature	Ambient	
UV detection	254/4 nm, reference 390/20 nm, 20 Hz	
Flow modulator	Mode M6, split ratio 5000:1, dilution factor 1:300	
MSD	Spray chamber	Agilent Jet Stream Electrospray
	Make up solvent	0.1% formic acid in methanol/water (70/30, v/v)
	Make up flow rate	1.50 mL/min
	Signal	Positive scan, *m/z* 100 to 500 Fragmentor 125 V
	Drying gas flow	12 L/min
	Nebulizer pressure	35 psig
	Drying gas temperature	300 °C
	Sheath gas temperature	350 °C
	Sheath gas flow	11 L/min
	Capillary voltage	4000 V
	Nozzle voltage	600 V
Fraction collection	Peak-based from 0.00 to 3.25 min, UV and MSD connected with AND condition UV: threshold 5 mAU MSD: threshold 5000 cps	

Analytical-scale LC method

Parameter	Value
Column	Agilent Polaris C18-A, 4.6 × 150 mm, 5 µm
Solvent	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Gradient	0.00 min – 3% B 0.50 min – 3% B 20.5 min – 50% B 20.6 min – 100% B Stop time: 25 min Post time: 5 min
Injection	Injection volume: 1 µL, sample temperature: 8 °C, 3 s needle wash with acetonitrile/water (50/50, v/v)
Flow rate	1.00 mL/min
Temperature	25 °C
Detection	UV, 254/4 nm, reference 390/20 nm, 20 Hz

Results and discussion

Analytical-scale LC of the crude sample

The analytical-scale LC analysis of the diluted crude sample, employing a 1260 Infinity II LC, is shown in Figure 1. Seven impurities (impurities A, D, E, F, I, J, and K) were separated from the target compound paracetamol. Based on the areas of peaks detected in the UV signal at 254 nm, a 57% purity of the crude sample can be calculated.

Purification of the target compound from the crude sample

Purification of the target compound, paracetamol, from the crude sample was achieved using the 1290 Infinity II Preparative LC/MSD System. The method was scaled up to preparative-scale LC conditions using the same column type as in the analytical-scale LC analysis, but with a larger internal diameter, and a focused gradient was employed. With the chosen injection volume of 1000 µL, the preparative-scale LC method enabled purification of 100 mg of the 100 mg/mL crude sample in a single injection. Mass-based fraction collection was used to enable highly specific peak triggering, with the mass of paracetamol (151.1 Da) set as the target mass. Peak-based fraction collection triggered by the UV and MSD signals connected by a logical AND combination was employed. This combination triggers a fraction start as soon as both detector signals have exceeded the user-defined threshold/slope settings. Collection continues until one of the detector signals drops below the settings. Peak-based fraction collection was disabled after the elution of the target compound paracetamol because impurity A eluting after paracetamol has the same mass as paracetamol.

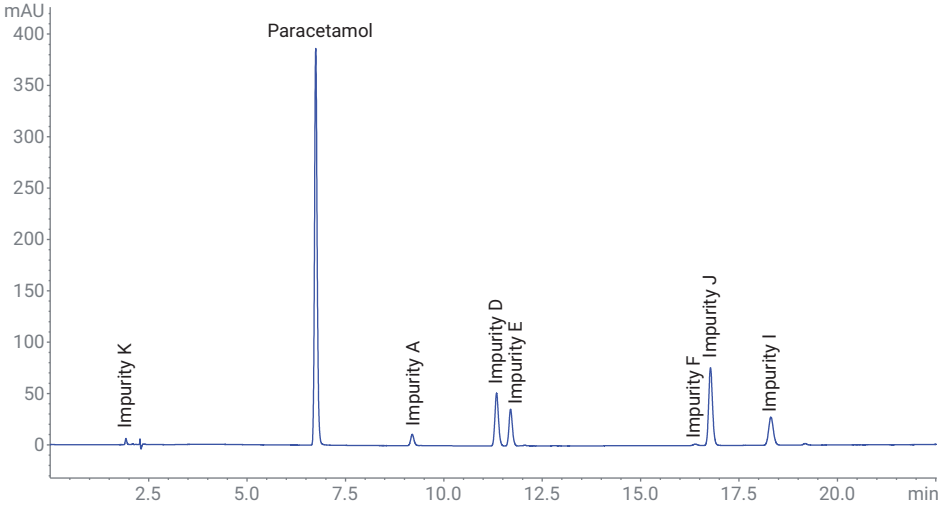

Figure 1. Analytical-scale LC analysis of the diluted crude sample.

Figure 2 shows the purification of the target compound paracetamol from the crude sample based on the UV signal at 254 nm (Figure 2A) and the extracted ion chromatogram (EIC) of the mass of paracetamol (Figure 2B).

Determination of purity and recovery of the purified target compound

For determination of the purity and recovery of the purified target compound, the collected fractions were diluted in volumetric flasks and the target compound paracetamol was quantified by analytical-scale LC analysis on a 1260 Infinity II LC. Recovery was calculated as the percentage of the quantified amount of the target compound in the diluted fraction with reference to the total amount of target compound injected into the purification system. The total amount of target compound injected to the purification system was determined from the analytical-scale LC analysis of the diluted crude sample. The purity of the fractions was determined based on the ratio of the target compound and the total peak area at 254 nm. Figure 3 shows the analytical-scale LC analysis of the diluted fraction that was collected as shown in Figure 2.

No impurities were detected in the analytical-scale LC analyses of the collected fractions from purification of the target compound paracetamol from the crude sample. Table 1 shows the purity and recovery determined after fivefold purification of 100 mg of the crude sample. Highest purity and excellent recovery were achieved using the 1290 Infinity II Preparative LC/MSD System. Furthermore, excellent reproducibility of the amount of the target compound paracetamol purified was observed.

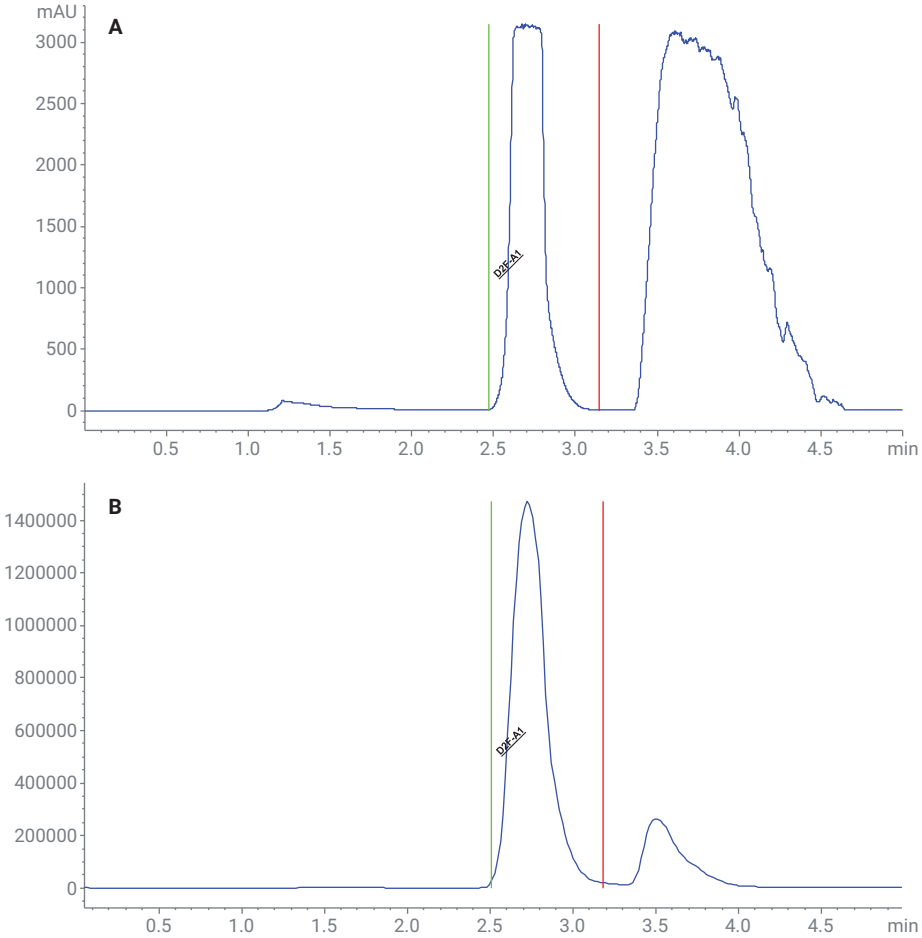

Figure 2. Purification of the target compound paracetamol from the crude sample; (A) UV detection at 254 nm; (B) extracted ion chromatogram (EIC) of the *m/z* of the $[M+H]^+$ ion of paracetamol.

Table 1. Purity and recovery determined from purification of the target compound paracetamol from 1000 µL injection of the crude sample, equivalent to 100 mg of crude sample purified (N = 5).

Fraction	Recovery (%)	Purity (%)
Average	95.9	>99%
RSD (%)	0.2	

Conclusion

The 1290 Infinity II Preparative LC/MSD System including the 1290 Infinity II Preparative Open-Bed Sampler/Collector was used for purification of paracetamol from an example crude sample. 100 mg of crude sample were purified with a single injection resulting in highest purity (>99%) of the collected fractions with excellent recoveries (>95%). The high purity of collected fractions obtained using mass-based fraction collection with the 1290 Infinity II Preparative LC/MSD System makes this system ideally suited for purification in early-stage drug discovery.

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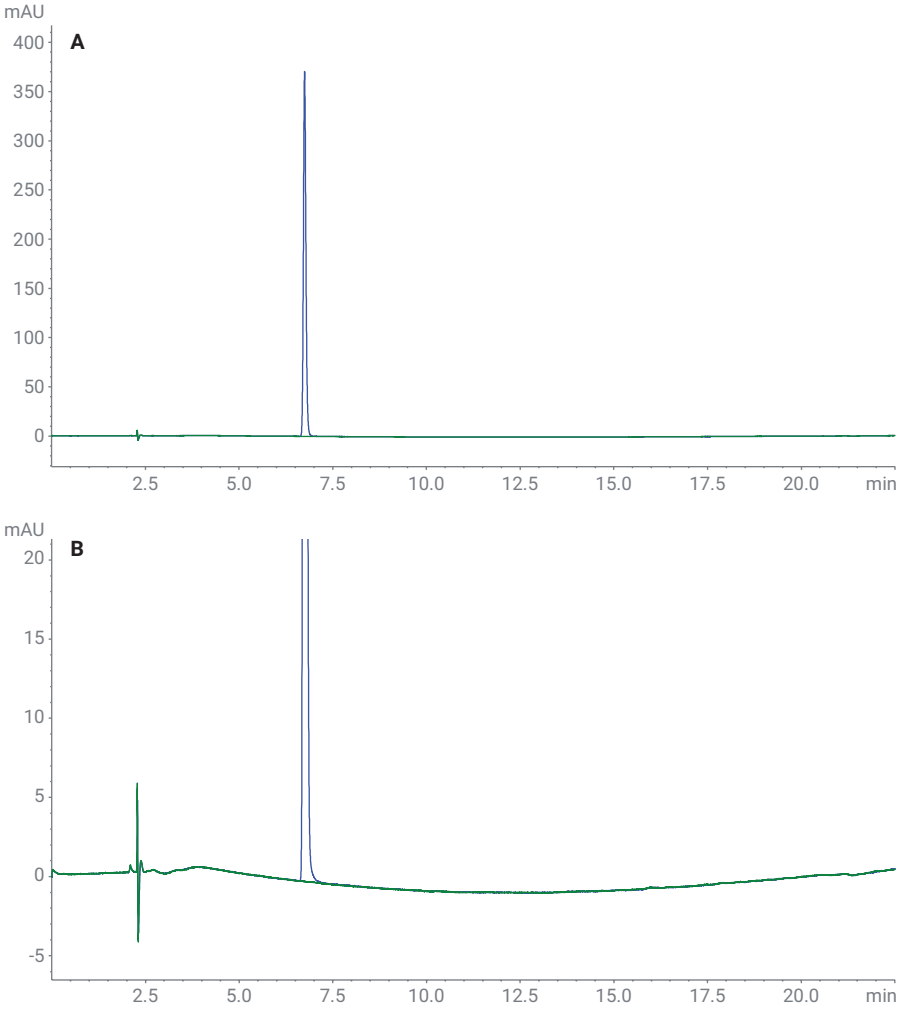

Figure 3. Analytical-scale LC analysis of the purified target compound paracetamol (blue) with UV detection at 254 nm, overlaid with a blank injection (green); (A) full scale; (B) zoom.

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