High-Throughput LC/MS Purification of Pharmaceutical Impurities

Application Note

Small Molecule Pharmaceuticals

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Abstract

Legal regulations demand that organic impurities in pharmaceuticals must be identified and characterized. The isolation of impurities from a pharmaceutical product can be done by preparative-scale liquid chromatography (LC). This Application Note demonstrates the purification of acetaminophen and six known impurities using an Agilent 1290 Infinity II Preparative LC/MSD System. For high throughput and sample load, a column of 50 mm inside diameter (id) is operated at a solvent flow of 118 mL/min. Mass-based fraction collection is combined with UV detection for highest selectivity. The performance of the fraction collection is assessed by determining the purity of collected fractions and the recovery of the active pharmaceutical ingredient (API).

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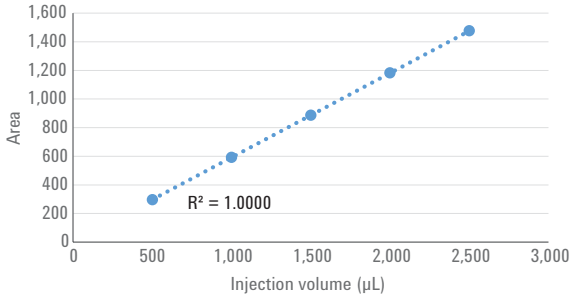
Introduction

Organic impurities in drug manufacturing can present a major challenge for pharmaceutical companies. Byproducts and intermediates originating from the chemical synthesis can be present in significant amounts, even in an optimized production process. Regulations such as the ICH Guideline Q3A(R2) demand identification and monitoring of all impurities exceeding a given threshold[1]. The isolation of the different impurities from the active pharmaceutical ingredient (API) for characterization can be done by preparative-scale LC. With precious pharmaceuticals, however, it is key to collect not only the impurities, but to recover as much of the API as possible.

This Application Note demonstrates the separation and purification of acetaminophen (also known as paracetamol) and six common impurities listed in the European and United States Pharmacopoeias[2,3]. A total amount of 500 mg of the API and 0.5 mg of each impurity (equaling 0.1 %) were separated on an Agilent Load & Lock column of 50 mm inside diameter (id). For this purpose, an Agilent 1290 Infinity II Preparative LC/MSD System was used. This system can handle the high flow rates necessary to operate a column of these dimensions, and enables fraction collection triggered by UV and MSD signals for high selectivity. This system is one of the Agilent InfinityLab LC Purification Solutions[4], featuring modules that ensure safe and easy operation while improving the purification outcome. The Agilent 1260 Infinity II Preparative Autosampler delivers fast injection cycle times with lower carryover and injections from 2- and 5-mL vials. It supports injection volumes from microliters up to 3.6 mL, and has an excellent injection linearity (Figure 1).

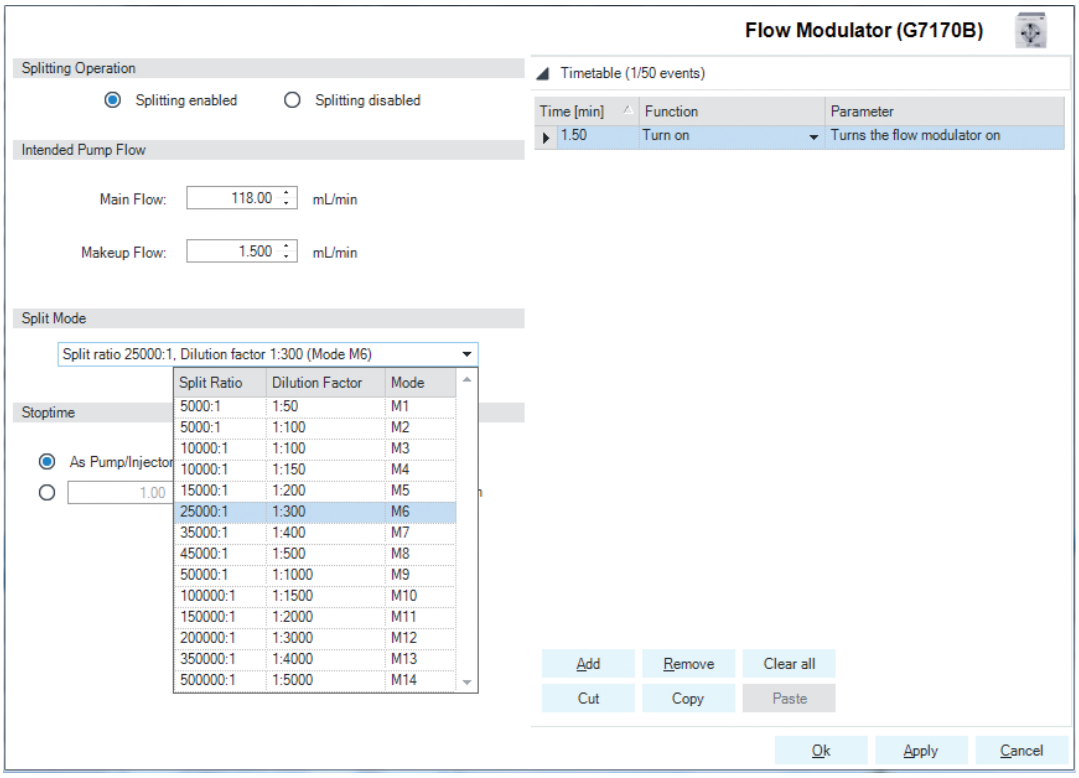
Another module is the Agilent 1290 Infinity II MS Flow Modulator. This module actively splits part of the main flow to the MSD, and integrates seamlessly into Agilent InfinityLab LC Purification Systems, both from a software and hardware point of view. Split ratio and dilution, as well as start and stop times are set and saved directly within the LC method. Depending on the makeup and main solvent flow rate, split ratios are calculated on-the-fly, and can be set between 100:1 and 500,000:1 (Figure 2). Delay coils, which are necessary to retard the flow to the fraction collector during MSD data processing, are stored in a dedicated Delay Coil Organizer. This module features a closed compartment with an inlet/outlet interface and a leak detector, designed to have the delay coils well arranged, and shut down the system in case of a leak. An additional external leak sensor can be connected to the system and placed at critical positions on or beneath the table. The external leak sensor was placed on the floor underneath the column.



Method and sample details

Sample	Caffeine in water/acetonitrile (ACN) 98/2 (v/v), 20 µg/mL
Column	Agilent 5 Prep-C18, 21.2 × 50 mm, 5 µm (p/n 446905-102)
Eluent	Water/ACN (85/15) isocratic, degassed
Flow rate	30.0 mL/min
Draw speed	900 µL/min
Eject speed	3,000 µL/min

Figure 1. Injection linearity of the Agilent 1260 Infinity II Preparative Autosampler with needle seat extension (p/n G7157-68711) between 500 and 2,500 µL.



Split Ratio	Dilution Factor	Mode
5000:1	1:50	M1
5000:1	1:100	M2
10000:1	1:100	M3
10000:1	1:150	M4
15000:1	1:200	M5
25000:1	1:300	M6
35000:1	1:400	M7
45000:1	1:500	M8
50000:1	1:1000	M9
100000:1	1:1500	M10
150000:1	1:2000	M11
200000:1	1:3000	M12
350000:1	1:4000	M13
500000:1	1:5000	M14

Figure 2. Method settings of the Agilent 1290 Infinity II MS Flow Modulator, displaying the different split ratios calculated based on the main and makeup flow.

Experimental

Instrumentation

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

- Agilent 1290 Infinity II Preparative Pump (G7161B)
- Agilent 1260 Infinity II Preparative Autosampler (G7157A)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A)
- Agilent 1290 Infinity II Preparative Open-Bed Fraction Collector (G7159B)
- Agilent 1290 Infinity II MS Flow Modulator (G7170B)
- Agilent 1260 Infinity II Delay Coil Organizer (G9324A)
- Agilent 1260 Infinity II Isocratic Pump (G7110B)
- Agilent InfinityLab LC/MSD (G6125B)
- Agilent External Leak Sensor Assembly (p/n 5067-6149)

Column

Agilent Load & Lock 4002 column, 50 × 500 mm (p/n PCG93LL500X50), packed with Agilent Prep C18, 10 µm bulk media (p/n 420910-902), compressed to a bed length of 243 mm

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, version C.01.07 SR3 [465]

Solvents and samples

All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak). Sample components and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich, Taufkirchen, Germany. The sample was prepared in DMSO at a concentration as described in Table 1.

Results and Discussion

A sample containing 500 mg of acetaminophen and 0.5 mg of each of the six impurities was successfully separated on a 50 mm id column; see Figure 1. Each compound was collected in a distinct fraction; the API was split into two fractions because of the large peak width and the resulting high fraction volume. Collection was triggered by a combination of UV and MSD signals at the target masses of the sample components; see Table 1. This way, a highly specific collection of the impurities was possible, and the collection of the solvent peak, visible at 220 nm, was avoided. UV signal threshold and slope settings enabled separation of closely eluting peaks into two fractions even when the peaks were not perfectly separated at the baseline (for example, impurities E/D and F/J). To avoid saturation of the MSD signal by the highly concentrated API, fraction collection was not triggered on the target mass of acetaminophen (151.1 Da) but on the de-acetylated fragment (109.1 Da), which was created during ionization. This meant a conservative MSD threshold of 10,000 cps could be used without triggering false fraction collection. Extracted ion chromatograms (EICs) of the applied target masses demonstrate that the API and all impurities were detected with high selectivity (see Figure 4), which in turn enabled highly selective fraction collection.

Table 1. Sample composition and target masses.

Name	Compound	Target mass (Da)	Concentration (mg/mL)
API	Acetaminophen	109.1	200
Impurity A	2-Acetamidophenol	109.1	0.2
Impurity D	Acetanilide	135.1	0.2
Impurity E	4'-Hydroxyacetophenone	136.1	0.2
Impurity F	4-Nitrophenol	139.0	0.2
Impurity J	4'-Chloroacetanilide	169.0	0.2
Impurity K	4-Aminophenol	109.1	0.2

Table 2. Chromatographic conditions.

Parameter	Description
Mobile phase	A) 0.1 % Formic acid in water B) 0.1 % Formic acid in acetonitrile
Makeup solvent	0.1 % Formic acid in methanol:water (70:30, v:v)
Makeup solvent flow rate	1.5 mL/min
Preparative flow rate	118 mL/min
Preparative gradient	0.0 minutes – 2 %B 9.0 minutes – 50 %B 11.0 minutes – 98 %B 12.0 minutes – 2 %B
Stop time	17 minutes
Injection volume	2,500 µL
Detection/Trigger	220 nm Peak width >0.05 minutes (1 second response time) 5 Hz Data rate
Flow modulator	Mode M6, split ratio 25,000:1, dilution factor 1:300
Fraction collection	Peak-based, UV and MSD connected with AND condition UV: threshold 8 mAU, upslope 0.5 mAU/s, downslope 1.0 mAU/s MSD: threshold 10,000 cps

Table 3. MSD Spray chamber and signal settings.

Parameter	Description
Spray chamber	Agilent Electrospray
Signal 1	Positive scan 100–600 Fragmentor 125 V
Signal 2	Negative scan 100–600 Fragmentor 125 V
Nebulizer pressure	40 psig
Drying gas temperature	300 °C
Drying gas flow	9.0 psig
Capillary voltage	±4,000 V

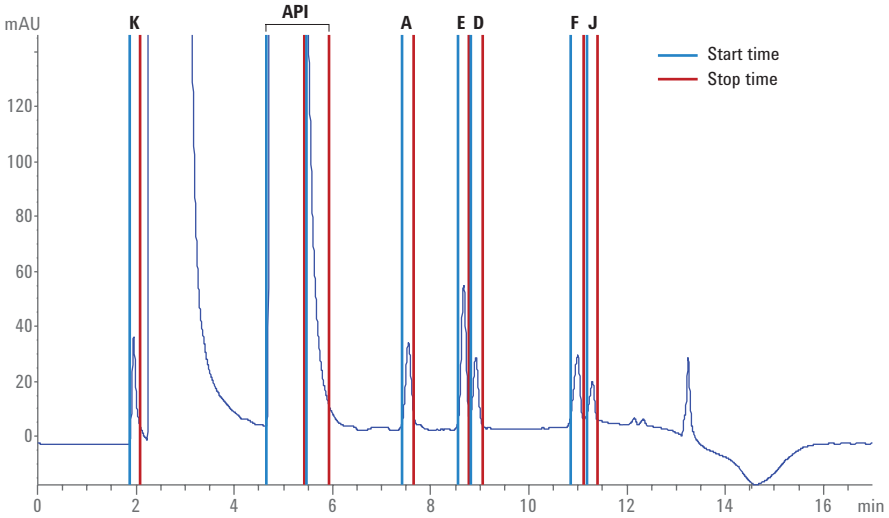


Figure 3. Separation and fraction collection of 500 mg of acetaminophen (API) with six 0.1 % impurities (UV chromatogram, 220 nm). Fraction start and stop times are shown as blue and red vertical lines, respectively.

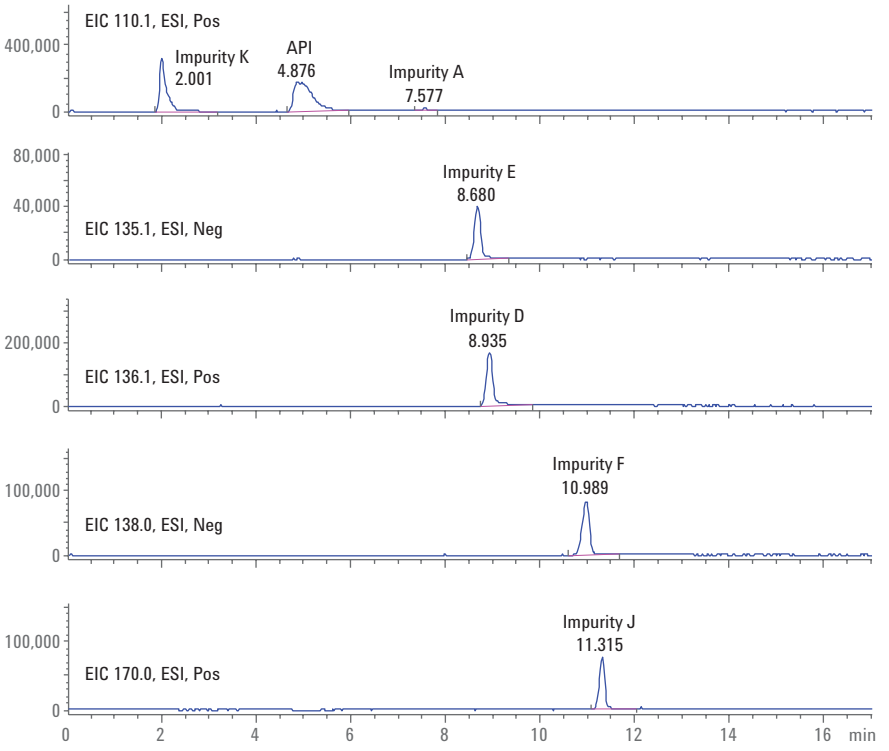


Figure 4. Extracted ion chromatograms (EICs) of the monitored target masses.

The purity of all collected fractions was determined by re-analysis on an Agilent 1260 Infinity II LC System. With the exception of impurity D, the purity of each fraction was 99 % or higher; see Table 4. This demonstrates the high selectivity of the fraction collection when triggered by a combination of the UV with the MSD signal. To determine the amount of API collected, the two fractions of the API were combined, and the solvent was evaporated. Over a series of three collections, the average amount of the API in the fractions was found to be 99 % of the injected amount.

Table 4. Purity of collected fractions.

Peak name	Compound	Target mass (Da)	Purity
Impurity K	4-Aminophenol	109.1	>99 %
API	Acetaminophen	109.1	>99 %
Impurity A	2-Acetamidophenol	109.1	>99 %
Impurity E	4′-Hydroxyacetophenone	136.1	>99 %
Impurity D	Acetanilide	135.1	81 %
Impurity F	4-Nitrophenol	139.0	99 %
Impurity J	4′-Chloroacetanilide	169.0	99 %

Conclusion

The Agilent 1290 Infinity II Preparative LC/MSD System enabled the separation and purification of a pharmaceutical sample. A 500 mg amount of active pharmaceutical ingredient with impurities at 0.1 % was injected and successfully separated on a 50 mm id Agilent Load & Lock column. Fraction collection was triggered by a combination of UV and MSD signals, which enabled a highly specific collection of all sample components by their respective target mass. The purity of the collected fractions was generally high (99 % or greater), with the exception of one compound. The fraction of the active pharmaceutical ingredient was dried and weighed to determine the recovery, which was found to be 99 % of the injected amount.

References

1. ICH Guideline Q3A(R2): Impurities in New Drug Substances, **2006**.
2. Paracetamol, Pharmacopoeia Europaea 9.0, **2017**.
3. Acetaminophen, United States Pharmacopoeia [USP 39], **2016**.
4. Agilent InfinityLab LC Purification Solutions, *Agilent Technologies Brochure*, publication number 5991-8009EN, **2017**.

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