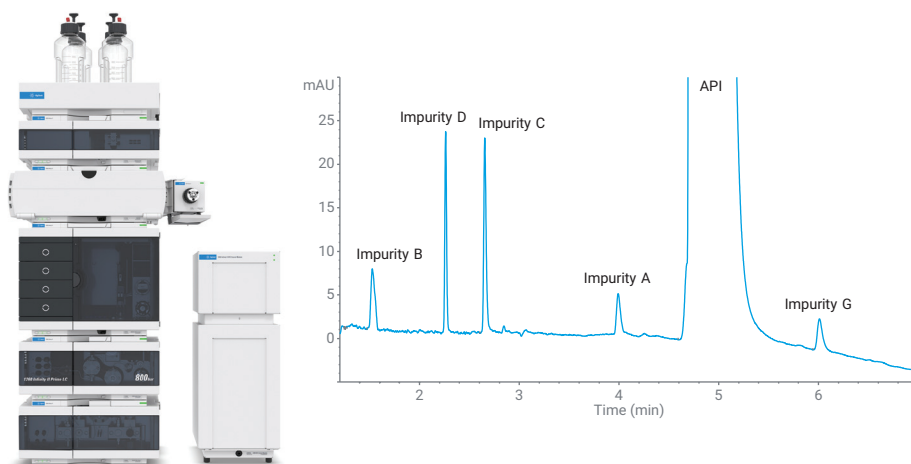


# Orthogonal Separation of Pharmaceutical Impurities by the Agilent 1260 Infinity II SFC/UHPLC Hybrid System

SFC and UHPLC separations in one system  
enables higher possibility to detect impurities in  
pharmaceutical APIs

## Author

Edgar Naegele  
Agilent Technologies, Inc.



## Abstract

This Application Note demonstrates the use of the Agilent 1260 Infinity II SFC/UHPLC Hybrid System with the Agilent 1260 Infinity II Variable Wavelength Detector (VWD) for the determination of pharmaceutical low-level impurities in SFC and UHPLC modes. Instrument selectivity changes depending on the use of SFC mode or UHPLC mode, which can lead to a better identification of impurities by changing the elution pattern.

## Introduction

In active pharmaceutical ingredients (API), impurities caused by the production process, formulation, or degradation must be monitored and documented. The International Conference of Harmonization (ICH) published guidelines on different reporting, identification, and quantification levels for impurities depending on the maximum daily dose of a drug substance.<sup>1</sup> For instance, a daily intake of more than 1 g requires a reporting level for all impurities of 0.05%. As shown in this Application Note, this requirement can easily be achieved with the Agilent 1260 Infinity II VWD SFC configuration.<sup>2</sup> When identifying impurities in APIs, different chromatographic separation techniques such as HPLC, GC, or SFC are applied due to their differing separation selectivities. This increases the possibility of identifying inherent impurities.

This Application Note describes using the 1260 Infinity II SFC/UHPLC Hybrid System<sup>3</sup> with the 1260 Infinity II VWD to determine low-level impurities in an API. Using the VWD, it is possible to detect impurities at a very low level to fulfill the regulations described in the ICH guideline Q3B(R2). The influence of different selectivities in SFC mode or UHPLC mode on the separation will also be shown.

## Experimental

### Instrumentation

Agilent 1260 Infinity II SFC/UHPLC Hybrid System comprises:

- Agilent 1260 Infinity II SFC Control Module (G4301A)
- Agilent 1260 Infinity II SFC Binary Pump (G4782A)
- Agilent 1260 Infinity II SFC Multisampler (G4767A)
- Agilent 1290 Infinity II Multicolumn Thermostat (MCT) (G7116B) with Agilent InfinityLab Quick Change eight-column selection valve (G4239C)
- Agilent 1260 Infinity II Quaternary Pump (G7111B)
- Agilent 1290 Infinity Valve Drive (G1170A) with 2-position/10-port valve (G4232C)
- Agilent 1260 Infinity II Variable Wavelength Detector (G7114A) with high-pressure flow cell (G1314-60182)

| Method for SFC mode |  |
|---------------------|--|
| Solvents            | A) CO <sub>2</sub><br>B) MeOH + 10 mM ammonium formate   |
| Flow Rate           | 1.5 mL/min   |
| Gradient            | 0.0 minutes – 1% B,<br>3.0 minutes – 30% B,<br>6.0 minutes – 50% B,<br>8 minutes – 70% B<br><br>Stop time: 8 minutes<br>Post time: 3 minutes |
| Injection Volume    | 3 µL   |
| Feed Speed          | 100 µL/min   |
| Overfeed Volume     | 2 µL, solvent: MeOH  |
| Needle Wash         | 3 seconds, solvent: MeOH   |
| Column Temperature  | 55 °C  |
| BPR Temperature     | 60 °C  |
| BPR Pressure        | 150 bar  |
| VWD                 | 270 nm, data rate 20 Hz  |

| Method for UHPLC Mode |  |
|-----------------------|--|
| Solvents              | A) Water + 0.1 formic acid,<br>B) ACN + 0.1% formic acid                                       |
| Flow Rate             | 1.0 mL/min   |
| Gradient              | 0.0 minutes – 5% B,<br>10 minutes – 45% B<br><br>Stop time: 10 minutes<br>Post time: 3 minutes |
| Injection Volume      | 1 µL   |
| Needle Wash           | 3 seconds, solvent: MeOH   |
| Column Temperature    | 30 °C  |
| VWD                   | 270 nm, data rate 20 Hz  |

### Columns

- **SFC Mode:** ZORBAX RxSil, 3.0 × 100 mm, 1.8 µm
- **UHPLC Mode:** ZORBAX SB-C18, 3.0 × 100 mm, 1.8 µm

### Software

- Agilent OpenLab CDS ChemStation Edition for LC and LC/MS Systems, Rev. C.01.08

### Samples

- Stock solutions: Metoclopramide (10 mg/mL in MeOH) and its impurities A, B, C, D, and G (2 mg/mL, MeOH, each)
- Method development sample: Mixture of metoclopramide and its impurities at a final concentration of 200 µg/mL each in MeOH
- Impurity sample: 0.03% impurities spiked in metoclopramide

## Chemicals

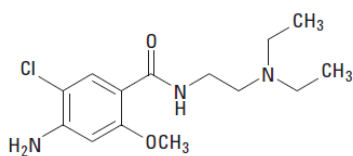
- All solvents were purchased from Merck, Germany.
- Fresh ultrapure water was obtained from a Milli-Q integral system equipped with LC-Pak polisher and a 0.22 µm membrane point of use cartridge (Millipak).
- Metoclopramide was purchased from Sigma-Aldrich, Germany.
- Metoclopramide impurities were bought from LGC Standards, Germany.

## Results and discussion

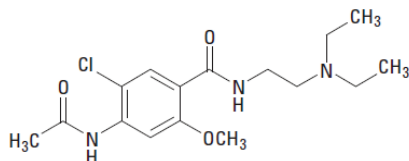
### Detection of impurities in metoclopramide using UHPLC mode

The test mixture used for this Application Note comprises the API metoclopramide and some of its impurities (Figure 1).

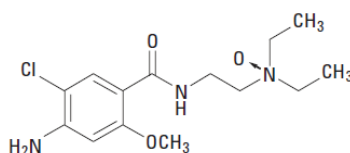
Two impurities (Figure 1: EP A and EP G) are structurally very similar to the main compound, and three impurities (Figure 1: EP B, EP C and EP D) are the product of a major degradation.



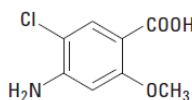
Metoclopramide



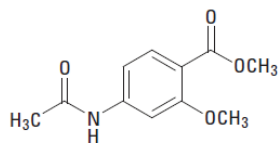
4-(Acetylamino)-5-chloro-N-2-(diethylaminoethyl)-2-methoxybenzamide (EP A)



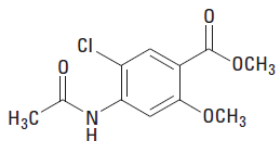
4-Amino-5-chloro-N-2-(diethylaminoethyl)-2-methoxybenzamide N-oxide (EP G)



4-Amino-5-chloro-2-methoxybenzoic acid (EP C)



Methyl 4-(acetylamino)-2-methoxybenzoate (EP D)



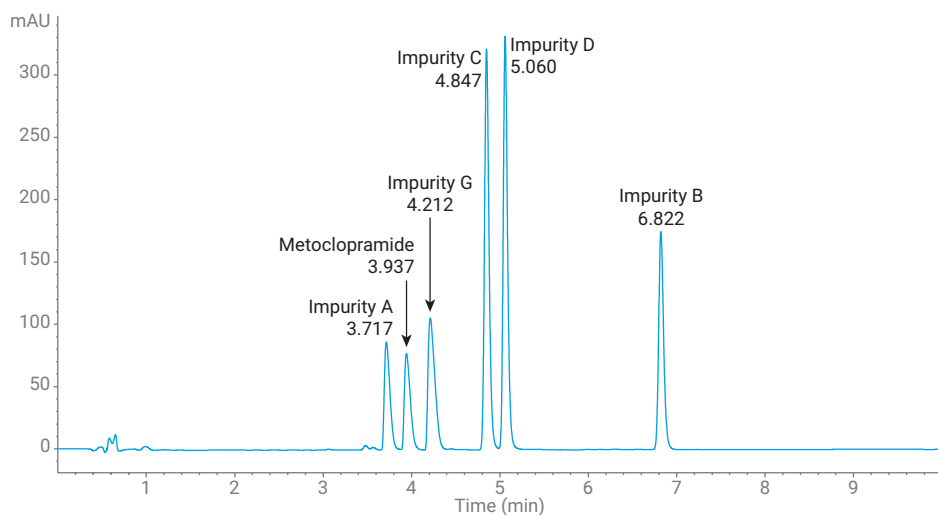
Methyl 4-(acetylamino)-5-chloro-2-methoxybenzoate (EP B)

**Figure 1.** Formulae of metoclopramide and the impurities used in this study.

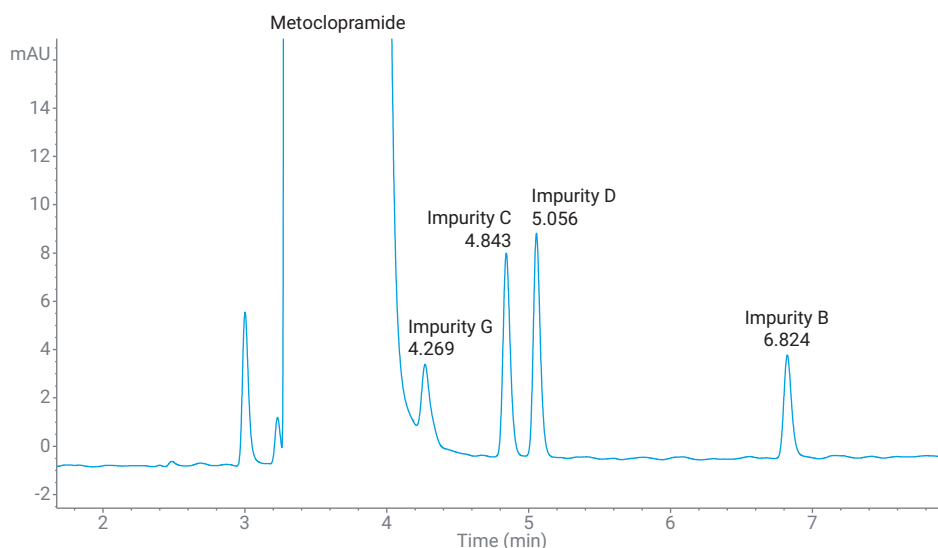
For the development of the UHPLC separation method, a mixture of metoclopramide and its impurities (200 µg/mL each, in MeOH) was used. The optimum separation was achieved on a C18 stationary phase with a water-acetonitrile gradient (Figure 2). The group of compounds, including metoclopramide and the structurally similar impurities A and G, eluted first, between 3.7 and 4.2 minutes. The degradation impurities B, C, and D eluted later in the chromatogram, and the impurities C and D eluted close together.

To demonstrate the detection of low-level impurities in the presence of a main API, the impurities were diluted at a level of 0.03% in a solution of metoclopramide (10 mg/mL, MeOH). In the resulting separation, the degradation impurities B, C, and D were well separated and clearly detected (Figure 3). Unfortunately, impurity A, which eluted at 3.717 minutes (Figure 2) was completely hidden under the main peak of metoclopramide and could be not found in the sample of the API (there are also two unknown impurities at 3.00 and 3.23 minutes). Impurity G eluted in the tailing of the major API peak and was detectable. However, as the peaks of the API and impurity G are not baseline separated, quantification was not possible.

With these results, it is necessary to search for a method, which provides an orthogonal separation and different selectivity. The 1260 Infinity II SFC/UHPLC Hybrid System offers the opportunity to switch seamlessly to SFC mode to analyze identical samples under orthogonal separation conditions.



**Figure 2.** A mixture of metoclopramide and impurities (200 µg/mL each, in MeOH). The separation was developed using UHPLC mode with VWD detection.

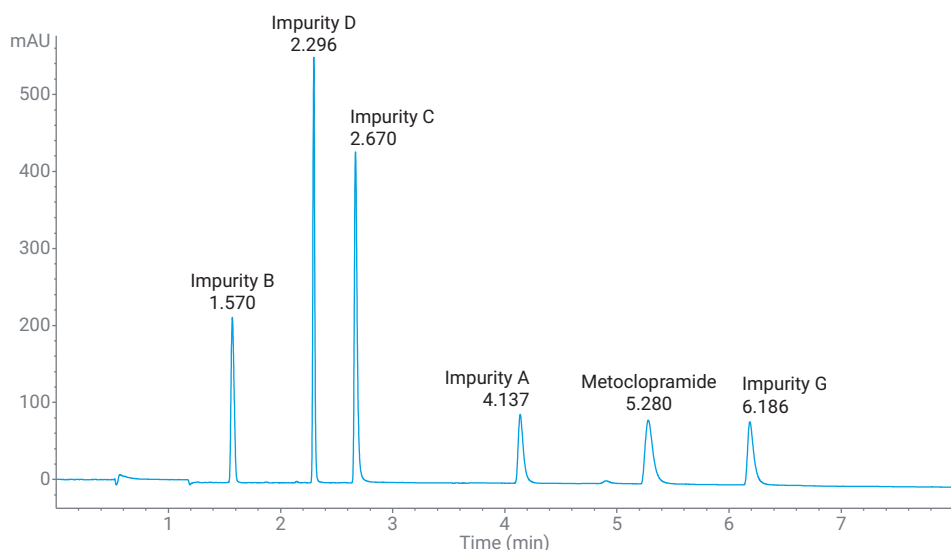


**Figure 3.** Separation of metoclopramide from its impurities at a 0.03% trace level by UHPLC mode with VWD detection.

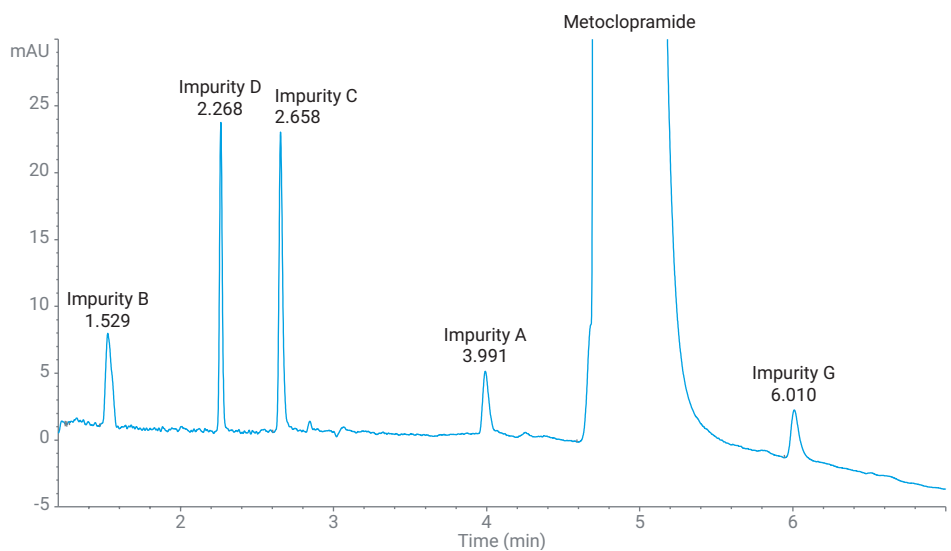
## Detection of impurities in metoclopramide using the SFC mode

The mixture of equally concentrated metoclopramide and its impurities has also been used for the development of a separation method by means of SFC mode. After method development, an Agilent ZORBAX Rx-SIL column with methanol/10 mM ammonium format showed the best separation results (Figure 4). The best peak shapes were achieved at elevated temperatures. The selectivity of the separation showed that compounds with a chemical structure similar to the API (impurities A and G) eluted in the second half of the chromatogram between 4.0 and 6.2 minutes. The compounds that were the product of API degradation (impurities B, C, and D) eluted in the first half of the chromatogram between 1.5 and 2.7 minutes. There was also higher resolution in the elution times between impurities A and G to the API compared to the separation in the UHPLC mode.

To detect impurities at a trace level of 0.03% in an API in SFC mode, the impurities were spiked in a highly concentrated solution of metoclopramide (10 mg/mL, MeOH). Figure 5 shows the result of the separation of the trace level impurities from the highly concentrated API. Figure 5 and Table 1 show that the measured signal-to-noise (S/N) was far above the detection limit (S/N = 3) for reliable detection and determination of the identity of the impurities. The measured RSD values of the retention times were typically below 0.2%, and the RSD values of the peak areas were below 2%. The RSD of 2.10% for the last eluting Impurity G was due to its tailing behavior.



**Figure 4.** A mixture of metoclopramide and its impurities (200 µg/mL each, in MeOH). Separation results were developed by means of SFC mode with VWD detection.



**Figure 5.** Detection of impurities at 0.03% trace level in the API metoclopramide in SFC mode with VWD detection at a data rate of 20 Hz. The noise was taken in the gradient between 3.2 and 3.4 minutes (n=10 for calculation of RSDs).

**Table 1.** S/N and RSD for API impurities.

| Compound   | R.T. RSD (%) | S/N  | Area RSD (%) |
|------------|--------------|------|--------------|
| Impurity B | 0.19         | 23.9 | 1.63         |
| Impurity D | 0.17         | 80.9 | 1.62         |
| Impurity C | 0.12         | 79.1 | 1.67         |
| Impurity A | 0.40         | 16.7 | 2.02         |
| Impurity G | 0.37         | 12.9 | 2.10         |

## Conclusion

This Application Note demonstrates the capability of the Agilent 1260 Infinity II SFC/UHPLC Hybrid System with Agilent 1260 Infinity II VWD to be used for the detection and determination of low-level trace impurities in an API. In an example, the orthogonal separation in UHPLC and SFC mode by their inherent different selectivities was described. In this particular example, better separation and performance characteristics were achieved in SFC mode. The impurities showed a retention time RSD below 0.2%, and the area RSD were typically below 2%. The S/N ratios were sufficiently above the detection limit for all compounds at the low 0.03% trace level. Operating in two orthogonal separation modes enables intelligent screening for the best-suitable method. This capability, provided by the 1260 Infinity II SFC/UHPLC Hybrid System, delivers comprehensive information on complex mixtures for higher productivity and confidence in your results.

## References

1. ICH Harmonized Tripartite Guideline: Impurities in New Drug Products Q3B(R2)
2. Improved Sensitivity for Low-Level Impurity Detection with the Agilent 1260 Infinity II SFC System Featuring an Agilent 1260 Infinity II Variable Wavelength Detector, *Agilent Application Note*, publication number 5994-1351EN
3. Orthogonal Chromatographic Separations using the Agilent 1260 Infinity II SFC/UHPLC Hybrid System, *Agilent Technical Overview*, publication number 5991-8276EN

[www.agilent.com/chem](http://www.agilent.com/chem)

This information is subject to change without notice.