Application Note

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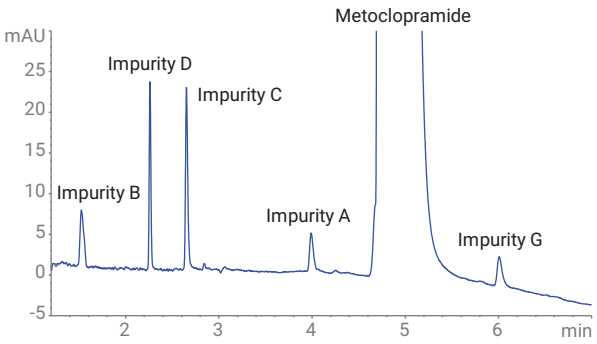
Improved Sensitivity for Low-Level Impurity Detection with the Agilent 1260 Infinity II SFC System Featuring an Agilent 1260 Infinity II Variable Wavelength Detector



Authors

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Abstract

This Application Note demonstrates the optimum field of application for the combination of the Agilent 1260 Infinity II SFC System with an Agilent 1260 Infinity II Variable Wavelength Detector (VWD) for the detection of low-level pharmaceutical impurities in an active pharmaceutical ingredient (API). Especially at low SFC flow rates with elution gradients at elevated temperature and smaller id columns, the VWD shows lower noise, and signal-to-noise (S/N) ratios up to five-fold higher than with the Agilent 1260 Infinity II Diode Array Detector (DAD). These characteristics enable the determination of API impurities at the lowest levels.

Introduction

Any API could be polluted with potentially harmful organic impurities due to degradation during the production process of the API or even during shelf storage after final formulation due to environmental influences.[1] To control the amount of impurities in new drug products, the International Conference of Harmonization (ICH) published guidelines of different reporting, identification, and quantification levels for impurities depending on the maximum daily dose of a drug substance.[2] The ICH guideline Q3B(R2) refers to degradation impurities and their reporting levels. For instance, a daily intake of more than 1 g requires a reporting level for all impurities of 0.05%.

This threshold requires highly sensitive detection, especially if UV-based detection is applied for the quality control of the API. Due to the different design of the optical bench and thus better S/N values, VWDs are the UV detector of choice for high-sensitivity measurements, rather than DADs. As a matter of fact, the 1260 Infinity II VWD is specified with an ASTM noise three times lower than the 1260 Infinity II DAD WR.[3]

This Application Note presents the optimum field of application of the 1260 Infinity II VWD in a 1260 Infinity II SFC System for the detection of low-level impurities in an API at a level as low as 0.03%. A 3.0 mm id column was used at a low flow rate of 1.5 mL with a gradient separation, and comparison made with a workflow involving the 1260 Infinity II DAD. In addition, the behavior of both detectors at higher flow rates is discussed.

Experimental

Instrumentation

Agilent 1260 Infinity II SFC 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 8-column selection valve (G4239C)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with high-pressure SFC flow cell (G4301-60200) or
- Agilent 1260 Infinity II Variable Wavelength Detector (G7114A) with high-pressure flow cell (G1314-60182)

Columns

- **Column for low flow rate:** Agilent ZORBAX Rx-SIL, 100 × 3.0 mm, 1.8 µm
- **Column for high flow rate:** Agilent ZORBAX Rx-SIL, 150 × 4.6 mm, 5 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 (10 mg/mL)

Methods for low and high SFC flow rates

Parameter	Value
Solvents	A) CO_2 B) MeOH + 10 mM ammonium formate
Low Flow Rate	1.5 mL/min
High Flow Rate	3.5 mL/min
Gradient	0.0 minutes – 1% B 3.0 minutes – 30% B 6.0 minutes – 50% B 8.0 minutes – 70% B Stop time: 8 minutes Post time: 3 minutes
Injection Volume	3 µL
Feed Speed	100 µL/min at low flow rate and 400 µL/min at high flow rate
Overfeed Volume	2 µL at low flow rate and 4 µL at high flow rate, 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
DAD	270/4 nm; reference 360/100 nm; slit 8 nm; data rate 20 Hz

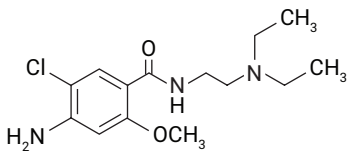
Chemicals

All solvents were purchased from Merck, Germany. Metoclopramide was purchased from Sigma-Aldrich, Germany. Metoclopramide impurities were bought from LGC Standards, Germany.

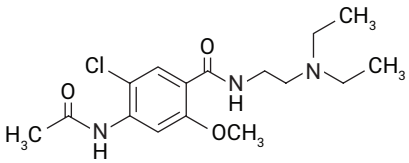
Results and discussion

As a test mixture, the API metoclopramide and a selection of its impurities were selected for this study (Figure 1). In the group of impurities, A and G were chosen as being structurally similar to the API. Three impurities (B, C, and D) are degradation products of the API.

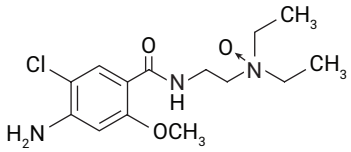
To develop the SFC separation method, an equally concentrated mixture of metoclopramide and the impurities was tested on different columns with a gradient elution of CO_2 against several modifier/additive combinations. The combination of ZORBAX Rx-SIL solid phase material with a CO_2-methanol/10 mM ammonium formate gradient up to 70% B gave the best separation result (Figure 2). The peak shapes improved at an elevated column temperature of 55 °C. The column used for the low flow experiments had dimensions of 100 × 3.0 mm, 1.8 µm. The separation showed that compounds that have a similar chemical structure to the API (impurities A and G) were eluting later. Impurities B, C, and D, which are degradation products of the API, eluted earlier.



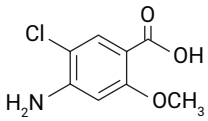
Metoclopramide



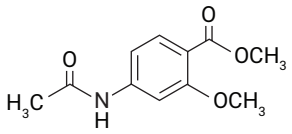
4-(Acetylamino)-5-chloro-N-2-(diethylaminoethyl)-2-methoxybenzamide (EP A)



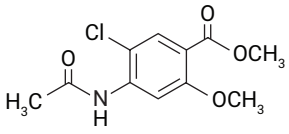
4-Amino-5-chloro-N-2-(diethylaminoethyl)-2-methoxybenzamide N-oxide (EP G)



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.

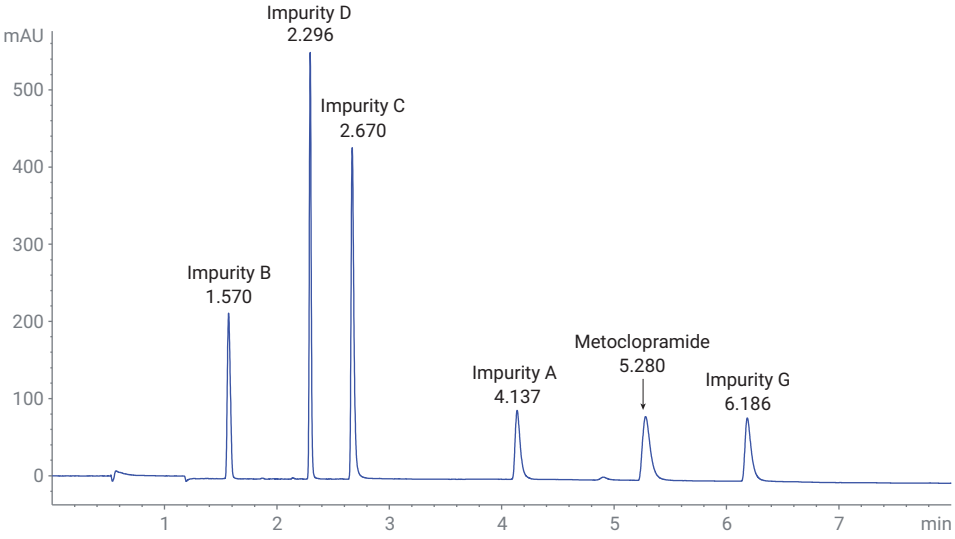
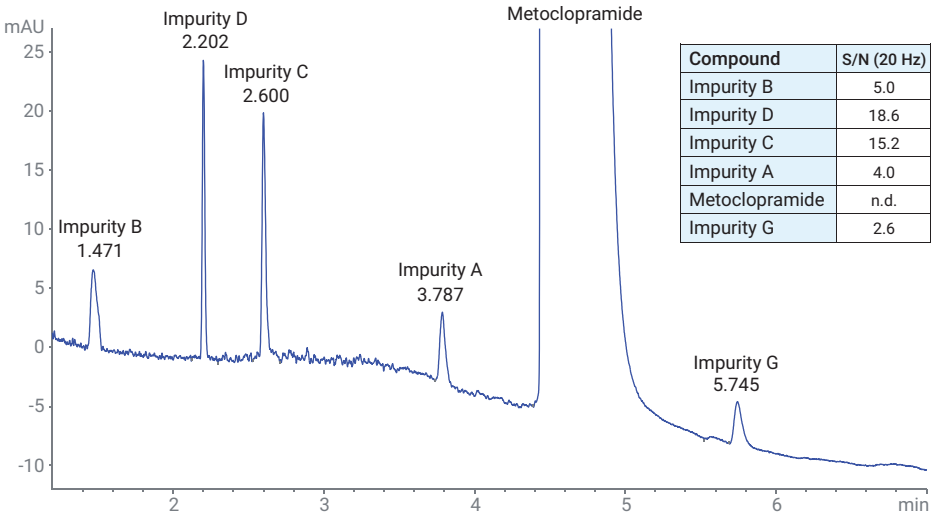


Figure 2. Mixture of metoclopramide and its impurities for the development of the separation method by SFC with VWD detection (200 µg/mL each in MeOH).

The determination of a UV detector noise specification is typically done under optimized isocratic conditions. These conditions gave a peak-to-peak noise of 60 µAU for the combination of the 1260 Infinity II DAD WR and the 1260 Infinity II SFC System.[4] However, under gradient conditions, lower flow rates or elevated column temperatures and other effects, such as changes from laminar to turbulent flow or changes in the refractive behavior of the eluent, refractive index effects come into play with the changes occurring in the gradient.[5] This can result in higher noise values and compromised S/N ratios. A typical application where this becomes important is in the determination of compounds at trace levels, such as the determination of impurities in an API (for example, metoclopramide (Figure 3). The detection of the impurities at the 0.03% trace level with the DAD showed an S/N around the limit of detection (LOD, S/N = 3) for impurities B, A, and G, while the S/N for impurities D and C was above the limit of quantification (LOQ, S/N >10).

To overcome this problem, the S/N behavior of the VWD, equipped with a VWD specific high pressure flow cell, was investigated for this application. Typically, a VWD should operate at a lower noise level and show analytes with better S/N. As shown in Figure 4, all impurity compounds were easily detected at S/N levels above the LOD and well above the LOQ. The comparison of S/N values for both detectors revealed that the VWD delivers S/Ns up to five times better than those delivered by the DAD. This enhancement leads to an improved detection of trace level compounds, such as impurities in an API. Additionally, the retention time RSDs of the impurities were typically below 0.02% and the area RSD below 2% (table in Figure 4, n = 10).



Compound	S/N (20 Hz)
Impurity B	5.0
Impurity D	18.6
Impurity C	15.2
Impurity A	4.0
Metoclopramide	n.d.
Impurity G	2.6

Figure 3. Detection of impurities at 0.03% trace level in the API metoclopramide by means of a DAD at a data rate of 20 Hz; the noise was taken in the gradient between 3.2 and 3.4 minutes.

Compound	S/N (20 Hz)	RT RSD (%)	Area RSD (%)
Impurity B	23.9	0.19	1.63
Impurity D	80.9	0.17	1.62
Impurity C	79.1	0.12	1.67
Impurity A	16.7	0.40	2.02
Impurity G	12.9	0.37	2.10

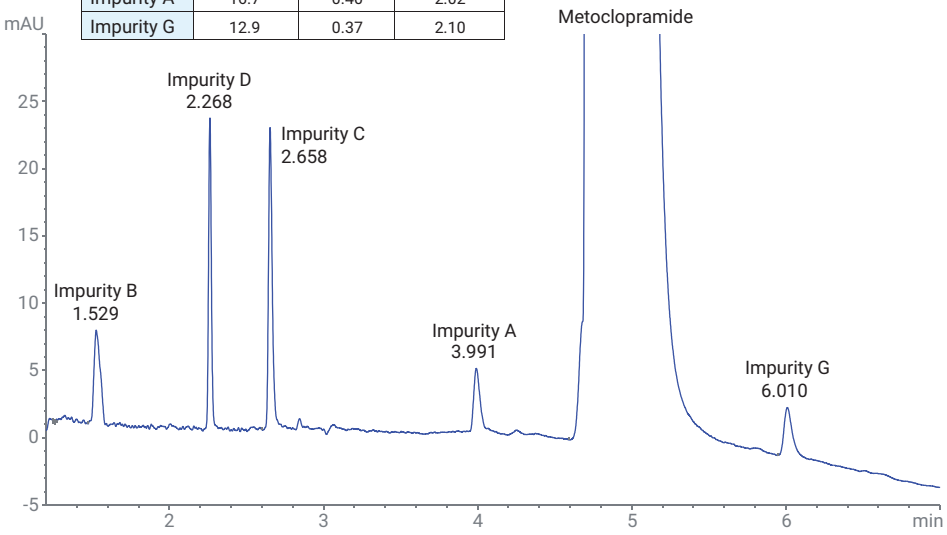


Figure 4. Detection of impurities at 0.03% trace level in the API metoclopramide by means of a VWD at a data rate of 20 Hz; the noise was taken in the gradient between 3.2 and 3.4 minutes.

For a final comparison, the method used for the narrow bore column at 3.0 × 100 mm with 1.8 µm material was transferred to a standard 4.6 × 150 mm column with 5 µm material. The flow rate was increased from 1.5 to 3.5 mL/min. The VWD and the DAD were used under standard flow conditions for the described separation. The compounds eluted at identical retention times from the standard column, with lower intensity compared to the lower flow rate method. Table 1 outlines the retention times and the S/N values obtained for VWD and DAD detection under standard conditions. Comparison shows that, under standard high flow conditions, the DAD delivers slightly better S/N values. This indicates that, at lower flow conditions, combined with a narrow bore column, the VWD is the detector of choice for the detection of traces such as impurities in an API. Conversely, under standard flow conditions (>3 mL/min), the DAD is the more suitable detector, with its additional multiwavelength detection capabilities. This behavior is probably due to different refractive indices at different flow rates because of the mixing performance and the different technical principles of the DAD and VWD.

Table 1. Metoclopramide and its impurities measured under standard conditions at 0.03%, by VWD and DAD detection with a high flow method.

Compound	RT (min)	S/N (VWD, 20 Hz)	S/N (DAD, 20 Hz)
Impurity B	1.492	13.7	16.5
Impurity D	2.212	39.1	40.7
Impurity C	2.604	39.3	36.3
Impurity A	3.844	7.1	12.1
Metoclopramide	n.d.	n.d.	n.d.
Impurity G	6.148	4.8	5.3

Conclusion

This Application Note demonstrates the use of the Agilent 1260 Infinity II VWD with the Agilent 1260 Infinity II SFC system for the detection of lower trace level impurities in APIs in comparison to DAD detection at lower flow rates and the use of 3.0 mm id columns. It has been shown that the detection of trace level impurities with the VWD is approximately four to five times more sensitive when comparing the respective S/N values. At elevated flow rates and with use of a standard 4.6 mm id column, the S/N values obtained from detection by VWD and DAD are in a comparable range. For the detection of trace level compounds with lower flow rates and narrower id columns, the VWD is the detector of choice.

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