Impurity Testing of Fixed-Dose Combination Drugs Using the Agilent 1290 Infinity II HDR-DAD Impurity Analyzer Solution

Application Note

Small Molecule Pharmaceuticals

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Abstract

The Agilent 1290 Infinity II High Dynamic Range Diode Array Detection (HDR-DAD) Impurity Analyzer Solution combines the signals from two diode array detectors with different path length Max-Light cartridge cells to increase the linear dynamic UV-range. This solution can be used to detect and quantify main and trace compounds in a single run without exceeding the linear UV-range. Using one DAD with a 10-mm flow cell, typically two injections with different injection volumes are needed to determine low-dose, high-dose, and trace compounds. This Application Note demonstrates that one injection is adequate to reliably quantify low-dose, high-dose, and trace compounds of a fixed-dose combination drug using the Agilent 1290 Infinity II HDR-DAD Impurity Analyzer Solution.

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Introduction

Fixed-dose combination drugs are used in the medication of various disease patterns. The composition percentage of the active ingredients can vary depending on the desired physiological effect. If high-dose and low-dose ingredients are combined, the analysis using conventional HPLC and UHPLC diode array detectors may need at least two injections. This ensures that all compounds are quantified within the linear range of the detector with reliable integration and quantification of trace compounds.

This Application Note shows that with the Agilent 1290 Infinity II HDR DAD Impurity Analyzer Solution, high-dose and low-dose compounds, as well as their impurities can be determined in a single run with high sensitivity without exceeding the linear UV-range.

Experimental

Equipment

The deployed Agilent 1290 Infinity II LC system consisted of the following modules:

- Agilent 1290 Infinity II High-Speed Pump (G7120A)
- Agilent 1290 Infinity II Multisampler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (2x G7117B) with 3.7-mm, 10-mm and 60-mm Max-Light cartridge cells

Column

Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm (p/n 959996-902)

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, version C.01.07 [27]

Solvents and samples

Solvent A
Water + 0.1 TFA

Solvent B
Acetonitrile + 0.09 % TFA

Sample
A fixed-dose combination drug with the following ingredients was used: Paracetamol and chlorphenamine at a ratio of 1:80, vitamin C, caffeine, and a small amount of unknown impurities (Figure 1).

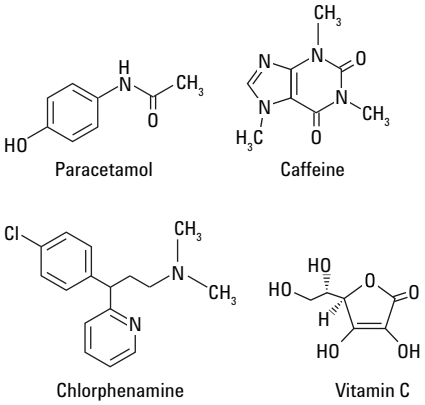


Figure 1. Ingredients of the fixed-dose combination drug: vitamin C, paracetamol, caffeine, and chlorphenamine.

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). The fixed-dose combination drug was bought in a local pharmacy.

Sample preparation

1. Two capsules of a cold medication were opened and dissolved in 20 mL of distilled water.
2. Extract with an ultrasonic bath for 5 minutes.
3. Filter with an Agilent Premium Syringe Filter Nylon, 0.45 µm, 15 mm (p/n 5190–5091)
4. Clear liquid was filled and stored in 1.5-mL LC vials containing:
   - 250 ng/µL chlorphenamine
   - 20,000 ng/µL paracetamol
   - 2,500 ng/µL caffeine
   - 15,000 ng/µL vitamin C

Table 1. Chromatographic conditions.

Parameter	Value
Mobile phase	A) Water + 0.1 % TFA B) Acetonitrile + 0.09 % TFA
Flow rate	1 mL/min
Gradient	0 minutes – 5 %B 0.5 minutes – 5 %B 6.1 minutes – 40 %B 6.5 minutes – 95 %B
Stop time	8 minutes
Post time	5 minutes
Injection volume	1 and 5 µL for 10-mm flow cell 5 µL for HDR DAD solution
Column temperature	40 °C
Wavelength	254/20 nm, reference 380/80 nm Peak width > 0.025 minutes (0.5-seconds response time) (10 Hz)

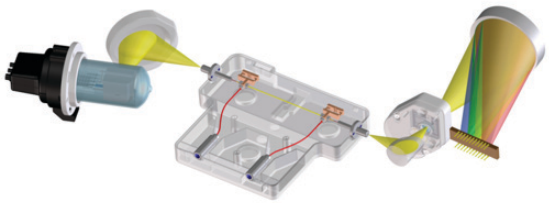
5. Dilution 1:20 with water containing:
    - 12.5 ng/µL chlorphenamine
    - 1,000 ng/µL paracetamol
    - 125 ng/µL caffeine
    - 750 ng/µL vitamin C
6. This solution was injected.

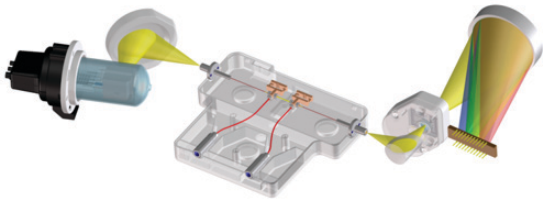
Principle of the Agilent 1290 Infinity II HDR-DAD Impurity Analyzer Solution

The Agilent 1290 Infinity II HDR-DAD Impurity Analyzer Solution provides more than three times wider linear dynamic UV-range (typically 6.7 AU at 265 nm) compared to one 1290 Infinity II DAD with a 10-mm Max-Light cartridge cell (greater than 2.0 AU at 265 nm). By combining the signals from two diode array detectors with different path length Max-Light cartridge cells, the HDR detection solution detects and quantifies components with significantly different concentrations in a single run. Two 1290 Infinity II DADs are clustered (Figure 2). One 1290 Infinity II DAD is equipped with a 60-mm path length cell to analyze lower concentrated compounds, and the second 1290 Infinity II DAD is equipped with a 3.7-mm path length cell to analyze highly concentrated compounds. The 60-mm cell must be the first in the flow path followed by the 3.7-mm cell. The output signal is a combined signal, normalized to a 10-mm path length.

The high dynamic range (HDR) tool is configured during instrument configuration. Both detectors are clustered, and the delay volume of the capillary connecting both detectors is entered. In the user interface, both detectors appear as one detector (Figure 3).

Detector 1, with a 60-mm path length flow cell for the detection of low concentrations





Detector 2, with a 3.7-mm path length flow cell for the detection of high concentrations

Figure 2. Clustered Agilent 1290 Infinity II DADs.

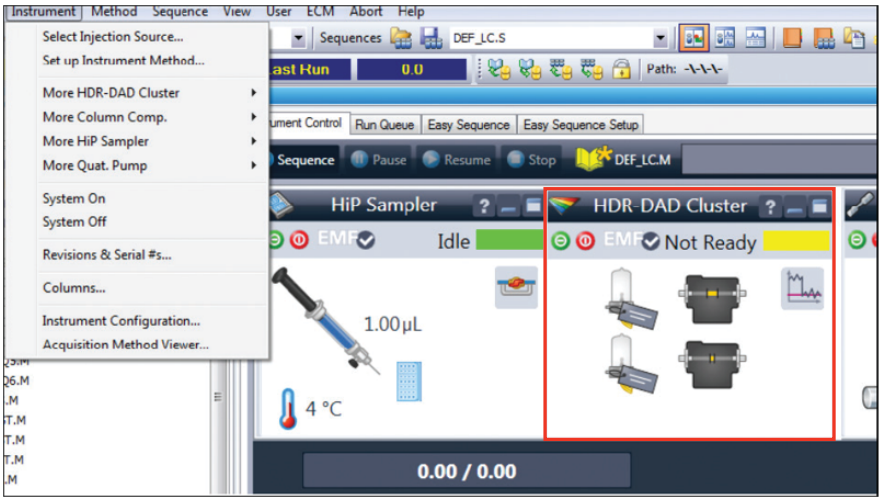


Figure 3. Activating the HDR tool in the Agilent ChemStation software.

Results and Discussion

To evaluate the performance differences between the 1290 Infinity II DAD and the 1290 Infinity II HDR-DAD Impurity Analyzer Solution, the following experiments were performed:

- Analysis of a fixed-dose combination drug on a 1290 Infinity II LC system with DAD and a 10-mm Max-Light cartridge cell using two injections of 1 and 5-µL volumes to determine high-dose and low-dose drugs and further trace compounds.
- Analysis of a fixed-dose combination drug on a 1290 Infinity II LC system with the 1290 Infinity II HDR-DAD Impurity Analyzer Solution using one 5-µL injection of to determine low-dose and high-dose drugs and further trace compounds within the typical linear UV range of 6.7 AU.

For the first experiment, the 1290 Infinity II DAD with the 10 mm Max-Light cartridge cell was used. Two injections were necessary to determine all compounds and trace impurities within the linear UV range (Figures 4 and 5).

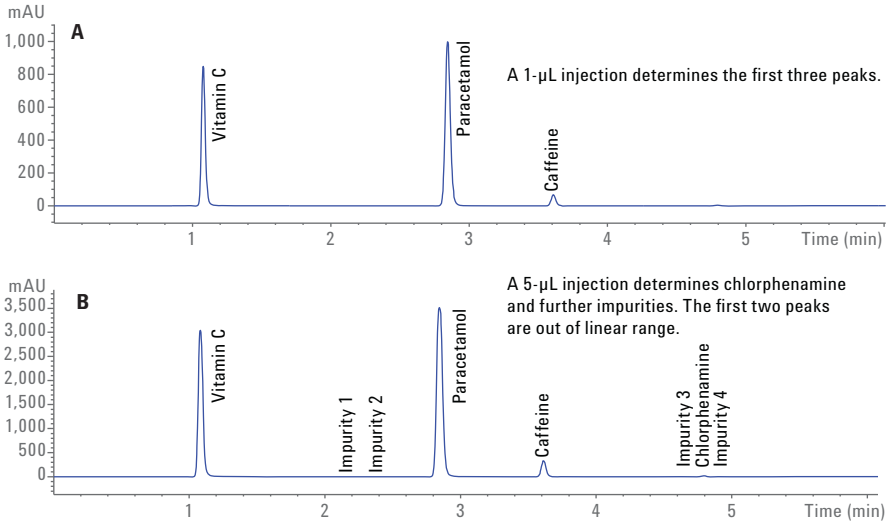


Figure 4. Chromatograms of the drug using two injections with different injection volumes using the Agilent 1290 Infinity II DAD with 10-mm Max-Light cartridge cell.

Vitamin C, paracetamol, and caffeine were determined by injecting 1 µL. The maximum peak height was approximately 1,000 mAU, which is within the linear UV range of the 1290 Infinity II DAD. The other compounds were determined by injecting 5 µL. Vitamin C and paracetamol were now at a maximum peak height of 3,000 and 3,500 mAU, and, therefore, out of the linear UV range.

Figure 5 shows, enlarged, the 1-µL (red) and 5-µL (blue) injections using the 1290 Infinity II DAD. It was possible to reliably identify and determine chlorphenamine and further trace compounds with the 5-µL injection only. For chlorphenamine, the peak height for the 1-µL injection was as low as 3.8 mAU, whereas for the 5-µL injection, approximately 19 mAU were achieved.

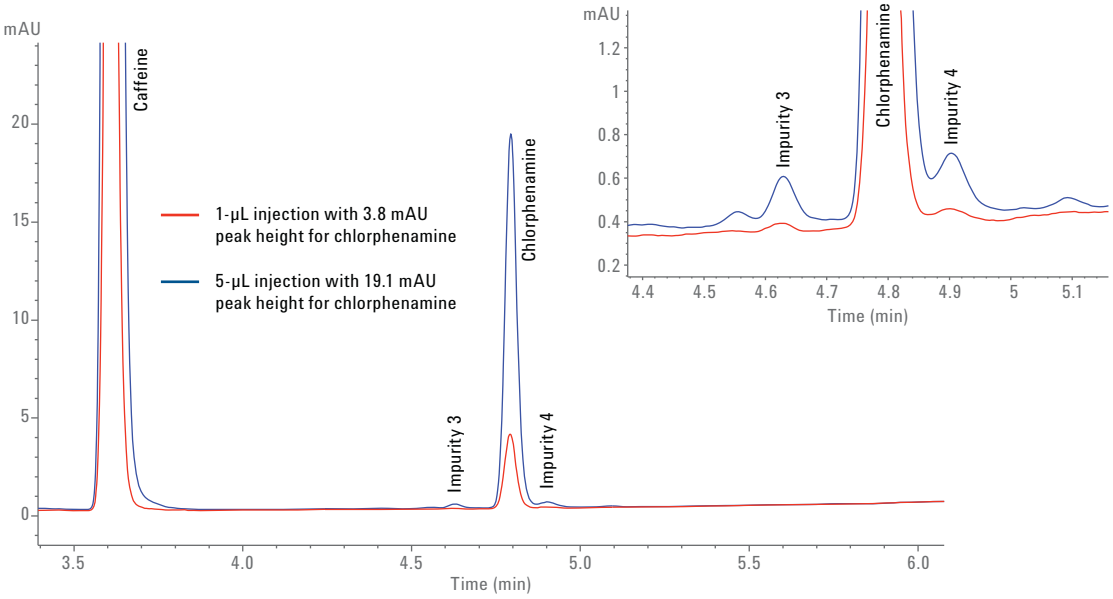


Figure 5. Enlargement of the 1-µL (red) and 5-µL (blue) injections for the Agilent 1290 Infinity II DAD with a 10-mm Max-Light cartridge cell.

This procedure ensured easier integration and, subsequently, more reliable quantification. Impurities 3 and 4 could only be analyzed by injecting 5 µL, and Impurities 1 and 2 elute earlier in the chromatogram.

In a second experiment, 5 µL of the fixed-dose combination drug was injected using the 1290 Infinity II HDR-DAD Impurity Analyzer Solution. Figure 6 shows an overlay of the 5-µL injection of the 1290 Infinity II DAD signal (red) and the 1290 Infinity II HDR DAD signal (blue). Using the 1290 Infinity II HDR DAD signal, all peaks were within the linear range, and could be precisely quantified, while the signals of vitamin C and paracetamol were saturated using the 10 mm Max-Light cartridge cell. The 1290 Infinity II HDR DAD signal approaches 4,350 mAU, which is within the linear UV range for the 1290 Infinity II HDR DAD of approximately 6,700 mAU. Consequently, vitamin C, paracetamol, and caffeine could be quantified without problems by saturated signals.

Figure 7 shows an enlargement of the 5-µL injection for the conventional and the 1290 Infinity II HDR DAD signal. As expected, the 1290 Infinity II HDR DAD signal allowed the evaluation of chlorphenamine and Impurities 1 to 4 simultaneously with the determination of the high-dose compounds.

The limit of detection (LOD) with signal-to-noise (S/N) = 3 of the compounds was evaluated for the 1290 Infinity II DAD and the 1290 Infinity II HDR DAD solution (Table 2). The LODs for the 1290 Infinity II HDR DAD signal were approximately 30 to 50 % lower than the 1290 Infinity II DAD signal.

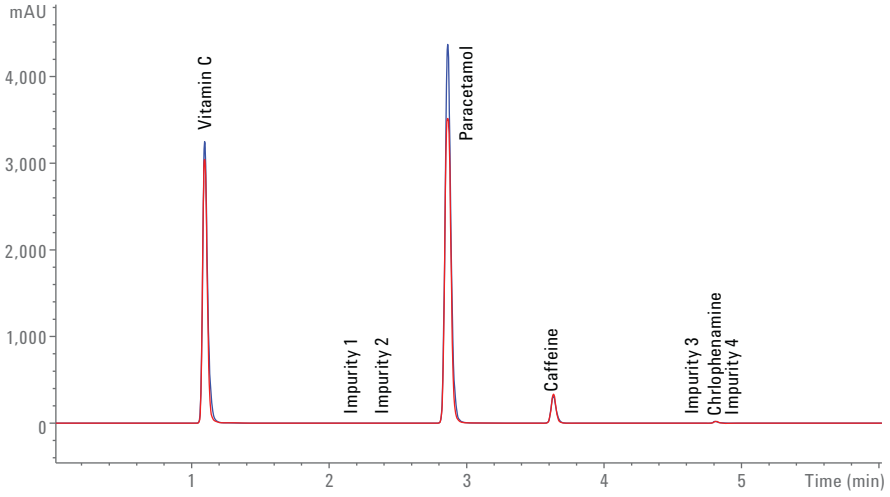


Figure 6. Overlay of 5-µL injection of the Agilent 1290 Infinity II DAD signal (red) signal and the Agilent 1290 Infinity II HDR DAD signal (blue).

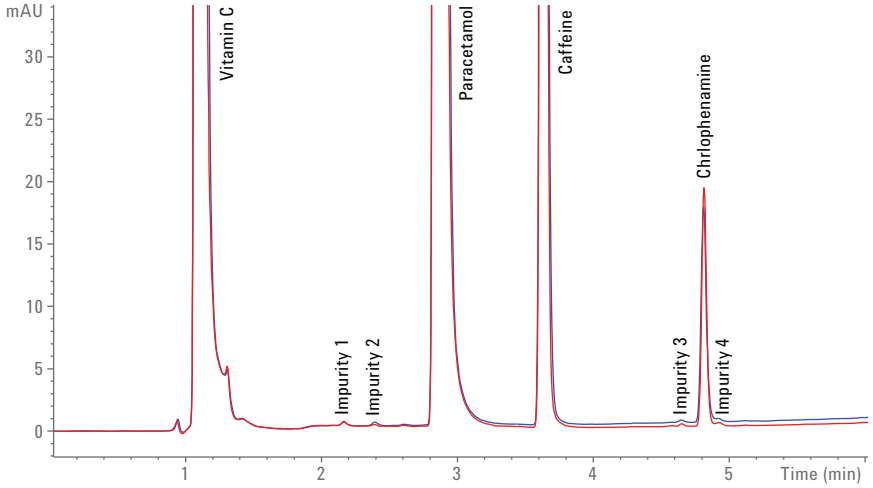


Figure 7. Enlargement of 5-µL injection of the Agilent 1290 Infinity II DAD signal (red) and the Agilent 1290 Infinity II HDR DAD signal (blue).

Table 2. Sensitivity.

	Agilent 1290 Infinity II DAD LOD (pg on column)	Agilent 1290 Infinity II HDR DAD LOD (pg on column)	Improvement in %
Vitamin C	44.8	26.6	40
Paracetamol	51.5	26.4	49
Caffeine	67.5	47.2	30
Chlorphenamine	118.5	84.4	29

Conclusion

With the Agilent 1290 Infinity II HDR DAD Impurity Analyzer Solution, determination of low-dose and high-dose drugs and additional impurities present in a fixed-dose combination drug was possible in one run. The complete determination of all compounds using the 1290 Infinity II DAD with a 10-mm Max-Light cartridge cell required two injections with different injection volumes to avoid saturated signals for the high-dose drugs.

In addition, lower detection limits were achieved with the 1290 Infinity II HDR DAD Impurity Analyzer Solution compared to the Agilent 1290 Infinity II DAD. The LOD for chlorphenamine was 30 % lower using the 1290 Infinity II HDR DAD Impurity Analyzer Solution.

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Published in the USA, May 1, 2015
5991-5743EN

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