Highly Sensitive UV Analysis with the Agilent 1290 Infinity LC for Fast and Reliable Cleaning Validation – Part 2

Monitoring a cleaning validation procedure using a DAD equipped with standard or high sensitivity flow cell

Application Note

Pharmaceutical and Chemical Industry

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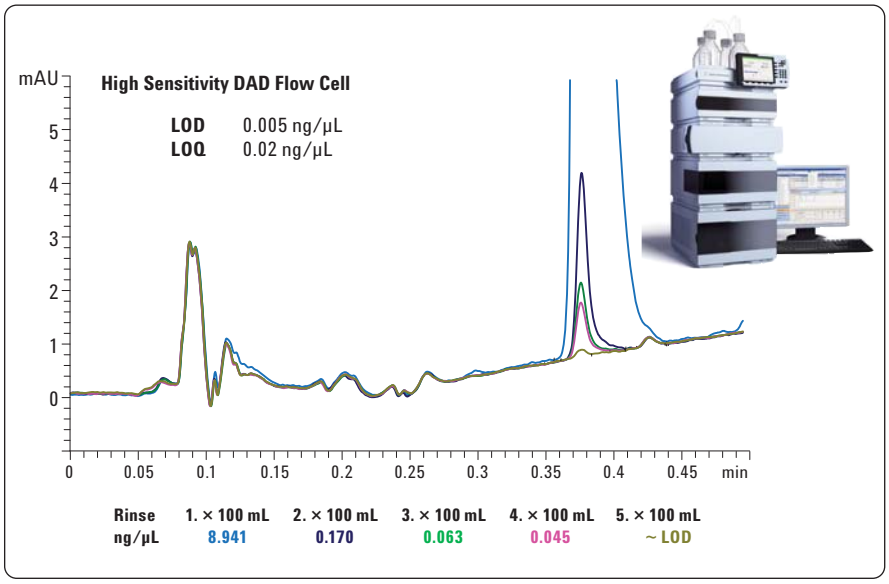

Abstract

This Application Note demonstrates the use of the Agilent 1290 Infinity LC in cleaning validation studies for high sensitivity monitoring of active pharmaceutical product residues in cleaning solutions. It shows that fast analysis is highly useful for cleaning validation, providing fast results and lower production equipment downtime.

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Introduction

Cleaning validation is a process of providing documented evidence that cleaning methods employed within a facility consistently limit potential carryover of products to a level that is below predetermined levels[1]. Validation of cleaning procedures can be initiated by a change of customer requirements, regulatory requirements or internal control and compliance. In active pharmaceutical products (APP), different types of contaminants can be found, such as byproducts, previous products, solvents, cleaning agents or micro-organisms.

Cleaning validation includes a number of steps. Acceptance criteria must first be established, then a cleaning procedure, an analytical method, and sampling procedures must be defined. This is followed by validation, the generation of a protocol and the final report.

One approach for setting acceptance criteria for contamination of an APP with another APP is based on the pharmacological dose. The amount of contaminant must not be higher than 1/1000 of the normal dose of an APP present (APP1) per typical dose of the subsequent product (APP2). Another option is to define a general limit for any contaminant that can be present in the subsequent product (10 ppm up to 0.1%). A typical cleaning procedure of production equipment can be a swabbing or a rinsing process, while monitoring the contaminants in the extraction solvent of the swab or rinse solution.

A series of three Application Notes describes a complete quality control workflow including cleaning validation and final product quality control. This Application Note, which is Part 2 of the series, describes detection and quantitation of a contaminant in the rinsing solvent during a cleaning procedure by the Agilent 1290 Infinity LC equipped with a DAD with a standard (10 mm) or high sensitivity (60 mm) flow cell. As a result, the 60 mm cell exhibits five times higher sensitivity than the standard cell.

Part 1 of the series describes the measurement of calibration curves for APP1, method validation and determination of LOD and LOQ with the Agilent 1290 Infinity LC and DAD with a standard or high sensitivity flow cell[2].

Part 3 describes the determination of contamination of APP2 with remains of APP1. It is demonstrated that detection of very low level amounts of contaminant with the 60 mm cell shows five times higher sensitivity than with the standard cell[3].

Experimental

One-step rinse

An experimental simulation of a cleaning procedure for a reaction vessel was performed twice; as a one-step rinse and as a multistep rinse. Each individual experiment was run in duplicate to provide fresh samples for measurement with individual DAD configurations. The distribution of residual APP1 in the cleaning solutions was measured with individual DAD configurations with the 10 mm standard cell and the 60 mm high sensitivity cell. The initial APP1 concentration in the reaction vessel was 1.0 g/L.

In the first experiment, the solution was poured out from the reaction vessel. The vessel was then cleaned with 500 mL methanol and the concentration of APP1 in the rinse solvent was measured in repeated experiments with both DAD configurations (Figure 1).

Multistep rinse

The disadvantage of using only one rinse to clean a reaction vessel is that the remaining amount is unknown and can contaminate the following reaction. Therefore, multiple rinse steps should be applied and the decreasing amount of APP1 should be monitored during the process in all rinse fractions.

In the multiple rinse the reaction vessel was rinsed five times with 100 mL and the experiment was performed twice (Figure 1).

Chromatographic analysis was done with an Agilent 1290 Infinity LC and DAD equipped with a standard 10 mm flow cell or high sensitivity 60 mm flow cell, respectively (for details of the

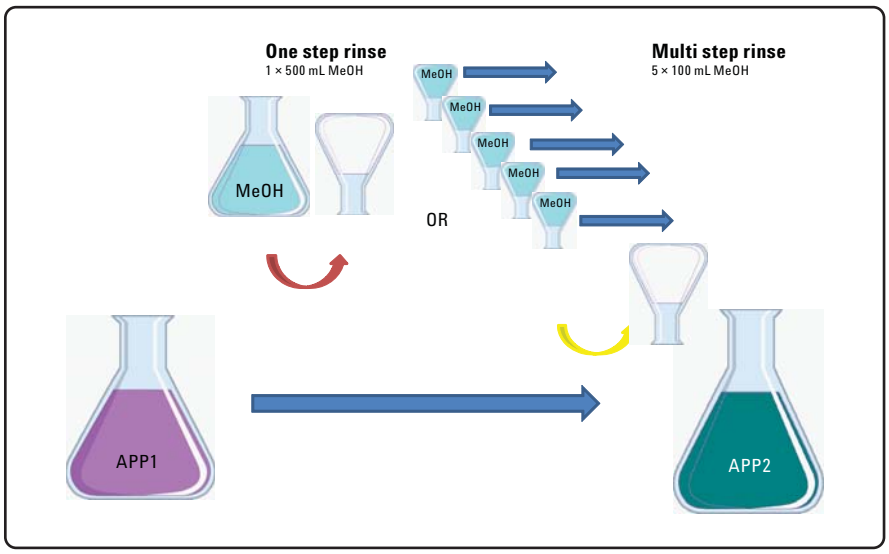

Figure 1
Schematic of the cleaning process during batch exchange in production of APPs.

chromatographic method see Part 1[2]) and the quantitative results from both configurations were compared.

Results and discussion

One-step rinse

In the one-step rinse experiment, the APP1-containing solution was poured out of the reaction vessel. Then the vessel was cleaned with 500 mL methanol. The remaining concentration of APP1 in the rinse solvent was measured with both DAD configurations (Figure 3). A concentration of 1.58 ng/µL (1.58 mg/L) was determined with the 10 mm DAD cell, and 1.52 ng/µL (1.52 mg/L) was determined with the 60 mm DAD cell. The results for both replicate experiments and subsequent measurements were similar, but the signal intensity of the 60 mm cell was about five times higher than for the 10 mm cell.

Multistep rinse

In a multistep rinse, the reaction vessel was rinsed five times with 100 mL and the experiment was performed twice.

In the first experiment, the residual amount of APP1 was measured in each 100 mL rinse fraction with the 10 mm cell (Figure 4). The amount of APP1 in the third fraction was 0.092 ng/µL (92 µg/L), which is below LOQ_{10} and therefore is at a level of uncertainty. The measured concentration in the fourth fractions cannot be confirmed. In accordance with an LOD_{10} of 0.04 ng/µL (40 µg/L) there was no signal detected for APP1 in the fifth fraction.

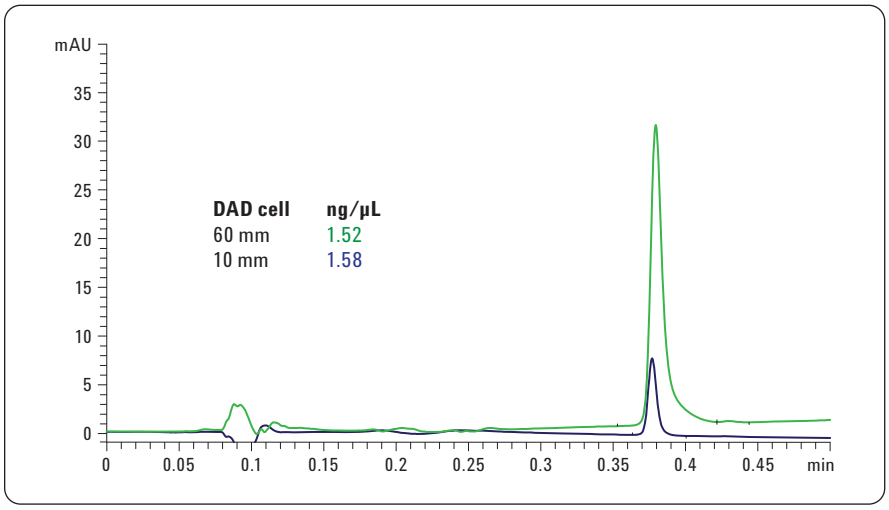

Figure 3
Residual active pharmaceutical product 1 in equipment rinse solution after one application of 500 mL MeOH.

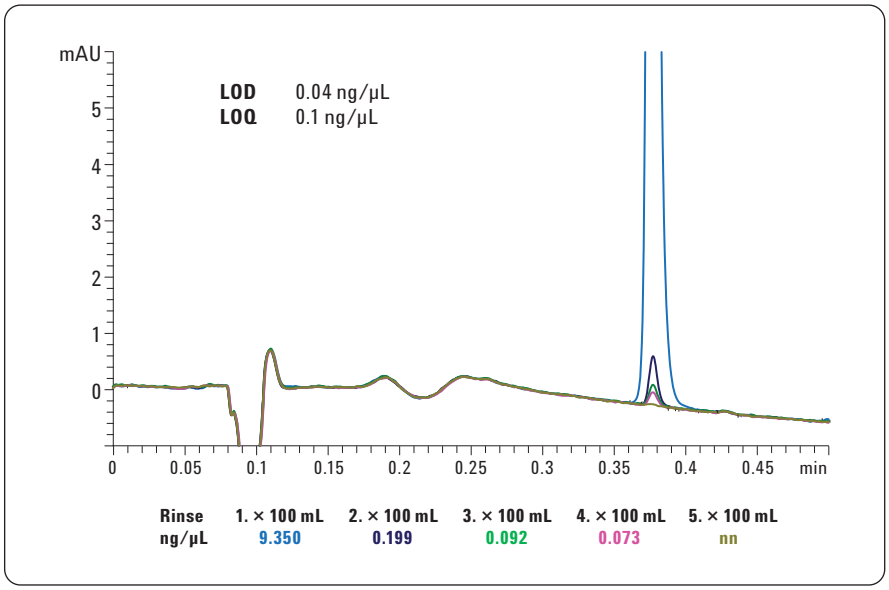

Figure 4
Residual active pharmaceutical product 1 in equipment rinse solution after five applications of 100 mL MeOH measured with the Agilent 1290 Infinity DAD with 10 mm cell.

The 60 mm cell was used for the second experiment (Figure 5). Here, the amount of APP1 in the fourth fraction was determined to be 0.045 ng/µL (45 µg/L), which is twice the LOQ_{60}. For the fifth fraction it was possible to detect a trace of APP1 at the LOD_{60} (0.005 ng/µL or 5 µg/L).

In comparison to the detection with the high sensitivity cell, it is possible to detect down to the LOQ_{10} at about 0.1 ng/µL (100 µg/L) of APP1 in the third fraction with the 10 mm cell. Using the 60 mm cell it was possible to detect APP1 reliably in one more fraction (fraction four) down to an LOQ_{60} of 0.02 ng/µL (20 µg/L) and even to detect a trace at LOD_{60} of 5 µg/L in fraction five (Figure 5). The 60 mm cell is five times more sensitive than the 10 mm cell offering more reliability and safety for the detection of residues in cleaning validation.

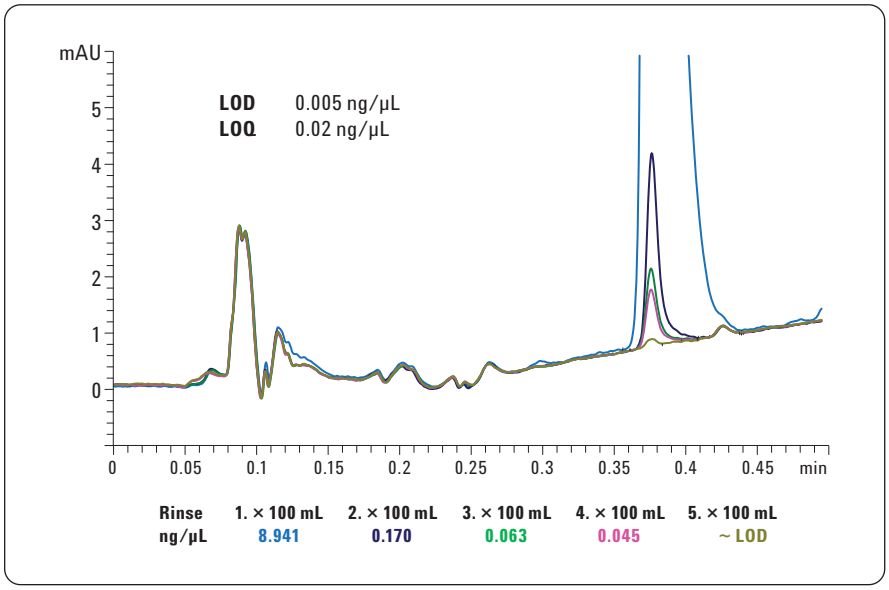

Figure 5
Residual active pharmaceutical product 1 in equipment rinse solution after five applications of 100 mL MeOH measured with the Agilent 1290 Infinity DAD with 60 mm cell.

Conclusion

The Agilent 1290 Infinity LC with DAD and standard flow cell is an excellent instrument to determine residual amounts of APPs during a cleaning validation. If it is necessary to determine trace levels of contaminants, the high sensitivity 60 mm flow cell gives better certainty of results. It exhibits higher sensitivity and therefore helps to monitor cleaning procedures with higher reliability and safety.

In addition to the increased sensitivity for detection of residuals, the analysis time per sample was only one minute. The entire analysis took less than 30 minutes, including all replicates, quality controls, and system suitability samples. This allows faster decisions about production equipment use. Finally, it decreases downtime of equipment leading to higher productivity and reduced costs.

References

1.
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3.
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