Highly Sensitive UV Analysis with the Agilent 1290 Infinity LC System for Fast and Reliable Cleaning Validation – Part 3

Determination of residual active pharmaceutical impurities from a previous production batch using a DAD with standard or high sensitivity flow cell

Application Note

Pharmaceutical and Chemical Industry

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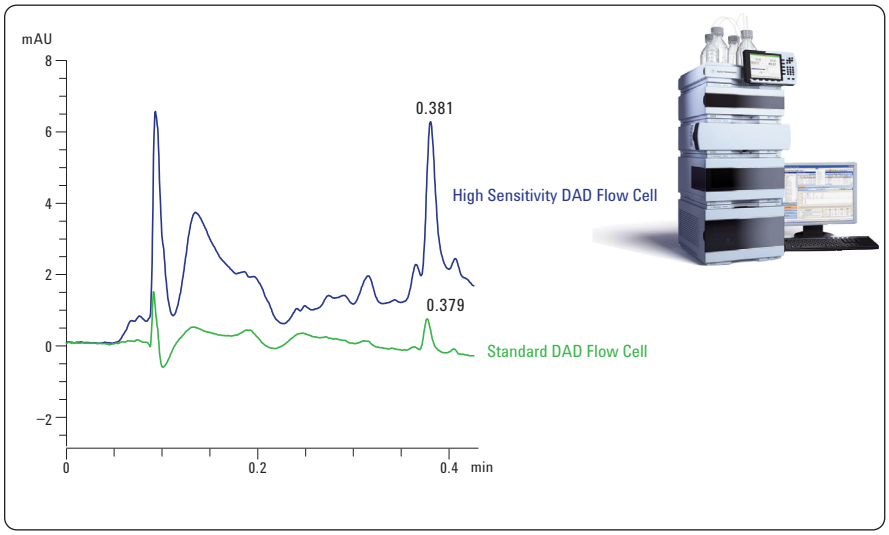

Abstract

This Application Note studies detection of the contamination of an active pharmaceutical product (APP) with the remains of another APP. It demonstrates that high sensitivity detection of very low level amounts of contaminant with the Agilent 1290 Infinity LC DAD equipped with a high sensitivity 60 mm flow achieves about five times higher sensitivity than the standard cell.

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Introduction

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

Cleaning validation includes a number of steps. Acceptance criteria must first be established, then a cleaning procedure, 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) (Figure 1). Another option is to define a general limit for any contaminant that could be present in the subsequent product (10 ppm up to 0.1%). A typical cleaning procedure for production equipment can be a swabbing or a rinsing process with monitoring of 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 3[2] of the series, describes the determination of APP2 contamination with remains of APP1. It is demonstrated that high sensitivity detection of very low level amounts of contaminant with the Agilent 1290 Infinity LC DAD equipped with a 60 mm cell shows 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 2 simulates the cleaning process of a reaction vessel. The difference in detection limits between different flow cells is also discussed. As a result, the high sensitivity DAD cell can detect compounds in the lowest concentrations. Therefore cleaning procedures can be monitored with higher reliability and safety.[3]

Experimental

In this study two pharmaceutical compounds (APP1 and APP2) of the same class of active pharmaceutical products with equal therapeutic daily dosage were used. The residual amount of APP1 was determined in the subsequent pharmaceutical product APP2 (Figure 1). Chromatographic analysis was conducted with an Agilent 1290 Infinity LC and DAD equipped with a standard 10 mm flow cell or high sensitivity 60 mm flow cell, respectively (the chromatographic method is given in part 1[2]). Quantitative results from both configurations were compared.

Results and discussion

After a change in the production of an active pharmaceutical product from APP1 to APP2, it is important that the contamination by APP1 does not exceed a certain limit in the following batch of APP2. Contaminations can occur due to improper cleaning of the production equipment used. Typical limits are the 0.1% impurity rule or that a daily dose of APP2 must not contain 1/1000 of the daily dose of APP1.

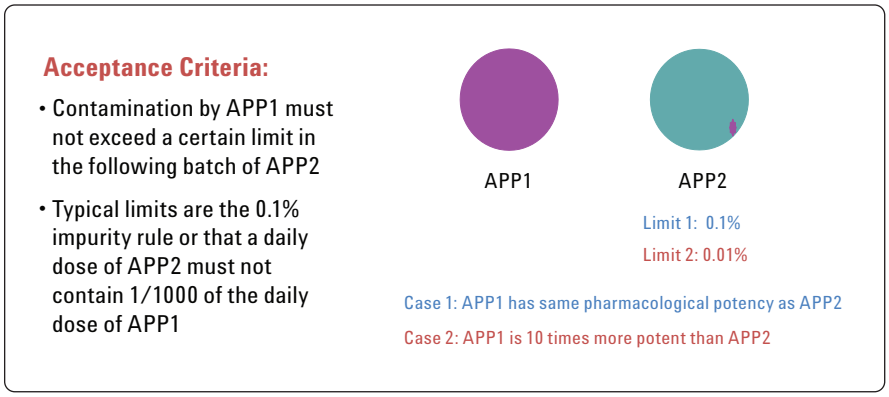

Figure 1
Schematic of the required detection limits according to the pharmacological daily doses of APP1 and APP2.

In the sample, as described in the experimental part, a 0.1% contamination of APP2 by APP1 was determined with a signal-to-noise ratio of 101 by measurement with the standard 10 mm DAD cell (Figure 2). This would be acceptable if the daily dose of APP1 and APP2 were the same amount. It would also meet the requirement of 1/1000 of the daily dose of APP2 (1 ng/µL APP1 in 1000 ng/µL APP2 or 1 µg APP1 in 1 mg APP2, dissolved in 1 mL). In the case, 0.01% of APP1 is contained in APP2, the signal-to-noise ratio is 13 for APP1. This is the limit of quantitation, where quantitation is no longer reliable or safe. This is especially important if the daily dose of APP1 is only 1/10 of the daily dose of APP2. In this case, contamination can pose a significant health risk, because the pharmaceutical potency of APP1 would be 10 times higher than that of APP2.

The experiment was repeated with the DAD configuration containing the 60 mm high sensitivity cell to demonstrate reliable detection of small amounts of contaminant by highly active APPs in APPs applied in a higher daily dose (Figure 3). In this experiment, the 0.1% contamination of APP2 by APP1 was detected with a signal-to-noise ratio of 500. This is a 1 to 1000 ratio for compounds with equal daily doses and there is additional dynamic range for quantitation in case the daily doses differ. In the case, APP1 has 10 times more pharmaceutical potency than APP2 and the daily dose is 1/10 of APP2. A contamination of 0.01% APP1 in APP2 must be detected, which is easily achieved with the 60 mm high sensitivity cell. The detection of the contamination of 0.01% APP1 is possible with a signal-to-noise ratio (S/N) of about 80 (Figure 3, inserts).

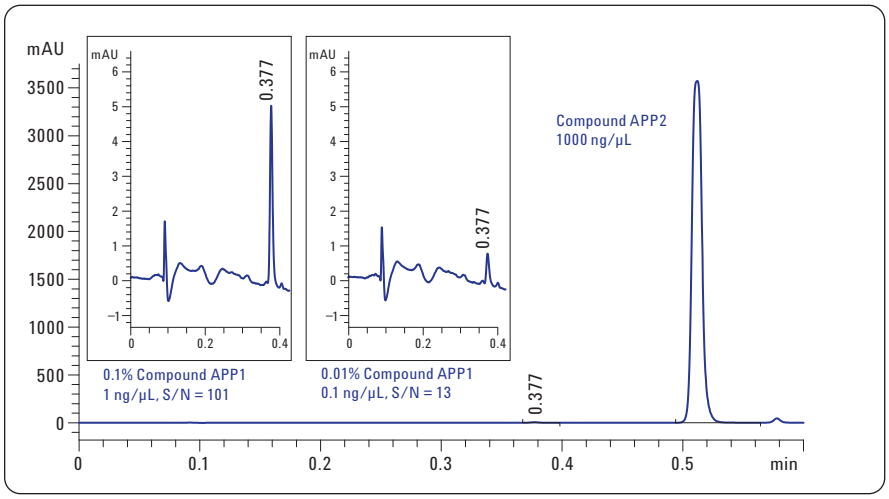

Figure 2
Residual active pharmaceutical product 1 in product 2 at 0.1% and 0.01% measured with the Agilent 1290 Infinity DAD with 10 mm standard flow cell.

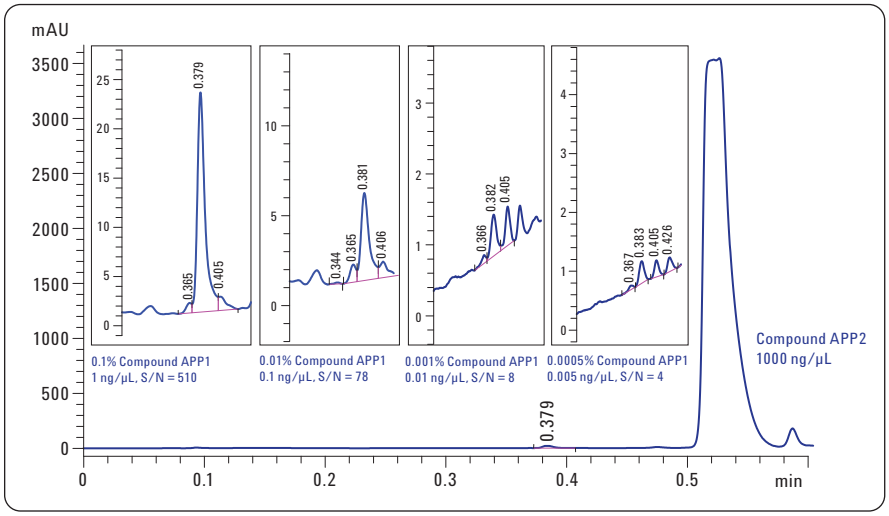

Figure 3
Residual active pharmaceutical product 1 in product 2 at 0.1% to 0.0005% measured with the Agilent 1290 Infinity DAD with 60 mm high sensitivity flow cell.

Quantitatively, it is 0.1 ng/µL or equivalent to 0.1 µg per 1 mg APP2, which is 1/10.000 of the daily dose of APP2 and therefore meets the pharmacological requirements. The LOQ_{60} is reached for a contamination at 0.001%, which is equivalent to 10 ng APP1/1 mg APP 2 (10 ppm). Even a contamination at the level of 0.0005% (5 ppm) can be seen at LOD_{60}. This shows that high sensitivity detection provides very reliable results for trace contaminations of APP2 by other APPs, even when their daily doses and pharmaceutical potency differ by a factor of 100.

Comparison of signal intensity of the 0.01% level contamination of APP2 by APP1 shows a S/N of 13 for the 10 mm standard cell and S/N of 78 for the 60 mm high sensitivity cell. This is an improvement of a factor of 6 for measurements at this low level (Figure 4).

Conclusion

This Application Note discusses measurement of contamination of an APP by a previous active pharmaceutical product. A significant sensitivity improvement of the 60 mm high sensitivity cell allows detection of highly active pharmaceutical compounds in another APP at very low levels. This is important when very low detection levels of APP1 are required, such as defined by an acceptance criteria of 1/1000 of daily dose in subsequent APP 2. The Agilent 1290 Infinity LC equipped with a 60 mm high sensitivity flow cell is an instrument suitably designed for this purpose. The 60 mm high sensitivity cell exhibits about six times higher sensitivity compared to the 10 mm standard DAD flow cell.

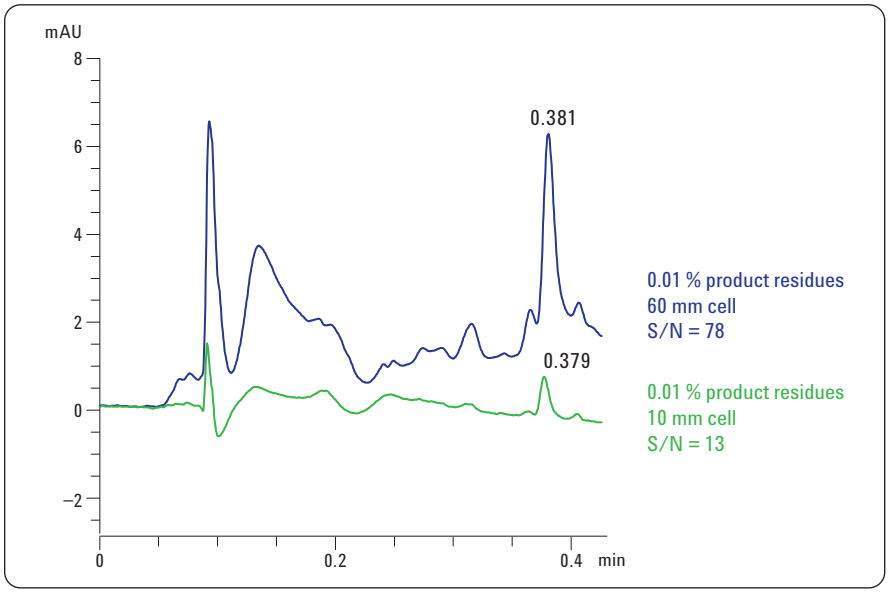

Figure 4
Comparison of the signal intensity measured with the DAD configuration using a 60 mm cell (blue) and a 10 mm cell (green) for a 0.01% impurity of APP1 in active pharmaceutical product 2.

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1.
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