Highly Sensitive UV Analysis with the Agilent 1290 Infinity LC System for Fast and Reliable Cleaning Validation – Part 1

Measurement of calibration curves, determination of LOD and LOQ and method validation using a DAD equipped with standard or high sensitivity flow cell

Application Note

Pharmaceutical and Chemical Industry

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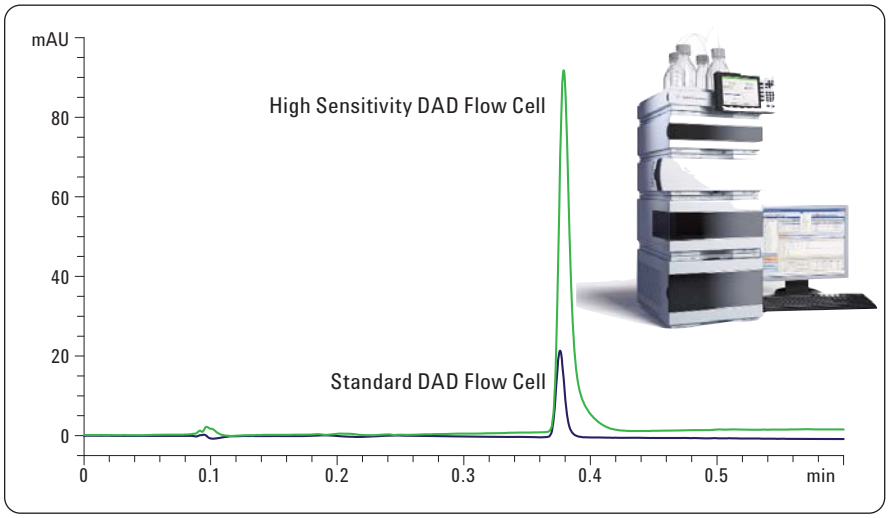

Abstract

This Application Note demonstrates high sensitivity measurement of pharmaceutical compounds with the Agilent 1290 Infinity LC. It also demonstrates a performance comparison of different flow cells with the Agilent 1290 Infinity LC Diode Array Detector (DAD) for highly sensitive UV measurement including calibration, validation, and determination of LOD and LOQ.

Verified for Agilent
1290 Infinity II LC

Agilent Technologies

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]. A validation of cleaning procedures can be initiated by a change of customer requirements, regulatory requirements, or internal control and compliance. In an active pharmaceutical product (APP), different types of contaminants can be found, such as by-products, 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 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, 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 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 standard or high sensitivity flow cell.

Part 2 simulates the cleaning process of a reaction vessel (Figure 1). The difference in detection limits between different flow cells is also discussed. As a result, the high sensitivity DAD cell can detect compounds in low concentrations. Therefore cleaning procedures can be monitored with higher reliability and safety[2].

Part 3 describes the determination of contamination of APP2 with remains of APP1 (Figure 1). 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

In this study two pharmaceutical compounds (APP1 and APP2) of the same class of active pharmaceutical products with equal therapeutic daily dosage were used. A method for compound separation was developed (Table 1) and a calibration curve for quantitation was obtained in the first step. The method was validated and the LOD and LOQ were determined. This was performed for both DAD configurations (standard

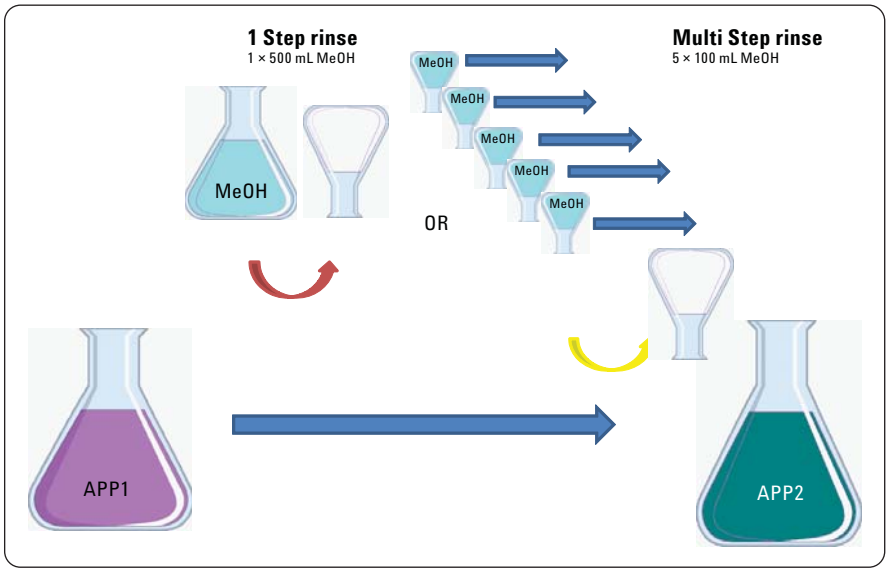

Figure 1
Schematic of the cleaning process during batch exchange in production of APPs.

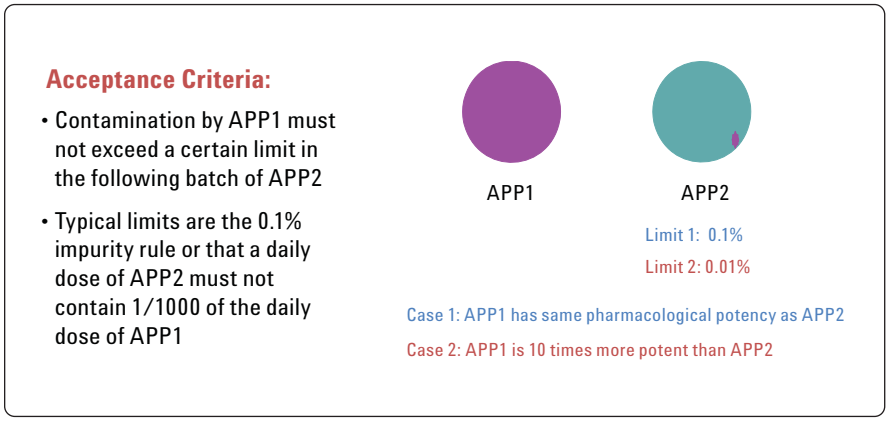

Figure 2
Schematic of the required detection limits according to the pharmacological daily doses of APP1 and APP2.

10 mm or high sensitivity 60 mm cell). Both calibrations and validations were compared and discussed.

Results and discussion

A calibration curve of APP1 was obtained for the two DAD flow cells, 10 mm and 60 mm (Figure 3).

Figure 3a shows the calibration curve of the 10 mm DAD cell. An injection of 1 µL of 5 ng/µL of APP1 produced an intensity of 22 mAU. The calibration curve had a range from 0.04 to 1000 ng/µL (shown: 0.04 to 10 ng/µL). And the low concentration section of the calibration curve was calculated separately from the respective LOQ_{10} (0.1 ng/µL) up to 50 times the LOQ_{10} (5 ng/µL) with five levels for a more precise quantitation of lower concentrations falling in this range (Figure 3a: A–C).

Figure 3b shows the calibration curve of the 60 mm DAD cell. An injection of 1 µL of 5 ng/µL of APP1 produced an intensity of 93 mAU. The calibration curve had a range from 0.005 to 1000 ng/µL (shown: 0.005 to 5 ng/µL). And the low concentration section of the calibration curve was calculated separately from the limit of quantification (LOQ_{60}, 0.02 ng/µL) up to 25 times the LOQ (0.5 ng/µL) with five levels for a more precise quantitation of lower concentrations (Figure 3b).

Agilent 1290 Infinity LC System	Product Number	Parameter	
Agilent 1290 Infinity Binary Pump	G4220	Mobile phase gradient	A: Water + 0.1% TFA. B: ACN + 0.08% TFA 0 min – 10% B, 1 min – 95% B
		Flow rate	1.5 mL/min
Agilent 1290 Infinity Autosampler	G4226	Injection volume	1 µL
		Needle wash	20 s Flush Port: MeOH/Water 50/50
Column		ZORBAX SB C18 Rapid Resolution HD	2.1 mm × 50 mm, 1.8 µm
Agilent 1290 Infinity Column Compartment	G1316C	Column temperature	25 °C
Agilent 1290 Infinity DAD	G4212A	Wavelength	270/4 nm; Ref: 380/40 nm Slit: 4 nm
		Flow cells	10 mm and 60 mm path length
		Peak width	40 Hz data rate
ChemStation Software	G2170BA	For data acquisition	Rev. B. 04.02 SP1 and data analysis
Sample		APP1	APP2 (subsequent compound)

Table 1
Equipment and chromatographic method.

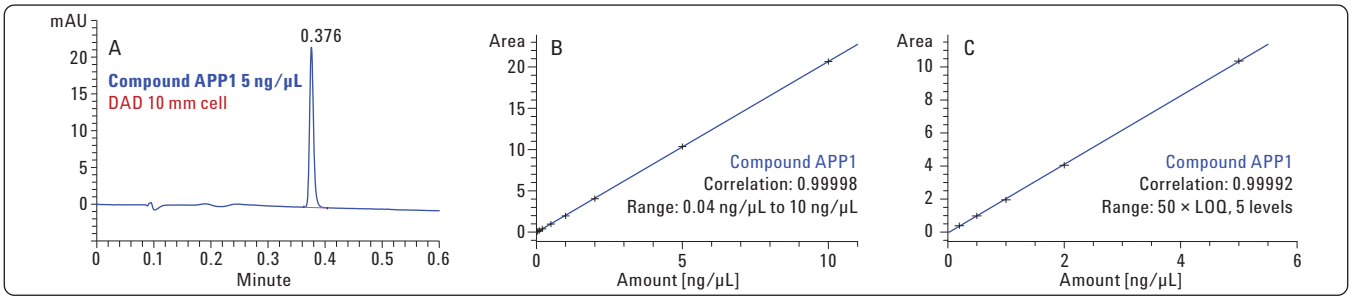

Figure 3a
Calibration curve for the compound APP1 with 10 mm DAD cell. A) Signal from 1 µL injection of 5 ng/µL with 22 mAU. B) Calibration curve 0.04 to 10 ng/µL. C) Calibration curve from LOQ (0.1 ng/µL) up to 50 x LOQ with five levels.

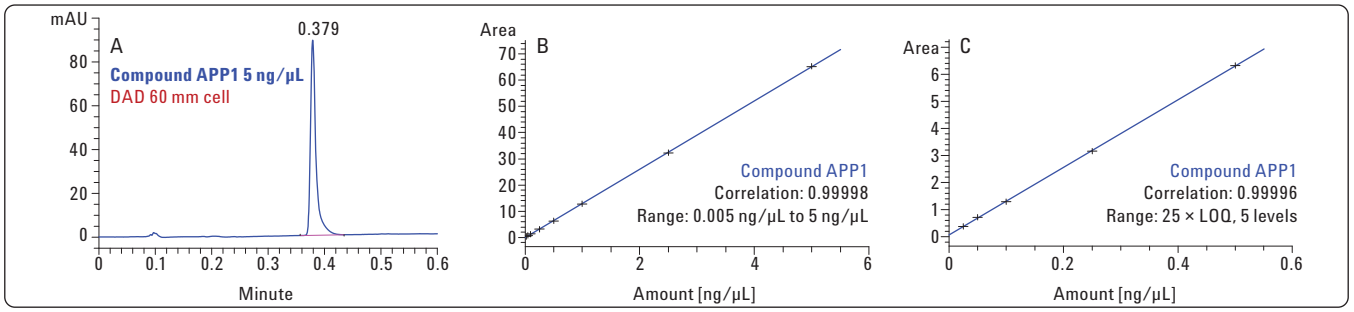

Figure 3b
Calibration curve for compound APP1 with 60 mm DAD cell. A) Signal from 1 µL injection of 5 ng/µL with 95 mAU B) Calibration curve 0.005 to 5 ng/µL C) Calibration curve from LOQ (0.02 ng/µL) up to 25 times the LOQ with five levels.

The analytical method for quantitation of APP1 was validated. Relevant validation parameters, such as retention time stability and area precision from replicate injections (n=10) of 5 ng/µL are shown in Table 2. Relative Standard

	Compound APP1			
	10 mm cell		60 mm cell	
n=10	RT	area	RT	area
Mean	0.377	10.679	0.379	61.253
SD	0.001	0.065	0	0.359
RSD	0.150	0.610	0	0.586

Table 2
Method validation for compound APP1 with 10 mm DAD cell and 60 mm DAD cell.

Deviation (RSD) of retention times for both cells was less than 0.15%, whereas area precision was less than 0.61%. Typical expected values are < 0.25 %RSD for the retention time and < 2 %RSD for the area precision. The linearity coefficient was between 0.99992 and 0.99998. LOD_{10} at a signal-to-noise ratio (S/N) of 3 was 0.04 ng/µL, and LOD_{60} was 0.005 ng/µL. LOQ_{10} (S/N=10) was 0.1 and LOQ_{60} was 0.02 ng/µL (Table 3).

	Compound A	
ng/µL	10 mm cell	60 mm cell
LOD	0.04	0.005
LOQ	0.1	0.02

Table 3
Limit of detection (LOD) at signal-to-noise ratio of 3 (S/N=3) and limit of quantification (LOQ) at signal-to-noise ratio of 10 (S/N=10) for compound APP1 measured with the 10 mm DAD cell and with the 60 mm DAD cell.

A comparison of the signals of APP1 at a concentration of 5 ng/µL showed a signal increase of about 4.5 times when changing from the 10 mm cell to the 60 mm cell (Figure 4). Due to higher signal detection achieved with the 60 mm DAD cell, the LOQ_{60} is five times lower than the 10 mm standard cell values.

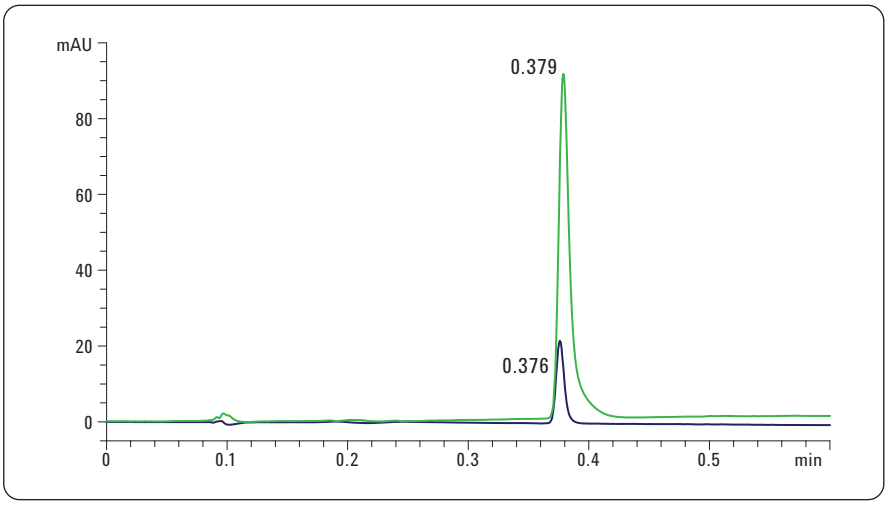

Figure 4
Comparison of the signal of compound APP1 at a concentration of 5 ng/µL measured with the Agilent 1290 Infinity DAD with the 10 mm standard cell (blue) and the 60 mm high sensitivity cell (green).

Conclusion

This Application Note demonstrates the use of the Agilent 1290 Infinity LC for calibration, determination of LOD and LOQ, as well as method validation. These data can be used for cleaning validation and determination of residual active pharmaceutical products in other active pharmaceutical products (see Part 2[2] and Part 3[3]). The 1290 Infinity DAD equipped with either a 10 mm standard or 60 mm high sensitivity cell was used. The gain in performance sensitivity and improvement in LOQ and LOD are shown for calibration and method validation.

References

1.
Cleaning validation in active pharmaceutical ingredient manufacturing plants, APIC Active Pharmaceutical Ingredients Committee, September 1999, http://apic.cefic.org/pub/4CleaningVal9909.pdf.

2.
"Highly Sensitive UV Analysis with the Agilent 1290 Infinity LC for Fast and Reliable Cleaning Validation – Part 2. Monitoring of a cleaning validation procedure using a DAD equipped with standard or high sensitivity flow cell," Agilent Technologies publication 5990-6930EN.

3.
"Highly sensitive UV Analysis with the Agilent 1290 Infinity LC System for Fast and Reliable Cleaning Validations – Part 3. Determination of residual active pharmaceutical impurities from a previous production batch using a DAD with standard or high sensitivity flow cell," Agilent Technologies publication 5990-6931EN.

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September 15, 2014
Publication Number 5990-6929EN

Agilent Technologies