Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System for the Quantification of Trace Level of Genotoxic Impurity

A case study with degraded omeprazole drug product

Application Note

Small Molecule Pharmaceuticals & Generics

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Abstract

This Application Note showcases the use of an Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System to quantify trace level genotoxic impurities formed during degradation of pharmaceutical compounds. Peroxide-degraded omeprazole drug product was used as an example to demonstrate the quantification of an alerting genotoxic impurity with an aromatic N-oxide structure found in degraded omeprazole. The drug product was treated with different concentrations of hydrogen peroxide to cause degradation and formation of the genotoxic impurity. The alerting genotoxic impurity (N-oxide impurity) formed during degradation was quantified using an Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System. A linearity curve was constructed using API spiked with N-oxide impurity standard at trace levels.

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Introduction

Oxidative degradation of some drugs may lead to the formation of genotoxic impurities. As per guidelines from the regulatory authorities, a rigorous control has been emphasized on genotoxic impurities in pharmaceutical drug products. Thus, it is important to monitor genotoxic impurities at trace levels. Degradation of omeprazole drug product resulted in the formation of an alerting aromatic N-oxide impurity[1]. Aromatic N-oxide can be a potential genotoxic alerting functional group[2]. Reliable quantitation of trace level genotoxic impurities is hard to perform using conventional diode array detectors. Auto integration of trace level peaks is often challenging, especially when there is baseline drift[3]. Higher injection volumes may improve the peak height of trace level impurity, but usually saturates the main drug peak and makes it difficult to use the data for quantitation. With the use of an Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System, these issues can be eliminated. This study demonstrates the quantitation of trace level potential genotoxic impurity formed during oxidation degradation of omeprazole using HDR-DAD. The HDR-DAD Impurity Analyzer System expands the linear dynamic range, allowing identification of trace level impurities while maintaining the main peak under the saturation limit.

Experimental

Reagents and chemicals

Omeprazole capsules were purchased from a local drug store, and the N-oxide impurity standard was purchased from Clearsynth. Hydrogen peroxide, monobasic sodium phosphate, anhydrous dibasic sodium phosphate, acetonitrile, and so forth, were purchased from Sigma-Aldrich. The mobile phase was prepared following the pharmacopeia recommendations[4].

Instrument configuration and chromatographic conditions

A 1200 Infinity Series HDR-DAD Impurity Analyzer System based on an Agilent 1290 Infinity Binary LC System was used for the experiments. Tables 1 and 2 show instrument configuration and the chromatography conditions used for the experiments respectively. Agilent OpenLab CDS ChemStation C.01.05 software was used to operate LC system.

Sample preparation

Unspiked drug product sample

Omeprazole drug product sample was prepared at a concentration of 1 mg/mL using the mobile phase as diluent.

Linearity samples

API spiked with N-oxide impurity samples were used for linearity determination. Stock solution of omeprazole at 1,250 ppm and N-oxide impurity solution at 1,000 ppm were prepared. A specific amount of N-oxide impurity was spiked with omeprazole stock solutions to get various linearity levels. The N-oxide impurity linearity levels used for the study were 0.1, 0.2, 0.4, 0.8, 1, 1.5, and 2 ppm spiked with 1,000 ppm API. Mobile phase was used as the diluent for preparing the samples. Unspiked drug product solution was used as the blank. All spiked standards were vortexed, and then centrifuged at 13,000 rpm for 10 minutes, and supernatant was injected.

Table 1. Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System configuration.

Instruments	Model number
Agilent 1290 Infinity Binary Pump	G4220A
Agilent 1290 Infinity Autosampler	G4226A
Agilent 1290 Infinity Thermostatted Column Compartment	G1316C
Agilent 1290 Infinity DAD 1 with 60-mm path length flow cell (p/n G4212-60007)	G4212A
Agilent 1290 Infinity DAD 2 with 3.7-mm path length flow cell (p/n G4212-60032)	G4212A
Agilent 1200 Infinity Series HDR-DAD Solution Kit	G2199AA

Table 2. Chromatographic conditions.

LC parameters	Conditions
Mobile phase	Phosphate buffer[4]: Acetonitrile (3:1)
Column	Agilent ZORBAX Eclipse Plus C8, 4.6 × 150 mm, 3.5 µm (p/n 959963-906)
TCC temperature	25 °C
Needle wash	Acetonitrile for 6 seconds
Run time	30 minutes
Flow rate	0.8 mL/min
Injection volume	20 µL
Detection	280/4 nm, 5 Hz, Ref :off

Degradation samples

Omeprazole drug product was subjected to oxidation, and a known potential genotoxic N-oxide impurity (Figure 1) was monitored. Four different concentrations of hydrogen peroxide (1, 2, 3, and 4 %) were used to degrade 1 mg individual concentrations of omeprazole drug product. After treating the drug product with peroxide for a constant degradation time at room temperature, each sample was subjected to a speed vacuum for 30 minutes. All four samples were then adjusted to 2 mL, vortexed, and centrifuged at 13,000 rpm for 10 minutes. The supernatant clear liquid was taken out and injected in the LC system.

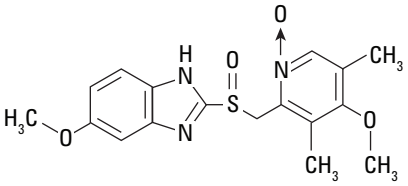

Figure 1. Omeprazole N-oxide impurity

Method

The details of the 1200 Infinity Series HDR-DAD Impurity Analyzer System are given in the User Manual[5]. UV data were acquired using two diode array detectors with different path length Max-Light flow cells. Detector 1 was equipped with a 60-mm path length cell for analyzing low concentration compounds, and Detector 2 was equipped with a 3.7-mm path length cell for analyzing high concentration compounds. The HDR-DAD signal is the combined signal, normalized to a 10-mm path length. Using this instrumentation, a linearity curve was constructed for API spiked with alerting genotoxic N-oxide impurity at trace levels. From this linearity data, the amount of genotoxic N-oxide impurity formed during the oxidation of omeprazole drug product was quantified.

Results and Discussion

Unspiked omeprazole drug product

Unspiked drug product sample was injected twice with varying injection volumes, 5 µL and 20 µL with the HDR feature disabled. The result showed that with a 5 µL injection volume (without HDR), the impurity peak was really trace level (area % 0.008) and was difficult to integrate. While, with a 20 µL injection volume, the impurity peak was significantly visible (area % 0.012), however, the main peak became saturated.

With HDR enabled, a 20 µL injection of the unspiked drug product resulted in a clear impurity peak (area % 0.009) without saturating the main peak. The result from the HDR was compared with the 5-µL and 20-µL injections of API without HDR. Figures 2A, 2B, and 2C show a comparison of the above mentioned analyses. Table 3 shows the RSD values for the API injections with and without HDR.

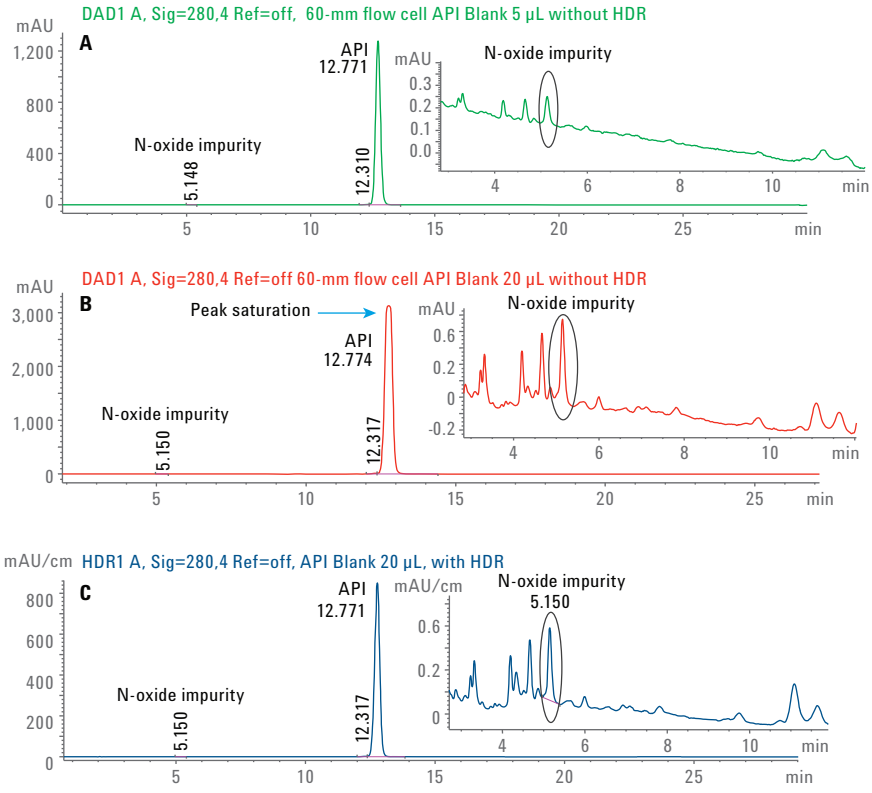

Figure 2. Chromatograms of unspiked omeprazole drug product sample. A) 5 µL injection volume without HDR, B) 20 µL injection volume without HDR, and C) HDR signal with 20 µL injection volume. Insets show zoomed impurity peak for each case. The impurity peak was clear in Figure 2C without saturating the main peak.

With the use of HDR, the noise level was decreased up to 88 %, which, in turn, improved the signal-to-noise (S/N) value. Observed noise values for 20 µL injection with and without HDR were compared and tabulated in Table 4.

Linearity curve from N-oxide impurity spiked samples

With HDR solution, extremely good linearity with regression coefficient (R^2 value) > 0.9989 was achieved, including all seven concentrations of spiked N-oxide impurity (Figure 3). Consistent retention times of N-oxide impurity observed in the study shows excellent RT reproducibility. Figure 4 shows the overlay of N-oxide impurity peaks from linearity samples.

Table 3. RSD values for impurity peak using 5 µL and 20 µL injection volumes with and without HDR.

Injection volume (µL)	Configuration	RSD % (n = 5) of impurity peak	
		Area	RT
5	HDR disabled	6.63	0.01
20	HDR disabled	0.56	0.04
20	HDR enabled	0.00	0.05

Table 4. Calculated noise values for impurity peak using 20 µL injection volume with and without HDR.

Injection volume (µL)	Configuration	Peak-to-peak noise (mAU/cm)
20	HDR disabled	0.0393
20	HDR enabled	0.0046

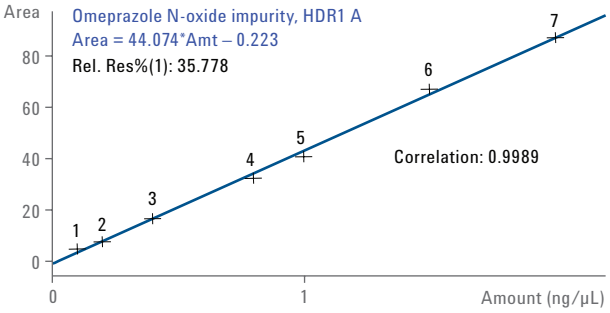

Figure 3. Linearity curve plotted for spiked N-oxide impurity from 0.1 to 2 ppm.

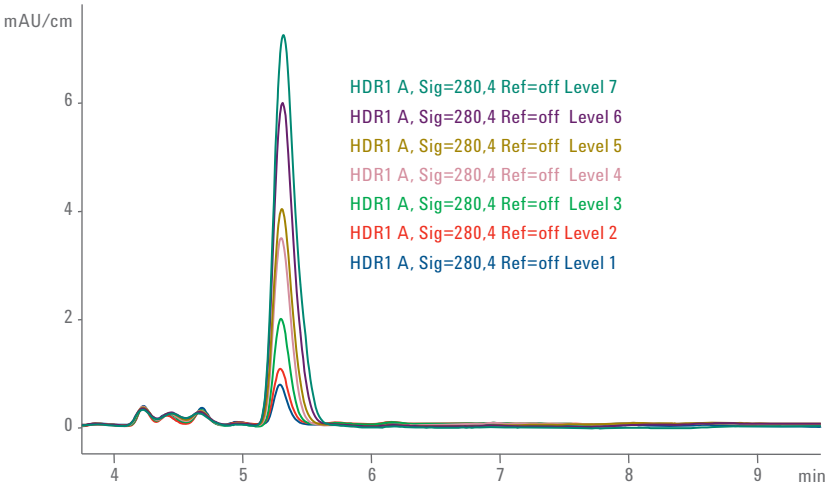

Figure 4. Overlaid chromatograms of N-oxide impurity peak from linearity samples.

Quantitation of N-oxide impurity from degradation samples

The degradation samples showed a linear increment in the impurity peak with an increase in the H_2O_2 concentration (Figure 5). The amount of impurity formed after degradation was calculated using the linearity curve generated from the standard spiked samples, and was found within the linearity range. The results are tabulated in Table 5.

Conclusion

This Application Note demonstrates the effective use of Agilent HDR-DAD solution for the quantitation of trace level potential genotoxic impurity formed during omeprazole peroxide degradation. Forced degradation using increased concentrations of hydrogen peroxide showed an increase in potential genotoxic N-oxide impurity formation. With the use of an Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System, higher volumes of samples could be injected, enabling better impurity peak detection without saturating the main peak. Trace level N-oxide impurity in HDR plots was integrated automatically with excellent area precision compared to manual integration while operating without HDR. The 1200 Infinity Series HDR-DAD Impurity Analyzer System also offers a reduction in noise and, thus, improved S/N for trace peaks. A linearity curve was plotted with seven levels of spiked N-oxide impurity samples, and excellent linearity with a regression coefficient > 0.9989 was observed.

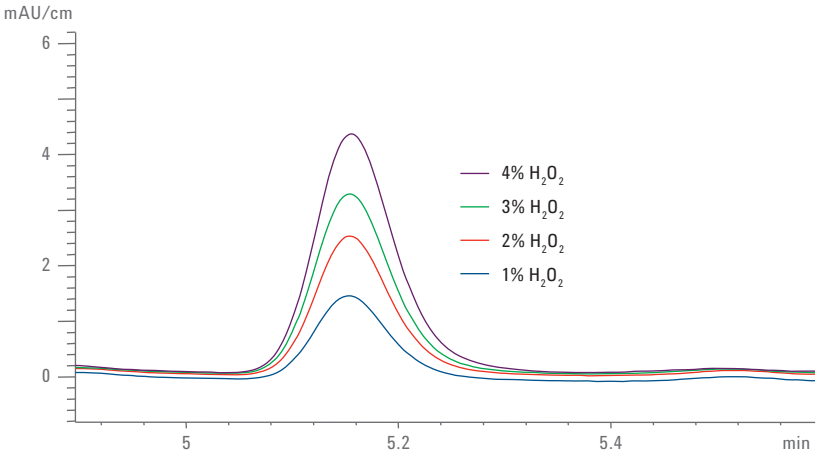

Figure 5. HDR signals of N-oxide Impurity formed during degradation with various concentrations of H_2O_2.

Table 5. Amount of potential genotoxic N-oxide impurity formed during degradation using various peroxide concentrations.

Degradation sample	Amount of N-oxide impurity (alerting genotoxic impurity) in ppm
1 % H_2O_2 treat	0.43
2 % H_2O_2 treat	0.69
3 % H_2O_2 treat	0.93
4 % H_2O_2 treat	1.32

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