Analysis of Degradation Products of Doxycycline in Solution Exposed to Light and Elevated Temperatures Using the Agilent 1200 Infinity Series High Dynamic Range Diode Array Detector Impurity Analyzer System

Application Note

Small Molecule Pharmaceuticals & Generics

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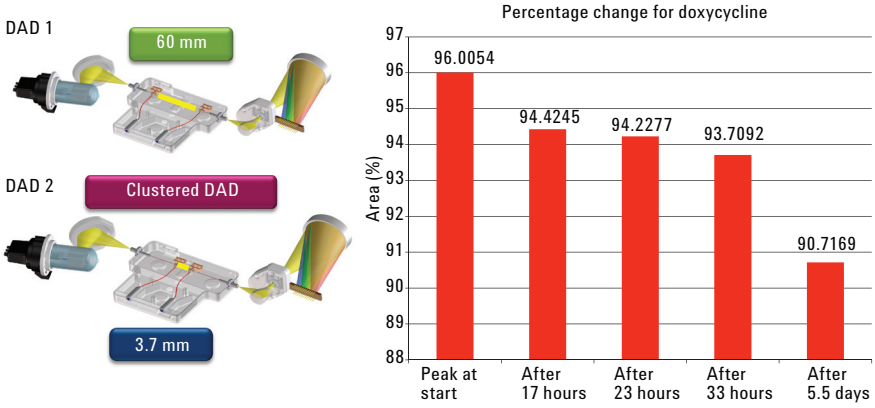
Abstract

Tetracyclines undergo epimerization and degradation if stored under inappropriate conditions. Stability experiments, which are important for the determination of the expiry date, were done using doxycycline. Doxycycline was exposed to light and elevated temperature for days. To determine decomposition products, the Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System was used. Using this analyzer, it was possible to detect peaks with peak heights < 0.2 mAU.



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Introduction

Tetracyclines are antiobiotics used in case of bacterial infections. Typical applications are prophlylaxis and therapy of human and animal infections. They are also used in animal feed as a growth promoter. When exposed to heat and light, tetracyclines undergo epimerization and degradation. Often, the degradation products have very low antibiotic activity, and some are toxic[1,2,3]. In the pharmaceutical industry, stability tests must be done for all pharmaceutical products on the market. Recommendations from the International Conference on Harmonization (ICH)[4] include long-term studies, where the product is stored at room temperature and humidity conditions. In further studies, the product was stored at more extreme conditions.

The1200 Infinity Series HDR-DAD Impurity Analyzer system offers the possibility to recognize degradation products at very low levels, due to the low noise behavior of this detector. In addition, the main compound can be analyzed within the same experiment due to the wide dynamic range of the system.

In the following experiment, doxycycline was dissolved and the solution was exposed to light at 40 °C for several days. From time to time, the solution was analyzed to discover additional impurities and the changes in the main compound and impurities, which were present in the starting solution.

Experimental

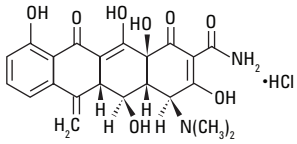
Instrumentation

Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System:

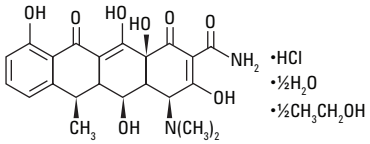
- Agilent 1290 Infinity DAD with 60-mm cell (G4212A) (clustered)
- Agilent 1290 Infinity DAD with 3.7-mm cell (G4212A) (clustered)
- Agilent 1200 Infinity HDR-DAD Kit (G2199AA)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Autosampler G4226A
- Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Quaternary Pump (G4204A)

Compound analyzed

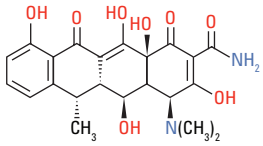
Doxycycline and impurities. Doxycycline was purchased from Sigma-Aldrich, Germany



Methacycline



Doxycycline hyclate



6-Epidoxycycline

Chromatographic conditions

Parameter	Value
Column	Agilent ZORBAX Eclipse XDB-C8, 3 × 100 mm, 3.5 µm (p/n 961967-302)
HDR-DAD	Wavelength = 350, 254, 280 nm, reference = off, data rate = 20 Hz
Flow rate	0.8 mL/min
Mobile phases	A=water, B=Acetonitrile, C=1%TFA in water
Gradient	at 0 minutes A = 65 %, B = 15 %, C = 20 %, at 5 minutes A = 37 %, B = 43 %, C=20 %, at 6 minutes A = 20 %, B=60 %, C=20 %
Stop time	6 minutes
Post time	3 minutes
Column temperature	40 °C
Injection volume	1 µL, 40 °C, needle wash for 3 seconds

Sample preparation

A 20 mg sample of doxycycline was dissolved in 1.5 mL water/ACN = 1:2, 1-µL injection volume contained 13.33 µg of doxycycline.

Acquisition and evaluation software

Agilent OpenLAB CDS ChemStation Edition Rev.C.01.05

Results and Discussion

The high dynamic range (HDR) system was configured during instrument configuration. Both detectors were clustered, and the delay volume of the capillary connecting both detectors was filled in. In the user interface, the clustered detectors appear as one detector (Figure 1).

For the analysis of doxycycline, three wavelengths were selected: 230, 280, and 350 nm, to ensure discovery of all impurities. In the data file, all three HDR-DAD signals were stored as well as the three signals of the 60-mm and the three signals of the 3.7-mm cell (Figure 2).

Having dissolved 20 mg of doxycycline, the followings experiments were done:

- Analysis of the fresh solution
- The solution was exposed to daylight at 40 °C for 17 hours and analyzed afterwards
- Analysis after 23 hours exposure
- Analysis after 33 hours exposure
- Analysis after 5.5 days exposure

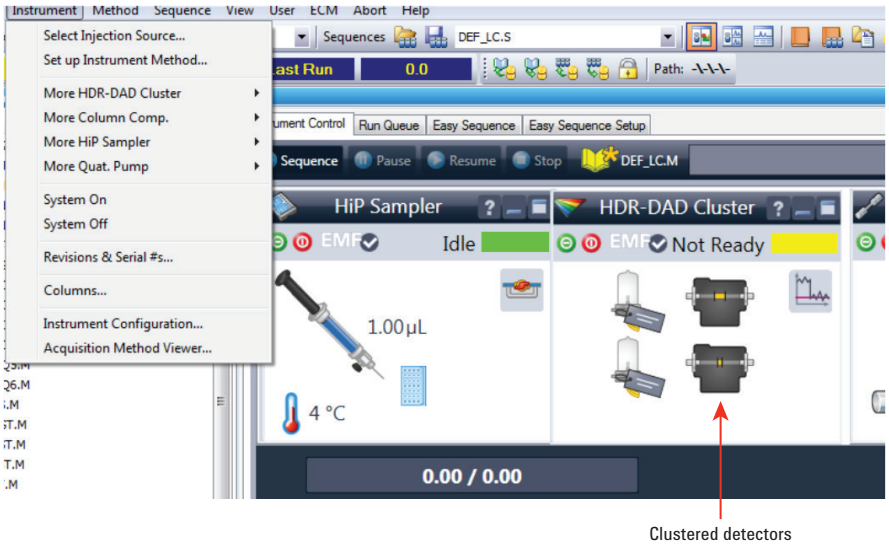


Figure 1. User interface in an Agilent OpenLAB CDS ChemStation.

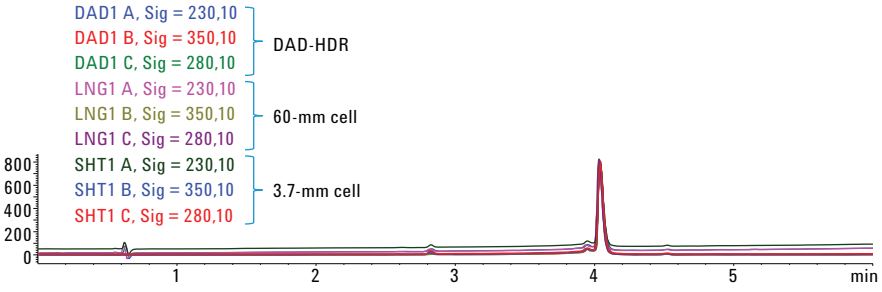


Figure 2. Stored signals.

One prerequisite for decomposition studies is that even very small decomposition peaks are detectable. Therefore, the noise level must be as low as possible. For the analysis of doxycycline, the P to P noise level was as low as 0.013 mAU (Figure 3). This enabled the detection of peaks with a peak height below 0.2 mAU.

Figure 4 shows chromatograms of the fresh and the exposed solution after 5.5 days.

Impurities 1, 2, and 3 were present from the beginning. Visibility of Impurity 4 started after 17 hours. After 5.5 days and exposure to light at 40 °C, Impurities 5 to 13 were detected.

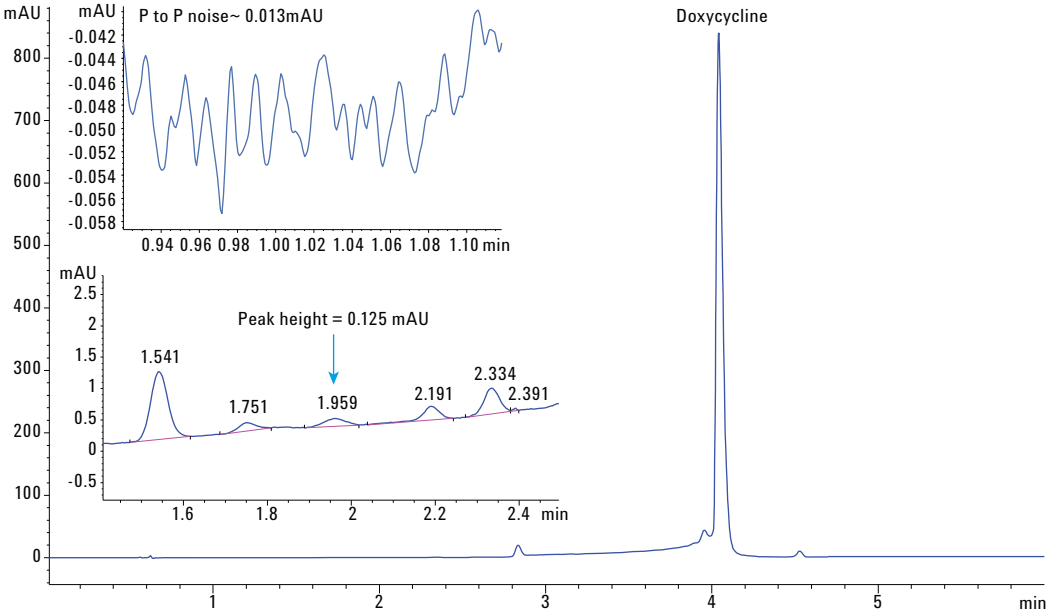


Figure 3. Analysis of doxycycline and determination of noise level.

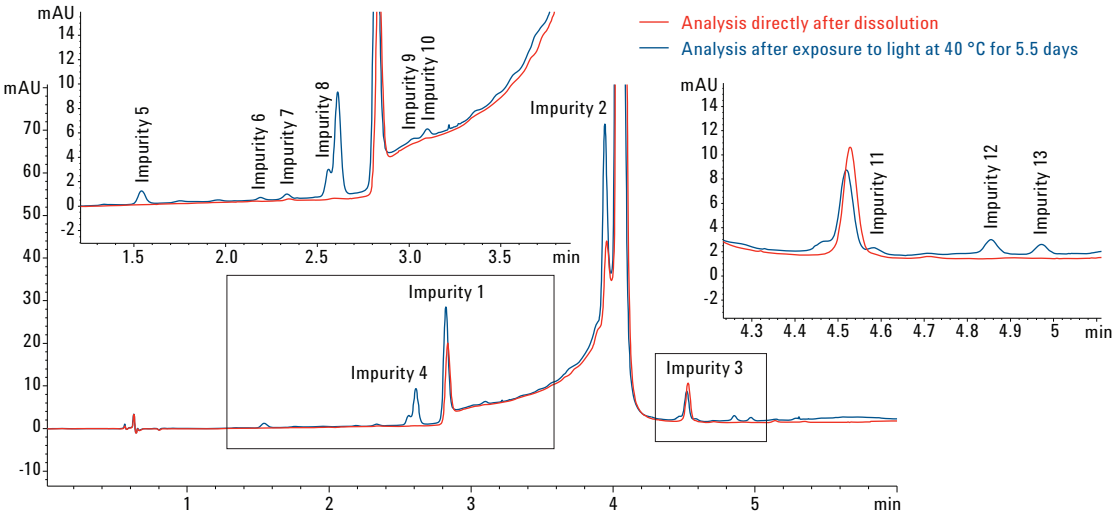


Figure 4. Degradation of doxycycline after 5.5 days, overlay of start chromatogram and degradation chromatogram.

Figure 5 shows the process of degradation after 17 hours, 23 hours, 33 hours, and 5.5 days.

The percentage of doxycycline decreased continuously, whereas Impurities 1 and 2 increased. Impurity 2 increased continuously, whereas Impurity 1 stagnated after the first increase. After 17 hours, Impurity 4 occurred and increased even further for the duration of the exposure. After 5.5 days, additional small impurities became visible.

Conclusion

Stability tests are frequently done in the pharmaceutical industry to determine the date of expiry for a pharmaceutical product. To detect degradation products at trace levels, the Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System was used for the decomposition process of doxycycline. Doxycycline was exposed to daylight at 40 °C over 5.5 days, and the degradation was monitored. It was possible to detect impurities with < 0.2 mAU of peak height. After 5.5 days of exposure, the percentage of doxycycline was reduced by 5.3 %, while additional impurities occurred and the amount of impurities already present in the original solution increased proportionally.

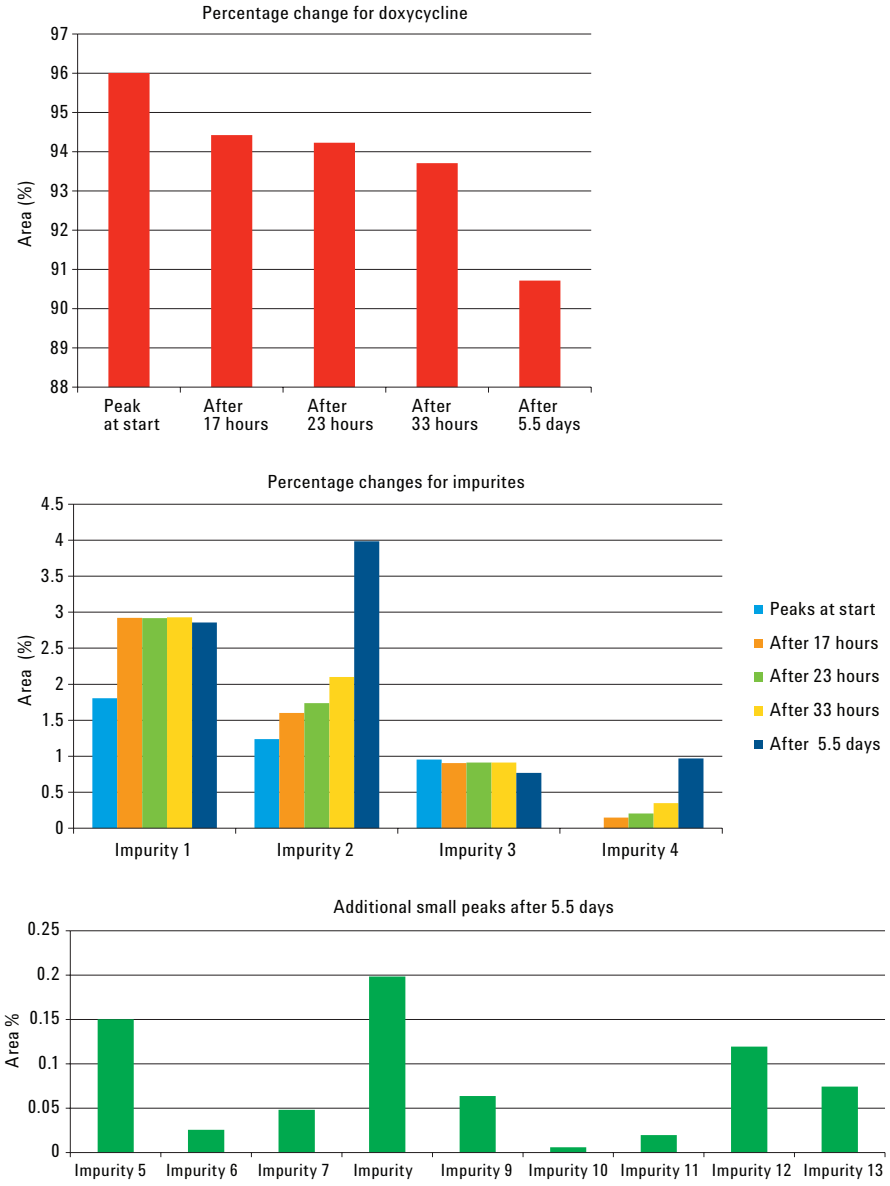


Figure 5. Process of degradation.

References

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3. Y. Liang, M.B. Denton, R.B. Bates "Stability studies of tetracycline in methanol solution" *Journal of Chromatography A*, 827: p.45–55 (**1998**).

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