Analysis of amoxicillin and five impurities on the Agilent 1220 Infinity LC System

LC analysis of impurities down to the 0.01% level with long sub-2-µm columns, high flow rates and back pressure greater than 400 bar

Application Note

Drug Development and Pharmaceutical QA/QC

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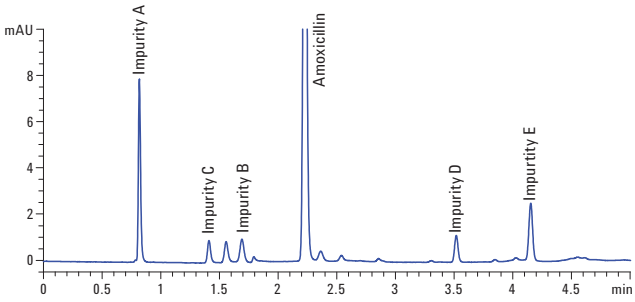

Abstract

Amoxicillin is an antibacterial drug widely used against gram-positive and gram-negative organisms. In pharmaceutical drug discovery and development, it is crucial to analyze impurities due to their potential for toxic effects on humans. Amoxicillin and its impurities were analyzed on the Agilent 1220 Infinity LC System on a 150 mm × 4.6 mm column with 1.8 µm particles. When using a flow rate of 2 mL/min (at 525 bar), the analysis was completed within seven minutes. Under these conditions, impurities down to a level of 0.01% could be detected. The Agilent 1220 Infinity LC System is an ideal instrument for impurity analysis in a pharmaceutical quality control laboratory.

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Introduction

In pharmaceutical drug discovery and development, it is crucial to analyze for impurities due to their potential for toxic effects on humans. An impurity could be the active pharmaceutical substance itself, a minor byproduct from the production process, a secondary substance in a drug isolated from a natural source, a metabolite created in the human body, or a degradation product of the pharmaceutical agent created under storage conditions. Regulatory agencies stipulate that in the final drug, the amount of an impurity is below 0.01% of the main compound.

Recently, a number of publications have dealt with the analysis of amoxicillin and its impurities by HPLC with UV or MS detection.[1,2,3] Amoxicillin is an antibacterial drug widely used against gram-positive and gram-negative organisms. It can degrade to several different byproducts, as illustrated in the pathways in Figure 1. Amoxicillin is a polar substance, and its separation requires a high amount of water in the mobile phase.

In this Application Note, we use a gradient method in combination with UV detection for the determination of amoxicillin and related impurities. Precision of areas and retention times as well as limits of detection (LOD) and limits of quantitation (LOQ) for the impurities were evaluated. Linearity in the low nanogram range (trace level) was evaluated for impurities. The Agilent 1220 Infinity LC System is an ideal instrument for impurity analysis in a pharmaceutical quality control laboratory.

Experimental

Instrumentation

The instrument used in this application is the Agilent 1220 Infinity LC System in the following configuration: Gradient Pump, Autosampler, Column Oven and Variable Wavelength Detector.

Analyzing amoxicillin requires a column that can tolerate 100% water as a starting condition. The Agilent ZORBAX SB-Aq column, which is compatible with the highest percentages of water in the mobile phase was used for all experiments. The software used was ChemStation, revision B.04.02.

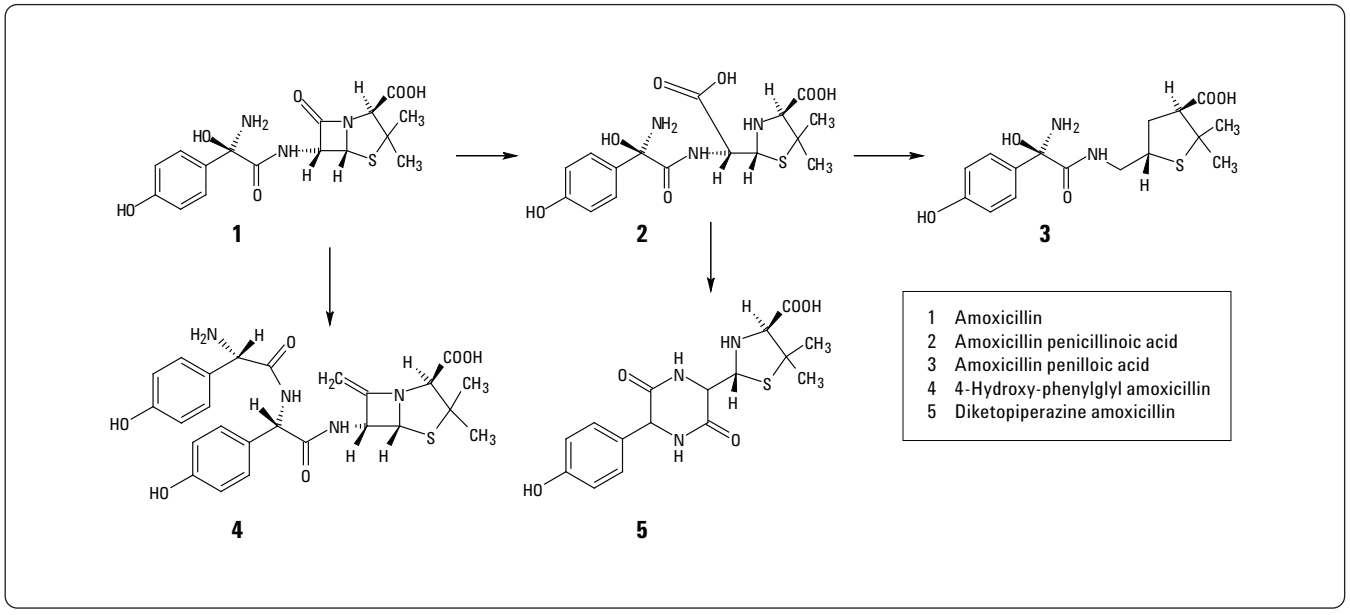

Figure 1
Degradation pathways of amoxicillin.

Chromatographic conditions

Sample:	Amoxicillin diluted in pure water
Column:	Agilent ZORBAX SB-Aq 4.6 mm × 150 mm, 1.8 µm p/n 829975-914
Mobile phase:	A: Water phosphate buffer (0.01 mol/L), pH=4.8 B: ACN

Gradient:

Time [min]	Component A [%]	Component B [%]
0	100	0
5	78	22
7	60	40

Flow rate:	2.0 mL/ min, max pressure 525 bar
Stop time:	7 min
Post time:	3 min
Detector:	VWD 229 nm
Peak width:	PW > 0.025 min, 20 Hz
Injection Vol:	1 µL
Column temp:	40 °C

Sample preparation

Sample compounds:

- Amoxicillin trihydrate
- Impurity A, p-hydroxy-Phenylglycine
- Impurity B, Amoxicillin Penicilloic acid
- Impurity C, 6-Aminopenicillanic acid
- Impurity D, p-hydroxy-Phenylglycyl Amoxicillin
- Impurity E, Diketopiperazine-Amoxicillin

A stock solution of amoxicillin was prepared in water with a concentration of 1 mg/mL. Solutions for the evaluation of impurity data were prepared in water (Table 1). Different concentration levels were used for testing the precision of retention times and areas for the evaluation of LOD, LOQ and linearity.

Level	1 Amount (ng/µL)	2 Amount (ng/µL)	3 Amount (ng/µL)	4 Amount (ng/µL)	5 Amount (ng/µL)	6 Amount (ng/µL)
Impurity A	6.9	3.45	1.38	0.69	0.345	0.138
Impurity B	6.96	3.48	1.392	0.696	0.348	0.1392
Impurity C	10.9	5.45	2.18	1.09	0.545	0.218
Impurity D	3.57	1.785	0.714	0.357	0.1785	0.0714
Impurity E	5.28	2.64	1.056	0.528	0.264	0.1056

Table 1
Concentratiion levels of impurities.

Results and discussion

Precision of retention times and areas

Precision of areas and retention times was evaluated with concentration level 1 (Figure 2). The injection volume was 1 µL. The resolution for impurity B is 1.97.

Other impurities and degradation products are observed in the chromatogram, but in Figure 2, only the impurities and degradation products shown in Table 1 were determined.

Table 2 shows the precision of retention times and areas of amoxicillin impurities.

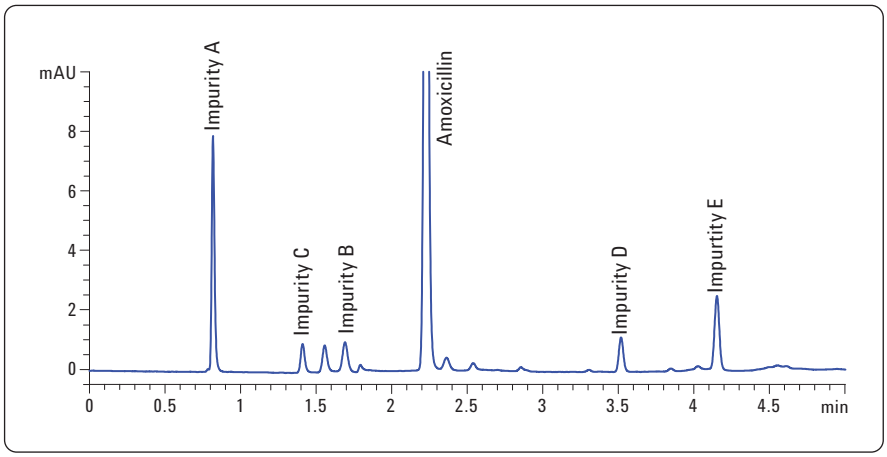

Figure 2
Concentration level 1 for testing precision of retention times and areas over eight runs, 1 µL injected, maximum pressure of 525 bar at 2 mL/min flow rate.

n=8	Impurity A Ret Time (min)	Impurity A Area (mAU's)	Impurity B Ret Time (min)	Impurity B Area (mAU's)	Impurity C Ret Time (min)	Impurity C Area (mAU's)	Amoxicillin Ret Time (min)	Amoxicillin Area (mAU's)	Impurity D Ret Time (min)	Impurity D Area (mAU's)	Impurity E Ret Time (min)	Impurity E Area (mAU's)
Mean:	0.818	10.670	1.404	1.884	1.686	2.373	2.224	60.416	3.515	2.487	4.152	5.675
S.D.:	2.890E-04	9.549E-02	3.330E-03	1.254E-02	2.560E-03	9.084E-02	2.240E-03	1.868E-01	7.860E-04	3.342E-02	4.220E-04	2.803E-02
RSD:	0.035	0.895	0.237	0.666	0.152	3.828	0.101	0.309	0.002	1.344	0.022	0.494

Table 2
Precision of RT and areas of amoxicillin and impurities for level 1 concentration.

The precision of retention times is between 0.002% and 0.24% RSD for all peaks.

The precision of areas is between 0.5% and 3.9% RSD for impurities with area counts between 1.9 and 11 mAU's.

Linearity

Linearity was tested over all six concentration levels (Figure 3). The linearity is better than 0.999 for the factor of correlation.

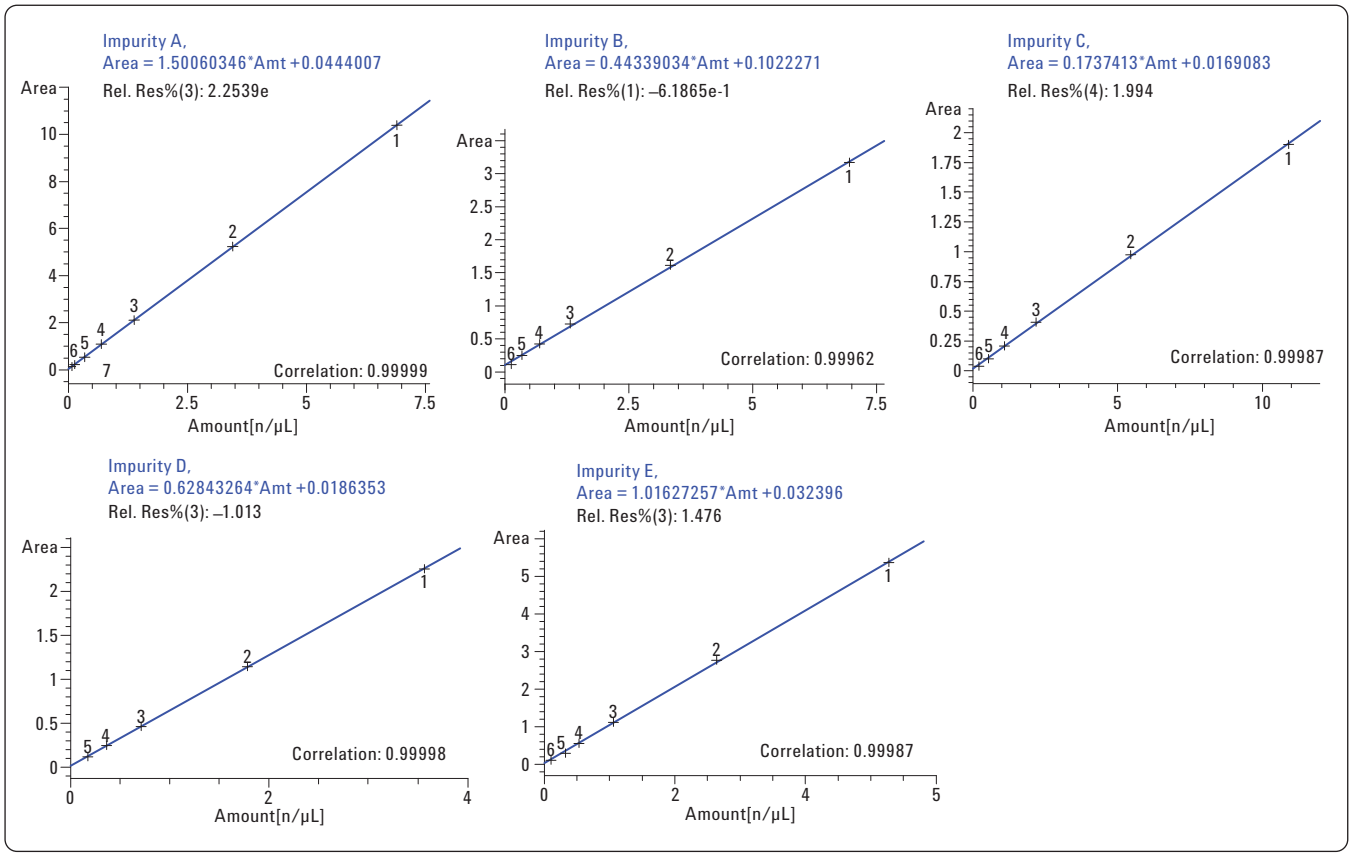

Figure 3
Linearity of impurities over all six concentration levels.

The limit of detection for the impurities was between 0.02 ng and 0.17 ng injected amount with a signal-to-noise ratio of 2 (Figure 4 red trace). Level 6 was used to determine the minimum detectable level. The limit of quantitation was 20 times higher, close to concentration level 4, (Figure 5 blue trace).

In Table 3, the results for LOD and LOQ are combined.

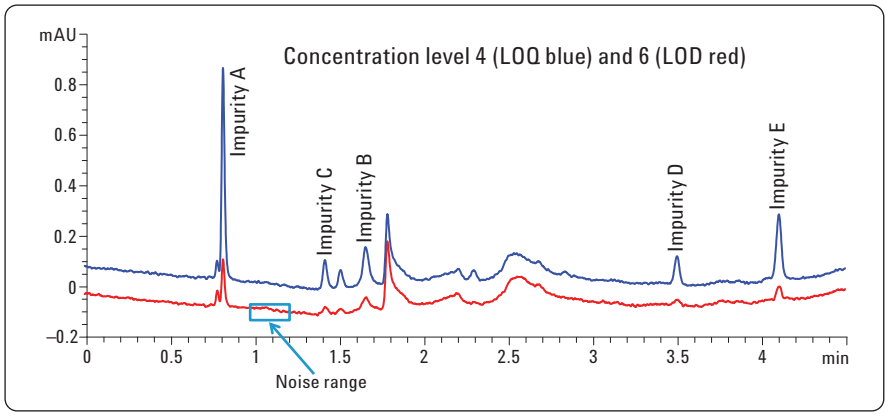

Figure 4
Concentration level 4 (blue trace) and level 6 (red trace), 1 µL injected.

	LOD amount (ng/µL)	Concentration level 6 amount (ng/µL)	LOQ (times 20 LOD) amount (ng/µL)	Concentration level 4 amount (ng/µL)
Impurity A	0.02	0.138	0.4	0.69
Impurity C	0.17	0.1392	3.4	0.696
Impurity B	0.06	0.218	1.2	1.09
Impurity D	0.075	0.0714	1.5	0.357
Impurity E	0.047	0.1056	0.94	0.528

Table 3
Results for LOD and LOQ.

Analysis of the amoxicillin sample

Figure 5 shows the chromatogram of the amoxicillin sample. The sample was dissolved in water to a final concentration of 1 mg/mL.

The results are presented in Table 4, showing that impurities in the 0.01% range can be detected. Amoxicillin is present at a percentage level of 96%. The percentage levels for the impurities are calculated in relation to this level.

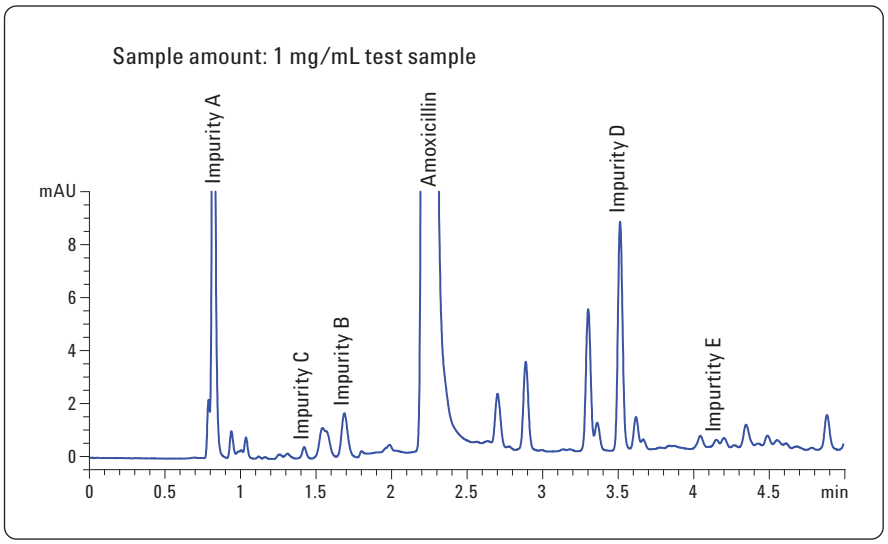

Figure 5
Amoxicillin sample, 1 µL injected.

	Area (mAU's)	ng/µL	Percentage
Impurity A	18.35269	12.20062	0.5
Impurity C	0.340461	1.86227	0.01
Impurity B	3.96829	8.71932	0.11
Impurity D	8.67657	13.77702	0.25
Impurity E	0.719148	0.675756	0.02
Amoxicillin	3360.63696		96

Table 4
Results of real life sample.

Conclusion

Amoxicillin and its impurities were analyzed on the Agilent 1220 Infinity LC System using a 150 mm × 4.6 mm column with 1.8 µm particles. At a flow rate of 2 mL/min (at 525 bar) the analysis was completed within seven minutes. Under these conditions, impurities down to a level of 0.01% could be detected. This shows that the Agilent 1220 Infinity LC System is an ideal instrument for impurity analysis in a pharmaceutical quality control laboratory.

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