High throughput LC/MS TOF analysis of drug degradation products

Agilent 1200 Series Rapid Resolution LC and Agilent 6210 Time-of-Flight MS with alternating column regeneration

Application Note

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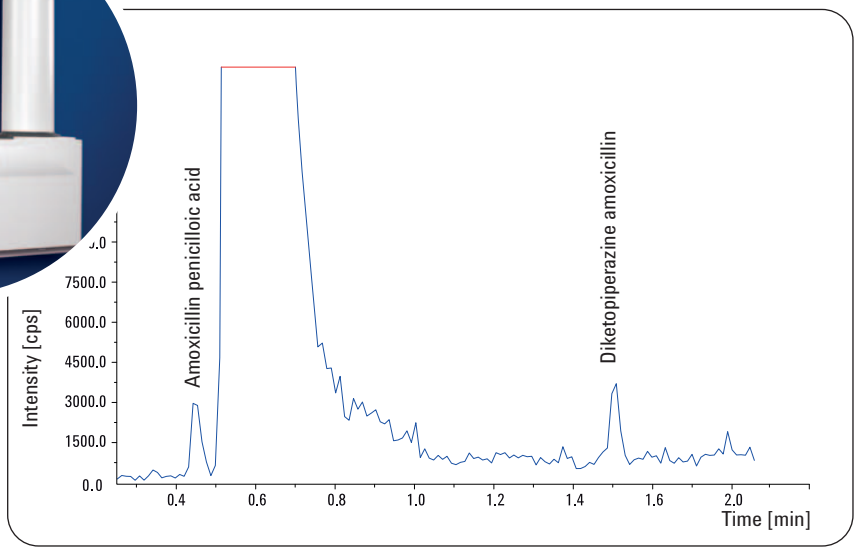

Abstract

This Application Note describes:

- The use of a high-throughput rapid resolution LC/MS TOF system for detection of impurities in a pharmaceutical formulation in more than 1000 samples per day.
- The instrument setup and a method for alternating column regeneration in a high-throughput LC system to reduce the overall analysis time by 50 %.
- The use of accurately measured molecular masses for formula confirmation and confident identification of impurities at levels below 0.1 %.

Agilent Equipment:
1200 Rapid Resolution LC system
6210 Time-of-Flight MS
MassHunter Workstation software

Application Area:
Pharmaceutical formulation trials and production

Agilent Technologies

Introduction

In modern pharmaceutical production processes it is crucial to monitor drug production or formulation processes for generation of by-products. Any compounds which emerge, even at very low concentrations, as a result of production or formulation conditions must be detected and confidently identified because of their potential toxicity to humans[1].

As early as possible during drug development, it is necessary to elucidate all possible by-products which could occur during production, formulation and degradation[2,3]. Continuous monitoring for these by-products ensures a high quality, final product.

However, monitoring of production processes and formulation trials generates a large number of samples for analysis, creating the need for a high-throughput LC/MS system, which is capable of analyzing over 1000 samples per day.

This Application Note describes the use of a high-throughput Agilent 1200 Series Rapid Resolution LC system with an Agilent 6210 Time-of-Flight mass spectrometer to monitor and identify degradation products emerging during a formulation trial of the antibiotic amoxicillin. The equipment details (figure 1), methodology and final result of the optimization with minimized by-products are described.

High throughput LC is achieved by alternating between two columns (packed with 1.8 µm particles for optimal resolution and speed[4,5]), using overlapping injections (figure 2). The LC system is directly connected to a TOF mass spectrometer capable of scanning at a high speed compatible with the LC system and providing the necessary dynamic range of at least 3 decades. With this configuration, total analysis time for a complete set of samples
was reduced by 50 %, degradation products were detected at levels below 0.1 % level and all degradation products which appeared in the formulation trial were identified by accurate mass measurement and empirical formula confirmation.

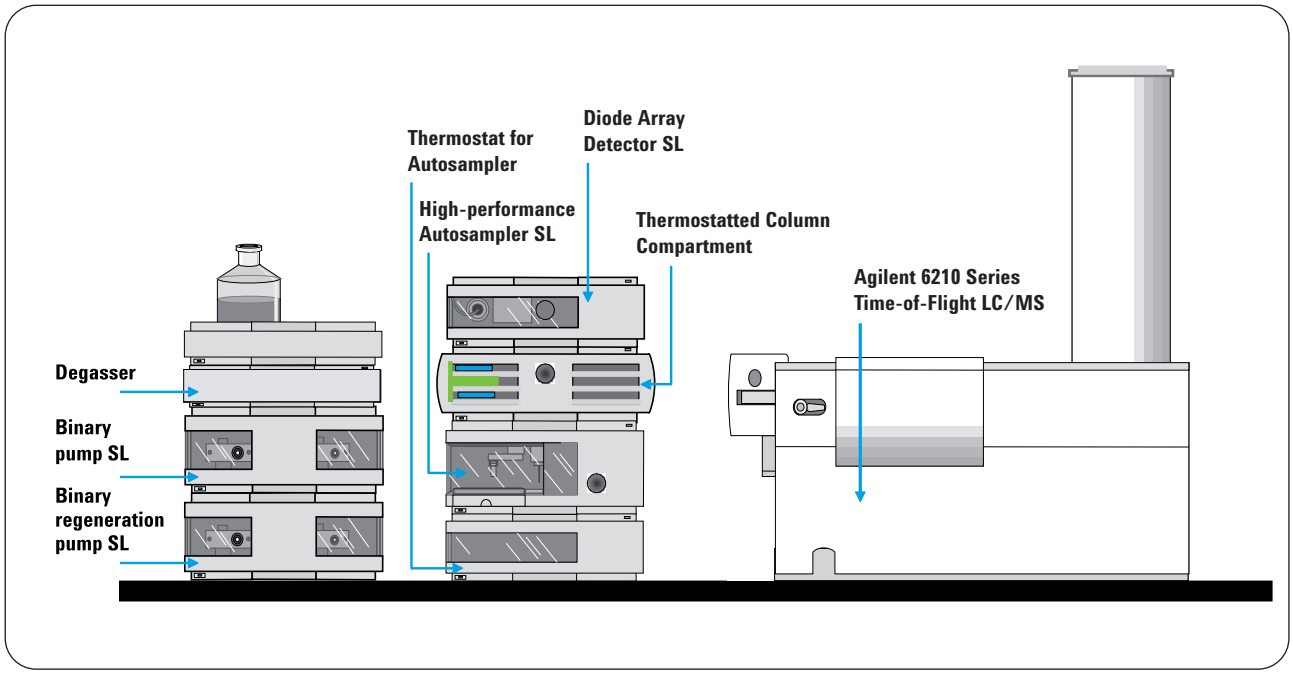

Figure 1
Instrument configuration for fast LC/TOF with alternating column regeneration.

Experimental

Equipment

- Agilent 1200 Series binary pump SL with degasser for high resolution HPLC analysis with a 1.8 µm particle size column.
- Agilent 1200 Series high performance autosampler SL with thermostat, designed to provide the lowest delay volume when used with the Agilent 1200 Series binary pump SL.
- Agilent 1200 Series thermostatted column compartment optimized for use with the Agilent 1200 Series binary pump SL and including a 2-position/10-port valve (figure 2) to enable column switching for alternating column regeneration and optional separate low dispersion heat exchangers and post column cooling for optimized delay volume conditions
- Agilent 1200 Series diode array detector SL, capable of acquiring data at a sampling rate up to 80 Hz and with built-in data storage capability.
- TOF instrument control software MassHunter Workstation A.02.00 for data acquisition, and Analyst software for data analysis.
- Agilent 6210 TOF orthogonal acceleration time-of-flight mass spectrometer with dual sprayer interface for mass calibration to acquire molecular masses with highest accuracy. Data acquisition rate 40 Hz and positive/negative switching.
- Columns: Two ZORBAX SB C18, 2.1 x 50 mm, 1.8 µm particle size.

Method

- Solvent A: Water + 5 mM ammonium formate, pH 4.3; Solvent B: ACN
- Gradient 1: 0 min, 0 %B; 0.2 min, 0 %B; 3 min, 25 %B
  Flow rate: 1 mL/min
  Stop time: 3 min.
  Post time: 2 min

  Gradient 2: 0 min, 0 %B; 0.2 min, 0 %B; 2 min, 25 %B
  Flow rate: 1 mL/min
  Stop time: 2 min
  Post time: 2 min.
- Column regeneration: Solvent A: Water + 5 mM ammonium formate, pH 4.3.
  Flow rate: 1 mL/min.
- Autosampler with automated delay volume reduction and overlapped injection functions; 1 µL sample injections with needle wash; samples cooled to 4 °C.
- Diode array detection: 210 nm ±4 nm, Ref. 360 ±16 nm with 2 µL flow cell, 3 mm path length.
- Column temperature: 50 °C, thermostatically controlled. At the end of a run, each column was switched into the alternate flow path for regeneration.
- MS analysis: ESI source in positive mode with dual spray for reference mass solution.
  Dry gas: 12.0 L/min
  Dry Temperature: 350 °C
  Nebulizer: 50 psi
  Scan: 50-1000 at 40 Hz
  Fragmentor: 200 V (300 V CID)
  Skimmer: 60 V
  Capillary: 5000 V

Sample:

Samples from a formulation trial of the antibiotic drug amoxicillin, collected at various time points for LC/MS analysis.

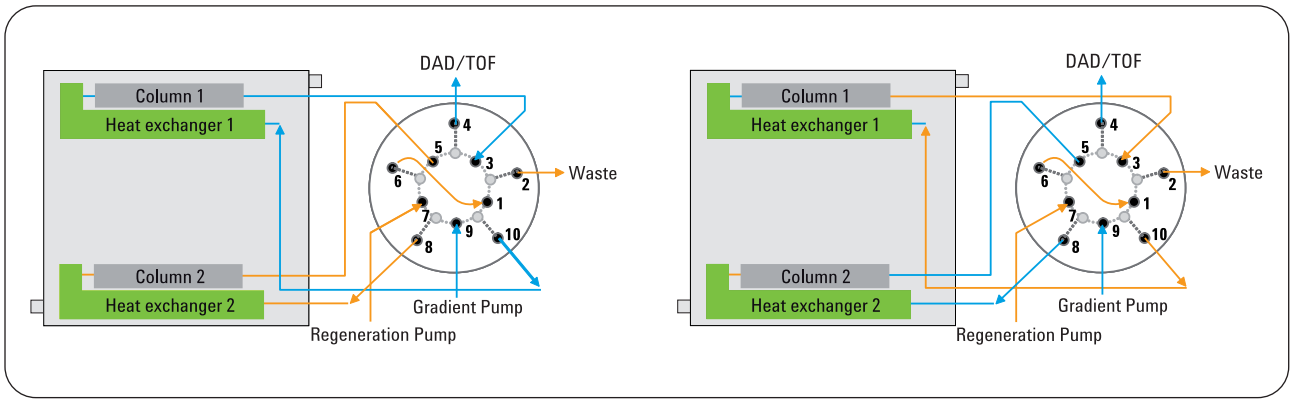

Figure 2
Column switching at the 2-position/10-port valve for fast LC/TOF with two columns (2.1 mm x 50 mm, 1.8 µL).

Results and Discussion

Reduction in total analysis time

By switching columns between alternate flow paths, so that one column was being regenerated while the other was being equilibrated, the total analysis time for a complete sample set was reduced by 50 %. Gradient and column regeneration times were 2 or 3 minutes, depending of the gradient used.

Monitoring of degradation

The emerging degradation products were identified in the MS TOF files by accurate mass measurement and empirical formula confirmation. The chromatogram obtained from the first sample shows only slight degradation (figure 3) with two degradation products at 0.95 minutes and 2.51 minutes retention time. After 30 minutes under stress conditions, significant degradation has occurred, with additional degradation products emerging at 1.92, 2.10 and 2.26 minutes (figure 4).

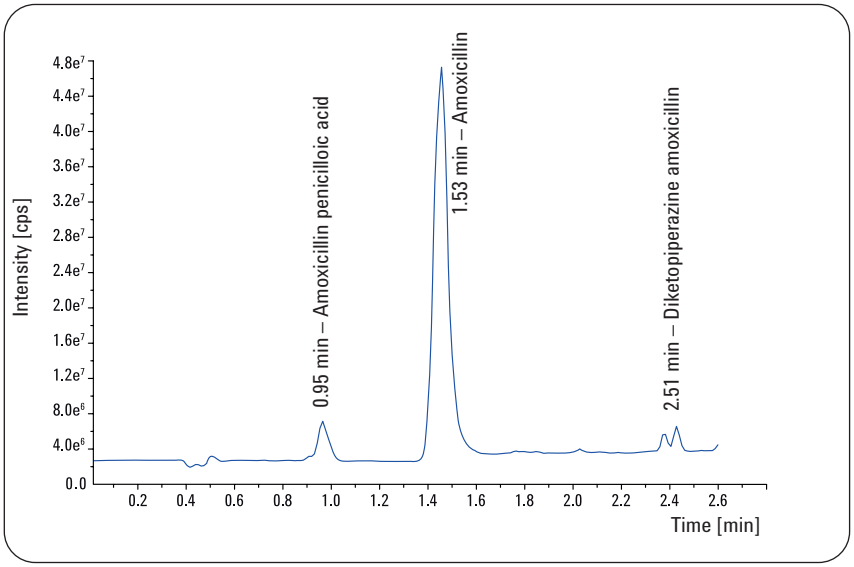

Figure 3
MS TOF TIC of the first time point in the degradation experiment of the antibiotic drug amoxicillin (gradient 1).

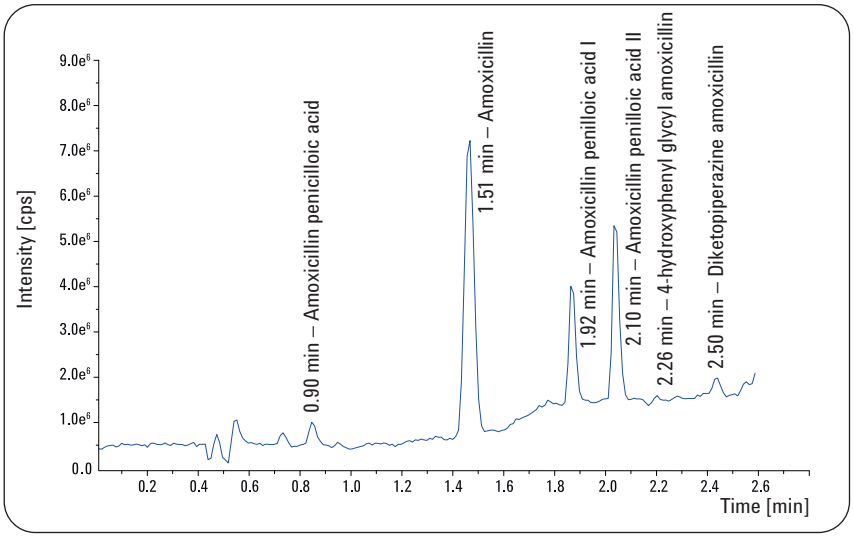

Figure 4
MS TOF TIC of the degradation experiment of the antibiotic drug amoxicillin with the identified degradation products (gradient 1).

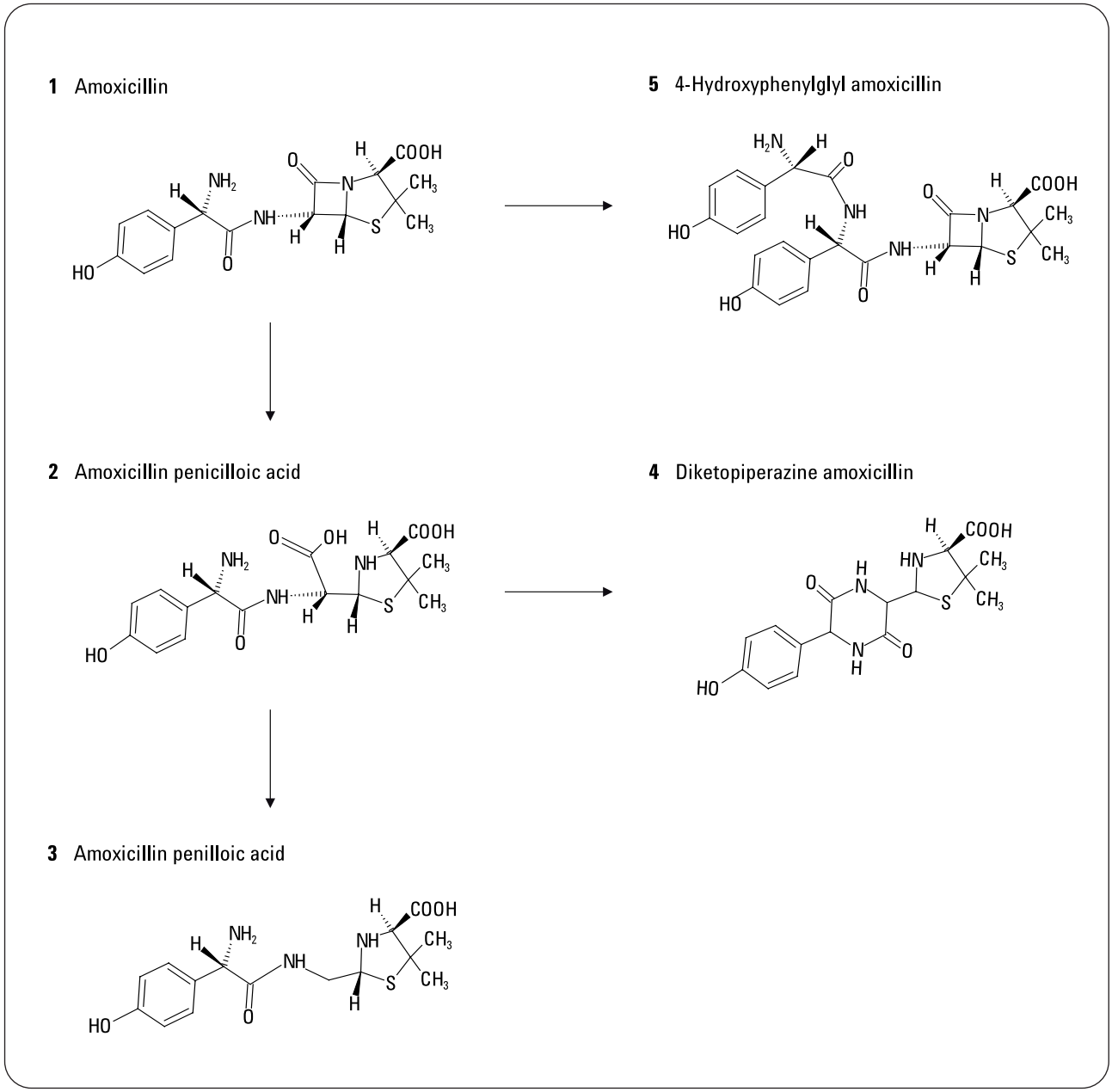

Figure 5
Degradation reactions of amoxicillin.

Identification of degradation products

The previously known degradation products were identified by empirical formula confirmation based on the highly accurate mass measurements. The degradation of amoxicillin (1) starts with an opening reaction of the four-membered beta-lactame ring and different subsequent reactions (figure 5). The first degradation product is amoxicillin penicilloic acid (2). This initial degradant undergoes further reactions.

After decarboxylation two stereoisomeric compounds amoxicillin penilloic acid (3) will be obtained or after new formation of a stable six-membered ring the stable product diketopiperazine amoxicilline (4) will be formed. The TOF mass spectrum of (2) shows the $[M+H]^+$ ion at m/z 384.1220 and the molecule fragment coming from a loss of NH_3 at m/z 367.0953 (figure 6).

Other typical CID fragments are also present, which allow identification by empirical formula confirmation (table 1).

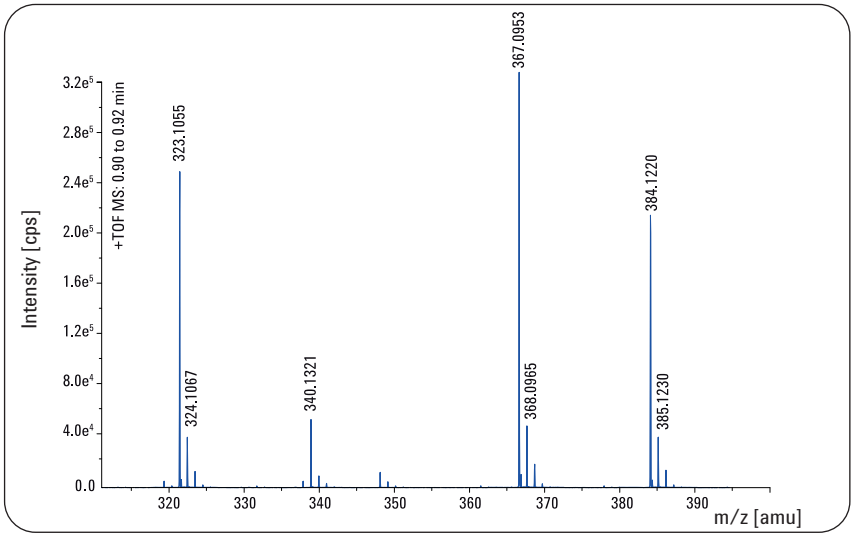

Figure 6
Extracted TOF mass spectrum of amoxicillin penicilloic acid (2).

Measured mass	Calculated mass	Formula	Mass accuracy [mDa]	Mass accuracy [ppm]
384.1220	384.1229	$C_{16}H_{22}N_3O_6S$	-0.90	2.43
367.0953	367.0964	$C_{16}H_{19}N_2O_6S$	-1.10	2.95
340.1321	340.1331	$C_{15}H_{22}N_3O_4S$	-1.00	2.94
323.1056	323.1066	$C_{15}H_{19}N_2O_4S$	-1.00	2.95

Table 1
Achieved mass accuracies and confirmed empirical formulas of fragments from amoxicillin penicilloic acid (2) from CID experiment.

The TOF mass spectrum of degradation product (4), which has the same isobaric mass as compound (1), shows the $[M+H]^+$ ion at m/z 366.1115 and the sodium adduct at m/z 388.0931 (figure 7 and table 2). Since the degradation of amoxicillin (1) starts with the opening reaction of the four-membered beta-lactame ring giving amoxicillin penicilloic acid (2) followed by the closure to the stable six-membered diketopiperazine ring in diketopiperazine amoxicillin (4), these are the first degradants which are detectable in minor amount in a formulation of amoxicillin (figure 8).

The empirical formulas of these minor by-products were calculated with mass accuracies of 2.68 ppm for $C_{16}H_{22}N_3O_6S$ (2) and with 3.19 ppm for $C_{16}H_{20}N_3O_5S$ (4).

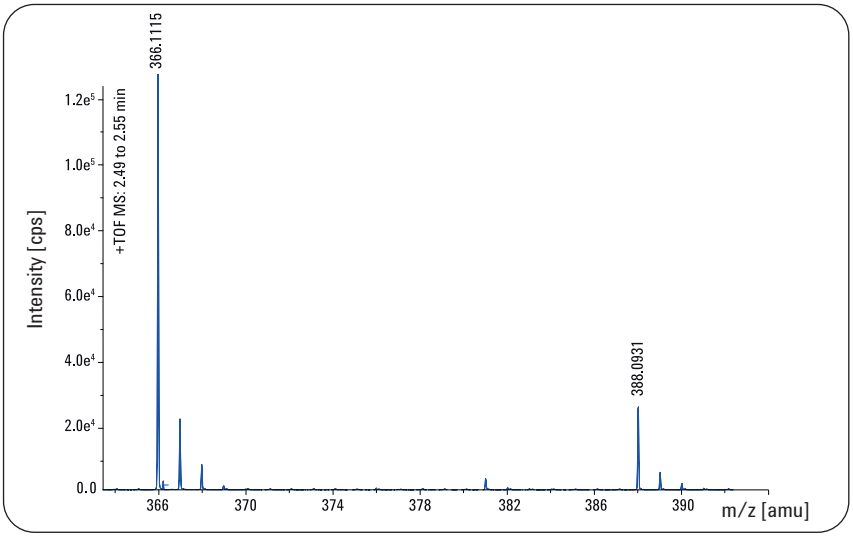

Figure 7
Extracted TOF mass spectrum of amoxicillin diketopiperazine amoxicillin (4).

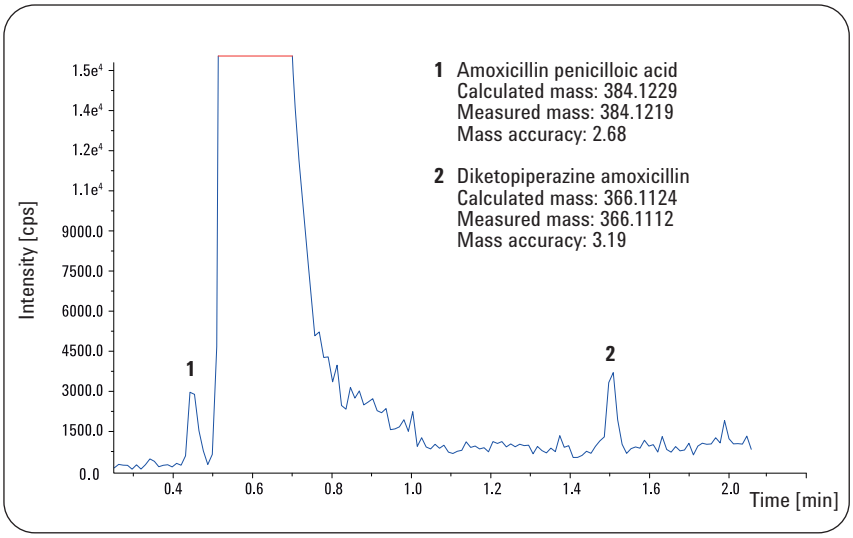

Figure 8
Detection of two 0.1 % minor impurities in a formulation of amoxicillin (gradient 2).

Measured mass	Calculated mass	Formula	Mass error [mDa]	Mass error [ppm]
388.0931	388.0943	$C_{16}H_{19}N_3O_5SNa$	-1.2	3.12
366.1115	366.1124	$C_{16}H_{20}N_3O_5S$	-0.9	2.37

Table 2
Achieved mass accuracies and confirmed empirical formulas of diketopiperazine amoxicillin (4) and its sodium adduct.

Conclusion

A high throughput LC/MS TOF method for identification of degradation products in a production process or formulation trial was developed successfully using an optimized configuration of the Agilent 1200 Series RRLC system and Agilent 6210 TOF MS. The method was tested using samples from a degradation experiment of the antibiotic drug amoxicillin. A significant reduction in analysis times was achieved and degradation products were confidently identified. Identification of two degradation products with content of 0.1% confirmed that the dynamic range of the TOF MS was sufficient for this analytical task.

References

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