Detection and identification of impurities in pharmaceutical drugs

Computer-assisted extraction, profiling and analysis of Q-TOF data for determination of impurities using Agilent MassHunter software

Application Note

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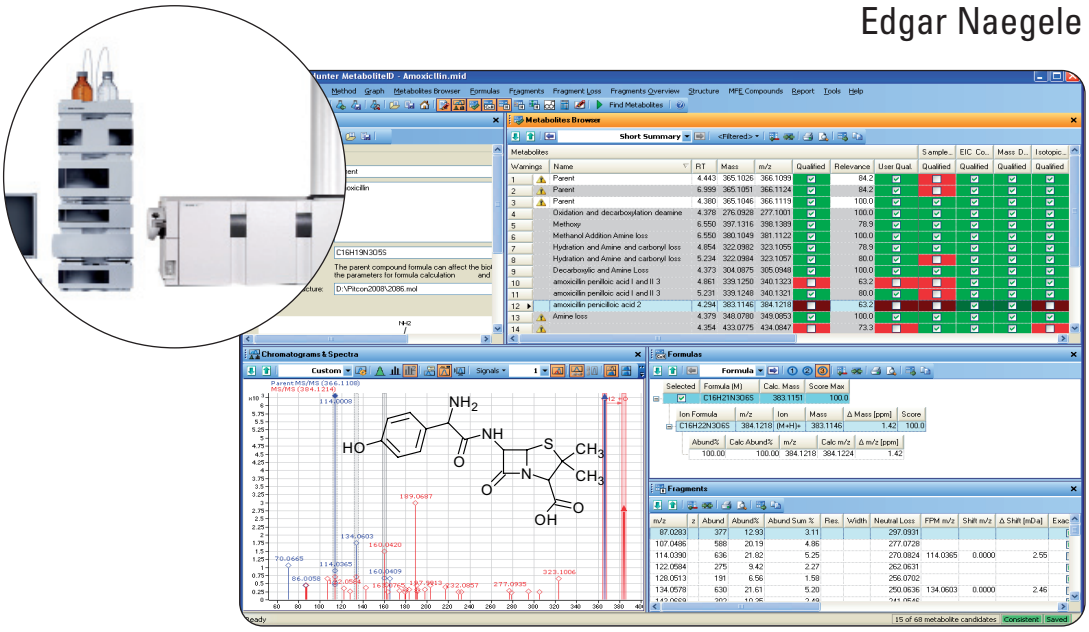


Abstract

This Application Note describes:

- Extraction of high resolution, accurate mass Q-TOF data from chemical background noise using the molecular feature extractor of the Agilent MassHunter software
- Differential analysis to identify impurities in pharmaceutical drugs using the Mass Profiler algorithm of the Agilent MassHunter Profiler software.
- Generation of molecular formulas for MS and MS/MS using the molecular formula generator of the Agilent MassHunter software
- Automated analysis of a degraded pharmaceutical drug and comparison with a non-degraded standard using the Agilent MassHunterMetID software

Agilent Equipment

- 1200 Series Rapid Resolution LC
- 6520 Accurate-Mass Q-TOF
- MassHunter software

Application area

Identification of degradation products in pharmaceutical drugs during drug development and quality control

Agilent Technologies

Introduction

In the production of pharmaceutical drugs, impurities can arise due to different means such as solvents, catalysts, synthesis building blocks or degradation. Besides these causes during the production process, impurities can arise in the final purified product by slow degradation during shelf storage in the sealed package under ambient temperature conditions. To identify potential degradation impurities, degradation studies with the pure substance are performed under various conditions[1]. With a basic knowledge of possible degradation products, studies with the final drug formulation under long time storage conditions are carried out. The LC/MS data obtained in such studies is often analyzed manually, which is a very time consuming process.

Modern software tools are available to improve data analysis. The first challenge is to extract the compounds of interest from the complex background that results form the matrix of the final drug formulation. This data extraction can be done with using the molecular feature extractor (MFE)[2] of the Agilent MassHunter software (figure 1). In the generation of the molecular features, which are the entity of molecular ions, isotopes and adducts at a certain retention time, chemical background is removed from the total ion chromatogram (TIC), and features are grouped and displayed in the processed TIC. Finally, the molecular formulas are generated for each feature, including information from isotopes and adducts generated by the molecular formula generation (MFG) algorithm of the Agilent MassHunter software. In the next step of the analysis of the differences between two groups, the impure group and the pure (control) group are analyzed (figure 2). For this analysis the Mass Profiler algorithm[3] of the Agilent MassHunter software compares a set of data of both groups and displays a plot that shows the unique features of each group. This makes it possible to find the degradation products that are unique to the impure group. The entire analysis can be performed by specialized data analysis software, comprising MFE, MFG, Mass Profiler and several other algorithms for data comparison and structure elucidation from Q-TOF MS and MS/MS information.

This Application Note describes the data analysis for detection and determination of low level impurities in final pharmaceutical drug formulations.

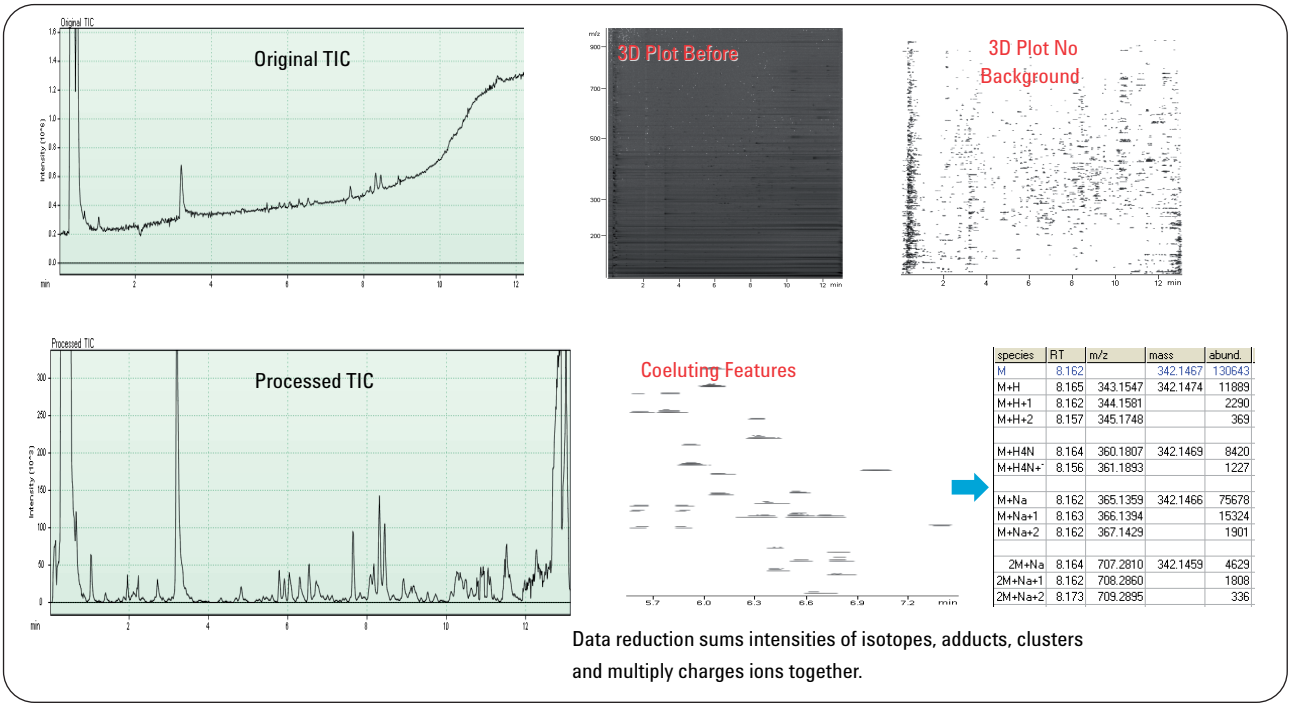


Figure 1
Automated data reduction using molecular feature extraction (MFE) of the Agilent MassHunter software.

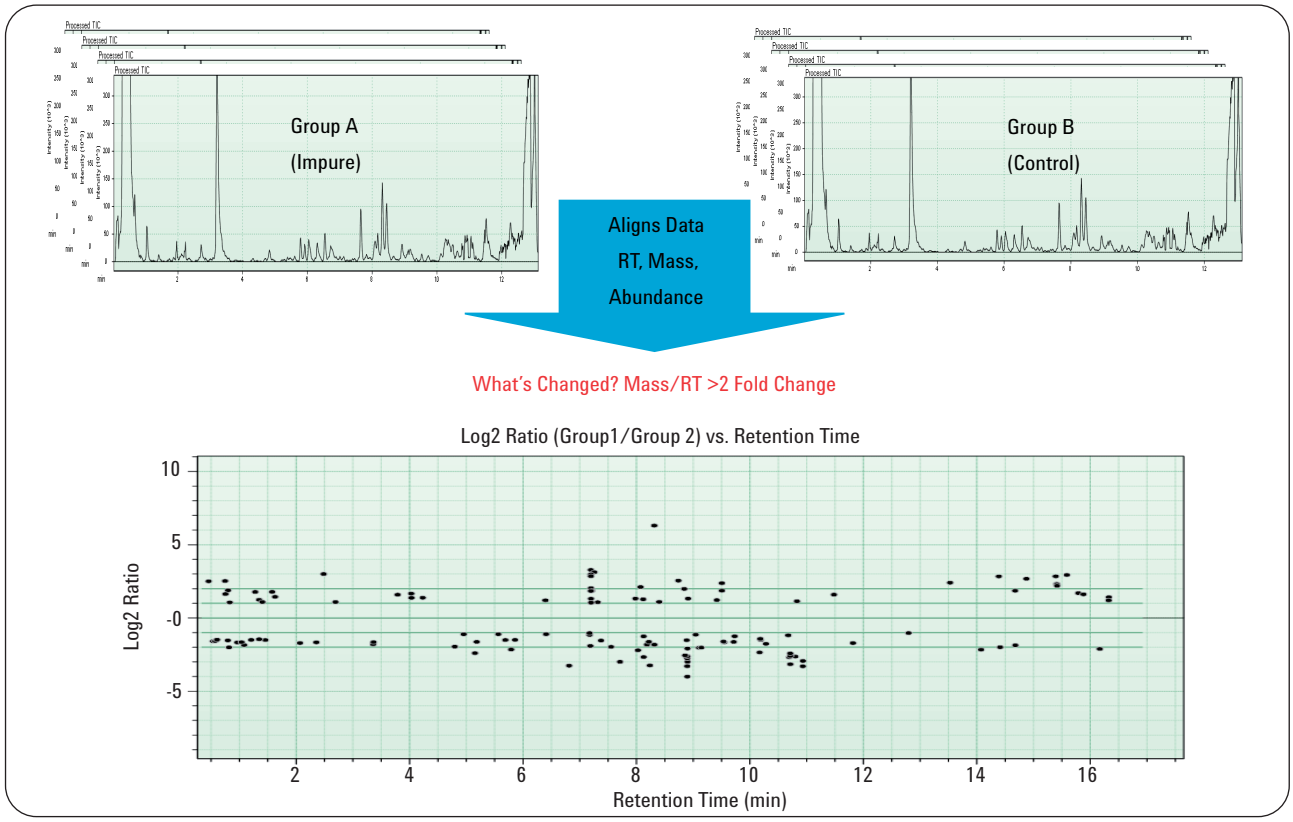


Figure 2
Impurity analysis using the Mass Profiler of the Agilent MassHunter software.

Experimental

Equipment

- Agilent 1200 Series Rapid Resolution LC system with binary pump SL and degasser, high performance autosampler SL with thermostat, and thermostatted column compartment SL
- Agilent 6520 Accurate-Mass Q-TOF
- Agilent MassHunter data acquisition software, qualitative software, MFE software, Mass Profiler software and MetID software
- ZORBAX Ecilpse Plus C18 column, 2.1 x 100 mm, 1.8 µm

Sample preparation

Tablets of the antibiotic drug amoxicillin (new and stored for 6 months at ambient temperature) were dissolved in 40 mL methanol/water (1/1 v/v) with 0.1 % formic acid, mixed for 20 minutes, centrifuged for 5 minutes at 14,000 rpm, and diluted 1:100 and 1:1000 with water.

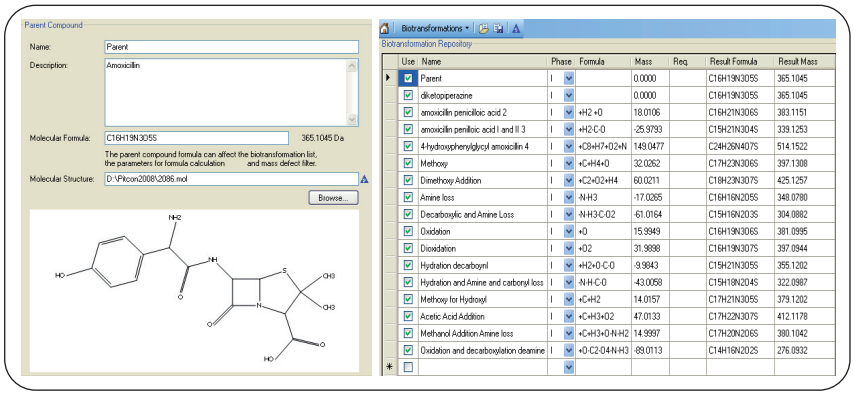


Figure 3
Method input for MetID software.

High resolution LC/MS method

Solvent A: Water, 5 mM NH_4Ac
Solvent B: Methanol
Flow rate: 0.4 mL/min
Gradient: 0 min, 5 %B;
13 min, 95 %B
Stop time: 13 min
Post time: 10 min
Inj. volume: 1-10 µL, needle wash, samples cooled to 4 °C
Column temp.: 50 °C.

Q-TOF MS and MSMS method

Source: ESI in positive mode with dual spray for reference mass solution
Dry gas: 10.0 L/min
Dry Temp.: 250 °C
Nebulizer: 45 psi
Mass range: 100-1000
Fragmentor: 165 V
Skimmer: 60 V
Capillary: 4000 V
Collision energy: 30 V
Data-dependent MS/MS:
2 compounds, 3 MS/MS spectra, exclusion for 0.25 minutes. For data acquisition the high resolution and the enhanced dynamic range modes were used.

Data analysis with MassHunter MetID software

The first step in the analysis comprised a comparison between the data from the degradation products (sample) and the data from the pure parent drug (control). All detectable mass signals were extracted from the MS level data using the molecular feature extraction (MFE) algorithm. Adduct masses of related compounds were grouped together into discrete molecular features and chemical noise was removed. The compound lists of the degraded sample and the control were then compared. All new compounds or those which increased in amount in the degraded sample were considered to be potential degradation products and subjected to further analysis by different user-specified algorithms. The molecular mass and structure information as well as the possible degradation reactions were introduced for the analysis (figure 3). The algorithms were able to identify and qualify new degradants or simply qualify degradation compounds found by another algorithm. The results of all compound identification algorithms were weighted and combined to a final identification relevance score. Degradation products were qualified when their final score was above a defined relevance threshold. The results from all algorithms were collated in a results table, which could be inspected at-a-glance. The workflow in the MetID software is summarized in table 1.

Results and discussion

Following data analysis, the identified potential degradation products were then displayed in a table (figure 4). The displayed compounds were identified by various algorithms in the software, for example, the compounds were new in the degraded sample and were identified by the Mass Profiler algorithm. Other algorithms checked the fit with an isotopic pattern comparative to the

1	Parent Compound	MW, Formula and Structure
2	Transformations	List Proposed Degradation Products
3	Identification Criteria	Vary Importance of Tests
4	Find Compounds by MFE	Molecular Feature Extraction
5	Find Compounds by AutoMSMS	For MSMS data
6	Sample Comparison	Mass Profiler What is Different
7	Isotope Pattern Filtering	Best for Halogenated Species
8	Mass Defect Filtering	From Proposed Compounds
9	EIC Generation	Confirms Presence of Compounds

Table 1
Overview of workflow in the MetID software.

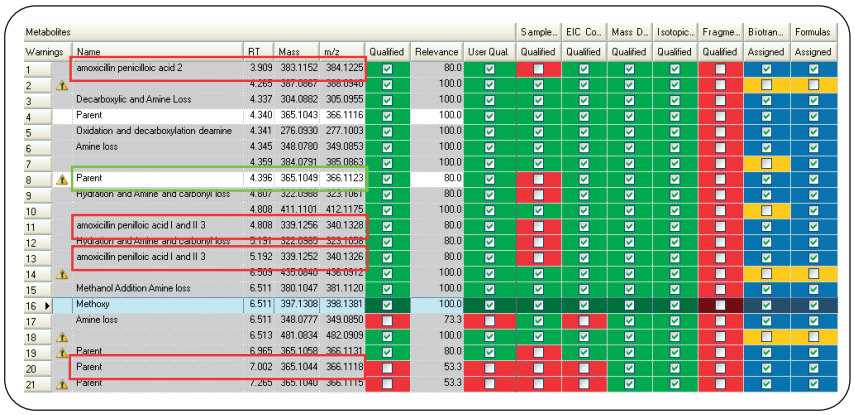


Figure 4
Proposed degradation products qualified by various algorithms.

RT [min]	Compound name	Formula	Calculated mass	Measured mass	Mass accuracy [mDa]	Mass accuracy [ppm]
3.90	amoxicillin penicilloic acid 2	$C_{16}H_{22}N_3O_6S$	384.1224	384.1225	-0.1	-0.31
4.39	amoxicillin 1	$C_{16}H_{20}N_3O_5S$	366.1118	366.1123	-0.5	-1.32
4.80 5.19	amoxicillin penilloic acid I and II 3	$C_{15}H_{22}N_3O_4S$	340.1325	340.1328 340.1326	-0.3 -0.1	-0.73 -0.14
7.00	diketopiperazine amoxicillin 4	$C_{16}H_{20}N_3O_5S$	366.11182	366.1118	0.02	0.05

Table 2
Measured accurate mass of amoxicliin degradation products and calculated formula (masses and accuracies are rounded).

pure parent drug and the fit in a mass defect window typical for the modifications by degradation of the parent drug. Finally, the reaction was assigned by comparison to the defined possible degradation reactions from the data analysis method.

The following known degradation products were identified in the degraded tablets:

Amoxicillin penicilloic acid (2)
at retention time
3.90 min and
m/z 384.1225

Amoxicillin penilloic acids I and II (3)
at retention times
4.80 and 5.19 min and
isobaric masses
m/z 340.1326 and
340.1328

Diketopiperazine amoxicillin (4)
at retention time
7.00 min and
m/z 366.1118

The parent drug amoxicillin (1) was found at retention time 4.39 minutes and *m/z* 366.1123 (figure 5). For confirmation the software calculated the molecular formula from the measured accurate mass (table 2). The parent compound amoxicillin (1) and the impurity (4) occurred with isobaric mass. To elucidate the structure of unknown or isomeric impure compounds, the MS/MS information was combined with the accurate mass measurements. For each MS/MS fragment a formula was calculated, which had to match with the calculated formula of the parent ion (figure 6).

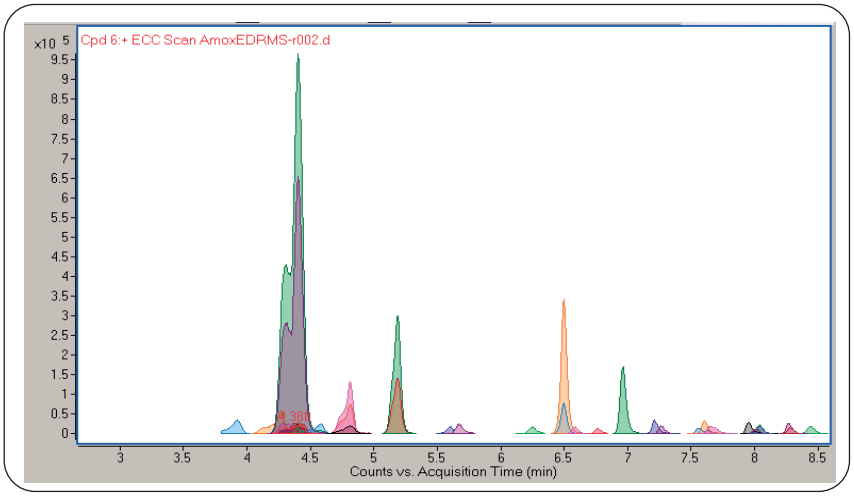
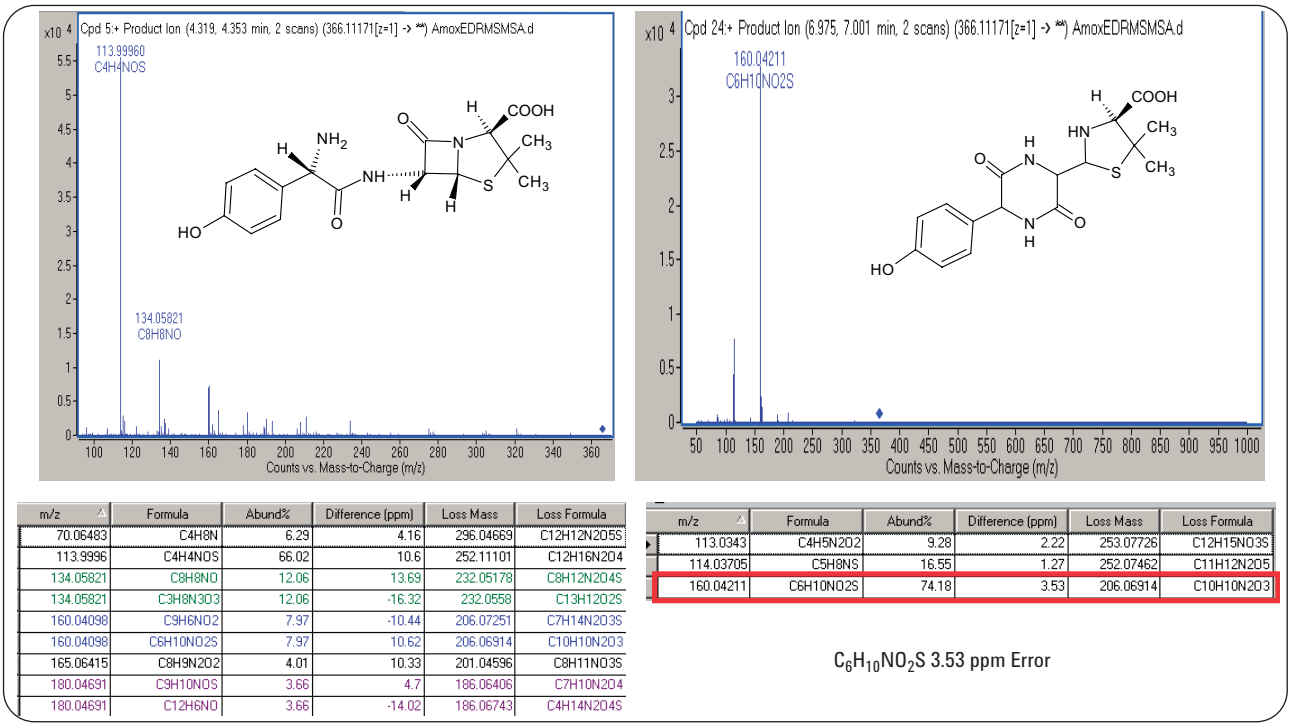


Figure 5
Extracted ion chromatograms of amoxicillin and degradation products.



m/z	Formula	Abund%	Difference (ppm)	Loss Mass	Loss Formula
70.06483	C4H8N	6.29	4.16	296.04669	C12H12N2O5S
113.9996	C4H4NOS	66.02	10.6	252.11101	C12H16N2O4
134.05821	C8H8NO	12.06	13.69	232.05178	C8H12N2O4S
134.05821	C3H8N3O3	12.06	-16.32	232.0558	C13H12O2S
160.04098	C9H6NO2	7.97	-10.44	206.07251	C7H14N2O3S
160.04098	C6H10NO2S	7.97	10.62	206.06914	C10H10N2O3
165.06415	C8H9N2O2	4.01	10.33	201.04596	C8H11NO3S
180.04691	C9H10NOS	3.66	4.7	186.06406	C7H10N2O4
180.04691	C12H6NO	3.66	-14.02	186.06743	C4H14N2O4S

m/z	Formula	Abund%	Difference (ppm)	Loss Mass	Loss Formula
113.0343	C4H5N2O2	9.28	2.22	253.07726	C12H15NO3S
114.03705	C5H8NS	16.55	1.27	252.07462	C11H12N2O5
160.04211	C6H10NO2S	74.18	3.53	206.06914	C10H10N2O3

$C_6H_{10}NO_2S$ 3.53 ppm Error

Figure 6
Structural information from MS/MS $C_{16}H_{19}N_3SO_5$.

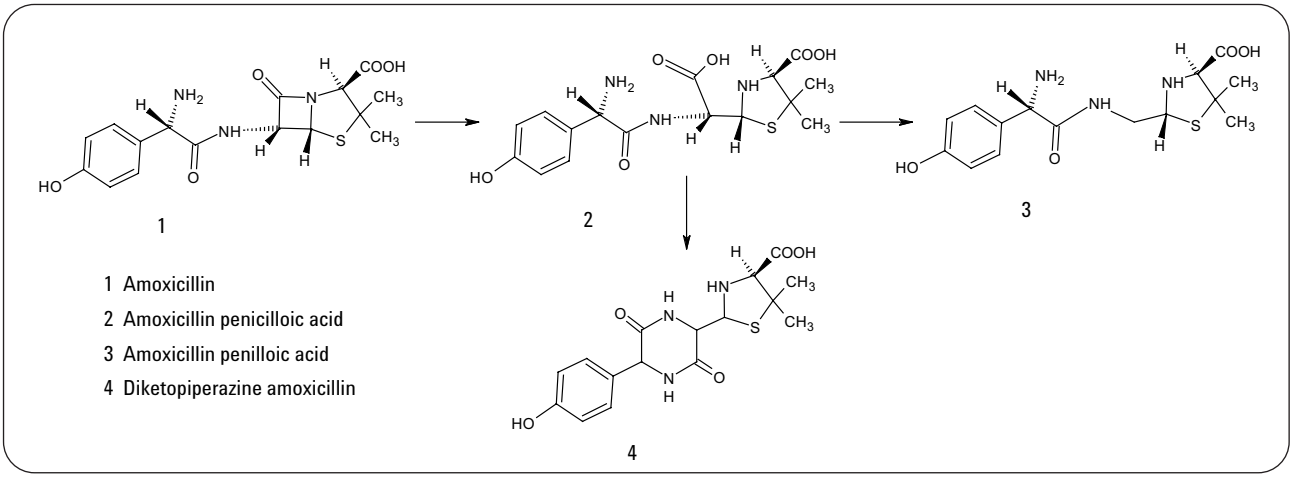


Figure 7
A possible degradation pathway.

For the isomeric compounds (1) and (4), the differences can be clearly seen from the MS/MS spectra and the calculated fragment formulas. The degradation product (4) had a main fragment at *m/z* 160.04211 with the formula $C_6H_{10}NO_2S$ and the remaining neutral loss had the formula $C_{10}H_{10}N_2O_3$, which fits with the parent formula $C_{16}H_{20}N_3O_5S$. The final degradation pathway for these impurities is shown in figure 7.

Conclusion

This Application Note demonstrated the use of the Agilent MassHunter MetID software for the identification of degradation products in the final formulation of the pharmaceutical drug amoxicillin. The degradation products were extracted from the Q-TOF data by the molecular feature extractor and newly emerging compounds were filtered out by the Mass Profiler algorithm. After isolation of the relevant compounds, they were classified by other algorithms such as mass defect filtering and isotopic pattern matching, and then assigned to possible degradation reactions. Finally, 17 degradation products were identified and an initial degradation pathway was assigned.

References

1.
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