Identification of antibiotic degradation products with UHPLC-QTOF system

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ABSTRACT

As emerging environmental pollutants, antibiotics are abundant in the environment. In order to effectively remove antibiotics, while reducing secondary pollution, there is need to study their degradation pathways. In this poster, a simple and widely applicable workflow was established to study the degradation products of antibiotics, using sulfamethoxazole as an example. Using this workflow, researchers can reduce the experimental time, simplify the data processing and improve workflow efficiency.

INTRODUCTION

Antibiotics are a group of secondary metabolites produced by microorganisms or other higher plants and animals. They are the most important type of antibacterial drugs used in the treatment and prevention of bacterial infections. The residual amount of antibiotics in the environment has increased due to the overuse of antibacterial drugs in people, as well as in the animals we consume. Sulfamethoxazole (SMX) as a representative of refractory and impediment antibiotics, has been detected from 0.01 μg/L to 2.0 μg/L in surface waters from different countries. In addition, the use of antibiotics has led to the rapid emergence of antibiotic-resistant bacteria and antibiotic resistant genes. Antibiotic resistance has been listed by the World Health Organization (WTO) as one of the major threats to global health.[1-3]

Antibiotics in the environment can undergo degradation reactions. While their metabolism and degradation products are often less active than the parent antibiotics, the toxicity might increase significantly. Antibiotics in aquatic environments are mainly from the discharge of domestic wastewater, pharmaceutical wastewater, medical wastewater and aquaculture wastewater. The effectiveness of antibiotic treatment in wastewater treatment plants has become a key link in controlling antibiotics into the environment. The removal rate of antibiotics and the risk of secondary pollution are the criteria for the effectiveness of sewage treatment. The amount of antibiotics used in China is much higher than that in western developed countries, so it is necessary to study the antibiotic degradation pathways and potentially harmful degradation products.

Traditionally, multiple injections are necessary to study the antibiotic degradation products. For example, multiple injections may be required to separately find the possible degradation products from the complex mass spectrum, obtain the MS/MS fragmentation spectrum of each product, and then finally to identify the structure of the degradation product from the MS and MS/MS spectrum. The final step often requires a high level of chemical knowledge for researchers. In this paper, we use sulfamethoxazole – a frequently detected antibiotic in the aquatic environment – as an example chemical to establish a simple, fast, and widely applicable experimental procedure for the study of antibiotic degradation pathways.[1, 4]

MATERIALS AND METHODS

Sample preparation: In this study, $CuFe_2O_4$ magnetic spinel nanoparticles (6 g/L) were synthesized with a sol–gel combustion method and coupled with hydroxylamine (HA, 1.0 mM) to degrade sulfamethoxazole (SMX, 10 μg/mL) in aqueous solution. 1 mL samples were taken and quenched with 50 μL of sodium thiosulfate immediately at 0.5 min, 2.5 min, 5 min and 10 min. The mixture of 4 time points were filtered with 0.45 μm filter membrane and stored at 4°C. A blank was prepared as described above except that SMX was not added.[5]

UHPLC conditions: Chromatography was performed with a SCIEX ExionLC AC system on a Kinetex F5 column (2.6 um, 2.1 × 100 mm) at 40°C. A 20 min gradient of mobile phase A (methanol) and mobile phase B (2 mM ammonium formate in water) was used at a flow rate of 400 μL/min. The injection volume was set to 10 μL.

MS/MS conditions: An SCIEX X500R QTOF system combined with SCIEX OS software was operated in positive ion mode electrospray ionization (ESI). Source conditions and the method settings for non-targeted, IDA-MS/MS acquisition methods are listed below:
Mass Range: TOF MS, 100-1000 Da; TOF MS/MS, 50-1000 Da
CE: 35 ± 15 V
IDA criteria: 10 most intense candidate ions, DBS ON

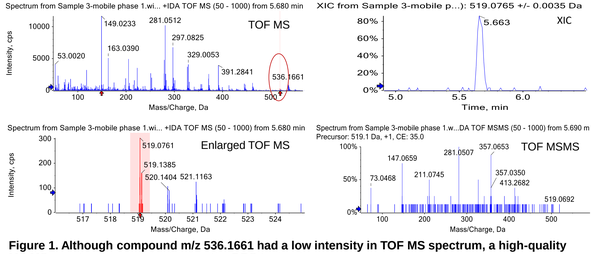

Figure 1. Although compound m/z 536.1661 had a low intensity in TOF MS spectrum, a high-quality MS/MS spectra was acquired using the DBS technology.

RESULTS

Due to the high scan speed and dynamic background subtraction (DBS) technology of the X500R QTOF system, more than 4,700 MS/MS spectra were acquired with a single injection. DBS effectively reduced the interference of complex matrices and ensured that the MS/MS spectra at low concentrations of suspected degradation products are also acquired with high-quality (Figure 1).

To discover and identify degradation products in the samples, suspected screening and non-targeted screening approaches were employed using SCIEX OS software. A suspect screening approach was used for degradation products that have been reported in the published literature. The confidence criteria used for screening were mass error and isotope ratio difference. A traffic light system where different colors were assigned to different performance levels provided a rapid method to assess the match quality. For example, in the case of mass error, green represented mass errors less than 5 ppm; orange, between 5 and 10 ppm; and red, larger than 10 ppm. A representative search result is also shown (Figure 2).

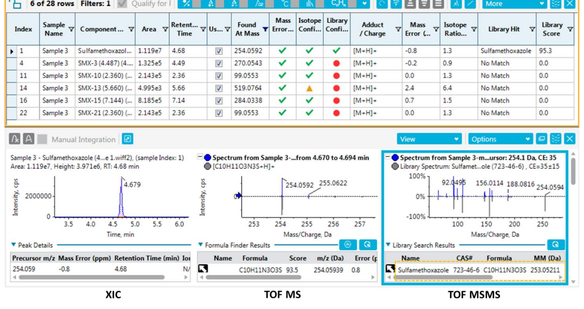

Figure 2. Identification of sulfamethoxazole using suspect screening.

For unknown degradation products, non-targeted screening was used to find compounds that are present in the sample but not in the blank, as well as their possible formulas. Using this technique several unexpected products were found. For example, two chlorinated intermediates were found and identified. Presumably, the Cl- was released from hydroxylamine hydrochloride. The representative search result is shown in Figure 3.

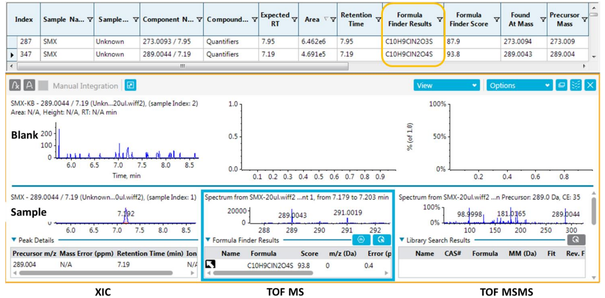

Figure 3. Non-targeted for screening for unknown degradation products of sulfamethoxazole.

To identify degradation products, the “Fragments” pane in the SCIEX OS software was used to compare the acquired MS/MS spectrum to that of the candidate structure (Figure 4). In addition, the potential formula was searched against the ChemSpider database. ChemSpider also contains candidate structures for each formula, asd well as their theoretical MS/MS spectra, which can be compared to the acquired MS/MS spectrum (Figure 5).

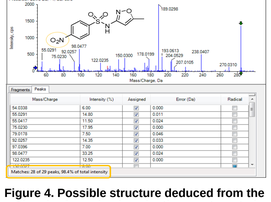

Figure 4. Possible structure deduced from the molecular formula given by the non-targeted screening, matched with the acquired MS/MS spectra through the “Fragments” pane.

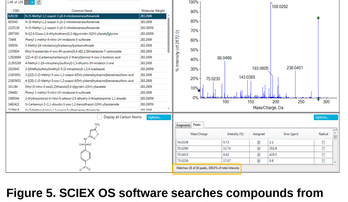

Figure 5. SCIEX OS software searches compounds from the ChemSpider website based on the calculated formula and performs simulation matching to acquired MS/MS spectra.

Fourteen degradation products of SMX in the $CuFe_2O_4$/HA system including P-284, P- 300, P-273, P-289, P-255, P-271, P-109, P-99, P-114, P-270 (RT: 4.8 min), P-286, P-270 (RT: 2.7 min), P-288, and P-272 were identified, and 6 possible pathways of SMX oxidation were presented (Figure 6). The C2, C4, C6, S7, N8, O9, O10, and N11 of SMX were the most favorable sites for free radicals attack by the results of fukui function calculations. [6-7]

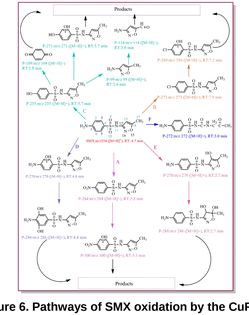

Figure 6. Pathways of SMX oxidation by the $CuFe_2O_4$/HA system.

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CONCLUSIONS

A simple and efficient DDA acquisition method was developed to study the degradation of antibiotics in the wastewater treatment process. Sulfamethoxazole was used as a test compound. This method used the high scanning speed of the SCIEX X500R QTOF system to acquire MS/MS spectra from a large quantity of potential degradation compounds. Further, the generated TOF MS and TOF MS/MS data can be retrospectively analyzed for increased compound identification. Coupled with the powerful data processing function of the SCIEX OS software, this method is beneficial to the comprehensive discovery of unknown and unexpected degradation products. Furthermore, this method is widely applicable to the study degradation products from other antibiotics, provided that the acquisition method scanning ranges are modified accordingly.

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