2.5: Applications of Mass Spectrometry

Learning Objectives

- Learn how mass spectrometry is applied in real-world applications
- Understand what mass spectrometry's use is in science

Mass spectrometry is applicable across diverse fields with specific applications including, but not limited to drug testing and discovery, food contamination detection, pesticide residue analysis, isotope ratio determination, protein identification, and carbon dating. One of the new ways that clinical mass spectrometry is being used is to quantitatively detect small amounts of proteins, biomarkers, or drug molecules, with very low concentrations. Some drugs have been given to patients in microdoses and with the ability to use small samples with low concentrations, it allows researchers to determine pharmacokinetic profiles of drugs that have been given. The reason for this is that it protects the patient from possible adverse effects of the drug while allowing scientists to determine what happens to the drug in the body. This will be especially helpful with pediatric patients. Below is a summary of a study detecting quinolones in animal food using mass spectrometry.

Application of LC/ESI-QTOF-MS in the Detection of Quinolones in Edible Animal Food

Quinolones are a family of common antibacterial veterinary medicine which can inhibit DNA-gyrase in bacterial cells. However, the residues of quinolone in edible animal products may be directly toxic or cause resistant pathogens in humans. Therefore, sensitive methods are required to monitor such residues possibly present in different animal-producing food, such as eggs, chicken, milk and fish. The molecular structures of eight quinolones, ciprofloxacin (CIP), anofloxacin methanesulphonate (DAN), enrofloxacin (ENR), difloxacin (DIF), sarafloxacin (SARA), oxolinic, acid (OXO), flumequine (FLU), ofloxacin (OFL), are shown in Figure 2.5.1.

Figure 2.5.1 The molecular structure of eight quinolones. Adapted from M. M. Zheng, G. D. Ruan, and Y. Q. Feng, *J. Chromatogr. A*, 2009, **1216**, 7510.

LC-MS is a common detection approach in the field of food safety. But because of the complex matrix of the samples, it is always difficult to detect those target molecules of low concentration by using single quadrupole MS. The following gives an example of the application of LC/ESI-QTOF-MS.

Using a quaternary pump system, a Q-TOF-MS system, a C18 column (250 mm × 2.0 mm I.D., 5 µm) with a flow rate of 0.2 mL/min, and a mixture of solvents as the mobile phase comprising of 0.3% formic acid solution and acetonitrile. The gradient phofile for mobile phase is shown in Table 2.5.1. Since at acidic pH condition, the quinolones carried a positive charge, all mass spectra were acquired in the positive ion mode and summarizing 30,000 single spectra in the mass range of 100-500 Da.

Table 2.5.1 The gradient phofile for mobile phase

Time (min)	Volume % of Formic Acid Solution	Volume % of Acetonitrile
0	80	20
12	65	35
15	20	80
20	15	85
30	15	85
30.01	80	20

The optimal ionization source working parameters were as follows: capillary voltage 4.5 kV; ion energy of quadrupole 5 eV/z; dry temperature 200 °C; nebulizer 1.2 bar; dry gas 6.0 L/min. During the experiments, HCO_2Na (62 Da) was used to externally calibrate the instrument. Because of the high mass accuracy of the TOF mass spectrometer, it can extremely reduce the matrix effects. Three different chromatographs are shown in Figure 2.5.2. The top one is the total ion chromatograph at the window range of 400 Da. It's impossible to distinguish the target molecules in this chromatograph. The middle one is at one Da resolution, which

is the resolution of single quadrupole mass spectrometer. In this chromatograph, some of the molecules can be identified. But noise intensity is still very high and there are several peaks of impurities with similar mass-to-charge ratios in the chromatograph. The bottom one is at 0.01 Da resolution. It clearly shows the peaks of eight quinolones with very high signal to noise ratio. In other words, due to the fast acquisition rates and high mass accuracy, LC/TOF-MS can significantly reduce the matrix effects.

Figure 2.5.2 Different chromatographs of 4 ng/g eight quinolones spiked in fish samples at different mass resolutions. Peaks: 1 = OFL; 2 = CIP; 3 =DAN; 4 = ENR; 5 = SARA; 6 = DIF; 7 =OXO; 8 = FLU. Adapted from M. M. Zheng, G. D. Ruan, and Y. Q. Feng, *J. Chromatogr. A*, 2009, **1216**, 7510.

The quadrupole MS can be used to further confirm the target molecules. Figure 2.5.3 shows the chromatograms obtained in the confirmation of CIP (17.1 ng/g) in a positive milk sample and ENR (7.5 ng/g) in a positive fish sample. The chromatographs of parent ions are shown on the left side. On the right side, they are the characteristic daughter ion mass spectra of CIP and ENR.

Figure 2.5.3 Chromatograms obtained in the confirmation of CIP (17.1 ng/g) in positive milk sample and ENR (7.5 ng/g) in positive fish sample. Adapted from M. M. Zheng, G. D. Ruan, and Y. Q. Feng, *J. Chromatogr. A*, 2009, **1216**, 7510.
