Fast and Comprehensive Analysis of Major use Antibiotics by UHPLC-High Resolution and High Mass Accuracy Hybrid Linear Ion-Trap-Orbitrap Mass Spectrometry

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Abstract
In this study, antibiotics of multiple classes (sulfonamides, quinolones, penicillins, macrolides, tetracyclines, pyrimidines) were selected to be accurately detected with modern chromatographic systems based on hybrid mass analyzers. For that purpose 13 antibiotics of major use were separated and detected with ultra-high performance liquid chromatography (UHPLC) high-resolution LTQ/Orbitrap mass spectrometry. The recent trend is focused toward the use of powerful high resolution MS detectors like Orbitrap which has become the technique of choice because of its high selectivity and sensitivity. Compounds were successfully identified in spiked samples from their accurate mass and LC retention times from the acquired full-scan chromatogram.
Keywords: antibiotics, residues, UHPLC-Orbitrap-MS

1. Introduction

Antibiotics constitute one of the most important emerging classes of environmental pollutants. This is due to their diverse use: in veterinary medicine to treat bacterial disease and protect the health of livestock as well as in human medicine to ensure human health safety. They are a prominent group of emerging contaminants frequently found in wastewater effluents and wastewater-impacted aquatic environments. However, the ubiquitous presence of antibiotic residues can create antibiotic-resistant bacteria that are harmful for the aquatic environment along with the consideration to be one of the most serious global threats to human health in the upcoming decades (Seifrtova et al., 2009, Senta et al., 2017).

It is well known that, for confirmatory purposes in chemical residue testing, mass spectrometry is the technique of choice. A chemical approach based on mass spectrometric detection brings the specificity needed to chemically identify an antibiotic compound, even at the screening step. In the last decade, many analytical methods based on liquid chromatography coupled to tandem mass spectrometry instruments LC–MS/MS have been developed for multi-antimicrobial residue screening.

More recently, new approaches using high resolution mass spectrometry (HRMS) have been reported for screening residual compounds with equipment such as time-of-flight mass detectors (TOF) or Orbital trap mass detectors (Orbitrap). These instruments allow full-scan acquisition of all signals obtained from the ionization source, without pre-selecting any compounds.

In this work, a screening method using LC–MS/MS has been developed for the identification of 13 antibiotics, belonging to the main antimicrobial families (Table 1). The sensitivity of the method was assessed through analysis of spiked samples. Data processing was implemented to allow the automatic identification of the compounds through the evaluation of their respective exact mass in combination with their retention times.

Table 1. Physicochemical properties of selected compounds

Antibiotic	Formula	logP	pKa
Sulfapyridine	$C_{11}H_{11}N_3O_2S$	0.35	8.43
Sulfamethizole	$C_9H_{10}N_4O_2S_2$	0.54	1.95
Sulfamethoxy-pyridine	$C_{11}H_{12}N_4O_3S$	0.30	6.32
Sulfamethazine	$C_{12}H_{14}N_4O_2S$	0.43	7.59
Sulfacetanamide	$C_8H_{10}N_2O_3S$	0.15	4.3
Sulfaquinoxaline	$C_{14}H_{12}N_4O_2S$	0.84	5.1
Sulfadiazine	$C_{10}H_{10}N_4O_2S$	0.25	3.16
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	0.89	1.97
Sulfathiazole	$C_9H_9N_3O_2S_2$	0.98	2.04
Erythromycin	$C_{37}H_{67}NO_{13}$	2.37	12.44
Oxolinic acid	$C_{13}H_{11}NO_5$	-0.2	5.94
Trimethoprim	$C_{14}H_{18}N_4O_3$	1.26	7.16
Oxytetracycline	$C_{22}H_{24}N_2O_9$	0.22	3.25

2. Experimental

2.1. Materials and reagents

All reagents and solvents used were of analytical- or HPLC-grade. Water was purified using a Milli-Q-System. Individual stock standard solutions (0.5 mg/ml) were prepared by dissolving the appropriate amount of each standard into methanol according to their solubility. All stock solutions were stored in a dark place. For spiking, dilute composite standard solutions were also prepared in ultra-pure water to obtain the desired concentrations.

2.2. Liquid chromatography–mass spectrometry (LC–LTQ-Orbitrap)

Chromatographic separations were performed on an Accela liquid chromatography U-HPLC system (Thermo Fisher, Bremen, Germany) equipped with a C18-diphenyl column 50mm × 2.1 mm; 2.6 μm particle size (Fortis Technologies, Cheshire, UK). The column was kept at a temperature of 27°C. The samples were separated at 40 °C, with 0.1% formic acid in water and 0.1% formic acid in methanol, used as mobile phases A and B, respectively. The elution gradient started at 95% A (initial conditions), remained 95% for 1 min and progressed to 0% in 7 min, stayed 0% for 1 min and returned to the initial conditions after 1 min with re-equilibration of the column set at 2 min. Total run time was 12 min. The flow rate was 0.4 mL/min with an injection volume of 5 μL.

Mass spectral analysis was carried out on LTQ-FT Orbitrap XL 2.5.5 SP1 (Thermofisher, Bremen, Germany) equipped with an electrospray ionization interface (ESI) and operated in the positive ion mode. The instrument was calibrated using the manufacturer's calibration solution consisting of three mass calibrators to reach mass accuracies in the 1–3-ppm range. auxiliary gas in the ion source. Full scan in positive ionization (PI) mode was acquired, for identification and quantification purposes, at mass resolving power of 60,000 FWHM, over a mass range of 100–500 Da. The following ionization parameters were applied: Spray voltage 3.7 kV, heater temperature 300 °C, tube lens 110 V, sheath gas flow rate 42 arbitrary units (au), auxiliary gas flow rate 11 au and capillary temperature 320 °C.

3. Results and Discussion

3.1. Methodology of processing acquired data and concept of validation

To evaluate the performance of the LC–HRMS screening method developed in our study, some characteristic parameters have been determined. The characteristics of performance that were determined were the detection capability of the method, also called CCβ, its selectivity/specificity against various interferences and its applicability/ruggedness/stability.

3.2. Perspective to further confirmation of chemical structures

From screening, the further step for definitively confirming the target analytes has been developed using the LTQ-Orbitrap LC–MS instrument. Indeed, the LTQ-Orbitrap offers some other possibilities; for example, to operate fragmentation of a selected precursor ion either in the linear ion trap (CID) or in the High Collision Dissociation cell (HCD). The detection of product ions can also be performed using either the linear ion trap detector or the Orbitrap detector. Therefore, there are at least three possible ways of obtaining further confirmation of a detected compound: a. CID with detection in ion trap leading to low resolution mass measurement of products ions, b. CID with detection in Orbitrap leading to high resolution mass measurement of products ions, c. HCD with detection in Orbitrap leading to high resolution mass measurement of products ions.

4. Conclusions

Mass accuracy of measured ions was calculated below 3 ppm at 50 ppb, in all cases. The selected conditions proved to be excellent within 8 min elution with the instrument limits of quantification down to low ppb level for the majority of the analytes. Detector linearity was proved to be excellent in all cases. The excellent analytical characteristics achieved, showed that the proposed analytical protocol is a promising trend that could be fully exploited in the routine monitoring of these antibiotics in several environmental substrates.

References

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