Combining Non-Targeted SWATH® MS/MSALL Acquisition with Highly Selective MRM^{HR} for the Analysis of Veterinary Drugs in Tissue Using the SCIEX X500R QTOF System

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Overview

A highly flexible, selective and sensitive LC-MS/MS method for the analysis of veterinary drugs in liver extract is presented, using the SCIEX X500R QTOF high resolution mass spectrometer together with the SCIEX OS software for a combined non-targeted and targeted screening workflow.

Introduction

Veterinary drugs are commonly used in livestock breeding to prevent or treat infections of the animals and to ensure their optimal growth. Legal regulations define waiting periods between the application of active pharmaceutical ingredients and the release of the animals for food manufacturing. Veterinary drugs which still find their way into human nutrition represent a potential risk to human health, e.g. in terms of possible allergenic reactions or reproductive dysfunctions. Furthermore, abuse of antibiotics in animals may also contribute to the development of antimicrobial resistance.

Therefore, European guidelines require to carefully and sensitively control residues of veterinary drugs in animal products [1]. Here we present a versatile and sensitive workflow on the SCIEX X500R QTOF system which combines a non-targeted screening workflow using SWATH® data acquisition looped with highly selective MRM^{HR} acquisition. Confident identification of veterinary drug residues according legal requirements [2] is achieved by accurate precursor and fragment mass measurement and their compound specific ion ratios, as reported in the SCIEX OS software.

Materials and Methods

Sample Preparation

Liver tissue was mixed with extraction solution (acetonitrile, water, formic acid) and homogenized. Following centrifugation for 5 minutes, a 5 mL aliquot from the supernatant was concentrated under nitrogen flow. After addition of 2.5 mL of solvent A, the extract was vortexed, centrifuged and filtered prior to injection. Aliquots of the extracts were spiked with a standard solution yielding final concentrations of 0.2, 1, 5, 10, and 50 ng/mL (corresponding to 0.08, 0.4, 2, 4, and 20 µg/kg liver).

Figure 1: SCIEX X500R QTOF system

LC Method

Veterinary drugs were chromatographically separated on a SCIEX ExionLC^{TM} AD UHPLC system, using a Phenomenex Kinetex C18 column (150 x 2.1 mm, 2.6 µm). Mobile phase A was water with 5% acetonitrile and 0.3% formic acid. Mobile phase B was acetonitrile with 5% water and 0.3% formic acid. Chromatographic separation was achieved using the gradient below. Oven temperature was set to 30 °C. Injection volume was 5 µL.

	A [%]	B [%]	Flow [mL/min]
0.0 min	100	0	0.4
2.0 min	100	0	0.4
7.0 min	70	30	0.4
11.0 min	0	100	0.4
11.1 min	0	100	0.8
12.5 min	0	100	0.8
12.6 min	100	0	0.4
14.0 min	100	0	0.4

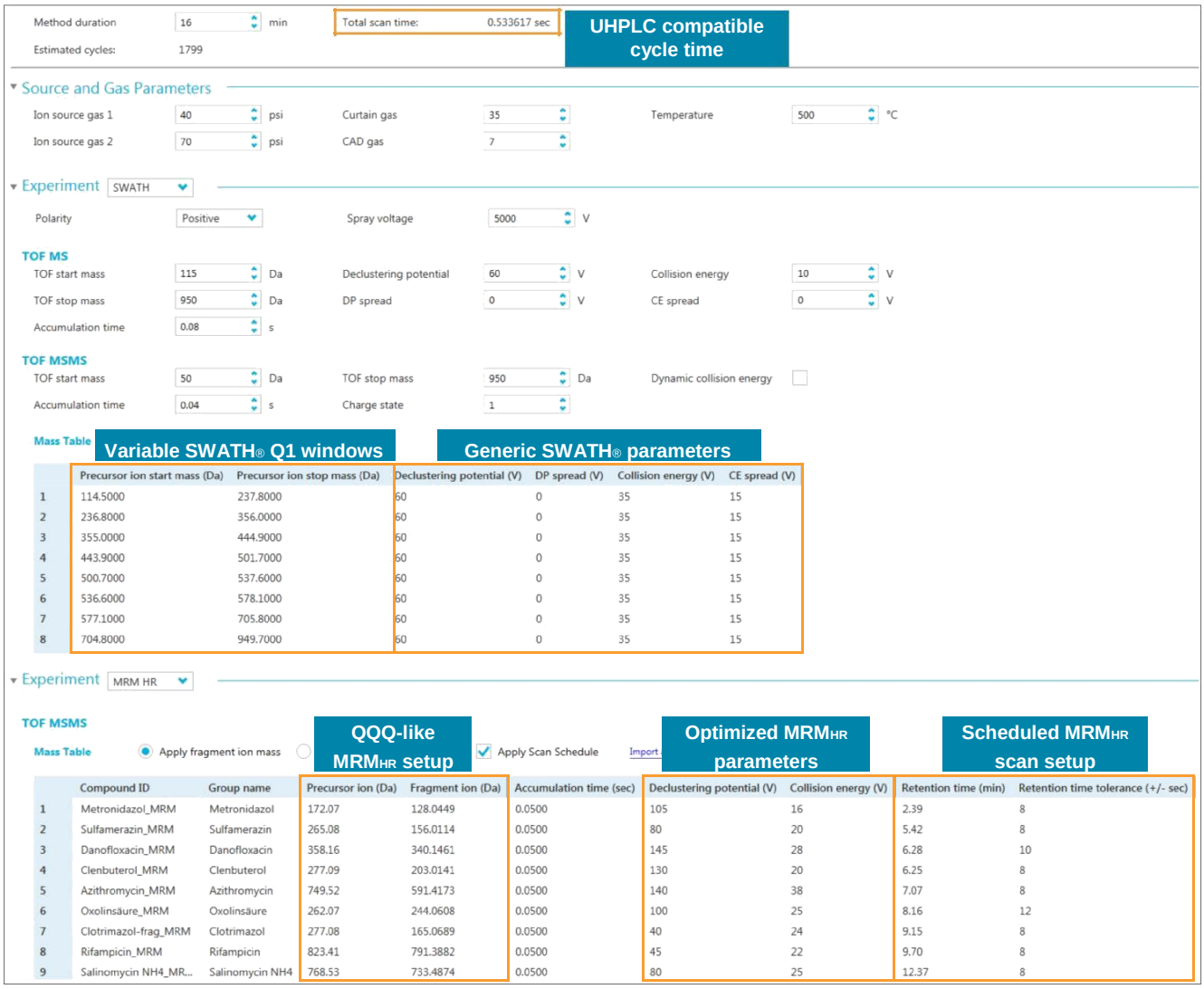

Figure 2: MS Method in SCIEX OS**.**

MS Method

The SCIEX X500R QTOF system was operated in positive mode with electrospray ionization. Data acquisition was performed using TOF-MS mode looped with eight SWATH® MS/MS experiments and scheduled MRM^{HR} acquisition. Variable SWATH® Q1 windows were used, calculated with the SCIEX SWATH® Variable Window Calculator. MRMHR experiments were acquired in fragment mode with a TOF scan window of 20 Da. Figure 2 shows the MS method as displayed in SCIEX OS. Data processing was done in SCIEX OS version 1.3.

Results & Discussion

Quantitative Results

On the SCIEX X500R QTOF system, TOF-MS mode is the standard acquisition mode for quantitation, providing non-targeted data collection which can be subsequently processed in SCIEX OS using a list of targeted compounds. For the 27 analytes of interest, TOF-MS mode provides excellent sensitivity in the standard solution at 1 ng/mL, as shown in figure 3.

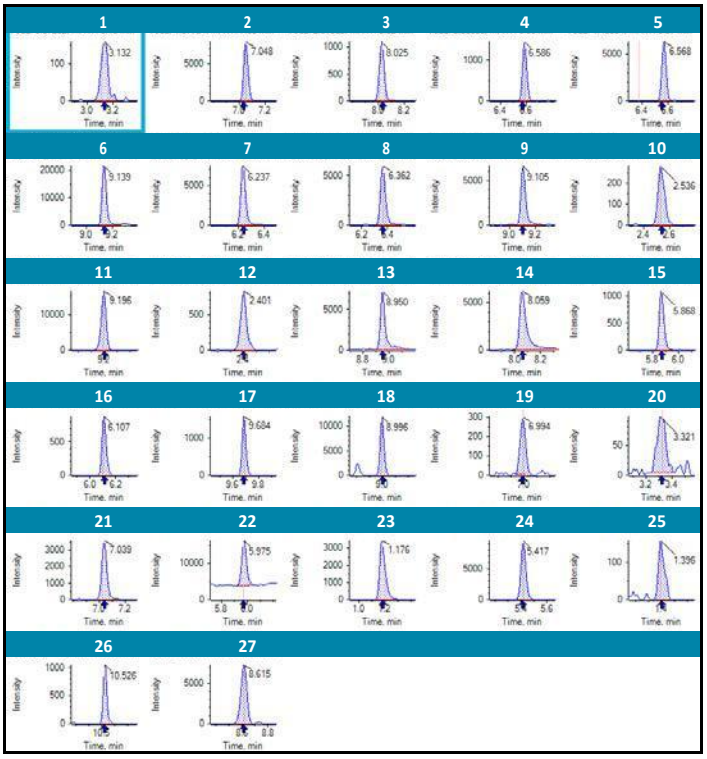

Figure 3: Extracted ion chromatograms of a standard solution of veterinary drugs at 1 ng/mL. 1 Amoxicillin. 2 Azithromycin. 3 Ceftiofur. 4 Chlortetracycline. 5 Clenbuterol. 6 Clotrimazole. 7 Danofloxacin. 8 Enrofloxacin. 9 Flumequine. 10 HMMNI. 11 Josamycin. 12 Metronidazole. 13 Nalidixic acid. 14 Oxolinic acid. 15 Oxytetracycline. 16 Penicillin G. 17 Rifampicin. 18 Roxythromycin. 19 Spiramycin. 20 Sulfacetamide. 21 Sulfachlorpyridazine. 22 Sulfadimidine. 23 Sulfagunidine. 24 Sulfamerazine. 25 Sulfanilamide. 26 Triclabendazole-sulfone. 27 Tylosin A.

However, in very complex matrices such as liver extracts, interferences may hamper the sensitive detection of certain analytes. For example, the signal for azithromycin in matrix spiked at 0.2 ng/mL shows a shoulder from a matrix interference which is not chromatographically resolved, and which makes an accurate integration and thus quantitation difficult (left panel in figure 4). In such a case, quantitation can be alternatively performed using the comprehensive MS/MS traces from SWATH® acquisition, a unique – as low matrix interfered – MS/MSALL technology. Using the MRM-like higher selectivity of SWATH® fragments, the interference observed in the TOF-MS trace can be removed (middle panel in figure 4). If even higher selectivity and sensitivity is needed, true MRM^{HR} provides even better signal-to-noise ratios (right panel in figure 4). The increase of signal-to-noise performance is due to the fact that MRM^{HR} uses compound specific collision energy, CE, and declustering potential, DP, voltages, while SWATH® is a generic method. Furthermore, transmission of the precursor ion as well as the fragment ion on their way through the mass spectrometer is optimized. Finally, the high selectivity in MRM^{HR} decreases the noise in the chromatogram to its minimum.

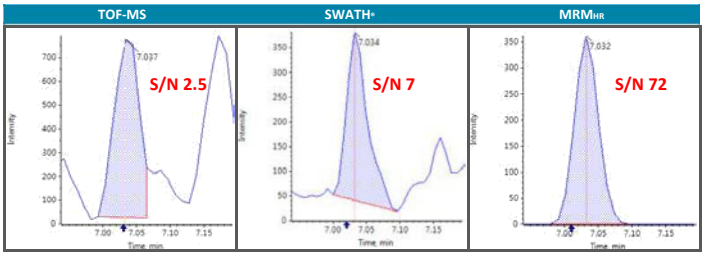

Figure 4: Extracted ion chromatograms of azithromycin spiked at 0.2 ng/mL in liver extract from different acquisition experiments. Left panel: TOF-MS (m/z 749.5158). Middle panel: SWATH®-MS/MS (749.52 > 591.4215). Right panel: MRMHR (749.52>591.4215).

Qualitative Results

SCIEX OS displays several parameters allowing the confident identification of a detected signal, meeting the European Union criteria of identification points [2]. First, it calculates mass errors of the precursor ion as well as of the fragment ions. Second, the ion ratio measured in unknown samples is compared to the one calculated from standards. Both the mass error and the ion ratio confidences are clearly displayed with a traffic light system, using a green checkmark for signals which meet the identification criteria. This allows the user to easily review large data sets and filter for positively detected compounds (figure 5).

Typically, the ion ratio can be calculated from the area of the precursor ion and the area of one fragment. Alternatively, if the TOF-MS trace is disturbed by interferences, two MS/MS fragments can be used. MS/MS fragments can be taken either from the SWATH® experiment or, if higher selectivity is needed, from a looped MRM^{HR} experiment.

Figure 5: Quantitative and qualitative results for Danofloxacin as shown in SCIEX OS. Upper left panel: Results table with confidence display for ion ratio and mass errors of precursor and fragment. Upper right panel: Calibration curve. Lower Panel: Extracted ion chromatograms of standard solutions and matrix samples. Quantifier (TOF-MS) is displayed in pink. Qualifier (MS/MS fragment from SWATH®) is displayed in blue. Expected ion ratio is shown as blue solid line, tolerances (±30%) as dotted line.

Conclusion

The SCIEX X500R QTOF system is a powerful instrument for the sensitive analysis of veterinary drugs in complex matrices, with a unique combination of versatile acquisition modes for different requirements:

1) TOF-MS data as standard trace used for quantitation.

2) Concurrent acquisition of untargeted SWATH® MS/MS data, used for identification with the help of accurate fragment masses and compound specific ion ratios as required by official guidelines. Furthermore, SWATH® MS/MS fragment can be used for quantitation, if the TOF-MS trace shows interferences.

3) Concurrent acquisition of targeted MRM^{HR} data increased selectivity for analytes which show interferences both in TOF-MS and SWATH® MS/MS mode.

References

1) European Commission, Commission Decision 37/2010/EU of 22 December 2002. ABl. :L15/1-72 (2002)

2) European Commission, Commission Decision 2002/657/EC of 12 August 2002. ABl. L221, 8-36 (2002)

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