

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

Jasmin Meltretter

SCIEX, Landwehrstraße 54, Darmstadt, Germany

## 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<sup>HR</sup> 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



Figure 1: SCIEX X500R QTOF system

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).

### LC Method

Veterinary drugs were chromatographically separated on a SCIEX ExionLC™ 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

Method duration: 16 min Total scan time: 0.533617 sec UHPLC compatible cycle time

Estimated cycles: 1799

▼ Source and Gas Parameters

Ion source gas 1: 40 psi Curtain gas: 35 Temperature: 500 °C  
 Ion source gas 2: 70 psi CAD gas: 7

▼ Experiment SWATH

Polarity: Positive Spray voltage: 5000 V

**TOF MS**

TOF start mass: 115 Da Declustering potential: 60 V Collision energy: 10 V  
 TOF stop mass: 950 Da DP spread: 0 V CE spread: 0 V  
 Accumulation time: 0.08 s

**TOF MSMS**

TOF start mass: 50 Da TOF stop mass: 950 Da Dynamic collision energy: ☐  
 Accumulation time: 0.04 s Charge state: 1

**Mass Table**

Variable SWATH® Q1 windows			Generic SWATH® parameters			
	Precursor ion start mass (Da)	Precursor ion stop mass (Da)	Declustering potential (V)	DP spread (V)	Collision energy (V)	CE spread (V)
1	114.5000	237.8000	60	0	35	15
2	236.8000	356.0000	60	0	35	15
3	355.0000	444.9000	60	0	35	15
4	443.9000	501.7000	60	0	35	15
5	500.7000	537.6000	60	0	35	15
6	536.6000	578.1000	60	0	35	15
7	577.1000	705.8000	60	0	35	15
8	704.8000	949.7000	60	0	35	15

▼ Experiment MRM HR

**TOF MSMS**

Mass Table ☒ Apply fragment ion mass ☐ **QQQ-like MRM<sub>HR</sub> setup** ☒ Apply Scan Schedule [Import](#) **Optimized MRM<sub>HR</sub> parameters** **Scheduled MRM<sub>HR</sub> scan setup**

	Compound ID	Group name	Precursor ion (Da)	Fragment ion (Da)	Accumulation time (sec)	Declustering potential (V)	Collision energy (V)	Retention time (min)	Retention time tolerance (+/- sec)
1	Metronidazo_MRM	Metronidazol	172.07	128.0449	0.0500	105	16	2.39	8
2	Sulfamerazin_MRM	Sulfamerazin	265.08	156.0114	0.0500	80	20	5.42	8
3	Danofloxacin_MRM	Danofloxacin	358.16	340.1461	0.0500	145	28	6.28	10
4	Clenbuterol_MRM	Clenbuterol	277.09	203.0141	0.0500	130	20	6.25	8
5	Azithromycin_MRM	Azithromycin	749.52	591.4173	0.0500	140	38	7.07	8
6	Oxolinsäure_MRM	Oxolinsäure	262.07	244.0608	0.0500	100	25	8.16	12
7	Clotrimazol-frag_MRM	Clotrimazol	277.08	165.0689	0.0500	40	24	9.15	8
8	Rifampicin_MRM	Rifampicin	823.41	791.3882	0.0500	45	22	9.70	8
9	Salinomycin NH4_MR...	Salinomycin NH4	768.53	733.4874	0.0500	80	25	12.37	8

Figure 2: MS Method in SCIEX OS.

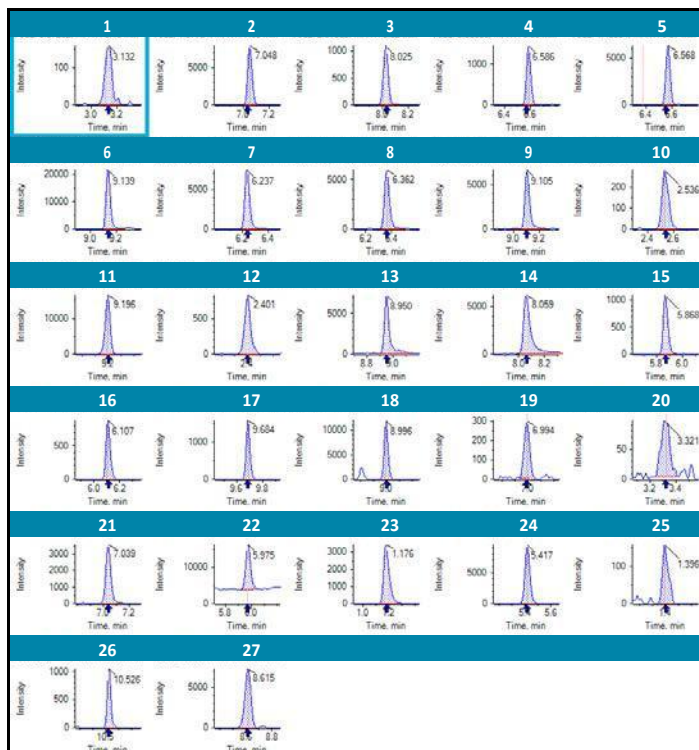
## MS Method

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<sub>HR</sub> acquisition. Variable SWATH® Q1 windows were used, calculated with the SCIEX SWATH® Variable Window Calculator. MRM<sub>HR</sub> 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

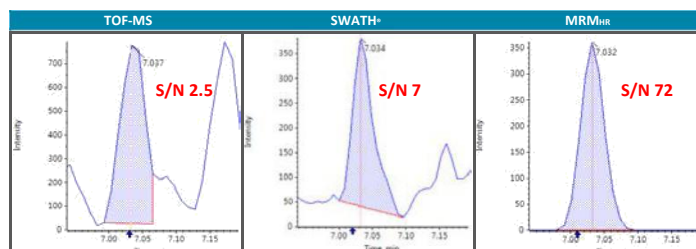
On the SCIEX X500R QTOF system, TOF-MS mode is the standard acquisition mode for quantitation, providing non-targeted data collection which can be subsequently processed in SCIEX OS using a list of targeted compounds. For the 27 analytes of interest, TOF-MS mode provides excellent sensitivity in the 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 Ceftriaxone. 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 Sulfaguanidine. 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<sup>HR</sup> 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<sup>HR</sup> 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<sup>HR</sup> decreases the noise in the chromatogram to its minimum.

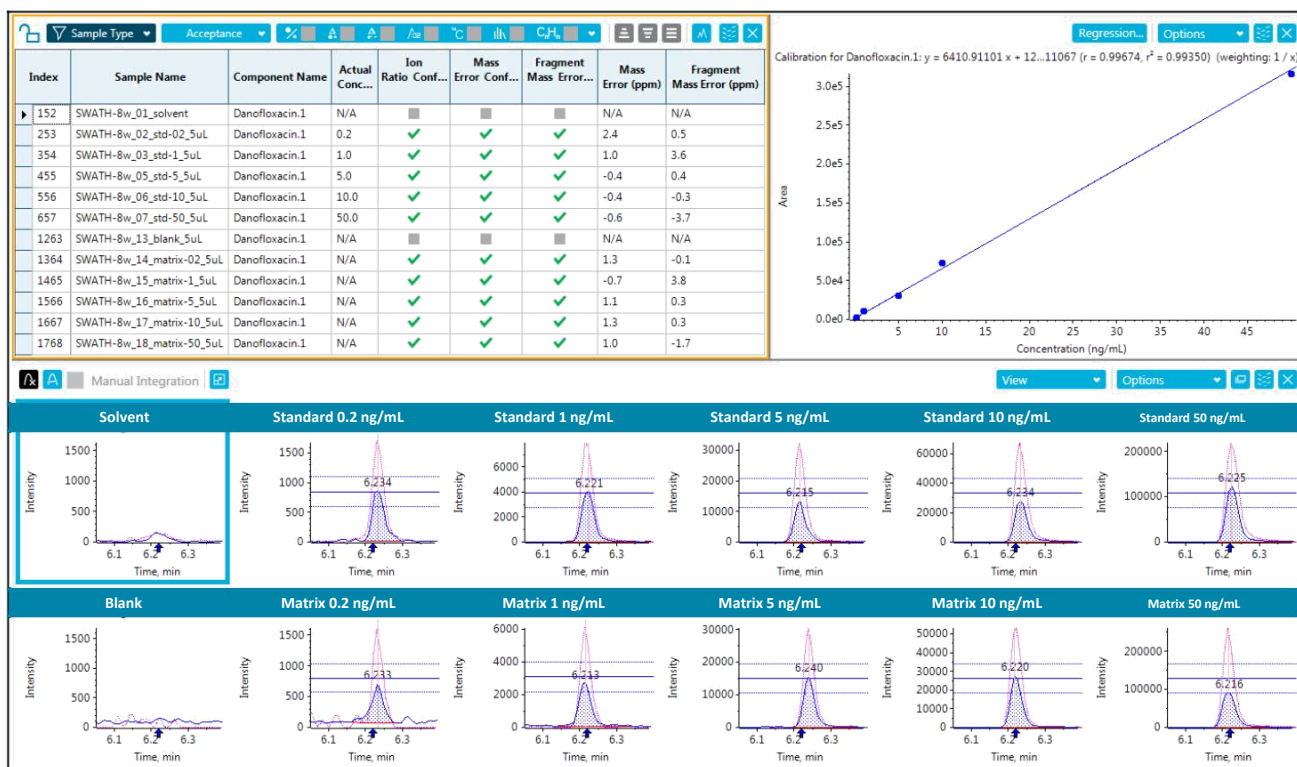


**Figure 4:** Extracted ion chromatograms of azithromycin spiked at 0.2 ng/mL in liver extract from different acquisition experiments. Left panel: TOF-MS (m/z 749.5158). Middle panel: SWATH®-MS/MS (749.52 > 591.4215). Right panel: MRMHR (749.52 > 591.4215).

### 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<sup>HR</sup> 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 ( $\pm 30\%$ ) 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<sup>HR</sup> data increased selectivity for analytes which show interferences both in TOF-MS

AB Sciex is doing business as SCIEX.

© 2017 AB Sciex. For Research Use Only. Not for use in diagnostic procedures. The trademarks mentioned herein are the property of AB Sciex Pte. Ltd. or their respective owners. AB SCIEX™ is being used under license.

Document number: RUO-MKT-02-6409-A

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)

## Acknowledgements

We would like to thank Anton Kaufmann from the Cantonal Laboratory of Zurich, Switzerland, who provided the liver extracts and the chromatographic method.