ASMS 2013

WP-370

Multi-residue Screening and Confirmation of Veterinary Drugs in Tissue Samples by LC/MS/MS with New Triggered MRM Acquisition

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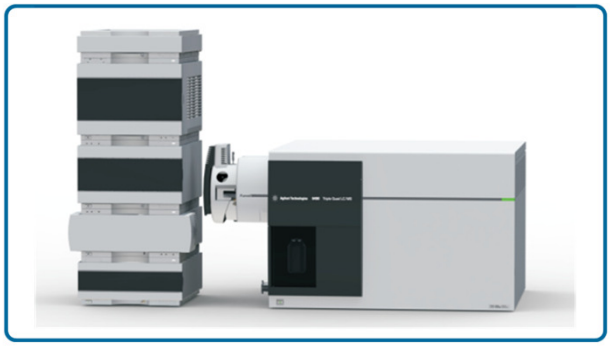
Introduction

Industrial livestock farming requires the use of veterinary drugs including antibiotics to prevent the propagation of infectious diseases. However, the extensive use and incorrect dosage of antibiotics can result in the formation of multi-resistant pathogens. To control the proper use of antibiotics and to protect consumers from veterinary drug residues, animal products need to be analyzed for pharmacologically active compounds. Large multi-residue methods for the screening and quantitation of veterinary drugs in food are not yet established. This is partly due to the absence of a generic extraction method, and partly due to the wide range of regulated maximum residue limits from very low for banned compounds to very high for other compounds.



Modern triple quadrupole mass spectrometers can achieve low limits of detection and a linear dynamic range which covers up to five orders of magnitude. In addition they can deal with hundreds of MRM transitions without a loss in performance. Screening methods for the detection of veterinary drugs should be designed to avoid false compliant results. While false negative results are generally not acceptable, false positive results increase the number of samples which need to go into confirmatory analysis and thus generate additional labour and costs. By the data dependent acquisition of additional confirmatory transitions and the comparison of the resulting spectra with a library, triggered MRM has the potential to reliably eliminate false positives.

Here we show the development of a multi-residue target screening method for veterinary drugs in food of animal origin. This method was applied to a variety of food matrices which were spiked with a comprehensive mixture of veterinary drugs to evaluate the applicability of this screening method for a routine control laboratory. Data of this evaluation study are shown.

Experimental

A UHPLC/MS/MS method for the analysis of more than 200 relevant veterinary drugs was developed for the routine screening and quantitation of pharmaceutically active compounds in food. An Agilent 1290 Infinity UHPLC system was coupled to a 6490 LC/MS instrument. The QQQ was operated in positive and negative Dynamic MRM acquisition mode with two primary and up to 6 confirmatory ions the collision cell operating at 3 different collision energies (0, 20, and 40 V). MRM transitions and conditions were taken from a database containing parameters for more than 500 compounds.

Table 1: UHPLC method parameters.

UHPLC column	Phenomenex Aqua C-18 column, 150 x 2 mm, 3 µm @ 25°C
Mobile phase	A: 5 mM NH_4 formate + 0.1% formic acid B: 5 mM NH_4 formate + 0.1% formic acid in methanol
Gradient program	Min % B 0 10 2 10 13.0 60 18.0 95 23.0 95 24.0 10 Stop time 27 min
Flow rate	0.30 mL/min
Injection volume	1 µL

Pork muscle, liver, eggs and honey were purchased from a local market and were extracted according to established extraction techniques for antibiotics in these matrices. Porcine urine was extracted according the method for ß-agonists. Blank extracts were spiked with a comprehensive veterinary drug standard at four relevant concentrations immediately before analysis. Triggered MRM in combination with reference library matching was used for compound confirmation and library match scores were compared to the identification by the qualifier to quantifier ratio.

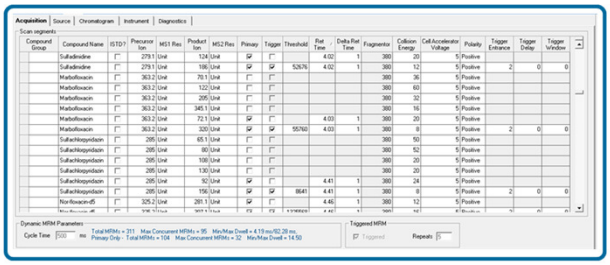


Figure 1: Screenshot of the MassHunter Acquisition for QQQ software which illustrates the setup of a Triggered MRM method.

Results and Discussion

UHPLC/MS/MS Screening Method for Veterinary Drugs

A UHPLC/MS/MS method for the analysis of more than 200 veterinary drugs from 25 different compound classes has been developed and applied to 5 different animal-derived matrices. Figure 2 shows the chromatogram of a comprehensive standard containing 215 veterinary drugs.

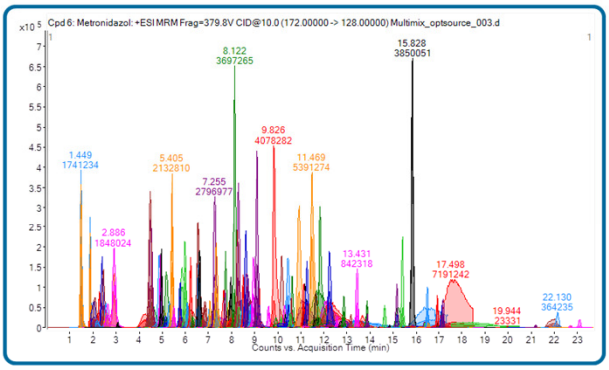


Figure 2: Chromatograms of the primary transitions for 215 veterinary drugs in a solvent standard.

Peak shapes for most of the compounds were acceptable with the exception of some dyes (brilliant green, malachite green), sedatives (promethazine), fluorquinolones (ciprofloxacin), and anticoccidials (decoquinate). Further optimization for these compounds is required.

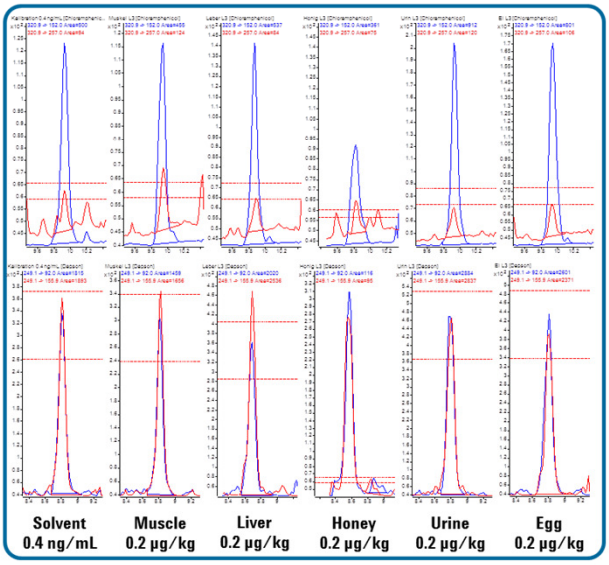


Figure 3: Chromatograms for chloramphenicol (negative ionization) and dapsone (positive ionization) below the MRPL in solvent and in matrix.

More than 95% of all compounds could be quantified comfortably at the 10 µg/kg level and more than 80% of all tested compounds even at the 1 µg/kg level. Figure 3 shows the chromatograms for chloramphenicol (negative ionization) and dapsone (positive ionization) for which low Minimum Required Performance Limits (MRPL) are in place.

Triggered MRM with Library Matching

Due to the use of optimized collision energies for each transition and reasonably long dwell times, tMRM produces high quality spectra even at very low concentrations. Figure 4 shows the MRM chromatograms of dimetridazole spiked into muscle extract corresponding to a concentration of 2.5 µg/kg (MRPL pig muscle: 3 µg/kg), together with the tMRM spectrum acquired at the same concentration. The signal-to-noise ratio of the quantifier transition was 47.7 (P2P, based on signal height) and thus was well above the LOQ, while the qualifier transition showed several interfering signals and therefore had a lower S/N ratio. However, verification of the compound was still possible since the triggered MRM spectrum was in very good agreement with the reference library spectrum, with a library match score of 99.3. Library match scores above 80 were observed for all compounds for which more than 4 fragments were acquired even at the lowest spiking levels and in the most complex matrices.

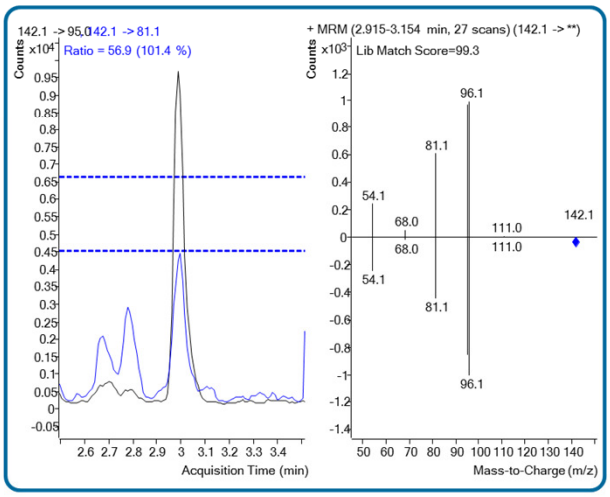
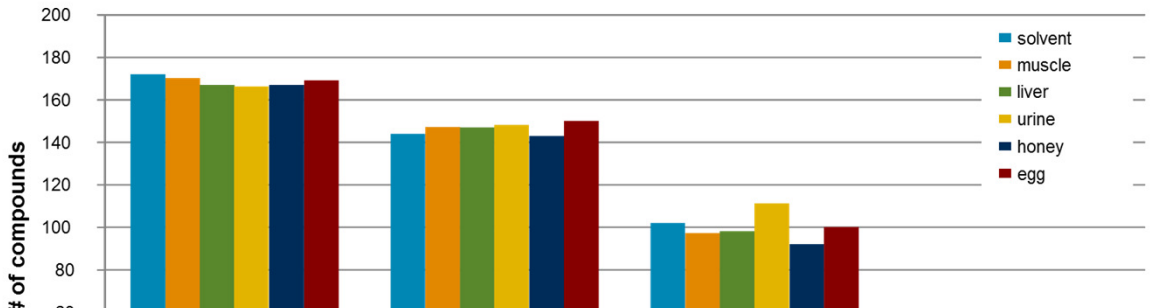


Figure 4: Chromatograms and tMRM spectra of dimetridazole in a meat extract corresponding to 2.5 µg/kg. The spectrum on top is the acquired spectrum whereas the spectrum below shows the reference library spectrum.

Results and Discussion



	Solvent		Muscle		Liver		Urine		Honey		Egg	
	> LOQ*	> LOD*	> LOQ*	> LOD*	> LOQ*	> LOD*	> LOQ*	> LOD*	> LOQ*	> LOD*	> LOQ*	> LOD*
10 µg/kg	172	6	170	5	167	9	166	6	167	6	169	7
1 µg/kg	144	20	147	19	147	15	148	16	143	16	150	11
0.2 µg/kg	102	39	97	40	98	41	111	30	92	42	100	45
0.05 µg/kg	42	56	39	50	48	52	46	57	37	52	50	50

Figure 5: Evaluation of compound detection with the LC/MS/MS targeted screening method above the LOQ (S/N>10, based on qualifier transition) in all tested matrices. The table also shows compounds which could be detected above the LOD (S/N>3, based on qualifier transition).

Evaluation of the LC/MS/MS Targeted Screening Method

Figure 5 shows the evaluation of compound detection with the LC/MS/MS targeted screening method above the LOQ in all tested matrices. The table also shows the compounds which could be detected above the LOD (S/N>3, based on qualifier transition).

Due to specific extraction protocols, the method performance was nearly independent of the sample matrix with honey being the most difficult and egg being the least complex matrix. The restricted stability of several groups of compounds like quinolones, tetracyclines, penicillins and cephalosporins turned out to be one of the limiting factors for a universal method, and finally 180 compounds could be evaluated in the spiked matrix samples. Several other compounds which showed lower performance would benefit from specific mobile phase conditions or stationary phases to improve their ionization behavior or chromatographic peak shapes.

Conclusions

Although it has been found that not all veterinary drugs can be analyzed with one generic method, the use of this comprehensive UHPLC/MS/MS method for qualitative target screening has the potential to eliminate several biological screening assays. The method showed good performance—even for quantitation—and excellent precision data for replicate injections showed that quantitation was not compromised when triggering additional transitions. The use of triggered MRM with library matching against a tMRM library with product ion spectra of more than 200 veterinary drugs resulted in higher confidence in the results with a low probability of false positives.

Further work is required on the development of an universal extraction and clean-up procedure. In addition, the chromatography needs to be optimized. Preliminary results on a fully endcapped ZORBAX Eclipse Plus C-18 UHPLC column look promising.