1. Introduction

Veterinary drugs are used for therapeutic, prophylactic, and prophylactic growth promotion purposes. To provide an assurance that food from animals is safe with regards to residues of veterinary medicines, regulatory authorities have established Maximum Residue Limits (MRLs) for certain drugs in target tissues and animal species and has also identified pharmacologically active compounds that are prohibited and considered a hazard at any level (EU regulation EC 37/2010; Commission Decision 2003/181/EC; 21CFR Part 556 Tolerances for Residues of New Animal Drugs in Food). In this work, we describe a method that delivers highly sensitive and selective triple quadrupole detection combined with MRM Spectro mode to reduce false positive and false negative reporting. MRM mode acquires a high number of fragment ions for each target compound generating a fragmentation spectra that could be used in routine library searching and compound verification using reference library match scores.

2. Materials and Methods

Samples of egg, milk, honey, and salmon were extracted and spiked in the calibration range 0.01-10ng/μL. Repeatability was assessed at low and high concentrations. Samples were measured using a Nexera UHPLC and the LCMS-8060 triple quadrupole detector (Table 1). Over-200 veterinary drugs were targeted, with more than 2,000 MRM transitions in both ESI- and the 120min gradient.

3. Results

3.1 Library identification using MRM Spectro mode

Table 1. LC and MRM acquisition parameters used to create the LC-MS/MS method

3.2. Quantitation using MRM Spectro mode

To assess the robustness of this approach the same sample was repeatedly injected using a method that comply with the EU guidelines SANTE/1194/2015 that requires the retention time and the ion ratio from at least 20MRA transitions to be within acceptable tolerance limits. The absolute response and signal variability was compared to a method using a higher number of fragment ions (Table 2). Both methods resulted in a variance of less than 4%US (10ng/mL injected spiked into sample matrix).

4. Conclusions

To reduce false negative and false positive reporting a larger number of MRM transitions were used for each veterinary drug target to increase the level of confidence in compound identification and verification. The number of fragment ion transitions monitored for each target compound was dependent upon the chemical structure with typically more than 10 fragment ions for each compound. MRM Spectro mode combines conventional MRM quantitation with the generation of a high number of fragment ions which could be used in routine library searching and compound verification using reference library match scores.

Multi-residue veterinary drug analysis of >200 compounds using MRM Spectro mode by LC-MS/MS

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