Ultra-high sensitivity quantification of veterinary drug residues in products of animal origin

Jack Steed[1], Yoann Fillatre[2], Michael Scherer[3], Aline Staub Spörri[4], Grégoire Bonvin[4], Jianru Stahl-Zeng[5]
[1]SCIEX, UK; [2]SCIEX, France; [3]SCIEX, Switzerland; [4]Official Food Control Authority and Veterinary Affairs of Geneva; [5]SCIEX, Germany

ABSTRACT

As regulations continue to tighten around food testing, it has become increasingly challenging to achieve the necessary levels of sensitivity during analysis while maintaining a high level of accuracy and precision. Within this method, LOQ values down to 0.005 ng/mL have been achieved while keeping high levels of accuracy and precision. When spiked into matrices, LOQs of 0.01 µg/kg in pork and chicken and 0.005 µg/kg in milk were observed. This high level of sensitivity allows routine laboratories to further dilute their samples to minimize any matrix effects observed. The linear range of each compound analyzed has been assessed, with ranges spanning up to 4 orders of magnitude and r values >0.99. sMRM acquisition helps ensure that both quantifier and qualifier transitions can be used to increase the specificity of the analysis without the need to compromise on data quality.

In addition to MRLs (maximum residue limits), minimum method performance requirements (MMPRs) are recommended by the EU for some prohibited compounds, which are summarized here. In these instances, the sensitivity of the analysis is paramount to ensure the MMPR is achieved or exceeded. In this method the MMPR has been met or improved upon, showing that even for the most difficult to analyze compounds, this method can achieve or improve on the recommended levels of sensitivity.

INTRODUCTION

The use of pharmacologically active substances in veterinary settings has been scrutinized for several years due to their sometimes inappropriate or intensive use. This is particularly worrying to authorities due to possible allergic reactions and an increase in antibiotic resistance which is becoming increasingly problematic both in livestock and human populations.[1] Therefore, the use of these substances needs to be controlled and limited to mitigate these known issues. One such way these controls are implemented is by analytical testing of products of animal origin, with a number of compounds receiving a MRL to reduce the levels of particular compounds. In addition to MRL values, some compounds have subsequently been prohibited due to their inherent toxicity. Therefore, in this case it is important to achieve LOQ values as low as is reasonably possible to limit these compounds in case of illegal use in the food industry.[2] Outside of this, minimum method performance requirement (MMPR) values have also been set for certain compounds to ensure than an acceptable level of sensitivity is reached.[3]

Here, a method for analyzing over 180 relevant compounds used in the veterinary industry has been developed, with LOQ values down to 0.005 ng/mL. See Figure 1 which highlights the sensitivity of the SCIEX 7500 System for the analysis of chlorpromazine, which is one of the compounds listed to be prohibited by the European Union.[4]

MATERIALS AND METHODS

Standard preparation: A mixed standard solution was provided by the official food control authority and veterinary affairs of Geneva at a concentration of 1 µg/mL. A series of dilutions were prepared to cover a range of 0.005 - 100 ng/mL.

Sample preparation: Each sample was homogenized before 5 g of each sample (pork, milk and chicken) was weighed. 0.1 M EDTA-McIlvaine buffer (4 mL for pork and chicken, 3 mL for milk) was added. All samples were then homogenized (pork and chicken for 1 min 15 secs using a FASTH21, milk for 10 minutes using a mechanical shaker). To each solution acetonitrile and ammonium sulfate was added before being further homogenized and centrifuged for 5 min at 4700 rpm (4° C). 4 mL of the upper layer was removed and transferred into an evaporative vial, 400 µL of DMSO added to each. Evaporation of the solution was performed using nitrogen until a final weight of ~ 0.5 g was achieved. 1 g of H2O was added to each solution before vortexing for ~15 sec, centrifuging for 5 mins at 13,000 rpm and filtering through a 0.45 µm nylon filter.

Spiked sample preparation: 90 µL of prepared matrix sample (pork, chicken, milk) was added to 10 µL of a relevant standard solution. Three spike concentrations were prepared at 0.01, 0.1 and 1 ng/mL with standard solutions at 0.1, 1 and 10 ng/mL being used as spiking solutions.

Chromatography: An ExionLC AD system was used to perform the chromatographic separation along with a Phemomenex Kinetex Polar C18 (2.6 µm, 100 Å, 100 x 2.1 mm) column.

Mass spectrometry: The analysis was performed using the SCIEX 7500 system. The system was operated using time-scheduled multiple reaction monitoring (sMRM) mode using positive and negative electrospray ionization (ESI) switching. QⁿD optimization was performed to enhance some of the compound's signal.5 Data was acquired using SCIEX OS software.

Data Processing: Data was processed using SCIEX OS software, with the AutoPeak algorithm for peak integration.[6-7]

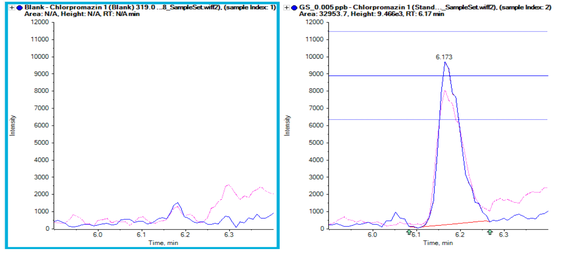

Figure 1. Overlaid XICs for chlorpromazine in solvent. Both the quantifier (blue) and qualifier (pink) MRM transitions of a chlorpromazine are shown in the solvent blank (left) and the LOQ of 0.005 ng/mL in solvent (right). The LLOQ image shows two MRM transitions for chlorpromazine overlaid along with ion ratio lines which outline the acceptable height (± 30%) of the qualifier peak.

Linear dynamic range

The linear range of an analyte is the span at which quantification is possible, while achieving acceptable levels of precision and accuracy. See Figure 2 which shows the linear dynamic range of three compounds across 4 orders of magnitude while still achieving accuracy levels between 70 – 130% and an r value >0.99.

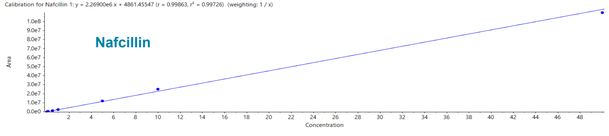

Figure 2. The linear ranges for three of the compounds analyzed. Each compound provides a linear range from 0.005 to 50 ng/mL, therefore highlighting a linear range over 4 orders of magnitude. The r value for each linearity was >0.99.

Sensitivity and specificity

Sensitivity and specificity are two of the main drivers of a successful analysis, with triple quadrupole mass spectrometers typically being used to achieve high levels of these metrics. See Figure 3 which shows albendazole at its LLOQ of 0.005 ng/mL in solution, highlighting the levels of sensitivity which can be achieved when using the SCIEX 7500 system. A blank injection has also been included in the figure to show that the blank is free of interferences and the peak observed at the LLOQ is genuine. In addition to sensitivity, the use of two MRM transitions (quantifier and qualifier) is shown within the figure and is used to provide an extra level of specificity to the analysis by implementing the use of ion ratios which can better confirm the identity of the peak when compared to a single MRM transition, especially in matrix. The ion ratio lines shown represent where the qualifier peak needs be within to be ±30% of the expected value.

Sensitivity in solvent is an important factor for any analysis however, what is more important is to carry this performance over to analysis in matrix. See Figure 4 which shows flubendazole spiked into a pork matrix. The XIC shown is at the lowest spike level analyzed (0.01 ng/mL) which equates to 0.01 µg/kg in the pork matrix, again showing the ultra-levels of sensitivity which can be achieved.

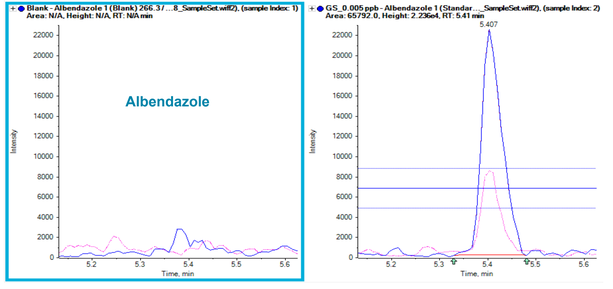

Figure 3. Overlaid XICs of the quantifier (blue) and qualifier (pink) MRM transitions of albendazole. The above images show the respective solvent blank (left) for each compound alongside the compounds' LLOQ (right). Albendazole achieved an LLOQ of 0.005 ng/mL.

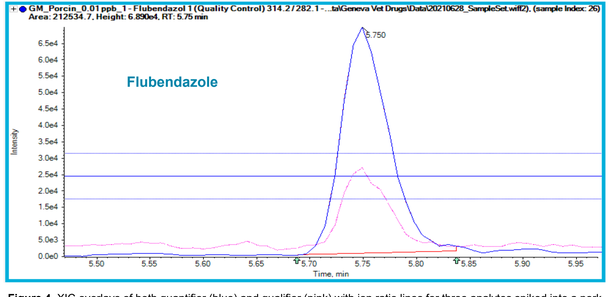

Figure 4. XIC overlays of both quantifier (blue) and qualifier (pink) with ion ratio lines for three analytes spiked into a pork matrix at the lowest spike concentration analyzed (0.01 ng/mL). This concentration equates to 0.01 µg/kg in sample.

Accurate and precise quantification

To ensure accurate and precise quantification, it is important that each individual peak has enough data points. Each peak was assessed and showed >10 data points which is consistent across the compounds analyzed.

Accuracy was also accessed at each point of the calibration curve, with all analytes providing accuracy values between ± 30 % at each level when back calculated against the curve. In addition, precision is paramount to show the consistency of the instrument both in solution and in sample. See Table 1 below which shows the %CV values achieved for the three compounds at the lowest concentration which was assessed for precision (0.01 ng/mL in solution and 0.01 µg/kg in pork and chicken, 0.005 µg/kg in milk) in solution and when spiked into matrix.

Table 1. The %CV (calculated concentration) values of chlorpromazine, metronidazole and triclabendazole sulfoxide at the lowest concentration assessed for precision (0.01 ng/mL in solution. 0.01 µg/kg in pork and chicken, 0.005 µg/kg in milk) (N=5).

Compound name	Solvent	Pork	Milk	Chicken
Chlorpromazine	*4.00*	*4.15*	*5.67*	*6.60*
Metronidazole	*5.33*	*5.24*	*5.84*	*6.99*
Triclabendazole sulfoxide	*12.11*	*9.89*	*11.36*	*5.99*

CONCLUSIONS

- An ultra-sensitive and fast method for the quantification of over 180 pharmacologically active compounds has been showcased
- Analysis has been performed in three relevant food matrices down to 0.01 µg/kg for pork and chicken, 0.005 µg/kg for milk
- Linear dynamic ranges which span up to 4 orders of magnitude without the use of internal standard
- Accurate and precise quantification including the use of ion ratios to increase the specificity of the analysis
- The assigned MRL or MMPR values can be achieved for all compounds analyzed.

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