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

## *Using the SCIEX 7500 system*

Jack Steed[1], Yoann Fillatre[2], Michael Scherer[3], Aline Staub Spörri[4], Grégoire Bonvin[4] and 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*

The use of pharmacologically active substances in veterinary settings has been scrutinized for several years due to their sometimes inappropriate or excessive use. This is particularly worrying to authorities due to possible allergic reactions and increasing antibiotic resistance in both livestock and human populations.[1] Therefore, the use of these substances must be controlled and limited to mitigate these issues. One way this control is implemented is through testing products of animal origin. In these analyses, compounds of interest have a set maximum residue limit (MRL), intended to reduce the levels of these compounds. Some compounds have been prohibited altogether, due to their inherent toxicity. While performing this analytical testing, it is therefore crucial to achieve very low LLOQ values to limit the misuse of compounds 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 that reached LLOQ values as low as 0.005 ng/mL was developed and implemented to analyze more than 180 relevant compounds used in the veterinary industry.

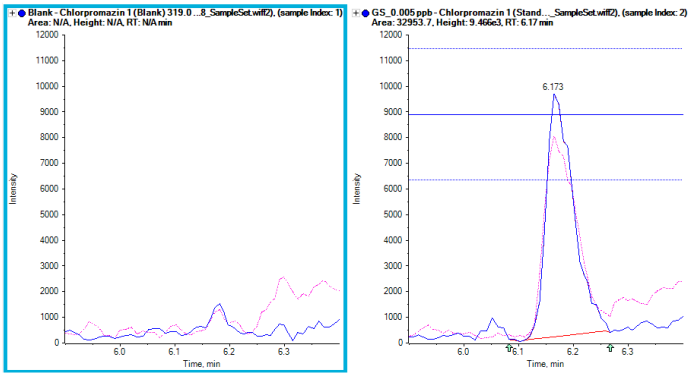


**Figure 1. Overlaid XICs for chlorpromazine in solvent.** Both the quantifier (blue) and qualifier (pink) MRM transitions for chlorpromazine are shown in the solvent blank (left) and in solvent at the LOQ of 0.005 ng/mL (right). The LLOQ image shows 2 MRM transitions for chlorpromazine overlaid with ion ratio lines that outline the acceptable height (±30%) of the qualifier peak.

Figure 1 highlights the sensitivity of the SCIEX 7500 system for the analysis of chlorpromazine, which is prohibited by the European Union.[4] Tables 1 and 2, included at the end of this technical note, provide an overview of the LLOQs, precision data and linear ranges achieved for each compound analyzed.

## Key features the SCIEX 7500 system for the analysis of veterinary drug residues in products of animal origin

- The sensitivity of the SCIEX 7500 system allows for LLOQ values as low as 0.005 ng/mL
- Demonstrated ability to analyze more than 180 relevant compounds in pork, chicken and milk food matrices, at concentrations as low as 0.01 µg/kg in pork and chicken and 0.005 µg/kg in milk
- The use of quantifier and qualifier transitions allows for the use of ion ratios to increase specificity of the analysis
- Linear dynamic ranges span up to 4 orders of magnitude
- Scheduled MRM algorithm ensures accurate quantification with enough data points for each compound analyzed.

## 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 was performed to cover a range of concentrations from 0.005 to 100 ng/mL.

***Sample preparation:*** Each sample was homogenized prior to weighing. A 5 g sample of pork, milk or chicken was combined with 0.1M EDTA-McIlvaine buffer (4 mL for pork and chicken, 3 mL for milk). Pork and chicken samples were then homogenized for 75 seconds using a FASTH21, whereas milk samples were homogenized for 10 minutes using a mechanical shaker. Pork and chicken solutions were then combined with 16 mL acetonitrile and 5 g ammonium sulfate. Milk solutions were combined with 8 mL acetonitrile and 5 g ammonium sulfate. All samples were then further homogenized and centrifuged for 5 minutes at 4,700 rpm at 4°C. A 4 mL sample of the upper layer was removed and transferred into an evaporative vial with 400 µL of DMSO. Nitrogen was used to evaporate the sample at 40°C until a final weight of approximately 0.5 g was achieved. Then, 1 g of $H_2O$ was added to each solution before vortexing for approximately 15 seconds, centrifuging for 5 minutes at 13,000 rpm and filtering through a 0.45 µm nylon filter prior to analysis.

***Spiked sample preparation:*** 90 µL of prepared pork, chicken or milk matrix sample 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 with a Phenomenex 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 the time-scheduled multiple reaction monitoring (sMRM) mode (see Figure 2) using positive and negative electrospray ionization (ESI) switching. Q0D optimization was performed to enhance the signal of some compounds.[5] Data were acquired using SCIEX OS software.

***Data processing:*** Data were processed using SCIEX OS software with the AutoPeak algorithm for peak integration.[6]

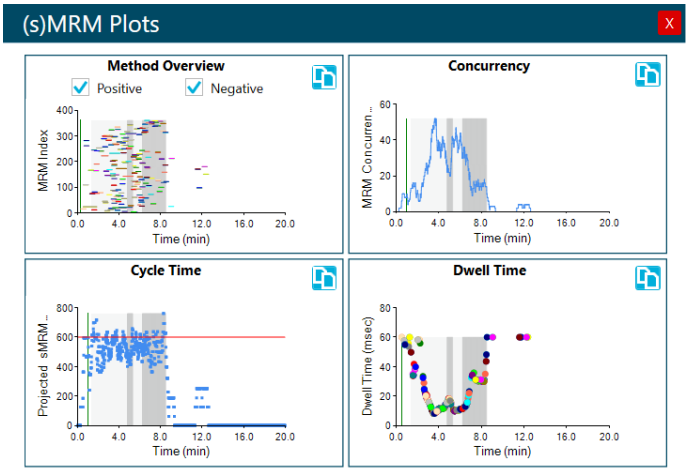


**Figure 2. Scheduled MRM algorithm plots in SCIEX OS software.** The scheduled MRM algorithm enables the analysis of hundreds of compounds in a single analysis with high quantitative accuracy. Each MRM transition is acquired during a short retention time window around its known elution time. This allows more MRM transitions to be monitored in a single LC run, while still maintaining maximized dwell times and optimized cycle times.[6] In SCIEX OS software, new visualization plots are available to help with method development and improve overall data quality.

## Chromatographic separation

When analyzing many compounds in a single analytical method, chromatographic separation is an important factor to ensure that the number of compounds analyzed at a particular time is minimized. This allows the balance between the cycle time of the mass spectrometer and the dwell time for each analyte to be optimal. Figure 3 shows the chromatographic separation achieved in both positive and negative ion acquisition modes. It is important to note that baseline separation of compounds is not necessary in most cases, due to the specificity of a triple quadrupole mass spectrometer operated in MRM acquisition mode. Chromatography helps to minimize analyte concurrency and to reduce interferences that may occur from the sample matrix.

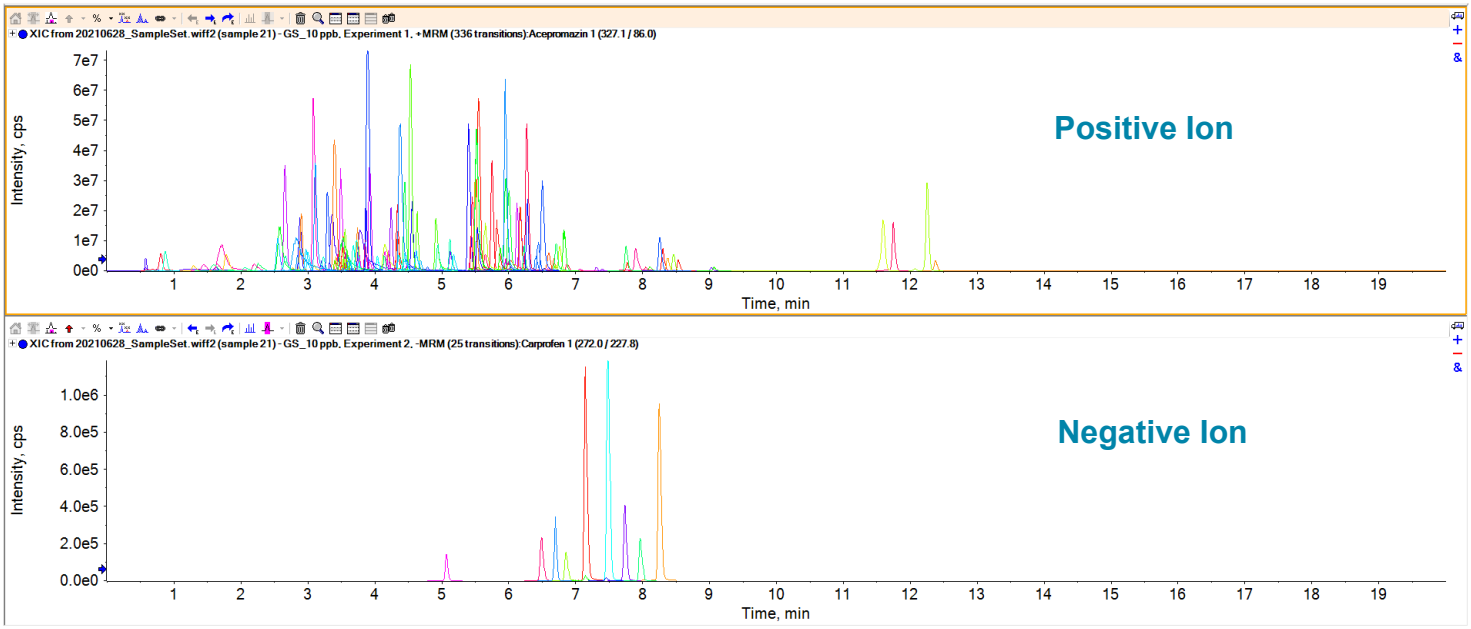


**Figure 3. Positive and negative XIC overlays of all compounds analyzed.** The above image illustrates the chromatographic separation achieved using the method, allowing for accurate quantification of each compound by achieving enough data points across each peak. Figure 6 highlights the amount of data points achieved for a subset of the compounds analyzed.

## Sensitivity and specificity

Sensitivity and specificity are crucial for a successful analysis and triple quadrupole mass spectrometers are typically used to achieve high levels of these metrics. Figure 4 highlights the levels of sensitivity that can be achieved using the SCIEX 7500 system for 3 representative analytes at their respective LLOQs of 0.005 ng/mL in solution. The blank injection is free of interferences, therefore indicating that the peak observed at the LLOQ is genuine. The use of quantifier and qualifier MRM transitions enhances the specificity of the analysis in matrix by using ion ratios to better confirm the identity of the peak, relative to a single MRM transition. The ion ratio lines highlight the acceptable range within ±30% of the expected value for the qualifier peak.

Although sensitivity in solvent is an important factor for analysis, it is crucial to maintain the quality of analyses in complex matrices. The same 3 compounds analyzed in solvent in Figure 4 were spiked into the pork, milk and chicken matrices. The results of these analyses are shown in Figure 5. The XICs shown are for the lowest spike level analyzed (0.01 ng/mL), which equated to 0.01 µg/kg of compound in the pork or chicken samples and 0.005 µg/kg of compound in milk. These results demonstrate the ultra-high level of sensitivity that can be achieved in this assay.

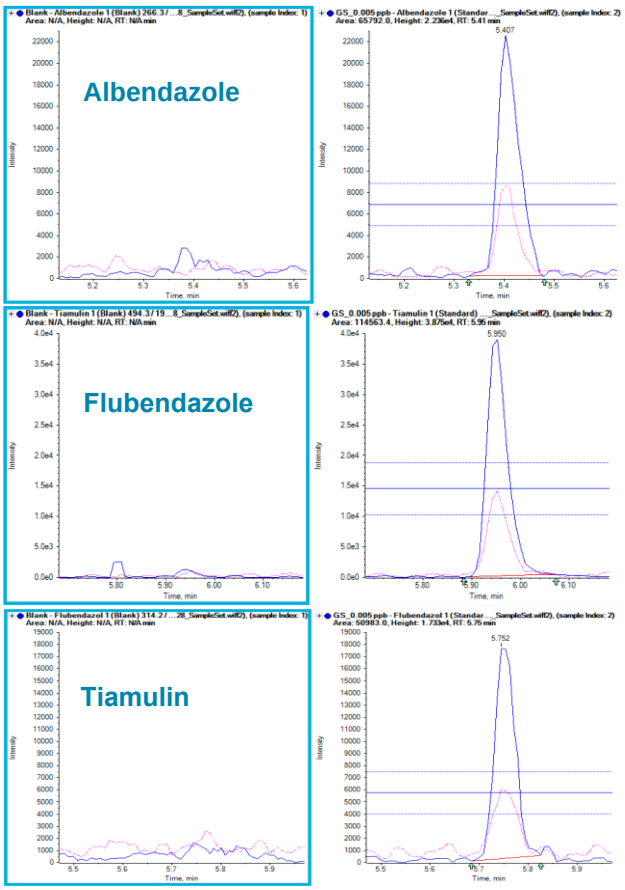


**Figure 4. Overlaid XICs of the quantifier (blue) and qualifier (pink) MRM transitions of albendazole (top), flubendazole (middle) and tiamulin (bottom).** The above images show the respective solvent blank (left) for each compound alongside the MRM transitions obtained at the LLOQ (right). Each compound achieved an LLOQ of 0.005 ng/mL.

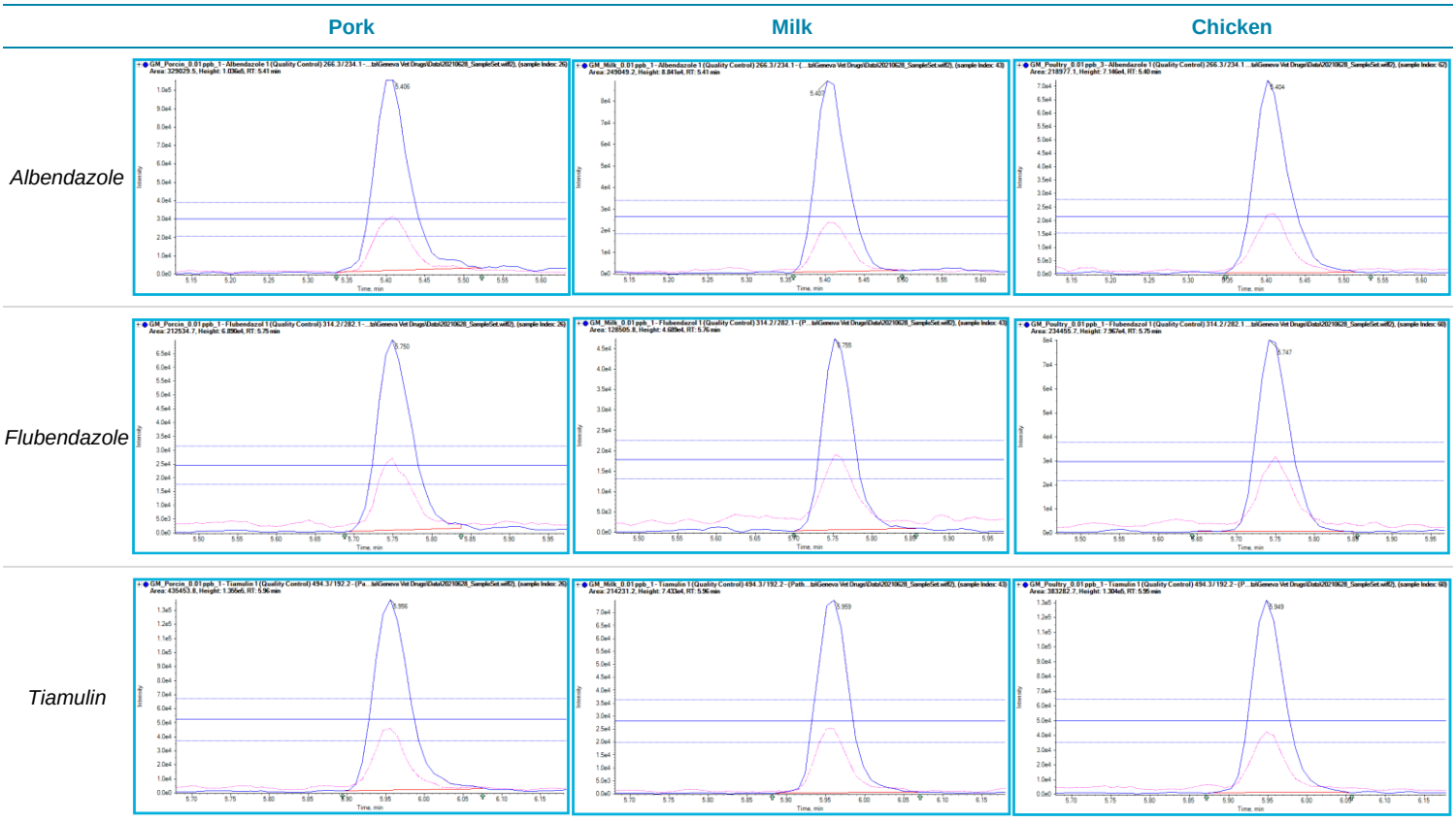


**Figure 5. XIC overlays of 3 analytes spiked into 3 different food matrices at the lowest spike concentration analyzed (0.01 ng/mL).** Quantifier and qualifier MRM transitions are shown in blue and pink, respectively, with ion ratio lines. The concentration analyzed equates to 0.01 µg/kg in sample for pork and chicken and 0.005 µg/kg for milk.

## 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. Each calibration curve analyzed achieved an r value >0.99 with accuracy between ±30% of the expected value. The linear dynamic ranges are indicated in Tables 1 and 2, included at the end of this technical note, for compounds analyzed in positive and negative ion modes, respectively. Representative examples of 3 compounds with linear dynamic ranges spanning 4 orders of magnitude are shown in Figure 6.

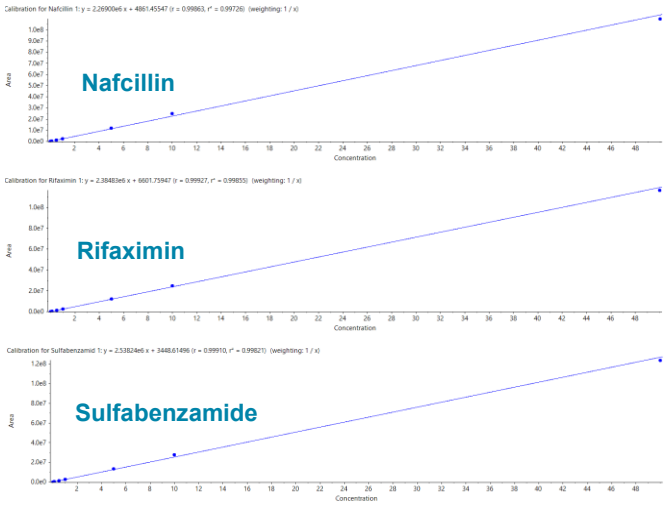


**Figure 6. The linear ranges for 3 of the compounds analyzed.** Each compound had a linear dynamic range that spanned 4 orders of magnitude, between 0.005 and 50 ng/mL. The r value for each compound was >0.99.

## Accurate and precise quantification

To ensure accurate and precise quantification, individual peaks must have enough data points. For the compounds analyzed, each peak consisted of more than 10 data points. Representative quantifier and qualifier ion MRM transitions are shown in Figure 7 for 3 analytes at a concentration of 10 ng/mL in solution.

Accuracy was assessed at each point of the calibration curves. Accuracy values for all analytes were between ±30% of the expected value at each concentration.

Precision is paramount to show the consistency of the instrument for analyses in solution and in sample. Table 3 outlines the %CV values achieved for the 3 compounds highlighted in Figure 7. Precision was assessed for compounds in solvent and spiked in matrix. Precision was calculated for the lowest concentrations tested: 0.01 ng/mL in solvent, 0.01 µg/kg in pork and chicken and 0.005 µg/kg in milk.

**Table 3. The calculated concentration %CV values for chlorpromazine, metronidazole and triclabendazole sulfoxide.**

| 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* |

*Precision was assessed for each compound at 0.01 ng/mL in solvent, 0.01 µg/kg in pork or chicken and 0.005 µg/kg in milk. (N=5).*

## Minimum method performance requirements (MMPRs)

Many of the compounds analyzed in this study are prohibited for use in veterinary settings. For these compounds, it is paramount to achieve high levels of sensitivity to ensure that they are not used illegally. Prohibited compounds do not have MRL values, as their presence at any level is not permitted. Some of these prohibited compounds have been detailed in a specific EURL guidance that defines MMPR values, which correspond to the minimum compound concentrations that should be detected by official laboratories.[3] Table 4 presents a compiled list of the prohibited compounds analyzed in this study that have MMPR values. The method developed was capable of detecting each of the prohibited compounds analyzed at concentrations lower or equal to the MMPR value.

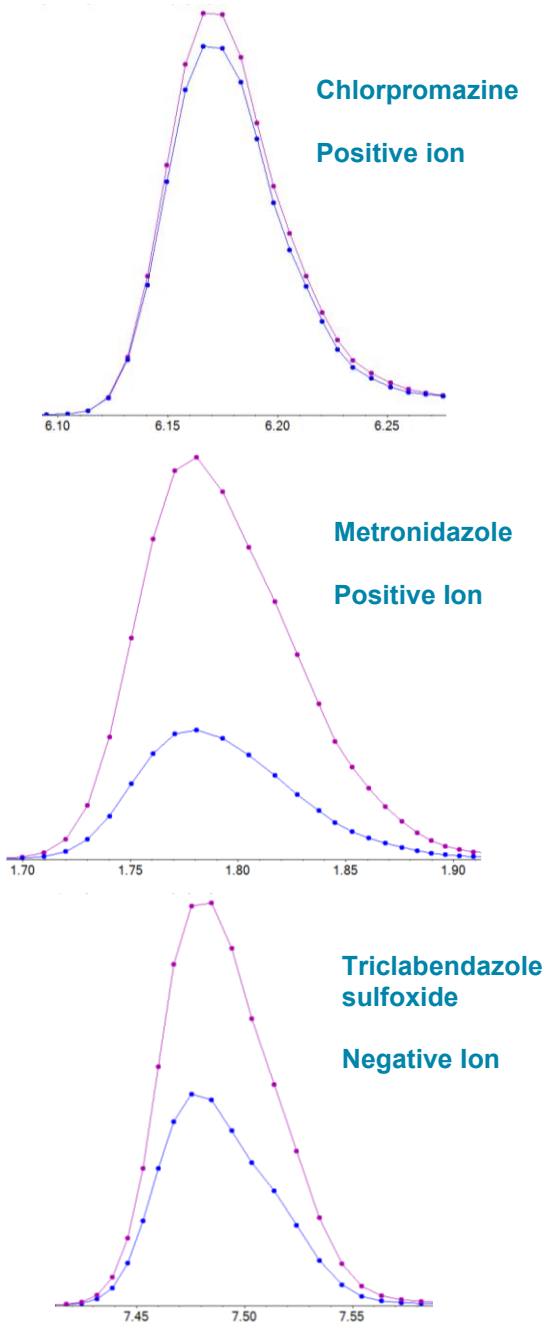


**Figure 7. Overlaid XICs of quantifier (purple) and qualifier (blue) MRM transitions, highlighting the number of data points acquired for each of the 3 compounds shown.** Over 10 data points were collected for both the quantifier and qualifier transitions across the peak, indicating that accurate and reproducible quantification can be performed.

## Conclusions

- An ultra-sensitive and fast method for the quantification of more than 180 pharmacologically active compounds was developed
- Analysis was performed in 3 relevant food matrices, at concentrations as low as 0.01 µg/kg for pork and chicken and 0.005 µg/kg for milk
- Linear dynamic ranges spanned up to 4 orders of magnitude without the use of internal standard
- Accurate and precise quantification were achieved, and ion ratios were used to increase the specificity of the analysis
- The assigned MRL or MMPR values were achieved for all compounds analyzed.

## References


1. Estelle Dubreil et al. (2017) Validation approach for a fast and simple targeted screening method for 75 antibiotics in meat and aquaculture products using LC-MS/MS, Food Additives & Contaminants: Part A, 34:4, 453-468.
2. Commission regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, 22nd December 2009.
3. EURL guidance on minimum method performance requirements (MMPRs) for specific pharmacologically active substances in specific animal matrices – September 2020.
4. EFSA - Scientific opinion on chloramphenicol in food and feed, 26th November 2014.
5. Enabling new levels of quantification. SCIEX technical note, RUO-MKT-02-11886-A.
6. The Scheduled MRM algorithm Pro. SCIEX technical note, RUO-MKT-02-8539-A.
7. SCIEX OS software – Transforming your samples into meaningful analytical answers.
8. Commission regulation (EU) No 2019/1871, 7th November 2019.
9. Commission implementing regulation (EU) No 2021/808, 22nd March 2021.


**Table 4. A comparison of the MMPR value and the lowest quantifiable concentration in a chicken matrix.** The chicken matrix was spiked at 0.01, 0.1 and 1 µg/kg.

| Compound Name | Lowest quantifiable concentration (µg/kg) | MMPR value (µg/kg) |
|---|---|---|
| *Brombuterol* | *0.1* | *0.1* |
| *Carbadox* | *0.1* | *5* |
| *Cimbuterol* | *0.01* | *0.1* |
| *Clenbuterol* | *0.1** | *0.1* |
| *Clenproperol* | *0.1** | *0.1* |
| *Dapsone* | *0.1* | *5* |
| *Dimetridazol-hydroxy* | *0.1* | *1* |
| *Flufenamic acid* | *0.1* | *10* |
| *Metronidazole-hydroxy* | *0.1* | *1* |
| *Mefenamic acid* | *0.1* | *10* |
| *Ronidazole* | *0.1* | *1* |
| *Tulobuterol* | *0.1** | *0.1* |
| *Zeranol* | *1* | *1* |
| *Phenylbutazone* | *0.1* | *5* |
| *Salbutamol* | *0.1* | *0.5* |

**= Peak observed in the blank matrix injection prevented assessment at lower concentrations.*



**Headquarters**
500 Old Connecticut Path | Framingham, MA 01701 USA
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**Table 1. All compounds analyzed in positive ion mode.** The table contains the LLOQ value achieved for each compound, the calculated concentration %CV at the 3 concentrations assessed and the associated linear dynamic range.

| Compound name | LLOQ (ng/mL) | %CV – 0.01 ng/mL | %CV – 0.1 ng/mL | %CV – 1 ng/mL | Linear range (ng/mL) |
|---|---|---|---|---|---|
| *Acepromazine* | 0.005 | 10.3 | 2.1 | 4.0 | 0.005 - 5 |
| *Albendazole* | 0.005 | 4.5 | 2.6 | 3.3 | 0.005 - 10 |
| *Albendazole sulfone* | 0.005 | 6.7 | 2.1 | 2.6 | 0.005 - 10 |
| *Albendazole sulfone amide* | 0.005 | 8.6 | 4.1 | 1.0 | 0.005 - 10 |
| *Albendazole sulfoxide* | 0.005 | 4.1 | 3.8 | 2.0 | 0.005 - 10 |
| *Amoxicillin* | 0.05 | N/A | 3.2 | 2.4 | 0.05 - 100 |
| *Ampicillin* | 0.01 | 12.4 | 4.0 | 4.7 | 0.01 - 100 |
| *Amprolium* | 0.01 | N/A | 5.9 | 6.5 | 0.1 - 10 |
| *Azaperol* | 0.01 | 9.4 | 1.1 | 1.9 | 0.01 - 10 |
| *Azaperone* | 0.05 | N/A | 7.7 | 6.7 | 0.05 - 10 |
| *Azithromycin* | 1 | N/A | N/A | 5.5 | 1 - 100 |
| *Beclometasone dipropionate* | 0.05 | N/A | 3.6 | 1.0 | 0.05 - 10 |
| *Beclomethasone* | 0.5 | N/A | N/A | 6.1 | 0.50 - 50 |
| *Benzocaine* | 0.05 | N/A | 6.7 | 2.0 | 0.05 - 100 |
| *Betamethasone* | 0.05 | N/A | 3.5 | 4.1 | 0.05 - 10 |
| *Brombuterol* | 0.05 | N/A | 5.9 | 3.3 | 0.05 - 10 |
| *Budenoside* | 0.05 | N/A | 7.6 | 4.4 | 0.05 - 50 |
| *Carbadox* | 0.05 | N/A | 7.6 | 4.9 | 0.05 - 10 |
| *Cefaclor* | 5 | N/A | N/A | N/A | 5 - 100 |
| *Chlorpromazine* | 0.005 | 4.0 | 4.0 | 2.6 | 0.005 - 10 |
| *Chlortetracycline* | 0.5 | N/A | N/A | 10.5 | 0.5 - 100 |
| *Cimbuterol* | 0.005 | 7.1 | 2.7 | 3.4 | 0.005 - 5 |
| *Ciprofloxacin* | 0.1 | N/A | 8.7 | 6.0 | 0.1 - 1 |
| *Clarithromycin* | 0.005 | 6.5 | 2.7 | 3.7 | 0.005 - 10 |
| *Clenbuterol* | 0.005 | 9.4 | 3.8 | 1.2 | 0.005 - 5 |
| *Clenproperol* | 0.01 | 20.2 | 3.6 | 2.4 | 0.01 - 5 |
| *Clindamycin* | 0.01 | 14.7 | 5.4 | 8.4 | 0.01 - 100 |
| *Cloxacillin* | 0.05 | N/A | 11.6 | 3.3 | 0.05 - 100 |
| *Corticosterone* | 0.5 | N/A | N/A | 4.9 | 0.50 - 100 |
| *Cortisone* | 0.5 | N/A | N/A | 7.0 | 0.50 - 100 |
| *Danofloxacin* | 0.05 | N/A | 6.7 | 3.6 | 0.05 - 1 |
| *Dapson* | 0.05 | N/A | 2.8 | 3.3 | 0.05 - 10 |
| *Demeclocycline* | 0.05 | N/A | 1.4 | 1.1 | 0.05 - 100 |
| *Dexamethasone* | 0.05 | N/A | 3.5 | 1.1 | 0.05 - 50 |
| *Diaveridine* | 0.005 | 6.4 | 3.1 | 2.4 | 0.005 - 5 |
| *Dicloxacillin* | 0.05 | N/A | 3.7 | 2.5 | 0.05 - 100 |

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|---|---|---|---|---|---|
| *Dicyclanil* | 0.05 | N/A | 4.8 | 2.5 | 0.05 - 10 |
| *Diethylcarbamazine* | 0.05 | N/A | 2.1 | 2.3 | 0.05 - 10 |
| *Difloxacin* | 0.05 | N/A | 4.7 | 3.3 | 0.05 - 1 |
| *Dimetridazole* | 0.05 | N/A | 3.5 | 2.9 | 0.05 - 100 |
| *Dimetridazole-OH* | 0.5 | N/A | N/A | 2.8 | 0.50 - 100 |
| *Diphenylamine* | 0.5 | N/A | N/A | 4.7 | 0.50 - 100 |
| *Doxycycline* | 0.5 | N/A | N/A | 10.4 | 0.50 - 100 |
| *Enoxacin* | 0.5 | N/A | N/A | 4.4 | 0.50 - 50 |
| *Enrofloxacin* | 0.05 | N/A | 3.0 | 1.5 | 0.05 - 50 |
| *Erythromycin* | 0.005 | 12.1 | 3.0 | 2.7 | 0.005 - 10 |
| *Erythromycin anhydride* | 0.05 | N/A | 6.2 | 4.4 | 0.05 - 100 |
| *Ethoxyquin* | 0.5 | N/A | N/A | 2.5 | 0.50 - 100 |
| *Ethoxyquine dimer* | 0.01 | 3.5 | 1.1 | 0.6 | 0.01 - 1 |
| *Febantel* | 0.05 | N/A | 5.1 | 1.4 | 0.05 - 50 |
| *Fenbendazole* | 0.005 | 3.2 | 3.1 | 0.9 | 0.005 - 10 |
| *Fenbendazole sulfone* | 0.005 | 5.5 | 2.1 | 3.0 | 0.005 - 10 |
| *Fenbufen* | 0.05 | N/A | 5.9 | 3.1 | 0.05 - 100 |
| *Fleroxacin* | 0.05 | N/A | 4.3 | 4.1 | 0.05 - 5 |
| *Florfenicol amine* | 1 | N/A | N/A | 5.1 | 1 - 100 |
| *Flubendazole* | 0.005 | 10.0 | 1.7 | 2.8 | 0.005 - 10 |
| *2-Aminoflubendazole* | 0.005 | 10.8 | 3.6 | 4.3 | 0.005 - 10 |
| *Fludrocortisone acetate* | 0.5 | N/A | N/A | 11.2 | 0.5 - 100 |
| *Flufenamic acid* | 0.1 | N/A | 8.7 | 3.3 | 0.1 - 100 |
| *Flumequine* | 0.05 | N/A | 4.2 | 6.1 | 0.05 - 50 |
| *Fluticasone propionate* | 0.005 | 6.5 | 3.3 | 3.8 | 0.005 - 10 |
| *Haloperidol* | 0.005 | 2.5 | 2.7 | 2.1 | 0.005 - 5 |
| *Hydrocortisone* | 0.1 | N/A | 13.1 | 5.4 | 0.10 - 100 |
| *Indomethacin* | 0.05 | N/A | 4.0 | 3.3 | 0.05 - 100 |
| *Indoprofen* | 0.01 | 8.1 | 3.1 | 4.3 | 0.01 - 10 |
| *Ipronidazole* | 0.05 | N/A | 1.4 | 2.9 | 0.05 - 10 |
| *Ipronidazole-OH* | 0.5 | N/A | N/A | 2.9 | 0.5 - 50 |
| *Isoxicam* | 0.05 | N/A | 1.7 | 4.4 | 0.05 - 100 |
| *Josamycin* | 0.005 | 6.2 | 3.6 | 1.6 | 0.005 - 5 |
| *Ketoprofen* | 0.05 | N/A | 6.3 | 2.4 | 0.05 - 100 |
| *Leucomycin* | 5 | N/A | N/A | N/A | 5 - 100 |
| *Lidocaine* | 0.005 | 6.7 | 1.0 | 2.6 | 0.005 - 10 |
| *Lincomycin* | 0.005 | 6.1 | 3.3 | 1.0 | 0.005 - 10 |
| *Lomefloxacin* | 0.05 | N/A | 4.2 | 1.6 | 0.05 - 1 |

| | | | | | |
|---|---|---|---|---|---|
| *Maduramicin* | 0.01 | 5.4 | 3.5 | 4.4 | 0.01 - 10 |
| *Marbofloxacin* | 0.1 | N/A | 4.6 | 1.6 | 0.1 - 10 |
| *Mebendazole* | 0.005 | 6.6 | 2.5 | 2.9 | 0.005 - 10 |
| *Mebendazole-hydroxy* | 5 | N/A | N/A | N/A | 5 - 100 |
| *Meclofenamic acid* | 0.5 | N/A | N/A | 2.0 | 0.5 - 100 |
| *Methylprednisolone* | 0.5 | N/A | N/A | 3.7 | 0.5 - 100 |
| *Meticlorpindol, (Clopidol)* | 0.5 | N/A | 8.7 | 3.2 | 0.05 - 10 |
| *Metronidazole* | 0.005 | 5.3 | 3.2 | 2.3 | 0.005 - 10 |
| *Metronidazole-OH* | 0.05 | N/A | 2.2 | 1.9 | 0.05 - 100 |
| *Minocycline* | 0.5 | N/A | N/A | 2.8 | 0.50 - 100 |
| *Monensin* | 0.005 | 2.5 | 1.6 | 0.7 | 0.005 - 10 |
| *Morantel* | 0.1 | N/A | 7.0 | 3.1 | 0.1 - 50 |
| *Nafcillin* | 0.005 | 4.3 | 2.5 | 2.5 | 0.005 - 50 |
| *Nalidixic acid* | 0.1 | N/A | 5.8 | 6.6 | 0.1 - 50 |
| *Naproxen* | 0.5 | N/A | N/A | 7.9 | 0.5 - 10 |
| *Narasin* | 0.005 | 0.9 | 1.2 | 1.4 | 0.005 - 10 |
| *Natamycin* | 0.5 | N/A | N/A | 11.0 | 0.5 - 100 |
| *Norfloxacin* | 0.5 | N/A | N/A | 1.9 | 0.5 - 50 |
| *Novobiocin* | 0.005 | 8.4 | 2.1 | 2.9 | 0.005 - 50 |
| *Ofloxacin* | 0.01 | 12.8 | 3.7 | 2.6 | 0.01 - 1 |
| *Olaquindox* | 0.5 | N/A | N/A | 1.3 | 0.50 - 100 |
| *Oleandomycin* | 5 | N/A | N/A | N/A | 5 - 100 |
| *Oxacillin* | 0.1 | N/A | 5.6 | 1.6 | 0.1 - 100 |
| *Oxfendazole* | 0.005 | 10.8 | 5.0 | 3.9 | 0.005 - 10 |
| *Oxibendazole* | 0.005 | 5.6 | 1.3 | 1.4 | 0.005 - 5 |
| *Oxolinic acid* | 0.01 | 4.4 | 2.3 | 3.4 | 0.01 - 10 |
| *Oxytetracycline* | 0.05 | N/A | 4.1 | 4.4 | 0.05 - 100 |
| *Pefloxacin* | 0.5 | N/A | N/A | 2.2 | 0.5 - 100 |
| *Penicillin G* | 0.1 | N/A | 7.8 | 3.7 | 0.1 - 100 |
| *Penicillin V* | 0.1 | N/A | 13.6 | 5.2 | 0.1 - 100 |
| *Phenylbutazone* | 0.05 | N/A | 2.6 | 2.0 | 0.05 - 100 |
| *Phoxime* | 0.05 | N/A | 5.9 | 3.9 | 0.05 - 100 |
| *Piroxicam* | 0.05 | N/A | 3.5 | 3.1 | 0.05 - 100 |
| *Praziquantel* | 0.01 | 5.7 | 2.0 | 2.9 | 0.01 - 10 |
| *Prednisolone* | 0.5 | N/A | N/A | 7.6 | 0.50 - 100 |
| *Prednisone* | 0.5 | N/A | N/A | 7.9 | 0.50 - 100 |
| *Procaine* | 0.005 | 2.9 | 0.9 | 2.8 | 0.005 - 10 |
| *Promazine* | 0.005 | 4.1 | 5.6 | 1.3 | 0.005 - 10 |

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|---|---|---|---|---|---|
| *Promethazine* | 0.005 | 4.8 | 2.0 | 3.5 | 0.005 - 10 |
| *Propionylpromazine* | 0.005 | 3.9 | 1.8 | 2.6 | 0.005 - 10 |
| *Propyphenazone* | 0.005 | 6.0 | 1.2 | 2.1 | 0.005 - 5 |
| *Pyrantel* | 0.05 | N/A | 2.7 | 0.8 | 0.05 - 50 |
| *Pyrimethamine* | 0.005 | 7.8 | 1.6 | 2.3 | 0.005 - 5 |
| *Rifaximin* | 0.005 | 11.6 | 1.5 | 1.5 | 0.005 - 50 |
| *Robenidine* | 0.005 | 7.5 | 6.7 | 1.7 | 0.005 - 10 |
| *Ronidazole* | 0.05 | N/A | 3.5 | 1.6 | 0.05 - 100 |
| *Roxarsone* | 1 | N/A | N/A | 4.5 | 1 - 100 |
| *Roxithromycin* | 0.005 | 7.1 | 5.6 | 2.4 | 0.005 - 10 |
| *Salbutamol* | 0.05 | N/A | 4.8 | 2.6 | 0.05 - 10 |
| *Sarafloxacin* | 0.05 | N/A | 3.2 | 2.0 | 0.05 - 1 |
| *Spiramycin* | 5 | N/A | N/A | N/A | 5 - 100 |
| *Sulfabenzamide* | 0.005 | 11.0 | 4.1 | 3.1 | 0.005 - 50 |
| *Sulfacetamide* | 0.5 | N/A | N/A | 4.6 | 0.5 - 100 |
| *Sulfachloropyridazine* | 0.05 | N/A | 6.0 | 5.7 | 0.05 - 100 |
| *Sulfadiazine* | 0.05 | N/A | 1.3 | 1.5 | 0.05 - 10 |
| *Sulfadoxine* | 0.01 | 6.7 | 4.7 | 4.0 | 0.01 - 50 |
| *Sulfaguanidine* | 0.005 | 7.8 | 1.1 | 2.4 | 0.005 - 10 |
| *Sulfaisoxazole* | 0.5 | N/A | N/A | 6.1 | 0.5 - 100 |
| *Sulfamerazine* | 0.05 | N/A | 4.8 | 3.6 | 0.05 - 50 |
| *Sulfameter* | 0.05 | N/A | 3.7 | 1.4 | 0.05 - 10 |
| *Sulfamethazine* | 0.05 | N/A | 3.7 | 2.9 | 0.05 - 50 |
| *Sulfamethizole* | 0.05 | N/A | 7.1 | 2.1 | 0.05 - 50 |
| *Sulfamethoxazole* | 0.05 | N/A | 2.5 | 2.3 | 0.05 - 10 |
| *Sulfamethoxypyridazine* | 0.05 | N/A | 1.1 | 2.7 | 0.05 - 100 |
| *Sulfamonomethoxine* | 0.05 | N/A | 7.3 | 4.5 | 0.05 - 100 |
| *Sulfamoxole* | 0.05 | N/A | 5.6 | 3.6 | 0.05 - 100 |
| *Sulfanilamide* | 0.5 | N/A | N/A | 3.3 | 0.5 - 50 |
| *Sulfapyridine* | 0.05 | N/A | 3.2 | 4.0 | 0.05 - 50 |
| *Sulfaquinoxaline* | 0.01 | 12.8 | 3.1 | 3.1 | 0.01 - 10 |
| *Sulfathiazole* | 0.01 | 8.2 | 4.0 | 3.3 | 0.01 - 10 |
| *Sulfisomdine* | 0.005 | 2.2 | 1.9 | 1.6 | 0.005 - 10 |
| *Sulfisoxazole* | 0.01 | 9.0 | 3.8 | 3.7 | 0.01 - 50 |
| *Tenoxicam* | 0.05 | N/A | 4.2 | 2.7 | 0.05 - 100 |
| *Ternidazole* | 0.5 | N/A | N/A | 6.3 | 0.5 - 100 |
| *Tetracycline* | 0.05 | N/A | 10.0 | 2.2 | 0.05 - 10 |
| *Tetramisole* | 0.005 | 4.1 | 1.8 | 0.9 | 0.005 - 10 |

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|---|---|---|---|---|---|
| *Thiabendazole* | 0.005 | 4.2 | 1.6 | 3.0 | 0.005 - 10 |
| *Thiabendazole-5-hydroxy* | 0.005 | 10.3 | 4.3 | 3.6 | 0.005 - 5 |
| *Tiamulin* | 0.005 | 3.6 | 5.3 | 8.8 | 0.005 - 5 |
| *Ticlopidine* | 0.005 | 5.6 | 2.1 | 2.7 | 0.005 - 5 |
| *Tilmicosin* | 1 | N/A | N/A | 3.8 | 1 - 100 |
| *Tolbutamide* | 0.05 | N/A | 3.8 | 3.5 | 0.05 - 100 |
| *Tolfenamic acid* | 0.5 | N/A | N/A | 3.2 | 0.5 - 100 |
| *Tolmetin* | 0.05 | N/A | 5.8 | 3.8 | 0.05 - 50 |
| *Triamcinolone* | 0.05 | N/A | 8.6 | 4.5 | 0.05 - 100 |
| *Triclabendazole* | 0.005 | 6.5 | 1.4 | 2.3 | 0.005 - 10 |
| *Triflupromazine* | 0.005 | 3.8 | 2.2 | 2.6 | 0.005 - 10 |
| *Trimethoprim* | 0.005 | 9.3 | 3.7 | 0.8 | 0.005 - 5 |
| *Tulobuterol* | 0.005 | 5.5 | 3.1 | 5.4 | 0.005 - 1 |
| *Tylosin A* | 0.05 | N/A | 6.3 | 4.3 | 0.05 - 1 |
| *Tylosin B* | 0.05 | N/A | 9.7 | 4.4 | 0.05 - 1 |
| *Valnemulin* | 0.005 | 6.5 | 3.1 | 2.5 | 0.005 - 10 |
| *Virginiamycin* | 0.1 | N/A | 11.5 | 7.8 | 0.1 - 10 |
| *Xylazine* | 0.5 | N/A | N/A | 2.7 | 0.5 - 50 |

**Table 2. All compounds analyzed in negative ion mode.** The table contains the LLOQ value achieved for each compound, the %CV at the 3 concentrations assessed and the associated linear dynamic range.

| Compound name | LLOQ (ng/mL) | %CV – 0.01 ng/mL | %CV – 0.1 ng/mL | %CV – 1 ng/mL | Linear range (ng/mL) |
|---|---|---|---|---|---|
| Carprofen | 5 | N/A | N/A | N/A | 5 - 100 |
| Diclofenac | 0.5 | N/A | N/A | 3.1 | 0.5 - 100 |
| Dinitrocarbanilide | 0.005 | 4.1 | 3.3 | 1.6 | 0.005 - 10 |
| Ethopabate | 0.5 | N/A | N/A | 1.5 | 0.5 - 100 |
| Flunixin | 0.1 | N/A | 8.6 | 6.0 | 0.1 - 50 |
| Isoxicam | 0.05 | N/A | 2.1 | 1.2 | 0.05 - 10 |
| Mefenamic acid | 0.1 | N/A | 3.4 | 1.4 | 0.05 - 50 |
| Meloxicam | 0.5 | N/A | N/A | 6.5 | 0.5 - 50 |
| Nicarbazin | 0.005 | 7.4 | 1.6 | 1.8 | 0.005 - 10 |
| Sulfanitran | 0.5 | N/A | N/A | 3.5 | 0.5 - 10 |
| Triclabendazole sulfone | 0.05 | N/A | 5.3 | 3.1 | 0.05 - 10 |
| Triclabendazole sulfoxide | 0.005 | 12.1 | 3.4 | 5.1 | 0.005 - 5 |
| Zeranol | 0.5 | N/A | N/A | 5.2 | 0.5 - 100 |