BRIEF COMMUNICATION: Rapid analysis of moxidectin in plasma
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Introduction

Intestinal parasites are a significant impediment to the productivity of grazing livestock in New Zealand (Scott et al. 2008). They are regarded by New Zealand sheep farmers as their most important animal health issue (Lawrence et al. 2007). The New Zealand deer industry estimated that parasites cost as much as 5% of the total farm revenue (Mackintosh & Wilson 2003). On most farms controlling parasites in order to minimise their impact on animal performance relies heavily on the use of anthelmintics (Lawrence et al. 2007), but their continued usefulness is threatened by the development of worm populations resistant to these drugs (Waghorn et al. 2006a, 2006b). Studies aimed at developing more sustainable worm management practices will be important for maintaining the future productivity and profitability of many farming operations.

Moxidectin is a commercially important semisynthetic product used in the treatment of parasites in a variety of livestock, particularly sheep and cattle. It is produced by derivitization of nemadectin, a macrocyclic lactone produced by Streptomyces cyanogriseus (Asato & France 1990). Moxidectin is a potent anthelmintic agent active against a wide variety of nematode and arthropod parasites. Previous studies have shown that it can be detected at very low concentrations in plasma after dosing (Alvinerie et al. 1995). Current moxidectin products on the market can be administered orally, by injection, or as a pour-on solution.

Previous methods for analysis of moxidectin have involved a labour intensive solid phase extraction clean up, followed by derivatization and high performance liquid chromatography (HPLC) with fluorescence detection (Alvinerie et al. 1995; Durden 2007; Wang et al. 2009). This procedure has many limitations such as low sample throughput and dynamic range. Other detection methods have utilised high performance liquid chromatography-mass spectrometry (HPLC-MS) using an ion trap mass spectroscopy system to measure residues in liver tissue (Howells & Sauer 2001), or HPLC-MS/MS triple quadrupole instruments as used to monitor moxidectin residues in milk (Turnipseed et al. 2005). These methods also require extensive sample clean up which reduces throughput significantly. This low throughput is both time-consuming and expensive. It is a significant disadvantage when applied to large sample sets and can act as a limitation as to what is economically feasible within a research programme.

This paper describes a method which focuses on minimal sample preparation to decrease preparation time and increase accuracy, coupled with ultra high performance liquid chromatography (UHPLC) with selected reaction monitoring using a triple quadrupole MS/MS instrument, for selectivity, sensitivity, and speed.

Materials and methods:

Sample preparation

Plasma samples were collected as part of a larger study in which the performance of three moxidectin administration techniques, namely injection, oral or pour-on, were compared in cattle (Leathwick & Miller 2013). On a subset of four farms, six animals from each treatment group and two untreated Control animals were bled at regular intervals over a 21 day period after treatment.

Plasma samples were stored frozen at -20°C until analysis. Prior to analysis the samples were thawed and 100 µL of plasma was placed in an eppendorf tube and 400 µL of cold acetonitrile was added to precipitate the protein. The sample was shaken briefly and then centrifuged for five minutes in a Minifuge at 12,000 rpm. A 200 µL aliquot of the supernatant was transferred to an auto-sampler vial for subsequent instrumental analysis.

Instrumental analysis

The instrument consisted of a Thermo Accela 1250 ultra high pressure pump, a Thermo PAL autosampler coupled to a Thermo TSQ triple quadrupole mass spectrometer. A 5 µL injection of sample was made onto an Agilent SB-C8 column (50 x 2.1 mm, 1.9 µm particle size) held at 25°C, fitted with two Phenomenx C18 guard cartridges. The HPLC solvents used were: Solvent A = 0.1 % formic acid in MilliQ® water; Solvent B = 0.1% formic acid in acetonitrile. The flow rate was 600 µL/min. The gradient started at 40% B, increasing to 95% B over four minutes, then back to 40% B over 30 seconds, and held to re-equilibrate for 30 seconds for a total run time of five minutes. Mass spectra were acquired using a heated electrospray ionisation probe at 400°C in positive mode. The first 90 seconds of flow from the HPLC were diverted to waste. The mass spectrometer was programmed to perform a selected reaction monitoring experiment for 640.3 m/z (mass to charge
Table 1 Calibration curve for moxidectin standards.

<table>
<thead>
<tr>
<th>Moxidectin standard concentration (ng/mL)</th>
<th>Mean peak area ± standard deviation (Arbitrary units)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>1,077 ± 215</td>
<td>20</td>
</tr>
<tr>
<td>1</td>
<td>13,783 ± 2,425</td>
<td>18</td>
</tr>
<tr>
<td>10</td>
<td>160,752 ± 837</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>1,615,801 ± 8,440</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 1 Mean concentrations of moxidectin in six subsamples of cattle plasma following three different methods of administering the moxidectin. Error bars are standard error of mean.

The developed method was applied to the experimental samples to determine the concentrations of moxidectin over time in cattle plasma for the three different administration techniques (Figure 1).

Administration by injection resulted in the highest concentrations of moxidectin, with mean concentrations approximately five times higher than that of the oral administration. Oral administration, in turn, resulted in higher drug concentrations in plasma than the pour-on route. Both the oral and injection treatments reached maximum concentration one day after administration. The pour-on treatment resulted in a maximum blood concentration of approximately 1 ng/mL and generally peaked between two and four days after application.

Results and discussion

The calibration standards gave good linearity and reproducibility over a wide dynamic range of four orders of magnitude between 0.1 ng/mL and 100 ng/mL (Table 1). The limit of detection was 0.01 ng/mL with a signal to noise ratio of 3:1, with a limit of quantitation of 0.03 ng/mL with a signal to noise ratio of 9:1. Plasma spiked with moxidectin standard to attain a series of concentrations from 1–100 ng/mL, and then prepared in the manner described previously. External standard calibration standards were run after every 50 experimental samples to ensure detector response was stable.

For linearity tests, standard solutions were serially diluted with 4:1 acetonitrile:water to recreate similar conditions to the sample matrix, and run in triplicate. A 100 mL sample of control plasma samples from each of the undosed animals were spiked with moxidectin standard to attain a series of concentrations from 1–100 ng/mL, and then prepared in the manner described previously. External standard calibration standards were run after every 50 experimental samples to ensure detector response was stable.

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Five moxidectin spiked plasma samples (100 ng/mL) were prepared and run to establish an intra-day variance of 6.0%. Another set of 5 spiked plasmas gave an inter-day variance of 11.6%.

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Interestingly, these plasma profiles did not correlate with measured efficacy with the oral treatment that resulted in higher efficacy than the other two routes of administration (Leathwick & Miller 2013). As a result, future studies will attempt to measure moxidectin concentrations in fluids and tissues where the parasites are found. The method described here is currently being adapted for this purpose.

The analytical method developed here requires very little sample preparation and an instrumental analysis time of five minutes, giving a combined laboratory preparation and instrument throughput of approximately 250 samples per day. This is in marked contrast to previously published methods with limits of 30 samples per day (Wang et al. 2009). Both the detection sensitivity and recovery are well within acceptable limits for the range of concentrations found in plasma samples. The same analytical methodology could potentially be extended to other drugs of a similar nature. Experimental data using this method of analysis, have been reported elsewhere (Leathwick & Miller 2013).

References


