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A sensitive automated online SPE-LC-MS/MS method for the quantitative determination of ivermectin B_{1a} in bovine plasma

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Introduction

Ivermectin:

- Mixture of two homologous compounds: ivermectin B_{1a} and ivermectin B_{1b} (Figure 1).
- Routinely administered as antiparasitic drug to millions of cattle per year.
- The topical "pour-on" formulation is displacing the conventional injectable formulation in farming practices.

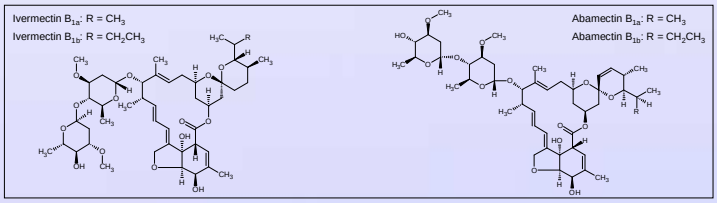

Figure 1: Chemical structure of ivermectin and abamectine (IS).

Sensitive analytical methods are required for the quantitative determination of ivermectin for pharmacokinetic purposes in bovine plasma samples.

Goal:

The objective of the present study was to develop and validate a sensitive and reliable bioanalytical LC-MS/MS method suitable for the determination of ivermectin B_{1a} in bovine plasma.

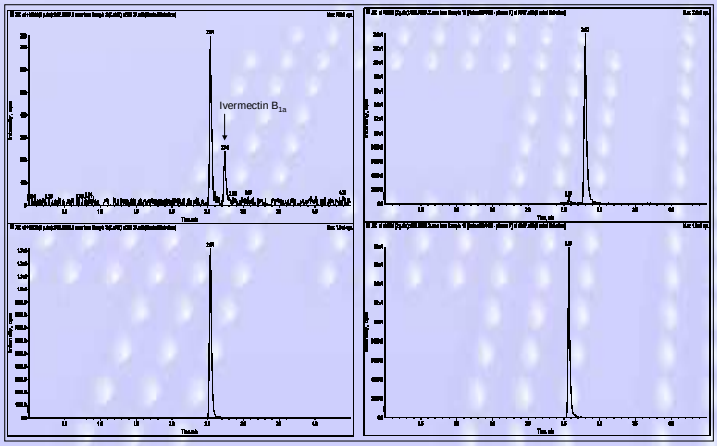

Figure 2: LC-MS/MS chromatograms of ivermectin B_{1a} (upper panel) and abamectin (lower panel). Calibrator at the LLOQ level (left panel) and a bovine plasma study sample (right panel).

Materials and Methods

- Bovine plasma samples were supplemented with the internal standard (IS, abamectin) and subjected to a protein precipitation using acetonitrile followed by online solid phase extraction (SPE) on HySphere™ C_8 EC-SE cartridges using a Symbiosis™ Pharma system. The extracted samples were introduced into the LC-MS/MS system for quantification.
- The samples were chromatographed on a Zorbax Eclipse XDB-C_8 column (3.5 µm, 75 x 4.6 mm). The mass-spectrometer consisted of a Sciex API 4000 equipped with an atmospheric pressure chemical ionization interface and was operating in the positive ion mode.
- A full validation of the method was performed according to the current guidelines for bioanalytical method validation[1,2]. The validation included the determination of the parameters: calibration, accuracy and precision, recovery, specificity, dilution, and stability.

Results

Calibration: The assay was validated in the concentration range of 0.200 – 200 ng/mL ivermectin B_{1a} in bovine plasma. A lower limit of quantitation (LLOQ) of 0.200 ng/mL was achieved in bovine plasma (Figure 2).

Accuracy and precision: The method showed acceptable accuracies (expressed as bias) and precisions (expressed as CV) (Table 1).

Table 1: Accuracy and precision in bovine plasma.

QC level	n	Mean (ng/mL)	Accuracy (%)	Bias (%)	Intra CV (%)	Total CV (%)
LLOQ (0.200 ng/mL)	18	0.204	102.1	2.1	9.5	12.4
Low (0.600 ng/mL)	18	0.658	109.6	9.6	5.1	5.7
Medium (8.00 ng/mL)	18	8.17	102.1	2.1	6.4	8.4
High (160 ng/mL)	18	157	98.1	-1.9	8.2	9.6
OQC# (2000 ng/mL)	18	2000	100.0	0.0	6.0	7.9

The "Over the curve" (OQC) samples were analysed after 20-fold dilution with blank matrix.

Recovery: The method was found valid with respect to recovery (protein precipitation step).

Specificity: No relevant interferences from endogenous compounds at the retention time of ivermectin B_{1a} and the IS were observed.

Dilution: The 20-fold dilution of plasma samples with the blank matrix was considered valid.

Stability: 24h storage of samples on the bench and in the refrigerator, 72h storage of samples on the autosampler, and three additional freeze/thaw cycles were successfully validated.

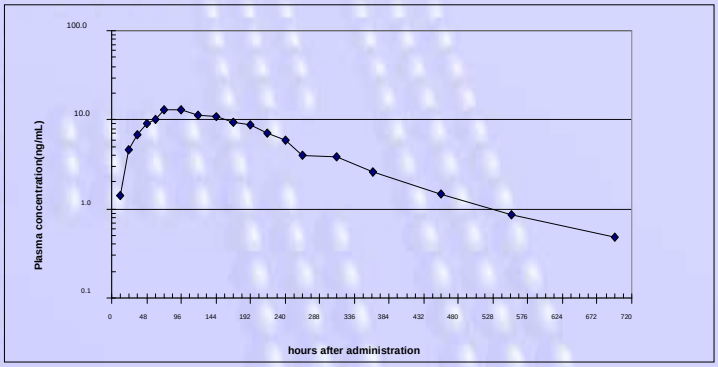

Figure 3: Plasma concentration time curve of ivermectin B_{1a} after a single topical administration of an ivermectin containing pour-on formulation at a dose rate of 0.5 mg/kg bw. Data is obtained from a study performed by FRAH.

Discussion and Conclusions

- At ABL, a sensitive bioanalytical LC-MS/MS method for the determination of ivermectin B_{1a} in bovine plasma has been developed and validated successfully.
- An automated online SPE technique was used for the first time, resulting in highly accurate and precise results and a very low LLOQ (0.200 ng/mL) was achieved in comparison to more conventional methods (usually with an LLOQ of 1.00 ng/mL).
- The assay appeared to be extremely suitable for the quantification of ivermectin B_{1a} in bovine plasma samples (Figure 3), thereby offering a competitive alternative to the methods available for the investigation of the pharmacokinetics of ivermectin B_{1a} in plasma.
- A similar method can be successfully applied for the determination of ivermectin B_{1a} in other species, such as horse, dog and cat.

References

1. Shah et al. Bioanalytical Method Validation. A revisit with a decade of progress. Pharmaceutical Research 2000; 17: 551-1557.
2. Guidance for Industry, Bioanalytical Methods Validation. U.S. Department of Health and Human Services, FDA. June 2001.