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A Review of Moxidectin Macrocyclic Lactone Derivative

Ramesh Babu Bodla, Ritesh Pathania*, Shaik Khasimbi

Department of Quality Assurance, Delhi Institute of Pharmaceutical Sciences and Research, DPSRU, Sector-3, M.B road, Puspvihar, New Delhi, India

***Corresponding author:** Ritesh Pathania
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Abstract **Review Article**

Moxidectin is a macrocyclic lactones derivative are probably the anti-parasitic agents most widely used in the treatment of food producing animals, poultry, aquaculture and crops. A number of alternative products such abamectin, doramectin, emamectin, eprinomectin, milbemycin and selamectin, have been marketed since. The increase in the number of macrocyclic lactones drugs, there has been a steady increase in the number of published analytical methods for determination of their residues. Methodologies for determination of moxidectin residues in biological matrices are described in terms of extraction and clean-up methods used for different matrices. Detection systems for determination of moxidectin residues are discussed with a particular emphasis placed on new developments in screening technologies and different chromatography with fluorescence or mass spectrometry.
Keywords: Moxidectin, Avermectins, Milbemycins, UV-Spectroscopy, HPLC –HPTLC Fluorescence; LC–MS/MS Spectroscopy.

INTRODUCTION

In the late 1980s, an American Cyanamid Company agronomist discovered the *Streptomyces* a bacterium from which moxidectin (As shown in figure1) is derived in a soil sample from Australia. Two companies filed patents for moxidectin: Glaxo Group and the American Cyanamid Company, in 1988, all patents were transferred to American Cyanamid. In 1990, the first moxidectin product was sold in Argentina [1].

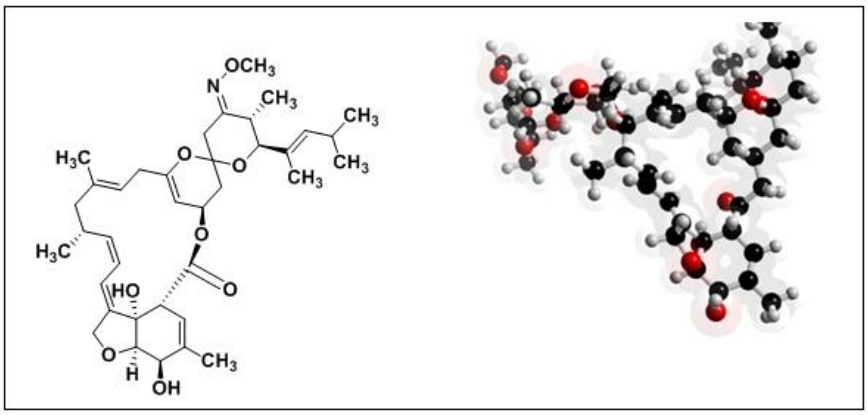

Fig-1: Structutre of the Moxidectin and Ball-and-stick model of the moxidectin molecule

Moxidectin contains a 16-membered macrolactone ring as part of a pentacyclic network. It is similar in structure to the well-known drug ivermectin, B1 and differs in the absence of the disaccharide attached at C13 and the presence of an olefin-containing chain at C25 as well as a methoxime moiety at C23 [2]. Quantitative combustion of moxidectin samples confirmed the % composition of C, H, N, and O against the values presented in Merck Index [3]. The name of the moxidectin are (6R,25S)-5-O-Demetyl-28-deoxy-25-[(E)-1,3-dimetyl-1-butenyl]-6,28-epoxy-23-oxomilbemycin B 23-(E)-(O-metyloxime) (WHO), Milbemycin B, 5-O-demety l-2 8-deoxy-25-(1,3-dimetyl-1-butenyl)-6,28-epoxy-23-(metoxyimino)-,[6R,23E,25S(E)]-USAN [4]. It is a semisynthetic derivative of nemadectin, which is a fermentation

product of the bacterium *Streptomyces cyanogriseus* [5].

Moxidectin was approved for onchocerciasis (river-blindness) in 2018 for people over the age of 11 in the United States based on two studies [5]. There is a need for additional trials, with long-term follow-up, to assess whether moxidectin is safe and effective for treatment of nematode infection in children and women of childbearing potential l[6]. Moxidectin is predicted to be a helpful to achieve elimination goals of this disease [7]. Prevention of heartworm. In combination with imidacloprid to treat sarcoptic mange [8]. Treatment of parasites including *Strongylus vulgaris*, and stomach bots such as *Gasterophilus intestinalis* [9]. Treatment of parasites such as gastrointestinal nematode *Ostertagia ostertagi*, and the lungworm *Dictyocaulus viviparus* [10] Treatment of the nematodes *Teladorsagia circumcincta* and *Haemonchus contortus*[11] Nematodes can develop resistance between moxidectin and other similar parasiticides, such as ivermectin, doramectin and abamectin (As shown in Figure 2).

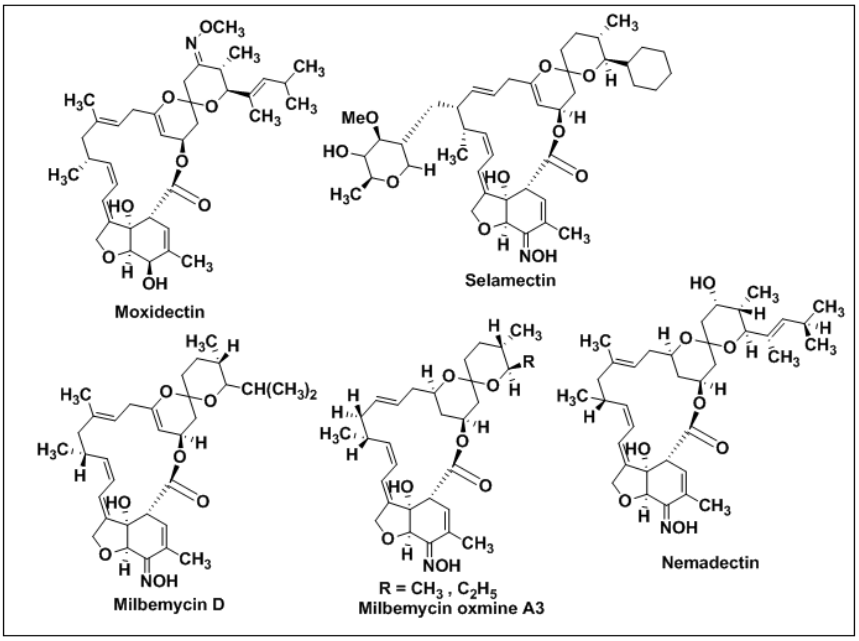

Fig-2: Marketed drugs of lactones derivatives

Other Names: 23-(O-Methyloxime)-F28249-α, Moxidectin technical CL301, 423[2], Cydectin ®1

Trade Names: Cydectin® Cattle Pour-On consists of a 0.5% moxidectin solution.
CAS Registration Number 113507-06-5

Characterization and Composition

Moxidectin is a semisynthetic macrolide antibiotic, the methyloxime derivative of nemadectin [12].

Properties [13, 14]

APPEARANCE AND ODOR:	White/Yellow Powder
MELTING POINT:	Liquefies at 145-154C
VAPOR PRESSURE:	< 3.2 x 10>-8< TORR-Limit of Detection
% VOLATILITY (BY Negligible VOL.):	OCTANOL / HO 58,300
EVAPORATION RATE:	Negligible
SOLUBILITY IN WATER	0.51 mg/L at 25C

Synthesis of Moxidectin

G. Asato *et al*. The work of Asato and France demonstrated a successful synthesis of moxidectin using nemadectin as a precursor [15]. by applying typical steps such as (a) protection of C5-OH using t-butyldimethylsilyl (TBDMS) acetate/ chloride and a base; (b) reaction with an oxidizing agent such as pyridinium chlorochromate (PCC) in dimethyl sulfoxide (DMSO), forming a C23-oxo derivative; (c) addition of p-toluenesulfonic acid (p-TSA) in methanol to remove the TBDMS unit and dilute sodium hydroxide (NaOH) solution to remove any acetate groups; (d) reaction with methoxylamine hydrochloride (MH) to yield C23-methoximino-nemadectin (moxidectin), or alternatively, TBDMS can be removed from the C23-oxo derivative, which can be directly converted into moxidectin; and finally (e) washing with solvents and drying over magnesium sulfate (MgSO4)

to collect amorphous moxidectin powder [16]. (As shown in Figure3)

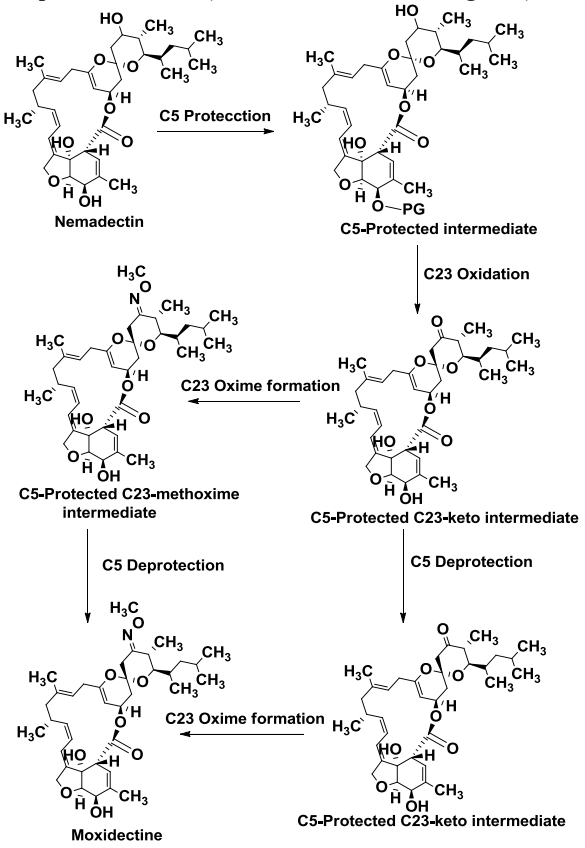

Fig-3: Schematic representation of the conversion of nemadectin into moxidectin

Patricia C. Tway *et al.* development Precolumn derivatization with fluorescent detection provides greater sensitivity and selectivity than UV detection, and therefore, fluorescence is often preferred to UV for detection of these residues at low concentrations. The derivatization procedure involves reacting the moxidectin and other macrocyclic lactones with nonfluorescent reagents to produce fluorescent derivatives, such as the aromatic didehydro-moxidectin [17]. J.W. Tolan *et al.* a number of derivatization procedures have been developed over the past 20 years based on this principle and an early trend was to use more reactive reagents to shorten the reaction time to reduce the derivatization temperature, and to eliminate the need for postderivatization clean up [18] (As shown in figure4).

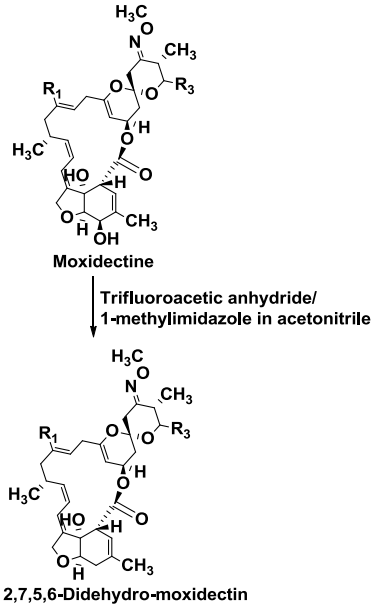

Fig-4: Derivatization of moxidectin into the fluorescent didehydro derivative

Different Methods of Development and Validation of Moxidectin

Nageh Abotaleb *et al*. were validated chromatographic methods for simultaneous determination of Triclabendazole (TCL) and Moxidectin (MOX) in combined dosage forms with no prior separation or interference from excipients. The first method was an isocratic HPLC method on a BDS phenyl C18 column using acetonitrile: methanol: 5mM ammonium dihydrogen phosphate solution (60:30:10, by volume) as a mobile phase at wavelength 242nm, retention times were found to be 1.9 min and 3.9 min for TCL and MOX, respectively. The second method was a simple HPTLC method where separation was performed on HPTLC silica gel 60 F254 plates using ethyl acetate: toluene: formic acid 85%: (50:45: 5, by volume) as a developing system, the developed bands were scanned at 242nm, R_f values were found to be 0.60 and 0.90 for TCL and MOX, respectively. The linear ranges of the first method were found to be 1-200 µg/mL and 0.5-100 µg/mL, while those of the second method were found to be 0.5 -20 µg/band and 0.1-2 µg/band for TCL and MOX, respectively. Both methods were validated and applied for the determination of the two drugs in pure raw material and combined dosage form with no interference from reported excipients and were found to be suitable for quality inspection of combined dosage forms[19]. (As shown in Table.no:1).

Table-1: Analysis of TCL and MOX in marketed formulation by HPTLC densitometry and application of standard addition technique

Product	Standard addition					
	Proposed method% recovery	**Taken Amount (µg)**	**Added Amount (µg)**	***Total Found (µg)**	***Standard Found (µg)**	***% Recovery of added**
Cydectin Triclamox sheep oral drench® Labeled to contain 1mg MOX and 50mg TCL / 1mL		7.5	0	7.5±0.04	-	
		7.5	5	12.5±0.09	5±0.05	100±1
	TCL 99.66±0.66	7.5	7.5	14.94±0.10	7.44±0.008	99.2±0.106
		7.5	9	16.48±0.12	8.98±0.08	99.77±0.88
		Mean ±RSD*				99.66±0.66
	MOX 99.64±0.62	0.15	0.0	0.15±0.003	-	-
		0.15	0.1	0.249±0.005	0.0996±0.002	99.6±0.02
		0.15	0.15	0.299±0.005	0.149±0.0004	99.33±1.33
		0.15	0.2	0.35±0.004	0.201±0.0003	100 ±0. 5
		Mean ±RSD*				99.64±0.62

They were reported HPTLC method was accuracy, simplicity, time and cost effectiveness confirm their suitability for use as routine quality control methods for both drugs in their combined dosage forms without prior separation or interference from reported excipients.

Yashpal S. Chhonker *et al*. was developed and validated a LC-MS/MS method of MOX in mice, monkey, and human, monkey and mouse plasma. The separation was achieved on an ACE C18 (50 × 50 mm, 3µ) column with isocratic elution using 0.1% acetic acid and methanol: acetonitrile (1:1, v/v) as mobile phase. MOX was quantitated using MS/MS with electrospray ionization source operating in negative MRM mode. The MRM precursor ion → product ion transitions for MOX and abamectin (IS) were m/z 638.40 →236 .30 and m/z 871.50→565.35 respectively. The MS/MS response was linear over the concentration range from 0.1-1000 ng/mL in plasma with a correlation coefficient (r2) of 0.997 or better. The within- and between-day precision (relative standard deviation, % RSD) and accuracy were within the acceptable limits per FDA guidelines [20]. (As shown in Table.no2 and Table.no3)

Table-2: Mean extraction recoveries of the MOX from human, monkey and mouse plasma

Matrix	% Extraction recoveries (Mean ± SD, n=5)		
	LQC	MQC	HQC
Human Plasma	67.1 ± 5.2	64.2 ± 5.0	67.4 ± 6.1
Monkey Plasma	62.3 ± 8.6	68.3 ± 4.9	63.3 ± 7.1
Mouse Plasma	70.5 ± 5.8	66.3 ± 4.3	66.5 ± 4.2

Table-3: Mean stability recoveries of the MOX at different storage conditions in human plasma

Analyte	% Stability recoveries (Mean ± SD)			
	Freeze-thaw (–80 ± 5℃ after three cycle)	**Long-term (–80 ± 5℃, 30 days)**	**Auto-sampler (4℃, 36 hrs)**	**Bench-top (room temperature, 4 hrs)**
LQC	97.2 ± 7.6	85.2 ± 1.0	95.3 ± 7.8	92.4 ± 12.0
MQC	100.5 ± 3.6	87.4 ± 1.2	94.5 ± 5.5	92.0 ± 9.0
HQC	103.0 ± 9.9	89.9 ± 3.1	97.5 ± 9.8	96.5 ± 9.6

S. Croubels *et al*. a novel, sensitive and specific method for the quantitative determination of ivermectin B1 (As shown in Figure.5) in animal plasma using liquid chromatography combined with positive electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) is presented. Abamectin was used as the internal standard. Extraction of the samples was performed with a deproteinization step using acetonitrile. Chromatographic separation was achieved on a Nucleosil ODS 5 µm column, using gradient elution with 0.2% (v/v) acetic acid in water and 0.2% (v/v) acetic acid in acetonitrile. Calibration curves using plasma fortified between 1 and 100ng ml−1 showed a good linear correlation (r ≥ 0.9989, goodness-of-fit coefficient ≤8.1%). The trueness at 2 and 25ng ml−1 (n = 6) was +4.2 and −17.1%, respectively. The trueness and between-run precision for the analysis of quality control samples at 25ng ml−1 was −4.0 and 11.0%, respectively (n = 16). The limit of quantification of the method was 1.0ng ml−1. Using a signal-to-noise ratio of 3: 1, the limit of detection was calculated to be 0.2 ng ml−1. The specificity was demonstrated with respect to ivermectin B1b [21] (As shown in Table. No: 4).

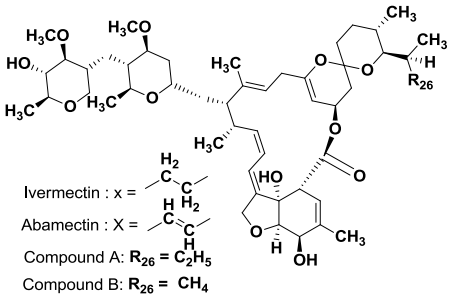

Fig-5: Structures of ivermectin and abamectin

Table-4: Validation results for the determination of ivermectin B1a in calf plasma by LC/ESI-MS/MS

	Concentration	Trueness (%)	Precision (RSD, %)
Calibration curve r = 0.9989 g= 8.1%	0 -100ng ml^{-1}	within -50 to +20% depending on the concentration	
Trueness and precision:			
within-run	2ng ml^{-1}	+4.2	25.8
within-run	25ng ml^{-1}	-17.1	12.9
between-run	25ng ml^{-1}	-4.0	11.0
Limit of quantification	1ng ml^{-1}	+13.3	14.4
Limit of detection	0.2ng ml^{-1}		
Specificity	no interference of endogenous compounds no interference of analogous compounds (ivermectin B1b)		

P Sathish Babu *et al*. were development accurate and validated UV Spectrophotometry method has been developed to determine Moxidectin in bulk drug and synthetic mixture. The Calibration graphs were plotted over the range of 8-22 µg/ml with correlation coefficient value of 0.9994. The Limit of Detection (LOD) and Limit of Quantification (LOQ) for Moxidectin were 0.0264 and 0.08 µg/ml. The percentages RSD for precision of the method were found to be less than 2%. The assay percentages were found to be 100.8%. The newly developed method was validated according to the ICH guidelines with respect to linearity, accuracy, precision and specificity [22] (As shown in Table.no 5).

Table-5: % RSD calculation of synthetic mixture of moxidectin

Concentration (µg/Ml)	Precision	Standard Deviation
18	0.7285	0.00004
18	0.7292	0.0001
18	0.7284	0.0006
18	0.7287	0
18	0.729	0.0006
18	0.7289	0.0001
MEAN/ AVERAGE	0.7287	0.00029

%RSD (Relative Standard Deviatin)=0.0397% (which is less than 2%)

Hsiu-Kuan Chou *et al*. Abamectin, Doramectin, Moxidectin, Ivermectin, Milbemectin A3 and Milbemectin A4 are similar macrocyclic lactone chemicals used as parasiticides or acaricides. A method using high performance liquid chromatography (HPLC) with fluorescence detection is presented for the simultaneous determination of the residue amounts of these compounds in bovine muscle. samples are extracted using acetonitrile and cleaned up with solid phase extraction using a C18 column, followed by fluorescence-derivatized with 1-methylimidazole and trifluoroacetic anhydride in acetonitrile. the analogue was measured by Hplc with fluorescence detector at 365nm excitation and 470nm emission wavelengths. the limits of quantification are below the stipulated taiwan maximum residue limit for each compound. The recoveries of this method in bovine muscle ranged from 73.3 to 110%, with a rsd from 2.11 to 16.57%. The detection limit of those 6 compounds in bovine muscle was 5 ppb. no any above compounds were detected in 50 samples of bovine muscle tested. Therefore, the developed method can be used for rapid screen of macrocyclic lactones in bovine muscle [23].

Table-6: The recoveries and relative standard deviation of macrocyclic lactones from spiked bovine muscle (n=3)

Compound	Theoretical concentration (µg/kg)	Recovery (%) (mean±SD) standard	Relative standard deviation[%]
Milbemectin A3	300	90.0 ± 6.9	7.70
	100	93.4 ± 6.7	7.13
	20	94.0 ± 8.4	8.95
	5	96.9 ± 10.6	10.93
Moxidectin	300	79.8 ± 6.8	8.59
	100	85.6 ± 6.7	7.89
	20	81.3 ± 4.3	5.30 5
	5	86.7 ± 9.0	10.41
Milbemectin A4	300	86.3 ± 5.5	6.51
	100	93.1 ± 8.0	8.63
	20	82.0 ± 1.7	2.11
	5	100.7 ± 9.0	8.96
Abamectin	300	86.0 ± 5.0	5.88
	100	85.3 ± 3.5	4.13
	20	82.7 ± 3.8	4.62 5
	5	90.0 ± 13.1	14.57
Doramectin	300	81.8 ± 6.5	7.99
	100	89.9 ± 14.9	16.57
	20	97.3 ± 5.1	5.27 5
	5	92.7 ± 14.1	15.31
Ivermectin	300	79.2 ± 5.2	6.53
	100	81.7 ± 3.8	4.76
	20	80.8 ± 3.6	4.56 5
	5	98.7 ± 11.0	11.16

Martin Danaher *et al.* a multi-residue method had been developed for the quantitative determination of moxidectin, abamectin, doramectin and ivermectin in liver samples, with capability for qualitative identification of the presence of eprinomectin. Liver samples are extracted with isooctane, followed by clean-up on alumina-N solid phase extraction (SPE) cartridges. Extracts are derivatised and determined by high-performance liquid chromatography (HPLC) with fluorescence detection. The method was validated using bovine liver fortified at levels of 4 and 20 mg kg21 with the drugs. The mean recovery from bovine liver ranged between 90 and 96%. The intra and inter-assay variations showed RSD typically of < 5% and < 10%, respectively. The procedure was applied also to ovine and porcine liver, giving similar results. A robustness study, carried out on the alumina clean-up step, indicated that the step is relatively insensitive to method changes. However, significant differences overall were found for the type of alumina and/or commercial SPE cartridge used. The limit of quantitation of the method is 2 mg kg21 (ppb) [24]. (As shown in Table.No:7).

Table-7: Effect of different SPE alumina cartridges on recovery from samples fortified at 20 mg kg 21 (n = 3).

Analyte	Mean recovery ± s (%)			
	Laboratory	Isolute™	Bond-Elut Jr™	Sep-Pak™
Moxidectin	84 ± 2.6	87 ± 0.9	88 ± 3.4	68 ± 6.7
Abamectin	81 ± 3.0	83 ± 1.3	84 ± 2.9	93 ± 3.5
Doramectin	86 ± 3.1	90 ± 1.4	87 ± 2.3	96 ± 3.1
Ivermectin	83 ± 3.1	89 ± 1.8	87 ± 2.1	96 ± 4.4
Analysis of variance— Source of variation		F test significance		
Cartridge Analyte Cartridge*analyte		P < 0.01 P < 0.001 P < 0.001		
*SPE cartridge prepared in laboratory.				

Dennis Kitzman *et al.* was accurate, sensitive and selective high-performance liquid chromatography (HPLC) method for the quantitation of ivermectin in human plasma that separates the parent drug from metabolites. Ivermectin and the internal standard, moxidectin, were extracted from 0.2 ml of human plasma using Oasis HLB solid phase extraction cartridges. After extraction, fluorescent derivatives of ivermectin and moxidectin were made by reaction with trifluoroacetic anhydride and N-methylimidazole (As shown in Figure 6). column with a mobile phase composed of tetrahydrofuran–acetonitrile–water (40:38:22 v/v/v). Detection is by fluorescence, with an excitation of 365 nm and emission of 475 nm. The retention times of ivermectin and internal standard, moxidectin are approximately 24.5 and 12.5 min, respectively. The assay is linear over the concentration range of 0.2–200ng/ml of ivermectin in human plasma (r = 0.9992, weighted by 1/concentration). Recoveries of ivermectin are greater than 80% at all concentrations. The analysis of quality control samples for ivermectin 0.2, 25, and 200ng/ml demonstrated excellent precision with coefficient of variation of 6.1, 3.6 and 2.3%, respectively (n=6). The method is accurate with all intra-day (n = 6) and interday (n=12) mean concentration within 10% of nominal values at all quality control sample concentrations. Storage stability for 30 days at −80°C and after three freeze–thaw cycles are within acceptable limits [25].

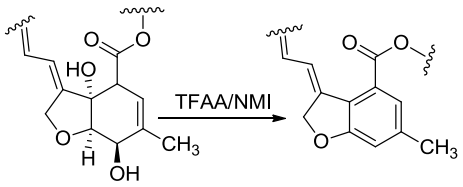

Fig-6: Derivatization reaction of ivermectin and moxidectin with trifluoroacetic anhydride (TFAA) and N-methylimidazole (NMI)

Mathieu Varache *et al.* a reversed-phase (RP) high-performance liquid chromatography (HPLC) method for the content determination of IR780-oleyl (IRO) dye in lipid nanoparticles were developed and validated. Chromatographic separation was performed on a RP C18 column with a gradient program of water and acetonitrile both with 0.1% (v/v) TFA, at a flow rate of 1.0 mL/min and a total run of 21 min. IRO dye detection were made by fluorescence at emission wavelength of 773 nm (excitation wavelength: 744 nm). According to ICH guidelines, the developed method was shown to be specific, linear in the range 3–8mg/ mL (R2 = 0.9998), precise at the intra-day and inter-day levels as reflected by the coefficient of variation (CV 1.98%) at three different concentrations (4, 6 and 8 mg/mL) and accurate, with recovery rates between 98.2–101.6% and 99.2–100.5%. The detection and quantitation limits were 0.41 and 1.24mg/mL, respectively. Stability studies of sample processing showed that IRO dye was stable after 24 h in the autosampler or after three freeze/thaw cycles. Combined with fluorescence measurements, the developed method was successfully applied to optimize the loading capacity of IRO dye in the core of lipid nanoparticles [26]. (As shown in Table.No:8).

Table-8: Concentration of IRO dye for a concentration of lipids at 100 mg/mL, number of dyes per nanoparticle (NP), dye loading (DL) and entrapment efficiency (EE). For each concentration, results are expressed as average and SD of three independent samples analysed in duplicate. The number of IRO dyes per nanoparticle was calculated from the concentration of dye measured on the final product and by considering a particle diameter of 50 nm and a lipid density of 1.05 g/cm3

$[Dye]^{theo}$ (mg/mL)	DLt^{heo} (%)	$[Dye]^{exp}$ (mg/mL)	DL^{exp} (%)	EE (%)	$Dyes/NP^{exp}$
67	0.07	52.5 ± 6.4	52.5 ± 6.4 0	78.6± 9.4	22.0 ± 2.7
133	0.13	108.1 ± 4.1	0.108 ± 0.004	81.0 ± 2.9	45.4 ± 1.7
267	0.27	217.8 ± 10.9	0.217 ±0.011	81.7± 4.0	91.4± 4.6
533	0.53	436.5 ± 50.5	0.43 ± 0.050	82.1 ± 9.6	2 ± 21.2
798	0.79	728.8 ± 16.2	0.723 ± 0.016	91.6 ± 1.9	305.8 ± 6.8
1,060	1.04	925.1 ± 66.1	0.855 ± 0.108	187.3± 6.1	362.5 ± 45.7
1,322	1.31	133.4 ± 20.5	1.119 ± 0.020	85.5 ± 1.6	475.6 ± 8.6

C. Paraud *et al*. Resistance to ivermectin and moxidectin were explored by a faecal egg count reduction test in two sheep flocks with suspected anthelmintic resistance. With a mean percentage of reduction in egg excretion within the treated groups of 0% for ivermectin (CI 95%: −228 to 58) and 13% for

moxidectin (CI 95%: −152 to 70). An experimental infection of 18 naïve lambs were set up using infective larvae isolated from this flock (5000 L3/lamb). Compared to the control group, abomasal worm burdens (Teladorsagia circumcincta) were reduced by 90% [CI 95%: 81.5–94.8] and 85% [CI 95%: 72.4–92.2] after ivermectin ($p < 0.05$) and moxidectin ($p < 0.05$) treatment respectively. Again, compared to the control group, there were a reduction for intestinal strongyles (Trichostrongylus colubriformis) of 100% and 99% [CI 95%: 97.5–99.7] for ivermectin and moxidectin respectively. No difference was found between the efficacy of moxidectin and ivermectin. Pharmacokinetic values indicated that the strongyles were submitted to anthelmintic concentrations usually lethal to them. This trial demonstrated the first multiple resistance of ovine strongyles in France [27]. (As shown in Table.No:9).

Table-9: mean strongyle numbers in abomasums, small intestine and caecums in the control, ivermectin (IVM) and moxidectin (MOX) groups 10 days after administration of anthelmintics (n = 6 lambs per group) and percentage of reduction in the treated groups compared to the control group

	Group	Number of infested lambs	Mean burden(min-max)	% reduction (CI 95%)
Abomasum	Control IVM MOX	6 6 6	578[a] (220–670) 57[b] (10–110) 85[b] (30–200)	- 90.2 [81.5–94.8] 85.3 [72.4–92.2]
Small intestine	Control IVM MOX	6 0 3	1088[a] (770–1430) 0[b] 8[b] (0–30)	- 100 99.2 [97.5–99.8]
Large intestine	Control IVM MOX	6 0 0	60[a] (12–118) 0[b] 0[b]	- 100 100
Total worm burden	Control IVM MOX	6 6 6	1727[a] (1272–2268) 57[b] (10–110) 93[b] (40–200)	- 94.6 [90.5–96.9] 96.7 [94.2–98.1]

Michelle Del Bianchi A *et al.* were The development and validation of a throughput method for the quantitation of moxidectin residues in lamb target tissues (muscle, kidney, liver and fat) was conducted using ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The chromatographic separation was achieved using a Zorbax Eclipse plus C18 RRHD column with a mobile phase comprising 5 mM ammonium formate solution + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B) in a linear gradient program. Method validation was performed based on the Commission Decision 2002/657/EC and VICH GL49. To quantify the analyte, matrix-matched analytical curves were constructed with spiked blank tissues, with a limit of quantitation of 5ng g-1 and limit of detection of 1.5ng g-1 for all matrices. The linearity, decision limit, detection capability accuracy, and inter- and intra-day repeatability of the method are reported. The method was successfully applied to incurred lamb tissue samples (muscle, liver, kidney and fat) in a concentration range from 5 to 200ng kg-1, which demonstrated its suitability for monitoring moxidectin residues in lamb tissues in health surveillance programs, as well as for pharmacokinetics and residue depletion studies [28] (As shown in Table.N.10).

Table-10: Method validation parameters for quantitation of moxidectin (MOX) in lamb target tissues by UHPLC-MS/MS

Parameters	Specifications*	Muscle	Kidney	Liver	fat
Range of work (ng g-1)		0 – 200	0 – 200	0 – 200	0 – 200
Equation of the analytical curve		y =0,0087x+ 0,0108	y=0,0591x-0,0069	y =0,0591x - 0,0069	y = 0.0289x–0.1534
Linearity	$r \geq 0.98$	r = 0.9998	r = 0.9929	r = 0.9991	r = 0.9969
LOD (µg/kg)		1.5	1.5	1.5	1.5
LOQ (µg/kg)		5	5	5	5

J.C. Williams *et al*. Twenty male Holstein calves averaging 105 kg in weight and naturally infected with gastrointestinal nematodes and small numbers of lungworm and hookworm, were given experimental infections with the two latter species to provide adult and larval stages for anthelmintic evaluation. Following random allotment, one group of 10 calves was injected subcutaneously with moxidectin at a dosage of 0.2 mg kg-~ of body weight. A second group of 10 was injected subcutaneously with unmedicated blank vehicle at a dosage of 1 ml per 50 kg of body weight. Fecal samples were examined

before treatment and at 7 and 13 days after treatment. The 20 calves were necropsied for worm recovery at 13 and 14 days after treatment. All calves were positive for lungworm and hookworm on the treatment date. Treatment was 100% effective in elimination of hookworm eggs and lung worm larvae and 99.9% in reducing total egg counts at both 7 and 13 days after treatment [29] (As shown in Table No11).

Table-11: Geometric mean worm counts and efficacy of moxidectin against gastrointestinal nematodes and lungworm in calves

Parasite	Control (n= 10)	(No. infected)	Moxidectin (n= 10)	Percent reduction	P value
Ostertagia ostertagi adult	2260	6	0	100	<0.01
Ostertagia ostertagi early L	21	5	0	100	<0.01
Ostertagia lyrata adult male	8	10	0	100	<0.01
Trichostrongylus axei adult	218	10	0	100	<0.01
Haemonchus placei adult	512	10	0	100	<0.01
Cooperia punctata adult male	865	9	0	100	<0.01
Cooperia pectinata adult male	354	6	0	100	<0.01
Cooperia pectinata adult male	17	10	0	100	<0.01
Cooperia spp. adult female	2320	9	0	100	<0.01
Bunostomum phlebotomum adult	75	8	0	100	<0.01
Oesophagostomum radiatum adult	75	10	0	100	<0.01
Trichuris discolor adult	239	7	0	100	<0.01
Dictyocaulus viviparus adult	27	10	0	100	<0.01
Dictyocaulus viviparus imm. adult	31	10	0	100	<0.01

Andreia Freitas *et al*. A multi-residue quantitative screening method covering 41 antibiotics from 7 different families, by ultra-highperformance–liquid-chromatography tandem mass spectrometry (UHPLC–MS/MS), is described. Sulfonamides, trimethoprim, tetracyclines, macrolides, quinolones, penicillins and chloramphenicol are simultaneously detected after a simple sample preparation of bovine muscle optimized to achieve the best recovery for all compounds. A simple sample treatment was developed consisting in an extraction with a mixture of acetonitrile and ethylene diaminetetraacetic acid (EDTA), followed by a defatting step with n-hexane. The methodology was validated, in accordance with Decision 2002/657/EC by evaluating the required parameters:decision limit (CCα), detection capability (CCβ), specificity, repeatability and reproducibility. Precision in terms of relative standard deviation were under 20% for all compounds and the recoveries between 91% and 119%. CCα and CCβ were determined according the maximum residue limit (MRL) or the minimum required performance limit (MRPL), when required [30].

Andressa Camargo Valese *et al*. a sensitive method for the simultaneous residues analysis of 62 veterinary drugs in feeds by liquid chromatography-tandem mass spectrometry has been developed and validated in accordance to Commission Decision 657/2002/EC. Additionally, limits of detection (LOD), limits of quantitation (LOQ), matrix effects and measurement uncertainty were also assessed. Extractions were performed for all analytes and respective internal standards in a single step and chromatographic separation were achieved in only 12 min. The top-down approach was adequate for the calculation of measurement uncertainty for all analytes, except the banned substances, which should be rather assessed by the bottom up approach. A high throughput screening/confirmatory method for the residue analysis of several veterinary drugs in feeds was proposed as a helpful control tool [31]. (As shown in Table.No:12).

Table-12: Results of residue analysis of multi-class veterinary drugs in samples of feeds for pigs, cattle and poultry

Analytes	Quantifiable samples	Minimum concentration*	Maximum concentration*
Abamectin	1	476.7 ± 52.6 µg kg^{-1}	
Amoxicilin	2	3.56 ± 0.05 mg kg^{-1}	44.4 ± 0.1 mg kg^{-1}
Amprolium	1	51.4 ± 11.0 µg kg^{-1}	
Clopidol	1	Detected	
Chlortetracycline	2	414.2 ± 26.3 µg kg^{-1}	
Florfenicol	1	130.2 ± 26.0 µg kg^{-1}	
Lincomycin	2	451.2 ± 52.6 µg kg^{-1}	8360 ± 50 µg kg^{-1}

Monensin	1	12.9 ± 4.6 µg kg^{-1}	
Narasin	1	190.8 ± 35.4 mg kg^{-1}	
Nicarbazin	4	15.6 ± 3.9 µg kg^{-1}	> 400 µg kg^{-1}
Norfloxacin	2	186.2 ± 46.9 µg kg^{-1}	4050 ± 50 µg kg^{-1}
Ractopamine	3	1.96 ± 0.05 µg kg^{-1}	> 400 µg kg^{-1}
Salinomycin	2	133.3 ± 0.5 µg kg^{-1}	> 400 µg kg^{-1}
Sulfamerazine	1	717.6 ± 52.6 µg kg^{-1}	
Tiamulin	4	36.2 ± 19.0 µg kg^{-1}	> 400 µg kg^{-1}
Tilmicosin	1	105.2 ± 39.4 µg kg^{-1}	
Thiamphenicol	1	1 105.2 ± 26.5 µg kg^{-1}	
Tylosin	6	63.4 ± 26.0 µg kg^{-1}	2.0 ± 0.05 mg kg^{-1}
Trimethropim	1	104.0 ± 36.3 µg kg^{-1}	

*Measurement uncertainty was calculated using an effective degree of freedom that corresponds to a probability of coverage factor of approximately 95.45%.

G.D. Almeida *et al*. Ivermectin (IVM) resistance of Cooperia spp. in cattle has become an increasing and global problem. The early detection of anthelmintic resistance (AR) is importantto propose strategies to slow down the development of resistance and requires sensitive, reliable, economic high-throughput and practical tests. The purpose of the present study was to apply a larval migration inhibition test (LMIT) for evaluating IVM and MOX efficacy against well characterized field isolates of Cooperia spp. infecting cattle in Brazil The LMIT used in the present study can be a useful tool for in vitro evaluation of IVM, but not of MOX. However, such methodology cannot be used in large-scale studies yet. The isolates of Cooperia spp. showed various degrees of resistance to IVM, though remaining susceptible to MOX [32] (As shown in Tabble.No13).

Table-13: Larval migration inhibition test results for moxidectin against Cooperia field isolates, with the half maximal effective concentration (EC50), confidence interval (95% CI), coefficient of determination (R2), hill slope (HS), resistance factor (RF) and P values (comparison to the susceptible isolate).

Isolate	EC50(µmol)	95% Cl (µmol)	P-value	R^2	HS	RF
Susceptible	0.75	0.596–0.964	-	0.92	1.17	-
Campo Grande BNA	0.93	0.465–1.865	0.1143	0.81	9.24	1.24
Campo Grande TBR	0.36	0.286–0.466	0.0351	0.92	0.48	0.48
Nova Alvorada do Sul II	2.57	1.517–4.363	0.1125	0.75	0.75	3.43
Bandeirantes	1.43	0.700–2.912	0.01062	0.61	0.66	1.91
Campo Grande II	1.08	0.632-1.870	<0.0001	0.74	0.75	1.41
Porto Murtinho	0.49	0.307-0.788	<0.0001	0.77	0.98	0.68

Gabriel Rübensama *et al.* was simple and inexpensive sample preparation method based on solvent extraction, followed by low temperature cleanup, were demonstrated to be applicable for the determination of avermectin and milbemycin residues in bovine muscle by liquid chromatography-tandem mass spectrometry (LC-MS/ MS) and liquid chromatography with fluorescence (LC-FL) detection. The analytical methodology was validated according to the Commission Decision 2002/657/EC, using LC-MS/MS for confirmatory and LCFL for quantitative purposes.Method precision led to satisfactory values of decision limits ($CC\alpha$) and detection capabilities (CC_β). The proposed was method has been applied in the Brazilian National Residue Control Plan since 2010 for the determination of avermectins and milbemycin residues in bovine muscle samples. A total of 760 samples were analyzed and none of them presented residues at concentrations above the permitted levels established by the more recently applied directives [33] (As shown in Table.No:14).

Table-14: Results of the Brazilian monitoring program for avermectins and milbemycin analysis in bovine muscle samples carried out from 2010 to 2011

Analytes	LOD	LOQ	MRL[a]	n < LOD	LOD < n < LOQ	LOQ < n < MRL[a]	MRL[a] < n
ABA	0.2	0.6	10	680	72	8	0
DOR	0.6	1.9	10	711	44	5	0
EPR	1.1	3.4	100	754	6	0	0
IVR	0.3	0.9	10	601	124	35	0
MOX	0.1	0.4	20	721	26	13	0

Legend: (n) number of occurrences; (LOD), (LOQ), and (MRL) are respectively limits of detection, limits of quantification, and maximum residue limits, expressed in mg kg1; (n < LOD) results below the detection limits, (LOD < n < LOQ) results between detection and quantification limits, (LOQ < n < MRL) results between quantification limits and MRL, and (MRL < n) results above the MRL. A MRL values established by Brazilian legislation [6].

Maria Angela Machado Fernandes *et al*. was to determine the presence of antiparasitic drug residues in 42-days old lamb serum and tissues, submitted to three endoparasite control programs: preventive treatment (PT) using moxidectin (MOX) at every 28 days; selective treatment (FEC) using MOX when fecal egg count was greater than or equal to 700; and selective treatment (FMC), using MOX when FAMACHA/FMC score was 3 and above. For this purpose, MOX residues were quantified in serum, muscle, fat, liver and kidney. Lambs were slaughtered when reaching 30 kg of body weight, and after a 28-day MOX withdrawal period. The quantitation of MOX residues were performed using liquid chromatography tandem mass spectrometry (LC-MS/MS). In conclusion was all weaned lambs, produced in continuous grazing and subjected to gastrointestinal parasite control programs via selective (FEC and FMC) or preventive (PT) treatment, displayed a low risk (less than 1%) of MOX residues above the MRL in muscle, fat, kidney, and liver [34].

Emiliano Felici *et al.* sensitive, specific, robust and environmentally friendly analytical methods are still required. In this paper, a new automatized pre-concentration methodology followed by microemulsion electrokinetic chromatography (MEECK) analysis were developed for the simultaneous separation and determination of the most used macrocyclic lactones, ivermectin (IVM) and moxidectin (MXD) in environmental water. XAD-4 resin was employed as adsorbent for the preconcentration process and ethanol was used as the eluent. In contrast to traditional analysis for IVM and MXD in this methodology non-polluting solvents were involved during the whole process and therefore, it could be considered as a contribution to green analytical [35].

CONCLUSIONS

A mini-review is presented on methodology for determination of moxidectin residues in biological matrices. Recommendations are made on multi-residue methods that are considered to be most suitable for surveillance of macrocyclic lactone residues in food. This approach could lead to a reduction in the extent of sample clean-up required prior to analysis. LC fluorescence and LC–MS are presently the techniques of choice for determination of moxidectin residues. LC fluorescence has advantages over mass spectrometry in terms of cost but mass spectrometry is more sensitive and specific. At present, the widespread application of immunochemical methods is restricted by the limited cross-reactivity of antibodies, particularly between avermectins and milbemycins. There have been a number of developments in methodology for determination of ML residues in recent years, particularly in multi-residue applications. In the future, it is expected that developments will continue in the areas of sample preparation and detection. In particular, research should focus on the development of automated or on-line clean-up procedures that allow unattended purification of sample extracts. Alternatively, 96-well plate technology that has found application in plasma analysis may find application in testing for moxidectin residues in milk and tissues. Such an antibody may be applied in a biosensor assay to give equivalent sensitivity to chromatographic detection systems. Automated pre-column derivatisation has not found widespread application in multiresidue methods. Many of the latest multi-residue methods developed for determination of MLs use LC–MS/MS for detection of residues. It is expected that researchers will develop LC–MS/MS methods offering improved reproducibility and reliability.

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