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A Review on Different Analytical Techniques for Quantification of Moxidectin

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Abstract: Nemadectin, a macrocyclic lactone of the milbemycin class, is a fermentation by-product of the bacteria *Streptomyces cyanogriseus subsp*. *non-Cyanogenus.* Moxidectin is a semi-synthetic derivative of nemadectin. River blindness, also known as onchocerciasis, is treated with moxidectin in patients 12 years of age and older. This condition is brought on by the parasitic worm *Onchocerca volvulus* and is subjected to intense itching, skin conditions that are disfiguring, and impaired vision brought on by the larvae of the worm. Some of the most common internal and exterior parasites are killed by moxidectin by selectively binding to their glutamate-gated chloride ion channels. In this review article, various pieces of equipment, such as a UV spectrometer, HPLC, LC-MS, and UPLC-MS, are used to determine moxidectin as well as its related compounds. The QuEChERS method was also used in the sample preparation according to the literature survey. The report also offers an overview of the pharmacodynamics, pharmacokinetics, and medication interactions of moxidectin.

Keywords: Moxidectin, Anthelmintic, River Blindness, Macrocyclic Lactones

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1. INTRODUCTION

The drug moxidectin (MOX) is a member of the macrocyclic lactones (MLs) class. It is created by the bacteria *Streptomyces cyanogriseus* or *S. hygroscopicus* and is primarily used in veterinary medicine to treat a variety of parasite infections in animals used for food production. Various regulatory organizations have defined maximum residue limits and tolerance limits for veterinary drugs to protect humans from being exposed to harmful residual levels through the consumption of edible products produced from treated animals. The liver and other tissues including the kidney and the muscle regulate the maximum levels of these drugs. (1) Because MLs are harmful, the European Union Council decided to set a maximum residual level for this substance out of concern for consumer safety. To identify ML residues in goods of animal origin, extremely sensitive and selective procedures are needed because the concentration of interest was frequently quite low. (2).

1.1. Pharmacodynamics

Moxidectin has been reported to be extremely effective against *Onchocerca volvulus* than other drugs. Moxidectin remains to be more efficient than a variety of Ivermectin-resistant nematode species when given to infected individuals. While still safe to be utilized in mass drug administration, the levels of microfilaria were reduced to undetectable levels, and moxidectin's efficacy is significantly higher when compared to other drugs. (4)

1.2 Pharmacokinetics

Moxidectin was more quickly absorbed from the injection site than other drugs (absorption half-life = 0.7 days). Moxidectin's highest plasma concentration (C_{max}) had occurred much earlier (T_{max} = 0.4 days). However, moxidectin's mean residence time was longer than doramectin's (9.4 days) and had a higher region under the concentration-time curve (475 ng day/mL) than doramectin's (198 ng day/ mL) (4). No variations in C_{max} values were seen. Moxidectin has a

shorter T_{max} but a longer C_{max} and AUC. It has limited oral bioavailability and this small species' early emergence of resistance may be connected. (5)

1.3 Drug interaction studies

1.3.1. Clinical Study using Midazolam (CYP3A4 substrate)

In healthy individuals, taking moxidectin tablets in combination with a single 8 mg dosage had no impact on the pharmacokinetics of midazolam. Moxidectin and CYP3A4 substrates can be administered together. (5)

2. HIGH-PERFORMANCE LIQUID CHROMATO-GRAPHIC (HPLC) METHOD

HPLC is used to separate the complex molecular mixtures that can be found in biological and chemical systems so that each function of the component can be thoroughly understood. The HPLC method can achieve the required precision and has great selectivity. The drug has been analyzed by using HPLC with UV, MS, or MS/MS detection modes. Utilizing high-performance liquid chromatography-based techniques, Animal tissues like liver, muscle, and fat as well as milk, urine, feces, and plasma have been found to contain moxidectin. (1) The following table 1 lists the results of the quantitative measurement of moxidectin using HPLC techniques.

Table 1: Determination of moxidectin by HPLC method.

Sr No	Drug	Mobile phase	Stationary phase	Parameters	Reference
1.	Moxidectin	Acetonitrile, ethyl acetate, and Water (90:4:6 *v/v/v*)	Waters Sun Fire C_8 column (150 mm x 4.6 mm, 5 µm, Waters, USA)	Selectivity, linearity, trueness, LOD, LOQ, decision limit (CCα), and detection capability (CCβ).	1
2.	Moxidectin	85% Methanol and 15% water	Waters PAH C_{18} (50 mm × 4.6 mm × 3 µm from Waters Mild ford, MA, USA)	Recovery, trueness, precision, decision limit, and detection capability	2
3.	Moxidectin	Acetonitrile and water (90:10 *v/v*), Acetonitrile and Isopropyl alcohol (85:15 *v/v)*	Zorbax Eclipse XDB-C_{18} column (150mm × 4.6 mm × 1.8 µm, Agilent, USA)	Linearity, LOD, LOQ, decision limit, detection capability, precision	3
4.	Moxidectin	Acetic acid - Methanol – Acetonitrile (4:15:31, *v/v/v*).	a Supelcosil C_8 column	Linearity, precision, recovery, LOD, LOQ	6
5.	Moxidectin	Tetrahydrofuran–Acetonitrile–Water (40:38:22 *v/v/v*)	Alltech Ultra sphere C_{18} (5µm, 4.6 × 250 mm) column	Selectivity, precision, accuracy, recovery, linearity	7
6.	Moxidectin	0.2% Acetic acidic in water/methanol Acetonitrile (1.6:60:38.4, *v/v/v*)	Reverse Phase C_{18} column (5 µm, 4.6 mm × 250 mm)	C_{max}, T_{max}, $t_{1/2}$, AUC, standard deviation	8
7.	Moxidectin	Acetonitrile and Water (94/6, v/v)	Merck Lichrospher 100RP-18E, (5 µm, 125mm × 4 mm)	Recovery test, LOD, LOQ, precision	9
8.	Moxidectin	Acetic Acid: Methanol: Acetonitrile (20:40:40, *v/v/v*).	Supelcosil C_{18} column (5 µm, 4.6mm ×150mm)	Recovery, radiopurity	10

9.	Moxidectin	Acetonitrile: methanol: water (55:27:18, *v/v/v*)	Gemini C_{18} column (150 mm × 4.60 m m, 5 µm)	Precision, accuracy, recovery tests, robustness	11
10.	Moxidectin	Acetic acid-methanol-acetonitrile	Supelcosil C_{18} column (5 µm 150 mm x 4.6 mm)	Cmax, Tmax, mean residence time (MRT)	12
11.	Moxidectin	Acetonitrile (A) and water (B)	Luna C_8 (150 mm × 3.0 mm, 5 µm)	Specificity, linearity, recovery, precision, LOD, LOQ	13
12	Moxidectin	Acetonitrile, Tetrahydrofuran, water (52:25:23 *v/v/v*).	C_{18} column (150 × 5mm, 5 µm)	LOD, LOQ, recovery, correlation coefficient	14

Fabio Macedo et al. developed and verified a method for measuring four macrocyclic lactones in butter simultaneously by combining liquid chromatography (LC) with fluorescence detection. The mobile phase consisted of 10 ml of a (90:4:6 *v/v/v*) ACN, ethyl acetate, and water solution. For the separation, a Sun Fire C_8 (150 x 4.6 mm, 5 m) column was used. The examined samples had ML residues, according to the method's applicability to actual samples. The recovery, repeatability, within-laboratory reproducibility, and LOD values were satisfactory for the intended application. The technique proved straightforward to apply and performed well for the simultaneous measurement of Macrocyclic lactone residues in butter. (1)

David Pimentel-Trapero et al. established a procedure employing QuEChERS and HPLC with fluorescence detection to measure the macrocyclic lactones in the liver of cows. An analytical Waters PAH C_{18} column of (50 mm × 4.6 mm, 3 µm) was used for the separation. Methanol comprised about 85% of the mobile phase and 15% of it was water. The analytes' fluorescent derivatives were generated by subjecting the sample extracts to a mixture of trifluoroacetic anhydride, triethylamine, and 1-methylimidazole. Results for recovery of analytes, trueness, repeatability, LOD, and LOQ were found to be following EU Decision 2002/657's requirements. (2)

Ana Maria de Souza Santos et al. created and verified a very sensitive analytical technique for the detection of five macrocyclic lactone residues in cheese. For the separation, a Zorbax Eclipse XDB-C_{18} column (150 mm × 4.6 mm, 1.8 µm) has been used. ACN and water made up the mobile phase (ACN: H2O, 90:10 v/v), and acetonitrile and isopropyl alcohol made up the reaction mixture (ACN: ISOPOH, 85:15 *v/v*) (B). Excellent mean recoveries, repeatability, limits of quantification, and intermediate precisions were obtained. (3)

Bengone-Ndong et al. analyzed the kinetics of the plasma of moxidectin and doramectin in zebu gobra in the field settings following cutaneous application of available commercial formulations for cattle at 0.2 mg/kg. When compared to doramectin, the highest plasma concentration of moxidectin was achieved significantly earlier. The C_{max} values were constant, but moxidectin had a larger area under the concentration-time curve (475 ng day/ml) than doramectin (198 ng day/ml) and its average residence time was extended (13.4 days) than doramectin's (9.4 days). The outcomes revealed information about the bioavailability of doramectin and moxidectin in zebu gobra, which have a pharmacological property similar to that of other cattle. (4)

Escudero et al. investigated how doramectin and moxidectin act pharmacologically in goats and followed by oral or subcutaneous at a dosage of 0.2 mg/kg. Both compartmental and non-compartmental approaches were employed to analyze the data on drug plasma concentration over time. The maximal plasma concentrations of moxidectin were reached to a greater extent than doramectin. Moxidectin's mean residence time (MRT), when delivered subcutaneously or orally did not differ significantly. This small species' early emergence of resistance and its low oral bioavailability may be related. These findings should be compared to efficacy research to optimize the dosage needs for endectocides in this species. (5)

Alvinerie et al. reported that using fluorescence-enhanced high-performance liquid chromatography as a technique to detect moxidectin in plasma. By using this technique, moxidectin can be converted into a fluorescent derivative rapidly. Using a Supelcosil C_8 column with fluorescence detection, the separation was performed, and the mobile phase consisted of acetic acid/methanol/ACN (4:15:31, *v/v/v*). The limitations of the analyte recovery, precision, linearity, and quantification limits were found to be within the expected limits. The approach

has worked well for the pharmacokinetic study of moxidectin administered to cows subcutaneously. (6)

Dennis Kitzman et al. devised an HPLC approach for the detection of ivermectin and moxidectin in human plasma that is precise, sensitive, and selective and separates the parent drug from metabolites. The mobile phase consisted of tetrahydrofuran/acetonitrile/water (40:38:22 *v/v/v*) for separation on an Alltech ultra-sphere C_{18} (5µm, 4.6 × 250 mm) column. For all concentrations, the assay was linear over the range of 80% and above. With the coefficient of variation, it showed excellent precision. Studies on precision and storage stability were within acceptable limits. This technique was reliable and appropriate for pharmacokinetic clinical research. (7)

Mercedes Lloberas et al. worked to determine the drug concentrations in plasma, target tissues, and parasites were measured by HPLC. An RP C_{18} (5µm, 4.6 mm × 250 mm) column with a mobile phase of 0.2% of acetic acid in water/methanol/ACN (1.6:60:38.4, v/v/v) was used for the separation at a flow rate of 1.5 ml/min. Moxidectin concentrations showed a longer plasma persistence ($P < 0.05$). By using the least squares linear regression analysis, calibration curves were developed. Correlation coefficients and coefficient of variation were determined, and the results were found to be within the limits. (8)

Hsiu-Kuan chou et al. developed a technique for simultaneously determining the compound residue quantities (abamectin, doramectin, moxidectin, ivermectin, milbemycin) using HPLC and fluorescence detection in bovine muscle. The mobile phase consisted of 940 mL of acetonitrile and 60 mL of water (94/6, v/v). Acetonitrile was used to extract the samples, and then a C_{18} column (5 µm, 125mm × 4 mm) was employed for solid phase extraction to clean them up. The quantification thresholds are below the specified limit residue threshold for each compound. The detection limits and recovery studies both were within accepted limits. Hence, the suggested method can be utilized to quickly screen for macrocyclic lactones in cow muscle. (9)

Alvinerie et al. examined how the enzyme cytochrome P450 contributes to the *in vitro* metabolism of moxidectin in homogenates of adult *Haemonchus contortus* stages. After phosphate buffer homogenization, 2 ml of the homogenates were cultured with 5 microorganisms, MOX, at 37 °C for 24 hours. To separate MOX and its metabolites, HPLC with Supelcosil C_{18} (5 µm, 4.6 mm ×150 mm) column with radio detection online was utilized. The mobile phase consisted of acetic acid/ methanol/ acetonitrile (20:40:40, *v/v/v*), and was pumped at a flow rate of 1 ml/min. One molecule was found, and carbon monoxide prevented the formation of that metabolite. The results showed that the cytochrome P450 system in the milbemycin-resistant H. contortus metabolizes moxidectin. (10)

Roseanne Andrade Teixeira et al. conducted comprehensive research on Poly (1-vinyl imidazole-co-trimethyl propane trimethacrylate) which was employed in pipette-tip nanostructured polymeric solid phase extraction as a selective adsorbent. For the chromatographic separation, the mobile phase was comprised of acetonitrile, methanol, and water (55:27:18, v/v/v), and the analytical Phenomenex® Gemini C_{18} column was employed (150 mm × 4.60 mm, 5 µm). The stability, robustness, recovery, sensitivity, precision, and accuracy performance criteria have been evaluated and were determined to be within the suggested limits. The results demonstrated the good potential of the HPLC-UV coupled PT-MIP-SPE technique for the extraction of macrocyclic lactones from water, grape juice, and other substances. (11)

Patricia Esmeralda Vazquez-Quintal et al. developed a method using spectrofluorometry and HPLC with fluorescence detection to measure moxidectin (MOX) and abamectin (ABA) levels in bovine plasma. The separation was carried out on a supelcosil C_{18} column (150 mm x 4.6 mm, 5 µm) with acetic acid, methanol, and acetonitrile serving as the mobile phase. The developed procedures' total analysis time was comparable to or less than that of the published analytical procedures. The linear range and recovery studies were within the ranges. (12)

Roberta Galarini et al. developed a technique for simultaneously detecting the anti-parasitic veterinary drugs (abamectin, doramectin, emamectin, ivermectin, and moxidectin) in foods and feed. With the help of a Luna C8 (150mm x 3.0mm, 5 µm) column, the separation was accomplished. Acetonitrile (A) and water made up the mobile phase (B). The recovery studies, repeatability, and reproducibility within the lab were satisfactory. Since it completely satisfies the criteria, national control strategies for both food and feed have effectively used this multi-analyte strategy. (13)

Craven et al. investigated if the composition of the animal body at the time of treatment affected the pharmacokinetics of both MOX and IVM. Following a 300 g/kg intravenous infusion of IVM or MOX, samples of blood were collected regularly, and the plasma was analyzed using fluorescence-HPLC on a C_{18} column (150 × 5 mm, 5 µm) eluted with the mobile phase consisting of acetonitrile, tetrahydrofuran, and water (52:25:23 *v/v/v*). IVM kinetics showed significant differences from MOX kinetics regardless of body composition, allowing MOX to be detectable in plasma for more than 40 days. MOX had more extended distribution and elimination half-lives than IVM, as well as a slower clearance rate. In this investigation, changes in body composition had no noticeable effect on the kinetic distribution of IVM. MOX was dispersed more evenly inside the lean animals and eliminated quickly than from fat animals, even though still there was no change in the area under the curve and the volume of dispersion. (14)

3. LIQUID CHROMATOGRAPHY-MASS SPECTROSCOPY (LC-MS)

One of the most significant developments of the preceding part of the twentieth century is the combination of mass spectrometry and liquid chromatography. It has become the preferred method for analytical sustenance during several stages of pharmaceutical quality control and assurance. It has been demonstrated that tandem mass spectrometry (MS/MS) was a useful method for describing the structural characteristics of unknown compounds. (15) The quantitative measurement of moxidectin using LC-MS methods is listed in the following table 2.

Table 2: Determination of moxidectin using LC-MS/MS.

Sr No	Drug	Mobile phase	Stationary phase	Parameters	Reference
1.	Moxidectin	Methanol and water	Whatman RAC I1 Partisil5-C_8 (4.6 mm x 10 cm)	Concentration ion	15
2.	Moxidectin	78% acetonitrile, 22% water, 0.1%triethylamine	3 mm 'Luna' C_{18} stationary phase	Recovery studies, CCα, CCβ, precision	16
3.	Moxidectin	3mM ammonium formate or 0.05% formic acid prepared in pure water.	Luna C_8 column (30 × 2.0 mm, 3 µm particle size, 100 Å)	Accuracy, precision, selectivity, sensitivity, linearity	17
4.	Moxidectin	The mixture of (A) 0.05% TEA in acetonitrile and (B) 0.05% TEA in water (70:30 *v/v*)	C_{18} (5 µm, 2.1 x150 mm, Waters, Milford, MA, USA).	Specificity, selectivity, precision, stability, ruggedness, CCα, CCβ	18
5.	Moxidectin	50% water (A), 45% ACN (B), and 5% 50 mM ammonium acetate buffer pH 5 (C)	Phenomenex Luna C_{18} column (150 mm x 2.1 mm, 5 µm)	LOD, LOQ, selectivity, retention, CCα, CCβ	19
6.	Moxidectin	Acidic: contained 4 mM ammonium formate and 0.1% formic acid in methanol and water. Basic: 10 mM ammonium acetate in methanol and water	Aqueous C_{18} column (3 µm, 100 x 2.1 mm)	Accuracy, linearity, precision, ruggedness, CCα, CCβ	20
7.	Moxidectin	(A) MeOH: ACN (1:1) with 0,1% formic acid, and eluent (B) ammonium formate solution with 0.1% formic acid.	A Waters Acquity UPLC BEH C_{18} column (100 mm x 2.1 mm, 1.7 µm)	Selectivity, linearity, retention, accuracy, precision, LOD, LOQ, CCα, CCβ	21
8.	Moxidectin	Methanol/water (90:10 *v/v*)	Agilent Zorbax SB-C_{18} (3 mm × 150 mm, 3.5µm)	Average recovery, %RSD	22

9.	Moxidectin	Ammonium acetate: water: methanol	Whatman RACII Partisil 5-C_8 (10 cm x 4.6 mm)	Linearity, % recovery, RSD	23
10.	Moxidectin	ACN: water (50:50 *v/v*)	Waters Xterra RP_{18}, (3.5micron, 3 mm × 100 mm)	CCα, CCβ, LOD, LOQ, %recovery, sensitivity, precision.	24
11.	Moxidectin	50 mM ammonium acetate buffer: acetonitrile (5:95, *v/v*)	Luna C_{18} column (50 mm × 2.1 m m, i.d., 5 µm)	Precision, accuracy, CCα, CCβ, LOQ, LOD, recovery test	25
12.	Moxidectin	(A) ACN and (B) MeOH: H_2O (50:50 *v/v*) and TEA	Reverse phase analytical column C_{18} (50 × 2.1 mm, 2.7 µm)	Linearity, sensitivity, selectivity, precision, accuracy	26
13.	Moxidectin	A- methanol B- 10mM ammonium formate	Kinetex EVO C_{18} (100mm x 4.6mm, 5 µm)	Linearity, precision, accuracy, stability	27
14.	Moxidectin	0.1% acetic acid and methanol–acetonitrile (1:1, *v/v*)	ACE C_{18} (50× 3.0 mm, 3 µm)	Precision, RSD, correlation coefficient, linear range	28

J. Steven Stout et al. utilized significant amounts of metabolites produced in vitro from incubations of liver microsomes to study the *in vivo* metabolism of moxidectin in cattle, sheep, and rats. To estimate the molecular weight of the unidentified metabolites using liquid chromatography/tandem mass spectroscopy and to produce daughter ion spectra of molecular species, both metabolites produced *in vitro* and *in vivo* were characterized. It was discovered that the principal metabolites of moxidectin originate from the monohydroxylation of the parent. Less commonly occurring processes were O-demethylation, monohydroxylation, and dihydroxylation. Little metabolic differences have been observed between the species. (15)

Howells et al. established a multi-residue technique for measuring and verifying the presence of avermectins as well as moxidectin residues in the liver of cattle. Using C_8 solid-phase cartridges, the target analytes were isolated from the liver homogenate and detected by using an atmospheric pressure chemical ionization (APCI) interface and ion-trap mass spectrometry in the negative ion mode. The approach allowed for the detection of abamectin, doramectin, moxidectin, and ivermectin at levels of 3.1, 3.2, 2.2, 4.0, and 3.2 ng g^{-1} liver, respectively, which is lesser than each of their respective maximum residue levels. (16)

Daniela Hofmann et al. developed a method using LC-MS to compare the levels of moxidectin from human blood collected using capillary sampling and the micro-sampling technology mitra® to blood and plasma collected using venous samples. Using a Luna C_8 (30 × 2.0 mm, 3 µm) column, moxidectin was discovered. The mobile phase consisted of 3 mM ammonium formate or formic acid (mitra®) produced in ultrapure water at a concentration of 0.05%. For venous and capillary blood, the quantification limits were 0.5 and 2.5 ng/ml respectively. All of the following were validated: sensitivity, selectivity, linearity, stability, recovery, and haematocrit effect. It has been shown that the quick and efficient capillary method using mitra® micro-sample is suitable for moxidectin pharmacokinetic research. (17)

Herlinde Noppe et al. established a selective and precise approach for the simultaneous evaluation of one milbemycin (MOX) and five avermectins in fish, beef meat, and pig liver. Acetonitrile was used for extraction, while C_{18} SPE was employed for purification. A mixture of 70% A: 30% B, consisting of (A) Triethylamine (TEA) in acetonitrile and (B) TEA in water, made up the mobile phase. A negative-mode LC-MS equipped with APCI was employed for detection. Considering the application of analytical techniques and interpretation of outcomes, the method has been verified according to the standards stated in EC/2002/657 Commission Decision. Additionally, all compounds showed good precision results, linear response, detection limits, and quantification limits. (18)

Gabriel Rubensam et al. detected the presence of ivermectin and milbemycin residues in cattle muscle by (LC-MS/MS) and (LC-FL) detection by using easy and affordable sample preparation, a solvent extraction-based process that is followed by low-temperature clean-up. Using Phenomenex Luna C_{18} column (150 mm × 2.1 mm, 5 µm), the separation process occurs that involves the use of a solvent, made up of (A) 50% water, (B) 45% ACN and 5%

ammonium acetate buffer pH 5 (C). The recovery studies, variation coefficient for repeatability and reproducibility, and precision led to satisfactory results. A total of 760 samples were analyzed in total, but none of them revealed residual concentrations above those permitted by the current regulations. (19)

Chris Sack et al. did collaborative validation of the QuEChERS technique for LC-MS/MS detection of pesticides in food to detect 173 pesticides in under 20 minutes, seven FDA pesticide laboratories worked together. For the examination of oranges, carrots, and spinach, the LC-MS/MS method was employed. Aqueous C_{18} column was employed, and the mobile phases were basic (10 mM ammonium acetate in methanol and water) and acidic (4 mM ammonium formate and 0.1% formic acid). Recoveries of the pesticides were found to be satisfactory. Thus, the method was suitable to detect pesticides in food. (20)

Ioulia Moschou et al. utilized the LC-ESI-MS/MS method to simultaneously estimate avermectins and moxidectin in fish tissue. Using the Waters Acquity UPLC BEH C_{18} (100 mm × 2.1 mm, 1.7 µm) column, the target analytes were separated and the mobile phase was made up of (A) 0.1% HCOOH in ACN-MeOH and (B) 0.1% HCOOH in 1 mM $HCOONH_4$. The LOD and relative standard deviations were all within the acceptable ranges, indicating the method's excellent sensitivity. Fish samples from aquaculture were successfully processed using the established technology. (21)

Sherri Turnipseed et al. used a variety of ionization methods, such as atmospheric pressure photoionization (APPI), to detect ivermectin, doramectin, and moxidectin residues in milk. Using Agilent Zorbax SB-C_{18} column (3 mm × 150 mm, 3.5 µm) and (90:10 *v/v*) methanol/water was the mobile phase used to achieve separation. Electrospray, APPI, Atmospheric Pressure Chemical Ionization (APCI), and a combination of APPI and APCI were all used to determine the ionization responses of these chemicals. The responses of these compounds were evaluated by operating the APCI/APPI source in positive ion mode without supplying the corona needle with any discharge current. An MS-MS approach was developed using this mode of ionization to track the ions of sodiated molecular salt product ion scans utilizing an ion trap apparatus. Relative standard deviations and good recovery studies were observed for the residues. (22)

Khunachak et al. used liquid chromatography-mass spectrometry with fluorescence detection to determine moxidectin in cattle tissue using Whatman RACII Partisil 5-C_8 column (10 mm x 4.6 mm) and the solvent was made up of ammonium acetate/water/methanol. Following the ACN-hexane partitioning phase, the underivatized parent compound was identified using the LC/MS confirmatory method with an average recovery of 108% at the 250 ppb level in cow fat. (23)

David Durden. quantified antiparasitic endectocide medicines (abamectin, doramectin, and ivermectin) in milk with LC-MS-MS techniques using positive and negative electrospray. The mobile phase consisted of ACN: water (50:50 *v/v*) which was employed on Waters Xterra RP_{18} (3 mm × 100 mm, 3.5 µm) column for separation. The LOD results were obtained within the limits. The positive ion signals were stronger and had low detection limits, the negative ion technique produced more linear responses and more accurate performance. (24)

Gabriel Rubensam et al. created a method and validated it using liquid chromatography with fluorescence detection to examine the macrocyclic lactones in cow's milk. Using an isocratic mobile phase made up of 50 mM ammonium acetate buffer: acetonitrile (5:95, *v/v*), the separation was accomplished on the Luna C_{18} (50 mm × 2.1 mm, 5 µm) column. The quantification limits were much lesser than the maximum allowable limits, and the recovery rates fell within the ranges. The suggested approach has proven to be simple and inexpensive, enabling for high-volume analysis of several samples per day. (25)

Rafaela Baptista et al. established a method using high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry for detecting MOX in lamb serum. For the chromatographic separation, a reverse phase C_{18} (50 mm × 2.1 mm, 2.7 µm) analytical column was employed. The sample solvent was made up of ACN, MeOH: H2O (50:50 *v/v*), and TEA was added in required quantities to achieve a pH of 8. The outcomes of precision, accuracy, LOD, and LOQ were found to be within the limits, according to the method validation. (26)

Subbarao et al. validated a rapid bioanalytical approach for the determination of moxidectin in cattle hair. Using a liquid-liquid extraction, moxidectin was isolated from cow hair using methyl tert-butyl ether as the solvent and Sorenson's buffer as the digesting solvent for incubation. The separation was achieved using a Kinetex EVO C_{18} (100 mm x 4.6 mm, 5 µm) column and the sample solvent made up of methanol: 10 mM ammonium formate. Studies on validation characteristics like linearity, accuracy, precision, and stability have provided reliable results. The method for calculating the amount of moxidectin in cattle hair was successfully utilized. (27)

Yashpal singh chhonker et al. created a bioanalytical LC-MS technique to identify moxidectin in mouse, monkey, and human plasma. On the ACE C_{18} (50 mm x 50 mm, 3 µm) column, the separation has been accomplished using the sample solvent made of methanol: acetonitrile (1:1, *v/v*) and 0.1% acetic acid. With a correlation coefficient of 0.997 or above, over the range of 0.1-1000 ng ml^{-1}, MS/MS response was linear in plasma. The accuracy and precision were within acceptable bounds. A study of moxidectin's in vitro metabolic stability using this technique proved effective. (28)

4. UV- SPECTROPHOTOMETRIC METHOD

Spectrophotometry is the quantitative measurement of a material's wavelength-dependent transmission or reflection properties. The advantage of these methods is that it requires minimal time and effort. These methods also offer good precision. The following table 3 lists the quantitative measurements of moxidectin made using UV spectrophotometric techniques.

Table 3: Determination of moxidectin using UV spectrophotometer.

SR No:	Drug	Instrument	λ max (nm)	Parameters	References
1.	Moxidectin	UV spectrophotometer	244.89 nm	Linearity, Accuracy, Precision, LOD, LOQ, Specificity, Assay	29

Sathish Babu et al. developed a sensitive, accurate, and validated UV spectrophotometry method to determine moxidectin in bulk drug and synthetic mixture. With a correlation coefficient of 0.9994, the calibration curve was plotted in the range of 8-22 g/ml. LOD and LOQ findings were within the expected range. The RSD for the method's precision was found to be less than 2% as a percentage. A 100.8% assay percentage was discovered. The newly developed method's linearity, accuracy, precision, and specificity were all validated following the ICH requirements. (29)

5. ULTRA HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (UHPLC)

Liquid chromatographic separations using columns that encapsulate particles smaller than the commonly used HPLC particle size range of 2.5-5 µm are referred to as "ultra-high-performance liquid chromatography". Similarly, to HPLC, UHPLC operates under the governing principle that efficiency and therefore, resolution increase with a decrease in the column packing particle size. In the modern world, all chromatographic techniques that support separations using shorter columns, faster flow rates for high speed, and better resolution and sensitivity are available through UHPLC. (30) The following table 4 lists the results of the quantitative analysis of moxidectin using UHPLC.

Table 4: Determination of moxidectin using the UHPLC method.

Sr. No	Drug	Mobile Phase	Stationary Phase	Parameters	Reference
1.	Moxidectin	5 mM ammonium formate solution + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B)	Zorbax Eclipse Plus C_{18} RRHD column (1.8 µm, 2.1 mm x 50 mm)	Linearity, sensitivity, precision, accuracy, CCα, CCβ, LOQ and LOD	30
2.	Moxidectin	Ammonium acetate: acetonitrile (10:90, v/v)	Acquity UPLC BEH C_{18} analytical column (2.1 × 50 mm, 1.7 µm)	Linearity, precision, LOD, LOQ, accuracy.	31
3.	Moxidectin	5 mM ammonium formate in water with 0.1% formic acid and B acetonitrile: in water with 0.1% formic acid	Acquity UPLC® BEH C_{18} column (50 mm x 2.1 mm, 1.7 µm)	Linearity, selectivity, matrix effect, CCα, CCβ, LOD, LOQ, accuracy, precision, robustness	32
4.	Moxidectin	(A) water: acetonitrile (98:2, *v/v*), containing 10 mmol L^{-1} aqueous ammonium formate, and (B) methanol:	Waters Acquity UPLC™ analytical column HSS-T3 (100 × 2.1 mm,1.8 µm particle size)	Accuracy, precision, CCα, CCβ, linearity, RSD.	33

Sr. No	Drug	Mobile Phase	Stationary Phase	Parameters	Reference
		acetonitrile (75:25, v/v), with 0.1% formic acid.			
5.	Moxidectin	Acetonitrile and methanol	Acquity BEH C_{18} column (50 × 2.1 mm, dp: 1.8 µm) and an Acquity HSS-T3 column (100 × 2.1 mm)	Linearity, LOD, LOQ, precision, accuracy, carry-over, specificity, stability	34
6.	Moxidectin	0.1% solution of formic acid in water (A)/acetonitrile (B)	RP ACQUITY UPLC BEH C_{18} (1.7 µm, 100 mm × 2.1 mm).	Precision, CCα, CCβ, % recovery	35
7.	Moxidectin	A consisted of a 5 mM ammonium formate in water and acetonitrile: water (95:5 v/v) with 0.1% formic acid	Acquity UPLC® BEH C_{18} column (50 mm × 2.1 m m, 1.7 µm)	Linearity, selectivity, CCα, CCβ, accuracy, precision, LOD, LOQ,	36
8.	Moxidectin	Mobile phase A, 0.01% HOAc in water: MeCN (90:10, v/v) and Mobile phase B, 5 mM ammonium formate in MeOH: MeCN (75:25, *v/v*).	A stainless steel HSS T3 analytical column (100 mm × 2.1 mm, particle size 1.8 µm)	Sensitivity, linearity, precision, accuracy, CCα, CCβ,	37
9.	Moxidectin	5 mM and 10 mM ammonium buffer and 0.1% formic acid	C_{18} column	Precision, LOD, LOQ, recoveries	38
10.	Moxidectin	5 mM ammonium formate + 0.01% formic acid, pH 4.00) and mobile phase B (acetonitrile: mobile phase A, 95:5 v/v)	Waters Acquity BEH UPLC® C_{18} column (100 × 2.1 mm ID, 1.7 µm)	Selectivity, linearity, CCα, CCβ, recovery, LOD, LOQ	39
11.	Moxidectin	A- Water B- Methanol	Poroshell 120 EC-C_{18}, (150 mm × 3.0 m m, 2.7 µm column)	LOD, LOQ, precision, recovery, linearity, trueness	40

Michelle Del Bianchi Cruz et al. established a throughput approach (UHPLC-ESI-MS/MS) for the identification of moxidectin residues in the target tissues of lambs. The sample was prepared using a modified QuEChERS technique to increase the analyte's high recovery from the matrices. Using Zorbax Eclipse Plus C_{18} (1.8 µm, 2.1 mm x 50 mm) column and a linear gradient program were used to carry out the chromatographic separation. The sample solvent was made up of (A) 5 mM ammonium formate solution + formic acid and (B) acetonitrile + 0.1% formic acid. Results for the method's linearity, accuracy, and precision were found to be adequate. (30)

Fabrício de Oliveira Ferreira et al. reported a technique for determining the presence of moxidectin residues in soils using online SPE-UHPLC-TMS and extraction from solids. Using Acquity UPLC BEH C_{18} (2.1 mm × 50 mm, 1.7 µm) analytical column was used for separation. The optimal conditions were: a sample solvent of water: methanol (40:60, *v/v*); a solvent of water/methanol (96:4, *v/v*); a sample volume of 2 × 250 µL; and with the back flush of mobile phase composed of 5 mmol l ammonium acetate/ ACN (10:90, v/v), eluting the analytes retained on the SPE column. Results from this method were within the acceptable ranges in terms of accuracy, precision, linearity, LOD, and LOQ. (31)

Guilherme Resende da Silva et al. created and validated a multi-residue approach by combining QuEChERS extraction and UHPLC-MS/MS to identify avermectins, benzimidazoles, and nitroimidazoles in bovine muscle tissue. The separation was achieved on the Waters Acquity UPLC BEH C_{18} (50 mm x 2.1 mm, 1.7 µm) column. Mobile phase (A) was made up of 5 mM ammonium formate in water with 0.1% formic acid and ACN/water (95:5 *v/v*) with 0.1% formic acid was used as mobile phase (B) with a flow rate of 0.4 ml/min at a column temperature of 35 °C. All the studied analytes performance results, including those for accuracy, linearity, selectivity, LOD, LOQ, precision, and robustness, were satisfactory. (32)

Tiele Rizzetti et al. developed a straightforward and quick multi-class technique for detecting veterinary drugs in the muscle, liver, and kidney of cattle. Using the sample solvent made up of water/ACN (98:2, *v/v*), with 10 mol l1 of ammonium formate; (B) methanol: acetonitrile (75:25, *v/v*), with formic acid, separation was carried out on Waters Acquity UPLC™ analytical column HSS-T3 (100 mm x 2.1 mm, 1.8 µm particle size). The results of linearity, accuracy, precision, detection limits, and capability limits fell within the acceptance limits. According to European Commission Directive 2002/657, the approach has been verified and provided satisfactory results for 69 veterinary pharmaceuticals in the liver and 68 chemicals in the muscle and kidney. (33)

Gemechu Zeleke et al. created a quick, accurate, and safe UHPLC-MS/MS technique for determining moxidectin simultaneously in bovine plasma. For the chromatographic separation of the analytes in plasma, two RP UPLC columns, the BEH C_{18} column (50 × 2.1 mm, 1.8 µm) and the Acquity HSS-T3 column (100 mm × 2.1 mm, 1.8 µm), were assessed with mobile phased made up of 0.01% acetic acid in ACN and methanol. All the analytes had limits for quantification of 1 ng/ml, although the detection limits of IVER, DORA, and MOX were 0.02 ng/ml, 0.03 ng/ml, and 0.58 ng/ml, respectively. The intraday (RSD 6.50%) and inter-day (RSD 8.10%) results were proved to be within the limits. (34)

Bayer et al. conducted a study to establish the MS/MS detection parameters to assess the application of UHPLC-MS and to specify the validation requirements for the investigation of the residual content of avermectins and moxidectin in milk. The samples were chromatographed in gradient mode using the Acquity UPLC BEH C_{18} (100 mm × 2.1 mm, 1.7 µm) RP analytical column and a system of formic acid solution in water (A) and acetonitrile (B). The data collected to evaluate the results' suitability, accuracy, and reproducibility complies with the European Directive's (2002/657/EC) standards. The effective UHPLC-MS technology, which has been devised and adopted for widespread use in veterinary medicine laboratories, enables the detection of residual levels of the five avermectins used in animal breeding to prevent helminthiasis. (35)

Victor Pastore et al. utilized a QuEChERS technique to establish a quantitative and confirmatory approach to measure the macrocyclic lactone residues, monensin, and fipronil present in the liver of cattle. The separation was accomplished by Acquity UPLC® BEH C_{18} (50 mm × 2.1 mm, 1.7 µm) column. The mobile phase (A) was ammonium formate in water at a concentration of 5 mM, while the mobile phase (B) was ACN and water at a ratio of (95:5 *v/v*) with 0.1% formic acid. The method presented linearity and selectivity with a coefficient of correlation, LOD and LOQ were within the ranges. The accuracy, precision, CCα, CCβ, and uncertainty results showed a satisfactory level of performance. The method proved successful in determining MLs, monensin, and fipronil residues in the livers of chickens and cattle. (36)

Michelle Whelan et al. devised and tested a modified QuEChERS-type extraction technique to detect 38 anthelmintic medication residues, including benzimidazoles and avermectins, in milk. For the separation, a stainless steel HSS T3 (100 mm × 2.1 mm, 1.8 µm) analytical column was utilized and the mobile phase (A) was made up of 0.01% HOAc in water: MeCN (90:10*, v/v*) and mobile phase (B) was composed of 5 mM ammonium formate in MeOH/MeCN (75:25, *v/v*). The decision limits of the method were found to be within the limits. By taking part in a proficiency study, the method's effectiveness for benzimidazoles and levamisole was successfully confirmed. (37)

Mirta Zrncic et al. established a method to investigate the presence of 10 anthelmintic medications in surface water using quadrupole linear ion trap mass spectrometry linked to UHPLC (UHPLC-QqLIT-MS). To achieve chromatographic separation, a C_{18} column was employed. The organic phase contains 0.1% formic acid in methanol or ACN, whereas the aqueous phase is made up of 5 mM and 10 mM ammonium buffers. At two concentration levels, most of the analytes displayed analyte recoveries from spiked samples > 75%. The devised technique was applied to study the anthelmintics in the Lobregat River and its principal tributaries. All the samples were analyzed, and eight of the 10 anthelmintics were found at quantities below the ngl (-1) threshold. The technique covers a gap in analytical methods for environmental anthelmintic drug identification. (38)

Marianna Ramos dos Anjos et al. developed a method using QuEChERS for extraction and clean-up, coupled with detection and quantification by UPLC-MS/MS, for the evaluation of moxidectin, abamectin, doramectin, ivermectin, and aflatoxin M1 in whole raw milk. The Waters Acquity BEH UPLC C_{18} (100 mm x 2.1 mm ID, 1.7 µm) column was used for chromatographic separation. The mobile phase (A) was made up of 5 mM ammonium formate/ formic acid and the mobile phase B was ACN: mobile phase (A) (95:5 *v/v*). Within the suggested working ranges of recovery studies, standard deviation, LOD, and LOQ, it was shown that this technique was suitable for measuring these analytes. Animals treated with abamectin, doramectin, and ivermectin samples were analyzed using an established method. (39)

Heng Zhou et al. devised a technique for qualitative screening and quantitative analysis of 569 pesticides in honeysuckle utilizing UHPLC with quadrupole-orbitrap high-resolution mass spectrometry and an internal executable chemical database that contains theoretical masses of precursor and fragment ions and retention times. A Poroshell C_{18} column (150 mm × 3.0 mm, 2.7 µm) from the infinity lab was used for the separation. Water and methanol were employed as the mobile phases, and both contained 5 mM ammonium formate and 0.1% formic acid. The method showed good repeatability, linearity, and precision. Among the 82 samples analyzed, pesticides were found in 75 of them, with some of the most severely polluted samples having pesticide amounts as high as 4116.9 g/kg. To analyze honey, and suckle samples both qualitatively and quantitatively, UHPLC-Full MS/ddMS2 was thought to be the best procedure. (40)

6. CONCLUSION

Onchocerciasis is treated with moxidectin, a potent second-generation endectocide macrocyclic lactone (river blindness). For the assessment of moxidectin in pharmacological dose forms, various analytical methods, including UV, HPLC, and hyphenated techniques such as LC-MS/MS and UPLC-MS/MS were reported. Among these techniques, LC-MS and UPLC-MS yielded successful results due to their greater resolution and sensitivity. Also, the HPLC methods were desirable due to no sample preparation or extraction step. Of all the HPLC methods, David Pimentel-Trapero et al. established a greener method compared to others by using methanol and water as mobile phase and also the use of the QuEChERS method made it much more productive. The use of the QuEChERS method made the analytical technique minimize the usage of chemical solvents and a non-labor-intensive process. Additionally, there were more HPLC methods available for evaluating moxidectin in pharmaceutical dosage forms that are available in bulk. Among the UHPLC methods, Heng Zhou et al. developed a greener method compared to others using methanol and water as mobile phase. The current study provides a brief overview of the analytical techniques used to evaluate moxidectin in pharmaceutical formulations.

7. CONFLICT OF INTEREST

The authors declare no competing interests.

8. ACKNOWLEDGMENTS

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