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METHOD DEVELOPMENT AND VALIDATION OF MOXIDECTIN IN SYNTHETIC MIXTURE USING UV-SPECTROPHOTOMETRY

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ABSTRACT

A sensitive, accurate and validated UV Spectrophotometry method has been developed to determine Moxidectin in bulk drug and synthetic mixture. The Calibration graph was 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 percentage RSD for precision of the method was found to be less than 2%. The assay percentage was found to be 100.8%. The newly developed method was validated according to the ICH guidelines with respect to linearity, accuracy, precision and specificity.

KEYWORDS: Moxidectin, Validation, Synthetic mixture, ICH Guidelines.

INTRODUCTION: Moxidectin is a broad-spectrum, potentendectocide which acts against a wide range of insects, nematodes and acari. It is worldwide used as a parasiticide in a variety of mammalian species. Moxidectin is a semi-synthetic methoxime derivative of LL F-2924α, commonly referred as F-alpha or nemadectin, a 16-member pentacyclic lactone of the milbemycin class. F-alpha is a product of fermentation of Streptomyces cyaneogriseus subspecies. F-alpha possesses strong anthelmintic activity but has limited ectoparasiticide activity. Moxidectin is the result of chemical optimization of F-alpha. The mode of action of Moxidectin on parasites has been demonstrated that these compounds act by binding to ligand-gated chloride channels, more specifically the subtypes that are gamma-aminobutyric (GABAA) mediated and glutamate-gated.[1] The consequence of ML binding and activation is

an increased permeability, leading to an influx of chloride ions and flaccid paralysis of the parasite leading to death.[2]

Moxidectin has been determined by HPLC,[3-9] HPLC-MS/MS,[10,11] RP-HPLC,[12] UHPLC-MS/MS,[13]LC-MS/MC,[14]],LC-MS/MS,[15-17] LC-MSD Quad &Ion Trap[18] and HPLC-UV spectroscopy[19]methods in single and combined dosage form. Literature reveals that few chromatographic methods in biological fluids and tissues were reported along with other anthelmintic drugs. Even though various methods has been reported to carry out Moxidectin in individual / bulk drug or combination with other drugs, no method has been so far evolved for estimation of Moxidectin as an individual drug in UV spectroscopy. The present study was aimed to carry out the estimation of Moxidectin in synthetic mixture by UV-Spectrophotometry method.

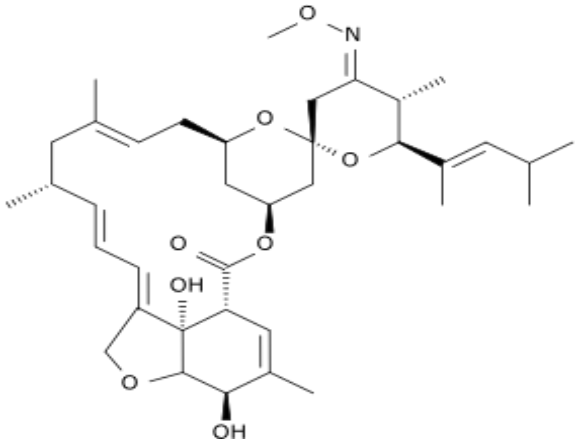

Fig No.1: Structure of Moxidectin

IUPACNAME: Spiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]-benzodioxacyclo-octadecin-13,2'-[2H]pyran-17-one]-6'-[1,3-dimethyl-1-butenyl]3',4'5,6,6',7,10,11,14,15,17a, 20,20a,20b-dihydro-4'-[methoxyimino]- 5, 6,8,19 -tetra-methyl-[6R [2aE,4E,5'S*,6R*,6'S* (E),8E,11R*,13R*,15S*,17aR*,20R*,20aR*,20bS*]

MOLECULAR FORMULA: $C_{37}H_{53}NO_8$

MATERIALS AND METHODS

Experimental Section

Moxidectin used in the study was procured as a gift sample. Ethanol-99.9% (AR grade) and all other analytical grade reagents obtained from Fischer and were used. Milli-Q water purification system and glass distillation was done to obtain water of HPLC grade.

Instrumentation

UV Spectrophotometry analysis was carried out using Perkin Elmer UV visible spectrophotometer (Mode- Lambda 35). For weighing purpose SH Imad 2U Electronic Analytical Balance was used. Sonication was done using Ultrasonic bath Sonicator(Make-3.5L (H).

Preparation Of Standard Stock Solution

A quantity of 50mg of Moxidectin was accurately weighed and dissolved in 50 ml of ethanol to get a concentration of 1000µg / ml. Further dilution was made using water to obtain the desired concentration of the solution.

Preparation Of Synthetic Mixture

Moxidectin was mixed with Magnesium Carbonate, Starch, Magnesium Stereate and Carboxy Methyl Cellulose (CMC). Synthetic mixture of about 200mg was prepared and from which a quantity of the powder equivalent to 50 mg of Moxidectin was accurately weighed and transferred to 50 ml volumetric flask, mixed with 30 ml of ethanol and sonicated for 20 min with occasional shaking. The required quantity was made up to 50ml with Ethanol. The solution was filtered using 0.45µm whattmann filter paper. Further dilutions were made using water to get the desired concentrations.Fig. No.2 represents the chromatogram of Moxidectin.

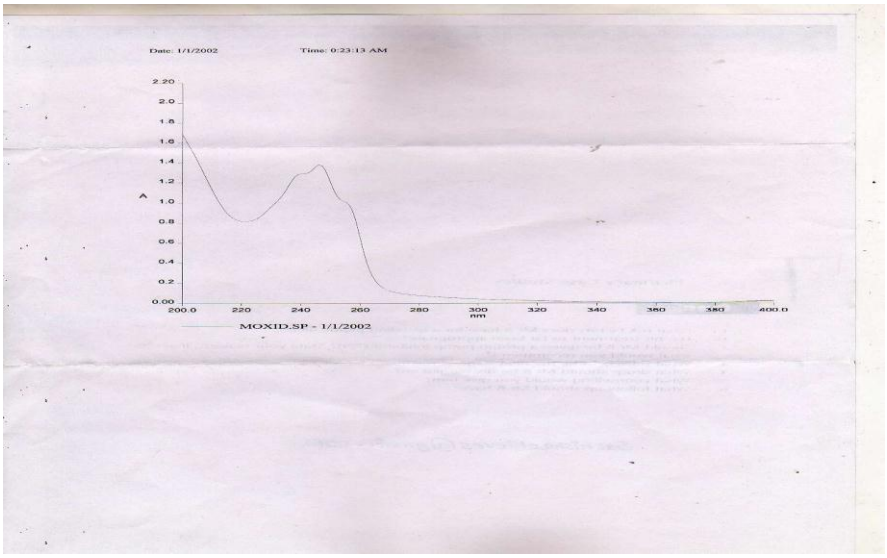

Fig.no.2: Chromotagram of moxidectin

Methodology

UV spectrophotometry condition was optimized and a working standard solution of Moxidectin was prepared in Ethanol of concentration of 18µg/ml. The solution was then

analysed in the wavelength range of 200 to 400nm against Ethanol as standard. The maximum absorbance was measured at 244.89nm. This wavelength can be employed for the determination of Moxidectin without any interference from the other components in their synthetic formulations.

Validation Of The Proposed Method

The proposed method was validated with respect to Linearity, Accuracy, Precision, Specificity, Limit of detection and limit of quantification according to the guidelines prescribed by the International Conference on Harmonization (ICH).[20]

Linearity

In developed UV method, calibration curve was found to be linear in the concentration range from 8 -22µg/ml. 8 different concentrations were used to determine the calibration curve. Three sets of standard solution were prepared. Each set was analyzed to plot a calibration graph. The absorbance of the solution was measured at 244.89nm against Ethanol as blank. To ascertain linearity, Slope, Intercept and coefficient of determination (R^2) of calibration curves were calculated by constructing the calibration curve, plotting peak area versus concentrations. (Fig. No.3)

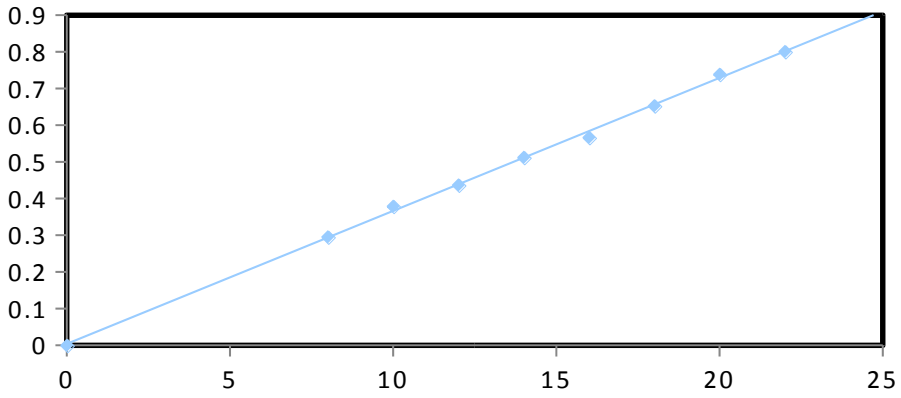

Fig. No. 3: Linearity of moxidectin

Table No.:1: Linear regression analysis of calibration curve of Moxidectin

CONCENTRATION (µg/ml)	ABSORBANCE (nm)
8	0.2956
10	0.3791
12	0.437

14	0.5125
16	0.5666
18	0.6531
20	0.739
22	0.8009
SLOPE	0.036264
INTERCEPT	0.003562
R^2	0.999384

Limit of detection and Limit of Quantification

By calculating the signal-to-noise ratio using the equations designated by the International Conference on Harmonization (S/N, i.e., 3.3 for LOD and 10 for LOQ), the Limit Of Detection (LOD) and Limit Of Quantification (LOQ) of the drug was calculated. The LOD and LOQ values for the concentration range between 8 -22µg/ml of Moxidectin were evaluated from the calibration graph.

Table No.: 2: Formulas for LOD and LOQ calculation

LOD=3.3 X σ/S
LOQ=10 X σ/S

Where, σ = standard deviation of the response and S = slope of the calibration curve.

Accuracy

By calculating recovery of Moxidectin by the standard addition method, the accuracy of the method was determined. The sample was spiked using synthetic mixture with 80%, 100% and 120% standard solution. The amount was estimated by applying obtained values to the respective regression line equations. A triplicate repetition of the experiment was done.

Table No.: 3: Recovery study of synthetic mixture of Moxidectin

Amount Added (%)	Actual Concentration Taken (Ml)	% Recovery (%)
80	0.4	100.25
100	0.6	100.62
120	0.8	100.81

Precision

By carrying out six independent assays of the test sample, precision was calculated by the proposed method. %RSD (Relative Standard Deviation) of six assays obtained were calculated from the calibration curves prepared in medium which was run triplicate in the same day for three days. The %RSD was found to be less than 2%.

Table No.: 4: % 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%)

Specificity

The method was found to be specific by assessing the values obtained from the sample solution. There was no interference of the materials used in the synthetic mixture with the analytes.

Assay

A sample of 16 µg/ml was prepared and the mean absorbance was measured at 244.89nm and the assay value was calculated.

RESULT AND DISCUSSION

A summary of the validation parameters are tabulated (Table no.5). Linear regression data over a concentration range of 8 -22µg/ml showed good linear relationship for Moxidectin. The correlation coefficient value was 0.9994. The Limit of detection (LOD) and limit of quantification (LOQ) for Moxidectin were 0.0238 and 0.07996µg/ml respectively. Accuracy was calculated in three different concentrations like 80%, 100% and 120% standard solution. The average percentage recovery was found to be 100.25%, 100.62% and 100.8 1% respectively. The method id found to be more sensitive from the values obtained. Precision (%RSD) was calculated and it was found to be 0.0397%. It indicates that the method is more précised. Assay was carried out using synthetic mixture and the value was found to be 100.8%. The proposed UV Spectrophotometry method is accurate, precise, specific and rapid for the estimation of Moxidectin in synthetic mixture.

Table No.5 SUMMARY OF THE VALIDATION PARAMETERS

PARAMETERS	RESULTS
Results Accuracy (% of recovery) at 80% 100% 120%	 100.25 100.62 100.81
Precision % of RSD	0.0397
Linearity concentration	8-22µg/ml
Correlation coefficient	0.9994
Specificity	Specific
LOD	0.02638µg/ml
LOQ	0.07996 µg/ml
Assay	100.8%

CONCLUSION

The developed UV Spectrophotometry method used to determine Moxidectin quantitatively in bulk and synthetic mixture was found to be simple, accurate, precise and economical.The method showed a percentage recovery of 100% and % RSD of less than 2%. The method wasvalidated as per ICH guidelines and can be employed for routine analysis in quality control laboratories.

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