METHOD DEVELOPMENT AND VALIDATION OF IVERMECTIN AND CLORSULON IN THEIR COMBINED DOSAGE FORM

*LIMBANI RAJEN K.¹, MODI JIGNASA², PASHA T. Y.³

ABSTRACT

The RP-HPLC method has been developed for simultaneous estimation of Ivermectin and Clorsulon in their Combined Dosage Form. For RP-HPLC method, all the standard and sample solutions were prepared in Methanol: Water. A RP-HPLC method has been developed and subsequently validated for simultaneous estimation of Ivermectin and Clorsulon in their combination product. The proposed RP-HPLC method utilizes a Hypersil BDS C18 (25cm x 4.6mm, 5 μm) column, mobile phase consisting of Buffer and Methanol in the proportion of 55: 45 (v/v) with apparent pH adjusted to 4, and UV detection at 258 nm. The described method was linear over a range of 2.5-7.5 μg/ml for Ivermectin and 25-75 μg/ml for Clorsulon. Validations of the proposed method were carried out for its accuracy, precision, linearity and range, specificity, LOD and LOQ according to ICH guidelines. The method has been successfully applied for the analysis of drugs in formulation.

KEYWORDS

RP-HPLC, Ivermectin and Clorsulon.

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INTRODUCTION

Ivermectin (IVM) is a macrocyclic lactone that has been known as a potent, effective, and safe antiparasitic drug for 20 years. It is widely used as an antiparasitic agent in domestic animals and is considered the drug of choice for lymphatic filariasis and river blindness (onchocerciasis) in humans. IVM is a member of the Avermectins; this group includes natural compounds produced by fermentation of the soil-dwelling actinomycete *Streptomyces avermitilis*. IVM, a semi-synthetic derivative of avermectin B1, consists of an 80:20 mixture of the equipotent homologous 22, 23 dehydro B1a and B1b.

Clorsulon (CLO) is an antihelminthicum. It is used against the adult forms of parasitic flatworms in cattle, in particular from the liver fluke *Fasciola hepatica*, and against *Fasciola gigantica*. Clorsulon is chemically 4-Amino-6-(trichlorvinyl) benzen-1,3-disulfonamide. The structures of IVM and CLO are shown in Fig. 1.

Literature survey reveals a few spectrophotometric and chromatographic methods for the estimation of both drugs as a single component and in combination with other drugs [1-15]. However no method has been reported for analysis of these drugs in combined dosage form. The objective of present communication is to develop simple, rapid and precise spectrophotometric method for the estimation of Ivermectin and clorsulon in combined pharmaceutical dosage form.

EXPERIMENTAL SECTION

Apparatus and instruments

The HPLC system (Shimadzu VP, Japan), consisted of a system controller (SCL-10AVP), on-line degasser (DGU-14A), low-pressure gradient flow control valve (FCV-10ALVP), solvent delivery module (LC-10ADVP), auto injector (SIL-10 ADVP), column oven (CTO-10AVP), UV – VIS and PDA detector (SPD-10AVP) and CLASS – VP software version 6.14 SP1. Sonicator (5510, Branson Ultrasonics Corporation, USA) was used in the study.
Material

IVR and CLO bulk powder was gifted by Kamdhenu stermed, Ahmadabad, Gujarat, India. The commercial fixed dose combination product was procured from the local market. All other reagents used were of HPLC grade.

Method Development and Optimization for Selection of detection wavelength

The standard solution of Ivermectin and Clorsulon were scanned over the range of 200 nm to 400 nm wavelengths. The overlay spectra were recorded and the intersection point wavelength was selected. It was observed at 258 nm. So the wavelength, 258 nm, was selected for the determination of Ivermectin and Clorsulon.

Selection of mobile phase

The mobile phase was selected on the basis of best separation, peak purity index, peak symmetry, theoretical plate etc. So no. of trials was taken for the selection of mobile phase. A specific ml of Methanol and water was filtered through 0.45μm membrane filter paper separately and as per need they were mixed together and sonicated for 10 min. After sonication; mobile phase was filtered through 0.45μm membrane filter paper. pH was adjusted with Ortho Phosphoric Acid and Triethylamine. Acetonitrile: water was prepared as per same procedure of Methanol : water.

Selection of pH

pH was adjusted with dilute Phosphoric acid.

Selection of column

For HPLC, various columns are available, but C18 column was preferred over other columns. Hypersil BDS C18 column (250 mm x 4.6 mm i.d., 5 μm particle size) was chosen to give good peak shape, good lifetime and high resolution compared to other C18 columns.

Selection of column temperature

The column temperature (30°C) has minimized variation of retention and made the peak sharp. It has also shortened the run time.

Chromatographic Conditions

- **Column** – Hypersil BDS C18 (25cm x 4.6mm, 5 μm)
- **Column temp.** - 25°C
- **Buffer**- 6.8 gm Potassium Dihydrogen phosphate in 1000 ml water, pH 4 adjusted with dilute Phosphoric acid
- **Mobile Phase** - Buffer : Methanol (55 : 45)
- **Flow Rate** – 1 ml per minute
- **Detector Condition** – 258 nm
- **Injection Volume** – 20 μl
Buffer Preparation

Phosphate buffer containing 50mM was prepared by accurately weighing 6.8 gm. of potassium dihydrogen phosphate in 1L volumetric flask and make it up to the volume with HPLC grade water. The pH of the buffer was adjusted with ortho phosphoric acid to the required pH.

Standard Stock preparation:

Accurately weighed 50 mg of standard Ivermectin and 500 mg of standard Clorsulon API were transferred to a 100 ml volumetric flask and dissolved in 25 ml mobile phase. The flask was shaken and volume was made up to the mark with mobile phase to give a solution containing 50μg/ml Ivermectin and 500μg/ml Clorsulon. From this solution 10ml solution was taken and diluted up to 100 ml with mobile phase to give solution of 5μg/ml and 50μg/ml Ivermectin and Clorsulon respectively. Stock solution was diluted with mobile phase to give working standard solution containing 5μg/ml Ivermectin and 50μg/ml Clorsulon.

Table 1: Data for system suitability test for Ivermectin and Clorsulon.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Proposed method</th>
<th>Standard value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ivermectin</td>
<td>Clorsulon</td>
</tr>
<tr>
<td>Retention time (R&lt;sub&gt;t&lt;/sub&gt;)</td>
<td>3.247±0.150 min</td>
<td>5.699±0.143 min</td>
</tr>
<tr>
<td>Resolution (R&lt;sub&gt;s&lt;/sub&gt;)</td>
<td>-</td>
<td>12.252</td>
</tr>
<tr>
<td>Theoretical plates (N)</td>
<td>7778.667</td>
<td>8120.667</td>
</tr>
<tr>
<td>Tailing factor (T)</td>
<td>1.174</td>
<td>1.319</td>
</tr>
</tbody>
</table>

Assay preparation

Sample equivalent to 5 mg Ivermectin and 50 mg Clorsulon was accurately weighed and transferred to volumetric flask of 100ml capacity. 100 ml of methanol was transferred to this
volumetric flask. The flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through whatman filter paper (0.45μ). These solutions give a solution containing 5 μg/ml Ivermectin and 50 μg/ml Clorsulon. This solution was analysed by RP-HPLC and the content of Ivermectin and Clorsulon from dosage form was calculated from calibration curve.

RESULTS AND DISCUSSION

Selection of Wavelength

The detection was carried out in the UV region and wavelength selected for detection was 258 nm in mobile Phase.

Validation of RP-HPLC Method

As per the ICH guidelines\textsuperscript{16}, the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation.

Linearity and Range

The linearity of the method was determined for the formulation at five concentration levels ranging from 2.5 to 7.5 μg/ml for Ivermectin and 25- 75 μg/ml for Clorsulon. The equation for regression line was $y = 78.529X - 4.35$ $(R^2 = 0.999)$ for Ivermectin and $y = 38.324X - 23.339$ $(R^2 = 0.999)$ for Clorsulon.

![Calibration curve for Ivermectin](image)

**Fig. 3:** Calibration curve for Ivermectin.

![Calibration curve for Clorsulon](image)

**Fig. 4:** Calibration curve for Clorsulon.
Table 2: Linearity of Ivermectin and Clorsulon by RP-HPLC method.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration (μg/ml)</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ivermectin</td>
<td>Clorsulon</td>
</tr>
<tr>
<td>1.</td>
<td>2.5</td>
<td>25</td>
</tr>
<tr>
<td>2.</td>
<td>3.75</td>
<td>37.5</td>
</tr>
<tr>
<td>3.</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>4.</td>
<td>6.25</td>
<td>62.5</td>
</tr>
<tr>
<td>5.</td>
<td>7.5</td>
<td>75</td>
</tr>
</tbody>
</table>

The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

Accuracy and Precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels of 80, 100 and 120% and the percentage recovery was calculated and presented in Table 3.

Table 3: Recovery studies of Ivermectin and Clorsulon.

<table>
<thead>
<tr>
<th>Level of % Recovery</th>
<th>Mean % Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ivermectin</td>
<td>Clorsulon</td>
</tr>
<tr>
<td>80</td>
<td>100.32</td>
<td>100.09</td>
</tr>
<tr>
<td>100</td>
<td>100.02</td>
<td>99.71</td>
</tr>
<tr>
<td>120</td>
<td>100.02</td>
<td>99.78</td>
</tr>
</tbody>
</table>

Table 4: Intraday precision data for analysis of Ivermectin and Clorsulon.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (μg/ml)</th>
<th>Mean Conc. ± %RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ivermectin</td>
<td>2.5</td>
<td>2.51 ± 0.3984</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.01 ± 0.3047</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>7.48 ± 0.1336</td>
</tr>
<tr>
<td>Clorsulon</td>
<td>25</td>
<td>24.99 ± 0.2400</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>49.95 ± 0.1007</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>74.97 ± 0.0600</td>
</tr>
</tbody>
</table>
Table 4: Interday precision data for analysis of Ivermectin and Clorsulon.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (μg/ml)</th>
<th>Mean Conc. ± %RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ivermectin</td>
<td>2.5</td>
<td>2.45 ± 1.4210</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.98 ± 0.6024</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>7.45 ± 0.8089</td>
</tr>
<tr>
<td>Clorsulon</td>
<td>25</td>
<td>24.92 ± 0.4614</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>49.89 ± 0.2216</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>74.88 ± 0.1737</td>
</tr>
</tbody>
</table>

The results obtained accurately fall within the limit of acceptance criteria. Hence, the method can be termed as precise.

**Limit of detection and limit of qualification**

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated:

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S}
\]

\[
\text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where, \( \sigma \) = the standard deviation of the response,

\( S \) = slope of the calibration curve.

LOD and LOQ for Ivermectin are found to be 0.002 μg/ml and 0.030 μg/ml respectively. LOD and LOQ for Clorsulon are found to be 0.006 μg/ml and 0.093 μg/ml.

**CONCLUSION**

From the results obtained, it is obvious that the proposed method is applicable for the determination of Ivermectin and Clorsulon without interference and with good sensitivity. The results obtained indicate that the proposed method for the estimation of Ivermectin and Clorsulon is simple, specific, rapid, linear, accurate, precise, robust, sensitive and suitable for intended use. These merits suggest the use of the proposed method in routine and quality control analysis of Ivermectin and Clorsulon without interference from commonly encountered excipients and additives.

**REFERENCES**


