UV SPECTROSCOPIC METHOD DEVELOPMENT AND VALIDATION OF FIRST DERIVATIVE METHOD FOR SIMULTANEOUS ESTIMATION OF PRAZIQUANTEL AND ABAMECTIN IN FINISHED DOSAGE FORM

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(Received 27 July 2020) (Accepted 15 June 2021)

ABSTRACT
In the present investigation, methanol is employed as the solubilizing agent to solubilize poorly water-soluble drugs such as praziquantel and abamectin. UV spectrophotometric method has been developed for simultaneous estimation of praziquantel and abamectin in bulk drug and in their combined pharmaceutical dosage form by first order derivative method. This method utilizes methanol as a common solvent and \( \lambda_{\text{max}} \) of praziquantel and abamectin selected for analysis was found to be at 248 nm (at ZCP of abamectin) and 274 nm (at ZCP of praziquantel), respectively. Linearity was observed in the concentration range of 25-150 µg mL\(^{-1}\) for praziquantel (\( r^2 = 0.9984 \)) and 1-11 µg mL\(^{-1}\) for ABAM (\( r^2 = 0.9986 \)). The accuracy and precision were determined and found to comply with ICH guidelines. This method shows good reproducibility and recovery with % RSD in the desired range. Developed method was applied for marketed formulation. The results were found to be within acceptance criteria according to ICH guideline.

Keywords: Praziquantel, abamectin, Simultaneous estimation, first derivative method, validation

INTRODUCTION
Chemistry and mechanism of drug\(^1\)-\(^3\)

Praziquantel (Fig. 1) is chemically (11bRS)-2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4\(H\)-pyrazino[2,1-a] isoquinolin-4-one. It is an anthelminthic. Praziquantel increases the permeability of the membranes of schistosome cells towards calcium ions. The drug thereby induces contraction of the parasites, resulting in paralysis in the contracted state.

Abamectin (Fig. 2) is chemically (10E,14E,16E) (1R,4S,5′S,6S,6′R,8R,12S,13S,20R,21R,24S)-6′-[(S)-sec-butyl]-21,24-dihydroxy-5′,11,13,22-tetramethyl-2-oxo 19-(3,7,19-trioxatetracyclo [15.6.1.14,8.020,24] pentacos-10,14,16,22-tetraene)-6-spiro-2′-(5′,6′-dihydro-2′H-pyran)-12-yl 2,6-dideoxy-4-O-(2,6-dideoxy-3-O-methyl-\(\alpha\)-L-arabinohexopyranosyl)-3-O-methyl-\(\alpha\)-L-arabino-hexopyranoside. It is also from the anthelminthic class of drugs. Abamectin blocks the transmission of electrical activity in invertebrate nerve and muscle cells,

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https://doi.org/10.53879/ld.60.05.12653
mostly by enhancing the effects of glutamate at the invertebrate-specific glutamate-gated chloride channel, with minor effects on gamma-aminobutyric acid receptors. This causes an influx of chloride ions into the cells, leading to hyperpolarisation and subsequent paralysis of invertebrate neuromuscular systems; comparable doses are not toxic for mammals because they do not possess glutamate-gated chloride channels.

In the present study, the first derivative zero crossing applied for the simultaneous analysis of the tablet formulation containing praziquantel and abamectin as the first derivative method allows for the selection of the defined analytical wavelengths of highest value due to the presence of lot of maxima and minima and provides a high sensitivity and accuracy.

The review of the literature revealed that various analytical methods involving spectrophotometry, HPLC and dissolution have been reported for praziquantel alone and in combination with other drugs. However, to the best of our knowledge, no spectrophotometric method is published for the simultaneous determination of PRAZI and ABAM in tablet dosage form. The present work describes the development of a simple, precise, accurate, and reproducible spectrophotometric method for the simultaneous estimation of PRAZI and ABAM in combined dosage forms. The developed method was validated in accordance with ICH guideline and successfully employed for the assay of PRAZI and ABAM in combined tablet dosage form.

MATERIALS AND METHODS

Instrumentation

Double beam UV-visible spectrophotometer (Shimadzu 1800) having two matched quartz cells with 1 cm light path. An electronic analytical balance (Mettler Toledo) was used in the study.

Materials and reagents

HPLC grade methanol used as solvent for both drugs. Active pharmaceutical ingredients (API) praziquantel and abamectin were purchased from the market.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guideline Q2 (R1).

Selection of wavelength

Standard drugs of praziquantel and abamectin were scanned separately in the range of 200-400 nm. Data was obtained by overlain spectra of PRAZI and ABAM. Data was obtained as 248 nm (ZCP of praziquantel) and 274 nm (ZCP of abamectin) maxima wavelength for simultaneous estimation method.

Preparation of solutions

Preparation of standard stock solution of praziquantel

The standard stock solution of praziquantel was prepared by accurately weighed 100 mg of praziquantel transferred to a 10 mL volumetric flask, dissolved in and diluted up to the mark with methanol to obtain a standard stock solution (1000 µg mL⁻¹).

Preparation of standard stock solution of abamectin

The standard stock solution of abamectin was prepared by accurately weighed 10 mg of abamectin transferred to a 100 mL volumetric flask, dissolved in and diluted up to the mark with methanol to obtain a standard stock solution (100 µg mL⁻¹).

Preparation of calibration standards

Calibration standards for praziquantel

The calibration curve for praziquantel was constructed using different concentrations of standard solutions ranging from 25-150 µg mL⁻¹. Working standard solutions of praziquantel (0.25, 0.5, 0.75, 1, 1.25, 1.5 mL) were transferred into a series of 10 mL volumetric flasks and diluted up to the mark with methanol (25, 50, 75, 100, 125, 150 µg mL⁻¹).

Calibration standards for abamectin

Calibration curve for abamectin was constructed using different concentrations of standard solutions ranging from 1-11 µg mL⁻¹. Working standard solutions of abamectin (0.1, 0.3, 0.5, 0.7, 0.9, 1.1 mL) were transferred into a series of 10 mL volumetric flasks and diluted up to the mark with methanol (1, 3, 5, 7, 9, 11 µg mL⁻¹).

Method validation

Linearity and range

The linearity response was determined by analyzing concentrations in the range of 25-150 µg mL⁻¹ for PRAZI and 1-11 µg mL⁻¹ for ABAM. Accurately measured standard stock solutions of each PRAZI (0.25, 0.5, 0.75, 1, 1.25, 1.5 mL) and ABAM (0.1, 0.3, 0.5, 0.7, 0.9, 1.1 mL) were transferred into 10 mL volumetric flask and volume made......
up with distilled water to obtain concentrations 1:25, 3:50, 5:75, 7:100, 9:125, 11:150 µg mL⁻¹ of PRAZI and ABAM respectively. Absorbance of each solution was measured at 248 nm (ZCP of praziquantel) and 274 nm (ZCP of abamectin). Range in term which calibrations curve constructed by plotting graph absorbance vs. concentration.

**Precision**

**Repeatability**

Repeatability of PRAZI and ABAM checked by repeated measurement of absorbance of solution (n=6) of 75 µg mL⁻¹ (PRAZI) and 5 µg mL⁻¹ (ABAM) measured and % RSD was calculated.

Acceptance criteria: - %RSD should be less than 2

**Intraday precision**

Three replicates of three concentrations of PRAZI and ABAM using that 3 concentrations (75, 100, 125 µg mL⁻¹) and (5, 7, 9 µg mL⁻¹) were prepared, thus, total nine determination were analyzed at same day within short time intervals and absorbances were measured and % RSD was calculated.

Acceptance criteria: - %RSD should be less than 2

**Interday precision**

Three replicates of three concentrations of PRAZI and ABAM using that 3 concentrations (75, 100, 125 µg mL⁻¹) and (5, 7, 9 µg mL⁻¹) were prepared thus total nine determination were analyzed at three consecutive day and first derivative absorbance were measured and %RSD was calculated.

Acceptance criteria: - %RSD should be less than 2

**Different instrument**

Three replicates of three concentration of PRAZI and ABAM using that 3 concentration (75, 100, 125 µg mL⁻¹) and (5, 7, 9 µg mL⁻¹) were prepared, thus, total nine determination were analyzed in two different instruments (UV-1800, UV-1700) on the same day and absorbances were measured and % RSD was calculated.

Acceptance criteria: - % RSD should be less than 2

**Robustness**

Three replicates of three concentrations of PRAZI tablets and ABAM using 3 concentrations (75, 100, 125 µg mL⁻¹) and (5, 7, 9 µg mL⁻¹) were prepared and analyzed at different wavelengths. The solutions of PRAZI were analyzed at 247 nm, 248 nm, and 249 nm and of ABAM of 273 nm, 274 nm, and 275 nm. Absorbances at each wavelength were measured and % RSD was calculated.

Acceptance criteria: - %RSD should be less than 2

**Limit of detection**

The LOD was estimated from the set of five calibration curves used to determine method linearity. The LOD may be calculated as,

\[
LOD=3.3 \left( \frac{SD}{Slope} \right)
\]

where SD = Standard deviation of the Y- intercepts of the 5 calibration curves and

\[
slope= \text{Mean slope of the 5 calibration curves}
\]

**Limit of quantification**

The LOD was estimated from the set of 5 calibration curves used to determine method linearity. The LOD may be calculated as,

\[
LOQ= 10 \left( \frac{SD}{Slope} \right)
\]

where, SD= Standard deviation of the Y- intercepts of the 5 calibration curves and

\[
Slope= \text{Mean slope of the 5 calibration curves}
\]

**Accuracy**

The accuracy of the method was determined by calculating % recovery of praziquantel and abamectin by standard addition method. Known amounts of standard solutions of PRAZI and ABAM corresponding to 50, 100 and 150 % of target concentration were spiked with pre - analyzed sample solution. The amounts of PRAZI and ABAM were estimated by applying obtained values to regression equation of calibration curve.

**Analysis of drug in marketed formulation**

The response of sample solution was measured at 248 nm and 274 nm. The amounts of PRAZI and ABAM present in sample solution were calculated.

**Preparation of sample solution**

The average weight of 20 tablets was calculated and the powdered. Weight equivalent to 6.25 mg of praziquantel and 0.25 mg of abamectin was transferred into 100 mL volumetric flask. 20 mL of methanol was added and sonicated for 15 minutes. The volume was
adjusted with methanol up to the mark. The solution was then filtered through Whatman filter paper number 1, to get final concentration 62.5 μg mL⁻¹ of praziquantel and 2.5 μg mL⁻¹ of abamectin. From this stock solution, 5 mL was pipetted out and transferred into 10 mL volumetric flask and volume made up to the mark with distilled water to get concentration 50 μg mL⁻¹ of PRAZI and 2 μg mL⁻¹ of ABAM. The samples were scanned in UV region and absorbance noted at 248 nm and absorbance at 274 nm. At this point PRAZI and ABAM concentration were quantified.

RESULTS AND DISCUSSION

METHOD VALIDATION

Selection of solvent

Praziquantel and abamectin were soluble in methanol, so methanol used was as solvent for drug analysis.

Selection of analytical wavelength

The standard solutions of praziquantel (25-150 μg mL⁻¹) and abamectin (1-11 μg mL⁻¹) were scanned separately in the UV range of 200-400 nm. The zero order spectra obtained were then processed to obtain first order derivative spectra and overlaid. At 248 nm, praziquantel showed zero absorbance and abamectin, showed reasonable absorbance, while at 274 nm, abamectin showed zero absorbance and praziquantel, showed reasonable absorbance so these two wavelengths were selected for measurement of the respective drugs.

Fig. 3 Overlain zero order spectra of standard solution of praziquantel (75 μg), and abamectin (5 μg)

Linearity and range

Calibration curve constructed was linear over the selected range of 25-150 μg mL⁻¹ for praziquantel and 1-11 μg mL⁻¹ for abamectin at λ.max of 248 nm and 274 nm, respectively. Each concentration measurement was repeated three times. The assays were performed according to experimental conditions and the linearity of the calibration graphs.

The optical characteristics of praziquantel and abamectin as shown in Table I.

Table I: Optical characteristics of praziquantel and abamectin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Praziquantel</th>
<th>Abamectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ.max (nm)</td>
<td>248</td>
<td>274</td>
</tr>
<tr>
<td>Linearity range (μg mL⁻¹)</td>
<td>25-150</td>
<td>1-11</td>
</tr>
<tr>
<td>Correlation coefficient (R²)</td>
<td>0.9984</td>
<td>0.9986</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = -0.0001x - 0.0024</td>
<td>y = -0.0028x - 0.0001</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.0024</td>
<td>0.0001</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>-0.0001</td>
<td>-0.0028</td>
</tr>
</tbody>
</table>

Precision

Precision was determined by studying the repeatability which indicates the precision under the same operating conditions over a short interval time. The experiments were repeated for six times for precision. The developed method was found to be precise for intraday and inter day precision on the basis of % RSD values for both drugs.

Intradays precision

It was performed by taking three replicates of standard solution of PRAZI and ABAM and using that 3 concentrations (75, 100, 125 μg mL⁻¹) and (5, 7, 9 μg mL⁻¹) were prepared thus total nine determination were analysed within the short period of time interval. The % RSD was found to be < 2.
### Table II: Results of intraday precision studies

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Conc. (µg mL⁻¹)</th>
<th>Praziquantel intra-day precision (n=3)</th>
<th>Abamectin intra-day precision (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>% RSD</td>
</tr>
<tr>
<td>1</td>
<td>75</td>
<td>-0.0125±0.0001</td>
<td>0.0008</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>-0.0161±0.0001</td>
<td>0.9468</td>
</tr>
<tr>
<td>3</td>
<td>125</td>
<td>-0.0188±0.0001</td>
<td>0.6120</td>
</tr>
</tbody>
</table>

Results are mean of three readings and expressed as mean ± standard deviation.

### Table III: Results of inter-day precision studies

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Conc. (µg mL⁻¹)</th>
<th>Praziquantel intra-day precision (n=3)</th>
<th>Abamectin intra-day precision (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>% RSD</td>
</tr>
<tr>
<td>1</td>
<td>75</td>
<td>-0.0125±0.0001</td>
<td>0.0008</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>-0.0161±0.0002</td>
<td>1.2876</td>
</tr>
<tr>
<td>3</td>
<td>125</td>
<td>-0.0189±0.0001</td>
<td>0.7482</td>
</tr>
</tbody>
</table>

Results are mean of three readings and expressed as mean ± standard deviation.

### Table IV: Results of different instrument data

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Conc. (µg mL⁻¹)</th>
<th>Praziquantel different instrument data [UV 1800, UV 1700] (n=3)</th>
<th>Abamectin different instrument data [UV 1800, UV 1700] (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>% RSD</td>
</tr>
<tr>
<td>1</td>
<td>75</td>
<td>-0.0126±0.0001</td>
<td>1.1223</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>-0.0161±0.0001</td>
<td>0.8783</td>
</tr>
<tr>
<td>3</td>
<td>125</td>
<td>-0.0199±0.0001</td>
<td>0.7482</td>
</tr>
</tbody>
</table>

Results are mean of three readings and expressed as mean ± standard deviation.

### Table V: Results of different wavelength

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Conc. (µg mL⁻¹)</th>
<th>Praziquantel at different wavelength [247, 248, 249 nm] (n=3)</th>
<th>Abamectin at different wavelength [273, 274, 275 nm] (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>% RSD</td>
</tr>
<tr>
<td>1</td>
<td>75</td>
<td>-0.0125±0.0001</td>
<td>1.2187</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>-0.0159±0.0001</td>
<td>1.2876</td>
</tr>
<tr>
<td>3</td>
<td>125</td>
<td>-0.0188±0.0002</td>
<td>1.0638</td>
</tr>
</tbody>
</table>

Results of intraday precision studies as shown in Table II.

**Interday precision**

Three replicates of 3 concentrations of standard solution of PRAZI and ABAM, total nine determinations were analyzed on three consecutive days and first derivative absorbances were measured at 274 nm and 248 nm. %RSD was calculated.

The results of inter-day precision studies as shown in Table III.
Different Instrument

Three different concentrations of PRAZI and ABAM were analyzed on UV 1800 and UV 1700 spectrophotometers and the absorbances at 274 nm and 248 nm were recorded. %RSD was calculated as shown in Table IV.

**Robustness**

Robustness carried by changing wavelength ± 1.0 nm. %RSD PRAZ and ABAM was calculated. Robustness at different wavelength 247, 248, 249 nm for praziquantel and for abamectin at 273, 274, 275 nm results as mention in Table V.

**Limit of detection and limit of quantification**

[LOD and LOQ]

Calibration curves were repeated for five and standard deviation of intercept was calculated, then LOD and LOQ were calculated as shown in Table VI.

**Accuracy**

From marketed formulation at three levels of standard addition, accuracy of the method was confirmed by recovery study. % recoveries of PRAZI and ABAM were found between 98% and 100 % The

**Table VI: Results of LOD and LOQ**

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>LOD (µg mL⁻¹)</th>
<th>LOQ (µg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Praziquantel</td>
<td>1.65</td>
<td>5</td>
</tr>
<tr>
<td>Abamectin</td>
<td>0.1184</td>
<td>0.0359</td>
</tr>
</tbody>
</table>

**Table VII: % Recovery data for PRAZI and ABAM**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Amount added (%)</th>
<th>Praziquantel % recovery ± S.D.</th>
<th>% RSD</th>
<th>Abamectin % recovery ± S.D.</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>99.55±0.5315</td>
<td>0.5339</td>
<td>99.66±1.1547</td>
<td>1.1585</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>98.67±0.0115</td>
<td>0.0117</td>
<td>99.66±0.6650</td>
<td>0.6672</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>98.92±0.2003</td>
<td>0.2025</td>
<td>99.66±0.3818</td>
<td>0.3831</td>
</tr>
<tr>
<td>4</td>
<td>150</td>
<td>99.226±0.0378</td>
<td>0.0381</td>
<td>99±0.87178</td>
<td>0.8805</td>
</tr>
</tbody>
</table>

**Table VIII: Analysis of PRAZIMEC-C Tablet**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Label claim (mg)</th>
<th>Amount found (mg)</th>
<th>% Label claim (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Praziquantel</td>
<td>Abamectin</td>
<td>Praziquantel</td>
</tr>
<tr>
<td>1</td>
<td>6.25</td>
<td>0.25</td>
<td>6.11</td>
</tr>
<tr>
<td>2</td>
<td>6.25</td>
<td>0.25</td>
<td>6.0</td>
</tr>
<tr>
<td>3</td>
<td>6.25</td>
<td>0.25</td>
<td>6.0</td>
</tr>
<tr>
<td>4</td>
<td>6.25</td>
<td>0.25</td>
<td>6.10</td>
</tr>
<tr>
<td>5</td>
<td>6.25</td>
<td>0.25</td>
<td>6.14</td>
</tr>
<tr>
<td>6</td>
<td>6.25</td>
<td>0.25</td>
<td>6.22</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
% recovery data for praziquantel and abamectin as shown in Table VII.

**Analysis of drug in marketed formulation**

Analysis of marketed formulation [PRAZIMEC-C Tablet] by UV Spectroscopy as shown in Fig. 5. The analysis of marketed formulation [PRAZIMEC-C tablet] was found within standards limit as shown in Table VIII.

**DISCUSSION**

The present paper describes the estimation of praziquantel and abamectin in bulk and tablet dosage form by simultaneous estimation using first derivative UV method. Solubility studies indicated that both drugs show better solubility and stability in methanol. The Beer-Lambert's concentration range was found to be 25-150 µg mL⁻¹ for praziquantel and 1-11 µg mL⁻¹ for abamectin at 248 nm and 274 nm, respectively. Co-efficient of correlation was found to be 0.9984 for praziquantel and 0.9986 (Table I) for the proposed method. Precision was determined by studying the interday and intraday precision. The standard deviation and relative Standard deviation (% RSD) were found less than 2.0%, which indicates good intermediate precision (Table III and IV). The values LOD and LOQ were 1.65 µg mL⁻¹ and 5 µg mL⁻¹ for praziquantel and 0.1184 and 0.0359 µg mL⁻¹ for abamectin, respectively (Table VI). Percentage estimation of praziquantel and abamectin in tablet dosage form were 97.52 % and 99.66 % by the proposed method respectively (Table VII).

**ACKNOWLEDGEMENT**

We are thankful to Pure Chem. Pvt. Ltd., Ankleshwar for providing praziquantel as a free gift sample.

**REFERENCES**