Simultaneous Estimation of Atorvastatin and Ramipril by First Derivative Spectrophotometric method

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ABSTRACT

A simple, precise and economical procedure for the simultaneous estimation of Atorvastatin (ATV) and Ramipril (RAM) in tablet formulation has been developed. ATV is an anticholesterolic agent as well as hydroxymethylglutaryl-CoA reductase inhibitor and RAM is an antihypertensive agent belonging to category of angiotensin-converting enzyme inhibitor. The formulation of Atorvastatin (ATV) and Ramipril (RAM) (Stator-R 5) in a combination mixture is very good pharmacologically since it enhances the efficacy of the individual drugs. The present study involving the simultaneous estimation done by first derivative method. ATV has absorbance maxima at 246nm in methanol and RAM has absorbance maxima at 208nm in methanol. Both these drugs obey Beer’s law in the concentration range employed for the present method. The result of analysis has been validated statistically by recovery studies. The slope and intercept for ATV were -0.00183 and -0.001 and for RAM were 0.0036 and -0.046 respectively as determined by the method of least squares. The results were found satisfactory and reproducible. The method was applied successfully for the estimation of ATV and RAM simultaneously in tablet dosage form without the interference of common excipients.

Keywords: Atorvastatin; Ramipril; first derivative spectrophotometric method.

INTRODUCTION

Tablet containing ATV, categorized for hypercholesterolemia in combination with RAM, an antihypertensive agent found to be therapeutically very effective in cardiac patients (Anonymous, 1992). This combination is widely started to use in India. A to Z Life Sciences, Pondicherry, India marketed this formulation in the trade name Stator-R 5, which contain ATV 10 mg and RAM 5 mg. The local quality analysis requirement is that each Tablet should contain a minimum of 93.0% and maximum of 107.0% of the declared amount of the Tablet as per USP. Even though various analytical methods were reported for the estimation of ATV and RAM in their individual dosage forms as well as in combination with other drugs1-18, none of the methods reported for simultaneous estimation of both compounds in the mixture by the present developed methods, which are simple, rapid and reliable method of assaying quality control of ATV and RAM containing Tablet simultaneously. We have applied simultaneous estimation by first derivative to determine the concentration of each drug in the mixture. Mixtures of known composition were used as standards to minimize errors due to the presence of both components in the solution.

The objective of the present work was to assess whether derivative spectrophotometry could be used to avoid the overlapping spectral bands of the components, which occurs on zero order spectrum and hence use it for routine assessment of the drug ATV and RAM.

Materials & Methods:-

Analytical Grade Methanol (Qualigens) was used as solvent. Shimadzu UV Spectronic 1700 model was used for the spectrophotometric determinations. Gift samples of Atorvastatin (ATV) and Ramipril (RAM) were obtained from Ajantha Pharmaceutical Ltd. Aurangabad.

Experimental part:-

Study of Spectra and selection of wavelengths

The aliquot portions of standard stock solutions of ATV and RAM were diluted appropriately, with methanol to obtain a concentration 20µg/ml of ATV and 20µg/ml of RAM respectively. The solutions were scanned in the range of 400-200nm in 1.0 cm cell against reagent blank. The results are shown in Figure 1.

The overlain zero order spectra of ATV and RAM shows that the absorption maxima of RAM was overlapping with the absorption maxima of ATV, rendering the zero order determinations inaccurate. So a first order spectrum of both drugs in combination was developed.

First Derivative Spectra

1. Standard working solutions:-

An accurately weighed quantity of ATV (100mg) and RAM (100mg) was dissolved in methanol in 100 ml volumetric flask and volume was made up to mark with methanol (1000µg/ml). The aliquot portions of standard stock solutions of ATV and RAM were diluted appropriately, with methanol to produce conc. 100µg/ml ATV and 100µg/ml of RAM respectively. Five serial dilutions of Atorvastatin
and Ramipril, were prepared in triplicate, from the stock solutions in the conc. range of 10–50µg/ml and 10–50µg/ml respectively. The curves of the solutions were scanned in the range of 200nm–300nm against methanol as the blank. The following derivative ($D^1$) values were obtained.$^{19}$

ATV shows zero crossing points at 208nm, 223nm and 246nm. So, 208nm was selected for the estimation of RAM because at this wavelength, ATV shows zero absorbance. The results are shown in Figure2.

RAM shows zero crossing points at 215nm and 246nm. So, 246nm was selected for the estimation of ATV, because at this wavelength RAM shows zero absorbance. The results are shown in Figure 3.

This technique involves the differentiation of the normal spectrum with respect to the wavelength. The first derivative $D^1$ of the ATV spectrum shows a zero crossing point at 208nm RAM is measured at this wavelength. And the first derivative $D^1$ of the RAM spectrum shows a zero crossing point at 246nm ATV is measured at this wavelength. The method is satisfactory and the calibrated graph indicates that method is linear. The results are shown in Table 1, figure 4 and Figure 5.

2. Tablet assay solution

Procedure

Twenty tablets were accurately weighed and powdered in a mortar. An amount of powder equivalent to the weight of 10 mg of ATV and 5mg of RAM was mixed in 100ml volumetric flask. Appropriate dilution made with methanol, filtered and the same used for preparation of the tablet assay solution. The $D^1$ curve of the solutions (n=5) were obtained and Atorvastatin and Ramipril determined as described above. The results are shown in Table 2. The mean RSD value indicate the repeatability or precision of the method.

3. Recovery Studies

Procedure

An accurately weighed quantity of preanalysed tablet powder equivalent to 100 mg ATV was taken in 100 ml volumetric flask and pure drug of RAM (50 mg) weighed quantity was added and mixed thoroughly. To it standard solution was added in different proportions. Then the volume was adjusted up to the mark with methanol and the solution was filtered through Whatman filter paper No.41. The aliquot portion of the filtrate was further diluted to get final concentration. The $D^1$ value sample solution was measured at 246nm and 208nm. The result are shown in Table 3. The percent recovery value near to 100%, indicate accuracy of the method and non interference of the excipients used.

RESULTS

The average derivative values produced when the different concentrations of Atorvastatin and Ramipril were scanned are indicated. Plotting these derivative values against the respective concentrations, the curves yielded the equation $D^1 • 246 = 0.00183x – 0.00095$ (correlation coefficient of 0.993) for ATV and $D^1 • 208 = 0.0036x – 0.046$ (correlation coefficient of 0.915) for RAM. Absorption measurements made by scanning the solution of ATV, RAM and the mixture of ATV and RAM on zero order are presented. Their overlapping spectral bands are shown in Figure 1. Derivative spectral bands for the individual components ATV and RAM are shown in Figure 2 and Figure 3 respectively. In figure 6, first derivative spectra ($D^1$) are represented by 1 for ATV, 2 for RAM and 3 for the mixture. $D^2$ of the ATV shows a peak at 246nm, where the $dA/d?, for RAM is zero, so ATV can be specifically measured at this wavelength. RAM on the other hand, has a near trough at 208nm where $dA/d?$, for ATV is zero; hence RAM is exclusively measured at that wavelength. The figure also shows that other zero crossings of the two compounds do appear. To prove the validity and applicability of the proposed method, synthetic mixtures of different ratios in the concentration ranges stated in assay table. The results were computed against the previously constructed standard curve. Table 2 presents the results of the assay of the mixtures. As can be seen, satisfactory results were obtained with a mean recovery of 99.1±0.077 and 101±0.214 for ATV and RAM respectively.

Commercial tablet

Similar method as above was applied to the determination of ATV and RAM in a commercial tablet preparation. Table 3 gives the results of this application. The results obtained were in satisfactory agreement with the label claims, within the acceptable limits.

DISCUSSION

As can be seen in figure 1, absorption measurements of each antihyperlipidemic in a binary mixture appears to be quite impossible because of the total overlap of bands. The band overlap might be solved by Vierordt’s simultaneous equation method$^{20}$. However this may be influenced by differences between the sample and reference, or by the matrix in the pharmaceutical formulations leading to erroneous results. Since the mid seventies (1970s), workers like O’Haver$^{21}$ and Fell$^{22}$ found that to resolve the problem of closely overlapping spectra, derivative spectroscopy, with digital processing together with zero crossing, offered the best option. This technique involves the differentiation of the normal spectrum with respect to the wavelength. The first derivative $D^1$ of the ATV spectrum shows a peak at 246nm, where the $dA/d?$, for RAM is zero, so ATV can be specifically measured at that wavelength. RAM on the other hand, has a near trough at 208nm where $dA/d?$, for ATV is zero; hence RAM is specifically measured at that wavelength.

In the derivative zero crossing method, it is possible to get two or more ‘zero crossings’. In such a case, meticulous assessment of the best crossing point is possible, basing partly on the strength of the signal. Indeed the present work found other ‘zero-crossings’ of the two compounds. However, they appear either at lower wave-
Table 1. Calibration data of ATV and RAM

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration(µg/ml)</th>
<th>Average D-value n=5</th>
<th>Equation</th>
<th>Correlation coefficient</th>
<th>S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atorvastatin</td>
<td>10</td>
<td>-0.02</td>
<td></td>
<td>D^1=-0.00183X-0.001</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>-0.04</td>
<td></td>
<td></td>
<td>0.000707</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>-0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>-0.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ramipril</td>
<td>10</td>
<td>0.01</td>
<td></td>
<td>D^1=0.0036X-0.046</td>
<td>0.915</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.02</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>50</td>
<td>0.16</td>
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</table>

Table 2. Assay of ATV and RAM in tablet formulation

<table>
<thead>
<tr>
<th></th>
<th>Equivalent taken (µg/ml)</th>
<th>D^1-246nm found (µg/ml) (%)</th>
<th>Purity (µg/ml) (%)</th>
<th>Equivalent taken (µg/ml)</th>
<th>D^1-208nm found (µg/ml) (%)</th>
<th>Purity (µg/ml) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>-0.063</td>
<td>30.31</td>
<td>101.2</td>
<td>20</td>
<td>-0.024</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>-0.062</td>
<td>30.1</td>
<td>100.4</td>
<td>20</td>
<td>-0.022</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>-0.065</td>
<td>29.7</td>
<td>98.8</td>
<td>20</td>
<td>0.023</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>-0.066</td>
<td>30.2</td>
<td>100.8</td>
<td>20</td>
<td>-0.022</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>-0.068</td>
<td>30.3</td>
<td>101.2</td>
<td>20</td>
<td>-0.021</td>
</tr>
</tbody>
</table>

Mean±R.S.D 98.74±0.171

Table 3. Recovery of ATV and RAM in tablet formulation.

<table>
<thead>
<tr>
<th></th>
<th>Theoretical Found</th>
<th>Recovery %</th>
<th>Theoretical Found</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATV</td>
<td></td>
<td></td>
<td>RAM</td>
<td></td>
</tr>
<tr>
<td>1:1</td>
<td>-0.023</td>
<td>10.01</td>
<td>100.1</td>
<td>101.02</td>
</tr>
<tr>
<td>1:1</td>
<td>-0.042</td>
<td>20.03</td>
<td>100.3</td>
<td>102.02</td>
</tr>
<tr>
<td>1.5:1</td>
<td>-0.061</td>
<td>30.04</td>
<td>101.8</td>
<td>101.10</td>
</tr>
<tr>
<td>2:1</td>
<td>-0.084</td>
<td>39.82</td>
<td>99.1</td>
<td>104.10</td>
</tr>
<tr>
<td>2.5:1</td>
<td>-0.093</td>
<td>49.96</td>
<td>99.8</td>
<td>104.10</td>
</tr>
</tbody>
</table>

Mean±R.S.D 99.1±0.077

Figure 1. Overlay zero order spectra of ATV and RAM

Figure 2. First Derivative spectra of ATV with 10µg/ml solution

Figure 3. First Derivative spectra of RAM with 10µg/ml solution

Figure 4. Calibration graph for ATV for first derivative spectra

Figure 5. Calibration graph for RAM for first derivative spectra
REFERENCES


CONCLUSION

In conclusion, the successful application of the proposed method confirms that derivative spectrophotometry offers a simple, rapid and direct method of determining this binary mixture, with some advantages over other separation techniques, such as in the official USP-high-performance liquid chromatography. Admittedly, the HPLC method is expensive in both the hardware and chromatographic reagents.

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