DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF VALSARTAN AND NIFEDIPINE IN BULK AND SYNTHETIC MIXTURE
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Abstract

The present manuscript describes simple, sensitive, rapid, accurate, precise and cost effective First derivative spectrophotometric method for the simultaneous estimation of Valsartan and Nifedipine in Synthetic Mixture. The first order derivative absorption at 248.43 nm (zero crossing point for Valsartan) was used for Nifedipine and 216.52 nm (zero crossing point for Nifedipine) was used for Valsartan. The linearity was obtained in the concentration range of 5-25 μg/ml for Valsartan and 2-10 μg/ml for Nifedipine with correlation coefficient (R^2) 0.9994 and 0.9963, respectively. The mean % recoveries were found to be in the range of 99.50-100.50% and 100.30-100.50% for Valsartan and Nifedipine, respectively. The suitability of these methods for the quantitative determination of Valsartan and Nifedipine was proved by validation. The proposed method has been validated as per ICH guideline and successfully applied to the simultaneous estimation of Valsartan and Nifedipine in their Synthetic Mixture. The results of analysis have been validated statistically and by recovery studies.

Keywords: Valsartan, Nifedipine, First order derivative, Synthetic Mixture, Validation method.

Introduction

Valsartan is Chemically N-(1-Oxopentyl1)-N-[[2’ –(1H-tetrazol-5-yl)][1,1’ –biphenyl]-4-yl]-L-:N-\{p-(o-1H-tetrazol-5-ylphenyl)benzyl1\} -N-valeryl-L-valine: (S)N-(1-car-boxy-2-methylprop-1-yl) -N-petanoyl-N-[2’ –(1H-tetrazol-5-yl) -bi-phenyl-4-ylmethyl] amine\[1\]. Valsartan is vasodilator agents, phosphodiesterase inhibitors, bronchodilator agents, respiratory smooth muscle relaxant. Valsartan competitively inhibits type III and type IV phosphodiesterase (PDE), the enzyme responsible for breaking down cyclic AMP in smooth muscle cells, possibly resulting in bronchodilator \[3\].

Nifedipine is chemically, 1, 4-Dihydro-2, 6-dimethyl-4-(2-nitrophenyl)-3, 5-pyridinecarboxylic acid dimethyl ester; 4-
(2-nitrophenyl) 2, 6-dimethyl-3, 5-dicarboxmethoxy-1, 4-dihydro-pyridine \[^2\]. Nifedipine is a direct acting sympathomimetic with predominantly active precursor of the Selective \(B_2\) adrenergic agonist \[^2, 4\]\. Nifedipine is Calcium channel blocker \[^4\]\. The overriding action of Nifedipine is arteriolar dilation, BP falls \[^4\]\. The direct depressant effect on heart requires much higher does, but a weak negative inotropic action can be unmasked after \(\beta\) blockade \[^4\]\.

Valsartan and Nifedipine are commercially available in various dosage forms an individual formulation. Combination of Valsartan and Nifedipine are study under Clinical Trial phase. Identifier No: NCT00993109 by Bayer \[^{11}\] (Chine & Korea FDA). Combination of Valsartan and Nifedipine are useful in reduce the blood pressure. Valsarant is official in IP \[^5\], USP-NF \[^6\] and NF \[^7\]. Nifedipine is official in IP \[^8, 10\], USP-NF \[^9\] (From Literature Survey, various method (UV, HPLC, HPTLC, GC and Colorimetric) were reported for the analysis of individual drug in combination with other drug but no method were reported for simultaneous estimation of Valsartan and Nifedipine. Hence, the purpose of the present work was to develop and validate first order derivative spectrophotometric method for simultaneous estimation of Valsartan and Nifedipine in synthetic mixture.

**Material and methods**

**Instruments**

Spectrophotometric measurements were performed on Shimadzu UV –visible double beam spectrophotometer (Model- 1800). All weighing were done on electronic analytical balance (Wensar Dab220).

**Chemicals and Reagents**

The bulk drug, Valsartan was obtained from Torrent Pharmaceuticals, Ahmedabad and Nifedipine was obtained from Mediwin Pharmaceuticals, Ahmedabad. Fixed dose of synthetic mixture of Valsartan 80 mg and Nifedipine 30 mg were prepared in laboratory scale as pilot batch. Analytical grade methanol was procured from Merck Fine chemicals (Mumbai).

**Selection of a Solvent**: Methanol was selected as solvent for studying spectral characteristic of drugs.

**Preparation of Standard Stock Solution**

Accurately weighed 10 mg of Valsartan and 10 mg of Nifedipine standard were transferred to separate 100 ml volumetric flask and dissolved in 100 ml methanol. The flasks were shaken and volume was made up to the mark with Methanol to give solution containing 100 \(\mu\)g/ml Valsartan and Nifedipine. For Nifedipine preparation Umbel color volumetric flack were used.
Preparation of Working Standard Solution of Valsartan and Nifedipine

From above solution of Valsartan pipette out 0.5, 1.0, 1.5, 2.0, 2.5 ml of the stock solution were further diluted to 10 ml volumetric flasks individually with methanol to get concentrations 5, 10, 15, 20, 25 μg/ml. From above solution of Nifedipine pipette out 0.2, 0.4, 0.6, 0.8, 1.0 ml of the stock solution were further diluted to 10 ml volumetric flasks individually with methanol to get concentrations 2, 4, 6, 8, 10 μg/ml.

Selection of Analytical Wavelength

Standard 5-25 μg/ml solutions of Valsartan and 2-10 μg/ml solutions of Nifedipine were prepared in methanol by appropriate dilution and spectrum was recorded between 200-400 nm. All zero order spectrum (D₀) were converted to first derivative spectrum (D¹) using delta lambda 2.0 and scaling factor 10. The overlain first derivative spectrums of Valsartan and Nifedipine at different concentration were recorded. The zero crossing point (ZCP) of Valsartan was found to be 248.43 nm and ZCP of Nifedipine was found to be 216.52 nm.

Assay of Synthetic Mixture

The quantity of synthetic mixture powder equivalent to 80 mg of Valsartan and 30 mg of Nifedipine was transferred in to 100 ml volumetric flask, containing methanol. The volume was made up to the mark with methanol and the solution filtered through 0.45μm Whatmann filter paper. An aliquot of this solution (1.0 ml) was transferred in to 10 ml volumetric flask and volume was made up to the mark with methanol to obtain final concentration of 80 μg/ml Valsartan and 30 μg/ml Nifedipine. Absorbance of a sample solution recorded using first order derivative spectroscopy at 248.43 nm (ZCP of Valsartan) and 216.52 nm (ZCP of Nifedipine) for determination of Nifedipine and Valsartan, respectively. The analysis procedure was repeated three times with synthetic mixture.

Method Validation

Method validation was performed following ICH guidelines. The proposed method has been extensively validated in terms of linearity, accuracy and precision, limit of detection and limit of quantification.

Linearity (Calibration curve)

Appropriate volume of aliquot from Valsartan and Nifedipine standard stock solution was transferred to 10 ml volumetric flask. The volume was made up to the mark with methanol to give solution containing 5-25 μg/ml Valsartan and 2-10 μg/ml Nifedipine. All D¹spectra were recorded using above spectrophotometric condition. D¹ absorbance at 248.43 nm and 216.52 nm were recorded for Nifedipine and Valsartan, respectively (n=6).
Calibration curve were constructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves. The linear regression equation of Valsartan was \( y = -0.0035x - 0.0036 \) \((R^2= 0.999)\) and Nifedipine was \( y = -0.0025x - 0.0009 \) \((R^2= 0.9963)\).

**Accuracy**

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the prequantified sample preparation at three different concentration levels 80%, 100% and 120%, taking into consideration percentage purity of added drug sample. The amounts of Valsartan and Nifedipine were estimated by applying obtained values to the respective regression line equations. Each concentration was analyzed 3 times and average recoveries were measured.

**Precision**

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was verified as repeatability, intra-day, inter-day and reproducibility.

The repeatability was evaluated by assaying 6 times of sample solution of 15 μg/ml Valsartan and 6 μg/ml Nifedipine prepared for assay determination without changing the parameter. The intra-day and inter-day precision study of Valsartan and Nifedipine was carried out by estimating different concentration of Valsartan (10, 15, 20 μg/ml) and Nifedipine (4, 6, 8 μg/ml), 3 times on same day and on 3 different day (first, second and third).

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the \(3.3 \times (SD/Slope)\) and \(10 \times (SD/Slope)\) criteria, respectively; where SD is the standard deviation of y-intercept of regression line and S is the slope of the calibration curve.

**Result and Discussion**

A reliable first order derivative spectrophotometric method was developed for simultaneous estimation of Valsartan and Nifedipine in synthetic mixture by UV Spectrophotometric. Beers law was obeyed in concentration range of 5-25 μg/ml for Valsartan and 2-10 μg/ml for Nifedipine at 248.43 nm and 216.52 nm wavelengths, respectively. The correlation coefficient Valsartan and Nifedipine was found to be \(R^2= 0.999\) and 0.9963. The mean % recoveries
were found to be in the range of 99.50-100.50 % and 100.30-100.50 %, respectively. Precision (% RSD) of Valsartan and Nifedipine was found to be 1.57624-1.8563 % and 1.49171-1.87728 %, respectively. The LOD and LOQ were 0.6369 μg/ml and 1.93 μg/ml of Valsartan and 0.1584 μg/ml and 0.48 μg/ml of Nifedipine, respectively. The proposed method was precise, accurate and reproducible and acceptable recovery of the analytes, which can be applied for the analysis of Valsartan and Nifedipine in synthetic mixture.

**Table-1: Regression analysis data and summary of validation parameters for the proposed method.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First-Derivative UV Spectrophotometric</th>
<th>Valsartan</th>
<th>Nifedipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration range (μg/ml)</td>
<td></td>
<td>5-25</td>
<td>2-10</td>
</tr>
<tr>
<td>Regression equation</td>
<td></td>
<td>(y = -0.0035x - 0.00036)</td>
<td>(y = -0.0025x - 0.0009)</td>
</tr>
<tr>
<td>Slope</td>
<td></td>
<td>-0.0035</td>
<td>-0.0025</td>
</tr>
<tr>
<td>Intercept</td>
<td></td>
<td>-0.00036</td>
<td>-0.0009</td>
</tr>
<tr>
<td>Correlation Coefficient ((R^2))</td>
<td></td>
<td>0.9994</td>
<td>0.9963</td>
</tr>
<tr>
<td>Accuracy (% recovery, n=3)</td>
<td></td>
<td>99.50-100.50</td>
<td>100.30-100.50</td>
</tr>
<tr>
<td>Repeatability (%RSD, n=6)</td>
<td></td>
<td>1.7987</td>
<td>1.7915</td>
</tr>
<tr>
<td>Intraday (%RSD, n=3)</td>
<td></td>
<td>1.57624-1.76508</td>
<td>1.49171-1.60309</td>
</tr>
<tr>
<td>Interday (%RSD, n=3)</td>
<td></td>
<td>1.64455-1.8563</td>
<td>1.53538-1.87728</td>
</tr>
<tr>
<td>LOD (μg/ml)</td>
<td></td>
<td>0.6369</td>
<td>0.1584</td>
</tr>
<tr>
<td>LOQ (μg/ml)</td>
<td></td>
<td>1.93</td>
<td>0.48</td>
</tr>
</tbody>
</table>

**Table-2: Recovery data of proposed method.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Level (%)</th>
<th>Test amount (μg/ml)</th>
<th>Spiked STD Amount (μg/ml)</th>
<th>Total Amount Recovered (μg/ml)</th>
<th>%Mean recovery ± RSD. (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valsartan</td>
<td>80</td>
<td>10</td>
<td>8</td>
<td>7.96</td>
<td>99.50 ± 0.18604</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>10</td>
<td>10.10</td>
<td>100.10 ± 1.05155</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>10</td>
<td>12</td>
<td>12.06</td>
<td>100.50 ± 0.89347</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>80</td>
<td>4</td>
<td>3.2</td>
<td>8.04</td>
<td>100.50 ± 1.248</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4</td>
<td>4</td>
<td>10.04</td>
<td>100.40 ± 1.52892</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>4</td>
<td>4.8</td>
<td>12.01</td>
<td>100.30 ± 1.14826</td>
</tr>
</tbody>
</table>

**Table-3: Analysis of Valsartan and Nifedipine by proposed method.**

<table>
<thead>
<tr>
<th>Synthetic Mixture</th>
<th>Label claim (mg)</th>
<th>Mean amount found (mg)</th>
<th>% Label claim ± RSD (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valsartan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nifedipine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>30</td>
<td>80</td>
<td>30.08</td>
</tr>
<tr>
<td>120</td>
<td>4</td>
<td>12</td>
<td>12.01</td>
</tr>
</tbody>
</table>

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Figure 3: UV Spectrum for Valsartan (10 μg/ml) at 248.43 nm and Nifedipine (8 μg/ml) at 216.52 nm in Methanol.

Figure 4: D¹ spectrum of Valsartan (5-25 μg/ml) in Methanol.

Figure 5: Overlaid D¹ spectrum of Nifedipine (2-10 μg/ml) in Methanol.

Figure 6: Overlaid D¹ spectrum of Valsartan (5-25 μg/ml) and Nifedipine (2-10 μg/ml) in Methanol.

Figure 7: Calibration curve of Valsartan at 216.52 nm in Methanol.

\[
\text{Conc (μg/ml)}
\]

\[
\begin{align*}
\text{Abs} & = y = -0.0035x - 0.0036 \\
R^2 & = 0.9994
\end{align*}
\]

Figure-8: calibration curve of Nifedipine at 248.43nm in methanol.
Conclusion

The results of present study indicate that the proposed UV spectroscopic method is simple, rapid, precise and accurate.

The developed UV spectroscopic method was found suitable for determination of Valsartan and Nifedipine in bulk drug and synthetic mixture without any interference from the excipients. Statistical analysis proves that the method is repeatable and selective for the analysis of Valsartan and Nifedipine in combination. It can therefore be concluded that the developed analytical method is precise & accurate and can be use for routine Analysis of both the drug in combination.

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