Determination of Amlodipine and Valsartan in Binary Mixture Using Derivative- Ratio Spectrophotometric, Chemometric and High Performance Liquid Chromatographic-UV Methods

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Abstract
Three methods were developed for simultaneous determination of amlodipine and valsartan without previous separation. The first method depends on first derivative of the ratios spectra (DD1) by measurements of the amplitudes at 234.5 and 247 nm for amlodipine using 30μg/mL of valsartan as a divisor and at 282 and 292 nm for valsartan using 80μg/mL of amlodipine as a divisor. Calibration graphs were established in the range of 10-100 μg/mL and 5-40 μg/mL for amlodipine and valsartan, respectively. The second method describes the use of multivariate spectrophotometric calibration for the simultaneous determination of the analyzed binary mixture, where the resolution is accomplished by using partial least squares (PLS) regression analysis. In the third method, High performance liquid chromatography (HPLC), separation was performed by using reversed phase column and a mobile phase composed of acetonitrile: KH\textsubscript{2}PO\textsubscript{4} (50:50v/v) adjusted to pH 3.5 by phosphoric acid. The proposed methods were validated and results obtained by adopting the three methods were statistically analyzed and compared with those obtained from compendial method.

Keywords: Amlodipine; Derivative-Ratio; HPLC; PLS; Valsartan.

1. Introduction
Amlodipine Besylate, chemically described as (R, S) 3- ethyl-5 -methyl-2-(2-aminoethoxy-methyl) - 4 - (2-chlorophenyl) - 1, 4- dihydro - 6 -methyl-3,5-pyridinedicarboxylate benzene-sulfonate; is a long acting calcium channel blocker (dihydropyridine class) used as anti hypertensive and in the treatment of angina. Like other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall decreasing total peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart muscle [1].

Valsartan, chemically described as N-(1-oxopentyl)-N-[(2'- (1H-tetrazol-5-yl) [1, 1'-biphenyl] -4-yl] methyl]- L-valine is an...
angiotensin II receptor antagonist with particularly high affinity for the type I (AT1) angiotensin receptor. By blocking the action of angiotensin, valsartan dilates blood vessels and reduces blood pressure [2]. Chemical structures of the examined drugs are shown in Fig.1.

Fig. 1. Chemical structures of amlodipine and valsartan.

Literature survey revealed that HPLC [3-6], RP-HPLC [7-9], HPTLC [10, 11], LC-MS [12] LC-MS/MS [13] and UV – spectrophotometric methods [14-16] are reported for the estimation of amlodipine alone or in combination with other anti-hypertensive agents. Methods such as HPLC [6,9,17-20], LC/MS [21-23], capillary electrophoresis [24] and simultaneous UV – spectrophotometric methods [16, 25, 26] are reported for the estimation of valsartan alone or in combination with other agents. Quantitative spectroscopy has been greatly improved by the use of multivariate statistical methods [27-29]. Multivariate calibrations are useful in spectral analysis because of the simultaneous inclusion of multiple spectral intensities which can greatly improve the precision and applicability of quantitative spectral analysis [30]. The aim of the present study is to investigate the utility of derivative ratio spectrophotometry, multivariate methods in addition to a fast and sensitive HPLC technique in the assay of amlodipine and valsartan in pharmaceutical preparation without the necessity of sample pre-treatment. The proposed methods were optimized and validated for this purpose. According to the ICH description, the developed methods were accurate and precise [31].

2. Experimental

2.1. Drug Substances and Dosage Form

2.1.1. Amlodipine and valsartan pure substances were kindly supplied by Novartis Pharma, Egypt.

2.1.2. Exforge® Tablets (Novartis Pharma, Egypt) claim to contain 5 mg amlodipine and 160 mg valsartan, Batch No. Y0004.

2.2. Reagents

Acetonitrile (Fisher Scientific, UK). Potassium dihydrogen phosphate (KH$_2$PO$_4$) (Sigma-Aldrich Chemie, Germany). Orthophosphoric acid (H$_3$PO$_4$) (Sigma-Aldrich Chemie, Germany). Methanol (Sigma-Aldrich Chemie, Germany). Acetonitrile is HPLC grade and all other chemicals are analytical reagent grade.

2.3. Instrumentation

Agilent 1200 series isocratic quaternary pump HPLC instrument connected to 1200 multiple wavelength- UV detector. Separation was performed on 150 x 4.6 mm Zorbax® Extend-C18 column 5µm particle size (Agilent, Germany). Chromatographic peaks were electronically integrated and recorded using Chemstation software (Germany). A Shimadzu (Japan) 1601 PC double-beam UV-visible...
spectrophotometer. PLS was modeled using PLS toolbox 2.0 software under MATLAB® 6.5. pH/mv Meter with double junction glass electrode (Fisher, USA).

2. 4. Procedure
2. 4. 1. Preparation of stock solutions and calibration
2. 4. 1. 1. For derivative ratio method (DD1): amlodipine (1 mg/mL) and valsartan and (0.5 mg/mL) stock solutions in methanol were prepared by dissolving 100 mg of amlodipine and 50 mg of valsartan in 100 mL volumetric flasks. Series of working solutions of both drugs were prepared by the appropriate dilution of the stock solutions with same solvent to reach concentration ranges of 10-100 μg/mL for amlodipine and 5-40 μg/mL for valsartan. According to the theory of the ratio- spectra derivative method, the stored UV absorption spectra of standard solutions of amlodipine were divided wavelength-by-wavelength by a standard spectrum of valsartan (30μg/mL). The first derivative was calculated for the obtained spectra with Δλ= 5. The amplitudes at 234.5 and 247 nm were measured. For valsartan, the stored UV absorption spectra of standard solutions of valsartan were divided wavelength-by-wavelength by a standard spectrum of amlodipine (80 μg/mL). The first derivative was calculated for the obtained spectra with Δλ= 5. The amplitudes at 282 and 292 nm were measured.

2. 4. 1. 2. For multivariate method: In order to obtain the calibration matrix for applying PLS analysis fifteen solutions of each of the pure components (amlodipine and valsartan) were prepared in a concentration range of 40-80 μg/mL and 10-30 μg/mL for amlodipine and valsartan, respectively. These ranges were previously verified to obey Beer's law for each of the studied drugs in methanol. The absorption data in the range of 220-450 nm (digitized every 0.5 nm) of 15 different laboratory prepared mixtures containing amounts of amlodipine and valsartan in different proportions according to the multilevel multifactor design were subjected to partial least squares analysis in order to obtain the calibration matrix. A Validation set composed of 10 different laboratory prepared mixtures containing different concentrations of the two drugs were prepared in order to assess the prediction ability of the proposed PLS method for the analysis of such mixture.

2. 4. 1. 3. For HPLC method: amlodipine and valsartan stock solutions in methanol were prepared by dissolving 10 mg of both drugs in 100 mL volumetric flasks (0.1mg/mL). Series of working solutions of amlodipine and valsartan were prepared by the appropriate dilution of the stock solutions with same solvent to reach the concentration ranges of 5-40 μg/mL for amlodipine and 2.5 -25 μg/mL for valsartan.

Triplicate 20 μL injections were made for each concentration using the following chromatographic conditions: Mobile phase consisting of acetonitrile: KH₂PO₄ (50:50 v/v) adjusted by phosphoric acid to pH 3.5. Detector wavelength: 238 nm for amlodipine and 210 nm for valsartan, flow rate: 1 mL/min, Column temperature: ambient temperature (20-22  °C).

Peak area of each concentration was plotted against the corresponding concentration to obtain the calibration graph.

2. 4. 2. Application to Pharmaceutical Dosage Form
For all methods, five tablets labeled to contain 5 mg amlodipine and 160 mg valsartan were powdered. Amount of powder equivalent to one tablet content was accurately weighed, transferred into 100 mL volumetric flask
containing about 50 mL methanol, sonicated for 10 min then filtered. After filtration a calculated amount of standard amlodipine was added to reach the desired stock concentration, diluted to volume with additional methanol and working solutions were prepared by transferring suitable aliquots of clear filtrate and diluting with appropriate solvent. The assay was completed as under section (calibration). Amlodipine and valsartan concentrations were calculated from the corresponding regression equations.

To study the accuracy of the proposed methods, and to check the interference from excipients present in the dosage form, recovery experiments were carried out by standard addition method in which different amounts of amlodipine and valsartan were added to a known concentration of commercial tablets. The resulting mixtures were analyzed as described under section (calibration).

3. Results and Discussion

DD1 method: As shown in Fig. 2 the zero-order spectra of standard drugs were found to be overlapped making their simultaneous determination difficult, ratio-spectra derivative spectrophotometric method permits the determination of each component in their mixture at the wavelengths corresponding to a maximum or minimum. The main advantage of DD1 method is the chance of easy measurements in correspondence to peaks so it permits the use of the wavelength of highest value of analytical signals (maximum or minimum). Moreover, the presence of a lot of maxima and minima is another advantage by the fact that these wavelengths give an opportunity for the determination of active compounds in presence of other active compounds or excipients which possibly interfere with the analysis. The influence of Δλ for obtaining the first derivative of the ratio spectra as well as, the effect of divisor concentration on the calibration graphs for the proposed mixture was studied in order to select the best factors affecting the determination. Results indicated that Δλ = 5 nm was the most suitable one.

![Absorption spectra of amlodipine (........), 60μg/mL and valsartan (----), 25 μg/mL in methanol.](image-url)
Determination of both drugs was done by dividing the absorption spectra of amlodipine by that of standard solution of valsartan (30 μg/mL) while the absorption spectra of valsartan were divided by that of standard solution of amlodipine (80 μg/mL).

The first derivative of the developed ratio spectra were calculated with Δλ = 5 nm. Fig. 3 shows that amlodipine can be determined by measuring the amplitude at 234.5 and 247 nm. Fig. 4 shows that valsartan can be determined by measuring the amplitude at 282 and 292 nm. The proposed method is applicable over a concentration range of 10-100 μg/mL for amlodipine and 5-40 μg/mL for valsartan. The characteristic parameters and necessary statistical data of the regression equation, limit of quantitation (LOQ), limit of detection (LOD), repeatability and reproducibility data are collected in Table 1.

In order to demonstrate the validity and applicability of the proposed DD1 method, recovery studies were performed by analyzing laboratory prepared mixtures of amlodipine and valsartan with different composition ratio. The validity of the method was further assessed by applying the standard addition technique; results are presented in Table 2.

Multivariate method: PLS method was applied for the determination of amlodipine and valsartan in their binary mixture. Mixtures with different concentrations of both drugs were used as calibration samples to construct the model. The spectra of these mixtures were collected and examined. The selection of the optimum number of factors was a very important pre-construction step because if the number of factors retained was more than required more noise would be added to the data. On the other hand, if the number retained was too small meaningful data that could be necessary for the calibration might be discarded. Different methods can be used to determine the optimum number of factors [32, 33]. In this study, the leave-one-out cross-validation method was used and the root mean square error of calibration (RMSEC) values of different developed models was compared. The model selected was that with the smallest number of factors such that RMSEC for that model was not significantly greater than RMSEC from the model with additional factor.
As the difference between the minimum RMSEC and other RMSEC values became smaller, the probability that each additional factor was significant became smaller. Four factors were found suitable as shown in Fig. 5.

![Fig. 5. RMSEC plot of the cross validation results of the training set as a function of number of principal components used to construct the PLS calibration for amlodipine and valsartan.](image)

The characteristic parameters and necessary statistical data of the regression equation, limit of detection (LOD), repeatability and reproducibility data are collected in Table 1.

Ten laboratory-prepared mixtures containing different ratios of both drugs were subjected to the PLS analysis in order to confirm the suitability of the calibration model for determination of studied drugs in the pharmaceutical sample solutions, where satisfactory results were obtained Table 2.

The validity of the method was further assessed by applying the standard addition technique.

HPLC Method: In order to perform the simultaneous elution of amlodipine and valsartan peaks, different chromatographic conditions were optimized. The composition of the mobile phase was studied by trying acetonitrile and KH₂PO₄ (0.05 M) in different ratios, best peak shape and adequate separation of the two drugs were obtained by a final composition of acetonitrile-KH₂PO₄ (50:50 v/v) adjusted to pH 3.5 by phosphoric acid.

Different flow rates (0.5-1.2 mL/min) were tested good resolution was obtained using 1 mL/min. Four wavelengths were tried (210, 230, 240 and 280 nm); much sensitive detector response was obtained at 238 nm for amlodipine and 210 nm for valsartan.

System suitability parameters were calculated and retention times were found to be 1.67 min for amlodipine and 3.22 min for valsartan.
valsartan, Fig. 6. Resolution and selectivity factors for this system were found to be 2.22 and 2.51 for amlodipine and valsartan, respectively. Tailing and capacity factors were obtained as 1.21 and 0.91 for amlodipine and 1.51 and 1.55 for valsartan. Results obtained from system suitability tests are in agreement with the USP requirements.

### 3.2. Application to Pharmaceutical Dosage form:

The three proposed methods were successfully applied for the simultaneous determination of both drugs in Exforge® tablet without interference of the excipients present and without prior separation. The utility of the three proposed methods was verified by replicate estimations of the pharmaceutical preparation and results obtained are evaluated statistically Table 2.

A statistical comparison of the results obtained by the proposed methods and the reported HPLC method [6] is shown in Table 2. The values of the calculated t and F are less than the tabulated ones, which reveals that there is no significant difference with respect to accuracy and precision between the proposed and compendial method.

**Table 1.** Spectral Data for the Determination of Amlodipine and Valsartan by the Proposed Method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Amlodipine</th>
<th>Valsartan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DD1</td>
<td>PLS</td>
</tr>
<tr>
<td>Linearity range (μg/mL)</td>
<td>10-100</td>
<td>0.012</td>
</tr>
<tr>
<td>Slope</td>
<td>0.012</td>
<td>0.013</td>
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<tr>
<td>Intercept</td>
<td>0.065</td>
<td>0.0994</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.9991</td>
<td>0.9991</td>
</tr>
<tr>
<td>Repeatability (R.S.D %)</td>
<td>0.36-0.55</td>
<td>0.19-0.90</td>
</tr>
<tr>
<td>Reproducibility (R.S.D %)</td>
<td>0.36-0.55</td>
<td>0.19-0.90</td>
</tr>
<tr>
<td>LOQ (μg/mL)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>LOD (μg/mL)</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*LOQ and LOD were determined practically*
Table 2. Determination of Amlodipine and Valsartan in Authentic, laboratory, Prepared Mixtures and Pharmaceutical Dosage Form Using DD1, PLS, HPLC and Compendial Method [6].

<table>
<thead>
<tr>
<th>Standard Solution</th>
<th>Compendial</th>
<th>DD1  (\lambda 234.5)</th>
<th>DD1  (\lambda 247)</th>
<th>PLS</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Authentic amlodipine (\lambda 234.5)</td>
<td>99.30±1.77</td>
<td>100.62±1.69</td>
<td>99.93±1.21</td>
<td>100.02±1.73</td>
<td>99.95±1.71</td>
</tr>
<tr>
<td>N</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>t</td>
<td>-</td>
<td>1.18(1.94)*</td>
<td>0.61(1.94)*</td>
<td>0.81(1.73)*</td>
<td>0.62(1.83)*</td>
</tr>
<tr>
<td>F</td>
<td>-</td>
<td>1.52(5.19)*</td>
<td>2.50(5.19)*</td>
<td>1.04(4.62)*</td>
<td>1.07(4.95)*</td>
</tr>
<tr>
<td>Authentic valsartan (\lambda 234.5)</td>
<td>100.01±1.11</td>
<td>100.62±1.69</td>
<td>99.93±1.21</td>
<td>100.04±1.24</td>
<td>99.55±1.67</td>
</tr>
<tr>
<td>N</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>t</td>
<td>-</td>
<td>0.12(1.86)*</td>
<td>0.27(1.86)*</td>
<td>1.07(1.73)*</td>
<td>0.67(1.83)*</td>
</tr>
<tr>
<td>F</td>
<td>-</td>
<td>1.19(5.19)*</td>
<td>1.17(5.19)*</td>
<td>2.04(4.62)*</td>
<td>2.26(4.39)*</td>
</tr>
<tr>
<td>Amlodipine in laboratory prepared mix.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Valsartan in laboratory prepared mix.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amlodipine in Exforge® tablet</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Valsartan in Exforge® tablet</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard addition technique for amlodipine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard addition technique for valsartan</td>
<td>-</td>
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<td>-</td>
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</tbody>
</table>

* Theoretical values at 95% confidence limit

4. Conclusions

The DD1, Multivariate (PLS) and HPLC methods enable the quantitation of amlodipine and valsartan binary mixture with good accuracy and precision, either in laboratory prepared samples or in pharmaceutical dosage form. The proposed HPLC method has the advantage of being faster than the two reported HPLC methods used for the simultaneous estimation of amlodipine and valsartan in their binary mixture. All the proposed procedures are rapid, precise and direct. The good recoveries obtained in all cases as well as the reliable agreement with the compendial method proved that the proposed methods could be applied efficiently for determination of amlodipine and valsartan binary mixture with quite satisfactory precision and could be easily used in quality control laboratory for their analysis.

References