HPLC method development for the simultaneous analysis of amlodipine and valsartan in combined dosage forms and in vitro dissolution studies

Mustafa Çelebier¹, Mustafa Sinan Kaynak²,³, Sacide Altınöz¹*, Selma Sahin²

¹Department of Analytical Chemistry, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey, ²Department of Pharmaceutical Technology, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey, ³Department of Pharmaceutical Technology, Faculty of Pharmacy, İnönü University, Malatya, Turkey

A simple, rapid and reproducible HPLC method was developed for the simultaneous determination of amlodipine and valsartan in their combined dosage forms, and for drug dissolution studies. A C₁₈ column (ODS 2, 10 µm, 200 x 4.6 mm) and a mobile phase of phosphate buffer (pH 3.6, 0.01 mol L⁻¹):acetonitrile:methanol (46:44:10 v/v/v) mixture were used for separation and quantification. Analyses were run at a flow-rate of 1 mL min⁻¹ and at ambient temperature. The injection volume was 20 µL and the ultraviolet detector was set at 240 nm. Under these conditions, amlodipine and valsartan were eluted at 7.1 min and 3.4 min, respectively. Total run time was shorter than 9 min. The developed method was validated according to the literature and found to be linear within the range 0.1 – 50 µg mL⁻¹ for amlodipine, and 0.05 – 50 µg mL⁻¹ for valsartan. The developed method was applied successfully for quality control assay of amlodipine and valsartan in their combination drug product and in vitro dissolution studies.


INTRODUCTION

Amlodipine (as besylate, mesylate or maleate) is a long-acting calcium channel blocker used as an anti-hypertensive agent and in the treatment of angina (Haria, Wagstaff, 1995; Murdoch, Heel, 1991). Akin to other calcium channel blockers, amlodipine (AMD) acts by relaxing the smooth muscle in the arterial wall, decreasing peripheral resistance and hence reducing blood pressure. In angina it increases blood flow to the heart muscle. Valsartan (VAL) is an angiotensin II receptor antagonist, acting on the AT₁ subtype (Markham, Goa, 1997; Remuzzi et al., 1998). VAL keeps blood vessels from narrowing, which lowers blood pressure and improves blood flow. VAL is used to treat hypertension. It is sometimes given together with other blood pressure medications. It was noted that

*Correspondence: S. Altınöz. Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry. 06100 - Ankara- TURKEY. E-mail: saltinoz@hacettepe.edu.tr
the combination of an angiotensin receptor blocker and a calcium channel blocker constitutes an effective option for patients with hypertension (Boutouvrie et al., 2008; Plosker, Robinson, 2008).

In the literature, several HPLC methods have been described for the determination of AMD in pharmaceutical preparations when used alone (Avadhanulu et al., 1996; Basavaiah et al., 2005; Fang et al., 2007; Kamat, Chaturvedi, 2005; Li et al., 2006; Patki et al., 1994; Shang, Shang, 1996; Ustun, Atay, 2006; Xiao, Wang, 2007) or in combination with other compounds such as atorvastatin, atenolol, benazepril, losartan, ramipril and enarapril maleate (Aravindraju et al., 2006; Barman et al., 2007; Dhanda, Shetkar, 1999; Dongre et al., 2008; Freddy, Chaudhari, 2005; Gowri et al., 2002; Halkar et al., 1998; Luksa et al., 1997; Mohammadi et al., 2007; Rajeswari et al., 2006; Rajkondawar, 2006; Rao et al., 2002; Sankar et al., 1997; Shah et al., 2006; Si- vakumar et al., 2007; Zarapkar, Kanyawar, 2002; Zarapkar et al., 1997). HPLC methods for the determination of VAL in pharmaceutical preparations have also been described (Carlucci et al., 2000; Doshi et al., 2008; Han et al., 2007; Jin et al., 2006; Satana et al., 2001; Tatar, Saglik, 2002).

There is an HPLC method described for simultaneous determination of AMD and VAL (Figure 1) in pharmaceutical preparations (Chitlange et al., 2008). In addition, there is another method reported for simultaneous determination of these drugs for liver perfusion studies (Celebier et al., 2008). However, both methods are not developed for dissolution studies while the dissolution profile of AMD and VAL from the combination drug product has not hitherto been reported in the literature. In order to elucidate the dissolution profiles of AMD and VAL, a validated HPLC method is required for simultaneous determination of these drugs in dissolution matrix.

Therefore, the aim of this study was to develop and validate an efficient HPLC method for simultaneous determination of AMD and VAL and to introduce the dissolution profiles of these drugs. Moreover, this new method could also be used for the routine analysis of AMD and VAL in pharmaceutical dosage forms, provided it is completely validated and rapid. The newly developed method was totally different both in terms of methodology and aim in comparison to previously reported methods in the literature.

The method was validated according to guidelines (Thompson et al., 2002) and applied for assay of AMD and VAL from their combination tablet dosage form. Also, in vitro dissolution of AMD and VAL containing tablets were performed to validate the suitability of the proposed method.

MATERIAL AND METHODS

Chemicals

Standard AMD and VAL were supplied by Refik Saydam National Public Health Agency. Methanol and acetonitrile were of HPLC grade from Merck (Darmstadt, Germany) and all other reagents were analytical grade. Water obtained from the Milli-Q water system (Barnstead, USA) was used for the preparation of buffer and other aqueous solutions. Commercially available tablets (Exforge®) containing 5 mg of AMD (as equal to 6.94 mg of AMD besylate) and 160 mg of VAL were purchased from a local pharmacy.

Solutions

Standard stock solutions

Standard stock solutions of AMD and VAL were prepared separately by dissolving 50 µg of AMD besylate and 50 mg of VAL in 50 mL methanol, and stored protected from daylight at 4°C until use. These solutions were prepared freshly every week, during method development and application period.

Phosphate buffer solution

1.74 g of K2HPO4 was dissolved in 1 L of deionized water and pH was adjusted to 3.6 with orthophosphoric acid.

Calibration standards

Calibration standards for AMD (as besylate salt) and VAL (1.25, 2.0, 3.0, 4.0, 10.0, 20.0, 30.0, 40 and 50.0 µg mL⁻¹) were daily prepared from standard stock solutions by appropriate dilution processes using mobile phase.

Instrumentation

The HPLC system consisted of a Waters 2690 Separation Module equipped with a Waters 2996 Photodiode
Array Detector (Waters, USA). A C₁₈ column (Waters Spherisorp (PSS832514) ODS 2, 10 µm, 200 x 4.6 mm; USA) was used for separation and quantification. The mobile phase consisted of phosphate buffer (pH 3.6 , 0.01 mol L⁻¹); acetonitrile : methanol (46:44:10 v/v/v) and was filtered through a 0.45 µm filter and degassed before use. The injection volume was 20 µL and the ultraviolet detector was set at 240 nm. Analyses were run at a flow-rate of 1 mL min⁻¹ at an ambient temperature (25 °C). The peak areas were integrated automatically by using Empower® software. Under these conditions, VAL and AMD were eluted at 3.4 min and 7.1 min, respectively. Total run time was shorter than 9 min.

**In vitro dissolution studies**

*In vitro* dissolution of six tablets containing AMD and VAL was performed using phosphate buffer (pH 6.8) as the dissolution media at 50 rpm using an USP Apparatus II. The dissolution study was carried out in a 900 mL volume of phosphate buffer at 37 °C (± 0.5) using the paddle method. One mL of sample was withdrawn and replaced with fresh dissolution medium at the time intervals of 0, 5, 10, 15, 20, 30, 45, and 60 minutes. The sample (250 µL) was diluted with mobile phase (750 µL) and then filtered through a 0.45 µm membrane filter. The concentrations of AMD and VAL in samples were determined by the proposed HPLC method. According to the FDA Guidance (Qui, Xu 2007) no less than 85% of the active ingredients of the labeled claim should be dissolved within 30 minutes.

**Analysis of dosage forms**

Ten tablets were weighed to determine their mean weight, and finely powdered in a mortar. An amount of powdered mass equivalent to one tablet was accurately weighed and transferred to a 100 mL volumetric flask. A 50 mL volume of methanol was added then sonicated for 15 min to ensure complete extraction of the drugs. The flask was then diluted to volume with methanol. An aliquot from this solution was centrifuged at 5000 rpm for 10 min. Clear supernatant was transferred to another flask. Appropriate aliquots from the sample solution were suitably diluted with mobile phase so as to achieve concentrations of 1.25 µg mL⁻¹ and 40.0 µg mL⁻¹ for AMD and VAL, respectively. Twenty µL of this solution was injected into the HPLC system after filtration through a 0.45 µm membrane filter.

**Synthetic tablet solutions**

For preparation of the synthetic tablets, common inactive ingredients (microcrystalline cellulose, povidone, talc, magnesium stearate, starch, iron oxide) and standard AMD besylate (6.94 mg) and VAL (160 mg) at amounts equivalent to one tablet were weighed and transferred to a 50 mL volumetric flask. The same procedure described in Section 2.5 was then applied to prepare the synthetic tablet solutions containing 1.25 µg mL⁻¹ of AMD and 40.0 µg mL⁻¹ of VAL, respectively. In order to prepare blank tablet solutions, the synthetic tablet solutions were prepared in the absence of AMD and VAL.

**RESULTS AND DISCUSSION**

**Method optimization**

Due to the relatively apolar properties of AMD and VAL, a reversed phase HPLC system was used to analyze both compounds with a sufficient separation and fine peak shapes within a short analysis time. Therefore, all the experiments were carried out on a C₁₈ column (Waters Spherisorp ODS 2, 10 µm, 200 x 4.6 mm) by trying various mobile phase conditions systematically. After the initial experiments, the optimum conditions were found to be the mobile phase of phosphate buffer (pH 3.6, 0.01 mol L⁻¹): acetonitrile: methanol (46:44:10 v/v/v) mixture pumped at 1 mL min⁻¹ flow rate and 240 nm UV detection wavelength. Under the optimum conditions, VAL and AMD were eluted at 3.4 min and 7.1 min, respectively.

**System suitability**

A suitability test was applied to the chromatograms taken under optimum conditions to check various parameters such as column efficiency (plates), peak tailing, retention factor, and resolution. Suitable resolution (>2) and column efficiency (>1500 for both compounds) were achieved for the analysis method. The peak symmetries for both compounds were <1.2 , whereas the capacity factors were >1.5 . The analysis time was shorter than 9 minutes.

**Method validation**

The proposed method was validated as to linearity range, sensitivity, repeatability, precision, accuracy, and specificity according to the literature (Ermer, 2001; Ermer, Ploss, 2005; Taverniers et al., 2004a; Taverniers et al., 2004b; Thompson et al., 2006).

**Linearity range**

The solutions of AMD and VAL at different con-
centrations were analyzed. According to the observations, the peak shapes worsened after 50 µg ml\(^{-1}\) for both compounds. The graph of the peak area against concentration proved linear up to 50 µg mL\(^{-1}\). Therefore, eight different concentrations of AMD and VAL within the linear range were analyzed. This process was repeated six times to calculate the regression equations. The results are given in Table I.

**Limit of detection (LOD) and limit of quantitation (LOQ)**

The signal-to-noise ratios of 3:1 and 10:1 were taken as LOD and LOQ, respectively (Taverniers et al., 2004a; Taverniers et al., 2004b). The values of LOD and LOQ for both AMD and VAL are given in Table I.

**Repeatability**

The repeatability of the developed HPLC method was calculated by 10 consecutive injections made with standard solutions containing 20.00 µg mL\(^{-1}\) of AMD and VAL. The results were evaluated by considering retention time and peak area. Neither the peak areas, nor the retention time changed more than 2 %, indicating that the method was highly repeatable.

**Precision and accuracy**

Three different concentrations of standard AMD and VAL solutions (within the linear range) were analyzed on three consecutive days (inter-day precision) and three times within the same day (intra-day precision). The obtained values for relative standard deviation (RSD) and Bias of intra- and inter-day studies indicated that the precision and accuracy of the method were satisfactory (Taverniers, et al. 2004a; Taverniers, et al. 2004b). The results are summarized in Table II.

**Recovery**

To verify the accuracy of the method, recovery experiments were performed. The synthetic tablets and synthetic tablet solutions were analyzed by the developed method. The results were 98.5 ± 0.8 and 99.6 ± 0.6 (mean ± SE where SE is standard error) for AMD and VAL, respectively.

**Selectivity**

The chromatograms obtained from standard solutions were identical to those obtained from tablet solutions containing an equivalent concentration of AMD and VAL.

### Table I - Linearity data of the developed method (n=6)

<table>
<thead>
<tr>
<th></th>
<th>AMD besylate</th>
<th>VAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression equation*</td>
<td>(y = 39604 x + 2682)</td>
<td>(y = 42497 x + 12845)</td>
</tr>
<tr>
<td>Standard error of intercept</td>
<td>308</td>
<td>99</td>
</tr>
<tr>
<td>Standard error of slope</td>
<td>758</td>
<td>761</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9992</td>
<td>0.9991</td>
</tr>
<tr>
<td>Linearity range (µg mL(^{-1}))</td>
<td>0.1 - 50</td>
<td>0.05 - 50</td>
</tr>
<tr>
<td>Number of data points</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>LOD (µg mL(^{-1}))</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>LOQ (µg mL(^{-1}))</td>
<td>0.1</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*where \(y\) is peak area and \(x\) is concentration in µg mL\(^{-1}\)

### Table II - Precision and accuracy of the developed method

<table>
<thead>
<tr>
<th></th>
<th>Intra-day</th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added (mg mL(^{-1}))</td>
<td>Found (mg mL(^{-1}))</td>
</tr>
<tr>
<td>Amlodipine bes.</td>
<td>3</td>
<td>2.95 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20.38 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>39.28 ± 0.30</td>
</tr>
<tr>
<td>Valsartan</td>
<td>3</td>
<td>2.86 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20.72 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>39.84 ± 0.14</td>
</tr>
</tbody>
</table>

Found: mean ± standard error (n=6); RSD: Relative standard deviation; Bias: \([(\text{Found} - \text{Added})/\text{Added}] \times 100)
The representative chromatograms (Figure 2) show no other peaks in retention time of AMD and VAL and retention times did not change. In addition, when the solution prepared from the blank tablet was injected into the HPLC system, no co-eluting peaks were obtained at the retention time of AMD and VAL.

Based on these results, the proposed methods can be considered selective.

**Tablet analysis results**

The assay results of AMD and VAL in tablet dosage form were comparable with the label value claimed (5 mg AMD and 160 mg VAL (Exforge®). The results presented in Table III indicate the suitability of the method for routine analysis of AMD and VAL from their combination drug products.

**TABLE III** - Assay of AMD and VAL from its tablet dosage form

<table>
<thead>
<tr>
<th>Tablet Solutions</th>
<th>Exforge® Tablets (160 mg VAL and 5 mg AMD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VAL</td>
</tr>
<tr>
<td>1</td>
<td>151.93</td>
</tr>
<tr>
<td>2</td>
<td>156.71</td>
</tr>
<tr>
<td>3</td>
<td>160.34</td>
</tr>
<tr>
<td>4</td>
<td>152.47</td>
</tr>
<tr>
<td>5</td>
<td>152.32</td>
</tr>
<tr>
<td>6</td>
<td>153.58</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>154.56 ± 1.35</td>
</tr>
<tr>
<td>RSD</td>
<td>2.15</td>
</tr>
<tr>
<td>Bias</td>
<td>3.40</td>
</tr>
</tbody>
</table>

**In vitro dissolution studies**

The average percentage drugs released within 60 min as detected by the proposed HPLC method after *in vitro* dissolution of tablets containing combination drug product are depicted in Figure 3. VAL was completely released within 10 min, whereas 85% of AMD was released within 15 min. The dissolution pattern complies with the FDA Guidance (Qui and Xu 2007) standards, indicating suitability of the proposed method for the dissolution study of the two drugs.

**CONCLUSION**

It is a well known that validation procedure is an integral part of the analytical method development. Therefore, the developed method was validated according to the guidelines. Based on the results, it can be concluded that there is no other co-eluting peak with the main peaks and that the method is specific for estimation of AMD and VAL. The proposed method has linear response in the stated range and is accurate and precise. To our knowledge, the developed HPLC method is the first reported method for simultaneous determination of AMD and VAL from their combination drug product. Taken together, these results clearly showed that this method can be used for assay of AMD and VAL in their combination drug product. The developed method can also be conveniently adopted for dissolution testing of tablets containing AMD and VAL.

**REFERENCES**

Reverse phase HPLC determination of Amlodipine besylate in drug and its pharmaceutical dosage forms. *Indian Drugs*, v.33, p.36-40, 1996.


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