DEVELOPMENT OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE SIMULTANEOUS ANALYSIS OF AMLODIPINE AND VALSARTAN IN COMBINED DOSAGE FORM AND *IN VITRO* DISSOLUTION STUDIED

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ABSTRACT

Objective: A simple, rapid, and reproducible high-performance liquid chromatography method was developed for the simultaneous determination of amlodipine and valsartan in their combined dosage forms and for drug dissolution studies.

Methods: A C_{18} column (Zorbax Eclipse XDB-C18, 5 μm, 2.1 mm × 150 mm) and a mobile phase of water:acetonitrile:trifluoroacetic acid (55:45:0.1 v/v/v) mixture were used for separation and quantification. Analyses were run at a flow rate of 0.4 mL/min and at ambient temperature. The injection volume was 5 μL and the ultraviolet detector was set at 265 nm. The method was validated as per ICH guidelines.

Results: Under these conditions, amlodipine and valsartan were eluted at 1.64 min and 4.08 min, respectively. Total run time was shorter than 7 min. The results were 99.6 ± 0.6 and 98.5 ± 0.8 for amlodipine and valsartan, respectively. Valsartan was released within 15 min (98.32%) and amlodipine was also released within 30 min (96.16%) both at a pH of 6.8.

Conclusion: The developed method was applied successfully for quality control assay of amlodipine and valsartan in their combination drug product and *in vitro* dissolution studies.

Keywords: Amlodipine besylate, Valsartan, High-performance liquid chromatography, Validation, Dissolution study.

INTRODUCTION

The "dissolution" test for solid dosage forms is one of the main pharmacotechnological tests used in the development, as well as during the life cycle of the finished medicinal product. The main attention is paid attention to the study of the kinetics of the dissolution of the active substance (or substances), which is key in the selection and evaluation of the composition of the preparation in the development process. Kinetics or dissolution profiles are the dependence on the concentration of the active substance on the release time. The study of dissolution profiles is also used to prove the *in vitro* equivalence of generic drugs (according to the "Bioveyver" procedure), as well as when introducing registry changes (introducing an alternative substance, changing the technology and composition of the drug, introducing a new dosage, etc.). For two or more component products, it is important to develop a method for the simultaneous determination of the active components since it is not only convenient in the routine process and significantly reduces the resources but also makes it possible to estimate the release kinetics of the active components from the same unit (tablets, capsules). This is extremely important in the study of laboratory series in the formulation and *in vitro* kinetics studies for a product series for which bioequivalence (*in vivo*) is planned. However, such a task is rather complicated, since active components tend to have different physicochemical properties (solubility, chromatographic response, etc.) and are the part of the drug at various concentrations. Chromatographic techniques are most suitable for such purposes since they allow the components to be separated between themselves and the components of placebo.

There is an high-performance liquid chromatography (HPLC) method described for simultaneous determination of amlodipine and valsartan (Fig. 1) in pharmaceutical preparations. In addition, there is another method reported for simultaneous determination of these drugs for liver perfusion studies [1-3]. However, both methods are not developed for dissolution studies while the dissolution profile of amlodipine and valsartan from the combination drug product has not hitherto been reported in the literature. To elucidate the dissolution profiles, amlodipine and valsartan, 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 amlodipine and valsartan and to introduce the dissolution profiles of these drugs. Moreover, this new method could also be used for the routine analysis of amlodipine and valsartan in pharmaceutical dosage forms provided it is completely validated and rapid.

The method was validated according to guidelines and applied for the assay of amlodipine and valsartan from their combination tablet dosage form. Furthermore, *in vitro* dissolution of amlodipine and valsartan containing tablets was performed to validate the suitability of the proposed method.

METHODS

Chemicals
Standard amlodipine and valsartan were supplied by Refik Saydam National Public Health Agency.

Trifluoroacetic acid 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 (company Farmak, Kyiv City, Ukraine)

containing 10 mg of amlodipine and 160 mg of valsartan were purchased from a local pharmacy.

Solutions

Standard stock solutions

Standard stock solutions of amlodipine and valsartan were prepared separately by dissolving 50 μg of amlodipine besylate and 50 mg of valsartan in 50 mL acetonitrile and water (1:1). These solutions were prepared freshly every week, during method development and application period.

Phosphate buffer solution

1.74 g of K_2HPO_4 was dissolved in 1 L of deionized water and pH was adjusted to 3.6 with orthophosphoric acid.

Calibration standards

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

Instrumentation

The HPLC system consisted of Agilent 1200. A C18 column (Zorbax Eclipse XDB-C18, 5 μm, 2.1 mm × 150 mm) was used for separation and quantification. The mobile phase consisted of water:acetonitrile:trifluoroacetic acid (55:45:0.1 v/v/v) and was filtered through a 0.45 μm filter and degassed before use. The injection volume was 5 μL and the ultraviolet detector was set at 265 nm. Analyses were run at a flow rate of 0.4 mL/min at an ambient temperature (25°C). The peak areas were integrated automatically using Empower® software. Under these conditions, amlodipine and valsartan were eluted at 1.64 min and 4.08 min, respectively. Total run time was shorter than 7 min.

In vitro dissolution studies

In vitro dissolution of six tablets containing amlodipine and valsartan was performed using phosphate buffer (pH 1.2; 4.5; 6.8) as the dissolution media at 50 rpm using an USP Apparatus II. The dissolution study was conducted in a 900 mL volume of phosphate buffer at 37°C (±0.5) using the paddle method. 1 mL of sample was withdrawn and replaced with fresh dissolution medium at the time intervals of 5, 10, 15, 20, 30, 45, 60, 90, and 120 min. The concentrations of amlodipine and valsartan in samples were determined by the proposed HPLC method.

According to the FDA guidance (Qui, Xu 2007), not less than 85% of the active ingredients of the labeled claim should be dissolved within 30 min.

RESULTS AND DISCUSSION

Method optimization

Due to the relative apolar properties of amlodipine and valsartan, 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 (Zorbax Eclipse XDB-C18, 5 μm, 2.1 mm × 150 mm) by trying various mobile phase conditions systematically [4-9]. After the initial experiments, the optimum conditions were found to be the mobile phase of water:acetonitrile:trifluoroacetic acid (55:45:0.1 v/v/v) mixture pumped at 0.4 mL/min flow rate and 265 nm ultraviolet detection wavelength. Under the optimum conditions, amlodipine and valsartan were eluted at 1.64 min and 4.08 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 7 min.

Method validation

The proposed method was validated as to linearity range, sensitivity, repeatability, precision, accuracy, and specificity according to the ICH guidelines [10].

Linearity range

The solutions of amlodipine and valsartan at different concentrations were analyzed. According to the observations, the peak shapes worsened after 50 μg/mL for both compounds. The graph of the peak area against concentration proved linear up to 50 μg/mL. Therefore, eight different concentrations of amlodipine and valsartan within the linear range were analyzed. This process was repeated to calculate the regression equations. The results are given in Figs. 2-7.
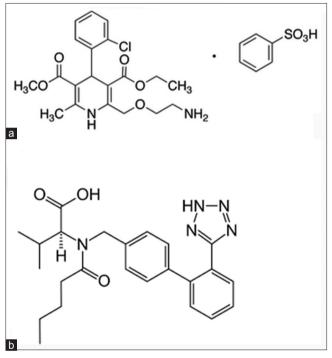
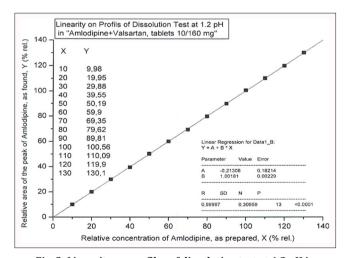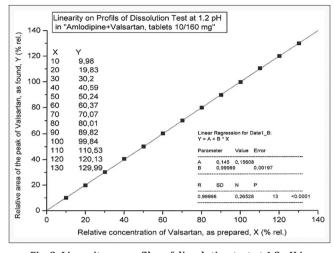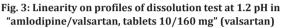**Fig. 1: The chemical structures of amlodipine besylate (a) and valsartan (b)**

Fig. 2: Linearity on profiles of dissolution test at 1.2 pH in "amlodipine/valsartan, tablets 10/160 mg" (amlodipine)
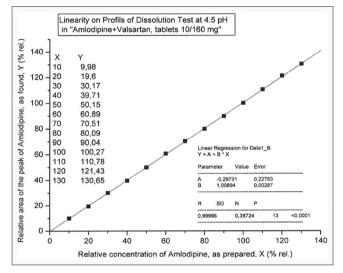
Fig. 3: Linearity on profiles of dissolution test at 1.2 pH in "amlodipine/valsartan, tablets 10/160 mg" (valsartan)

Fig. 4: Linearity on profiles of dissolution test at 4.5 pH in "amlodipine/valsartan, tablets 10/160 mg" (amlodipine)

Fig. 5: Linearity on profiles of dissolution test at 4.5 pH in "amlodipine/valsartan, tablets 10/160 mg" (valsartan)

Fig. 6: Linearity on profiles of dissolution test at 6.8 pH in "amlodipine/valsartan, tablets 10/160 mg" (amlodipine)

Repeatability

The repeatability of the developed HPLC method was calculated by 10 consecutive injections made with standard solutions containing 20.00 μg/mL of amlodipine and valsartan. 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 amlodipine and valsartan solutions (within the linear range) were analyzed on three consecutive days (interday precision) and 3 times within the same day (intraday precision). The obtained values for relative standard deviation and bias of intra- and inter-day studies indicated that the precision and accuracy of the method were satisfactory. The results are summarized in Table 1.

Recovery

To verify the accuracy of the method, recovery experiments were performed. The results were 99.6 ± 0.6 and 98.5 ± 0.8 (mean ± SE where SE is standard error) for amlodipine and valsartan, respectively.

Selectivity

The chromatograms obtained from standard solutions were identical to those obtained from tablet solutions containing an equivalent concentration of amlodipine and valsartan.
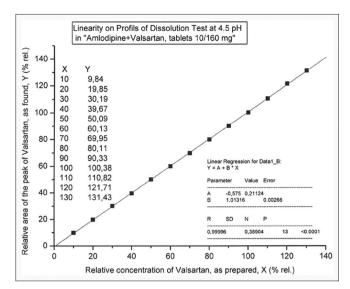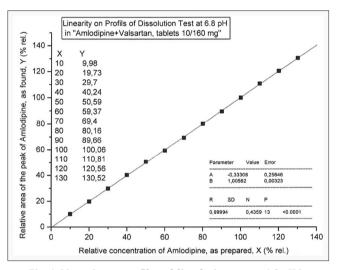
The representative chromatograms (Fig. 8) show no other peaks in retention time of amlodipine and valsartan and retention times did not change. In addition, when the solution prepared from the blank tablet was injected into the HPLC system, no coeluting peaks were obtained at the retention time of amlodipine and valsartan.

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

Tablet analysis results

The assay results of amlodipine and valsartan in tablet dosage form were comparable with the label value claimed (10 mg amlodipine and 160 mg valsartan [Farmak]). The results presented in Table 2 indicate the suitability of the method for routine analysis of amlodipine and valsartan from their combination drug products.

In vitro dissolution studies

The average percentage drugs released as detected by the proposed HPLC method after *in vitro* dissolution of tablets containing combination drug product are depicted in Figs. 9-14.

Valsartan was released within 15 min (98.32%) at 6.8 pH; amlodipine was released within 30 min (96.16%) at 6.8 pH. The

dissolution pattern complies with the FDA standards, indicating suitability of the proposed method for the dissolution study of the two drugs.

CONCLUSION

It is 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 coeluting peak with the main peaks and that the method is specific for the estimation of amlodipine and valsartan. The proposed method has linear response in the stated range and is accurate and precise. To the best of our knowledge, the developed HPLC method is the first reported method for simultaneous determination of amlodipine and valsartan from their combination drug product. Taken together, these results clearly showed that this method can be used for the assay of amlodipine and valsartan in
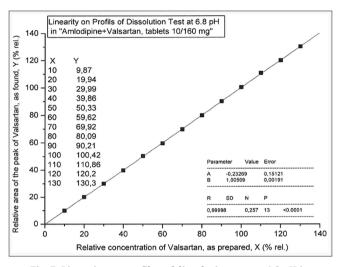

Fig. 7: Linearity on profiles of dissolution test at 6.8 pH in "amlodipine/valsartan, tablets 10/160 mg" (valsartan)
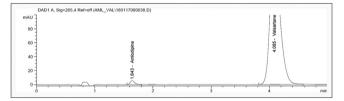

Fig. 8: Representative chromatograms obtained from standard and tablet solutions of amlodipine and valsartan
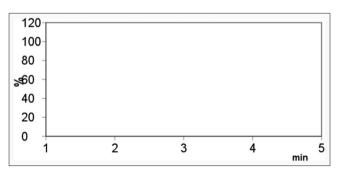

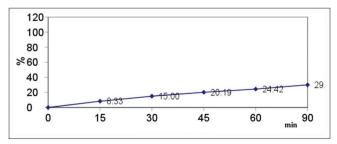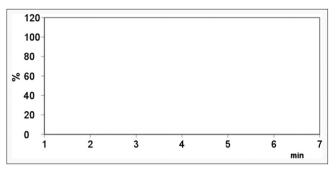Fig. 9: *In vitro* dissolution profiles of amlodipine at 1.2 pH

Fig. 10: *In vitro* dissolution profiles of valsartan at 1.2 pH

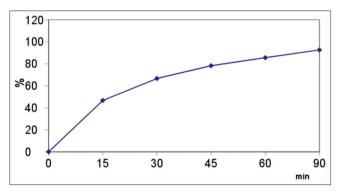Fig. 11: *In vitro* dissolution profiles of amlodipine at 4.5 pH

Fig. 12: *In vitro* dissolution profiles of valsartan at 4.5 pH
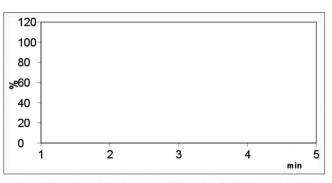

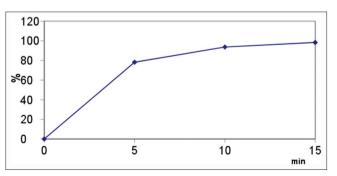Fig. 13: *In vitro* dissolution profiles of amlodipine at 6.8 pH

Fig. 14: *In vitro* dissolution profiles of valsartan at 6.8 pH

Table 1: Precision and accuracy of the developed method

API	Intraday				Interday		
	Added (mg/mL)	Found (mg/mL)	Precision RSD %	Accuracy Bias %	Found (mg/mL)	Precision RSD %	Accuracy Bias %
Amlodipine besylate	3	2.86±0.03	2.1	−4.7	2.71±0.03	3.6	−6.1
	20	20.72±0.18	2.1	4.1	20.58±0.08	0.9	4.2
	40	39.84±0.14	0.9	−1.6	40.03±0.27	1.7	−2.0
Valsartan	3	2.95±0.02	1.5	−3.3	2.86±0.02	2.1	−4.7
	20	20.38±0.22	2.6	2.8	20.83±0.08	0.9	5.4
	40	39.28±0.30	1.9	−3.2	40.24±0.27	1.6	−1.5

Found: Mean±standard error (n=6), RSD: Relative standard deviation, Bias: [(found - added)/added] ×100

Table 2: Assay of amlodipine and valsartan from its tablet dosage form

Tablet solutions	Farmak tablets (160 mg valsartan and 10 mg amlodipine)	
	Amlodipine	Valsartan
1	9.74	151.93
2	9.81	156.71
3	10.07	160.34
4	9.74	152.47
5	9.74	152.32
6	9.80	153.58
Mean±SE	9.82±0.05	154.56±1.35
RSD	2.61	2.15

their combination drug product. The developed method can also be conveniently adopted for dissolution testing of tablets containing amlodipine and valsartan.

AUTHORS CONTRIBUTION

Olga Yuryeva conceived and designed the experiments.

Yuliya Kondratova prepared the samples and performed the experiments.

Liliya Logoyda, Yuliya Kondratova, Olga Yuryeva worked together on the development of the ideas presented in this paper, and contributed to the data analysis and manuscript writing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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