Determination of *in vitro* Dissolution Profiles of Amlodipine Besylate and Olmesartan Medoxomil Using a New "HPLC Method"

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The aim of this study was to develop an HPLC method for simultaneous determination of these active compounds and to apply this method to determine the dissolution of AML and OLM from a commercially available tablet. Valsartan (VAL) was used as an internal standard (IS). Separation of AML, OLM and VAL was performed using Phenomenex C_{18} column (Luna 5 µ, 100A, 250x4.6 mm; California, USA) protected with a Phenomenex C_{18} guard column (4.0x3.0 mm; California, USA). The chromatographic separation operated isocratically at room temperature using a mobile phase consisted of phosphate buffer (pH 4.0, 0.04 mol/L):methanol:acetonitrile (40:45:15, v/v/v) delivered at a flow rate of 0.8 mL/min and injection volume was 10 µL. The diode array detector was set at 234 nm and 205 nm wavelengths for the quantification of AML and OLM respectively. In vitro dissolution studies revealed that 85% of the labeled amounts of AML and OLM were released within 25 min from their fixed combination tablet dosage form. The developed HPLC method was validated according to the ICH guidelines and it is proposed for dissolution studies of the combination dosage forms of these compounds.

Keywords: Amlodipine besylate, dissolution, HPLC method, Olmesartan medoxomil

Drug absorption from a solid dosage form (e.g. tablet, capsule) after oral administration depends on three different factors. These are the release of the drug substance from the drug product, the dissolution or solubilization of the drug under physiological conditions, and the permeability across the gastrointestinal tract. Since the first two steps are critical , *in vitro* drug dissolution study results are useful to predict the *in vivo* performance of the drug. The dissolution test is intended to measure the time required for a given drug in an oral solid dosage form to go into solution under specified set of conditions. It is used to assess the batch-to-batch quality of a drug product, guide to development of a new formulation, ensure continuing product quality and support the bioavailability of a new product, bioequivalence of an essentially similar product [1, 2].

Amlodipine (as besylate; AML; fig. 1) is a long-acting calcium channel blocker used as an anti-hypertensive and in the treatment of angina [3, 4]. Olmesartan medoxomil (OLM; fig. 1) is a prodrug which is hydrolyzed to olmesartan during absorption from the gastrointestinal tract [5, 6]. Olmesartan is a selective AT1 subtype angiotensin II receptor antagonist. Combining the calcium channel blocker AML with an angiotensin receptor blocker OLM, produces significant mean reductions in seated systolic and diastolic blood pressure in patients with hypertension [7].

To our knowledge, three different HPLC methods have been reported in the literature up to date, for simultaneous determination of AML and OLM [8-10]. However, none of these methods is suitable for the simultaneous determination of AML and OLM for drug dissolution studies. The reported methods are not specific to avoid some undesirable peaks coming from matrix components close to the dead volume peak, and also there is no internal standard used to avoid some potential errors associated with injection or sample preparation [11].

Therefore, the aim of this study was to develop a specific HPLC method for the simultaneous determination of AML and OLM, and to apply the developed method for the measurement of in vitro release of the compounds from their fixed combination tablet dosage forms (5/40 mg; AML/OLM). Since the system suitability parameters of the HPLC method are within the acceptable range as recommended by FDA, the HPLC method proposed in this study could be also recommended for dissolution studies of other formulations for these compounds. .

Experimental part
Materials and methods
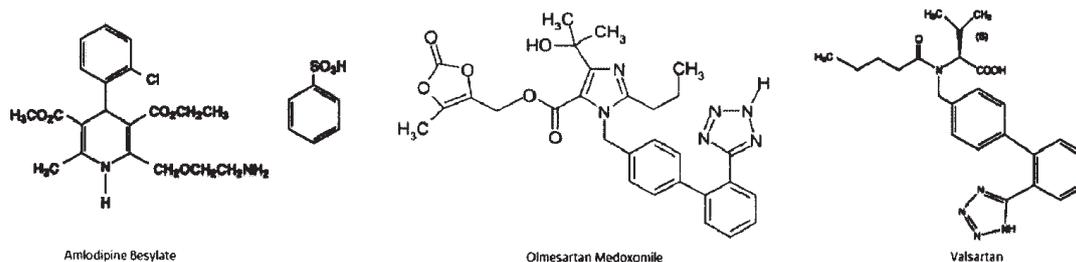Standard AML (as besylate salt) and valsartan (VAL, fig. 1) were supplied by Refik Saydam National Public Health

Fig. 1. Chemical structures of amlodipine besylate, olmesartan medoxomile and valsartan.

Agency (Turkey). Standard OLM was supplied by Daiichi Sankyo Pharmaceutical Company (Japan). Methanol and acetonitrile were purchased from Merck (Darmstadt, Germany) and were of HPLC grade. All other reagents were of analytical grade and obtained from Merck (Darmstadt, Germany). Water obtained from the Milli-Q water purification system (Barnstead, USA) was used for the preparation of buffer and all other aqueous solutions. Commercially available tablet containing 5 mg of AML and 40 mg of OLM (Sevicar®) was kindly supplied by Daiichi Sankyo Pharmaceutical Company.

The HPLC equipment was comprised of a solvent delivery system (Shimadzu 10 ATVP, Tokyo, Japan), an auto sampler (Shimadzu SIL 10 ADVP Tokyo, Japan), a column oven (Shimadzu CTO 10 ASVP, Tokyo, Japan) and a photodiode array detector (Shimadzu M 10VP, Tokyo, Japan). ChromQuest software (Shimadzu Technologies) was used for data acquisition and evaluation.

A Mettler Toledo MA 235 pH meter (USA) connected with a Mettler Toledo Inlab 413 pH electrode was used to measure the pH of the aqueous mobile phase and the dissolution medium.

Standard Stock Solutions
Standard stock solutions of AML, OLM and VAL (1000 μg/mL) were prepared separately in different flasks by dissolving 50 mg of AML besylate, 50 mg of OLM and 50 mg VAL in 50 mL acetonitrile. All these solutions were prepared freshly every week, during the method development process and application of the method. They were protected from daylight and stored at 4°C until they are used.

Calibration Standards
Calibration standards for AML and OLM were prepared daily in a concentration range between 1.0 to 50.0 μg/mL by appropriate dilution of standard stock solutions with the mobile phase. Dilutions of these standards were performed in a vial (1 mL total volume) after the addition of 20 μg/mL VAL as internal standard.

Preparation of Phosphate Buffer for in vitro Dissolution Studies
Based on FDA guidance, phosphate buffer (pH 6.8, 900 mL) was chosen as the dissolution medium for the dissolution studies of AML and OLM. Dissolution medium (pH 6.8 phosphate buffer) was prepared by dissolving 20.40 g of K_2HPO_4 and 21.18 g of NaH_2PO_4 in 6 L of deionised water. The pH of the medium was adjusted to 6.8 (±0.5) with 0.1 M sodium hydroxide solution or 0.1 M phosphoric acid solution.

Chromatographic Separation
AML, OLM and VAL were separated by a Phenomenex C_{18} column (Luna 5μ C_{18} 100A 250 x 4.6 mm; California, USA) protected with a Phenomenex C_{18} guard column (4 × 3.0 mm; California, USA). The mobile phase was consisted of phosphate buffer (pH 4.0, 0.04 mol/L):methanol:acetonitrile (40:45:15 v/v/v). The flow rate was 0.8 mL/min and the injection volume was 10 μL. The diode array detector was set at 234 nm and 205 nm wavelengths for the quantification of AML and OLM, respectively. The total run time was shorter than 16 min. Developed and validated HPLC method was applied successfully for the *in vitro* dissolution studies of AML and OLM.

In-Vitro Dissolution Studies
Dissolution studies on the fixed combination tablet formulation (5 mg AML and 40 mg OLM; Sevicar®, Daiichi Sankyo Pharmaceutical Company) were conducted using USP Apparatus II (paddle method) with six replicates at 37±0.5°C (Sotax, Switzerland). Phosphate buffer (pH 6.8) was used as the dissolution medium (900 mL) and the paddle rotation speed was kept at 50 rpm. At predetermined time intervals (5, 10, 15, 20, 25, 30, 45 and 60 min), 1 mL sample was withdrawn and replaced with an equal volume of fresh medium to maintain a constant total volume. After the filtration of the dissolution samples, 500 μL sample was mixed with 20 μL of internal standard solution (1000 μg/mL) and 480 μL of mobile phase. The concentrations of AML and OLM in these samples were determined simultaneously by the developed and validated HPLC method. For both compounds (AML and OLM), the percent dissolved at each time point was calculated using the following eq.1.

$$\% \, Dissolved = \frac{Amount \; dissolved}{Labeled \; amount} x 100 \qquad (1)$$

All tabulated results were expressed as mean ± standard error (S.E.)

Results and discussions
Method Development
Various mobile phase systems were tried to find the optimum conditions for the separation of AML, OLM and VAL with an acceptable resolution and a short analysis time. The optimum separation of these three compounds were achieved by using acetonitrile and methanol mixture with phosphate buffer (pH: 4.0). Thus, the final composition of the mobile phase was chosen as phosphate buffer (pH 4.0, 0.04 mol/L):methanol:acetonitrile (40:45:15 v/v/v). Since the sample solutions pH is lower than the pH of the mobile phase, the concentration of the phosphate buffer was 0.04 mol/L in order to improve pH resistance of the mobile phase against the retention time shifting of compounds. The chromatograms taken under these conditions were given in figure 2. It can be seen in figure . 2 that the system suitability parameters were within the acceptable values as recommended by FDA[12]. Plates (N) were more than 2400 and the peak symmetries were less than 1.25 for all compounds (AML, OLM, VAL). Capacity factors (k') for these three compounds were in between 2 and 10. The resolution values for the peaks were more than 1.5 required for a good separation. Following ten consecutive injections from the same vial, the repeatability values determined for both the peak areas and the retention times were less than 1 percent.

Since the quantification results were not satisfactory for the developed method by using single wavelength, the diode array detector was set at 234 nm and 205 nm wavelengths for determination of AML and OLM, respectively. At these wavelengths, peak areas of AML and VAL were divided to the peak areas of VAL and peak area ratios were used to construct the calibration curve and quantify the AML and OLM contents in the dissolution samples. Thus, the precision and accuracy of the developed method were improved. The developed method was validated according to the ICH guideline [13].

Stability

The stabilities of AML and OLM have already been reported in the literature [14-17]. However, there is no information available in regard to the stability of these compounds either in dissolution medium or during the analysis when they are to be kept in an autosampler. Therefore, in this study, we focused on the stability of AML and OLM both in the dissolution medium and the samples kept in an autosampler. When they were protected from daylight, AML and OLM were stable for at least 4 h at ambient temperature whether they were in the dissolution medium or kept in the autosampler. However, when they were kept in dissolution medium or in autosampler for overnight, they were not stable for 24 h period. The degradation of OLM can be easily identified by a reduction on the peak area of OLM and the appearance of unexpected peaks coming after dead volume and at 4.9 minutes which might be associated with the degradation product(s). Based on these results, the samples obtained from dissolution studies of AML and OLM were kept at -20⁰C and analyzed within 3 days by using daily calibrations.

Linearity

The calibration curves for AML and OLM were constructed under optimum conditions and the linearity of the method was determined by performing injections at eight different concentration levels in the linear range. The peak area ratios of AML and OLM to VAL were plotted against the corresponding nominal concentration to obtain calibration graph. The method was proved to be linear up to 50 μg/mL. The results were given in table 1.

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. The values of LOD and LOQ for both AML and OLM were given in table 1.

Precision and Accuracy

Three different concentrations of standard AML (2, 5 and 10 μg/mL) and OLM solutions (5, 20 and 40 μg/mL) within the linear range were analyzed three consecutive days (inter-day precision) and three times within the same day (intra-day precision). As seen in table 2, for the intra- and inter-day studies, the relative standard deviation values for AML and OLM were varied from 0.98 to 4.66 and 0.03 to 3.02 %, respectively. Bias values were varied from -2.63 to 10.99 % for AML and -1.54 to 0.83% for OLM.

In-vitro Dissolution Studies

Drug dissolution testing is a quantitative analytical technique for assessing drug release from pharmaceutical products, in particular solid oral dosage forms such as tablets and capsules. The reason for conducting the test is that generally for a drug to be absorbed, usually from the gastrointestinal tract, the drug should be in solution form. Thus, evaluation of dissolution becomes useful and necessary. Since the developed HPLC method is validated, it could be proposed for drug dissolution studies on the further formulation studies of AML and OML combined dosage forms [18].

	AML	OLM
Used wavelength*	234 nm	205 nm
Regression equation**	y = 0.0673x + 0.0475	y = 0.0784 x + 0.0397
Standard error of intercept	0.0023	0.0044
Standard error of slope	0.0008	0.0012
Correlation coefficient (r)	0.9997	0.9991
Linearity range (μg/mL)	0.1 - 50	0.1 - 50
Number of data points	8	8
LOD (μg/mL)	0.1	0.1
LOQ (μg/mL)	0.05	0.05

Table 1
LINEARITY DATA OF THE DEVELOPED METHOD (n = 6)

* Used wavelength for measuring peak areas of the compound and the IS
**where y is peak area ratio (Peak area of compound/ Peak area of internal standard at selected wavelenght) and x is concentration in μg/mL

	Added (μg/mL)	Inter-day			Intra-day		
		Found* (μg/mL)	Precision** RSD%	Accuracy Bias%	Found* (μg/mL)	Precision** RSD%	Accuracy Bias%
AML	2	1.95±0.04	2.00	-2.63	1.78±0.09	4.54	10.99
	5	4.90±0.07	1.39	-1.95	4.98±0.23	4.66	-0.49
	10	10.20±0.10	0.98	1.98	10.20±0.10	0.98	2.01
OLM	5	4.97±0.09	1.75	-0.65	4.92±0.08	1.58	-1.54
	20	20.13±0.07	0.36	0.65	20.16±0.01	0.03	0.80
	40	40.33±0.25	0.62	0.83	39.70±1.21	3.02	-0.74

Table 2
INTER- AND INTRA-DAY PRECISION AND ACCURACY RESULTS OF DEVELOPED METHOD

*mean±standard error (n=3)
**Relative standard deviation
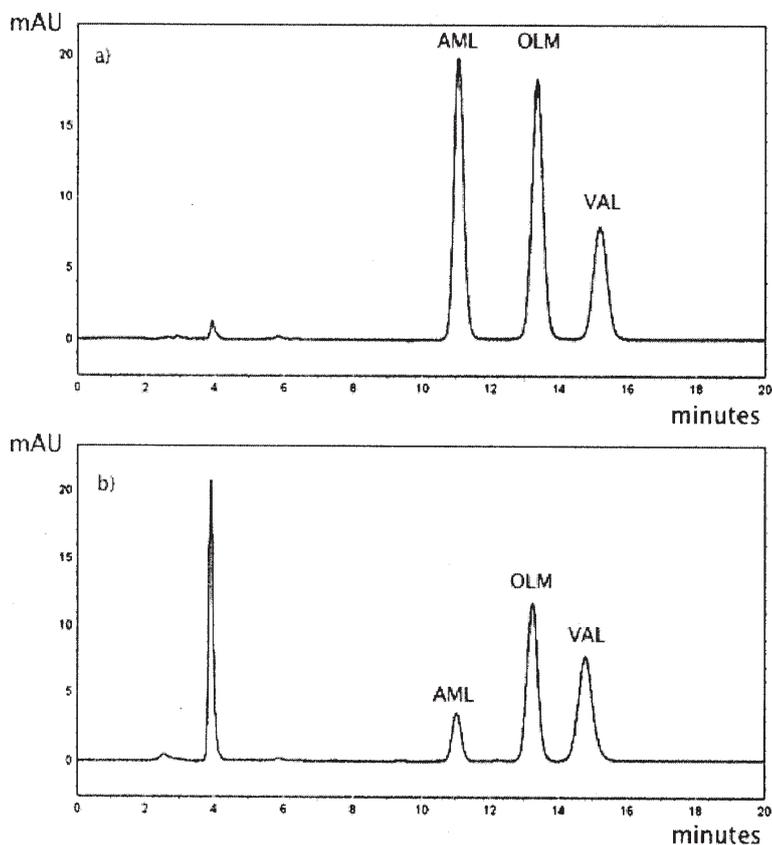Bias %= [(Found-Added)/Added] x 100

Fig. 2. HPLC Chromatograms of amlodipine besylate (AML), OLM (olmesartan medoxomile) and internal standard valsartan (VAL) from stock solution (a) and from dissolution sample (b).
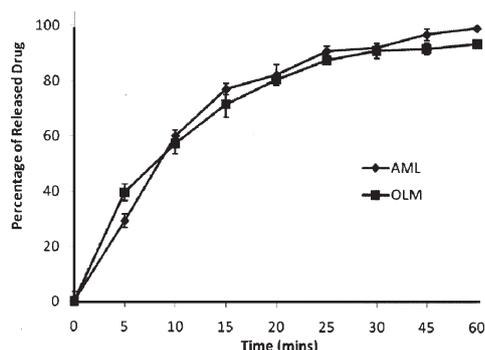

Fig. 3. Release profiles of AML and OLM from combined dosage form

The developed HPLC method was successfully used for determination of average percentage drugs released within 45 min for *in-vitro* dissolution of tablets containing combination drug product. Our *in-vitro* dissolution studies revealed that 85% of labeled amounts of AML and OLM (fig. 2) were released within 25 min from their fixed combination tablet dosage form. At the end of the dissolution test (45 min) released% of the drugs were found 97 and 92% for AML and OLM respectively. The dissolution pattern complies with the FDA Guidance standards indicating suitability of the proposed method for the dissolution study of the two drugs.

Conclusions

In this study, a simple fast and reliable HPLC method was developed and validated for the simultaneous determination of AML and OLM in dissolution samples. The method showed good performance with respect to linearity, sensitivity, accuracy, precision, selectivity. The developed method was successfully applied for the analysis of AML and OLM in dissolution samples indicating that this can be successfully applied to the dissolution studies.

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