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HPLC method development and *in vitro* dissolution kinetics of amlodipine tablets under biowaiver conditions

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Abstract: A biowaiver means that *in vivo* bioavailability and/or bioequivalence studies may be waived (not considered necessary for product approval). Instead of conducting expensive and time consuming *in vivo* studies, a dissolution test could be adopted as the surrogate basis for the decision as to whether the two pharmaceutical products are equivalent. The biowaiver approach based on BCS is intended to replace bioequivalence *in vivo* studies. The aim of the study was to study dissolution kinetics of amlodipine tablets in order to assess their equivalence under conditions *in vitro* according to the biowaiver. The study of dissolution kinetics of drugs in the form of amlodipine tablets has been carried out in accordance with the requirements of the "biowaiver" procedure, the recommendations of the SPhU and the WHO requirements in order to assess the possibility of replacing the pharmacokinetic studies *in vivo* by tests *in vitro*. The possibility to use the recommendations of the "biowaiver" procedure for the registration of generics amlodipine tablets has been found. The studies conducted have shown that amlodipine can be referred to class I of the biopharmaceutical classification system, i.e. substances with a high biopharmaceutical solubility and a high penetration rate. It will allow conducting comparative studies *in vitro* to confirm the equivalence of drugs. The evaluated amlodipine drugs fulfill biowaiver criteria for drugs containing BCS Class I active pharmaceutical ingredients. Both drugs are "rapidly dissolving," both meet the criteria of dissolution profile similarity, f_2 (i.e., the dissolution profile of the test product is similar to that of the reference product in pH 1.2, 4.5, and 6.8 buffers using the paddle method at 75 rpm), and both are considered to be *in vitro* equivalent without *in vivo* evaluation. The proposed chromatographic methods are simple, rapid and accurate for the determination of amlodipine in pharmaceutical dosage forms and can be used for routine quality control of drugs and *in vitro* dissolution study.

Keywords: amlodipine; high-performance liquid chromatography; method development; validation; dissolution; tablets.

1. Introduction

A biowaiver means that *in vivo* bioavailability and/or bioequivalence studies may be waived (not considered necessary for product approval). Instead of conducting expensive and time consuming *in vivo* studies, a dissolution test could be adopted as the surrogate basis for the decision as to whether the two pharmaceutical products are equivalent. The biowaiver approach based on BCS is intended to replace bioequivalence *in vivo* studies. Bioequivalence is a vital concern in drug development even more significant in the case of Narrow Therapeutic

Index (NTI) drugs. In the clinical development of New Chemical Entities (NCE), bioequivalence studies necessitate being performed when the formulation of the pharmaceutical dosage form has been changed. *In vivo* pharmacokinetic data can be used as surrogate parameters for *in vivo* solubility and permeability data. The Biopharmaceutics Classification System (BCS) has emerged as a helpful tool in product development by alluding to the in vivo performance of the active substance. The bio-relevance of the BCS properties and the in vitro release are best expressed through a correlation between in vitro and *in vivo* data. Recently BCS has been implemented for waiving bioequivalence studies on the basis of the solubility and gastrointestinal permeability of drug substances and can be strategically deployed to save time and resources during generic drug development. The BCS has been adopted as a very useful tool for *in vivo* drug design and development worldwide, particularly in terms of regulatory standards. A BCS-based biowaiver has become an important and cost-saving tool in the approval of generic drugs [1-9].

Amlodipine is used alone or in combination with other medications to treat high blood pressure and chest pain (angina). Amlodipine is in a class of medications called calcium channel blockers. It lowers blood pressure by relaxing the blood vessels so the heart does not have to pump as hard. It controls chest pain by increasing the supply of blood to the heart. If taken regularly, amlodipine controls chest pain, but it does not stop chest pain once it starts. Its structure is shown in Fig. 1. The chemical name is 3-*O*-ethyl 5-*O*-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate**.**

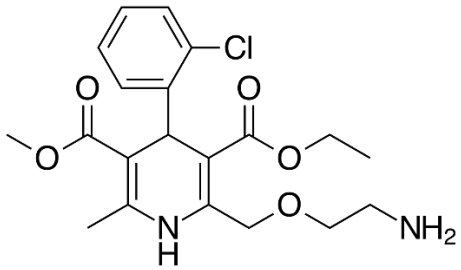

Figure 1. Chemical structure of amlodipine.

Amlodipine used in the product complies with its European Pharmacopoeia monographs. Amlodipine besilate is described as slightly soluble in water, freely soluble in methanol, sparingly soluble in anhydrous ethanol, slightly soluble in 2-propanol**.** Ukraine has marketing authorizations for amlodipine as an immediate-release dosage form in strengths of 5 and 10 mg. According to the Caco-2 test results (permeability), amlodipine appeared to have moderate to high permeability. Caco-2 permeability values for amlodipine is in agreement with BCS Class I and high bioavailability in humans. In Ukraine amlodipine tablets are produced by different manufacturers [10-15]. The aim of our research was to study dissolution kinetics of amlodipine tablets in order to assess their equivalence under conditions *in vitro* according to the biowaiver

2. Materials and Methods

Innovator amlodipine l immediate-release tablets, used as reference product, and a generic version (test product) marketed in Ukraine were evaluated. The study of dissolution kinetics was conducted in accordance with the monograph of the SPhU, Supplement 2 “5.N.2. Studies on bioavailability and bioequivalence of generic medicines”, Guidance on bioavailability and bioequivalence research, methodological recommendations, as well as the

WHO Guide in three media with different pH values: hydrochloric acid solution with pH 1.2, acetate buffer solution with pH 4.5 and phosphate buffer solution with pH 6.8. All buffer solutions were prepared according to the SPhU [2].

2.1. Equipments.

All dissolution studies were performed using USP Apparatus 2 (Erweka DT 600, Frankfurt, Germany) the device with basket was used; the volume of the dissolution medium – 1000 ml; the temperature of the dissolution medium – (37.0+0.5)°C; the rotation speed of the basket – 100 rpm. Sampling was carried out in 15, 30 and 45 min manually with a 10.0 ml pipette from the plot midway between the surface of the dissolution medium and the basket at the distance of 2 cm from the wall of the dissolution vessel. The samples obtained were filtered through a filter paper with a pore size of 2 to 3 μm. 5.0 Ml of the filtrate obtained was diluted to the volume of 100.0 ml with the corresponding dissolution medium. The volume selected was compensated by the corresponding dissolution medium. To obtain statistically reliable results the test was carried out on 12 samples of each of the study objects.

2.2. Chromatographic conditions.

Twelve tablets of each preparation were studied to obtain statistically significant results. Dissolution profile comparisons were made according to WHO Guidances. The chromatographic analysis of amlodipine performed on liquid chromatograph Agilent 1290 Infinity II LC System. Chromatography is performed on liquid chromatograph with spectrophotometric detector under the following conditions:Ascentis C18 column size 4,6×150 mm with a particle size of 5 microns; mobile phase: acetonitrile R - 0.1% solution of trifluoroacetic acid R (40:60); the rate of mobile phase: 1.0 ml/min; column temperature: 30° C; detection wavelength: 237 nm. Statistical treatment was carried out using Microsoft Excel software. The equivalence of dissolution kinetics of drugs in the form of amlodipine tablets was assessed by the value of the similarity factor (f_2), which should be from 50 to 100, in order to make a conclusion about conformity of the kinetic curves. For each time interval, the standard deviation of the mean value (SD) was calculated. It must keep the following requirements: should be less than 10% starting from the second to the last point of control; less than 20% for the first time point.

3. Results and Discussion

The HPLC method was developed to provide a specific procedure for the rapid quality control analysis for the dissolution test [16-18]. This method provides the sensitivity of the technique and allows to separate of API with the impurities and components of the placebo. To find the appropriate HPLC conditions for the separation of the examined drug and impurities, various columns, isocratic and gradient mobile phase systems were tried, and successful attempts were performed using Ascentis C18 column size 4,6×150 mm with a particle size of 5 microns and mobile phase composed of acetonitrile R - 0.1% solution of trifluoroacetic acid R (40:60), at a flow rate of 1.0 ml/min with λ_{max} at 237 nm. Fig. 2 shows a typical chromatogram. The retention time is 4.55, plate number -12215 and a tailing factor - 1.12. The obtained values of the entire system suitability parameters are within the limits of the agreeable range, which shows that the developed method is fit for the detection of amlodipine in the tablet

form. The optimum chromatographic conditions and system suitability parameters are tabulated in Table 1.

Figure 2. Representative chromatogram of amlodipine using UV detection at 237 nm.

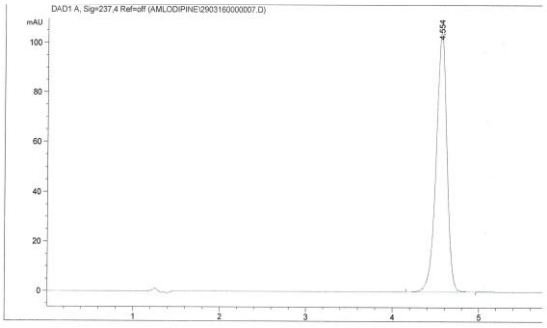

Figure 2. Representative chromatogram of amlodipine using UV detection at 237 nm.

Table 1. Optimized chromatographic conditions and system suitability parametrs.

Parameter	Chromatographic conditions
Instrument	Agilent 1290 Infinity II LC System
Column	Ascentis C18 column size 4,6×150 mm with a particle size of 5 microns
Mobile phase	acetonitrile R:0.1% solution of trifluoroacetic acid R (40:60, v/v)
Flow rate	1.0 ml/min
Detection wavelength	UV at 237 nm
Runtime	5 min
Column temperature	30° C
Volume of injection loop	10 µl
Retention time	4.55 min

3.1. Linearity.

Calibration curve representing the relation between the concentrations of drugs versus the peak area was constructed. In triplicate run from which the linear regression equation was calculated. The results of linearity are present in Table 2.

Table 2. Analytical parametrs of proposed method.

Parameter	Value	Criterion	Conclusion
Slope (m)	155585.776	–	
Intercept (b)	-1.14818e-1	–	
Correlation coefficient (r)	0.99998	> 0.99236	Responds

The results obtained were processed by the least squares method. The correlation coefficient of amlodipine r^2 was noted as 0.99998 which states that the method was good linear to the concentration versus peak area responses. Results indicate high sensitivity of the proposed HPLC method.

3.2. Specificity.

Commonly used tablet excipients did not interfere with this method. It shows that the method is specific. Furthermore, the well shaped peaks also indicate the specificity of the method. The specificity results are tabulated in Table 3.

Table 3. Specificity study.

Name of the solution	Retention time (tR) min
mobile phase	No peaks
placebo	No peaks
amlodipine 0.5 mg/ml	4.55

3.3. Accuracy and Precision.

System precision is shown in Table 4. Intra-day and inter-day % RSD values lower than 2% clearly assuring that this method was found to be fairly precise and reproducible (Table 5). Regarding accuracy, a known amount of the standard drug was added to the fixed amount of preanalyzed sample solution % recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 70 %, 80 %, 90 %, 100 %, 110 %, 120 %, 130 % levels (Table 6). The high value of recoveries obtained for amlodipine indicates that the proposed method was found to be accurate.

Table 4. Results of system precision.

Sample	Concentration (µg/ml)	Peak area	Injection no.	RSD, %
Amlodipine	0.1	1086,213	1	0,07
		1086,486	2	
		1085,791	3	
		1084,526	4	
		1085,396	5	

Table 5. Intra-day and inter-day precision.

Day	Intra-day precision		Inter-day precision	
	Mean	R.S.D %	Mean	R.S.D %
1	99.82	0.311	100.76	0.364
2	100.41	0.647	99.27	0.390
3	100.82	0.336	100.53	0.572

Table 6. Accuracy study.

Model Solutions	The amount of amlodipine, %		Found,% to predetermined, $Z_i = (Y_i/X_i)\ 100\%$
	Predetermined quantity, $X_i = (m_i/m_{rs})\ 100\ \%$	Found quantity, $Y_i = (S_i/S_{rs})\ 100\ \%$	
M1	69.98	70.03	100.07
M2	80.01	80.12	100.14
M3	89.97	90.02	100.06
M4	95.01	95.09	100.08
M5	100.00	99.95	99.95
M6	104.95	105.04	100.09
M7	110.00	110.08	100,07
M8	120.08	120.17	100.07
M9	130.01	130.11	100.08
Average, *Z, %*			100.07
Standard deviation, S_z, *%*			0.05
Confidence interval of convergence of results (actual) $\Delta z = t(95\%,8) \cdot S_z = 2.3060 \cdot S_z, \%$			0.11
Critical value for the convergence of results $\Delta \leq max\Delta_{As} = 2.4\%$			Performed (< 2.4)
Systematic error $\delta = \lvert Z - 100 \rvert, \%$			0.07
Criterion of significance of systematic error $\delta \leq max\ \delta\%$			Performed (< 0.77)
The general conclusion about the technique:			Correct

3.4. Robustness.

The robustness of the developed method was evaluated by small deliberate changes in method parameters such as flow rate (+10 %) and temperature of column (± 3 %). The % RSD values of robustness which is less than 2 % reveal that the proposed method is robust. The results of robustness study results are shown in Table 7. Even though the small changes in the conditions did not significantly affect the retention time of amlodipine.

Biowaiver criteria for drugs containing BCS Class I active pharmaceutical ingredients are:

a. The dosage form is rapidly dissolving (dissolution amount is greater than 85% at 30 min in all media with pH 1.2, 4.5, 6.8) and the dissolution profile of the test product is similar to that of the reference product in pH 1.2, 4.5, and 6.8 buffers using the paddle method at 75 rpm or the basket method at 100 rpm and meet the criteria of dissolution profile similarity, $f_2 \geq 50$ (or equivalent statistical criterion);
b. If both the test and the reference dosage forms are very rapidly dissolving (dissolution amount is greater than 85% at 15 min in all media with pH 1.2, 4.5, 6.8) the two products are deemed equivalent, and a profile comparison is not necessary. Both evaluated drugs were "rapidly dissolving" (Table 8) because the active pharmaceutical ingredient release at time point 30 min was more than 85%.

Table 7. Results of the study of robustness.

Conditions of analysis	Retention time, min
Standard conditions	4,543
flow rate 1,1 mL/min, (+10 %)	4,602
flow rate 0,9 mL/min, (-10 %)	4,711
temperature of column 33°C	4,562
temperature of column 27°C	4,587

Table 8. Dissolution amount («rapidly dissolving», «very rapidly dissolving» or «not rapidly dissolving») for evaluated drugs.

Medium	Test product		Reference product	
	% dissolved 15 min	% dissolved 30 min	% dissolved 15 min	% dissolved 30 min
pH 1.2	90.34	94.21	89.18	92.59
pH 4.5	93.24	95.14	92.84	93.02
pH 6.8	87.21	90.63	88.99	93.67

Dissolution profiles and corresponding data are shown in Fig. 3–5 and Table 8.

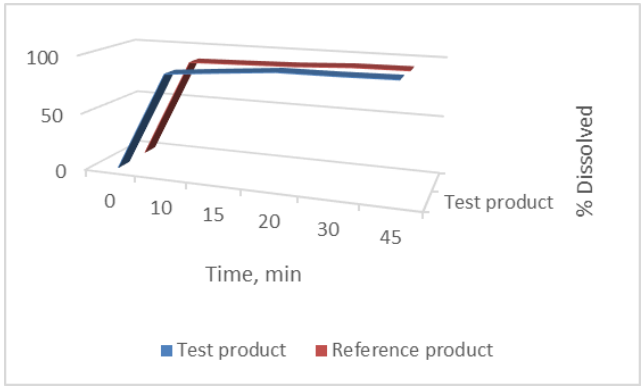

Figure 3. Dissolution profiles of test and reference drugs at pH 1.2.

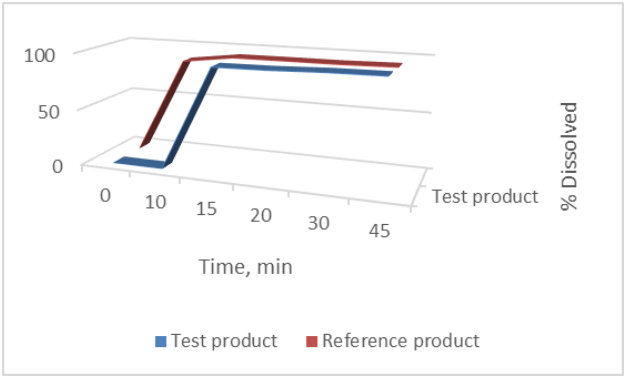

Figure 4. Dissolution profiles of test and reference drugs at pH 4.5.

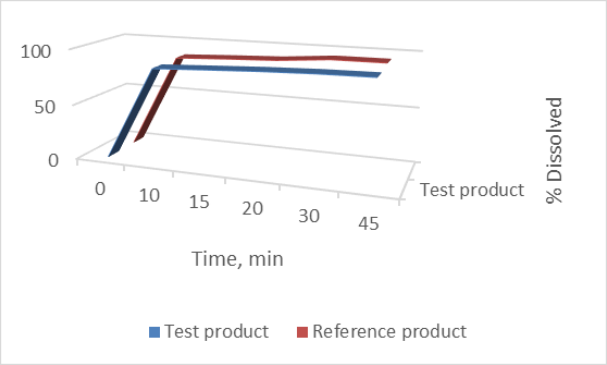

Figure 5. Dissolution profiles of test and reference drugs at pH 6.8

On the basis of the data obtained it has been found that the equivalence of dissolution profiles for all recommended dissolution media is observed (pH 1.2, 4.5 and 6.8) for the drugs studied. In all three dissolution media the release of amlodipine is more than 85% in 15 min (Table 8, 9), i.e. the drugs under research can be classified as "highly soluble", and their equivalence can be determined by the method *in vitro*. The percent relative standard deviation (% RSD) for all time points fulfills all requirements (≤10% for 15 min and other time points), so results are valid (Table 9). The studies conducted have shown that bisoprolol can be referred to class I of the biopharmaceutical classification system, i.e. substances with a high biopharmaceutical solubility and a high penetration rate. It will allow conducting comparative studies *in vitro* to confirm the equivalence of drugs.

Table 9. Dissolution test results.

Medium	Time, min	Test product		Reference product	
		% dissolved	RSD, %	% dissolved	RSD, %
pH 1.2	10	86.35	3.13	88.12	3.41
	15	90.34	2.56	89.18	3.21
	20	94.11	2.79	90.34	2.30
	30	94.21	3.01	92.59	2.86
	45	94.91	2.71	93.11	2.67
pH 4.5	10	86.87	3.07	86.91	3.37
	15	93.24	2.87	92.84	2.56
	20	93.68	2.61	92.99	2.87
	30	95.14	3.08	93.02	2.89
	45	95.61	3.23	93.87	3.24
pH 6.8	10	85.10	3.47	86.13	3.28
	15	87.21	2.89	88.19	3.13
	20	89.21	2.96	89.94	2.82
	30	90.63	3.14	93.67	2.99
	45	91.22	3.03	93.89	2.53

4. Conclusions

Chromatographic separation achieved isocratically on Ascentis C18 column size 4,6×150 mm with a particle size of 5 microns using mobile phase composed of acetonitrile R : 0.1% solution of trifluoroacetic acid R (40:60) at a flow rate of 1.0 ml/min with λ_{max} at 237 nm. Statistical analysis proves that the method is reproducible and selective for the simultaneous estimation of amlodipine.

The developed method was validated as per ICH guidelines in terms of accuracy, precision, linearity, robustness and specificity. Thus the study aimed at developing and validating the new HPLC method, being simple, accurate, selective, and sensitive and can be

applied for the estimation of amlodipine in pharmaceutical dosage forms and can be used for routine quality control of drugs and *in vitro* dissolution study.

The evaluated amlodipine drugs fulfill biowaiver criteria for drugs containing BCS Class I active pharmaceutical ingredients. Both drugs are "rapidly dissolving," both meet the criteria of dissolution profile similarity, f_2 (i.e., the dissolution profile of the test product is similar to that of the reference product in pH 1.2, 4.5, and 6.8 buffers using the paddle method at 75 rpm), and both are considered to be *in vitro* equivalent without *in vivo* evaluation.

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Conflicts of Interest

The authors declare no conflict of interest.

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