PHARMACIA

Pharmacia 69(1): 61–67
DOI 10.3897/pharmacia.69.e77319

Research Article

Study of the dissolution kinetics of drugs in solid dosage form with lisinopril and atorvastatin and intestinal permeability to assess their equivalence *in vitro*

Nataliia Shulyak[1,2], Kateryna Liushuk[2], Oksana Semeniuk[2], Nadiya Yarema[1], Tetyana Uglyar[1], Dariya Popovych[1], Andriy Sverstiuk[1], Roman Ciciura[1], Liliya Logoyda[1]

1 *I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine*

2 *Municipal Institution of Higher Education «Volyn Medical Institute» of the Volyn Oblast Counsil, Lutsk, Ukraine*

Corresponding author: Liliya Logoyda (logojda@tdmu.edu.ua)

Received 29 October 2021 ♦ **Accepted** 25 December 2021 ♦ **Published** 7 January 2022

Citation: Shulyak N, Liushuk K, Semeniuk O, Yarema N, Uglyar T, Popovych D, Sverstiuk A, Ciciura R, Logoyda L (2022) Study of the dissolution kinetics of drugs in solid dosage form with lisinopril and atorvastatin and intestinal permeability to assess their equivalence *in vitro*. Pharmacia 69(1): 61–67. https://doi.org/10.3897/pharmacia.69.e77319

Abstract

Atorvastatin and lisinopril are a successful combination for the treatment of patients with chronic heart failure and hypertension. Study of the dissolution kinetics of drugs in solid dosage form with lisinopril and atorvastatin and intestinal permeability to assess their equivalence *in vitro* were described. In medium with hydrochloric acid pH 1.2, in the medium of acetate buffer solution with a pH of 4.5 and in the medium phosphate buffer solution with a pH of 6.8 for 15 min more than 85% of the active substance passes into solution, hence the dissolution profiles these drugs in these environments are similar, and the drugs in them are "very quickly soluble". Among the *in vitro* models that make it possible to assess the degree of absorption of API, the most widely used culture of adenocarcinoma cells of the colon – Caco-2. The development of the analytical methodology and its validation is the final stage of both the dissolution study and the Caco-2 test, as well as the biowaver procedure. It plays the most important role in the reliability of the results for all the above procedures and tests. To study permeability, method LC-MS/MS was developed. According to the obtained results, atorvastatin and lisinopril showed low permeability. The values of recovery of transport of test and control substances through the monolayer of cells of the Caco-2 line indicate that the results of the experiment can be considered reliable. The equivalence of the drugs "Lisinopril", tablets of 10 mg and "Atorvastatin", tablets of 10 mg, belongs to class III BCS proven by *in vitro* studies.

Keywords

Atorvastatin, Dissolution study, Lisinopril, Recovery, Permeability

Introduction

Ensuring the quality of treatment largely depends on the bioavailability of the drug, which in turn is influenced by the correct and effective pharmaceutical development. Determination of bioavailability and assessment of bioequivalence are important elements in the development and state registration of a medicinal product. Understanding the process of drug delivery to an organ or target cell is one of the tasks of modern pharmacy. In order for the drug to reach the systemic bloodstream, it goes through many stages, namely release from the dosage form, dissolution in the physiological

environment of the gastrointestinal tract, absorption through the gastric membrane. Analytical methods should be developed and validated to quantify the concentration of API in solution. These are usually spectrophotometry and high performance liquid chromatography (HPLC). Carrying out the classical test "Dissolution" is insufficient for many purposes, it is necessary to study the dissolution kinetics of the drug. The trend of modern biopharmacy is the biowaver procedure, the approach of which on the basis of the biopharmaceutical classification system is designed to replace bioequivalence studies *in vivo*. Pharmacokinetic studies are complicated when, in order to increase the effectiveness of pharmacotherapy of any disease, the patient is prescribed several drugs simultaneously or the objects of study of bioequivalence are multicomponent dosage forms. Reliable and objective analytical studies at the stage of pharmaceutical development and implementation allow to ensure proper bioavailability and, as a consequence, the expected therapeutic effect of the drug. All this will optimize the pharmaceutical development of drugs and improve the effectiveness and safety of treatment, as well as patient compliance with pharmacotherapy.

Atorvastatin and lisinopril are a successful combination for the treatment of patients with chronic heart failure and hypertension (Naveed 2015; GoodRx undated). Analytical methods of analysis (Leis et al. 1998, 1999; El Gindy et al. 2001; Andreas et al 2003; El-Emam et al. 2004; Beasley et al. 2005; Rahman et al. 2005a, 2005b; Huang et al. 2006; Stanisz and Kania 2006; Chaudhari et al. 2007; Ivanovic et al. 2007; Shah et al. 2007; Basavaiah et al 2009; Chauhan et al. 2011; Jamakhandi et al. 2011; Sultana et al. 2011; Kumar et al. 2012; Naveed et al. 2012; Arayne et al 2013; Hafez et al. 2014; Lakshmi and Sreedevi 2014; Sathiyasundar and Valliappan 2014; Sbârcea et al. 2014; Shulyak et al. 2021a, 2021b, 2021c) have been developed for the determination of atorvastatin and lisinopril in medicines and biological liquids. However, many of these methods are limited in their usage. There is, therefore, a need for a rapid simple green spectrophotometric and chromatographic methods for the assay of atorvastatin and lisinopril for *in vitro* bioequivalence study.

The present paper describes the study of the dissolution kinetics of drugs in solid dosage form with lisinopril and atorvastatin and intestinal permeability to assess their equivalence *in vitro* with usage of spectrophotometric and chromatographic methods for the determination of atorvastatin and lisinopril.

Aim of work

We aimed to study the dissolution kinetics of drugs in solid dosage form with lisinopril and atorvastatin and intestinal permeability to assess their equivalence *in vitro*.

Materials and methods

Reagents and standards

All the chemicals were used of analytical reagent grade.

Atorvastatin calcium (purity 99.1%) and lisinopril (purity 99.3%) were purchased from Sigma-Aldrich (Switzerland).

The used dosage forms of lisinopril: Lisinopril (10 mg) (Tablets A), Lisinopril (10 mg) (Tablets B), Lisinopril (10 mg) (Tablets C).

The used dosage forms of atorvastatin: Atorvastatin (10 mg) (Tablets A), Atorvastatin (10 mg) (Tablets B), Atorvastatin (10 mg) (Tablets C).

Apparatus

A double – beam Shimadzu UV-Visible spectrophotometr, with spectral bandwidth of 1 nm wavelength accuracy ±0.5 nm, Model –UV 1800, Software UV-Probe 2.62, and a pair of 1 cm matched quartz cells, was used to measure absorbance of the resulting solution. Designed in accordance with the governing Japanese and European Pharmacopoeia, the new UV-1800 UV-VIS spectrophotometer achieves a resolution of 1 nm, the highest in its class, in a compact design.

All LC measurements were performed using Shimadzu VP HPLC system including vacuum degasser, gradient pumps, column oven and autosampler. The LC system was coupled with tandem mass spectrometer API 3000 (PE Sciex). The TurboIonSpray ion source was used in both positive and negative ion modes. Parameters of electrospray ionizer and MRM parametrs are listed in Table 1. Acquisition and analysis of the data were performed using Analyst 1.5.2 software (PE Sciex).

Table 1. Parameters of electrospray ionization.

Parameter	Value
Polarity	**Positive**
Nebulizer Gas (NEB, Gas 1)	15
Curtain Gas (CUR)	8
Collision Gas (CAD)	5
IonSpray Voltage (IS)	5000
Temperature (TEM)	400
Turbo IonSpray Gas	8
Horizontal Position	5.0
Lateral Position	1.0

Stationary phase was a reversed –phase column Discovery C_{18}, 50 × 2.1 mm, 5 µm. Eluent A: acetonitrile – H_2O – formic acid (5: 95: 0.1); eluent B: acetonitrile – formic acid (100: 0.1).

Parameters of acqusition method atorvastatin

Samples were chromatographed in gradient mode. The initial content of eluent B was 10%, which linearly increases to 100% in 1.0 min and in 1.11 min returns to the original 10%. Eluent consumption – 0.4 mL/min; column thermostat temperature – 30 °C; registration time – 2.5 minutes; the volume of the injected sample – 2 µl; autosampler thermostat temperature – 15 °C. Multiple reaction monitoring (MRM) parameters of atorvastatin are presented in Table 2.

Table 2. Multiple reaction monitoring (MRM) parameters of atorvastatin.

Analyte	Parent, m/z	Daughter, m/z	Time, ms	DP, V	EP, V	CE, V	CXP, V
Atorvastatin	559.234	440.300	120	96	370	11	29

* Abbreviations: DP, declustering potential; EP, entrance potential; CE, collision energy; CXP, collision cell exit potential.

Parameters of acqusition method lisinopril

Samples were chromatographed in gradient mode. The initial content of eluent B was 0%, which linearly increases to 100% in 1.2 min and in 1.31 min returns to the original 0%. Eluent consumption – 0.4 mL/min; column thermostat temperature – 30 °C; registration time – 2.8 minutes; the volume of the injected sample – 2 µl; autosampler thermostat temperature – 15 °C. Multiple reaction monitoring (MRM) parameters of lisinopril are presented in Table 3.

Table 3. Multiple reaction monitoring (MRM) parameters of lisinopril.

Analyte	Parent, m/z	Daughter, m/z	Time, ms	DP, V	EP, V	CE, V	CXP, V
Lisinopril	406.145	84.2	120	91	11	43	4

* Abbreviations: DP, declustering potential; EP, entrance potential; CE, collision energy; CXP, collision cell exit potential.

Parameters of acqusition method atenolol

Samples were chromatographed in gradient mode. The initial content of eluent B was 0%, which linearly increases to 90% in 1.1 min and up to 1.11 min returns to the original 0%. Eluent consumption – 0.4 mL/min; column thermostat temperature – 30 °C; registration time – 2.3 minutes; the volume of the injected sample – 3 µl; autosampler thermostat temperature – 15 °C. Multiple reaction monitoring (MRM) parameters of atenolol are presented in Table 4.

Table 4. Multiple reaction monitoring (MRM) parameters of atenolol.

Analyte	Parent, m/z	Daughter, m/z	Time, ms	DP, V	EP, V	CE, V	CXP, V
Atenolol	267.026	145.1	120	56	11	37	12

* Abbreviations: DP, declustering potential; EP, entrance potential; CE, collision energy; CXP, collision cell exit potential.

Parameters of acqusition method propranolol

Samples were chromatographed in gradient mode. The initial content of eluent B was 10%, which linearly increases to 100% in 1.0 min and to 1.01 min returns to the original 10%. Eluent consumption – 0.4 mL/min; column thermostat temperature – 30 °C; registration time – 2.2 minutes; the volume of the injected sample – 3 µl; autosampler thermostat temperature – 15 °C. Multiple reaction monitoring (MRM) parameters of propranolol are presented in Table 5.

Table 5. Multiple reaction monitoring (MRM) parameters of propranolol.

Analyte	Parent, m/z	Daughter, m/z	Time, ms	DP, V	EP, V	CE, V	CXP, V
Propranolol	260.1	183.4	100	56	11	27	16

* Abbreviations: DP, declustering potential; EP, entrance potential; CE, collision energy; CXP, collision cell exit potential.

Parameters of acqusition method quinidine

Samples were chromatographed in gradient mode. The initial content of eluent B 0%, which linearly increases to 100% in 1.0 min and up to 1.01 min returns to the original 0%. Eluent consumption – 0.4 ml / min; column thermostat temperature – 30 °C; registration time – 2.4 minutes; the volume of the injected sample – 3 µl; autosampler thermostat temperature – 15 °C. Multiple reaction monitoring (MRM) parameters of quinidine are presented in Table 6.

Table 6. Multiple reaction monitoring (MRM) parameters of quinidine.

Substance	Parent, m/z	Daughter, m/z	Time, ms	DP, V	EP, V	CE, V	CXP, V
Quinidine	325.2	172.2	150	61	11	47	28

* Abbreviations: DP, declustering potential; EP, entrance potential; CE, collision energy; CXP, collision cell exit potential.

Cultivation of Caco-2 cells

Caco-2 cells were cultivated in 75 cm^2 flasks to 70–80% of confluence according to the ATCC and Millipore recommendations in humidified atmosphere at 37 °C and 5% CO_2. Cells were detached with Trypsin/EDTA solution and resuspended in the cell culture medium to a final concentration of 2×10^5 cells/ml. 500 µl of the cell suspension was added to each well of HTS 24-Multiwell Insert System and 35 ml of prewarmed complete medium was added to the feeder tray. Caco-2 cells were incubated in Multiwell Insert System for 21 days before the transport experiments. The medium in filter plate and feeder tray was changed every other day. After 21 days of cell growth, the integrity of the monolayer was verified by measuring the transepithelial electrical resistance (TEER) for every well using the Millicell-ERS system ohm meter. The final TEER values were within the range 150–600 $\Omega\times cm^2$ as required for the assay conditions. 24-well insert plate was removed from its feeder plate and placed in a new sterile 24-well transport analysis plate. The medium was aspirated and inserts washed with PBS twice (Arena and Phillips 2003; Sevin et al 2013; Srinivasan et al 2015; Logoyda 2018).

Procedure

To determine the rate of compounds transport in apical (A) to basolateral (B) direction, 300 µL of the test compound dissolved in transport buffer at 10 µM (HBSS, 10 mM HEPES, pH=7.4) was added into the filter wells; 1000µL of buffer (HBSS, 10 mM HEPES, pH=7.4) was

added to transport analysis plate wells. The plates were incubated for 90 min at 37 °C with shaking at 100 RPM. 75 µL aliquots were taken from the donor and receiver compartments for LC-MS/MS analysis. All samples were mixed with 2 volumes of acetonitrile with following protein sedimentation by centrifuging at 10000 rpm for 10 min. Supernatants were analyzed using the LC system coupled with tandem mass spectrometer. Propranolol (high permeability), atenolol (low permeability) and quinidine (moderate permeability) were used as reference compounds (Arena and Phillips 2003; Sevin et al 2013; Srinivasan et al 2015; Logoyda 2018).

Permeability coefficient (P_{app}) was calculated for Caco-2 permeability assay using the following equation:

$$P_{app} = \frac{V_A}{\text{Area} \times \text{time}} \times \frac{[drug]_{acc}}{[drug]_{\text{initial donor}}} * 1000000$$

$\boldsymbol{V_A}$ – volume of transport buffer in acceptor well (1.0 cm^3 in the test A → B; 0.3 cm^3 in the test B → A),
Area – surface area of the insert (equals to effective growth area of the insert – 0.7 cm^2),
Time – time of the assay (5400 sec),
$\boldsymbol{[drug]_{acc}}$ – concentration of test compound in acceptor well,
$\boldsymbol{[drug]_{initial,d}}$ – initial concentration of test compound in a donor well.
$\boldsymbol{P_{app}}$ is expressed in 10^{-6} cm/sec.
n=3

The % recovery can be useful in interpreting the Caco-2 data. If the recovery is very low, this may indicate problems with poor solubility, binding of the compound to the test plate materials, metabolism by the Caco-2 cells or accumulation of the compound in the cell monolayer. The % recovery was calculated using the following equation:

$$\%\text{recovery} = \frac{C_{acc} \times V_{acc} + C_d \times V_d}{C_{\text{initial,d}} \times V_d} \times 100,$$

$\boldsymbol{V_{acc}}$ – volume of compound solution in acceptor well (1.0 cm^3 in the test A → B; 0.3 cm^3 in the test B → A),
$\boldsymbol{V_d}$ – volume of compound solution in donor well (0.3 cm^3 in the test A → B; 1.0 cm^3 in the test B → A),
$\boldsymbol{C_{acc}}$ – concentration of test compound in acceptor well (µM),
$\boldsymbol{C_{initial,d}}$ – initial concentration of test compound in a donor well (µM).
n=3

Sample preparation for dissolution study

Samples preparation for dissolution study were prepared in the requirements of Ph. Eur. regarding dissolution tests. The final concentrations were 0.02 mg/mL for atorvastatin (absorption maximum at λ = 247 nm) and 0.03 mg/mL for lisinopril (absorption maximum at λ = 212 nm).

In vitro dissolution of twelve tablets containing atorvastatin and lisinopril was performed using buffer solutions (pH 1.2; 4.5; 6.8) as the dissolution media at 50 rpm. The dissolution study was carried out in a 900 mL volume of buffer solution 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 5, 15 min. The concentrations of atorvastatin and lisinopril in samples were determined by spectrophotometric method.

Results and discussion

The main parameters that are analyzed in relation to the API for the possibility of the biowaver procedure are solubility and absorption. First of all, it is necessary to establish and discuss the pH-dependent solubility profile of API. A drug substance is considered to be highly soluble if the highest single dose of immediate-release drug is completely dissolved in 250 mL of buffer solutions in the range of pH 1–6.8 at 37 ± 1 °C. However, for confirmation, it is necessary to perform studies in three buffer solutions within this range (at pH 1.2, 4.5 and 6.8) and also additionally at pKa, if its value is in the above pH range. In the procedure, a biowaver based on Biopharmaceutics Classification System (BCS) gives preference to data of complete absorption in humans (Aggelos et al 2019). In this context, the absorption is considered complete if the measured absorption level is ≥ 85%. Complete absorption in most cases is associated with high permeability. Highly soluble drugs with incomplete absorption (class III according to BCS) are entitled to a biowaver procedure based on BCS, provided that certain preconditions regarding the composition of the drug and dissolution *in vitro* are met. In the process of developing a dosage form, dissolution tests are used as a way to identify influencing factors and can have a decisive influence on the bioavailability of the drug. After determining the composition and development of the production process, dissolution tests are used to control the quality of batches made by process scaling and industrial batches to ensure both reproducibility from batch to batch and that dissolution profiles remain similar to batches used in the main clinical trials. In addition, dissolution tests can in some cases be used to refuse *in vivo* bioequivalence studies.

Comparative dissolution profiles obtained by spectrophotomectic method of determination of atorvastatin and lisinopril in tablets are shown in Table 7. The drugs were found to obey beers law at the selected wavelength. The overlain spectra of the atorvastatin and lisinopril in methanol were recorded and λmax values of both drugs and isobestic wavelength were noted.

In all three dissolution media, the releases of atorvastatin and lisinopril of all dosage forms were more than 85% in 15 min (Table 7). The dissolution profile of all the selected brands was estimated to be within the standard limits and was acceptable. In medium with hydrochloric acid pH 1.2, in the medium of acetate buffer solution with a pH of 4.5 and in the medium phosphate buffer solution with a pH of 6.8 for 15 min more than 85% of the active substance passes into solution, hence the dissolution profiles these drugs in these environments are similar, and the drugs in them are "very quickly soluble".

Table 7. Comparative dissolution data of atorvastatin and lisinopril in selected brands.

Brand code	Medium	% dissolved 5 min	% dissolved 15 min
Lisinopril (10 mg) (Tablets A)	pH 1.2	85.67	88.38
	pH 4.5	88.45	91.06
	pH 6.8	87.49	92.09
Lisinopril (10 mg) (Tablets B)	pH 1.2	85.56	87.87
	pH 4.5	87.38	89.18
	pH 6.8	87.94	93.48
Lisinopril (10 mg) (Tablets C)	pH 1.2	85.56	86.38
	pH 4.5	87.85	90.05
	pH 6.8	86.82	92.04
Atorvastatin (10 mg) (Tablets A)	pH 1.2	85.34	95.26
	pH 4.5	85.76	94.94
	pH 6.8	86.39	96.82
Atorvastatin (10 mg) (Tablets B)	pH 1.2	86.95	93.67
	pH 4.5	87.76	94.01
	pH 6.8	90.57	94.27
Atorvastatin (10 mg) (Tablets C)	pH 1.2	85.76	91.69
	pH 4.5	89.19	93.45
	pH 6.8	87.63	92.31

Among the *in vitro* models that make it possible to assess the degree of absorption of API, the most widely used culture of adenocarcinoma cells of the colon – Caco-2.The development of the analytical methodology and its validation is the final stage of both the dissolution study and the Caco-2 test, as well as the biowaver procedure. It plays the most important role in the reliability of the results for all the above procedures and tests.

In our study, optimization and critical evaluation of mobile phase composition, flow rate, and analytical column were important to obtain good resolution of peaks, which in turn affect reproducibility of the method. The resolution of peaks was achieved with Discovery C_{18}, 50 × 2.1 mm, 5 µm column.

Typical multiple reaction monitoring chromatograms of atorvastatin and lisinopril shown in Figs 1, 2. Under these conditions, the peak of vatorvastatin was eluted for about 1.98 min, lisinopril – 1.58 min. The total chromatographic run time was 2.5 min, so the developed analytical method for the determination of atorvastatin and lisinopril to study intestinal permeability in the model of the Caco-2 test was express.

A-B permeability data for the test compound of atorvastatin, lisinopril and the three reference compounds are listed in the Table 8. A-B permeability data for all the reference compounds correspond to the literature data (HPA Culture Collections undated), thus validating this study.

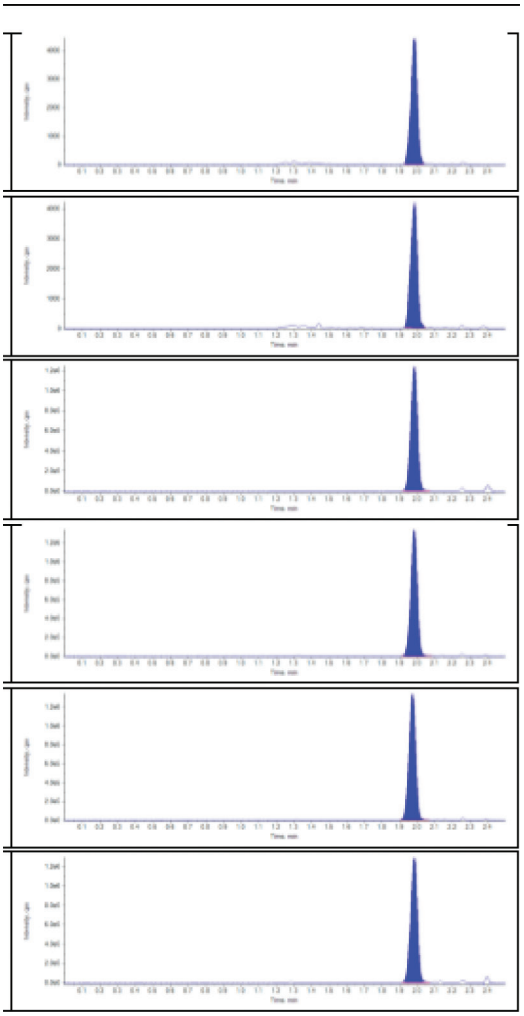

Figure 1. Typical multiple reaction monitoring chromatograms of atorvastatin.

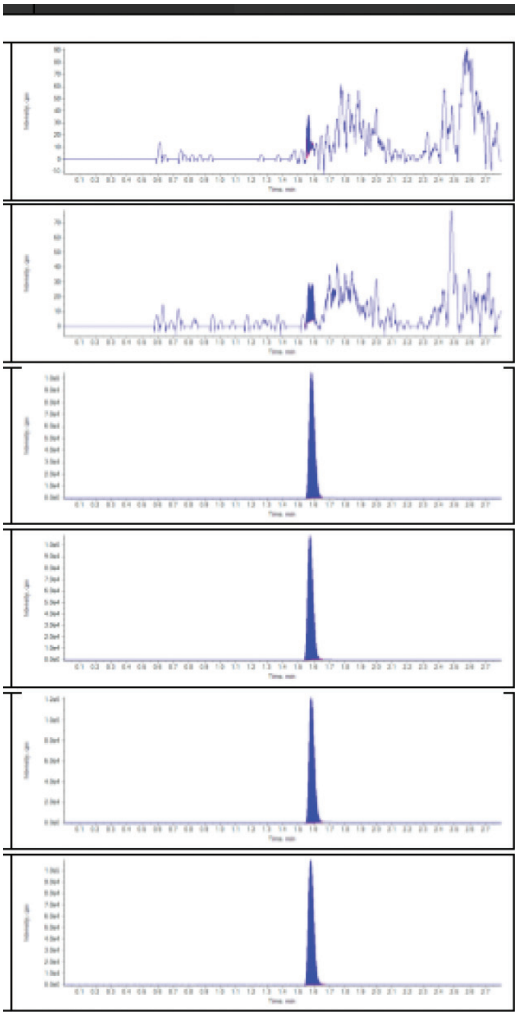

Figure 2. Typical multiple reaction monitoring chromatograms of lisinopril.

Table 8. Permeability coefficient (Papp) of atorvastatin, lisinopril and three reference compounds through the monolayer of Caco-2 cells in the apical-basolateral direction.

Analyte	Papp (AB), 10^{-6} cm/sec			
	1	2	M	m
Propranolol	16.4	16.1	16.2	0.2
Atenolol	0.6	0.7	0.6	0.02
Quinidine	4.2	6.7	5.5	1.8
Lisinopril	0.3	0.2	0.2	0.03
Atorvastatin	0.9	0.8	0.9	0.1

* Abbreviations: M is the arithmetic mean value of the permeability coefficient; m is the standard deviation.

Table 9. Recovery data of transport of atorvastatin, lisinopril and the three reference compounds through a monolayer of cells of the Caco-2.

Analyte	% recovery		
	1	2	M
Propranolol	63	69	66
Atenolol	108	106	107
Quinidine	78	74	76
Lisinopril	95	94	95
Atorvastatin	92	94	93

* Abbreviations: M is the arithmetic mean value of % recovery.

According to the results presented in Table 8, atorvastatin and lisinopril showed low permeability.

Recovery data of transport of atorvastatin, lisinopril and the three reference compounds through a monolayer of cells of the Caco-2 are listed in Table 9. The values of mass balance (recovery) of transport of test and control substances through the monolayer of cells of the Caco-2 line (Table 9) indicate that the results of the experiment can be considered reliable.

The equivalence of the drugs "Lisinopril", tablets of 10 mg and "Atorvastatin", tablets of 10 mg, belongs to class III BCS proven by *in vitro* studies.

Conclusion

Study of the dissolution kinetics of drugs in solid dosage form with lisinopril and atorvastatin and intestinal permeability to assess their equivalence *in vitro* were described. Comparative dissolution profiles obtained by spectrophotomectic method of determination of atorvastatin and lisinopril in tablets shows that in all three dissolution media the releases of atorvastatin and lisinopril of all dosage forms were more than 85% in 15 min. The dissolution profile of all the selected brands was estimated to be within the standard limits and was acceptable. To study permeability, method LC-MS/MS was developed. According to the obtained results, atorvastatin and lisinopril showed low permeability. The values of recovery of transport of test and control substances through the monolayer of cells of the Caco-2 line indicate that the results of the experiment can be considered reliable. The equivalence of the drugs "Lisinopril", tablets of 10 mg and "Atorvastatin", tablets of 10 mg, belongs to class III BCS proven by *in vitro* studies.

Acknowledgements

Authors are grateful to the Ministry of Health of Ukraine Fund for providing scholarship for studies related to solutions for development of original combinations of antihypertensive agents, their analysis and standardization (0120U104201).

References

Aggelos C, Maria S, Christel B, Panos M (2019) The Biopharmaceutics Classification System (BCS) and the Biopharmaceutics Drug Disposition Classification System (BDDCS): Beyond guidelines. International Journal of Pharmaceutics 20: 264–281. https://doi.org/10.1016/j.ijpharm.2019.05.041

Andreas T, Bairachtari K, Georgarakis M (2003) Development of a liquid chromatography–mass spectrometry method for monitoring the angiotensin converting enzyme inhibitor lisinopril in serum. Journal of Chromatography B 783: 425–432. https://doi.org/10.1016/S1570-0232(02)00704-3

Arayne MS, Sultana N, Zuberi MH, Siddiqui FA, Haroon U (2013) Simultaneous determination of metformin, captopril, lisinopril, and enalapril by RP-HPLC: its applications in dosage formulations and in human serum. Medicinal Chemistry Research 22: 5717–5722. https://doi.org/10.1007/s00044-013-0501-z

Arena A, Phillips J (2003) Optimization of Caco-2 cell growth and differentiation for drug transport assay studies using a 96 well MultiScreen Caco-2 Assay System. Millipore protocol note PC1060EN00P. https://www.sigmaaldrich.com/UA/en/technical-documents/technical-article/research-and-disease-areas/pharmacology-and-drug-discovery-research/multiscreen-caco-2-assay-system

Basavaiah K, Tharpa K, Hiriyanna SG, Vinay KB (2009) Spectrophotometric determination of lisinopril in pharmaceuticals using ninhydrin – a modified approach, Journal of Food and Drug Analysis 17: 93–99. http://lawdata.com.tw/File/PDF/J991/A04971702_093.pdf

Beasley CA, Shaw J, Zhao Z, Reed RA (2005) Development and validation of a stability indicating HPLC method for determination of lisinopril, lisinopril degradation product and parabens in the lisinopril extemporaneous formulation. Journal of Pharmaceutical and Biomedical Analysis 37: 559–567. https://doi.org/10.1016/j.jpba.2004.11.021

Chaudhari GB, Patel MN, Shah BP (2007) Stability indicating RP-HPLC method for simultaneous determination of atorvastatin and amlodipine from their combination drug products. Chemical and Pharmaceutical Bulletin 55: 241–246. https://doi.org/10.1248/cpb.55.241

Chauhan V, Prajapati ST, Patel CN (2011) A validated RP-HPLC method for simultaneous estimation of amlodipine and lisinopril in pharmaceutical dosage form. International Journal of Pharmaceutical Sciences and Research 2: 1712–1715. http://dx.doi.org/10.13040/IJPSR.0975-8232.2(7).1712-15

El Gindy A, Ashour A, Abdel-Fattah L, Shabana MM (2001) Spectrophotometric, Spectrofluorimetric and LC determination of lisinopril. Journal of Pharmaceutical and Biomedical Analysis 25: 913–922. https://doi.org/10.1016/S0731-7085(01)00376-4

El-Emam AA, Hansen SH, Moustafa MA, El-Ashry SM, El-Sherbiny DT (2004) Determination of lisinopril in dosage forms and spiked

human plasma through derivatization with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) followed by spectrophtometry or HPLC with fluorimetric detection. Journal of Pharmaceutical and Biomedical Analysis 34: 35–44. https://doi.org/10.1016/j.japna.2003.08.021

GoodRx (undated) 10-most-common-drug-combinations. https://www.goodrx.com/healthcare-access/medication-education/10-most-common-drug-combinations

Hafez HM, Elshanawany AA, Abdelaziz LM, Mohram MS (2014) Development of a stability-indicating HPLC method for simultaneous determination of amlodipine besylate and atorvastatin calcium in tablets. Austin Journal of Analytical and Pharmaceutical Chemistry 1: 1–11. https://austinpublishinggroup.com/analytical-pharmaceutical-chemistry/fulltext/ajapc-v1-id1028.php

HPA [Health Protection Agency] Culture Collections (undated) Permeability values and efflux ratios of test compounds atenolol, propranolol & loperamide in differentiated CACO-2 cultures. https://www.phe-culturecollections.org.uk/media/51860/Permeability_values_and_efflux_ratios_CACO-2.pdf

Huang J, Xu Y, Liu F, Gao S, Guo Q (2006) Developement of a liquid chromatography/tandem mass spectrometry assay for quantification of lisinopril in human plasma. Rapid Commun. Journal of Mass Spectrometry 20: 248–252. https://doi.org/10.1002/rcm.2309

Ivanovic D, Medenica M, Jancic B, Knezevic N (2007) Validation of an analytical procedure for simultaneous determination of hydrochlorothiazide, lisinopril and their impurities. Acta Chromatographica 18: 143–156. https://www.infona.pl/resource/bwmeta1.element.baztech-article-BAT8-0006-0026/tab/summary

Jamakhandi CM, Javali C, Disouza JI, Chougule US, Mullani AK (2011) Spectrophotometric determination of lisinopril dosage form by condensation reaction. International Journal of Pharmacy and Pharmaceutical Sciences 3: 185–187.

Kumar KK, Rao KC, Lakshmi VM, Mukkanti K (2012) A validated stability indicating RP-HPLC method for atorvastatin calcium. American Journal of Analytical Chemistry 3: 392–399. https://doi.org/10.4236/ajac.2012.35052

Lakshmi RV, Sreedevi B (2014) Simultaneous estimation of atorvastatin and olmesartan in bulk and pharmaceutical formulations by RP-HPLC method. International Journal of Innovative Research 2: 1559–1563. https://doi.org/10.5740/jaoacint.11-204

Leis HJ, Fauler G, Raspotnig G, Windischhofer W (1998) Quantitative determination of the angiotensin-converting enzyme inhibitor lisinopril in human plasma by stabile isotope dilution gas chromatography/negative ion chemical ionization mass spectrometry. Rapid Commun. Journal of Mass Spectrometry 12: 1591–1594. https://doi.org/10.1002/(SICI)1097-0231(19981115)12:21%3C1591::AID-RCM368%3E3.0.CO;2-C

Leis HJ, Fauler G, Raspotnig G, Windischhofer W (1999) An improved method for the measurement of the angiotensin-converting enzyme inhibitor lisinopril in human plasma by stabile isotope dilution gas chromatography/negative ion chemical ionization mass spectrometry. Rapid Commun. Journal of Mass Spectrometry13: 650–653. https://doi.org/10.1002/(SICI)1097-0231(19990430)13:8%3C650::AID-RCM536%3E3.0.CO;2-X

Logoyda L (2018) Quantitative determination of amlodipine from Caco-2 cell monolayers by HPLC MS/MS. Asian Journal of Pharmaceutical and Clinical Research 11(6): 204–207. https://doi.org/10.22159/ajpcr.2018.v11i6.24971

Naveed S (2015) Interaction Studies of ACE Inhibitors with Statins. Hypercholesterolemia, Sekar Ashok Kumar, IntechOpen. https://www.intechopen.com/chapters/48034

Naveed S, Sultana N, Arayne MS (2012) Simultaneous determination of lisinopril and H_2 antagonists in API, formulations and human serum by using two different HPLC systems. Medicinal Chemistry Research 21: 4037–4042. https://doi.org/10.4236/ajac.2012.32021

Rahman N, Anwar N, Kashif M (2005a) Application of π-acceptors to the spectrophotometric determination of lisinopril in commercial dosage forms. Il Farmaco 60: 605–611. https://doi.org/10.1016/j.farmac.2005.04.011

Rahman N, Singh M, Hoda Md N (2005b) Optimized and validated spectrophotometric methods for the determination of lisinopril in pharmaceutical formulation using ninhydrin and acid ascorbic. Journal of the Brazilian Chemical Society 16: 1001–1009. https://doi.org/10.1590/S0103-50532005000600018

Sathiyasundar R, Valliappan K (2014) An improved RP-HPLC method for the simultaneous estimation of aspirin, atorvastatin and clopidogrel in pharmaceutical formulation using experimental design methodology. International Journal of Pharmacy and Pharmaceutical Sciences 6: 279–283. https://innovareacademics.in/journals/index.php/ijpps/article/view/2716

Sbârcea L, Udrescu L, Drăgan L, Trandafirescu C, Szabadai Z, Bojiță M (2014) Spectrophotometric method for lisinopril determination using ninhydrin. Farmacia 62: 107–118. https://farmaciajournal.com/arhiva/201401/art-10-sbarcea%20107-118.pdf

Sevin E, Dehouck L, Fabulas-da Costa A (2013) Accelerated Caco-2 cell permeability model for drug discovery. Journal of Pharmacological and Toxicological Methods 68: 334–339. https://doi.org/10.1016/j.vascn.2013.07.004

Shah AD, Bhatt KK, Mehta SR, Shankar BM, Baldania LS (2007) RP-HPLC method for the determination of atorvastatin calcium and nicotinic acid in combined tablet dosage form. Indian Journal of Pharmaceutical Sciences 69: 700–703. https://doi.org/10.4103/0250-474X.38484

Shulyak N, Budzivula K, Kucher T, Kryskiw L, Poliak O, Logoyda L (2021a) Spectrophotometric methods for the determination of lisinopril in medicines. Pharmacia 68: 811–818. https://doi.org/10.3897/pharmacia.68.e72991

Shulyak N, Piponski M, Kovalenko S, Bakovska Stoimenova T, Drapak I, Piponska M, Rezk M, Donkor Abbeyquaye A, Oleshchuk O, Logoyda L (2021b) Chaotropic salts impact in HPLC approaches for simultaneous analysis of hydrophilic and lipophilic drugs. Journal of Separation Science 44: 2908–2916. https://doi.org/10.1002/jssc.202100168

Shulyak N, Piponski M, Kovalenko S, Stoimenova TB, Balkanov T, El-Subbagh HI, Drapak I, Omotosho JO, Logoyda L (2021c) Development of a Novel, Fast, Simple HPLC method for determination of atorvastatin and its impurities in tablets. Scientia Pharmaceutica 89: 1–16. https://doi.org/10.3390/scipharm89020016

Sultana N, Arayne MS, Naveed S (2011) Validated method for the simultaneous determination of lisinopril, pravastatin, atorvastatin and rosuvastatin in API, formulations and human serum by RP-HPLC. Chinese Journal of Chemistry 29: 1216–1220. https://doi.org/10.1002/cjoc.201190226

Srinivasan B, Kolli AR, Esch MB, Abaci HE, Shuler ML, Hickman JJ (2015) TEER measurement techniques for *in vitro* barrier model systems. JLab Autom. 20: 107–126. https://doi.org/10.1177/2211068214561025

Stanisz B, Kania L (2006) Validation of HPLC method for determination of atorvastatin in tablets and for monitoring stability in solid phase. Acta Poloniae Pharmaceutica 63: 471–476. https://pubmed.ncbi.nlm.nih.gov/17438862/