UDC 615.22:615.453.4]-092.4 https://doi.org/10.24959/ubphj.17.121

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Development of methodology for of simultaneous determination of amlodipine, captopril and bisoprolol identification in dosage forms

Topicality. During the sample preparation and chromatographic separation thin layer chromatography (TLC) plays important role. Thin layer chromatography, or TLC, is a method for analyzing mixtures by separating the compounds in the mixture. TLC can be used to help determine the number of components in a mixture, the identity of compounds, and the purity of a compound. For treatment of hypertonic disease almost uses not mono-theraphy but combination of different pharmacological group of medicines.

Aim. To improve to more rapid, simple, selective, more accurate, less expensive methods TLC analysis of simultaneous determination of amlodipine, captopril and bisoprolol and for using this method of analysis for development of bioanalytical methods.

Materials and methods. The present study is assessed mobile phases of amlodipine, captopril and bisoprolol for thin layer chromatography.

Results and discussion. Method of simultaneous identification of amlodipine, captopril and bisoprolol by TLC has been developed. Established that the most optimal Rf observed using mobile phases: ammonia (25 %)-propanol (30 : 70). We have explored the validation characteristics – specificity and suitability of the chromatographic system that met, the eligibility criteria established by the SPU.

Conclusions. We have been developed chromatographic methods for simultaneous determination of amlodipine, captopril and bisoprolol. Propects for future research will be aimed at developing bioanalytical methods of analysis.

***Key words**: amlodipine; captopril; bisoprolol; identification; thin layer chromatography; validation*

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Розробка методу ідентифікації одночасного визначення амлодипіну, каптоприлу та бісопрололу в лікарських формах

Актуальність. Під час пробопідготовки та хроматографічного розділення тонкошарова хроматографія (ТШХ) відіграє важливу роль. Тонкошарова хроматографія або ТШХ – це метод аналізу шляхом розділення сполук у суміші. ТШХ можна використовувати для визначення кількості компонентів у суміші, тотожності та чистоти сполуки. Для лікування гіпертонічної хвороби практично не використовується монотерапія, а комбінація різних фармакологічних груп лікарських засобів.

Метою дослідження було удосконалення більш швидких, простих, вибіркових, точніших, менш дорогих методів аналізу ТШХ для одночасного визначення амлодипіну, каптоприлу та бісопрололу та використання цього методу аналізу для розробки біоаналітичних методів аналізу.

Матеріали та методи. В даному дослідженні оцінюються рухливі фази амлодипіну, каптоприлу та бісопрололу для тонкошарової хроматографії.

Результати та їх обговорення. Розроблено метод одночасної ідентифікації амлодипіну, каптоприлу та бісопрололу за допомогою ТШХ. Встановлено, що найбільш оптимальна Rf спостерігається при використанні: аміаку (25 %) – пропанолу (30 : 70). Були вивчені валідаційні характеристики – специфічність та придатність хроматографічної системи, що відповідали критеріям прийнятності, встановленим ДФУ.

Висновки. Нами був розроблений хроматографічний метод одночасного визначення амлодипіну, каптоприлу та бісопрололу. План майбутніх досліджень буде спрямований на розробку біоаналітичних методів аналізу.

***Ключові слова:** амлодипін; каптоприл; бісопролол; ідентифікація; тонкошарова хроматографія; валідація*

Л. С. Логойда

Разработка методики идентификации одновременного определения амлодипина, каптоприла и бисопролола в лекарственных формах

Актуальность. Во время подготовки образца и хроматографического разделения важную роль играет тонкослойная хроматография (ТСХ). Тонкослойная хроматография или ТСХ представляет собой метод анализа смесей путем разделения соединений в смеси. ТСХ может использоваться для определения количества компонентов в смеси, идентичности соединений и чистоты соединения. Для лечения гипертонического заболевания не используется монотерапия, а комбинации фармакологических групп лекарств.

Целью исследования было улучшить более быстрые, простые, селективные, более точные и менее дорогостоящие методы ТСХ-анализа одновременного определения амлодипина, каптоприла и бисопролола и для использования этого метода анализа для разработки биоаналитических методов.

Материалы и методы. В исследовании оцениваются подвижные фазы амлодипина, каптоприла и бисопролола для тонкослойной хроматографии.

Результаты и их обсуждение. Был разработан метод одновременной идентификации амлодипина, каптоприла и бисопролола с помощью ТСХ. Установлено, что наиболее оптимальная Rf наблюдается с использованием подвижных фаз: аммиак (25 %) – пропанол (30 : 70). Мы изучили характеристики валидации – специфичность и пригодность хроматографической системы, которая соответствовала критериям отбора, установленным ГФУ.

Выводы. Мы разработали хроматографические методы для одновременного определения амлодипина, каптоприла и бисопролола. Перспективы будущих исследований будут направлены на разработку биоаналитических методов анализа.

***Ключевые слова:** амлодипин; каптоприл; бисопролол; идентификация; тонкослойная хроматография; валидация*

INTRODUCTION

Bioanalytical methods are used for the quantitative analysis of drugs and their metabolites in the biological media like saliva, urine, plasma, serum. Development and validation of bioanalytical method is important to understand the pharmacokinetics of any drug and/or its metabolites. Bioanalytical method development consists of three essential interrelated parts sample preparation, chromatographic separation and detection by using proper analytical method. During the sample preparation and chromatographic separation thin layer chromatography (TLC) plays important role. Thin layer chromatography, or TLC, is a method for analyzing mixtures by separating the compounds in the mixture. TLC can be used to help determine the number of components in a mixture, the identity of compounds, and the purity of a compound. By observing the appearance of a product or the disappearance of a reactant, it can also be used to monitor the progress of a reaction.

Hypertonic disease – pathology of the cardiovascular system developing as a result of a primary dysfunction of the superior centers of vascular regulation and of the neurohormonal and renal mechanisms, characterized by arterial hypertension and functional modifications (organic changes in advanced stages) in kidneys, heart, central nervous system, manifested through a high blood pressure over 140/90 mmHg, heart pain and palpitations, headache, dizziness, impaired vision, walking dyspnea, acrocyanosis, edema of soles and calves. For treatment of hypertonic disease almost uses not monotheraphy but combination of different pharmacological group of medicines. Amlodipine, (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate, sold under the brand name Norvasc among others, is a medication used to treat high blood pressure and coronary artery disease. Amlodipine is a member of the dihydropyridine class of calcium antagonists. Captopril, 1-[(2S)-3-mercapto-2-merhyl-1-oxopropyl]-L-proline, (2S)-1-[(2S)-2-Methyl-3-sulphanylpropanoyl]pyrrolidine-2-carboxylic acid, is a sulfhydryl-containing analog of proline with antihypertensive activity and potential antineoplastic activity. Bisoprolol, marketed under the trade name Zebeta or Concor among others, (RS)-1-{4-[(2-isopropoxyethoxy)methyl]phenoxy}-3-(isopropylamino)propan-2-ol, is a medication most commonly used for heart diseases. This specifically includes high blood pressure, chest pain from not enough blood flow to the heart, and heart failure. Bisoprolol is in the β-blocker family of medications and is of the β_1-selective type [1, 2]. A compound can often be measured by several methods and the choice of analytical method involves many considerations, such as chemical properties of the analyte, concentrations levels, sample matrix, cost of the analysis, and speed of the analysis, quantitative or qualitative measurement, and precision required and necessary equipment.

The **aim** is to study and to improve the more rapid, simple, selective, more accurate, less expensive methods TLC analysis of simultaneous determination of amlodipine, captopril and bisoprolol and for using this method of analysis for development of bioanalytical methods.

MATERIALS AND METHODS

Using this technique, we have analyzed medicines "Amlodipine" 10 mg (tablets containing 10 mg of amlodipine produced by "Farmak"), "Capropril" (tablets containing 25 mg of captopril produced by "Ternofarm"), "Bisoprolol-ratiopharm" (tablets containing 10 mg of bisoprolol produced by "Ratiopharm").

Analytical equipment. Scales AVT-120-5D, measuring vessel glass and reagents that meet the SPU requirements. TLC test was carried out using Silica gel, chromatographic plates 60 F254 "Merck" (Germany) and «Sorbfil» (Russia).

Sample preparation for investigation solution. Investigation solution from tablets "Amlodipine", "Capropril", "Bisoprolol-ratiopharm". To sample powder tablets or powder, equivalent to 10.00 mg amlodipine, 25.00 mg captopril, 10.00 mg bisoprolol, add 5.0 ml of *methanol R* and dilute with *methanol R* to 10.0 ml, mix and filter.

Reference solution of amlodipine. 10.00 mg Pharmacopoeial standard sample SPU of amlodipine besilate dissolved in *methanol R* and dilute with the same solvent to 10.0 ml.

Reference solution of captopril. 25.00 mg Pharmacopoeial standard sample SPU of captopril dissolved in *methanol R* and dilute with the same solvent to 10.0 ml.

Reference solution of bisoprolol. 10.00 mg Pharmacopoeial standard sample SPU of bisoprolol fumarate dissolved in *methanol R* and dilute with the same solvent to 10.0 ml.

Mobile phase: *ammonia (25 %)-propanol (30 : 70).*

Samples that are applied: 2 μl, applied the test solutions and investigation solutions.

Over a path of 10 cm from the starting line.

Detection: examination in ultraviolet light at 254 nm.

RESULTS AND DISCUSSION

The present study was assessed the different solvent extracts of amlodipine, captopril and bisoprolol for TLC. The chromatograms obtained with the test solution were detected at the main spot spots basic substance in the chromatograms obtained with reference solutions, corresponding in size and color. We had investigated various mobile phases (solvent system) in order to identify the optimal choice of amlodipine, captopril and bisoprolol investigation by TLC. The factors of mobility in the studied of simultaneous determination of amlodipine, captopril and bisoprolol in mobile phases, are listed in Table.

We have established that the most optimal Rf observed using mobile phases for amlodipine, captopril and bisoprolol: *ammonia (25 %)-propanol (30 : 70)*. The ana-

Table

CHROMATOGRAPHIC CHARACTERISTICS OF AMLODIPINE, CAPTOPRIL AND BISOPROLOL IN DIFFERENT SOLVENT SYSTEMS

Mobile phase	Stationary phase (plate) Rf on "Sorbfil" (Detection in ultraviolet light at 254 nm)		
	Amlodipine	Captopril	Bisoprolol
Chloroform – methanol (9 : 1)	0.37	0.80	0.85
Chloroform – ethanol (8 : 2)	0.25	0.82	0.80
Chloroform – methanol – ammonia (25 %) (4 : 4 : 2)	0.86	0.95	0.76
n-butanol – methanol (3 : 2)	0.65	0.85	0.68
Ammonia (25 %) – propanol (30 : 70)	0.67	0.80	0.47
ethyl acetate – methanol – ammonia (25 %) (4 : 4 : 2)	0.66	0.86	0.89
Propanol – water (70 : 30)	0.35	0.7	0.76

lysis considered probable, though the test requirements "Check suitability chromatographic system".

Chromatographic system is considered appropriate when:

- the chromatogram obtained with reference solution is a clearly visible spot;
- Rf principle spot in the chromatogram obtained with reference solution to be about 0.6.

The process of validating a procedure cannot be separated from its development as the analyst will not know whether the procedure and its performance are acceptable until validation has been performed. However, following best practices requires a highly formalised protocol. Thus, before outlining the validation protocol and the experimental design, it is necessary to qualify all instrumentation and equipment. All chemicals, TLC plates, and reference standards must be defined, specified, and tested. The analytical procedure must be developed, optimised, and documented. A validation protocol, which includes the acceptance criteria and the specified statistical approaches, must be agreed upon and signed. Validated analytical methods play an important role in achieving this goal [3-6]. The results from method validation can be used to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice. Validation of analytical methods is also required by most regulations and quality standards that impact laboratories.

According to the SPU and Note for guidance on validation of analytical procedures: text and methodology (CPMP/ICH/381/95) to test the Identification must be validated, to determine such characteristics as specificity and suitability of the chromatographic system. The maximum difference of Rf values in the same plate (for two series of plates) must not exceed the value of 0.02. Originally, plates were tested according to the requirements of SPU on chromatographic resolution. When checking for the stability of the solution at the time we started chromatography of amlodipine, captopril and bisoprolol freshly prepared test solution sustained, over time for 30 min. Visual assessment of spots on the size and intensity of staining confirms that they clearly appear as freshly cooked and seasoned in time solutions (for plates of different series). The solutions were stable over time and new areas, had been identified. Thus, we explored the validation characteristics – specificity and suitability of the chromatographic system that met, the eligibility criteria established by the SPU. Therefore the present study provided a suitable as well as accurate method for simultaneous determination of amlodipine, captopril and bisoprolol, which is of potential practical significance in development of bioanalytical methods.

CONCLUSIONS

We have developed TLC methods for simultaneous determination of amlodipine, captopril and bisoprolol. We have found that the most optimal Rf observed using mobile phases for simultaneous determination of amlodipine, captopril and bisoprolol: ammonia (25 %)-propanol (30 : 70). The validation study of the characteristics of specificity and suitability of the chromatographic system, confirmed that they meet the eligibility requirements under the SPU. Propects for future research will be aimed at developing bioanalytical methods of analysis.

Conflict of Interests: authors have no conflict of interests to declare.

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Рекомендовано д. біол. н., професором А. Л. Загайком
Надійшла до редакції 20.06.2017 р.