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L. S. Logoyda[1], T. A. Proniv[2], M. I. Dmutriv[2]
I. HORBACHEVSKY TERNOPIL STATE MEDICAL UNIVERSITY[1]
STATE SERVICE ON MEDICINAL PRODUCTS AND DRUGS CONTROL IN TERNOPIL REGION[2]

DEVELOPMENT OF METHODOLOGY FOR IDENTIFICATION OF SIMULTANEOUS DETERMINATION OF NIFEDIPINE, ENALAPRIL AND BISOPROLOL

Introduction. *Active pharmaceutical ingredient (API) 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. Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. TLC can be used to help determining 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.*

The aim of the study *– to develop more rapid, simple, selective, less expensive methods of TLC analysis of simultaneous determination of nifedipine, enalapril and bisoprolol and for using this method of analysis in future for development of bioanalytical methods and pharmacokinetic study.*

Research Methods. *The present study assessed mobile phases of nifedipine, enalapril and bisoprolol for TLC.*

Results and Discussion. *Method of simultaneous identification of nifedipine, enalapril and bisoprolol by TLC was developed. It was established that the most optimal Rf observed using mobile phases for simultaneous determination of nifedipine, enalapril and bisoprolol: chloroform-methanol (9:1). We explored the validation characteristics – specificity and suitability of the chromatographic system that met, the eligibility criteria established by the SPU.*

Conclusion. *We developed chromatographic methods for simultaneous determination of nifedipine, enalapril and bisoprolol. Prospects for future research will be aimed at developing bioanalytical methods of analysis.*

KEY WORDS: **nifedipine; enalapril maleate; bisoprolol fumarate; identification; thin layer chromatography; validation.**

INTRODUCTION. Active pharmaceutical ingredient (API) 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. Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. TLC can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance. The process is similar to paper chromatography with the advantage of faster runs, better separations, and the choice between different stationary phases. Different compounds in the sample mixture travel at different rates due to the differences in their attraction to the stationary phase, and because of differences in solubility in the solvent. By changing the solvent, or perhaps using a mixture, the separation of components (measured by the Rf value) can be adjusted. Also, the separation achieved with a TLC plate can be used to estimate the separation of a flash chromatography column. Separation of compounds is based on the competition of the solute and the mobile phase for binding places on the stationary phase. For instance, if normal phase silica gel is used as the stationary phase it can be considered polar. Given two compounds that differ in polarity, the more polar compound has a stronger interaction with the silica and is, therefore, more capable to dispel the mobile phase from the binding places. As a consequence, the less polar compound moves higher up the plate (resulting in a higher Rf value). If the mobile phase is changed to a more polar solvent or mixture of solvents, it is more capable of dispelling solutes from the silica binding places and all compounds on the TLC plate will move higher up the plate. It is commonly said that "strong" solvents (eluents) push the analyzed com-

pounds up the plate, whereas "weak" eluents barely move them. The order of strength/weakness depends on the coating (stationary phase) of the TLC plate [1].

For treatment of hypertonic disease almost uses not mono-therapy but combination of different pharmacological group of medicines.

The aim of the study – to develop more rapid, simple, selective, less expensive methods TLC analysis of simultaneous determination of nifedipine, enalapril and bisoprolol and for using this method of analysis in future for development of bioanalytical methods and pharmacokinetic study.

RESEARCH METHODS. Using this technique, we have analyzed "Nifedipine" 10 mg (tablets containing 10 mg of nifedipine produced by "Darnitsa"), "Enalozid mono" (tablets containing 10 mg of enalapril maleate produced by "Farmak"), "Bisoprolol" (tablets containing 10 mg of bisoprolol fumarate produced by "Farmak").

All solvents were obtained from Merck pharmaceuticals.

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 "Nifedipine", "Enalozid mono", "Bisoprolol". To sample powder tablets or powder, equivalent to 10.00 mg nifedipine, 10.00 mg enalapril maleate, 10.00 mg bisoprolol fumarate, add 5.0 ml of *methanol R* and dilute with *methanol R* to 10.0 ml, mix and filter.

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

Reference solution of enalapril. 10.00 mg Pharmacopoeial standard sample SPU of enalapril maleate 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: *chloroform-methanol (9:1).*

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

We established that the most optimal Rf observed using mobile phases for simultaneous determination of nifedipine, enalapril and bisoprolol: *chloroform-methanol (9:1).*

The analysis 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.

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 [2–4]. 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

Table – **Chromatographic characteristics for simultaneous determination of nifedipine, enalapril and bisoprolol in different mobile phases**

Mobile phase	Nifedipine	Enalapril	Bisoprolol
Chloroform-methanol (9:1)	0.30	0.56	0.85
Chloroform-ethanol (8:2)	0.93	0.47	0.80
Chloroform-methanol-ammonia (25 %) (4:4:2)	0.60	0.61	0.76
n-Butanol-methanol (3:2)	0.72	0.56	0.68
Ammonia (25 %)-propanol (30:70)	0.70	0.55	0.68
Ethyl acetate-methanol-ammonia (25 %) (17:2:1)	0.51	0.1	0.89
Chloroform-ethanol-ammonia (25 %) (20:5:1)	0.52	0.24	0.78

time we started chromatography of nifedipine, enalapril 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 [5, 6].

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 nifedipine, enalapril and bisoprolol, which is of potential practical significance in development of bioanalytical methods.

CONCLUSIONS. We developed TLC methods for simultaneous determination of nifedipine, enalapril and bisoprolol. We found that the most optimal Rf observed using mobile phases for simultaneous determination of nifedipine, enalapril and bisoprolol: *chloroform–methanol (9:1).* The validation study of the characteristics of specificity and suitability of the chromatographic system, confirmed that they meet the eligibility requirements under the SPU. Prospects for future research will be aimed at developing bioanalytical methods of analysis.

LITERATURE

1. Review on analytical method development and validation. Research and reviews / R. Patil, T. Deshmukh, V. Patil, K. Khandelwal // Journal of Pharmaceutical Analysis. – 2014. – № 3 (3). – P. 1–10.
2. ICH Topic Q2 (R1) Validation of analytical procedures: text and methodology.
3. Liliya L. Development of methods for identification of calcium channel blockers in medicines / L. Liliya, K. Dmutro, S. Stanislav // International Journal of Research Ayurveda Pharmaceutical. – 2016. – № 7 (2). – P. 88–91.
4. Logoyda L. S. Development and methodology for the estimation of bisoprolol in pharmaceuticals / L. S. Logoyda // Medical and Clinical Chemistry. – 2017. – № 2. – P. 34–37.
5. Logoyda L. Development and validation of new methods of analysis for the determination of different natural and synthetic original active pharmaceutical ingredients in medicines / L. Logoyda // Duphat. – 2015. – P. 48.
6. Logoyda L. Validation of chromatographic methods of analysis for the determination of active pharmaceutical ingredients in different medicines / L. Logoyda // Pharma School association for pharmaceutical development and scientific research. Egypt. – 2016. – P. 34.

REFERENCES

1. Patil, R., Deshmukh, T., Patil, V., & Khandelwal, K. (2014). Review on analytical method development and validation. Research and reviews. *Journal of Pharmaceutical Analysis, 3* (3), 1-10.
2. ICH Topic Q2 (R1) Validation of analytical procedures: Text and methodology.
3. Liliya, L. Dmutro, K., & Stanislav, S. (2016). Development of methods for identification of calcium channel blockers in medicines. *International Journal of Research Ayurveda Pharmaceutical, 7* (2), 88-91.
4. Logoyda, L.S. (2017). Development and methodology for the estimation of bisoprolol in pharmaceuticals. *Medical and Clinical Chemistry,* 2, 34-37.
5. Logoyda, L. (2015). Development and validation of new methods of analysis for the determination of different natural and synthetic original active pharmaceutical ingredients in medicines. *Duphat, 48*.
6. Logoyda, L. Validation of chromatographic methods of analysis for the determination of active pharmaceutical ingredients in different medicines. *Pharma. School Association for Pharmaceutical Development and Scientific Research, 34*.

Л. С. Логойда[1], Т. А. Пронів[2], М. І. Дмитрів[2]

ТЕРНОПІЛЬСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО[1]
ДЕРЖАВНА СЛУЖБА З ЛІКАРСЬКИХ ЗАСОБІВ ТА КОНТРОЛЮ ЗА НАРКОТИКАМИ У ТЕРНОПІЛЬСЬКІЙ ОБЛАСТІ[2]

РОЗРОБКА МЕТОДУ ІДЕНТИФІКАЦІЇ ОДНОЧАСНОГО ВИЗНАЧЕННЯ НІФЕДИПІНУ, ЕНАЛАПРИЛУ ТА БІСОПРОЛОЛУ

Резюме

Вступ. *Активний фармацевтичний інгредієнт часто можна визначати кількома способами, і вибір аналітичного методу включає в себе хімічні властивості аналіту, концентрацію, матрицю зразків, вар-*

тість аналізу та швидкість аналізу, кількісне або якісне вимірювання, необхідну точність і необхідне обладнання. Тонкошарова хроматографія (ТШХ) – це метод хроматографії, який використовують для розділення сумішей. Її можна застосовувати для визначення кількості компонентів у суміші, ідентичності сполук та їх чистоти. Спостерігаючи появу продукту або зникнення реагенту, метод також можна використовувати для контролю за ходом реакції.

***Мета дослідження** – розробити більш швидкі, прості, селективні, менш дорогі методи ТШХ-аналізу для одночасного визначення ніфедипіну, еналаприлу та бісопрололу і використати їх для розробки біоаналітичних методів аналізу і фармакокінетичних досліджень.*

***Методи дослідження.** У даному дослідженні було оцінено рухомі фази з метою одночасного визначення ніфедипіну, еналаприлу та бісопрололу для тонкошарової хроматографії.*

***Результати й обговорення.** Розроблено метод одночасної ідентифікації ніфедипіну, еналаприлу та бісопрололу за допомогою ТШХ. Найбільш оптимальний Rf спостерігали при проведенні одночасної ідентифікації ніфедипіну, еналаприлу та бісопрололу: хлороформ – метанол (9:1). Було вивчено характеристики валідації – специфічність та придатність хроматографічної системи, що відповідала критеріям прийнятності, встановленим ДФУ.*

***Висновок.** Ми розробили хроматографічний метод для одночасного визначення ніфедипіну, еналаприлу та бісопрололу. План майбутніх досліджень буде спрямований на розробку біоаналітичних методів аналізу.*

КЛЮЧОВІ СЛОВА: **ніфедипін; еналаприл; бісопролол; ідентифікація; тонкошарова хроматографія; валідація.**

Л. С. Логойда[1], Т. А. Пронив[2], М. И. Дмитрив[2]
ТЕРНОПОЛЬСКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ ИМЕНИ И. Я. ГОРБАЧЕВСКОГО[1]
ГОСУДАРСТВЕННАЯ СЛУЖБА ПО ЛЕКАРСТВЕННЫМ СРЕДСТВАМ И КОНТРОЛЮ ЗА НАРКОТИКАМИ В ТЕРНОПОЛЬСКОЙ ОБЛАСТИ[2]

РАЗРАБОТКА МЕТОДА ИДЕНТИФИКАЦИИ ОДНОВРЕМЕННОГО ОПРЕДЕЛЕНИЯ НИФЕДИПИНА, ЭНАЛАПРИЛА И БИСОПРОЛОЛА

Резюме

***Вступление.** Активный фармацевтический ингредиент часто можно определять несколькими способами, и выбор аналитического метода включает в себя химические свойства аналита, концентрацию, матрицу образцов, стоимость анализа и скорость анализа, количественное или качественное измерение, необходимую точность и необходимое оборудование. Тонкослойная хроматография (ТСХ) – это метод хроматографии, который используют для разделения смесей. Ее можно применять для определения количества компонентов в смеси, идентичности соединений и их чистоты. Наблюдая за появлением продукта или исчезновением реагента, метод также можно использовать для контроля за ходом реакции.*

***Цель исследования** – разработать более быстрые, простые, селективные и менее дорогостоящие методы ТСХ-анализа для одновременного определения нифедипина, эналаприла и бисопролола и использовать их для разработки биоаналитических методов анализа и фармакокинетических исследований.*

***Методы исследования.** В этом исследовании были оценены подвижные фазы с целью одновременного определения нифедипина, эналаприла и бисопролола для тонкослойной хроматографии.*

***Результаты и обсуждение.** Разработан метод одновременной идентификации нифедипина, эналаприла и бисопролола с помощью ТСХ. Наиболее оптимальный Rf наблюдали при проведении одновременной идентификации нифедипина, эналаприла и бисопролола: хлороформ – метанол (9:1). Было изучено характеристики валидации – специфичность и пригодность хроматографической системы, которая соответствовала критериям отбора, установленным ГФУ.*

***Вывод.** Мы разработали хроматографический метод для одновременного определения нифедипина, эналаприла и бисопролола. План будущих исследований будет направлен на разработку биоаналитических методов анализа.*

КЛЮЧЕВЫЕ СЛОВА: **нифедипин; эналаприл; бисопролол; идентификация; тонкослойная хроматография; валидация.**

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Address for correspondence: *L. S. Logoyda, I. Horbachevsky Ternopil State Medical University, Maidan Voli, 1, Ternopil, 46001, Ukraine, e-mail: logojda@tdmu.edu.ua.*