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DEVELOPMENT OF METHODOLOGY FOR SIMULTANEOUS DETERMINATION OF METOPROLOL AND MELDONIUM IN PHARMACEUTICALS BY USING TLC

©M. M. Horyn, L. S. Logoyda, N. O. Zarivna

I. Horbachevsky Ternopil National Medical University

SUMMARY. 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 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.

The aim of the study – to improve to more rapid, simple, selective, less expensive methods TLC analysis of simultaneous determination of metoprolol and meldonium.

Methods. The present study assessed mobile phases of metoprolol and meldonium for TLC.

Results and Discussion. Method of simultaneous identification of metoprolol and meldonium by TLC has been developed. We have established that the most optimal *Rf* observed using mobile phases for simultaneous determination of metoprolol and meldonium: *acetone – water (3: 2)*. We have explored the validation characteristics – specificity and suitability of the chromatographic system that met, the eligibility criteria established by the SPU.

Conclusion. We have developed chromatographic method for simultaneous determination of metoprolol and meldonium. Prospects for future research will be aimed at developing analytical methods of analysis.

KEY WORDS: metoprolol; meldonium; identification; thin layer chromatography; validation.

Introduction. In many experiments, it is important to be able to separate a mixture into its chemical components in order to isolate one compound or to assess the purity of the mixture. Thin layer chromatography (TLC) is one of the easiest and most versatile methods of doing this because of its low cost, simplicity, quick development time, high sensitivity, and good reproducibility. TLC is used by many industries and fields of research, including pharmaceutical production, clinical analysis, industrial chemistry, environmental toxicology, food chemistry, water, inorganic, and pesticide analysis, dye purity, cosmetics, plant materials, and herbal analysis. In its simplest form, glass plates are coated with a uniform layer of silica gel (SiO_2). The dissolved sample is placed on the plate, and the plate is inserted into a screw-top jar containing the developing solvent and a piece of filter paper. When the solvent has risen to near the top of the plate, the plate is removed, dried, and visualized using UV light. Variations on this protocol are used for different purposes, including pretreating the sample, changing the sorbent, plate material, the solvent system, the development techniques, and method of detection and visualization or by coupling TLC to other techniques [1, 2].

Combination therapy of meldonium and metoprolol is used in the treatment of various chronic cardio-vascular diseases and disorders of the cerebral circulation, as well as to improve mental and physical capacity.

The aim of the present study was to improve to more rapid, simple, selective, less expensive methods TLC analysis of simultaneous determination of metoprolol and meldonium.

Methods. Using this technique, we have analyzed medicines Metoprolol 50 mg (tablets containing 50 mg of metoprolol tartrate produced by Farmak), Vasopro (capsules containing 250 mg of meldonium produced by Farmak).

All solvents were obtained from Merck pharamaceticals.

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 Metoprolol, capsules Vasopro. To sample powder tablets, capsules or powder, equivalent to 10.00 mg metoprolol, 20.00 mg meldonium add 5.0 ml of *methanol R* and dilute with *methanol R* to 10.0 ml, mix and filter.

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

Reference solution of meldonium. 20.00 mg Pharmacopoeial standard sample SPU of meldonium dissolved in *methanol R* and dilute with the same solvent to 10.0 ml.

Mobile phase: *acetone – water (3:2).*

Samples that are applied: 20 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, 356 nm, Dragendorff's reagent.

Results and Discussion. The present study was assessed the different solvent extracts of metoprolol and meldonium 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 in order to identify the optimal choice of metoprolol and meldonium investigation by TLC. The factors of mobility in the studied of simultaneous determination of metoprolol and meldonium in mobile phases, are listed in Table 1, 2.

Table 1. Chromatographic characteristics of meldonium in different mobile phases

Mobile phase	Stationary phase (plate) *Rf* on "Sorbfil"	The limit of detection, micrograms	Detection in ultraviolet light at 254 nm	Detection in ultraviolet light at 365 nm	Detection Dragendorff's reagent
Chloroform-methanol (9:1)	–	–	–	–	–
Chloroform-ethanol (8:2)	–	–	–	–	–
Chloroform- methanol-ammonia (25 %) (4:4:2)	–	–	–	–	–
n-butanol-methanol (3:2)	–	–	–	–	–
Ammonia (25 %)-propanol (30:70)	0.23	0.2	–	–	brown
Propanol-water (70:30)	0.16	0.2	–	–	brown
n-butanol-Acetic acid-water (40:10:20)	–	–	–	–	–
Acetone – water (3:2)	0.39	0.2	–	–	brown

Table 2. Chromatographic characteristics of metoprolol in different mobile phases

Mobile phase	Stationary phase(plate) *Rf* on "Sorbfil"	The limit of detection, micrograms	Detection in ultraviolet light at 254 nm	Detection in ultraviolet light at 365 nm	Detection Dragendorff's reagent
Chloroform-methanol (9:1)	0.15	0.2	violet	–	brown
Chloroform-ethanol (8:2)	0.28	0.2	violet	–	brown
Chloroform-methanol-ammonia (25 %) (4:4:2)	0.96	0.2	violet	–	brown
n-butanol-methanol (3:2)	0.15	0.2	violet	–	brown
Ammonia (25 %)-propanol (30:70)	0.82	0.2	violet	–	brown
Propanol-water (70:30)	0.19	0.2	violet	–	brown
n-butanol-acetic acid-water (40:10:20)	0.55	0.2	violet	–	brown
Acetone – water (3:2)	0.26	0.2	violet	–	brown

We have established that the most optimal Rf observed using mobile phases for the simultaneous determination of metoprolol and meldonium: *acetone – water (3: 2).*

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 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 [5–8].

Thus, we have 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 metoprolol and meldonium, which is of potential practical significance in development of analytical methods.

Conclusions. We have developed TLC method for simultaneous determination of metoprolol and meldonium. We have found that the most optimal Rf observed using mobile phases for simultaneous determination of metoprolol and meldonium: *acetone – water (3:2)*. 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 analytical methods of analysis.

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*Огляди літератури, **оригінальні дослідження**, погляд на проблему, випадок з практики, короткі повідомлення*

РАЗРАБОТКА МЕТОДИКИ ИДЕНТИФИКАЦИИ ОДНОВРЕМЕННОГО ОПРЕДЕЛЕНИЯ МЕТОПРОЛОЛА И МЕЛЬДОНИЯ В ЛЕКАРСТВЕННЫХ СРЕДСТВАХ

©М. М. Горин, Л. С. Логойда, Н. О. Заривна

Тернопольский национальный медицинский университет имени И. Я. Горбачевского МОЗ Украины

РЕЗЮМЕ. Активный фармацевтический ингредиент (АФИ) часто можно измерить несколькими способами, и выбор аналитического метода включает в себя множество соображений, таких как химические свойства аналита, уровни концентраций, матрица образцов, стоимость и скорость анализа, количественные или качественное измерение, требуемое и необходимое оборудование. Тонкослойная хроматография (ТСХ) представляет собой метод хроматографии, используемый для разделения смесей. ТСХ может использоваться для определения количества компонентов в смеси, идентичности соединений и чистоты соединения. Наблюдая за появлением продукта или исчезновением реагента, его также можно использовать для контроля за ходом реакции.

Цель – улучшить более быстрые, простые, селективные и менее дорогостоящие методы ТСХ-анализа для одновременного определения метопролола и мельдония.

Методы. В исследовании оцениваются подвижные фазы для одновременного определения метопролола и мельдония для тонкослойной хроматографии.

Результаты. Разработан метод одновременной идентификации метопролола и мельдония с помощью ТСХ. Установлено, что наиболее оптимальная *Rf* наблюдается с использованием подвижных фаз: *ацетон-вода (3:2)*. Мы изучили характеристики валидации – специфичность и пригодность хроматографической системы, которая соответствовала критериям отбора, установленным ГФУ.

Вывод. Разработана хроматографическая методика для одновременного определения метопролола и мельдония. Перспективы будущих исследований будут направлены на разработку аналитических методов анализа.

КЛЮЧЕВЫЕ СЛОВА: метопролол; мельдоний; идентификация; тонкослойная хроматография; валидация.

РОЗРОБКА МЕТОДИКИ ІДЕНТИФІКАЦІЇ ОДНОЧАСНОГО ВИЗНАЧЕННЯ МЕТОПРОЛОЛУ ТА МЕЛЬДОНІЮ В ЛІКАРСЬКИХ ЗАСОБАХ

©М. М. Горин, Л. С. Логойда, Н. О. Зарівна

Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України

РЕЗЮМЕ. Активний фармацевтичний інгредієнт (АФІ) часто можна визначати кількома способами, а вибір аналітичного методу включає в себе багато міркувань, таких як хімічні властивості аналіту, концентрації, матриця зразків, вартість та швидкість аналізу, кількісне або якісне вимірювання, необхідна точність та необхідне обладнання. Тонкошарова хроматографія (ТШХ) – це метод хроматографії, який використовується для розділення сумішей. ТШХ можна використовувати для визначення кількості компонентів у суміші, ідентичності сполук та чистоти сполуки. Спостерігаючи появу продукту або зникнення реагента, його також можна використовувати для контролю прогресу реакції.

Мета – удосконалення більш швидких, простих, вибіркових, менш дорогих методів аналізу ТШХ для одночасного визначення метопрололу та мельдонію.

Матеріал і методи. В дослідженні оцінюються рухливі фази для одночасного визначення метопрололу та мельдонію для тонкошарової хроматографії.

Результати. Розроблено методику одночасної ідентифікації метопрололу та мельдонію за допомогою ТШХ. Встановлено, що найоптимальніша *Rf* спостерігається при використанні одночасної ідентифікації метопрололу та мельдонію: *ацетон-вода (3:2)*. Були вивчені валідаційні характеристики – специфічність та придатність хроматографічної системи, що відповідали критеріям прийнятності, встановленим ДФУ.

Висновок. Розроблена хроматографічна методика одночасного визначення метопрололу та мельдонію. План майбутніх досліджень буде спрямований на розробку аналітичних методів аналізу.

КЛЮЧОВІ СЛОВА: метопролол; мельдоній; ідентифікація; тонкошарова хроматографія; валідація.

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