DEVELOPMENT OF THE METHODOLOGY FOR THE ESTIMATION OF ENALAPRIL MALEATE IN MEDICINES

Introduction. 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.

The aim of the study – to develop a thin layer chromatography method for the estimation enalapril in medicines.

Research Methods. Analysis of enalapril maleate is described in the State Pharmacopeia of Ukraine 2.2 but the aim of our study consisted in the development of rapid, simple, selective, more accurate, less expensive methods for analysis of enalapril maleate and their use for development of bioanalytical methods.

Results and Discussion. Method of identification of enalapril maleate in medicines by TLC was developed. We established that the most optimal Rf observed using mobile phase: ammonia (25 %) – propanol (30:70). The detection limits of enalapril maleate in this system are 0.2 mcg. However, those mobile phase is the most express. We explored the validation characteristics – specificity and suitability of the chromatographic system that met, the eligibility criteria established by the SPU.

Conclusions. We developed chromatographic methods of identification of enalapril maleate in medicines. The proposed method is rapid, economical and simple.

KEY WORDS: enalapril maleate; identification; thin layer chromatography; validation.
(Kyiv, Ukraine). 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**

Investigation solutions from tablets “Enalozid mono”. To sample powder tablets or powder, equivalent to 10.0 mg enalapril maleate, add 5.0 ml of methanol R and dilute with methanol R to 10.0 ml, mix and filter.

Reference solution. 10.0 mg Pharmacopoeial standard sample SPU of enalapril maleate 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: 5 µl, applied the test solution and investigation solutions.

Over a path of 10 cm from the starting line.

Detection: a) examination in ultraviolet light at 254 nm;

b) spray with ninhydrin solution R and dry at 100 °C for 15 min.

**RESULTS AND DISCUSSION.** The present study assessed the different solvent extracts of enalapril maleate in medicines for TLC. We used examination in ultraviolet light at 254 nm and spray with ninhydrin solution R because in structure of enalapril there is a fragment similar to aminoacids. The chromatogram obtained with the test solution is detected at the main spot basic substance in the chromatogram 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 enalapril maleate in medicines investigation by TLC. The factors of mobility in the studied of enalapril maleate in mobile phases, are listed in Table.

We found that for identification by TLC it should be used a sensitive of all investigated solvents. We established that the most optimal RF was observed using mobile phases for enalapril maleate: ammonia (25%) – propanol (30:70). The detection limit is 0.2 mcg. However, the most express mobile phase is ammonia (25%) – propanol (30:70). 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.

We previously studied the behavior of placebo tablets in terms of methods of identification of enalapril maleate. It was established that the excipients are part of tablets and do not affect the sensitivity and specificity of enalapril detection.

Validated analytical methods play an important role in achieving this goal. 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 [3–6]. 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 enalapril freshly prepared test solution sustained, over time for 30 min. Visual

| Mobile phase                  | Stationary phase (plate) Rf on “Sorbfil” | The limit of detection, micrograms | Detection in ultraviolet light at 254 nm | Detection ninhydrin solution R |
|------------------------------|------------------------------------------|------------------------------------|----------------------------------------|--------------------------------|
| chloroform-methanol (9:1)    | 0.56                                     | 0.2                                | violet                                 | violet-blue                    |
| chloroform-ethanol (8:2)     | 0.47                                     | 0.2                                | violet                                 | violet-blue                    |
| chloroform-methanol-ammonia (25%) (4:4:2) | 0.61                          | 0.2                                | violet                                 | violet-blue                    |
| n-butanol-methanol (3:2)     | 0.56                                     | 0.2                                | violet                                 | violet-blue                    |
| ammonia (25%)-propanol (30:70) | 0.55                             | 0.2                                | violet                                 | violet-blue                    |
| ethyl acetate-methanol-ammonia (25%) (17:2:1) | 0.1                             | 0.2                                | violet                                 | violet-blue                    |
| chloroform-ethanol-ammonia (25%) (20:5:1) | 0.24                            | 0.2                                | violet                                 | violet-blue                    |
| propanol-water (70:30)       | 0.52                                     | 0.2                                | violet                                 | violet-blue                    |
| n-butanol-acetic acid-water (40:10:10) | 0.46                           | 0.2                                | violet                                 | violet-blue                    |
| n-butanol-acetic acid-water (40:10:20) | 0.61                           | 0.2                                | violet                                 | violet-blue                    |
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.

CONCLUSIONS. In conclusion, we developed TLC methods of identification of enalapril maleate in medicines. We found that enalapril identification by TLC using a sensitive of all investigated solvents. We established that the most optimal Rf observed using mobile phases for enalapril maleate: ammonia (25 %) – propanol (30:70). The detection limit is 0.2 mcg. The validation study of the characteristics of both specificity and suitability of the chromatographic system, confirmed that they meet the eligibility requirements under the SPU.

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Розробка методики визначення еналаприлу в лікарських засобах

Резюме

Вступ. Тонкошарова хроматографія – це метод аналізу суміші шляхом розділення сполук у суміші. Вона може використовуватися для визначення кількості компонентів у суміші, ідентифікації речовин та встановлення їх чистоти.

Мета дослідження – розробити метод тонкошарової хроматографії для ідентифікації еналаприлу малеату в лікарських засобах.

Методи дослідження. Метод аналізу еналаприлу малеату описано в Державній Фармакопеї України 2.2, але мета нашого дослідження полягала у використанні швидших, простих, вибіркових, точних, надійних, менш дорогих методів аналізу еналаприлу малеату та використанні їх для розробки біоаналітичних методів.

Результати й обговорення. Розроблено метод ідентифікації еналаприлу малеату в лікарських засобах за допомогою тонкошарової хроматографії. Найбільш оптимальний Rf встановлено при використанні рухомої фази: аміак (25 %) – пропанол (30:70). Межа виявлення еналаприлу малеату в цій системі становила 0,2 мкг. Однак дана мобільна фаза є найбільш вираженою. Було вивчено також деякі характеристики валидacji – специфічність та придатність хроматографічної системи, яка відповідала критеріям прийнятності, встановленим Державною Фармакопеєю України.

Висновок. Було розроблено хроматографічний метод ідентифікації еналаприлу малеату в лікарських засобах. Запропонований метод є швидким, економічним та простим.

Ключові слова: еналаприл малеат; ідентифікація; тонкошарова хроматографія; валидация.