ORIGINAL ARTICLE

Development of methods for the chromatographic identification of active pharmaceutical ingredient from group of angiotensin-converting enzyme inhibitors in pharmaceuticals

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Abstract

Introduction: Thin-layer chromatography (TLC) is a chromatography used to separate non-volatile mixtures. TLC can be used for monitoring the progress of a reaction, identification compounds present in a given mixture, and determination of purity of an active pharmaceutical ingredient (API). Analysis of API from group of angiotensin-converting enzyme inhibitors is described in pharmacopeia, but the aim of our researchers was to improve to more rapid, simple, and less expensive methods. TLC analysis of enalapril, captopril, fosinopril, and perindopril in pharmaceuticals and for using this method of analysis in future for the development of bioanalytical methods. **Materials and Methods:** The present study is assessed system solvents of enalapril, captopril, fosinopril, and perindopril for TLC. **Results:** We have established that the most perfect Rf observed using mobile phases: Ammonia (25%) - propanol (30:70) for enalapril, chloroform - methanol (9:1) for captopril, ammonia (25%) - propanol (30:70) for perindopril, and *n*-butanol - methanol (3:2) for fosinopril. However, those mobile phases are the most expressive. We have explored the validation characteristics - specificity and suitability of the chromatographic system according to SPU. **Conclusion:** We have developed chromatographic methods of identification of enalapril, captopril, fosinopril, and perindopril in pharmaceuticals. The developed methods are rapid, economical, simple, and applicable to the analysis of pharmaceutical dosage forms. These methods can also give excellent results and can be employed for the routine analysis. Prospects for future research will be aimed at developing of bioanalytical methods of determination of enalapril, captopril, fosinopril, perindopril in medicines, and for analysis of their metabolites.

Key words: Captopril, enalapril, fosinopril, perindopril, thin-layer chromatography, validation

INTRODUCTION

Thin-layer chromatography (TLC) is a chromatography used to separate non-volatile mixtures. TLC can be used for monitoring the progress of a reaction, identification compounds present in a given mixture, and determination of 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 due to differences in solubility in the solvent. By changing the solvent, or perhaps using a mixture, the separation of components can be adjusted.

An angiotensin-converting enzyme inhibitors (ACE inhibitors) are medicines used for the treatment of hypertension and congestive heart failure.[1-6]

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Analysis of active pharmaceutical ingredient (API) from group of ACE inhibitors is described in pharmacopeia, but the aim of our researchers was to improve to more rapid, simple, and less expensive methods. TLC analysis of enalapril, captopril, fosinopril, and perindopril in pharmaceuticals and for using this method of analysis in future for the development of bioanalytical methods.[6-8]

MATERIALS AND METHODS

We have analyzed tablets «Enalapril» (tablets containing 10 mg of enalapril maleate produced by «Zdorovja»), «Captopress» (tablets containing 25 mg of captopril produced by «Darnitsa»), «Perindopril» (tablets containing 4 mg of perindopril produced by «Serdia Pharmaceuticals»), and «Fosinace» (tablets containing 10 mg of fosinopril sodium produced by «Cipla Limited»).

All solvents were obtained from Merck pharmaceuticals.

Analytical Equipment

TLC test was carried out using silica gel, chromatographic plates 60 F254 «Merck» (Germany) and «Sorbfil» (Russia).

Sample Preparation of Enalapril

Investigation solutions from tablets «Enalapril». 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. About 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: Examination in ultraviolet light at 254 nm.

Sample Preparation of Captopril

Investigation solutions from tablets «Captopress». To sample powder tablets or powder, equivalent to 10.0 mg captopril, add 5.0 ml of methanol R and dilute with methanol R to 10.0 ml, mix and filter.

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

- Mobile phase: Chloroform - methanol (9:1).
- Samples that are applied: 5 µl applied the test solution and investigation solutions.
- Over a path of 10 cm from the starting line.
- Detection: Examination in ultraviolet light at 254 nm.

Sample Preparation of Perindopril

Investigation solutions from tablets «Perindopril». To sample powder tablets or powder, equivalent to 4.0 mg perindopril, add 5.0 ml of methanol R and dilute with methanol R to 10.0 ml, mix and filter.

Reference solution. About 4.0 mg pharmacopoeial standard sample of perindopril 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: Examination in ultraviolet light at 254 nm.

Sample Preparation of Fosinopril

Investigation solutions from tablets «Fosinace». To sample powder tablets or powder, equivalent to 10.0 mg fosinopril sodium, add 5.0 ml of methanol R and dilute with methanol R to 10.0 ml, mix and filter.

Reference solution. About 10.0 mg pharmacopoeial standard sample of fosinopril sodium dissolved in methanol R and dilute with the same solvent to 10.0 ml.

- Mobile phase: n-butanol - methanol (3:2).
- Samples that are applied: 5 µl applied the test solution and investigation solutions.
- Over a path of 10 cm from the starting line.
- Detection: Examination in ultraviolet light at 254 nm.

RESULTS

The present study was assessed the different solvent extracts of enalapril, captopril, fosinopril, and perindopril in medicines for TLC. For detection, we have used examination in ultraviolet at 254 nm. The chromatograms obtained with the test solutions are detected at the main spot spots basic substance in the chromatograms obtained with reference solutions, corresponding in size and color. We had investigated various system solvents to identify the optimal for investigation of enalapril, captopril, fosinopril, and perindopril in medicines investigation.[9-11] Rf of enalapril, captopril, fosinopril, and perindopril in mobile phases are listed in Tables 1–4. Representative chromatograms present in Figures 1–4.

DISCUSSION

In our study, in developing the methods of identification of enalapril, captopril, fosinopril, and perindopril in different

Table 1: Chromatographic characteristics of enalapril maleate («Enalapril»)

Mobile phase	Stationary phase Rf on «Sorbfil»	The limit of detection, micrograms	Detection in ultraviolet light at 254 nm
Chloroform - methanol (9:1)	0.56	0.2	violet
Chloroform - ethanol (8:2)	0.47	0.2	violet
Chloroform - methanol -ammonia (25%) (4:4:2)	0.61	0.2	violet
n-butanol - methanol (3:2)	0.56	0.2	violet
Ammonia (25%) - propanol (30:70)	0.55	0.2	violet
Ethyl acetate - methanol - ammonia (25%) (17:2:1)	0.1	0.2	violet
Chloroform – ethanol - ammonia (25%) (20:5:1)	0.24	0.2	violet
Propanol - water (70:30)	0.52	0.2	violet

Table 2: Chromatographic characteristics of captopril («Captopress»)

Mobile phase	Stationary phase Rf on «Sorbfil»	The limit of detection, micrograms	Detection in ultraviolet light at 254 nm
Chloroform - methanol (9:1)	0.60	0.4	violet
Chloroform – methanol - ammonia (25%) (4:4:2)	0.95	0.4	violet
n-butanol - methanol (3:2)	0.81	0.4	violet
Ammonia (25%) - propanol (30:70)	0.80	0.4	violet
Propanol - water (70:30)	0.75	0.4	violet

Table 3: Chromatographic characteristics of perindopril («Perindopril»)

Mobile phase	Stationary phase Rf on «Sorbfil»	The limit of detection, micrograms	Detection in ultraviolet light at 254 nm
Chloroform - methanol (9:1)	0.87	0.4	violet-blue
Chloroform – methanol - ammonia (25%) (4:4:2)	0.89	0.4	violet-blue
n-butanol - methanol (3:2)	0.80	0.4	violet-blue
Ammonia (25%) - propanol (30:70)	0.72	0.4	violet-blue
Propanol - water (70:30)	0.85	0.4	violet-blue

Table 4: Chromatographic characteristics of fosinopril («Fosinace»)

Mobile phase	Stationary phase Rf on «Sorbfil»	The limit of detection, micrograms	Detection in ultraviolet light at 254 nm
Chloroform - methanol (9:1)	0.87	0.4	violet
Chloroform - methanol - ammonia (25%) (4:4:2)	0.92	0.4	violet
n-butanol - methanol (3:2)	0.73	0.4	violet
Ammonia (25%) - propanol (30:70)	0.84	0.4	violet
Propanol - water (70:30)	0.85	0.4	violet

solvent systems, we have investigated the sensitivity of detection enalapril, captopril, fosinopril, and perindopril. The detection limits of enalapril, captopril, fosinopril, perindopril are given in Tables 1–4. We have found that enalapril, captopril, fosinopril, and perindopril identification by TLC using a sensitive of all investigated solvents. We have established that the most perfect Rf observed using system solvents: Ammonia (25%) - propanol (30:70) for enalapril, chloroform - methanol (9:1) for captopril, ammonia (25%) - propanol (30:70) for perindopril, and *n*-butanol - methanol (3:2) for fosinopril. The detection limits in those systems are 0.2–0.4 mcg. However, mobile phases are the most expressive.

According to test requirements «check suitability chromatographic system», chromatographic system is considered appropriate when:

- The chromatogram obtained with reference solution is a clearly visible spot;

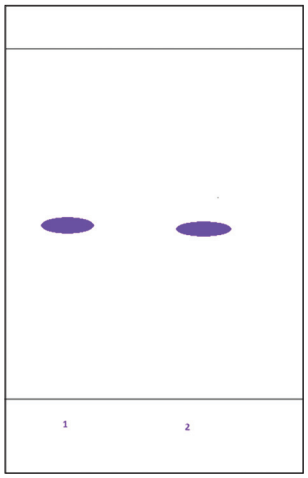

Figure 1: Representative chromatogram of enalapril maleate using ultraviolet detection at 254 nm, mobile phase - ammonia (25%) - propanol (30:70) (1 - solutions from tablets «Enalapril», 2 - solutions from pharmacopoeial standard sample SPU of enalapril maleate)

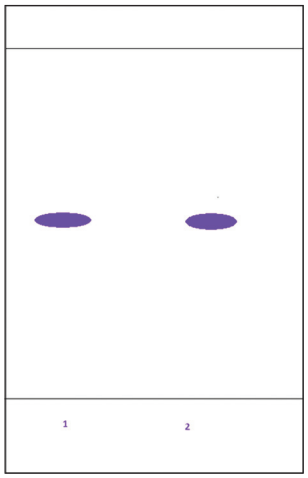

Figure 2: Representative chromatogram of captopril using ultraviolet detection at 254 nm, mobile phase – chloroform - methanol (9:1) (1 - solutions from tablets «Captopress», 2 - solutions from pharmacopoeial standard sample SPU of captopril)

- Rf principle spot in the chromatogram obtained with reference solution to be about 0.6.

We have studied the placebo of tablets in terms of identification of enalapril, captopril, fosinopril, and perindopril in tablets. It has established that the excipients and another API do not affect the sensitivity and specificity of enalapril, captopril, fosinopril, and perindopril detection.

According to validation procedures: Text and methodology to test the identification must be validated, to determine such characteristics as specificity and suitability of the chromatographic system.[9,12-15] The maximum difference of Rf values in the same plate (for two series of plates) must not exceed the number of 0.02. Originally, plates were tested

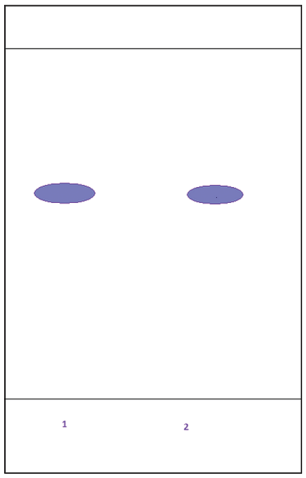

Figure 3: Representative chromatogram of perindopril using ultraviolet detection at 254 nm, mobile phase - ammonia (25%) - propanol (30:70) (1 - solutions from tablets «Perindopril», 2 - solutions from standard sample of perindopril)

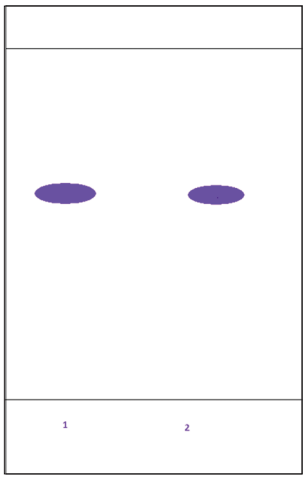

Figure 4: Representative chromatogram of fosinopril using ultraviolet detection at 254 nm, mobile phase - *n*-butanol - methanol (3:2) (1 - solutions from tablets «Fosinace», 2 - solutions from standard sample of fosinopril)

according to the requirements of SPU on chromatographic resolution. When checking for the stability of the solution at the time, we have started chromatography of enalapril, captopril, fosinopril, and perindopril 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.

We have explored the validation parameters - specificity and suitability according to SPU. Therefore, the present study provided a suitable as well as accurate method for determination of enalapril, captopril, fosinopril, and perindopril, which is of potential practical significance in the development of bioanalytical methods for those API.

CONCLUSION

We have developed chromatographic methods of identification of enalapril, captopril, fosinopril, and perindopril in tablets. We have established that the most optimal Rf observed using mobile phases: Ammonia (25%) - propanol (30:70) for enalapril, chloroform - methanol (9:1) for captopril, ammonia (25%) - propanol (30:70) for perindopril, and *n*-butanol - methanol (3:2) for fosinopril. The validation study of the characteristics of both specificity and suitability of the chromatographic system, according to SPU. These methods can also give excellent results and can be employed for the routine analysis. Prospects for future research will be aimed at developing of bioanalytical methods of determination of enalapril, captopril, fosinopril, perindopril in medicines, and for analysis of their metabolites.

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