DEVELOPMENT OF METHODOLOGY FOR THE SIMULTANEOUS DETERMINATION OF AMLODIPINE, CAPTOPRIL AND BISOPROLOL IDENTIFICATION IN DOSAGE FORMS

Topicality. During the sample preparation and chromatographic separation liquid layer chromatography (LIC) plays an 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-therapy 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. Prospects 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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Development of methods of identifying simultaneous determination of amlodipine, captopril and bisoprolol in drug forms

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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, chest pain from not enough blood flow detected at the main spot spots basic substance in the chromatograms obtained with test solution and reference solutions. We had investigated various mobile phases (solvent systems) in order to identify the optimal choice of amlodipine, captopril and bisoprolol for 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”), “Captopril” (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”, “Captopril”, “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-
Validation of 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. Prospects for future research will be aimed at developing bioanalytical methods of analysis.

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

**REFERENCES**

1. Electronic Medicines Compendium (eMC). – Available at: www.medicines.org.uk

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>Stationary phase (plate) Rf on “SorbFil”</th>
<th>Amlodipine</th>
<th>Captopril</th>
<th>Bisoprolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform – methanol (9 : 1)</td>
<td>0.37</td>
<td>0.80</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Chloroform – ethanol (8 : 2)</td>
<td>0.25</td>
<td>0.82</td>
<td>0.80</td>
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</tr>
<tr>
<td>Chloroform – methanol – ammonia (25 %) (4 : 4 : 2)</td>
<td>0.86</td>
<td>0.95</td>
<td>0.76</td>
<td></td>
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<tr>
<td>n-butanol – methanol (3 : 2)</td>
<td>0.65</td>
<td>0.85</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>Ammonia (25 %) – propanol (30 : 70)</td>
<td>0.67</td>
<td>0.80</td>
<td>0.47</td>
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</tr>
<tr>
<td>Ethyl acetate – methanol – ammonia (25 %) (4 : 4 : 2)</td>
<td>0.66</td>
<td>0.86</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Propanol – water (70 : 30)</td>
<td>0.35</td>
<td>0.7</td>
<td>0.76</td>
<td></td>
</tr>
</tbody>
</table>
REFERENCES

1. Electronic Medicines Compendium (eMC). Available at: www.medicines.org.uk

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