SELECTION AND VERIFICATION OF THE METHOD FOR PHYNLEFRINE HYDROCHLORIDE ASSAY IN SIMANOVSKY OINTMENT

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Phenylephrine hydrochloride – a synthetic substitute for adrenaline that has vasoconstrictive and α-adrenomimetic action [1, 2]. It is part of the eye drops [1, 3], solution for injection [3], nasal drops [2, 3], and powders and tablets for the treatment of colds [1, 2].

It is a synthetic substitute for adrenaline that has vasoconstrictive and α-adrenomimetic action [1, 2].

1. Introduction

Phenylephrine hydrochloride (Fig. 1) – a synthetic substitute for adrenaline that has vasoconstrictive and α-adrenomimetic action [1, 2].

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

Since the ointment is being prepared for stock, in accordance with the requirements of the State Pharmacopoeia of Ukraine (SPhU) necessary to develop a technological instruction of the ointment preparation with a description of the methods for assay of its active components. In addition, the methods of quantitative determination are necessary to increase the ointment shelf life during the analysis of its stability.

3. Analysis of recent studies and publications

Monographs on phenylephrine hydrochloride are part of many pharmacopoeias. SPhU [9], European [10] and British [11] Pharmacopoeias are recommending using alkalimetric titration with a potentiometric determination of the equivalence point for its quantitative determination. According to the Chinese [12], Japanese [13] and USP [14] Pharmacopoeias requirements assay of the phenylephrine hydrochloride substance is carried out using bromatometric reverse titration.

For the quantitative determination of phenylephrine hydrochloride in a variety of dosage forms it
is proposed to use the method of spectrophotometry. Direct spectrophotometry method recommended for its quantitative determination in solution for injection [1], drops for the nose [3], in capsules with chlorpheniramine maleate [2], in combined drops with dimethindene maleate [4]. It is also recommended to carry out a spectrophotometric determination of phenylephrine hydrochloride by reaction with diazotized metoclopramide hydrochloride [7], after interaction with 4-aminopyridine followed by the formation of a complex with copper (II) [8], by using haematoxylin [15], with alizarin dyes [16], by reaction with ninhydrin [17], with iron (III) ions and subsequent addition of 2,2'-bipyridyl [18].

Indirect spectrophotometric methods after adding of chloramine-T and rhodamine-B [19] and after adding of N-bromosuccinimide and indigo carmine for its assay were also proposed [20].

In addition, for the simultaneous quantitative determination of phenylephrine hydrochloride with other components of dosage forms, it is recommended to use the method of derivative spectrophotometry. For example, to determine it in combination with tropicamide in eye drops [21], cetirizine hydrochloride by simultaneous equation method and first order derivative spectroscopy [5], dual wavelength, absorbance ratio and mean centering of ratio spectra methods [22], absorbance ratio and area under curve methods [23], paracetamol by the graphical, simultaneous equation, first order derivative and absorbance ratio methods [6], paracetamol and chlorpheniramine maleate by simultaneous equation, absorbance ratio and area under curve methods [24], chlorpheniramine maleate [25] in combined dosage forms.

4. Allocation of unsolved parts of the general problem, which is dedicated to the article

Literature review of the developed methods for phenylephrine hydrochloride assay showed that there is no description of the methods for its quantitative determination in the composition of the Simanovsky ointment.

5. Formulation of goals (tasks) of the article

The task of the work was to select and verify the optimal method for phenylephrine hydrochloride assay in the Simanovsky ointment with the possibility of its further use for the analysis of the dosage form stability during storage.

6. Presentation of the main material of the research (methods and objects) with the justification of the results

Class A volumetric glassware, reagents which meet the requirements of the SPbU, analytical balance AXIS ANG 200 (Poland), spectrophotometer Evolution 60s (USA) with 1 cm cell, phenylephrine hydrochloride substance (series PPHLP60001 produced by Unichem Laboratories LTD, India) were used for the analysis.

Tested solution. 1.0000 g of ointment was heated in a water bath with 10 ml of 0.1 M hydrochloric acid, carefully mixed with a glass rod, cooled and filtered in the volumetric flask of 25.0 ml. The operation was repeated two more times, using 5 ml of 0.1 M hydrochloric acid. The volume of the solution was adjusted to the mark 25.0 ml with a 0.1 M hydrochloric acid and stirred.

Reference solution: 0.020 g of phenylephrine hydrochloride standard sample was dissolved in 0.1 M hydrochloric acid in the 25.0 ml volumetric flask, diluted to the mark 25.0 ml with the same solvent. 1.0 ml of this solution was placed into the 10.0 ml volumetric flask and adjusted to the mark with 0.1 M hydrochloric acid.

Compensation solution. 0.1 M hydrochloric acid.

The method of direct spectrophotometry was chosen for the phenylephrine hydrochloride assay in the studied ointment. Due to the fact that compound is a salt of weak base and strong acid, phenylephrine hydrochloride can be extracted from the ointment with 0.1 M hydrochloric acid. The possibility of using this solvent has been proven in the analysis of the solution for injections of phenylephrine hydrochloride [1], so it was chosen for extraction procedure. The optical density of the test and the reference solutions was measured at a wavelength of 273 nm relative to the compensation solution (Fig. 2).

![Fig. 2. Absorption spectra of solutions absorbance: 1 – standard sample of phenylephrine hydrochloride, 2 – ointment extract, 3 – placebo in 0.1 M hydrochloric acid](image)

For study the specificity of the method (δ<sub>noise</sub>, %), a solution of placebo was made using the method of the test solution preparing. During preparation the ointment without phenylephrine hydrochloride was used. The optical density of the placebo solution was determined three times with the removal of the cuvette with a paral-
lel measurement of the reference solution optical density. The resulting spectrum (Fig. 2) indicates the absence of influence of the other ointment components on the results of phenylephrine hydrochloride assay. Contribution of placebo was calculated by the following formula (1) and found that it has a negligible effect on the total absorption of the medicine.

\[
\frac{A_{\text{blank}}}{A_{\text{d}}} \times 100 = \frac{0.002}{0.429} \times 100 = 0.47 \% \leq \max \delta \leq 1.02 \%
\]

The next stage of the study was the verification of the robustness of the method. The stability of solutions in time has been studied for this purpose.

The optical density of the ointment test and reference solutions in the maximum absorption at a wavelength of 273 nm was measured. Measurements were carried out immediately after preparation of solutions, and then after 15, 30, 45 and 60 minutes. The obtained results indicate that the solution is stable for an hour (Table 1).

Linearity, accuracy and precision of the method in the concentration range of 80–120 % of the nominal concentration of the test substance in the ointment were also studied. In the chosen range, nine concentrations were investigated with a 5 % step. According to the results of the research, the parameters that characterize the linear dependence (Table 2) were calculated. The criteria for linear dependency parameters were calculated according to tolerances in the content of compounding ointments components (±10 %).

The obtained values indicate that the requirements for all parameters of linear dependence are met. According to the results of the linearity study, a calibration curve was constructed in normalized coordinates (Fig. 3).

### Table 1

<table>
<thead>
<tr>
<th>Solution</th>
<th>The term of the stability study (t, min.)</th>
<th>Mean</th>
<th>RSD, %</th>
<th>Δt, %</th>
<th>max δ, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tested</td>
<td>0.435</td>
<td>0.435</td>
<td>0.436</td>
<td>0.435</td>
<td>0.435</td>
</tr>
<tr>
<td>Reference</td>
<td>0.428</td>
<td>0.429</td>
<td>0.429</td>
<td>0.428</td>
<td>0.428</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Validation characteristic</th>
<th>Value</th>
<th>Permissible criteria</th>
<th>Conclusion on compliance</th>
</tr>
</thead>
<tbody>
<tr>
<td>( b )</td>
<td>0.98</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>( S_b )</td>
<td>0.012</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>((b-1))</td>
<td>0.020</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>( a )</td>
<td>2.31</td>
<td>statistical insignificance ( a \leq t(95%, n-2) \times S_a ) (( a \leq 2.32 )) practical insignificance ( a \leq 5.12 )</td>
<td>fulfilled by both criteria</td>
</tr>
<tr>
<td>( S_a )</td>
<td>1.22</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>( S_0 )</td>
<td>0.47</td>
<td>( \max S_0=1.69 )</td>
<td>correspond</td>
</tr>
<tr>
<td>( S_Y )</td>
<td>13.69</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>( r )</td>
<td>0.9994</td>
<td>( \min r=0.9924 )</td>
<td>correspond</td>
</tr>
</tbody>
</table>

Fig. 3. Graph of the linear dependence of optical density of the phenylephrine hydrochloride concentration in normalized coordinates
Simultaneously with the study of linearity, the study of the accuracy and precision parameters was performed. It was done using the obtained data during the study of the method linearity according to the standardized procedure (Table 3).

The obtained results testify the compliance of the validation parameters with the requirements of the SPhU. Thus, the method can be used to analyze the researched ointment and study its stability.

Table 3
Results of study of precision and accuracy of the spectrophotometric method of phenylephrine hydrochloride assay

<table>
<thead>
<tr>
<th>Validation characteristics</th>
<th>The obtained value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>100.20</td>
</tr>
<tr>
<td>S_Z</td>
<td>0.21</td>
</tr>
<tr>
<td>( \Delta_Z ) (0.39\leq\Delta_Z \leq3.20)</td>
<td></td>
</tr>
<tr>
<td>( \delta ) (0.20)</td>
<td></td>
</tr>
<tr>
<td>Criterion of practical insignificance ( \delta ), %\leq0.13</td>
<td></td>
</tr>
</tbody>
</table>

An ointment analysis using this method was conducted. Calculation of the quantitative content of phenylephrine hydrochloride in mg was carried out in two ways: by the standard method (2) and by the specific absorption index method (3) for determine the optimal method of calculation.

\[
X, \text{mg} = \frac{A \cdot m_{SS} \cdot V_{v.f.} \cdot V_{f.SS} \cdot m_{ss}}{A_{ss} \cdot m_{out sample} \cdot V_{f.SS}} \times 1000
\]  

(2)

\[
X, \text{mg} = \frac{A \cdot V_{f.SS} \cdot m_{out sample} \times 1000}{A_{m} \cdot V_{m} \cdot m_{int sample} \cdot 100}
\]  

(3)

where: \( A \) – optical density of the tested solution; \( A_{ss} \) – optical density of the reference solution; \( m_{SS} \) – sample weight of the phenylephrine hydrochloride standard sample, g; \( m_{out sample} \) – the weight of the ointment sample for the analysis, g; \( m_{int} \) – total mass of the ointment by prescription, g; \( V_{v.f.} \) – volume of the volumetric flask, ml; \( V_{f.SS} \) – volume of volumetric flask for dilution of the standard sample, ml; \( V_{f.SS} \) – volume of the pipette, ml; \( A_{m} \) – specific absorption index (equal to 92).

Results of determination of the phenylephrine hydrochloride quantitative content in the ointment by the standards method calculation (Table 4) and by the specific absorption index method (Table 5) testify that both methods give almost identical results. Thus, to reduce the analysis time and the cost of phenylephrine hydrochloride assay by spectrophotometry, the calculation of its quantitative content can be carried out by the method of specific absorption index.

Table 4
Results of determination of phenylephrine hydrochloride quantitative content in ointment (calculation by standard method (P=95; t (P, v)=2.0150))

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>( A_{ss} )</th>
<th>A</th>
<th>( m_{out sample} ), g</th>
<th>Found, mg</th>
<th>Metrological characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.722</td>
<td>0.676</td>
<td>1.0018</td>
<td>20.02</td>
<td>( \bar{X} = 19.73; \ S^2 = 0.34 ); ( S_z = 0.14 )</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.682</td>
<td>1.0032</td>
<td>20.17</td>
<td>( \Delta X = 0.69 ); ( \bar{X} = 0.28 )</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.678</td>
<td>1.0102</td>
<td>19.92</td>
<td>( \bar{E}, % = 1.42 )</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.663</td>
<td>1.0097</td>
<td>19.48</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0.659</td>
<td>1.0073</td>
<td>19.41</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>0.657</td>
<td>1.0048</td>
<td>19.40</td>
<td></td>
</tr>
</tbody>
</table>

Table 5
Results of determination of phenylephrine hydrochloride quantitative content in ointment (calculation by specific absorption index method (P=95; t (P, v)=2.0150))

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>( A_{1cm} ), %</th>
<th>A</th>
<th>( m_{out sample} ), g</th>
<th>Found, mg</th>
<th>Metrological characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92</td>
<td>0.676</td>
<td>1.0018</td>
<td>18.89</td>
<td>( \bar{X} = 18.62; \ S^2 = 0.11 ); ( S = 0.32; \ S_z = 0.13 )</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.682</td>
<td>1.0032</td>
<td>19.03</td>
<td>( \Delta X = 0.65 ); ( \bar{X} = 0.27 )</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.678</td>
<td>1.0102</td>
<td>18.79</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>0.663</td>
<td>1.0097</td>
<td>18.38</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td>0.659</td>
<td>1.0073</td>
<td>18.31</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td>0.657</td>
<td>1.0048</td>
<td>18.30</td>
<td></td>
</tr>
</tbody>
</table>

7. Conclusions and prospects for further research

The method of direct spectrophotometry was chosen as an optimal method for phenylephrine hydrochloride assay in the composition of the Simanovsky compounding ointment.

Validation characteristics of the chosen method were studied. The obtained parameters of linearity, accuracy, precision, specificity and robustness testify to its correctness and the possibility of use in other laboratories to determine the quantitative content of phenylephrine.
hydrochloride in the studied ointment and to analyze its stability.

The analysis of the studied ointment was carried out using the chosen method. An estimation of metrological characteristics was performed when calculating the quantitative content of phenylephrine hydrochloride by the standard method and the method of specific absorption index. The obtained results indicate the possibility of using both methods for determining the quantitative content of the test component in the ointment.

References
STUDY OF EXCIPIENTS QUANTITIES INFLUENCE IN THE COMPOSITION OF THE POWDER IN SACHET PACKAGES

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粉末在包装中被患者所喜爱，因为它们结合了片剂（精确剂量）和更容易吞服的片剂（不需要吞食片剂）[1]。2. Statement of the problem

在开发新药时，必须考虑到一些关键因素。毕竟，药物开发的目的是不仅创造一个有效且安全的药物，而且组织一个有效的生产过程，其结果能够保证其一致性。选择最佳的成分组合并开发技术，使可能的重量范围内的过程参数和配方成分的遵守保证最终产品符合规格的要求[2]。

为了开发具有抗炎效果的新药，选择了活性药物成分，根据其作用机制。当在装有0.325克丙氨酰胺、0.05克抗坏血酸和0.01克苯丙醇胺的药片中创建粉末时，可能会出现以下问题：

1. Introduction

粉末在包装中被患者所喜爱，因为它们结合了片剂（精确剂量）和更容易吞服的片剂（不需要吞食片剂）[1]。