Quantitative HPLC Determination of Antigrippin Drug Components

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An HPLC technique for simultaneous quantitative determination of antigrippin components ascorbic acid, paracetamol, and chlorpheniramine maleate has been developed. Optimum conditions are determined for chromatographic separation on a Symmetry C18 column of the poorly retained impurity 4-aminophenol and ascorbic acid. The proposed method provides high sensitivity and rapid analysis and ensures accurate and reproducible results in the quantitative determination of active components (ascorbic acid, paracetamol, and chlorpheniramine maleate), a controlled toxic impurity (4-aminophenol), and an excipient (sodium saccharinate) in antigrippin effervescent tablets for children and adults.

Key words: antigrippin, drug components, ascorbic acid, chlorpheniramine maleate, paracetamol.

Modern medicines for treating colds and the flu contain many drug components. Such preparations include antipyretics, analgesics, antihistamines, and drugs that affect exchange processes, the combined composition of which can effectively relieve the main symptoms of the flu and acute respiratory and viral diseases [1]. Effervescent tablets of antigrippin for children and adults are one type of such widely recommended agents [2]. They are composed of several active ingredients such as paracetamol, ascorbic acid, and chlorpheniramine maleate and several excipients such as sodium bicarbonate, citric acid, sorbitol, povidone, sodium saccharinate, sodium carbonate, macrogol, sodium laurylsulfate, and flavors. For this reason, it is difficult to determine simultaneously all active ingredients and impurities of antigrippin. Thus, preliminary labor-intensive separations of the analytes must be used. This requires a multi-step analysis in general and makes it tedious and costly [3, 4].

However, quality assurance and control of these medicines is very critical because of the presence of a highly active component (chlorpheniramine maleate), the large difference in the content of active ingredients of tens and hundreds of times, and the obligatory requirement for quantitative determination of the toxic impurity 4-aminophenol, a starting material for synthesizing paracetamol. HPLC plays a key role in contemporary quality control of multi-component drugs because of several advantages for analyzing complicated mixtures such as high accuracy and speed [5]. HPLC methods that have been described in regulations for analyzing antigrippin provide for the use of several eluents and do not resolve problems with simultaneous determination of the active ingredients and impurities because their quantitative content in drugs varies widely [3, 4]. Antigrippin also contains compounds that are poorly retained on widely used C18 columns with a bonded phase and a number of excipients that must be considered in determining the type of extraneous impurities.

Therefore, our goal was to develop an HPLC gradient method for determining the components of antigrippin tablets and the impurity 4-aminophenol.

Experimental Part

We used a Shimadzu LC-20 liquid chromatograph (Japan) consisting of a high-pressure gradient pump (LC-20AB), column thermostat (CTO-20A), Rheodyne injector, vacuum degasser, and diode-array matrix (SPD-M 20A) and fluorescence (RF-10AX1) detectors connected in series. Chromatograms were processed and recorded on a PC.
using LC Solution version 1.22 software. The separation was carried out in a Symmetry C18 reversed-phase column (4.6 mm × 150 mm, 5 μm) with a Symmetry C18 precolumn (3.9 mm × 20 mm, 5 μm). The eluents were phase A, aqueous trifluoroacetic acid (0.05%), and phase B, CH₃CN. The pump was operated using the program given in Table 1. Analytical determinations were carried out under the following conditions: flow rate 1.0 mL/min, injected sample volume 20 μL, column temperature 30°C.

Eluents were prepared using CH₃CN (high purity chromatographic, type 0, Kryokhrom, St. Petersburg), CH₃OH and CH₃CN (Lab-scan, Ultra Gradient, Ireland), and ultrapure water obtained from a MilliporWaters (USA) system using doubly distilled water. We used standard samples of ascorbic acid, paracetamol, chlorpheniramine maleate (Fluka), and 4-aminophenol (Sigma) as standards in addition to the excipients of pharmacopoeic purity. Working solutions of standards were prepared in de-ionized water with concentrations of ascorbic acid, paracetamol, chlorpheniramine maleate, and 4-aminophenol of 1, 5, 0.83, and 0.87 mg/mL, respectively.

**Analytical method.** Antigrippin tablets were broken and ground in order to prepare the analytical solutions. Powder of ground tablets (0.3 g, accurate weight) was weighed, dissolved in de-ionized water, transferred to a 50-mL volumetric flask, shaken vigorously, sonicated for 5 min, and adjusted to the mark with water. Samples were filtered through a membrane filter (0.45 μm pore size) before injection onto the column. A sample volume (20 μL) of the studied solutions or standards was injected into the chromatograph using a microsyringe. The mass of determined compounds in a single tablet was calculated using the formula:

\[ X = \frac{S_k m_{st}}{S_{st} m_w} \]

where \(S_k\) and \(S_{st}\) are the average peak areas of the determined components on chromatograms of test and standard samples, respectively; and \(m_{st}, m_w\), and \(m_a\) (all in g), the mass of the standard of the determined compound in the standard solution, the average mass of a tablet, and the mass of the weighed portion of ground tablets used to prepare the test solution, respectively.

**RESULTS AND DISCUSSION**

Preliminary experiments using the diode-array matrix detector (Fig. 1) determined the light absorption of the deter-

**TABLE 1.** Gradient Elution Scheme

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Flow rate, mL/min</th>
<th>Eluent A, %</th>
<th>Eluent B, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 3</td>
<td>1.0</td>
<td>99.8</td>
<td>0.2</td>
</tr>
<tr>
<td>3 – 13</td>
<td>1.0</td>
<td>99.8 → 45.0</td>
<td>0.2 → 55.0</td>
</tr>
<tr>
<td>13 – 15</td>
<td>1.0</td>
<td>45.0</td>
<td>55.0</td>
</tr>
</tbody>
</table>

where 4-Aminophenol (1), paracetamol (2), ascorbic acid (3) in mobile phase CH₃CN:aqueous trifluoroacetic acid (0.05%).

**Fig. 1.** Absorption spectra of antigrippin active ingredients using a diode-array matrix detector: chlorpheniramine maleate (1), paracetamol (2), ascorbic acid (3) in mobile phase CH₃CN:aqueous trifluoroacetic acid (0.05%).

**Fig. 2.** Antigrippin chromatogram: ascorbic acid (100 μg/mL, 1), paracetamol (500 μg/mL, 2), sodium saccharinate (3), chlorpheniramine maleate (6 μg/mL, 4). Mobile phase CH₃CN and aqueous trifluoroacetic acid (0.05%), gradient elution. Flow rate 1 mL/min. Detection wavelength of diode-array matrix detector 263 nm.