HPLC determination and quantification of pheniramine and pyrilamine in over the counter cold medicines

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Abstract

A method to quantitatively determine amounts of antihistamines in pharmaceuticals has been reproduced. Two antihistamines (pheniramine and pyrilamine) were quantified in two over-the-counter cold medications by use of HPLC. A set of standards was created for each antihistamine and a calibration plot was established for each based on peak areas. These calibration plots were then used to interpolate the concentration of the sample solutions, and thus the amount of each antihistamine per serving.

Introduction

An allergic response to a substance is defined as the body overreacting to a substance by initiating an immune response. This response causes the symptoms we associate with allergies such as itchy eyes and runny nose.\(^1\) Antihistamines, sometimes referred to as H1 blockers, are a class of drugs that actively target and inhibit the body's receptors for allergens, which are usually found in the intestines and lungs.

A large portion of modern medications list antihistamines as either a major or minor active ingredient. Antihistamines have become incorporated into the daily lives of people around the world within the last ten to fifteen years at a staggering rate. Due to this, along with the fact that many of these drugs degrade very slowly, bioaccumulation is possible.\(^2\) A reduction in the use/production of antihistamines is very unlikely as they gross a very large amount of money for pharmaceutical companies, and many people have become accustomed to their use.

The antihistamines pheniramine and pyrilamine are found in such medications as Theraflu\® and Pamprin®, respectively. Research has shown undesirable effects caused by these antihistamines. Pyrilamine has been found to cause liver cancer in rats when administered in large amounts.\(^3\) Both pyrilamine and pheniramine have been linked to cardio toxicity, meaning prolonged use can lead to excessive stress on the heart.\(^5\)

An experimental procedure was modeled after the method designed by Louhaichi, et al. HPLC was used to measure the absorbance peak areas for a set of known standards, which were then used to develop calibration plots. Amounts of each antihistamine in both medications were then quantified by HPLC in an attempt to determine if the amount of each chemical was significantly different than was stated on the contents label. The intention was to determine if either excessive amounts or inadequately low amounts of the active ingredients were being incorporated into the medications that many of us use on a regular basis.\(^4\)

Experimental

Materials and chemicals

Reference standards of pheniramine maleate and pyrilamine maleate were purchased from Sigma-Aldrich. Antihistamine-containing samples, Pamprin® and Theraflu®, were purchased from a local grocery store. Theraflu®, a flu and sore throat medication (Pheniramine maleate 20 mg)
was distributed by Novartis consumer Health, Inc. Pamprin®, a menstrual pain relief medication (Pyrimidine maleate 15 mg) was distributed by Chattem, Inc.

Standard preparation

A 1000-ppm pheniramine standard stock solution was prepared by dissolving 0.1000 g of sample in a 100-mL volumetric flask with ultrapure H₂O. This stock was then diluted with ultrapure H₂O to create 10, 20, 30, 40, and 50 ppm standard solutions.

A 1000-ppm pyrilamine standard stock solution was prepared with ultrapure H₂O by dissolving 0.1000 g of sample in a 100-mL volumetric flask. This stock was then diluted with ultrapure H₂O to 50, 150, 250, and 350-ppm solutions in 1-L volumetric flasks, which were then diluted again with ultrapure H₂O to final concentrations of 10, 30, 50, and 70 ppm.

Sample preparation

The contents of one single-serving Theraflu® packet was dissolved in 1000 mL ultrapure H₂O using sonication for 10 minutes, giving an expected pheniramine concentration of 20 ppm. Approximately 10 mL of solution was filtered with a 25-mm Fisher Brand, nylon non-sterile, 0.45-µm syringe filter.

The contents of one Pamprin® tablet were ground into a fine powder using a mortar and pestle. The powder was dissolved in 100 mL ultrapure H₂O using five minutes of sonication. A 1:5 dilution of this solution was made, which produced an expected concentration of 30 ppm, and an appropriate amount was filtered with a 0.45-µm syringe filter.

Chromatographic conditions

A Varian ProStar HPLC system with Model 410 autosampler and reversed phase column (Varian C-18 Microsorb-MV 100 Å) with 3-µm particle size and column dimensions of 4.6 mm x 50 mm was used. The absorbance was collected at 220 nm and a column heater was used to keep the column at 35°C. A mobile phase consisting of methanol and 0.1 M KH₂PO₄ buffer in a ratio of 45:55 was used with a flow rate of 0.70 mL/min, which corresponds to a system pressure of 920 psi. The pH of the buffer was adjusted to 3.00 with phosphoric acid and degassed prior to analysis.

Results and Discussion

The standard solution data was collected and plotted to show differentiation in peak area tabulated in both Fig. 1 and Fig. 2 for pyrilamine and pheniramine samples respectively. Standard curves were then plotted for the respective data of pyrilamine and pheniramine in order to determine the relative concentrations of sample solutions in Fig. 3. Chromatograms representing peak areas for sample solutions were collected. Although three pheniramine sample solutions were tested, only one sample produced quantitative chromatographic results.

In the figures below, the sample peak areas are directly related to the amount of analyte in each sample. These peak areas were used with the calibration curves to quantify the amount of analyte in each sample.

Two assumptions were made when determining the area under the curves for both pyrilamine and pheniramine. The first assumption was made in making the total area under the curve positive in order to quantitatively assign a value to it. This was done by finding the equation for the line that connected the time where the absorbance started and
where it ended. The value of this line was then added to the absorbance at each point on
the line.

The second assumption is that the trapezoid rule is a good estimate for determining
the area under the new curve. The area for each adjusted curve was calculated with this
rule as the computer software was not generating reasonable areas.

Figure 1. Standard solutions of differing concentrations of PHEN.

Figure 2. Standard solutions of differing concentrations of PA.

Figure 3. The calibration curve of PA (shown on the right) and the calibration curve of PHEN (shown on left).

Figure 4. PA Sample 1 chromatogram

Figure 5. PA Sample 2 chromatogram
Table 1. Area under PA sample curves

<table>
<thead>
<tr>
<th>PA Sample 1</th>
<th>PA Sample 2</th>
<th>PA Sample 3</th>
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<tbody>
<tr>
<td>77.68</td>
<td>83.55</td>
<td>71.39</td>
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Table 2. Area under PHEN sample curves

<table>
<thead>
<tr>
<th>PHEN Sample 1</th>
<th>PHEN Sample 2</th>
<th>PHEN Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.17</td>
<td>*</td>
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Table 3. Conversion of peak areas into concentrations per serving

<table>
<thead>
<tr>
<th></th>
<th>Average Area</th>
<th>Average Concentration per serving (mg)</th>
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<tbody>
<tr>
<td>PA</td>
<td>77.54 (+/- 4.82)</td>
<td>13.36 (+/- 1.19)</td>
</tr>
<tr>
<td>PHEN</td>
<td>28.17 (+/- 2.54)</td>
<td>13.38 (+/- .46)</td>
</tr>
</tbody>
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Conclusions

A method to quantitatively determine the amount of antihistamines in pharmaceuticals has been reproduced, although with surprising results. Through HPLC analysis, the amounts of pyrilamine and pheniramine were determined. The observed pyrilamine value of 13.36 (±1.19) mg is slightly lower, but comparable to the labeled amount in Pamprin®. The observed 13.38 (± .46) mg of pheniramine is much lower than the labeled amount Theraflu®. The less than desirable results are more than likely due to the assumptions and derivations used to develop the results.

Possible future research would involve testing more samples and researching different antihistamines. Using this method, acquired data could provide insight to the quality control exhibited by pharmaceutical companies.
References