Analysis of dextromethorphan, guaifenesin, benzoate, and saccharin in cough syrup using high-performance liquid chromatography

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Abstract

A method for analysis and quantification of both active and inactive ingredients in cough syrups namely dextromethorphan (DM), guaifenesin, benzoic acid and saccharin has been developed. Over the counter cough syrups Robitussin Dm and Vicks (Nyquil cough and chest congestion) were analyzed using HPLC. The HPLC conditions included phosphate buffer and different wavelengths. A set of standards for each active ingredient developed calibration plots which were used to verify the amount of active ingredient in each cough syrup.

Introduction

Coughs usually do not require treatment as they go away on their own, but cough syrups help if the coughing happens to be consistent. Robitussin and Vicks cough syrup are two of the many other syrups that are OTC (Over-the-counter), i.e. that can be obtained in stores without prescription. OTC syrups are generally safe to take at a reasonable or recommended level; however they have side effects when consumed in large amounts such as hallucination or dissociative behaviors. The 2006 National Survey on Drug Use and Health (NSDUH) states that about 3.1 million persons aged 12 to 25 (5.3 %) had over used an over-the-counter (OTC) cough and cold medication to get high (i.e., “misused” the drug), and nearly 1 million (1.7 percent) had done so in the past year. Robitussin DM is the most misused cough syrup as it contains a high amount of dextromethorphan (cough suppressant). This research analyses the main compounds found in the cough syrups that could be responsible for the over consumption hence addiction of the syrup.

The main ingredients of cough syrups are dextromethorphan, a commonly used antitussive (cough suppressant) which relieves cough by blocking the cough reflex system in the brain. Guaifenesin, an active ingredient acts as an expectorant. It allows the loosening and thinning of bronchial secretions, hence decreasing cough productivity. Guaifenesin exists as two enantiomers. The compound is in the racemic mixture form in both the syrup and the tablet. Both dextromethorphan and guaifenesin act as the active ingredients and the excess absorption of dextromethorphan is responsible for the hallucinations or “highness” obtained from excessive consumption of cough syrups. Sodium benzoate is an inactive ingredient which is found in a small amount as a preservative the acidity of the cough syrups. Saccharin acts as a sweetening agent in the cough syrups also inactive. This paper focuses on determining the amounts of these ingredients using HPLC.
Experimental

Materials and Chemicals

Standards for reference of dextromethorphan (DXM) hydrobromide, guaifenesin, saccharin and sodium benzoate were purchased from Sigma- Aldrich. Over the counter cough syrups Robitussin DM Cough and Chest Congestion and Vicks Nyquil Cough were purchased from a local pharmacy.

Instrument Conditions

A Varian Prostar HPLC system with a reversed phase column of particle size 3-µm and an autosampler was used. The column heater kept the temperature stable at 35°C. The mobile phase used consisted of phosphate buffer of pH = 2.8 (25 mM phosphoric acid, Triethylamine (TEA) to bring to 2.8) and acetonitrile in a 75:25 ratio with an isocratic flow rate of 1ml/1min. Wavelengths 250 nm and 290 nm were used for absorbance as guaifenesin absorbs at 250 nm while dextromethorphan (DXM), benzoic acid and saccharin absorb at 290 nm. Two columns were used for analysis. The first was of length 250 mm x 4.6 mm (wide), with particle size 5 µm, pore size 80 Å and injection volume of 100 µL. The second column had a length of 50 mm x 4.6 mm (wide), particle size of 2.6 µm, pore size of 100 Å and injection volume of 10 µL.

Standard Preparation

Pure working standards of analytes were prepared by weighing 50.2 mg of dextromethorphan HBr, 500.1 mg guaifenesin, 87.5 mg sodium benzoate and 62.4 mg of saccharin. Each analyte was dissolved with distilled (DI) water into a 25 ml volumetric flask forming the stock solution. The standard solutions were prepared by pipetting 1, 2, 3, 5 and 10 mL of each analyte into 25-mL volumetric flasks and diluted with DI water. HPLC auto-injection vials were filled with each standard for HPLC identification.

Sample preparation

2.5 mL of each sample, Robitussin DM and Vicks cough syrups was diluted with DI water into a 25-mL volumetric flask. Using a 0.45-µm syringe filtration disk, an aliquot of each sample was filtered into HPLC vials.

Results and discussion

Standard Results

Data from the standard preparation was used in generating a calibration plot for each working standard. Each calibration plot was obtained by plotting the area (mAU.min) under the peak verses the increasing concentrations of the standards (ppm). These plots were generated to assist in determining the concentrations of each analyte in the samples of cough syrups. A peak absorbance for dextromethorphan could not be obtained in this
experiment using the column 1 of length and width 250 mm x 4.6 mm. The chromatograms obtained showed only the baseline with no absorbances. Parameters such as increasing the concentration of dextromethorphan by 5 times the initial mass used, changing the diluant from DI water to the mobile phase and increasing the run time to about 45mins per standard and sample showed no results. Column 2 on the other hand showed an absorbance for dextromethorphan for its standards and cough syrup samples.

Figures 1, 2, 3, and 4 represent the chromatographs for analytes guafenesin, dextromethorphan, benzoic acid and saccharin. Table 1 shows the linearity and correlation coefficients generated from the regression equations of the calibration plots. The $R^2$ values show good linearity and as such can be used to determine the amounts of active ingredients in the cough syrup.

Figures 5, 6 show the chromatographs of the cough sample syrups. These were obtained using Column 1 as the data obtained from Column 2 showed all the analytes with additional unknown peaks attached. As the data could not be interpreted, Column 1 chromatographs were used and peaks identified.
Table 1: Linearity parameters from calibration plots

<table>
<thead>
<tr>
<th>Linearity</th>
<th>Dextromethorphan</th>
<th>Guaifenesin</th>
<th>Benzoic acid</th>
<th>Saccharin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards (ppm)</td>
<td>240.2 - 2402</td>
<td>80.7 - 806.6</td>
<td>140.16 - 140</td>
<td>100 - 500</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.01</td>
<td>1.346</td>
<td>0.631</td>
<td>0.0155</td>
</tr>
<tr>
<td>Intercept (b)</td>
<td>0.4472</td>
<td>3.3863</td>
<td>21.818</td>
<td>-0.0183</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9969</td>
<td>0.9986</td>
<td>0.9899</td>
<td>0.9997</td>
</tr>
</tbody>
</table>

Sample Results

![Chromatograph of Robitussin DM using Column 1](image1)

![Chromatograph of Vicks Nyquil Cough and Chest Congestant using Column 1](image2)

Table 2. Concentrations of the analytes in the samples determined using the calibration curves. Benzoic acid and saccharin could not be determined as their quantities in the cough syrups are little and a literature value could not be obtained to compare with.

<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>Robitussin DM</td>
<td>2000 ppm</td>
<td>NA</td>
<td>20000 ppm</td>
<td>500 ppm</td>
</tr>
<tr>
<td>Vicks</td>
<td>1000 ppm</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Conclusions

A method for determining the concentrations of both active and inactive ingredients in cough syrups has been developed and validated using HPLC. The expected concentrations were not equivalent to that labelled on the cough syrups. For instance in Robitussin DM, the observed concentration of guafenesin was 500 ppm while the expected was 20000 ppm. Guafenesin concentration could not be determined in Vicks as it contains doxylamine succinate as an expectorant instead of guafenesin. Its peak can however be
compared to that of guaifenesin in Robittusin DM. The unexpected results could been a result of not using a column that could effectively separate the peaks in the cough syrups.

Future research could involve using a gradient flow instead of isocratic as a parameter while running the samples with the HPLC. This could effectively separate the peaks and give better results. Perfecting this method could give an opportunity to determine the concentrations of active ingredients in other pharmaceutical syrups.

References


4) Drug Information Online. [www.drugs.com/mtm/robitussin-dm.html](http://www.drugs.com/mtm/robitussin-dm.html). (accessed 04/04/11)