Simultaneous Determination of Dextromethorphan and Promethazine in Pharmaceutical Syrups by Rapid HPLC Method

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Received November 11, 2014; accepted January 3, 2015

ABSTRACT
A simple, precise and accurate method is validated for the simultaneous determination of two nitrogenous compounds dextromethorphan hydrobromide and promethazine hydrochloride, which are commonly used as active ingredients in pharmaceutical syrups. The chromatographic conditions comprised of a classical C8-type stationary phase (250 × 4.6 mm, 5 μm) with a mobile phase consisting of 3 g of sodium lauryl sulfate in a mixture of 400 ml of water and 600 ml of acetonitrile along with 0.5 g of ammonium nitrate as additional agent. The solution pH 2.0 was adjusted with glacial acetic acid. The flow rate was 1 ml/min; the detection was carried out at 280 nm under temperature of 25°C. The retention time of dextromethorphan was 8.5 min and 9.9 min for promethazine. The method was validated for specificity, accuracy, precision and linearity. The results indicate that the drugs are susceptible to photodegradation. All the peaks of degraded products were resolved from the two active pharmaceutical ingredients with significant different retention times.

KEYWORDS: Dextromethorphan; Promethazine; Syrups; assay validation.

Introduction
Dextromethorphan (ent-3-methoxy-17-methylmorphinan hydrobromide monohydrate) is an opioid receptor agonist used as cough suppressant Fig. 1. Literature survey revealed that the recommended analytical method of dextromethorphan and its related substances was mentioned in the British Pharmacopeia (British Pharmacopeia, 2013). Many other analytical methods of dextromethorphan alone or with combination with other drugs in pharmaceutical dosage forms were reported in previous researches such as HPLC methods (Al-Rimawi, 2010; Tedesco et al., 2013; Rauha et al., 1996; Wilson et al., 1993; Louhaichi et al., 2009). Hydrophilic interaction liquid chromatographic procedure for the simultaneous determination of pseudoephedrine hydrochloride, diphenhydramine hydrochloride and dextromethorphan hydrobromide in cough-cold formulations was also reported (Shahid et al., 2007).

Carbon paste and PVC electrodes for the flow injection potentiometric determination of dextromethorphan was conducted by Elmorsy et al, 2010. A micellar electrokinetic capillary chromatography and capillary zone electrophoresis was investigated for simultaneous determination of dextromethorphan, diphenhydramine and phenylephrine in expectorant and decongestant syrups (Gomez et al., 2002). UV methods for the determination of dextromethorphan HBr has been reported (Tantishaiyakul et al., 1998; Tan et al., 1998).

Promethazine (2RS)-N,N-Dimethyl-1-(10H-phenothiazin-10-yl)propan-2-amine hydrochloride is a histamine H1 receptor antagonist Fig. 2. The recommended analytical method of promethazine and its related substances was reported in the British Pharmacopeia (British Pharmacopeia, 2013). Literature survey methods of promethazine in the pharmaceutical formulations alone or with combination with other drugs were reported in previous bibliography.

Fig. 1. Chemical structure of dextromethorphan.

Fig. 2. Chemical structure of promethazine.
Two new analytical procedures for fast and simultaneous determination of promethazine and codeine have been developed (Pereira et al., 2014). Another voltammetry method based on a composite biosensor MWCN/SiO$_2$/Al$_2$O$_3$/Nb$_2$O$_5$/DNA (MWCN/SiAlNb/DNA) was developed and validated (Marco et al., 2013). A simple and fast-automated method was developed and validated for the assay of promethazine hydrochloride in pharmaceutical formulations, based on the oxidation of promethazine by cerium in an acidic medium (Saleh et al., 2012). A highly accurate nephelometric titration for the determination of promethazine hydrochloride and its preparations was presented by Zhang et al. (2005).

The first flow injection spectroelectro-analytical method for the determination of promethazine hydrochloride has been developed (Daniel et al., 2003). A visible spectrophotometric method by Saif et al. (2005) was reported in which Promethazine-HCl reacts with potassium persulphate to give a pinkish red color complex exhibiting maximum absorbance at 515 nm. A bead injection spectroscopy-flow injection analysis (BIS-FIA) system for the spectrophotometric detection of promethazine and trifluoperazine was developed (Ruedas Rama et al., 2004).

A method using capillary zone electrophoresis (CZE) for quantitative analysis of three phenothiazines, thiazinamium methylsulphate (TMS), promazine hydrochloride (PMH) and promethazine hydrochloride (PTH) in pharmaceutical formulations, was developed and validated (Lara et al., 2005). Three chiral stationary phases based on macrocyclic antibiotics (teicoplanin, vancomycin and ristocetin A) have been tested for chiral separations of promethazine (Bobáková et al., 2002).

There are several international oral pharmaceutical preparations containing a combination of dextromethorphan hydrobromide and promethazine hydrochloride such as (Phenergan with Dextromethorphan, Pherazine DM, Prometh with Dextromethorphan, Promethazine; Destromethorphan, Dextromethorazine, Promethazine; Dextromethorphan, Dextromethorphan Compound and Promethazine; Destromethorphan).

The aim of this research was to develop and validate a simple, precise and accurate method for the simultaneous determination of dextromethorphan hydrobromide and promethazine hydrochloride using HPLC technique.

**Materials and Methods**

**Materials**

Working standard of Promethazine hydrochloride and Dextromethorphan hydrobromide (Purity 98.7% and 99.3% consequently) was provided as a gift from Shifa Pharmaceutical Industries, Syria and used without further purification. The syrup preparation was also a gift from Shifa Pharmaceutical Industries. All the other used reagents were of HPLC grade: Acetonitrile (Scharlau), Glacial acetic acid (SCP), Sodium Lauryl Sulfate (Roth), Ammonium nitrate (Sigma), Deionized Water for HPLC, Filters 0.45 µm.

**Instrumentation**

The HPLC instrument was from Agilent 1260 infinity, equipped with a UV detector. The UV instrument used was from Jasco V560. The pH meter used was from Crison.

**Reference Preparation**

Accurately weighed 10 mg of each of the two drugs was dissolved in 25 ml of the mobile phase, 1 ml of this solution is diluted in 10 ml of the mobilephase. Standards concentration obtained is 0.04 mg/ml for both Promethazine hydrochloride and Dextromethorphan hydrobromide. The analysis was repeated in triplicate.

**Method development and optimization of chromatographic conditions**

*Selection of detection wavelength:* Dextromethorphan hydrobromide solution was prepared in water at a concentration of 10 mg/100 ml and scanned in UV-Visible spectrophotometer a maximum wavelength is found at 280 nm Fig. 3.

Promethazine hydrochloride solution was prepared in water at a concentration of 10 mg/100 ml and scanned in UV-Visible spectrophotometer, two maximum wavelength are found at 300 nm and 262 nm Fig. 4. The common wavelength of the two medicaments utilized is at 280 nm.

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Column selection
- Chrometisil 120-5-C8 SH Reversed phase column, 250 × 4.6 mm is utilized.

Mobile phase preparation: The mobile phase is consisting of 3 g of Sodium Lauryl Sulfate in a mixture of 400 ml of water and 600 ml of acetonitrile, then 0.5 g of ammonium nitrate is added and apparent pH of 2.0 is adjusted with glacial acetic acid.

Degraded reference solution
The reference solution containing a mixture of Prome-thazine hydrochloride and Dextromethorphan hydro-bromide prepared above is standing at room temperature and sunlight for 7 days.

Syrup solution preparation
1 ml of the syrup is diluted in 25 ml of the mobile phase, the diluted syrup solution is injected directly in the HPLC system.

Results and Discussion
Selection of detection wavelength
As noticed in Fig. 3, dextromethorphan has a maximum wavelength at 280 nm. As noticed in Fig. 4, promethazine has two maximum wavelengths at 300 nm and 262 nm. Hence detection at 280 nm was selected for method development purpose.

HPLC analysis
The chromatographic conditions comprised of a classical C8-type stationary phase (250 × 4.6 mm, 5μ) with a mobile phase consisting of 3 g of Sodium Lauryl Sulfate in a mixture of 400 ml of water and 600 ml of acetonitrile, then 0.5 g of ammonium nitrate is added and apparent pH of 2.0 is adjusted with glacial acetic acid. The flow rate was 1 ml/min, the detection was carried out at 280 nm under temperature of 25°C. The reference solution was injected three times under the previous chromatographic conditions, the retention time of Dextromethorphan was 8.5 min and 9.9 min for Promethazine showed in Fig. 5. Since it give the best resolution of the two drugs in the chromatogram with no interference with impurities, or tailing in the peaks.

Analytical method validation
Method validation was performed under a variety of ICH and British Pharmacopeia recommended test conditions.

Linearity: Five standard solutions containing both dextromethorphan and promethazine were prepared with the concentrations (0.02, 0.03, 0.04, 0.05 and 0.06 mg/ml), each standard solution was prepared and injected three times in HPLC. Fig. 6 and Fig. 7 shows the linearity of dextromethorphan and promethazine with a correlation coefficient of 0.9997 and 0.9998 in order.

Fig. 5. Chromatogram of the reference solution.

Fig. 6. Linearity of dextromethorphan.
Tefi: Simultaneous Determination of Dextromethorphan and Promethazine in Pharmaceutical Syrups by Rapid HPLC Method

Fig. 7. Linearity of promethazine.

Range: Linearity was confirmed in the interval (0.02, 0.03, 0.04, 0.05 and 0.06 mg/ml) for dextromethorphan and promethazine.

Accuracy: Three levels of Concentration (0.03, 0.04, and 0.05 mg/ml) have been used to study the accuracy of Dex (each concentration level is prepared three times by three different analysts) Table 1. Results indicate that the individual recovery of dextromethorphan ranges from 96.5% to 98.3% with mean recovery of 97.4% and %RSD of 0.9%. The recovery of the dextromethorphan by proposed method is satisfactory as %RSD is not more than +5.0% and mean recovery between 95.0-105.0%.

<table>
<thead>
<tr>
<th>Conc. mg/ml</th>
<th>Area</th>
<th>Av. Calc. Conc.</th>
<th>Recovery %</th>
<th>Av. Recovery %</th>
<th>SD</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>115</td>
<td>0.0289</td>
<td>96.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>118</td>
<td>0.0293</td>
<td>96.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>118</td>
<td>0.0293</td>
<td>96.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.04</td>
<td>157.7</td>
<td>0.0389</td>
<td>97.4</td>
<td>97.4</td>
<td>0.9</td>
<td>0.92</td>
</tr>
<tr>
<td>0.04</td>
<td>164.3</td>
<td>0.0389</td>
<td>97.4</td>
<td>97.4</td>
<td>0.9</td>
<td>0.92</td>
</tr>
<tr>
<td>0.04</td>
<td>155.3</td>
<td>0.0389</td>
<td>97.4</td>
<td>97.4</td>
<td>0.9</td>
<td>0.92</td>
</tr>
<tr>
<td>0.05</td>
<td>198.7</td>
<td>0.0491</td>
<td>98.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>202.9</td>
<td>0.0491</td>
<td>98.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Three levels of concentration (0.03, 0.04, and 0.05 mg/ml) have been used to study the accuracy of Pro (each concentration level is prepared three times by three different analysts) Table 2. Results indicate that the individual recovery of Promethazine ranges from 97.8% to 98.8% with mean recovery of 98.1% and %RSD of 0.6%. The recovery of the promethazine by proposed method is satisfactory as %RSD is not more than +5.0% and mean recovery between 95.0-105.0%.

Precision: Repeatability and Intermediate Precision

The solution 0.04 mg/ml has been prepared three different times by three analysts at three weeks, each solution was injected three times. Standard deviation and relative standard deviation of the response have been calculated, and the results were illustrated in Table 3 and Table 4.

Table 1
Accuracy of dextromethorphan

The RSD% for intermediate precision of dextromethorphan is 2.5 Table 3. This shows that the intermediate precision of the method is satisfactory as RSD% is not more than +5.0%.

Table 2
Accuracy of promethazine

The RSD% for intermediate precision of promethazine is 2.3 Table 4. This shows that the intermediate precision of the method is satisfactory as RSD% is not more than +5.0%.
Repeatability and intermediate precision of promethazine

<table>
<thead>
<tr>
<th>Conc. mg/ml</th>
<th>Area</th>
<th>Calc. Conc.</th>
<th>SD</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.04</td>
<td>175.78</td>
<td>0.04090</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.04</td>
<td>175.28</td>
<td>0.040294</td>
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<td></td>
</tr>
<tr>
<td>0.04</td>
<td>175.29</td>
<td>0.040297</td>
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<td></td>
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<tr>
<td>0.04</td>
<td>166.64</td>
<td>0.038308</td>
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<tr>
<td>0.04</td>
<td>166.9</td>
<td>0.038368</td>
<td>0.00091</td>
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<td>0.04</td>
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<td>0.04</td>
<td>168.9</td>
<td>0.038826</td>
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<td></td>
</tr>
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</table>

Robustness: The method robustness and ruggedness were determined by analyzing the same sample (0.04 mg/ml) at normal operating conditions and also by changing some parameters such as temperature and mobile phase pH. As illustrated in Table 5, 6, 7 and 8. Results of modification of temperature at dextromethorphan shows a satisfactory RSD% not more than +2.0%. Results of modification of temperature at promethazine shows a satisfactory RSD% not more than +5.0%.

Temperature modification at dextromethorphan

<table>
<thead>
<tr>
<th>Modification of Temperature at Dex</th>
<th>No. of Injection</th>
<th>Peak area</th>
<th>Peak area of unchanged temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>T= 30°</td>
<td>1</td>
<td>165.14</td>
<td>158.94 164.32</td>
</tr>
<tr>
<td>T= 20°</td>
<td>2</td>
<td>165.77</td>
<td>159.80 164.44</td>
</tr>
<tr>
<td>T= 25°</td>
<td>3</td>
<td>164.82</td>
<td>160.98 164.30</td>
</tr>
<tr>
<td>AVR</td>
<td></td>
<td>165.25</td>
<td>159.91 164.36</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>0.63</td>
<td>3.15</td>
</tr>
<tr>
<td>RSD%</td>
<td></td>
<td>0.4</td>
<td>1.31</td>
</tr>
</tbody>
</table>

Temperature modification at promethazine

<table>
<thead>
<tr>
<th>Modification of Temperature at Pro</th>
<th>No. of Injection</th>
<th>Peak area</th>
<th>Peak area of unchanged temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>T= 30°</td>
<td>1</td>
<td>161.68</td>
<td>163.69 166.64</td>
</tr>
<tr>
<td>T= 20°</td>
<td>2</td>
<td>161.90</td>
<td>164.20 166.91</td>
</tr>
<tr>
<td>T= 25°</td>
<td>3</td>
<td>160.98</td>
<td>163.96 166.40</td>
</tr>
<tr>
<td>AVR</td>
<td></td>
<td>161.52</td>
<td>163.95 166.65</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>3.63</td>
<td>1.91</td>
</tr>
<tr>
<td>RSD%</td>
<td></td>
<td>2.18</td>
<td>1.14</td>
</tr>
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</table>

Mobile phase pH modification at dextromethorphan

<table>
<thead>
<tr>
<th>Modification of Mobile phase pH at Dex</th>
<th>No. of Injection</th>
<th>Peak area</th>
<th>Peak area of unchanged Mobile phase pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH = 1.5</td>
<td>1</td>
<td>153.21</td>
<td>155.09 157.68</td>
</tr>
</tbody>
</table>

Results of mobile phase pH modification at dextromethorphan shows a satisfactory RSD% not more than +5.0% when pH value is 2.5.while the results of mobile phase pH modification at promethazine shows a RSD% of 7.2% when pH value is 1.5. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters and by changing analytical operator has proven that the method is robust and rugged.

LOD and LOQ: The calculated LOD and LOQ for dextromethorphan were 1 µg/ml and 3.3 µg/ml respectively and the calculated LOD and LOQ for Pro were 0.9 µg/ml and 2.7 µg/ml respectively.

Specificity: The chromatogram of the reference sample indicated no additional peaks other than those of dextromethorphan and promethazine at 8.5 min and 9.9 min in order Fig. 3. The reference solution was standing and exposed to daylight for seven days then it was injected into HPLC. The chromatogram of the standing reference sample shows many additional peaks, but they are well resolved from the peaks of dextromethorphan and promethazine with a significant difference in the retention time Fig. 8. So this means that this method is well specific for the determination of dextromethorphan and promethazine in the presence of their degradation products.

Syrup analysis

The chromatogram of a syrup sample is presented in Fig. 9. The two peaks of dextromethorphan and promethazine are separated from other excipients peaks. The retention time of dextromethorphan was 8.5 min and 10.1 min for promethazine. The quantitative determination of the two active ingredients in this pharmaceutical form is simple, fast, precise and specific.
Conclusion

In conclusion, the developed HPLC technique has shown adequate separation of dextromethorphan and promethazine from each other in the presence of other components and their degradation products in the syrup. Thus, simultaneous determination of the two active ingredients in syrup products is simple, fast, accurate and precise.

References


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