EXTRACTIVE SPECTROPHOTOMETRIC DETERMINATION OF SOME ANTIHISTAMINIC DRUGS FROM PHARMACEUTICAL FORMULATIONS USING ROSE BENGAL

AKRAM M. EL-DIDAMONY¹, SAMEH M. HAFEEZ²

¹Chemistry Department, Faculty of Science, Zagazig University, Zagazig, 44519, Egypt, ²Ismailia Chemical Laboratory, Forensic Medicine Authority, Justice Ministry, Egypt

Email: ak_eldidamony@yahoo.com

ABSTRACT

Objective: Simple and sensitive an extractive-spectrophotometric method have been developed for the determination of four important antihistaminic drugs, namely desloratadine (DSL), chlorpheniramine maleate (CPM), diphenhydramine hydrochloride (DPH) and fexofenadine (FXO).

Methods: This method is based on the formation of colored ion-pair complexes between the basic nitrogen of the drugs and haloephoresin dyes, namely rose bengal (RB) dye in weak acidic medium. The formed complexes were extracted with dichloromethane measured spectrophotometrically at 550 nm.

Results: The reaction conditions were optimized to obtain the maximum color intensity. Beer’s law was obeyed with a good correlation coefficient (0.9963-0.9975) in the concentration ranges 1-6, 4-18, 6-16 and 2-22 μg/ml for DSL, CPM, DPH and FXO, respectively. The composition ratio of the ion-pair complexes was found to be 1:1 as established by Job’s method.

Conclusion: The proposed method was successfully extended to pharmaceutical preparations. Excipients used as additive in commercial formulations did not interfere in the analysis. The proposed method can be recommended for quality control and routine analysis where time, cost effectiveness and high specificity of analytical technique are of great importance.

Keywords: Desloratadine, Chlorpheniramine maleate, Diphenhydramine hydrochloride, Fexofenadine, Rose Bengal, Ion-pair complexes, Spectrophotometry.
(CPM), diphenhydramine (DPH) and fexofenadine (FXO) that supplied from Egyptian International Pharmaceutical Industries Company (EIPICo) 10th of Ramadan City, Egypt. Rose bengal was supplied from (Aldrich), sodium acetate and methylene chloride were supplied from (Egyptian, Adewic).

Tablets containing the drugs were obtained from the local market. The pharmaceutical preparations of desloratadine pharmaceutical preparations were delarex tablets 5 mg/tab produced by (global napi Pharm. Cairo –Egypt). The chlorpheniramine maleate pharmaceutical preparations were analle rge tablets 4 mg/tab (Kahira Pharm. Cairo –Egypt). Diphenhydramine hydrochloride is sultan tablets, 50 mg/tab produced by Pharazia pharmaceuticals. The fexofenadine hydrochloride pharmaceutical preparations were allerfen tablets 60 mg/tab produced by (Amoun Pharm. Cairo –Egypt).

Reagents and solutions

All chemicals and reagents used were of analytical-reagent grade and distilled water was used throughout the investigation.

Pure drugs

An accurately weighed quantity of the investigated drugs (20 mg) was dissolved in distilled water in a 100 ml measuring flask. Aliquots of the above prepared stock solution were further diluted to obtain 100 µg/ml working standard solutions.

Buffer solution

Citrate–phosphate buffer was prepared by adding 0.20 M disodium hydrogen phosphate (Fisher Scientific Co., Pittsburgh, PA) to 50 ml 0.1 M citric acid (Sigma Chemical Co., St. Louis, MO) to adjust the pH to 2–7 and the volumes were diluted to 100 ml with distilled water.

Dye stuff

A stock solution of 1 ×10⁻³ M rose bengal (4,5,6,7-Tetrachloro-3',6'-dihydroxy-2',4',5',7'-tetraiodo-3H-spiro[kobenzofuran-1,9'-xanthen]-3-one), was prepared by dissolving 97.367 mg from dye (99% purity) in distilled water and diluting to 100 ml in a measuring flask with distilled water.

Procedure for calibration curves

Into a series of separating funnels, accurately measured aliquots DSL, CPM, DPH or FXO in the concentration range shown in (table 1) were pitted out and then 2.0 ml of 1×10⁻³ M of RB dye was added. The solution was diluted to 10 ml with distilled water after the addition of 2.0 ml of acetate buffer of pH 6 for CPM, DPH or FXO but buffer of pH 6.8 for DSL was added. The ion-pairs were extracted with 10 ml of dichloromethane by shaking for 2.0 min and then, the combined dichloromethane extracts were dried over anhydrous sodium sulphate. The absorbance of colored ion-pair complexes were measured within 5.0 min of extraction against the reagent blank prepared in the same manner except addition of drugs.

Procedure for tablets

Ten tablets of each commercial pharmaceutical formulation were crushed, powdered, weighed out and the average weight of one tablet was determined. An accurate weight equivalent to 20 mg each drug and then active component was transferred into a 100 ml measuring flask. About 25 ml of distilled water was added and the mixture was shaken thoroughly for about 5 min. Then, it was diluted up to the mark with distilled water, mixed well and filtered using filter paper. An aliquot of this solution was diluted appropriately to obtain the working concentrations and analyzed as described under the standard procedure.

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectra of the ion-pair complexes were measured in the range 525-630 nm against dichloromethane (blank). Antihistamine cations were found to react with anions of rose bengal dye in acidic buffer and gave an intense color with a maximum absorption at 550 nm as shown in fig. 2. Therefore, all the following measurements are carried out at 550 nm against blank where the investigated drugs, dyes, buffer and dichloromethane have no absorption in this region. The optimum conditions were established by varying one variable and observing its effect on the absorbance of the colored product.
Effect of pH

The influence of pH on the ion-pair complex formations of DSL, CPM, DPH and FXO with RB dye has been studied using different types of buffers of different media. The optimum buffer associated with the maximum color intensity is disodium hydrogen phosphate-citric acid of pH=6 in case of DPH, CPA or FXO but pH=6.8 in case of DSL (fig. 3). Buffer volume was determined by applying the same experiment and variation the volume regularly (0.5-4.0 ml). The higher absorbance value obtained at using 2.0 ml.

Fig. 3: Effect of pH on the formation of ion-pair complex between RB and the studied drugs at 550 nm

Choice of organic solvent

A number of organic solvents such as dichloromethane, chloroform, carbon tetrachloride, benzene and toluene were examined for extraction of the ion-pair complexes in order to provide an applicable extraction procedure. Dichloromethane was found to be the most suitable solvent for extraction of colored complex yielding maximum absorbance intensity and it was also, observed that only one extraction was adequate to achieve a quantitative recovery of the complex and very low absorbance of the reagent blank and shortest time to reach the equilibrium between both phases.

Effect of RB dye concentration

Keeping other conditions unaltered, the influence of 1×10⁻³ M RB dye concentration on absorbance was investigated. The results showed that the maximum absorbance was at using 3.0 ml from RB dye for DSL, CPM, DPH and FXO. After this volume, the absorbance remains constant by increasing the volume of RB dye. So any excess of reagents has no effect on the determination of the drugs.

Effect of shaking time

Shaking time of 1.0-4.0 min provided a constant absorbance and hence, 2.0 min was used as an optimum shaking time throughout the experiment. The ion-pair complexes were quantitatively recovered in one extraction only and were, also stable for at least 24 h without any change in color intensity.

Sequence of addition

The sequence of addition of drugs, buffer, and dye were studied via the formation of the colored complexes. The optimum sequence of addition was similar in all cases by starting with drug, then dye and at last buffer. Other sequences gave lower absorbance values under the same experimental conditions.

Effect of temperature and stability time

The effect of temperature on colored complexes was studied over the range 20-35 °C. It was found that the absorbance of the ion pair complex was constant up to 30 °C. At higher temperatures, the drug concentration was found to increase due to volatile nature of the dichloromethane. Therefore, the temperature chosen was 30 °C as the best temperature for micro-determination of the drugs under study in pure and pharmaceutical formulations. The stability time of the four extracted ion-pair complexes was more than 120 min.

Stoichiometric ratio

In order to establish the molar ratio between DSL, CPM, DPH, FXO drugs on one side and RB reagent used on the other, Job’s method of continuous variation was applied [62]. In this method, 5×10⁻³ M solutions of drug and reagent were mixed in varying volume ratios in such a way that the total volume of each mixture was the same. The absorbance of each solution was measured and plotted against the mole fraction of the drug. This procedure showed that a (1: 1) complex was formed through the electrostatic attraction between the positively charged drug, D⁺ ions and negatively charged reagent, R⁻ ions. The extraction equilibrium can be represented as follows:

\[ D_\text{aq}^+ + R_\text{aq}^- \leftrightarrow D_\text{org}^+ R_\text{org}^- \]

Where D⁺ and R⁻ represent the protonated drug and the anion of the reagent, respectively and the subscripts “aq” and “org” refer to the aqueous and organic phases, respectively.

Quantification

Under the optimum conditions described above, the calibration graphs for the investigated drugs were constructed by plotting absorbance versus concentration in μg/ml (fig. 4). Conformity with Beer’s law was evident in the concentration ranges cited in table 1. Regression equations, intercepts, slopes and correlation coefficients for the calibration data were presented in table 1.

The high molar absorptivities of the resulting colored complexes indicated high sensitivity of the method [2.35×10⁴–6.28×10⁴]. The small values of Sandell’s sensitivity indicate the high sensitivity of the proposed method in the determination of the drugs under investigation. The limit of detection (LOD) and limit of quantitation (LOQ) are calculated according to ICH guidelines [63] and the results are tabulated in (table 1).
Accuracy and precision

In order to determine the accuracy and precision of the recommended procedure, five replicate determinations at three different concentrations of the studied drugs were carried out. Precision and accuracy were based on the calculated relative standard deviation (RSD, %) and relative error (RE, %) of the found concentration compared to the theoretical one, respectively, indicating that the proposed method is highly accurate and reproducible (Table 2).

Table 1: Analytical parameters and optical characteristics with RB dye

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DSL</th>
<th>CPM</th>
<th>DPH</th>
<th>FXO</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>550</td>
<td>550</td>
<td>550</td>
<td>550</td>
</tr>
<tr>
<td>pH</td>
<td>6.8</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Beer’s law limit, µg/ml</td>
<td>1-6</td>
<td>4-18</td>
<td>6-16</td>
<td>2-22</td>
</tr>
<tr>
<td>Molar absorptivity, 1 mol⁻¹ cm⁻¹</td>
<td>( 6.28 \times 10^4 )</td>
<td>( 2.69 \times 10^4 )</td>
<td>( 2.35 \times 10^4 )</td>
<td>( 2.84 \times 10^4 )</td>
</tr>
<tr>
<td>Sandell’s sensitivity, ng/cm²</td>
<td>4.94</td>
<td>14.52</td>
<td>12.41</td>
<td>18.90</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9938</td>
<td>0.9945</td>
<td>0.9947</td>
<td>0.9926</td>
</tr>
<tr>
<td>Linear regression equation*</td>
<td>( y = 0.066x + 0.056 )</td>
<td>( R^2 = 0.994 )</td>
<td>( y = 0.056x + 0.086 )</td>
<td>( R^2 = 0.994 )</td>
</tr>
</tbody>
</table>

*\( A = a + bC \), where \( A \) is the absorbance and \( C \) is the concentration of drug in µg/ml.

Table 2: Evaluation of intra-day accuracy and precision of the proposed method

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Drug taken µg/ml</th>
<th>Drug found* µg/ml</th>
<th>Recovery, %</th>
<th>RSD, %</th>
<th>RE, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSL</td>
<td>1.0</td>
<td>0.999</td>
<td>99.994</td>
<td>3.410</td>
<td>-0.001</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>2.999</td>
<td>99.996</td>
<td>2.104</td>
<td>-0.033</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4.999</td>
<td>99.994</td>
<td>1.152</td>
<td>-0.020</td>
</tr>
<tr>
<td>CPM</td>
<td>4.0</td>
<td>3.999</td>
<td>99.994</td>
<td>4.255</td>
<td>-0.025</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>7.999</td>
<td>99.994</td>
<td>6.075</td>
<td>-0.012</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>13.999</td>
<td>99.996</td>
<td>2.542</td>
<td>-0.007</td>
</tr>
<tr>
<td>DPH</td>
<td>2.0</td>
<td>1.999</td>
<td>99.994</td>
<td>4.214</td>
<td>-0.050</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>5.999</td>
<td>99.994</td>
<td>1.401</td>
<td>-0.016</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.999</td>
<td>99.996</td>
<td>1.759</td>
<td>-0.010</td>
</tr>
<tr>
<td>FXO</td>
<td>2.0</td>
<td>1.999</td>
<td>99.996</td>
<td>3.871</td>
<td>-0.050</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.999</td>
<td>99.994</td>
<td>0.949</td>
<td>-0.010</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>17.999</td>
<td>99.996</td>
<td>3.759</td>
<td>-0.005</td>
</tr>
</tbody>
</table>

*Mean value of five determinations, *RE: Relative error.

Analysis of dosage forms

To evaluate the validity and reproducibility of the method, known amounts of the DSL, CPM, DPH and FXO drugs were added to the previously analyzed pharmaceutical preparations and the mixtures were analyzed by the proposed method. The percent recoveries are given in Table 3. Interference studies revealed that the common excipients and other additives such as lactose, starch, gelatin, talc and magnesium trisilicate, that are usually present in the tablet dosage forms did not interfere at their regularly added levels.
The proposed spectrophotometric method is simple, sensitive, and suitable for the determination of DSL, CPM, DPH and FXO drugs in bulk and pharmaceutical dosage forms. The proposed method offers the advantages of accuracy and time saving as well as simplicity of reagents and apparatus. The developed method may be recommended for routine and quality control analysis of the investigated drugs in pharmaceutical preparations.

Table 3: Recovery of the studied drugs in pharmaceutical formulations using the proposed method

<table>
<thead>
<tr>
<th>Drug formulations</th>
<th>Drug taken μg/ml</th>
<th>Drug foundμg/ml</th>
<th>Recovery, %</th>
<th>RSD, %</th>
<th>RE, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>sultan tablets,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.999</td>
<td>99.994</td>
<td>1.232</td>
<td>-0.050</td>
<td></td>
</tr>
<tr>
<td>50 mg/tab, 6</td>
<td>5.999</td>
<td>99.994</td>
<td>1.761</td>
<td>-0.016</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9.999</td>
<td>99.999</td>
<td>2.929</td>
<td>-0.010</td>
<td></td>
</tr>
<tr>
<td>allerfen tablets, 4 mg/tab</td>
<td>3.999</td>
<td>99.994</td>
<td>4.134</td>
<td>-0.025</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>7.999</td>
<td>99.996</td>
<td>2.910</td>
<td>-0.012</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>13.999</td>
<td>99.994</td>
<td>1.579</td>
<td>-0.007</td>
<td></td>
</tr>
<tr>
<td>allerfen tablets, 60 mg/tab</td>
<td>5.999</td>
<td>99.994</td>
<td>4.762</td>
<td>-0.016</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>13.999</td>
<td>99.996</td>
<td>2.936</td>
<td>-0.007</td>
<td></td>
</tr>
<tr>
<td>delarex tablets, 4 mg/tab</td>
<td>3.999</td>
<td>99.994</td>
<td>2.375</td>
<td>-0.011</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.999</td>
<td>99.996</td>
<td>4.377</td>
<td>-0.050</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4.999</td>
<td>99.996</td>
<td>3.466</td>
<td>-0.025</td>
<td></td>
</tr>
</tbody>
</table>

*Mean value of five determinations, **RE: Relative error.

CONCLUSION

The proposed spectrophotometric method is simple, sensitive, and suitable for the determination of DSL, CPM, DPH and FXO drugs in bulk and pharmaceutical dosage forms. The proposed method offers the advantages of accuracy and time saving as well as simplicity of reagents and apparatus. The developed method may be recommended for routine and quality control analysis of the investigated drugs in pharmaceutical preparations.

CONFLICT OF INTERESTS

Declared None

REFERENCES


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