Validated stability indicating liquid chromatographic method for the determination of fexofenadine hydrochloride in presence of its degradation products. Application to tablets and content uniformity testing.

Department of Analytical Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura, 35516, Egypt.
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ABSTRACT

A simple, stability - indicating, reversed phase liquid chromatographic method has been developed for the determination of fexofenadine hydrochloride in the presence of its forced alkaline, acidic and oxidative degradation products. Reversed phase chromatography was conducted using an ODS C18 (150 x 4.6 mm id) column at ambient temperature with UV-detection at 225 nm. A mobile phase consisting of potassium dihydrogen phosphate buffer: acetonitrile (35:65, v/v) adjusted to pH 5.5 with phosphoric acid, has been used for the separation of the studied drug and its degradation products at a flow rate of 1 ml/min. The calibration curve was rectilinear over the concentration range of 2-20 µg/ml with a detection limit (LOD) of 0.92 µg/ml, and quantification limit (LOQ) of 1.5 µg/ml. The proposed method was successfully applied for the analysis of fexofenadine hydrochloride in its dosage forms, the obtained results were favorably compared with those obtained by a comparison method. Furthermore, content uniformity testing of the studied pharmaceutical formulations was also conducted. The drug was exposed to forced alkaline, acidic and oxidative degradation according to the ICH Guidelines. Moreover, the method was utilized to investigate the kinetics of the different degradation products of the drug. The first-order rate constant, half-life time, and activation energy of the degradation reactions were calculated.

Key words: HPLC; Fexofenadine; degradation; stability study; content uniformity

1. INTRODUCTION

Fexofenadine, α, α - dimethyl-4-[1-hydroxy-4-[4-(hydroxydiphenyl-methyl)-1 piperidinyl]butyl]-benzene acetic acid [1], is a second generation antihistamine drug useful to available treatments of allergic diseases with a wide safety margin [2].

Several analytical methods were reported for the determination of fexofenadine (FXD) including spectrophotometry [3-5], spectrofluorometry [6], and High Performance Liquid Chromatography (HPLC) [6-10].

Photo degradation of FXD was previously studied [11] where elucidation of structure of the resultant degradation product was performed. On the other hand, acidic and basic degradation of the drug was mentioned in another report [12], but the method did not provide any separation of the degradation products, nor did it elucidate their structure or perform any stability kinetic studies. As a consequence, this paper is trying to focus on the development of an efficient liquid chromatographic method for the determination of FXD in the presence of its different degradation products in a short chromatographic run, and to study the results kinetically to prove the stability-indicating property of the method.

2.EXPERIMENTAL

2.1.Materials and reagents

Fexofenadine hydrochloride (FXD); of purity 99.68%; was kindly provided El-Obour Modern Pharmaceutical Industries Company, Cairo, Egypt. Acetonitrile and methanol (Sigma-Aldrich), HPLC grade.

• Phosphate buffer pH 5.5 (0.03 M) was prepared in distilled water. The pH was adjusted to 5.5 using 0.03 M phosphoric acid and 0.03 M sodium hydroxide.
• Sodium hydroxide (2M solution), hydrochloric acid (2M solution), hydrogen peroxide (6% v/v solution); (BDH, Poole,UK).

2.2.Pharmaceutical preparations

• Fastofen® tablets (Batch # 7065), labeled to contain 60 mg FXD / tablet and Fastofen® tablets (Batch # 109108), labeled to contain 120 mg FXD / tablet, El-Obour Modern Pharmaceutical Industries Company, Cairo, Egypt.
• Fexoxide® capsules (Batch # 308134), labeled to contain 180 mg FXD / capsule, Memphis Company for Pharmaceutical and Chemical Industries, Cairo, Egypt.

2.3.Apparatus

Separation was performed with a Shimadzu C-R6A Chromatopac equipped with a Rheodyne injector valve with a 20 µL loop and a UV/VIS detector.

• A Shimadzu UV 1601 PC Spectrophotometer equipped with a pair of 1 cm matched cells, recording range : 0-2; wavelength: 200-400 nm; factor:1; number of cells:1; cycle time:0.1 min was used.

2.4.Columns and mobile phases

Separation was achieved on an EC nucleosil C18-SN : 4115568 column (150 mm x 4.6 mm id) at ambient temperature with UV-detection at 225 nm. A mobile phase consisting of potassium dihydrogen phosphate buffer: acetonitrile in a ratio of 35:65 v/v. The mixture was then sonicated for 30 minutes. The resulting mobile phase was filtered through a 0.45 µm membrane filter (Millipore, Ireland).

2.5.Standard solutions

A stock solution containing 10.0 mg/ml of FXD was prepared in methanol and further diluted with the same solvent to obtain the working concentration range for the spectrophotometric measurements, and diluted with the mobile phase for the HPLC measurements. This solution was found to be stable for at least two weeks when kept in the refrigerator.A stock solution containing 1.0 mg/ml of diphenhydramine internal standard was prepared in methanol and further diluted with the mobile phase to obtain a final concentration of 10.0 µg/ml.

2.6.Preparation of the degradation products

For the spectrophotometric measurements, aliquots of FXD stock solution (10.0 mg/ml) were transferred into a series of 25 ml volumetric flask to obtain a final concentration of 400 µg/ml, the volume was completed with 2 M sodium hydroxide, 2 M hydrochloric acid, or 6 % hydrogen peroxide to prepare the alkaline, acidic, or oxidative degradation product respectively. The solutions were left in a boiling water bath for 20 minutes (acidic and alkaline degradation), and for 30 minutes (oxidative degradation). Aliquot volumes of the degraded solutions were transferred to a series of 10 ml volumetric flasks, neutralized with 2 M hydrochloric acid or 2 M sodium hydroxide for alkaline and acidic degradation respectively, the solutions were completed to volume with methanol, and the absorption spectra were measured at 225 nm.

For HPLC measurements, the above solutions were completed to the volume with the mobile phase, and the material was tested for degradation by the appearance of peaks at retention times of 5.5 minutes for the alkaline degradation product, 4.8 and 6 minutes for the acidic degradation products, 5 and 6.1 minutes for the oxidative degradation products, while the rest of the intact un-decomposed drug appear at 4 minutes.
2.7. Calibration curve

Aliquots of FXD standard solution covering the working concentration range were transferred into a series of 10 ml volumetric flasks, mixed with 100 µg/ml aliquots of diphenhydramine internal standard and diluted with the mobile phase to the mark. Twenty µl aliquots were injected (in triplicates) and eluted with the mobile phase under the reported chromatographic conditions. The calibration curve was constructed by plotting the peak area ratio against the final concentration of the drug (µg/ml). Alternatively, the corresponding regression equation was derived.

2.8. Analysis of tablets

Twenty tablets were weighed and pulverized. An accurately weighed quantity of the powder equivalent to 100 mg of FXD was transferred into a small conical flask, and extracted three successive times each with 30 ml of methanol. The extract was filtered into 100 ml volumetric flask. The conical flask was washed with few millilitres of methanol and completed to the mark with the same solvent. The procedure was followed as described under “Calibration Curve”. The nominal contents of the tablets were calculated using either the calibration graph or the corresponding regression equation.

3. RESULTS AND DISCUSSION

The mobile phase utilized in this study was chosen after several trials with various proportions of phosphate buffer and acetonitrile at different pH values, different molar strengths of buffer, different flow rate values, and various columns. Under the described chromatographic conditions complete base line separation with satisfactory resolution between the peaks was achieved in a short chromatographic run of less than 10 minutes. A collective study of experimental parameters affecting the chromatographic separation is summarized in Table 1.

### Table 1. Effect of experimental parameters on chromatographic performance

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of theoretical plates (a)*</th>
<th>Peak area ratio (FX) (a)*</th>
<th>% Error (b)*</th>
<th>Limit of quantitation (LOQ) (µg/ml) (d)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 6.0</td>
<td>3963 6300 7121 9400 71 0.73 0.84 0.45 0.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 5.5</td>
<td>3963 6300 7121 9400 71 0.73 0.84 0.45 0.72</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>pH 5.0</td>
<td>3963 6300 7121 9400 71 0.73 0.84 0.45 0.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 4.0</td>
<td>3963 6300 7121 9400 71 0.73 0.84 0.45 0.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 3.0</td>
<td>3963 6300 7121 9400 71 0.73 0.84 0.45 0.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 2.5</td>
<td>3963 6300 7121 9400 71 0.73 0.84 0.45 0.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 2.0</td>
<td>3963 6300 7121 9400 71 0.73 0.84 0.45 0.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 1.5</td>
<td>3963 6300 7121 9400 71 0.73 0.84 0.45 0.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 1.0</td>
<td>3963 6300 7121 9400 71 0.73 0.84 0.45 0.72</td>
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</tr>
<tr>
<td>pH 0.5</td>
<td>3963 6300 7121 9400 71 0.73 0.84 0.45 0.72</td>
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<td></td>
</tr>
<tr>
<td>pH 0.0</td>
<td>3963 6300 7121 9400 71 0.73 0.84 0.45 0.72</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a* is FXD, b* is the oxidative degradation product, c* is alkaline degradation product, d* is the acidic degradation product, **A is acetaminol, B is phosphate buffer

### 3.1. Analytical performance and applications

Detection limit (LOD) and quantification limit (LOQ) were calculated according to the USP[11] and listed in Table 2 together with other performance parameters.

### 3.2. Validation of the method

3.2.1. Accuracy

The accuracy of the proposed method was evaluated by analyzing standard solutions of FXD. The results obtained by the proposed method were favorably compared with those obtained by the comparison method[10], and found to be in good agreement where the % recovery ± SD was found to be 100.02 ± 0.59, and calculated t and F values of 0.58 and 2.07 respectively which shows the high accuracy of the method keeping in consideration that the tabulated t and F values are 1.83 and 3.86 respectively.

### 3.2.2. Precision

#### Repeatability and intermediate precision

They were performed according to ICH guidelines[12]. The results are shown in Table 3.

### 3.3. Pharmaceutical applications

3.3.1. Dosage forms analysis

The proposed method was successfully applied to the determination of FXD in its commercial tablets. The average percentages found of different concentrations were based on the average of three replicate determinations. The results showed % recovery ± SD of 99.92 ± 0.98, 100.18 ± 0.56, and 99.98 ± 0.67 for Fastofen® 60mg, Fastofen® 120mg, and Fexodine® respectively with % RSD ±SD of 0.72% (0.23 ± 0.0103), 0.72% (0.23 ± 0.0103), and 0.72% (0.23 ± 0.0103) respectively.

### 3.3.2. Content uniformity testing

Due to the high precision of the method, and its ability to rapidly estimate the concentration of the drug in a single tablet extract with sufficient accuracy, the method is suited for content uniformity testing which is a time consuming process when using conventional assay technique. The steps of the test were adopted according to the USP[11] procedure. The results demonstrated reasonable drug uniformity (mean % of recovery of 99.98, 100.47, and 100.12% for Fastofen® 60mg, Fastofen® 120mg, and Fexodine® respectively with % RSD ±SD of 0.72% (0.23 ± 0.0103), 0.72% (0.23 ± 0.0103), and 0.72% (0.23 ± 0.0103) respectively).

### 3.4. Interferences

Many drugs which are frequently co-administered with FXD such as erythromycin and ketoconazole were carefully tested. The studied drugs were found to be adequately separated from FXD and its degradation products as exemplified by the chromatograms represented in Figures 1 (A-C) illustrating the separation of erythromycin from FXD and its different degradation products. Similar results were obtained for the separation of ketoconazole which appeared at 8 minutes.

![Figure 1A: Chromatogram showing a) solvent front, b) internal standard (10µg/ml), c) remaining FXD, d) minor acidic degradation product, e) major acidic degradation product, f) erythromycin (10µg/ml)](image)

![Figure 1B: Chromatogram showing a) solvent front, b) internal standard (10µg/ml), c) remaining FXD, d) alkaline degradation product, e) erythromycin (10µg/ml)](image)
3.5. Degradation kinetics study

The degradation was found to be temperature dependent. The first order degradation rate constant and the half life time at each temperature were calculated (Table 4).

Plotting log \(K_\text{obs} \) values versus 1/T, the Arrhenius plots were obtained; a representative plot showing the alkaline degradation is illustrated in Figure 2. The activation energy for each type of degradation was also calculated.

Table 4. Effect of temperature on the kinetic parameters of FXD

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Alkaline degradation (k)</th>
<th>Acidic degradation (k)</th>
<th>Oxidative degradation (k)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.001400 495</td>
<td>0.002028 342</td>
<td>0.001400 495</td>
</tr>
<tr>
<td>60</td>
<td>0.00267 260</td>
<td>0.004929 141</td>
<td>0.00267 260</td>
</tr>
<tr>
<td>70</td>
<td>0.004089 169</td>
<td>0.008971 71</td>
<td>0.004089 169</td>
</tr>
<tr>
<td>80</td>
<td>0.005943 117</td>
<td>0.01338 52</td>
<td>0.005943 117</td>
</tr>
</tbody>
</table>

\(E_a = (KJ/\text{Cal mol}^{-1})\) 10.7 14.46 10.7

3.6. pH profile study

The effect of different pH values on the degradation of FXD was studied using Britton-Robinson buffer of pH values ranging from 2-10 at 100°C for different time intervals. The first order degradation rate constants were calculated and plotted versus various pH values resulting in pH profile curve (Fig.3). The lowest degradation rate constant is at pH 7.0.

3.7. Pathway of degradation

In order to isolate the different degradation products, complete degradation has to be attained first, this was accomplished by heating FXD with either 2M sodium hydroxide or 2M hydrochloric acid for two hours to get the alkaline or acidic degradation products respectively, and with 6% hydrogen peroxide for 3 hours to get the oxidative degradation product. Complete degradation was followed by HPLC, where the peak corresponding to the intact drug at 4 minutes completely disappeared. Fractions containing the degradation products were successively purified by preparative TLC using methylenechloride: methanol (90:10 v/v), The solvent was removed by evaporation under reduced pressure, and the purity of the products was tested by HPLC and TLC. The TLC was performed using chloroform: methanol (80:20 v/v) as a developing solvent for all the products, where the Rf of FXD, alkaline, acidic and oxidative degradation products were 0.84, 0.45, 0.65 and 0.59 respectively.

After confirmation of the purity of the degradation products, infra red (IR) and mass spectroscopy (MS) were performed to elucidate the structure of the resultant products.

The alkaline degradation is proposed to proceed as in scheme 1a, where decarboxylation occurs as confirmed by MS (Fig.4a) where a molecular ion peak of m/z 456 appears, which undergoes further fragmentation to form the base peaks characterized by the structural formula suggested in the scheme and having m/z values of 120 and 336. Intra Red spectroscopy shows the disappearance of the carbonyl stretching peak that appears at 1700-1760 cm⁻¹, confirming the decarboxylation of the drug.

Acidic degradation is suggested to proceed as in scheme 1b, where breakage of the heterocyclic ring occurs yielding two degradation products appearing at m/z of 275 and 226 (Fig. 4b). Oxidative degradation presented in scheme 1c explains the degradation pathway to occur through the formation of the N-oxide derivative in the fragment representing the major degradation product which appears in MS at m/z of 282 and another minor degradation product at m/z of 219 (Fig.4c).

Scheme 1: pathway of degradation of FXD in a) alkaline, b) acidic, c) oxidative conditions

4. Conclusion

A simple, rapid, stability - indicating, reversed phase liquid chromatographic method has been developed to determine FXD in the presence of its different
degradation products. The method succeeded to analyze the drug in its dosage forms, content uniformity testing of the studied pharmaceutical formulations was also performed. The kinetics of the different degradation products of the drug was investigated. The fist-order rate constant, half-life time, and activation energy of the degradation reactions were calculated. Moreover, different degradation products were isolated to elucidate the pathway of degradation.

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REFERENCES


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