Difference spectrophotometric method for the determination of Fluphenazine hydrochloride in tablets using peroxomonosulfate

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Keywords: spectrophotometric method, Fluphenazine hydrochloride, optical density, peroxomonosulfate.

The oxidative derivatization method using potassium hydrogenperoxomonosulfate for the indirect spectrophotometric determination of Fluphenazine hydrochloride is presented. Potassium hydrogenperoxomonosulfate is introduced as a derivatizing agent for Fluphenazine hydrochloride, yielding the sulfoxide. This reaction product was successfully used for the spectrophotometric determination of the Fluphenazine hydrochloride. The UV spectroscopic detection of the sulfoxide proved to be a more robust and sensitive method. The elaborated method allowed the determination of Fluphenazine hydrochloride in the concentration range of 0.2-30 µg mL⁻¹. The molar absorptivity at 349 nm is 5.6×10³ (dm³cm⁻¹mol⁻¹). The limit of quantification, LOQ (10S) is 0.24 µg/mL. A new spectrophotometric technique was developed and the possibility of quantitative determination of Fluphenazine hydrochloride in tablets 5.0 mg was demonstrated. The present method is precise, accurate and excipients did not interfere. RSD for Fluphenazine Hydrochloride 5.0 mg tablets was 1.37 %.

Introduction

Fluphenazine hydrochloride (FPh), 2-[4-[3-(2-(trifluoromethyl)phenothiazine-10-yl)propyl]piperazine-1-yl]ethanol dihydrochloride (Figure 1) belongs to the piperazine class of phenothiazines [1], and is a typical antipsychotic drug used for the treatment of psychoses such as schizophrenia, manic phases of bipolar disorder, agitation, and dementia [2]. In addition, as a serotonin antagonist, this agent may inhibit lymphocyte and myeloma cell proliferation [3].

Figure 1. The molecular structure of Fluphenazine hydrochloride
Numerous analytical methods have been noticed in the literature for the determination of FPh, either in pure form or pharmaceutical preparations and biological fluids. These methods include spectrophotometry [4-5], spectrofluorimetry [6-8], voltammetry [9-11], high performance liquid chromatography (HPLC) [12-17], densitometric high performance thin layer chromatography (HPTLC) [18] capillary electrophoresis [19], and chemiluminescence [20].

The British Pharmacopoeia (BPh) [21] recommended a non-aqueous potentiometric method for the determination of FPh using perchloric acid as a titrant; the LOQ was 6.3 mg mL\(^{-1}\). For analysis of its tablets, the British Pharmacopoeia (BPh) and the European Pharmacopoeia (Ph Eur) recommended a spectrophotometric method based on recording second-derivative ultraviolet absorption spectra of the working and reference solutions in the range 230 to 300 nm. For each solution, the amplitude was measured from the peak at about 266 nm to the peak at about 258 nm [22].

The United States Pharmacopoeia (USP) on the other hand, described an HPLC method for the determination of FPh in pure form, Fluphenazine Hydrochloride Injection and Fluphenazine Hydrochloride Tablets using UV detection at 254 nm, where the LOQ was 2.4 µg mL\(^{-1}\) [23].

However, HPLC method involves sophisticated and expensive equipment or solvents and is time-consuming. Therefore, there is a need for an alternative substitute to the HPLC methods, and the method of derivatization difference spectrophotometry by virtue of its high sensitivity and selectivity can be a promising substitute [24, 25].

The application of the method of derivatization spectrophotometry with the use of potassium hydrogenperoxomonosulfate (oxone) as a derivatizing reagent is currently very promising. The light absorption of the oxidation product by this reagent, the corresponding FPh sulfoxide (Figure 2), can be successfully used to develop a relatively simple method for the quantitative determination of FPh in pharmaceuticals, in particular in 5 mg tablets.

![Figure 2. Scheme of FPh oxidation by oxone](image)

In difference spectroscopy, a component in a mixture is analyzed by carrying out a reaction
which is selective for the analyte, this chemical transformation, an oxidation for example, inducing a wavelength shift.

Excipients do not undergo appreciable shifts whereas FPh does. Figure shows the spectrum of the extract from tablets in 0.001 M H$_2$SO$_4$. In fact, there is relatively minor background noise at the wavelength used for the determination of FPh but by using the sample in H$_2$SO$_4$ in place of a blank in the reference cell, one can be sure that background noise from excipients will be eliminated.

Figure 3 shows the difference spectra of the tablets extract in 0.001 M H$_2$SO$_4$ in the reference cell and the tablets extract in 0.001 M H$_2$SO$_4$ with oxone in the sample cell. The absorbance at 349 nm is thus wholly due to FPh.

![Figure 3](image_url)

**Figure 3.** Electronic spectra of FPh hydrochloride (1) and FPh sulfoxide (2). $c$(FPh) = 2.6 × 10$^{-5}$ mol L$^{-1}$; $c$(FPh sulfoxide) = 2.6 × 10$^{-5}$ mol L$^{-1}$; $c$(H$_2$SO$_4$) = 1.0 × 10$^{-3}$ mol L$^{-1}$

The problem remains of how to quantify the analyte in such a sample. This can be readily carried out using through the addition of a known amount of FPh as a standard and comparison of the absorbance of the original sample extract with the absorbance of the spiked sample.

**Experimental part**

**Materials and methods**

As an analytical reagent, potassium hydrogenperoxomonosulfate (oxone) in the form of a stable triple salt Oxone®, monopersulfate (2KHSO$_5$·KHSO$_4$·K$_2$SO$_4$) (SIGMA-ALDRICH, CAS: 70693-62-8, the content of active oxygen 4.5 %) was used.

**Oxone solution.** 600 mg of oxone was dissolved in a 100 mL volumetric flask by twice distilled water and mixed thoroughly. The exact content of potassium hydrogen peroxymonosulfate in the solution was monitored by iodometric titration [24, 26].

Fluphenazine dihydrochloride (FPh) (Sigma-Aldrich, qualification: Pharmaceutical Primary Standard). Synonym: 4-[3-[2-(Trifluoromethyl)-10H-phenothiazin-10-yl]propyl]-1-piperazineethanol dihydrochloride, CAS number 146-56-5, C$_{22}$H$_{26}$F$_3$N$_3$OS·2HCl, molecular weight 510.44 g mol$^{-1}$.

It is a chemical reference substance (CRS Pharmacopoeial standard (EPh), purity not less than 99.5 % (titer)). All other reagents were "pure for analysis" or "chemically pure" and used without further purification.

**Fluphenazine working standard solution, 0.10 mg mL$^{-1}$, was prepared by volume-weight method. A sample of the preparation with a known content of the main substance containing 10.0 mg of FPh in terms of FPh hydrochloride, was dissolved in 100 mL of 0.001 M sulfuric acid solution.**
The object of study was tablets Fluphenazine (HCl) 5mg, production Mediphar Laboratories (Dbayeh, Lebanon), batch number 8273. The average weight of the tablet ($n=15$) was 0.3177 g. Stated content in the tablets: Fluphenazine Hydrochloride 5.0 mg and inactive ingredients: hydroxypropyl methylcellulose, lactose monohydrate; polyethylene glycol; polysorbate 80, povidone, stearic acid, and titanium dioxide

Procedure for obtaining results of the calibration graph. Using a pipette, different volumes of Fluphenazine working standard solution (1.00–15.00 mL) were taken and transferred to a 50 mL volumetric flask. 0.5 mL of 0.1 M sulfuric acid solution, 1.0 mL of $2 \times 10^{-2}$ mol L$^{-1}$ oxone solution are added thereto and this solution was diluted to the volume with twice distilled water, cork and mix thoroughly for 5 minutes. Fill the cuvette with the resulting solution and measure the light absorption on a spectrophotometer at a wavelength of 349 nm.

The graph was positioned in the following coordinates: the absorbance (A) on the ordinate axis and corresponding concentration of FPh, $C$ in $\mu$g mL$^{-1}$ on the abscissa axis (Fig. 4). The graph equation coefficients have been calculated by least square method.

Procedure for determining the content of Fluphenazine in tablets of 5 mg (by the addition method). Analysis was carried out by difference spectrophotometry. A one-point standard calibration for the determination of FPh in a standard stock solution was used.

Test solution was prepared by dissolving 0.64 g (accurate weight) of powdered tablets (5 mg each), which corresponds to the average weight of two tablets, with 1.0 mL of 0.1 M sulfuric acid solution and 20-30 mL of water. Mix thoroughly on a shaker for 30 minutes, filter through a "blue tape" filter, rinse the residue thoroughly on the filter with twice distilled water and, combining the filtrates, transfer the solution quantitatively into a 100 mL volumetric flask. The solution was diluted to the volume with twice distilled water and mixed thoroughly. Using a pipette, 10.0 mL of the resulting solution is taken and transferred to a 50 mL volumetric flask, 0.5 mL of 0.1 M sulfuric acid solution, 1.0 mL of $2 \times 10^{-2}$ mol L$^{-1}$ oxone solution are added thereto and this solution was diluted to the volume with twice distilled water, cork and mix thoroughly for 5 minutes. Fill the cuvette with the resulting solution and measure the light absorption on a spectrophotometer at a wavelength of 349 nm. Readings were taken at 349 nm of the sample solution without standard addition versus reference solution 1.

The reagent solution, which contained the solvent in which the analyte was solubilized and the reagent added to the sample prior to measurement, showed no absorbance at 349 nm.

Similar procedures are performed with a solution with the addition of a working standard sample. Using a pipette, 10.0 mL of the resulting
solution is taken and transferred to a 50 mL volumetric flask. 5.0 mL of standard solution of FPh, 0.5 mL of 0.1 M sulfuric acid solution, 1.0 mL of $2 \times 10^{-2}$ mol L$^{-1}$ oxone solution are added thereto and this solution was diluted to the volume with twice distilled water, cork and mix thoroughly for 5 minutes. Fill the cuvette with the resulting solution and measure the light absorption on a spectrophotometer at a wavelength of 349 nm. Readings were taken at 349 nm of the sample solutions with standard addition versus reference solution 2.

The content of fluphenazine in terms of fluphenazine dihydrochloride in mg in one tablet is calculated by the formula:

$$X = \left( C_{st} \times A_x \times k \times V \times m \right) / \left( (A_{x+st} - A_x) \times m_s \right)$$

where $A_x$ – optical density in the experiment with the test solution of tablets;

$A_{x+st}$ – optical density in the experiment with the investigated solution of tablets and FPh standard solution;

$C_{st}$ – the concentration of fluphenazine hydrochloride in the cuvette in the experiment with the test solution of tablets and FPh standard solution, mg mL$^{-1}$;

$m_s$ – weight of a sample of tablet powder, g;

$m$ – the average weight of the tablet, g;

$V$ – volumetric flask volume;

$k$ – dilution factor.

**Results and discussions**

The linear dependence of the absorbance is observed in the FPh concentration range 1-30 µg mL$^{-1}$, the calibration curve equation was

$$A = (1.12 \pm 0.005) \times 10^{-2} \times C \quad (r = 0.999)$$

(Figure 4).

Analytical characteristics of the calibration graph for the quantification of FPh hydrochloride as the corresponding sulfoxide are given in Table 1.

![Figure 4](image.png)

**Figure 4.** Calibration graph for the quantification of FPh hydrochloride as the corresponding sulfoxide. $c(\text{H}_2\text{SO}_4) = 1.0 \times 10^{-3}$ mol L$^{-1}$

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration range (µg mL$^{-1}$)</td>
<td>0.2-30</td>
</tr>
<tr>
<td>Correlation coefficient ($r$)</td>
<td>0.999</td>
</tr>
<tr>
<td>Linear regression equation</td>
<td>$y = 0.0112x - 0.0002$</td>
</tr>
<tr>
<td>Slope ($b \pm \Delta b$)</td>
<td>$0.0112 \pm 0.00005$</td>
</tr>
<tr>
<td>Intercept ($a \pm \Delta a$)</td>
<td>$-0.0002 \pm 0.0008$</td>
</tr>
<tr>
<td>S.D. of slope ($S_b$)</td>
<td>0.00002</td>
</tr>
<tr>
<td>S.D. of intercept ($S_a$)</td>
<td>0.0003</td>
</tr>
<tr>
<td>LOD (3S) (µg mL$^{-1}$)</td>
<td>0.071</td>
</tr>
<tr>
<td>LOQ (10S) (µg mL$^{-1}$)</td>
<td>0.238</td>
</tr>
</tbody>
</table>

The molar absorptivity (dm$^3$cm$^{-1}$mol$^{-1}$) at 349 nm is $5622.7 \pm 32.6$; LOQ = 0.24 µg mL$^{-1}$.

The results of the quantitative determination of FPh hydrochloride in 5 mg tablets are given in Table 2. Fluphenazine tablets contain from 90.0 % to 110.0 % of the specified...
amount of fluphenazine hydrochloride \((C_{22}H_{26}F_{3}N_{3}OS \cdot 2HCl)\).

Table 2. Results of the quantitative determination of fluphenazine hydrochloride in tablets of 5 mg

<table>
<thead>
<tr>
<th>Taken</th>
<th>Found</th>
<th>Metrological characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/one tablet</td>
<td>mg/one</td>
<td>P=0.95</td>
</tr>
<tr>
<td>0.64000 g tablets powder</td>
<td>4.90</td>
<td>(\bar{x} = 4.95)</td>
</tr>
<tr>
<td>Fluphenazine hydrochloride 5 mg, Mediphar Laboratories (Dbayeh - Lebanon)</td>
<td>4.89</td>
<td>(R=100.40%)*</td>
</tr>
<tr>
<td></td>
<td>5.06</td>
<td>(S = 0.0676)</td>
</tr>
<tr>
<td></td>
<td>4.94</td>
<td>(\Delta x = 0.0841)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(RSD = 1.37%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(\delta^* = +0.41%)</td>
</tr>
</tbody>
</table>

Notes: * The calculation is based on the average content found by the method of USP 39 (4.93 mg / tablet - 98.60 ± 10% (μ). \(\delta = (\bar{x}-\mu) 100\% / \mu\)

Precision and accuracy

The levels of precision and accuracy of the measurement method were confirmed by calculation of the relative standard deviation (%RSD) and percentage recovery (%R) using five replicate measurements of a drug sample; the trueness of the measurement method was investigated by comparing the accepted reference value (according to the certificate of analysis) with the results given by the measurement method. In general, good levels of precision were obtained for API with perfect value of 1.37% RSD, as shown in Table 2. The results obtained for the API analysis using the differential spectrophotometric method were highly comparable to the certified value. Analytical recovery value is 100.40% for API determined, as reported in Table 2.

In conclusion, the proposed method provided a sensitive, specific and inexpensive analytical procedure for Fluphenazine hydrochloride. It can be applied for quality control testing and drug stability monitoring. This subject is now under investigation.

Conclusions

For the first time, a procedure for the indirect spectrophotometric determination of Fluphenazine hydrochloride as its S-oxide obtained by reaction with potassium hydroperoxomonsulfate was proposed for use in pharmaceutical analysis. The proposed spectrophotometric method for the determination of Fluphenazine hydrochloride is simple, reliable, sufficiently sensitive and accurate, is faster and does not require expensive and relatively toxic solvents needed for HPLC procedures. A new spectrophotometric technique was developed and the possibility of quantitative determination of Fluphenazine hydrochloride in tablets 5.0 mg was demonstrated. The present method is precise, accurate and excipients did not interfere. RSD for Fluphenazine hydrochloride 5.0 mg tablets was 1.37%.

References


