The quantitative determination of Perphenazine in tablets by the spectrophotometric method as its sulfoxide obtained with diperoxyazelaic acid

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The oxidative derivatization method using diperoxyazelaic acid for the indirect spectrophotometric determination of Perphenazine dihydrochloride is presented. Diperoxyazelaic acid is introduced as a derivatizing agent for Perphenazine, yielding sulfoxides. This reaction product was successfully employed for the spectrophotometric determination of Perphenazine dihydrochloride. The UV spectroscopic detection of sulfoxide has been proven to be the more robust and selective. The method developed allowed determination of Perphenazine dihydrochloride in the concentration range of 1–40 μg/mL. The limits of quantification (LOQ=10S) is 3.3 μg·ml-1. A new spectrophotometric method has been developed, and the possibility of the quantitative determination of Perphenazine dihydrochloride in Perphenazine Tablets has been demonstrated. The present method is precise, accurate and other inactive excipients of the drug do not interfere. RSD = 2.00%; δ=($\bar{x}$ -μ) 100%/μ = – 0.85 %).

Introduction

Perphenazine (PER), 2-[4-[3-(2-chlorophenothiazin- 10- yl) propyl] piperazin-1- yl] ethanol, chemically belongs to the piperazinyl phenothiazine family of drugs with the chemical formula – $C_{21}H_{26}CIN_3OS$ (Fig. 1). Because of its neuroleptic and antidepressive effects it is often prescribed for the treatment of mental illnesses, such as schizophrenia and schizoaffective psychosis in order to decrease restlessness, aggressiveness and impulsive behavior. It is also found to be effective in the treatment of Parkinson's disease [1]. It is available as oral tablets containing 2 mg, 4 mg, 8 mg, and 16 mg of perphenazine.

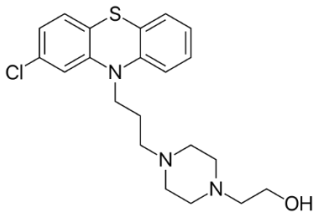

Figure 1. The chemical formula of Perphenazine base (*syn*. Etaperazine)

Perphenazine hydrochloride, as well as other 2,10-substituted phenothiazine derivatives, possesses some interesting chemical properties. It is very susceptible to oxidation by many oxidizing agents, such as H_2O_2, $Cr_2O_7^{2-}$, VO^{3-},

Ce^{4+}, Fe^{3+}, $[Fe(CN)_6]^{3-}$, Au^{3+}, chloramine T, etc. [2]. The oxidation reaction of Perphenazine proceeds in two steps. The first step is the loss of one electron giving a violet semiquinone free radical cation. In the second step of reaction, the free radical cation loses another electron giving the colorless Perphenazine sulfoxide (Ph-SO). The described process can be presented as follows (see Scheme in **Figure 2**.) [3].

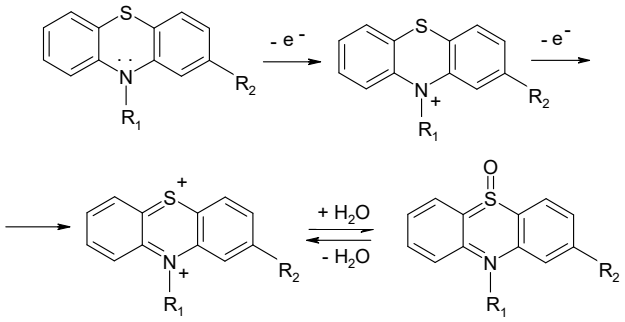

Figure 2. The scheme of Phenothiazine derivatives oxidation

Thus, the final oxidation product is Phenothiazine S-oxide. The monitoring of Perphenazine is important for quality assurance in pharmaceutical industry and for obtaining the optimum therapeutic concentrations in the body fluids to minimize the risk of toxicity. Therefore, it is important to develop simple and sensitive methods for the determination of this drug. A wide variety of analytical procedures are available for the determination of perphenazine in pharmaceutical preparations and biological samples, such as HPLC [4,5], mass spectrometry [6], spectrophotometry [7,8], electrochemistry [9-11], electrophoresis [12] fluorescence [13] and chemiluminescence [14].

The official method for the assay of Perphenazine in tablets described by the British Pharmacopoeia (BPh) [15] is a spectrophotometric method, in which the ultraviolet absorption spectra of the second derivative of the following solutions are recorded in the range from 210 to 290 nm, and the amplitude is measured from the peak at 265 nm to the trough at 255 nm. The $C_{21}H_{26}N_3OS$ content was calculated using the declared $C_{21}H_{26}N_3OS$ content in BPCRS perphenazine. In return, for the assay of perphenazine injection, syrup and tablets Perphenazine the United States Pharmacopoeia (USP) [16] recommends applying the procedure with the acid-alcohol solution and Palladium chloride solution with the subsequent measurement of the optical density at a wavelength of about 480 nm. The content is found by the standard method. One of the promising areas in improving the selectivity and sensitivity of the determination of phenothiazine derivatives is an approach based on their determination in the form of a functional derivative - S-oxidation product produced from the analytical reagent using a peroxide derivative of organic acid. Chemical conversion covers only the functional group of the active substance molecules, and therefore, the selectivity of the active substance determination is achieved [17,18].

We have offered to perform the Perphenazine assay by using indirect spectrophotometry in the form of the

corresponding sulfoxide produced with diperoxyazelaic acid as an analytical oxidizing reagent.

The scheme of Perphenazine oxidation with diperoxy azelaic acid in the acidic medium is given in **Figure 3**.

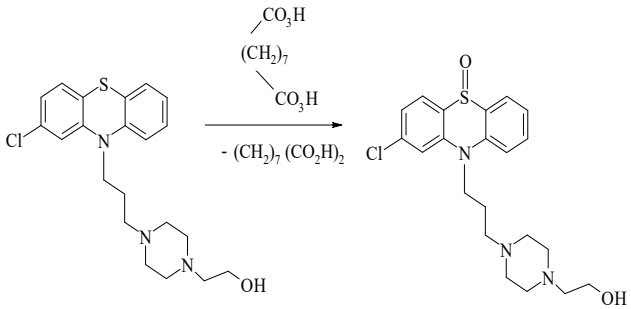

Figure 3. The scheme of Perphenazine oxidation with diperoxy azelaic acid in the acidic medium.

Experimental part

Material and methods

Reagents

Diperoxyazelaic acid. Diperoxyazelaic acid was prepared by acylation of hydrogen peroxide with azelaic acid in the concentrated sulfuric acid according to the Swern method [18].

Potassium iodide solution. Dissolve 10 g of potassium iodide in fresh boiled and cooled water, and dilute the solution to the volume of 100 mL. The solution should be colorless.

0.1 mol L-1 sodium thiosulfate standard solution was prepared using the titer–fixanal ampoule.

The hydrochloric acid solutions: c(HCl) =2 mol L^{-1}; 0.4 mol L^{-1} and 0.2 mol L^{-1}.

The determination of Perphenazine was made using commercial tablets available in Russia: “Ethaperazine” (Perphenazine) film-coated tablets, 10 mg, No. 50, by “Tatchempharmpreparaty” JSC (Kazan, Russia), batch number 10218. According to Data Sheet No. 458, the drug content was 0.0092 g per tablet (tolerances of no less than 0.0085 g per tablet and no more than 0.0115 g per tablet, i.e. 85-115%). The dose of Perphenazine in a pharmaceutical preparation, was 10 mg/tablet. Thus, ten tablets were powdered and a suitable quantity of the sample was accurately weighed and dissolved in 0.4 mol L^{-1} of hydrochloric acid solution. The solubility was increased by using an ultrasonic bath.

Preparation of 0.0085 mol L^{-1} diperoxyazelaic acid. Weigh 0.2 g of diperoxy azelaic acid powder and dissolve in 100 mL of 50% ethanol. The content is determined by iodometric titration.

The standard solution (0.1 mg ml-1) of perphenazine dihydrochloride (Sigma, St. Louis, Missouri, USA) was prepared by dissolving a weighed amount of the drug (containing 10.0 mg of the Perphenazine base, $C_{21}H_{26}ClN_3OS$) in 100.0 mL of 0.4 mol L^{-1} hydrochloric acid solution at +20°C. It was stored in a refrigerator and protected from light.

All chemicals used were of the analytical reagent grade, and double-distilled water was used throughout the experiment.

Apparatus

Registration of spectra of Perphenazine hydrochloride solutions and products of its oxidation, as well as measurement of absorbance of solutions, was performed in a 1 cm quartz cuvette on an Evolution 60S UV-Visible Thermo-Scientific Spectrophotometer (USA) against a solution without the Phenothiazine derivative analyzed or double-distilled water (compensation solution).

The pH of the solution was measured by the electrometric method using an "Ionmeter I-130" potentiometer with an ESL-43-07 glass electrode.

A 10 ml class 2 microburette was used to measure the volume of the titrant solution.

Procedure

Preparation of the calibration curve

Using a pipette sample 2.00; 5.00; 10.00; 15.00 and 20.00 mL of Perphenazine standard solution and transfer to 50 mL flasks, then sequentially add 10 mL of 2 mol L^{-1} hydrochloric acid solution and 1.0 mL of 0.0085 mol L^{-1} diperoxy azelaic acid solution to each flask, and dilute to the volume with double distilled water capped and thoroughly mixed by upturning the flask 7-10 times. The absorbance was measured at 342 nm using double distilled water as a compensation solution.

Procedure for Pharmaceutical Preparation

Determination of the Perphenazine content in Ethaperazine film-coated tablets, 10 mg.

Powder 20 tablets and accurately weigh approximately 0.31 g of the sample and mix with 50 mL of 0.4 mol L^{-1} hydrochloric acid solution and thoroughly shake for 30 min, filter through a glass microfiber paper filter, rinse the residue carefully on the filter (3 times by 10 mL) with the recommended solution, and once the filtrates are combined, transfer the solution to a 100 mL flask. Dilute the solution volumes with the recommended solvent and thoroughly stir up. Using a pipette sample 20 mL of the Perphenazine solution obtained and transfer to 50 mL flasks, then sequentially add 10 mL of 2 mol L^{-1} hydrochloric acid solution and 1.0 mL of 0.0085 mol L^{-1} diperoxy azelaic acid solution, and dilute to the volume with double distilled water capped and thoroughly mixed by upturning the flask 7-10 times. The absorbance was measured at 342 nm using double distilled water as a compensation solution.

The similar procedure was performed with the standard solution (SS) according to instructions to the method starting with "…sample 20 mL of the Perphenazine solution obtained…".

The content of Perphenazine dihydrochloride expressed as the Perphenazine base ($C_{21}H_{26}ClN_3OS$) (X) per mg in the tablet is calculated according to the following formula:

$$X=(C_{st}\times A_x\times 100\times \bar{m})/(A\times m)$$

where A_x is the absorbance of the test solution;

A is the absorption in the experiment with the SS;

C_{st} is the drug content in the SS, mg mL^{-1};

m is the weighed mass of the tablet powder, g;

100 is the flask volume for pharmaceutical or standard solution preparation;

$\overline{m}$ is the average mass of a tablet, g.

Results and discussion

Figure 4 shows the UV-spectrum of Perphenazine (lower curve) and its S-oxidation product – the corresponding sulfoxide (upper curve).

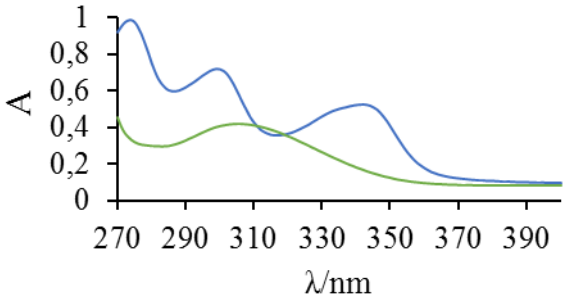

Figure 4. The UV-spectrum of Perphenazine (lower curve) and its S-oxidation product – the corresponding sulfoxide (upper curve). C=40 µg mL^{-1}, 0.4 mol L^{-1} HCl

The linear dependence of the absorbance was observed in the concentration range of 1 to 40 µg mL-1 of Perphenazine (A=0.0134±0.0006)C – (0.004±0.01), r=0.999) where C – the concentration in µg mL-1 (**Figure 5** and **6).**

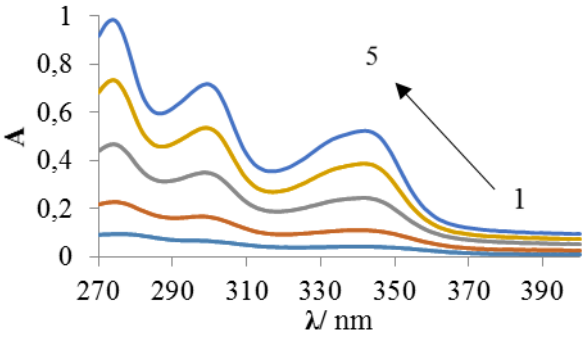

Figure 5. The concentration dependent UV-spectra of the S-oxidation product of Perphenazine (Perphenazine base), C, µg mL^{-1}:1 – 4; 2 – 10; 3 – 20; 4 – 30; 5 – 40. 0.4 mol L^{-1} HCl

At 342 nm, the molar absorptivity was $5.45 \cdot 10^3$ L·mol^{-1}·cm^{-1}. LOD(3S) =0.6 µg mL^{-1}; LOQ(10S)=1.9 µg mL^{-1}.

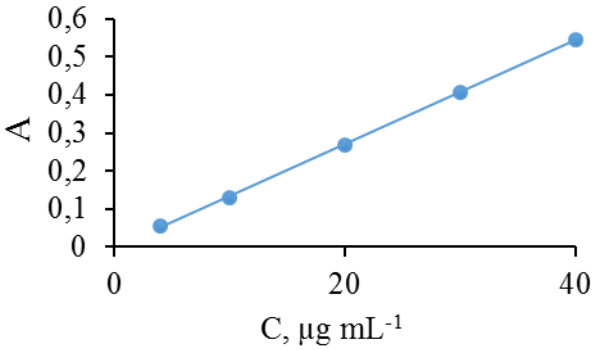

Figure 6. The dependence of absorption on the Perphenazine concentration

Various quantities of interfering compounds that were possible during production were added to a fixed amount of perphenazine dihydrochloride (10 mg) studied, and the recommended procedure for the spectrophotometric determination was followed (**Table 1**). Other compounds, such as ascorbic acid, microcrystalline cellulose, polyethylene glycol and potassium hydrogen sulfite did not show any interference as well.

The data obtained showed a prospect of the possibility of using the method developed for the analysis of the tablet form of the drug. **Table 2** shows the results of the analysis of Perphenazine in film-coated tablets (10 mg) obtained by the spectrophotometric method proposed. It has been proven that the method developed allows accurately determining Perphenazine in a dosage form.

Table 1. The quantitative assessment of tolerable amounts of the possible interference

Possible interfering Inactive Excipients of the drug	The amount without interfering[a] (mg)
Excipients :	
Lactose monohydrate	125.0
Potato starch	13.5
Corn starch	13.5
Calcium stearate	1.5
Magnesium stearate	1.5
Shell Excipients:	
Sucrose	93
Magnesium hydroxy carbonate	50
Povidone	2
Colloidal silicon dioxide	3.3
Tropeolin O	0.01
Yellow quinoline	0.13
Indigo carmine	0.01
Titanium dioxide	1.5
Bee wax	0.22

[a] The value is mg of the drug excipient with respect to 10 mg of perphenazine, which does not cause absorption changes by more than +0.005.

The RSD does not exceed ± 2 %. The results are in good agreement with the analysis of Perphenazine tablets according to the Specification. The determination of Perphenazine in the form of the corresponding sulfoxide obtained with the use of diperoxyazelaic acid as an analytical reagent is quite selective, as well as faster and less laborious and cheaper compared to the methods based on the formation of phenothiazine free radicals [3] or the complex with Palladium salt [16], respectively. The official method of analysis of Perphenazine tablets recommended by the British Pharmacopoeia (BPh), in our opinion, is also relatively difficult to implement and takes a lot of time.

Table 2. The results of the assay for Perphenazine film-coated tablets, 10 mg

Taken	Found	Metrological characteristics
	mg per tablet	P=0.95
0.33701 g (9.2 mg Perphenazine per tablet ± 10%) "ETAPERAZINE" "Tatchempharm-preparaty" JSC (Kazan, Russia)	8.90 9.15 9.35 9.25 9.28 9.00 8.92	$\bar{x}$ = 9.12 S = 0.18 $S_{\bar{x}}$ = 0.07 $\Delta\bar{x}$ = 0.17 *RSD* = 2.00 % δ* = – 0.85 %

Notes: * The calculation is made using the μ analysis specification data, $\delta = (\bar{x} - \mu)\ 100\%/\mu$

Conclusions

On the example of the determination of Perphenazine in a dosage form using diperoxyazelaic acid as an oxidizing agent the method for the indirect spectrophotometric determination of phenothiazine derivatives with piperazine rings in side chains in ition 10 has been proposed. The linear dependence of the absorbance was observed in the concentration

range of 40 μg mL^{-1} of Perphenazine (A=0.0134 C, r=0.999) where C – the concentration in μg mL-1. At 342 nm, the molar absorptivity was $5.45 \cdot 10^3$ L·mol-1·cm^{-1}. LOD(3S) =1.1 μg mL^{-1}; LOQ(10S)=3.3 μg mL^{-1}. The assay of Perphenazine in the form of the corresponding sulfoxide obtained with the use of diperoxyazelaic acid as an analytical reagent is quite selective, as well as faster and less laborious and cheaper compared to methods based on the formation of phenothiazine free cation-radicals or the complex with Palladium salt, respectively.

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