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APPLICATION OF AMMONIUM PEROXIDISULFATE AND METAVANADATE FOR SPECTROPHOTOMETRIC DETERMINATION OF PROTHIPENDYL HYDROCHLORIDE

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Abstract: Prothipendyl hydrochloride reacts with $(NH_4)_2S_2O_8$ and NH_4VO_3 forming the coloured oxidation products which exhibit maximum absorbance at λ = 372 nm and 374 nm, respectively. The optimum conditions of the reaction have been established. It was found that Beer's law is obeyed in the concentration range 3–95 μg/ml and 3–90 μg/ml in PTP–$(NH_4)_2S_2O_8$ and PTP–NH_4VO_3 system, respectively.

Keywords: prothipendyl, spectrophotometry, oxidation.

Prothipendyl, i.e. [10–(3–dimethylaminopropyl)–1–azaphenothiazine] belongs to important group of compounds– azaphenothiazine derivatives. It is known for its sedative and antiemetic activities and its use has therapeutic importance for the treatment of diseases of the central nervous system.

Due to the characteristic molecular structure prothipendyl shows a number of interesting analytical properties. It exhibits similar properties to phenothiazines, especially to promazine (1). It easily oxidized in an acid medium by some oxidizing agents, e.g. NH_4VO_3, $(NH_4)_2S_2O_8$, with the formation of coloured products. This property may be exploited for the spectrophotometric determination of prothipendyl hydrochloride. Prothipendyl is available in dosage forms mainly tablets and injections. Among methods applied for the determination of the drug, chromatographic and electrochemical methods prevail (2–8). Chromatographic methods have adequate sensitivity for determining of the typically low therapeutic levels of prothipendyl in biological fluids. Use of these methods is adequate when the sample matrix is rather complex and the drug concentration is low. In pharmaceutical analysis, where analyte concentration levels are fairly high, the main aim is to develop rapid, simple, reproducible and inexpensive methods that can readily find applications in routine analysis and in the quality control laboratories. Spectrophotometry, due to its simplicity is very useful in qualitative and quantitative analysis of drugs in pharmaceuticals.

Number of the spectrophotometric methods for the determination of the cited drug is very limited, so the authors have taken the task to elaborate new and simple methods for the determination of prothipendyl hydrochloride.

As a continuation of our previous studies in the field of tricyclic psychotropic drugs (9–13) the present work investigates application of reactions of $(NH_4)_2S_2O_8$ and NH_4VO_3 with prothipendyl in order to establish conditions of quantitative analysis of the drug. Under the recognized properties the new spectrophotometric methods for determination of prothipendyl hydrochloride in pure form and its pharmaceutical preparations have been elaborated.

EXPERIMENTAL

Reagents and Apparatus

Prothipendyl hydrochloride (PTP.HCl) stock solutions. An aqueous (10^{-2} mol/l) stock solution of prothipendyl hydrochloride was prepared from the pure product (Fluka) by dissolving an appropriate weighed amount (3.2171 g) in 1 litre of distilled water. Stock solution was kept in the refrigerator. Working solutions were freshly prepared by appropriate dilution of the stock solution.

Ammonium peroxidisulfate (POCH, Poland) 10^{-2} mol/l solution was prepared by dissolving appropriate amounts in distilled water.

Ammonium metavanadate (Fluka, Switzerland) 10^{-2} mol/l solution was prepared by dissolving appropriate amounts of NH_4VO_3 (0.5850 g) in 500 ml of 0.5 mol/l sulphuric acid.

Solutions of acids. Appropriate concentrations of acid solution were prepared by dilution of concentrated acid (POCH, Poland).

Dominal Forte tablets (ASTA Medica, Frankfurt). Solution with the concentration 1000 μg/ml

of prothipendyl was prepared by dissolving an amount of crushed and powdered tablets equivalent to the required amount of prothipendyl in distilled water. The mixture was filtered and made up to volume in a 50 ml volumetric flask. Working solutions were obtained by appropriate dilution.

Injection fluid of prothipendyl Dominal Forte. Working solutions were prepared by appropriate dilution of the fluid as a stock solution without any treatment.

All reagents used were of analytical grade.

Experiments were carried out using following equipment:

Spectrophotometer UV/VIS CECIL CE 8020, Milton Technical Centre, Cambridge CB4 6AZ; Hewlett Packard Model 8452 A diode–array spectrophotometer; Spekol 11, Carl–Zeiss, Jena.

Procedure of investigation

In a 10 ml volumetric flask place 1 ml of 10^{-2} mol/l solution of oxidant ($(NH_4)_2S_2O_8$ or NH_4VO_3), 2 ml of 10 mol/l acetic, phosphoric, sulphuric or perchloric acid, 1.5 ml of 10^{-3} mol/l prothipendyl hydrochloride and dilute to 10 ml with distilled water. Measure the absorbance after 2 min at λ = 372 nm and 374 nm against a reagent blank prepared in the same way but without the drug in system PTP – $(NH_4)_2S_2O_8$ and PTP – NH_4VO_3, respectively.

RESULTS AND DISCUSSION

Prothipendyl hydrochloride reacts in acidic medium with $(NH_4)_2S_2O_8$ and NH_4VO_3 forming yellow oxidation products. The general behaviour of prothipendyl using both oxidants was found to be similar. A suggestion for the sequence of oxidation reaction, as predicted from literature reports (1,14) using H_2O_2 as oxidizing agent, is shown in Figure 1.

The oxidation of prothipendyl takes place by a two– electron mechanism via the radical cation to the sulphoxide. Prothipendyl undergoes a one–electron reversible step to form the intensely colored free radical, which is stable in acidic solution. The free radical is further oxidized irreversibly by excess of oxidant to a colourless sulphoxide (1,14). Using $(NH_4)_2S_2O_8$ and NH_4VO_3 as oxidizing agents, prothipendyl is oxidized to the coloured free radical and then to sulphoxide.

Maximum absorption bands of coloured products were recorded at λ = 372 nm in system PTP – $(NH_4)_2S_2O_8$, in system PTP – NH_4VO_3 at λ = 374 nm.

Coloured oxidation products are stable for at least 2 h for oxidants $(NH_4)_2S_2O_8$ and NH_4VO_3.

Reaction Conditions and Stoichiometry

Effects of time, acidity of the solution and excess of oxidant on the reactions in PTP – $(NH_4)_2S_2O_8$ and PTP – NH_4VO_3 systems were investigated.It was found that the absorbance grows to a constant value during 2 minutes after mixing of PTP with $(NH_4)_2S_2O_8$ or NH_4VO_3.

Effects of concentration of mineral acids (H_2SO_4, H_3PO_4, $HClO_4$,CH_3COOH) were studied for each system. It was observed that the influence of examined acids on the absorbances produced in PTP – $(NH_4)_2S_2O_8$ and PTP – NH_4VO_3 systems are significant. Highest and stable absorbances were recorded in the presence of H_2SO_4, H_3PO_4, $HClO_4$ or CH_3COOH in the reaction solutions. Results showed that the optimum concentrations for colour development in the system PTP – $(NH_4)_2S_2O_8$ were at 1.5–7 mol/l of H_2SO_4, 1–7 mol/l of H_3PO_4, 1.5–6 mol/l of $HClO_4$ and 1.5–7 mol/l of CH_3COOH. In the system PTP – NH_4VO_3 a significant increase in intensity and

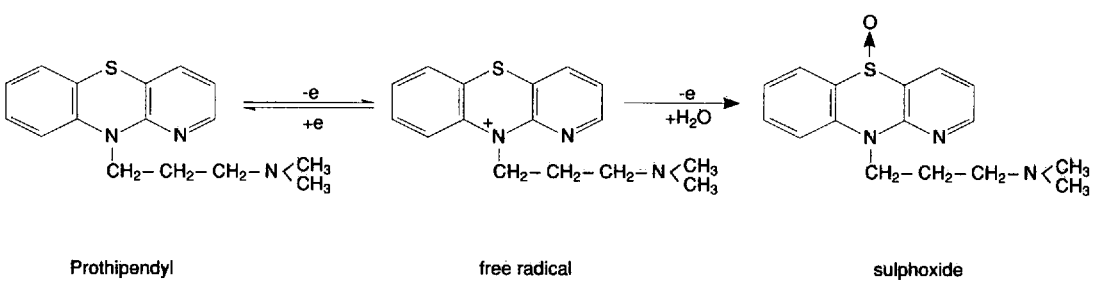

Figure 1. Oxidation of prothipendyl

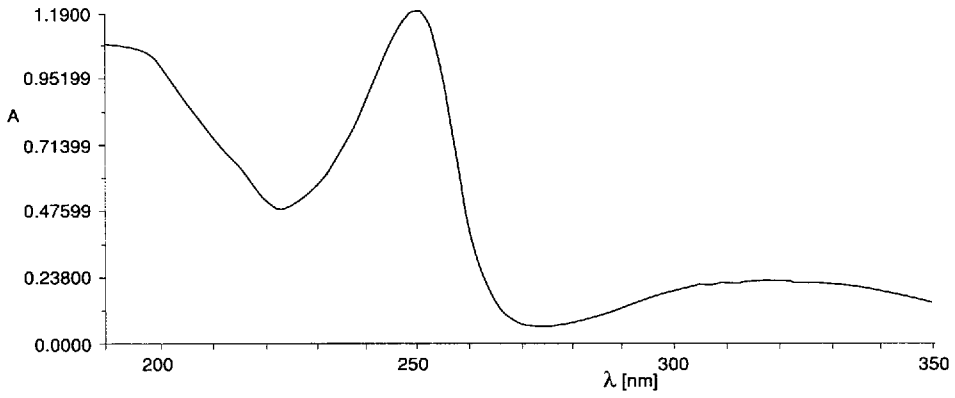

Figure 2. Absorption spectrum of PTP
C=5·10^{-5} mol/l

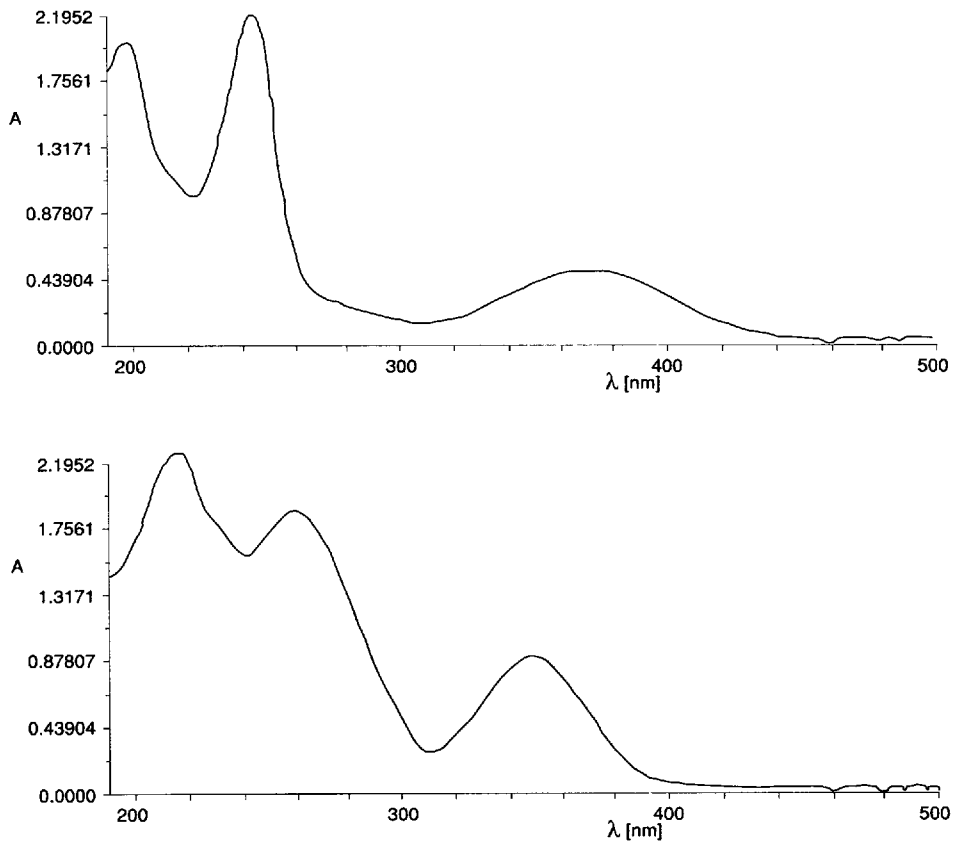

Figure 3. Absorption spectra in PTP–$(NH_4)_2S_2O_8$ system
a) coloured product of PTP oxidation, b) colourless product of PTP oxidation, $C_{PTP}=2\cdot10^{-4}$ mol/l, $C_{(NH_4)_2S_2O_8}=4\cdot10^{-3}$ mol/l

stability of absorbance were obtained at 2–4 mol/l of H_2SO_4, 1–3.5 mol/l of H_3PO_4, 1–6 mol/l of $HClO_4$ and 1–7 mol/l of CH_3COOH.

Analytical measurements for further investigations were made in phosphoric acid medium in the range 1–3.5 mol/l in PTP – $(NH_4)_2S_2O_8$ and PTP – NH_4VO_3 systems.

For the used range 10–60 μg/ml PTP in the final solution, 2 ml of 10^{-2} mol/l of oxidant will give maximum absorbance of coloured oxidation products.

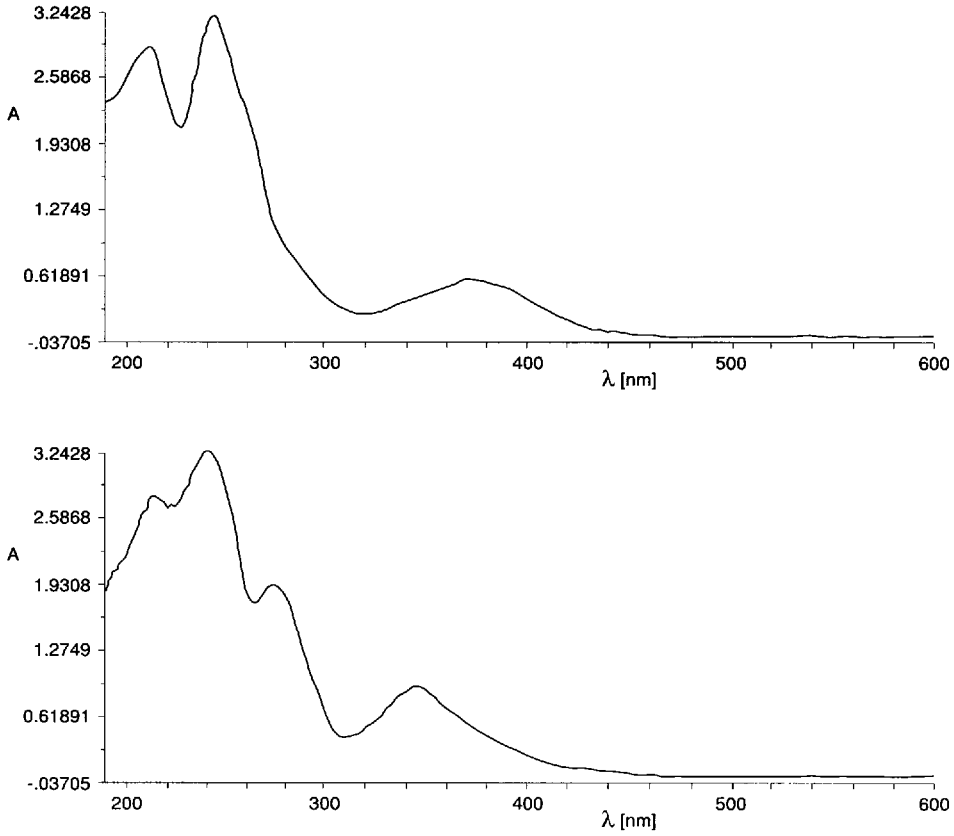

Figure 4. Absorption spectra in PTP–NH_4VO_3 system
a) coloured product of PTP oxidation, b) colourless product of PTP oxidation, $C_{PTP}=2\cdot10^{-4}$ mol/l, $C_{NH_4VO_3}=8\cdot10^{-4}$ mol/l

Table 1. Spectrophotometric determination of prothipendyl hydrochloride in pure form using $(NH_4)_2S_2O_8$, λ = 372 nm, n = 6

Concentration of PTP, μg/ml	$\bar{A}$	Standard deviation S	Standard deviation of the mean $\bar{S}$	Confidence interval $u = \bar{A} \pm t\,\bar{S}$
3.2	0.080	0.0035	0.0020	0.080 ± 0.005
16.0	0.385	0.0040	0.0023	0.385 ± 0.006
29.9	0.672	0.0030	0.0017	0.672 ± 0.004
41.8	0.979	0.0045	0.0026	0.979 ± 0.007
54.7	1.273	0.0010	0.0005	1.273 ± 0.001
67.6	1.580	0.0020	0.0011	1.580 ± 0.003
80.4	1.898	0.0025	0.0015	1.898 ± 0.004
95.3	2.192	0.0025	0.0015	2.192 ± 0.004

Absorption Spectra

As mentioned previously, prothipendyl is oxidized by $(NH_4)_2S_2O_8$ and NH_4VO_3 in an acidic media, forming yellow oxidation products with maximum absorbance at λ = 372 nm and 374 nm, respectively.

The nature of oxidation products was also proved by UV spectra. Coloured oxidation products, similary as colourless oxidation products (sulphoxi-

Table 2. Results of spectrophotometric determination of PTP in pure form using NH_4VO_3, λ = 374 nm, n = 6

Concentration of PTP µg/ml	$\bar{A}$	Standard deviation S	Standard deviation of the mean $\bar{S}$	Confidence interval $u = \bar{A} \pm t\,\bar{S}$
3.2	0.096	0.003	0.0017	0.096 ± 0.004
16.0	0.348	0.002	0.0005	0.348 ± 0.001
27.9	0.655	0.004	0.0020	0.655 ± 0.005
41.8	0.928	0.003	0.0017	0.928 ± 0.004
54.7	1.273	0.004	0.0015	1.273 ± 0.004
67.6	1.566	0.003	0.0017	1.566 ± 0.004
80.5	1.915	0.004	0.0020	1.915 ± 0.005
93.3	2.194	0.002	0.0005	2.194 ± 0.001

Table 3. Spectrophotometric determination of prothipendyl hydrochloride in some pharmaceutical preparations, n=6.

Sample	Content found (mg) by methods				Relative error** (%)		RSD (%)	
	proposed*		reference					
	A	B	A	B	A	B	A	B
Dominal Forte–Injection liquid, Amp. 40 mg/ml	39.92	39.89	39.99	39.84	0.2	0.28	0.12	0.16
Dominal Forte, 80 mg/tab.	80.08	80.10	80.14	80.07	0.1	0.2	0.09	0.06

* A – using $(NH_4)_2S_2O_8$, B – using NH_4VO_4

** versus reference method

des) exhibit absorption maxima in UV – region. Spectra of the coloured products of PTP formed with $(NH_4)_2S_2O_8$ or NH_4VO_3 show main absorption maxima at λ = 238 nm and λ = 242 nm, respectively. Spectra of sulphoxides of PTP have main maxima absorption at λ = 262 nm and λ = 274 nm for $(NH_4)_2S_2O_8$ and NH_4VO_3, respectively.

Spectrophotometric Determination of Prothipendyl Hydrochloride in Pure Form and Pharmaceutical Preparations

Ammonium peroxidisulfate and metavanadate have been tested as reagents for spectrophotometric determination of prothipendyl hydrochloride. Calibration curves have been prepared according to procedures described above. Beer's law is obeyed in the range 3–95 µg/ml and 3–90 µg/ml in PTP – $(NH_4)_2S_2O_8$ and PTP – NH_4VO_3 systems, respectively. Some results are given in Table 1 and 2. Least square regression analysis was carried out for slope, intercept and correlation coefficient of calibration graphs. The slope and intercept are: 0.0234 μg^{-1} cm^{-1} ml, 0.0050 for method with $(NH_4)_2S_2O_8$ and 0.0238 μg^{-1} cm^{-1} ml, 0.0049 for method with NH_4VO_3 and correlation coefficients: 0.9998 and 0.9996, respectively. RSD is less than 0.5%.

Elaborated methods were applied succesfully to determination of prothipendyl hydrochloride in the tablets and injections of Dominal Forte (Table 3). Results were in accordance with the declared amounts and with those obtained by applying reference method, GC – chromatography [8]. The statistical comparison of mean, values obtained by described and reference method, were carried out using the test equality of mean values. The values of t coefficients were: 1.982 (inj.), 1.126 (tab.) for method with $(NH_4)_2S_2O_8$ and reference and 1.826 (inj.), 1.369 (tab.) for method with NH_4VO_3 and reference and they were smaller than the critical value $t_{\alpha,f}$ = 2.228 (test t– Student for f=6+6–2=10). The information suggests that the difference between the results, obtained by two methods, are inessential and caused only by accidental errors.

The proposed methods are simple, rapid and accurate, and can therefore be used to the deter-

mination of the drug alone and in the pharmaceutical preparations. Described methods of the indirect determination of prothipendyl hydrochloride may be complementary to the methods in use. Reagent consumption is inconsiderable, instrumentation is available to any analytical laboratory and the expensive laboratory equipment is not necessary. Precision and reproducibility of the adapted spectrophotometric methods are good, the RSD is low and recovery is high. Results obtained permit to conclude that described spectrophotometric methods can be recommended for routine analysis of prothipendyl hydrochloride.

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