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Short communication

Extractive-spectrophotometric determination of some phenothiazines with dipicrylamine and picric acid

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Abstract

Dipicrylamine and picric acid have been tested as reagents for the determination of promethazine and perphenazine. They react in neutral media with these drugs forming the coloured compounds. The compounds are sparingly soluble in water and quantitatively extracted into organic solvents. The extracts are intensely coloured and very stable. These properties have been exploited for the extractive spectrophotometric determination of promethazine and perphenazine in pure solutions and pharmaceuticals. Linear calibration graphs were obtained in the concentration range 4–40, 3–30 μg ml^{-1} of promethazine and 4–80, 8–60 μg ml^{-1} of perphenazine for picric acid and dipicrylamine, respectively. The relative standard deviation (RSD) is less than 0.8%.

Keywords: Spectrophotometric; Promethazine; Perphenazine; Pharmaceutical preparations

1. Introduction

2,10-Disubstituted phenothiazines belong to a large group of tricyclic aromatic compounds. These drugs are frequently used for treating different kinds of depression due to their high physiological activity and versatile pharmacological action [1]. They have been intensely studied in a number of fields of chemical, biological and medical research.

This group is also interesting from an analytical point of view. The most important of these properties are:

- the ability to oxidation by means of many oxidizing agents, e.g. Ce(IV), V(V), Cr(VI), Mn(VII), IO_4^-, IO_3^-, BrO_3^-, NO_2^-, H_2O_2, chloramine T with the formation of coloured oxidation products [2,3]; these properties enable certain phenothiazines to be used as indicators in various redox titrations [4,5] and as reagents for spectrophotometric determinations [2,6];
- formation of coloured sparingly a soluble ion-association complexes with acidocomplexes of

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metals, e.g. Co(II), Bi(III), Cr(III), Ti(IV), Nb(V), Mo(V), U(IV) [3,7,8];
- formation of coloured crystalline species with several organic compounds, e.g. flavianic acid, alizarin S [9–11].

In the present work the reactions of some 2,10-disubstituted phenothiazines with dipicrylamine and picric acid have been described. We found that promethazine and perphenazine react in neutral medium with dipicrylamine and picric acid forming coloured compounds, which are insoluble in water but can be quantitatively extracted into chloroform or benzene. The extracts are intensely coloured and stable about 2–6 days. These properties may be applied for extractive spectrophotometric determination of promethazine and perphenazine in pure forms and in their pharmaceutical formulations.

Since the introduction of 2,10-disubstituted phenothiazines as antipsychotic agents in the late 1950s, several analytical methods have been suggested for their determination. The official methods are based on non-aqueous titration [12], a variety of manual spectrophotometric methods on coloured complex formation or oxidation reactions [13,14].

Several methods for the analysis of 2,10-disubstituted phenothiazines have been reported in the literature by Blažek et al. [15], Fairbrother [16], Belikov and Moiseeva [17], Puzanowska-Tarasiewicz and Karpińska [18].

A variety of HPLC methods [19,20] are now widely used in routine application owing to their sensitivity, specificity and low cost. The methods have adequate sensitivity in order to quantify low concentrations of the drugs in biological fluids, e.g. human plasma. Use of these methods is justified when sample matrix is rather complex and the phenothiazines concentration low, as is usually the case with clinical samples. However, in pharmaceutical analysis, where the sample matrix is usually less complex and analyte concentration levels are fairly high, the main aim is to develop fast, simple, inexpensive methods, that can readily be adapted for routine analysis at relatively low cost to the different requirements of analytical problems.

Reviews of analytical methods used for the determination of phenothiazines [15–18,21] show that the literature on the extractive spectrophotometric determination of 2,10-disubstituted phenothiazines is very scanty though methods are very useful for their determination in pharmaceuticals.

The composition of the compounds of promethazine and perphenazine with dipicrylamine and picric acid was established. The infrared spectra were performed.

2. Experimental

2.1. Reagents and apparatus

Promethazine hydrochloride (PMT·HCl) (EGYT, Budapest), Perphenazine (PPZ) (SCHERING, USA). Stock solutions containing 1×10^{-1} M phenothiazine derivatives were prepared by dissolving suitable amount of each drug separately in distilled water and diluting to 100 ml with distilled water. Working solutions were prepared by suitable dilution of the stock solution with distilled water and standardised by spectrophotometric method in UV range [22].

Dipicrylamine ammonium salt (DPA), (FLUKA, Switzerland), 5×10^{-3} M solutions. Picric acid (PA), (FLUKA, Switzerland), 5×10^{-2} M solutions.

All chemicals were of analytic-reagent grade.

Pharmaceutical preparations: PHENERGAN tablets (SPECIA, France), PERPHENAZIN tablets (SCHERING, USA).

Hewlett Packard model 8452A (France) and Spekol 11 (Carl Zeiss, Jena, Germany) spectrophotometers were used with 1-cm cells.

2.2. Procedures for determination of promethazine and perphenazine

In a 50 ml separating funnel was placed:
- 0.5–5.0 ml of 2×10^{-4} M solution of PMT·HCl or 0.5–4.0 ml of 4×10^{-4} M solution of PPZ and 5 ml of 5×10^{-4} M solution of DPA,

- 0.25–2.5 ml of 5×10^{-4} M of PMT·HCl or 0.2–4 ml of 5×10^{-4} M of PPZ and 1 or 2 ml of 4×10^{-3} M solution of PA, respectively,

and made up to 10 ml with distilled water. The mixture was shaken vigorously until a district precipitate appeared. Then 10 ml in portions 4–5 ml of chloroform (in the case of DPA) or benzene (in the case of PA) were added to each separating funnel and the contents were shaken for 1–2 min. The extracts were combined in 10-ml volumetric flasks. The absorbance was measured at appropriate wavelength for the drug (Table 1), against a blank treated similarly. Absorbance of the separated extracts was stable for more than 2 days.

The methods are applicable to analysis for some phenothiazine drugs in commercial pharmaceutical preparations.

2.3. Tablets analysis

Ten tablets were weighted and powdered. The powder equivalent to 50 mg of active ingredient was transferred into a 100-ml standard flask. About 50 ml of methanol was added and shaken vigorously for about 10 min. The mixture was dissolved and made up to volume with methanol, mixed well and filtered using of quantitative hard filter. First portion (25 ml) of filtrate was thrown away, equivalent amounts of the next portion of filtrate was vaporized to dryness. Dry residue was dissolved with distilled water, transferred into 50 ml standard flask and made up to the mark. A suitable aliquot (0.5–2 ml) of this solution was transferred into separating funnel and assayed by the procedure described under calibration graph. The amount of each drug present was calculated from its calibration graph or regression equation.

The results are comparable to those obtained by the official method [23] (Table 2).

3. Results and discussion

It was found that promethazine and perphenazine react with dipicrylamine and picric acid forming ion-associated compounds. The composition of these compounds was established. The spectroscopic studies in IR region were performed and the structures of compounds were proposed.

The studied compounds precipitated from neutral media solution in the form of an orange–brown sediment, which can be quantitatively extracted with chloroform or benzene. Taking advantage of these properties the extractive spectrophotometric methods for the determination of promethazine and perphenazine have been elaborated.

3.1. Compounds stoichiometry

The method of continuous variations was employed to establish the stoichiometry of compounds in the system promethazine–dipicrylamine, promethazine–picric acid, perphenazine–dipicrylamine, perphenazine–picric acid. The results indicated that molar ratio of phenothiazines: dipicrylamine (or picric acid) in compounds is 1:1 and it is formed independent of the phenothiazine nature.

3.2. Infrared spectra

Infrared spectra of the compounds were measured (KBr discs) in the region 450–4000 cm^{-1}

Table 1
Optimal condition for the extraction of compounds of promethazine and perphenazine with dipicrylamine and picric acid

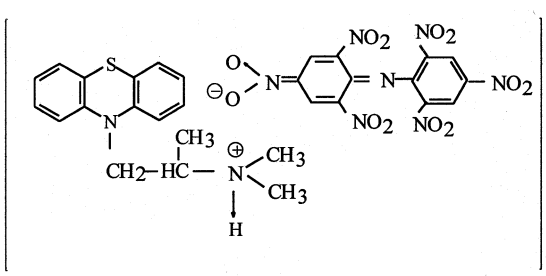
Parameters	PMT–DPA system	PPZ–DPA system	PMT–PA system	PPZ–PA system
Reagents concentrations	2×10^4 M	4×10^4 M	5×10^4 M	5×10^4 M
	5×10^4 M	5×10^4 M	4×10^3 M	4×10^3 M
Medium	Neutral	Neutral	Neutral	Neutral
Organic solvent	$CHCl_3$	$CHCl_3$	C_6H_6	C_6H_6
The colour of extracts	Yellow	Yellow	Yellow	Yellow
Stability of extracts, days	6	4	2	2
Wavelength, nm	420	420	407	407

with a Unicam SP-200 spectrophotometer. The spectra of the PD picrates and PD dipicrylamines in the region 450–1700 cm^{-1} are the sum of the spectra of the reagents, with the PA or DPA spectrum predominant. Only an insignificant shift (~ 30 cm^{-1}) is observed as well as a decrease in the intensity of ν_{as} (NO_2) and ν_s (NO_2) appearing in the PA or DPA spectrum at 1540 and 1350 cm^{-1}.

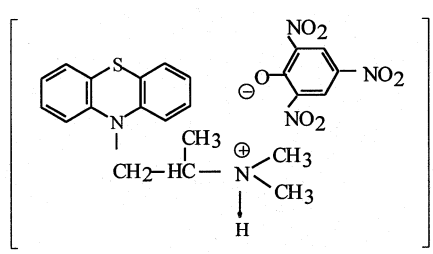
Significant changes in the spectra of the compounds are observed in the region 2300–2650 cm^{-1}. In this region, a wide band characteristic of vibrations of the $\equiv NH^+$ group in the PD spectra, is shifted considerably (~ 300 cm^{-1}) towards higher frequencies in the spectra of the compounds, while its intensity decreases.

3.3. Structure of the compounds

On the basis of the results it can be concluded that OH group of PA (NO_2 group of DPA) and the nitrogen of the chain of PD are responsible for the formation of PD picrates (PD–DPA complexes). For example the compounds of promethazine with dipicrylamine and picric acid can be represented as follows (I and II):



I



II

We suggested that the compounds formed between PD and PA or DPA have an ion-association character. This interpretation is in fair agreement with the results of the examination of PD compounds and anionic forms of other organic substances [9,11,24–26].

3.4. Optimisation of the methods

The compounds of phenothiazine derivatives precipitated from neutral aqueous solutions as crystalline orange–brown sediments, which are insoluble in water, but can be quantitatively extracted with organic solvents. The effect of the concentration acids (HCl, H_2SO_4, CH_3COOH) and reagents (DPA, PA) on the absorbance of the extracts of these compounds was examined. It was found that the absorbance of the extracts decreased with increasing concentration of the acid in aqueous phase. The constant and maximum absorbance readings were obtained for 2–30 excess and 5–50 excess of DPA with respect to PMT and PPZ, respectively, and for 4–40 excess of PA with respect to PD. The optimum conditions for the formation and extraction of the compounds studied are summarized in Table 1.

3.5. Analytical evaluation

Dipicrylamine and picric acid have been tested as reagents for the extractive spectrophotometric determination of promethazine and perphenazine.

Typical calibration graphs for determination of the drugs obtained under the optimized conditions and from these results were linear over the range 3–30 and 8–60 μg ml^{-1} of PMT·HCl and 4–40 and 4–80 μg ml^{-1} of PPZ using of dipicrylamine and picric acid, respectively. The molar coefficients and the equations of regression lines are shown in Table 2. Limits of detection were estimated: 1.00, 1.75 μg ml^{-1} for promethazine and 1.80, 1.34 μg ml^{-1} for perphenazine determination using picric acid and dipicrylamine, respectively. The value of y at the limit of detection was found to be $y_{LOD} = a + 3s_{y/x}$ [28].

The reproducibility of the measurements, expressed as relative standard deviation (RSD) is 0.4–0.8% (Table 2).

Table 2
Characteristics of the methods for the determination of promethazine hydrochloride (PMT·HCl) and perphenzine (PPZ)

Sample	Reagent	Linearity range μg ml^{-1}	ε, l mol^{-1} cm^{-1}	Intercept	Slope	Correlation coefficient	RSD %	$t_{0.95}$·S
PMT·HCl	DPA	3–30	1.83×10^4	−0.0038	0.0570	0.9999	0.72	0.0387
PPZ	DPA	8–60	1.09×10^4	−0.0001	0.2085	0.9999	0.68	0.0292
PMT·HCl	PA	4–40	9.80×10^3	+0.0050	0.0279	0.9990	0.81	0.0425
PPZ	PA	4–80	7.60×10^3	+0.0081	0.0153	0.9994	0.47	0.0527

3.5.1. Effect of interferences

In order to evaluate the selectivity of the developed methods for the analysis pharmaceutical preparations, the effect of the presence of several species, which can occur in real samples with promethazine and perphenazine was investigated. It was found that the presence of the common expicients of tablets (e.g. talc, saccharin, starch, lactose, glucose) does not interfere in the determination of the studied drugs. The oxidizing agents, e.g. ascorbic acid or sodium sulfite, interfere seriously.

3.6. Application to pharmaceutical preparations

The proposed methods were applied to determine promethazine and perphenazine in pharmaceuticals. The measurements were carried out according to the procedure described in Section 2. Phenothiazines contents were determined following the same procedures as those for the calibration graphs. The pharmacopoeial method [23] was the comparative method. The summary results are presented in Table 3. The results obtained using the proposed methods agree well with those obtained by the pharmacopoeial method. The low relative RSD and the error values (less than $\pm$ 1%) permit recommendation of these methods for promethazine and perphenazine determination in tablets PHENERGAN and PERPHENAZINUM, respectively.

Satisfactory recovery values ranging from 99.74 to 100.52% in phenergan and from 99.8 to 101.12% in perphenazinum were obtained for promethazine and perphenazine investigated from pharmaceutical preparations using picric acid and dipicrylamine, respectively.

Table 3
Results of the extractive spectrophotometric determination of promethazine and perphenazine in pharmaceutical preparations, $n = 5$

Sample	Nominal value mg per tablet	Found (mg per tab or mg ml^{-1}) by method			Error % [b]		RSD %	
		Proposed		Official [a]	PA	DPA	PA	DPA
		PA	DPA					
Promethazine HCl PHENERGAN tablets	25	25.06	25.13	25.24	−0.71	−0.44	0.65	0.78
Perphenazine PERPHENAZINE tablets	25	24.95	25.28	25.20	−0.99	+0.32	0.76	0.42

[a] PP [23].
[b] Relative to official method.

4. Conclusion

The proposed methods are superior to pharmacopoeia method, e.g. non-aqueous titration in glacial CH_3COOH and anhydride of CH_3COOH medium [12] (more difficult procedure) and to extractive spectrophotometric methods with chromeazurol S [27] (more difficult procedure and less stability of extracts). The stability of the complexes formed favours this method and was found to be more advantageous than many methods involving oxidation in which a highly unstable radical product was monitored for quantification.

The proposed methods are simple, rapid, precise and accurate and can be used for the routine determination of the promethazine and perphenazine. They are cheap and do not need any complicated apparatus.

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