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## ANALYSIS

# SPECTROFLUOROMETRIC DETERMINATION OF 2– AND 10–DISUBSTITUTED PHENOTHIAZINES

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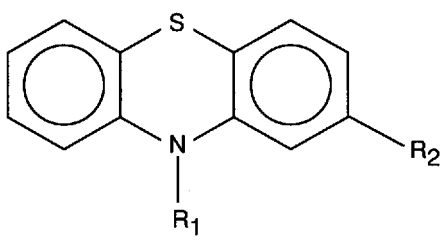
**Abstract:** Spectrofluorometric method for determination of promethazine hydrochloride, thioridazine hydrochloride and perazine dimaleate in the pure form and in drugs is described. Fluorescence excitation spectra of series of aqueous solutions were measured. The fluorescence signal was found to be a linear function of the promethazine hydrochloride (PTM) concentration in the range: 0.30–20.02 ppm, thioridazine hydrochloride (TR): 0.43–21.70 ppm and perazine dimaleate (PDM): 0.85–50.60 ppm. The excitation spectra were used for determination of phenothiazine derivatives in pharmaceutical formulations such as: Diphergan, Thioridazin and Pernazinum. The influence of $K^+$, $Na^+$, $Mg^{2+}$ i $Ca^{2+}$ cations on the fluorescence intensity of phenothiazine derivatives was also studied.

**Keywords:** spectrofluorometry, phenothiazine derivatives, Diphergan, Thioridazin and Pernazinum drugs.

Phenothiazine derivatives (PT) are the constituents of a pharmacologically diverse group of neuroleptics revealing antiemetic, antipsychotic, sedative, antipruritic, antidyskinetic, analgesic and antihistaminic properties (1–3). Moreover, 2, 10–substituted phenothiazines are used in analytical chemistry. The structural formula of phenothiazine derivatives is:

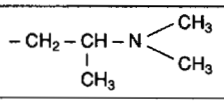
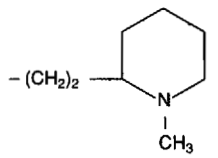
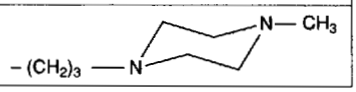



Scheme 1.

PT determination has been described by many authors (4–18). Among others, the Sequential Injection Analysis (SIA) technique was applied for promethazine determination in 50–400 ppm range using $10^{-3}$ M Pd(II) solution (4), whereas for thioridazine hydrochloride low injection method has been proposed. The later one is based on the oxidation of thioridazine by potassium dichromate and iron(III) chloride in acidic medium in the range of 10–210 ppm (5, 6). Ion – selective electrodes were also applied for determination of promethazine in pharmaceuticals (7). Spectrofluorometric determination of promethazine in bulk or in pharmaceuticals was based on fluorescent Au(III) complexes (8). The method has been used with quinolinum chlorochromate in phosphoric acid and with potassium persulfate in the presence of p–aminobenzoic acid. These methods were applied for the concentration range of 2–30 ppm and 8–60 ppm respectively (9). In suspension, promethazine hydrochloride was determined in an UV range using extraction with chloroform and bromocresol green as the reagent (10). Perazine in 40–130 μg/mL range and thioridazine in 50–150 μg/mL range were analysed with cerium(IV) in the visible range at 510 nm and 640 nm, respectively (11). Perizine and thioridazine react also with Reinecke salt giving red reineckates with absorption maximum at λ=520 nm (68–680 ppm) (12, 13). The flavonic and picric acids were applied for spectrophotometric determination of thioridazine hydrochloride (6–70 ppm) and perazine (7–60 ppm) (14, 15). A potassium hexacyanoferrate(III) was used for the indirect spectrophotometric determination of some phenothiazines in pure and in pharmaceutical samples (16). On the opposite, perazine was found to be a sensitive reagent for the spectrophotometric determination of mercury (II) (17). A liquid chromatographic method was developed for the concurrent assay of R(+) and S(–) promethazine from human urine and serum using solid – phase extraction and fluorescence detection (18).

Among the methods presented above, only

Table 1. Phenothiazine derivatives

| Chemical name | Trivial name | Substituents mean | |
|---|---|---|---|
| | | $R_1$ | $R_2$ |
| 10-[2-(dimethylamino)propyl] phenothiazine | Promethazine (PMT) | – H |  |
| 10-[2-(1-methyl-2-piperidyl)ethyl]-2-(methylthio) phenothiazine | Thioridazine (TR) | $-SCH_3$ |  |
| 10-[3-(4-methylpiperazin-1-yl)propyl] phenothiazine | Perazine (PDM) | – H |  |

one used spectrofluorescence for PT derivatives. Majority of determinations has applied the absorption spectra of complexes. PT also react with thiocyanate and halide complexes of metals as well as some organic substances, and form ion–association compounds. Coordination compounds used in VIS spectroscopy referred to the large value of molar extinction coefficients. It suggests that observed band can be assigned to Charge Transfer Transitions (CT).

Reviews of analytical methods for the determination of PT show that, first of all, spectrophotometric methods are very useful for the determination of phenothiazines in pharmaceuticals and body fluids. Spectrophotometric methods referred mainly to such factors as: type of extraction solvent, nature of coordination agent, acidity of the solution, temperature and time of reaction. Moreover, majority of the above mentioned methods is relatively complex and time–consuming and is less suitable for the routine measurements in the drug control laboratory. In the presented paper, the fluorescence properties of promethazine hydrochloride (PTM), thioridazine hydrochloride (TR) and perazine dimaleate (PDM) were applied for their quantitative determination in pharmaceuticals.

The spectrofluorometric method was applied for the determination of studied drugs in the suitable pharmaceutical preparations and to test the influence of several cations added to the performance of the determination procedure.

Our spectrofluorometric method does not require any additional chemical reactions. The method is very simple, fast and inexpensive, allowing the routine analysis of drugs of commercial drug formulations.

## EXPERIMENTAL

### Chemicals

PMT (99%) were purchased from Sigma, whereas PDM (99,9%) was obtained from Labor (Wrocław, Poland) and they all were used without further purification. Pharmaceutical formulations: Diphergan dragées 25 mg (Pharmaceutical Works Polfa of Starogard), Thioridazin dragées 25 mg (Pharmaceutical Works Polfa of Starogard), Thioridazin dragées 25 mg (Pharmaceutical Works Jelfa S.A. of Jelenia Góra), Pernazinum tablets 25 mg (Pharmaceutical Works Labor of Wrocław) were used as received.

Sodium chloride, potassium chloride, magnesium chloride and calcium chloride solutions, $2.36\times10^{-3}$ mole·l$^{-1}$.

All other chemicals used were of analytical–reagent grade.

### Sample preparation

Stock solutions of PMT ($3.12\times10^{-4}$ mole·l$^{-1}$), TR ($3.55\times10^{-4}$ mole·l$^{-1}$) and PDM ($2.95\times10^{-4}$ mole·l$^{-1}$) were pepared by dissolving the corresponding compounds in redistilled water and stored in dark at ambient temperature. Working solutions were obtained by serial dilution of stock stolutions and the results of measurements of relative fluorescence intensity were used for preparation of the calibration curves. A linear dependence between the relative fluorescence intensity and the concentration was found in the range of $6.24\times10^{-5}$ – $9.36\times10^{-7}$ mole·l$^{-1}$ for PMT, $5.33\times10^{-5}$ – $1.06\times10^{-6}$ mole·l$^{-1}$ for TR and $8.85\times10^{-5}$ – $1.48\times10^{-6}$ mole·l$^{-1}$ for PDM.

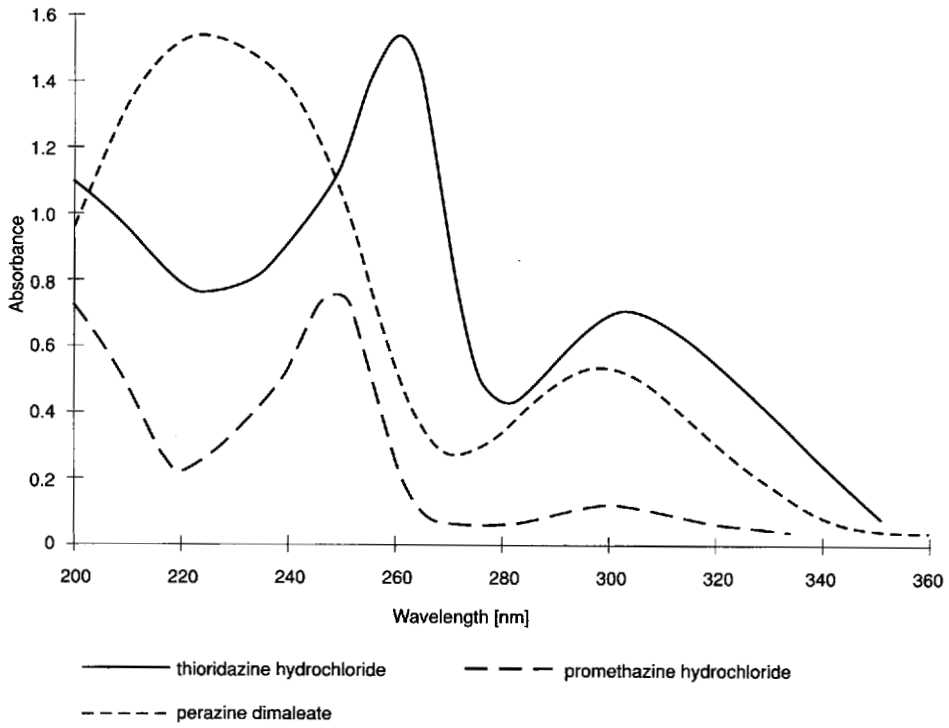



Figure 1. Absorption spectra of promethazine hydrochloride ($c=3.12\times10^{-4}$ mol·l$^{-1}$), thioridazine hydrochloride ($c=3.55\times10^{-4}$ mol·l$^{-1}$) and perazine dimaleate ($c=2.95\times10^{-4}$ mol·l$^{-1}$).

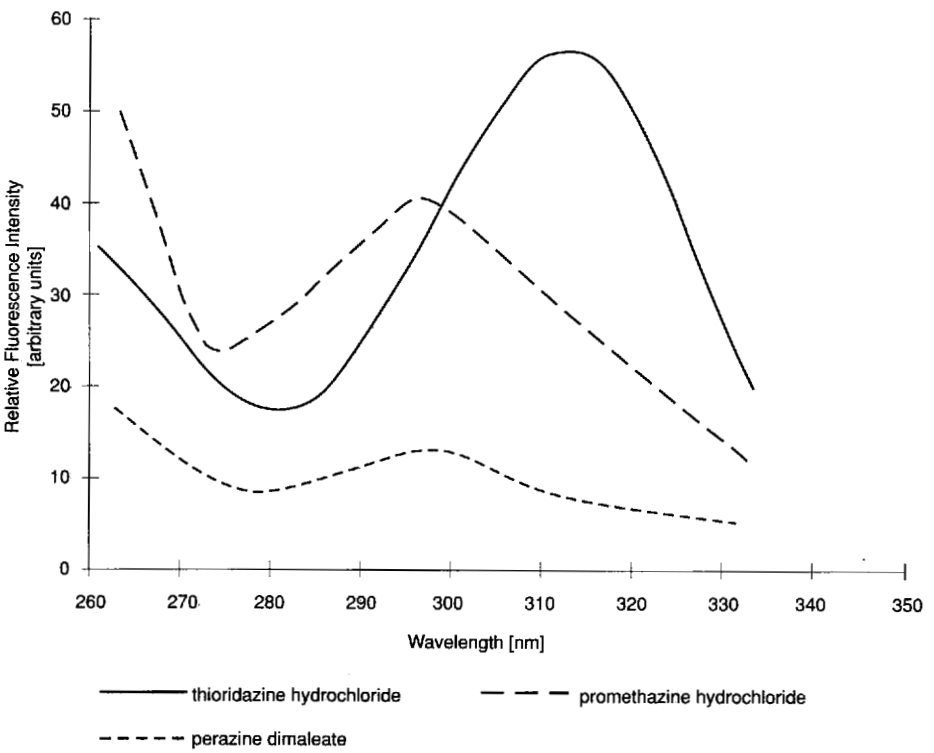



Figure 2. Excitation spectra of promethazine hydrochloride ($c=3.12\times10^{-5}$ mol·l$^{-1}$) thioridazine hydrochloride ($c=3.55\times10^{-5}$ mol·l$^{-1}$) and perazine dimaleate ($c=2.95\times10^{-5}$ mol·l$^{-1}$).

**Absorption spectra**

Absorption spectra of an aqueous solutions, in the range of 200–360 nm, were recorded with a SPECORD M40 (Zeiss, Jena) in a quartz cell (volume 5 $cm^3$, path 1 cm) and is presented in Figure 1.

**Excitation spectra**

Excitation spectra were recorded with a SPECORD M40 spectrophotometer equipped with a adapter for fluorescence intensity measurements. This spectrophotometer records a total fluorescence intensity in the studied range. Samples (aqueous solutions) and a fluorescence standard ($1 \cdot 10^{-3}$ g $ml^{-1}$ Rhodamine B in ethylene glycol) were excited in 263.16–333.33 nm range, and is presented on Figure 2 (19). Rhodamine B in ethylene glycol was used as reference solution because its spectral range is: 250–590 nm (20). The fluorescence intensity of samples and reference solutions was referred to fluorescence intensity of Rhodamine B in ethylene glycol. This solution maintains a constant ratio of quanta absorbed to quanta emitted (21). The WK38 cut–off filter was used for elimination of the scattered exciting light. The wavelengths of 298.51 nm for PMT, PDM and 312.50 nm for TR were chosen as the analytical peaks because these are maximum excitation peaks.

## RESULTS AND DISCUSSION

PTM, TR and PDM exhibit a relative fluorescence intensity in aqueous solutions with maximum excitation wavelengths at 298.51, 312.50 and 298.51 nm, respectively. The absorption spectra were obtained in order to determine the wavelengths ranges to be used for excitation spectra measurements. The long wavelength excitation peaks detected at 298.51 and 312.50 nm appear to be concentration independent and identical with those in the absorption band. The relation between the fluorescence intensity $I_f$ and the molar concentration $c$ is:

$$I_f = kQI_0(1-e^{-\varepsilon lc})$$

For the very dilute solutions this equation reduces itself to the following:

$$I_f = kQI_0\varepsilon lc$$

where $k$ is a constant, $Q$ is the quantum efficiency, $I_0$ is the intensity of incident radiation, $l$ is the cell length, $c$ is the molar concentration and $\varepsilon$ is the molar absorptivity of the compound (21).

The ranges of the linear relationship between the psychotropic drugs fluorescence intensity in water and their concentrations are listed in Table 2. The data were elaborated using a linear least squares method.

The relative standard deviation (RSD) was found to be less than 1%, indicating reasonable reproducibility (22). The method gave the possibility of determining phenothiazine derivatives with good precision in the range of 0.30–20.02 ppm for PTM, 0.43–21.70 ppm for TR and 0.58–50.60 ppm for PDM.

**Determination of phenothiazine derivatives in pharmaceutical formulations**

In order to show the applicability of the proposed spectrofluorometric method, the phenothiazine derivatives were analyzed in pharmaceutical formulations using a standard addition procedure.

Pure drugs were determined by excitation spectra in ten tablets (dragées) of pharmaceutical formulations in aqueous solutions. One tablet (dragée) was transferred into a 100 ml standard flask, followed by the addition of 50% excess of pure drug to the declared content and dissolved in water. Next 0.5 ml of the solution (tablet plus pure drug) was made up with distilled water to 10 ml in the volumetric flask. The resulting solution was fil-

Table 2. Analytical parameters for the spectrofluorometric determination of phenothiazine derivatives

| Phenothiazine derivatives | Concentration range [$\mu g \cdot ml^{-1}$] | Regression equation | Correlation coefficient | RSD* [%] |
|---|---|---|---|---|
| Promethazine hydrochloride | 0.30 – 20.02 | $1.28 \cdot 10^6x + 0.2502$ | 0.9986 | 0.81 |
| Thioridazine hydrochloride | 0.43 – 21.70 | $1.70 \cdot 10^6x + 0.2734$ | 0.9974 | 0.52 |
| Perazine dimaleate | 0.85 – 50.60 | $0.50 \cdot 10^6x + 0.4827$ | 0.9973 | 0.75 |

RSD – Relative standard deviation (five replicate determinations)

Table 3. Determination of phenothiazine derivatives in pharmaceutical formulations

| Pharmaceutical formulation (declared content) | Total found[a] | | | | SD[a] [mg] | RSD[a] [%] | Recovery[a] [%] | Confidence limit[b] $u_{95}=m_x \pm t_{95}s$ [mg] |
|---|---|---|---|---|---|---|---|---|
| | $m_t$ [mg] | $I_f$ [a.u.] | $c_f \times 10^5$ [mole·l$^{-1}$] | $m_f$ [mg] | | | | |
| Diphergan (dragées, 25) | 37.50 | 74.53 | 5.80 | 37.19 | 0.19 | 0.51 | 99.17 | 37.19 ± 0.24 |
| Thioridazin (dragées, 25) | 37.50 | 77.92 | 4.58 | 37.24 | 0.16 | 0.43 | 99.31 | 37.24 ± 0.19 |
| Pernazinum (tablets, 25) | 37.50 | 16.73 | 3.23 | 36.95 | 0.17 | 0.46 | 98.53 | 36.95 ± 0.21 |

[a] Average of ten determinations, [b] Probability level = 0.95, SD – Standard deviation, RSD – Relative standard deviation, $m_t$ – declared content plus 50% excess of pure drugs, $I_f$ – Relative fluorescence intensity.

Table 4. Influence of physiologically active cations on phenotiazine derivatives determination

| Cations $c_{PH}:c_{cation}$ | | Promethazine hydrochloride | Thioridazine hydrochloride | Perazine dimaleate |
|---|---|---|---|---|
| $Na^+$ | 1:0.2 | 1.4 [a] | 0 | 3.9 [a] |
| | 1:2 | 7.2 [a] | 0 | 5.6 [a] |
| | 1:20 | 2.9 [a] | 0 | 7.8 [a] |
| $K^+$ | 1:0.2 | 1.4 [a] | 2.7 [a] | 1.9 [a] |
| | 1:2 | 1.4 [a] | 5.4 [a] | 2.2 [a] |
| | 1:20 | 1.4 [a] | 7.1 [b] | 3.9 [a] |
| $Ca^{2+}$ | 1:0.2 | 5.8 [a] | 0 | 5.6 [a] |
| | 1:2 | 4.3 [a] | 0 | 7.8 [a] |
| | 1:20 | 31.9 [b] | 0 | 9.8 [a] |
| $Mg^{2+}$ | 1:0.2 | 4.3 [a] | 2.7 [a] | 9.8 [b] |
| | 1:2 | 4.3 [a] | 2.7 [a] | 17.7 [b] |
| | 1:20 | 5.8 [a] | 3.6 [b] | 17.7 [b] |

a– quenching [%], b – increase in the peak intensity [%]

tered before measurements. A reference solutions of 37.50 mg pure PTM, TR, PDM were prepared in the same way (resulting in the concentrations of $5.85\times10^{-5}$, $4.61\times10^{-5}$ and $3.28\times10^{-5}$ mole·l$^{-1}$ respectively) and their relative fluorescence intensity were found to be 75.15, 78.46 and 16.98 (arbitrary units) respectively.

The relative fluorescence intensity values, concentration per tablet (dragée) of drugs and recovery are listed in Table 3.

The relative standard deviations were found to be less than 1% (22), indicating a rather similar spread of the phenothiazine derivatives content in individual tablets (dragée). Also, satisfactory recovery values were obtained as follows: 99.17% for PTM in Diphergan, 99.31% for TR in Thioridazin and 98.53% for PDM in Pernazinum. According to the data in Table 3, the spectrofluorometric method has adequate precision and accuracy to carry out reliable analysis of phenothiazine derivatives in pharmaceutical formulations (RSD<1%, recovery values about 99%) (23).

Precision and accuracy of the proposed method was studied in order to determine the validity of the method.

**Influence of physiologically active cations on phenothiazine derivatives determination**

The influence of physiologically important cations such as: sodium, potassium, magnesium and calcium occurring in solutions of the psychotropic drugs, on the fluorescence intensity was examined and is presented in Table 4.

The addition of $CaCl_2$ (to promethazine hydrochloride solution), KCl and $MgCl_2$ (to thioridazine hydrochloride solution) in twenty–fold excess resul-

ted in the peak intensity increase. When $MgCl_2$ was added to the perazine dimaleate solution the enhancement of fluorescence was observed. Sodium and potassium cations caused a lightly decrease in peak intensity of PTM and PDM. Also $Ca^{2+}$ and $Mg^{2+}$ were an ion–quenchers of PDM and PTM fluorescence, respectively. An uncertainty of 5% in the intensity values is considered tolerable in the literature (24). The results show that cations $Na^+$ and $Ca^{2+}$ do not influence the thioridazine hydrochloride fluorescence intensity. The pronounced effect of above mentioned cations may have important implications for the phenothiazine derivatives therapy and indicates that simultaneous administration of drug containing psychotropic drugs and magnesium, calcium, potassium and sodium should be checked.

## CONCLUSION

A simple, accurate, precise, equally and inexpensive spectrofluorometric method for the determination of three phenothiazine derivatives: promethazine hydrochloride, thioridazine hydrochloride and perazine dimaleate has been proposed. This spectrofluorometric procedure can be successfully applied over the wide range of concentrations because of the linear relationship. In the laboratory practice the analytical determination of phenothiazine derivatives may be sufficiently well performed using excitation spectra as well as applying the bidistilled water as a solvent. Satisfactory results (high recovery and the low RSD) demonstrate the benefit of using this method for the routine analysis of phenothiazine derivatives in pharmaceutical formulations. It allows to reduce extremely the quantity of used sample mass for analysis. The influence of some added, physiologically important cations occurring in solution of studied drugs, appears that the simultaneous administration of phenothiazine derivatives and calcium, magnesium, sodium and potassium containing drugs should be checked. This should be taken into account in the psychotropic drugs therapy.

The spectrofluorometric method may be generally recommended for the determination of fluorescent components in pharmaceutical formulations in drug control laboratories. The drug could be deterined by this method rapidly and with enough accuracy and precision.

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