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The determination of phenothiazine drugs in pharmaceutical preparations by a difference spectrophotometric method

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A method is described for the rapid determination of phenothiazine drugs in a wide variety of pharmaceutical preparations. The drugs are determined by a difference spectrophotometric technique based upon the absorbance of the sulphoxide derivative of the drug relative to the absorbance of a solution of the underivatized drug. The sulphoxide derivatives are formed rapidly and quantitatively at room temperature by the addition of a solution of peroxyacetic acid, prepared by the slow reaction of hydrogen peroxide and glacial acetic acid on standing. The difference absorbance of the solutions is proportional to the concentration of the phenothiazine drug in the preparation and is specific for the intact drug in the presence of oxidative and photochemical decomposition products, colouring and flavouring agents, excipients and most co-formulated drugs.

Phenothiazine drugs are currently formulated in a variety of dosage forms either as the sole medicament or in combination with one or more other drugs. Among the methods used to assay the phenothiazine drugs in pharmaceutical preparations are titrimetric (Soliman, Abdine & Zakhari, 1975), chromatographic (Sperling, 1967; Smith, 1972), ultraviolet (Flanagan, Lin & others, 1959) and visible spectrophotometric procedures (Ryan, 1959; Murty & Baxter, 1970).

The simple spectrophotometric methods used for example by the British Pharmacopoeia (1973) usually involve the extraction or dilution of the preparation followed by a measurement of absorbance in the ultraviolet region. These procedures lack specificity and are subject to interference from other ultraviolet absorbing drugs, colouring and flavouring agents or the oxidation products of the phenothiazine drugs which have been shown to be the corresponding phenothiazine sulphoxide and sulphone (Massie, 1954).

This paper describes a difference spectrophotometric assay for phenothiazine drugs in a variety of commercial preparations. The assay may be performed as rapidly as a direct spectrophotometric method and it is specific for the intact phenothiazine drug. The method involves oxidizing an aliquot of a solution of the drug with peroxyacetic acid (generated by the reaction of hydrogen peroxide and acetic acid) to form the phenothiazine sulphoxide and measuring the absorbance of the solution around 345 nm using an unoxidized solution of the drug of equal concentration in the reference cell. The resultant difference absorbance is proportional to the intact phenothiazine drug and is unaffected by the presence of excipients, decomposition products or co-formulated drugs. Provided these substances remain unaltered by the oxidizing reagent, their concentration in the test and reference solutions is identical and their difference absorbance is zero.

MATERIALS AND METHOD

Spectrophotometric measurement. Absorbance values were measured in 2 cm quartz cells matched for equal transmission and pathlength on a spectrophotometer operating on the double-beam mode.

Phenothiazine reference compounds. The reference phenothiazine drugs were obtained from a variety of manufacturers as follows: promethazine hydrochloride, dimethothiazine mesylate, trimeprazine tartrate, trifluoperazine dihydrochloride, chlorpromazine hydrochloride, prochlorperazine mesylate (May & Baker, Ltd), thioridazine hydrochloride (Sandoz-Wander), promazine hydrochloride (Wyeth Ltd). All the drugs except dimethothiazine were of B.P. or B.P.C. quality, as appropriate, and all were shown by thin-layer chromatography (Korczak-Fabierkiewicz, Kofoed & Lucas, 1965) to contain less than 0·5% of the corresponding sulphoxide. Promethazine sulphoxide and promethazine sulphone were kindly donated by May and Baker, Ltd.

Oxidizing reagent. Dilute hydrogen peroxide (100 volumes; 5 ml) to 500 ml with glacial acetic acid. Allow the solution to stand at room temperature

overnight or heat the solution at 70° for 1 h. The reagent may be kept for at least two months.

Standard reference solutions

Dissolve the reference phenothiazine drug (about 60 mg, accurately weighed) in water and dilute to 100 ml. Transfer an aliquot (5 ml) to two volumetric flasks (100 ml). Dilute the contents of one flask to volume with water. To the other flask add oxidizing reagent (5 ml) and dilute the contents to 100 ml with water. Measure the absorbance of the oxidized solution using the unoxidized solution in the reference cell at the difference maximum in the wavelength range 335–355 nm.

When assaying chlorpromazine suspension prepare all the solutions using glacial acetic acid instead of water as the solvent.

When assaying trifluoperazine sustained-release capsules, prepare the solutions as follows. Dissolve trifluoperazine dihydrochloride (about 60 mg, accurately weighed) in water (800 ml). Add N hydrochloric acid (100 ml) and dilute the solution to 1 litre with water. Transfer an aliquot (20 ml) to two volumetric flasks (25 ml). To one flask add oxidizing reagent (2 ml) and dilute the contents of both flasks to volume with water. Measure the difference absorbance of the solutions at 353 nm.

General procedure for dosage forms

Syrups, linctuses and elixirs. Determine the weight ml^{-1} of the preparation. Accurately weigh an amount of the preparation equivalent to about 12 mg of the phenothiazine drug into a volumetric flask (100 ml) and dilute to volume with water. Prepare an oxidized and an unoxidized solution by diluting aliquots (25 ml) to 100 ml as described under *standard reference solutions* and measure the difference absorbance of the solutions at the same wavelength as was used for the appropriate standard reference solutions.

Tablets. Weigh and pulverize 20 tablets. Shake an accurately weighed portion of the powdered tablets, equivalent to about 12 mg of the phenothiazine drug with 0·02 N hydrochloric acid (100 ml) for 15 min. Clarify the solution by passing it through a No. 4 sintered glass filter. Prepare an oxidized and an unoxidized solution as described for *syrups, linctuses and elixirs* and measure the difference absorbance at the appropriate wavelength.

Injections. Dilute a volume of the injection equivalent to about 12 mg of the phenothiazine drug to 100 ml with water and proceed as described for *syrups, linctuses and elixirs.*

Modified procedure for specific preparations

Sustained-release capsules containing trifluoperazine. Weigh and thoroughly pulverize the contents of 20 capsules. Shake an accurately weighed portion of the powdered capsules, equivalent to about 6 mg trifluoperazine dihydrochloride with 0·1N hydrochloric acid (100 ml) for 30 min. Clarify the solution by passing it through a No. 4 sintered glass filter. Transfer an aliquot (20 ml) to two volumetric flasks (25 ml). To one flask add oxidizing reagent (2 ml) and dilute the contents of both flasks to 25 ml with water. Measure the difference absorbance of the solutions at 353 nm.

Suppositories containing chlorpromazine (100 mg, as the base). Weigh 10 suppositories and cut them into small pieces. Dissolve in a beaker an accurately weighed portion of the sample equivalent to 25 mg chlorpromazine base, in light petroleum (boiling range 60–80°; 25 ml) with the aid of gentle heating on a hot water bath at 50°. When the solution is cool, transfer it to a separating funnel (250 ml), rinsing the beaker with light petroleum (10 ml) and adding this to the separating funnel. Shake the light petroleum solution with 0·02 N hydrochloric acid (100 ml, accurately pipetted) and allow the phases to separate. Transfer an aliquot (10 ml) of the acidic solution to two volumetric flasks (100 ml). Add oxidizing reagent (5 ml) to one flask and dilute the contents of both flasks to 100 ml with water. Measure the difference absorbance of the solutions at 343 nm.

Suspension containing chlorpromazine embonate (2·9% w/v). Determine the weight ml^{-1} of the preparation. Weigh an amount of the preparation equivalent to about 40 mg chlorpromazine base into a volumetric flask (100 ml) and dilute to volume with glacial acetic acid. Stir the solution for 15 min and filter through a No. 3 sintered glass filter. Pipette an aliquot (5 ml) into two volumetric flasks (100 ml). Add oxidizing reagent (5 ml) to one flask and dilute the contents of both flasks to 100 ml with glacial acetic acid. Measure the difference absorbance of the solutions at 343 nm.

Treatment of the results

The concentration of the phenothiazine drug in the dosage form as mg per unit dose is given by

$$\frac{\Delta A_T}{\Delta A_S} \times C_S \times D \times \frac{\text{Weight of unit dose}}{\text{Weight of sample}}$$

where ΔA_T and ΔA_S are the difference absorbances of the sample and the standard solutions respectively at the wavelength of maximum difference absorbance

around 345 nm, C_S is the concentration of the standard reference solutions in mg per 100 ml and D is the dilution factor of the sample solutions.

RESULTS AND DISCUSSION

The ultraviolet absorption spectra of equimolar solutions of promethazine hydrochloride and promethazine sulphoxide, prepared by adding oxidizing reagent to a solution of promethazine hydrochloride, are given in Fig. 1a. The difference absorption spectrum (Fig. 1b) of the promethazine sulphoxide solution using the solution of promethazine hydrochloride in the reference cell shows peaks at 269, 292 and 337 nm. The difference absorption maximum at 337 nm was chosen for the determination of promethazine because at this wavelength the oxidizing reagent gives zero absorbance (Fig. 1a) and therefore does not contribute to the difference absorbance of the drug. The other phenothiazine drugs show similar difference spectra although there are individual differences in the wavelength of maximum difference absorbance and in absorptivity (Table 1).

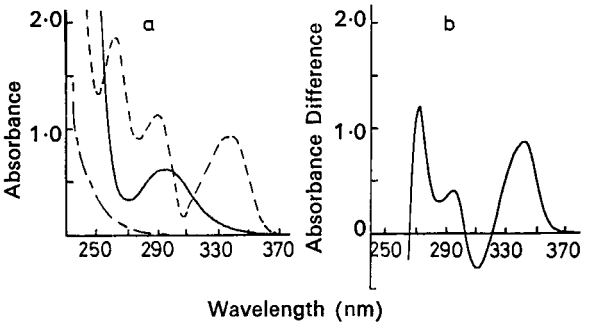

FIG. 1. (a) The ultraviolet absorption spectra of aqueous solutions (0·10 m mol litre^{-1}) of promethazine hydrochloride (———), promethazine sulphoxide (– – –) and oxidizing reagent diluted (1 + 19) with water (— · —). (b) The difference absorption spectrum of a solution of promethazine sulphoxide (0·10 mmol litre^{-1}) relative to a solution of promethazine hydrochloride (0·10 mmol litre^{-1}).

Oxidizing reagent

The sulphoxides of phenothiazine drugs have been prepared previously (De Leenheer, 1973; Breyer, 1969) by heating a solution of the drug with hydrogen peroxide solution and glacial acetic acid which react together slowly to form the oxidant peroxyacetic acid (Swern, 1970). The oxidizing reagent used in the present work is a solution of peroxyacetic acid prepared before the addition to the phenothiazine drug by allowing glacial acetic acid and hydrogen peroxide solution to equilibrate for 16 h at room temperature or 1 h at 70°. The concentration of peroxyacetic acid in the oxidizing reagent determined by the method of Greenspan & MacKellar (1948) is sufficiently high under these conditions to oxidize the phenothiazine drugs to the sulphoxides rapidly and quantitatively without heating the solution.

The product formed by the reaction of promethazine hydrochloride with oxidizing reagent under the conditions of the assay was confirmed as promethazine sulphoxide by comparison of its R_F values in two solvent systems (Korczak-Fabierkiewicz & others, 1965; Mital & Jain, 1970) and its ultraviolet absorption spectrum with those of authentic promethazine sulphoxide.

Adherence to Beer's law

Beer's law plots (Fig. 2) for promethazine hydrochloride, chlorpromazine hydrochloride and trifluoperazine dihydrochloride show that the ΔA values measured at the appropriate wavelength of maximum difference absorption are proportional to the concentration of drug in the oxidized and unoxidized solutions in the concentration range 0–0·007% (m/v).

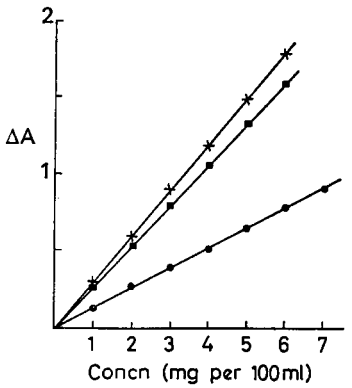

FIG. 2. The variation of difference absorbance (△A) values with changes in concentration for promethazine hydrochloride (–×–×–), chlorpromazine hydrochloride (–■–■–) and trifluoperazine dihydrochloride (–●–●–).

The concentrations of the drugs in the standard and sample solutions have been chosen to give ΔA values of approximately 0·86 which is the absorbance giving least relative error in spectrophotometers equipped with phototube detectors (Wybourne, 1960).

Specificity

A number of substances which may be present in preparations of phenothiazine drugs, either as decomposition products or as co-formulated drugs, was examined under the conditions of the assay for difference absorbance around 345 nm. The following substances give zero difference absorbance and therefore do not interfere in the assay: promethazine sulphoxide, promethazine sulphone, amylobarbitone, butobarbitone, ephedrine hydrochloride, pholcodine, phenylpropanolamine hydrochloride and dexamphetamine sulphate.

The effect of coloured photodecomposition products of promethazine on the ΔA values was also investigated. An aqueous solution of promethazine hydrochloride (0·006% m/v) in a clear glass container was exposed to actinic light for four weeks. Analysis of the coloured solution by the difference method indicated that no intact promethazine remained and this was confirmed by thin-layer chromatography (Korczak-Fabierkiewicz & others, 1965) of a chloroform extract of the degraded solution basified with sodium hydroxide. When various amounts of promethazine hydrochloride were added to the degraded solution, the ΔA values obtained by the procedure were in good agreement with the theoretical values showing that the coloured products do not interfere in the assay.

Any phenothiazine preparation may be assessed for non-specific absorption by comparison of the difference spectrum with that of the appropriate standard reference solutions. All the phenothiazine drugs in this study show negative difference absorbance in the region around 315 nm. There are thus two wavelengths (isosbestic points) in the region 300–330 nm where there is zero difference absorbance due to the oxidized and unoxidized solutions having equal absorbance. If the spectral properties in this region of any other substance present in the preparation are altered by the oxidizing reagent then the isosbestic points will differ from those of the standard reference solutions. It is a reasonable assumption, therefore, that if the standard and sample solutions have zero difference absorbance above 370 nm and the isosbestic points are identical then the absorbance at the difference maximum around 345 nm is also free from interference.

Accuracy

To assess the accuracy of the method in the presence of oxidation products, the concentration of promethazine was determined in several standard solutions containing promethazine hydrochloride, promethazine sulphoxide and promethazine sulphone. The results given in Table 2 show good recovery of intact promethazine in these mixtures and confirm that

Table 1. *The levels of phenothiazine drugs in commercial preparations.*

Drug	$\Delta A^{1\%}_{1\,cm}$	λmax nm	Dosage form	Declared amount per unit dose	Co-formulated drug and amount per unit dose		Found % declared difference method	Found % declared Ryan's (or B.P.C.) method
Promethazine hydrochloride	149·6	337	Elixir	5 mg/5 ml	—		99·2	99·7 (B.P.C.)
			Linctus	3·6 mg/5 ml	Ephedrine HCl Codeine phosphate	7·2 mg 9·0 mg	96·4	97·0 (B.P.C.)
			Linctus	1·5 mg/5 ml	Phenylpropanolamine HCl Pholcodeine	5·0 mg 1·5 mg	98·1	95·0 (B.P.C.)
			Tablet	25 mg	—		98·4	98·0
			Tablet	15 mg	Butobarbitone	75 mg	97·1	97·3
			Tablet	12·5 mg	Amylobarbitone	50 mg	94·7	94·6
Chlorpromazine hydrochloride	135·1	343	Syrup	25 mg/5 ml	—		96·0	95·0
			Suspension	89·7 mg base/5 ml as embonate salt	—		99·1	—
			Suppository	100 mg base	—		100·6	101·0
			Tablet	100 mg	—		99·7	100·6
			Tablet	25 mg	Amylobarbitone	50 mg	98·3	100·1
Trifluoperazine hydrochloride	89·3	353	Capsule	2 mg base (as di HCl)	—		99·9	—
			Capsule	2 mg base (as di HCl)	Dexamphetamine sulphate	10 mg	101·4	—
			Capsule	2 mg base (as di HCl)	Amylobarbitone	65 mg	105·0	—
Thioridazine hydrochloride	110·0	353	Syrup	27·5 mg/5 ml	—		99·9	99·6
Trimeprazine tartrate	133·8	342	Syrup	7·5 mg/5 ml	—		100·7	99·9
			Tablet	10 mg	—		100·0	98·5
Dimethothiazine mesylate	56·2	354	Elixir	10 mg base/5 ml (as mesylate)	—		101·2	—
Promazine hydrochloride	166·1	345	Injection	100 mg/2 ml	—		98·1	98·0
Prochlorperazine mesylate	77·5	345	Injection	12·5 mg /ml	—		85·6	84·8

the assay is specific for unchanged drug in the presence of its oxidation products.

Precision

To measure the precision of the procedure, an elixir of promethazine hydrochloride available commercially, containing 5 mg per 5 ml was assayed 10 times by the procedure. The mean concentration and relative standard deviation were determined to be 4·96 mg per 5 ml and 1·08% respectively.

Assay results

Several commercially available phenothiazine preparations containing a variety of co-formulated drugs and excipients were assayed by the difference method. For comparison, the preparations were also assayed by the colorimetric procedure of Ryan (1959) which involves the formation of a coloured phenothiazine-palladium complex in ion-pair combination with lauryl sulphate anion.

Table 2. *The levels of promethazine determined in standard mixtures containing promethazine hydrochloride, I, promethazine sulphoxide, II, and promethazine sulphone, III.*

Composition of standard mixture			I	
I mmol litre^{-1}	II mmol litre^{-1}	III mmol litre^{-1}	found mmol litre^{-1}	Recovery %
1·870	0	0	1·870	100·0
1·683	0·187	0	1·693	100·6
1·496	0·374	0	1·511	101·0
1·122	0·748	0	1·122	99·1
0·748	1·122	0	0·746	99·7
1·683	0·093	0·093	1·698	100·9
1·496	0·187	0·187	1·495	99.9
1·122	0·374	0·374	1·205	98·8
0·374	0·748	0·748	0·363	97·1

During this comparative study a number of difficulties were encountered in the application of Ryan's method to certain preparations. The elixir and linctuses containing promethazine gave low results and it was found that the complex was not fully formed as further increases in absorptivity could be achieved by the addition of more magnesium lauryl sulphate. Consequently the results obtained with the difference method are compared with those obtained using the procedure of the British Pharmaceutical Codex (1973) described for promethazine elixir B.P.C. in which the absorbance at 472 nm of the phenothiazine-palladium chloride complex is measured without the addition of the magnesium lauryl sulphate. It is essential, however, that the absorbance at 472 nm is corrected for the absorbance due to the colouring agents in these preparations. Ryan's method is also unsuitable for the determination of dimethothiazine or trifluoperazine in preparations as these substances give very low absorbances (less than 0·06) under the stated conditions.

The levels of phenothiazine drugs in the preparations (Table 1) are in good agreement with the declared amounts and, where appropriate, with the results using the procedure of Ryan (1959) or the B.P.C. (1973). An injection of prochlorperazine mesylate, which was 15 years old, showed a loss of drug when assayed by Ryan's method and the difference method. The principal decomposition product in this injection was established as prochlorperazine sulphoxide by the R_F values of the components in the injection. In addition, a shoulder at 343 nm, characteristic of the sulphoxide, was present in the ultraviolet absorption spectrum of an aqueous dilution of the injection. These findings further validate the application of the difference spectrophotometric procedure for the determination of intact phenothiazine drug in degraded samples.

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