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The determination of sulphoxide in degraded phenothiazine formulations by difference spectrophotometry*

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A method is described for the assay of phenothiazine sulphoxides which may be formed in phenothiazine formulations during storage under unfavourable conditions. The assay is based upon the measurement of difference absorbance of the sulphoxide solution in 0·2 M hydrochloric acid relative to an equimolar solution reduced with zinc dust. A solvent extraction procedure avoids interference from colouring agents and coloured photolytic breakdown products of the phenothiazines. The assay is specific in the presence of intact drug, sulphone and co-formulated drugs for the formulations examined and is sensitive to 0·5% of the total phenothiazine present as sulphoxide. Many aqueous formulations stored in partially filled containers have been shown to contain up to 31·5% of the total phenothiazine as sulphoxide.

During the development of a difference spectrophotometric assay for phenothiazine drugs in pharmaceutical formulations (Davidson, 1976), it was observed that certain samples, particularly liquid preparations stored in partially filled containers, gave low results while similar samples from newly opened containers gave good recoveries of intact drug. A shoulder around 345 nm in the absorption spectra of aqueous solutions of the degraded samples indicated the presence of the corresponding phenothiazine sulphoxide in agreement with the findings of Kapadia, Barber & Martin (1970). Thin-layer chromatography of the components in extracts of these formulations confirmed the presence of the sulphoxides which are thought to be formed by aerial oxidation of the parent phenothiazine. A procedure for the determination of phenothiazine sulphoxides in the presence of the intact drug is therefore required which would permit the direct assessment of degradation in pharmaceutical preparations. Chromatographic (West, Rosenblum & others, 1974) and polarographic (Porter & Beresford, 1966) procedures for sulphoxides have been published although their application to formulations was not reported.

The assay of phenothiazine sulphoxides now described is based upon a difference spectrophotometric measurement of the absorbance of a solution of the sulphoxide in 0·2 M hydrochloric acid relative to an equimolar solution reduced with zinc dust to give the parent phenothiazine. The difference spectrum shows an absorption peak around 345 nm which is proportional to the concentration of the sulphoxide in the unreduced solution. The assay is specific for the sulphoxide in the presence of the intact drug, co-formulated drugs and most excipients. Colouring agents and coloured photolytic products of the phenothiazines are however simultaneously reduced to colourless products which interfere in the assay. To avoid this interference, the sulphoxides in coloured samples are extracted from alkaline solution into chloroform and then re-extracted into 0·05 M hydrochloric acid giving colourless extracts of the sulphoxide.

* The preliminary results of this investigation were presented at the British Pharmaceutical Conference, Sheffield in September, 1977.

MATERIALS AND METHODS

Spectrophotometric measurement. Absorbance values were measured in 1, 2 or 4 cm matched quartz cells using a recording spectrophotometer with the absorbance range selected to give the maximum scale expansion of the small ΔA values.

Reference compounds. The sulphoxides and sulphones of promethazine, chlorpromazine, prochlorperazine and trimeprazine were kindly donated by May and Baker Ltd. Promethazine hydrochloride, dimethothiazine mesylate, prochlorperazine mesylate, trimeprazine tartrate, chlorpromazine hydrochloride (May and Baker Ltd.) and promazine hydrochloride (Wyeth, Ltd.) were chromatographically pure and were used as received.

Zinc dust (not less than 99% pure; Koch Light Ltd.) Other reagents and solvents were of Analytical Reagent quality.

Procedure

Protect the solutions from strong actinic light throughout the assay.

Standard reference solutions. Dissolve the appropriate phenothiazine reference sulphoxide (about 0·2 mmol accurately weighed) in 0·2M hydrochloric acid to produce 100 ml of solution. Pipette an aliquot (5 ml) into a volumetric flask (100 ml) and dilute the solution to volume with 0·2 M hydrochloric acid. Transfer 50 ml of the solution to a conical flask (50 ml) and while stirring with a bar magnet add zinc dust (50 mg). Stir the solution for 5 min, add another 50 mg zinc dust and continue stirring for a further 10 min. Allow the solution to stand for 5 min and carefully decant the clear supernatant liquid into a 2 cm quartz cell. Measure the difference absorbance of the unreduced aliquot of the sulphoxide solution in a 2 cm cell relative to the reduced solution in the reference cell at the difference maximum around 345 nm.

Injections, syrups and linctuses. Determine the weight ml^{-1} of viscous preparations. Transfer to a separating funnel a quantity of the preparation equivalent to up to 0·05 mmol of total phenothiazine. Dilute the sample volume of viscous preparations with at least three volumes of water. Basify the solution with 20% m/V sodium hydroxide solution (1 ml) and extract it three times with chloroform (15 ml). Re-extract the combined chloroform solution three times with 0·05 M hydrochloric acid (30 ml) and combine the acidic extracts in a volumetric flask (100 ml). Add hydrochloric acid (35%; 1·4 ml) and dilute the solution to 100 ml with water. Remove the suspended chloroform from the extract by gently shaking it with light petroleum (b.p. 40–60°; 10 ml). Reduce an aliquot (50 ml) of the acidic extract as described for *standard reference solutions* and measure the difference absorbance of the solutions at the appropriate wavelength of maximum difference absorbance.

Injections and other samples yielding colourless solutions in which the content of the sulphoxide exceeds 5% of the total phenothiazine may be assayed directly without preliminary extraction, as follows. Transfer an amount of the preparation equivalent to 0·01 mmol of total phenothiazine to a volumetric flask (100 ml). Add M hydrochloric acid (20 ml) and dilute the solution to 100 ml with water. Reduce an aliquot (50 ml) as described for *standard reference solutions* and measure the difference absorbance of the solutions.

Tablets. Weigh and pulverize 20 tablets. Extract a quantity of the powder containing 0·1 mmol of total phenothiazine with 0·05 M hydrochloric acid (80 ml) for 30 min and dilute to 100 ml with water. Filter the extract through a No. 3 sintered glass filter, discarding the first 20 ml of filtrate. Basify a volume of the filtrate up to 50 ml, depending on the concentration of sulphoxide in the sample, with 20% m/V sodium hydroxide solution and continue the assay as described for *injections, syrups and linctuses* from the words 'and extract it three times with chloroform . . . '.

Treatment of the results

The concentration of phenothiazine sulphoxide in mg per unit dose of the formulation is given by

$$\frac{\Delta A \times \text{mol. wt} \times \text{weight of unit dose} \times 100}{\Delta\epsilon \times d \times \text{weight of sample}}$$

where ΔA is the difference absorbance of the sample solutions, $\Delta\epsilon$ is the molar difference absorption coefficient of the appropriate reference phenothiazine sulphoxide, mol. wt is the molecular weight of the phenothiazine or its salt, d is the pathlength of the cells and where the weights are in g.

RESULTS AND DISCUSSION

Fig. 1a shows the effect of zinc dust and hydrochloric acid on the ultraviolet absorption spectrum of a solution prepared by diluting a partially degraded prochlorperazine injection. The major decomposition product has been shown by thin-layer

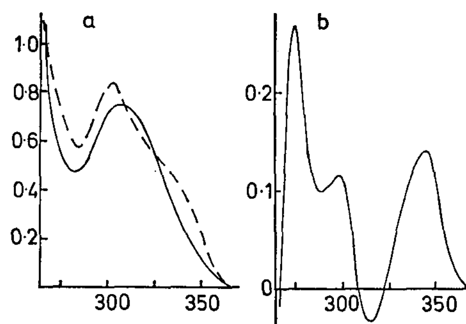

FIG. 1. (a) The ultraviolet absorption (ordinate) spectra of a partially decomposed prochlorperazine injection diluted with 0·2 M hydrochloric acid (- - -) and reduced with zinc dust (——). (b) The difference absorption (ordinate) spectrum of the solution of the prochlorperazine injection in 0·2 M hydrochloric acid relative to the equimolar solution reduced with zinc dust. Abscissa: Wavelength (nm).

chromatography to be prochlorperazine sulphoxide (Davidson, 1976). The shoulder at 343 nm characteristic of the sulphoxide is abolished after reduction and the resultant spectrum is that of prochlorperazine. The difference spectrum (Fig. 1b) of the unreduced solution relative to the reduced solution is identical in form to that given by standard solutions of prochlorperazine sulphoxide.

The difference spectra and $\Delta\epsilon$ values at the wavelengths of maximum difference absorbance of the sulphoxides of chlorpromazine, prochlorperazine, trimeprazine and promethazine (Table 1) are almost identical to those obtained by oxidizing the parent phenothiazine with peroxyacetic acid (Davidson, 1976). Thus $\Delta\epsilon$ values obtained by the peroxyacetic acid oxidation of the parent phenothiazines may be used in the assay of sulphoxide concentrations if authentic sulphoxide reference substances are not available.

The rate of reduction of the sulphoxides depends on the concentrations of hydrochloric acid and zinc dust. At high concentrations of the reagents a fast reduction occurs but problems are encountered due to the formation of coloured products in highly acidic solutions and at high zinc levels adsorption of the phenothiazine on to the zinc occurs which increases the measured difference absorbance of the solutions. The conditions for the reduction have been chosen to give a moderately fast rate of reduction and to minimize errors due to adsorption effects and colour formation.

Coloured photolytic products of the phenothiazines and many of the colouring agents used in the formulations are also reduced by the zinc and hydrochloric acid resulting in a distortion of the difference spectrum. The problem is overcome by extraction of the sulphoxide from an alkaline solution into chloroform followed by re-extraction into 0·05 M hydrochloric acid. The sulphoxides give quantitative recovery under these conditions but many of the parent phenothiazines show poor extraction from the chloroform into 0·05 M hydrochloric acid (Table 2). This separation of the sulphoxide from most of the unchanged drug enables a greater sample weight of the formulation to be assayed with a resultant increase in the sensitivity of the procedure. Concentrations of sulphoxide equivalent to 0·5% may be determined in a sample containing 0·05 mmol of total phenothiazine carried through the extraction procedure.

Adherence to Beer's Law

Beer's Law graphs for the reduction of the sulphoxides of promethazine, chlorpromazine, prochlorperazine and trimeprazine give rectilinear plots passing through the origin and show that ΔA values at the appropriate wavelengths of maximum difference

Table 1. *The concentrations of phenothiazine sulphoxides and intact phenothiazines in old formulations.*

Formulation	Declared amount per unit dose	Storage conditions*	Found unchanged phenothiazine % label	Found phenothiazine sulphoxide (as unchanged phenothiazine) % label	$\Delta\varepsilon$ Phenothiazine sulphoxide	λmax nm
Chlorpromazine syrup	25 mg/5 ml	9/10 full bottle; 26 months	98·0	3·3	4816	343
Chlorpromazine syrup	25 mg/5 ml	½ full bottle; 11 months	84·3	15·9		
Chlorpromazine syrup	25 mg/5 ml	1/4 full bottle; 18 months	78·1	21·6		
Chlorpromazine syrup	25 mg/5 ml	1/5 full bottle; 18 months	72·2	28·6		
Chlorpromazine tablets	10 mg	5 years	100·6	N.D.		
Prochlorperazine injection	12·5 mg ml⁻¹	1 ml ampoules; 16 years	81·2	16·2	4497	345
Trimeprazine syrup	7·5 mg/5 ml	4/10 full bottle; 18 months	98·7	1·3	4960	342
Trimeprazine linctus	2·5 mg/5 ml	6/10 full bottle; 18 months	66·8	31·5		
Promethazine syrup	5 mg/5 ml	8/10 full bottle; 19 months	87·2	11·0	4834	337
Promethazine linctus	3·6 mg/5 ml	6/10 full bottle; 5 months	86·5	13·0		
Promethazine tablets	25 mg	18 months	95·4	0·5		
Dimethothiazine elixir	10 mg/5 ml	3/10 full bottle; 14 months	92·0	6·7	2740†	354
Promazine syrup	25 mg/5 ml	3/10 full bottle; 27 months	95·3	3·7	5330†	345
Promazine injection	12·5 mg ml⁻¹	1 ml ampoules; 6 years	100·6	N.D.		
Promazine tablets	25 mg	18 months	98·6	0·5		

* Syrups and linctuses were stored in 125 ml amber glass bottles.
Tablets were stored in the manufacturer's metal container.
Injections were stored in clear glass ampoules protected from light.
† Calculated from $\Delta A1\%/1cm$ of peroxyacetic acid oxidized phenothiazines (Davidson, 1976).
N.D. None detected.

Table 2. *Recovery of phenothiazines, phenothiazine sulphoxides and phenothiazine sulphones in the extraction procedure.*

Drug	Phenothiazine sulphoxide %	Parent phenothiazine %	Phenothiazine sulphone %
Promethazine	100·5	25·6	92·9
Chlorpromazine	100·8	13·5	88·5
Trimeprazine	99·2	18·8	93·7
Prochlorperazine	100·2	92·1	98·6

absorbance are proportional to the concentration of the sulphoxide in the range 0–0·15 mM.

Specificity

The specificity of the procedure was assessed by examining the effect of zinc and hydrochloric acid on the ultraviolet absorption spectra of several phenothiazine sulphones which may be present in badly degraded samples, parent phenothiazines and certain other drugs found in the formulations listed in Table 1. The following substances gave $\Delta A = 0$ and therefore do not interfere in the assay of sulphoxides: promethazine, promazine, chlorpromazine, prochlorperazine, dimethothiazine, trimeprazine, codeine, ephedrine, phenylpropanolamine, guaiphenesin and the sulphones of promethazine, chlorpromazine, prochlorperazine and trimeprazine. Evidence for the specificity of the method in the presence of the formulation excipients was provided by the observation that the isosbestic points in the 300–330 nm region were identical in the difference spectra of the sample and reference solutions (see Davidson, 1976).

Accuracy

Mixtures of promethazine sulphoxide, promethazine hydrochloride and promethazine sulphone prepared to simulate formulations in various stages of oxidative decomposition were assayed for the sulphoxide by reduction of the acid extracts obtained by the solvent extraction procedure. The results given in Table 3 show good recovery of the sulphoxide and confirm that the assay is specific for the sulphoxide in the presence of the unchanged drug and the sulphone. Similar satisfactory recoveries of the sulphoxides of prochlorperazine, chlorpromazine and trimeprazine were also obtained in mixtures with the appropriate unchanged phenothiazine and the sulphone.

Precision

A sample of chlorpromazine hydrochloride syrup (25 mg/5 ml) stored in a quarter-full amber glass bottle for 18 months gave a mean concentration of chlorpromazine sulphoxide of 5·09 mg/5 ml with a relative standard deviation of 1·8% in 10 determinations.

Table 3. *The concentrations (μmol litre^{-1}) of promethazine sulphoxide (PSO) determined in mixtures with promethazine sulphone (PSh) and promethazine HCl (PHCl).*

Composition of the mixture			PSO	
PHCl	PSh	PSO	found	%
502	200	301·0	299·0	99·3
803	100	100·3	102·3	102·0
904	50	50·2	51·2	102·0
964	20	20·0	19·6	98·0
984	10	10·0	9·7	97·0

Assay results

To test the application of the method, sulphoxides were assayed in several formulations which had been stored for various periods of time in contact with different volumes of air in their containers. Most of the formulations had been shown to contain the declared amounts of intact phenothiazine when the containers were first opened. The assay of sulphoxide in formulations of prochlorperazine, chlorpromazine, promethazine and trimeprazine was based upon $\Delta\epsilon$ values obtained by the reduction of standard solutions of the reference sulphoxide. Since promazine and dimethothiazine sulphoxides were not available during the present investigation, the assay of these was based on the $\Delta\epsilon$ values obtained by the oxidation of the appropriate reference phenothiazine with peroxyacetic acid. As a check on the assay for sulphoxides the formulations were also assayed for intact drug by the peroxyacetic acid oxidation procedure (Davidson, 1976).

The results recorded in Table 1, show that in all the formulations, the total phenothiazine concentrations (i.e. intact phenothiazine + sulphoxide) were in good agreement with the declared amounts of the phenothiazine. The concentrations found in promazine and dimethothiazine formulations confirm the validity of $\Delta\epsilon$ values obtained by oxidation of the reference phenothiazine for use in the assays of intact drug and sulphoxide. Although three tablet preparations were found to contain only low sulphoxide concentrations even after several years storage, many of the aqueous formulations were found to contain concentrations of sulphoxide which repre-

sent a significant proportion of the total phenothiazine. The results thus demonstrate the application of the method to formulations stored under unfavourable conditions and confirm that sulphoxide formation is the major route of decomposition in aqueous preparations in contact with air. Although this experiment was not designed as a stability study it is apparent that the concentration of sulphoxides in these formulations depends on the period of storage and on the relative volume of air in the container.

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