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Quantitation of pharmaceutically important phenothiazines by oxidimetry

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Abstract

A new spectrophotometric method for the assay of phenothiazines in pure form as well as in pharmaceutical formulations with the chromium(VI)–metol–sulfanilic acid system has been developed. The method is based on the oxidation of the drugs by a known excess of chromium(VI) and subsequent determination of the unreacted oxidant by interacting with metol and sulfanilic acid. The reacted oxidant corresponds to the drug content. The coloured species exhibits maximum absorbance at 530 nm. Beer's law is obeyed over the concentration range 5–60 μg ml^{-1} and the relative standard deviation is found to be less than 2%. The apparent molar absorptivities are in the range 3.77×10^3–3.98×10^3 l mol^{-1} cm^{-1}, the detection limits being in the range 0.6133–1.1349 μg ml^{-1}. The method was successfully applied to the determination of the studied drugs in their formulations and the mean percentage recoveries were found to be 97.32–102.80%.

Keywords: Phenothiazines; Spectrophotometry; Cr(VI)–metol–sulfanilic acid system oxidimetry; Pharmaceutical formulations

1. Introduction

Phenothiazines form a large class of basic drugs used primarily for the treatment of moderate to severe mental disorders. They are also used as antiemetics, sedatives, antipruritics, antidyskinetics, analgesics and antihistaminics [1].

Several methods have been described for the determination of phenothiazines in pharmaceuticals and biological fluids. They include titrimetry [2–4], polarography [5], voltammetry [6], potentiometry [7], gas chromatography [8], high-performance liquid chromatography [9,10], ESR spectroscopy [11], spectrofluorimetry [12] and radioimmunoassay [13]. Reviews of methods for the determination of phenothiazines have also been published [14–16]. Several spectrophotometric methods based on radical cation formation [17], charge-transfer complexation [18], diazocoupling [19], oxidative coupling [20], complex formation [21], ion-associate complexation [22] and condensation [23] reactions have also been applied for the assay of phenothiazines. The official methods [24] for the assay of phenothiazines include non-aqueous titrimetry for pure drugs and UV spectrophotometry for formulations.

Most of the spectrophotometric methods developed have one or other disadvantage. For example, the method employing *N*-bromosuccinimide [17] as the chromogenic agent requires a high concentration of sulfuric acid as the reaction medium, whereas several procedures [25] require heating of the reaction mixture in a boiling-water bath for over 15 min. The reaction with chloranilic acid [18] should be carried out at ice-cold temperature and that with chromeazurol S [26] requires adjustment of pH and incubation of the reaction mixture for 30 min. The diazocoupling method [19] uses a large volume of concentrated hydrochloric acid as the diluent. The method proposed by Dembinsky [27] based on the use of Reinecke salt involves precipitation, filtration and measurement in acetone medium besides lacking sensitivity (linear range 68–680 μg ml^{-1}). The method of Aman et al. [28] uses an uncharacterised coloured product and is poorly sensitive, the determination range being 0.01–1.25 mg ml^{-1}. The aim of the present work was to develop a simple and

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convenient method for the determination of phenothiazines in pharmaceutical formulations. The procedure is based on the oxidation of the drugs by a known excess of potassium dichromate in acidic conditions and subsequent determination of unreacted dichromate via charge-transfer reaction involving metol and sulfanilic acid. The proposed method is reasonably sensitive, accurate and precise and free from many drawbacks found in other procedures previously cited. The coloured species is unusually stable (stability >24 h versus 10–15 min reported earlier) [29–33].

2. Results and discussion

One of the sensitive methods developed for the determination of primary aromatic amines involves the formation of a purple–red colour when primary aromatic amines are made to react with metol and an oxidising agent [34,35]. Of the several oxidising agents employed for this study, chromium(VI) [29,30] has been found to be convenient because of its moderate oxidising power. Of late, the oxidant–metol–primary amine combination has been used for the indirect spectrophotometric determination of many reducible substances like thiazide diuretics [32], methotrexate [33] and thiols [36]. In the present paper, we describe the application of Cr(VI)–metol–primary amine combination to the determination of six phenothiazine drugs.

The method is based on the oxidation of the drugs by a known excess of chromium(VI) in acidic conditions and subsequent determination of the unreacted chromium(VI) by interacting with metol and the primary aromatic amine, sulfanilic acid. Phenothiazines, when added in increasing amounts, consume dichromate and consequently there will be a concomitant fall in the dichromate concentration. This is observed as a proportional decrease in the absorbance of the reaction mixture on increasing the concentration of the drugs.

To start with, the upper limit of dichromate, which can be determined by metol–sulfanilic acid combination, was found by treating different amounts of dichromate with metol and sulfanilic acid under the conditions described in the Section 3.3. Beer's law was obeyed up to a concentration of 10 μg ml^{-1} potassium dichromate. Hence, different amounts of the drug were treated with 1 ml of 100 μg ml^{-1} $K_2Cr_2O_7$ and the unreacted dichromate was determined as described in Section 3.3.

Since the various parameters for the determination of the oxidant are well established [32,33,36], the conditions for the oxidation of the drugs by dichromate prior to its determination were optimised. A total of 1 ml of 10 M sulfuric acid was found to be adequate in a total volume of 5 ml for the oxidation of the drugs to their respective sulfoxides over a reasonable time period (Table 1). The reaction was slow in hydrochloric or phosphoric acid medium. Since the characteristic colour is not produced in the acidic condition employed for the oxidation of the drug [29–36], the pH was raised by adding an optimum volume of 2 ml of 1:1 ammonia after the addition of metol and sulfanilic acid. Higher volumes up to 4 ml had no effect on the absorbance.

Two blanks were prepared for this system. The reagent blank, which contained optimum concentrations of all reagents except the drug, gave maximum absorbance. The other blank was prepared in the absence of dichromate and the drug to determine the contribution of other reagents to the absorbance of the system. Since the absorbance of this second blank was negligible, the absorbance of the developed colour was measured against water. The colour was stable for more than 24 h. The reaction time, i.e. the time taken for the complete oxidation of the drugs to their respective sulfoxides, is not critical. Any delay up to 2 h in the determination of unreacted dichromate has no effect on the absorbance. However, a 10 min contact time after the addition of metol is critical. Any delay in the addition of amine results in decreased absorbance values.

Table 1
Analytical data for the determination of phenothiazines using Cr(VI)–metol–sulfanilic acid combination

Parameters	CPH	PH	TPH	TFPH	PCPM	PCPMS
Beer's law limits (μg ml^{-1})	5–35	5–30	5–35	5–45	7.5–6.0	7.5–60
Detection limit (μg ml^{-1})	0.6759	0.6133	0.7467	0.6681	1.1349	0.7212
Molar absorptivity $\times 10^3$ (l mol^{-1} cm^{-1})	3.88	3.91	3.85	3.77	3.90	3.98
Sandell sensitivity (μg cm^{-2} per 0.001 A unit)	0.0091	0.0081	0.0100	0.0127	0.0155	0.0170
Regression coefficient, r	−0.9997	−0.9979	−0.9998	−0.9984	−0.9968	−0.9972
Regression equation, y [a]						
Intercept (a)	0.4020	0.4007	0.3999	0.4081	0.4020	0.4002
Slope (b)	−0.0110	−0.0122	−0.0100	−0.0112	−0.0066	−0.0058
Reaction time (min)	15	5	15	10	10	10
Relative standard deviation (%)	1.03	0.69	1.24	0.72	0.84	1.04
Range of error (%) (95% confidence limit)	±1.08	±0.72	±1.30	±0.75	±0.88	±1.08

[a] $y = a + bx$ where 'y' is the absorbance for concentration 'x' in μg ml^{-1}.

Table 2
Results of analysis of pharmaceutical preparations containing the studied phenothiazines

Drug and preparation	Label claim, mg/tablet or mg/ml	Found [a]		Student's t-value [b]	F-value [c]
		Proposed method % recovery ± SD	BP method % recovery ± SD		
CPH					
CPH tablets [d]	25	98.60 ± 0.64	98.90 ± 1.20	0.34	3.51
Megatil tablets [e]	50	100.30 ± 0.62	102.20 ± 1.50	2.83	5.85
Emetil tablets [f]	100	97.65 ± 0.73	98.04 ± 0.86	0.78	1.38
CPH injections [d]	25	99.04 ± 0.59	98.68 ± 1.06	0.69	3.23
PH	10	98.38 ± 1.62	100.02 ± 0.81	2.13	4.00
Phenergan tablets [g]	25	98.96 ± 1.52	100.60 ± 0.86	2.17	3.12
Phenergan injections [g]	25	102.80 ± 1.63	101.30 ± 0.72	2.01	5.12
TPH					
Siquil tablets [h]	10	98.08 ± 0.62			
Siquil injections [h]	10	99.62 ± 0.92			
TFPH					
Trazine tablets [d]	5	97.32 ± 1.14	98.64 ± 0.96	1.98	1.41
Neocalm tablets [e]	5	100.40 ± 0.32	99.60 ± 0.32	3.95	1.00
PCPM					
Emydoxin tablets [i]	5	99.60 ± 0.17	99.80 ± 0.31	1.31	3.32
Stemetil tablets [g]	5	99.80 ± 0.21	100.20 ± 0.32	2.38	2.32
PCPMS					
Stemetil injections [g]	12.5	97.80 ± 0.32	98.50 ± 0.46	2.83	2.06

[a] Average value of five determinations + standard deviation.
[b] Tabulated value at 95% confidence level is 9.
[c] Tabulatd value at 95% confidence level is 9.
[d] Marketed by Sun Pharma.
[e] Marketed by Intas.
[f] Marketed by L.A.Pharma.
[g] Marketed by Rhone–Poulenc.
[h] Marketed by Ind-Swift.
[i] Marketed by Sarabhai Chemicals.

2.1. Analytical parameters

The reaction times, Beer's law limits, detection limits, molar absorptivities and Sandell sensitivities are given in Table 1. The slope and intercept values obtained by using a linear least-squares treatment of the results of the systems are also presented in Table 1. The correlation coefficients of the calibration plots are in the range -0.9968 to -0.9998, confirming a linear decrease in absorbance with increasing concentration of the drugs. The accuracy and precision of the method were established by six replicate determinations of 20 μg ml^{-1} of each drug. The relative standard deviation (%) and range of error (% at 95% confidence limit) obtained are also given in Table 1.

2.2. Application

The method was applied to the assay of the studied drugs in tablets and injections. The results obtained were found to be in good agreement with those of the official methods [24]. The calculated t- and F-values did not exceed the theoretical values, indicating that there is no significant difference between the methods in the mean values obtained and their precision (Table 2). Recovery experiments were carried out by adding known amounts of pure drug to preanalysed formulations and the results are presented in Table 3. Common excipients such as talc, starch, glucose, alginate, stearate and bisulfite did not interfere as indicated by the results of the recovery study.

2.3. Mechanism

The chemistry of the colour reaction may be suggested on the basis of a previously reported mechanism [37,38]. It is probable that the *p*-*N*-methylbenzoquinone monoimine formed in situ from the metol–Cr(VI) reaction, being a good electron acceptor, forms a charge-transfer complex with the amine as electron donor. The probable reaction scheme is shown in Fig. 1.

Table 3
Results of recovery of pure drug added to formulations

Formulation	Drug	Drug initially present as formulation (μg)	Pure drug added (μg)	Amount of pure drug recovered (μg)	Recovery (%)[a]
CPH tablets (25 mg)	CPH	100	150	147.25	98.17
		100	200	200.40	100.20
CPH injections (25 mg)	CPH	100	150	149.40	99.60
		100	200	199.60	99.80
Phenergan tablets (10 mg)	PH	100	150	146.86	97.91
		100	175	173.07	98.90
Phenergan injections (25 mg)	PH	100	150	147.99	98.66
		100	175	174.65	99.80
Siquil tablets (10mg)	TPH	100	150	145.83	97.22
		100	200	197.44	98.72
Trazine tablets (5 mg)	TFPH	150	200	196.44	98.22
		150	250	250.50	100.20
Neocalm tablets (5 mg)	TFPH	150	200	200.06	100.03
		150	250	249.87	99.95
Stemetil tablets (5 mg)	PCPM	150	225	226.26	100.56
		150	300	296.76	98.92
Stemetil injections (12.5 mg)	PCPMS	150	300	298.80	99.60
		150	375	375.07	100.02

[a] Mean value of three determinations.

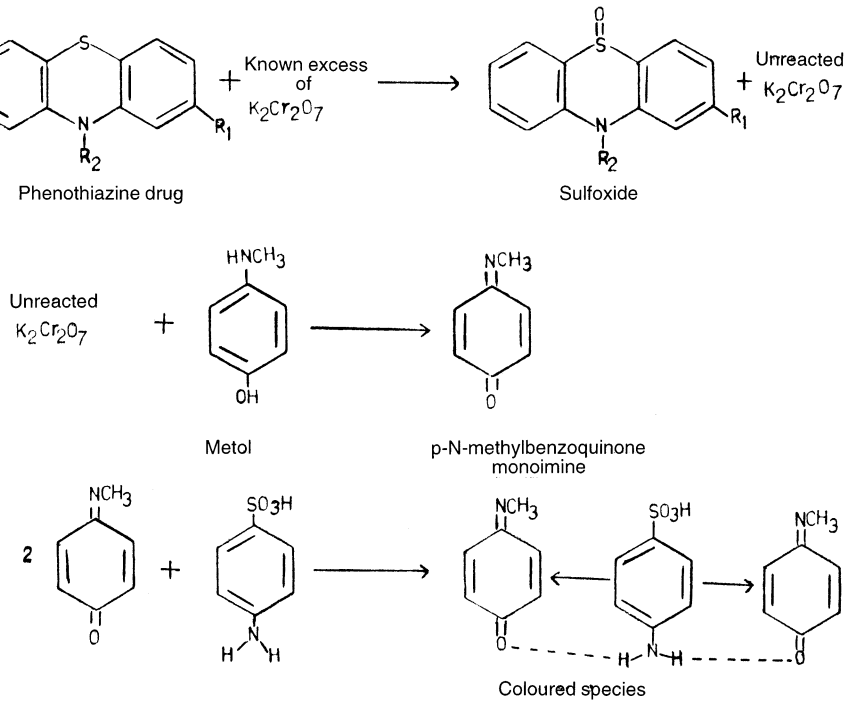

Fig. 1. Probable reaction scheme.

3. Experimental

3.1. Apparatus

An Elico model SL-171 digital spectrophotometer with 1 cm glass cells was used for absorbance measurements.

3.2. Reagents and solutions

All chemicals used were of analytical-reagent grade and double distilled water was used throughout the study. A stock solution of potassium dichromate (1000 μg ml^{-1}) was prepared by dissolving 0.1 g of reagent (S.d. Fine Chemicals, India) in water and diluting to 100 ml in a standard flask. The solution was further diluted to get 100 μg ml^{-1}. A freshly prepared 0.2% aqueous solution of metol (BDH) was always used. Aqueous solutions of 0.2% sulfanilic acid (Ranbaxy Fine Chemicals Ltd.) and 1:1 ammonia (S.d. Fine Chemicals, India) were prepared in the usual way.

3.2.1. Standard drug solutions

Pharmaceutical grade phenothiazines were kindly provided by various pharmaceutical companies and were used as received. About 100 mg of chlorpromazine hydrochloride, CPH (British Pharmaceuticals); promethazine hydrochloride, PH; prochloperazine maleate, PCPM and prochlorperazine mesylate, PCPMS (Rhone–Poulenc); triflupromazine hydrochloride, TPH (Sarabhai Chemicals) and trifluoperazine hydrochloride, TFPH (SmithKline Beecham) was accurately weighed and dissolved in water and diluted to 100 ml in a standard flask. (In the case of PCPM a few drops of 0.1M HCl were used to aid dissolution before diluting to the mark.) The solutions were kept in amber coloured bottle and stored in a refrigerator. Working solutions containing 300 μg ml^{-1} PCPM and PCPMS, and 200 μg ml^{-1} in the case of others were prepared by appropriate dilution before use.

3.3. Procedure

A 1 ml portion of 100 μg ml^{-1} potassium dichromate solution was placed in a series of 10 ml calibrated flasks followed by acidification by adding 1 ml of 10 M sulfuric acid. Then, different aliquots of the drug solution (0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 ml (200 μg ml^{-1}) TPH or TFPH; 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 ml (300 μg ml^{-1}) PCPM; 0.25, 0.50, 0.75, 1.00, 1.25, 1.50 and 1.75 ml (200 μg ml^{-1}) CPH; 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 ml (200 μg ml^{-1}) PH or 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 ml (300 μg ml^{-1}) PCPMS) were accurately measured into the flasks, the overall volume was adjusted to 5 ml with water and the flasks were let stand for 5–15 min depending on the individual phenothiazine drug (Table 1) with occasional shaking, for the oxidation of the drug to complete as indicated by the disappearance of the orange or purple colour of the radical cation. Then, 1 ml of 0.2% metol solution was added to each flask and after 10 min, 1 ml of 0.2% sulfanilic acid solution and 2 ml of 1:1 ammonia were added and diluted to the mark with water, mixed well and the absorbance of the coloured solution measured at 530 nm against double distilled water. A blank experiment was also carried out omitting the drug. The decrease in absorbance was plotted against the drug concentration and the concentration was read from the appropriate calibration graph or calculated from the linear regression equation.

3.4. Determination of phenothiazines in formulations

Twenty tablets were weighed and powdered. A weighed portion of the powder equivalent to about 50 mg of drug was transferred to a beaker and extracted with 60 ml of distilled water for 20 min; the mixture was filtered and diluted to 100 ml. (For PCPM a few drops of 0.1 M HCl were used for extraction.) The solution was diluted to get a working concentration (300 μg ml^{-1} for PCPM and PCPMS, and 200 μg ml^{-1} for others). The sample was then ready for treatment as described in Section 3.3.

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