Indirect Titrimetric and Spectrophotometric Methods for the Determination of Some Phenothiazine Psychotropics

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A titrimetric and a spectrophotometric method herein described for the determination of phenothiazine psychotropics. In the titrimetric method, phenothiazines are reacted with a known excess of potassium dichromate under acidic conditions and after the completion of the reaction, unreacted dichromate is backtitrated with ammonium ferrous sulphate. In the spectrophotometric method, the unreacted dichromate is reacted with s-diphenylcarbazide under acidic conditions and the absorbance measured at 540 nm. The proposed methods were successfully applied to the determination of phenothiazines in pharmaceutical preparations. The reliability of the assays was established by parallel determination by the official methods of British pharmacopoeia.

Key words phenothiazine; psychotropics; titrimetry; spectrophotometry; determination

Phenothiazines are compounds with well known neuroleptic activity. Chlorpromazine remains the most widely used psychotropic drug throughout the world and continues to serve as a standard by which other neuroleptics are compared.[1)] Other phenothiazine analogues possess antidepressive rather than neuroleptic action and are widely used for the treatment of depressive states. Of the various phenothiazines, the piperazine derivatives were found to be potent antiemetics. The increasing need to determine low concentrations of these drugs has led to the development of a number of methods.

Official methods used for the determination of phenothiazines in pharmaceutical preparations are usually based on extraction as a free base and subsequent determination by UV-spectrophotometry.[2,3)] Many organic compounds, drug excipients and diluents as well as various organic bases, strongly interfere. Several titrimetric procedures based on the redox properties of s-atom using hexacyanoferrate(III),[4)] sodium metavanadate[5)] and potassium bromate[6)] as oxidimetric titrants have been proposed. However, these require high acidic conditions and the use of a screening indicator.[4)] Methods based on the protonated nitrogen atom of the molecules, including non-aqueous titration,[7)] two-phase titration,[8,9)] and titration with picrates of lead, copper, cadmium and zinc,[10)] have also been used for the estimation of phenothiazines. Non-aqueous titrimetry requires a scrupulously anhydrous medium, which is inconvenient in practice, whereas complexometric procedures[10)] involve the filteration of the complex before titrating the unreacted metal, which is cumbersome.

The determination of phenothiazines by spectrophotometric methods has been proposed based on oxidation with persulphate,[11)] iron,[12)] chloramine-T,[13)] and *N*-bromosuccinimide[14)] to give products that can be determined either directly in the visible region or after coupling/condensing with *p*-aminobenzoic acid,[15)] 3-methyl-2-benzothiazolinone hydrazone (MBTH),[16)] haematoxylin[17)] and morpholine.[18)] Other chromogenic reactions use diphenylpicryl hydrazyl,[19)] chlorophenolred,[20)] ammonium molybdate[21)] and nitroprusside.[22)] These methods involve a time-consuming extraction step and require strictly controlled reaction conditions.

Phenothiazines in pharmaceutical preparations have been determined by polarographic and potentiometric techniques. The polarographic method is based on the prior conversion of phenothiazines into polarographically active derivatives through nitration,[23)] followed by measuring the redox wave. Most of the potentiometric methods are based on the extraction of the free base, followed by titration with standard acids in non-queous media.[24)] Other methods include stripping voltammetry,[25)] HPLC[26)] and spectrofluorimetry[27)] but involve expensive experimental setup.

The purpose of this investigation was to develop simple, accurate, precise and inexpensive methods for the assay of some phenothiazine psychotropics in pharmaceutical formulations.

Experimental

A systronics model 106 Spectrophotometer with 1 cm pathlength quartz cells was used for all absorbance measurements.

Reagents All reagents were of analytical-reagent grade and distilled water was used for preparing all solutions.

Potassium Dichromate Solution (0.003 mol l^{-1}): Prepared by dissolving 0.8825 g potassium dichromate in 1000 ml of water. Suitable dilutions were made to obtain a 10 μg ml^{-1} chromium(IV) working standard solution for spectrophotometry.

Ammonium Ferrous Sulphate (0.02 mol l^{-1}): This was prepared by dissolving about 3.92 g of the reagent in 50 ml of 2 mol l^{-1} sulphuric acid and diluting it to one litre with distilled water. The solution was then standardized by a titration with standard solution of potassium dichromate using diphenylamine sulphonic acid salt as an indicator.

Indicator (0.2%): Prepared by dissolving 0.2 g of barium salt of diphenylamine sulphonic acid in 100 ml of distilled water.

s-Diphenylcarbazide (0.25%): Prepared by dissolving 0.25 g of reagent in 100 ml of acetone.

Sulphuric Acid (10 mol l^{-1}): Diluted 280 ml of concentrated sulphuric acid (sp. gr. 1.84) to 500 ml with distilled water. It was further diluted to get 4 mol l^{-1} for the spectrophotometric method.

Sample Solutions Pure drug samples were kindly provided by several pharmaceutical companies and used as received. Stock standard solutions containing 2000 μg ml^{-1} drug were prepared by dissolving accurately weighed 500 mg amounts of chlorpromazine hydrochloride, CPH (British Pharm.); promethazine hydrochloride, PH (Rhone-Poulenc); prochlorperazine maleate, PCPM (SmithKline Beecham); triflupromazine hyrochloride, TPH (Sarabhai Chemicals); trifluoperazine hydrochloride, TFPH (SmithKline Beecham) and thioproperzine mesylate, TPPM (Rhone-Poulenc) in distilled water in 250 ml calibrated flasks. A few drops of diluted hydrochloric acid were used for dissolving PCPM. Each solution was further diluted stepwise to get 40 μg ml^{-1} of CPH, PH, TPH & TFPH and 80 μg ml^{-1} of PCPM and TPPM for spectrophotometric work.

Procedure Titrimetry: An aliquot (5 to 10 ml) containing 2—20 mg

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Table 1. Analytical Data for the Titrimetric Determination of Phenothiazines

Drug	Reaction time (min)	Molar ratio Drug : Oxidant	Range (mg)	Recovery[a)] (%)	RSD (%)
CPH	2	3 : 1	2—20	99.70	0.80
PH	2	3 : 1	2—20	99.30	1.01
PCPM	3	3 : 1	2—20	99.80	1.12
TPH	2	3 : 1	2—20	100.50	1.39
TFPH	3	3 : 1	2—16	100.20	1.31
TPPM	5	3 : 1	2—16	97.80	0.96

a) Average of six determinations each containing 10 mg of drug.

CPH, PH, TPH, or PCPM, 2—16 mg TFPH or TPPM was transferred to a 100 ml titration flask followed by the addition of 1 ml of 10 mol l^{-1} sulphuric acid and 10 ml of 0.003 mol l^{-1} potassium dichromate. The reaction mixture was shaken occasionally, and after a specified time (Table 1) the unreacted dichromate was titrated with 0.02 mol l^{-1} standardized ammonium ferrous sulphate using 5 drops of barium salt of diphenylamine sulphonic acid indicator. A reagent blank was run under identical conditions using all the reagents except the drug.

Determination of Reacting Ratio and Minimum Reaction Time: A 5 ml aliquot containing 2—16 mg of each drug was allowed to react with a known excessive volume of potassium dichromate solution (0.003 mol l^{-1}). After the complete disappearance of the orange or purple color, the unreacted oxidant was back titrated. The reacting ratio (moles of dichromate per mole of drug) was calculated for each test. The findings are presented in Table 1.

Spectrophotometry: In each of a series of 10 ml calibrated flasks was placed 1 ml of 10 μg ml^{-1} chromium(VI) solution followed by acidification by the addition of 1 ml of 1 mol l^{-1} sulphuric acid. After adding 0—2.5 ml of CPH or TPH (40 μg ml^{-1}), 0—2.0 ml PH (40 μg ml^{-1}), 0—2.0 ml of PCPM or TPPM (80 μg ml^{-1}) or 0—3.0 ml of TFPH (40 μg ml^{-1}), the overall aqueous volume was adjusted to 5 ml by the addition of requisite amount of distilled water, mixed well and allowed to stand for 20—30 min, *i.e.*, until the disappearance of the orange or purple color. Subsequently, 1 ml of 0.25% s-diphenylcarbazide reagent was added, diluted to the mark with distilled water, mixed well and absorbance was recorded at 540 nm against the distilled water blank. The concentration of the drug was calculated from the calibration graph constructed under the same conditions or the regression equation in each case.

Analysis of Pharmaceutical Preparations Tablets: Ten to forty tablets depending on content per tablet were weighed and ground into a fine powder. An amount of the powder equivalent to 100 mg of active component was weighed into a 100 ml volumetric flask, about 60 ml of distilled water was added and shaken thoroughly for about 20 min. The volume was increased to the mark with distilled water, shaken and filtered using quantitative filter paper. A 10 ml aliquot of this filtrate was analyzed by titrimetry. For spectrophotometric determination, the filtrate was diluted sequentially to get 40 μg ml^{-1} in respect to CPH, PH, TPH and TFPH and 80 μg ml^{-1} of drug in the case of TPPM and PCPM. A suitable portion was then used for analysis.

Injections: The contents of twenty ampoules were mixed and an accurately measured volume equivalent to 100 mg of drug was transferred into a 100 ml calibrated flask and diluted to the mark with distilled water. Steps described under the tablets were then followed. The samples were also analyzed by the official methods of British Pharmacopoeia and the results are given in Table 3.

Results and Discussion

Titrimetry The method is based on the fact that dichromate in an acidic medium directly oxidizes phenothiazines first to purple or orange phenothiazonium free radicals and finally to colorless sulphoxides.[28)] The effect of variables such as oxidant concentration, strength of sulphuric acid, temperature and reaction time were studied. It was found that 10 ml of 0.003 mol l^{-1} solution of dichromate in 0.5 mol l^{-1} sulphuric acid was suitable for a quantitative reaction. It was also observed that the reaction was complete at room temperature in 1—5 min depending on the amount of drug taken.

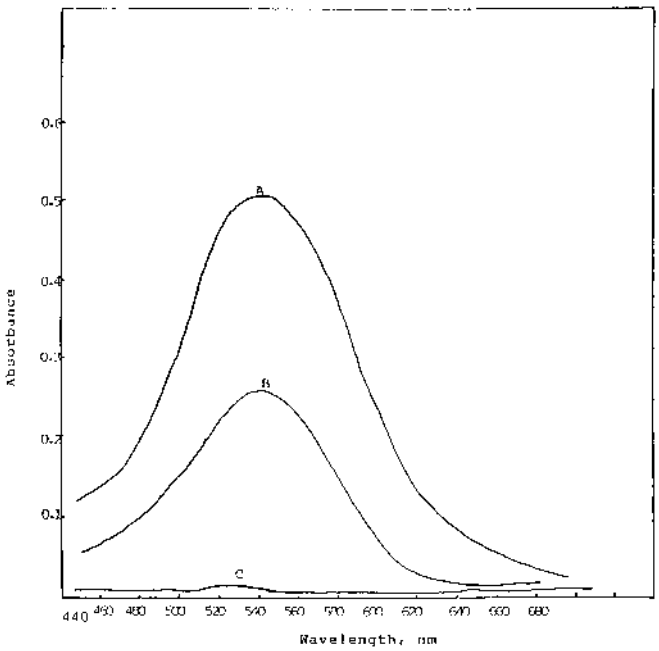

Fig. 1. Absorption Spectra Measured against Water
A, blank; B, 5 μg ml^{-1} of CPH; C, without chromium(VI) solution.

The number of moles of dichromate consumed per mole of the drug was 0.333 in all cases, in conformity with the formation of sulphoxides.[28)] Any delay up to 60 min in the back titration of the unreacted dichromate did not result in the consumption of more than a stoichiometric amount of dichromate in the case of pure drugs, indicating that the oxidation of drugs stopped at sulphoxide formation. However, in the case of such formulations, especially tablets, when the back titration of the unreacted dichromate was delayed beyond 15 min, more dichromate than the stoichiometric amount was consumed. This is possibly due to the oxidation of sugar which will be present in the tablets as an excipient. Hence, in respect to formulations, it is recommended that the unreacted dichromate be backtitrated immediately after the decoloration of the radical cation. Other additives and excipients such as starch, talc, gelatin, alginate, stearate and metabisulphite in levels found in formulations did not interfere under the described experimental conditions. This is due to the preferential reaction of phenothiazines with dichromate and the self-indication system provided by the phenothiazonium free radicals.

Spectrophotometry In an acid solution, s-diphenylcarbazide (1,5-diphenylcarbohydrazide) gives a soluble, strongly colored red-violet product with hexavalent chromium.[29)] This reaction has been the basis of the best method for the determination of the most minute amounts (0.2—1.0 μg ml^{-1}) of chromium.[30)] In this work, known but excessive dichromate was employed to oxidize phenothiazines in sulphuric acid medium to colorless sulphoxides, and the unreacted dichromate was determined by reacting it with s-diphenylcarbazide in the same acidic condition. This formed the basis for the determination of microquantities of phenothiazine drugs.

Phenothiazines, when added in increasing amounts, consume dichromate; consequently there is a concomitant fall in chromium(VI) concentration. This is observed as a proportional decrease in the absorbance of the reaction mixture on

Table 2. Optical Characteristics, Precision and Accuracy

Drug	Reaction time (min)	Beer's law limits ($\mu g\,ml^{-1}$)	Detection limit[a] ($\mu g\,ml^{-1}$)	Molar absorptivity $\times 10^4$ ($l\cdot mol^{-1}\,cm^{-1}$)	Slope	Intercept	Correlation co-efficient	RSD[b] (%)
CPH	20	0—9	0.013	1.772	−0.0491	0.505	−0.99	0.76
PH	20	0—8	0.068	1.774	−0.0552	0.507	−0.99	1.11
PCPM	30	0—16	0.045	1.781	−0.0288	0.507	−0.99	0.85
TPH	30	0—10	0.099	1.768	−0.0450	0.507	−0.99	0.46
TFPH	30	0—12	0.089	1.772	−0.0376	0.510	−1.00	1.32
TPPM	30	0—16	0.061	1.739	−0.0275	0.512	−0.99	0.64

a) $C_L = 3S_B/m$; C_L, detection limit; S_B, standard deviation of blank; m, slope of calibration. *b*) Average of seven determinations each containing 5 $\mu g\,ml^{-1}$ of CPH, PH, TPH or TFPH and 8 $\mu g\,ml^{-1}$ of PCPM or TPPM.

Table 3. Assay of Phenothiazines in Pharmaceutical Preparation by Proposed and Reference Methods

Preparation	mg/tablet or mg/ml	Proposed methods: Titrimetry Found (mg)	Titrimetry Recovery[a] (%)	Titrimetry S.D.	Spectrophotometry Found (mg)	Spectrophotometry Recovery[a] (%)	Spectrophotometry S.D.	Reference Method Found (mg)	Reference Method Recovery[a] (%)	Reference Method S.D.	*t*-value[b] T	*t*-value[b] S	*F*-value[c] T	*F*-value[c] S
CPH														
Chloropromazine tablet	100	99.9	99.90	0.82	99.2	99.2	0.58	99.5	99.5	0.63	0.91	0.81	1.69	1.17
	25	24.89	99.56	0.37	25.51	102.0	0.50	25.42	101.7	0.50	1.82	0.30	2.01	1.00
Chlorpromazine injection	25	25.6	102.1	0.66	25.21	100.84	0.32	25.3	101.2	0.42	0.91	0.40	2.46	1.72
PH														
Phenergan tablet	10	10.02	100.2	0.63	10.04	100.4	0.42	10.01	100.1	0.82	0.002	0.05	1.69	3.81
Phenergan injection	25	24.77	99.1	0.35	25.30	101.2	0.26	25.21	100.84	0.54	2.07	0.37	2.38	4.33
TPH														
Siquil tablet	10	9.93	99.3	0.14	9.90	99.00	0.16	—	—	—	—	—	—	—
Siquil injection	10	9.99	9.99	0.17	9.92	99.2	0.32	—	—	—	—	—	—	—
TFPH														
Neocalm	5	4.97	99.5	0.10	5.02	100.4	0.32	4.98	99.6	0.32	0.18	0.11	0.26	3.16
Trazine	5	4.96	99.2	0.15	5.01	100.2	0.46	4.99	99.8	0.20	0.23	0.08	3.48	2.69
PCPM														
Stemitil injection (As mesylate)	12.5	12.40	99.2	0.94	12.46	99.6	0.82	12.44	99.2	0.54	0.08	0.05	3.03	2.30
TPPM														
Majeptil	5	4.93	98.6	0.92	4.95	99.0	0.65	—	—	—	—	—	—	—

a) Average of five determinations; *b*) Tabulated value 2.78; *c*) Tabulated value 6.39; T stands for titrimetry and S for spectrophotometry. Recovery means recovery of active component/content from the co-formulated substances.

increasing the concentration of phenothiazines.

The various parameters involved in the study were optimized. Suitable acidity for the chromium(VI)–diphenylcarbazide reaction is 0.025—0.1 $mol\,l^{-1}$. At low acidities the color does not develop immediately, but in 0.1 $mol\,l^{-1}$ H_2SO_4 solution, maximum color intensity is attained within a few seconds. Solutions having an acidity of greater than 0.1 $mol\,l^{-1}$ are not desirable because the color is less stable in these.[31] In 0.1 $mol\,l^{-1}$ H_2SO_4, the color is quite stable[32] (15 h). Although 0.1 $mol\,l^{-1}$ of sulphuric acid was adequate for the reaction between chromium(VI) and s-diphenylcarbazide, a slightly higher concentration (0.4 $mol\,l^{-1}$) of sulphuric acid was employed in the present investigation to facilitate complete oxidation of phenothiazines to respective sulphoxides in a reasonable time without affecting the sensitivity and stability of the colored product. At acidities lower than 0.4 $mol\,l^{-1}$, the reaction between drug and dichromate was slow, taking more than 30 min. By employing higher acidities (>0.4 $mol\,l^{-1}$), the reaction time and thereby the analysis time could be reduced, but higher acidities are undesirable since the reddish-violet product subsequently formed between chromium(VI) and diphenylcarbazide is less stable (less than 2 h).

Two blanks were prepared for this system. The reagent blank, which contained optimum concentrations of all the reagents except phenothiazines, gave maximum absorbance (Fig. 1). The other blank was prepared in the absence of dichromate to determine the contribution of other reagents to the absorbance of the system. As the absorbance of this second blank was comparable to that of water, the absorbance of the developed color was measured against distilled water. The developed color was stable for more than 1 h. The decreasing absorbance values at 540 nm were plotted against increasing concentrations of phenothiazines to obtain the cal-

ibration graphs. Beer's law was obeyed over the concentration ranges given in Table 2. Correlation coefficients, intercepts and slopes for the calibration data are also presented in Table 2. The wide difference in the slopes of the calibration curves of different phenothiazine drugs is due to the varying molecular weights of the drugs, from 320.89 for PH to 638.86 for TPPM. The linear Beer's law plots for the investigated drugs can also be used to calculate concentrations. The apparent molar absorptivities are around 1.77×10^4 $1\cdot mol^{-1}\cdot cm^{-1}$, and the detection limit[33] is in the range of 0.013—0.099 $\mu g\ ml^{-1}$.

To examine the precision of the procedure, seven replicate determinations were made on the same solution containing 5 $\mu g\ ml^{-1}$ of CPH, PH, TPH or TFPH and 8 $\mu g\ ml^{-1}$ of PCPM or TPPM; coefficients of variation of 0.76, 1.11, 0.46, 1.32, 0.85 and 0.64, respectively, were obtained.

Among the concomitant substances, only glucose was found to interfere with the determination. This could be avoided by reacting the unreacted chromium(VI) with s-diphenylcarbazide immediately after the disappearance of the color due to a phenothiazonium radical, *i.e.*, immediately after the specified time (Table 2).

Application The proposed methods were applied to the determination of studied drugs in their dosage forms. The results in Table 3 indicate that the methods give good accuracy and precision, with satisfactory agreement with the results obtained by the official method.

Statistical analysis of the results by *F*- and *t*-tests showed no significant difference in accuracy and precision between the proposed and official methods.

Conclusion

Although phenothiazines have been determined by a variety of techniques, the methods described here are simple, highly sensitive (spectrophotometry), convenient and don't require special working conditions, unlike many other reagents. Moreover, owing to the stability of the solid reagent as well as aqueous solution, dichromate can be used for routine analysis.

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References

1) Kaiser R. C., Settler P. E., "Burger's Medicinal Chemistry," Part III, Wolff M. F., (ed.), Chapter 56, Wiley, New York, 1981.
2) "British Pharmacopoeia 1993," Her Majesty's Stationery Office, London, 1993.
3) "United States Pharmacopoeia 23," National Formulary 18, US Pharmacopoeial Convention,, Rockville, MD, 1995.
4) Issa A. S., Mahrous M. S., *Talanta*, **31**, 287—288 (1984).
5) Stan M., Dima F., Ghimisescu, *Rev. Mad.-Chir.*, **82**, 337—340 (1978).
6) Blazek J., *Cslka Farm*., **15**, 200—205 (1996).
7) Milne J., *J. Pharm. Assoc*., **48**, 117—120 (1959).
8) Blazek J., Travnickova M., *Cslka Farm.*, **24**, 100—108 (1975).
9) Albert F. M., Aftalion H., Simionovici R., *Rev. Chim*. (Bucharest), **19**, 283—286 (1968).
10) Gajawska M., *Chem. Anal*. (Warsaw), **18**, 651—656 (1973).
11) Emmanuel J., Quenim U. S., *East. Pharma.*, **33**, 386—388 (1990).
12) Karpinska J., Kojlo A., Grudniewska A., Puzanowska-Tarasiewicz H., *Pharmazie*, **51**, 950—954 (1996).
13) Puzanowska-Tarasiewicz H., Kojlo A., *Farm. Pol.*, **39**, 141—142 (1983).
14) Mohammed A. L., *Talanta*, **44**, 1173—1782 (1997).
15) Emmanuel J., Quenim U. S., *East. Pharm*., **33**, 117—118 (1990).
16) El-Komos M. E., Emara K. M., *Analyst.*, **113**, 1267—1271 (1988).
17) Sastry C. S. P., Tipirneni A. S. R. P., Suryanarayana M. V., *Indian Drugs*, **26**, 351—353 (1989).
18) El-Shabouri S. R., Yousef A. I., Mohammed F. A., Rageh A. M. I., *J. Assoc. Off. Anal. Chem*., **69**, 821—824 (1986).
19) Emara K. M., *Anal. Lett.*, **25**, 99—100 (1992).
20) Seetharamappa J., Shubha B. K., Nagaraja P., *Indian J. Pharm. Sci.*, **58**, 258—261 (1996).
21) Basavaiah K., Krishnamurthy G., *Anal. Lett*., **31**, 1036—1047 (1998).
22) Basavaiah K., Krishnamurthy G., Manajunathaswamy J., *East. Pharm*., **41**, 107—110 (1998).
23) Dumortier A. G., Patriarche J., *Z. Anal. Chem*., **264**, 153—158 (1973).
24) Golabi S. M., Showkati-Shishevan M., *Talanta*, **38**, 1253—1256 (1991).
25) Zhang Z. Q., Chen Z. G., Zhang H., *Microchem. J*., **53**, 282—289 (1996).
26) Abdel-Moety E. M., Al-Deeb O. A., *Eur. J. Pharm. Sci*., **5**, 1—5 (1997).
27) Dembinski B., Szyldowska-Czerniak A., Kurzawa M., *Acta Pol. Pharm*., **54**, 415—419 (1997).
28) Merkle F. M., Discher C. A., *J. Pharm. Sci*., **53**, 620—626 (1964).
29) Cazeneuse P., *Bull Soc. Chim*., **23**, 701—705 (1900); *Idem*, *ibid*., **25**, 761—766 (1901).
30) Moulin A., *Bull. Soc. Chim. France*, **31**, 295—301 (1904).
31) Sandell E. B., "Colorimetric Determination of Traces of Metals," 3rd ed., Interscience Publishers, Inc., New York, p. 393 (1959).
32) Rowland G. P., *Ind. Eng. Chem.*, *Anal. Ed*.., **11**, 422—445 (1939).
33) Recommendations for the definition, estimation and use of the detection limit; Analytical Methods Committee, *Analyst*, **112**, 199—204 (1987).