Titrimetric and Spectrophotometric Determinations of Some Phenothiazine Psychotropics Using Chloramine-T

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A simple, convenient and accurate indirect titrimetric method and a rapid, sensitive and precise indirect spectrophotometric method for the determination of six phenothiazine drugs using chloramine-T as an oxidant are described. The titrimetric method is based on the oxidation of the drugs in sulphuric acid medium by a known excess of chloramine-T and iodometric determination of the unreacted chloramine-T. In the spectrophotometric method, the unreacted oxidant is made to react with metol in the presence of an aromatic amine to produce a purple-red product with the absorption maximum at 520 nm. The absorbance is found to decrease linearly with increasing concentration of drugs, which is corroborated by the calculated correlation coefficient values in the range -0.9920 to -0.9999. The systems obey Beer's law for 5-150 μg ml^{-1} of drugs. The molar absorptivities are around 2.00 x 10^3 1 mol^{-1} cm^{-1} and relative standard deviations (RSD) are less than 2% (n=7). The proposed methods were successfully applied in the determination of studied phenothiazines in pharmaceutical formulations. The reliability of the assay methods was established by parallel determination by the BP method.

Introduction

N-substituted phenothiazine derivatives have a profound psychotherapeutic activity[1] and hence are used in the treatment of various mental illnesses. They also possess sedative, antihistaminic, antiemetic, antipruritic and anaesthetic properties[2]. The importance of phenothiazine tranquillisers has prompted many investigators to look for methods for their rapid determination. Numerous methods developed for the determination of these biological important compounds have been reviewed[3−5]. The official method is based on non-aqueous titration or spectrophotometry of the pure drug or its dosage forms[6,7]. Many titrimetric methods based on oxidation by oxidizing agents such as hexacyanoferrate[8], ammonium metavanadate[9], N-bromosuccinimide and other brominating agents[10] and l-chlorobenzotriazole[11] have been proposed. Most other methods are based on oxidation prior to analytical measurement. Typical oxidants used are potassium iodate[12], ammonium molybdate[13], ammonium metavanadate[14], hexacyanoferrate(III)[15], nitroprusside[16],

N-bromophthalimide[17] iron (III) chloride[18] and iodic acid[19] and the final products are determined by spectrophotometry. Spectrofluorimetry has also been used after oxidation by N-bromosuccinimide[20] and cerium (IV)[21]. Even potentiometry[22] with an oxidative column in a flow injection system has been applied. High-performance liquid chromatography (HPLC)[23] with electrochemical detection has been proposed for the determination of phenothiazines in pharmaceuticals and human serum. Other methods proposed for the assay of phenothiazines include potentiometry[24,25], voltammetry[26,27], differential spectrophotometry[28] and chemiluminescene spectroscopy[29,30].

The use of chloramine-T as an oximetric reagent was first proposed by Noll[31] and, since then, a large number of compounds of pharmaceutical interest[32−38] have been determined using chloramine-T. A titrimetric procedure[39] based on the oxidation of drugs with iodine monochloride in strong hydrochloric acid medium and titrating the liberated iodine with chloramine-T by the Andrews method has been proposed for phenothiazines. The procedure is highly cumbersome. It employs highly acidic conditions (20 ml concentrated HCl for 10 ml drug solution), uses the presence of alcohol and requires vigorous shaking. In addition the procedure is applicable only to 20-100 mg of drugs. Issa *et al.*[40] have used chloramine-T as a chromogenic reagent for the spectrophotometric determination of some phenothiazines. However the method has many disadvantages, including the use of alcoholic solutions, heating in a water bath for a long time and, above all, poor sensitivity.

In this work, a simple and convenient titrimetric procedure and a rapid, accurate and sensitive spectrophotometric method utilising chloromine-T as an oximetric reagent are proposed. The methods are applicable to the determination of bulk drugs and dosage forms.

Experimental

Apparatus

A Systronics model 106 digital spectrophotometer with 1 cm matched quartz cells was used for absorbance measurements. An Elico model L1 digital pH meter was used for pH measurements.

Reagents

All chemicals used were of analytical-reagentgrade.

Chloramine-T (0.015 M and 500 μgml^{-1}): A 0.015 M chloramine-T solution was prepared by dissolving 4.22 g of the reagent in distilled water and diluting to one litre. This solution was standardised iodometrically and then diluted stepwise to get 500 μgml^{-1} solution for spectrophotometric work.

Sodium thiosulphate (0.02 M): This was prepared by dissolving 5.00 g of the sample in distilled water and diluting to one litre with the same solvent. This solution was standardised iodometrically using potassium dichromate.

Starch indicator (1%): A paste of 1.0 g of soluble starch with a little distilled water was made and it was poured, with constant stirring, into 100 ml of boiling water, boiled for a minute and cooled.

Sulphuric acid (5 M): Prepared by diluting 556 ml of concentrated sulphuric acid (d = 1.84) to two litres with distilled water.

Walpole buffer (pH 2.71): This was prepared by mixing 50 ml of 1 M sodium acetate and 50 ml of 1 M hydrochloric acid and diluting to 250 ml with distilled water[41].

Metol (0.2%): This was prepared by dissolving 0.2 g of the sample in 100 ml of distilled water just before use.

Sulphanilic acid (0.1%): This was prepared by dissolving 0.1 g of the sample in 100 ml of distilled water just before use.

Standard solutions of phenothiazine drugs

Pure drug samples were kindly provided by many pharmaceutical companies and were used as received. Stock standard solutions containing 2000 μgml^{-1} drugs were prepared by dissolving accurately weighed amounts of chlorpromazine hydrochloride (CPH), promethazine hydrochloride (PH), triflupromazine hydrochloride (TPH), trifluoperazine hydrochloride (TFPH), thioproperazine mesylate (TPPM) and prochlorperazine mesylate (PCPMS) in distilled water. The solutions were kept in amber-coloured bottles and stored in a refrigerator. Each solution was further diluted suitably to obtain 400-800 μgml^{-1} drug solutions for spectrophotometric work.

Procedures

Titrimetry: A 10 ml aliquot of drug solution containing 1-10 mg of TPH, PH or TFPH or 1-7 mg of CPH or 2-14 mg of TPPM or PCPM was transferred into a 100 ml titration flask followed by the addition of 2-5 ml of 5 M sulphuric acid (Table 1) and 10 ml of 0.015 M chloramine-T solution. The reaction mixture was shaken occasionally and, after a specified time (Table 1), 5 ml of 10% potassium iodide was added and the liberated iodine was titrated against 0.02 M sodium thiosulphate solution using starch indicator. A reagent blank was also run under under identical conditions using all the reagents except the drug. The amount of the drug was calculated from the number of equivalents of chloramine-T consumed in the reaction with phenothiazines.

Table 1. Analytical data for the titrimetric determination of phenothiazines

Drug Studied	Volume of 5 M H$_2$SO$_4$	Reaction time (min)	Molar ratio, Drug: oxidant	Range (mg)	Recovery* (%)	RSD (%) (n=6)
CPH	3	30	1:2	1-7	100.00	1.31
PH	5	25	1:2	1-10	100.86	1.67
TPH	3	30	1:2	1-10	99.40	1.02
TFPH	3	30	1:2	1-10	100.20	2.58
TPPM	2	30	1:2	2-14	100.00	1.96
PCPMS	2	25	1:2	2-14	99.70	1.60

* Average of six determinations each containing 5 mg of CPH, PH, TPH or TFPH, 10 mg of TPPM or PCPM

Spectrophotometry: In each of the series of 10 ml calibrated flasks was placed 1.5 ml of 500 μgml^{-1} chloramine-T solution followed by 0.0-2.0 ml of TPH, TFPH or PCPMS (500 μgml^{-1}), PH or CPH (400 μgml^{-1}) or TPPM (800 μgml^{-1}). The contents were mixed well and set aside for 1 min or until the disappearance of the red, reddish-purple or orange colour. Subsequently, 2 ml of buffer of pH 2.71 was added to each flask followed by 1 ml each of 0.2% metol and 0.1% sulphanilic acid solutions. The volume was made up to the mark with distilled water and the absorbance of the resulting purple colour was measured at 520 nm against the reagent blank prepared under identical conditions but without the drug. Calibration

graphs were constructed by plotting absorbance versus concentration of the drug in μgml^{-1}. Concentration of the drug in test solutions was calculated from the calibration graph or the regression equation computed from the Beer's law data.

Procedure for Pharmaceutical Preparations

Twenty to 80 tablets containing the phenothiazine drugs studied were ground into a fine powder. An amount of the powder equivalent to 100 mg of pure drug was weighed into a 100 ml standard flask, and about 60 ml of distilled water was added and shaken thoroughly for about 20 min. The volume was made up to the mark with distilled water, mixed well and filtered using quantitative filter paper. A 5 or 10 ml aliquot of this filtrate was then used for analysis by titrimetry. For the spectrophotometric method, the filtrate was diluted appropriately before taking a suitable aliquot for assay. In the case of injections, the contents of 20 ampoules were mixed and an accurately measured volume equivalent to 100 mg of the drug was transferred into a 100 ml calibrated flask and diluted to the mark with distilled water. The steps described under the tablets were then followed. The samples were also analysed by the official methods of the British Pharmacopoeia[6] and the results are given in Table 3.

Results and Discussion

Titrimetry: The procedure is based on the fact that chloramine-T in acidic solution oxidises phenothiazines first to red, reddish-violet or orange coloured radical cations and finally to colourless sulphoxide[42]. The effect of variables such as oxidant concentration, strength of sulphuric acid and the reaction time were studied. It was found that 10 ml of 0.015 M chloramine-T in 1 M sulphuric acid was suitable for quantitative oxidation. The reaction was not stoichiometric in hydrochloric or phosphoric acid medium. The oxidation of drugs as indicated by the disappearance of the colour was found to be completed at room temperature in 20-30 min depending on the individual phenothiazine derivative.

Two moles of chloramine-T per mole of each drug were required for complete reaction. Prolongation of the reaction time up to 60 min, had no effect on the stoichiometry and the results in respect of pure drugs, but the formulations, tablets in particular, were found to consume more oxidant if the back titration of the unreacted chloramine-T was delayed beyond 15 min. producing erroneous results, due to interference from sugars. Other additives and excipients like starch, talc, gelatin, alginate and stearate in levels found in formulations did not interfere under the experimental conditions described. This is due to the preferential reaction of phenothiazines with chloramine-T and the self-indicating system provided by the phenothiazinium free radicals.

Spectrophotometry: In mildly acidic conditions, when primary aromatic amines were made to react with 4-N-methylaminophenol (metol) and chloramine-T, a purple-red colour[43] is produced. In the present investigation, the above observation was utilised for estimating microgram quantities of chloramine-T, which formed the basis for the indirect spectrophotometric method for phenothiazines. In the proposed method, phenothiazines were oxidised to colourless sulphoxides by means of a known and excessive amount of chloramine-T and the unreacted chloramine-T was determined by metol-sulphanilic acid (primary aromatic amine) as a chromogenic agent.

Phenothiazines, when added in increasing amounts to a fixed amount of chloramine-T, consume the oxidant proportionately and, consequently, there is a concomitant fall in the chloramine-T concentration and this is indicated by the proportional decrease in the absorbance of the chromogen when fixed amounts

of metol and sulphanilic acid are was used. This decrease in absorbance is proportional to phenothiazine concentration.

The reaction between phenothiazines and chloramine-T was fast as indicated by the disappearance of red, reddish-purple or orange colour, which took barely 2-3 min. The various parameters involved in the formation of the chromogen were optimised. In order to determine Beer's law limits for chloramine-T, the absorbance at 520 nm of a series of solutions (10 ml) containing varying amounts of chloramine-T, 2 ml of Walpole buffer of pH 2.71 and specified concentrations of metol and sulphanilic acid (as given in the procedure) were used. The system was found to obey Beer's law in the concentration range 5-90 μgml^{-1}.

In the determination of drugs, varying amounts of drugs were reacted with fixed amounts of chloramine-T (750 μg), metol (1 ml of 0.2%) and sulphanilic acid (1 ml of 0.1%) throughout at pH 2.71 in a total volume of 10 ml.

After the oxidation of phenothiazines, the first step in colour development is the oxidation of metol to 1, 4-benzoquinone-methylimine, which has a short life in aqueous solution, being prone to hydrolysis to 1, 4-benzoquinone and methylamine[44], and then its coupling with sulphanilic acid. Neither 1, 4-benzoquinone nor the mixture of 1,4-benzoquinone and the oxidant will couple with sulphanilic acid to give the colour. Since chloramine-T is found to react with primary aromatic amines[43], metol should be added first and sulphanilic acid later, otherwise lower absorbance values will be obtained. Thus, metol should be added first and then sulphanilic acid. Because of the appreciable loss of 1,4-benzoquinone methylimine by its rapid hydrolysis, any delay in the addition of the amine also results in low (and variable) absorbance values[43,45].

Two blanks were prepared for this system. The reagent blank, which contained optimum concentrations of all the reactants except phenothiazines, gave maximum absorbance. The other blank was prepared in the absence of chloramine-T to determine the contribution of other reagents to the absorbance of the system. As the absorbance of the second blank was comparable to that of water, the absorbance of the developed colour was measured against distilled water. Full colour development took 30 min and was stable for 30 min. The decreasing absorbance values at 520 nm were plotted against the increasing concentrations of phenothiazines to obtain the calibration 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 apparent molar absorptivities are around 2x10^3 1 mol^{-1} cm^{-1}.

Table 2. Optical characteristics, precision and accuracy data of spectrophotometric method

Drug	Beer's law limit (μgml^{-1})	Molar absorptivity ($10^3 \times 1$ mol^{-1}cm^{-1})	Correlation coefficient (r)	Slope (b)	Intercept (a)	Recovery* (%)	RSD (%) (n=7)
CPH	5-90	1.992	-0.9900	-0.0056	0.5190	98.85	0.93
PH	5-80	2.018	-0.9999	-0.0063	0.5280	99.05	1.04
TPH	5-90	2.035	-0.9999	-0.0052	0.5270	100.65	0.63
TFPH	5-120	1.900	-0.9920	-0.0039	0.5196	98.81	0.44
TPPM	10-150	1.808	-0.9999	-0.0029	0.5085	100.01	1.07
PCPMS	5-100	2.033	-0.9920	-0.0036	0.5220	100.21	1.53

* Average of seven determinations each containing 60 μgml^{-1} of CPH, PH or TPH, 70 μgml^{-1} of PCPM, 80 μgml^{-1} of TFPH or 100 μgml^{-1} of TPPM

To examine the precision of the procedure, seven replicate determinations were performed on the same solution containing 60 μgml^{-1} of CPH, PH or TPH, 70 μgml^{-1} of PCPMS, 80 μgml^{-1} of TFPH or 100 μgml^{-1} of TPPM and % RSD of 0.93, 1.04, 0.63, 0.44, 1.07 and 1.53 respectively, were obtained.

The concomitant substances usually associated with the drugs in the pharmaceutical preparations did not interfere in the determination.

Application of the methods

The proposed methods were applied to the determination of the studied drugs in pharmaceutical preparations. 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.

Table 3. Assay of phenothiazines in pharmaceutical preparations by the proposed and official methods

Drug and formulation analysed[$]	Content per tablet or per ml (mg)	Found, % Recovery*±SD			Student's t-value[#]		F-value[@]	
		Proposed methods						
		Titrimetry	Spectrophotometry	BP method	T	S	T	S
CPH								
Megatil tablets[a]	25	97.46±1.26	98.58±0.88	98.85±1.06	1.89	0.44	1.41	1.45
	50	100.42±1.02	99.98±0.64	100.04±0.85	0.64	0.12	1.44	1.76
Megatil injections[a]	25	98.76±1.02	99.04±0.96	98.94±0.84	0.30	0.17	1.47	1.30
PH								
Phena tablets[b]	10	101.16±1.28	100.98±0.95	99.85±1.12	1.72	1.72	1.30	1.38
	25	98.52±1.64	99.03±1.26	98.74±0.76	0.28	0.45	4.65	2.74
Phenergan injections[c]	25	100.14±1.34	99.85±0.63	99.26±0.85	1.27	1.26	2.48	1.82
TPH								
Siquil tablets[d]	10	97.96±1.48	98.24±0.96					
Siquil injections[d]	10	99.26±1.02	99.68±0.76					
TFPH								
Trazine tablets[e]	10	98.58±1.36	99.04±0.66	100.06±0.72	2.25	2.33	3.56	1.19
Neocalm tablets[a]	10	98.27±1.02	98.73±0.84	99.17±0.53	1.83	1.01	3.70	2.51
TPPM								
Majeptil tablets[c]	5	99.28±1.54	101.42±1.16					
PCPMS								
Stemetil injections[f]	12.5	100.42±0.94	101.03±1.26	100.86±0.84	0.78	0.25	1.25	2.25

* Average of five determinations

Tabulated t-value at 95% confidence level is 2.75

@ Tabulated F-value at 95% confidence level is 6.39

$ Marketed by: a=Intas, b=Ind-Swift, c=Rhone-Poulenc, d=Sarabhai Chemicals, e=Sun Pharma, f=SmithKline Beecham

'T' stands for titrimetry 'S' stands for spectrophotometry

Statistical analysis of the results by Student's t-test and F-test showed no significant difference in accuracy and precision between the proposed and official methods.

Conclusion

The salient features of the proposed methods are simplicity, sensitivity (spectrophotometry) and long dynamic range of determination. The methods do not require special working conditions unlike many other procedures and thus are suitable for routine analysis of phenothiazines in bulk drugs as well as formulations.

Acknowledgements

References

1. A. G. Gilman, L. S. Goodman, T. W. Rall and F. Murad, **Las bases farmacologicas de la terapeutica**, Panamericana, Madrid (1989).

2. C. O. Wilson, O. Gisvold and R. F. Doerge, **Text book of organic medicinal and pharmaceutical chemistry**, 7th edn. (J B Lippincott) pp 338 (1977).

3. J. Blazek, A. Dymes and Z. Stejskal, **Pharmazie**, **31**, 681-703 (1976).

4. J. E. Fairbrother, **Pharm. J., 222** , 271-275 (1978).

5. L. F. S. Changoda and J. S. Millarship, **J. Pharm. Biomed. Anal.**, **7**, 271-278 (1989).

6. **The British Pharmacopoeia 1993**, Her Majesty's Stationery Office, London (1993).

7. **The United States Pharmacopoeia 23,** National Formulary 18, US Pharmacopoeial Convention, Rockville, MD (1995).

8. K. Basavaiah and G. Krishnamurthy, **Talanta, 47**, 59-66 (1998).

9. K. Basavaiah and G. Krishnamurthy, **Mikrochim. Acta**, **130**, 197-201 (1999).

10. M. I. Walash, M. Rizk, A. M. Abou-Ouf and F. Belal, **Analyst**, **108**, 626-632 (1983).

11. M. I. Walash, M. Rizk, S. S. Toubar, S. M. Ahemed and N. A. Zukhari, **Bul. Fac. Pharm. (Cairo Univ.)**, **34**, 71-75 (1996).

12. K. Basavaiah and P. G. Ramappa, **Indian J. Pharm. Sci., 47**, 125-127 (1985).

13. K. Basavaiah and G. Krishnamurthy, **Anal. Lett., 31**, 1037-46 (1998).

14. S. Singh, I. C. Shukla and S. Shukla, **Indian J. Pharm. Sci., 50**, 278-279 (1988).

15. K. Basavaiah and G. Krishnamurthy, **Ann. Chim. (Rome)**, **89**, 623-629 (1999).

16. K. Basavaiah, G. Krishnamurthy and J. Manjunatha Swamy, **East. Pharm., 41**, 107-109 (1998).

17. M. M. El-Kerdawy, S. M. Hassan and S. M. El-Ashry, **Mikrochim. Acta.**, **108**, 323-328 (1992).

18. H. Zhang, Q. Kang and Y. Liu, **Yaowu Fenxi Zazhi**, **13**, 124-125 (1993).

19. A. G. Sajjan, J. Seetharamappa and M. B. Melwanki, **Indian J. Pharm. Sci.**, **63**, 61-64 (2001).

20. F. A. Mohmed, **Anal. Lett., 28**, 2491-2501 (1995).

21. T. Perez-Ruiz, C. Martinezo-Lozano, V. Thomas and C. Sidrachdo Cardona, **Talanta, 40**, 1361-1365 (1993).

22. A. Kojlo, **Anal. Lett.**, **30**, 2553-2563 (1997).

23. A. R. Fox and D. A. McLonghlin, **J. Chromatogr.**, **631**, 255-261 (1997).

24. M. H. Pournaghi Azar and Farhadi Kh, **Talanta**, **44**, 1773-1781 (1997).

25. J. L. F. C. Lima, M. Motenegro, B. S. M. Conceicao., M. Sales and F. Goreti, **J. Pharm. Sci., 86**, 1234–1238 (1997).

26. I. Biryol, Z. Senturk, S. A. Ozkan, S. Dermis and B. Uslu, **Port. Electrochim. Acta.**, **15**, 5–15 (1997).

27. Z. Senturk, S. A. Ozkan, B. Uslu and L. Biryol, **J. Pharm. Biomed. Anal., 15**, 365–370 (1996).

28. L. Long and Y. Zhang, **Yaowu Fenxi Zazhi.**, **1**, 45-46 (1995).

29. J. J. Lopez Paz and A. Tounshend, **Anal. Commun.**, **33**, 31-33 (1996).

30. A. A. Alwarthan, S. A. Al-Tamrah and A. A. Akel, **Anal. Chim. Acta., 282**, 169–174 (1993).

31. A. Noll, **Chem Zty**, **48**, 845 (1924); **64**, 303 (1940).

32. K. Nikolic, B. Stankovic and M. Bogavac, **Boll. Chim. Farm**., **134**, 254-257 (1995).

33. C. S. P. Sastry, M. Aruna and A. S. R. P. Tipirneni, **Chem. Anal. (Warsaw), 36**, 153-158 (1991).

34. K. I. Nikolic, B. S. Stankovic and M. R. Bogavac, **J. Serb. Chem. Soc.**, **60**, 251-255 (1995).

35. S. Arora, **Natl. Acad. Sci. Lett. (India), 22**, 8-11 (1999).

36. K. K. Verma, P. Tyagi, and A. K. Gulati, **J. Pharm. Biomed. Anal., 5**, 51-58 (1987).

37. K. K. Verma and A. K. Gulati, **Anal. Chem**., **52**, 2336-38 (1980).

38. N. V. Rajashekaran and N. C. G. Ramachandran, **Anal. Chim. Acta**., **57**, 429-434 (1971).

39. A. N. Nayak, Rangaswamy, H. S. Yathirajan and P. G. Ramappa, **Indian. Drugs.**, **29**, 202-205 (1982).

40. A. S. Issa, Y. A. Beltagy and M. S. Mahrous, **Talanta**, **25**, 710-713 (1978).

41. Walpole, **Biochem. J**., **105**, 2501, 2521 (1914).

42. F. M. Merkle and C. A. Discher, **J. Pharm. Sci., 53**, 620-626 (1964).

43. R. R. Krishna, P. Siraj and C. S. P. Sastry, **Acta Cinec Indica, Ser. Chem.**, **6**, 140–143 (1980).

44. D. Amin, **Analyst, 110**, 211-214 (1985).

45. R. R. Krishna and C. S. P. Sastry, **Talanta, 26**, 861-865 (1979).