All the test compounds exhibited moderate to good antibacterial and antifungal activities. The compounds VII and IX exhibited equipotent activity with the standard ciprofloxacin against *S. flexneri* and *P. aeruginosa*; and the compound VIII exhibited equipotent activity with ciprofloxacin against *S. subtilis* and *C. freundii*. The compounds V, VI and IX were found to be equipotent with the standard clotrimazole against *A niger* and *M. gypseum*; and the compound VI was equipotent with standard clotrimazole against T. mentagrophytes.

ACKNOWLEDGEMENTS

The author is grateful to S.B. College of Pharmacy, Sivakasi-626 130, for providing facilities to carry out this research work.

REFERENCES

1. Saravanan, K., Mohan, S. and Manjunatha, K.S., **Indian J. Heterocycl. Chem.**, 1998, 8, 55.
2. Chao, P., Deng, Qi. H., Lynn, C., Shih, G., Hsienchengi Leoni, S., Lorenzom, V., Genini, V., Davide, K., Carson, Y., Dennis, A., Lottam, R. and Howard, B., **J. Med. Chem.**, 1999, 42, 3860.
3. Ibrahim, M.K., **Egypt. J. Pharm. Sci.**, 1999, 39, 519.
4. Kovalenko, S.I., **Farm. Zh. (kiev).**, 1999, 1, 64.
5. Pandey, V.K. and Raj, N., **Curr. Sci.**, 1985, 55, 785.
6. Desai, N.C., Bhatt, J.J. and Undavia, N.K., **Farmaco**, 1996, 51, 361.
7. Alagarsamy, V., Prabakaran, L., Duraimurugan, R., Gurumurthy, G., Bindu, P., Arun kumar, M. and Bothiraja, C., **Acta Pharm. Turc.**, 2000, 42, 33.
8. Anil, K. and Saxena, M., **Arznelm. Forsch.**, 1992, 39, 35.
9. Garcia, J.D. Somanathan, R., Rivero, I.A., Aguirre, G. and Hellberg, L.H., **Synth. Commun.**, 2000, 30, 2707.
10. Rivero, I.A., Somanathan, R. and Hellberg, L.H., **Synth. Commun.**, 1998, 28, 2077.
11. Lipunova, G.N., Mokrushina, E.V., Sidorova, L.P. and Charushin, V.N., **Pharm. Chem. J.**, 2000, 34, 19.
12. Pandey, V.K. and Negi, H.S., **Indian Drugs**, 1999, 36, 37.
13. Manabu, H., Ryvichi, I. and Hideaki, H., **Chem. Pharm. Bull.**, 1990, 38, 618.
14. Pandeya, S.N., Sriram, D. and Alagarsamy, V., **Acta Pharm. Turc.**, 1999, 41, 391.
15. Alagarsamy, V., Meena, S., Vijayakumar, S. and Revathi, R., **Farmaco**, 2002, 57, (In press).
16. Green wood, D., Slack, R.C.B. and Peutherer, J.F. In; Medical Microbiology 14th Edn., ELBS, London, 1992, 1.

Spectrophotometric Investigations on the Assay of Phenothiazine Drugs

A. G. SAJJAN, K. C. RAMESH[1], J. SEETHARAMAPPA* AND J. KESHAVAYYA.

Department of Chemistry, Karnatak University, Dharwad-580 003

[1]Department of Chemistry, Kuvempu University, Shankarghatta-577 451

Accepted 17 July 2002

Revised 1 July 2002

Received 22 December 2000

A rapid, accurate and sensitive spectrophotometric method for the quantitative determination of four phenothiazine drugs either in pure form or in pharmaceutical preparations has been developed. The method is based on the development of red coloured products by the interaction of phenothiazines with diazotised anthranilic acid in hydrochloric acid medium. The reaction proceeds via the oxidation of the phenothiazine nucleus into a semiquinonoid radical. The optimum reaction conditions and other analytical parameters are evaluated. The common excipients employed do not interfere in the determination of phenothiazine drugs. Results of analysis of pure drugs and their dosage forms by the proposed method are in good agreement with those of the official method.

Phenothiazines are widely used as anticholinergic, antihistaminic and antipsychotic drugs[1]. Phenothiazine drugs are analysed spectrophotometrically using various analytical reagents, which include, hexacyanoferrate (III)[2], N-

***For correspondence**

bromophthalimide[3], 1,2-naphthaquinone-4-sulphonic acid[4], morpholine and N-bromosuccinimide[5], 3-methylbenzothiazolin-2-one hydrazone and ammonium iron (III) sulphate[6], haematoxylin and chloramine-T[7], fast green FCF[8], amaranth and fast red-E[9], bromocresol green[10], sodium nitroprusside[11], fast red AL[12], diazotized p-nitroaniline[13], ammonium molybdate[14], iodic acid[15], p-benzoquinone[16], N-chlorosuccinimide[17], dapsone and m-aminophenol[18], diazotised dapsone[19]. But some of these spectrophotometric methods suffer from disadvantages such as the need for heating for colour development[5-7,14], require extraction[8-10], have less sensitivity[3,4,7,15,17], need standing for a certain period for colour development[7,11,16,18], applicable only at higher concentrations of the drugs[12,19] or less stable[15]. In the present paper, a rapid, sensitive and accurate spectrophotometric method for the determination of four phenothiazine drugs, propericiazine [2-Cyano-10-(4^{11}-hydroxy-N,-3^{1}-piperidinopropyl-1^{1}) phenothiazine] (PPC), methdilazine hydrochloride [10-(1^{1}-methyl-3^{1}-pyrrolidinomethyl) phenothiazine] (MDH), diethazine hydrochloride [10-(2^{1}-diethylaminoethyl) phenothiazine] (DH) and chlorpromazine hydrochloride [2-Chloro-10-(3^{1}-dimethylamino-1^{1}- propyl) phenothiazine] (CPH) is described using diazotised anthranilic acid (DAA) in hydrochloric acid medium. The method is based on the interaction of phenothiazines with DAA to yield red coloured products. The method has been successfully applied to the assay of phenothiazines in pharmaceutical preparations.

A Hitachi UV/vis spectrophotometer model U-2001 with 1 cm matched quartz cells was used for all absorbance measurements. All chemicals used were of analytical reagent grade or pharmaceutical grade and high purity quartz processed water was used throughout. Aqueous solution of phenothiazines obtained from different firms were prepared by dissolving requisite amounts of pure phenothiazine drugs in distilled water either in cold or warm conditions. Insoluble propericiazine was dissolved by addition of a few drops of dilute sulphuric acid. Working solutions were prepared as required by dilution.

The DAA was prepared by dissolving about 25 mg of anthranilic acid in 1 ml concentrated sulphuric acid in a 25 ml standard flask. The solution was cooled in an ice bath and 0.5 ml of ice cold solution of sodium nitrite solution (0.2%) was added. After 5 min, 0.2 ml of 10% sulphamic acid solution was added and the contents were diluted upto the mark with distilled water, mixed well and stored in an ice-bath. The reagent should be used within 5 h.

Aliquots of the phenothiazine solution were transferred into a series of 10 ml standard flasks. To this was added 0.5 ml of DAA for PPC or 1 ml of DAA for MDH, DH or CPH and mixed well. The volume was made up to the mark with 10 M HCl. The absorbances were measured at 517 nm for DH, at 530 nm for CPH, at 514 nm for MDH and at 513 nm for PPC against the corresponding reagent blank. The calibration graph was drawn or regression equation calculated.

Twenty tablets containing MDH, DH or CPH were weighed and powdered. An accurately weighed portion of the powder equivalent to 25 mg of the drug was transferred into a 100 ml standard flask and the volume was made upto

TABLE 1: OPTICAL CHARACTERISTICS AND PRECISION DATA

Parameter	CPH	DH	MDH	PPC
λ_{max} (nm)	530	517	514	513
Beer's law range, mg/ml	1.5-26	2-27	1-28	2-26
Molar absorptivity 10^3 l/mol/ cm	7.48	7.43	7.15	8.79
Sandell's sensitivity ng / cm^2	42.6	40.1	41.4	41.5
Regression equation (A)**				
Slope (b)	0.0233	0.0240	0.0247	0.0249
Intercept (a)	-0.0020	-0.0182	-0.0225	-0.0125
Correlation coefficient (r)	0.9996	0.9972	0.9955	0.9978
Relative Standard Deviation *, %	1.02	1.05	0.86	0.59

**A=a+bc, where c is the concentration in μg/ml. * Average of five determinations.

the mark with distilled water, shaken well and filtered. Suitable aliquots of the solution were taken into a series of 10 ml volumetric flasks, DAA (0.5 ml for PPC or 1 ml for MDH, DH or CPH) was added, diluted with 10 M HCl and mixed well. The absorbances were recorded against the corresponding reagent blank and the amount of drug was deduced from the standard curve.

For the analysis of injection, requisite volume of the drug was transferred into a 100 ml standard flask and diluted up to the mark with distilled water. Suitable aliquots of the solution were taken into a series of 10 ml volumetric flasks and analysed by following the procedure for the analysis of bulk sample or tablets.

Phenothiazine undergoes one-electron reversible oxidation in acid medium in the presence of DAA and water to form a red coloured species, which is believed to be a radical cation[20]. The nature of the radical cation was confirmed by ion-exchange studies. The red coloured species was retained only by cation exchange resin but not on an anion-exchange resin indicating the cationic nature of the coloured species. Moreover, the λ_{max} values of the coloured products formed are in good agreement with those of the respective radical cations obtained by one electron reversible oxidation of phenothiazines[15-17]. As DAA is less soluble in hydrochloric acid, 1ml sulphuric acid was used for the preparation of DAA. Many solvents have been tested as diluents. Dilution with methanol, ethanol, water, 2-propanol or 1,4-dioxan gave an unstable colour and dilution with dilute sodium hydroxide, ammonia or potassium hydroxide solution resulted in immediate disappearance of the colour and the formation of a precipitate. Dilution with sulphuric acid, hydrochloric acid, phosphoric acid, glacial acetic acid and nitric acid were checked at different concentrations. Except for 8-9.5 M overall concentration of hydrochloric acid, all other acids gave non-reproducible results or lower colour intensity or less stable coloured species. It was observed that a volume of 0.5 ml of DAA for PPC or 1 ml of DAA for MDH, DH and CPH in a total volume of 10 ml was required for the development of constant and maximum colour intensity. The order of addition of reagents had no effect on absorbance. The coloured species was stable for 30-40 min.

The applicability of the method was also checked by analyzing synthetic mixtures of each drug (20 mg) prepared separately in the laboratory containing the following amounts of excipients (mg): talc (30-40), sucrose (30-40), starch (30-50), gelatin (40-50), lactose (30-40) and magnesium stearate (40-50). Suitable amount of each synthetic mixture was

TABLE 2: ANALYSIS OF PHENOTHIAZINES IN PHARMACEUTICAL PREPARATIONS

Phenothiazine	Label claim (mg or mg/ml)	Amount found Official method* (mg)	Proposed method** (mg)	Recovery* ± SD,% by proposed method
DH				
Tablet 1[a]	10	9.99	9.92	99.9±0.89
				F=1.22, t=1.11
CPH				
Tablet[b]	50	49.1	49.2	99.9±1.19
				F=1.06, t=1.21
Injection[c]	25	24.8	24.9	98.9±0.79
				F=1.25, t=1.02
MDH				
Tablet[d]	10	9.94	9.98	99.0±1.03
				F=1.23, t=1.08

*Average of five determinations. **Determination of DH, CPH and MDH in pharmaceutical preparations by the proposed method. [#]British Pharmacopoeia, HMSO, London, 1993. [a] Marketed by EGYT, Budapest. [b,c]Marketed by Intas, India. [d]Marketed by Glaxo Allenburys, India.

analysed following the procedure for the analysis of the tablets. The above excipients did not cause any interference in the determination of phenothiazine drugs. The percentage recovery of the drug was found to be in the range of 99.3 - 99.6 with RSD values less than 1.0 for 5 replicates.

Beer's law range, molar absorptivity, Sandell's sensitivity, slope, intercept and correlation coefficient obtained by linear least squares treatment of the results are presented in Table 1. The precision and accuracy of the proposed method was checked using 20 μg/ml of phenothiazines and the RSD values were found to be less than 1.2.

The methods were applied to the determination of phenothiazines in commercial pharmaceutical formulations such as tablets and injection. The results of analyses presented in Table 2 are in good agreement with those of official method[21-23]. The results were also compared statistically using the Student's t-test and the variance ratio F-test with those obtained by official method[21-23]. The Student's t- values at 95 % confidence level did not exceed the theoretical value indicating that there was no significant difference between the proposed and the reported method. It was also observed that the variance ratio F-values calculated for p=0.05 did not exceed the theoretical value indicating that there was no significant difference between the precision of the proposed and the official method. The results are tabulated in Table 2. The reagent utilized in the proposed method is cheaper, readily available and does not require elaborate experimental conditions such as heating, need of extraction or tedious sample preparation.

REFERENCES

1. Gupta, R.R., In; Phenothiazines and 1,4-phenothiazines-Chemical and Biochemical Aspects, Elsevier, Amsterdam, 1988, 357.
2. Basavaiah, K. and Krishnamurthy, G., **Chem. Anal.**, 1999, 44, 67.
3. El-Kerdawy, M.M. and Hassan, S.M., **Mikrochim. Acta.**, 1992, 108, 323.
4. Nagaraja, P. and Seetharamappa, J., **Indian J. Pharm. Sci.**, 1995, 57, 68.
5. El-Shabouri, S.R., Youssef, A.F., Mohamed, F.A. and Rageh, A.M.I, **J. Assn. Anal. Chem.**, 1986, 69, 821.
6. El-Kommos, M.E. and Emara, K.M., **Analyst**, 1988, 113, 1267.
7. Sastry, C.S.P., Tipirneni, A.S.R.P. and Suryanarayana, M.V., **Indian Drugs**, 1988, 26, 351.
8. Sastry, C.S.P., Prasad, T.A.S.R. and Suryanarayana, M.V., **Mikrochim. Acta**, 1990, 1, 107.
9. Bhongade, S.L. and Kasture, A.V., **Indian J. Pharm. Sci.**, 1993, 55, 151.
10. Basavaiah, K. and Krishnamurthy, G., **Talanta**, 1998, 46, 665.
11. Basavaiah, K., Krishnamurthy, G. and Swamy, J.M., **Eastern Pharmacist**, 1998, 41, 107.
12. Mohamed, H.A. and Khashaba, P.Y., **Bull. Fac. Pharm.**, 1994, 32, 13.
13. El-Shabouri, S.R., **Talanta**, 1985, 32, 999.
14. Basavaiah, K. and Krishnamurthy, G., **Anal. Lett.**, 1998, 31, 1037.
15. Sajjan, A.G., Seetharamappa, J. and Melwanki, M.B., **Indian J. Pharm. Sci.**, 2001, 63, 61.
16. Srinivasamurthy, K.C. and Seetharamappa, J., **Indian J. Pharm. Sci.**, 2000, 62, 273.
17. Rangaswamy, Yathirajan, H.S., Nagaraja, P., Jayarama and Mohan, B.M., **Indian J. Pharm. Sci.**, 1984, 46, 172.
18. Mangala, D.S. and Sastry, C.S.P., **Indian J. Pharm. Sci.**, 1984, 46, 154.
19. Sastry, C.S.P., Rekha, T.V. and Satyanarayan, A., **J. Inst. Chem.**, 1998, 70,12.
20. Dwivedi, P.C., Gurudath Rao, K., Bhat, S.N. and Rao, C.N.R., **Spectrochim. Acta**, 1975, 31, 129.
21. The British Pharmacopoeia, HMSO, London, 1993, 290.
22. The British Pharmacopoeia, HMSO, London, 1993, 552.
23. The British Pharmacopoeia, HMSO, London, 1993, 1079.