The results shown in the fig.1 indicated that in 0.1 N HCl, degradation starts rapidly from the begining. Addition of ascorbic acid does not prevent degradition in acidic medium. Unlike in 0.1 N HCl, in phosphate buffer of pH 7.4, degradition started slowly. This was prevented by the addtion of ascorbic acid (200 µg/ml) and the drug is stable for more than 24 h. Basing on these results phosphate buffer pH 7.4 containing 0.02% w/v of ascorbic acid was selected and dissolution studies were carried out.

The dissolution rate of pure bulk drug and marketed products were studied by the developed dissolution medium. The amount of rifampicin dissolved from pure drug, marketed product A and marketed product B are 88.8±0.11, 74.01±0.58 and 78.6±0.23%, respectively. The results indicated that the dissolution rates were within the USP[6] limits (not less than 75% (Q) of the labelled amount of $C_{43}H_{58}N_4O_{12}$ is dissolved in 45 min).

In conclusion, a dissolution medium of pH 7.4 phosphate buffer containing 0.02% w/v ascorbic acid was found to be most suitable for studying the release profiles of rifampicin from rifampicin sustained release formulation without significant degradition.

ACKNOWLEDGEMENTS

One of the authors, Mr. B. Sreenivasa Rao, thank the Council of Scientific and Industrial Research (CSIR), New Delhi, for the award of a Senior Reserch Fellowship. The authors greatly acknowledge M/s Aristo Pharmaceuticals Ltd, Madhya Pradesh for the supply of rifampicin as a gift sample.

REFERENCES

1. Gharbo, S.A., Cognion, M.M., and Williamson, M.J., **Drug Dev. Ind. Pharm.**, 1989, 15, 331.
2. Awata, N., Iwane, I., Makagwana, H. and Sugimoto, E., **Pharm. Res. Cent. Canebo** 1978, 38, 145.
3. Nicklasson, M., Brodin, A. and Nyar Vist, H., **Acta. Pharm. Suec.**, 1981, 18, 119.
4. Nicklasson, M. and Brodin, A., **Int. J. Pharm.**, 1984, 18, 149.
5. Florey, K., Eds., In; Analytical profiles of drug substances, Vol. 5 ., Academic Press, New York, 1976, 489.
6. The United States Pharmacopoeia, 23rd Edn., Pharmacopoeial Convection, Inc., Rockvillie. MD., USA, 1995, 1382.

Spectrophotometric Methods for the Determination of Chlorzoxazone in Tablets

C. S. P. SASTRY* AND R. CHINTALAPATI
Foods & Drugs Laboratories, School of Chemistry
Andhra University, Visakhapatnam-530 003

Accepted 18 April 2001
Revised 10 April 2001
Received 8 May 2000

Three simple and sensitive spectrophotometric methods (A-C) in visible region have been developed for the determination of chlorzoxazone. The reactions in all the three methods (A-C) are stoichiometric oxidations when the drug is treated with an excess of oxidant [nitrous acid (HNO_2), method A; N-bromosuccinimide (NBS), method B; chloramine T (CAT), method C] in acidic medium. The unreacted oxidant is then estimated colorimetrically by using an oxidisable dye [cresyl fast violet acetate CFVA), method A; celestine blue (CB), method B; Gallocyanine (GC), method C]. Beer's law limits for methods A, B and C are 0.4 – 4.0 µg/ml, 0.4 – 5.0 µg/ml and 2-12 µg/ml respectively. No interference was observed from tableting additives and the applicability of the methods was examined by analyzing tablets containing chlorzoxazone.

Chlorzoxazone (CZZ) is a central muscle relaxant employed in the treatment of painful musculo skeletal conditions. The existing analytical procedures reported for its determination are based mainly on either HPLC[1-7] or UV spectrophotometry[7-18], while other methods include GLC[16-18] and titrimetry[19,20]. The only reported visible

*For correspondence
9-36-4, Opp, N.C.C. Office, Andhra Bank Road, Pitapuram Colony, Visakhapatnam - 530 003

spectrophotometric procedure[21] for its determination so far is based on a diazocoupling reaction of the CZZ hydrolysis product (HCZZ) with BM reagent. Apart from being a too general one, this procedure suffers from the disadvantage of low absorption maximum (410 nm). As part of our continuing efforts to develop simple, sensitive and selective visible spectrophotometric analytical procedures for bulk drugs and their formulations, attention was focussed on CZZ molecule, keeping in view the relative lack at such methods for its estimation. It was soon realized that rather than the native molecule itself, its hydrolysis product (generated from CZZ by a simple hydrolysis procedure) is more amenable to the development of some analytical procedures based on visible spectrophotometry. This paper presents three procedures involving the oxidation of either HCZZ (method A) or CZZ (methods B and C).

All the three methods are indirect procedures, involving the addition of excess oxidant and determination of the unreacted oxidant by measuring the decrease in absorbance of dye as suggested by Sastry *et al.* (HNO_2/CFVA[22], NBS/CB[23] and CAT/GC[24]).

A Milton Roy Spectronic 1201 UV-Visible spectrophotometer and a Systronics 106 digital spectrophotometer with 1 cm matched quartz cells were used for all absorbance measurements. An Elico LI-120 digital pH meter was used for pH measurements. Aqueous solutions of $NaNO_2$ (0.2889×10^{-3} M), CFVA (1.556×10^{-4} M), HCl (5.0 M), NBS (0.5168×10^{-3} M), CB (0.2748×10^{-3} M), CAT (0.1100×10^{-3} M) and GC (0.2969×10^{-3} M) were prepared.

In method A, about 100 mg of CZZ was accurately weighed and treated with 10 ml of 1 M NaOH solution and the contents were mixed well and kept at room temperature for 15 min. Subsequently the solution was treated with 1M HCl for bringing down the pH to almost neutrality (6.5–7.0) and then diluted with distilled water to 100 ml to obtain a 1 mg/ml HCZZ stock solution.

In methods B and C, 1 mg/ml stock solution of CZZ was prepared by dissolving 100 mg of the pure drug initially in 0.02 N NaOH and making up to 100 ml with distilled water. Working standard solutions (20 µg/ml for methods A and B and 50 µg/ml for method C) were obtained by appropriate dilution of the stock solution with distilled water. Sample solutions for Tablets were prepared exactly in the same manner as given under the standard solutions with prior filtration if necessary before making up the final volume and analyzed as described for pure samples.

To aliquots of hydrolyzed CZZ (HCZZ) solution (0.5–5.0 ml, 20 µg/ml) taken into a series of 25 ml calibrated flasks, 1.25 ml of 5.0 M HCl and 2.0 ml of $NaNO_2$ (20 µg ml^{-1}) were added and the volume was made up to 15 ml with distilled water. After 3 min 10.0 ml of CFVA was added, mixed thoroughly and the absorbencies were measured after 5 min at 560 nm against distilled water. Blank experiment was carried out in a similar manner omitting the drug. The decrease in absorbance corresponding to consumed HNO_2, which in turn to drug concentration was obtained by subtracting the absorbance of the blank solution from that of the test solution. The amount of drug present was calculated from its calibration graph.

To aliquots of compound CZZ solution (0.5–5.0 µl, 20 mg/ml) taken into a series of 25 ml calibrated flasks, 1.25 ml of 5.0 M HCl, 2.0 ml of NBS were added and the volume was made up to 20.0 ml in each flask. After 15 min 5.0 ml of CB was added and mixed thoroughly. After 5 min the absorbencies were measured at 540 nm against distilled water. The blank (omitting drug) and the dye (omitting drug and oxidant) solutions were prepared in a similar manner and their absorbencies corresponding to consumed NBS and in turn to drug concentration, were obtained by subtracting the decrease in absorbance of test solution (dye-test) from that of the blank solution (dye-blank). The amount of drug was calculated from its calibration graph.

To the aliquots of CZZ solution (1.0–6.0 ml, 50 µg/ml) taken into a series of 25 ml calibrated flasks 1.25 ml of 5.0 M HCl and 2.0 ml of CAT (200 µg/ml) were added and the volume was made up to 20 ml with distilled water. After 25 min, 5.0 ml of GC was added and mixed thoroughly and the absorbencies were measured after 5 min at 540 nm against distilled water. Blanks were prepared appropriately. The decrease in absorbance corresponding to consumed CAT, which in turn corresponds to the drug quantity was obtained by subtracting the absorbance of the blank solution from that of the test solution. The calibration graph was drawn by plotting the decrease in the absorbance of the drug (GC), against the amount of drug. The amount of drug in the sample was computed from its calibration graph.

Beer's law limits, molar extinction coefficient, Sandell's sensitivity, correlation coefficient, slope and

TABLE 1: OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY

Parameters	Methods		
	A	B	C
Beer's law limits (mg/ml)	0.4 – 4.0	0.4 – 5.0	2.0 – 12.0
Molar absorptivity (1 $mole^{-1}cm^{-1}$)	1.61×10^4	2.03×10^4	5.33×10^3
Sandell's sensitivity (mg/cm^2/0.001 absorbance unit	0.008	0.008	0.031
Regression equation[1] (Y = a + bC)	1.12×10^{-1}	1.19×10^{-1}	3.15×10^{-2}
Slope (b)	-7.3×10^{-4}	2.35×10^{-4}	-3.3×10^{-4}
Intercept (a)	0.9999	0.9999	0.9998
Correlation coefficient (r)	0.67	0.68	0.35
% RSD[2]	0.70	0.71	0.38
% Range of error (95% confidence limits)			

[1]Y = a + bC, where C is concentration of analyte (mg/ml) and Y is absorbance unit.
[2] Calculated from six determinations.

TABLE 2: ANALYSIS OF TABLETS USING PROPOSED AND OFFICIAL METHODS

Name of the formulation	Label claim	Amount found[1] (mg)			Official	% Recovery[2]		
		Method A	Method B	Method C	Method[7]	Method A	Method B	Method C
Tablet I	500	499.1	499.5	499.8	499.5	99.7	99.8	99.6
Tablet II	250	249.3	249.2	249.5	249.7	99.6	99.2	99.8
Tablet III	250	249.5	249.7	249.7	249.1	99.9	99.7	99.4
Tablet IV	250	249.9	249.4	249.1	249.5	99.5	99.9	99.4

[1]Average of six determinations
[2]Recovery of amount added to the pharmaceutical formulation (average of three determinations)

intercept of regression analysis using least squares method, precision and accuracy of the analysis of six separate samples containing 3/4th of the amount of upper Beer's law limits in each method were summarized in Table 1. The results of analysis of tablets by the proposed and reported methods and recovery studies are presented in Table 2.

All the diluents, excipients and colouring matters that are usually present in the dosage forms did not interfere with the proposed methods. The results indicate that the proposed methods are sensitive, accurate, precise and reproducible and can be used as alternative ones to the existing methods.

REFERENCES

1. Frye, R.F. and Shiff, D.D., **J. Chromatogr. B. Biomed. Appl.**, 1996, 686, 296.
2. Sodhi, R.A., Chawla, J.L. and Sane, R.T., **Indian Drugs**, 1996, 33, 126.
3. Dai, R., Du, L. and Xi, W., **Shenyang Yaoke Dauxue Xuebao**, 1997, 14, 126.
4. Tanaka, E., **J. Pharm. Biomed. Anal.**, 1998, 16, 899.
5. Ravisankar, S., Vasudevan, M., Nanjan, M.J., Bijukurian and Suresh, B., **Indian Drugs**, 1997, 34, 663.
6. Ravisankar, S., Vasudevan, M., Gandhimathi, M. and Suresh, B., **Talanta**, 1998, 46, 1577.

7. US Pharmacopoeia XXIII, USP Convention, INC., Rockville, MD, 1993, 364.
8. Bhatia, M.S. and Dhaneswar, S.R., **Indian Drugs**, 1995, 32, 446.
9. Bhatia, M.S., Kakshedkar, S.G. and Chaturvedi, S.C., **Indian Drugs**, 1997, 34, 149.
10. Bhatia, M.S., Kakshedkar, S.G. and Chaturvedi, S.C., **Indian J. Pharm. Sci.**, 1997, 59, 45.
11. Ravisankar, S., Vasudevan, M., Duraiswamy, B., Suresh, B., and Abraham, B., **Indian Drugs**, 1997, 34, 450.
12. Kotak, L.N., Mahadik, K.R., More, H.N. and Kadam, S.S., **East. Pharm.**, 1998, 41, 121.
13. Mashru, R.C. and Banarjee, S.K., **East. Pharm.**, 1998, 41, 141.
14. Xiao-Ping, G and Ling, C., **Zhongguo Yixao Gonggye Zazhi**, 1999, 30, 30.
15. Ding, L., Yang, C., Wu, H., Zhan, W. and Guo, J., **Hebei Yike Daxue Xuebao**, 1999, 20, 13.
16. Avadhanulu, A.B., Pantulu, A.R.R. and Anjaneyulu, Y., **Indian Drugs**, 1994, 3, 201.
17. Ojanpera, I., Rasanen, I. and Vaori, E., **J. Anal. Toxicol**, 1991, 15, 204.
18. Christ, D.W., Noomano, P., Rosas, M. and Rhone, D., **J. Anal. toxicol**, 1988, 12, 84.
19. Schnekenburger, J. and Quade-Henkel, M., **Dtsch. Apoth-Zeg**, 1984, 124, 1167.
20. Beral, H., Gamentzy and Bucur, I., **Rev. Chim.**, 1965, 16, 322.
21. Sanghvi, N.M., Joshi, N.G. and Dubal, K.S., **Indian Drugs**, 1981, 18, 299.
22. Sastry, C.S.P., Srinivas, K.R., and Krishna Prasad, K.M.M., **Anal. Lett., 1996, 29, 1639.**
23. Sastry, C.S.P., Srinivas, K.R. and Krishna Prasad, K.M.M., **Mikrochimika Acta**, 1999, 122, 77.
24. Sastry, C.S.P., Srinivas, K.R. and Krishna Prasad, K.M.M., **Talanta**, 1996, 43, 1625.