gen. To each test tube 5 ml of the mobile phase was volumetrically added, stoppered and mixed well. A volume of 20 µl each of standard and sample solutions were injected into the stabilised HPLC system. Detection was initially kept at 250 nm and changed to 280 nm after elution of CPM peak. The retention times for PPH, CPM, DMH were found to be approximately 2, 6 and 12 min, respectively. The respective peak areas of standard and sample for each ingredient were used for quantification.

The assays were carried out by the proposed method and the results are tabulated in Table 1. Accuracy of the method was established by spiking the placebo (PL) samples with active ingredients at four levels and the results are tabulated in Table 2. Linearity and range of the method was carried out by injecting five mixed standard solution containing 60-300 µg/ml for PPH, 10-50 µg/ml for CPM and 25-125 µg/ml for DMH. The calibration curves were plotted using peak areas versus concentration. Linearity coefficients obtained are given in Table 3. Precision of the method was demonstrated by repeatability studies. This was done by five replicate analysis of the composite sample. Percentage RSD was calculated and given in Table 3. The system suitability studies were carried out to determine resolution factor, symmetry factor and precision of the instrument. Results are tabulated in Table 3. The advantage of this extraction procedure is to avoid column loading, column contamination or interference of many excipients present in the formulation. Ruggedness of the method was confirmed by analysis of different batches (n=5) of the product on different instruments (HP and Waters), by different analysts on different days. RSD was found to be 1.45, 1.49 and 1.77 for PPH, CPM and DMH, respectively.

REFERENCES

1. Indian Pharmacopoeia, Vol. I, The controller of publications. New Delhi, 1996, 176
2. British Pharmacopoeia, Vol. I, Her Majesty's Stationery Office, London, 1998, 325.
3. The United States Pharmacopoeia 24, United States Pharmacopoeial convention. Inc., Rockville, 2000, 392.
4. Indian Pharmacopoeia, Vol. I, The controller of publications. New Delhi, 1996, 237.
5. British Pharmacopoeia, Vol. I, Her Majesty's Stationery Office, London, 1998, 452.
6. The United States Pharmacopoeia 24, United States Pharmacopoeial convention. Inc., Rockville, 2000, 530.
7. British Pharmacopoeia, Vol. I, Her Majesty's Stationery Office, London, 1998, 1032.
8. The United States Pharmacopoeia 24, United States Pharmacopoeial convention. Inc., Rockville, 2000, 1319.
9. Das Gupta, V. and Heble, A.R., **J. Pharm. Sci.,** 1984, 73, 1553.
10. Heidmann, D.R., Groon, K.S. and Smith, J.M., **L.C.-G.C.,** 1987, 5, 422.
11. Momani, K.A., and Jalal, I.M., **Microchem. J.,** 1987, 36, 391.
12. Lau, OW. and Mok, C.S., **J. Chromatogr.,** 1995, 693, 45.
13. Fanq-GW, and Eickhoff- WM., **Int. J. Pharm.,** 1989, 53, 91.
14. Wilson, T.D., Jump, W.G., Neumann, W.C. and San Martin, T., **J. Chromatogr.,** 1993, 641, 241.
15. Gobbler, K.H., **Deutsche-Apotheker-Zeitung,** 1971, 3,1291.
16. Al-kayasi, H.N. and Salem, M.S., **Anal. Lett.,** 1986, 19, 915.
17. Saha, L.K. and Sharma, A.K. **Indian J. Pharm. Sci.,** 2000, 62, 205.

Spectrophotometric Determination of Cefoperazone Sodium Using Imidazole-Mercury (II) Reagent

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A rapid sensitive and simple spectrometric method is developed for the estimation of cefoperazone sodium. It is based on the reaction with imidazole-mercury (II) reagent in slightly acidic medium and heating at 83° for 20 min. The solution has an absorption maxima at 352 nm and obeyed Beer's law in the concentration range of 24-96 µg/ml. Result of analysis were validated statistically and by recovery studies.

***For correspondence**

Cefoperazone sodium is a third generation semi-synthetic cephalosporin, which is used in the treatment of mild to moderate infections caused by susceptible microorganisms[1]. This drug is official in USP[2]. Chemically, cefoperazone sodium is 7[{(4-ethyl-2,3-dioxo-1-pieperazinyl) carbonyl} amino] (4-hydroxy phenyl) acetyl (amino)-3-[(1-methyl-1H-tetrazol-5-yl) thio methyl]-8-oxo-5-thia-1-azabicyclo-oct-2-ene-2-carboxylic acid[3]. A few colorimetric methods for the estimation of this drug have been reported the proposed method is based on the reaction with imidazole mercury (II) reagent, and heating at 83° for 20 min. Beer's law is obeyed in the concentration range of 24-96 µg/ml.

A Shimadzu UV/Vis spectrophotometer with 1 cm quartz cell was used in the present investigation. The standard solution was prepared by the following method. Sixty milligrams of cefoperazone sodium was accurately weighed and dissolved in 100 ml of imidazole stock solution in a 100 ml standard volumetric flask. Three grams of imidazole (AR) was weighed and dissolved in 70 ml of distilled water. The pH was adjusted to 6.8±0.05 using 0.01 N hydrochloric acid and diluted to 100 ml with distilled water to produce imidazole stock solution. Imidazole-mercury (II) reagent was prepared by transferring 2.7 ml of 1% w/v mercuric chloride solution into a 100 ml volumetric flask and the volume was made upto 100 ml with imidazole stock solution. The addition should be drop wise with continuous shaking. This solution is stable for only 1 h.

The following procedure has been adopted for obtaining the standard curve. In a series of 25 ml volumetric flasks, aliquots of cefoperazone sodium solution (1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml), were placed. To these solutions, 3.2 ml of imidazole-mercury (II) reagent was added. The tubes were stoppered, allowed to stand in a water bath at 83° for 20 min. The solutions were then cooled in an ice-bath for a short time to bring them to room temperature. After centrifugation, the supernatant solution was transferred into a 25 ml volumetric flask quantitatively and volume made up with distilled water. The absorbances were measured at 352 nm against the reagent blank.

The above method was extended to cefoperazone sodium in formulation. An accurately weighed powder to cefoperazone sodium equivalent to 60 mg was dissolved in 100 ml imidazole stock solution to give a concentration of 0.6 mg/ml. In a 25 ml of volumetric flask, to 3 ml of the sample solution 3.2 ml of imidazole-mercury (II) reagent was added and stoppered. The solution was allowed to stand in a water bath at 83° for 20 min. The solution was then cooled in an ice-bath. After centrifugation the supernatant solution was transferred into a 25 ml volumetric flask quantitatively and the volume was made up with distilled water. The absorbance was measured at 352 nm against a reagent blank. The proposed method has been compared with the UV method.

Accurately weighed 100 mg of cefoperazone sodium was dissolved in 100 ml of distilled water in a volumetric flask. In a series of 25 ml volumetric flasks, aliquots of cefoperazone sodium solution (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml) were placed. The volume was made upto 25 ml with distilled water. The absorbence of the solutions were measured at 230 nm against a reagent blank.

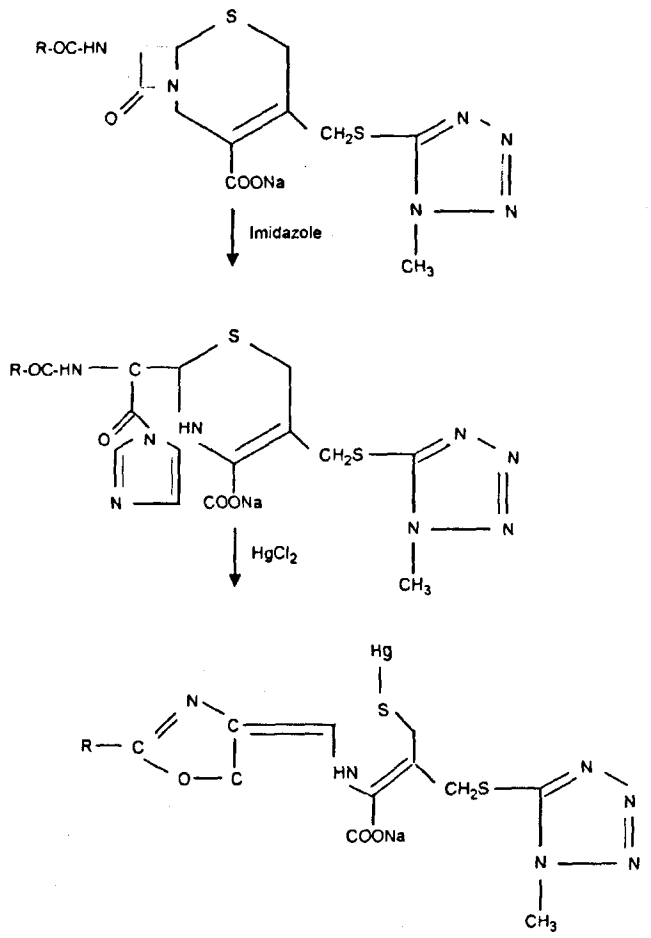
To ensure the accuracy and reproducibility of the results obtained, recovery experiments were carried out by adding a known amount of standard drug to previously analysed pharmaceutical preparations. The result are recorded in Table 1.

The reaction mechanism of cefoperazone sodium with imidazole-mercury (II) reagent is possibly similar to those for penicillins. It depends on the catalytic effect of the imidazole on β-lactam ring and the formation of an oxazolinne ring in the side chain. Binding of mercury (II) to the thiol group which is freed by the cleavage of dihydrothiazine ring stabilizes the product (fig. 1). This product shows maximum absorption at 352 nm. The optimum conditions were established by varying one parameter and keeping others fixed and observing the effect produced on the absorbance of the solution. The effect of reagent concentration, order of addition of reagents with respect to maximum sensitivity, effect of pH of solution and adherence to Beer's law have been

TABLE 1: ESTIMATION OF CEFOPERAZONE SODIUM IN PHARMACEUTICAL FORMULATIONS.

Pharmaceutical formulation	Label claim (mg/vial)	Amount found (mg)	% Recovery	Amount found by UV method (mg)	% Recovery
Injection-1	1000	990±0.5	104.5±0.5	990±0.5	104.5±0.5
Injection-2	1000	990±0.5	100.0±0.5	990±0.6	100.0±0.6

* Average of six replicate readings.



studied through controlled experiments and optimum conditions were incorporated in the procedure. The optical characteristics and figures of merit are given in Table 2. The precision was found by analysing six replicate samples contaning known amount of drug and the result are summarised in Table 2. As a check on accuracy of the method, recovery experiments were performed and percent recovery values were also tabulated (Table 1). Comparison of the data obtained in the proposed and UV method of analysis of cefoperazone sodium showed good agreement. This

TABLE 2: OPTICAL CHARACTERISTICS OF THE PROPOSED METHOD.

λmax (nm)	352
Beer's Law range (μg/ml)	24-96
Molar absorptivity (ϵ)	$3.09x10^3$
Sandell's sensitivity	5.39
(μg/cm² for 0.001 abs. unit)	
Slope (b) Intercept (a)	$4.6x10^{-4}$ $2x10^{-3}$
Standard deviation of standard cefoperazone sodium determination	0.165
Variance of standard cefoperazone determination	0.027

systematic study revealed that the proposed method for the determination of cefoperazone sodium is simple, sensitive, with good precision and accuracy. This method can be used for the routine determination of cefoperazone sodium.

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REFERENCES

1. Reynolds, J.E.F. Eds., In; Martindale, Extra Pharmacopiea, 29th Edn., The pharmaceutical press, London, 1989, 142.
2. The United States Pharmacopoeia, XXIII, U.S. Pharmacopoeial convention, Rockville, M.D. 1985, 284.
3. Alwarthan, A. A. and Metwally, A., **Anal. Letters,** 1993, 12, 2619.
4. Walash, M., **Anal. Letters,** 1994, 27, 2513.
5. Issopoulous, P.B., **Acta Pharma.,** 1991, 61, 205.
6. Bucourt, C., Bormann, D., Heymes, R, and Perronnet, M., **J. Chemotherapy,** 1980, 6, A, 63.
7. Fesengun, I. and Ulas, K., **Talanta,** 1986, 33, 363.