LIST OF PUBLICATIONS

1. "A rapid and precise colorimetric method for estimation of 5-Fluorouracil in bulk drug and dosage forms", Banerjee S.K. and Sumathi A. in Indian Journal of Pharmaceutical Sciences, 55(1), 42-44, (1993).
2. "A rapid and precise colorimetric method for estimation of 5-Fluorouracil in dosage forms", Banerjee S.K. and Sumathi V. Rao in Indian Drugs, 31(3), 90-92, (1994).
3. "A simple and rapid colorimetric method for estimation of 5-Fluorouracil in dosage forms", Banerjee S.K. and Sumathi V. Rao recently accepted for publication in Indian Drugs.

within the range 0.4-11.0 μg/ml. The method is statistically validated and results compared favourably with those obtained by the reported method.

5-Fluorouracil is a potent anticancer agent used in treatment of various types of cancers particularly those of GIT, breast, liver and pancreas[1]. 5-FU has been estimated by titrimetry[2], spectorphotometry[3], fluorimetry[4], HPLC[5].

Uracils, thiouracils and propyl thiouracils react with divalent metal ions like copper and cobalt to give coloured complexes in non-aqueous media in an alkaline pH[6]. This fact is used as the basis for developing a simple and sensitive colorimetric procedure for estimating 5-FU from bulk and also from dosage forms. Here the drug is reacted with copper acetate in 3:2 chloroform : methanol medium and diethylamine is added to make the pH alkaline. The intensity of coloured complex is measured at 350 nm.

5-FU U.S.P. was obtained as a gift sample from Biochem Industries Ltd., Bombay. The percentage purity was found to be 100.4%[2].

All the other chemicals used were of G.R. grade of E.merck.

A 500 ug/ml copper acetate solution was prepared in 3:2 chloroform: methanol. A 5% solution of diethylamine in 3:2 chloroform: methanol was prepared. All spectral measurements were made on Systronic UV-108 spectro-photometer.

A stock solution of 800 μg/ml of 5-FU was prepared in 3:2 CHCl3: MeoH. An aliquot of the drug solution was transferred into 10 ml volumetric flask. 1 ml Of 500 μg/ml metal solution and 1 ml of DEA solution were added and the volume made upto 10 ml with 3:2 CHCl3: MeoH. The absorbance was measured at 350 nm against the reagent blank similarly prepared by taking 1 ml of metal soluiton and 1 ml of DEA solution.

5-FU was analysed in commercially available injection. A volume of the injection equivlent to 100 mgs of the drug was diluted in sufficient water to produce 100 ml. The pH was adjusted to 4 with glacial acetic acid. A 2ml aliquot was pipetted out and extracted successively with five quantities each of 5 ml of ethyl acetate. The combined extracts were evaporated under vacuum and the residue was dissovled in 25 ml of 3:2 CHCl3 : MeoH and the colour was developed according to the proposed method.

The absorption spectrum of the yellowish green coloured complex of 5-FU-copper acetate shows an absorption maxima at 350 nm.Diethylamine was added to make the pH alkaline and the optimum concentration required for the estimation was found to be 0.5%. The optimum metal concentration was found to be 500 μg/ml.

The reaction was carried out at room temperature and colour obtained was stable for several hours. The intensity of colour was not affected by higher temperature.

Calibration curve was obtained within the range of 0.4-11.0 μg/ml. The line of

regression for the calibration curve was drawn and the regression coefficent was determined from the equation Y = a + bx where a = 0.085, b =0.031 and the regression coefficient r =0.992. Molar absorptivity was found to be 5.27×10^3.

The result of the estimation of 5-FU from the injectable preparation are presented in Table (1). The percentage recoveries have been compared with the I.P. method.

Thus 5-FU forms a stable coloured complex with copper acetate. The molar absorptivity of the complex is 5.27×10^3 which makes the method very sensitive and precise. On the basis of the statistical analysis of the data for the estimation of the drug from marketed product (Table-1) it may be concluded that the method is reproducible (S.D =1.025) and comparable with the pharmacoepoeial method.

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Table -1 Estimation of 5-FU from Injection

Reported Method		Proposed Method	
Concentration in µg/ml	% Recovery	Concentration in µg/ml	% Recovery
10.08	100.80	9.85	98.50
10.00	100.00	10.02	100.02
10.10	101.00	10.05	100.52
10.05	100.50	10.13	101.31
Standard deviation	0.376	Standard deviation	1.025

REFERNCES

1. Martindale, the Extra Pharmacopoeia, 29th Edition The Pharmaceutical Press London, 1989, 628.
2. Indian Pharmacopoeia, III Ed. Vol I, Controller of publications, New Delhi, p. 1985, 217.
3. K. Florey, Analytical Profiles of Drug Substances, 1988, Vol 18, p 599.
4. Bueyuekimikin N, Sci Pharm, 1983, 51 (1), p 26
5. Rustum Y.M. Anal Biochem, 1978, 90 (1) pa, 289.
6. Snell F.D and Snell C.T Colorimetric Methods of Analysis, 3rd edition, 1964, Vol. IV, p 99.

A RAPID AND PRECISE COLORIMETRIC METHOD FOR ESTIMATION OF 5-FLUOROURACIL IN DOSAGE FORMS

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ABSTRACT

The proposed colorimetric method for estimation of 5- fluorouracil (5FU) is based on the reaction between the drug and cobalt acetate in dry 3:2 chloroform; methanol ($CHCl_3$:MeOH) mixture in presence of isopropylamine. The purple coloured complex shows a λmax at 570nm and calibration curve was obtained within the range 20- 200μg/ml. The method is statistically validated and results compared favourably with those obtained by the reported method.

5-Fluorouracil, a potent anticancer agent has been estimated by titrimetry[1], spectrophotometry[1], colorimetry[2,3], fluorimetry[4] and HPLC[5].

The compounds having -CO NH CO-, -CO NH CS- groups have been reported to react with divalent metal ions like copper, and cobalt to give coloured complexes in non aqueous alkaline medium[6]. This fact is used as the basis for developing a simple and sensitive colorimetric procedure for estimating 5FU from dosage forms. The principle of reported method[2] involves precipitation of drug as hydrazone in acidic medium and subsequently dissolving the precipitate to obtain a violet coloured solution in alkaline medium. In the proposed method the procedure has been much simplified because it involves simple metal ligand complexation in non aqueous medium which can be subjected to direct colorimetric measurement.

The percentage purity of 5-FU was found to be 100.4%[7]. All the other chemicals used were of G.R. grade of E.Merck. Anhydrous solvents were used.

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All spectral measurements were made on Systronics UV-Visible Spectrophotometer 108.

A stock solution of 1mg/ml of 5-FU was prepared in 3:2 chloroform; MeOH. A 0.3% w/v solution of cobalt acetate and 20% v/v solution of isopropylamine were also prepared in 3:2 $CHCl_3$: MeOH. To an aliquot of the drug solution, 1ml each of 0.3% w/v solution of metal and 20% v/v solution of isopropylamine solution were added and volume made upto 5 ml with 3:2 $CHCl_3$: MeOH. The absorption spectrum of the purple coloured complex was scanned against the reagent blank. λmax was found to be 570 nm.

5-FU was estimated from commercially available injection. A volume of injection equivalent to 100 mg of drug was diluted with sufficient water to produce 100 ml. A 2 ml aliquot was pipetted out, it's pH was adjusted to 7 with glacial acetic acid and then it was extracted thrice with 10 ml volumes of ethyl acetate- isopropanol (7:3). The organic extracts were mixed together and then passed through anhydrous sodium sulphate and later evaporated to dryness on a water bath. Residue was dissolved in 10ml of 3:2 $CHCl_3$ -MeOH and colour was developed according to the proposed method.

Table I
Estimation of 5FU from Injection

	Reported Method			Proposed Method	
Conc.in liquot g/ml	Conc. Recovered µg/ml	% Recovery	Conc. in aliquot µg/ml	Conc. recovered µg/ml	% Recovery
10	9.88	98.8	100	99.5	99.5
10	9.85	98.5	100	99.1	99.1
10	10.00	100.0	100	100.0	100.0
10	9.88	98.8	100	98.8	98.8
10	9.91	99.1	100	99.5	99.5
		s.d. = 0.576			s.d. = 0.571

TABLE II
Estimation of 5FU from cream

	Reported Method			Proposed Method	
Conc. in aliquot µg/ml	Conc. recovered µg/ml	% Recovery	Conc. in aliquot µg/ml	Conc. recovered µg/ml	% Recovery
10	9.701	97.01	100	100.04	100.04
10	9.79	97.9	100	99.1	99.1
10	9.88	98.8	100	98.2	98.2
10	9.88	98.8	100	96.5	96.5
10	9.97	99.7	100	97.3	97.3
		s.d. = 0.9146			s.d. = 1.24

5-FU was also estimated from a 5% cream prepared in PEG base (U.S.P.). A portion of cream equivalent to 500 mg of 5FU was accurately weighed and the drug was extracted from the cream base as per U.S.P.[7]. Further operations were carried out as described in the injection starting from the words "A 2 ml aliquot.."

The absorption spectrum of the purple coloured complex of 5FU- cobalt acetate shows an absorption maxima at 570 nm. Isopropylamine was added to make the pH alkaline and optimum concentration required for the estimation was found to be 1ml of 20%v/v. The optimum metal concentration was found to be 1ml of 0.3% w/v. The reaction was carried out at room temperature and colour obtained was stable for several hours. The intensity of colour was not affected by temperature, but presence of moisture reduced its intensity.

Calibration curve was obtained within the range of 20- 200μg/ml. The line of regression for the calibration curve was drawn and regression coefficient was determined from the equation Y=a+bx, were a = 0.016 and b = 0.0021 and correlation coefficient r = 0.99.

The results of the estimation of 5-FU from the injectable preparation is presented in Table-1. The percentage recoveries have been compared with the I.P. method[1]. The result of the estimation of 5FU from a 5% cream is presented in Table-2. The percentage recoveries have been compared with the U.S.P. method[7].

Thus 5-FU forms a stable coloured complex with cobalt acetate. The molar absorptivity of the complex is 0.6 x 10^3 $1mol^{-1}$ which makes the method very sensitive and precise. On the basis of analysis of the data for the estimation of the drug from the formulations (Table 1&2) it may be concluded that the method is reproducible and comparable with the pharmacoepial method.

REFERENCES

1. Pharmacopoeia of India, III Ed. vol. 1, Controller of Publications, New Delhi, (1985), p. 217.
2. Florey K., **Analytical Profiles of Drug Substances**, vol. 18, (1988), p. 599-602.
3. Banerjee S.K. and Sumathi A., **Ind. J. Pharm. Sci.**, 55(i), (1993), p. 42-44.
4. Bueyuekimikin N, **Sci. Pharm.**, 51(1) (1993), p. 26-28.
5. Rustum Y.N., **Anal Biochem.**, 90(1), 1978, p. 289-291.
6. Snell F.D. and Snell C.T., **Colorimetric Methods of Analysis**, Third edition, vol. IV, 1965, p. 293-294.
7. United States Pharmacoepia, vol XXII, United States Pharmacoepial Convention, INC, Rockville, U.S.A., 1990, p. 581- 582 and 1963.