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Short Communication

Comparison of accelerated stability data for 5-fluorouracil obtained by a newly developed colorimetric method and by the standard spectrophotometric method

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Introduction

5-Fluorouracil (5 FU) is a potent anticancer agent used in the treatment of various types of cancers particularly those of the gastro-intestinal tract, breast, liver and pancreas [1]. It is available as an injection containing 500 mg 10 ml^{-1} stabilized at pH 9 [2]. The shelf-life of this product is 3 years [3]. The analytical method that has been used for stability testing is UV spectrophotometry [4].

Recently the authors reported a rapid and sensitive colorimetric method for the estimation of 5 FU in bulk drugs as well as in the parenteral formulation [5]. In this method, 5 FU was complexed with copper acetate in chloroform–methanol (3:2, v/v) and made alkaline with 0.5% v/v diethylamine. The absorbance of the yellowish green complex was measured at 350 nm. The molar absorptivity of the coloured complex was 7.27×10^3 and the calibration curve was linear in the range of 0.4–11.0 $\mu g\ ml^{-1}$.

The aim of the present investigation was to evaluate whether or not the method was stability indicating.

Materials and Methods

Materials

5 FU USP (gift from Biochem Industries, India) sodium hydroxide, chloroform, methanol, diethylamine, anhydrous sodium sulphate and copper acetate (Qualigens, India) were used in the present investigation. All chemicals were of AR grade and were used without further purification. The purity of 5 FU was found to be 100.4% [2].

Assay of 5 FU injection by colorimetric method

The assay was carried out as described previously [5]. In brief, an aliquot of the drug was acidified with glacial acetic acid at pH 4 and extracted with ethyl acetate. The extract was evaporated under vacuum and the residue was dissolved in chloroform–methanol (3:2, v/v). The colour was developed by complexing the drug with 500 $\mu g\ ml^{-1}$ of copper acetate in chloroform–methanol (3:2, v/v) in the presence of 0.5% v/v diethylamine.

Assay of 5 FU injection by UV spectrophotometry

The assay was carried out by the method of the Indian Pharmacopoeia [2]. All spectral measurements were made on a Hitachi 2000 UV–vis spectrophotometer.

Stability testing

A solution of 5 FU was prepared in water for injections and the pH was adjusted to pH 11 with sodium hydroxide solution Ind. P. The final concentration of 5 FU was 500 mg 10 ml^{-1}. The solution was aseptically filtered and

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Table 1
Data for the stability of 5 FU at pH 11

Time (days)	% Drug remaining undegraded (±SD)					
	Room temp.		45°C		60°C	
	UV method λ_{max} 266 nm	Colorimetric method λ_{max} 350 nm	UV method λ_{max} 266 nm	Colorimetric method λ_{max} 350 nm	UV method λ_{max} 266 nm	Colorimetric method λ_{max} 350 nm
0	100	100	100	100	100	100
	(-)	(-)	(-)	(-)	(-)	(-)
1	100	100	97.72	98.85	97.72	96.21
	(-)	(-)	(1.96)	(1.95)	(1.95)	(1.15)
2	100	99.85	95.49	95.49	87.09	93.76
	(-)	(2.49)	(1.90)	(2.39)	(2.17)	(1.87)
3	97.72	97.72	91.20	83.16	52.48	59.88
	(1.95)	(2.93)	(2.28)	(2.33)	(1.57)	(1.29)
5	95.49	91.20	83.18	75.85	39.81	47.43
	(3.34)	(3.19)	(3.39)	(2.65)	(3.67)	(2.78)
7	91.20	87.09	72.44	72.44	25.12	36.31
	(2.92)	(3.48)	(3.62)	(3.07)	(3.89)	(2.54)
15	79.43	72.44	45.71	47.86	15.85	23.18
	(3.97)	(3.63)	(2.51)	(1.67)	(3.71)	(3.08)
30	63.09	60.11	25.11	22.38	6.92	5.49
	(3.34)	(3.01)	(1.75)	(1.75)	(3.65)	(3.49)

Table 2
Stability rate constants for 5 FU

Temp. (°C)	Rate constants	
	UV method	Colorimetric method
25	0.273	0.282
45	0.715	0.716
60	1.305	1.215

10 ml was filled into glass ampoules under laminar flow and stored at three different temperatures: room temperature (25°C), 45°C and 60°C in incubators. At specific time intervals, samples were withdrawn and assayed by the colorimetric method as well as by the spectrophotometric method. Each analysis was carried out in triplicate. Data for the concentration of the drug remaining at each sampling time point together with the standard deviation are presented in Table 1. A plot of log % drug remaining against time was a straight line indicating first-order degradation of 5 FU. The stability rate constants obtained by the proposed method and UV spectrophotometric methods are reported in Table 2.

Results and Discussion

5-Fluororuacil undergoes hydrolysis in alkaline solution, with the probable formation of barbituric acid, which rapidly degrades to urea, fluoride and an aldehyde [3].

The results of the stability study of 5 FU at pH 11 are summarized in Table 1. At constant pH and temperature, the overall disappearance of 5 FU displayed first-order kinetics according to the equation:

$$k = \frac{2.303}{t} \log \frac{a}{a - x}.$$

The observed first-order rate constants (k) for degradation were calculated from the slopes of the regression lines obtained by plotting the logarithm of drug remaining against time (days) (Fig. 1). The percentage of the drug remaining at each sampling time interval determined by the colorimetric method and by the spectrophotometric method were compared statistically using Student's t-test. From Table 1 and Fig. 1 it may be inferred that there is no statistically significant difference between the degradation patterns observed by both methods. The differences in stability rate constants (Table 2) obtained by the two methods are not statistically significant.

Experimentally, the reaction rate constant was observed to have an exponential dependence on temperature. This is given by the equation:

$$\log k = \log A - E_a/2.303\, RT,$$

where k = the reaction rate constant, E_a = the activation energy and T = the absolute temperature in Kelvin.

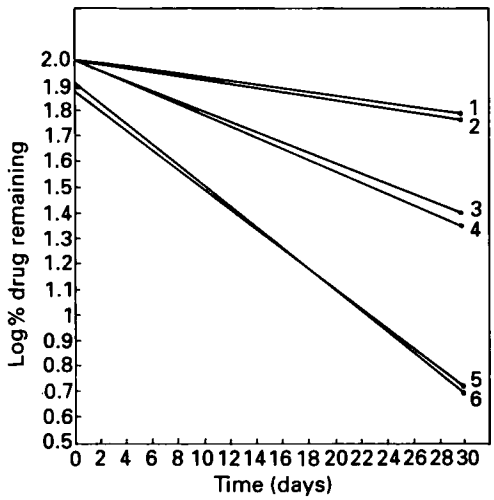

Figure 1
Log % of drug (5 FU) remaining as a function of time. 1, Regression line for stability data at room temperature by the UV method; 2, regression line for stability data at room temperature by the colorimetric method; 3, regression line for stability data at 45°C by the UV method; 4, regression line for stability data at 45°C by the colorimetric method; 5, regression line for stability data at 60°C by the UV method; and 6, regression line for stability data at 60°C by the colorimetric method.

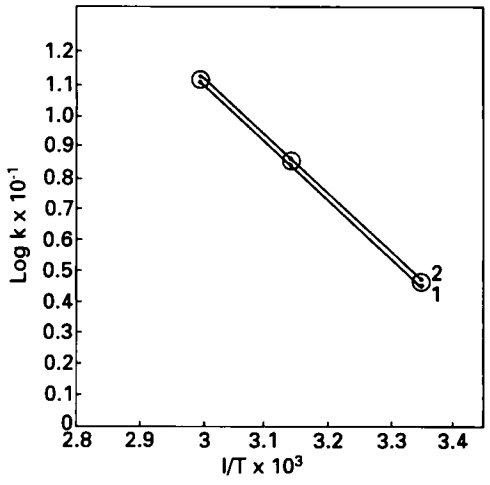

Figure 2
Arrhenius plot for degradation of 5 FU. 1, Line of regression for UV method; and 2, line of regression for colorimetric method.

The activation energy values were determined from the Arrhenius plot of log k against $1/T$. From the slope of the straight lines the activation energies were calculated (Fig. 2). The E_a values were 25.16 and 24.98 kJ mol^{-1} for the UV method and the colorimetric method, respectively.

Conclusions

The colorimetric method [5] is rapid, sensitive and selective. Hence, it was thought necessary to further validate the method. The results of the experiments indicate that the method is stability indicating.

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