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UTILITY OF DERIVATIVE SPECTROPHOTOMETRY FOR THE DETERMINATION OF FLUOROURACIL IN COMBINATION WITH ITS ALKALI - INDUCED DEGRADATION PRODUCTS

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ABSTRACT

A simple, rapid and direct spectrophotometric method for the determination of fluorouracil in presence of its alkali-induced degradation products is presented. The method is based on measuring the absolute value of ^1D and ^2D of the absorption curve at the chosen wavelengths. The validity of the method was confirmed using laboratory prepared mixtures of the intact drug with its alkali-induced degradation products. The method has been also applied for the assay of some pharmaceutical preparations. Moreover, alkaline degradations has been studied and a derivative spectrophotometric method for quantitative determination of these degradations, even in presence of the intact molecule is presented.

INTRODUCTION

Several methods have been reported for the determination of fluorouracil. These included pharmacological[1], potentiometric[2], nuclear magnetic resonance[3], gas chromatography[4], high pressure liquid chromatography[5] and spectrophotometric ones[6-10]. In the present paper, we report a simple and direct spectrophotometric method for fluorouracil in the presence its alkali-induced degradation products.

EXPERIMENTAL

Apparatus: A Shimadzu recording spectrophotometer UV 260; Digisense pH meter, Model NO. 5986.

Materials: Fluorouracil: Roche Co., Switzerland, acetate buffer, [11] (pH 4.7), sodium hydroxide, hydrochloric acid: Nasr Co.; Egypt, and fluorouracil ampoules; Roche Co.; Switzerland labeled to contain 250 mg fluorouracil per ampoule.

Standard Solutions :

Stock solution A : 100 mg % of reference fluorouracil in acetate buffer solution (pH 4.7) was prepared.

Working solutions A : serial dilutions within the concentration range 1-6 mg % were prepared by pipetting separately 0.5-3.0 ml aliquots of stock solution A into 50 ml volumetric flasks and completing to volume with acetate buffer solution (pH 4.7).

Sample Preparations :

1- Fluorouracil alkali-induced degradation products :

Fluorouracil (1.0 g) was accurately weighed and transferred into a 200 ml conical flask 50 ml of 2 N sodium hydroxide solution was added and the solution was heated under reflux for three hours, then cooled. The solution, was transferred into a 100 ml volumetric flask and completed to volume with 2N sodium hydroxide solution. Stock solution B was prepared by transferring 10 ml aliquot of the above prepared solution

into a 100 ml volumetric flask, neutralizing with 2N hydrochloric acid and diluting to volume with acetate buffer solution (pH 4.7). Working solutions B were prepared by pipetting separately 0.5-3.0 ml aliquots of stock solution B into 50 ml volumetric flasks and completing to volume with acetate buffer solution (pH 4.7).

2- Laboratory prepared mixtures of authentic Fluorouracil and alkali-induced degradation products :

a- Six serial dilutions were prepared by pipetting 0.5-3.0 ml, aliquots of stock solution A then 3 ml of stock solution B, into 50 ml volumetric flasks and completing to volume with acetate buffer solution (pH 4.7).

b- Six serial dilutions were prepared by pipetting, 0.5-3.0 ml aliquots of stock solution B accompanied by 3 ml of stock solution A, into 50 ml volumetric flasks and completing to volume with acetate buffer solution (pH 4.7).

3- Fluorouracil ampoules :

Two ml aliquot of fluorouracil ampoule (equivalent to 100 mg fluorouracil) was transferred into a 100 ml volumetric flask and completed to volume with acetate buffer solution (pH 4.7). 0.5-3.0 ml aliquots of the above solution were transferred into 50 ml volumetric flasks and completed to volume with acetate buffer solution (pH 4.7).

RESULTS AND DISCUSSION

Figure 1a shows that the fluorouracil absorption maximum at 266 nm is overlapped by the alkaline-induced degradation products absorption spectrum. As it appears from Figures 1b & 1e fluorouracil has maxima at the wavelengths $^1D\ 1 =$ 279 nm and $^2D\ 1 =$ 266 nm, at which Fluorouracil alkali-induced degradation products do not interfere. Thus at these wavelengths fluorouracil can be determined in presence of its alkali-induced degradation products. Under the described experimental conditions, the graphs obtained by plotting ^1D and ^2D values versus concentration, in the range stated in Table 1, show linear relation.

For comparison, the A_{max} method[12] was applied for the determination of the intact drug. Statistical analysis of the results (Table 2) show that all the suggested procedures are as precise and accurate as the A_{max} method[12].

In order to prove the validity and the applicability of the proposed method, six different dilutions of a mixture of Fluorouracil and its alkali-induced degradation products were analyzed for the intact fluorouracil. For comparison, the modified Vierodt's method[13] was also applied and statistical analysis of the results obtained are shown in Table 3. The suggested first and second derivative spectrophotometric measurements are as precise and accurate as the modified Vierordt's method.

Fluorouracil ampoules were assayed for their fluorouracil content using the proposed methods and compared with the modified Vierordt's method (Table 3). The suggested procedures are as precise and accurate as the modified Vierordts's method.

Moreover, accuracy of the suggested method was further checked by applying standard addition technique. The added amounts are 0-5 times that found in the ampoules. The results obtained (Table 3) revealed high degree of accuracy.

Stability and Degradation :

Fluorouracil is stable in solutions which are not strongly basic (pH less than 9). When subjected to strongly basic conditions, fluorouracil is hydrolysed to urea, fluoride, and aldehyde. Some of the urea formed on hydrolysis reacts further giving ammonia and carbon dioxide[12].

Figure 1a reveals that the alkali-induced degradation products have maximum absorption at 282 nm which may be attributed to the presence of carbonyl group. However, that maximum is over-

Table (1) : Optimum parameters for calibration curves construction

Standard Solution of	Measure-ments	Conc. range mg%	λ nm	Regression analysis			C.V. %
				B	K	R	
Fluorouracil	^1D	1 - 6	279	0.0257	17.6024	0.9999	0.29
	^2D	1 - 6	266	0.1034	90.4531	0.9979	0.24
Fluorouracil alkali-induced degradation products	^1D	1- 6	262	0.0314	13.3140	0.9994	0.28
	^2D	1- 6	247	0.0865	79.9815	0.9998	0.33

Table (2): Statistical analysis of the results obtained for assay of Fluorouracil using first and second derivative spectrophotometric measurements (Zero-Crossing technique of measurements) compared with A_{max} method.

	A_{max} method	^1D (λ=279nm)	^2D (λ=266nm)
	Recovery %	Recovery %	Recovery %
X	100.1	100.2	100.4
± S.D.	0.19	0.29	0.24
N	6	6	6
V	0.04	0.08	0.06
t		0.72 (1.812)	1.57 (1.812)
F		2.00 (4.28)	1.50 (4.28)

Table (3): Statistical analysis of the results obtained for the assay of fluorouracil using first and second derivative spectrophotometric measurements (Zero-Crossing technique of measurements) compared with modified Vierordt's method.

		Modified Vierordt's method	^1D (λ=279nm)	^2D (λ=266nm)
		Recovery %	Recovery %	Recovery %
Laboratory prepared mixture of Fluorouracil and its alkali-induced degradation products	X ± S.D. N V t F	100.1 0.38 6 0.14	100.2 0.57 6 0.32 0.38 (1.812) 2.29 (4.28)	100.2 0.32 6 0.10 0.49 (1.812) 1.40 (4.28)
Fluorouracil ampoules	X ± S.D. N V t F	100.3 0.25 6 0.06	100.3 0.27 6 0.07 0.00 (1.812) 1.17 (4.28)	100.3 0.49 6 0.24 0.00 (1.812) 4.00 (4.28)
Fluorouracil ampoules applying standard addition technique	X ± S.D. N V t F		100.2 0.42	100.0 0.68

lapped by the intact fluorouracil spectrum. Meanwhile, in the corresponding ^1D and ^2D curves, the degradation maxima are located at zero-crossing point of fluorouracil (Figures 1b & 1c). At these maxima, the degradation can be detected and quantitatively determined without fluorouracil interference. Alkaline degradation of fluorouracil was here investigated as a function of sodium hydroxide concentration and time of reflux.

Figure 2 reveals that there is a regular decrease in the intact drug concentration, parallel to a regular increase in the degradation concentration, and an increase in sodium hydroxide concentration. Maximum degradation concentration, accompanied with nil concentration of intact drug, is achieved using 2N sodium hydroxide under the described experimental conditions.

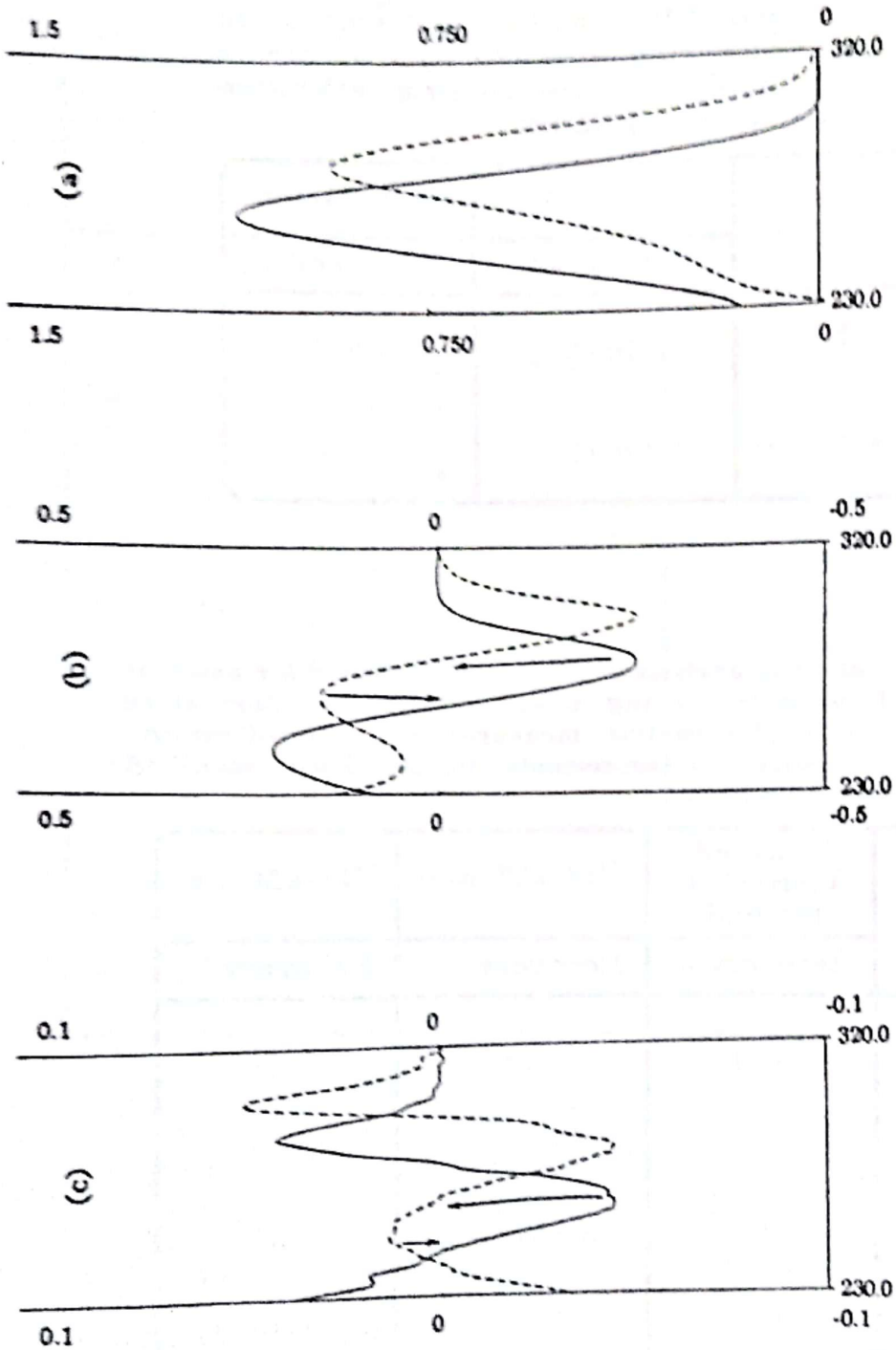
Figure 3 shows that there is a regular increase in the degradation concentration, parallel to a regular decrease in the intact drug concentration, when increasing time of reflux. Maximum degradation accompanied with nil concentration of intact drug, is achieved after 2.5 hours refluxing. The absorption is constant for another hour of refluxing, then starts to decrease indicating further degradation

Table (4): Determination of fluorouracil alkali-induced degradation products using first and second derivative spectrophotometric measurements applying Zero-Crossing technique of measurements.

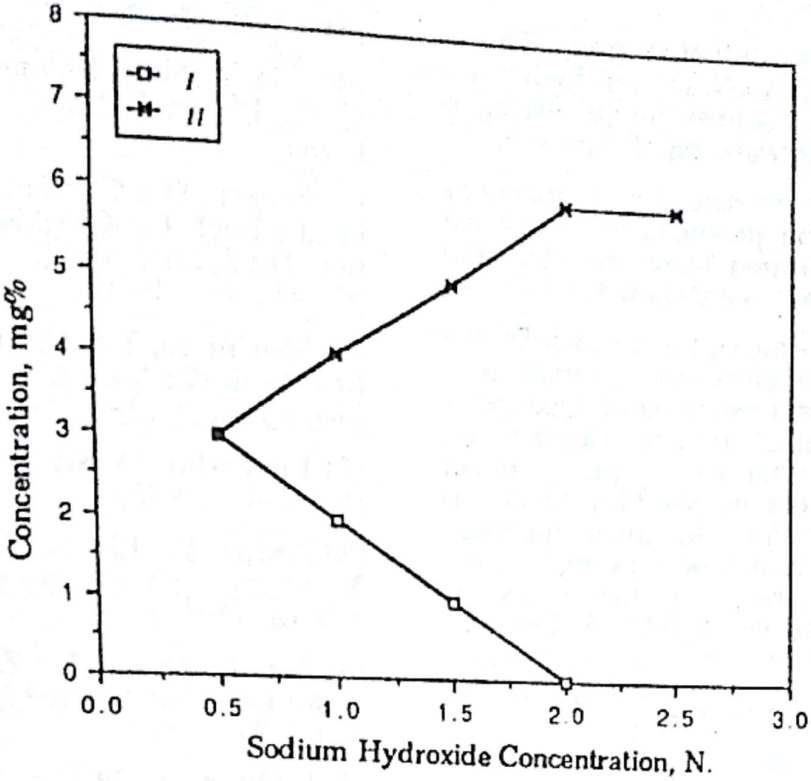
	^1D (λ=262nm)	^2D (λ=247nm)
	Recovery %	Recovery %
X	100.5	100.2
± S.D.	0.28	0.33

Table (5): Statistical analysis of the results obtained for assay of fluorouracil using first and second derivative spectrophotometric measurements (Zero-Crossing technique of measurements) compared with modified Vierordt's method.

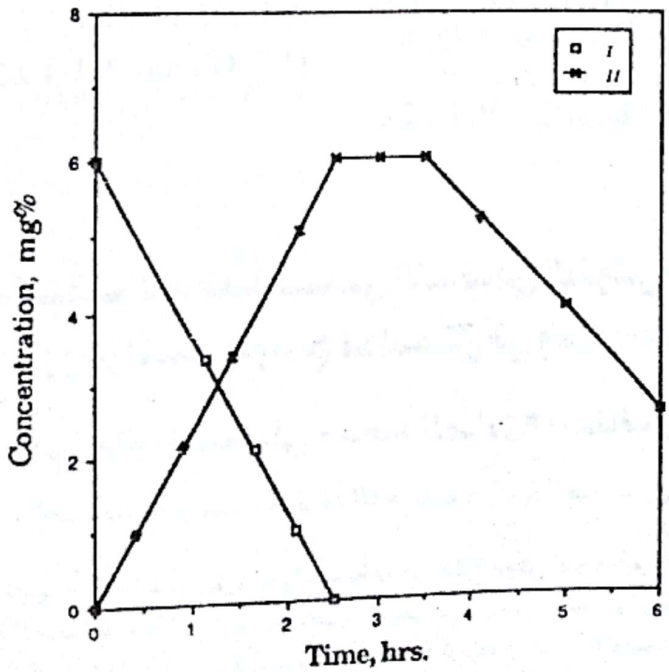
	Modified Vierordt's method	^1D (λ=279nm)	^2D (λ=266nm)
	Recovery %	Recovery %	Recovery %
X	99.9	99.4	99.6
± S.D.	0.32	0.23	0.39
N	6	6	6
V	0.10	0.05	0.15
t		3.2 (1.812)	0.96 (1.812)
F		2.0 (4.28)	1.50 (4.28)



Figure(1): U.V. absorption (a), ^1D determination of Fluorouracil at λ = 279 nm and of its alkali-induced degradation products at (b) 262 nm (b) and ^2D determination of Fluorouracil at λ = 266 nm and of its alkali-induced degradation products at λ = 247 nm (c). Fluorouracil 6 mg % (—) and Fluorouracil
alkali-induced degradation products 6 mg % (----).



Figure(2): Stability study of the concentration, mg% of intact Fluorouracil (I) and its alkali-induced degradation products (II) versus sodium hydroxide concentration applied for degradation step.



Figure(3): Stability study of the concentration, mg% of intact Fluorouracil (I) and its alkali-induced degradation products (II) versus time of reflux.

of the absorbing carbonyl group. Therefore, refluxing in 2N sodium hydroxide for 3 hours was here recommended here for complete degradation of fluorouracil.

Spectrophotometric measurements of the degradation products at the above mentioned maxima were recorded and the results obtained are shown in Table 4.

In order to prove the validity of the proposed procedures, six different dilutions of a laboratory prepared mixture of fluorouracil alkali-induced degradation products, together with 6 mg % intact fluorouracil were analyzed for the degradation content. For comparison, the modified Vierordt's method was applied too and statistical analysis of the results obtained were shown in Table 5. The suggested first and second derivative measurements are as precise and accurate as the modified Vierordt's method.

However, fluorouracil ampoules, even the stored ones, fail to give any absorption at the previously mentioned maxima of degradation, proving its absence.

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استخدام المشتقات التفاضلية لمنحنى الامتصاص الضوئى لتعيين مركب الفلوروبوراسيل ونواتج تحلله كل فى وجود الآخر

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فى هذا البحث تم تقدير مركب الفلوروبوراسيل المستخدم فى علاج بعض الأورام السرطانية فى وجود نواتج تحلله باستخدام طريقتى المشتقة التفاضلية الأولى والثانية لمنحنى الامتصاص الضوئى. كما تم قياس نواتج هذا التحلل الدوائى كمياً فى وجود المادة الأصلية باستخدام الطرق الطيفضوئية السابقة الذكر بدون تداخل طيفضوئى بين الدواء ونواتج تحلله. ولقد اوضحت النتائج المطبقة على مخاليط معملية من مركب الفلوروبوراسيل مع نواتج تحلله وامبولات الفلوروبوراسيل أن الطرق المقترحة بسيطة وتماثل الطرق المنشورة فى دقتها وتكرارها.