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FLUOROMETRIC DETERMINATION OF ZIDOVUDINE IN SPIKED HUMAN PLASMA

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ABSTRACT

A highly sensitive fluorometric procedure for determination of zidovudine is described. The procedure depends on the interaction between zidovudine with 7-hydroxy-4-methylcoumarin where a fluorophore of high fluorogenic activity is produced exhibiting wavelengths of maximum excitation and emission of 340 and 450 nm, respectively .The procedure is used for the determination of zidovudine in human plasma in the range of 2-8 $\mu g\ ml^{-1}$ in a rectilinear relationship .The mean percentage recoveries were 99 . 193± S.D 1.928 in case of bulk drug while it was 99 . 832± S.D 1.809 in case of 7 concentrations in human plasma.

INTRODUCTION

Zidovudine (1) , 3' - Azido - 3'- deoxythymidine is an antiviral drug having an inhibitory effect against HIV-1(Human Immuno- Defficiency Virus Type-1),the etiologic agent of AIDS (Acquired Immuno- Deficieny Syndrome) [1,2] . In the last few years zidovudine emerged as the only prescription product in the United States for adult AIDS patients [3].

Different analytical procedures for the quantitation of zidovudine have been reviewed [3]. Recent methods for the determination of zidovudine included HPLC [4,5]and electrochemical methods has been reported.[6] . The electrophoresis technique was also used for zidovudine monitoring in serum[7] .

In the present work, a highly sensitive fluorometric procedure is suggested for the rapid determination of zidovudine in spiked human plasma .

EXPERIMENTAL

Apparatus :

Schimadzu RF 500 Spectrofluorophotometer was used during the coarse of this work.

Materials :

Zidovudine (Wellcom Co) ; 7 - hydroxy - 4- methylcoumarin (Aldrich) and acetonitrile (Analytical grade). Blank human plasma was delivered by the local hospital blood bank.

Reagents and Solutions :

1- Zidovudine stock standard solution was prepared by dissolving 10 mg of zidovudine in 10 ml of distilled water .
2- Zidovudine working standard solutions were prepared by diluting aliquots from the stock solution with distilled water to obtain 2 - 8 $\mu g\ ml^{-1}$ concentrations
3- Zidovudine spiked human plasma samples were prepared by diluting aliquots from the stock solution of zidovudine with blank human plasma to obtain concentrations ranging from 0.2- 0.8 $mg\ ml^{-1}$
4- the 7 - Hydroxy -4- methyl lcoumarin solution was prepared in distilled water to contain 10 $\mu g\ ml^{-1}$.

Procedures :

1- Treatment of Zidovudine spiked human plasma samples :

100 µl aliquots of zidovudine spiked human plasma samples (0.2-0.8 $mg\ ml^{-1}$) were transferred into a 10 ml centrifuge tubes .The volumes were completed to 1.0 ml with acetonitrile and well mixed . The tubes were centrifuged at 5000 r.p.m for 5 minutes .The clear supernatant layer was filtered through millipore filter (0.45 µm) . 100 µl aliquots from the filtrate were completed to 1.0 ml with distilled water and used in the fluorometric procedure as zidovudine standard plasma solutions.

100 µl of blank plasma was treated by the same procedure , completed to 1.0 ml with distilled water and used as a blank experiment in the general fluorometric procedure.

2- General fluorometric procedure :

One ml of zidovudine solution (2-8 µg ml^{-1}) in water or from plasma samples was transferred into a 10 ml test tube . The content of the tube was mixed with 1.0 ml of 7-hydroxy-4-methyl-coumarin solution. The fluorescence of the resulting solution was measured at the wavelengths of maximum excitation and emission at 340 and 450 nm , respectively .The concentrations of zidovudine were calculated from the regression equation of the corresponding calibration graph, using zidovudine working standard solutions or zidovudine standard plasma solutions .

RESULTS AND DISCUSSION

The development of highly sensitive analytical methodology for the quantitation of zidovudine (1) in biological fluids became of great importance due to the increasing use of the drug for the adult AIDS(Acquired Immuno- Deficiency Syndrome) patients .

It was found that the addition of an aqueous solution of 7- hydroxy -4-methylcoumarin , HMC (II) , to an aqueous solution of zidovudine, produces a highly fluorogenic product. Although HMC has inherent fluorescence,the addition of zidovudine increased greatly the fluorescence intensity .The resulting fluorescence was found to be proportional with zidovudine concentrations.The produced fluorescence exhibited maximum excitation emission at 340 and 450 nm, respectively (Figure 1) .

Different reaction conditions were studied to obtain maximum sensitivity . Maximum fluorescence intensity was obtained by using 1.0 ml of 10 µg ml^{-1} HMC solution .In addition, the use of different neutral buffers did not enhance fluorescence production .Acids and alkalies interferred with the reaction between HMC and zidovudine.

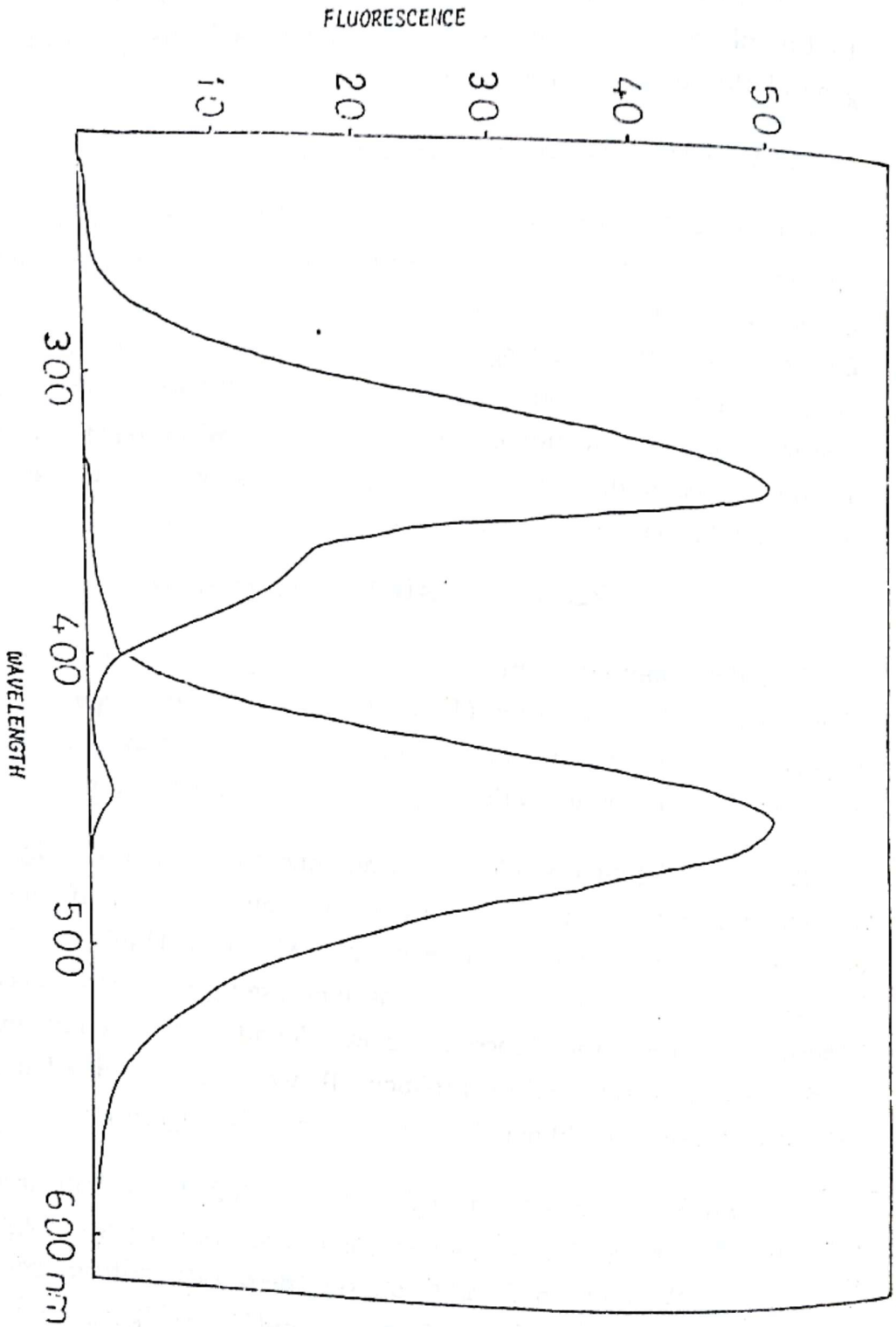


FIG 1: UNCORRECTED EXCITATION AND EMISSION SPECTRA OF 5 μg/ml of ZIDOVUDINE

A rectilinear relationship was obtained in the range of 2 - 8 $\mu g\ ml^{1}$.The good linearity of the method was indicated by the regression equation:

$$Y = -0.9642 + 10.464\ C\ (r = 0.9994)$$

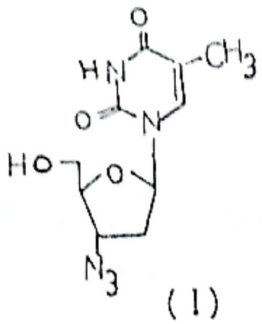
Where : y is the fluorescence intensity = intersept + slope x conc .($\mu g\ ml^{-1}$) and r is the correlation coefficient. The mean percentage recovery from triplicate determinations of 7 concentrations (lies in the same range 2-8 $\mu g\ ml^{-1}$) were 99.193 ± S.D 1.928 (Table 1).

The utility of the method for the determination of zidovudine in biological fluids was established by spiking blank human plasma with zidovudine and its subsequent determination by the proposed method. The determination have been done in plasma ather deproteinization with acetonitrile prior to the application of the fluorometric procedure. The mean percentage recovery was 99.832 ±S.D 1.809 (Table 1) .The concentrations were calculated from a regression equation of the calibration graph prepared simultaneously.

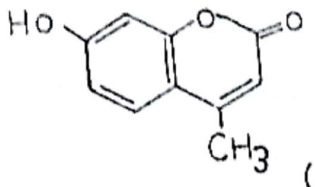
$$Y = -0.4285 + 10\ C\ (r = 0.9993)$$

The nature of the interaction of zidovudine and HMC was not investigated . However, in a previous work 7- hydroxycoumarin carboxylic acid derivative has been applied for the fluorometric determination of amphetamine [8].

Thus, the presented method is rapid and simple in comparison with HPLC and RIA methods .The accuracy, precision and sensitivity of the results suggests the method to be recommended for the determination of zidovudine in biological fluids for the purpose of bioavailability, bioequivalency and drug monitoring studies .



(I)



(II)

Table 1 : Results of Recovery Experiments of Zidovudine

Zidovudine Aqueous Solutions			Zidovudine Spiled Human Plasma		
Added Conc $\mu g\ ml^{-1}$	Found * $\mu g\ ml^{-1}$	% Recovery	Theoritical conc. $\mu g\ ml^{-1}$	Found * $\mu g\ ml^{-1}$	% Recovery
2	2.0034	100.17	2	1.9428	97.14
3	2.8635	95.45	3	3.0428	101.43
4	3.9146	97.86	4	3.9428	98.57
5	4.9659	99.32	5	5.0428	10085
6	6.0170	100.28	6	6.1428	102.38
7	7.0682	100.97	7	6.9428	99.18
8	8.0239	100.30	8	7.9428	99.28
Mean % Recovery 99.193 ± S.D 1 .928			Mean % Recovery 99.832±S.D 1 .809		

* Mean of 3 Experiments

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طريقة لصيفة لتقييم عقار الزيدوفيودين في البلازما البشرية

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تم استحداث طريقة لصيفة ذات حساسية عالية لتقييم عقار الزيدوفيودين والذي يوصف لمرض الايدز وتعتمد الطريقة علي اضافة محلول مائي من الزيدوفيودين الي محلول مائي من كاشف ٧ - هيدروكسي - ٤ ميثل كومارين حيث ينتج مركب ذو خواص لصيفه قوية تم قياسه عند طول موجه انبعاث ٤٥٠ نانوميتر بعد استثارته عند ٣٤٠ نانوميتر . وقد طبقت الطريقة أولاً لتقييم الزيدوفيودين في محاليل مائية ثم في البلازما البشرية . وقد تم الحصول علي علاقة خط مستقيم بين شدة الوميض وتركيزات الدواء في مدي ٢-٨ ميكروجرام لكل ملليتر بنسبة استرجاع مئوي ١٩٣ ، ٩٩± ١,٩٢٨ في حالة المحاليل المائية و ٩٩,٨٨٢ ± ١,٨٠٩ في حالة محاليل البلازما البشرية المضاف اليها الدواء في نفس مدي التركيزات (٢-٨ ميكروجرام لكل ملليتر)

والطريقة الجديدة شديدة الحساسية والبساطة وتصلح لتقييم الدواء في السوائل الحيوية المختلفة وفي مستحضراته الصيدلية في حالة توافرها.