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FLUOROMETRIC DETERMINATION OF ACYCLOVIR IN SPIKED HUMAN PLASMA

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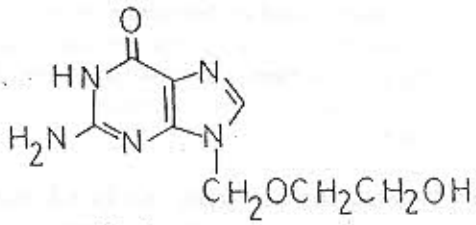
ABSTRACT

A highly sensitive fluorometric method for determination of acyclovir is described. The method depends on the interaction between acyclovir and 7-hydroxy-4-methylcoumarin where a fluorophore of high fluorogenic activity is formed which has wavelengths of mximum excitation and emission of 342 and 452,respectively. The method is used for determination of acyclovir in human plasma in the range of 5-10 ng m^{-1} in a rectilinear relationship. The mean percentage recovery was 99.954 S.D. ± 1.450 in case of bulk drug while it was 99.893 S.D.+ 0.711 in case of 6 concetrations of human plasma. The method is recommended for drug monitoring of acyclovir.

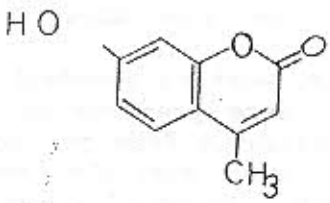
INTRODUCTION

Acyclovir, (I), 9-[(2- hydroxyethoxy) methyl] guanine, mol. wt. 225, also referred to as acycloguanosine, is an antiviral drug with strong and selective activity against herps simplex and varcilla zoster viruses. This drug is used clinicaly in the treatment of a variety of herps virus infection[1].

So far, only little work has been done on the analysis of acyclovir in biological fluids. The reported methods include, high performance liquid chromatography procedures[2-7], radioimmunoassay (RIA)[8], and competitive enzyme linked immunosorbent assay[9].



(I)



(II)

Following administration of acyclovir to man it was found that most of the published HPLC methods lacked selectivity and sensitivity for the analysis of the drug in plasma and urine[4-9].

Although RIA has the relevant sensitivity and specificity, occasional problems in analysing plasma and urine from patietns were encountered due to the possible cross-reactivity with the antiserum[4]. For this reason the same author has suggested a sensitive HPLC method for acyclovir determination as ion pair with heptanesulfonic acid[4].

In this work highly sensitive fluorometric pocedure was suggested for the rapid determination of acyclovir in spiked human plasma.

EXPERIMENTAL

I-Apparatus

Schimadzu RF 500-Spectrofluorophotometer

II-Materials:

Acyclovir [Wellcome Co.], 7-hydroxy-4-methylcoumarin(Aldrich), acetonitrile (analytical grade). Blank human plasma was delivered by the local hospital blood bank.

III-Reagents and solutions:

1-Acyclovir stock standard solution : was perepared by dissolving 10 mg of acyclovir in 10 ml of distilled water.

2-Acyclovir, working standard solutions: were prepared by diluting aliquots from the stock solution with distilled water to obtain 5-10 ng ml^{-1} concentrations.

3-Acyclovir spiked human plasma samples: were prepared by diluting aliquots from the stock solution of acyclovir with blank human plasma to obtain concentrations ranging from 0.5-1 µg ml^{-1}.

4-**7-Hydroxy-4-methylcoumarin solution** : was prepared in distilled water to contain 0.01 µg ml^{-1}.

IV-Procedures :

1-Treatment of acyclovir spiked human samples:

100 ul aliquots of acyclovir spiked human plasma samples (0.5-1µg ml^{-1}) were transferred into a 10 ml centrifuge tubes. The volumes were completed to 1 ml with acetonitrile and well mixed. The tubes were centrifuged at 5000 r.p.m for 5 minutes. 100 µl aliquots from the clear supernatant solutions were completed to 1 ml with distilled water and used in the fluorometric procedure as acyclovir standard plasma solutions.

100 µl of blank plasma were treated by the same procedure completed to 1 ml with distilled water and used as a blanka experiment in the general fluorometric procedure.

2-General fluorometric procedure:

One ml. of acyclovir solution (5-10 ng ml-1 in water or from plasma samples) is transferred into a 10 ml test tube. The content of the tube was mixed with 1 ml of 7-hydroxy -4-methylcoumarin solution. The fluorescence of the resulting soultion was measured at the wavelenghts of maximum excitation and emission, 342,452, nm respectively. The concentrations of acyclovir were calculated from the regression equation of the corresponding calibration graph, using acyclovir working standard solutions or acyclovir standard plasma solutions.

RESULTS AND DISCUSSION

Interest in developing analytical methedology for determination of acyclovir has arised from the importance of the drug as antiviral agent of increasing current use and the lack of reported analytical methods capable of determining its nanogram level in biological fluids.

Acyclovir is poorly absosrbed from gastrointestinal tract. The maximum acyclovir blood level was reported to be about 0.590 µg ml-1 after two hours from oral adminstration of 200 mg[1].

Most of the reported HPLC methods are lacking sensitivity to de-

tect the drug at this ultimate low level[4,9].

It was reported that acyclovir can form water soluble complexes with several organic ligands of different structures at neutral pH [10]. None of these ligands has been used for quantitative determination of acyclovir.

Upon the screening of possible measurable interaction between acyclovir and other reagents, the 7-hydroxy-4-methylcoumarin, HMC (11), was found to produce a highly fluorogenic product with the drug.

Although, HMC, has a native fluorescence, the addition of acyclovir produced hunderdth manyfolds increase in fluorescence compared with that of HMC. The resulting fluorescence was found to be proportional with acyclovir concentrations.

The reaction is so simple, spontaneous and takes place upon the addition of aqueous solution of HMC to the acyclovir aqueous solution. The produced fluorescence shows wavelengths of maximum excitation and emission at 342 and 452 nm respectively. (Figure 1).

The optimum reaction conditions was studied and it was found that the use of one ml of 0.01 µg ml^{-1} aqueous HMC solution produces maximum fluorescence intensity. The reaction takes place in aqueous medium, no advantage was observed upon applying different buffering solutions of neutral pH over the use of water. Acids and alkalies interefere with the interaction between HMC and acyclovir. Decreasing the polarity of the solvent reduces greatly the resulting fluorescence.

The method can detect as little as 5 ng ml^{-1}. of acyclovir upon using the maximum technical capabilities of the instrument.

The method has been applied first on aqueous acyclovir solutions of different concentrations. A rectilinear relationship was obtained in the range of 5-10 ng ml^{-1}. The good linearity of the method was indicated by the regression equation:

Y=-0.304+8.543 C (C.C=0.9887) Equation 1

Where Y is the fluorescence intensity=intercept+slope x conc., ng ml^{-1} and C.C is the correlation coefficient. The mean percentage recovery from triplicate determinations of six concentrations lies in the same range (5-10 ng ml^{-1}) was 99.954 S.D.$\pm$ 1.45 (Table 1).

To assess the applicability of the method for the determination of acyclovir in human plasma, acyclovir spiked plasma samples were analyzed by the proposed method after deproteinization with acetonitrile.

The mean percentage recovery was 99.893 S.D. $\pm$ 0.711 (Table 1). The concentrations were calculated from the regression equation of the calibration graph prepared simultaneously:

Y=-2.47+7.285 C (C.C.0.9994) Equation 2

It was observed that the fluorescence produced from plasma samples are somewhat lower than that obtained from the aqueous solutions but still correlates linearly with acyclovir concentrations as indicated by the comparison of the slopes in equations 1 and 2. Although the intercept in equation 2 is somewhat higher than that of equation 1, the good correlation coefficient indicates the precision of the calibration data.

The relatively lower fluorescence intensity and the relatively high negative intercept value may be

attributed to quenching effect of certain constituents of plasma. However the negative value of the intercept is relatively small if compared with the full scale of fluorescence range (0-100).

The nature of the interaction of acyclovir and HMC is not investigated. However, in a previous work 7-hydroxycoumarin carboxylic acid has been applied for the fluorometric determination of some amines via coumarin-amine salt formation[11].

The method has an advantage over the reported HPLC and RIA methods in being not requiring separation step for acyclovir from plasma samples before interaction with the reagent or separation of the reaction product prior to fluorescence measurment. Furthermore, the method is so sensitive in comparison to HPLC methods and more simple in comparison to the RIA method.

From the accuracy, precision and sensitivity of the results, the method can be recommended for the determination of acyclovir in biological fluids for the purpose of bioavailability bioequivalency and drug monitoring studies.

Table 1 : Results of Recovery Experiments of Acyclovir from Acyclovi Aqueous Solutions and Acyclovir Spiked Human Plasma.

Acyclovir Aqueous Solutions			Acyclovir Spiked Human Plasma		
Added conc. ng ml^{-1}	Found* ng ml^{-1}	%Recovery	Theortical conc ng ml^{-1}	Found**	% Recovery
5	4.905	98.100	5	5.052	101.040
6	6.005	100.083	6	5.967	99.450
7	7.176	102.514	7	7.020	100.285
8	7.995	99.937	8	7.934	99.175
9	8.932	99.244	9	8.940	99.333
10	9.985	99.850	10	10.080	100.080
Mean % Recovery S.D ±		99.954±1.450	Mean % Recovery S.D ±		99.893±0.711

* Mean of three determinations and calculated from the regression equation of the standard curve Y = 0.304 ± 8.543 C (.CC = 0.9987)

** Mean of three determinations and calculated from the regression equation of the standard plasma acyclovir curve Y = 2.47 ± 7.285 C (.C.C = 0.9994).

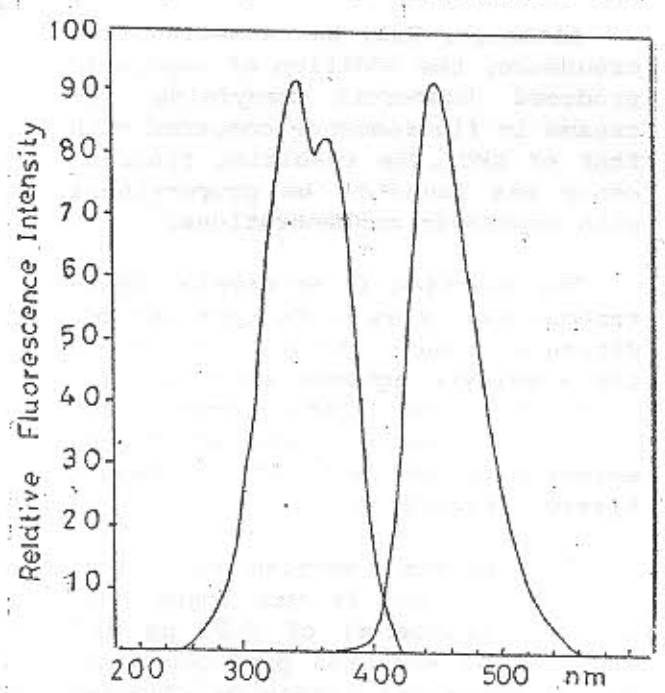


Fig. 1 : Uncorrected Excitation and Emission Spectra of Acyclvir-HMC Reaction Product.

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تقدير الاسيكلوفير فى البلازما البشرية المطعمة به بطريقة لصيفة

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استحدثت طريقة فائقة الحساسية لتعيين الاسيكلوفير. تعتمد تلك الطريقة على تفاعل الاسيكلوفير مع ٧-هيدروكسى-٤-ميثيل الكومارين حيث تكون ناتج ذو خاصية لصيفة مرتفعة للغاية وكان الناتج ذو طاقة انبعاث عند ٤٥٢ نانوميتر بعد استثارته عند ٣٤٢ نانوميتر.

استخدمت الطريقة لتعيين الاسيكلوفير فى البلازما البشرية فى معدل يتراوح من ٥-١٠ نانوجرام لكل ميلليتر حيث كانت العلاقة بين الانبعاث للطيف وتركيز الاسيكلوفير خطيا. وكانت نسبة الاسترداد المئوى للاسيكلوفير ٩٩,٩٥٤٪ ± ١,٤٥ فى حالة العقار النقى ونسبة استرداد المئوى للاسيكلوفير من البلازما المطعمة به ٩٩,٨٩٣٪ ± ٠,٧١١.

والنتائج ترشح الطريقة للتطبيق فى متابعة العقار فى السوائل الحيوية.

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