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# RAPID AND SENSITIVE METHOD FOR A KINETIC AND PREFORMULATION STUDY OF AN INVESTIGATIONAL ANTIVIRAL DRUG

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## ABSTRACT

Win 51711 is an oxazoline compound investigated for antiviral activity against rhinovirus. The drug is not very soluble in water (<1 mcg/mL) above pH 5 but is quite soluble at more acid pH's due to salt formation with the oxazoline nitrogen. Formulation of the drug depends on selective use of excipients. Acidic excipients destabilize the drug whereas neutral and basic ingredients have no adverse effect on stability. The present study reports on an HPLC method developed to separate the drug and the products under stressed conditions. The products of hydrolysis were identified and found to have little or no antiviral activity. A simple spectrophotometric method was also developed. This method was found to have an advantage of assaying solutions of Win 51711 and its hydrolysis product without separation. The HPLC method was used in selecting compatible excipients which resulted in a formulation with long term stability.

## INTRODUCTION

Win 51711 is chemically known as 5-[7-[4-(4,5-Dihydro-2-oxazolyl) phenoxy] heptyl]-3-methyl-isoxazole and has shown antipicornavirus activity



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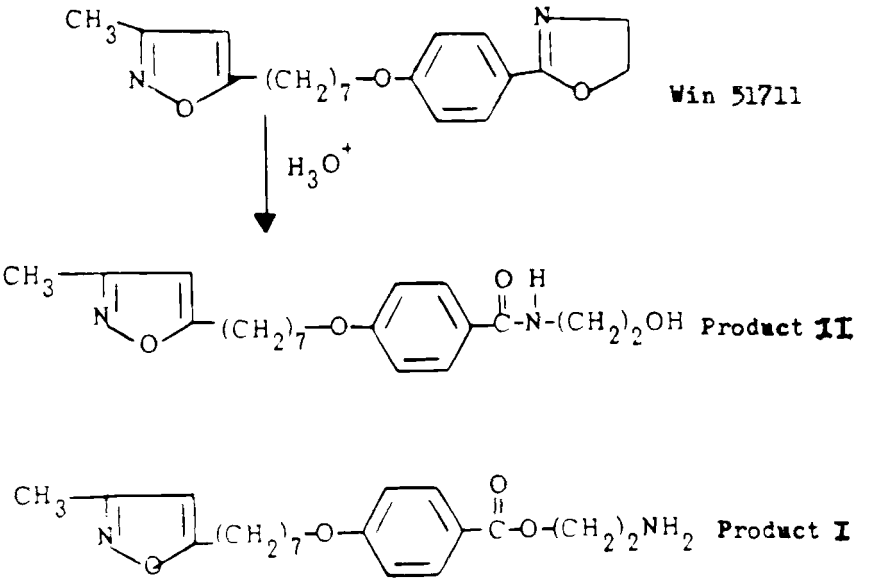


SCHEME I

Decomposition of Win 51711 to Compound I and II in acidic medium.

(1,2,3,4,5). A fresh acidic solution of Win 51711 shows an UV peak at 289 nm. Upon storage, the UV peak at 289 nm slowly disappears with a new peak forming at 260 nm. The conversion was due to the formation of an amino-ester product (compound I) which absorbs at 260 nm. HPLC assays shows that Win 51711 is predominantly converted to I with just a trace of II (Scheme I). A similar ring-opening reaction of 2-oxazolines in the presence of dilute acids has been described (6).

## EXPERIMENTAL SECTION

### Method 1-HPLC Assay Method

The chromatographic system consisted of a Varian 5000 Liquid Chromatograph, Vari-Chrom variable wavelength detector and a Varian Model 9176 recorder. The mobile phase consisted of 40% B and 60% A (A-acetic acid 40 mL, sodium octanesulfonate 2.6 g and sufficient water to make a liter; B - 100% acetonitrile). Flow rate was maintained at 2 mL/min and the UV detector was set at 270 nm. Attenuation was 0.1 AUFS. Retention time for

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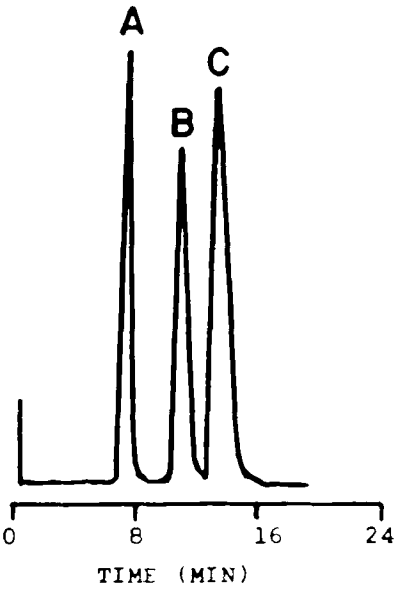


FIGURE 1

Chromatogram of Win 51711 and the 2 hydrolysis products. A-compound II; B-compound I; C-Win 51711.

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Win 51711 is 14.4 minutes and that of the ester and amide product are 12 and 8.3 minutes respectively (Fig. 1). In the absence of compound II, retention time for Win 51711 may be shortened. Retention time for Win 51711 may be shortened to 4 minutes and that of compound I to 2.8 min by increasing the acetonitrile to 70% in the mobile phase.

Standard solutions were prepared by diluting stock solutions of Win 51711 and compound I in 0.1 N HCl with 90% volume of HPLC solvent to give the following concentrations of Win 51711 and ester product respectively: (1) 0, 0 (2) 10, 15 (3) 20, 30 (4) 40, 60 (5) 60, 90 and (6) 80, 0 mcg/mL. These mixtures were analyzed using the HPLC method plotting the peak heights (mm) versus concentrations. Good linearity of standard curve was obtained for both Win 51711 and compound I (Figure 2 and 3). Because of

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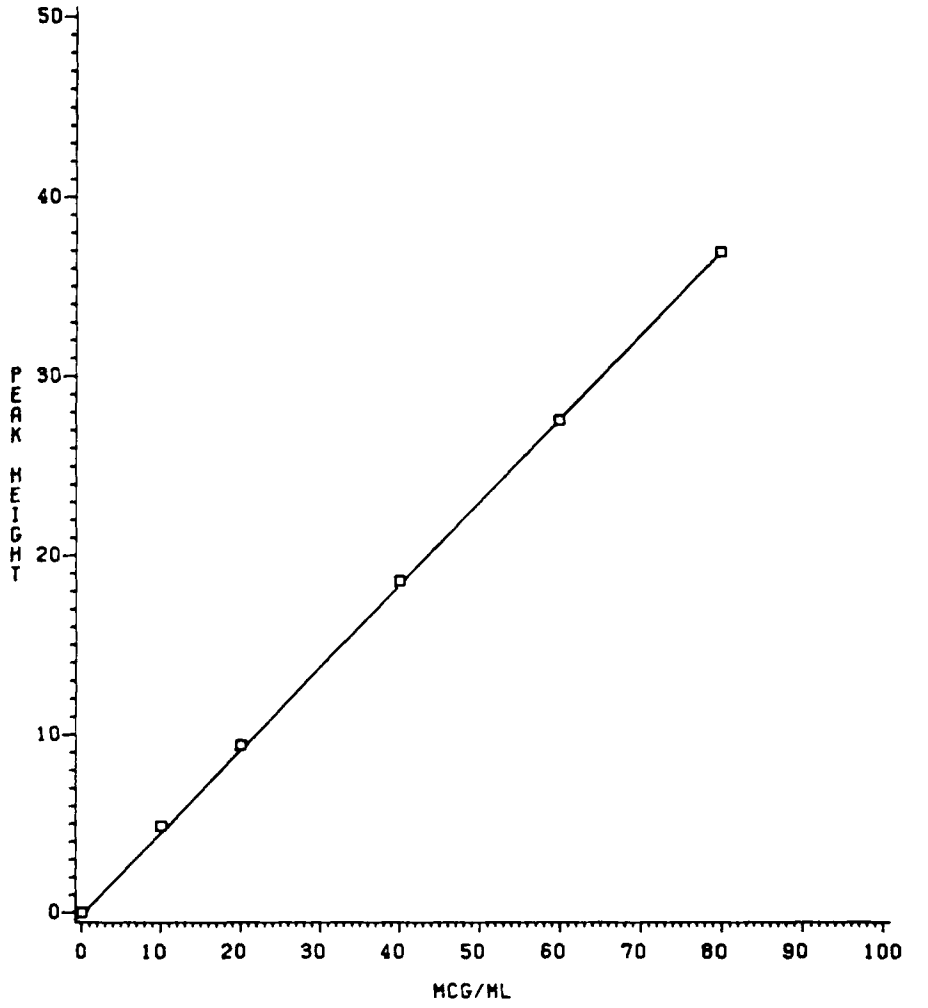


FIGURE 2

HPLC Peak Height vs. Win 51711 concentration.

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rapid decomposition of Win 51711, all assays should be completed within one hour so that hydrolysis does not affect the assay results.

Method 2 - Spectrophotometric Method

Freshly prepared aqueous solutions of Win 51711 and compound I in 0.1 N HCl were prepared. The solutions were immediately scanned using a

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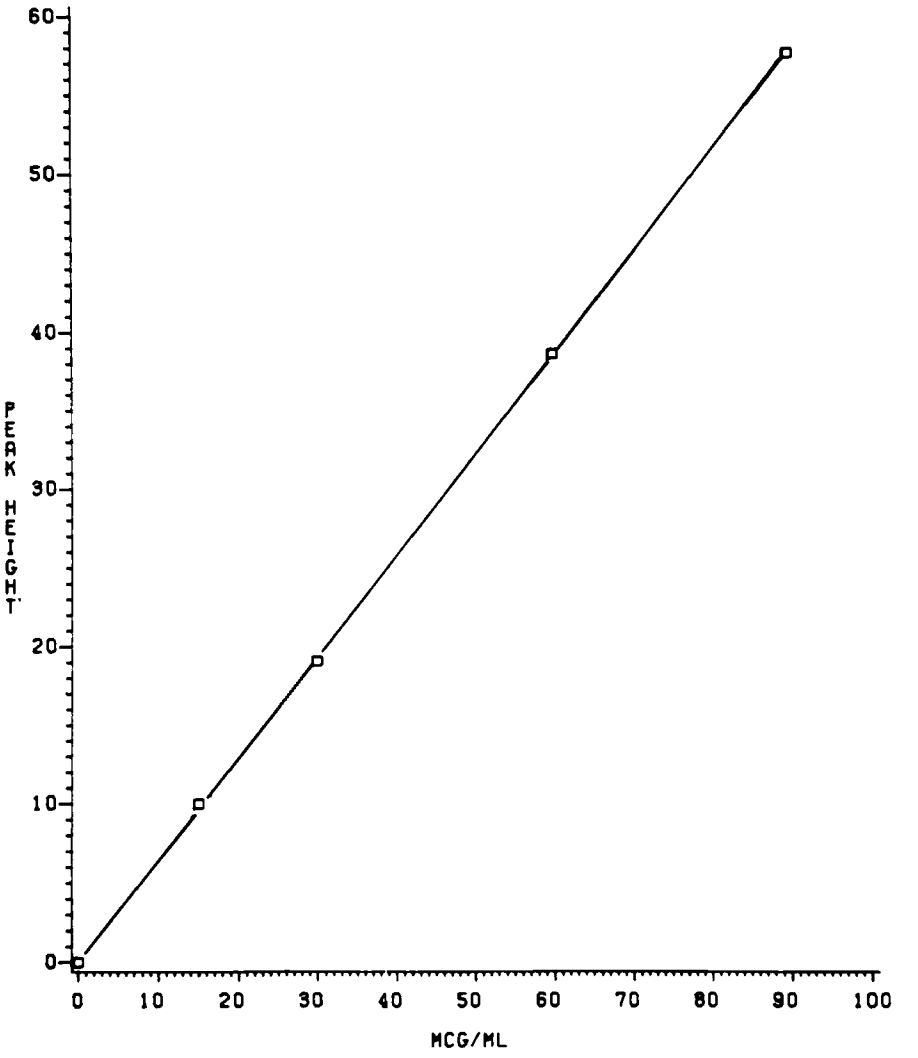


FIGURE 3

HPLC Peak Height vs. Compound I concentration.

Spectronic 2000 Spectrophotometer. Win 51711 shows a UV peak at 289 nm (absorptivity = 71). Compound I shows a UV peak at 260 nm (absorptivity = 45.5). The absorptivity of Win 51711 at 260 nm is 24. The absorptivity of the compound I at 289 nm is 4. The separation of UV peaks of the two components (Fig. 4) allow the mixture components to be calculated as

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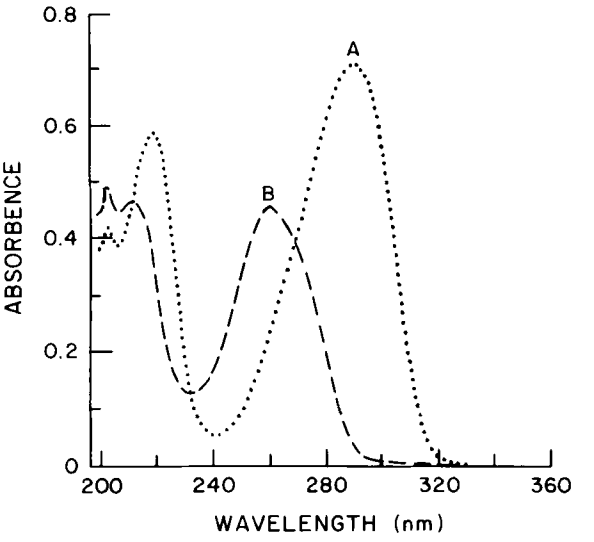


FIGURE 4

Absorbance vs. Wavelength Spectra for Win 51711 (A) and Compound I

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follows: In a binary system, absorbance of a sample (Ab) at a given wavelength is the sum of absorbance contributed by the two components (7). At the two wavelengths of interest i.e. 260 and 289 nm,

$$Ab^{260} = E_1^{260}C_1 + E_2^{260}C_2 \qquad \text{Eq (1)}$$

$$Ab^{289} = E_1^{289}C_1 + E_2^{289}C_2 \qquad \text{Eq (2)}$$

Where

$Ab^{260}$ = Sample absorbance at 260 nm

$Ab^{289}$ = Sample absorbance at 289 nm

$C_1$ = Concentration of component 1 in mixture

$C_2$ = Concentration of component 2 in mixture

$E_1^{260}$ = Absorptivity of component 1 at 260 nm

$E_2^{260}$ = Absorptivity of component 2 at 260 nm

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$E_1^{289}$ = Absorptivity of component 1 at 289 nm

$E_2^{289}$ = Absorptivity of component 2 at 289 nm

Rearranging the two equations and solving for C results in:

$$C_2 = \frac{Ab^{289} E_1^{260} - Ab^{260} E_1^{289}}{E_2^{289} E_1^{260} - E_1^{289} E_2^{260}} \qquad \text{Eq (3)}$$

$$C_1 = \frac{Ab^{260} - E_2^{260} C_2}{E_1^{260}} \qquad \text{Eq (4)}$$

Equations (3) and (4) were used to calculate concentrations of Win 51711 and compound I in the sample mixture. Actual calculations were performed using a SAS program on the IBM computer (8). This method is only valid at pH's below 5 where the only product is the aminoethylester derivative (compound I). In addition, all absorption readings for standards should be made within an hour to avoid error due to decomposition.

Table 1. Stability of Win 51711 5 mg/mL in Various Suspending Vehicles at 70°C

| Excipient | % Drug Remaining 2 days - 70°C |
|---|---|
| 10% Veegum K | 4.8% |
| Carbopol 1% | 68.8% |
| Keltrol 1% | 90.8% |
| CMC (7 HF) 2% | 92.1% |
| Methylcel K 15 M 1.5% | 93.3% |
| Klucel HF 2% | 90.0% |

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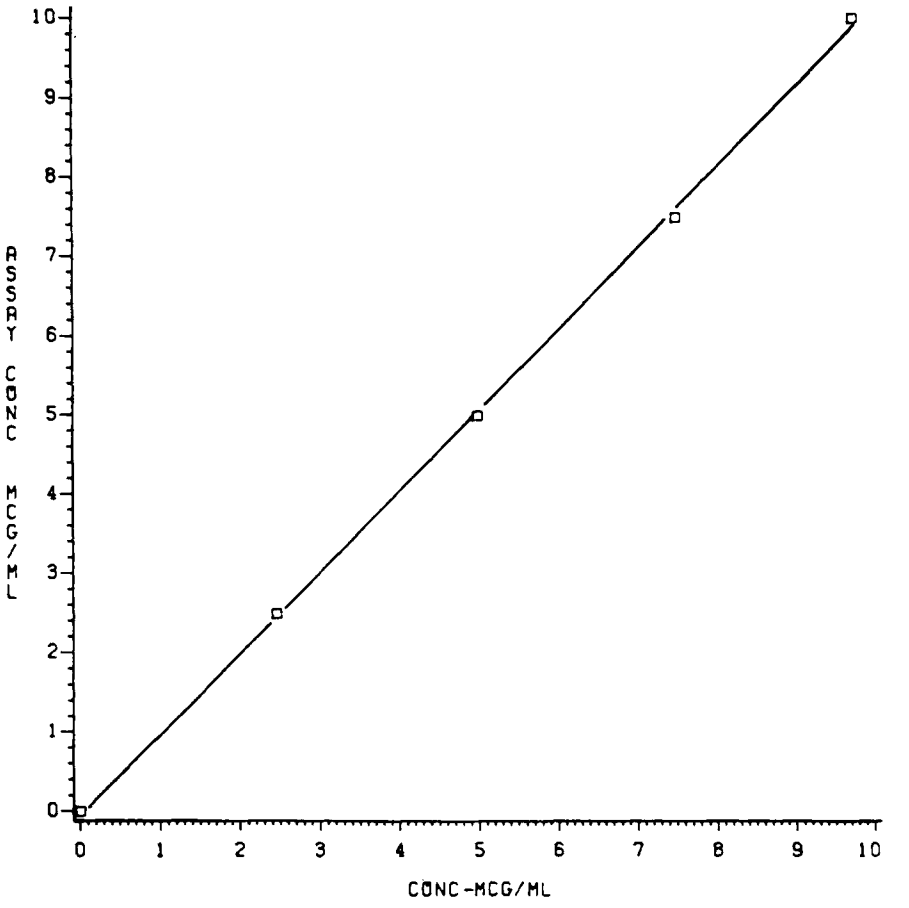


FIGURE 5

Experimental Values vs. Prepared Concentrations for Win 51711 in 4 Equal Mixtures of Win 51711 and Compound I.

## RESULTS AND DISCUSSION

### HPLC Method

The peak height versus concentrations of a series of Win 51711 and compound I solution mixtures were plotted in Fig. 2 and Fig. 3 (see Experimental Section - Method 1 for composition of mixtures). Correlation coefficients of 0.999 were obtained for both plots using linear regression analysis. The HPLC method also was used successfully to analyze a number

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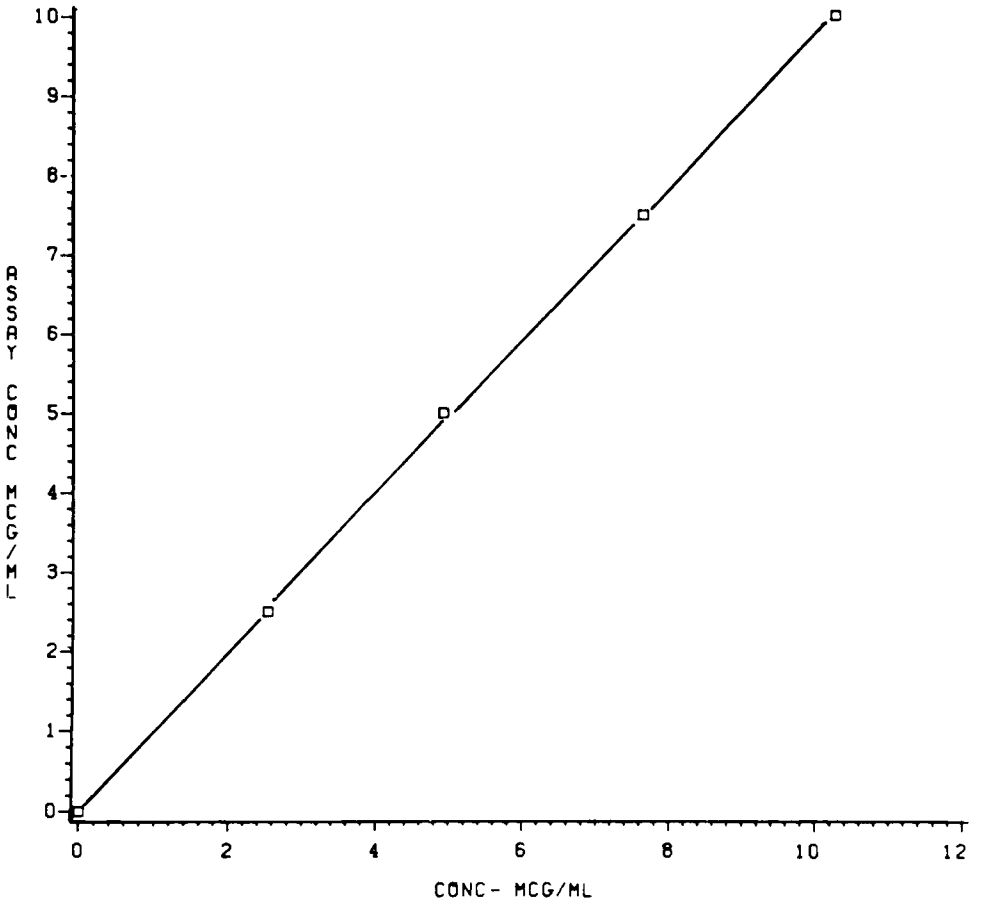


FIGURE 6

Experimental Values vs. Prepared Concentrations for Compound I in 4 Equal Mixtures of Win 51711 and Compound I.

of suspension dosage forms involving keltrol, Veegum, methylcellulose and Carbopol 934 (Table 1). Substantial instability involving 2-oxazoline (70°C) ring hydrolysis was found with Veegum and Carbopol 934 vehicles at accelerated conditions. A stable formulation was possible with non acidic vehicles such as Keltrol and methylcellulose, but was not possible with acidic Veegum and Carbopol 934.

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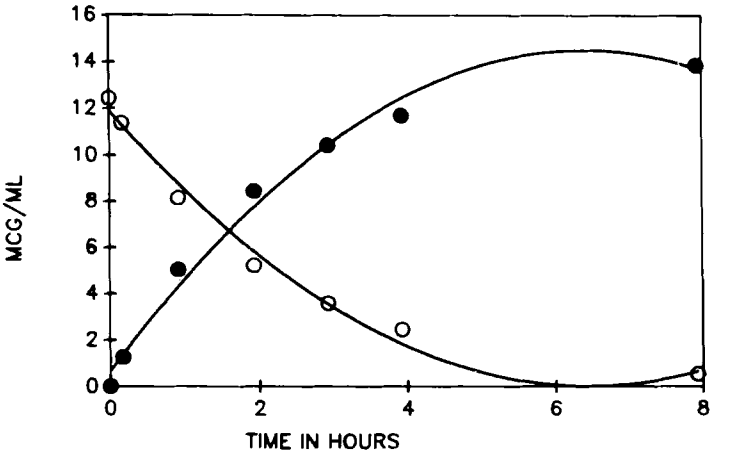


FIGURE 7

Concentration of Win 51711 remained (open circle) and compound I (dark circle) formed expressed as equivalent of Win 51711 in a solution of Win 51711 in 0.01 N HCl at 25° C.

Spectrophotometric Method

Four samples containing equal amount of both Win 51711 and product I (0, 2.5, 5.0, 7.5 and 10.0 μg/mL) in 0.1 N HCl were prepared. These mixtures were assayed using the spectrophotometric method based on Equations (3) and (4) (Figures 5-6). Linear regression analysis of concentration by UV assay versus prepared concentration for both compounds were performed. The correlation coefficient relating assayed concentration to prepared concentration for Win 51711 is 0.999. The slope is 0.98 and the intercept is 0.03. The correlation coefficient relating assayed compound I concentration to prepared concentration is 0.999. The slope is 1.02 and the intercept is 0.06. The proximity of the regression slope to 1 and the intercept to zero indicate good agreement between assayed concentration and prepared actual concentration for both compounds.

After the absorptivity for Win 51711 and compound I are determined, all samples may be assayed rapidly by simply measuring the absorbances of

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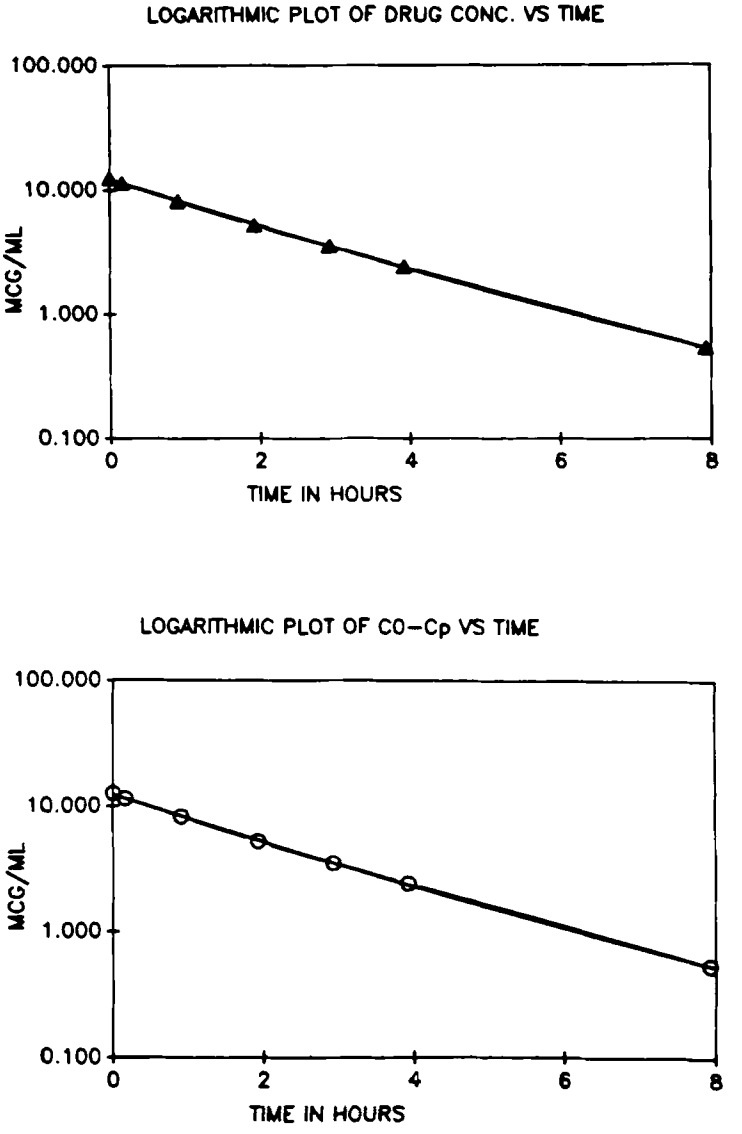


FIGURE 8

Semi-logarithmic plots of Win 51711 concentration (Top) and Co-Cp concentration (bottom) versus time for a Win 51711 solution in 0.01 N HCl at 25°C

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the samples at 260 and 289 nm, and keying these values into the computer. The concentration of both compounds were printed out using the SAS program (8).

An application of this method was used to study the kinetics of Win 51711 in 0.01 N HCl at 25°C. The concentration of Win 51711 (12.5 μg/mL) was seen to decrease gradually whereas that of the compound I increased gradually yielding a stoichiometric amount of the compound I (Figure 7). A plot of log concentration of Win 51711 versus time is linear, yielding a first order half life of 1.769 day (Figure 8).

Alternatively, when the product data was plotted, a logarithmic plot of Co-Cp versus time yield a half life of 1.768 days. Co is the initial concentration of Win 51711, and Cp is the corrected product (compound I) concentration by assay. By converting product concentration into equivalent drug concentration, the concentration of drug remaining at any time, Co-Cp was obtained and plotted to yield a rate constant. The log linear regression in both cases yield correlation of coefficient of 0.999.

## CONCLUSION

Two methods were developed for the monitoring of stability of Win 51711. The methods were found to be useful in assaying the drug in excipient combinations as well as for performing kinetic studies.

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