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Research Papers

# A HIGH PRESSURE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF TRIFLUOROTHYMIDINE DEGRADATION IN AQUEOUS SOLUTION

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## SUMMARY

Trifluorothymidine (I) is shown to be hydrolyzed in aqueous solution to form 5-trifluoromethyl-1,2,3,4-tetrahydropyrimidine-2,4-dione (III). An HPLC method for the determination of III is described, and using this method the rates of decomposition of I in aqueous solution have been investigated. A 1% solution showed 2% breakdown after 2 months at 20°C. From accelerated stability studies it is found that solutions stored at 4°C for 2 years would show 1% degradation.

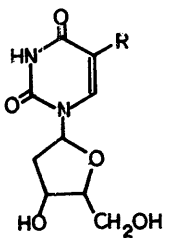
## INTRODUCTION

Trifluorothymidine (I) and analogue of idoxuridine (II) has been used as an alternative to idoxuridine for the treatment of herpes simplex viral ulcers of the cornea. It has been shown to be more effective than idoxuridine in this treatment (Welling et al., 1972).

It has previously been demonstrated that heat sterilization is unsuitable for trifluorothymidine; a 1% solution was almost totally hydrolyzed when autoclaved and 40% breakdown had occurred following steaming (Lee et al., 1978). Since at that time, no data were available concerning the stability of trifluorothymidine when stored in aqueous solution, freeze-drying following aseptic filtration was considered the most suitable formulation for eye drops containing the drug.

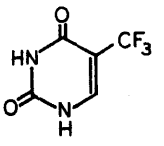
The breakdown products were not identified in this earlier work and estimation of the decomposition product was carried out using a semi-quantitative TLC method. Such at method would not be suitable for accurate quantitative determinations and HPLC was thought more suitable. This study was therefore undertaken to identify the degradation products of trifluorothymidine and to develop an HPLC method to be used to determine the stability of the drug in aqueous solution.

SCHEME 1



(I) R = $CF_3$

(II) R = I



(III)

## MATERIALS AND METHODS

Mass spectra were run on a Varian CH5 mass spectrometer. Inlet temperatures were 180°C with an electron beam energy of 70 eV. High resolution studies were carried out at a resolving power of 8000 (10% valley).

Infra-red (I.R.) spectra were recorded on a Perkin-Elmer 137 spectrophotometer and ultra-violet (U.V.) spectra were run on a Pye-Unicam S.P. 1800 spectrophotometer.

*Preparation of 5-trifluoromethyl-1,2,3,4-tetrahydropyrimidine-2,4-dione*

5-Trifluoromethyl-1,2,3,4-tetrahydropyrimidine-2,4-dione was crystallized from a solution of trifluorothymidine which had been autoclaved at 121°C for 20 min and was recrystallized from chloroform/methanol.

m.p. = 240°C (decomposition)

I.R. ($cm^{-1}$) 3350, 3200, 1730, 1670, 1490, 1380, 1130

U.V. in water $\lambda_{max}$ = 261 nm; (1%, 1 cm) = 445

U.V. in 0.1 N NaOH sq $\lambda_{max}$ = 282 nm (1%, 1 cm) = 445

mass spectrum m/e 180.0179 ($m^+$; 100%), 137 (40%), 118 (8%), 110 (29%), 109 (11%), 108 (5.5%), 91 (12%), 90 (9%), 82 (5.4%), 70 (6%), 69 (4.5%).

*HPLC analysis.* Chromatography was carried out on a Pye L.C. 3 liquid chromatograph using a 250 mm × 3.2 mm i.d. Partisil-10 ODS column. Methanol–water (20 : 80) was used for the mobile phase at a flow rate of 1 ml/min and detection was by U.V. at 254 nm and either 0.2 or 0.5 a.u.f.s. The sample volume of 20 μl was delivered from a sample injection valve.

Standard solutions (0.0005%–0.060%) were prepared by serial dilution from 3 stock solutions of III (0.25%, 0.2%, 0.15%).

*Preparation of samples*

Solutions of trifluorothymidine (1%, 10 ml) were prepared and stored at 5 different temperatures (20°C, 37°C, 58°C, 75°C, 90°C). Aliquots of 0.5 ml were taken at appropriate time intervals, diluted 1 in 10 with water and this solution was used for chromatographic analysis.

## RESULTS AND DISCUSSION

### *Identification of degradation product*

The decomposition product was identified from its I.R., U.V. and mass spectra, as 5-trifluoromethyl-1,2,3,4-tetrahydropyrimidine-2,4-dione (III). The mass spectrum is shown in Fig. 1. The molecular formula was confirmed by high resolution studies and the major fragment ions at m/e 137 and 110 are due to M-(-CO-NH-) and M-(-CO-NH-CO-) respectively. The only major change in the I.R. spectrum compared with that for trifluorothymidine was the loss of absorption bands at 3370 $cm^{-1}$ corresponding to the -OH group of the deoxyribose moeity. The U.V. spectrum in water was similar to that of trifluorothymidine with a max at 261 nm as compared with 263 nm. However, unlike trifluorothymidine the maximum in alkali was shifted the 282 nm. From these observations it can be concluded that there is little modification of the pyrimidine ring except that the ring nitrogen is no longer substituted.

Trifluorothymidine must therefore be hydrolyzed at the ribose–pyrimidine linkage to yield III and probably 2-deoxyribose.

### *Assay procedure*

Trifluorothymidine degradation can be analyzed successfully by HPLC. The breakdown product III is well resolved from the parent compound and 0.1% degradation can be quite easily detected. (Fig. 2). U.V. analysis following hydrolysis confirmed that the formation of III from the trifluorothymidine was quantitative and so it was possible to directly relate the concentration of III to trifluorothymidine degradation.

The results of a statistical evaluation of the HPLC procedure are given in Table 1. They have been compiled from daily measurements of the standard solutions and therefore reflect day to day variations in peak heights. These, as can be seen from the coeffi-

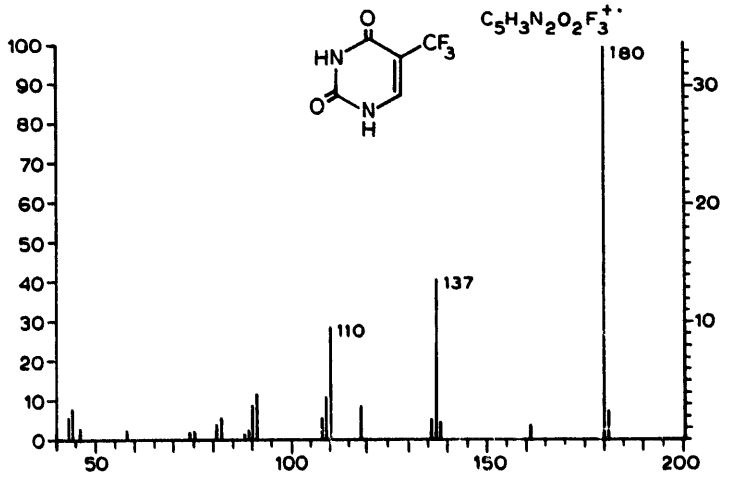



Fig. 1. Mass spectrum of 5-trifluoromethyl-1,2,3,4-tetrahydropyrimidine-2,4-dione.

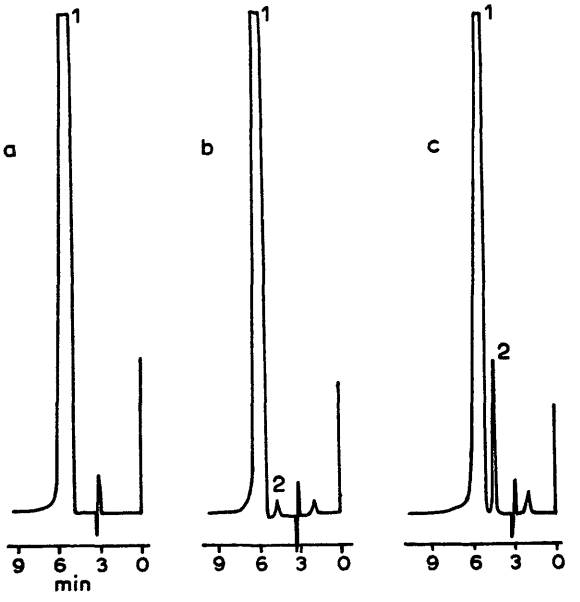



Fig. 2. HPLC traces of trifluorothymidine (1) and its degradation product (2). (a) blank (t = 0); (b) 0.1% breakdown; (c) 1% breakdown.

cients of variance are low throughout the calibration range and the linear correlation between concentration and mean peak height is excellent for this particular concentration range. The correlation coefficient was 0.995 over the wider range 0.001–0.06% as compared with 0.999 for the range quoted in the Table

*Trifluorothymidine degradation*

The results of the studies on the degradation of trifluorothymidine at various temperatures are shown in Table 2. Graphs of log concentration against time were plotted for

TABLE 1

COEFFICIENTS OF VARIANCE FOLLOWING REPEATED INJECTIONS OF VARYING CONCENTRATIONS OF III

| Concentration | Coefficient of variance (%) (Peak heights, 0.2 a.u.f.s.) |
|---|---|
| 0.0005 | 3.94 (8 results) |
| 0.001 | 3.08 (8 results) |
| 0.0025 | 5.25 (8 results) |
| 0.005 | 3.38 (8 results) |
| 0.0075 | 5.34 (6 results) |
| 0.001 | 4.50 (6 results) |
| 0.0015 | 5.04 (6 results) |

$r = 0.999$.

TABLE 2

DEGRADATION OF TRIFLUOROTHYMIDINE AT VARYING TEMPERATURES

| 20° | | 37° | | 58° | | 76° | | 90° | |
|---|---|---|---|---|---|---|---|---|---|
| Time (days) | Concn. % | Time (days) | Concn. % | Time (h) | Concn. % | Time (h) | Concn. % | Time (h) | Concn. % |
| 4 | 0.999 | 1 | 0.9972 | 2 | 0.996 | 1 | 0.986 | 0.5 | 0.983 |
| 7 | 0.9975 | 2 | 0.9918 | 4 | 0.989 | 2 | 0.966 | 1.0 | 0.953 |
| 14 | 0.9961 | 3 | 0.9875 | 6 | 0.984 | 3 | 0.957 | 2 | 0.820 |
| 21 | 0.994 | 4 | 0.9852 | 8 | 0.978 | 4 | 0.936 | 2.5 | 0.781 |
| 32 | 0.9906 | 7 | 0.9734 | 24 | 0.929 | 5 | 0.918 | 3.25 | 0.674 |
| 44 | 0.9875 | 11 | 0.9589 | 28 | 0.915 | 6 | 0.901 | 4 | 0.600 |
| 56 | 0.9854 | 14 | 0.9548 | 32 | 0.900 | 7 | 0.887 | 5 | 0.513 |
| | | 17 | 0.9368 | 48 | 0.869 | 8 | 0.873 | 6 | 0.443 |
| | | 21 | 0.928 | 56 | 0.838 | | | 7 | 0.400 |
| | | | | 72 | 0.803 | | | 8 | 0.339 |
| $r = -0.998$ | | $r = -0.996$ | | $r = -0.999$ | | $r = -0.997$ | | $r = -0.999$ | |
| intercept = 1.00 | | intercept = 0.999 | | intercept = 1.001 | | intercept = 1.004 | | intercept = 1.089 | |
| $k = 0.0001576$ days$^{-1}$ | | $k = 0.00357$ days$^{-1}$ | | $k = 0.0742$ days$^{-1}$ | | $k = 0.423$ days$^{-1}$ | | $k = 3.51$ days$^{-1}$ | |

each temperature. All gave straight line plots with a high degree of correlation indicating that the breakdown was a 1st order process. This was confirmed from the results at 90°C which gave a linear plot for log concentration against time but the graph of concentration against time was a curve. The intercepts at each temperature were computed and in all but one case they agreed with the known concentration of 1%. At 90°C there was a lag time of 25 min whilst the solution reached the required temperature and this led to a

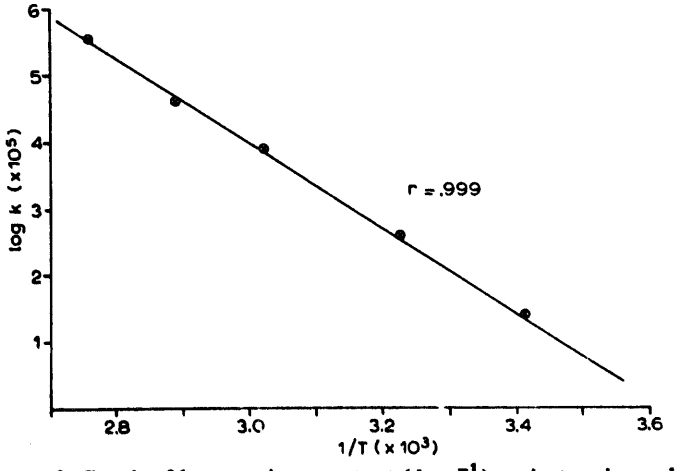



Fig. 3. Graph of log reaction constant (days$^{-1}$) against reciprocal temperature (°K).

false result when extrapolating back to time t = 0.

From the above results it was possible to construct the Arrhenius plot of log $k$ against reciprocal temperature (Fig. 3). The linear correlation coefficient again confirms the precision of the graph. By extrapolation, the reaction constant at 4°C was found to be $1.576 \times 10^{-5}$. This figure represents a very slow rate of decomposition and this result was confirmed by measuring the degradation in samples stored at 4°C. These showed 1.10% breakdown after 30 months as compared to a predicted value of 1.14% degradation.

Trifluorothymidine is less stable in aqueous solution than its analogue, idoxuridine. Idoxuridine was stable for 12 months when stored at 5°C and room temperature (Ravin and Gulosich, 1964), whereas trifluorothymidine shows 1% breakdown after 1 month at room temperature and 1% breakdown after 2 years at 4°C. The usual pharmacopoeial limits for such preparations are 95% of stated strength and it can be predicted that 5% breakdown would have occurred after 179 days at 20°C and 8.9 years at 4°C. However, since little is known of the toxicity of the degradation products it is preferable to minimize its production wherever possible. The freeze-dried preparation would therefore still appear to be the most suitable formulation for trifluorothymidine eye drops. Following reconstitution they can be given a 1 month expiry date when stored in a refrigerator.

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