Journal of Chromatography, 348 (1985) 299–303
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 104

Note

Determination of ophthalmic therapeutic trifluorothymidine and its degradation product by reversed-phase high-performance liquid chromatography

G. BALANSARD*
Laboratoire de Pharmacognosie–Homéopathie, Faculté de Pharmacie, 27 Boulevard J. Moulin, 13385 Marseille Cedex 5 (France)
G. SCHWADROHN
Laboratoires Dulcis, "Le Mercator", rue de l'Industrie, Monaco (Monaco)
and
E. VIDAL and R. ELIAS
Laboratoire de Pharmacognosie–Homéopathie, Faculté de Pharmacie, 27 Boulevard J. Moulin, 13385 Marseille Cedex 5 (France)
(First received March 13th, 1985; revised manuscript received August 13th, 1985)

The chemical structure of trifluorothymidine (Fig. 1a), or 2'-deoxy-5-trifluoromethyluridine, is that of a fluorinated pyrimidine nucleoside[1].

Trifluorothymidine (TFT) has important pharmacological properties[2,3]. It is a powerful inhibitor of a number of viruses, in particular type I and II simplex viruses, responsible for herpes. This antiviral action justifies its use in ophthalmology[4].

A possible impurity resulting from TFT synthesis is 5-trifluoromethyluracil (TFMU) (Fig. 1b). When TFT is stored in on aqueous medium, TFMU is also encountered as a breakdown product.

As a result of its use in ophthalmological preparations, it is important to be able to test the purity of TFT by a highly specific, sensitive and reproducible analytical method. Reserved-phase high-performance liquid chromatography is the method of choice for the qualitative and quantitative determination of TFT in the presence of its degradation product.

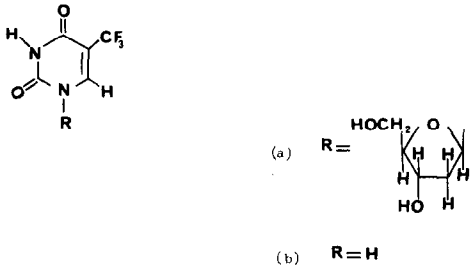

Fig. 1. (a) Structure of trifluorothymidine and (b) structure of trifluoromethyluracil.

0021-9673/85/$03.30

EXPERIMENTAL

A Waters Model 6000 A pump equipped with a U6K universal syringe injector was used in combination with a UV Model 480 spectrophotometer. A Data Module Model 833 integrator (Merck, Hitachi, France) was used to calculate retention times and peak surfaces.

Separation was carried out under isocratic conditions with a μBondapak C_{18} column (30 cm $\times$ 4 mm I.D.). The mobile phase was water–acetonitrile–acetic acid

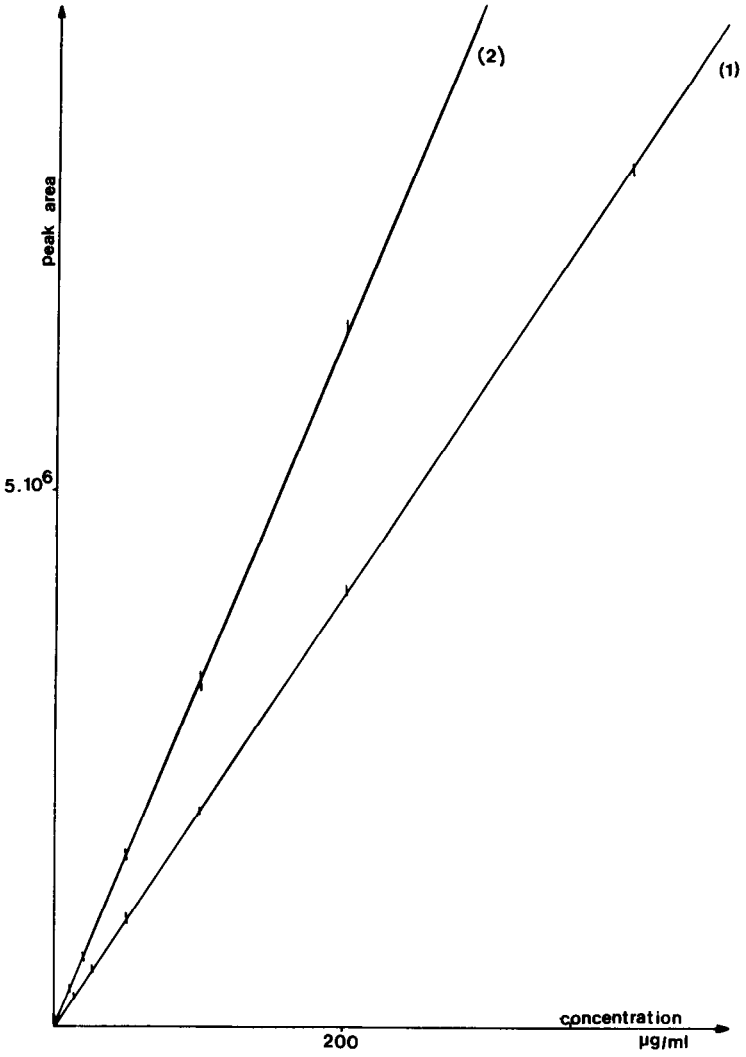

Fig. 2. Standard curves of TFT (1) and TFMU (2) *versus* peak area. Data represent mean ± S.D. of replicate samples (N = 4) run on the same day. Equations for the lines were calculated by linear regression analysis. Peak areas were expressed in integrator units.

(450:50:5), containing 0.0925 g of sodium hexane sulphonate. The flow-rate was maintained at 2 ml/min and detection was at 254 nm. Sensitivity was 1 for the assay of TFT and 0.5 for the assay of TFMU. A 20-μl aliquot of each sample was injected.

Standards

The following standards were used: reference TFT at 20 mg/50 ml of distilled water; reference TFMU at 20 mg/50 ml of distilled water.

Samples

The following samples were investigated: ready-to-use eye-drop solution dosed at 1% TFT and diluted 1/25 in distilled water (after 1 year of storage); lyophilized eye-drop solution after 1 year of storage at room temperature (50 mg dissolved in 5 ml of solvent, 5 ml of this solution diluted 1/25 in distilled water) and ointment containing 2% TFT including the anhydrous excipient (trifluorothymidine extraction was performed after dispersing 2 g of the ointment in 50 ml of petroleum ether, with 10-ml aliquots of distilled water; this solution was diluted 1/50).

RESULTS AND DISCUSSION

A high-performance liquid chromatographic (HPLC) method was set up for assaying TFT and TFMU. The retention times of TFT and TFMU were 5.61 and 4.69 min respectively. The method was linear for the concentration of TFT from 6.25 to 400 μg/ml and for the concentration of TFMU from 2.50 to 400 μg/ml (Fig. 2). The coefficients of variation are presented in Table I. The practical limits of detection for TFT and TFMU are 6 and 1 μg/ml, respectively. As can be seen from the chro-

TABLE I

COEFFICIENTS OF VARIATION FOR REPLICATE SAMPLES (N = 4) OBTAINED ON THE SAME DAY

Compound	*Concentration (μg/ml)*	*Mean peak area**	*S.D.*	*Coefficient of variation (%)*
TFT	6.25	135777	14576	10.7
	12.50	258688	13258	5.1
	25.00	545445	22594	4.1
	50.00	1016265	33846	3.3
	100.00	2025771	13962	0.7
	200.00	4094012	39338	1.0
	400.00	7975919	34255	0.4
TFMU	2.50	78944	285	0.4
	5.00	162505	4056	2.5
	10.00	317565	4557	1.4
	20.00	627422	1311	0.2
	50.00	1563792	10501	0.7
	100.00	3243745	144509	4.5
	200.00	6589220	27659	0.4
	400.00	12464175	64428	0.5

* Peak areas are expressed as arbitrary electric impulsion units of the integrator.

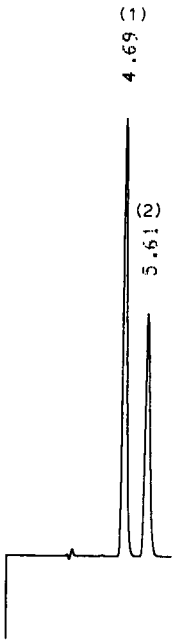

Fig. 3. Representative chromatogram of TFMU (1) and TFT (2) (c = 100 and 200 μg/ml, respectively).

matogram in Fig. 3, each compound was well resolved (R = 1.71) and had symmetrical peaks. Assays for eye-drop solutions and ointment are presented in Table II. The assay of TFT and TFMU was performed by comparing peak areas of the reference substances to those of each sample of eye-drop solutions or ointment. No interference peak was noted with other constituents of eye-drop solutions and ointment (Fig. 4).

The TFT content of ready-to-use eye-drop solutions stored for 1 year at room temperature (20°C) decreased by about 14%, with the appearance of an equivalent quantity of TFMU (Fig. 5). The importance of supplying the preparation in lyophilized form is noted, since this leads to satisfactory storage of TFT for 1 year. Similarly, TFT in the ointment, whose excipient is free of water, underwent no degradation.

TABLE II

TFT AND TFMU CONTENT OF EYE-DROP SOLUTIONS AND OINTMENT (%)

Sample	*(%) TFT*	*(%) TFMU*
Ready-to-use eye drops dosed at 1% TFT	86.8	14.6
Lyophilized eye drops dosed at 1% TFT	99.2	0
Ointment dosed at 2% TFT	99.2	0

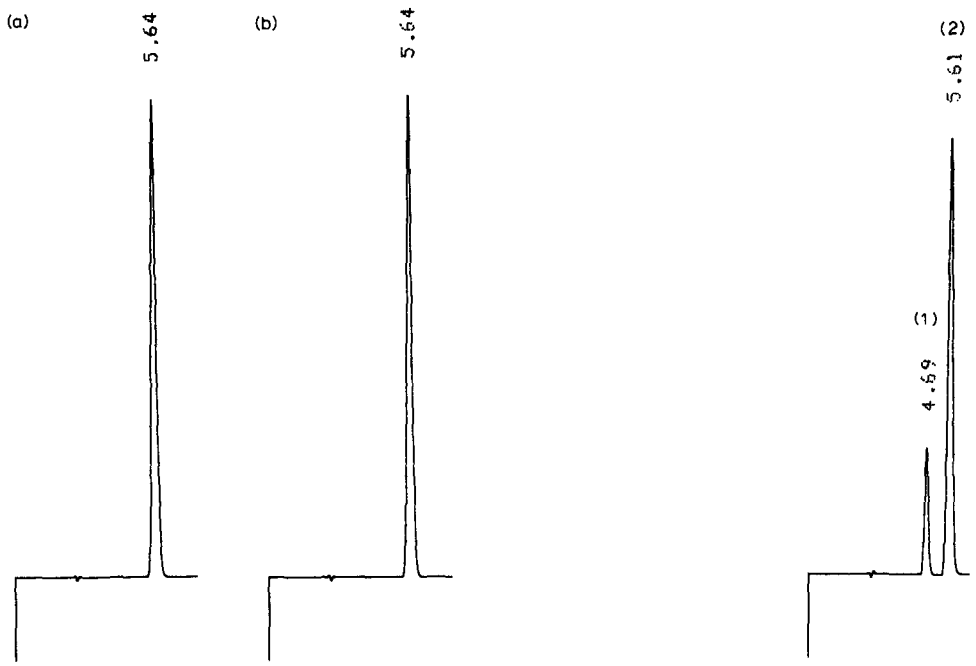

Fig. 4. (a) Typical chromatogram of a diluted lyophilized eye-drop solution containing 1% TFT; (b) typical chromatogram of a diluted ointment solution containing 2% TFT.

Fig. 5. Chromatogram of a ready-to-use eye drop solution stored for 1 year at room temperature (20°C). 1 = TFMU; 2 = TFT.

TFT conservation is best in anhydrous preparations: lyophilized eye lotions and non-aqueous excipient for ointment.

A rapid, reliable and sensitive procedure was developed for the analysis of TFT and TFMU in ophthalmological preparations and should provide a means for routine control measurements of the products.

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