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Short Communication

Studies of iododoxorubicin by HPLC*

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Introduction

Iodoxorubicin [1], identified by the laboratory code FCE 21954 (Fig. 1), belongs to the anthracycline group and shows a marked antineoplastic activity [2, 3]. FCE 21954 differs from the anthracycline drug, doxorubicin [4], by the presence of an iodine atom instead of a hydroxyl group in position 4′ of the aminosugar daunosamine. This well-known group of antitumoral compounds shows a marked toxicity on cardiac tissue as the main undesired side-effect. The iododerivative FCE 21954 shows an interesting decrease in the cardiac toxicity which could be related to the presence of the iodine atom increasing the lipophylicity of the compound. Furthermore, iododoxorubicin has shown its antitumoral activity also when administered *per os*, and as such represents a really marked improvement in a therapeutic area in which mainly injectable formulations are used, due to the poor oral absorption of these active drug substances. Our studies were aimed at the development of an ion-pair HPLC method capable of distinguishing FCE 21954 from

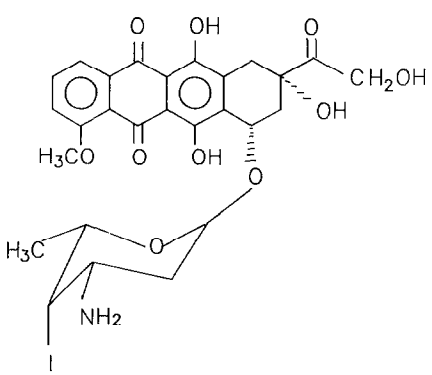

Figure 1

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its related compounds, allowing us to perform physico-chemical studies on the raw material characteristics, in order to set up a suitable oral formulation. The HPLC procedure was based on that published in United States Pharmacopeia Monograph for Doxorubicin HCl [5].

The first part of the physico-chemical studies was aimed at determining the different kinetic trends among raw material batches under stressed conditions; then the different stability shown by the active drug substances submitted to different physical treatment was evaluated. Lastly, the influence of the packaging on the raw material stability was studied using two kinds of stoppers: chlorobutyl rubber and silica gel filled plastic.

Experimental

Materials

Iodoxorubicin·HCl was kindly supplied by Chemical Research and Development Department of Farmitalia Carlo Erba. Acetonitrile and water were HPLC grade. Sodium laurylsulphate was analytical grade, 85% phosphoric acid was ACS grade. 0.1 M hydrochloric acid, 0.1 M sodium hydroxide and 3% hydrogen peroxide were prepared from ACS grade reagents.

Apparatus

The experiments were carried out on a liquid chromatograph Varian model LC 5000 equipped with a Zorbax TMS column (length 250 mm; internal diameter 4.6 mm; average particle size 6 μm) supplied by DuPont Instruments, and using an injection valve Rheodyne model 7125 fitted with a 5 μl loop, a detector Knauer model SP 87 and an integrating recorder Varian model CDS 401. During the experiments the mobile phase was filtered through a 0.22 μm porosity membrane filter and deaerated before using. High precision glassware was used in all our trials.

Chromatographic conditions

The mobile phase consisted of water–acetonitrile, 58:42 (v/v), with 1 g l^{-1} sodium laurylsulphate and adjusted to pH 2.0 with 85% phosphoric acid. The mobile phase flow rate used was 1.4 ml min^{-1} and the analytical column maintained at room temperature. The detector, connected to the integrator for maximum sensitivity was set up at the wavelength of 254 ± 1 nm. The quantitation was performed by peak area determination and internal normalization. The chart speed was 1.0 cm min^{-1}. The concentration of the iododoxorubicin solutions usually injected was about 1 mg ml^{-1}. Under these analytical conditions the FCE 21954 peak showed a retention time of about 8 min (Fig. 2).

Method performances

Linearity. Eight calibration solutions prepared, spanning a concentration range of 20–140% of the amount of FCE 21954 usually injected, showed a linear relationship between the peak areas and the amount injected, with a correlation coefficient r = 0.99983 (Fig. 3).

Accuracy. Solutions made up extemporaneously with FCE 21954 in quantities close to the upper and the lower limits of acceptance (90–110% of the label claim) showed an accuracy of 99.70% (mean of 12 determinations).

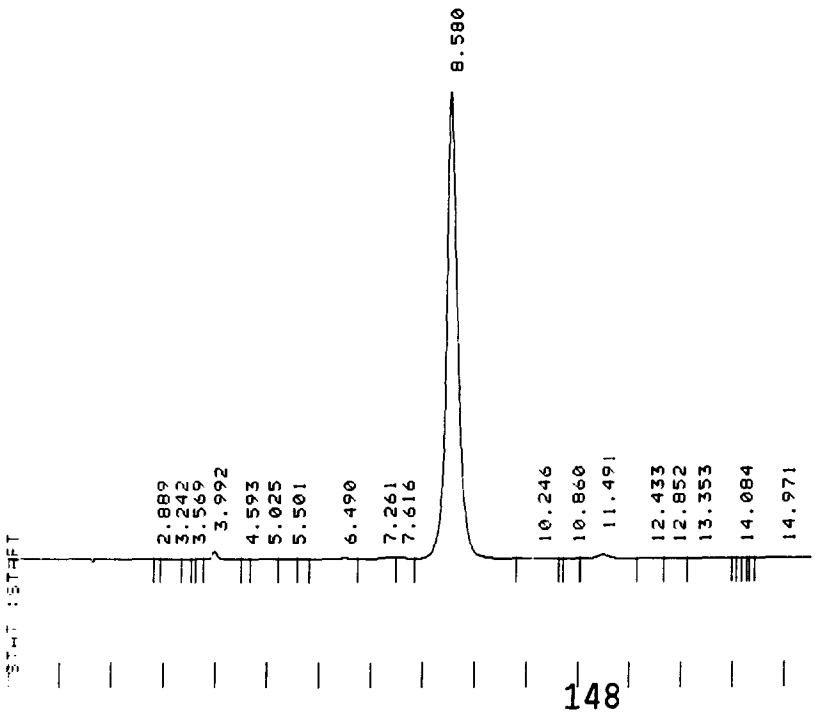

Figure 2

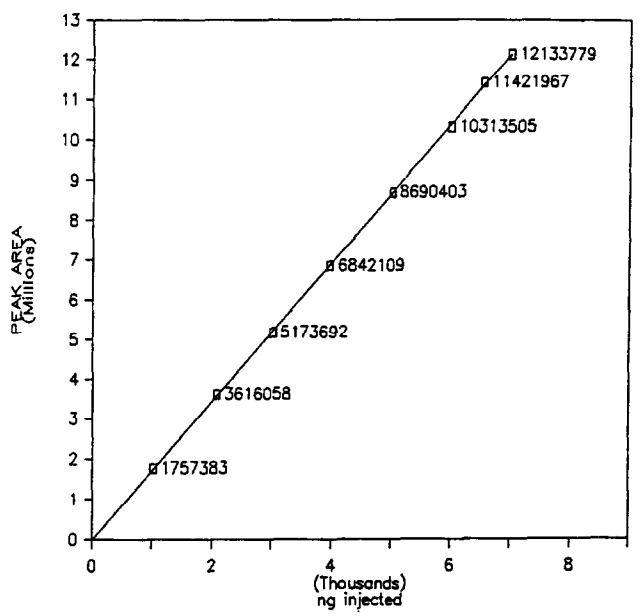

Figure 3

Table 1
Stability-indicating nature of the HPLC assay method for FCE 21945

Time	0.1 M HCl	0.1 M NaOH	2000 F.C.	3% H_2O_2
Initial	1.071 mg ml ^{1} (100.0%)			
After 336 h	0.962 mg ml^{-1} (89.8%)			
Initial		0.989 mg ml^{-1} (100.0%)		
After 30 min		0.494 mg ml^{-1} (49.9%)		
Initial			1.083 mg ml^{-1} (100.0%)	
After 168 h			0.689 mg ml^{-1} (63.6%)	
Initial				1.020 mg ml^{-1} (100.0%)
After 24 h				0.736 mg ml^{-1} (72.2%)

Precision. Repeated determinations on six different samples of the same batch of FCE 21954 drug product showed a RSD = ±0.958%.

Sensitivity. The HPLC method was able to detect an injection of about 50 ng of FCE 21954, equivalent to about 1% of the amount injected for a typical sample.

Stability-indicating power. The method allowed the quantitative determination of FCE 21954 on samples forcibly degraded in acidic (0.1 M hydrochloric acid) and basic (0.1 M sodium hydroxide) conditions, under intense white light (2000 foot-candles) and in presence of a strong oxidizing agent (3% hydrogen peroxide) (Table 1).

Results

Stability comparison among different raw materials

Three different batches of FCE 21954 raw material, (batches Nos GF6358/43B — GF6358/11B — GF6358/29), were checked for stability studies. Samples from each batch were put in glass vials sealed with rubber stoppers and stored at 45°C for 60 days. During the stability period, the samples were analysed at prefix times, and the results obtained are shown in Table 2. The different stability profiles of the raw materials tested are due to their dissimilar water content, resulting from different purification procedures.

Stability comparison among different physical treatments on a selected raw material

To investigate the possible physical behaviour of a standard batch of active drug substance, the raw material showing an intermediate stability trend (batch No. GF6358/43B) was investigated as is, after vacuum treatment and after lyophilization,

Table 2
Comparative stability of three batches of FCE 21954 stored at 45°C

	%FCE 21954 remaining (% degradation products) Batch no.		
Time (days)	GF6358/43B	GF6358/11B	GF6358/29
Initial	100.0 (4.4)	100.0 (3.7)	100.0 (4.4)
7		99.0 (5.6)	98.5 (6.6)
15	85.4 (12.7)	96.6 (9.0)	84.3 (16.2)
21	72.8 (24.2)	91.7 (10.9)	
25		88.2 (10.9)	22.1 (67.8)
30	42.6 (55.3)	86.3 (13.2)	9.5 (77.8)
40	30.5 (67.7)	83.0 (16.3)	2.1 (87.0)
50	11.0 (83.9)		1.3 (85.3)
60		71.9 (26.6)	1.2 (83.6)

Table 3
Comparative stability of FCE 21954 after various pretreatments and stored at 35°C

	% FCE 21954 remaining (% degradation products) Batch no. GF 6358/43B		
Time (months)	As is	Under vacuum	Lyophilized
1	95.5 (4.5)	78.3 (22.2)	85.0 (16.0)
2	72.4 (30.1)	22.7 (73.3)	49.5 (55.9)
3	16.6 (82.7)	9.0 (85.7)	24.4 (75.0)

with the aim of determining the most satisfactory physical treatment to be applied for the development of an oral formulation. The stability trends were studied at 35°C for 6 months, in glass vials sealed with rubber stoppers and examined at prefixed times. The results obtained are shown in Table 3.

Influence of the packaging on the stability of raw material

The three distinct physical presentations of the raw material batch No. GF6358/43B were studied with the aim of checking the influence of two kinds of stoppers on the product stability. The active drug substance, as is, under vacuum and lyophilized, was studied at 45°C for 2 months, in glass vials sealed either with chlorobutyl rubber stoppers or with silica gel filled plastic stoppers. The results obtained are shown in Table 4.

Table 4
Influence of packaging on stability of FCE 21954

	% FCE 21954 remaining (% degradation products) Batch no. GF 6358/43B					
	As is		Under vacuum		Lyophilized	
Time (days)	A	B	A	B	A	B
15	95.3	68.1	87.1	80.0	79.8	90.5
	(7.8)	(33.3)	(16.3)	(23.4)	(19.6)	(12.7)
30	75.9	43.6	28.1	49.1	55.5	85.8
	(18.3)	(49.9)	(60.5)	(42.5)	(42.0)	(16.9)
60	9.0	34.4	6.4	31.5	28.8	79.8
	(80.0)	(53.3)	(79.6)	(58.5)	(69.5)	(25.2)

A = Chlorobutyl rubber stopper.
B = Silica gel filled plastic stopper.

Conclusions

The HPLC method allowed a stability investigation to be conducted on the antitumoral compound under investigation. The studies carried out on FCE 21954 raw material submitted to different physical treatment highlighted a better stability trend for the lyophilized active drug substance. Furthermore, the stability of the drug was increased using a silica gel filled plastic stopper for the packaging. These results made possible the realization of further studies involving the compatibility among the active drug and different excipients, which led to the development of a pharmaceutical presentation of FCE 21954 for the oral administration of this new antineoplastic drug.

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