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Analysis of FCE 23762 (methoxymorpholinodoxorubicin hydrochloride), a new antitumour agent, by HPTLC and scanning densitometry*

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Abstract: A simple, rapid and reproducible high-performance thin-layer chromatographic (HPTLC) method using UV or fluorescence scanning densitometry has been developed for the assay and purity control of methoxymorpholinodoxorubicin hydrochloride (FCE 23762). With a mobile phase of chloroform–methanol–acetic acid (93:6:1, v/v/v) and a silica gel plate, all potential impurities were separated from the main component and from each other. Detection limits at a signal-to-noise ratio of 2:1 were a few nanograms for UV detection and <1 ng for fluorescence emission. The RSD values for the examined compounds were all <3%.

Keywords: *Methoxymorpholinodoxorubicin hydrochloride (FCE 23762); high-performance thin-layer chromatography (HPTLC); densitometry; purity control.*

Introduction

Methoxymorpholinodoxorubicin hydrochloride is 3′-deamino-3′-[2-(*S*)-methoxy-4-morpholino]-doxorubicin hydrochloride (FCE 23762), a new antitumour compound [1] undergoing clinical trials. It is derived from doxorubicin (Dx) and developed, in an attempt to obtain new anticancer agents with improved efficacy and therapeutic index [2], by the Research and Development Laboratories of Farmitalia Carlo Erba.

FCE 23762 is characterized by higher potency than that of the parent Dx compound; indeed efficacy on Dx-resistant cells has been observed [1]. The improved potency is directly related, at molecular level, with the presence of the methoxy-morpholinyl group at position 3′ of the sugar moiety (Fig. 1, I); such a group plays a key role in the lipophilic properties of the drug.

Quality control and drug monitoring of very active compounds require analytical methods of high specificity, sensitivity and accuracy. Various chromatographic methods for the determination of doxorubicin and doxorubicin analogue compounds [3–12] in pharmaceutical formulations and in biological fluids have been reported. Among these methods only two HPLC procedures were described for the determination of FCE 23762 in plasma [11] and for stability studies [12].

The present study describes an HPTLC method with UV- or fluorescence-densitometry suitable for the determination of FCE 23762 and its related compounds (Fig. 1, II–III) in bulk materials and in pharmaceutical formulations. Complete resolution of FCE 23762 and impurities has been achieved.

Experimental

Materials

Reference standards of FCE 23762 (I), its *R*-isomer (II), adriamycinone (III) and the synthetic intermediate doxorubicin (Dx) were kindly supplied by the Chemical Research and Development Department of Farmitalia Carlo Erba. The purity of FCE 23762 reference standard, attested by Farmitalia Carlo Erba, was methoxymorpholinodoxorubicin hydrochloride 90.0%, water 7.0%, adriamycinone 0.4%, *R*-isomer 1.6% and unknown impurities 0.8%. Solvents and reagents were of analytical grade.

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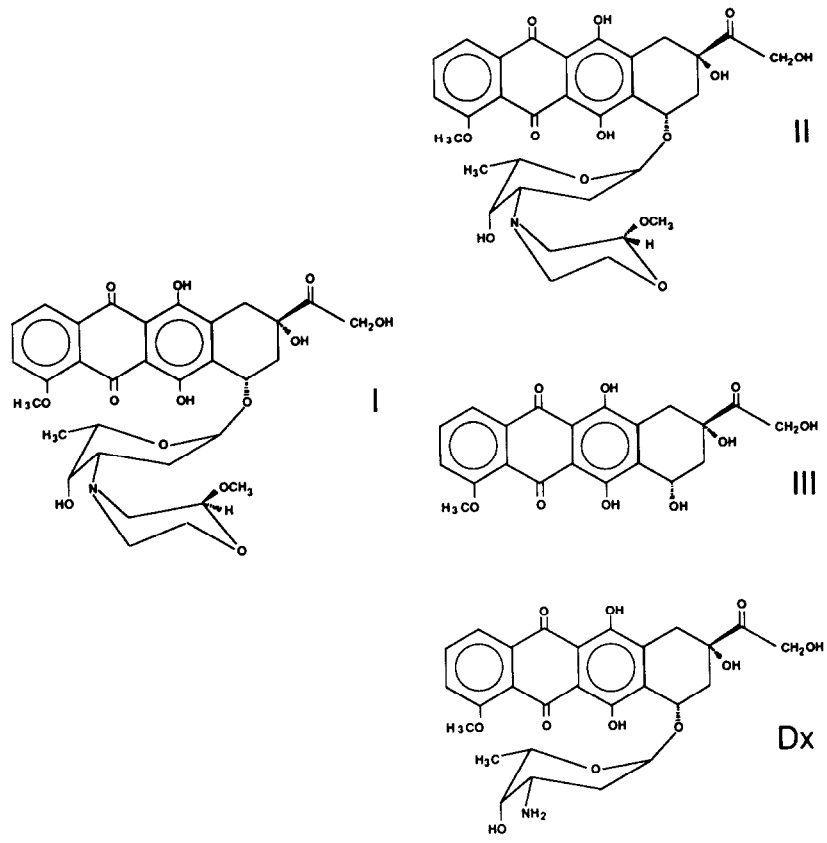

Figure 1
Chemical structure of FCE 23762 (I), its *R*-isomer (II), adriamycinone (III) and doxorubicin (Dx).

Procedure

Standard solutions. Methanolic standard solutions of FCE 23762 (200 ng μl^{-1}), its *R*-isomer (50 ng μl^{-1}), doxorubicin (50 ng μl^{-1}) and adriamycinone (50 ng μl^{-1}) were prepared immediately before use for the determination of the R_f values and, after appropriate dilutions, for quantitative purposes.

Sample solutions. The contents of a vial were diluted with water–methanol (1:1, v/v) to obtain a final concentration of 200 ng μl^{-1} (solution A) and 500 ng μl^{-1} (solution B). The assay of FCE 23762 required the subsequent dilution of 1 ml of solution A with 9 ml of methanol.

TLC densitometry. Chromatography was performed on 20 × 10 cm analytical HPTLC plates precoated with silica gel 60 of 0.2 mm thickness (Merck, Darmstadt, Germany). Before use the plates were cleaned by development with methanol and then activated for 1 h at 110°C. Standards and samples (aliquots of 1–10 µl each) were alternately applied as spots to HPTLC plates (8 mm from the lower edge of the plates) by means of a Linomat IV applicator (Camag, Muttenz, Switzerland) (rate of delivery 6 s μl^{-1}; 16 spots per plate). Plates were developed for about 9 cm from the baseline (development time, about 25 min) in saturated horizontal developing chambers (Camag, Muttenz, Switzerland) with chloroform–methanol–acetic acid (93:6:1, v/v/v). Developed plates were dried with a stream of cold air and quantified by linear scanning in the reflection mode either in UV absorbance at 254 nm (deuterium lamp) or in fluorescence emission (λ_{ex} 254 nm; mercury lamp; secondary cut-off emission filter at 400 nm) with a HPTLC-Scanner (Camag, Muttenz, Switzerland) that was computer aided (CATS software residing in an IBM PS/2 computer). The analysis conditions were: bandwidth 10 nm; slit 0.6 × 8 mm; and scanning time 10 mm s^{-1}.

Qualitative and quantitative analysis. The qualitative and quantitative analysis of FCE 23762 and its impurities has been carried out on two lots of vials each containing 500 µg of

lyophilized drug. Quantitative analysis was performed with the aid of a computer to compare the peak areas of standards and samples. Complete drying of the plate was important to ensure accurate and reproducible measurements. Spots were stable for 2 days at room temperature. However all the quantitative measurements were carried out immediately after developing and drying the plates.

Results and Discussion

Scanning profiles of HPTLC chromatograms for FCE 23762, its *R*-isomer, adriamycinone and doxorubicin are presented in Fig. 2(a). A second development with the same eluent is recommended for better resolution and consequently correct integration of the *R*-isomer peak [Fig. 2(b)]. Scanning profiles from fluorescence or UV absorbance detection, on the same plate, do not show any significant difference. The examined compounds were identified from their R_f values (Fig. 2) and UV spectra obtained on the spot (Fig. 3).

Linearity, reproducibility and detectability data are presented in Table 1. For each compound the linearity was checked for absorption and fluorescence detection procedures by linear regression analysis. Correlation coefficients (r) were also calculated; values of r were greater than 0.9900. The reproducibility expressed as RSD was calculated for compound I, II and III, by applying 10 spots of the same amounts and volumes of standards on a single plate. The RSD values of the peak areas are reported in Table 1.

Lower detection limits at the signal-to-noise ratio of 2:1 were a few nanograms for UV detection and <1 ng for fluorescence emission (Table 1). The lower limit of quantitation is approximately three times the detectability.

The method has been applied to the quantitative determination of FCE 23762, its *R*-isomer and adriamycinone in 500-μg vials of lyophilized drug. The results in respect of four vials for each of the two batches analysed (mean values of duplicate assays from UV and fluorescence detection) are summarized in Table 2. Satisfactory agreement was observed between the two different procedures.

The proposed HPTLC–densitometric method provides accuracy, reproducibility and selectivity for the identification and deter-

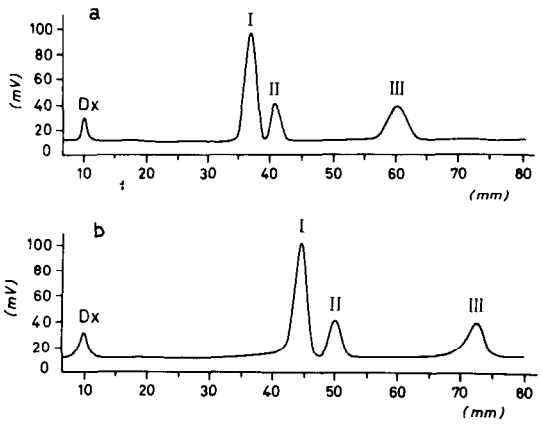

Figure 2
Scanning profiles of HPTLC chromatograms of a solution containing I, II, III and Dx: (a) the R_f values are 0.33, 0.38, 0.60 and 0.03, respectively; (b) improvement of *R*-isomer (II) resolution after a second run.

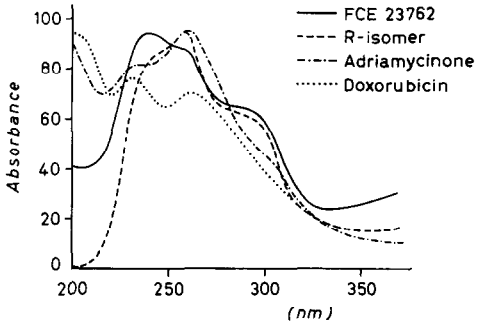

Figure 3
In situ UV spectra of FCE 23762 and related compounds on silica gel.

Table 1
Comparison of linearity, repeatibility and detectability using UV absorption and fluorescence detection

	UV absorption			Fluorescence emission		
Parameters	I	II	III	I	II	III
Linear range (ng)	50–750	10–200*	10–200*	10–75	10–50*	10–50*
Correlation coefficient	0.9976	0.9924	0.9949	0.9985	0.9977	0.9940
RSD	1.82	2.42	2.54	1.84	2.11	2.20
Detectability† (ng spot^{-1})		~3			<1	

*Concentration range examined, corresponding to ng applied to the plate.
†Signal-to-noise ratio >2.

Table 2
Control analyses* of FCE 23762 vial samples using UV and fluorescence detection

	Batch A		Batch B	
	UV	Fl	UV	Fl
FCE 23762	104.3 (1.96)	106.3 (1.73)	106.8 (2.18)	105.5 (1.48)
R-Isomer	0.42 (0.03)	0.33 (0.04)	0.32 (0.03)	0.28 (0.02)
Adriamycinone	0.75 (0.05)	0.64 (0.02)	0.64 (0.02)	0.52 (0.05)

*Values (in %) represent the mean of four experiments (four vials for each batch); SD values are given in parentheses. The analysis of FCE 23762 must be carried out immediately after the preparation of working solutions because after 24 h unknown decomposition compounds may be formed.

mination of FCE 23762, its *R*-isomer and adriamycinone; in addition, it enables good resolution of the major and minor impurities of FCE 23762 and it can be successfully used not only for the assay but also to test the stability of the drug in raw materials or in pharmaceutical forms. Four duplicate samples and eight standards (four different concentrations in duplicate) allow, within a few hours and for each examined compound, separation and specific quantitation.

The lower detection limits obtained using the fluorescence detection suggest the usefulness of this procedure for the determination of FCE 23762 in biological fluids and trace amounts of II and III in pharmaceutical forms.

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