Journal of Pharmaceutical & Biomedical Analysis
Vol. 9, Nos 10–12, pp. 1165–1168, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00

Short Communication

Analysis of iododoxorubicin and its major impurity*

A. FARINA,† M.G. QUAGLIA,‡§ E. BOSSÙ‡ and P. MELCHIORRE‡

†*Istituto Superiore di Sanità, Viale Regina Elena 299, Rome, Italy*
‡*Dipartimento Studi Farmaceutici, Università "La Sapienza", P. le A. Moro 5, 00185 Rome, Italy*

Keywords: *RP-HPLC; fluorescence detection; diode array detector.*

Introduction

Because of the effectiveness of anthracycline antibiotics in the treatment of human malignancies, research into the chemistry of anthracyclines has been given particular attention. Furthermore, the serious toxicity exhibited by these compounds, among which cardiotoxicity is the worst, has resulted in continuous research for analogue compounds which show greater potencies coupled with reduced side-effects. One of these new compounds, currently undergoing clinical investigation, is iododoxorubicin [1] (FCE 21954, Fig. 1) which shows a marked antineoplastic activity [2, 3].

The introduction of an iodine atom instead of the hydroxyl group in the 4′ position of the amino sugar (daunosamine) decreases the toxicity on cardiac tissue [4, 5]. The decrease of this undesired side-effect probably could be related to the presence of the iodine atom which increases the lipophilicity of the compound [6]. A further advantage is the unchanged pharmacological activity of iododoxorubicin if it is administered *per os* [7].

The potential toxicity of this anticancer drug and, more importantly, its impurities, related to the synthesis and hydrolysis reactions, makes the development of specific and sensitive analysis methods particularly necessary. Some impurities are always present in the iododoxorubicin samples, but the main one is doxorubicinone, a degradation product. So, suitable methods are necessary not only in the quality control of the bulk drug substance but also for stability testing.

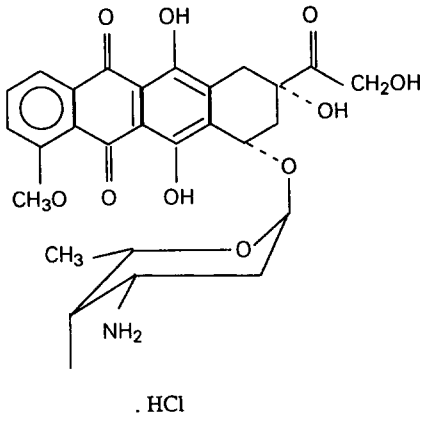

Figure 1
(7*S*,9*S*)-9-hydroxyacetyl-4-methoxy-7,8,9,10-tetrahydro-6,7,9,11-tetrahydroxy-7-*O*-(2,3,4,6-tetradeoxy-3-amino-4-iodo-α-L-lyxo-exopyranosyl)-5,12-naphthacendione hydrochloride.

Many chromatographic methods have been proposed for the analysis of anthracycline antibiotics such as doxorubicin, daunorubicin, etc. [8–11], but for iododoxorubicin only two HPLC methods have been proposed to test the stability of drug and to determine the iododoxorubicin metabolites in plasma samples [12, 13]. The purpose of this work was to develop a suitable method which allows the separation of the active drug from the impurities, generally present in the bulk material, and the simultaneous determination of iododoxorubicin and its major impurity.

* Presented at the "Third International Symposium on Pharmaceutical and Biomedical Analysis", April 1991, Boston, MA, USA.

§ Author to whom correspondence should be addressed.

A sensitive, precise and accurate RP18-HPLC method for iododoxorubicin and doxorubicinone analysis has proposed. As this compound is fluorescent, a fluorimetric and a diode array detectors were used to investigate the more sensitive detection system.

Experimental

Apparatus

A high-performance chromatograph Waters Model 6000 A (Waters Assoc., Milford, MA, USA), equipped with a fluorimetric detector HP 1046A, with a xenon light source, and a diode array detector HP 1040 M, controlled by a computer HP 9000 Model 310 (Hewlett–Packard, WA, USA), was used. Standard and sample solutions were injected manually via a U6K loop valve injector (Waters Assoc.).

Reagents and chemicals

Reference standards of iododoxorubicin HCl and doxorubicinone were kindly supplied by Farmitalia Carlo Erba (Milan, Italy). All other chemicals and solvents, obtained from Merck (Darmstadt, Germany), were of analytical or HPLC grade and were used without further purification.

Chromatographic conditions

The chromatographic separation of iododoxorubicin from doxorubicinone, its main impurity, was achieved by a 5 μm ODS-Hypersil RP-18, (100 × 4.6 mm i.d.) column. The chromatographic analysis was carried out isocratically using a mixture of acetonitrile–water (1:1, v/v) with 0.08% acetic acid (pH 3.5) as eluent at a flow rate of 1 ml min^{-1}.

The detectors conditions were as follows. Fluorescence detector: excitation 472 nm, emission 547 nm; Pmtgain 12; lamp frequency 220 Hz; response time 2000 msec; analogue zero 5% of full scale; diode array detector: λ values 233, 253 and 495 nm; acquisition rate of spectra 1280 msec; bandwidth for each channel 4; sensitivity range 50; reference wavelength 600; reference bandwidth 4.

Procedure

Standard solutions. Iododoxorubicin reference standard was used for quali-quantitative analysis. The purity of this reference standard was attested by Farmitalia-Carlo Erba (certificate no. 00347, December 1990, stock G.F.6358/45). The analysis data concerning this iodoxorubicin reference standard were: iododoxorubicin 86.8%, water 8%, total organic residual solvents 1.4% and total organic impurity 4.2% (the nature of organic impurities was not declared). About 15 mg of the standard described above were weighed accurately with an analytical balance situated in an air-conditioned room and equipped with a suitable drier. This amount was then transferred to a 25 ml volumetric flask and diluted to volume with HPLC mobile phase (sol. a). A 10.4 mg mass of doxorubicinone pure standard was transferred to a 100 ml volumetric flask which was then made up to the mark with HPLC mobile phase. A 5 ml volume of this solution was similarly diluted (×2) by transferring to a 10 ml volumetric flask (sol. b). Both solutions were used to make the calibration curves.

Calibration curves

The calibration curves of iododoxorubicin and doxorubicinone were obtained by injection of 5 μl of reference standard solutions a and b diluted to have different concentrations between 0.21 and 0.52 mg ml^{-1} for iododoxorubicin and 0.021 and 0.052 mg ml^{-1} for doxorubicinone. The graphs obtained from peak area vs amounts injected showed linear relationships ($r = 0.994$ for iododoxorubicin and $r = 0.997$ for doxorubicinone) with zero intercepts.

Qualitative and quantitative analysis

The qualitative and quantitative analysis of iododoxorubicin and its impurity have been carried out on three different lots of vials containing lyophilized iododoxorubicin hydrochloride. The declared amount of antibiotic in each vial was 10 mg.

Sample solution. The vial content was accurately transferred to a 25 ml volumetric flask containing about 10 ml of mobile phase, stirred for 5 min and up to mark with the same solvent (sol. c). This solution was used for quantitative analysis of doxorubicinone in the sample. A 2 ml volume of solution a was diluted to 3 ml with mobile phase and used for the determination of iododoxorubicin (sol. d).

Chromatographic analyses. The same volume of reference standards and sample solutions were injected alternately. The

amounts of iododoxorubicin and doxorubicinone in the samples examined were calculated by the calibration curves. Four vials for each lot were analysed.

Results and Discussion

Iododoxorubicin shows a good fluorescence and therefore the sensitivity of the method was increased using a fluorescence detector. Figures 2 and 3 show the chromatograms obtained by injecting the same quantity of iododoxorubicin, added with a known amount of doxorubicinone, using a diode array or a fluorescence detector. A slight difference between the best λ excitation and λ emission values of doxorubicinone (472 nm ex, 547 nm em) and iododoxorubicin (472 nm ex, 583 nm em) was noted (Fig. 4). In the analysis of the samples, the best conditions for the impurity were chosen.

As doxorubicinone is the most probable degradation product, only this impurity was determined. However, the proposed method can also be used for the analysis of all impurities giving rise to peaks in the chromatogram. The quantitative analysis was performed using an external standard method because of the difficulty in obtaining suitable, structurally-related internal standard. The calibration curves obtained by the analysis of iododoxorubicin and doxorubicinone were linear over the concentrations range considered. The correlation coefficients and relative standard deviations, obtained from 10 determinations, were 0.994 ± 1.6% for iododoxorubicin and 0.997 ± 1.3% for doxorubicinone. The proposed method was checked by analysing laboratory samples prepared by adding known amounts of doxorubinone to iododoxorubicin

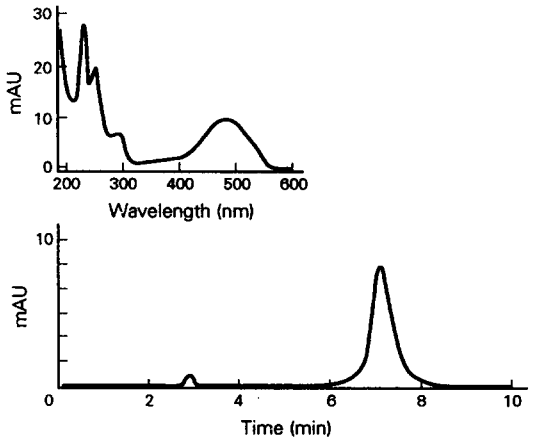

Figure 2
Chromatogram of iododoxorubicin, containing adriamycinone as impurity, and its UV spectrum obtained using a diode array detector.

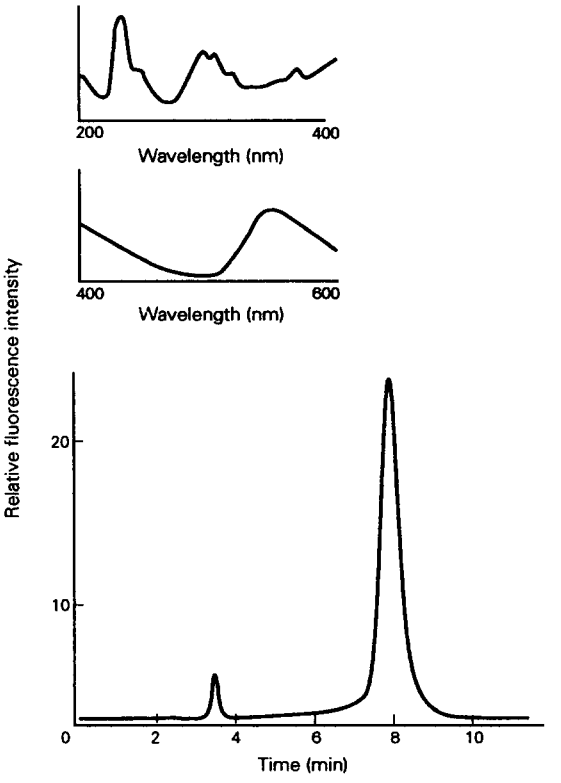

Figure 3
Chromatogram of iodoxorubicin, containing adriamycinone as impurity, and its fluorescence spectrum obtained with a fluorimetric detector.

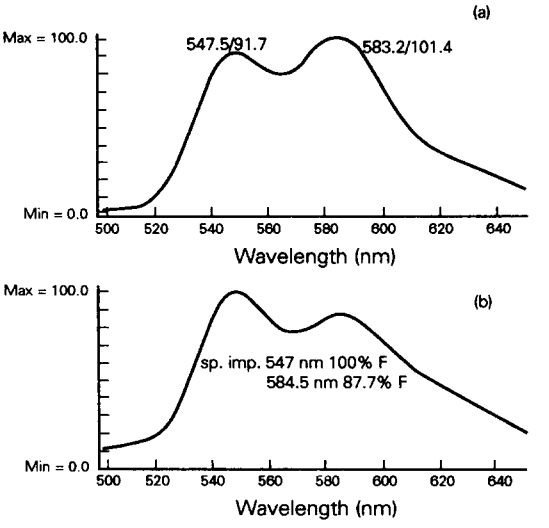

Figure 4
Chromatogram of lyophilized sample: the first peak is related to adriamycinone.

reference standard. The minimum detectable amount of impurity is about 4 ng ml^{-1} using fluorimetric detection and about 8 ng ml^{-1} using the diode array detector.

The quantitative analysis was carried out on the vials containing a declared amount of 10 mg of lyophilized iododoxorubicin. The amount of antibiotic in the vial takes into account the iododoxorubicin strength in the original bulk material. The quantitative data obtained from the analysis is summarized in Table 1.

Table 1
Control analyses of iododoxorubicin vial samples

	Batch A	Batch B	Batch C
Iododoxorubicin	91.6	92.1	92.15
Doxorubicinone	4.5	4.2	4.3

The results are expressed as percentages and each one is the average of three determination.
The analyses were carried out intentionally on the old samples.

The analysis of iododoxorubicin must be carried out immediately after the preparation of working solutions because after 24 h, it is possible to note the formation of unknown compounds insoluble in the usual polar and non polar solvents.

The proposed chromatographic analysis allows a good separation of iododoxorubicin not only from doxorubicinone, but also from the other related compounds. Therefore this can be a useful method to test the purity and the stability of this anthracycline antibiotic.

Acknowledgement — This work has been supported by a grant from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica italiano.

References

[1] The 79th Annual Meeting of the American Association for Cencer Research, New Orleans, May 25–28, 1102 (1988).
[2] R. Brown, *Prog. Med. Chem.* **15**, 125–164 (1978).
[3] R. Brown and S.H. Iman, *Prog. Med. Chem.* **21**, 170–236 (1984).
[4] B. Schott, P. Vrignau, C. Ries, J. Robert and D. Londos-Gagliardi, *Br. J. Cancer* **61**, 543–547 (1990).
[5] Y. Singh, L. Ulrich, D. Katz, P. Bowen and G. Krishna, *Toxicol. Appl. Pharmacol.* **100**, 9–23, (1989).
[6] A.M. Isetta, M.C. Fornasiero, M. Ferrari and D. Trizio, *Int. J. Immunopharmacol.* **12**, 665–673 (1990).
[7] R. Mariani, M. Farina and W. Sfreddo, *J. Pharm. Biomed. Anal.* **7**, 1877–1882 (1989).
[8] A. Vigevani and M.J. Williamson, *Anal. Prof. Drug Subst.* **9**, 245–274 (1980).
[9] S. Eksborg, H. Ehrsson, B. Anderson and M. Beran, *J. Chromatogr.* **153**, 211–218 (1978).
[10] C.M. Riley and A.K. Runyan, *J. Pharm. Biomed. Anal.* **5**, 33–43 (1987).
[11] E. Moro, V. Bellotti, M.G. Jannuzzo, S. Stegnjaich and G. Valzelli, *J. Chromatogr.* **274**, 281–287 (1983).
[12] R. Mariani, M. Farina and W. Sfreddo, *J. Pharm. Biomed. Anal.* **7**, 1877–1882 (1989).
[13] K. Mross, U. Mayer, K. Hammand and D.K. Hossfeld, *J. Chromatogr.* **530**, 192–199 (1990).

[Received for review 29 April 1991; revised manuscript received 3 September 1991]