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Review

Anthracyclines: recent developments in their separation and quantitation

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Abstract

Anthracyclines are among the most widely used anticancer agents. Notwithstanding the large efforts to develop new drugs with a better pharmaceutical profile, daunorubicin, doxorubicin, epirubicin and idarubicin are still the most used in clinical practice. Many efforts are now ongoing to reduce the side effects by using pharmaceutical formulations able to release the drug in the most appropriate way and monitoring the quantity of anthracyclines and their metabolites in the body fluids or tissues frequently and in every patient to maintain the drug concentration within the expected range. This review describes the most recent developments in the separation and quantitation of the above clinically useful drugs, together with their principal metabolites. Some less widely used derivatives will also be considered.

Keywords: Reviews; Anthracyclines; Daunorubicin; Doxorubicin

Contents

1. Introduction

Anthracyclines were isolated from a pigment-producing *Streptomyces* [1]. They have been used for

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more than 30 years and they can still be considered among the most useful anticancer agents ever developed: most patients treated with systemic cancer chemotherapy receive an anthracycline at some time during the treatment. From the 1960s to the present day more than 200 naturally occurring anthracyclines have been identified and many hundred derivatives have been synthesized [2,3] in order to overcome their high toxicity and the multi-drug resistance (MDR). Among the undesirable side effects are myelosuppression, stomatitis, nausea and vomiting; but the most severe side effect is a cumulative dose-related cardiotoxicity, commonly attributed to a radical damage on the cardiac tissue [4]. As a consequence of this very large research effort eight anthracyclines have been marketed and more than six new derivatives and three formulations are in clinical trials. Most of them bear a morpholino group at position 3′ or 4′ of the sugar residue, while others have a hydrophilic group attached to the side chain in position 9 (Fig. 1).

To the best of our knowledge, at the present the clinically most used anthracyclines are: daunorubicin (DNR — daunomycin, rubidomycin), doxorubicin (DXR — adriamycin), epirubicin (EPI) and idarubicin (IDA). As a consequence they will be discussed individually in this review while some of the others anthracyclines will be treated as a group. The chemical structure of the anthracyclines that will be considered and their principal metabolites are shown in Fig. 1.

Although many other anthracycline analogs are being tested [5–7] and many laboratories are working to elucidate the mechanism of action at the molecular level [8–12], DNR, DXR, IDA and EPI are still the most used in clinical practice. As a consequence of the lack of introducing new anthracyclines in the common clinical practice, the main interest is actually in the determination of the concentration of the drugs in biological samples using a fast and reliable method. It has been shown that the clinical efficacy of the anthracyclines is related to their actual concentration in the tumor tissue [13] and this parameter varies from patient to patient and has to be evaluated for everyone. Another topic that is still very deeply investigated is the way of administration. The interest for methods of administration avoiding high peaks in the plasma concentration of the drug stems from the observation that the cardiotoxicity of doxorubicin and daunorubicin is considerably reduced when the drug is administered by continuous infusion or linking it to a polymer or using a liposomal encapsulation that releases the drug very slowly [4,14–16].

As a consequence, almost all the papers published in the period 1995–2001 deal with the analysis of the anthracycline content in biological samples or in pharmaceutical preparations.

2. Daunorubicin (DNR) and doxorubicin (DXR)

Many publications are concerned both with DNR and DXR and for this reason they are considered together in this review.

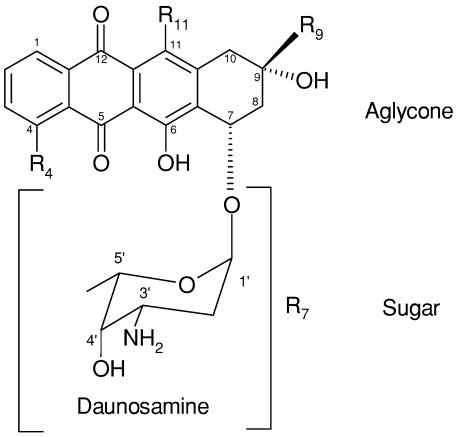
DNR was the first anthracycline introduced in the market as an antineoplastic agent in 1967 and it is now used for the treatment of acute granulocytic and acute lymphocytic leukemias. DNR is administered intravenously and has a half-life of 30 min, it is metabolized primarily in the liver to daunorubicinol, an active metabolite, and other metabolites where the aglyconic part is separated from the sugar [17,18].

DXR, although slightly different from DNR, displays activity against a wide range of human neoplasms, including a variety of solid tumors. It is used concurrently with cyclophosphamide, vincristine, bleomycin and prednisone.

2.1. Determination of daunorubicin and doxorubicin in biological fluids

2.1.1. Capillary electrophoresis separation

The requirement of determining the concentration of the drug in very diluted samples without preconcentration has been investigated recently using capillary electrophoresis and different detectors. Very interesting is the use of capillary zone electrophoresis coupled with an amperometric detector [19]. In this case to a sample of urine DNR was added in amounts ranging from 4.00×10^{-6} *M* to 1.00×10^{-4} *M* and the quantity of dissolved DNR was determined by measuring the height of the oxidation peak of the two phenolic hydroxyl systems. The recovery ranged from 109.0 to 93.2%, the linear range was (0.002–0.2) mmol/l and the detection



	Anthracycline	R_4	R_9	R_7
1	**Daunorubicin (DNR)**	OCH_3	$-C(=O)CH_3$	Daunosamine
1a	daunorubicinol	OCH_3	$-CH(OH)CH_3$	Daunosamine
1b	daunorubicinone	OCH_3	$-C(=O)CH_3$	OH
1c	7-deoxydaunorubicinone	OCH_3	$-C(=O)CH_3$	H
2	**Doxorubicin (DXR)**	OCH_3	$-C(=O)CH_2OH$	Daunosamine
2a	doxorubicinol	OCH_3	$-CH(OH)CH_2OH$	Daunosamine
2b	doxorubicinone	OCH_3	$-C(=O)CH_2OH$	OH
2c	7-deoxydoxorubicinone	OCH_3	$-C(=O)CH_2OH$	H
2d	7-deoxydoxorubicinolone	OCH_3	$-CH(OH)CH_2OH$	H
2e	doxorubicinolone	OCH_3	$-CH(OH)CH_2OH$	OH
3	**Idarubicin (IDA)**	H	$-C(=O)CH_3$	Daunosamine
3a	idarubicinol	H	$-CH(OH)CH_3$	Daunosamine
3b	idarubicinone	H	$-C(=O)CH_3$	OH (S configuration)
3c	epi-idarubicinone	H	$-C(=O)CH_3$	OH (R configuration)
4	**Epirubicin (EPI)**	OCH_3	$-C(=O)\ CH_2OH$	Daunosamine (4' epimer)
4a	epirubicinol (13-S-epirubicin)	OCH_3	$-CH(OH)CH_2OH$	Daunosamine (4' epimer)
4b	epirubicinone	OCH_3	$-C(=O)\ CH_2OH$	OH
4c	7-deoxydoxorubicinone (2c)	OCH_3	$-C(=O)CH_2OH$	H
4d	7-deoxydoxorubicinolone (2d)	OCH_3	$-CH(OH)CH_2OH$	H
4e	epirubicin-glucoronide	OCH_3	$-C(=O)CH_2OH$	Daunosamine (4' O-Glucuronide)
4f	epirubicinol-aglycon	OCH_3	$-CH(OH)CH_2OH$	OH
5	**Pirarubicin**	OCH_3	$-C(=O)CH_2OH$	$O(CH\text{-}(CH_2)_4\text{-}O)$
6	**Zorubicin**	OCH_3	$-C(CH_3)=N\text{-}NH\text{-}COC_6H_5$	

Fig. 1. Chemical structure of anthracyclines and their metabolites.

limit 0.8 μmol/l. Perez-Ruiz and co-workers used the capillary electrophoresis technique to separate DRN, DXR and IDA in serum samples, deproteinized by addition of acetonitrile and water and filtering through a 0.45 μm filter, using a laser-induced fluorescence detector [20]. In particular they examined the excitation/emission wavelengths, the pH and the composition of the mobile phase. Using optimal conditions, the method showed linearity in the range 10–500 ng/ml and in the interval 25–250 ng/ml the RSDs ($n=3$) were below 4% for the three anthracyclines examined. A related paper was published by Siméon and co-workers [21]: they determined the content of DNR in biopsies and plasmas from patients having a Kaposi sarcoma at different times using laser-induced fluorescence (LIF) and

rhodamine as the internal standard. Comparison of a doxorubicin/daunorubicin (1:1) mixture separated with HPLC using conventional fluorescence detection and LIF detection is reported in Fig. 2. The advantage of using LIF is clearly shown. The first paper describing capillary electrophoresis (CE) with laser-induced fluorescence (LIF) detection was published in 1992 [22]. In this paper the quantitation of a test mixture of doxorubicin (DOX) and EPI with a daunomycin (DAU) internal standard in plasma samples is described. Critical for the success of the method is the presence of acetonitrile both in the extracting and in evaluating the plasma samples. The effects of the injection volume, of the excitation/detection wavelengths and of the laser power on the efficiency and sensitivity (MDC, minimum detectable concentration) were examined. The concentration range was (0.5–10) ng/ml, the inter-day inaccuracy was from 1.4 to −1.5% for EPI and from 2.2 to −0.8% for DOX with a coefficient of variation (C.V.) of 1.0–6.9% while the intra-day inaccuracy was from 2.9 to 1.9% for EPI and from 2.1 to −2.1% for DOX with a C.V. of 1.7–5.4% and detection limits in the pg/ml range.

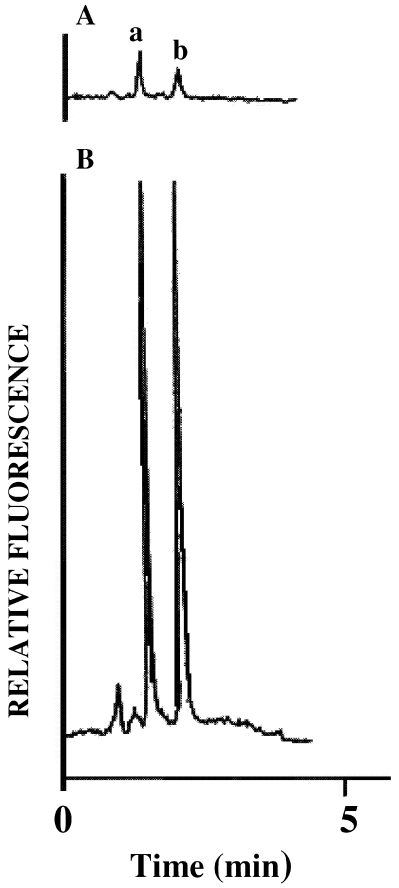


Fig. 2. Comparison of a 5 μl injection of a 1.8×10^{-9} *M* doxorubicin (a)–daunorubicin (b) (1:1) mixture separated with HPLC using (A) conventional fluorescence detection and (B) LIF detection. The chromatograms are presented with the same noise intensity. Adapted from Ref [21].

2.1.2. UV–visible spectroscopy quantitation

Four attractive methods for the quantitation of DXR hydrochloride using visible spectrophotometry is proposed by Sastry and Rao [23]. In the first protocol they oxidize the phenols with Fe(III) and determine the reduced Fe(II) with 1,10-*ortho*-phenantroline; in the second they substitute the iron with Folin–Ciocalteu reagent and read the absorbance of the solution at 770 nm; finally in the third and fourth protocol they oxidize the drug with periodate to get formaldehyde and dialdehyde and react the aldehydes either with 3-methyl-2-benzothiazolinone hydrazone or with phenylhydrazine and they read the absorbance of the formed hydrazones at 620–670 nm or 510 nm. The detection limit is in the interval 0.034–0.42 μg/ml and the RSD in the range of 0.70–0.44%.

2.1.3. HPLC separation

Some methods have been published for the determination of DNR, DXR, IDA and EPI and their rubicinol metabolites in biological fluids in which the substances were separated by HPLC and detected by different techniques. In the first method [24], the anthracyclines were added to human plasma from healthy volunteers and the pH adjusted to 8.4. The solutions were extracted with chloroform–1-heptanol (9:1 v/v) and the separated organic layer was extracted with water containing 0.1 *M* orthophosphoric acid. The anthracyclines were separated using a Supelcosil LC–CN, 5 μm column and observed by a fluorescence detector with λ_{ex}=480 nm and λ_{em}= 560 nm. The linearity was checked in the interval 0.4–10^4 ng/ml, the accuracy was in the range of 91–107% for all compounds and the precision values

of the method were <10%. The recoveries were 93–109% for DNR/daunorubicinol and IDA/idarubicinol, 67–109% for DXR/doxorubicinol and 61–109% for EPI/epirubicinol. The chromatograms obtained injecting 50 μl of 2.5 ng/ml of anthracyclines and some of their metabolites are shown in Fig. 3 (in abscissa the time-scale is in minutes).

In the second method the same anthracyclines were examined but aclarubicin was added as the internal standard and the detector was a mass spectrometer and two or three ions per anthracycline were evaluated [25]. The mass spectra obtained for anthracyclines and active metabolites are presented in Fig. 4, and a total ion chromatogram of an extract of a serum sample is reported in Fig. 5. To the serum were added the compounds and they were separated using SPE (solid-phase extraction) and then a RP18, 3.5 μm column. The limit of quantitation was 2.5 ng/ml for DXR, EPI and daunorubicinol and 5 ng/ml for DRN, IDA, doxorubicinol and idarubicinol; the linearity has been checked within 0–2000 ng/ml for the drugs and 0 –200 ng/ml for their rubicinol metabolites and the RSD, within-day and intra-day, was less than 15%.

In the third method [26], only DXR and metabolites **2a**, **2d** and **2e** (Fig. 1) were examined, but in many murine tissues and in blood. DNR was used as the internal standard and to the homogenized tissues DXR and metabolites were added. The samples were extracted with chloroform–1-propanol (4:1 v/v), the solvent evaporated and the solution reconstituted with acetonitrile–tetrahydrofuran (40:1) and eluted in a RP8, 7 μm, column with a mobile phase of water (pH=2.05)–acetonitrile–tetrahydrofuran (80:30:1 v/v/v). The eluent was monitored fluorimetrically with λ_{ex}=460 nm and λ_{em}=550 nm. The accuracy and precision were strongly dependent from the matrix and accurate tables are given. Ricciarello and co-workers [27] developed an alternative system of detection based on an electrochemical detector, composed of an amperometric electrode coupled with a coulometric electrode, for the simultaneous determination of DXR and EPI and their active metabolites **2a** and **4a** (Fig. 1) in human plasma.

The determination of DXR and some metabolites in plasma has been described in other similar papers [28–30]. In two of them the deproteinization was carried out using zinc sulphate and in all of them RP columns and fluorescence detector were used.

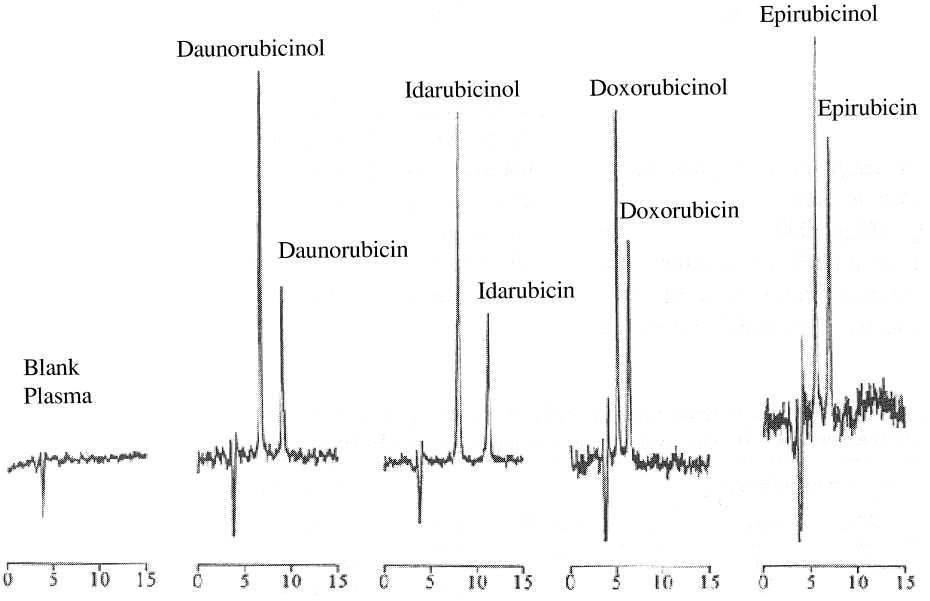


Fig. 3. Chromatograms obtained after injecting 50 μl of a 2.5 ng/ml solution. Adapted from Ref [24].

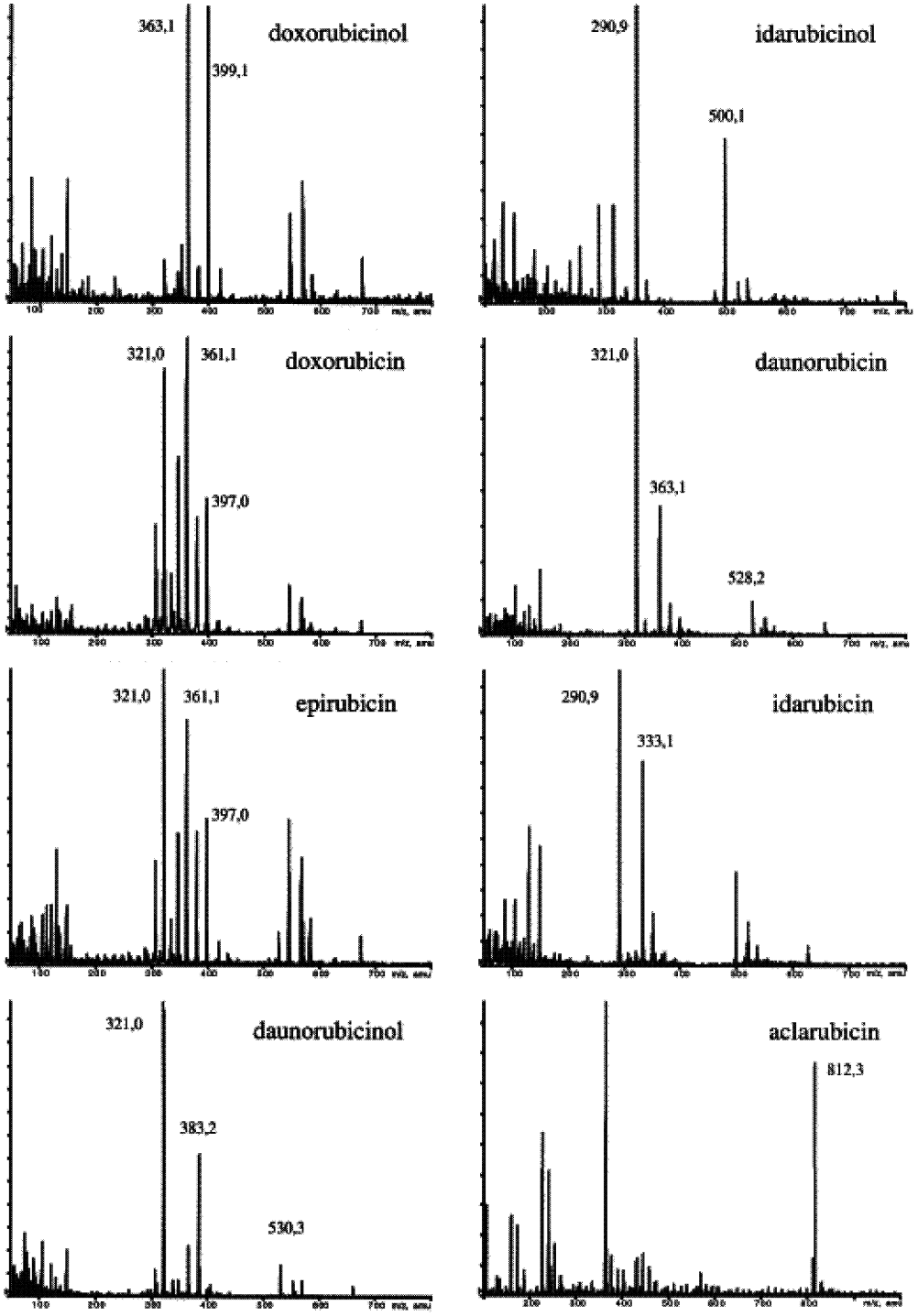


Fig. 4. Mass spectra of the anthracyclines and active metabolites analyzed. Adapted from Ref [25].

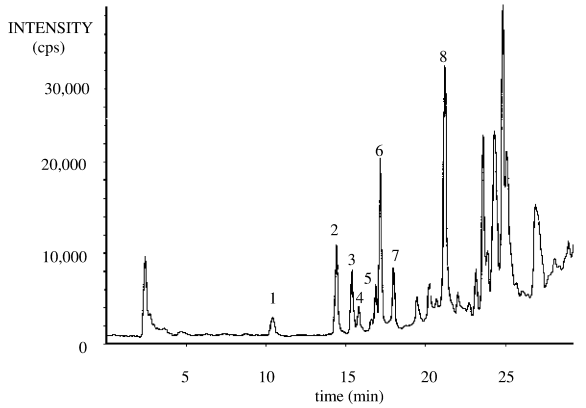


Fig. 5. Total ion chromatogram of an extract of a serum sample spiked at 50 ng/ml for the parent drugs and 20 ng/ml for the metabolites [1=doxorubicinol; 2=doxorubicin; 3=epirubicin; 4=daunorubicinol; 5=idarubicinol; 6=daunorubicin; 7=idarubicin; 8=aclarubicin (I.S.)]. Adapted from Ref. [25].

A summary of conditions for anthracycline separation by HPLC is reported in Table 1.

2.2. Bioconjugates and prodrugs

In the last few years many polymer-bound anthracyclines have been prepared that, following cellular uptake via pinocytosis and the linker cleavage by lysosomal enzymes, are released as intratumoral drug [31,32]. A few papers have been published on this topic. Configliacchi et al. [33] utilize a methacrylamide polymer to which DXR (anticancer part) and D-galactosamine (a liver targeting molecule) are bound. Their method allows the determination of DXR, as the contaminant product, and of the hydrolysis product **2c**. For DXR within the interval 1–50 μg/ml the average recovery was 98.7% and the RSD was 0.75%. The authors investigated also the hydrolysis condition and 1 *M* HCl, 50°C, 1.5 h was recognized as the best compromise between maximal conversion (>99%) and the stability of the formed product. A good linearity was observed in the interval 5–50 μg/ml for **2b** and the average recovery was of 96.9%.

Fraier's et al. [34] determination of DXR, **2a**, **2c**, **2d**, **2e**, **2f**, from a methacrylamide-bound DXR, in human plasma and urine is very interesting. DNR and **1c** were used as internal standards since the first was extracted with DXR at physiological pH with 2-propanol–chloroform (25:75 v/v) while the second was extracted at pH 8.4 with **2a**, **2c**, **2d**, **2e**, **2f** with the same extraction mixture. The organic phases were pooled and the solvents evaporated, the residue was taken up in methanol–0.5 *M* H_3PO_4 (50:50 v/v) and washed with hexane. The authors are also able to evaluate the polymer-bound and free drug. The method is accurate and precise for all the metabolites, the recovery ranges from 68% up to 89% for the different metabolites and DXR. In plasma, the limit of quantification ranges from 0.31 to 5.1 ng/ml, while in urine the corresponding interval is 9.78–25.50 ng/ml.

Another prodrug examined is glucoronyl–DXR (HMR 1826) [35] having the daunosamine residue linked to a group (Fig. 6) that gives a non-toxic compound. Such prodrug can be activated by an enzyme catalyzed hydrolysis in the tumor tissue.

To determine their efficacy and the selectivity of a particular prodrug therapy, the quantification of the

Table 1
Summary of some representative results for HPLC separation of anthracyclines

Compound(s)	Sample	Separation method	Detection method	Linear range mol/l	Recoveries %	Sensitivity mol/l	Precision or accuracy	Ref.
DNR	Urine	CZE	AD	$(0.002–0.2)\times10^{-3}$	93.2–109.0	8×10^{-7}	RSD<3.1%	[19]
DNR, DXR, IDA	Serum	CE	LIF	$(0.02–0.9)\times10^{-6}$	94–98	2×10^{-9}	RSD 1.0–3.2 intra-day	[20]
DNR (DXR)	Biopsies, plasma	CE	LIF	10^{-10}–10^{-8}	–	5×10^{-11}	RSD<0.6 plasma	[21]
DXR, EPI	Plasma	CE	LIF	–	45–70	$\sim0.2\times10^{-9}$	RSD 1.0–6.9 intra-day	[22]
DXR	Pure	–	UV–vis	$\sim0.7\times10^{-6}$–4×10^{-5}	–	0.6×10^{-6}	RSD 0.44–0.63	[23]
DNR, DXR, IDA, EPI, metabolites	Plasma	HPLC	Fluorescence	0.7×10^{-9}–2×10^{-5}	61–109	0.7×10^{-9}	RSD<10 accuracy >91%	[24]
DNR, DXR, IDA, EPI, metabolites	Serum	HPLC	ES–MS	5×10^{-9}–4×10^{-6}	85–105	5×10^{-9}	RSD 2–30 intra-day	[25]
DXR, metabolites	Tissues, blood	HPLC	Fluorescence	–	60.8–77.0	0.2×10^{-9}	RSD<13 intra-day	[26]

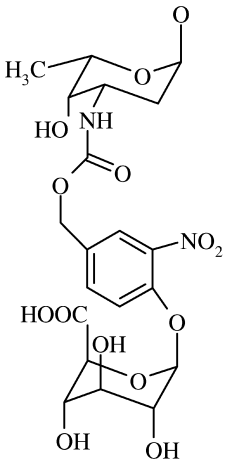


Fig. 6. Chemical structure of the *N*-(4-β-glucuronyl-3-nitrobenzyloxycarbonyl)daunosamine residue.

prodrug, drug and metabolites is an essential issue. In this paper the prodrug HMR 1826, DXR and the metabolites **2a–2e** were homogenized with normal and tumor lung tissue and EPI was added as the internal standard. After elimination of proteins and DNA, the clear solution was injected in a RP18, 5 μm column. The separation was performed with 20 m*M* citric acid (+0.14% triethylamine; pH 2.4)–acetonitrile–methanol–tetrahydrofuran (100:50:25:5 v/v/v/v) and the substances were detected fluorimetrically with λ_{ex}=490 nm and λ_{em}=590 nm. For concentrations of 0.05, 1, 10, 20, 300 μg/g the recoveries were in the range of 90–94% for DXR, 91–88% for the prodrug, 89–88% for **2a**, 94–97% for **2e**, 96–97% for **2d**, 99–97% for **2b** and 98–97% for **2c** with a good precision intra-assay and inter-assay. The liposomal and free forms of DNR and of his metabolite daunorubicinol administered as liposomal formulation (DaunoXome) were separated and quantified by HPLC using a laser-induced fluorescence detector [14]. For this purpose the plasma was flowed through an Sep-pak C_{18} cartridge equilibrated with a buffer phosphate to separate the liposomal (eluate) from free DNR and his metabolite that were then eluted with methanol, the liposome were disrupted with Triton X-100 and the DNR released purified as described above. The methanol was evaporated and to the reconstituted solution DXR was added as internal standard then the mixture was separated using a C_{18} Radial Pak column and detected with a laser-induced fluorescence detector. The total recovery was in the range 80.8±8.9% and the limit of detection for DNR and daunorubicinol was of 1 ng/ml. The within-day precision varied between 3 and 10% while the between-day precision varied in the range of 5 to 17%.

The development of MDR is still a major drawback in the chemotherapy of cancer, for this reason mixtures of non cross-resistant drugs are often used. It is known that a membrane-associated P-glycoprotein hyperexpression causes this effect. In their paper, Tassin and co-workers describe an HPLC method which enables the simultaneous determination of three cytotoxic drugs (DNR, DXR, vicristine) and two modulators (S 9788 and verapamil) in two cell lines [36].

3. Idarubicin

IDA is a synthetic anthracycline lacking a methoxy group in position 4 of the aglycone. The drug is less cardiotoxic and it is indicated for use in combination with cytarabine (Ara-C) for induction therapy of acute myelogenous leukemia (AML). IDA was approved by the FDA in 1990. IDA is currently administered by slow intravenous injection and it is converted, by a ketoreductase, to the active metabolite idarubicinol. IDA metabolism occurs mainly in the liver, however plasma clearance values suggest extensive extrahepatic metabolism.

An HPLC method for the simultaneous determination of IDA and idarubicinol in rat blood samples has been published recently [37]. After deproteinization with acetonitrile, IDA and its metabolite were separated on a RP18, 5 μm column and were detected with a fluorescence spectrometer, λ_{ex}=485 nm and λ_{em}=542 nm. An external calibration curve with nine points, from 1.0 to 500.0 ng/ml, was used. The mean recovery was 95.6 for IDA and 90.7 for idarubicinol while daily RSD was 3.2% for IDA and 4.4% for the metabolite.

Another method for the determination of IDA and idarubicinol in plasma but using capillary electrophoresis to separate the parent drug from the metabolite has been published [38]. This method is particularly useful for pharmacokinetic studies. Plasma was withdrawn from young children, where the

blood volume collected should be kept to a minimum. Capillaries were pretreated in order to remove the interaction of the anthracycline with the glass and DNR was used as the internal standard. Sodium phosphate buffer (pH 7.4) was added to the plasma and the internal standard, then the mixture was extracted with chloroform. The separated chloroform was evaporated and the residue taken up in acetonitrile–water (95:5). After separation, the substances were detected by a laser-induced fluorescence. The recovery was 70% for IDA and 68% for the metabolite and the RSD at 1 ng/ml was 2.3% and 2.5%, respectively.

4. Epirubicin

EPI is the 4′-epimer of DXR and a semi-synthetic derivative of DNR. EPI has similar response rates to DXN in non-small cell and small cell lung cancer, non-Hodgkin's lymphoma, ovarian cancer, gastric cancer and hepatocellular carcinoma. At equimolar doses EPI is less myelotoxic than DXR and has a lower incidence of cardiotoxicity. The FDA approved EPI in 1999. EPI is administered intravenously and it is rapidly and extensively metabolized by the liver and other tissues, including red blood cells. Metabolism of EPI occurs through four major routes: (1) reduction of the C-13 keto-group in R_9 with the formation of the 13(*S*)-dihydro derivative, epirubicinol, (2) conjugation of both the unchanged drug and epirubicinol with glucuronic acid, (3) loss of the amino sugar moiety through a hydrolytic process with the formation of doxorubicin and doxorubicinol aglycones, and (4) loss of the sugar moiety through a redox process with the formation of 7-deoxy-doxorubicin aglycone. To find an analytical procedure for EPI, **4a**, **4c**, **4d** and **4e** that is rapid, robust and has a high sensitivity for EPI and its metabolites in plasma samples, was the target of Barker and co-workers [39]. Plasma from blood of patients with multiple myeloma was separated by centrifugation and the proteins separated. Then the EPI and metabolites were separated by HPLC on a RP18, 5 μm column and detected fluorimetrically with λ_{ex}=480 nm and λ_{em}=560 nm. Having an external standard calibration curve, the method was validated in the concentration range 5–100 ng/ml for EPI and the RDS was 5–9% in this range. Recovery of metabolites at 20 ng/ml ranged from 94 to 104%.

Recently, new pharmaceutical preparations are being tested with an embolizing agent that leads to the reduction of blood flow. Thus the local drug concentration and the contact time of the anthracycline with the target organ are augmented. A specific and fully automated coupled-column LC method for the determination of EPI and **4a**, **4b**, **4c**, **4f** in human plasma, liver homogenate and liver tumor homogenate has been developed by Rudolphi and co-workers [40]. The authors coupled a sample processing precolumn [41] to an analytical RP column and a fluorescence detector with λ_{ex}=445 nm and λ_{em}=560 nm. In this procedure they avoided the separation of the proteins from the sample. The precision and the accuracy of the method were found to be very good. In a related paper Yamazoe and co-workers determine the content of carboplatin, EPI and mitomycin C in a Lipiodol® solution [42] to check the chemical stability of these drugs in the lipidic emulsion and they measure the drug-release profile. Lipiodol is an embolizing agent showing a selective distribution and retention in liver tumors. To determine the drugs content of the emulsion, hydroquinone was added as the internal standard, and then Tween® was added to obtain a clear solution. The mixture was then applied to purification by HPLC and the drugs determined spectrophotometrically with a multi-wavelength detector. The accuracies for the dissolved drugs and for the drug release were in the ranges of 96–104% and 98–108%, respectively, and the RSD<8% and <2%, respectively.

EPI and DXR were simultaneously determined in human plasma by Ricciarello [27] as described previously.

5. Other anthracyclines

As outlined above, slow administration of anthracyclines reduces their unwanted side effects. The stability of zorubicin in pharmaceutical formulations for continuous intravenous infusion has been studied by Benaji and collaborators [43]. Zorubicin is generally administered when the patients show resistance to DXR and DNR. Zorubicin is highly unstable

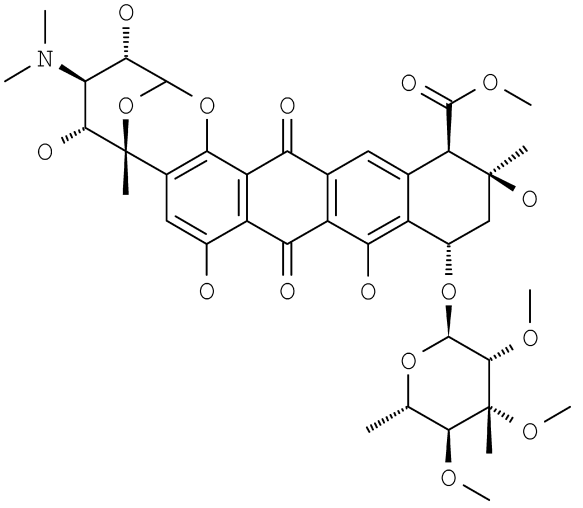


Fig. 7. Chemical structure of nogalomycin.

under mildly acidic conditions: at 5<pH<6 degradation of Zorubicin was observed, at pH 6.8 the solution was stable. However 6–10% contamination of DNR from the hydrazone hydrolysis is always present.

Nogalomycin, an anthracycline from *Streptomyces nogalator*, has been withdrawn from the clinical trials for its toxicity. Its structure is shown in Fig. 7.

Finally the voltammetric behavior of nogalomycin was determined with a hanging mercury drop electrode and a cathodic adsorptive stripping voltammetric method for the determination of the drug was found [44]. Two peaks characterize the cyclic voltammograms of nogalomycin: one peak is attributed to the reduction of the quinoid system and the other to the reduction of a Hg–nogalomycin complex which forms at the electrode surface. The first can be used for concentrations in the range 1.30–40 mg/l (RSD=3.6%) while the second in the interval of 0.10–1.45 mg/l (RSD=1.5%).

6. Conclusions

The efficacy of the conventional anthracyclines as anti-tumor drugs coupled with the difficulty of obtaining new and better drugs in this class has prompted the research in two directions: the monitoring of the drug concentration and the search for new pharmaceutical formulations. To monitor the drug concentration in the body, fast and sensitive determinations are needed. For this purpose, methods were developed simplifying the procedure for the separation of the drug or its metabolites from the matrix and allowing the use of small quantities of material, e.g., capillary electrophoresis. The analytical methods developed in the period covered by this review allow the precise and accurate determination of the anthracyclines and their metabolites in polymers, in lipid suspensions and in small biological samples.

7. Nomenclature

AD	amperometric detection
AML	acute myelogenous leukemia
CE	capillary electrophoresis
C.V.	coefficient of variation
CZE	capillary zone electrophoresis
DNR	daunorubicin, daunomycin, rubidomycin
DXR	doxorubicin, adriamycin
EPI	epirubicin
FDA	Food and Drug Administration
IDA	idarubicin
LIF	laser-induced fluorescence
MDC	minimum detectable concentration
MDR	multi-drug resistance
RP	reversed-phase
RSD	relative standard deviation

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