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Determination of idarubicin and idarubicinol in plasma by capillary electrophoresis

Georg Hempel[a,*], Stephan Haberland[b], Petra Schulze-Westhoff[a], Nora Möhling[a], Gottfried Blaschke[b], Joachim Boos[a]

[a]*Universitäts-Kinderklinik, Abt. Hämatologie/Onkologie, Albert-Schweitzer-Str. 33, 48129 Münster, Germany*
[b]*Institut für pharmazeutische Chemie der Universität Münster, Hittorfstr. 58-62, 48149 Münster, Germany*

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Abstract

A rapid and sensitive capillary electrophoretic method for the determination of idarubicin and its metabolite idarubicinol in plasma has been developed and validated. Plasma is extracted by liquid–liquid extraction using chloroform. Idarubicin, idarubicinol and the internal standard daunorubicin can be separated in less than 5 min using a phosphate buffer of pH 5 with 70% acetonitrile. Laser-induced fluorescence detection with an Ar ion laser operated at 488 nm provides a sensitive and selective detection method without interferences from biological fluids. The small sample volume of 100 μl is of particular advantage for studies in pediatric oncology. The reproducibility of the method has been shown to be sufficient for drug monitoring or pharmacokinetic studies. The limit of quantification for idarubicin in plasma is 0.5 ng/ml. © 1997 Elsevier Science B.V.

Keywords: Idarubicin; Idarubicinol

1. Introduction

Idarubicin (4-demethoxydaunorubicin, Fig. 1) is an anthracycline which exerts antitumour activity in several solid tumors and hematological malignancies [1]. Because the molecule is more lipophilic than the other anthracyclines it can be administered orally [2]. The drug seems to be less cardiotoxic than doxorubicin and daunorubicin, especially when given by mouth. Unlike most other anthracycline metabolites, idarubicinol, the C-13 alcohol metabolite of idarubicin (Fig. 1), is equipotent to the parent drug in its antineoplastic effect [3]. The elimination half-life of idarubicinol is longer and systemic exposure to the metabolite is greater compared with the parent drug. In addition, idarubicinol penetrates into the cerebrospinal fluid to a greater extent than idarubicin. After oral administration, idarubicin bioavailability is about 40% [4].

Previously published studies have described several methods of high-performance liquid chromatography (HPLC) for the simultaneous quantification of idarubicin and idarubicinol [3,5–7]. Usually, 0.4–1 ml of plasma is needed for the preparation of each sample. This amount is too large for pharmacokinetic

*Corresponding author.

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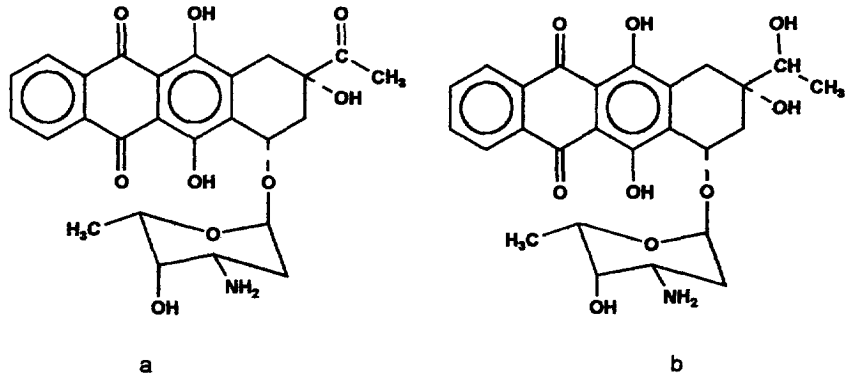

Fig. 1. Idarubicin (a) and idarubicinol (b).

studies in young children, when the blood volume collected should be kept to a minimum. Our aim was to develop an analytical method which would require only small sample volumes for pharmacokinetic studies of idarubicin and idarubicinol in children at different routes of administration and varying dosing schedules.

Capillary electrophoresis (CE) is a separation technique that is characterized by high efficacy, short analysis time and small sample volumes. The usefulness of CE in cancer research has been demonstrated [8]. However, when using UV detection, the sensitivity of capillary electrophoresis is insufficient to monitor drugs in biological fluids given in low mg amounts. Hence, we used a highly sensitive detector system based on laser-induced fluorescence (LIF).

Reinhoud et al. [9] have reported an assay to determine doxorubicin and epirubicin in human plasma by capillary electrophoresis with laser-induced fluorescence detection. However, the required sample volume of 1 ml was too large for our purpose and metabolites were not quantified. We modified the conditions of electrophoresis and the extraction procedure to quantify idarubicin and its main metabolite in a plasma volume as small as 100 μl. In contrast to the method of Reinhoud et al., we used a CE instrument equipped with an autosampler, a commercially available detector and a less expensive Ar ion laser thus adapting to the requirements of clinical routine. Currently, the method is used to study the pharmacokinetics after oral and i.v. administration of idarubicin in children.

2. Experimental

2.1. Equipment

A Model 5510 P/ACE (Beckman Instruments). equipped with an air-cooled argon ion laser operating at 488 nm was used (5 mW, Beckman Instruments, Palo Alto, CA, USA). Detection was carried out with a Beckman LIF detector equipped with a 520-nm band pass filter. A fused-silica capillary of 40 cm effective length and an inner diameter of 50 μm was used (O.D. 375 μm, Beckman Instruments). Separation was done at 532 V/cm (25 kV) applied voltage resulting in a typical current of about 12 μA. Samples were applied to the capillary by electrokinetic injection at 10 kV for 5 s. Between runs, the capillary was rinsed with sodium hydroxide 100 m*M* for 1 min and the running buffer for 2 min.

2.2. Chemicals and reagents

Idarubicin, idarubicinol and daunorubicin were kindly supplied by Farmacia (Erlangen, Germany). All chemicals used were of analytical grade. Acetonitrile and sodium hydroxide solution (0.1 *M*) were purchased from Baker (Deventer, The Netherlands); spermine tetrahydrochloride from Aldrich (Steinheim, Germany); chloroform and sodium dihydrogen phosphate from Merck (Darmstadt, Germany). Purified water was prepared on a Millipore-Q-UF system. The running buffer was gained by adjusting a solution of 100 m*M* sodium dihydrogen

phosphate with 100 m*M* phosphoric acid to a pH value of 5.0. Subsequently, spermine was added to obtain a concentration of 60 μ*M* and the solution was mixed with acetonitrile to a final concentration of 70% (v/v). All solutions for CE were filtered through a 0.45-μm filter.

2.3. Preparation of standard solutions

Stock solutions containing 100, 10, or 1 ng/ml idarubicin and idarubicinol were prepared in acetonitrile. Daunorubicin was dissolved in acetonitrile to obtain a concentration of 20 ng/ml. The stock solutions were stored at −20°C and could be used for up to 12 weeks.

For the determination of idarubicin and idarubicinol after oral administration of 5 mg/m^2 standard solutions of 0.5, 1, 2, and 7 ng/ml were prepared from the stock solutions by dilution with blank plasma. For the drug monitoring after i.v. administration of idarubicin (30 mg/m^2) standard solutions of 1, 5, 10, and 20 ng/ml were prepared in the same manner. Dilutions of 0.5, 1 and 5 ng/ml in blank plasma served as quality control samples.

2.4. Sample preparation

Blood samples were collected in polypropylene tubes and immediately centrifuged to separate the plasma fraction. Samples were stored at −20°C until analysis. One hundred μl plasma (spiked plasma or patient plasma), 100 μl sodium phosphate buffer, pH 7.4 (100 m*M*), 50 μl internal standard (daunorubicin 20 ng/ml in acetonitrile), and 1 ml chloroform were vortexed for 1 min and then centrifuged at 1500 *g* for 5 min. Eight hundred μl of the organic phase were transferred into an Eppendorf vial and dried under a gentle stream of nitrogen at 35°C. The residue was reconstituted to 50 μl acetonitrile–water (95:5) and an aliquot was placed into 30-μl polycarbonate vials.

3. Results and discussion

3.1. Method development

Anthracyclines have been known to interact with the capillary wall due to their great affinity to glass surfaces [10]. During CE separation the interaction was decreased by modifying the electrophoresis buffer with acetonitrile. Spermine interacts with the silanol groups on the glass surface and thus reduces the interaction of the analytes with the capillary wall. Furthermore, the electroosmotic flow (EOF) is lowered because the free silanol groups are occupied. The spermine coverage is probably incomplete, but nevertheless necessary, to achieve a good peak shape. Omitting the between-run rinse with sodium hydroxide increases the migration time, and after a few runs EOF reversal can be achieved due to accumulation of spermine to the capillary wall. When 70% acetonitrile and spermine were added to the buffer, the peak shape and the resolution of idarubicin, daunorubicin and idarubicinol were sufficient (Fig. 2).

Pretreatment of the sample results in a sample matrix of low ionic strength (acetonitrile–water, 95:5). This is needed in order to increase the sensitivity by a strong zone-sharpening effect during electrokinetic injection. The influence of the amount of acetonitrile in the sample matrix using electrokinetic injection is demonstrated in Fig. 3. When injecting anthracycline solutions with acetonitrile amounts from 90 to 100%, the peak area increases almost five-fold.

In the present assay we used an internal standard (daunorubicin) for quantification because of its similarity to the analytes. Further, it is unnecessary to adjust for the discrimination effect which occurs when electrokinetic injection is applied. Moreover, deviations due to the extraction procedure and due to evaporation of the sample in the autosampler before injection can be corrected.

3.2. Quantification

Plasma concentrations of idarubicin and idarubicinol were calculated from calibration lines obtained by analysing spiked plasma samples. Calibration lines were computed using the corrected peak area (peak area/migration time) ratio of the respective analyte to the internal standard. We decided to keep the calibration range small and use two overlapping calibration ranges depending on the expected concentration of the samples. The procedure avoids sample dilution, which may introduce unnecessary

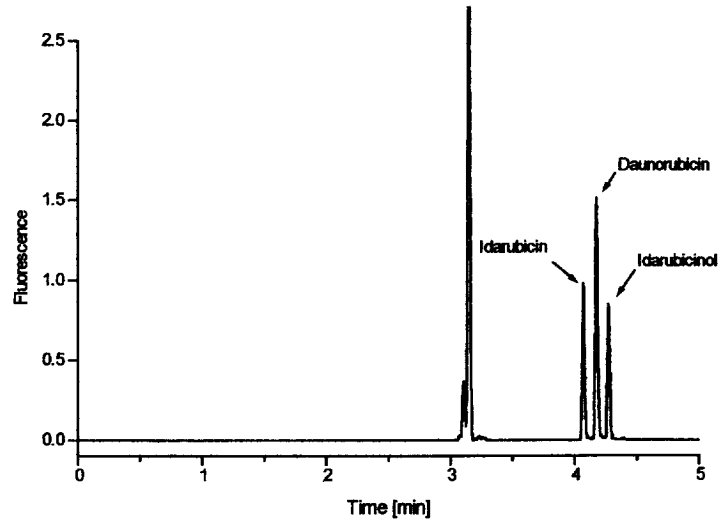

Fig. 2. Separation of idarubicin, idarubicinol and daunorubicin. Conditions: 50 μm; 47 cm-long capillary; phosphate buffer, pH 5.0; 60 μ*M* spermine; 70% acetonitrile; 532 V/cm.

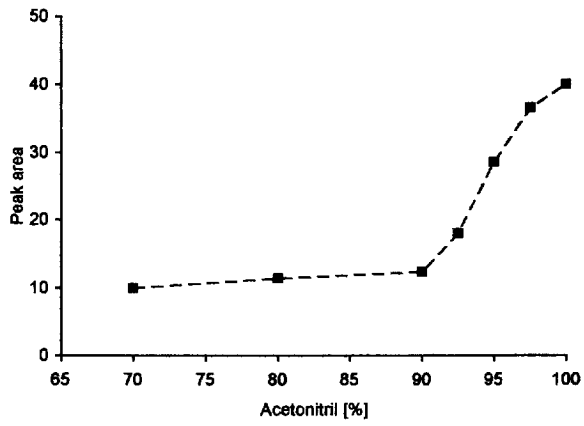

Fig. 3. Effect of the amount of acetonitrile in the sample matrix on the peak area. Conditions the same as in Fig. 2. Idarubicin (100 ng/ml) dissolved in different matrices.

errors to the assay [11]. This is of special importance for CE with electrokinetic injection, where the amount injected depends on the conductivity of the sample matrix. Typical parameters of the calibration lines are given in Table 1. The limit of quantification (LOQ) for idarubicin and idarubicinol, defined as the lowest concentration measured with acceptable precision and accuracy [12], was 0.5 ng/ml.

3.3. Reproducibility

The absolute recovery of idarubicin and idarubicinol was 70±5.5% and 68±8.5%, respectively, from spiked plasma samples determined at a concentration of 10 ng/ml with pressure injection. At lower concentrations, however, electrokinetic injection is needed due to insufficient sensitivity.

Table 1
Typical parameters of the calibration lines (S.E., standard error)

Range (ng/ml)	Analyte	Slope	S.E.	Intercept	S.E.	r^2
0.5–7	Idarubicin	0.5693	0.0260	−0.0008	0.1129	0.996
	Idarubicinol	0.4097	0.0125	0.0342	0.0541	0.998
1–20	Idarubicin	0.8393	0.0117	0.2925	0.1341	0.999
	Idarubicinol	0.6777	0.0214	0.4292	0.2459	0.998

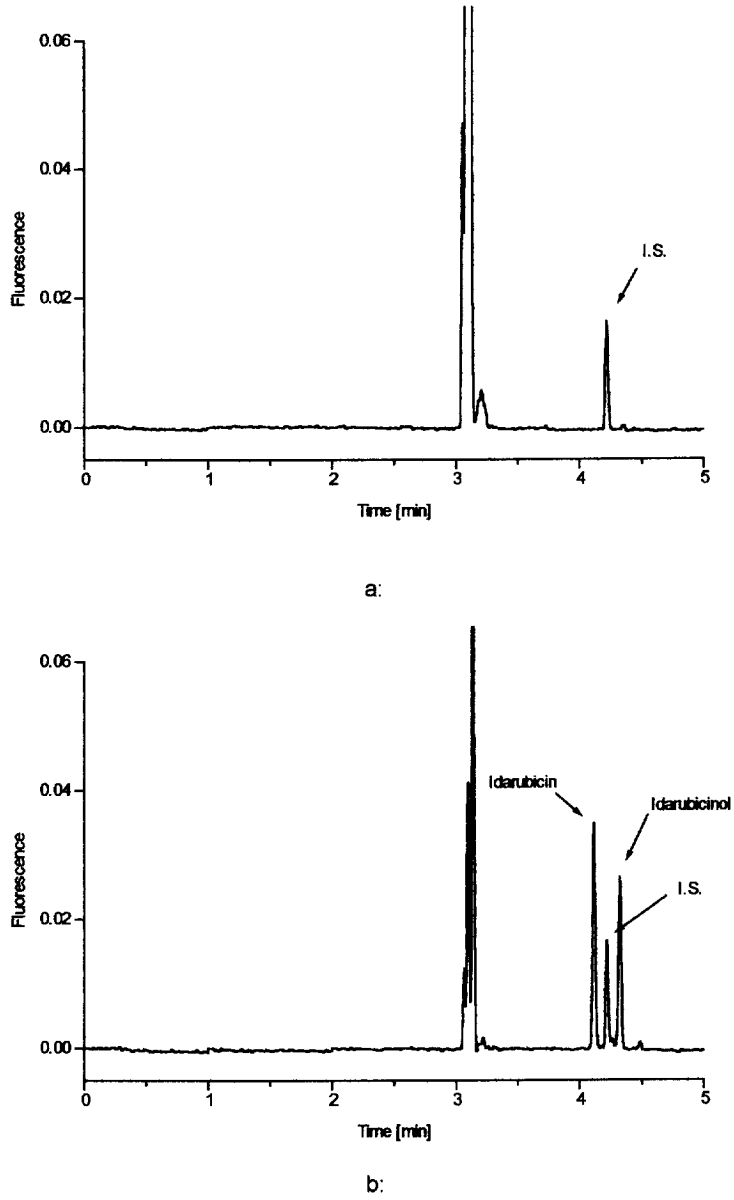

Fig. 4. Electropherogram of a patient plasma sample. Conditions the same as in Fig. 2. (a) Blank plasma with daunorubicin as internal standard, (b) patient plasma 3 h after oral administration of 5 mg/m^2 idarubicin. Conc.: idarubicin 3.76 ng/ml, idarubicinol 4.46 ng/ml.

With electrokinetic injection the extraction efficacy could not be detected accurately, because the peak areas are a function of the ionic strength in the sample matrix.

The accuracy and precision data of the method are shown in Table 2. It is apparent that the method meets the requirements for a bioanalytical method [12].

Table 2
Accuracy and precision of the assay for idarubicin and idarubicinol in plasma

	Spiked conc. (ng/ml)	Mean measured conc. (ng/ml)	Accuracy (%)	Rel. standard deviation (%)	*n*
Idarubicin	5	4.93	1.4	3	5
	1	1.05	5.0	2.3	5
	0.5	0.41	15.7	15.7	7
Idarubicinol	5	5.09	1.8	2.04	5
	1	1.05	5.0	2.47	5
	0.5	0.42	16.0	14.3	7

3.4. Clinical applications

Fig. 4 shows the electropherogram of a plasma sample from a patient treated with 5 mg/m^2 oral idarubicin. The idarubicin and idarubicinol plasma concentration over time following the oral administration of 5 mg/m^2 idarubicin in a patient with soft tissue sarcoma are plotted in Fig. 5. It is seen that the active metabolite idarubicinol has a longer half-life than the parent compound and is thus a major parameter of systemic exposure in patients treated with idarubicin.

4. Conclusions

A fast and sensitive CE method for the determination of idarubicin and its main metabolite idarubicinol in plasma has been presented. Compared with the established HPLC methods, the analysis time as well as solvent waste and the required sample volume could be reduced. Idarubicin and idarubicinol can be accurately measured using only 100 μl of plasma. The sensitivity is satisfactory with LIF detection. Studies are ongoing to further modify the method to determine doxorubicin, daunorubicin, epirubicin and their respective metabolites.

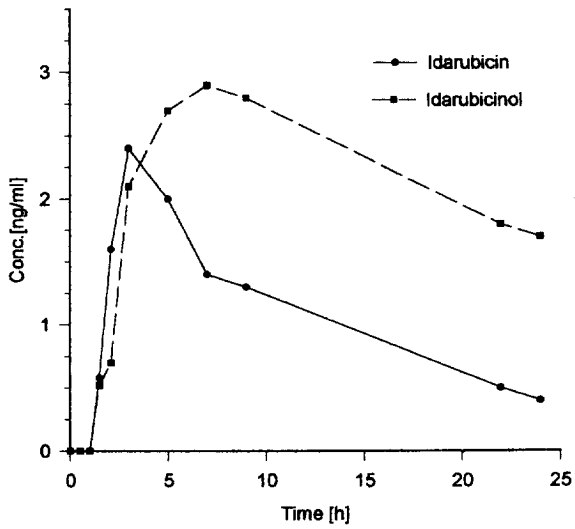

Fig. 5. Plasma concentration vs. time curve after oral administration of 5 mg/m^2 idarubicin.

Acknowledgements

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