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Note

Improved high-performance liquid chromatographic method using loop–column extraction for analysis of idarubicin and idarubicinol in plasma

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Idarubicin (4-demethoxydaunorubicin) is a relatively new anthracycline anticancer drug that has activity against adult and pediatric acute leukemia [1]. A specific feature of idarubicin is that it is adequately absorbed from the gastro-intestinal tract and therefore can be administered orally [1,2]. Idarubicin has also been reported to be less cardiotoxic than other anthracyclines [3,4].

After oral or intravenous administration, idarubicin is metabolized by hydroxylation, forming 4-demethoxy-13-hydroxydaunorubicin (idarubicinol) [1]. The latter compound has shown cytotoxic activity in preclinical studies [5]. The terminal half-life of idarubicin in plasma has been reported to average 34.7 h in adults and 11.3 h in children [6,7].

In published studies, different high-performance liquid chromatographic (HPLC) methods have been used for the quantitation of idarubicin and idarubicinol in biological fluids. Usually, at least 1 ml of plasma is needed for the preparation of each sample [6–9]. This amount of plasma may present a problem in young children, where the volume of blood used for clinical studies should be kept to a minimum. Pharmacokinetic studies require blood samples to be taken at multiple times and each sample should ideally be assayed in duplicate. Thus, in pediatric pharmacology there is a need for an assay that requires a minimal amount of plasma, while maintaining good sensitivity. We developed

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a new HPLC assay for the determination of idarubicin and idarubicinol that requires only 100 μl plasma per injection (for concentrations <5 ng/ml, 400 μl) and has a limit of detection of 0.1 ng/ml. Also, in this assay, loop–column extraction rather than liquid–liquid extraction is used, which represents a significant simplification of the sample preparation process.

EXPERIMENTAL

Idarubicin and idarubicinol were obtained from Adria Labs. (Columbus, OH, U.S.A.). Distilled water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.). HPLC-grade acetonitrile was purchased from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). ACS-certified monobasic sodium phosphate was purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.).

Idarubicin and idarubicinol concentrations in plasma were determined by reversed-phase HPLC with fluorescence detection. The chromatographic system consisted of an isocratic pump (Beckman 110B solvent delivery system, Beckman Industries, Fullerton, CA, U.S.A.) with a Rheodyne Model 7125 injector, fitted at ports 1 and 4 with a 2.3 cm×3.9 mm I.D. column, dry-packed with Waters Bondapak Phenyl 37–50 μm packing (Waters Assoc., Milford, MA, U.S.A.) instead of the customary volumetric loop [10]. The analytical column was a Waters Nova-Pak Phenyl column, particle size 4 μm, 15 cm×3.9 mm I.D. Proximal to the analytical column a 2.3 cm×3.9 mm I.D. guard column with Bondapak Phenyl packing was used. Column effluent was monitored with a Spectroflow 980 programmable fluorescence detector (ABO Analytical, Kratos Division, Ramsey, NJ, U.S.A.) with a 5-μl flow cell. The excitation wavelength was set at 250 nm and a 550-nm emission filter was used. An SP 4100 computer integrator (Spectra-Physics, Mountain View, CA, U.S.A.) was used for measuring peak heights and preparing multi-level calibration lines. The mobile phase consisted of a solution of 0.2 *M* NaH_2PO_4–acetonitrile (73:27, v/v). The sodium phosphate solution was first adjusted to pH 4.0 with a 40% solution of phosphoric acid. The mobile phase was filtered through a Millipore vacuum filtration system and degassed by sparging with helium. The flow-rate was 1.4 ml/min. Operating pressure with this system was about 210 bar.

Plasma sample preparation for injection consisted only of removal of gross particulate matter by centrifugation at 12 000 *g* for 2 min. Before each sample injection, the loop column was washed with 500 μl of filtered deionized water to remove mobile phase from the loop column. Plasma samples (100 or 400 μl) were injected directly into the loop column, as previously described [10]. With the injector remaining in the load position, an additional 500 μl of filtered deionized water was injected to remove potentially interfering substances such as proteins. The components of interest were then eluted from the loop column

by switching the injector to the inject position. For the quantitation of patient samples and controls, external calibration was used.

RESULTS

Representative chromatograms obtained from a blank plasma and from a 400-μl plasma sample spiked with 2.5 ng/ml idarubicinol and idarubicin are shown in Fig. 1. Retention times were 3.81 and 6.46 min, respectively. The peaks of interest were completely separated from one another and from the plasma peak. For multi-level calibration, two separate standard curves were made using least-squares linear regression. For idarubicin and idarubicinol concentrations between 5 and 50 ng/ml, 100 μl of plasma were used, and 400 μl for concentrations below 5 ng/ml. In practice this was handled as follows. For the quantitation of a patient's sample 100 μl of plasma were injected. Only in those few cases that the idarubicin or idarubicinol concentration was lower than 5 ng/ml, the injection was repeated using 400 μl of plasma. Thus, for the majority of patient samples, 200 μl of plasma were used for the duplicate measurement of idarubicin and idarubicinol. The calibration lines for idarubicin and idarubicinol were linear in both concentration ranges (0.5–5 and 5–50 ng/ml). The r^2 values were at least 99.9%. The smallest detectable concentration of the compounds of interest, defined as at least three times the baseline noise, was 0.1 ng/ml (i.e. only 0.04 ng on-column).

To determine precision and accuracy of the assay, plasma samples of a known concentration were assayed in ten replicates by an individual unaware of the spiked concentration. The results are summarized in Table I. Fig. 2 shows the concentration–time profile of idarubicin and idarubicinol measured by this method in a patient with relapsed acute leukemia following oral administration

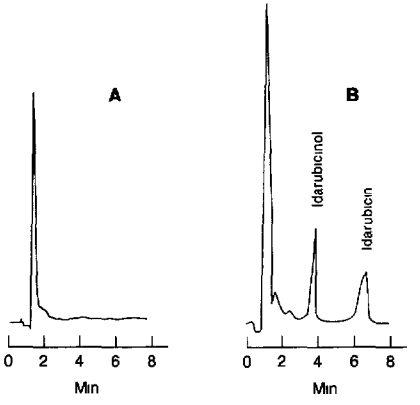

Fig. 1. Chromatograms from blank plasma (A) and a plasma sample of 400 μl spiked with 2.5 ng/ml idarubicin and idarubicinol (B).

TABLE I

ACCURACY AND PRECISION OF THE ASSAY FOR IDARUBICIN AND IDARUBICINOL ($n=10$)

Compound	Spiked concentration (ng/ml)	Mean measured concentration (ng/ml)	Accuracy (%)	Coefficient of variation (%)
Idarubicin	0.50	0.52	104.0	10.1
	25.0	24.85	99.4	3.4
Idarubicinol	0.50	0.46	92.0	6.8
	25.0	25.53	102.1	3.6

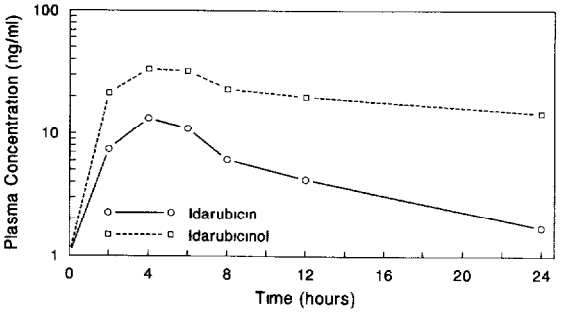

Fig. 2. Semilogarithmic plot of idarubicin and idarubicinol plasma concentrations versus time following oral administration of 40 mg/m^2 idarubicin to a patient with relapse acute leukemia.

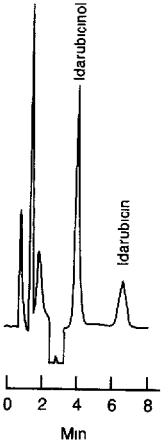

Fig. 3. Typical patient chromatogram 4 h after oral administration of 40 mg/m^2 idarubicin.

of 40 mg/m^2 idarubicin. Fig. 3 presents a typical patient chromatogram. The negative deflections were due to refractive index changes and were not seen in any spiked samples. They did not interfere with quantitation.

DISCUSSION

Accuracy and precision of the new HPLC assay described in this paper are similar and the sensitivity is better than that of previously reported assays for idarubicin and idarubicinol. However, the amount of plasma required for each determination is substantially smaller, which is a major advantage for pharmacokinetic studies of idarubicin in a pediatric population.

A convenient feature of the assay is the loop–column extraction, which eliminates the need for organic extraction of biological samples before injection into the chromatographic system. The loop–column extraction method is easy to perform and eliminates the variability associated with liquid–liquid extraction [10].

During the development of this assay, the use of external calibration proved to be superior to an internal standard method, using doxorubicin or daunorubicin as internal standard. We think that the minimization of extraction variability accounts for the excellent results of the external standard method. Also, one must appreciate the potential shortcomings of the internal standard technique, as extensively discussed by Haefelfinger [11], who showed that external calibration often is advantageous to the internal standard technique.

In summary, idarubicin and idarubicinol concentrations >5 ng/ml can now be accurately measured using only 100 μl of plasma, while concentrations as low as 0.1 ng/ml can be accurately quantitated using only 400 μl of plasma. This makes the new assay particularly well suited for pediatric pharmacokinetic studies.

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