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Note

High-performance liquid chromatographic determination of adriblastin in human plasma, urine, saliva and liver punctuate by column switching for drug monitoring studies

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Adriblastin (Fig. 1), an antitumour antibiotic, is isolated from cultures of *Streptomyces peuceticus* var. *caesius*. The mechanism of action has been studied extensively and appears similar to that of other antineoplastics such as mitomycin C and daunorubicin. Unfortunately, the drug shows severe side-effects, such as cardiotoxicity [1–3], and therefore proper dosage to reduce toxicity is very important and control of its levels in different body fluids is necessary. The drug is biotransformed to several metabolites, especially its main metabolite adriblastinol, but the metabolic pathway has not been elucidated totally. This paper describes a rapid and very sensitive high-performance liquid chromatographic (HPLC) method for the determination of adriblastin in various body fluids, and includes direct sample injection.

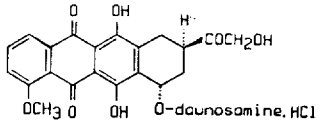

Fig. 1. Structure of adriblastin, 10-[(3-amino-2,3,6-trideoxy-α-1-lyxohexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione hydrochloride.

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EXPERIMENTAL

Apparatus

UV spectra were measured with an HP 8451 A photodiode-array spectrophotometer including a 7470 A plotter and an 82901 M floppy disc drive (Hewlett-Packard, Vienna, Austria). Fluorescence spectra were recorded with an SFM 23 spectrofluorimeter (Kontron, Vienna, Austria).

Chromatographic equipment and parameters

The whole chromatographic system was obtained from Kontron. Pumps were of the 420 type with a mixing chamber (1.5 ml) connected with a 460 intelligent autosampler with a column switching module. Stainless-steel columns (loop column, 20 mm × 4.6 mm I.D., packed with Spherisorb C_8, 30 μm; pre-column, 20 mm × 4.6 mm I.D., packed with Spherisorb ODS II, 10 μm; analytical column, 100 mm × 4.6 mm I.D., packed with Spherisorb ODS II, 3 μm) were connected by a Bischoff "eco tube" cartridge system. The chromatographic detectors consisted of an LC-4A amperometric detector (Bioanalytical Systems, West Lafayette, IN, U.S.A.) with a glassy carbon working electrode and an Ag/AgCl reference electrode, an LC 725 photometric detector and an SFM 23 spectrofluorimetric detector. Chromatograms were recorded with an Anacomp 220 controller and integration unit.

Chemicals

Adriblastin was obtained from Carlo Erba (Berne, Switzerland) (lyophilized powder, containing 60 mg of lactose per 10 mg of adriblastin). For the mobile phase tetrahydrofuran, methanol (both HPLC grade) (Merck, Vienna, Austria) and water (deionized and doubly distilled) were used. *n*-Pentanesulphonic acid disodium salt (Serva, Heidelberg, F.R.G.) served as an ion-pair-forming agent. Ammonium acetate and glacial acid (Merck) were used for aqueous buffer (pH 4.0).

Drug administration and sample preparation

A solution containing 10 mg of adriblastin per 1.0 ml of isotonic and sterile sodium chloride solution was used. A dose of 1.0 mg/kg body weight was given by chemobolization of the hypogastrica sinistra artery. Blood samples were taken by venipuncture from adriblastin-treated patients at various time intervals. Samples were mixed with 100 μl of sodium citrate solution and centrifuged at 1000 *g* for 5 min. Saliva samples of 1 ml were collected at the same time as venipuncture and urine was pooled for 8 h. Liver punctuate was obtained from one patient and treated in the same way as plasma samples. All samples were stored at $-70°C$ until analysis and centrifuged at 4000 *g* for 2 min prior to injection.

Extraction

Extraction from the matrix was performed by column switching. Prior to injection the loop column was flushed with distilled water containing 0.1% *n*-pentanesulphonic acid for 5 min (flow-rate 1 ml/min). Then the column was switched

to the solvent stream, followed by injection of 0.5 ml of sample. After 30 s the loop column was switched to *n*-pentanesulphonic acid solution to remove matrix constituents (1 ml/min for 3 min). Then the column was re-switched to the eluent and the chromatogram was measured.

Liquid chromatography

Adriblastin was separated from its metabolites and coextracted compounds as described [4, 5] on a reversed-phase Spherisorb column by isocratic elution with water–tetrahydrofuran–methanol (55:1:30, v/v/v) containing 8.4 g of ammonium acetate and 400 mg of *n*-pentanesulphonic acid sodium salt per 1000 ml. The absorbance of the eluate was measured at 254 or 280 nm (0.005 a.u.f.s.), electrochemical detection was performed at 700 mV oxidation potential (2 nA full scale) and fluorimetric detection at an excitation wavelength of 470 nm and an emission wavelength of 555 nm (sensitivity "high" on the detector).

RESULTS AND DISCUSSION

Photometric detection

The chromophoric shift of adriblastin at different pH values of aqueous solutions is depicted in Fig. 2. UV measurement at 560 nm showed an increased selectivity, but loss of sensitivity. Therefore, measuring at 254 or 280 nm was preferred, and also there are no spectral shifts at these wavelengths.

Fluorimetric detection

Fig. 3 depicts a typical emission spectrum of adriblastin (dissolved in the eluent) with its maximum at 555 nm. There is no quenching by the solvent with the fluorophore of adriblastin under these conditions.

Amperometric oxidation

Adriblastin was oxidized in the range 0.1–1.2 V in steps of 100 mV and chromatograms were recorded at each potential to determine the corresponding peak height. Fig. 4 shows the voltammogram obtained with characteristic turning points at 400 and 800 mV.

Table I lists the peak heights in mV for the described detection modes at three different concentrations of adriblastin.

Extraction procedure

Extraction was performed with a mixture of toluene and *n*-butanol [6] or chloroform and isopropyl alcohol [7] followed by evaporation. The residue was dissolved in the mobile phase and injected into the HPLC apparatus. In earlier studies, the isolation of adriblastin from the biological matrix was performed by injection of samples in a loop column of a Rheodyne 7125 valve [8] or by extraction with silica cartridges [9].

In our procedure for adriblastin, the drug was isolated from the biological matrix by column switching. The polar constituents of the biological matrix were removed nearly completely by flushing the column with *n*-pentanesulphonic acid.

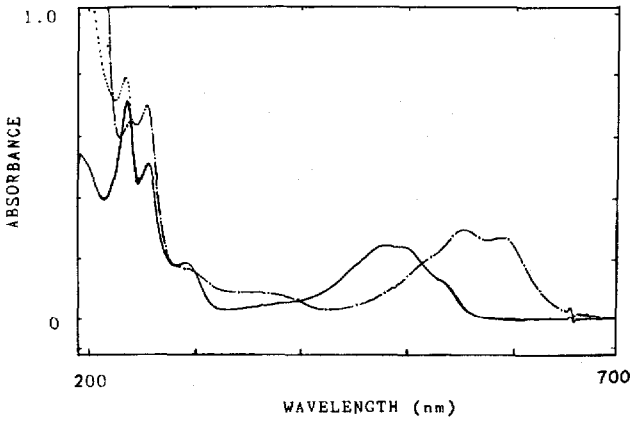

Fig. 2. UV spectra of adriblastin (1 mmol in distilled water) at different pH values. Dashed line, disodium hydrogenphosphate (1 mmol) (pH 8.0) with sodium hydroxide; solide line, distilled water (pH 6.8); dotted line, sodium citrate buffer (1 mmol) (pH 3.0) with hydrochloric acid.

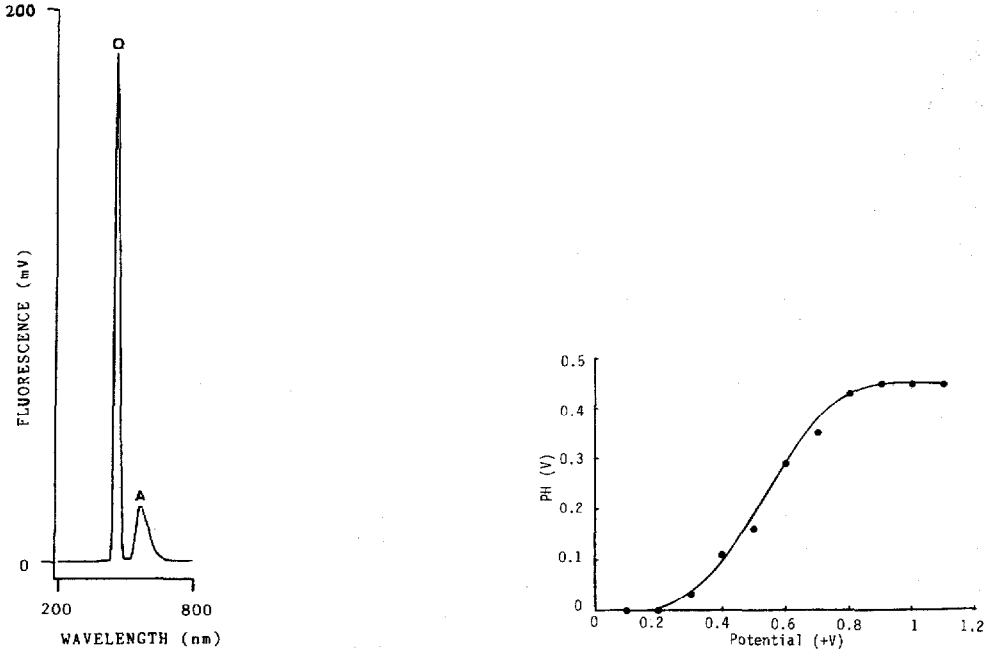

Fig. 3. Fluorescence emission spectrum of adriblastin (1 mmol in the mobile phase) at an excitation wavelength of 470 nm. Q, quenching; A, adriblastin fluorophore at 555 nm maximum.

Fig. 4. Voltammogram of adriblastin (1 mmol in the mobile phase); x-axis, oxidation potential of working electrode; y-axis, peak height (PH) of adriblastin on chromatogram.

By this on-column enrichment it is possible to detect very small amounts of the drug in different biological matrices. The loop column has to be changed after 50 injections owing to a decrease in efficiency. There is no need for time-consuming sample preparation and the whole procedure can be performed automatically.

TABLE I

PEAK HEIGHTS AND SENSITIVITY FACTORS FOR THE DETERMINATION OF ADRIBLASTIN WITH PHOTOMETRIC, FLUORIMETRIC AND AMPEROMETRIC DETECTION

Conc., concentration of adriblastin in solutions A, B and C (A, 1.0 mg/ml; B, 1.0 μg/ml; C, 1.0 ng/ml); *s*, standard deviation; FL, fluorimetric detection (excitation at 470 nm, emission at 555 nm); ECD, amperometric oxidation (0.7 V); UV_1, photometric detection at 280 nm; UV_2, photometric detection at 254 nm.

Parameter	Conc.	FL	*s*	ECD	*s*	UV_1	*s*	UV_2	*s*
Peak heights (mV)	A	11256	4.0	40686	4.9	9507	1.6	3921	11.5
	B	946	5.4	2376	10.2	192	2.7	347	11.7
	C	187	7.0	1409	14.6	88	4.3	185	11.9
Factors (reference $UV_{280\,nm}$)	A	1.2		4.3		1		0.4	
	B	4.9		12.4		1		1.8	
	C	2.1		16.0		1		2.1	

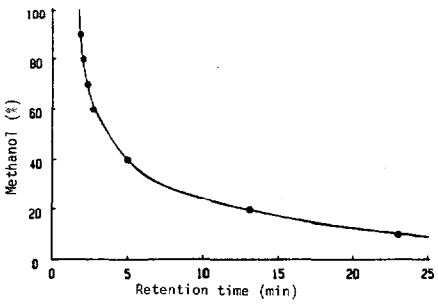

Fig. 5. Relationship between percentage of methanol in the mobile phase and retention time of adriblastin.

Chromatographic isolation

The retention of adriblastin is strongly dependent on the concentration of organic modifiers in the mobile phase, such as acetonitrile or methanol (Fig. 5). In the proposed method methanol was preferred as the modifier owing its lower toxicity. Adriblastin in plasma, urine, saliva and liver punctuate was completely isolated from its metabolites and endogenous compounds using a mobile phase containing 30% methanol and 1% of tetrahydrofuran in an aqueous buffer (Fig. 6).

Quantitative analysis and recovery

Adriblastin is metabolized into its hydroxylated main product adriblastinol, which elutes close to the parent compound. Addition of an internal standard that is suitable for electrochemical detection at an oxidation potential of 0.7 V (e.g., dopamine) lead to interferences with either adriblastin and its metabolites or the matrix peak in front of the chromatogram. The time required for analysis becomes very long if dihydroxybenzoic acid is used, so after a series of twenty sam-

ples a six-point calibration was run under the same conditions as the samples. These standard runs additionally indicate possible peak shifts in a large series of samples, caused by a reduced column efficiency. Quantitation was based on peak-area measurement and calculated automatically by the Anacomp 220 using the equation of the calibration graph ($y=59.5x+3.69$; $r=0.996$; $n=9$). The recovery of the drug was 97% from plasma (S.D. 4.1%), 92% from urine (S.D. 5.8%), 100.5% from saliva (S.D. 2.0%) and 89% from liver punctuate (S.D. 6.3%).

Detection limit

The detection limit is in the range 50–100 pg/ml, limited by a signal-to-noise ratio of 3:1 (injection volume 0.5 ml, amperometric oxidation). A higher sensitivity may be obtained by injection of a larger volume of sample.

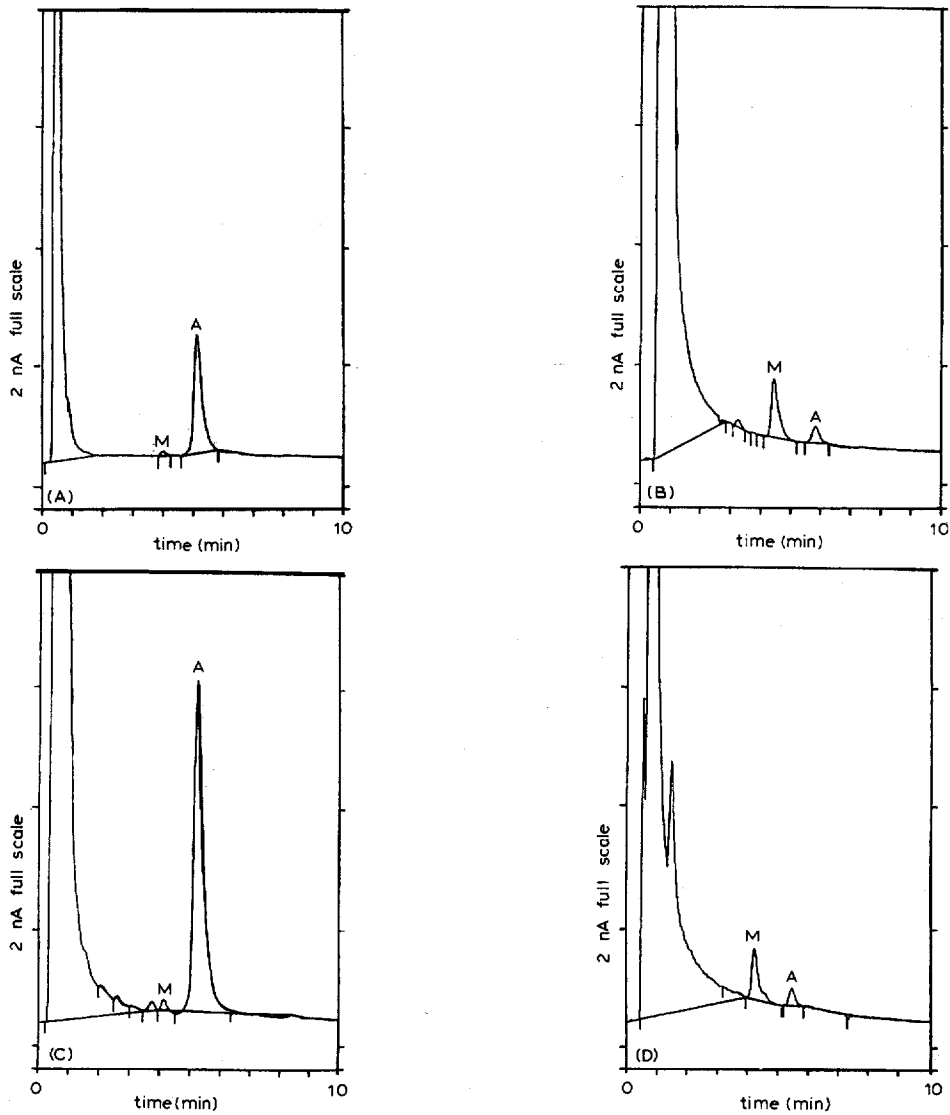

Fig. 6.

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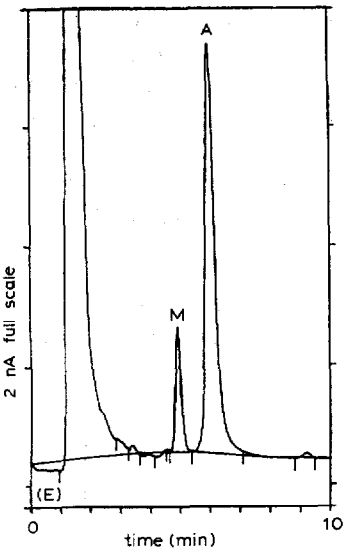

Fig. 6. Determination of adriblastin (A) and its main metabolite (M) by amperometric detection (0.7 V). Chromatograms of extracts from (A) standard solution, (B) plasma, (C) saliva, (D) liver punctuate and (E) urine samples. Adriblastin concentration in A, B, C, D and E: 500, 280, 1200, 108 and 1467 ng/ml, respectively.

Precision of assay

The coefficient of variation for twenty determinations, carried out within the same day, was 3.1–4.0% for the concentration range 100–500 ng/ml; it was 3.6–6.1% when calculated every other day within a period of six weeks (frozen samples, 25 determinations per day).

CONCLUSIONS

The proposed method provides excellent sensitivity and accuracy for the automated extraction and HPLC determination of adriblastin in human plasma, urine, saliva and liver punctuate. The procedure is very selective and therefore suitable for all types of drug monitoring and distribution studies in the human organism.

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