

CHROMSYMPO. 089

## DETERMINATION OF THE UNDERIVATIZED ANTINEOPLASTIC DRUGS CYCLOPHOSPHAMIDE AND 5-FLUOROURACIL AND SOME THEIR METABOLITES BY CAPILLARY GAS CHROMATOGRAPHY COMBINED WITH ELECTRON-CAPTURE AND NITROGEN-PHOSPHORUS SE- LECTIVE DETECTION

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### SUMMARY

A rapid and sensitive method for the determination of cyclophosphamide (CP) and 5-fluorouracil (5-FU) and some of their metabolites in one analysis has been developed. Surface-coated open-tubular OV-275 columns were combined with electron-capture detection and nitrogen-phosphorus selective detection. The influence of the column diameter on the separation is shown. Extraction with 2-propanol-diethyl ether (22:77) allows the isolation of CP, 5-FU and their analogues in one extraction step. The assay was applied to some pharmacokinetic experiments with chemotherapeutically treated patients and with a WAG/Rij rat.

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### INTRODUCTION

The determination in body fluids of cytostatic drugs and their metabolites is of great importance in optimizing cancer chemotherapy regimens. For over 20 years CP and 5-FU have been among the most frequently used cytostatic drugs in human cancer chemotherapy. A prodrug of 5-FU, 5'-deoxy-5-fluorouridine (5-DFUR), was recently introduced as an antitumour drug with lower systemic toxicity than 5-FU<sup>1</sup>.

Several assays have been described for routine pharmacokinetic studies on unchanged CP and 5-FU. 5-FU can be determined by means of gas chromatography<sup>2</sup> and high-performance liquid chromatography<sup>3</sup>, whereas for CP gas chromatography is mostly preferred<sup>4</sup>. As CP and 5-FU are often administered in a combined therapy, it seemed worthwhile to develop an assay for the determination of CP and 5-FU in one analysis. Moreover, both CP and 5-FU are intensively metabolized, and pharmacological studies today generally require information about metabolism too. This new assay should also permit the determination of metabolites of CP and 5-FU.

The new antitumour drug 5-DFUR is metabolized by thymidine phosphorylase (in man) and by uridine phosphorylase (in small animals) to 5-FU. 5-DFUR is now used in clinical trials in single-agent therapy; the determination of 5-DFUR, 5-FU and 5-fluorodihydrouracil (FDHU) appears to be of interest in following the pharmacokinetics of single-agent therapy.

## EXPERIMENTAL

### *Chemicals*

CP, 4-ketocyclophosphamide (4-KCP), carboxyphosphamide (CCP), 4-hydroxycyclophosphamide (4-HPCP) and iphosphamide (IP) as reference substances were kindly supplied by ASTA-Werke (Bielefeld, F.R.G.).

5-FU, FDHU and 5-DFUR were gifts from Hoffmann-La Roche (Mijdrecht, The Netherlands) and 5-chlorouracil (5-CU, internal standard) from Calbiochem (Los Angeles, CA, U.S.A.).

### *Extraction of plasma samples*

To 50  $\mu$ l of human or rat plasma in a polythene tube, 500  $\mu$ l of a mixture of 2-propanol–diethyl ether (22:77) and 5-CU as internal standard were added. During mixing on Vortex mixer (5 min), 50  $\mu$ l of acetone were added gradually to the mixture. After centrifugation for 5 min at 1000 g, the monolayer liquid phase was transferred into a second tube, which was placed at 291°K under a gentle stream of nitrogen. The residue was dissolved in 100  $\mu$ l of 2-propanol–diethyl ether (22:77) and 10  $\mu$ l of the solution were transferred to the stainless-steel needle of the solid-sample injector. All handling was carried out with protection against light as far as possible. If only 5-FU, FDHU and 5-CU must be determined, pre-extraction with chloroform can be used in order to remove interfering compounds, followed by the extraction of 5-FU, FDHU and 5-CU with ethyl acetate<sup>5</sup>.

### *Apparatus*

Two gas chromatographs were used: a Packard Becker Model 420 (Packard Becker, Delft, The Netherlands), equipped with a Model 18-789 A nitrogen–phosphorus selective detector (Hewlett-Packard, Avondale, PA, U.S.A.), and a Hewlett-Packard Model 5713A, equipped with a <sup>63</sup>Ni pulse-modified electron-capture detector. The samples were introduced by means of a solid-sample injection system.

### *Columns*

Support-coated open-tubular (SCOT) OV-275 columns (10 or 7 m  $\times$  0.4 mm I.D. and 10 or 7 m  $\times$  0.31 mm I.D.), made of Duran 50 glass, were prepared as described in the literature<sup>6</sup>.

### Temperatures

Unless a temperature programme was used, the oven temperature was set at 478°K for the determination of all compounds mentioned. The injection-port temperature was set at 328°K and the detector temperatures were 573°K (nitrogen-phosphorus selective detector) and 623°K (electron-capture detector). The plasma concentrations were calculated by means of calibration graphs, obtained by analysing blank plasma samples spiked with known amounts of the compounds of interest. 5-CU was used as an internal standard.

Blood samples were collected in heparinized polythene tubes, taken at certain time intervals before and after starting the treatment. The samples were centrifuged at 1000 g within 1 h after collection, and then the plasma was taken off and stored at 258°K until analysed.

### RESULTS AND DISCUSSION

Fig. 1a is a chromatogram of a test mixture of 5-FU, FDHU, CP, 4-KCP, CCP and 5-CU. The FDHU and CP separation was not optimal and, if greater resolution is required, reduction of the column diameter from 0.40 to 0.31 mm will solve the problem, as is demonstrated in Fig. 1b.

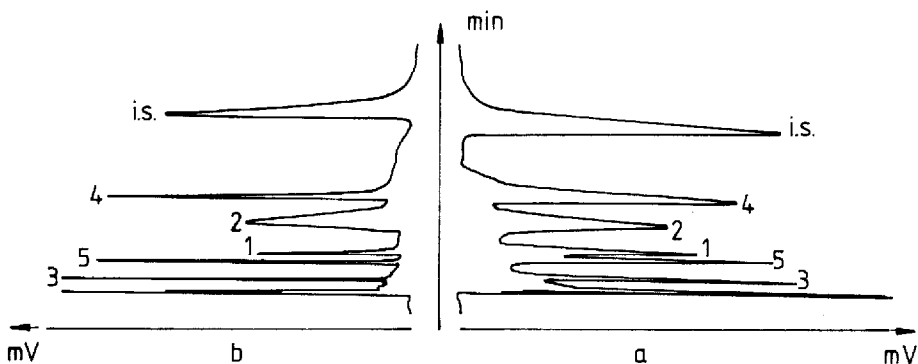


Fig. 1. Chromatograms of a test mixture of CP (1), 4-KCP (2), CCP (3), 5-FU (4), FDHU (5) and the internal standard (i.s., 5-CU). Nitrogen-phosphorus specific detector. Column diameter: (a) 0.40 mm; (b) 0.31 mm.

Fig. 2 is a chromatogram of a 2-propanol-diethyl ether-extracted plasma spiked with all the compounds of interest. This chromatogram demonstrates the feasibility of the extraction procedure. The detection limit for 4-KCP is relatively unfavourable, owing to its anomalous chromatographic behaviour. This effect may be accounted for by the keto-enol equilibrium, which has been confirmed by gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry experiments. The retention times of 5-FU and 5-CU can be shortened by using a temperature programme (Fig. 3), resulting in analysis times of less than 12 min for all compounds. In the same system compounds related to CP such as iphosphamide can be chromatographed.

The assay for the determination of CP, 5-FU and some metabolites, based on the 2-propanol-diethyl ether extraction, was applied to monitoring CP, 4-KCP, CCP,

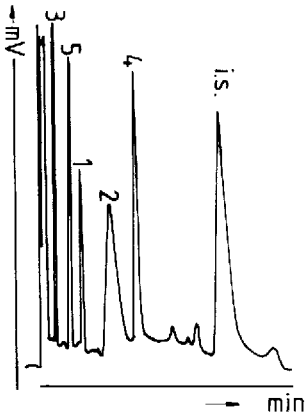


Fig. 2. Chromatogram of plasma extracted with 2-propanol-diethyl ether. Peaks: 1 = CP; 2 = 4-KCP; 3 = CCP; 4 = 5-FU; 5 = FDHU.

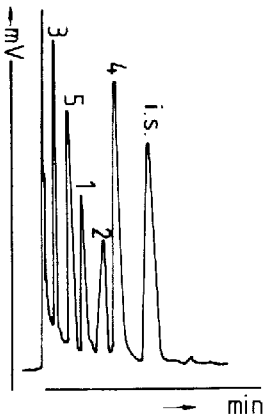


Fig. 3. Chromatogram of the test mixture. Temperature: programmed from 478 to 493°K at 2°K/min. Temperature programme started 6 sec after injection.

5-FU and FDHU plasma concentrations of a WAG/Rij rat that had received CP and 5-FU intravenously (Fig. 4a), the determination of CP, 5-FU and FDHU in the plasma of a patient treated with CP and 5-FU (Fig. 4b) and the determination of 5-FU and FDHU in the plasma of a patient treated with 5-DFUR (Fig. 4c).

It can be concluded that the nitrogen-phosphorus selective detector is the most suitable for monitoring all substances, because of the relatively large linearity. However, when measurements of 5-FU and FDHU between 1 and 50 ng/ml plasma are necessary, electron-capture detection must be applied.

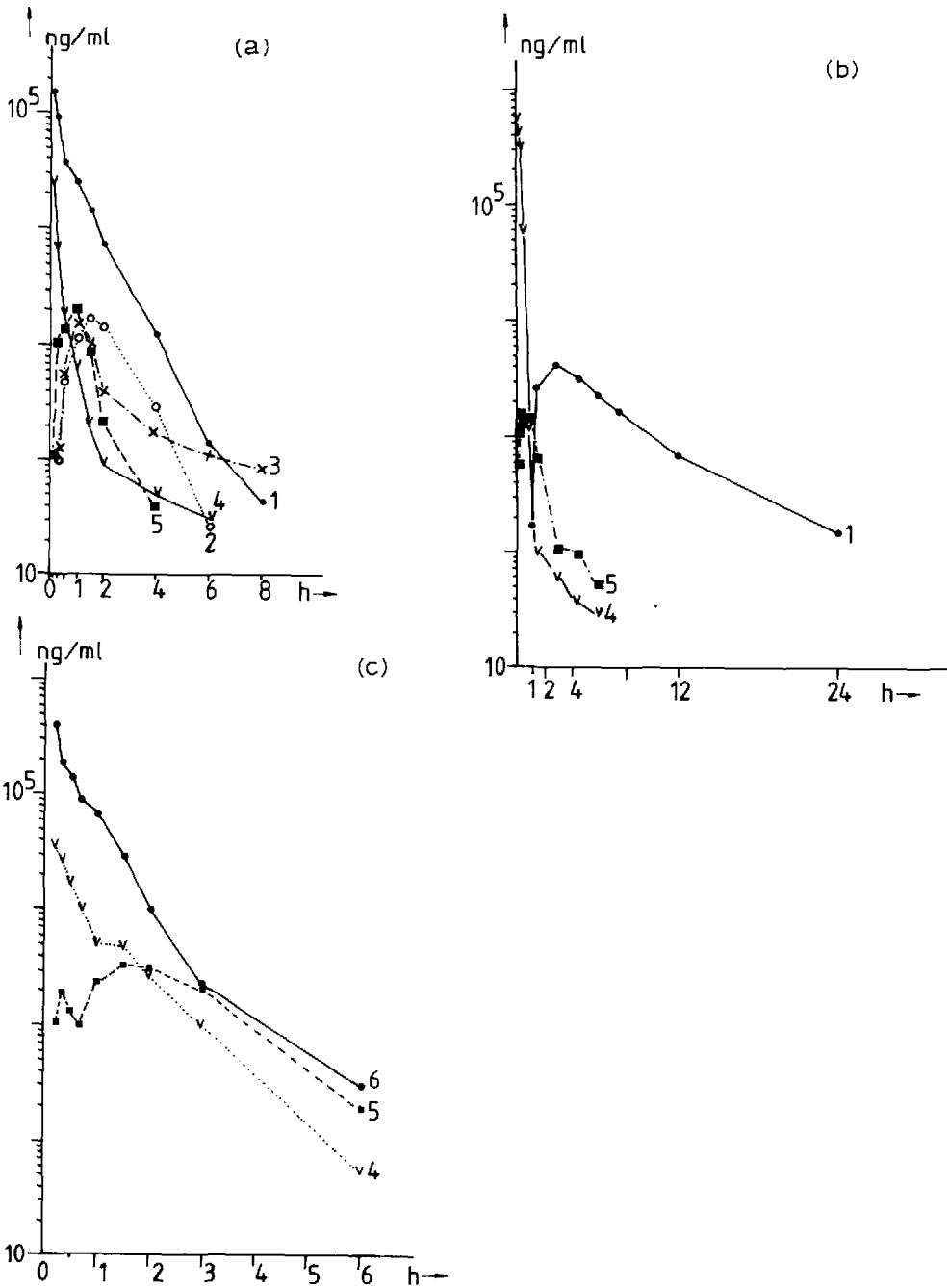


Fig. 4. Plasma concentration versus time curves for CP, 4-KCP, CCP, 5-FU and FDHU in different samples. (a) WAG/Rij rat. (1) CP; (2) 4-KCP; (3) CCP; (4) 5-FU; (5) FDHU. Dose: 70 mg/kg (CP) and 20 mg/kg (5-FU). (b) Patient 1. (1) CP (dose 100 mg/m<sup>2</sup>); (4) 5-FU (dose 500 mg/m<sup>2</sup>); (5) FDHU. (c) Patient 2. (6) 5-DFUR (dose 3000 mg/m<sup>2</sup>); (4) 5-FU; (5) FDHU. 5-DFUR concentrations were measured by high-performance liquid chromatography with UV detection.

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