Journal of Chromatography, 145 (1978) 311—314
Biomedical Applications

CHROMBIO.119

Note

An improved gas—liquid chromatographic assay for 5-fluorouracil in plasma

H.W. VAN DEN BERG, R.F. MURPHY, R. HUNTER and D.T. ELMORE

Department of Biochemistry, Queen's University, Belfast BT9 7BL (N. Ireland)

(Received June 27th, 1977)

5-Fluorouracil plays an important role in the chemotherapeutic management of certain forms of cancer [1]. Detailed pharmacokinetic studies could provide the clinician with information necessary to devise optimal drug schedules, combining maximum therapeutic response with minimum toxicity. Such studies require a rapid, specific and sensitive method of drug assay. The spectrophotometric assay for 5-fluorouracil lacks specificity and sensitivity [2], whilst the microbiological method lacks accuracy and is unsuitable for large numbers of samples [2].

In recent years three gas—liquid chromatographic (GLC) assay methods for 5-fluorouracil have been described. Two of these techniques [3, 4] were based on detection of a silyl derivative of the drug using a flame ionisation detector. A third method by Rao et al. [5] entailed flash-methylation of 5-fluorouracil followed by GLC separation and flame ionisation detection.

The sensitivity of all these methods was of the order of 1 μg 5-fluorouracil per ml of plasma. Recent pharmacokinetic studies in our own laboratory have shown that a more sensitive assay was required. We therefore investigated the possibility of preparing a derivative of 5-fluorouracil which would be sensitive to electron-capture detection. In this paper we wish to report a GLC assay method which is capable of detecting 10 pg 5-fluorouracil as a chloromethyldimethylsilyl derivative. This technique results in a lower limit of detection of 5-fluorouracil in plasma of 20 ng/ml.

MATERIALS AND METHODS

Gas—liquid chromatography

GLC was carried out using a Pye Unicam GCV chromatograph equipped with a ^{63}Ni electron-capture detector (Pye-Unicam, Cambridge, Great Britain). The column was a glass tube, 213 cm × 4 mm I.D., packed with 80—100 mesh Chromosorb W HP coated with 3% OV-1, (Pierce, Rockford, Ill., U.S.A.). Operating conditions were: column oven temperature, 230°; injection port temperature, 230°; electron-capture detector temperature 280°. Nitrogen was

used as the carrier gas at a flow-rate of 40 ml/min. The column was conditioned for 16 h before use at the operating temperature.

Extraction of 5-fluorouracil from plasma

5-fluorouracil (Sigma, St. Louis, Mo., U.S.A.) was extracted from 1-ml plasma samples with 15 ml of a solution of 16% *n*-propanol in ether according to the method of Cohen and Brennan [4]. The extract (0.5 to 7.5 ml depending on the concentration of 5-fluorouracil in the plasma) was evaporated to dryness in sample tubes under a gentle stream of nitrogen. Standards were prepared by evaporating aqueous solutions (50 μl) of the drug. Thymine (Calbiochem, San Diego, Calif., U.S.A.) was used as an international standard. Depending upon the anticipated concentration of 5-fluorouracil, 50 or 625 ng thymine in aqueous solution (50 μl) was added to each sample tube. The water was removed by evaporation prior to derivatization of 5-fluorouracil and internal standard.

Derivatization

1,3-Bis (chloromethyltetramethyldisilazane) (100μl) and chloromethyldimethylchlorosilane (50μl) (Pierce and Warriner, Chester, Great Britain), was added to the evaporated sample. The reaction mixture was heated at 75° for 10 min in a water bath. The tubes were cooled to room temperature and 2 ml redistilled spectroscopic-grade ethyl acetate (Koch-Light, Colnbrook, Great Britain), was then added. Addition of this solvent was necessary in order to prevent saturation of the detector by chlorinated reaction by-products. The resultant precipitate of ammonium chloride was removed by centrifugation and 1 μl of the supernatant was injected into the chromatograph.

RESULTS AND DISCUSSION

Fig. 1 shows chromatograms of derivatized extracts of plasma and of plasma-containing 1.25-μg/ml 5-fluorouracil. In agreement with Cohen and Brennan [4] we have found that 80—85% of 5-fluorouracil added to plasma is recovered in a single extraction with 16% *n*-propanol—ether. The peak corresponding to the derivative of 5-fluorouracil has a retention time of 9.4 min and the internal standard, thymine, emerges 10.0 min after injection. Although thymine is not well separated from 5-fluorouracil under the conditions used, reproducible calibration curves based on peak height ratios are obtained. No interfering peaks arising from endogenous plasma components or reaction by products were observed. A linear relationship between 5-fluorouracil concentration and peak height ratio was obtained provided the amount of 5-fluorouracil injected into the chromatograph fell within the range of 10—1000 pg. The standard deviation of the mean peak height ratio for a given sample was less than 3% of the mean within a run and less than 7% of the mean between runs.

We have been using this technique to monitor plasma levels of 5-fluorouracil in patients undergoing chemotherapy for breast cancer and the results of these studies will be reported separately. Although we have found thymine to be an acceptable internal standard it would clearly be inappropriate in studies directed towards quantitative analysis of 5-fluorouracil in biological specimens containing the naturally occuring pyrimidine.

The method described in this paper allows quantitative analysis of a wide range of drug concentrations in plasma, with a lower limit of detection of

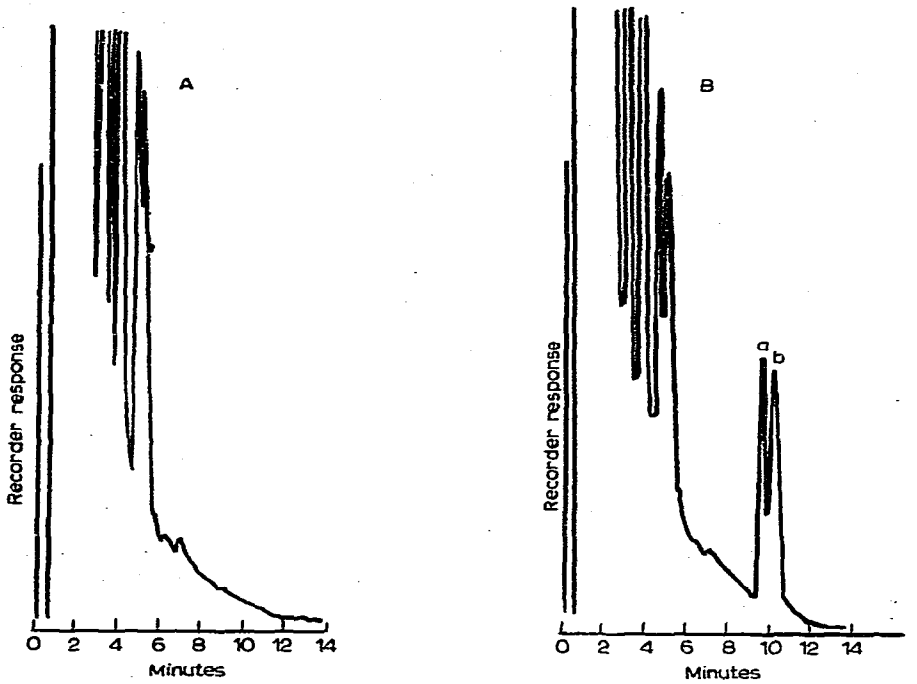

Fig. 1. Gas chromatograms of plasma extracts. A, Plasma blank; B, plasma containing 1.25 μg/ml 5-fluorouracil (peak a), with 625 ng thymine as internal standard (peak b). 7.5 ml of the *n*-propanol—ether extract was evaporated prior to derivatization.

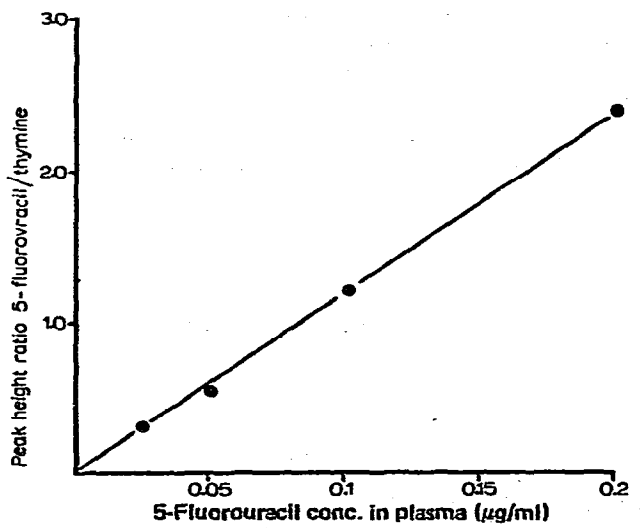

Fig. 2. Calibration curve for 5-fluorouracil extracted from serum at low 5-fluorouracil concentrations. 7.5 ml of the *n*-propanol—ether extract was evaporated prior to derivatization and the final reaction mixture contained 50 ng thymine as the internal standard.

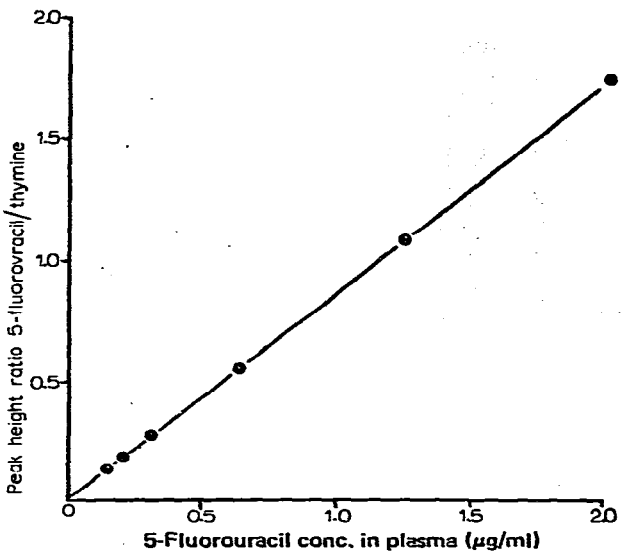

Fig. 3. Calibration curve for 5-fluorouracil extracted from serum over a wide concentration range. 7.5 ml of the *n*-propanol—ether extract was evaporated prior to derivatization and the final reaction mixture contained 625 ng thymine as the internal standard.

20 ng 5-fluorouracil per ml of plasma. The volumes of *n*-propanol—ether extract evaporated and ethyl acetate added prior to sample injection can be adjusted so that the amount of the 5-fluorouracil derivative injected into the chromatograph falls within the range of 10—1000 pg, for which linear detection response is obtained. The sensitivity afforded by the technique described has a number of important practical advantages over pre-existing GLC methods. For most analyses the amount of endogenous plasma material or reaction by products injected into the chromatograph can be kept to a minimum by the appropriate dilutions described. This greatly facilitates quantitative assay and reduces the incidence of detector contamination, a severe problem with earlier methods based on detection of the silyl derivative of 5-fluorouracil [3, 4]. In addition the method could be adapted to allow 5-fluorouracil measurement in plasma volumes of less than 1 ml, an important consideration in many clinical situations.

ACKNOWLEDGEMENT

We would like to thank Action Cancer for their generous financial support.

REFERENCES

1 C. Heidelberger, Cancer Res., 30 (1970) 1549.
2 B. Clarkson, A. O'Connor, L. Winston and D. Hutchinson, Clin. Pharmacol. Ther., 5 (1974) 581.
3 J.J. Windheuser, J.L. Sutter and E. Auen, J. Pharm. Sci., 61 (1972) 301.
4 J.L. Cohen and P.B. Brennan, J. Pharm. Sci., 62 (1973) 572.
5 K.V. Rao, K. Killion and Y. Tanrikut, J. Pharm. Sci., 63 (1974) 1328.