Journal of Pharmaceutical & Biomedical Analysis
Vol. 9, No. 1, pp. 19–21, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00

The analysis of 5-fluorouracil in human plasma by gas chromatography–negative ion chemical ionization mass spectrometry (GC–NICIMS) with stable isotope dilution

C.D. BATES,† D.G. WATSON,*† N. WILLMOTT,† H. LOGAN† and J. GOLBERG‡

† *Department of Pharmacy, University of Strathclyde, Glasgow G1 1XW, UK*
‡ *Department of Surgery, Glasgow Royal Infirmary, Glasgow G4, UK*

Abstract: 5-Fluorouracil (5-FU) was extracted from plasma and converted to its di-*N*-ditrifluoromethylbenzyl (DTFMBz) derivative by treatment with DTFMBzBr. Under negative ion chemical ionization (NICI) conditions the derivative yielded a mass spectrum in which most of the ion current was carried by the M^--DTFMBz ion (*m/z* 355). Selected ion monitoring enabled detection of this derivative in amounts <1 pg, and the practical limit of detection for 5-FU extracted from plasma was *ca* 400 pg ml^{-1}. 5-FU was quantified by adding a fixed amount of [$^{15}N_2$]5-FU to samples of plasma before extraction, and comparing the ratio of the ions of *m/z* 355 and 357 against a calibration curve constructed over the concentration range under investigation. The method was used to measure the variation in the concentration of 5-FU with time during continuous infusion of the drug via three, different protocols in patients with hepatic tumour.

Keywords: *Gas chromatography–negative ion chemical ionization mass spectrometry; 5-fluorouracil; di-N-ditrifluoromethylbenzyl derivative.*

Introduction

A number of methods have been developed for the measurement of 5-fluorouracil (5-FU) levels in plasma by gas chromatography and gas chromatography–mass spectrometry (GC–MS) [1–5]. These methods have employed alkylation [1, 2] or trimethylsilylation [3, 4] and using GC–MS with selected ion monitoring limits of sensitivity between 1–10 ng in plasma were established. Methods based upon HPLC analysis in general have ranges of sensitivity of 10–100 ng ml^{-1} for the analysis of 5-FU in plasma [6]. Recently, a method has been developed in which 5-FU is converted to its di-*N*-pentafluorobenzyl derivative and then analysed by GC–MS with negative ion chemical ionization [5], limits of detection for 5-FU using this method were *ca* 0.4 ng ml^{-1} of plasma. Most methods have employed 5-chlorouracil (5-CU) as an internal standard; [1,3-$^{15}N_2$]5-FU was used in the analysis of 5-FU as its di-trimethylsilyl derivative by GC–MS with positive ion chemical ionization [4]. A procedure is described here in which the advantages of the sensitivity and specificity of gas chromatography–negative ion chemical ionization mass spectrometry (GC–NICIMS) are combined with the quantitative accuracy afforded by the use of an isotopomeric internal standard and the method applied to the measurement of 5-FU in the plasma of patients treated for hepatic tumour.

Materials and methods

Chemicals

Chemicals were obtained from the following sources: 5-FU and 5-CU, Sigma Chemical Co. (Dorset, UK); [1,3-$^{15}N_2$]5-FU, MSD Isotopes (Croydon, UK); 3,5-ditrifluoromethylbenzyl bromide, Fluorochem (Derbyshire, UK); and solvents were glass distilled grade (Rathburn Chemicals, Peebleshire, UK).

Extraction of plasma and derivatization of extracts

[$^{15}N_2$]5-FU (50 ng in 50 μl of acetonitrile) was added to 0.5 ml of plasma, and the sample was then acidified with 0.5 ml of 0.2 M sodium acetate buffer (pH 5), and extracted with 7.5 ml of ether–isopropanol (4:1) by shaking for 30 min and then centrifuging (15 min, 1500 rpm). The solvent was then removed by flash

* Author to whom correspondence should be addressed.

evaporation and stored at −20°C until analysis. The residue was then dissolved in ethyl acetate (1 ml) and transferred to a reactivial. The ethyl acetate was removed under a stream of nitrogen and the residue was dissolved in acetonitrile (30 μl), and this was followed by addition of ditrifluoromethylbenzyl bromide (10 μl) and triethylamine (10 μl). The solution was allowed to stand at room temperature (15 min) and then ethyl acetate (100 μl) followed by hexane (900 μl) were added, and the solution was left for 5 min while a precipitate formed. The solution was passed through a column of Sephadex LH20 (*ca* 3 cm in a Pasteur pipette) which was washed with a further 1 ml of hexane. The eluent was concentrated to 0.5 ml and an aliquot (2 μl) was injected into the GC–MS.

Instrumentation

GC–MS in the NICI mode was carried out using a Hewlett–Packard 5988A gas chromatograph–mass spectrometer interfaced with a HP RTE-6/VM data system. The following mass spectrometric conditions were used: the instrument was tuned in the NICI mode to the ions at *m/z* 452, 595 and 633 from the perfluorotributylamine calibrant, source temperature was 140°C, electron energy 200 eV and methane reagent gas was introduced to give a source pressure *ca* 1 torr. The gas chromatograph was fitted with a BP-1 aluminium clad fused silica column, 12.5 × 0.25 mm i.d. (S.G.E., Burke Electronics, Glasgow, UK), helium carrier gas was used with a head pressure of 0.33 bar.

The GC conditions were as follows: injector temperature 250°C, transfer line temperature 280°C, the oven temperature was maintained at 100°C for 1 min then programmed at 20°C min^{-1} to 300°C. Injections were made using a Grob splitless injection system.

Results and Discussion

The mass spectrum obtained for the di-*N*-ditrifluoromethylbenzyl (DTFMBz) derivative of 5-FU under NICI conditions is shown in Fig. 1. Most of the ion current is carried by the ion at *m/z* 355 which arises via loss of a DTFMBz moiety from the molecular ion. This type of mass spectrum is of the same type obtained for the di-*N*-pentafluorobenzyl (PFBz) derivative of 5-FU by previous workers [7]. We employed the PFBz derivative at the beginning of our study but found that this derivative of 5-FU produced a poor peak shape upon GC–MS analysis unless the column was quite new and not contaminated by residues from previous runs; this decreased the precision and sensitivity of the method. We have previously encountered a tendency for the PFBz moiety to produce a poor peak shape when introduced into molecules in the course of derivatization [7, 8] and this is presumably due to the high polarity of the group.

In our earlier work we observed that derivatization of biogenic amines with ditrifluoromethylbenzoyl chloride resulted in sharper peaks compared with the derivatives formed by reaction with pentafluorobenzoyl chloride [7]. Similarly, in the present case we found that the peak shape of the DTFMBz derivative of 5-FU was much better than that of the corresponding PFBz derivative.

A calibration curve was constructed by spiking plasma with a fixed amount of [$^{15}N_2$]5-FU (50 ng) and varying amounts of 5-FU (1–200 ng), the calibration curve was linear over this range (correlation coefficient 0.998).

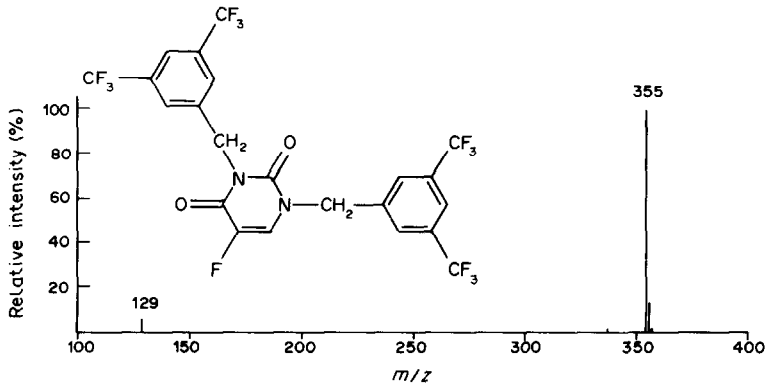

Figure 1
NICI mass spectrum of the 5-FU DTFMBz derivative.

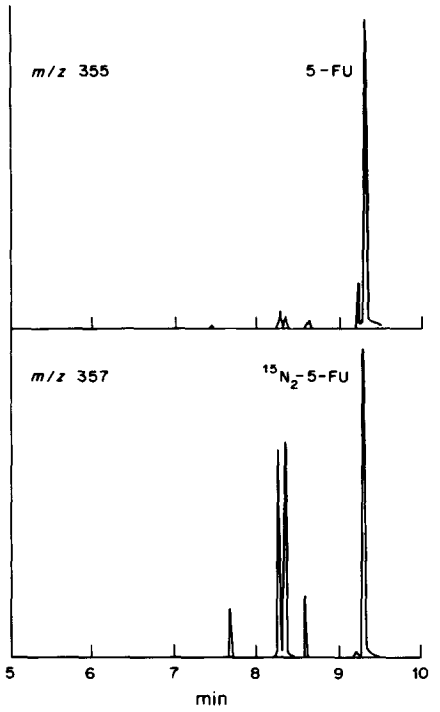

Figure 2
SIM traces of 5-FU (22 ng) and [$^{15}N_2$]5-FU (50 ng) extracted from plasma (0.5 ml) taken after 24 h of intra-arterial continuous infusion.

Figure 2 shows a selected ion monitoring trace of an extract from the plasma of a patient treated for 24 h by continuous intra-arterial infusion of 5-FU. Fifty nanograms of [$^{15}N_2$]5-FU were added prior to extraction and the extract was treated to form DTFMBz derivatives, the ions for the di-DTFMBz derivatives of 5-FU (*m/z* 355) and [$^{15}N_2$]5-FU (*m/z* 357) were monitored. Prior to obtaining [$^{15}N_2$]15-FU, 5-CU was used as an internal standard but, although this compound has been widely used as an internal standard in the measurement of 5-FU [1, 2, 5], we found it to be completely unsatisfactory in our procedure for the following reasons:

(1) the relative response of the DTFMBz derivatives of 5-FU and 5-CU varied widely (±200%) from day to day when they were analysed by GC–NICIMS, this may be due to a difference in electron capturing properties brought about by the presence of a chlorine atom in 5-CU combined with slight variations in the conditions within the ion source;
(2) the relative recoveries of 5-FU and 5-CU from plasma of patients treated with 5-FU were extremely variable, 5-CU sometimes being recovered to a much lesser extent than 5-FU. This led to a drift, over a period of weeks, in the quantitation of 5-FU in the same sample of plasma of over an order of magnitude. No such problems were experienced when [$^{15}N_2$]5-FU was used as an internal standard.

Conclusion

We have used this procedure to compare plasma concentration–time profiles of 5-FU during continuous infusion over 24 h by three protocols in patients with advanced colorectal liver metastases; the procedure was completely satisfactory for quantifying 5-FU over the range found in plasma samples, *ca* 1–200 ng ml^{-1}. The results of the clinical study have been reported elsewhere [9].

It is of interest that in a separate study we found that DTFMBzBr was a less effective alkylating reagent for thiouracils, and in an investigation of the uptake of these compounds by melanoma cell spheroids we achieved better results by using PFBzBr as a derivatizing agent [10].

Acknowledgement — We thank the SHHD for financial support.

References

[1] H. Isomura, S. Higuchi and S. Kawamura, *J. Chromatogr.* **224**, 423–429 (1981).
[2] A.P. De Leenheer and M.C. Cosyns-Duyck, *J. Chromatogr.* **174**, 325–339 (1979).
[3] D.B. Lakings, R.H. Adamson and R.B. Diasio, *J. Chromatogr.* **146**, 512–517 (1978).
[4] T. Marunaka and Y. Umeno, *J. Chromatogr.* **221**, 382–386 (1980).
[5] R.M. Kok, A.P.J.M. De Jong, C.J. Van Groeningen, G.J. Peters and J. Lankelma, *J. Chromatogr.* **343**, 59–66 (1985).
[6] S. Eksborg and H. Ehrsson, *J. Chromatogr.* **340**, 31–72 (1985).
[7] J.M. Midgley, J. MacLachlan and D.G. Watson, *Biomed. Environ. Mass Spectrom.* **15**, 535–539 (1980).
[8] J.M. Midgley, D.G. Watson, T. Healey and C.N.J. McGhee, *Biomed. Environ. Mass Spectrom.* **18**, 657–661 (1989).
[9] J. Golberg, D. Kerr, D.G. Watson, N. Willmott, C.D. Bates, H. Logan, J.H. McKillop and C.S. McArdle, *Br. J. Cancer* **61**, 613–615 (1990).
[10] G.S. Skellern, C.D. Bates, D.G. Watson, S. Martin, R. Mairs and D. Byrne, *J. Pharm. Pharmacol.* **41**, 19 (1989).

[*Received for review 7 December 1989*]