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Note

Determination of 5-fluorouracil and pyrimidine bases in plasma by gas chromatography—chemical ionization-mass fragmentography

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1-(Tetrahydro-2-furanyl)-5-fluorouracil (FT), named Ftorafur or FT-207, has been widely used in cancer chemotherapy. 1,3-Bis(tetrahydro-2-furanyl)-5-fluorouracil (FD-1) [1,2], and uracil plus FT (UFT; 4:1, mol/mol) [3—7] have been recently developed as an antitumor agent with more effective activity. We have reported the determination of 5-fluorouracil (5-FU) as an active metabolite of FT and FD-1 in plasma and visceral tissues by gas chromatography—electron impact-mass fragmentography (GC—EI-MF) [8,9], and further the simultaneous determination of 5-FU and uracil in plasma and visceral tissues after administration of UFT by a combination of GC—EI-MF and gas chromatography—electron impact-mass spectrometry for total ion monitoring (GC—EI-MS) [10]. Furthermore, a method for the simultaneous assay of uracil, thymine and cytosine present in biological materials as pyrimidine bases by a combination of GC—EI-MF and GC—EI-MS has been reported [11]. Pantarotto et al. [12] and Min and Garland [13] determined 5-FU as its methylated derivative after administration of 5-FU in plasma or serum by gas chromatography—chemical ionization-mass fragmentography (GC—CI-MF). This paper describes the use of GC—CI-MF in determining the plasma levels of 5-FU and pyrimidine bases, uracil, thymine and cytosine, as their trimethylsilylated (TMS) derivatives.

EXPERIMENTAL

Materials

5-FU, uracil, thymine and cytosine were obtained from Sigma (St. Louis, MO, U.S.A.). 5-Chlorouracil was synthesized in our laboratory. [1,3-$^{15}N_2$]-5-FU and [1,3-$^{15}N_2$]uracil (each 95% enrichment) were purchased from PCR (Gainesville, FL, U.S.A.). N,O-Bis(trimethylsilyl)acetamide (BSA), tri-

methylchlorosilane (TMCS) and pyridine for the silylating agents and solvent were from Pierce (Rockford, IL, U.S.A.). The other chemicals used were liquid-chromatographic and analytical grade materials.

Instrumentation

A JEOL JMS D300 mass spectrometer (EI/CI ion source) connected to a JEOL JGC-20KP gas chromatograph (Tokyo, Japan) was used.

The coiled glass column (1 m × 2 mm I.D.) of the gas chromatograph was packed with 3% OV-17 on Chromosorb W AW (80—100 mesh) (Gaschro Kogyo, Osaka, Japan). The temperatures of the injector and ion source were 230°C and 160°C, respectively, and analyses were carried out with an initial column temperature of 150°C and a temperature rise of 10°C/min. The carrier gas was helium and the flow-rate 30 ml/min. The mass spectrometer was operated under the following conditions: ionization energy, 190 eV; ionization current, 300 μA; accelerating voltage, 3.0 kV; ion multiplier voltage, 1.8 kV; reagent gas, ammonia; and the pressure of reagent gas, 1.0 Torr. The fragment ions detected were the protonated molecular ion peaks, $(M+1)^+$, of *m/z* 275, 257, 271, 256, 277, 259 and 291 for the TMS derivatives of 5-FU, uracil, thymine, cytosine, [1,3-$^{15}N_2$]-5-FU, [1,3-$^{15}N_2$]uracil and 5-chlorouracil.

Analytical procedure

The analytical procedure for GC—CI-MF was as described previously [8—11] except that for silylation a freshly prepared solution of 100 μl of pyridine containing 25% BSA and 1% TMCS was added to the residue extracted with ethyl acetate, and this solution was kept at 80°C for 20 min to allow silylation to occur.

[1,3-$^{15}N_2$]-5-FU and [1,3-$^{15}N_2$]uracil were used as an internal standard for determination of 5-FU and uracil; 5-chlorouracil was the internal standard for thymine and cytosine. The calibration curves were prepared by plotting the ratio of the peak height of each TMS derivative to that of the TMS derivative of the internal standard against the concentration (1.0, 0.5, 0.25, 0.10, 0.05, 0.01 and 0.005 μg/ml for each compound was added to 1.0 ml of plasma).

RESULTS AND DISCUSSION

The CI mass spectra of 5-FU, pyrimidine bases and their internal standards using ammonia as a reagent gas are shown in Fig. 1. The mass spectra of 5-FU, uracil, thymine, [1,3-$^{15}N_2$]-5-FU, [1,3-$^{15}N_2$]uracil and 5-chlorouracil showed an $(M+1)^+$ ion but no other fragment ions, while the spectrum of cytosine showed an $(M+1)^+$ ion and one fragment ion. The CI mass spectra of these compounds using isobutane as a reagent gas, on the other hand, showed an $(M+1)^+$ ion and many fragment ions.

To obtain suitable GC—CI-MF conditions for determination of 5-FU and pyrimidine bases in plasma after administration of FT or FD-1 plus pyrimidine base, the derivation procedure, the reagent gas and the ions detected by the mass spectrometer were investigated. The silylation procedure with pyridine

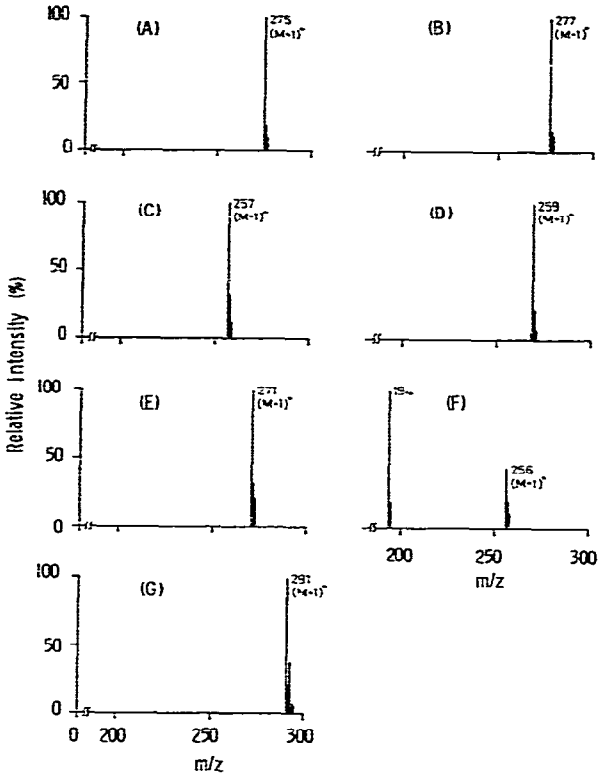

Fig. 1. CI mass spectra of the TMS derivatives of (A) 5-FU, (B) [1,3-$^{15}N_2$]-5-FU, (C) uracil, (D) [1,3-$^{15}N_2$]uracil, (E) thymine, (F) cytosine and (G) 5-chlorouracil using ammonia as a reagent gas.

containing BSA and TMCS, the use of ammonia as a reagent gas and the detection of an $(M+1)^+$ ion were found to result in better separation of 5-FU and pyrimidine bases from plasma constituents and a higher detection sensitivity.

The GC—CI-MF separation pattern of authentic samples of 5-FU and uracil, and of these compounds extracted from plasma after the administration of UFT, using the respective stable isotope-labeled compounds as internal standard, are shown in Fig. 2. The retention times of the TMS derivatives of 5-FU and uracil were 1.5 and 1.7 min, respectively, and the detection limit was 0.001 μg/ml of plasma for each compound.

The GC—CI-MS separation of human plasma extracted with known amounts of added 5-FU and thymine, and 5-FU and cytosine, using 5-chlorouracil as an internal standard, are shown in Fig. 3. The retention times were 2.1, 3.7 and 2.5 min for the TMS derivatives of thymine, cytosine and 5-chlorouracil, respectively. The detection limits for thymine and cytosine in plasma were 0.001 μg/ml and 0.050 μg/ml, respectively.

The precision of the method was ± 3.1%. This assay method appears to be satisfactory for the determination of these compounds in plasma.

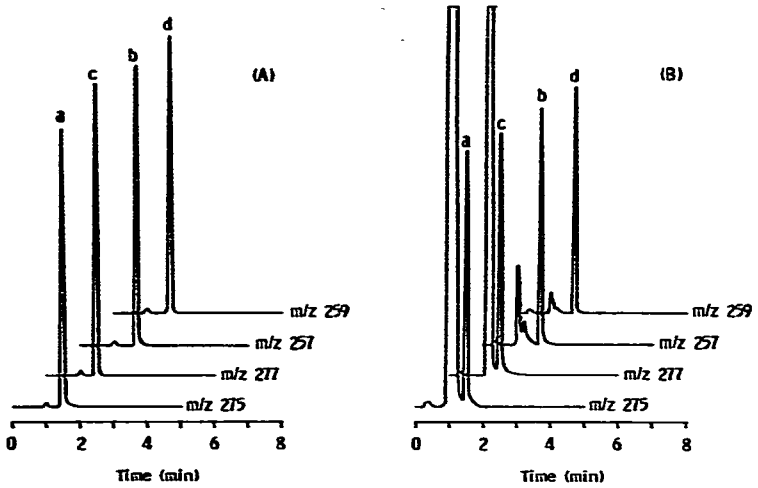

Fig. 2. Separation by GC—CI-MF of (A) authentic samples of 5-FU (a), uracil (b) and the internal standards, [1,3-$^{15}N_2$]-5-FU (c) and [1,3-$^{15}N_2$]uracil (d), and (B) these compounds extracted from human plasma. Results are for the TMS derivatives.

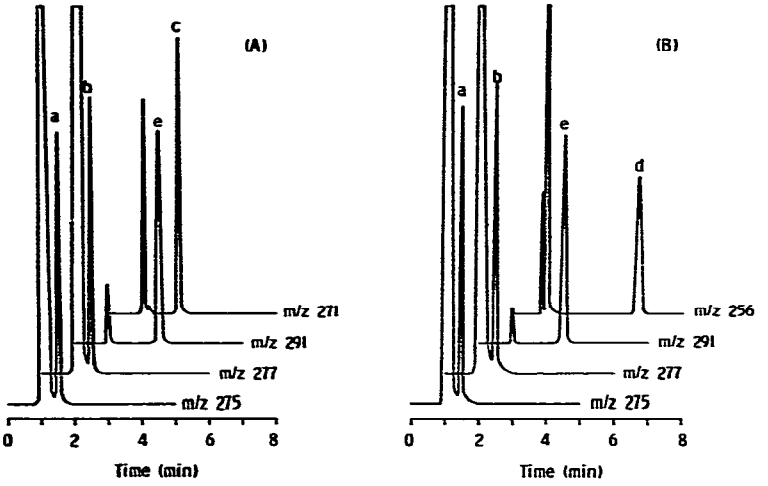

Fig. 3. Separation by GC—CI-MF of (A) 5-FU (a) and thymine (c), and (B) 5-FU (a) cytosine (d), and the internal standards, [1,3-$^{15}N_2$]-5-FU (b) and 5-chlorouracil (e), extracted from human plasma. Results are for the TMS derivatives.

The precision and sensitivity of GC—CI-MF for determination of 5-FU and pyrimidine bases were compared with those of GC—EI-MF reported previously [8—11]. The results obtained by GC—CI-MF were no less than those obtained by GC—EI-MF except for the sensitivity of cytosine. The GC—EI-MF procedure is useful for the simultaneous assay of both plasma and visceral tissue levels of 5-FU and pyrimidine bases; however, the GC—CI-MF method described in this paper could be applied to plasma but not to visceral tissues because of poor separation from visceral tissue constituents.

Thus, the present method should be useful as well as GC—EI-MF for measuring the plasma levels of 5-FU and pyrimidine bases after administration of FT or FD-1 plus uracil, thymine or cytosine.

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