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Note

A new sensitive and specific gas chromatographic method for the determination of the carcinostatic agent 6-chrysenamine in biological samples

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6-Chrysenamine (6-CA), EORTC 116, is an antitumour agent that has been demonstrated to be active on a mouse spontaneous mammary tumour in $(C_3H \times O_{20})F_1$ mice and on a rhabdomyosarcoma BA 1112 transplanted in inbred Wag/Rij rats[1–4]. The lack of a satisfactory analytical method for the assessment of the drug body concentrations[5] has prompted us to develop a sensitive and specific gas chromatographic method for 6-CA, which has been applied to the determination of the drug in mice urine and tumour of $(C_3H \times O_{20})F_1$ mice.

EXPERIMENTAL

Standards and reagents

6-CA was kindly supplied by Dr. P. Poitier (Gif sur Yvette, France), butropipazone by Dr. P. A. J. Janssen (Janssen Pharmaceutica, Beerse, Belgium) and diazepam by Ravizza S.p.A. (Milan, Italy).

The following reagents were used: diethyl ether, sodium hydroxide, ethyl acetate and acetone (Carlo Erba), and trifluoroacetyl anhydride (TFA) (Fluka).

Apparatus

Gas–liquid chromatography (GLC). GLC was carried out on a Carlo Erba Fractovap Model G1 gas chromatograph, equipped with a flame ionization detector (FID) or a ^{63}Ni electron-capture detector (ECD). In both cases glass columns were used, 1 m long and 4 mm I.D., packed with 100–120 mesh Chromosorb Q coated with 3% SE-30 (Applied Science Laboratories). Nitrogen was used as the carrier gas at a flow-rate of about 60 ml/min. Air and hydrogen flow-rates were adjusted to give the maximum response when operating with the FID.

The column oven was maintained at 240°, the flash heater at 280° and the ECD at 300°.

The ECD was used with a pulse current: excitation voltage, 10 V; pulse width, 3 μsec; period, 30 μsec; and scavenger gas (nitrogen) flow-rate, 60 ml/min.

Mass spectrometry (MS). An LKB Model 9000 mass spectrometer coupled with a gas chromatograph was used under the following conditions: energy of the ionization beam, 70 eV; ion source temperature, 290°; accelerating voltage, 3.5 kV; and trap current, 60 μA.

The sample was introduced by a gas chromatographic procedure on a glass column, 1 m long and 4 mm I.D., packed with 3% SE-30, on Gas-Chrom Q, 100–120 mesh, under the following conditions: injector temperature, 270°; oven temperature, 240°; helium (carrier gas) flow-rate, 30 ml/min; and detector, total ion monitor.

Mass fragmentography. The mass spectrometer was also used as a gas chromatographic detector by focusing the instrument upon the molecular ion of the trifluoroacetylated 6-CA at *m/e* 339. The minimum detectable amount by using this technique was 100 pg.

Extraction procedure

Extraction of 6-CA from urine. To 0.2 ml of urine were added 2.5 μg of butropipazone as internal standard, 0.5 ml of 1 *N* NaOH and 2 ml of diethyl ether. The test-tubes were subjected to gentle mechanical shaking for 10 min. After centrifugation at 4° for 5 min, 1.5 ml of the organic phase was transferred into a second test-tube and evaporated to dryness at room temperature on a rotating evaporator. A 50-μl volume of acetone was added and 1–2 μl injected into the gas chromatograph.

Extraction of 6-CA from tumour. To 1 ml of tumour (homogenized 1:5 with KCl) were added 100 ng of diazepam as internal standard, 0.5 ml of 1 *N* NaOH and 2 ml of diethyl ether.

The test-tubes were submitted to the same procedure as above, and, after evaporating to dryness, 25 μl of TFA and 50 μl of ethyl acetate were added and the stoppered tubes heated at 80° for 5 min. The samples were evaporated to dryness, 200 μl of acetone were added and 1–2 μl injected into the gas chromatograph.

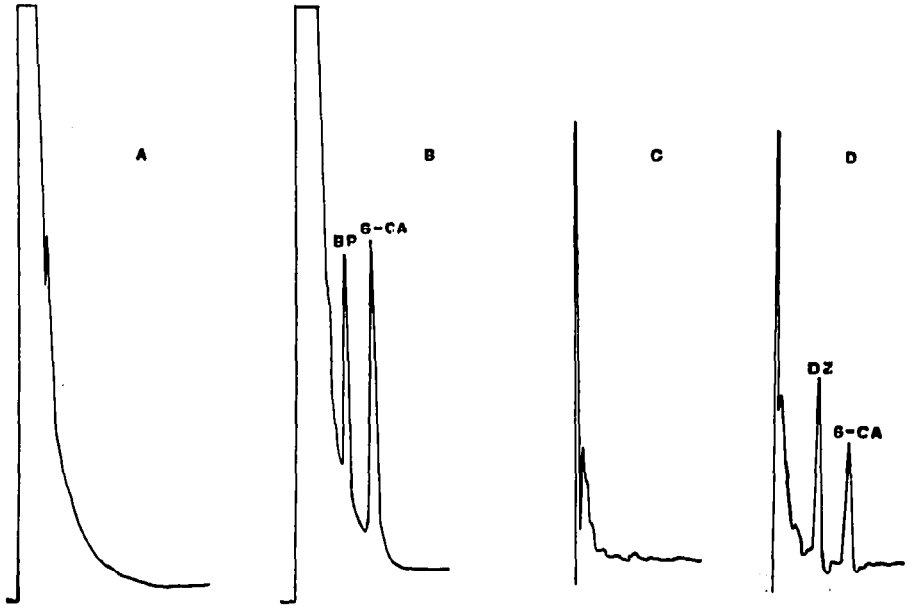

Fig. 1. Gas chromatograms of 6-chrysenamine (6-CA) using a flame ionization detector (A and B) and the 6-CA N-trifluoroacetyl derivative using an electron-capture detector (C and D). (A) Urine blank; (B) 6-CA extracted from urine; BP = butropipazone; (C) Tumour blank; (D) 6-CA extracted from tumour; DZ = diazepam.

RESULTS AND DISCUSSION

Fig. 1 shows some typical chromatograms of extracts from urine and tumour. No interfering peaks from endogenous substrates were noted.

The calibration curves for 6-CA in the urine, obtained by plotting ratios of the peak area of the drug to the internal standard against known amounts of the drug added to the biological specimens, are illustrated in Fig. 2.

The calibration curves for the 6-CA N-TFA derivative obtained in the same way using a halogenated internal standard (diazepam) are reported in Fig. 3. The linearity of the method ranges from 1.25 to 5 μg/ml using the FID and from 0.25 to 1 μg/ml using the ECD. The minimum detectable amount is 25 ng for 6-CA and 1 ng for the 6-CA N-TFA derivative. The recovery from urine and tumour is 90±2%.

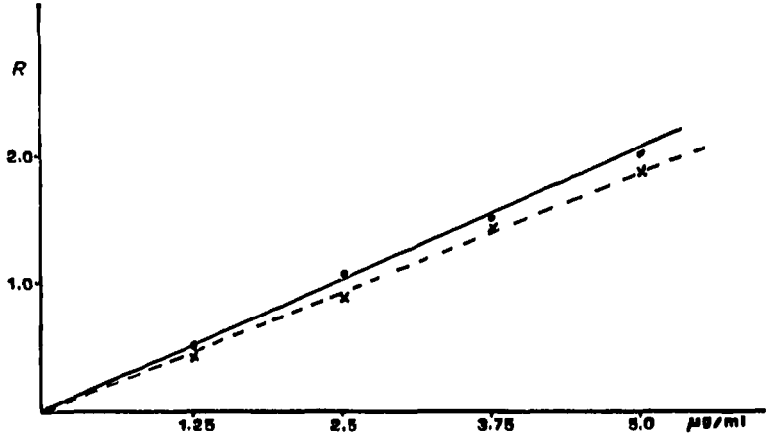

Fig. 2. Standard calibration curves of 6-chrysenamine (6-CA). Ordinate: peak area ratio (R) of 6-CA to the internal standard (butropipazone). Abscissa: drug concentration. ●—●, External calibration curve; ×---×, internal calibration curve (urine). The curves were obtained using a flame ionization detector.

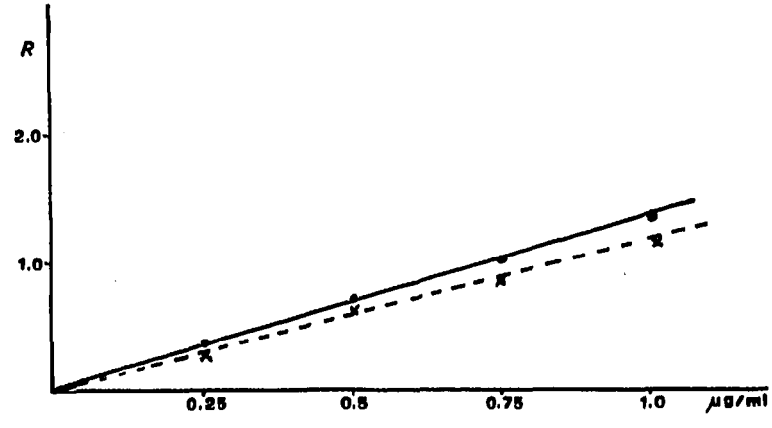

Fig. 3. Standard calibration curves of the 6-chrysenamine (6-CA) N-trifluoroacetyl derivative. Ordinate: peak area ratio (R) of 6-CA to the internal standard (Diazepam). Abscissa: drug concentration. ●—●, external calibration curve; ×---×, internal calibration curve (tumour). The curves were obtained using an electron-capture detector.

The identity of the gas chromatographic peaks obtained after GLC analysis of 6-CA and after reaction of 6-CA with TFA were checked by means of GLC–MS. The mass spectrum of 6-CA (Fig. 4) shows a molecular ion at *m/e* 243, and this peak, according to the aromatic nature of the molecule, is also the base peak. After the reaction of 6-CA with TFA, only one gas chromatographic peak was obtained. The mass spectrum (Fig. 4) showed a molecular ion at *m/e* 339, which also in this case is the base peak; this means that only one trifluoroacetyl group enters the molecule.

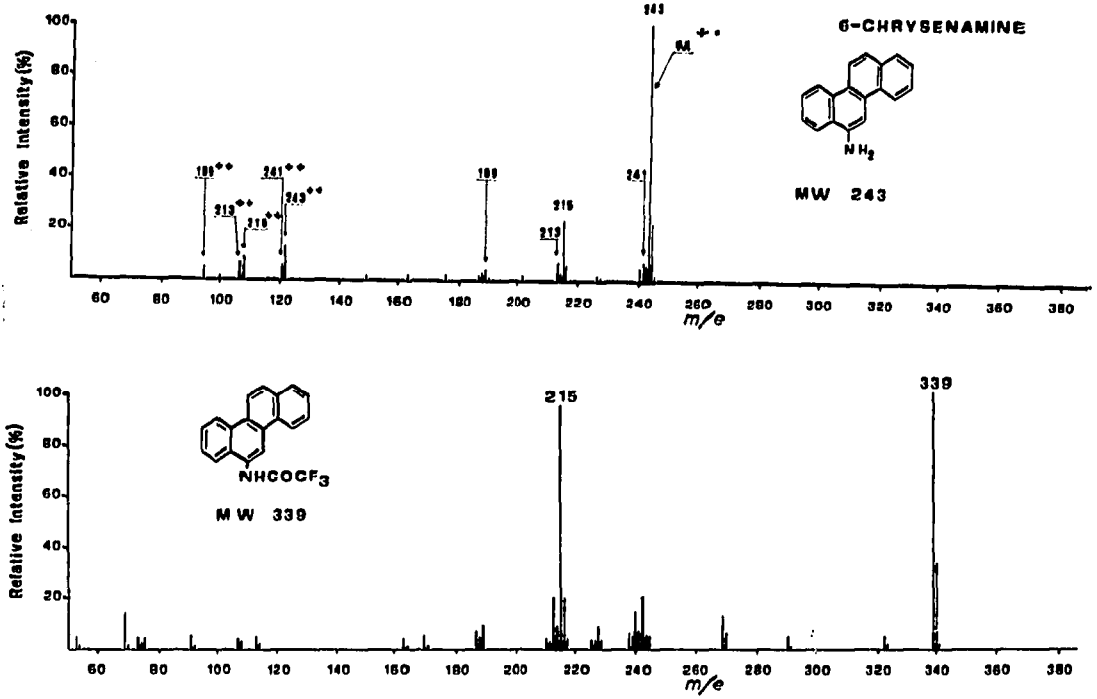

Fig. 4. Mass spectrum of 6-chrysenamine and its N-trifluoroacetyl derivative. For the experimental conditions, see text.

The high sensitivity and specificity of this analytical method makes it useful to study the pharmacokinetics and metabolism of 6-CA.

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