Journal of Chromatography, 275 (1983) 432—435
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1685

Note

Electron-capture—gas chromatographic analysis of ifosfamide in human plasma and urine

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(First received November 24th, 1982; revised manuscript received February 23rd, 1983)

Ifosfamide (IFA) is an anticancer agent that has recently been approved for use in the United States for treatment of non-oat cell bronchogenic carcinoma [1—4]. This drug has also been indicated for use in the treatment of a wide range of human cancers such as lymphoma, ovarian and testicular cancers [5]. Few analytical procedures have been developed for quantitation of this drug following human administration. Pharmacokinetics have been studied by radiochemical analysis of [^{14}C]ifosfamide [6] and by gas chromatography (GC) [4, 7]. This study describes the use of an electron-capture—gas chromatographic (EC—GC) method for IFA determination using heptafluorobutyric anhydride (HFBA) for derivatization.

EXPERIMENTAL

Chemicals

Ifosfamide and cyclophosphamide (CPA) (internal standard) were a gift from Mead Johnson (Evansville, IN, U.S.A.). The derivatizing agent heptafluorobutyric anhydride (HFBA) was obtained from Pierce (Rockford, IL, U.S.A.) and reagent-grade methylene chloride and pyridine were purchased from Mallinckrodt (St. Louis, MO, U.S.A.).

Instrumentation

A Hewlett-Packard 5730A gas chromatograph with a ^{63}Ni (15 mCi) electron-capture detector and 3390A integrator were used in all experiments. Chromatography was performed on a 1.8 m × 4 mm I.D. glass column with 3% SE-30 on Gas-Chrom Q, 100—120 mesh. The column temperature was maintained at 170°C, injection port at 250°C, EC detector at 300°C with a methane—argon (5:95) carrier gas flow-rate of 35 ml/min.

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Sample collection and extraction

Informed consent was obtained from patients with documented non-oat cell bronchogenic carcinoma. IFA doses of 1.2 g/m^2 body surface area were given intravenously (dissolved in 1 l of 5% dextrose in water). Blood samples were drawn in heparinized tubes and plasma was used for IFA analysis. Urine samples were collected periodically throughout the procedure. Plasma or urine (500 μl) was added to a 20-ml screw-capped glass tube containing 10 μg of internal standard dissolved in water (10 μg CPA per ml). To the tubes were also added 1.0 ml of 10% sodium bicarbonate and 10 ml methylene chloride. The samples were shaken for 10 min and centrifuged at 6300 *g* for 10 min. The aqueous layer was aspirated and 9.5 ml of the organic layer removed and added to a new screw-capped tube and dried under a stream of dry air. To the dried residue were added 200 μl of HFBA and the samples were heated at 80°C for 20 min. After derivatization the samples were again dried with a stream of dry air and 200 μl of pyridine added to each sample before injection.

Calculations

Calibration curves were constructed by plotting the peak area ratio IFA/CPA by the ratio of molar quantities of IFA and CPA. After determining the molar quantity of drug in a sample this was multiplied by the molecular weight of IFA (260 g/mol) and divided by the ml of sample to obtain final values in μg/ml plasma or urine.

RESULTS AND DISCUSSION

Fig. 1a and b presents typical chromatograms of normal blank plasma and a plasma sample 1 h following intravenous (i.v.) administration of IFA (internal standard included), respectively. The retention times for IFA and CPA are 4.84 and 6.67 min. Identification is achieved via retention time and peak superimposition, i.e., by injection of IFA and CPA standards (20—50 ng) along with previously extracted samples and observing the increased peak area and height at the corresponding retention times.

The limit of detection (signal-to-noise ratio of 2:1) of this assay was 1 ng/ml plasma or urine and the extraction recovery was 85 ± 5% (n = 25). Repetitive injections of standards and samples gave good reproducibility of retention times (coefficients of variation, C.V. ± 2.5% and 3.1%, respectively). Standard curves were linear in the range of 1—25 μg/ml media and day-to-day reproducibility varied less than 3.7% C.V. Standard stock solutions of the drugs were not stable when stored at —20°C for two days. Variable losses of 5—20% during this time were observed; therefore, fresh samples should be prepared daily. Samples extracted from biological media, derivatized, and stored at —70°C overnight were noted to have variable losses of up to 15%. All samples reported in this paper were analyzed within 4—6 h following extraction and derivatization.

A difference in the response of the electron-capture detector was noted by the use of methane—argon (5:95) as opposed to 100% nitrogen carrier gas. The methane—argon mixture produced approximately a 12% increase in signal

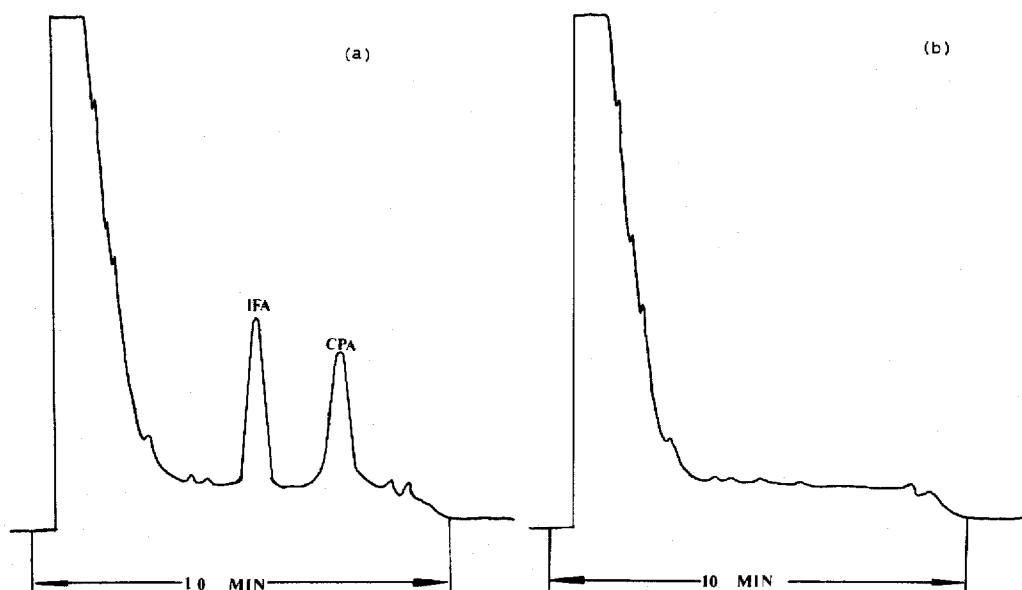

Fig. 1. (a) EC—GC chromatograms of IFA and CPA (internal standard) with retention times of 4.84 and 6.67 min, respectively, extracted from human plasma 2 h following i.v. administration. (b) EC—GC chromatogram of blank human plasma.

TABLE I

CONCENTRATION OF UNMETABOLIZED IFOSFAMIDE (IFA) IN HUMAN PLASMA AND URINE

Time* (h)	IFA**	
	Plasma	Urine
0	—	—
0.5	22.10 ± 1.35	2.41 ± 0.99
1	7.05 ± 0.97	6.02 ± 1.07
1.5	—	14.46 ± 1.39
2	5.36 ± 0.91	50.60 ± 1.72
7	4.16 ± 0.85	—
8	—	36.75 ± 1.60

*Time after an i.v. dose of 2.0 g IFA.
**Levels expressed in μg drug per ml media (average of three determinations ± S.D.).

intensity of the HFBA-derivatized drug. This was probably due to the greater amount of scavenger methane gas in the carrier gas that traps more thermal electrons [8]. Regardless, the nitrogen carrier gas gave an acceptable baseline for EC—GS analysis. Other derivatizing agents [trifluoroacetic anhydride (TFAA) and pentafluoropropionic anhydride (PFPA)] were evaluated for use in this procedure (obtained from Pierce). Following the derivatization procedure described in the Experimental section it was found that the HFBA derivative gave the greatest detector response. The HFBA response was 9% better than PFPA and 20% more intense than that of TFAA. It was also noted that the HFBA and PFPA derivatives were more stable upon standing at room

temperature for 6 h as opposed to TFAA (variable losses up to 15%). For these reasons, HFBA was chosen as the derivatization agent of choice.

In Table I are given the levels of μg/ml plasma or urine of IFA after an i.v. administration of 2.0 g IFA. The unmetabolized drug peaks in about 2—3 h in urine samples. It is still detectable in samples up to 24 h later. These data are within agreement of previously reported data from this laboratory using a flame ionization GC procedure for analysis [4].

In conclusion, this EC—GC method provides a specific and sensitive determination of IFA in human plasma and urine samples following therapeutic dosing with the drug. A number of improvements have been made in the analytical methodology (such as derivatization agent, carrier gas composition, extraction solvent, and stability studies) over the previously reported procedures [4, 7] that greatly increase this assay's general utility for clinical analysis.

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