Journal of Chromatography, 223 (1981) 202—207
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 786

Note

Determination of flurbiprofen in human plasma using gas chromatography—mass spectrometry with selected ion monitoring

KENJI KAWAHARA*, MISAKO MATSUMURA and KAZUHIKO KIMURA

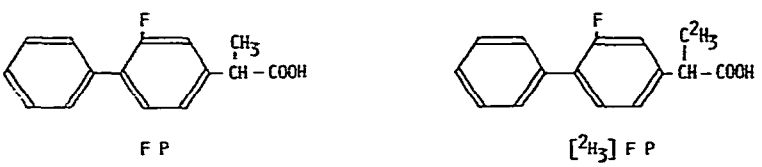
Research Laboratories, Kakenyaku-kako Co. Ltd., 14 Minami-kawaramachi, Shinomiya, Yamashina-ku, Kyoto 607 (Japan)

(Received August 26th, 1980)

Flurbiprofen (FP), 2-(2-fluoro-4-biphenylyl) propionic acid, provides a wide range of antiinflammatory, antipyretic and analgesic activities [1—3], and has the chemical structure shown in Fig. 1.

Kaiser et al. [4] and Kawahara et al. [5] have reported gas chromatographic (GC) methods for the determination of FP in human plasma after oral administration of FP. The minimum detectable concentration in each method was 50 ng/ml.

In a preliminary experiment of this study, it was found that the plasma levels of FP following application of FP plaster were lower than the limits of GC determination. This paper describes a highly sensitive gas chromatographic—mass spectrometric (GC—MS) method using selected ion monitoring (SIM) which allows the quantitation of plasma levels as low as ng/ml. The method established is applicable to pharmacokinetic and bioavailability investigations with respect to percutaneous absorption of FP in humans.



F P [$^2H_3$] F P

Fig. 1. Chemical structures of FP and [$^2H_3$]FP.

EXPERIMENTAL

Reagents and materials

Benzene, diethyl ether (for pesticide residue analysis), hydrochloric acid, sodium carbonate, sodium hydrogen carbonate and sodium hydroxide (ana-

0378-4347/81/0000—0000/$02.50

lytical grade) were obtained from Nakarai Chemical (Kyoto, Japan). Diazomethane ether solution (alcohol-free) was obtained from N-methyl-N-nitroso-*p*-toluenesulfonamide. A carbonate buffer (pH 9.5) was prepared with sodium carbonate and sodium hydrogen carbonate solutions (0.1 *M*).

[$^2H_3$]FP (see Fig. 1) was synthesized in this laboratory and 0.001 *N* sodium hydroxide solution containing 50 ng/ml [$^2H_3$]FP was used as the internal standard solution.

n-Tetracosane (TC), used as a secondary internal standard, was obtained from Applied Science Labs. (State College, PA, U.S.A.).

The plaster (10 cm × 13.6 cm), containing 40 mg of FP per sheet, was obtained from Lead Chemical (Toyama, Japan).

Gas chromatography—mass spectrometry conditions

GC—MS—SIM was carried out on an Hitachi 6MG gas chromatograph—mass spectrometer with an electron impact source and a multiple ion detector. The glass column (1 m × 3 mm I.D.) was packed with 1.5 % OV-17 on Chromosorb W AW DMCS (80—100 mesh). The column, injection port and molecular separator temperatures were 175°C, 220°C and 240°C, respectively. The carrier gas (helium) flow-rate was 50 ml/min. The ionizing energy and trap current were 20 eV and 60 μA, respectively. The ions of *m/e* 258 (FP) and *m/e* 261 ([$^2H_3$]FP) were chosen for SIM.

Sample preparation

In a glass-stoppered test-tube are placed 0.5—2 ml of plasma, 1 ml of the internal standard solution and 1 ml of 3 *N* hydrochloric acid solution. The mixture is extracted with 15 ml of benzene by shaking for 5 min and centrifuging for 5 min at 1660 *g*. The benzene layer is transferred to another test-tube and back-extracted with 5 ml of carbonate buffer solution (pH 9.5) by shaking for 5 min. After centrifuging for 5 min at 1660 *g*, the benzene layer is removed by aspiration. A 0.5-ml aliquot of 1 *N* sodium hydroxide solution is added to the remaining basic aqueous layer. The mixture is washed with 5 ml of benzene, acidified (pH 1) with 1 ml of 3 *N* hydrochloric acid solution and extracted with 10 ml of diethyl ether by shaking for 5 min and centrifuging for 5 min at 1660 *g*. The ether layer is evaporated to dryness in a flask under reduced pressure at 40°C. The residue is treated with 0.5 ml of diazomethane—ether solution. After reaction for 5 min at room temperature, the excess amount of reaction reagent is removed by evaporation under reduced pressure (21 mmHg) at 15°C. The residue is dissolved in 50 μl of methyl alcohol, and a 1—6-μl portion is injected into the GC—MS system.

Plaster application to volunteers

Six healthy male adults, 29—36 years, 57—63 kg, applied two sheets of FP plaster (80 mg/man) on both arms for 8 h.

A 5—10-ml volume of blood was withdrawn using a heparinized syringe at 0, 2, 4, 6 and 8 h after application of the plaster and 0.5—2 ml plasma, depending on the concentration of FP, was used for analysis.

RESULTS AND DISCUSSION

Selected ion monitoring

Trimethylsilylation and methylation have been appreciated as simple and common derivatization methods for GC analysis of carboxylic acids. Although both methods are also applicable to FP, this study employed methylation with use of diazomethane, because it gave rather cleaner chromatograms for plasma specimens. Fig. 2 shows the mass spectrum of the methyl ester of FP (Me-FP). The base peak of Me-FP at *m/e* 199 is about two times as intense as the molecular ion peak at *m/e* 258. The molecular and base ions of the methyl ester of [$^2H_3$]FP (Me-[$^2H_3$]FP) shifted, as expected, by three mass units from those of Me-FP; namely, from *m/e* 258 to 261 and from *m/e* 199 to 202 (Fig. 3). Although the base peak at *m/e* 199 seemed favorable for the

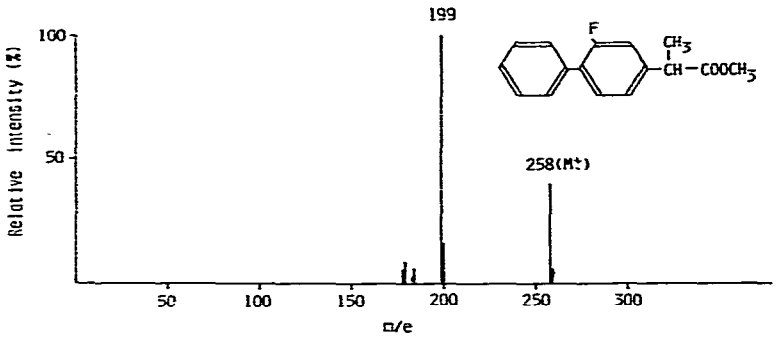


Fig. 2. Mass spectrum of methyl derivative of FP.

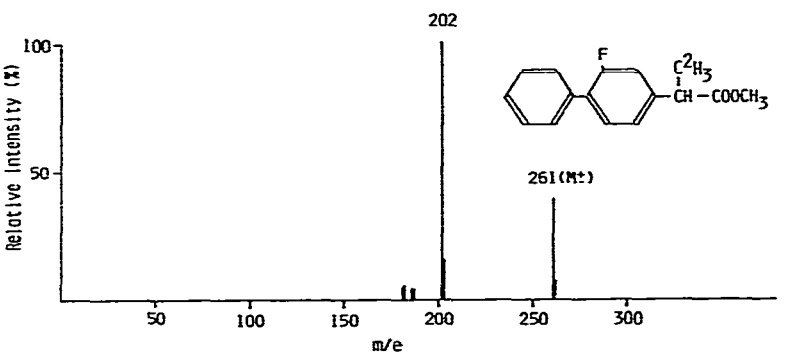


Fig. 3. Mass spectrum of methyl derivative of [$^2H_3$]FP.

SIM of FP, the particular mass fragmentogram suffered from interference by peaks of plasma components; one peak interfered with the separation of the FP peak and another peak had a long retention time. Such disadvantages were avoided by using *m/e* 258 for SIM. Fig. 4 shows a typical mass fragmentogram by SIM at *m/e* 258 and *m/e* 261 for a plasma specimen following application of FP plaster.

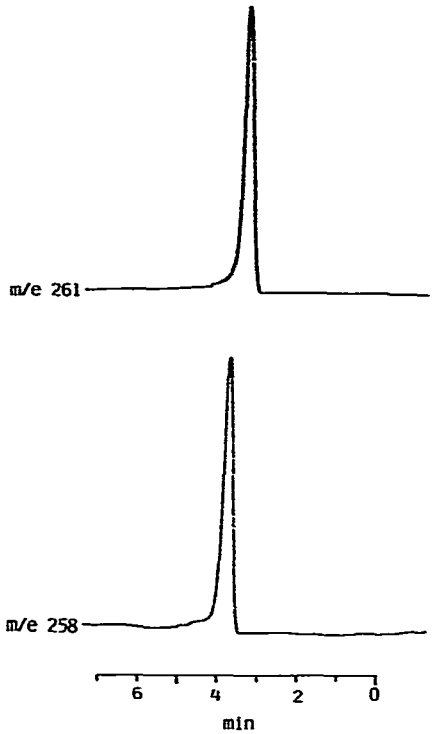


Fig. 4. Mass fragmentogram of plasma extract obtained after application of FP plaster (80 mg/man).

Sample preparation

The extraction procedure involving solvent extraction to organic phase, back-extraction to aqueous phase followed by re-extraction with organic solvent is tedious but often advantageous in providing clean samples of body fluids. The use of benzene for the extraction of FP from human plasma has the advantage of easy separation of the organic layer, whereas the diethyl ether used in the previous study [5] sometimes gave rise to an emulsion. The pH of the alkaline solution with which FP was back-extracted has an influence on the purity of the plasma extract. Carbonate buffer solution (pH 9.5) gave cleaner extracts than sodium hydroxide solution.

Time and temperature dependencies of the methylation of FP and [$^2H_3$]FP using ethereal diazomethane were examined by varying the reaction time (2, 5 and 10 min) and reaction temperature (0°C, 25°C and 35°C). TC was used as a secondary internal standard to evaluate the effect of those conditions on the yields of Me-FP and Me-[$^2H_3$]FP. The peak-height ratios of methyl esters vs. TC monitored at *m/e* 267 indicate that the yields of the methyl esters were independent of time and temperature, and the reaction was completed within 2 min at room temperature.

It was feared that Me-FP and Me-[$^2H_3$]FP might be partly lost during the evaporation of excess ethereal diazomethane under reduced pressure. This was examined by varying the evaporation temperature (15°C, 25°C and 35°C) and evaporation period (5, 10 and 15 min) at 21 mmHg. TC was again used as a secondary internal standard to estimate the loss of methyl esters. The results,

as shown in Fig. 5, indicate that the peak-height ratios of Me-FP and Me-[$^2H_3$]FP vs. TC remained constant when the evaporation temperature was 15°C or 25°C, but decreased with about 20 % loss of each product during evaporation between 5 and 15 min at 35°C.

These observations confirmed that methylation for 5 min at room temperature followed by evaporation of excess ethereal diazomethane at 15°C under reduced pressure (21 mmHg) is sufficient for the determination of FP.

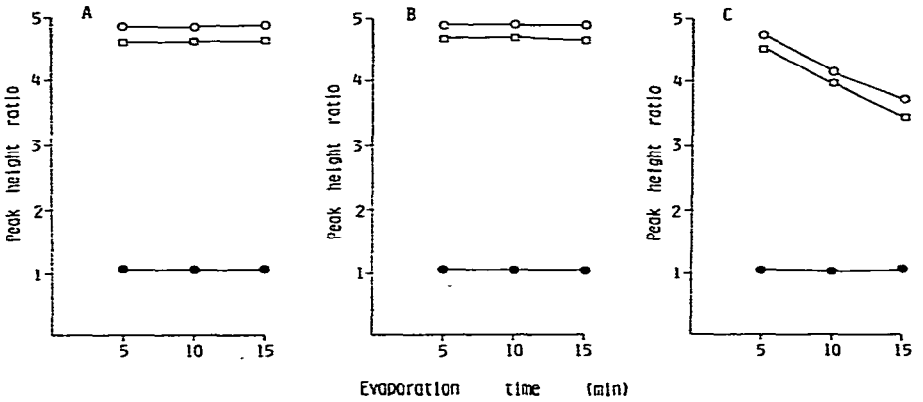


Fig. 5. Influence of evaporation time and temperature on the recoveries of methyl derivatives of FP and [$^2H_3$]FP. Evaporation temperatures: A, 15°C; B, 25°C; C, 35°C. ○, Peak-height ratio of FP to TC; □, peak-height ratio of [$^2H_3$]FP to TC; ●, peak-height ratio of FP to [$^2H_3$]FP.

Calibration graph

It is advantageous to use a stable isotope derivative as an internal standard for the GC—MS—SIM determination of the parent compound because retention times of both compounds are very close and, therefore, their response ratio is almost independent of the GC conditions. The calibration graph of FP against [$^2H_3$]FP was drawn using 2 ml of control plasma spiked with several known amounts of standard FP. The peak-height ratio (FP/[$^2H_3$]FP) thus obtained was linear over the FP concentration range 1.25—150 ng/ml with a correlation coefficient of 0.999. The statistical evaluation of the accuracy and precision of the present method is given in Table I, indicating satisfactory reproducibility over a wide range of plasma levels of FP.

Application of the method

Fig. 6 illustrates the time course of plasma levels of FP in six volunteers after application of two sheets of plaster (80 mg/man). Kaiser et al. [4] reported that the maximum mean level (1.32 ± 0.25 ng/ml) of FP in human plasma was observed at 1 h after a single oral administration of FP (10 mg/man) as a compressed tablet. However, the present results (Fig. 6) indicate a very slow and slight percutaneous absorption of FP with maxima at 6 h or more after application of the FP plaster. Since the present method allows specific and reproducible determinations of plasma levels of FP as low as ng/ml, the pharmacokinetics and bioavailability of FP following percutaneous absorption could be discussed by completing the time courses of Fig. 6. This will follow in the near future.

TABLE I

ACCURACY AND PRECISION OF THE DETERMINATION OF FP IN PLASMA

Amount of FP added to plasma (ng/ml)	Amount of FP found (ng/ml; mean of 3 experiments)	Precision (coefficient of variation, %)
2.5	2.4	10.5
5	5.1	3.3
12.5	12.4	6.0
25	25.2	1.1
50	50.1	1.3
100	101.2	4.5
150	149.4	3.3
300	296.6	5.8

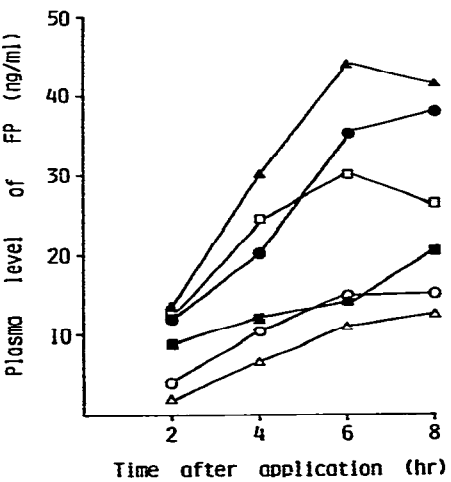


Fig. 6. Plasma levels of FP in human subjects after application of FP plaster (80 mg/man). The symbols specify the volunteers.

ACKNOWLEDGMENTS

The authors thank Professor T. Uno and Dr. T. Nakagawa of the Faculty of Pharmaceutical Sciences, Kyoto University, for many suggestions during this work, and Mr. Y. Doi of these laboratories for the synthesis of [$^2H_3$]FP.

REFERENCES

1 E.M. Glenn, N. Roholoff, B.J. Bowman and S.C. Lyster, Agents Actions, 3 (1973) 210.
2 S. Masumoto, Y. Ishii and Y. Mizushima, Pharmacometrics, 9 (1975) 363.
3 K. Takase, S. Masumoto and M. Okumura, Folia Pharmacol. Jap., 71 (1975) 573.
4 D.G. Kaiser, S.R. Skaw and G.J. Vangiessen, J. Pharm. Sci., 63 (1974) 567.
5 K. Kawahara, Y. Matsumura, Y. Ishii and T. Ofuji, Pharmacometrics, 10 (1975) 653.