Journal of Chromatography, 308 (1984) 329—333
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2093

Note

Sensitive method for the determination of diclofenac in human plasma by gas chromatography—mass spectrometry

H. KADOWAKI*, M. SHIINO and I. UEMURA

NRI Life Science, Kajiwara, Kamakura, Kanagawa 247 (Japan)

and

K. KOBAYASHI

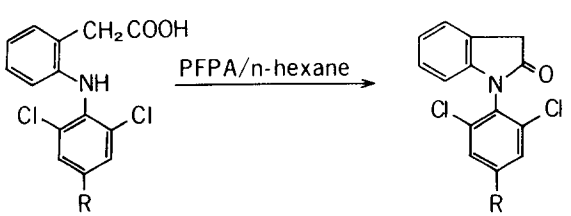
R & D Ciba-Geigy (Japan), Miyuki-cho, Takarazuka 105 (Japan)

(First received August 25th, 1983; revised manuscript received February 6th, 1984)

Diclofenac sodium (Voltaren®) is a widely used anti-inflammatory and analgesic agent.

Procedures have been described for the determination of unchanged diclofenac (Fig. 1, I) in biological materials by gas chromatography with electron-capture detection [1—6] and by liquid chromatography [7], but the lowest limit of detection attainable with these methods is 2 ng/ml of plasma [1].

These existing methods were consequently unsuitable for the measurement of the presumably very low concentrations of diclofenac reached in human



(I) R=H diclofenac (II) R=H
(III) R=OCH_3 internal standard (IV) R=OCH_3

Fig. 1. Formation of the indolinone derivatives.

0378-4347/84/$03.00

plasma after cutaneous application of 1% diclofenac sodium cream, and a more sensitive procedure therefore had to be developed for pharmacokinetic studies of this formulation.

The method proposed is capable of detecting diclofenac levels down to 0.2 ng/ml of plasma by gas chromatography—mass spectrometry (GC—MS) after conversion of diclofenac to the indolinone derivative (Fig. 1, II) through dehydration and cyclization with pentafluoropropionic anhydride (PFPA), as shown in Fig. 1.

EXPERIMENTAL

Materials

Diclofenac sodium (Voltaren active substance), 4′-methoxydiclofenac (internal standard; Fig. 1, III), and the indolinone derivative of diclofenac were supplied by Ciba-Geigy (Basle, Switzerland). Benzene and *n*-hexane were used as manufactured for pesticide-residue analysis by Wako (Tokyo, Japan), and ultrapure grade chloroform was obtained from Kanto Chemical Co. (Tokyo, Japan). PFPA was purchased from Gaskuro Kogyo (Tokyo, Japan). Other reagents used were all of analytical grade, manufactured by Wako.

Gas chromatography—mass spectrometry

A Shimadzu-LKB 9000 gas chromatograph—mass spectrometer and a Shimadzu 9060S multiple-ion-detector peak matcher were used. For GC, a glass column (0.5 m × 3 mm I.D.) was packed with silicone OV-1, 3% Chromosorb W AW DMCS, 80—100 mesh. The temperatures of the injection port, column oven, separator and ion source were set at 270°C, 200°C, 270°C and 290°C, respectively. Helium was used as carrier gas at a flow-rate of 30 ml/min.

For mass fragmentography, the parent ions *m/z* 277 of diclofenac and *m/z* 307 of the 4′-methoxydiclofenac derivative were monitored, and the gain ratio was adjusted at *m/z* 277/307 (1000/15). Accelerating voltage was set at 3.5 kV, electron-ionization energy at 20 eV, trap current at 60 μA, entrance slit width at 0.3 mm and collector slit width at 0.6 mm.

Extraction procedure

A sample of 1.0 ml plasma was taken, and 50 μl of 4′-methoxydiclofenac dissolved in methanol (22.0 ng/μl) were added as internal standard. After stirring for 2—3 sec, 1.0 ml of 1 *M* phosphoric acid and 7 ml of benzene were added as extraction solvent; the mixture was then shaken for 15 min and centrifuged (1870 *g*, 5 min). The benzene layer was taken, and 1.0 ml of 0.08 *M* sodium carbonate buffer (pH 9.6) added; the tubes were shaken for 10 min, then centrifuged. After the benzene layer had been aspirated and discarded, 1.0 ml of 1 *M* phosphoric acid and 7 ml of benzene were added; the mixture was shaken for 15 min and then centrifuged. The benzene layer was removed and evaporated to dryness in a water-bath at 50°C under a nitrogen stream. To the residue, 1.0 ml of *n*-hexane and 100 μl of PFPA were added, and the reaction mixture was left to stand at room temperature for 30 min. Upon the completion of the reaction, the resultant indolinone derivatives were dried in a water-bath at 40°C under a nitrogen stream. For GC—MS, 25 μl of

chloroform were added to the residue, 1—2.5 μl of which were injected for measurement.

Calibration curve

Diclofenac (0.5, 1.0, 2.4, 4.9 and 9.7 ng in a volume of 1.0 ml) was added to blank human plasma samples, and extraction was performed as described above. The peak-height ratio of the diclofenac derivative (*m/z* 277) to the internal standard derivative (*m/z* 307) in the mass fragmentogram was plotted against the concentration of added diclofenac, and a calibration curve prepared using the least-squares method.

RESULTS AND DISCUSSION

Mass fragmentography

In the mass spectra (Fig. 2) of the indolinone derivatives of diclofenac and internal standard, the parent ion peaks (*m/z* 277, *m/z* 307) are both base peaks.

Fig. 3 shows a mass fragmentogram obtained from a spiked human plasma sample. No interference peak derived from plasma was noted. The calibration curve of diclofenac in human plasma showed good linearity in the range of measurement. Table I summarizes the precision and accuracy of the determination procedure. The recovery of diclofenac in the extraction step from plasma was 83.6 ± 5.1% (n = 6).

Sensitivity

The limit of determination of diclofenac in this analysis using a 1-ml plasma sample was 0.2 ng/ml which is ten times lower than that of the most sensitive methods hitherto available. For the cyclization of diclofenac, Geiger et al. [1]

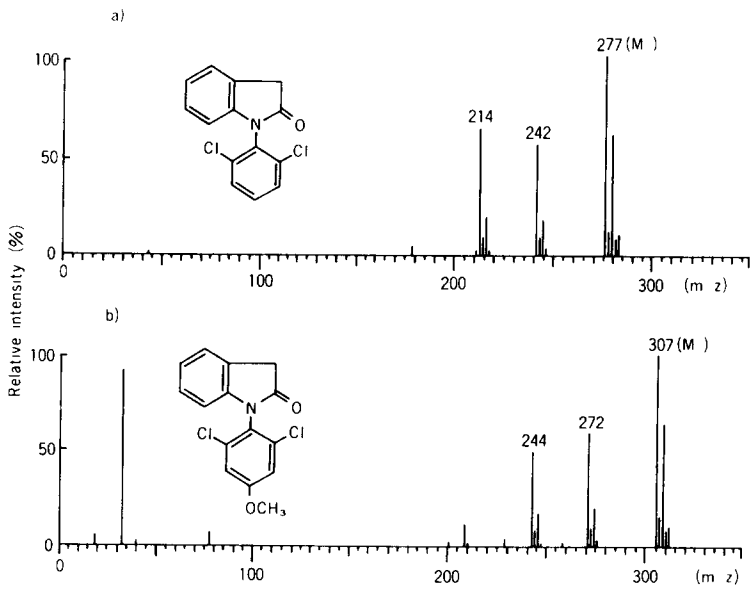


Fig. 2. Electron-impact mass spectra (20 eV) of indolinone derivatives: (a) diclofenac; (b) 4'-methoxydiclofenac (internal standard).

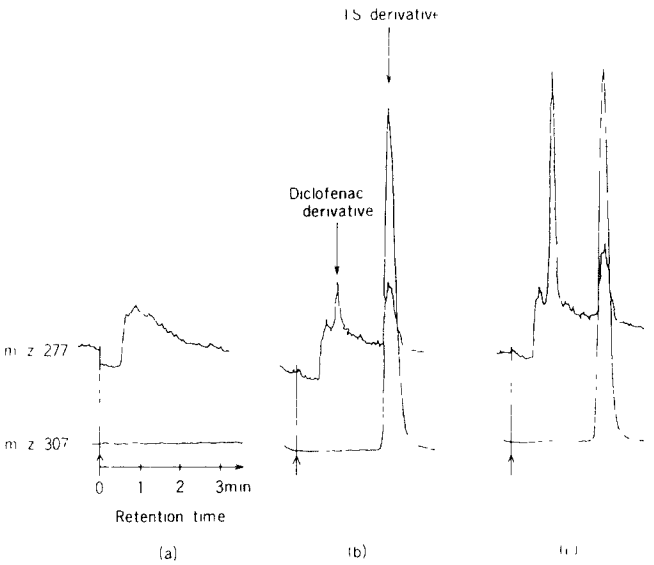


Fig. 3. Mass fragmentograms obtained from human plasma: (a) blank plasma; (b) 0.5 ng/ml diclofenac and internal standard(I.S.) in blank plasma; (c) 2.4 ng/ml diclofenac and internal standard in blank plasma.

TABLE I

PRECISION AND ACCURACY OF THE DETERMINATION OF DICLOFENAC IN HUMAN PLASMA

Actual concentration (ng/ml)	Found concentration (mean ± S.D., n = 6) (ng/ml)	C.V. (%)
0.2	0.23 ± 0.02	8.7
1.0	0.98 ± 0.05	5.1
1 9	1.92 ± 0.11	5.7
4.9	4.74 ± 0.28	5.9
7 4	7.41 ± 0.38	5.1

described a method using sulphuric acid in trifluoroethanol, but this method was unsuitable because of the appearance of an interference peak derived from plasma in the mass fragmentogram and the long time required for the cyclization reaction (more than 75 min). Further, in the method of extractive methylation published by Schweizer et al. [8] and Schneider and Degen [6], 4'-hydroxydiclofenac, a main metabolite, forms the same reaction product as 4'-methoxydiclofenac and so the method could not be applied. In the present study, PFPA [9] proved the most appropriate reagent, giving a rapid cyclization with no side-reaction and being easily removed afterwards. In addition, trifluoroacetic anhydride was tried but found unsatisfactory because of the appearance of an interference peak derived from plasma in the mass fragmentogram. PFPA was very reactive, forming by-products (considered to be enol esters) when no solvent was used, but none when n-hexane was used as solvent. The reaction was complete in 30 min at room temperature, and the indolinone

derivatives produced were stable at room temperature for at least four days. In the extraction procedure, a buffer solution at pH 9.6 was used in preference to 1 *M* sodium hydroxide for the back-extraction of diclofenac into the aqueous phase, this having proved a more effective means of removing interference peaks in the mass fragmentogram.

In case diclofenac cannot be determined down to 0.2 ng/ml in this analysis owing to contamination with other drugs taken simultaneously, it has been confirmed that a mass fragment is obtained without any interference peak if the cyclization products are redissolved in *n*-hexane and washed with sodium hydroxide solution.

Application

Diclofenac sodium (1%) cream was applied 28 times to the back of a healthy volunteer (2.5 g per 250—300 cm^2, three times daily), and blood samples were taken repeatedly. The results are shown in Fig. 4. Diclofenac was detectable in

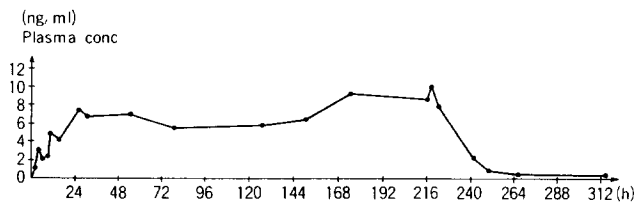


Fig. 4. Plasma concentration of diclofenac obtained after topical application of diclofenac sodium to the back of a human volunteer, three times daily (07:00, 15:00 and 23:00 h).

the plasma (1.3 ng/ml) 2 h after the initial application (in the morning of the first day); the plasma concentration subsequently remained steady between 6 and 10 ng/ml, decreasing to 0.4 ng/ml 98 h after the last application (in the morning of the tenth day). The described method is consequently well suited for studies of the percutaneous absorption of diclofenac sodium.

REFERENCES

1 U.P. Geiger, P.H. Degen and A. Sioufi, J. Chromatogr., 111 (1975) 293.
2 M. Ikeda, M. Kawase, M. Hiramatsu, K. Hirota and S. Ohmori, J. Chromatogr., 183 (1980) 41.
3 J. Kozaya, K. Tada and F. Shimojo, Clin. Rep., 6 (1972) 34.
4 D.B. Jack and J.V. Willis, J. Chromatogr., 223 (1981) 484.
5 M. Ikeda, M. Kawase, T. Kishie and S. Ohmori, J. Chromatogr., 223 (1981) 486.
6 W. Schneider and P.H. Degen, J. Chromatogr., 217 (1981) 263.
7 S.A. Said and A.A. Sharaf, Arzneim.-Forsch., 31 (1981) 2089.
8 A. Schweizer, J.V. Willis, D.B. Jack and M.J. Kendall, J. Chromatogr., 195 (1980) 421.
9 S. Ahuja, J. Pharm. Sci., 65 (1976) 163.