Journal of Chromatography, 147 (1978) 485–490
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 10,339

Note

Gas chromatographic method for the determination of alclofenac in plasma and urine

LAWRENCE T. SENNELLO, SOU-YIE CHU and JAMES W. BORCHERDING

Drug Analysis Section, Drug Metabolism Department, Abbott Laboratories, North Chicago, Ill. 60064 (U.S.A.)

(First received March 11th, 1977; revised manuscript received June 14th, 1977)

Alclofenac (4-allyloxy-3-chlorophenylacetic acid) is known to have good anti-inflammatory, analgesic, and antipyretic activities. The only published method for the determination of the drug in plasma or urine[1] involves trimethylsilylation of the alclofenac which has been extracted from the samples, with subsequent gas chromatographic (GC) analysis of the reaction product. The method frequently suffers from poor reproducibility because the trimethylsilyl (TMS) derivative is quite unstable in the presence of moisture. A sensitivity of 0.25 μg per injection, as reported in the literature, did not permit circulating drug levels to be monitored for a sufficiently long time post dosing for conducting detailed pharmacokinetic studies. To circumvent these problems, development of an alternate method was undertaken.

Preliminary studies showed that the methyl ester of alclofenac could readily be analyzed by GC using flame ionization detection, with a lower detection limit of approximately 10 ng of injected compound. Since the ester formed was considerably more stable toward moisture than was the TMS ether, the former derivative was considered the more desirable.

Several reagents such as diazomethane, methanol–boron trifluoride, methanol–sulfuric acid, and methanol–hydrochloric acid have been extensively used for the conversion of carboxylic acids to their methyl esters for GC analyses; however, esterifications with N,N-dimethylformamide dialkyl acetals have not yet been widely reported. Descriptions of the use of these acetal reagents in the GC analysis of mixtures of fatty acids[2] and amino acids[3] have appeared in the literature. Derivatizations were stated to be rapid and quantitative, with claims of added speed and convenience. The use of these reagents, however, has not yet been described for the analysis of drugs extracted from biological fluids. In the present study, N,N-dimethylformamide dimethyl acetal (DMF-DMA) was found to be a convenient reagent suitable for derivatization of alclofenac. A simple GC assay of the compound in biological fluids was developed based upon this reaction.

EXPERIMENTAL

All chemicals used were analytical-reagent grade. DMF-DMA was obtained from Aldrich (Milwaukee, Wisc., U.S.A.). Alclofenac and its methyl ester were avail-

able from Abbott Laboratories' stock of chemicals. A standard solution of alclofenac was prepared at 1 mg/ml in chloroform (Solution I). A standard alclofenac solution at 100 μg/ml was obtained by diluting Solution I (1:10) with chloroform.

A Varian Series 1400 gas chromatograph equipped with a hydrogen flame ionization detector was used. A 6-ft. × 2 mm I.D. coiled borosilicate glass column was silanized by treatment with a 10% solution of dimethyldichlorosilane in toluene for 30 min. The column was thoroughly rinsed with chloroform and dried. It was then packed with 33% OV-17 on Gas-Chrom Q, 100–120 mesh. The packed column was conditioned overnight at 300° with nitrogen carrier gas flowing at approximately 10 ml/min. The temperatures of the injection port, column, and detector were 200, 185, and 240°, respectively. The flow-rate of the nitrogen carrier gas was about 45 ml/min. Flow-rates of hydrogen and air for the flame ionization detector were set at 50 and 400 ml/min, respectively.

To a 2.0-ml aliquot of plasma or urine in a 15-ml screw-capped centrifuge tube were added 2 ml of 1 *N* hydrochloric acid and 5.0 ml of benzene. The mixture was gently shaken for 10 min, and then centrifuged for 10 min at *ca.* 1000 *g*. A 4-ml portion of the benzene layer was transferred to a clean conical centrifuge tube, and evaporated to dryness at 45° under a gentle stream of air. DMF-DMA (200 μl) was added to the residue, the tube was capped, and then heated in a water-bath at 80° for 30 min. After cooling to room temperature, a 4-μl aliquot was injected into the gas chromatograph and analyzed.

A set of samples prepared from control plasma or urine spiked with known amounts of alcolfenac was analyzed along with the unknown samples. Typically, an aliquot of 0, 10, 20, 40, 80, or 240 μl of the standard alclofenac solution at 1 mg/ml was transferred to a centrifuge tube, and the solution was evaporated to dryness at 45° under a gentle stream of air. To each tube was added 2.0 ml of control plasma or urine, and the contents were stirred briefly on a Vortex mixer. These plasma or urine samples to which alclofenac had been added at levels of 0, 5, 10, 20, 40, or 120 μg/ml were carried through the analytical procedure along with the unknown samples. From these data, a standard curve was constructed from peak heights *versus* the corresponding concentrations of alclofenac in the plasma or urine. The concentrations of the unknown samples were derived from the standard curve.

RESULTS AND DISCUSSION

After several attempts to chromatograph alclofenac as the free acid had failed, the methyl ester of the compound was examined for its GC properties. A single peak attributable to the injected alclofenac methyl ester was observed under various chromatographic conditions. It was found that although the compound contained a chlorine atom in its structure, it evoked a very poor response from a tritium-foil electron capture detector. Therefore, flame ionization detection was chosen and was shown to be able to detect approximately 10 ng of the injected alclofenac methyl ester. Such sensitivity was better than that reported for the TMS derivative of alclofenac (0.25 μg per injection[1]).

In the conversion of alclofenac to its methyl ester, reaction with diazomethane was found to be rapid and quantitative, but since the method under consideration was intended for the routine analysis of large numbers of biological samples, the hazardous

nature of diazomethane rendered its use less desirable. The reaction of alclofenac with DMF-DMA was subsequently examined. Alclofenac was heated in DMF-DMA, which served both as a reagent and a solvent. The solution was analyzed by GC using columns packed with various stationary phases such as 2% OV-1, 3% OV-17, and 3% Poly A-103. It was found that in every case a single peak was observed, which had a retention time identical to that of the methyl ester of alclofenac analyzed under the same GC conditions. GC–mass spectrometric analysis of the eluted peak substantiated that it was identical to that of a sample of the authentic methyl ester of alclofenac. Derivatization of alclofenac with DMF-DMA was quite rapid and quantitative, and GC results were comparable to those obtained from diazomethane treatment. The DMF-DMA reagent was chosen because it is easier and safer to handle than diazomethane.

In order to obtain adequate resolution of the alclofenac ester peak from some background peaks in normal plasma or urine samples, several columns were examined for the assay. Presilanized glass columns (6 ft. × 2.0 mm I.D.), one each packed with 2% OV-1, 10% OV-101, 1% OV-210, and 3% OV-225 on 100–120-mesh Gas-Chrom Q were all found to be unsatisfactory, because of lack of resolution of the desired peak from the interfering peaks. Among the columns tested, GC results obtained on 3% Poly A-103 and 3% OV-17 were found to be the best, and the results on a 20% OV-25 column were acceptable, yet slightly inferior because of a broader frontal peak from the solvent. Fig. 1 shows typical gas chromatograms of samples prepared from normal dog plasma, and blank dog plasma spiked with 10 μg/ml of alclofenac as analyzed on a 3% OV-17 column according to the conditions described

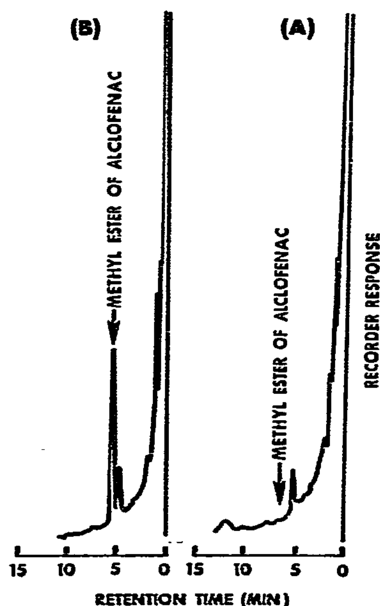

Fig. 1. Gas chromatograms of samples prepared from (A) normal dog plasma, and (B) normal dog plasma to which alclofenac had been added to a level of 10 μg/ml, on a 6-ft. 3% OV-17 column under conditions specified in the Experimental section.

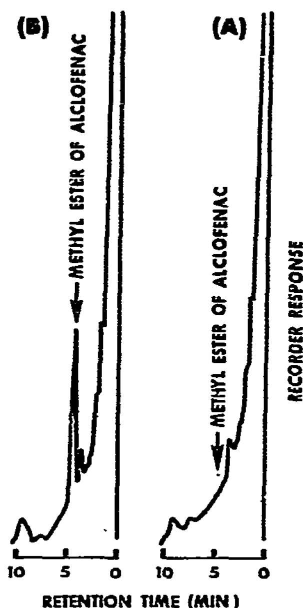

Fig. 2. Gas chromatograms of samples prepared from (A) control dog urine, and (B) blank dog urine to which alclofenac had been added to a level of 10 μg/ml, on a 3-ft. × 2 mm I.D. glass column packed with 3% Poly A-103 on Gas-Chrom Q, 100–120 mesh. The temperatures of column oven, injector port, and detector were 165, 230, and 240°, respectively. Flow-rates of nitrogen carrier gas, hydrogen and air for the flame ionization detector were 45, 40, and 400 ml/min, respectively.

in the Experimental section. Although gas chromatograms of samples prepared from urine are not shown, they were almost identical to those from plasma samples. No interfering peaks were observed in samples from either blank plasma or urine. Fig. 2 shows typical gas chromatograms of samples prepared from control dog urine, and blank dog urine to which alclofenac had been added to 10 μg/ml, as analyzed on a 3-ft. silanized glass column packed with 3% Poly A-103 on Gas-Chrom Q, 100–120 mesh. The column was operated at 165° with temperatures of injector port and detector being 230 and 240°, respectively. Nitrogen carrier gas was set to flow-rate of 45 ml/min. Under these conditions samples prepared from plasma gave nearly identical elution patterns to those shown for urinary samples in Fig. 2. These data from GC on both 3% OV-17 and 3% Poly A-103 columns indicate that either set of GC conditions could be used for the analysis. However, Poly A-103 stationary phase was found to decompose gradually at these temperatures, resulting in peak tailing and loss of resolution, necessitating repacking of the column quite frequently in order to maintain its suitability for the analyses. For this reason, OV-17 was chosen for routine analyses.

To study the precision and linearity of the method, seventeen 2-ml samples were prepared from control plasma to which known amounts of alclofenac had been added, six each at concentrations of 5 and 40 μg alclofenac per millilitre of plasma, and one each at 0, 2, 10, 20, and 200 μg/ml. These samples were carried through the analytical procedure as described in the Experimental section. Relative standard deviations from analyses of replicate samples at 5 and 40 μg/ml were $\pm$ 2.9 and $\pm$ 4.0%, respectively, indicating good reproducibility. A least-squares best-fit line

TABLE I

LINEARITY OF THE GC METHOD FOR THE ANALYSIS OF ALCLOFENAC IN PLASMA

Sample No.	*Methyl alclofenac peak height (arbitrary units)*	*Concentration in plasma (µg alclofenac per ml)*		*Percent of theory*
		Theoretical	*Found*	
1	0	0.00	0.12	—
2	9	2.00	1.92	96.0
3	26	5.00	5.32	106.4
4	50	10.0	10.1	101.2
5	99	20.0	19.9	99.6
6	197	40.0	39.5	98.8
7	1000	200.0	200.1	100.1

was calculated for peak heights, Y, *vs.* the corresponding alclofenac concentration, X, using the data listed in Table I. The equation of the resulting line was $Y = (5.001 \cdot X) -0.599$, in which the Y-intercept of -0.599 was not significantly different from zero. The correlation coefficient of 0.99999 clearly established the linearity of the data.

In the same manner, twenty-three 2-ml samples were prepared from control urine spiked with known amounts of alclofenac, five each at 6, 40, and 120 µg/ml, and one each at 0, 2, 4, 10, 20, 80, 160, and 200 µg alclofenac per millilitre urine. These samples were then carried through the analytical procedure, except that a column packed with 3% Poly A-103 on Gas-Chrom Q, 100–120 mesh, was used instead of a 3% OV-17 column ,as described above. The results are listed in Table II.

In conclusion, a GC method has been developed for the analysis of alclofenac n both plasma and urine. The method is based on derivatization of alclofenac with DMF-DMA, and the methyl ester thus formed is subsequently analyzed by GC. Using 2 ml of plasma or urine, the lower limit of detection of the analytical procedure

TABLE II

RESULTS FROM GC ANALYSIS OF ALCLOFENAC IN URINE

Sample No.	*Methyl alclofenac peak height (arbitrary units)*	*Concentration in urine (µg alclofenac per ml)*		*Percent of theory*
		Theoretical	*Found*	
1	0	0.00	0.02	—
2	9	2.00	1.93	96.5
3	18	4.00	3.85	96.2
4	28*	6.00	5.98	99.6
5	47	10.0	10.0	100.0
6	93	20.0	19.8	99.1
7	190*	40.0	40.5	101.2
8	368	80.0	78.4	97.9
9	588*	120.0	125.2	104.3
10	756	160.0	160.9	100.6
11	920	200.0	195.9	97.9

* Mean values from five replicate urine samples spiked with alclofenac at the levels of 6, 40, and 120 µg/ml. The relative standard deviations were ±2.6, ±1.5, and ±2.9%, respectively.

is approximately 0.7 μg of alclofenac per millilitre, with the quantification being linear up to at least 200 μg per millilitre of plasma. The method is simple and has better sensitivity and reproducibility than the published method involving the TMS derivative of alclofenac.

REFERENCES

1 R. Roncucci, M.-J. Simon and G. Lambelin, *J. Chromatogr.*, 62 (1971) 135.
2 J. P. Thenot, E. C. Horning, M. Stafford and M. G. Horning, *Anal. Lett.*, 5 (1972) 217.
3 J. P. Thenot and E. C. Horning, *Anal. Lett.*, 5 (1972) 519.