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Note

Determination of diclofenac in plasma and synovial fluid by high-performance liquid chromatography with electrochemical detection

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Diclofenac sodium, sodium *o*-(2,6-dichlorophenyl)aminophenylacetate (Voltaren), is a potent non-steroidal anti-inflammatory and analgesic drug, which has been used successfully for several years in the treatment of rheumatic deseases [1–3] This compound is metabolized in animals and humans to the corresponding mono- and dihydroxy and conjugated derivatives [4,5] Several methods have been described for its determination in body fluids, including gas chromatography with electron-capture detection [6,7], gas chromatography–mass spectrometry [8,9] and high-performance liquid chromatography (HPLC) with UV detection [10–2] The first two methods offer satisfactory sensitivity and specificity, but they require double extraction, derivatization and complex instrumentation, whereas HPLC methods have limited sensitivity

In this paper we describe a rapid, sensitive and specific procedure for the determination of diclofenac sodium in plasma and synovial fluid, involving rapid extraction and HPLC with electrochemical detection

EXPERIMENTAL

Materials

Diclofenac sodium and 4 -methoxydiclofenac (internal standard) (Fig 1) were kindly provided by Ciba-Geigy (Origgio, Italy) All chemicals and reagents were of analytical-reagent grade (Carlo Erba, Milan, Italy) Stock so-

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DIC **I.S.**

Fig 1 Structural formulae of diclofenac (DIC) and the internal standard 4'-methoxydiclofenac (I S)

lutions or diclofenac and internal standard (1 mg/ml) in methanol were prepared monthly and stored at 5 °C

Equipment and chromatographic conditions

A Series 10 Perkin-Elmer (Norwalk, CT, U S A) liquid chromatograph and an LC-4B/17 AT electrochemical detector (Bioanalytical systems, West Lafayette, IN, U S A) with a glassy carbon electrode were used An Erbasil 5-μm C_{18} column (15 cm×4 6 mm I D) (Carlo Erba) was operated at room temperature A potential of 0 95 V was applied versus a reference electrode. The mobile phase was 50 m*M* sodium acetate buffer–acetonitrile (60 40) at a flow-rate of 2 ml/min The buffer was adjusted to pH 3 0 with orthophosphoric acid

Extraction procedure

To 0 5 ml of plasma or synovial fluid sample were added 50 μl of internal standard solution in water (1 μg/ml) After rapid shaking, 1 ml of 1 *M* orthophosphoric acid and 5 ml of dichloromethane were added The tubes were shaken for 10 min and centrifuged at 1500 *g* for 10 min The aqueous layer was eliminated by aspiration and a 4-ml volume of the remaining organic phase was transferred into another tube and evaporated under a flow of nitrogen at 40 °C The residue was dissolved in 100 μl of mobile phase and 10–20 μl were injected into the chromatograph Plasma and synovial fluid calibration standards were prepared with 5, 25, 100, 200 and 500 ng/ml diclofenac sodium and 100 ng/ml internal standard, then repeated analyses were carried out to measure the recovery, precision and linearity of the method

Blood and synovial fluid samples

Venous blood sample was collected in heparinized tubes from patients given an intramuscular injection of 75 mg of diclofenac sodium Synovial fluid sample was obtained from the same patient who underwent arthrocentesis Both types of sample were centrifuged and stored at −40 °C until analysis

RESULTS AND DISCUSSION

Chromatograms of blank plasma and synovial fluid, obtained using the procedure presented here, do not show interfering peaks (Figs. 2A and 3A) The separation of diclofenac and the internal standard peaks is good in standard fluids and samples obtained as described above (Figs. 2B and C, and 3B and C)

Drug interference experiments were carried out by analysing samples containing aspirin, salicylic acid, phenylbutazone, *p*-hydroxyphenylbutazone, acetaminophen, indomethacin, naproxen, ibuprofen and ketoprofen No interference was found on injection of the above-mentioned drugs

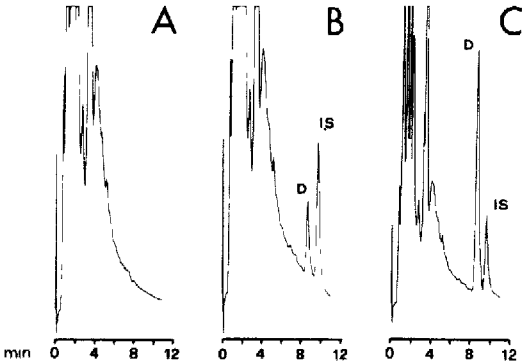

Fig 2 Chromatograms of plasma extracts processed according to the described methods (A) Blank, (B) standard containing 50 ng/ml diclofenac sodium (D) and 100 ng/ml internal standard (I S), (C) sample obtained from a patient 2 h after an intramuscular injection of 75 mg of diclofenac sodium and spiked with 100 ng/ml internal standard

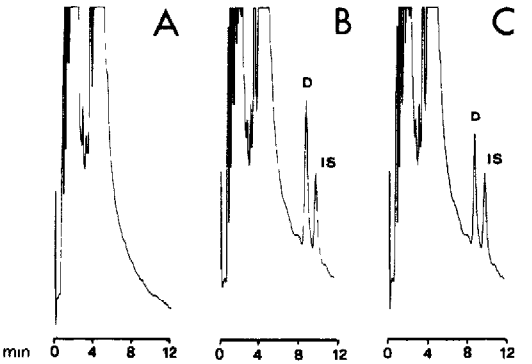

Fig 3 Chromatograms of synovial fluid extracts (A) Blank, (B) standard containing 200 ng/ml diclofenac sodium (D) and 100 ng/ml internal standard (I S), (C) sample taken from a patient given, 6 h before arthrocentesis, an intramuscular injection of 75 mg of diclofenac sodium and spiked with 100 ng/ml internal standard

A single-step extraction with dichloromethane was found to be satisfactory, as this solvent gave reliable recoveries without extracting interfering compounds and it can be evaporated more rapidly than benzene and toluene Indeed, the double extraction with benzene employed in previous assays was more

TABLE I

REPRODUCIBILITY OF DICLOFENAC MEASUREMENTS IN HUMAN PLASMA AND SYNOVIAL FLUID ($n=4$)

Sample	Concentration (ng/ml)	Between-day relative standard deviation (%)	Recovery (%)
Plasma	5	3 4[a]	83 7
	25	2 6	84 2
	100	2 9[a]	79 8
	200	2 5	83 3
	500	2 3	80 6
Synovial fluid	5	3 8	84 0
	25	3 0	81 8
	100	2 2	83 7
	200	2 7[a]	78 9
	500	2 7	80 1

[a] $n=5$

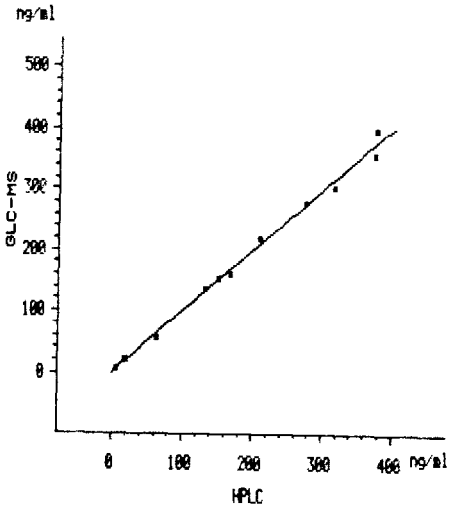

Fig 4 Comparison between the proposed method and GC-MS for the determination of diclofenac in plasma samples from patients

time-consuming [6–8,12], as was a single extraction with toluene [9] Recoveries were in the range 78 9–84 2% for both plasma and synovial fluid.

The possibility of detecting diclofenac through electrochemical oxidation is probably related to the presence in its structure of a diphenylamino group (Fig. 1) The applied potential was set at 0 95 V in order to achieve the highest response but keeping low the signals of endogenous compounds coextracted with diclofenac and the internal standard With this voltage, with equimolar amounts methoxydiclofenac provided a 1 1 response versus diclofenac This molecule has proved to be a reliable internal standard As it is structurally similar to diclofenac it shows similar electrochemical behaviour and the electron-donating effect of the methoxy group on the aromatic ring makes it more oxidizable

A good linear relationship between peak-height ratios and drug concentration in the range 5–500 ng/ml was found ($y=0\ 0403+0\ 0106x$, $r=0\ 998$ for plasma and $y=0\ 0021+0\ 0109x$, $r=0\ 999$ for synovial fluid) This procedure has a sensitivity of 1 ng/ml for both tested fluids, which is satisfactory for application in pharmacokinetic studies Only with a previously reported gas chromatographic–mass spectrometric (GC–MS) method can a better sensitivity be obtained [8]

Between-day relative standard deviations are given in Table I These were never higher than 3 8% even at concentrations as low as 5 ng/ml for both fluids used None of the previously reported HPLC methods [10–12] provides accurate measurements of concentrations below 20 ng/ml and no applications to the analysis of synovial fluid were given A further validation of the method presented here was performed by analysing a number of samples in comparison with mass spectrometry [8] There was a good correlation between the two procedures, as shown in Fig 4 ($y=-0\ 209+1\ 0095x$, $r=0\ 997$)

In our opinion, the present method is the method of choice for the determination of diclofenac in body fluids, as it does not require derivatization and complex instrumentation as in GC with electron-capture detection or GC–MS Extraction is also rapid and simple

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