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Note

Determination of ximoprofen in human plasma by gas chromatography

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Ximoprofen, 2-[4-(3-oximinocyclohexyl)phenyl]propionic acid is a new potent non-steroidal anti-inflammatory agent Ximoprofen belongs to the arylpropionic class of anti-inflammatory drugs, amongst which ibuprofen was the first in general clinical use [1] and is perhaps still the most widely used Studies in patients suffering from various rheumatic diseases have shown that ximoprofen is active at a dose level of 30 mg per day with a low incidence of gastro-intestinal side-effects [2]

This paper describes a novel analytical method, developed to measure concentrations of unchanged ximoprofen in the plasma of human subjects

EXPERIMENTAL

Materials

All reagents were of analytical grade (Fisons, Loughborough U K) unless otherwise stated, and all inorganic reagents were prepared in freshly glass distilled water

Diethyl ether, acetone and ethyl acetate were glass-distilled grade (Rathburn Chemicals, Walkerburn, U K) Benzyltrimethylammonium hydroxide was SLR grade (40%, w/w, in water, Fisons), pentafluorobenzyl bromide was derivatisation reagent grade (Phase Separations, Queensferry, U K) and *n*-dodecane was puriss grade (Fluka, Glossop, U K) Water was freshly glass-distilled

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Fig 1 Chemical structures of xımoprofen (left) and the ınternal standard, 2-[4-(benzo[*d*]thiazol-2-yl)phenyl]propionic acid (right)

The followıng solutıons were prepared cıtrate buffer (0 1 *M*, pH 4 5), benzyltrımethylammonıum hydroxıde (4%, v/v, ın methanol) and pentafluorobenzyl bromıde (2%, v/v, ın cyclohexane) Xımoprofen and the ınternal standard (13 442JL, Fıg 1), both kındly supplıed by Laboratoıres Jacques Logeaıs (Paris, France), were prepared ın methanol at concentratıons of 100 and 97 9 μg/ml, respectıvely, and stored at ca 4°C

Extractıon procedure

Dısposable screw-capped 10-ml culture tubes (Cornıng, through Fısons Scıentıfic Apparatus) were rınsed once wıth ethyl acetate and once wıth dıethyl ether, a tapered 10-ml centrıfuge tube (Cornıng) was rınsed once wıth acetone Plasma (1 ml) was pıpetted ınto a culture tube, and ınternal standard solutıon (5 or 10 μl, 0 49 or 0 98 μg, for calıbratıon ranges 0 04–1 or 0 1–2 0 μg/ml, respectıvely) followed by 0 1 *M* cıtrate buffer (1 ml) was added The mıxture was extracted wıth dıethyl ether (5 ml) by mıxıng on a recıprocatıng (rotary) mıxer (Eschmann, Shoreham-by-Sea, U K.) for 20 mın at 50 rpm The extract was centrıfuged for 5 mın at ca 2000 *g* (MSE Chılspın centrıfuge, Fısons Scıentıfic Apparatus) before the ether phase was transferred usıng a Pasteur pıpette to a tapered tube and evaporated to dryness under nıtrogen ın a water-bath (60°C) Acetone (1 ml) and benzyltrımethylammonıum hydroxıde solutıon (20 μl) were added and mıxed Pentafluorobenzyl bromıde solutıon (50 μl) was added and the tube was capped tıghtly and vortex-mıxed (Vortex mıxer, Fısons Scıentıfic Apparatus) for ca 2 s before beıng heated to 60 C for 20 mın ın a water-bath The tube contents were then evaporated to drvness under nıtrogen ın a water-bath (60°C) before heptane (2 ml) and water (1 ml) were added and the tube was capped and shaken on a box shaker for 10 mın An alıquot of the heptane phase (15 or 30 μl) was transferred to an autosampler vıal and evaporated to dryness under nıtrogen *n*-Dodecane (0 1 ml) was added to the resıdue and the vıal vortexed for ca 2 s before beıng capped An alıquot (1 μl) was ınjected ınto the chromatograph (automated ınjectıon)

Calıbratıon procedure

Two calıbratıon ranges were used to samples of drug-free plasma (1 ml) were added xımoprofen at concentratıons of 0 04, 0 25, 0 5, 0 75 and 1 00 μg/ml and ınternal standard at a fixed concentratıon of 0 49 μg/ml, or else xımo-

profen at concentrations of 0 1, 0 5, 1 0, 1 5 and 2 0 μg/ml and internal standard at a fixed concentration of 0 98 μg/ml The samples were taken through the extraction procedure described above

Instrumentation

The gas chromatograph was a Carlo Erba Fractovap 4160 Series (Erba Science, Swindon, U K), fitted with an automatic liquid injection system (Pye-Unicam PU 4700, Cambridge, U K) and an electron-capture detector (^{63}Ni, 10 mCi)

Chromatography

A 25 m × 0 31 mm I D fused-silica capillary column coated with cross-linked 5% phenylmethylsilicone gum (0 52 μm film thickness) was used (Hewlett-

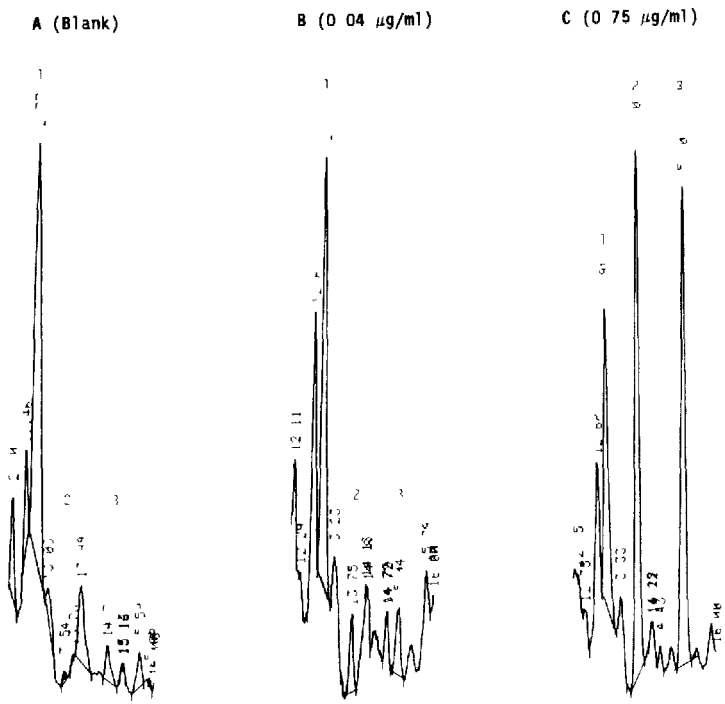

Fig 2 Representative chromatograms of extracts of plasma with internal standard (A) plasma blank, (B) plasma containing 0 04 μg/ml ximoprofen, (C) plasma containing 0 75 μg/ml ximoprofen Conditions column 25 m × 0 31 mm I D fused-silica with 5% cross-linked phenylmethylsilicone gum (0 52 μm film thickness), carrier gas (helium) flow-rate, 3 5 ml/min, temperature programme, 190 °C for 3 min, raised at 90 °C/min to 250 °C for 1 min, raised at 2 °C/min to 280 °C, then raised at 99 9 °C/min to 290 °C for 7 min Electron-capture detection at 300 °C The portion of the chromatogram recorded between 12 and 16 min after injection is shown Peaks 1 = internal standard (0 49 μg/ml), 2 = ximoprofen peak A (*Z* isomer), 3 = ximoprofen peak B (*E* isomer)

Packard ultra-performance column, Hewlett-Packard, Wokingham, U K) The sample was introduced into a split–splitless injector maintained at 300 °C and operated in the splitless mode for 0 67 min, on injection The initial column temperature of 190 °C for 3 min was raised to 250 °C for 1 min at a rate of 90 °C/min, thereafter to 280 °C at a rate of 2 °C/min and then to 290 °C for 7 min at a rate of 99 9 °C/min The detector was operated at a temperature of 300 °C Column gas flow-rate (helium) was 3 5 ml/min with a detector make-up gas flow-rate (oxygen-free nitrogen) of 20 ml/min Under these conditions, derivatised ximoprofen eluted (two peaks) with retention times of 13 8 and 15 1 min and the derivatised internal standard eluted with a retention time of 12 9 min (Fig 2). Peak analysis was achieved using a Trilab 2000 computing integrator (Trivector Scientific, Sandy, U K) The two derivatised ximoprofen peaks (due to geometric isomers Z and E) were summed for calibration purposes since their ratio was shown to remain constant in the plasma samples examined

Plasma samples

The method of analysis was applied to plasma samples obtained from twelve male volunteer subjects (age range 19–39 years, body weight range 61–83 kg) after each had ingested a 30-mg single oral dose of ximoprofen contained in gelatin capsules (2×15 mg) Blood was withdrawn into heparinised tubes at pre-dose and at several different times post-dose, and the resultant plasma stored at ca −20 °C until analysed The study was conducted under conditions similar to those described elsewhere [3].

RESULTS AND DISCUSSION

Ximoprofen was separated in two peaks, denoted peaks A and B, representing geometric isomers Z and E, respectively, this separation did not appear to adversely affect the accuracy of the procedure Least-squares regression lines of peak-height ratio of drug (peaks A+B) to internal standard (y) against concentration of drug in plasma (x) were constructed over the concentration ranges 0 04–1 00 and 0 1–2 0 μg/ml and were best described by the linear functions $y=0\ 160+4\ 122x$ and $y=0.101+2\ 049x$, respectively The coefficients of variation of the mean of five replicate analyses gave the precision of the method as 23% at 0 04 μg/ml, 11% at 0 1 μg/ml and 5% at 2 μg/ml (Table I). The mean of replicate measurements of ximoprofen at added concentrations of 0 1, 0 25, 0 5 and 1 0 μg/ml yielded measured concentrations that corresponded to 84, 105, 103 and 102% of the true values, respectively, providing an index of the accuracy of the method The mean (±S D) recovery of internal standard from plasma was 86±11% ($n=5$) at a concentration of 0 49 μg/ml. The mean (±S D) recovery of ximoprofen from plasma was 98±6% ($n=5$) determined at a concentration of 0 5 μg/ml, and was calculated by comparing peak-height

TABLE I

PRECISION OF THE ANALYTICAL METHOD

Concentration of ximoprofen added to plasma (μg/ml)	Mean peak-height ratio[a]	Coefficient of variation (%)
Range 0 04-1 00 μg/ml		
0 04	0 26	23
0 25	1 24	10
0 50	2 31	6
0 75	3 08	7
1 00	4 33	10
Range 0 10 2 00 μg/ml		
0 1	0 27	11
0 5	1 12	6
1 0	2 27	7
1 5	3 13	8
2 0	4 19	5

[a]Mean of at least four replicates

TABLE II

DRUG CONCENTRATION IN THE PLASMA OF TWELVE HUMAN SUBJECTS AFTER SINGLE ORAL DOSES OF 30 mg XIMOPROFEN

Time after administration (h)	Concentration (mean ± S D) (μg/ml)
0 5	0 76 ± 0 71
0 75	1 04 ± 0 77
1	1 10 ± 0 78
1 25	1 06 ± 0 65
1 5	1 10 ± 0 47
2	1 02 ± 0 32
3	0 64 ± 0 21
4	0 49 ± 0 14
5	0 29 ± 0 08
6	0 20 ± 0 05
7	0 16 ± 0 06
8	0 08 ± 0 06

ratio measurements of non-extracted standards with those of extracted standards corrected for the recovery of internal standard The analytical procedure was selective for ximoprofen, and the limit of detection was arbitrarily set at 0 04 μg/ml, being more than twice the background interference and the lowest datum point on the calibration line

This procedure has now been successfully applied to several pharmacokinetic studies of ximoprofen in human subjects, some typical results are presented in Table II

REFERENCES

1 A G Gilman, L S Goodman, T W Rall and F Murad (Editors), The Pharmacological Basis of Therapeutics, Macmillan, New York, 7th ed , 1985, pp 700-703
2 G Dorf, personal communication
3 R R Brodie, L F Chasseaud, A Darragh, R F Lambe, L Rooney and T Taylor Biopharm Drug Dispos , 7 (1986) 215