Journal of Chromatography, 533 (1990) 275–281
Biomedical Applications
Elsevier Science Publishers B V , Amsterdam

CHROMBIO 5470

Note

Rapid determination of xipamide in human plasma and urine by high-performance liquid chromatography

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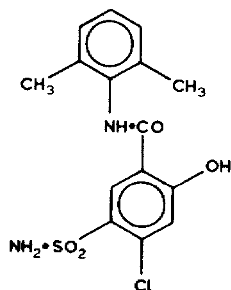
(First received April 19th, 1990, revised manuscript received June 27th, 1990)

Xipamide, N-(2,6-dimethylphenyl)sulphamido-5-chloro-4-salicylamide, is a salicylic acid derivative with the following molecular formula:



It is a new anti-hypertensive drug. Its pharmacodynamic profile shows a diuretic efficacy similar for a part of its activity to that of furosemide and a duration of action comparable with that of hydrochlorothiazide [1]. It has a mechanism of action that is relevant of a new concept of ionic transfer [2–4].

Current methods for assay of the drug from biological fluids are either scintillation counting with the ^{35}S-labelled molecule or a densitofluorimetric method [5,6].

In order to support pharmacokinetic studies, we have developed a selective reversed-phase high-performance liquid chromatographic (HPLC) method with UV detection of xipamide in plasma and urine samples. Its advantage is a rapid and easy extraction before a rapid separation.

EXPERIMENTAL

Reagents

Acetonitrile (RS per HPLC), methyl alcohol (RS per HPLC) and diethyl ether

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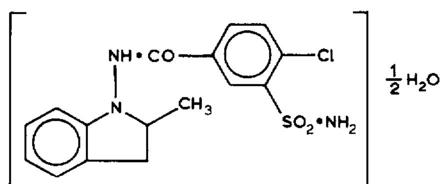
(RS per determinazione pesticidi) were all supplied by Carlo Erba (Milan, Italy). Potassium dihydrogenphosphate was supplied by E. Merck (Darmstadt, F.R.G.). Acetic acid (RP normapur) was supplied by Prolabo (Paris, France).

Xipamide (Lumitens®) was provided by Laboratoires de Thérapeutique Moderne LTM, and the internal standard, indapamide, was supplied by Sigma (La Verpillière, France); it has the following formula:



High-performance liquid chromatography

The HPLC system consisted of a pump (Kontron Model 420), an autosampler (Kontron Model 460) and a spectrophotometric detector (Kontron Model 430). This integrated system was monitored by Kontron MT 450 software (Kontron, St.-Quentin en Yvelines, France). The output signal generated by the spectrophotometer was processed by a computer (Kontron Data System 450). The chromatographic reports were recorded on a plotter (Kontron P 800).

The chromatographic separation was achieved on a 3-μm, 150 mm × 4.6 mm I.D. C_8 Hypersil column (S.F.C.C., Neuilly-Plaisance, France). The solvent used for plasma and urine was acetonitrile–methanol–water (45:5:50, v/v) containing 0.1% acetic acid. The flow-rate was 1 ml/min at ambient temperature The compounds eluted were detected at 231 nm.

Standard solutions

Stock solutions corresponding to 1 mg/ml in acetonitrile were prepared for xipamide and the internal standard.

Plasma standard samples were prepared at concentrations ranging from 0.02 to 1 μg/ml by spiking drug-free plasma with the appropriate concentration of xipamide. Urine standard samples were prepared in the same way at concentrations ranging from 0.05 to 5 μg/ml.

The stock solution of the internal standard was diluted to 2 μg/ml before being used to spike plasma and urine sample.

Extraction procedure

A volume of 0.5 ml of plasma or 0.2 ml of urine (standard or unknown) was transferred to a tube containing 50 μl of internal standard solution and 0.5 ml of 0.1 *M* phosphate buffer (pH 5). A volume of 5 ml of diethyl ether was added to each tube. The solutions were mixed on a vortex mixer for 0.5 min. The tubes were immediately frozen at −20°C and, after the solidification of aqueous phase,

the organic layer was transferred to a conical tube to be evaporated to dryness under a nitrogen stream at 40°C. The residue was dissolved in 0.5 ml of the mobile phase. A 50-μl aliquot was injected into the HPLC system

Calibration curves

The precision and the linearity of the method were validated by analysing calibration standards containing 0.02–1 μg/ml xipamide for plasma and calibration standards containing 0.02–5 μg/ml for urine. Each standard curve was replicated six times.

The standard curves were analysed according a weighted linear regression with a weighting factor $1/y^2$.

RESULTS

Typical chromatograms for drug-free plasma and urine, drug-free urine or plasma spiked with xipamide and the internal standard, and plasma and urine of healthy volunteers are shown in Figs. 1 and 2.

The recovery values of xipamide and the internal standard were 81.6 and 72.0%, respectively.

Precision, accuracy and limit of detection

The results of six consecutive standard curves for xipamide analysed on separate days were linear from 0.02 to 1 μg/ml for plasma, with a slope of 5.602. For urine the curves were linear from 0.05 to 5 μg/ml, with a slope of 2.781. The

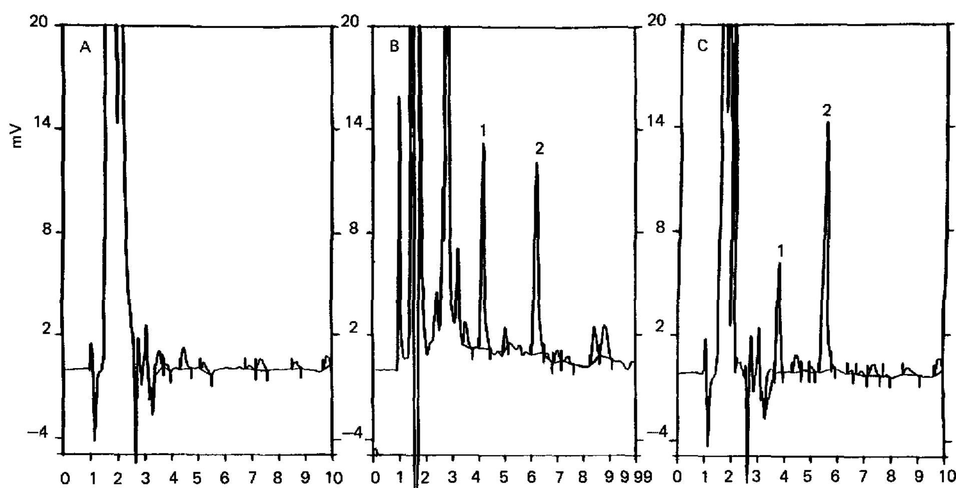


Fig 1 Chromatograms of control human plasma (A), control human plasma spiked with 0.5 μg/ml xipamide and 0.2 μg/ml I.S. (B) and a human plasma collected at 0.75 h after an oral dose of 20 mg (C) (*R* = 0.02 a.u.f.s., 20 mV) Peaks 1 = I.S. 2 = xipamide

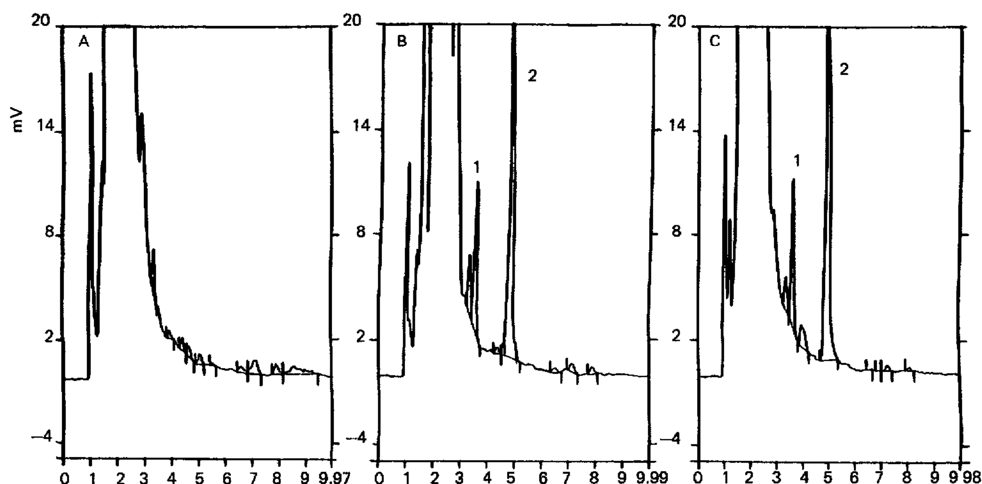


Fig 2 Chromatograms of a control human urine sample (A), a control human urine sample spiked with 2 µg/ml xipamide and 0 5 µg/ml I S (B) and a human urine sample collected during the period 8–24 h after an oral dose of 20 mg (C). (R = 0 02 a.u f.s , 20 mV) Peaks 1 = I.S , 2 = xipamide

linearity of the method was reflected by the correlation coefficient values: 0.9946 for plasma and 0.9942 for urine.

The equations were as follows: plasma, $y = 5.602x - 0.004$;
urine, $y = 2.781x + 0.021$.

Tables I and II show the reproducibility and accuracy of the calibration curves. The precision of the calibration standards in plasma, expressed as coefficient of variation (C.V.), ranged from 5.2 to 18.2% with a relative error within ±7.6%. For urine, the C.V. ranged from 1.4 to 9.5% with a relative error within ±9.4% for concentrations of 0.1–5 µg/ml. These values were higher for a concentration of 0.05 µg/ml.

TABLE I

MEAN PEAK-AREA RATIOS AND CALCULATED CONCENTRATIONS OF CALIBRATION STANDARD OF XIPAMIDE IN PLASMA (n = 6)

Concentration added (µg/ml)	Mean peak-area ratio	C V (%)	Mean concentration found (g/ml)	Relative error
0.020	0 11	18 2	0.021	+3 09
0.050	0 26	14 0	0.046	−7 59
0.100	0 52	13 1	0.093	−7 09
0.200	1 16	5 2	0.207	+3 58
0.500	2 88	9 7	0.514	+2 84
1.000	5 73	10 7	1.023	+2 32

TABLE II

MEAN PEAK-AREA RATIOS AND CALCULATED CONCENTRATIONS OF CALIBRATION STANDARD OF XIPAMIDE IN URINE (n = 6)

Concentration added (μg/ml)	Mean peak-area ratio	C V (%)	Mean concentration found (g/ml)	Relative error
0 05	0 124	20.2	0 052	+3.56
0 10	0 274	9.5	0 106	+5 78
0 20	0 483	7.0	0 181	−9 41
0 50	1 309	3.3	0 476	−4 89
1 00	2 974	2 0	1 077	+7 68
2 00	5 468	1.8	1 974	−1 31
5 00	13 380	1 4	4 819	−3 62

The limit of detection for both urine and plasma was 0.01 μg/ml, at a signal-to-noise ratio of 2.

DISCUSSION

The method described gave good separation between xipamide and the internal standard (indapamide). The concentration of acetic acid was chosen in order to reduce the retention time of the two compounds and to give better resolution of these peaks.

Extraction of xipamide was optimum at pH 5.0 ± 0.1. The percentage extraction was extremely dependent on the pH of the aqueous phase. The coefficient of extraction of indapamide was not an optimum but was reproducible. Some tests were carried out with ethyl acetate, but they showed much more endogenous interferences at the optimal pH 5. Because of its two pK_a values, 4.75 and 10.0, xipamide was not extracted at a good rate with the back-extraction method, owing to loss of ionized compound in alkaline solution. Some other tests performed with C_{18} solid-phase extraction did not show any improvements over the liquid extraction procedure

The C.V. for the lowest concentrations of xipamide in plasma or urine were higher because of the presence of other peaks in the samples. Determination of the baseline was more difficult in these chromatograms.

Application: study in healthy volunteers

A study was conducted in healthy volunteers to evaluate the kinetic parameters of xipamide Blood and urine samples were collected over 48 h after drug intake. Plasma and urine samples were immediately frozen and kept at −20°C.

Fig. 3A and B show a drug concentration–time profile for xipamide. These curves are the mean values of eight healthy volunteers. It allows us to calculate

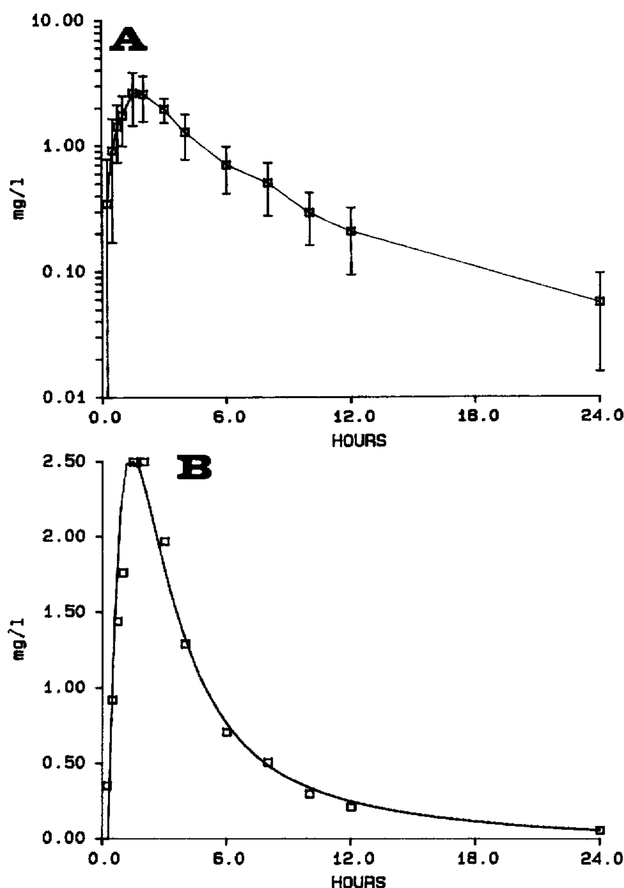


Fig 3 Plasma drug concentration (mean ± S.E M) in eight healthy volunteers following a single oral dose of 20 mg of xipamide

kinetic parameters after an oral dose of 20 mg of xipamide. The half-life of terminal elimination was 4.85 ± 1.16 h, with C_{max} of 2.89 ± 0.36 μg/ml and T_{max} of 1.63 ± 0.13 h. The area under the curve was 14.01 ± 1.61 μg/h/ml. This bi-exponential elimination profile is in accordance with those obtained with other compounds belonging to the thiazidic diuretic group.

CONCLUSIONS

A precise, sensitive and selective method for determination of xipamide in human plasma and urine has been developed. It was used successfully to determine the pharmacokinetics of xipamide in healthy volunteers and to compare the kinetics in cases of renal failure.

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