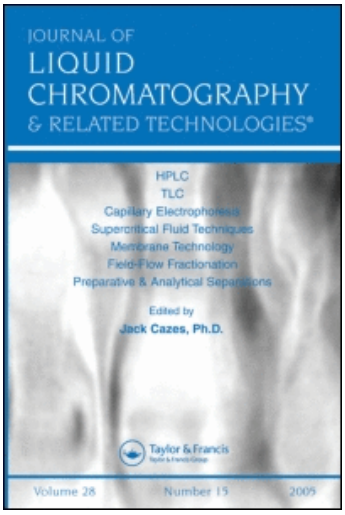
This article was downloaded by:
On: *25 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of piroximone in Plasma and urine by high performance liquid chromatography

N. Bernard[a]; M. Lush[b]; J. Probitts[b]; N. Ferry[a]; G. Cuisinaud[a]; J. Sassard[a]
[a] Department of Physiology and Clinical Pharmacology, URA CNRS 1483, Faculty of Pharmacy, Lyon, Cedex, France [b] Merrell Dow Research Institute, Winnersh Research Centre, Winnersh, Berkshire, United Kingdom

To cite this Article Bernard, N. , Lush, M. , Probitts, J. , Ferry, N. , Cuisinaud, G. and Sassard, J.(1994) 'Determination of piroximone in Plasma and urine by high performance liquid chromatography', Journal of Liquid Chromatography & Related Technologies, 17: 10, 2187 — 2197
To link to this Article: DOI: 10.1080/10826079408013540
URL: http://dx.doi.org/10.1080/10826079408013540

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(10), 2187-2197 (1994)

DETERMINATION OF PIROXIMONE IN PLASMA AND URINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

N. BERNARD[1], M. LUSH[2], J. PROBITTS[2], N. FERRY[1], G. CUISINAUD[1], AND J. SASSARD[1]

[1]Department of Physiology and Clinical Pharmacology
URA CNRS 1483, Faculty of Pharmacy
8 Avenue Rockefeller
F-69373 Lyon Cedex 08, France
[2]Merrell Dow Research Institute
Winnersh Research Centre
Reading Road
Winnersh, Berkshire, RG11 5HQ, United Kingdom

Downloaded At: 07:59 25 January 2011

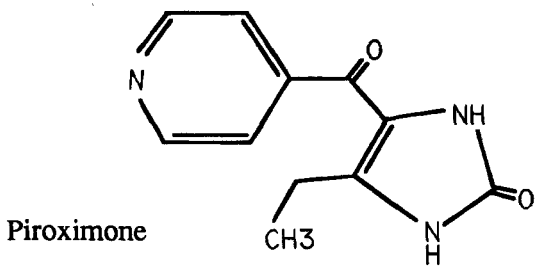
ABSTRACT

A reversed-phase high-performance liquid chromatographic (HPLC) method with UV detection was developed for quantifying piroximone (PI) in plasma and urine. A solid phase extraction of PI simultaneously with an internal standard with an average recovery higher than 86% was needed to avoid endogenous interferences especially in urines from patients with reduced renal functions. The sensitivity limit was 2 ng/ml in plasma and 100 ng/ml in urine. The method was reproducible with intra- and inter-assay coefficients of variations below 7 %. This method was applied to the determination of plasma and urine levels during a pharmacokinetic study in healthy subjects and renally impaired patients. It was found suitable to follow the concentrations until 24 h after a single intravenous infusion of 0.5 mg/kg body weight of piroximone.

Copyright © 1994 by Marcel Dekker, Inc.

INTRODUCTION

Piroximone, 4-ethyl-1,3-dihydro-5-(4-pyridinyl-carbonyl)-2H imidazol 2 one, MDL 19.205 (PI) is an imidazole derivative with inotropic and vasodilator properties used in congestive heart failure.



Piroximone

Downloaded At: 07:59 25 January 2011

The pharmacokinetic of PI has been studied in healthy subjects as well as in patients with heart failure at dosage ranging from 0.2 to 2 mg/kg after IV or oral administration(1-2). In man, metabolites have not been identified, and urinary excretion of native drug amounts to 67 % of administered drug in the first hours following administration. In rat(3) metabolites : isonicotinic acid and hydroxypiroximone never exceed 15% of amount excreted. The aim of this study was to provide an analytical method sensitive and specific enough to follow in humans the plasma and urine levels of PI after a single bolus of 0.5 mg/kg body weight. Previous analytical methods(4-5) did not distinguish PI from interferences often encountered in plasma and urine from renally impaired patients and were found insufficiently specific in our hands. The present report describes an HPLC technique with improved sensitivity and specificity for the simultaneous determination of PI in plasma and urine.

MATERIALS. METHODS

Chemicals

PI and internal standard (IS), 4-(3,4-dimethoxy-benzoyl)-1,3-dihydro-5-methyl-2H-imidazol-2-one;MDL 82.261 were supplied by Merrell Dow Laboratories. Methanol was purchased from Carlo Erba, Milano, Italy. Phosphoric acid, potassium dihydrogenphosphate and disodium hydrogenphosphate, dodecahydrate were purchased from Merck. All chemicals were of analytical grade. Columns for solid extraction : Bond Elut SPE ® 200mg/3ml from Analytichem International were distributed by Varian. Human plasma for laboratory use was obtained from CTS Lyon-Beysnot.

Plasma solutions were prepared by dissolving 10 mg of PI in 100 ml human plasma to obtain a 100 µg/ml concentration. Dilutions of 5, 1, 0.5, 0.1, 0.05, 0.01, and 0.002 µg/ml used for calibration and control were made by diluting previous solutions in the same unit of human plasma. Urine solutions were prepared in the same manner to obtain 100, 50, 10, 5 and 1 µg/ml concentrations. All standard solutions were frozen at least one week prior the assay under 2 ml volume in the conditions used for the samples of the pharmacokinetic study, that is −20°C, to avoid differences in extraction rate observed between fresh and frozen samples. Quality controls of plasma spiked with PI (50 and 500 ng/ml) and urine (5 µg/ml and 50 µg/ml) were stored from the beginning of the study in the same conditions. Solution of Internal Standard (100 µg/ml) was made by dissolving 10 mg of MDL 82.261 in 1 ml sodium hydroxide 1M in a volumetric flask and further completed to 100 ml with water. This solution was found stable only when stored at 4°C in darkness for a maximum of two weeks. Working solutions of IS were made just before extraction.

Downloaded At: 07:59 25 January 2011

Chromatographic Conditions

Reversed phase HPLC was performed at 22°C (air conditioned) using a high pressure pump, 414 model, equipped with two pulse damper 810 model (Kontron, Zürich, Switzerland), an injector : autosampler model MSI 660, equipped with a 90 µl loop (Kontron), two guard and analytical columns (30+150 x 4.6 mm) filled with Spherisorb ODS2 C18, 5 µm (Interchim, Montluçon France). The absorbance of the eluent was monitored by use of a spectrophotometer, model Uvikon 432, with wavelength set at 320 nm (4 nm slides) and equipped with a 8 µl flow cell with 5 mm pathlength (Kontron). The whole system was managed by a central station model MT2 450 (Kontron) for interfacing all the peripherics and recording of chromatograms.

Optimal separation of compounds was obtained using a mobile phase consisting of disodium hydrogenphosphate 0.04 M and potassium dihydrogenphosphate 0.026 M, (the pH of which is adjusted to pH = 3,2 by addition of phosphoric acid 45 N), methanol, 70/30, v/v.

Downloaded At: 07:59 25 January 2011

Extraction Procedure

For their conditioning, SPE columns placed on 10 ml polyethylene centrifuge tubes were twice rinsed with 0.5 ml methanol then 1 ml water. Centrifugation at 1000 rpm during 5 min was processed at each step of elution.

For plasma separation, on a conditioned Bond Elut column were applied 500 µl plasma (or standard or quality control) spiked with 1000 µl IS (0.50 µg/ml). Centrifugation at 2000 rpm was allowed during five minutes. Columns were washed two times with 1 ml of water followed by a 5 min centrifugation at 2000 r.p.m. Compounds were eluted in a conical glass tube with two 0.5 ml methanol fractions and the eluate was evaporated until dryness in a 37°C water-bath, under a stream of

nitrogen delivered by Pasteur pipettes in each tube. Residue was dissolved in 250 µl of mobile phase by vortexing process during 20 seconds. This solution is transferred into an Eppendorf microtube and 90 µl injected in the chromatograph, with a mobile phase flow of 1 ml/min.

For urine separation, on a conditioned Bond Elut column were applied 500 µl urine (or standard or quality control) spiked with 1000 µl IS (5 µg/ml). Centrifugation at 2000 r.p.m. was allowed during five minutes. Columns were washed two times with 1 ml water followed by a 5 min centrifugation at 2000 r.p.m. Compounds were eluted in a polyethylene tube with two 0.5 ml methanol fractions and the eluate was diluted with 2 ml of the buffer disodium hydrogenphosphate 0.04 M / potassium dihydrogenphosphate 0.026 M, (pH = 3,2) to obtain the composition of the mobile phase. This solution was transferred into an Eppendorf microtube and 90 µl injected in the chromatograph, with a mobile phase flow of 1 ml/min.

Downloaded At: 07:59 25 January 2011

Calculations

The PI concentrations were determined in biological samples by means of the ratio of the peak height of PI to the IS's one and calculated from the standard ratio determined with the spiked samples. As six determinations of the ratio are made at three different concentrations in each batch, the correlation is obtained by linear regression between ratio and concentration. Multiple regression analysis (Statgraphics STSC Inc.software) is performed with the reverse of concentration as weight. Correlation coefficient was always higher than 0.990. In each analytical run are introduced spiked samples (10, 100, 1000 ng/ml of plasma and 1, 10, 100 µg/ml of urine in duplicate) to determine the standard ratio and 4 quality controls (50 and 500

ng/ml of plasma and 5 and 50 µg/ml of urine in duplicate) to assess the reproducibility of the method.

RESULTS AND DISCUSSION

Preliminary assay of existing methods (4-5) revealed satisfactory when applied only to the plasma of healthy volunteers, but in our study, separation from endogenous interferences especially in urine or even in plasma was not sufficient. Indeed, patients included in this pharmacokinetic study exhibited reduced renal functions and received several comedications : acebutolol, allopurinol, aténolol, diltiazem, enalapril, furosemide, isradipine, lorazepam, nicardipine, nitrendipine, pindolol, pipemidic acid, prazosin, prednisolone, simvastatin, trazodone). Therefore exogenous and endogenous substances are at higher levels in plasma and in urine of renally impaired patients than in healthy volunteers. In addition in this study dosage was weak (0.5 mg/kg) and current kinetic studies require long time follow-up to ascertain the terminal part of the curve when concentrations are near the minimum quantity level. For these reasons specificity and sensitivity were enhanced by research and development of a chromatographic system and of an extraction procedure. The finalized method only is described here.

Chromatographic Conditions

The main chromatographic characteristics are in table 1.

This parameters table shows a large resolution of the two compounds with a good quality of peaks in the chromatographic conditions chosen (mobile phase: methanol/buffer pH = 3.2 (30/70), flow : 1 ml/min; column : Spherosil C8, 5 µm (150x4.6 mm); injected volume : 90 µl; detection UV wavelength : 320 nm).

Downloaded At: 07:59 25 January 2011

TABLE 1

Chromatographic Characteristics of a Mixture of Piroximone (1 µg/ml) and Internal Standard (0.5 µg/ml) in Mobile Phase.

PARAMETERS	COMPOUND	
	PIROXIMONE	INTERNAL STANDARD
Retention time (min)	4.82	10.05
Capacity factor (k')	1.41	4.03
Nr of theoretical plates (m^{-1})	19647	40900
Theoretical plate height (µm)	12.70	6.00
Asymetrical factor (As)	1.02	1.31
Resolution factor (Rs)	5.00	

Downloaded At: 07:59 25 January 2011

PI was the main compound observed in plasma as well as in urine of healthy volunteers and patients. These results are in accordance with recent pharmacokinetic studies(6). None of the compounds used as comedications were observed in this conditions.

Figure 1(a and b) shows chromatograms of extracts obtained from plasma (spiked with 500 ng/ml IS) and urine (spiked with 50 µg/ml IS) from healthy subject 1 hour after administration of PI (0.5 mg/kg). In this subject the values were 992 ng/ml and 13 µg/ml for plasma and urine concentrations respectively.

Extraction

The solid phase extraction procedure was highly efficient. For plasma extraction procedure, the extraction efficiency has been expressed by the ratio of peak heights obtained for plasma standards (500 ng/ml)

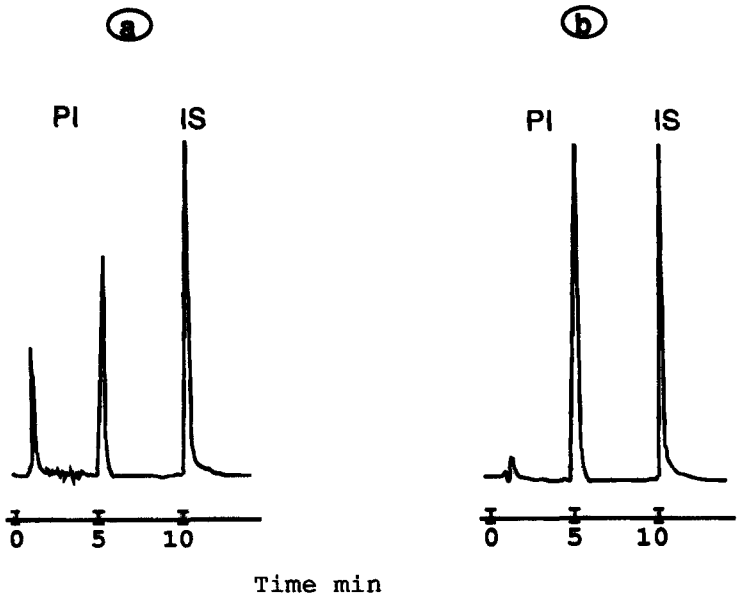


FIGURE 1. Chromatograms obtained with PI and IS in plasma (a) and urine (b) from patients 1 hour after I.V. administration of a 0.5 mg/kg dose of PI. (Full scale of the ordinate is 0.01 absorbance units for plasma and 0.1 absorbance units for urine).

Downloaded At: 07:59 25 January 2011

to the peak height of pure solutions at the same concentrations. The extraction rates expressed as % (X ± SD) obtained for n = 10 determinations were found at 88.9% ± 3.1% for PI and 96.9% ± 2.8% for IS. For urine extraction procedure, the extraction rates expressed as % (X ± SD) obtained for n = 10 determinations (at 50 µg/ml) were found at 86.3% ± 3.6% for PI and 99.3% ± 3.2% for IS.

Characteristics of the Method

The linearity was studied in the range of expected concentrations during the kinetic study with samples spiked with PI at seven concentrations (extracted eight

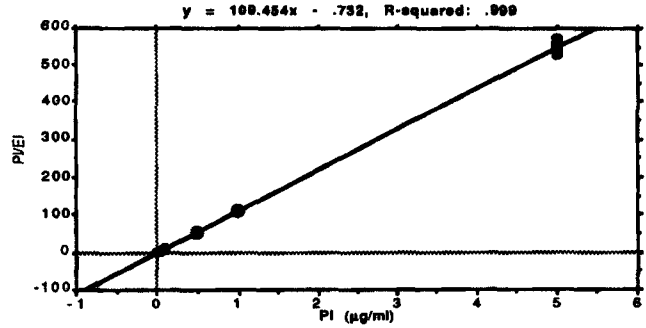


FIGURE 2. Linearity in plasma for PI concentrations (n = 8) from 2 to 5000 ng/ml.

Downloaded At: 07:59 25 January 2011

times) from 2 to 5000 ng/ml for plasma and at five concentrations (extracted eight times) from 1 to 500 µg/ml for urine. The ratios of peaks height were expressed as a function of PI concentrations. A test of linearity was applied and regression line obtained for plasma (r^2= 0.999) or urine (r^2= 0.996) are in figures 2 and 3. In both cases the low intercept ascertain of the purity of peaks measured.

For reproducibility estimation, the coefficients of variation intra- and inter-assay, expressed as SD % of the value, were determined from quality controls (biological samples spiked with PI at concentrations 50 and 500 ng/ml for plasma and 5 and 50 µg/ml for urine), average of 10 determinations in the same run or one determination on ten different days.

Plasma and urine quality controls stored at -20°C for up to 9 months showed no signs of decomposition suggesting that PI is stable under these storage conditions.

The sensitivity limit is determined as the quantity of extracted and injected compound which, in the experimental conditions, is responsible of a peak height 2 fold the residual noise. It was found in our

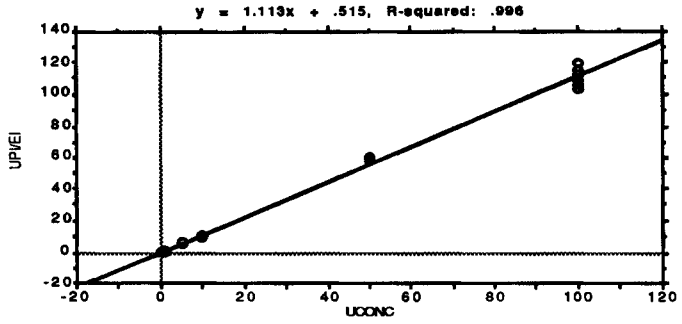


FIGURE 3. Linearity in urine for PI concentrations (n = 8) from 1 to 500 µg/ml.

TABLE 2

Intra- and inter-assay reproducibility of the method.

	Plasma		Urine	
	added	recovered	added	recovered
Intra assay	50	49,1 ± 1,9(3,9%)	5	5,3 ± 0,1(1,6%)
	500	491,5 ± 12,8(2,6%)	50	51,2 ± 0,6(1,1%)
Inter assay	50	48,0 ± 3,4(7,1%)	5	5,2 ± 0,2(3,1%)
	500	472,9 ± 15,1(3,2%)	50	51,6 ± 1,2(2,3%)

hands at 2 ng/ml of plasma and 0,1 µg/ml of urine (or less, depending on the dilution of urine).

The analytical technique is suitable for the determination of plasma samples in the range of 5 to 5000 ng/ml and urines in the range 1 to 100 µg/ml. This method was successfully applied to the determination of PI levels in plasma and urine of healthy and renally impaired patients after a single IV administration of

Downloaded At: 07:59 25 January 2011

0.5 mg/kg of PI. Neither endogenous nor exogenous compounds finally interfered with the detection of PI in plasma or urine even in patients. No metabolites could be identified.

ACKNOWLEDGMENTS

The authors are largely indebted to Mrs Dumas of Merrell Dow laboratories for supplying of drugs.

REFERENCES

1 - HAEGELE, K.D., BELZ, G.G., MEINICKE, T.T., SCHECHTER, P.J., Pharmacokinetics of Piroximone (MDL 19.205) in healthy volunteers. Eur. J. Clin. Pharmacol., 31, 239, 1986.

2 - Confidential reports from Merrell Dow : protocol 099-014, protocols 099-008 and 099-009.

3 - BERG-CANDOLFI, M., BORLAKOGLU, J.T., DULERY, B., JEHL, F., HAEGELE, K.D., Assessment of the biotransformation of the cardiotonic agent piroximone by high-performance liquid chromatography and gas chromatography-mass spectrometry. J. Chromatogr 593, 1, 1992.

4 - HOULDSWORTH, C., Merrell Dow Strasbourg research team. Actualized analytical method for pharmacokinetic studies. Personnal communication.

5 - KEELY, F.J., THEILE, J.D., GARTEIZ, D.A., WEINER, D.L., OKERHOLM, R.A., Method of analysis of the new cardiotonic agent, MDL 19205, in plasma and urine and its application in a dog pharmacokinetic study. J. Chromatogr., 278, 379, 1983.

6 - HAEGELE, K.D., HINZE, C., JODER-OHLENBUSCH, A.M., CREMER, G., BORLAK, J., Effects of a standardized meal on the pharmacokinetics of the new cardiotonic agent Piroximone. Arzneim.-Forsch./Drug Res. 41, 1225, 1991.

Received: November 1, 1993
Accepted: December 14, 1993

Downloaded At: 07:59 25 January 2011