Journal of Chromatography, 181 (1980) 67—75
Biomedical Applications

CHROMBIO. 442

DETERMINATION OF PLASMA MEPINDOLOL LEVELS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND ELECTROCHEMICAL DETECTION

W. KRAUSE

Department of Biodynamics, Schering AG, Berlin/Bergkamen, Müllerstrasse 170—178, 1000 Berlin 65 (G.F.R.)

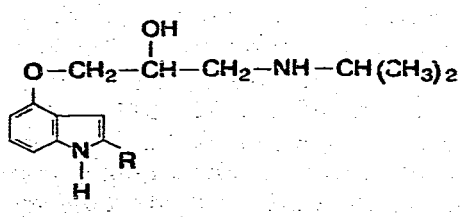
(Received June 21st, 1979)

SUMMARY

A method for determining the plasma concentration of the β-receptor blocking agent mepindolol by high-performance liquid column chromatography (paired-ion chromatography) and electrochemical detection is described. Pindolol is used as an internal standard and the detection limit after extraction of 1 ml of plasma is less than 1 ng of mepindolol. Reproducible results can be obtained with relative standard deviations from replicate analyses of 5 ng/ml plasma samples within ± 4.7%. The method is also suitable for quantification of plasma pindolol levels with mepindolol as an internal standard.

INTRODUCTION

Mepindolol sulphate (bis-[1-(isopropylamino)-3-(2-methylindol-4-yloxy)-2-propanol] sulphate) is a new, non-selective β-receptor blocking agent with slight intrinsic sympathomimetic activity. It is reported to be two to three times as potent as pindolol [1]. Its pharmacokinetic properties have been evaluated by a fluorimetric method [2] and by ^{14}C-labelled drug analysis [3]. Comparison



R = H : pindolol
R = CH_3 : mepindolol

of the two assays shows that, by spectrofluorimetry, (1) the plasma level of mepindolol is higher, (2) the elimination half-life in the plasma is longer, and (3) the amount of unchanged drug excreted with the urine is larger. The reason for this may be that metabolites similar to the drug molecule might have been assayed fluorimetrically together with mepindolol, thus showing higher and longer lasting drug concentrations in plasma and urine.

We therefore decided to develop a new method of detection which should be at least as sensitive as the fluorimetric assay mentioned above but without the use of radioactively labelled substances and without the need of derivatization.

EXPERIMENTAL

Subjects and medication

Four healthy female volunteers (age range 23—46 years, mean value 36 ± 11, and 54—66 kg in weight) were each given 20 mg of mepindolol sulphate orally as 10-mg tablets after a standardized breakfast. Blood samples were taken at 0, 1, 2, 3, 4, 5, 7, 9, 12 and 24 h after the drug administration. They were immediately centrifuged and the plasma stored frozen until analysis.

Reagents

Mepindolol and mepindolol sulphate were obtained from Schering, Berlin, G.F.R., and pindolol was a gift from Sandoz, Basle, Switzerland. All solvents (benzene, isoamyl alcohol, methanol, ethyl acetate and diethyl ether) were of analytical-reagent grade and were used without further purification. Sodium hydroxide, 0.1 *N* solution, and 0.1 *N* acetic acid were each prepared by dissolving one ampoule of "fixanal" (Riedel-de Haen, Hannover, G.F.R.) in 1 l of distilled water.

Standard solutions

Just before use, solutions of 10 μg of mepindolol and 100 μg of pindolol, each in 100 ml of methanol, were prepared.

Glassware

All glassware used in the extraction procedure was cleaned with chromic acid, washed with distilled water and methanol and dried at 150° before use.

Extraction procedure

One millilitre of plasma was pipetted into an 8-ml stoppered test tube and 5 ng of pindolol, 200 μl of 0.1 *N* sodium hydroxide solution and 5 ml of benzene—isoamyl alcohol (20:1, v/v) were added. After thorough mixing on a Vortex mixer for 1 min and centrifugation at 1200 *g* for 5 min, the organic phase was transferred to another test tube and re-extracted into 200 μl of 0.1 *N* acetic acid by mixing for 1 min and centrifugation for 5 min at 1200 *g*. The organic phase was discarded and the acetic acid extract washed twice with 500 μl of diethyl ether; 150 μl of the aqueous phase were used for analysis.

Standard samples prepared from blank plasma spiked with 5, 10 and 20 ng of mepindolol were analyzed along with the unknown samples.

The extraction efficiency was determined with 1-ml plasma samples containing 8 or 16 ng of ^{14}C-labelled mepindolol (n = 5 for each concentration), the ^{14}C-activity being measured in the aqueous phase of the extract.

Chromatographic system

The high-performance liquid chromatography (HPLC) system consisted of a solvent delivery pump (Knauer, Berlin, G.F.R. type 52.00), a LiChrosorb RP-18 chromatographic column (10 μm particle size, 250 mm × 4.6 mm; Knauer) and an electrochemical detector (E 611, cell EA 1096/2; Metrohm, Filderstadt, G.F.R.) using a glassy carbon working electrode and an Ag/AgCl/KCl reference electrode. Injection was accomplished with a Rheodyne RH 7120 system. The mobile phase consisted of methanol—water (65:35, v/v) with 0.01 *M* sodium dodecylsulphate and 2 ml of acetic acid per litre. The eluent was degassed under reduced pressure before use. The chromatographic system was operated at ambient temperature, with an eluent flow-rate of 2.0 ml/min. The electrochemical potential of the working electrode was set at +1.4 V against the reference electrode. The current range used was 5—20 nA according to the concentration of the drug. The detector signal was converted to a chromatographic trace by a W+W recorder at an input voltage of 1 V.

Calibration curve

The standard curve was constructed with 1-ml blank plasma samples containing 0, 1, 2.5, 5, 7.5, 10, 15, 20, 30 and 50 ng of mepindolol and 5 ng of pindolol. These samples were extracted by the method described above. Peak heights of internal standard and drug were measured and the calibration curve (peak height ratio of mepindolol:pindolol versus the concentration of mepindolol) was constructed.

Unknown plasma samples were processed together with three calibration points (5, 10, and 20 ng of mepindolol) which were used to correct for inter-assay variability.

The overall accuracy of the assay was calculated from two series of experiments: determination of five samples of 1 ml of plasma with 20 ng and with 5 ng of mepindolol.

Pharmacokinetic evaluation

To determine the absorption and elimination half-lives time courses of the plasma levels of each test subject were plotted separately on a semi-logarithmic scale. The elimination rate constant (k_e) was obtained from the slope of the terminal straight line. The absorption rate constant (k_a) was constructed by the "feathering" method [4]. t_{max} was calculated from

$$t_{max} = \frac{\ln (k_a/k_e)}{k_a - k_e}$$

RESULTS AND DISCUSSION

Assay

This report describes a highly sensitive and selective method for the deter-

mination of mepindolol in plasma utilizing HPLC with electrochemical detection. Extraction is performed in two steps by first using an organic solvent mixture (benzene—isoamyl alcohol) and then re-extracting into 0.1 *N* acetic acid and washing the aqueous phase with diethyl ether. The overall recovery of this procedure was found to be 69.9 ± 4.4% and 64.7 ± 6.2% for 8 and 16 ng of mepindolol, respectively, as determined by radioactivity measurements of the extract after spiking plasma samples with ^{14}C-labelled drug (see Table I). The mean value of recovery for the two concentrations was 67.3 ± 5.8%.

TABLE I

OVERALL EXTRACTION RECOVERIES OF MEPINDOLOL

Recoveries were determined by radioactivity measurements after spiking 1 ml plasma samples with ^{14}C-labelled drug.

Mepindolol (ng)	
Added to plasma	Recovered
8.00	5.66
	5.36
	5.45
	6.18
	5.32
16.00	11.33
	10.96
	9.74
	10.84
	8.93

Plasma constituents and possible co-extracted metabolites of mepindolol are then separated from the drug by HPLC using a reversed-phase system with ion-pair formation (see Fig. 1).

Detection is performed by means of an electrochemical detector in the oxidative mode. This is possible because the indole moiety can easily be oxidized or react with electrophiles. The main site of oxidation will always be the 3-position of the molecule [5]. The introduction of an additonal methyl group in position 2 as with mepindolol should enhance nucleophilicity at position 3 by an inductive effect thus making mepindolol even more susceptible to oxidation than pindolol. As can be seen from Fig. 2 (electrochemical response at varying potential settings) pindolol starts to be oxidized at somewhat higher voltages. The potential setting of +1.4 V used in the detection system described above is considerably higher than that applied in the determination of catecholamines and related compounds (+0.50 to +0.85 V) as described in the literature [6—11] and it may not be increased further because of electrolysis of the eluent.

Plasma concentrations of mepindolol were determined by comparing the peak heights of the drug and the internal standard added to the plasma specimens before extraction. A typical calibration curve of the assay is

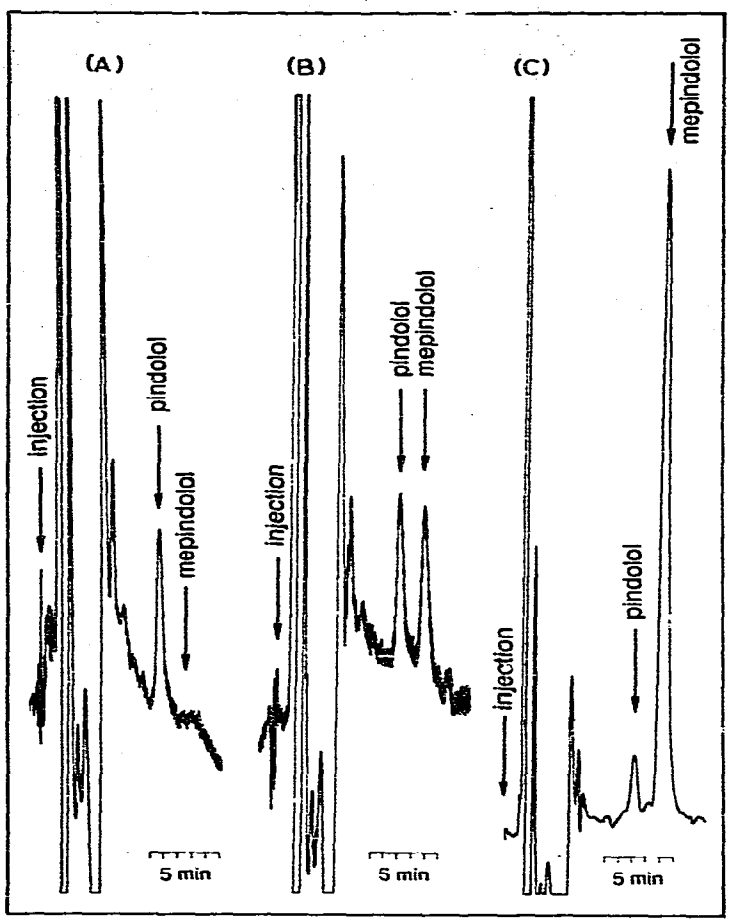


Fig. 1. HPLC chromatograms of (A) blank plasma samples spiked with 5 ng of pindolol, (B) 5 ng of pindolol + 7.5 ng of mepindolol, and (C) of a plasma sample obtained from test subject No. 4 2 h after oral administration of 20 mg of mepindolol sulphate.

illustrated in Fig. 3. The equation

$$\frac{\text{peak height of mepindolol}}{\text{peak height of pindolol}} = (0.133 \times \text{concentration of mepindolol}) - 0.061$$

was obtained, with the y-intercept of −0.061 being almost zero. The correlation coefficient was 0.998 and demonstrated the linearity of the data. This calibration curve was used for the analysis of unknown plasma samples. Concentrations of more than 50 ng/ml thus have to be regarded as estimates.

The overall accuracy of the assay expressed as standard deviation of five consecutive determinations of 5 and 20 ng of mepindolol per ml was 4.7 and 4.2%, respectively (see Table II). When assayed on four different days peak height ratios for 20 ng of drug per ml were obtained as 2.67, 2.90, 2.60 and 2.77, yielding a standard deviation of the inter-assay accuracy of 6.2%. Although this value was quite tolerable, standard samples prepared from blank plasma spiked with 5, 10 and 20 ng of mepindolol per ml were analyzed

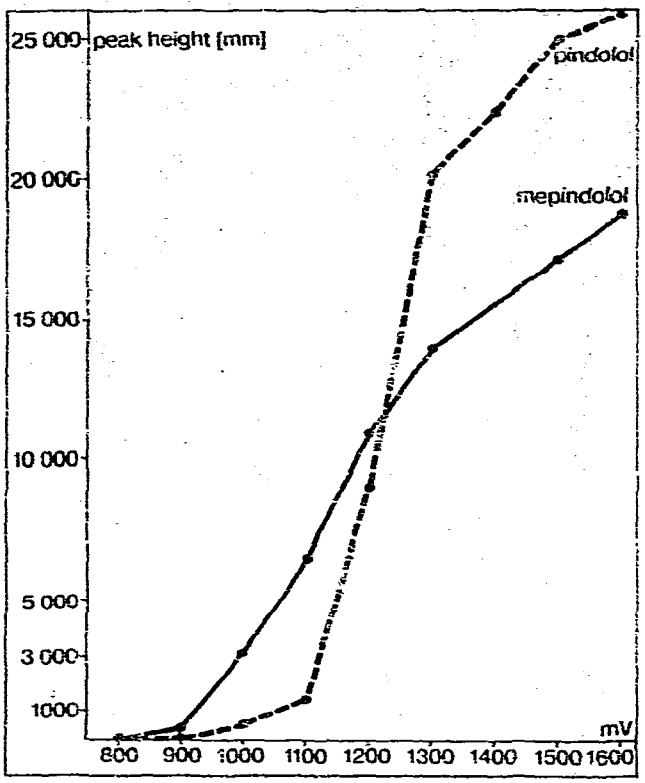


Fig. 2. Electrochemical response (peak height) of 2.5 μg each of pindolol and mepindolol at various potential settings.

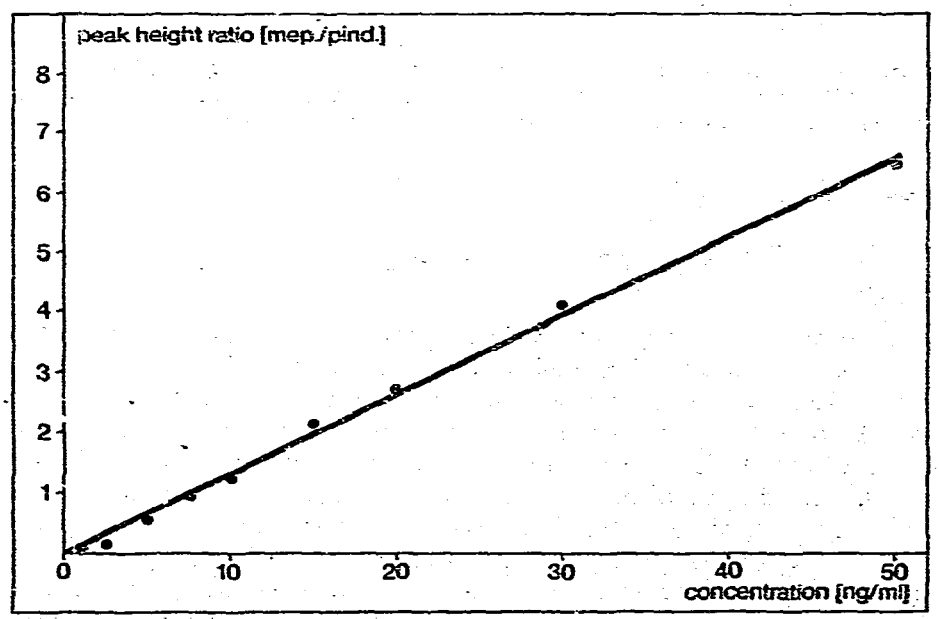


Fig. 3. Calibration curve for the determination of unknown mepindolol concentrations in 1 ml of plasma, obtained by spiking with 5 ng of pindolol and various amounts of mepindolol.

TABLE II

PRECISION OF THE ASSAY

Five consecutive determinations of 5 ng of mepindolol and 20 ng of mepindolol per ml of plasma were made.

Mepindolol (ng/ml)	Peak height (mm)			
	Pindolol	Mepindolol	Mep./Pind.	Mean ± S.D.
20	35	108	3.09	
	33	97	2.94	
	38	108	2.84	2.90 ± 0.12
	37	105.5	2.85	
	39	108	2.77	
5	66.5	29	0.44	
	63	30	0.48	
	80.5	40	0.50	0.47 ± 0.02
	58	27.5	0.47	
	71	34	0.48	

TABLE III

INDIVIDUAL PHARMACOKINETIC PARAMETERS OF FOUR TEST SUBJECTS AFTER ORAL ADMINISTRATION OF 20 mg OF MEPINDOLOL SULPHATE

Test subject	Absorption $t_{½}$ (h)	Concentration maximum		Elimination $t_{½}$ (h)
		(h)	(ng/ml)	
1	0.7	3	36.3	3.8
2	1.2	3	57.8	4.3
3	—	1	69.0	4.1
4	0.4	2	74.2	3.5
Mean ± S.D.	—	2.3 ± 1.0	59.3 ± 16.8	3.9 ± 0.4
Mean value *	0.4	1.5	47.3 ± 18.8	4.0

*Calculated from the mean values' curve of the four volunteers.

along with the unknown samples on different days to correct for the inter-assay variance.

The detection limit after extraction of 1 ml of plasma was less than 1 ng of mepindolol.

Study of plasma levels

Mepindolol was absorbed with a half-life of 0.4 h (calculated from a curve constructed with the mean values of the four test subjects) and reached its maximum at about 2 h (calculated: 1.5 h) at a level of 47.4 ± 18.8 ng/ml. The individual pharmacokinetic values of the four volunteers are listed in Table III (see also Fig. 4). As can be seen from Table IV, there is no difference in the results of the ^{14}C-assay and the method described above, especially when the data are corrected for the body weight of the test subjects. In that case — when taking the body weight of the male subjects (^{14}C-assay) as a standard — the mean value of the maximum concentration of the HPLC assay becomes 41.1 ± 16.3 ng/ml.

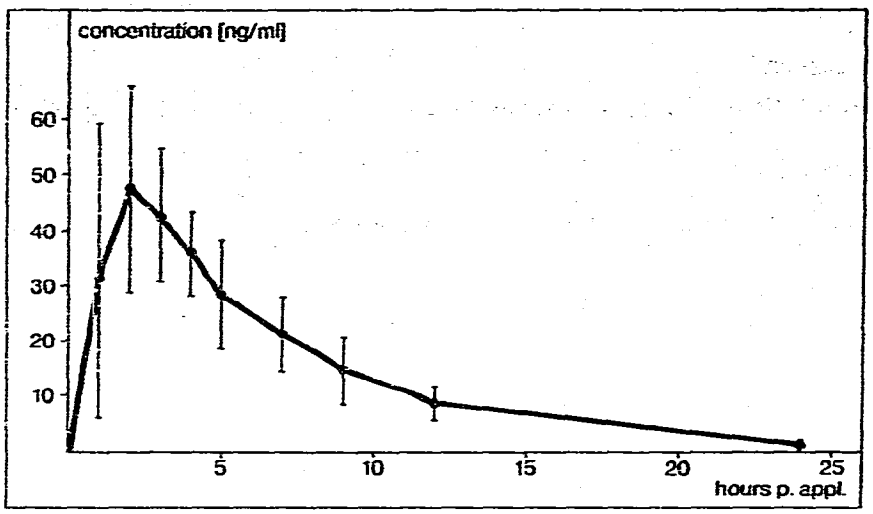


Fig. 4. Plasma level of mepindolol (mean ± S. D.) after oral administration of 20 mg of mepindolol sulphate to four healthy female volunteers.

TABLE IV

COMPARISON OF RESULTS OBTAINED BY THREE DIFFERENT METHODS AFTER ORAL ADMINISTRATION OF MEPINDOLOL SULPHATE TO HEALTHY VOLUNTEERS

Assay	Fluorimetry	^{14}C-method	HPLC
No. test subjects	5	5	4
Sex	Male + female	Male	Female
Dose (mg)	10	20	20
Absorption $t_{½}$ (h)	1.1	0.4	0.4
Concentration maximum			
(h)	2.8	1.6	1.5
(ng/ml)	41.4 ± 8.1	37.2 ± 20.7	47.3 ± 18.8
range (ng/ml)	33.4—54.3	21.9—71.6	36.3—74.2
Elimination $t_{½}$ (h)	4.6 ± 1.6	4.2 ± 1.3	3.9 ± 0.4

In the fluorimetric assay, however, an identical value is obtained with half the dose administered. The reason for this may be that metabolites chemically similar to the drug molecule might have been fluorimetrically assayed together with mepindolol, thus showing higher and longer lasting plasma concentrations.

The same holds for urinary excretion. In the report by Gugler et al. [2], 24 h after oral administration of 10 mg of mepindolol sulphate 17.4% of the dose was recovered in the urine in the unchanged form. ^{14}C-Labelled drug analysis [3], however, has shown that after double the dose less than 1% was to be found in the urine.

Thus, the HPLC assay of mepindolol described above differs from the fluorimetric method known in the literature by

(1) use of an internal standard for controlling the extraction procedure,
(2) separation of plasma constituents and mepindolol metabolites from the unchanged drug by HPLC (metabolic patterns will be reported elsewhere [12]),
(3) high sensitivity without the need of derivatization, and
(4) rapid sample preparation, thus permitting a higher efficiency in routine work.

The method will be used in further clinical pharmacokinetic studies in our laboratory and may also be applied to the determination of pindolol.

ACKNOWLEDGEMENT

The author thanks Prof. Dr. W. Schwartzkopff, Fett- und Stoffwechselambulanz der Freien Universität Berlin, for skillfully conducting the human study.

REFERENCES

1 W.H. Aellig, Brit. J. Pharmacol., 47 (1973) 621.
2 R. Gugler, L. Kreis and H.J. Dengler, Arzneim.-Forsch., 25 (1975) 1067.
3 J. Bonelli, G. Hitzenberger, W. Krause, H. Wendt and U. Speck, Int. J. Clin. Pharm. Biopharm., in press.
4 D.P. Vaughan, D.J.H. Mallard and M. Mitchard, J. Pharm. Pharmacol., 26 (1974) 508.
5 A.R. Katritzki and J.M. Lagowski, The Principles of Heterocyclic Chemistry, Methuen, London, 1967, p. 116.
6 P.T. Kissinger, R.M. Riggin, R.L. Alcorn and L.-D. Rau, Biochem. Med., 13 (1975) 299.
7 C. Hanson, G. Agrup, H. Rorsman, A.-M. Rosengren and E. Rosengren, J. Chromatogr., 162 (1979) 7.
8 T.P. Moyer and N.-S. Jiang, J. Chromatogr., 153 (1978) 365.
9 R.E. Shoup and P.T. Kissinger, Clin. Chem., 23 (1977) 1268.
10 R.M. Riggin and P.T. Kissinger, Anal. Chem., 49 (1977) 2109.
11 M.J. Cooper, R.F. O'Dea and B.L. Mirkin, J. Chromatogr., 162 (1979) 601.
12 W. Krause, in preparation.