Journal of Chromatography, 526 (1990) 240–245
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 5088

Note

Determination of mepindolol in plasma after transdermal drug delivery by high-performance liquid chromatography with electrochemical detection

I. MEINEKE*, S. HARTNACK and C. DE MEY

SK&F Institute for Applied Clinical Pharmacology, Hildebrandstrasse 10, D-3400 Gottingen (F.R.G.)

(First received July 7th, 1989; revised manuscript received October 14th, 1989)

Mepindolol (1-isopropylamino-3-(2-methylindol-4-yloxy)-2-propanol, Corindolan®, Schering) is a non-selective β-blocking agent with a mild intrinsic sympathomimetic effect. The pharmacokinetics of mepindolol after intravenous and oral administration have been evaluated with the aid of various analytical techniques including radiolabelling [1], high-performance liquid chromatography (HPLC) with fluorimetric detection [2] and HPLC with electrochemical detection [3]. All these methods reported limits of detection greater than 0.5 ng/ml. However, much lower drug concentrations are to be expected after transdermal delivery [4]. It was therefore our objective to develop an HPLC assay for mepindolol, which would allow the measurement of the compound in the pg/ml range, in support of clinical studies that assess transdermally delivered mepindolol.

EXPERIMENTAL

Materials

Mepindolol sulphate and pindolol (as free base) were obtained from Smith Kline Dauelsberg (Göttingen, F.R.G.). Sodium hydroxide, orthophosphoric acid, toluene, acetic acid, citric acid and acetonitrile were received from Merck (Darmstadt, F.R.G.). Triethylamine (97–99% purity) (TEA) and pentane-

0378-4347/90/$03.50 © 1990 Elsevier Science Publishers B.V.

sulphonic acid (PSS) were from Aldrich (Steinheim, F.R.G.). All chemicals were of analytical grade if not stated otherwise. Human plasma was obtained from a local bloodbank. Purified water (resistivity 18 MΩ cm) was produced by a Millipore-Q reagent water system (Millipore-Waters, Eschborn, F.R.G.).

Chromatography

The chromatographic system consisted of a Merck–Hitachi Model 655A-12 pump (Merck), a Merck–Hitachi Model 655A-40 autoinjector, a column oven (Waters TCM) and an ESA Coulochem Model 5100A electrochemical detector (Bischoff, Leonberg, F.R.G.) equipped with a Type 5011 dual-electrode analytical cell. The detector was operated at a nominal potential of +0.25 V applied to the upstream electrode and a potential of +0.55 V applied to the downstream electrode. The output signal of the second electrode was fed to a TRIO computing integrator (SES, Niederohm, F.R.G.).

For the chromatographic separation, a μBondapak C_{18} column (25 cm × 4.6 mm I.D., 10 μm particle size, Millipore-Waters) was used. The mobile phase was citrate–acetate buffer (0.0125 *M*:0.2 *M*) containing 0.017 *M* PSS, brought to pH 4.0 with sodium hydroxide–acetonitrile (77.5:22.5, v/v). The flow-rate was 1.0 ml/min. Mobile phases were filtered prior to use and degassed by sonication under vacuum. Columns were kept at 30°C.

Preparation of plasma samples

Samples were stored at −20°C prior to analysis. To 1 ml of plasma, 150 μl of an aqueous solution containing 10 ng/ml pindolol as internal standard were added, followed by 200 μl of 0.1 *M* sodium hydroxide. The sample was then extracted twice into 5 ml of toluene (10 min on a reciprocating shaker). After centrifugation at 4000 *g* for 10 min, 4.5 ml of the organic layer were collected. The combined organic phases were reextracted into 150 μl of 0.1 *M* phosphate buffer (pH 4.0). The organic solvent was then carefully removed, and 100 μl of the aqueous phase were pipetted into an Eppendorf-type vial. Typically, 40 μl of the sample were injected into the HPLC system.

Calibration and calibration samples

Standard solutions were prepared containing 100, 10 and 1 ng/ml mepindolol sulphate in water. Appropriate aliquots of these standard solutions were added to drug-free human plasma to give 1-ml samples containing 0.05–5.0 ng/ml mepindolol sulphate. In addition, a blank (human plasma, without mepindolol added) and a reagent blank (containing no internal standard) were worked up in each series. The calibration samples were processes as described above.

From the peak-height ratios (analyte/internal standard) and the corresponding concentrations, the calibration function was calculated by least-

squares regression analysis with weighting. The reciprocals of the concentrations were applied as weights.

The performance of the assay was monitored by including control samples in each series of analyses. The control samples were prepared to contain 0.2, 0.5 and 1.0 ng/ml mepindolol sulphate in plasma, and their aliquots were kept together with the unknowns.

RESULTS

The analytical method reported in this paper has been used for the analysis of several hundred plasma samples. Fig. 1 shows three chromatograms of calibration samples. The chromatograms show that the work-up procedure results in sufficiently clean samples (Fig. 1B) to allow the operation of the electrochemical detector with an excellent signal-to-noise ratio at the required gain to quantify concentrations of 0.05 ng/ml (Fig. 1C). Fig 1A depicts the chromatogram obtained after injection of a 1 ng/ml calibration sample. Under these experimental conditions the calibration function $y=ax+b$ (y is the peak-

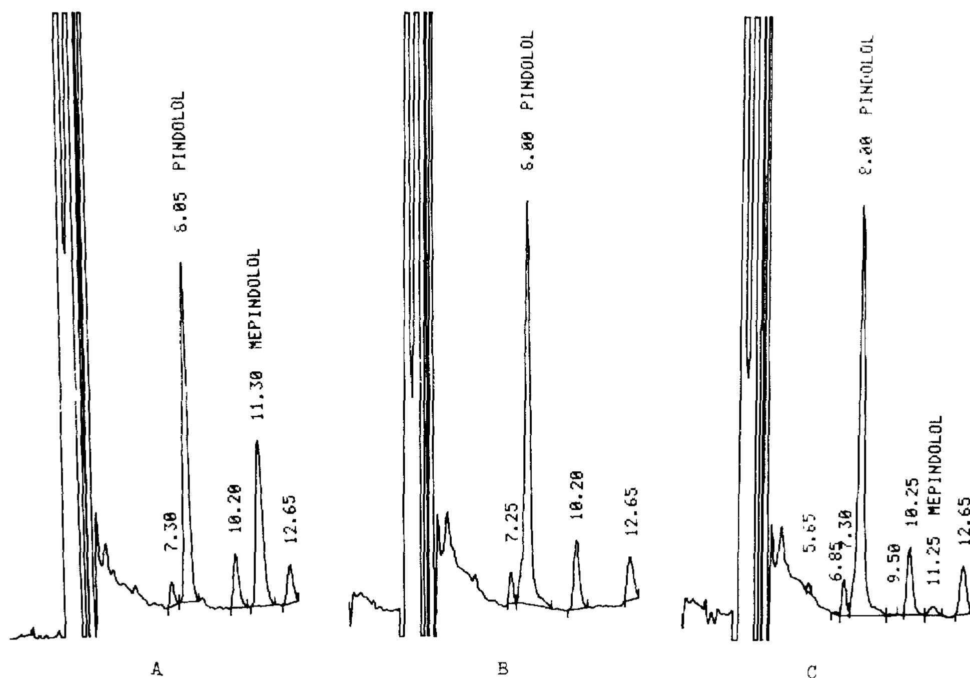

Fig. 1. Typical chromatograms of spiked samples. (A) A 1 ng/ml plasma calibration sample, peaks: 8 05 min, pindolol; 11 30 min, mepindolol. (B) Drug-free plasma sample; peak: 8.00 min, pindolol. (C) A 0.05 ng/ml plasma calibration sample; peaks: 8.00 min, pindolol; 11.25 min, mepindolol. Chromatographic conditions as described in Experimental (sensitivity, 8 5 nA full scale).

height ratio and x is the concentration) was linear over the range tested (0.05–5.0 ng/ml) with a slope of $a=0.502$ and an intercept of $b=-0.002$ calculated on the basis of 123 calibration samples. The coefficients of variation (C.V.) between batches ranged from 19% at 0.05 ng/ml to 6.9% at 5.0 ng/ml ($n>13$). For the control samples at the 0.2 ng/ml level, a value of 0.19 ng/ml was measured (S.D.=0.01, $n=3$), at the 0.5 ng/ml level a value 0.47 ng/ml was found (S.D.=0.01, $n=3$), and at the 1.0 ng/ml level the result was 0.95 ng/ml (S.D.=0.01, $n=3$).

Recoveries for mepindolol ranged between 82.3% and 89.2% (mean 85.8%, $n=10$) over the whole concentration range. The pindolol recovery was found to be somewhat lower at 75% (range 69–81%). Recoveries were evaluated by comparison of peak heights after injection of standard solution and calibration samples after work-up.

At the lower end of the calibration range (0.05–5.0 ng/ml) a signal-to-noise ratio of 6:1 was measured. Therefore the limit of detection of the method can be estimated to be 0.025 ng/ml.

Mepindolol plasma concentrations in humans after transdermal delivery of the drug were, as expected, much lower than after oral administration. After

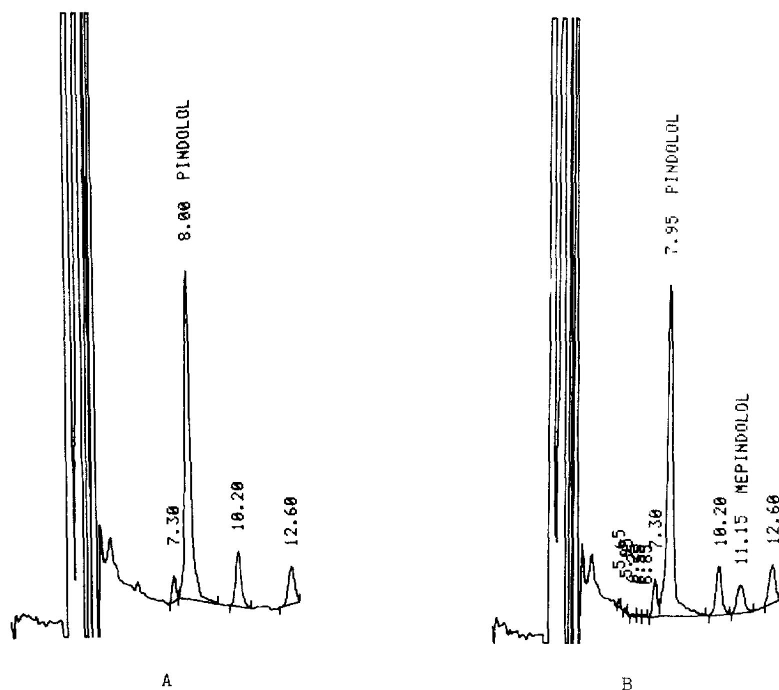

Fig. 2. Clinical study results. (A) Pre-dose day 1; peak: 8.00 min, pindolol. (B) Day 8 of chronic treatment; peaks: 7.95 min, pindolol; 11.15 min, mepindolol (0.14 ng/ml free base). Chromatographic conditions as described in Experimental (sensitivity, 8.5 nA full scale).

seven days of treatment, however, mepindolol was present in all samples taken. The concentrations measured, with a few exceptions, did not exceed 1 ng/ml. Typical examples are given in Fig. 2. The analytical procedure has also been employed in the evaluation of mepindolol concentrations in urine samples. Urine samples were diluted at least 1:10 with water prior to analysis and then processed as described for plasma. Chromatograms from urine samples showed more signals generated by endogenous material than did plasma samples. Occasionally such peaks obscured the analytes so that the sample needed to be reassessed. In these cases a mobile phase was used that contained less acetonitrile, by which means a separation could be achieved in all instances, at the cost of longer analysis times.

DISCUSSION

Although the sample preparation did not present unusual difficulties, the chromatographic separation needed to be carefully fine-tuned. At the beginning of the development work an Ultrasphere ODS column was tested for chromatographic purposes. As this brand of stationary phase has a relatively high proportion of free silanol groups on its surface [5], TEA is needed to counteract silanophilic interactions, in order to obtain narrow, symmetric peaks. The concentration of TEA in the mobile phase, however, must be kept within a very small optimum concentration. The same is true of the acetonitrile content. It was therefore necessary to adjust the prepared mobile phase in situ by addition of TEA and acetonitrile in order to achieve the desired separation. In one experiment, 18 ml of acetonitrile and 20 μl of TEA were required in 2000 ml of a mobile phase, which consisted of 0.02 *M* phosphate buffer (pH 4.0 with sodium hydroxide) containing 200 μl/l TEA–acetonitrile (81:19, v/v). Owing to these minor changes in the composition of the eluent the retention time of mepindolol was decreased by 0.8 min, and that of pindolol by 0.4 min. As a result of this strong influence of the mobile phase composition on the retention behaviour, retention times were more variable than usual from day to day. Intra-assay, i.e. with one batch of mobile phase, retention times showed good reproducibility.

As a consequence of the impact of variable retention times on peak heights, rather high C.V. between batches were found at the lower concentrations. Therefore, we used a μBondapak C_{18} column in the further development of the assay. On this stationary phase acceptable peak shape was obtained without a displacer. However, the separation of the analytes from three endogenous peaks (7.25, 10.20 and 12.65 min in Fig. 1B) could only be achieved by inclusion of an ion-pair reagent. Both the concentration of PSS and the percentage of acetonitrile in the mobile phase needed careful adjustment in order to produce

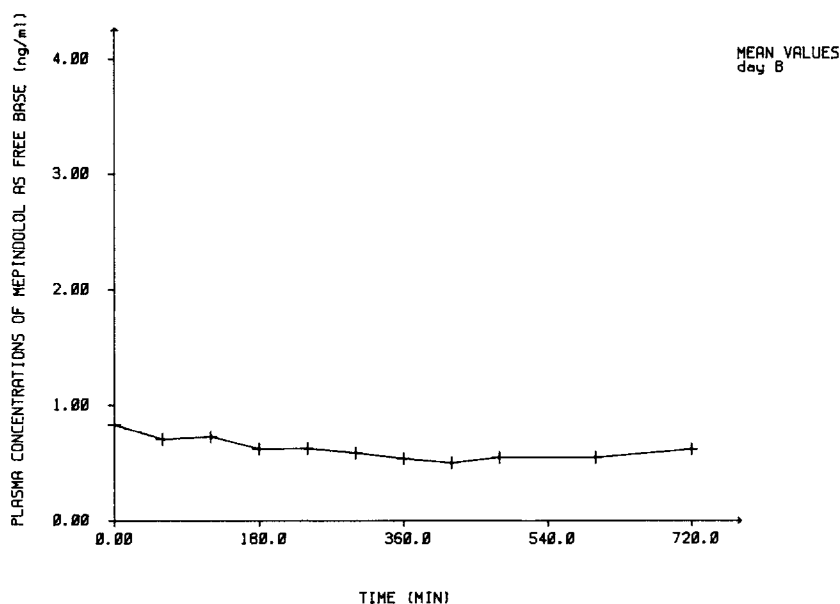

Fig. 3. Mean steady-state concentrations of mepındolol in human volunteers after seven days of chronic treatment with a transdermal delivery device.

complete separation of all peaks in the chromatogram. In our experiments we found, that depending on the individual column, the optimum PSS concentration varied between 0.015 and 0.017 *M*, and the acetonitrile fraction ranged from 22.5 to 23.5% (v/v). In contrast to Ultrasphere ODS, retention times on μBondapak showed good inter-assay reproducibility.

The measurement of plasma concentrations of mepindolol in human volunteers revealed, that a steady state is reached after seven days of treatment with the mepindolol transdermal delivery patch. Fig. 3 displays the mean plasma concentration profile of nine volunteers in the steady state. This indicates that very stable plasma levels can be sustained with the transdermal delivery system. The full pharmacokinetic and pharmacodynamic details will be reported elsewhere.

The method reported here would also be suitable for the sensitive analysis of pindolol, although this possibility was not pursued.

REFERENCES

1 J. Bonellı, G. Hitzenberger, W. Krause, H Wendtland and U. Speck, Int. J. Clin. Pharmacol. Ther Toxicol., 18 (1980) 169
2 R Gugler, L. Kreis and H.J. Dengler, Arzneim. Forsch./Drug Res., 25 (1975) 1067.
3 W. Krause, J. Chromatogr., 181 (1980) 67.
4 G Ridaut, G.C Santus and R.H Guy, Clin. Pharmacokın., 15 (1988) 114.