# A VALIDATED HPLC METHOD FOR ESTIMATION OF METOPROLOL IN HUMAN PLASMA

*M. Aqil*[1,*], *A. Ali*[1], *A. Ahad*[1], *Y. Sultana*[1], *A. K. Najmi*[1], and *N. Saha*[2]

[1]Faculty of Pharmacy, Hamdard University New Delhi, 110 062, India
[2]Ranbaxy Clinical Pharmacology Unit, Majeedia Hospital,
 New Delhi 110 062, India

## SUMMARY

A high-performance reversed-phase liquid chromatographic method for quantification of metoprolol tartrate (MT) in human plasma is presented. A $C_{18}$ column was used with acetonitrile–water–triethylamine 18:81:1 (*v/v*) as mobile phase and pinacidil monohydrate as internal standard (IS). UV detection was at 275 nm and MT and the IS were detected at retention times of 1.5 and 2.6 min, respectively. The method is sensitive with a limit of quantification of 20 ng mL$^{-1}$. The calibration plot for MT in spiked plasma was linear in the concentration range 20–200 ng mL$^{-1}$. Within-batch and total accuracy of the method ranged between 99.71% and 101.61%, and within-batch and total precision, expressed as the coefficient of variation, was 0.20–2.13%. Recovery of MT from spiked plasma was 97.00% and the freeze–thaw and bench-top stability of samples ranged from 77.92 to 105.62%. The method can be successfully used for analysis of MT in human plasma during pharmacokinetic studies.

## INTRODUCTION

Metoprolol tartrate (MT) is an established selective $\beta_1$-blocking drug used for management of moderate to severe essential hypertension [1]. Many dosage forms of MT are available commercially, including tablets. Because the drug undergoes hepatic first-pass metabolism in the body, leading to low oral bioavailability, a transdermal drug-delivery system has been developed to circumvent the problem. This work is part of clinical studies performed to ascertain the comparative bioavailability of MT from oral and transdermal dosage forms.

Several methods have been reported for quantification of MT in plasma using high-performance liquid chromatography (HPLC) with UV

or fluorescence detection [2–14]. Many of these methods involve a complex separation step and are non-reproducible. This paper reports a simple, rapid, sensitive, and reproducible method for analysis of MT in plasma, using pinacidil monohydrate as internal standard (IS). The method was validated for accuracy, precision, linearity, sensitivity, and stability.

## EXPERIMENTAL

### Chemicals and Reagents

Metoprolol tartrate MT (Fig. 1) was a gift from Astra-IDL, India. Pinacidil monohydrate PM was obtained from Leo Pharmaceuticals, Denmark. HPLC-grade acetonitrile and water were from Merck, India. All other chemicals and solvents were HPLC or AR-grade and were used as received.
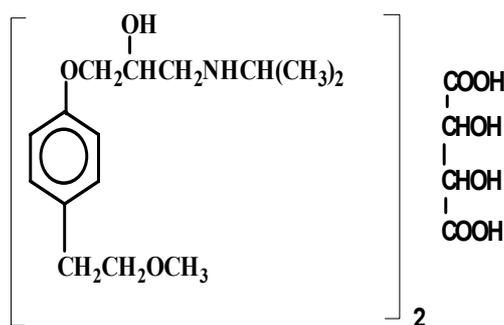


**Fig. 1**

The chemical structure of metoprolol tartrate

MT working standard (5 mg) was accurately weighed, transferred to a 50-mL volumetric flask, and diluted to volume with the mobile phase to produce a stock solution containing 100 μg mL$^{-1}$.

A solution of PM, the internal standard (IS), was prepared by dissolving 5 mg PM in mobile phase in a 50-mL volumetric flask and diluting to volume with the mobile phase to furnish a stock solution containing 100 μg mL$^{-1}$. For spiking plasma samples this stock solution was diluted to 2 μg mL$^{-1}$ with water.

### Chromatography

HPLC was performed with a Shimadzu (Japan) SCL10AT system comprising an LC-10 AT pump, an autosampler, and an SPD-10A detec-

tor. Data processing was by Shimadzu Class-VP software on a Hewlett–Packard computer. Compounds were separated on a 250 mm × 4 mm, 10-μm particle, Novapack C-18 column.

Before method development, column performance was tested by duplicate injection of a mixture of resorcinol, naphthalene, and anthracene (10 μL) with acetonitrile–water 55:45 as mobile phase at a flow rate of 1.0 mL min$^{-1}$. Detection was by UV absorption at 254 nm. System suitability was then tested by injecting a dilute solution of drug five times using the same chromatographic conditions.

The mobile phase used for the method was acetonitrile–water–triethylamine 18:81:1 (*v/v*), pH adjusted to 11 by use of phosphoric acid; before use the mobile phase was degassed and filtered through a 0.45-μm filter. The flow rate was 1.0 mL min$^{-1}$ and MT was detected by UV absorption at 275 nm.

## Calibration

Serial dilutions (1–10 μg mL$^{-1}$) of the MT stock solution were prepared in the mobile phase. Each of these dilutions (1 mL) was added to 49 mL screened plasma, to furnish concentrations in the range 20–200 ng mL$^{-1}$. These samples were analysed by HPLC and the areas of the MT peak were plotted against the concentration of MT. The calibration data obtained are listed in Table I and the calibration plot constructed from the data is shown in Fig. 2.

## Table I

Calibration data for metoprolol tartrate in water–acetonitrile–triethylamine 81:18:1 (*v/v*) after spiking of plasma with stock solutions

| No. | Drug concentration in spiked plasma (ng mL$^{-1}$), $X$ | Peak area[a] | Regressed value of peak area, $Y$ | *SD* |
|---|---|---|---|---|
| 1 | 20 | 154694 | 165856 | 3218 |
| 2 | 40 | 298620 | 303284 | 2873 |
| 3 | 80 | 596181 | 578141 | 1528 |
| 4 | 120 | 865487 | 852997 | 4384 |
| 5 | 160 | 1117645 | 1127853 | 3024 |
| 6 | 200 | 1398217 | 1402710 | 5387 |

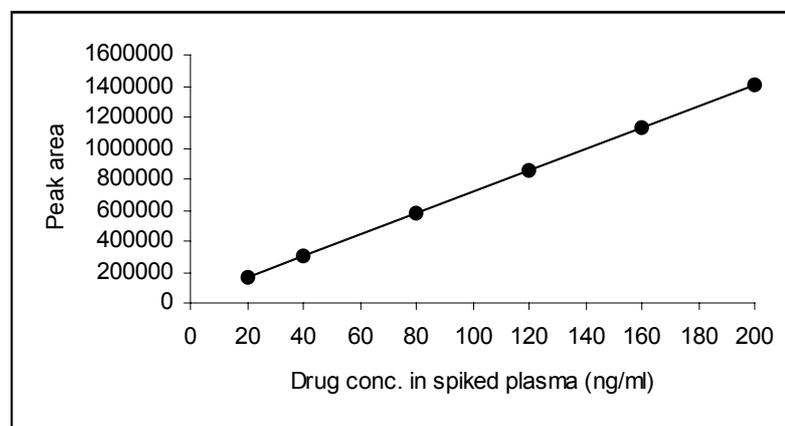[a]$n = 5$, i.e. results are means from five observations

**Fig. 2**

Calibration plot for metoprolol tartrate in water–acetonitrile–triethylamine 81:18:1 (*v/v*) after spiking of plasma with the pure drug. Regression equation, $Y = 6871X + 28428$, correlation coefficient, *r*, 0.9997

## Method Validation

The method was validated for accuracy, precision, sensitivity, recovery, linearity, and stability in accordance with ICH guidelines [15], using PM as IS. Linearity, precision, and accuracy were tested by analysis of five batches of spiked plasma quality-control (QC) samples. Each batch of spiked plasma samples included one complete set of calibration standards (comprising six different concentrations, blank, and blank with internal standard) and QC samples at low (40 ng mL$^{-1}$, LQC), middle (70 ng mL$^{-1}$, MQC), and high (100 ng mL$^{-1}$, HQC) concentrations.

Linearity was determined by regression analysis of the calibration data and determination of the coefficient of variation. Sensitivity was determined as the limits of detection (*LOD*) and quantification (*LOQ*). The accuracy of the method was evaluated as the MT-to-IS peak-area ratio. Precision was measured as the coefficient of variation (%) for the low, middle, and high QC samples during validation. Recovery (%) was determined by measuring the MT-to-IS peak-area ratio for the plasma QC samples (LQC, MQC, and HQC) with the MT-to-IS peak-area ratios of freshly prepared unextracted aqueous standards containing the same concentrations of MT. To determine the freeze–thaw stability of spiked plasma samples five replicates each of the low, middle, and high QC samples were analyzed after one and two freeze–thaw cycles. The freeze–thaw QC samples were

quantified by comparison with a calibration plot prepared after chromatography of freshly spiked samples. For assessment of bench-top stability, the concentrations of low, middle, and high QC samples were determined after 0, 2, and 4 h. The MT-to-IS peak-area response ratios after 2 and 4 h were compared with that after 0 h.

## RESULTS AND DISCUSSION

Under these chromatographic conditions MT and IS in a plasma matrix were resolved with retention times ($t_R$) of 1.5 and 2.6 min, respectively. The method was found to be suitable because reproducible results were obtained on each sample run. On validation of the above method using PM as the IS, the following results were obtained.

### Linearity

The linearity of the calibration plot for MT in human plasma at concentrations 20, 40, 80, 120, 160, and 200 ng mL$^{-1}$ was excellent
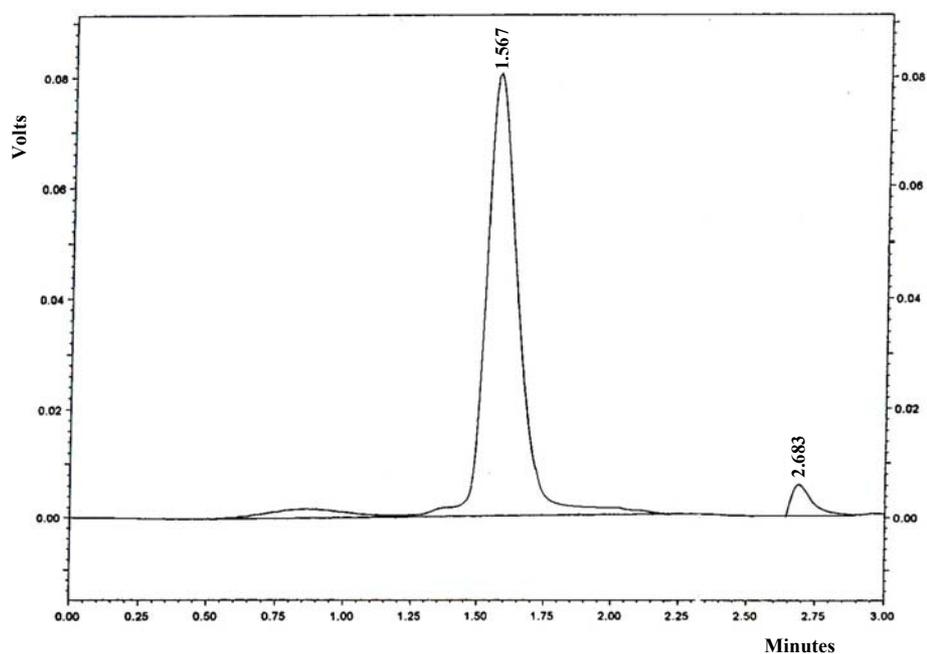
**Fig. 3**

Representative chromatogram obtained during quantification of metoprolol tartrate ($t_R = 1.5$ min) in human plasma with pinacidil monohydrate ($t_R = 2.6$ min) as IS

($r$ = 0.9997) over the concentration range investigated. A typical calibration plot obtained during plasma analysis could be described by the linear equation, $Y = 6871X + 28428$, where $Y$ is peak area and $X$ is concentration (ng mL$^{-1}$). Five calibration plots were constructed; the results obtained are presented in Table I and Figs 2 and 3.

**Sensitivity**

The limits of detection (*LOD*) and quantification (*LOQ*) for metoprolol tartrate were calculated by use of the formulae $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$, where $\sigma$ is the standard deviation of the response and $S$ is the slope of the calibration plot. For analysis of 1 mL spiked plasma the *LOD* was 5 ng mL$^{-1}$ and the *LOQ* 20 ng mL$^{-1}$.

**Accuracy**

The within-batch accuracy, determined by use of the internal standard area-ratio method, ranged from 99.71 to 101.61% (Table II). The between-batch or total accuracy, determined by use of the internal standard area-ratio method, was found to be 99.91 to 100.60% (Table II).

**Table II**

Within and between-batch precision and accuracy for analysis of metoprolol tartrate

| Actual | Nominal concentration (ng mL$^{-1}$) | | |
|---|---|---|---|
| | LQC[a], 40.4721 | MQC[a], 70.5232 | HQC[a], 100.7328 |
| QC1 | 40.3625 | 70.8324 | 100.3467 |
| QC2 | 40.6823 | 70.5983 | 100.5675 |
| QC3 | 39.9847 | 71.7345 | 100.8635 |
| QC4 | 41.2839 | 69.2384 | 99.9928 |
| Mean | 40.5783 | 70.6009 | 100.4426 |
| *SD* | 0.5500 | 1.032 | 0.3671 |
| *CV* (%) | 1.35 | 1.46 | 0.36 |
| Nominal (%) | 100.26 | 100.01 | 99.71 |
| QC5 | 40.4683 | 70.9395 | 100.9338 |
| QC6 | 39.8341 | 69.7682 | 99.9853 |
| QC7 | 40.7375 | 70.7555 | 100.752 |
| QC8 | 41.8382 | 70.3248 | 100.3253 |
| Mean | 40.7195 | 70.4470 | 100.4992 |
| *SD* | 0.8364 | 0.5207 | 0.4271 |
| *CV* (%) | 2.05 | 0.74 | 0.42 |
| Nominal (%) | 100.61 | 99.89 | 99.77 |

**Table II (continued)**

Within and between-batch precision and accuracy for analysis of metoprolol tartrate

| | Nominal concentration (ng mL$^{-1}$) | | |
|---|---|---|---|
| QC9 | 40.5354 | 70.6848 | 100.9461 |
| QC10 | 40.8252 | 69.5347 | 100.7877 |
| QC11 | 39.8411 | 70.3548 | 100.8089 |
| QC12 | 40.7231 | 71.4039 | 100.4040 |
| Mean | 40.4812 | 70.4945 | 100.7366 |
| *SD* | 0.3839 | 0.6715 | 0.2015 |
| *CV* (%) | 0.95 | 0.95 | 0.20 |
| Nominal (%) | 100.02 | 99.96 | 100.00 |
| QC13 | 40.1001 | 70.3845 | 101.3801 |
| QC14 | 39.9387 | 69.9128 | 100.9821 |
| QC15 | 41.8177 | 70.6599 | 100.5347 |
| QC16 | 40.9462 | 70.6848 | 100.7081 |
| Mean | 40.7007 | 70.4105 | 100.9013 |
| *SD* | 0.8659 | 0.3586 | 0.3685 |
| *CV* (%) | 2.13 | 0.51 | 0.36 |
| Nominal (%) | 100.56 | 99.84 | 100.17 |
| QC17 | 40.4076 | 71.9123 | 100.5157 |
| QC18 | 41.5347 | 70.8652 | 101.3738 |
| QC19 | 40.9995 | 69.3920 | 100.6598 |
| QC20 | 41.4696 | 70.5830 | 99.9991 |
| Mean | 41.1028 | 70.6881 | 100.6371 |
| *SD* | 0.5212 | 1.0362 | 0.5672 |
| *CV* (%) | 1.27 | 1.46 | 0.56 |
| Nominal (%) | 101.56 | 100.23 | 99.90 |
| | Total global statistics | | |
| Actual | LQC, 40.4721 | MQC, 70.5232 | HQC, 100.7328 |
| Mean | 40.7165 | 70.5282 | 100.6434 |
| *SD* | 0.6147 | 0.7119 | 0.3994 |
| *CV* (%) | 1.51 | 1.01 | 0.40 |
| Nominal (%) | 100.60 | 100.00 | 99.91 |

[a]LQC, low quality control; MQC, middle quality control; HQC, high quality control

## Precision

Within-batch precision, determined by use of the internal standard area-ratio method, ranged from 0.20 to 2.13% (Table II). The between-batch

or total precision, determined by use of internal standard area ratio method, ranged from 0.40 to 1.15% (Table II).

**Recovery**

Recovery (%) was determined by comparing the MT-to-IS peak-area ratio for the plasma QC samples (LQC, MQC, and HQC) with the MT-to-IS peak-area ratio for freshly prepared unextracted aqueous standards containing the same concentrations of MT. Total recovery of metoprolol tartrate was 93.95% and within batch recovery was 91.59 to 97.00% (Table III).

**Table III**

Absolute recovery of metoprolol tartrate from human plasma

| QC | Individual % recovery | | | Total recovery (%) |
|---|---|---|---|---|
| | LQC | MQC | HQC | |
| 1 | 91.5345 | 95.7266 | 97.4632 | |
| 2 | 99.6366 | 88.7573 | 94.3177 | |
| 3 | 90.3233 | 95.4163 | 94.7687 | |
| 4 | 87.4822 | 90.5341 | 98.9154 | |
| 5 | 88.9958 | 95.9528 | 99.5640 | |
| Mean | 91.5945 | 95.2774 | 97.0058 | 93.9592 |
| *SD* | 4.7423 | 2.9701 | 2.3786 | 4.0968 |
| *CV* (%) | 5.18 | 3.18 | 2.45 | 4.36 |
| *n* | 5 | 5 | 5 | 15 |

**Stability**

The freeze–thaw stability ranged from 77.92 to 103.02%. The stability through one and two freeze–thaw cycles was from 85.06 to 103.02% and from 77.92% to 89.76%, respectively. The freeze–thaw stability of MT over two cycles was therefore acceptable (Table IV). To meet acceptance criteria MT must be stable in plasma samples for up to 4 h. The bench-top stability ranged between 88.74 to 105.62% (Table V).

The results from method validation are summarised in Table VI. The method was used for quantification of MT in plasma to investigate comparative bioavailability after administration of the drug by the oral and transdermal routes [16]. Experimental values of pharmacokinetic data after oral administration were comparable with literature data [1].

**Table IV**

Freeze–thaw stability of metoprolol tartrate in human plasma

|  | Individual stability (%) | | |
|---|---|---|---|
|  | LQC | MQC | HQC |
| One cycle | | | |
| 1 | 91.3628 | 99.8230 | 99.7645 |
| 2 | 78.7324 | 95.1231 | 103.2015 |
| 3 | 83.7189 | 97.3842 | 110.3246 |
| 4 | 86.4112 | 89.5673 | 98.7843 |
| Mean | 85.0563 | 95.4744 | 103.0187 |
| *SD* | 4.56 | 3.79 | 4.5257 |
| *CV* (%) | 5.37 | 3.97 | 4.39 |
| *n* | 4 | 4 | 4 |
| Two cycles | | | |
| 1 | 85.1245 | 86.7321 | 91.8005 |
| 2 | 73.1911 | 88.2345 | 93.1295 |
| 3 | 72.8612 | 82.2265 | 88.8056 |
| 4 | 80.5162 | 79.3082 | 85.3098 |
| Mean | 77.9232 | 84.1253 | 89.7613 |
| *SD* | 5.1623 | 3.5528 | 3.0097 |
| *CV* (%) | 6.62 | 4.22 | 3.35 |
| *n* | 4 | 4 | 4 |

**Table V**

Bench-top stability of metoprolol in human plasma

| Time (h) | % Initial | | |
|---|---|---|---|
|  | LQC | MQC | HQC |
| 0 | 100.00 | 100.00 | 100.00 |
| 2 | 99.71 | 105.62 | 88.74 |
| 4 | 101.57 | 92.75 | 103.64 |
| *n* | 3 | 3 | 3 |

**CONCLUSION**

A simple, rapid, reproducible, and sensitive HPLC method has been developed for analysis of MT in human plasma. The short chromatographic run time of only 1.5 min makes this method suitable for processing of ma-

**Table VI**

Summary of results from method validation

| Method characteristic | Result |
|---|---|
| Analyte | Metoprolol tartrate |
| Biological Matrix | Human plasma |
| Detection | UV, 275 nm |
| Retention time (min) | |
| MT | 1.5 |
| IS | 2.6 |
| Linear range (ng mL$^{-1}$) | 20–200 |
| Limit of quantification (ng mL$^{-1}$) | 20 |
| Limit of detection (ng mL$^{-1}$) | 5 |
| Accuracy (%) | |
| Within batch | 99.71 to 101.61 |
| Total | 99.91 to 100.60 |
| Precision (%) | |
| Within batch | 0.20 to 2.13 |
| Total | 0.40 to 1.51 |
| Recovery (%) | |
| Within batch | 91.59 to 97.00 |
| Total | 93.95 |
| Stability (%) | |
| Freeze–thaw (1 cycle ) | 85.06 to 103.02 |
| Freeze–thaw (2 cycles) | 77.92 to 89.76 |
| Bench-top | 88.74 to 105.62 |

Validation was performed in accordance with ICH guidelines [15]

ny samples in limited time for pharmacokinetic and bioequivalence studies. The method was validated for analysis of MT in human plasma over the range 20 to 200 ng mL$^{-1}$.

## REFERENCES

[1] C. Dollery, Therapeutic Drugs, Churchill Livingstone, New York, 1991

[2] M.M. Bhatti and R.T. Foster, J. Chromatogr., **579**, 361 (1992)

[3] V.L. Herring, T.L. Bastian, and R.L. Lolende, J. Chromatogr., **567**, 221 (1991)

[4] S. Uzu, K. Imai, K. Nakashima, and S. Akiyama, Analyst, **116**, 1353 (1991)

[5] R.J. Straka, K.A. Johnson, P.S. Marshall, and R.P. Remmel, J. Chromatogr, **530**, 89 (1990)

[6] J. Hermansson and C. von Bahr, J. Chromatogr., **227**, 113 (1982)

[7] Y. Horai, T. Ishizaki, M. Kusaka, G. Tsujimoto, and K. Hashimoto, Ther. Drug Monit., **10**, 428 (1988)

[8] K. Balmer, P.O. Alagerstrom, B.A. Persson, and G. Schill, J. Chromatogr., **553,** 391 (1991)

[9] J. Shen, C.T. Hung, and A.R. Zoest, J. Liq. Chromatogr., **14**, 777 (1991)

[10] D. Scuster, M.W. Modi, D. Lalka, and F.M. Gengo, J. Chromatogr., **433**, 318 (1988)

[11] D.R. Rutledge and C. Garrick, J. Chromatogr. Sci., **27**, 561 (1989)

[12] V.L. Lanchote, P.S. Bonato, P.M. Cerqueira, V.A. Pereira, and E.J. Cesarino, Biomed. Sci. Appl., **738**, 27 (2000)

[13] A.J. Braza, P. Modamio, C.F. Lastra, and E.L. Marino, Biomed. Chromatogr., **16**, 517 (2002)

[14] P.M. Cerqueira, E.J. Cesariono, C. Bertucci, P.S. Bonato, and V.L. Lanchote, Chirality, **15**, 542 (2003)

[15] International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: methodology, adopted in 1996, Geneva

[16] M. Aqil, Ph.D. Thesis, Hamdard University, New Delhi, 2001