Mirtazapine Pharmacokinetics with Two Dosage Regimens and Two Pharmaceutical Formulations

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Purpose. To compare, in a clinical study of a special design, the pharmacokinetic profile of mirtazapine in 20 young healthy male volunteers on two treatment regimens with homothetic oral tablets at steady state: NOCTE (1 × 30 mg at 21.00 h) and BID (15 mg at 21.00 h and 15 mg at 09.00 h).

Methods. Pharmacokinetic parameters were calculated from mirtazapine plasma levels assayed by gas chromatography with nitrogen-sensitive detection. A special analysis of variance allowed interesting interactions to be distinguished.

Results. The steady state was reached after 4 and 6 days for NOCTE and BID respectively; the difference was presumably due to intersubject variability. In accordance with pharmacokinetic theory, the peak-to-trough ratio at steady state was significantly lower (twofold) for BID than for NOCTE. Within BID, a small difference (approx. 10%) was found in the extent of absorption between evening and morning administration. Although statistically significant, this difference meets strict bioequivalence requirements. The regimens NOCTE and BID were found to be bioequivalent for the steady-state area-under-the-curve-curve and the peak time. Bioequivalence testing for the peak level was not meaningful due to the difference in dosing regimens. The observed elimination half-lives of 19.7 ± 3.0 h and 20.8 ± 2.7 h (n = 20) for NOCTE and BID, respectively are in agreement with previous results.

Conclusions. Differences (if any) were found to meet strict bioequivalence requirements and were so small that they are of no clinical consequence.

KEY WORDS: Remeron; mirtazapine; Org 3770; antidepressant; pharmacokinetics.

INTRODUCTION

Mirtazapine is the pharmacologically active constituent of Remeron tablets, a novel antidepressant developed under the laboratory code Org 3770. It is a member of a chemical series of compounds known as piperazinoazepines, not related to any known class of psychotropic drugs. It has a unique pharmacological profile combining dual action on both the noradrenergic and serotonergic neurotransmitter systems with a specific action on particular serotonergic receptor subtypes (1–3). Considering this particular pharmacological profile, mirtazapine can be best described as the first Noradrenergic and Specific Serotonergic Antidepressant (2,3).

A number of pharmacokinetic studies on mirtazapine have been published elsewhere (4–6). Voortman and Paanakker (4) studied the absolute bioavailability of mirtazapine from standard Remeron tablets. The bioavailability at steady state was found to be 48 ± 7% (mean ± standard deviation), not significantly different from the bioavailability upon single dosing, which was 50 ± 8% (n = 8). These values closely approach the maximum attainable bioavailability of 56% for this pharmacokinetic profile, which indicates a very good *in vivo* performance of the pharmaceutical formulation of Remeron tablets.

In a pharmacokinetic dose-proportionality study (5), the steady state was attained on the fifth day of each period of increased dosing. The pharmacokinetic profile was essentially linear in the dose range of standard Remeron tablets studied (15, 30, 45, 60 and 75 mg per day). The elimination half-life was accurately determined as 21.5 ± 5.0 h (n = 27), range 13.1–33.6 h.

The effects of gender, age, and treatment regimen (single and multiple oral dosing) were assessed by Timmer et al. (6) with four groups of 8–9 volunteers (adult males, adult females, elderly males and elderly females). In all groups, chronic dosing resulted in approximately 10% higher plasma levels at steady state than those predicted from single-dose kinetics. This nonlinearity was so small that it was considered as clinically insignificant. In adult males, mirtazapine plasma levels were approximately 50% of the overall mean of the combined other groups. As a consequence, statistically significant effects of gender and age were observed. The differences, however, were not large enough to justify any dose adjustments.

The aim of the present study was to compare the pharmacokinetic profile of mirtazapine, at steady state, in 20 young healthy male volunteers on two treatment regimens: NOCTE (1 × 30 mg given orally at 21.00 h) and BID (2 × 15 mg given orally: 15 mg at 21.00 h and 15 mg at 09.00 h). The study design was of a special category: the periods in this two-treatment two-period crossover study were subdivided into blocks each with four volunteers. This allowed each block, representing a balanced complete two-period sub-crossover with four subjects, to be treated on different days. A special analysis of variance allowed a number of interesting interactions to be distinguished and analyzed.

MATERIALS AND METHODS

Active Constituent, Internal Standard and Pharmaceutical Formulations

The chemical name of mirtazapine, laboratory code Org 3770, the active constituent of Remeron tablets, is 1,2,3,4,10,14b-hexahydro-2-methylpyrazino[2,1-a]-pyrido[2,3-c][2]benzazepine, molecular weight 265.36.

Tritiated mirtazapine, synthesized by the Organic Synthesis Section, Department of Drug Metabolism & Kinetics, N.V. Organon, Oss, The Netherlands, as described by Kaspersen et al. (7), was used for recovery experiments in the analytical assay.

The internal standard for the analytical assay was Org 4606, an isomer of mirtazapine. Its chemical name is 1,2,3,4,10,14b-hexahydro-2-methylpyrazino [2,1-a]-pyrido[3,2-c][2] benzazepine (Z)-2-butenedioate (1:1), molecular weight 381.44. The internal standard was synthesized by the Department of Medicinal Chemistry, N.V. Organon, Oss, The Netherlands.

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The pharmaceutical formulations of mirtazapine tablets were prepared by the Department of Pharmacy, N.V. Organon, Oss, The Netherlands, lots CP 087064 and CP 087089. The tablets contained 15 and 30 mg of mirtazapine, respectively. Homothetic excipients were hydroxylpropyl cellulose, corn starch, magnesium stearate and colloidal silicon dioxide. Lactose was added up to a total tablet mass of 150 mg for lot CP 087064 and 300 mg for lot CP 087089.

Clinical Part

This was a randomized, open-label, active medication study with a balanced complete two-treatment crossover design of a special category. The principal investigator of the clinical part was Dr. S. Warrington, at Charterhouse Clinical Research Unit, London, U.K. The study protocol was approved by the local Ethics Committee and the study was performed in accordance with the principles of the Declaration of Helsinki. All subjects (20 normal, healthy young male volunteers) had given written informed consent. Demographic data of the subjects are given in Table I. On experiment days standard meals were provided. Alcoholic beverages were prohibited 24 hours prior to admission and during the study including the washout blood sampling days.

Each group of subjects received each treatment on two different occasions:

1. Treatment NOCTE: a regimen consisting of one daily oral dose of a 30-mg tablet of mirtazapine administered at 21.00 h for seven days;
2. Treatment BID: a regimen consisting of two daily oral doses of a 15-mg tablet of mirtazapine each administered at 21.00 and 09.00 h for seven days. For some comparisons, $BID_{evening}$ and $BID_{morning}$ were distinguished. The last (14th) dose was administered at 09.00 h on Day 8.

The treatment sequence for each subject is included in Table I. It should be noted that the first drug dosing for each regimen corresponds to different actual dates. In a balanced two-treatment crossover design the two subject groups usually are treated almost simultaneously. However, due to capacity limitations in the clinic, a sequence group was subdivided into five balanced complete subgroups (blocks) of two subjects each. There was at least a two-day interval between the start of the treatments and always a two-week interval between the periods within each block. The complete experimental design is shown in Table II. The blocks represent the replication at different times with different subjects; however, the periods in a block lag a few days behind those in the previous block. As can also be seen from Table II, the treatment times of Subject 19 do not fit in the design. Therefore, this subject was not included in the statistical analysis, although—for the purpose of documentation—his plasma levels were included in the reported means and standard deviations.

A 50 ml blood sample was taken from each volunteer for haematological/biochemical examinations and to provide a free-of-drug plasma sample for calibration purposes. Serial 5-ml blood samples were taken from an antecubital vein using heparinized vacutainers, as follows:

NOCTE: Day 4 (pre-dose); Day 5 (pre-dose); Day 6 (pre-dose); Day 7 (pre-dose) and at 0.25, 0.50, 0.75, 1, 1.25, 1.50, 2, 3, 4, 6, 8, 12, 16, 24, 48, 72, 96 and 120 hours after the last drug administration.

BID: Day 4 (pre-dose, twice); Day 5 (pre-dose, twice); Day 6 (pre-dose, twice); Day 7 (pre-dose, twice) and at 0.25, 0.50, 0.75, 1, 1.25, 1.50, 2, 3, 4, 6, and 8 h after dosing; Day 8 (pre-dose) and at 0.25, 0.50, 0.75, 1, 1.25, 1.50, 2, 3, 4, 6, 8, 12, 16, 24, 48, 72, 96 and 120 h after the last drug administration.

Plasma was separated from blood cells by centrifugation and stored at −20°C until analyzed.

Assay of Mirtazapine Plasma Levels

The assay method for mirtazapine comprised extraction of mirtazapine and its internal standard from alkalinized plasma with *n*-hexane. After evaporation of the hexane layer the residuals were dissolved in methanol and analyzed by capillary gas chromatography with nitrogen-sensitive detection. The validated assay method has been described in detail elsewhere (8).

Table I. Demographic Data on Subjects (all male) and Treatment Sequence

Sequence NOCTE-BID				Sequence BID-NOCTE			
Subj. (No.)	Age (yrs)	Height (cm)	Weight (kg)	Subj. (No.)	Age (yrs)	Height (cm)	Weight (kg)
1	23	172	57	2	28	175	67
4	22	181	72	3	21	179	70
6	22	180	70	5	25	173	64
7	19	175	70	8	20	178	82
10	24	180	72	9	21	166	68
11	30	185	70	12	25	178	70
14	25	173	70	13	26	188	70
15	29	179	67	16	23	183	70
18	22	175	65	17	23	179	67
20	27	182	68	19	23	177	67
Mean	24.3	178.2	68.1	Mean	23.5	177.6	69.5
SD	3.5	4.2	4.5	SD	2.5	5.8	4.8
n	10	10	10	*n*	10	10	10

Table II. Structure of Study Design

Sequence	Group size	Subj. Nos.	Period 1 Block 1	2	3	4	5	Period 2 Block 1	2	3	4	5
N–B	2	01 + 04	N					B				
B–N	2	02 + 03	B					N				
N–B	2	06 + 07		N					B			
B–N	2	05 + 08		B					N			
N–B	2	10 + 11			N					B		
B–N	2	09 + 12			B					N		
N–B	2	14 + 15				N					B	
B–N	1	13				B					N	
N–B	2	18 + 20					N					B
B–N	2	16 + 17					B					N
B–N	1	19					B					N

Note: N = NOCTE; B = BID; time interval between blocks ≥ 2 days.

Pharmacokinetic Parameter Estimation

The following pharmacokinetic (model-independent) parameters were calculated from the plasma levels of mirtazapine:

- The elimination half-life ($t_{1/2}$) was estimated by least-squares regression on the individual log-linear terminal plasma levels.
- The Area-Under-the-Curve (AUC) was calculated using the linear trapezoidal rule. For NOCTE, the AUC was obtained over the 24-h interval after the last dose. For $BID_{evening}$, the 12-h dosing interval of the last but one dose was used and for $BID_{morning}$ that of the last dose.
- The peak level (C_{max}), the peak time (t_{max}) and the trough level (C_{min}) were taken from the empirical plasma levels. For NOCTE, the trough levels were those on Days 4, 5, 6, and 7, and 24 h after the last dosing (Day 8); for BID these were the two pre-dose levels on Days 4, 5, 6 and 7, the pre-dose level on Day 8, and the level at 12 h after the last dosing (Day 8). The peak-to-trough ratio (PTR) was taken as C_{max} divided by the pre-dose C_{min}.
- The time to reach steady state (t_{ss}) and the minimum steady state concentration ($C_{ss,min}$) were estimated by using the Helmert Contrast Transformation on the C_{min}-values. In this analysis each pre-dose level is compared with the mean of all subsequent pre-dose levels. This determines the time point at which the pre-dose levels cease to change, i.e. on which day (t_{ss}) the steady state is reached. The values of $C_{ss,min}$ were taken as the mean of those of C_{min} from day t_{ss} onwards up to Day 8.

Statistical Analysis

Effects are indicated as significant if tail probabilities (p) from the appropriate tests were less than or equal to 0.05. Results are given as Mean ± SD. The statistical analysis comprised two major comparisons:

Treatment NOCTE versus $BID_{evening}$

This comparison served mainly to evaluate the bioequivalence of the 15-mg tablet, applied in a 2 × 15 mg BID regimen, versus the reference 30-mg tablet, applied in a 1 × 30 mg NOCTE regimen. As a rule, relevant parameters for bioequivalence assessment are AUC for extent of absorption, and C_{max} and t_{max} for rate of absorption (9,10). The use of AUC for extent of absorption has theoretically and practically been explored by Midha et al. (11). Bioequivalence testing for C_{max}, however, was not meaningful in the present study, since the daily time courses of plasma levels are unimodal and bimodal respectively.

As has been recommended by regulatory bodies (9,10), bioequivalence is concluded if the 90%-confidence interval for the median test-to-reference ratio of log-normally or normally distributed characteristics is fully included within predefined acceptance limits, usually 80–125% and 80–120% respectively. This is operationally identical to Schuirmann's two one-sided tests with $\alpha = 0.05$ for either test (12).

The two regimens were also compared in terms of C_{max}, C_{min}, C_{max}/C_{min}, and $t_{1/2}$. For all parameters of the BID regimen (except $t_{1/2}$), there were two estimates: one for the evening dose and the other for the morning dose. The parameters C_{max}, t_{max}, PTR, and $C_{ss,min}$ were compared between NOCTE and $BID_{evening}$, since they were estimated at the same times.

The parameter AUC of NOCTE was compared with the sum of the AUC of $BID_{evening}$ and that of $BID_{morning}$, both relating to a 24-h interval. The parameter $t_{1/2}$ of NOCTE could be only compared with that of $BID_{morning}$.

Model for the Comparison NOCTE versus $BID_{evening}$

For the design of the study of this special category the following statistical model was assumed:

$$y_{ijklm} = \mu + \phi_i + \delta_{ijk} + \tau_l + \pi_m + \beta_k + \beta\phi_{ik} + \beta\pi_{km} + \beta\tau_{kl} + \epsilon_{ijklm}$$

where

μ = overall mean,
ϕ_i = fixed effect of ith sequence,
δ_{ijk} = random effect of jth subject within ith sequence of kth block, normally distributed with mean 0 and variance σ_s^2,
τ_l = fixed effect of lth treatment,
π_m = fixed effect of mth period,
β_k = random effect of kth block with mean 0 and variance σ_{block}^2,
$\beta\phi_{ik}$ = random interaction effect of kth block and ith sequence, with mean 0 and variance $\sigma_{block\cdot seq}^2$,
$\beta\pi_{km}$ = random interaction effect of kth block and mth period, with mean 0 and variance $\sigma_{block\cdot period}^2$,
$\beta\tau_{kl}$ = random interaction effect of kth block and lth treatment, with mean 0 and variance $\sigma_{block\cdot trtmt}^2$,
ϵ_{ijklm} = random residual error, with mean 0 and variance σ_e^2, independent of the δ_{ijk}.

The indices i, l and m are not independent of each other, e.g., if the sequence i and treatment l are chosen, then the period m is determined. To facilitate the model notation, three separate indices were used. The effects *subject* and *block* were taken as random effects. It is commonly accepted to consider all interactions containing at least one random factor as random.

This model can be seen as (a) additive without any transformation, or (b) additive after logarithmic transformation of the characteristic analyzed. A log-normal distribution was assumed for AUC and C_{max} (9,10), and also for $t_{1/2}$, $C_{ss,min}$ and PTR. Since t_{max} follows a discrete distribution, a non-parametric analysis of this parameter was also performed by using Friedman's test (13), without taking into account sequence, period and block effects. An additional non-parametric analysis of t_{max} was performed by using the MS-DOS program BIOEQNEW (14), which provides non-parametric confidence intervals for treatment and period effects, including a test on sequence effects, but it necessarily ignores block effects.

The analyses of variance and all calculations to obtain treatment means were performed by using the GLM procedures of the SAS System (15) under the VAX/VMS Operating System V5.2 on a DEC/VAX-8700 computer.

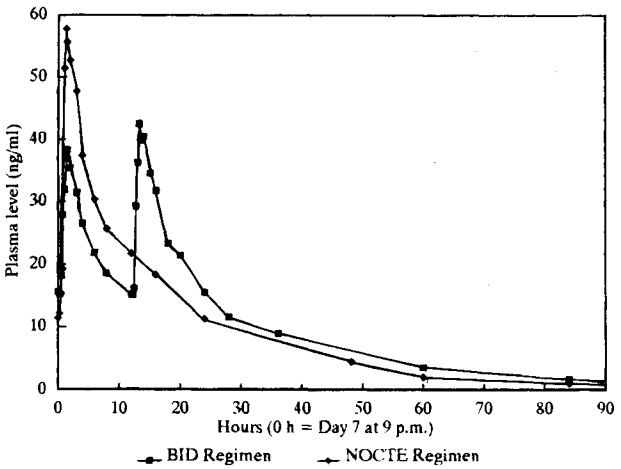

Fig. 1. Intersubject (n = 20) mean steady-state plasma levels of mirtazapine per sampling time, including washout period, from the last dose of the NOCTE regimen and the last two doses of the BID regimen.

Treatment $BID_{evening}$ versus $BID_{morning}$

The paired t-test served to evaluate mirtazapine pharmacokinetics after the evening and morning administration, for all parameters except $t_{1/2}$.

RESULTS AND DISCUSSION

Intersubject mean plasma levels of mirtazapine (n = 20) per sampling time are shown in Figure 1 for the last dose of the NOCTE regimen and the last two doses of the BID regimen, and in Figure 2 for the last $BID_{evening}$ dose and the last $BID_{morning}$ dose. A summary of pharmacokinetic parameters as Mean ± SD is given in Table III. The AUCs are the 24-hours steady-state interval for NOCTE, and the 12-hours steady-state interval for BID.

The replacement Subject 19 was not included in the statistical analysis (see Table II). Furthermore, for Subject 10 the BID regimen was found to be irregular. Therefore, all BID parameters were considered as missing for this subject. The missing cells were taken into account by the GLM procedure (15).

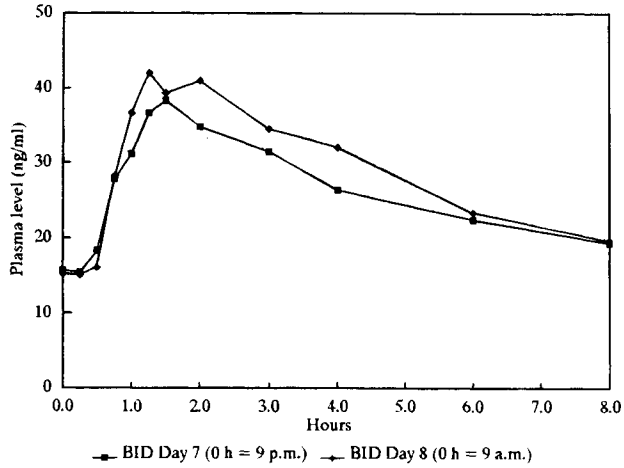

Fig. 2. Intersubject (n = 20) mean steady-state plasma levels of mirtazapine per sampling time, with the same time axis, from the last $BID_{evening}$ and the last $BID_{morning}$ dose.

Table III. Summary of Steady-State Pharmacokinetic Parameters Estimated from 20 Subjects

Parameter	Means ± Standard Deviation		
	NOCTE	BID (evening)	BID (morning)
AUC (ng·h/ml)	589 ± 142	275 ± 65	303 ± 71
C_{max} (ng/ml)	76 ± 33	47 ± 17	53 ± 19
t_{max} (h)	1.99 ± 1.65	1.78 ± 0.84	1.79 ± 0.92
Peak/Trough	6.94 ± 2.96	3.03 ± 0.77	3.50 ± 1.05
$C_{ss,min}$ (ng/ml)	11.4 ± 3.4	15.7 ± 4.1	16.1 ± 4.0
t_{ss} (days)	6[a]	4[a]	4[a]
$t_{1/2}$ (h)	19.7 ± 3.0	–[b]	20.8 ± 2.7

[a] Only a single estimation per treatment possible.
[b] No estimation possible without washout.

Estimation of the time to reach steady state using the Helmert transformation on the trough levels per individual volunteer appeared to be unreliable, due to large intrasubject variability. Therefore, the Helmert transformation on the treatment means of the trough levels was used instead. The results showed that the plateau was reached after 6 days for NOCTE and 4 days for BID. The difference between the two regimens is presumably due to intersubject variability.

The steady-state trough levels, $C_{ss,min}$, determined as the means from Day 6 onwards, are included in Table III. As could be theoretically expected, the mean trough level of $BID_{evening}$ was significantly higher than that of NOCTE. The mean values of the peak-to-trough ratio (PTR) are also included in Table III. As could be expected from the difference in regimens, these values were significantly higher (about twofold) for NOCTE as compared to BID.

In the comparison of $BID_{evening}$ and $BID_{morning}$, the paired t-tests on the log-transformed parameters C_{max}, t_{max}, $C_{ss,min}$ and PTR did not result in significant differences. The parameter AUC showed significantly higher steady-state levels from the morning doses (303 ± 71 $ng \cdot ml^{-1} \cdot h$) as compared to those from the evening doses (275 ± 65 $ng \cdot ml^{-1} \cdot h$). This difference may be due to circadian rhythms in either the extent of absorption or the total body clearance. However, the 90%-confidence interval for the morning-to-evening ratio of the AUC was calculated to be 106–114%. This means that strict bioequivalence requirements are met for the morning and evening AUCs within the BID regimen. This also implies that this difference is small and of no clinical consequence.

The results of the analyses of variance for the comparison of the BID (2 × 15 mg) regimen and the NOCTE (1 × 30 mg) regimen are given in Table IV. No significant block effects, sequence effects, block x sequence effects or period effects were found for any of the parameters. A significant interaction effect between block and treatment was only found for the AUC. This implies that confidence intervals for the population treatment means of AUC must be based on the block x treatment interaction variance (0.035361 with 4 degrees of freedom) instead of the residual (error) variance. Using log-transforms, the 90%-confidence interval of the median BID-to-NOCTE area was found to be 88–109%. This implies that the systemic

Table IV. Summary of Results from Analysis of Variance on Log-Transforms for NOCTE Regimen versus BID Regimen (no transformation for t_{max})

	EFFECT						
Parameter	Block	Seq.	Block x Seq.	Period	Block x Per.	Treatm.	Block x Treatm.
AUC	//	//	//	//	**	//	**
C_{max}	//	//	//	//	//	**	//
t_{max}	//	//	//	//	//	//	//
$C_{ss,min}$	//	//	//	//	//	***	//
PTR	//	//	//	//	//	***	//
$t_{1/2}$	//	//	//	//	//	//	//

Note: //, $p > 0.05$; *, $0.01 < p \leq 0.05$; **, $0.001 < p \leq 0.01$; ***, $p \leq 0.001$.

exposure, as measured by the steady-state AUC, meets strict bioequivalence requirements for the two regimens.

Non-parametric analysis of t_{max}, by means of the program BIOEQNEW (14) resulted in the absence of significant sequence or period effects ($p >> 0.05$). The point estimate of the median difference between the NOCTE and the BID regimens was less than 1 minute and the non-parametric 90%-confidence interval for the median difference ranged from −0.64 to +0.50 h, corresponding to 69% and 124% respectively when added to the mean t_{max} of the NOCTE regimen and subsequently expressed in terms of the latter. It follows that the whole 90%-confidence interval is of the same order of magnitude as the standard deviations of t_{max}, which are 0.88 and 1.65 h for the BID and NOCTE regimens respectively. It should also be considered that bioequivalence requirements for t_{max} do not exist (9,10). It is concluded that the two tablet formulations administered in the two regimens are clinically equivalent with respect to the parameter t_{max}.

The half-lives for the NOCTE regimen (19.7 ± 3.0 h) and the BID regimen (20.8 ± 2.7 h) found in the present study (with $n = 20$) are in excellent agreement with results reported earlier for healthy young male subjects. In a dose-proportionality study at steady state, Timmer *et al.* (5) found a mean value of 21.5 ± 5.0 h ($n = 27$). In a separate study (6), in which a subgroup of 9 healthy adult male volunteers participated, a mean value of 21.7 ± 4.2 h after single-dosing and 22.1 ± 3.7 h after multiple dosing was found.

CONCLUSIONS

With regard to the extent of absorption of mirtazapine, the relevant parameter is the AUC. For this parameter, bioequivalence was demonstrated for the 15-mg tablet applied in a 2 × 15 mg BID regimen, compared to the reference 30-mg tablet applied in a 1 × 30 mg NOCTE regimen. This implies that there were no statistically significant differences nor clinically relevant differences in systemic exposure per 24 h at steady state for the NOCTE and BID regimen. Similar conclusions could be drawn for t_{max}. Bioequivalence testing for C_{max} was not meaningful, since the daily time courses of plasma levels of the NOCTE and BID regimens are unimodal and bimodal respectively.

The steady state was reached after 4 and 6 days for the NOCTE regimen and the BID regimen respectively; the difference is considered to be due to interindividual variability. As expected from pharmacokinetic theory, the peak-to-trough ratio at steady state was significantly lower for the BID regimen compared to that of the NOCTE regimen.

Within the BID regimen the pharmacokinetic profiles of mirtazapine showed a difference of 10% in the extent of absorption between the evening and the morning administration, as measured by the AUC. This small difference, which may be due to circadian rhythms, meets strict bioequivalence requirements and is therefore considered to be of no clinical consequence.

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