Bioequivalence study of two different tablet formulations of donepezil using truncated areas under the curve

A single-center, single-dose, randomized, open-label, 2-way crossover study under fasting conditions

Susana Almeida[1,2], Augusto Filipe[1], Rita Neves[1], Isabelle Desjardins[3], Eric Shink[3], Araceli Castillo[4]

[1] Medical Department, Grupo Tecnimede, Sintra, Portugal
[2] Department of Pharmacology and Therapeutics, Universidad Autònoma de Barcelona, Spain
[3] Anapharm, Québec (Québec), Canada
[4] Anapharm Europe S.L., Barcelona, Spain

Correspondence to: Susana Almeida, MSc, Medical Department, Grupo Tecnimede, Sociedade Tecnico-Medicinal S.A., Zona Industrial da Abrunheira, R. da Tapada Grande, no. 2 Abrunheira, 2710-089 Sintra, Portugal; e-mail: dmed.ct@tecnimede.pt

Key words

- Acetylcholinesterase inhibitor
- CAS 120014-06-4
- Donepezil, bioequivalence, generic, pharmacokinetics
- Truncated AUC

Arzneimittelforschung 2010;60(3):116–123

Abstract

Background: **Donepezil hydrochloride (CAS 120014-06-4) is a piperidine-based, reversible inhibitor of the enzyme acetylcholinesterase (AChE). It is postulated to exert its therapeutic effect by enhancing cholinergic function. This is accomplished by increasing the concentration of acetylcholine (ACh) through reversible inhibition of its hydrolysis by AChE.**

Objective: **The aim of this study was to assess the bioequivalence of a new donepezil 10 mg formulation (test formulation) vs. the reference product, as required by European regulatory authorities for the marketing of a generic product. Additionally, the applicability of the truncated area under the plasma concentration curve (AUC) approach to this drug and under these test conditions was determined.**

Methods: **This was a single center, randomized, single-dose, open-label, 2-way crossover study in healthy volunteers under fasting conditions. Plasma samples were collected up to 288 h post-dosing and (+)-donepezil and (–)-donepezil plasma levels were determined by reverse liquid chromatography and by tandem mass spectrometry detection (ie, the LC-MS/MS method). Pharmacokinetic parameters were calculated using non-compartmental analysis. Area under the concentration-time curve from time zero to the time of the last non-zero concentration (AUC_{last}) and maximum observed concentration (C_{max}) were the main evaluation criteria, while area under the concentration-time curve from time zero to infinity (AUC_{inf}) was also analyzed for additional information. For the assessment of the applicability of the truncated AUC approach, AUCs truncated at 24, 48, 72, 96, 144, 192, 240, and 288 h were calculated. All of the abovementioned pharmacokinetic parameters were analyzed using 90 % geometric confidence interval of the ratio (T/R) of least-squares means from the ANOVA of the ln-transformed parameter. Tolerability was monitored using physical examination, including vital sign measurements and laboratory analysis.**

Results: **According to the classical approach, the 90 % geometric confidence intervals obtained by analysis of variance for AUC_{last}, C_{max} and AUC_{inf} were within the predefined ranges (80.00–125.00 %) for both analytes. Truncated AUCs were also in all cases within the predefined ranges for acceptance of bioequivalence.**

Conclusion: **Bioequivalence between test and reference formulations, both in terms of rate and extension of absorption, under fasting conditions, was concluded according to European guidelines. Both formulations were well tolerated. The conclusion of bioequivalence was also supported using the truncated AUCs approach.**

1. Introduction

Donepezil hydrochloride (CAS 120014-06-4) is a piperidine-based, reversible inhibitor of the enzyme acetylcholinesterase (AChE). It is postulated to exert its therapeutic effect by enhancing cholinergic function. This is accomplished by increasing the concentration of acetylcholine (ACh) through reversible inhibition of its hydrolysis by AChE. If this proposed mechanism of action is correct, effect of donepezil may lessen as the disease process advances and fewer cholinergic neurons remain functionally intact [1, 2].

Donepezil is indicated for the symptomatic treatment of patients with mild to moderate dementia of the Alzheimer's type. The usual daily dosage is 5 to 10 mg. Adverse events associated with donepezil are listed in the product monograph [1, 2].

Donepezil is well absorbed with a relative oral bioavailability of 100 %. After oral administration of a single oral dose of 10 mg donepezil (film-coated tablets) under fasting conditions, the maximum observed concentration (C_{max}) is reached at 2.4 to 4.4 h (T_{max}) [3–7]. Pharmacokinetics are linear over a dose range of 1–10 mg given once daily. Neither food nor time of administration (morning vs. evening dose) influences the rate or extent of absorption of donepezil film-coated tablets [1, 2]. The elimination half life of donepezil is about 70 h [1] and can thus be considered a long half-life drug. Sex, race and smoking history have no clinically significant influence on plasma concentrations of donepezil [2].

Donepezil is extensively metabolised and is also excreted in the urine as parent drug. The rate of metabolism of donepezil is slow and does not appear to be saturable. Donepezil is metabolised by CYP450 isoenzymes 2D6 and 3A4 and undergoes glucuronidation. Donepezil is primarily metabolised by CYP450 isoenzymes 2D6 and 3A4 and undergoes first-pass metabolism [4, 8]. There are 4 major metabolites, 2 of which are known to be active. 6-O-desmethyl donepezil, which has similar activity to that of the parent compound, was found in plasma at concentrations equal to about 20 % of donepezil [8]. Donepezil and its metabolites are primarily eliminated by renal excretion in humans [4, 8]. Approximately 57 % of the total administered radioactivity is recovered from the urine and 15 % is recovered from the feces (total recovery of 72 %) over a period of 10 days [1, 2].

According to the currently effective European guideline [9] the biological sampling schedule should cover the concentration vs. time curve long enough to provide a reliable estimate of the extent of absorption, which would be generally achieved by covering at least 80.00 % of the total area under the curve. However, truncation of AUCs may be used as a strategy to shorten the length of blood sample collection, especially in the case of drugs with a long half-life. In these cases, the total collection time should be justified and sample collection should ensure an adequate comparison of the absorption phase [11].

The present study aimed to compare the relative bioavailability of a new donepezil 10 mg formulation manufactured by Grupo Tecnimede (Sintra, Portugal) and that of a reference formulation so that bioequivalence could be assessed, as required by the European regulatory authorities for the marketing of a generic product.

Additionally, the results obtained by the truncated area under the curve (AUC) approach were obtained and conclusions compared to those obtained by the conventional approach.

2. Subjects and methods

2.1 Study protocol

An independent ethics committee, Institutional Review Board Services, Ontario, Canada, approved the clinical study protocol and a letter of no-objection was obtained from the Canadian authorities. The study was performed in accordance with the Helsinki Declaration and Good Clinical Practice Guideline [10] and an informed consent from participants was obtained before any study related procedures commenced. The clinical part of the study was conducted at the Anapharm Clinical Research Facility (Montréal, Québec, Canada) and the bioanalytical part at Anapharm Europe, SL facility (Barcelona, Spain).

2.2 Subjects

Subjects enrolled in this study were members of the community at large. Subject screening procedures included informed consent, inclusion/exclusion check, demography, medical history, medication history, physical examination, height, weight, body mass index, a concomitant medication check, vital signs measurements (blood pressure, pulse rate, respiratory rate, and oral temperature), a 12-lead electrocardiogram (ECG), a urine drug screen, a urine pregnancy test (female subjects), hematology, biochemistry, urinalysis, and Human Immunodeficiency Virus (HIV) and hepatitis B and C testing. All participating subjects were judged eligible for the study when assessed against the inclusion and exclusion criteria.

Subjects were male or female, light smoker or non-smoker, ≥ 18 and ≤ 55 years of age, capable of consent with a body mass index (BMI) ≥ 20.0 and < 25.0 kg/m^2.

Tolerability was monitored using physical examination (including vital sign measurements) and ECG performed at screening and laboratory analysis including biochemistry tests, hematology tests, and urinalysis, which were performed at screening and during the study conduct.

Clinical laboratory tests (hematology, biochemistry, and urinalysis) were performed for each subject at the time of the screening and post-study procedures. Laboratory tests performed included the following: Hematology: complete blood count with differential, hemoglobin, hematocrit; Biochemistry: albumin, alkaline phosphatase, alanine aminotransferase (ALT/ SGPT), aspartate aminotransferase (AST/ SGOT), urea nitrogen, calcium, chloride, glucose, phosphorus, potassium, creatinine, sodium, total bilirubin, total protein; Urinalysis: macroscopic examination, pH, specific gravity, protein, glucose, ketones, bilirubin, occult blood and cells, nitrite, urobilinogen, leukocytes, microscopic examination (performed on abnormal findings unless otherwise specified). Electrocardiograms and physical examinations were performed at the time of screening only.

Vital signs measurements (blood pressure, heart rate, respiratory rate, and oral temperature) were performed at the

time of screening, and post-study procedures. In addition, seated blood pressure and heart rate measurements were performed prior to dosing and approximately 2, 4, 8, and 24 h post-dose, in each period. Vital signs measurements were repeated at least once as soon as possible after the initial scheduled measurement under the following conditions: systolic blood pressure measurement lower than 90 mmHg or higher than 140 mmHg, diastolic blood pressure measurement.

Subjects were confined to the Anapharm Clinical Research Facility from at least 10 h prior to drug administration until after the 24-h post-dose blood draw, in each period. The treatment phases were separated by a washout period of 35 days.

2.3 Drug products

Donepezil hydrochloride film-coated tablet, 10 mg manufactured by Tecnimede, Sociedade Tecnico-Medicinal (batch no. 80585, expiry date 01/2010) was compared to the Portuguese reference product, 10 mg (batch no. 7115904; expiry date 11/2010), acquired from a local pharmacy.

2.4 Study design

This was a single centre, randomized, single-dose, open-label, 2-way crossover bioequivalence study to compare the rate and extent of absorption of test donepezil *versus* a reference donepezil, under fasting conditions. Prior to study commencement, subjects were randomly assigned to a treatment in accordance with the computer generated block randomization scheme (block size = 4, randomly variable) generated by Anapharm.

After a supervised overnight fast of at least 10 h, the subjects were administered the test or reference medication as a single oral dose of 1 film-coated tablet containing 10 mg of donepezil with 240 mL of water. Subjects were dosed as specified in the protocol and subsequently fasted for a period of at least 4 h.

Blood samples were collected prior to study drug administration and at 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.25, 3.50, 3.75, 4.00, 4.50, 5.00, 6.00, 8.00, 12.0, 24.0, 48.0, 72.0, 96.0, 144, 192, 240, and 288 h post-dose in each period.

2.5 Randomization and blinding

The randomization scheme was generated using SAS® program version 8.2 (SAS Institute Inc., Cary, NC, USA), performed by a biostatistician. The computer-generated sequence ensured equal distribution of treatments. After the random definition of the starting value blocks of 10 were generated from the list of the subjects' assignments.

The subjects were enrolled by a qualified investigator. When arriving at the clinical facility for the first confinement, subjects were assigned numbers, which corresponded to a previously generated randomization scheme.

Volunteers were administered the test or reference medication as per the randomization scheme which was kept unavailable to the Bioanalytical Division of Anapharm Europe S.L. until completion of the clinical and analytical phases. The investigator and clinical staff were blinded to each subject's treatment until after subjects qualified as eligible for the study. The pharmacist who prepared the treatment per subject in accordance with the predetermined randomization plan was not blinded to the randomization scheme, but kept no contact with the conduct of the study.

2.6 Drug analysis

All blood samples were drawn into blood collection tubes (1 × 2 mL) containing sodium-heparin, prior to drug administration and at 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.25, 3.50, 3.75, 4.00, 4.50, 5.00, 6.00, 8.00, 12.0, 24.0, 48.0, 72.0, 96.0, 144, 192, 240, and 288 h post-dose in each period (3 mL for each sampling time). The total volume of blood drawn from each subject completing this study did not exceed 139 144 mL. Blood samples were cooled in an ice bath and were centrifuged at 3,000 rpm for at least 10 min at approximately 4 °C. Two aliquots were dispensed into polypropylene tubes and were stored at –20 ± 5 °C. At the end of the study, the frozen plasma aliquots (1 of 2) from the Montréal Clinical Facility were sent to the analytical facility (Anapharm Europe S.L., Barcelona, Spain).

The method involved a liquid-liquid extraction procedure with tert-butyl methyl ether:hexane (85 : 15). (+)-Donepezil, (–)-donepezil and deuterated internal standard were measured by reversed phase high performance liquid chromatography coupled to a tandem mass spectrometry detector (LC/MS/MS). The modular liquid chromatographic system was made up of: an HTC autosampler (CTC-PAL), high pressure binary pump (Agilent 1200 series) and a mass spectrometer detector API 4000 (MDS Sciex, Applied Biosystems, Toronto, Canada). Separations were performed on a quiral column (Chiralcel OD-RH, 4.6 × 150 mm, 5 µm, Daicel Chemical Ind., Ltd.). The chromatographic separation was gradiently performed at room temperature. The mobile phase A was formic acid 0.125 % prepared in milli-Q water:acetonitrile (62.5 : 37.5) and mobile phase B was formic acid 0.125 % prepared in acetonitrile. The mass spectrometer was equipped with an ESI source and operated in MRM positive mode.

Calibration curves were obtained by using (1/X2) least-squares linear regression analysis of the peak area ratio (analyte/internal standard) *versus* nominal concentrations of the calibration standards. Regressions and figures were generated by Analyst version 1.4.2 software (MDS Sciex).

The validation scheme involved the analysis of calibration curves and quality controls at different concentrations to determine linearity, within-run and between-run precision and accuracy, limit of quantification, dilution factor, selectivity, recovery and stability. The analytical phase was performed in compliance with Good Laboratory Practices (GLP).

2.7 Pharmacokinetic analysis

The mean, standard deviation, coefficient of variation and range for AUC_{last}, AUCinf, C_{max}, residual area, T_{max}, $T_{1/2}$ and K_{el} pharmacokinetic parameters were calculated from plasma concentrations of (–)-donepezil and (+)-donepezil using a non-compartmental method. Truncated AUCs at times greater than or equal to 24 h were obtained by the linear trapezoidal method.

Pharmacokinetic calculations were made using Bioequiv (release 3.50), a proprietary software developed and tested for bioequivalence studies at Anapharm, which performs non-compartmental analyses of pharmacokinetic parameters and statistical analyses (via SAS release 8.2, SAS Institute Inc., Cary, NC, USA), and also using WinNonLin (5.2.1, Pharsight, Mountain View, CA, USA) for truncated AUCs.

2.8 Statistical analysis

ANOVA was performed on ln-transformed AUC_{last}, AUCinf and C_{max} and untransformed K_{el} and $T_{1/2\ el}$ at an alpha level of 0.05. All ANOVAs were performed with the SAS (release 8.2 for Windows) General Linear Models Procedure and Bioequiv (proprietary software version 3.50). The model included sequence, sub-

Arzneimittelforschung 2010;60(3):116–123

ject within sequence, period and treatment as factors. The sequence effect was tested using subjects within sequence effect as the error term. The treatment and period effects were tested against the residual mean square error. All sums of squares (Types I, II, III and IV) were reported. Probability (p) values were derived from Type III sums of squares. Truncated AUCs were analyzed using WinNonLin (version 5.2.1, Pharsight) using a similar statistical procedure.

For all analyses, effects were considered statistically significant if the probability associated with 'F' was less than 0.05. Based on pairwise comparisons of the ln-transformed AUC_{last}, AUC_{inf} and C_{max} data, the ratios of the least-squares means, calculated according to the formula "e (X-Y) × 100", as well as the 90 % geometric CI for ln-transformed AUC_{last}, AUC_{inf}, C_{max} and truncated AUCs were determined.

3. Results

3.1 Drug analysis

Results obtained during the validation of the analytical method demonstrated its suitability for the determination of (+)-donepezil and (–)-donepezil in human sodium heparinized plasma and its application to clinical studies.

The lower limit of quantitation (LLOQ), i. e. the lowest standard level with a coefficient of variation less than 20 %, was set at 99.40 pg/mL with a coefficient of variation of 9.37 % and a percentage of nominal concentration of 86.70 % for (+)-donepezil and at 49.80 pg/mL with a coefficient of variation of 3.92 % and a percentage of nominal concentration of 99.57 % for (–)-donepezil.

Calibration curves were found to be consistently accurate and precise over the range of 99.40 to 19 880.00 pg/mL for (+)-donepezil and 49.80 to 9960.00 pg/mL for (–)-donepezil. The determination coefficients (r^2) were greater than or equal to 0.9960 and 9972 for (+)-donepezil and (–)-donepezil), respectively.

The between-run precision (expressed as coefficient of variation, CV) ranged between 2.58 and 4.08 % for (+)-donepezil and 2.90 and 3.99 % for (–)-donepezil. The between-run accuracy ranged between 98.62 and 100.70 % for (+)-donepezil and 92.47 and 94.36 % for (–)-donepezil.

The within-run precision (expressed as coefficient of variation, CV) ranged between 2.70 and 6.27 % for (+)-donepezil and 2.25 and 4.19 % for (–)-donepezil. The within-run accuracy ranged between 98.68 and 109.52 % for (+)-donepezil and 85.33 and 94.45 % for (–)-donepezil.

Analysis of drug concentrations was performed by Anapharm Europe (Barcelona, Spain), according to GLP standards. Method used for the analysis showed good precision, accuracy and sensitivity and was suitable for the determination of (+)-donepezil and (–)-donepezil in human plasma samples.

3.2 Subjects

Nineteen (9 females and 10 males) healthy volunteers were enrolled and randomized (mean age 35 ± 10 years [range 19–52 years]; their BMI was 23.1 ± 1.3 kg/m^2 [range 20.1–24.8]). As one subject withdrew, safety analysis included 19 volunteers and 18 were considered for statistical analysis (Fig. 1). Baseline subject characteristics are presented in Table 1.

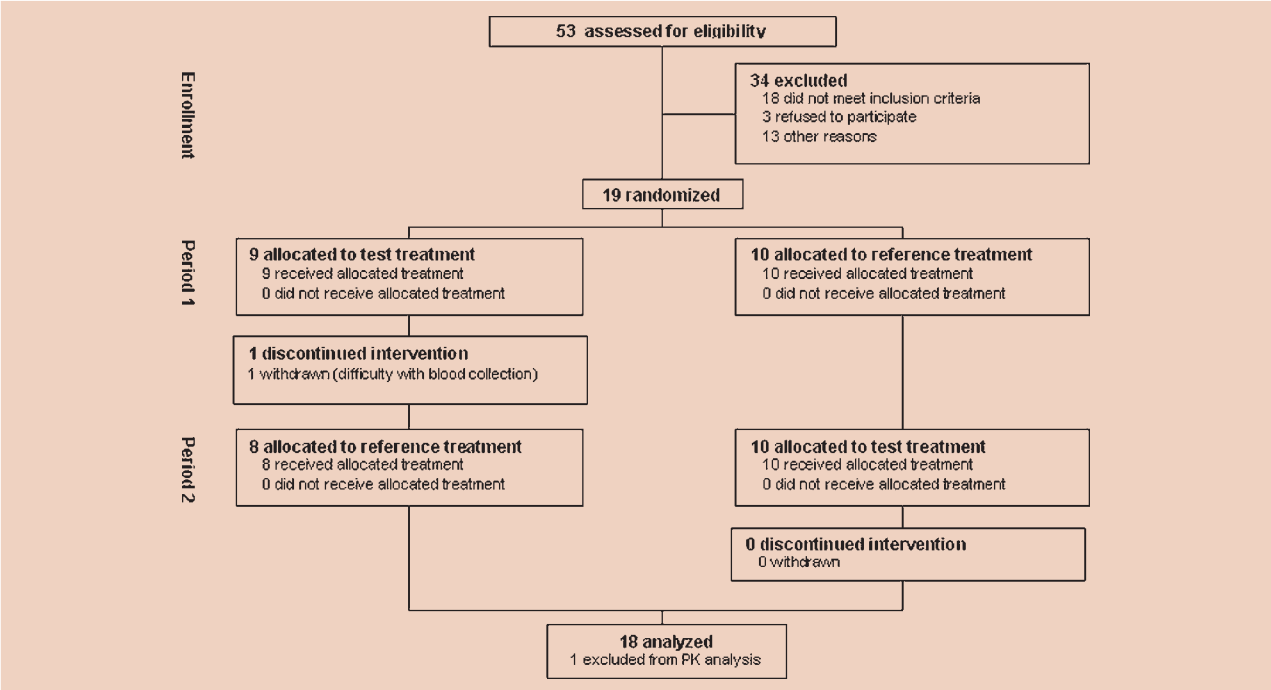

Fig. 1: Disposition of subjects.

Table 1: Baseline demographic characteristics of clinical trial subjects.

Category		Safety population
Age (years)	Mean ± SD Range Median	35 ± 10 19 – 52 36
Gender	Female Male	9 (47.4 %) 10 (52.6 %)
Race	Asian Black White Other	0 1 (5.3 %) 18 (94.7 %) 0
Height (cm)	Mean ± SD Range Median	168.3 ± 9.3 154.0 – 186.0 170.0
Weight (kg)	Mean ± SD Range Median	65.7 ± 8.9 50.2 – 82.0 67.5
BMI (kg/m^2)	Mean ± SD Range Median	23.1 ± 1.3 20.1 – 24.8 23.4

3.3 Pharmacokinetic and bioequivalence analysis

Mean plasma concentrations for (+)-donepezil and (–)-donepezil are presented in Fig. 2 and Fig. 3, respectively. Pharmacokinetic parameters were calculated for (+)-donepezil and (–)-donepezil are presented in Table 2.

For (+)-donepezil the mean ± SD of C_{max} for the test formulation was 19 480.32 ± 5818.59 pg/mL and for the reference formulation, 17 991.41 ± 4956.44 pg/mL. For (–)-donepezil the values were 8108.54 ± 2651.53 pg/mL and 7674.14 ± 2799.91 pg/mL, respectively. The mean values for $t_{1/2}$ for the test and reference formulation were 78.94 ± 12.32 h and 78.55 ± 12.57 h, respectively, for (+)-donepezil and 75.74 ± 12.08 h and 76.41 ± 15.51 h, respectively, for (–)-donepezil. The median (interquartile range) of T_{max} was 2.25 (1.75) h and 2.00 (0.88) h for the test and reference formulations for (+)-donepezil and 3.50 (1.38) h, and 2.50 (0.75) h for (–)-donepezil. The mean residual area was below 7 % for both (+)-donepe-

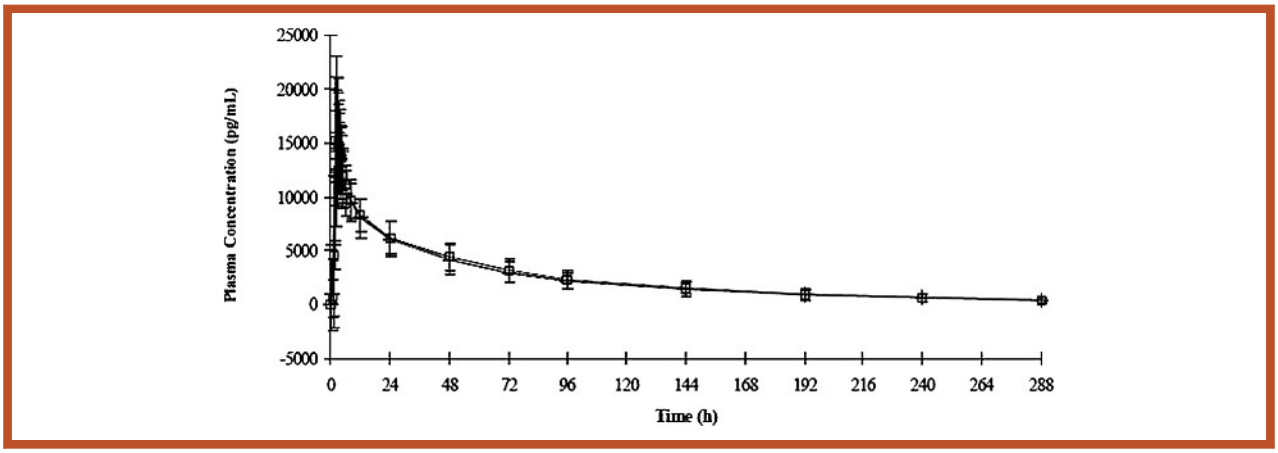

Fig. 2: Mean plasma (+)donepezil concentrations obtained for the test (⊟) and reference (+) formulation following a 10 mg dose (n = 18).

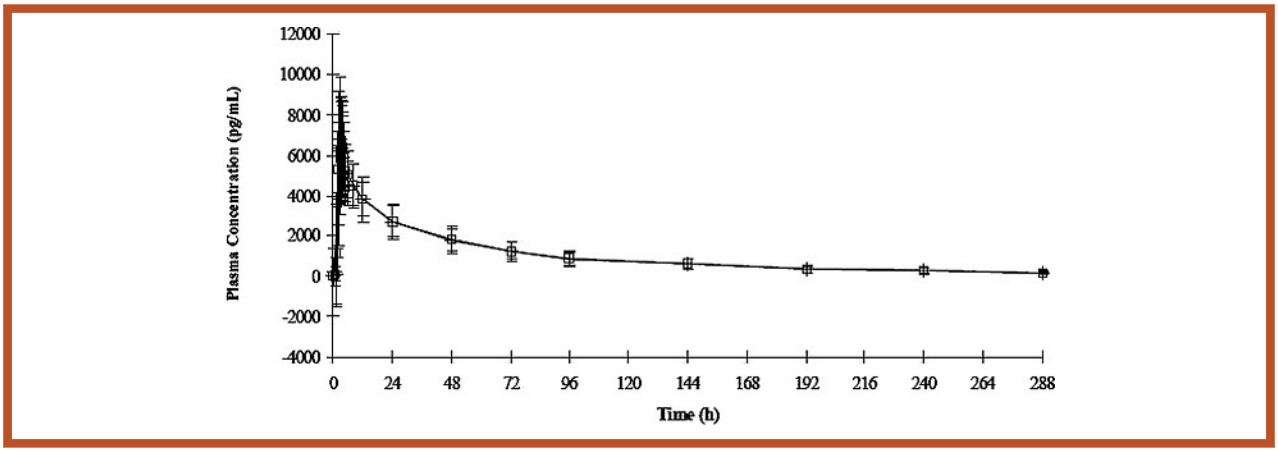

Fig. 3: Mean plasma (–)donepezil concentrations obtained for the test (⊟) and reference (+) formulation following a 10 mg dose (n = 18).

Arzneimittelforschung 2010;60(3):116–123

Table 2: Mean (SD) pharmacokinetic parameters determined after oral administration of donepezil to 18 healthy subjects under fasting conditions.

	Test (Treatment A)		Reference (Treatment B)	
	Arithmetic mean ± SD	CV (%)	Arithmetic mean ± SD	CV (%)
(+)-Donepezil				
AUC_{last} (pg · h/mL)	706 903.70 ± 221 430.42	31.32	674 262.57 ± 205 442.17	30.47
AUC_{inf} (pg · h/mL)	760 442.12 ± 56 299.91	33.70	723 700.96 ± 234 873.04	32.45
C_{max} (pg/mL)	19 480.32 ± 5818.59	29.87	17 991.41 ± 4956.44	27.55
Residual area (%)	6.38 ± 2.51	39.32	6.26 ± 2.52	40.35
T_{max} (h)	2.58 ± 1.13	43.66	2.29 ± 0.92	40.34
T_{max} [1)] (h)	2.25 (1.75)	–	2.00 (0.88)	–
K_{el} (h^{-1})	0.0090 ± 0.0015	16.74	0.0091 ± 0.0016	17.97
$t_{1/2}$ (h)	78.94 ± 12.32	15.61	78.55 ± 12.57	16.00
(–)-Donepezil				
AUC_{last} (pg · h/mL)	293 183.89 ± 91 650.76	31.26	285 791.52 ± 929 53.22	32.52
AUC_{inf} (pg · h/mL)	311 827.15 ± 100 843.70	32.34	304 838.08 ± 103 548.26	33.97
C_{max} (pg/mL)	8108.54 ± 2651.53	32.70	7674.14 ± 2799.91	36.48
Residual area (%)	5.67 ± 1.87	32.89	5.84 ± 2.61	44.65
T_{max} (h)	3.29 ± 1.04	31.51	3.00 ± 1.46	48.76
T_{max} [1)] (h)	3.50 ± 1.38	–	2.50 ± 0.75	–
K_{el} (h^{-1})	0.0094 ± 0.0015	15.98	0.0095 ± 0.0020	21.59
$t_{1/2}$ (h)	75.74 ± 12.08	15.95	76.41 ± 15.51	20.30

[1)] Median (interquartile range).

zil and (–)-donepezil for both formulations and analytes. The least-squares means ratios and the 90 % CIs are presented in Table 3. For both analytes, AUC_{last}, AUC_{inf} and C_{max} 90 % CIs were within 80.00 % and 125.00 %.

For truncated AUCs (Table 3), all of the 90 % CIs were also within the acceptance range of 80.00 – 125.00 % irrespective of the period of time.

Table 3: 90 % CIs for relevant pharmacokinetic parameters (bioequivalence acceptance range 80.00 – 125.00 %).

	Ratio (%)	Lower limit 90 % CI (%)	Upper limit 90 % CI (%)
(–)-Donepezil			
Ln C_{max}	105.18	95.81	115.47
Ln AUC_{inf}	104.36	100.39	108.49
Ln AUC_{last}	104.69	100.79	108.74
Ln AUC_{24}	102.95	99.91	106.09
Ln AUC_{48}	103.67	100.73	106.69
Ln AUC_{72}	104.21	101.14	107.37
Ln AUC_{96}	104.44	101.19	107.79
Ln AUC_{144}	104.50	100.94	108.19
Ln AUC_{192}	104.57	100.84	108.43
Ln AUC_{240}	104.66	100.84	108.63
Ln AUC_{288}	104.70	100.80	108.76
(+)-Donepezil			
Ln C_{max}	105.58	93.81	118.83
Ln AUC_{inf}	102.86	98.67	107.23
Ln AUC_{last}	103.38	99.69	107.20
Ln AUC_{24}	102.20	98.66	105.88
Ln AUC_{48}	102.86	99.63	106.19
Ln AUC_{72}	102.99	99.58	106.53
Ln AUC_{96}	103.12	99.58	106.78
Ln AUC_{144}	103.45	99.75	107.28
Ln AUC_{192}	103.53	99.84	107.36
Ln AUC_{240}	103.42	99.77	107.19
Ln AUC_{288}	103.40	99.72	107.21

3.4 Tolerability analysis

Twenty-seven adverse events (AEs) were reported by 9 of the 19 subjects who received at least one dose of the study medication (safety population). The AEs distribution by treatment group was as follows: 12 AEs reported by 31.6 % (n = 6) of the 19 subjects who received treatment A, 15 AEs reported by 38.9 % (n = 7) of the 18 subjects who received treatment B. The most commonly reported AEs were "Post-procedural swelling" reported by 21.1 % (n = 4) of subjects who constituted the safety population and "Procedural pain" and "Headache" reported each by 10.5 % (n = 2) of subjects. "Post procedural swelling" and "Procedural pain" were related to the study procedures and therefore not expected to be associated with the study medication. "Headache" was expected following the administration of the medication and both AEs were judged to be possibly and unlikely related to the medication. The remaining AEs reported during the study were reported by a single subject for each AE. All AEs reported were graded as mild. Of the 27 AEs reported, 15 were judged as "possible" and 12 as "unlikely"; the expectedness of 14 AEs was judged as "expected" and 13 as "not expected". No serious or severe adverse events were reported during this study. Upon conclusion of the clinical portion of the study, the results from the subjects who completed post study procedures, including laboratory tests and vital signs measurements, confirmed the absence of significant changes in the subjects' state of health. MedDRA classification of AEs and incidence are presented in Table 4.

Table 4: MedDRA, version 11.1, characterization of the post-dose adverse events experienced by subjects.

System Organ Class Preferred Term	Treatment group	
	Test	Reference
Number of subjects dosed	66	70
Ear and labyrinth disorders	1 (1.5 %)	
Ear discomfort	1 (1.5 %)	
Eye disorders	1 (1.5 %)	
Conjunctivitis	1 (1.5 %)	
Gastrointestinal disorders	3 (4.5 %)	
Abdominal pain	1 (1.5 %)	
Diarrhoea	1 (1.5 %)	
Nausea	1 (1.5 %)	
Toothache	1 (1.5 %)	
General disorders and administration site conditions	2 (3.0 %)	
Asthenia	2 (3.0 %)	
Infections and infestations	1 (1.5 %)	1 (1.4 %)
Oral herpes		1 (1.4 %)
Rhinitis	1 (1.5 %)	
Injury, poisoning and procedural complications	3 (4.5 %)	2 (2.9 %)
Post procedural complication	1 (1.5 %)	
Procedural dizziness	1 (1.5 %)	2 (2.9 %)
Procedural pain	1 (1.5 %)	1 (1.4 %)
Investigations	9 (13.6 %)	9 (12.9 %)
Blood pressure decreased	6 (9.1 %)	5 (7.1 %)
Blood pressure increased	1 (1.5 %)	
Haematocrit decreased	1 (1.5 %)	1 (1.4 %)
Heart rate decreased	1 (1.5 %)	1 (1.4 %)
Heart rate increased		1 (1.4 %)
Protein urine present		1 (1.4 %)
White blood cell count increased		1 (1.4 %)
Musculoskeletal and connective tissue disorders	3 (4.5 %)	3 (4.3 %)
Back pain	1 (1.5 %)	1 (1.4 %)
Musculoskeletal discomfort		1 (1.4 %)
Musculoskeletal stiffness	1 (1.5 %)	
Myalgia		1 (1.4 %)
Pain in jaw	1 (1.5 %)	
Nervous system disorders	6 (9.1 %)	5 (7.1 %)
Dizziness	1 (1.5 %)	
Dizziness postural	3 (4.5 %)	1 (1.4 %)
Headache	4 (6.1 %)	3 (4.3 %)
Poor quality sleep	1 (1.5 %)	
Somnolence	1 (1.5 %)	1 (1.4 %)
Tremor		1 (1.4 %)
Psychiatric disorders	1 (1.5 %)	
Depressed mood	1 (1.5 %)	
Respiratory, thoracic and mediastinal disorders		3 (4.3 %)
Cough		1 (1.4 %)
Oropharyngeal pain		1 (1.4 %)
Voice alteration		1 (1.4 %)
Skin and subcutaneous tissue disorders	1 (1.5 %)	
Hyperhydrosis	1 (1.5 %)	
Vascular disorders		1 (1.4 %)
Hot flush		1 (1.4 %)
Total	19 (28.8 %)	19 (27.1 %)

4. Discussion

Bioequivalence studies are general requirements for the registration of generic products. A randomized cross-over design is typically employed in bioequivalence studies, and was considered to be the most appropriate for this study. The study was open-label in nature, because blood concentration levels can not be influenced by knowledge of the identity of the treatment. The sampling schedule was adequate in the characterization of both enantiomers' absorption phase, determination of AUC and C_{max} parameters, and sufficient in the assessment of the enantiomers' elimination phase, as the 288 h sampling period covered a sufficient period of time to allow the characterization of the elimination phase as can be seen by the low residual area.

Even though plasma concentrations of donepezil after oral dosing are dose-proportional between 1 and 10 mg, there is no evidence of dose linearity for each enantiomers taken separately. Therefore, a chiral assay was performed to analyze (+)-donepezil and (–)-donepezil in plasma samples.

The mean residual area was less than 20 % for all treatments indicating that a sampling over a period of 288 h was sufficient for both (+)-donepezil and (–)-donepezil. A washout period of at least 35 days that represents more than 10 times the half-life of donepezil was chosen to allow the complete elimination of the drug between the two periods and to avoid carry-over effects.

The results obtained using the truncated AUC approach were in full agreement with those obtained using the classical approach without truncation. The new guideline [11] suggests that a sampling period longer than 72 h is not considered necessary for any immediate release formulation. Hence, for drugs with a long half-life, comparison of extent of exposure using truncated AUCs at 72 h is acceptable. Given the physiological characteristics of the absorption and the results obtained in this study, truncation at 72 h seems to be adequate for the determination of bioequivalence for donepezil.

Bioequivalence of the formulations was shown as the 90 % CIs of the ratio of least-squares means of the test to reference product of ln-transformed AUC_{last} and C_{max} were within the acceptance range of 80.00 % to 125.00 % for (+)-donepezil and (–)-donepezil, following administration of a 10 mg film-coated tablet under fasting conditions.

5. Conclusion

Overall, the study design was adequate to detect differences between the test and reference formulations of donepezil hydrochloride under fasting conditions. Bioequivalence between formulations, in terms of rate and extent of absorption, was therefore demonstrated.

Both formulations were well tolerated, with no relevant differences in safety profiles, particularly with respect to the number and characteristics of adverse events.

Truncation of AUC at 72 h was shown to lead to the same conclusions regarding bioequivalence of donepezil under these test conditions.

Conflict of Interest

This research was sponsored by Tecnimede, Sociedade Técnico-Medicinal SA and authors are either employees of the sponsor (Tecnimede) or of Contract Research Organizations (Anapharm and Anapharm Europe).

Arzneimittelforschung 2010;60(3):116–123

References

[1] Electronic Medicines Compendium (eMC). Datapharm Communications Ltd. [online]. [cited 2008/07/09]; available from: *www.medicines.org.uk*

[2] Canadian Pharmacists Association, 2008. Compendium of Pharmaceuticals and Specialties, online version (e-CPS). [online]. 2008 [cited 2008/07/09]; available from: *https://www.e-therapeutics.ca/*

[3] Rogers SL, Friedhoff LT. Pharmacokinetic and pharmacodynamic profile of donepezil HCl following oral doses. *Br J Pharmacol* 1998;46(Suppl 1):1–6.

[4] Tiseo PJ, Perdomo CA, Friedhoff LT. Metabolism and elimination of 14C-donepezil in healthy volunteers: A single-dose study. *Br J Pharmacol* 1998;46(Suppl 1):19–24.

[5] Xie Z, Liao Q, Xu X, Yao M, Wan J, Liu D. Rapid and sensitive determination of donepezil in human plasma by liquid chromatography/tandem mass spectrometry: application to a pharmacokinetic study. *Rapid Commun Mass Spectrometry* 2006;20:3193–8.

[6] Park EJ, Lee HW, Ji HY, Kim HY, Lee MH, Park ES, *et al.* Hydrophilic interaction chromatography-tandem mass spectrometry of donepezil in human plasma: application to a pharmacokinetic study of donepezil in volunteers. *Arch Pharm Res* 2008;31(9):1205–11.

[7] Seltzer B. Donepezil: an update. *Expert Opin Pharmacother* 2007;8(7):1011–23.

[8] Jann MW, Shirley KL, Small GW. Clinical pharmacokinetics and pharmacodynamics of cholinesterase inhibitors. *Clin Pharmacokinet* 2002;41(10):719–39.

[9] Note For Guidance on the Investigation of Bioavailability and Bioequivalence. CPMP (Committee for Proprietary Medicinal Products), 2001.

[10] Good Clinical Practice Consolidated Guideline. International Conference on Harmonisation, E6. 1996.

[11] Guideline on the investigation of bioequivalence. Committee for Medicinal Products for Human Use (CHMP), Doc. Ref. CPMP/EWP/QWP/1401/98 Rev. 1, 2010.