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Classification of torasemide based on the Biopharmaceutics Classification System and evaluation of the FDA biowaiver provision for generic products of Class I drugs

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Abstract

The biopharmaceutical properties of an in-house developed new crystal modification of torasemide (Torasemide N) were investigated in comparison with the most well known crystal modification form of torasemide (Torasemide I) in order to classify the drug according to the Biopharmaceutics Classification System (BCS), and to evaluate the data in line with current US Food and Drug Administration (FDA) guidance (with biowaiver provision for Class I drugs) to determine if the biowaiver provision could be improved. The solubility profiles of Torasemide I and Torasemide N were determined, and tablets prepared from both forms of the drug were studied for in-vitro release characteristics in media recommended by the current FDA guidance for biowaiver of generic products, and in other media considered more appropriate for the purpose than the ones recommended by the FDA. Two separate bioequivalence studies in healthy humans (following oral administration) were performed with two test products (both prepared from Torasemide I) against a single reference product (prepared from Torasemide N). The absorption profiles of the drug from the tablets were determined by deconvolution for comparison with the in-vitro release profiles to determine the appropriateness of some dissolution media for predicting in-vivo performance and to determine the comparative rate and extent of absorption. The drug was absorbed from the tested products quickly and almost completely (about 95% within 3.5 h of administration). However, one test product failed to meet the bioequivalence criteria and had a significant initial lower absorption rate profile compared with the reference product ($P \leq 0.05$), whereas the other product was bioequivalent and had a similar absorption profile to the reference product. A dissolution medium at pH 5.0, in which torasemide has minimum solubility, was found to be more discriminatory than the media recommended by the FDA. Torasemide has been classified as a Class I drug according to the BCS up to a maximum dose of 40 mg and the data suggest that the current FDA guidance could be improved by giving more emphasis to selection of appropriate dissolution media than is given in its current form for approving biowaiver to generic products of Class I drugs.

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Introduction

The Biopharmaceutics Classification System (BCS) classifies drugs into four categories on the basis of their solubility and permeability characteristics. The drugs with high solubility and permeability are grouped as Class I drugs, for which the US regulatory authority, the Food and Drug Administration (FDA), recently waived its requirements for bioequivalence testing of generic products against a reference product already available on the market. According to the current FDA guidance, generic products of Class I drugs can get marketing authorization without bioavailability and bioequivalence studies if they meet certain criteria of solubility and permeability testing (CDER 2000). A few studies have suggested that selection of an appropriate dissolution medium is not so crucial for in-vitro testing of Class I drugs for predicting their bioavailability and bioequivalence since dissolution is not considered to be the rate limiting factor for absorption of the drug (CDER 1995; Galia et al 1998).

Torasemide, a well known loop diuretic, belongs to the 3-pyridine sulfonylurea group. The chemical structure of torasemide is shown in Figure 1. Different crystal modifications

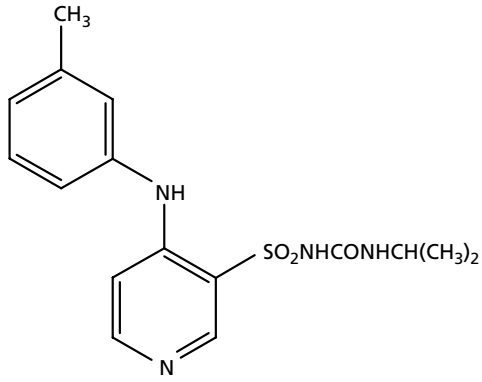

Figure1 Chemical structure of torasemide.

(polymorphs) of torasemide have been reported in the literature (Dupont et al 1978a, b), and these crystal modifications are known to differ significantly in their physical characteristics, including solubility. We have developed a new crystal modification of torasemide, Torasemide N (Danilovski et al 2001; Filić et al 2002). According to the current FDA draft guidance on pharmaceutical solid polymorphism (CDER 2004), it is recommended that, for each drug substance, the existence of different polymorphic forms should be investigated. The in-house developed Torasemide N has been fully characterized according to this FDA draft guidance, including determination of its crystal and molecular structure by single crystal X-ray diffraction. Although different methods can be used to characterize different polymorphic forms (Brittain 1999), only the demonstration of non-equivalent crystal structures by single crystal X-ray diffraction is currently regarded as definitive evidence of polymorphism (CDER 2004). We also found that the intrinsic dissolution rates of Torasemide I and Torasemide N differ.

The objectives of the present study were: (i) to investigate the physicochemical and biopharmaceutical properties of Torasemide N in comparison with Torasemide I; (ii) to classify torasemide according to the BCS; and (iii) to investigate if the FDA guidance for a biowaiver (CDER 2000) for generic products of Class I drugs is appropriate or if it could be improved. Both Torasemide I and Torasemide N were formulated as tablets and tested for bioavailability. In addition, in-vivo absorption profiles of the drug were determined from the bioavailability data obtained in humans for selecting an appropriate in-vitro release testing method for the tablets.

Materials and Methods

Materials

Torasemide I, prepared according to the in-house developed method (Filić et al 2004), was identical to an authentic sample as reported in the literature (Dupont et al 1978a). Torasemide N, was prepared in-house as previously reported (Danilovski et al 2001; Filić et al 2002). Tablet excipients used were of standard pharmacopoeial quality and all the chemical reagents used were of analytical grade.

Determination of solubility

The solubility profile was tested in dilute HCl solutions (pH 1.2 and 2.0), USP acetate buffer (pH 4.5), phosphate buffers (pH 5.0, 5.5 and 7.0), and in USP borate buffer (pH 8.5), to cover the entire physiological pH range of the gastrointestinal tract. Excess amounts of the drug were added in the media to obtain saturated solutions. For each medium, solutions were prepared in triplicate and shaken in a water bath (Köttermann, Uetze-Hänigsen, Germany) at 37°C for 24 h to reach equilibrium saturation. Samples were then centrifuged and the supernatant diluted (when necessary) and analysed using validated spectrophotometric methods.

Characterization of Torasemide N

The crystal and molecular structure of Torasemide N was solved and refined according to X-ray diffraction data collected on a Philips PW1100 automatic four-circle diffractometer (Stoe/Cie upgrade) using graphite mono-chromatized MoKα radiation ($\lambda = 0.71069$ Å) at room temperature, as previously reported (Danilovski et al 2001).

The intrinsic dissolution rates for both forms of torasemide were studied in the medium (pH 5.0) considered to be the most discriminatory, that is the medium in which the solubility of the drug was the lowest. Discs (surface area 0.57 cm^2) were prepared by compressing about 100 mg of the drug on a hydraulic press (Carver, Inc., Wabash, IN, USA) under a pressure of 1.5 ton. Experiments were performed in 900 mL of medium, pre-warmed to 37°C and stirred at 100 rev min^{-1}. Aliquots (10 mL) were withdrawn (with replacement) every 30 min for up to 6 h, filtered (35-μm filters; VanKel, NC, USA) and analysed by a validated spectrophotometric method ($\lambda = 288$ nm) for torasemide content.

Preparation of tablets

Tablets containing 10 mg of torasemide were prepared by direct compression using appropriately selected pharmacopeial excipients suitable for the method of manufacture, which are not known to have any influence on the absorption process of the drug. The test products (Test 1 and Test 2) were prepared from Torasemide I using the same formulation, but the active substance (Torasemide I) used in the Test 2 product was further processed to reduce the particle size (without inducing any polymorphic change) to be able to manipulate the drug release using a method discriminatory enough to predict the in-vivo release profiles. The reference product was prepared using exactly the same formulation as the Test 1 and Test 2 products but with the active substance being Torasemide N. The tablets were compressed on a rotary press. X-ray powder diffraction analysis revealed no change in polymorphic form of the drug during compression.

Dissolution testing

The dissolution tests were performed using an automated dissolution apparatus (VanKel 7010; VanKel) attached to a Varian Cary 100 UV-vis spectrophotometer and calibrated

according to the USP 24 method. Experiments were performed using the USP Apparatus 2 with the paddle rotating at 50 rev min^{-1} in 900 mL of the following media: pH 1.2 (diluted HCl); pH 2.0 (diluted HCl); pH 4.5 (USP acetate buffer); pH 5.0 (phosphate buffer); and pH 6.8 (USP phosphate buffer).

The pH 1.2, 4.5 and 6.8 media were selected as per the FDA guidance for biowaiver (CDER 2000). The other two media were selected based on the solubility data (pH 5.0) and the physiological stomach conditions (pH 2.0) under which standard bioequivalence studies are performed in the fasting state. The tablets were put in dissolution vessels filled with the media at 37°C and samples were automatically collected at pre-determined time points (every 2 min up to 10 min and every 10 min from 20 to 60 min). The pooled samples were filtered through VanKel's full flow 35-μm filters (previously checked for absorption). The spectrophotometer was set to measure the absorbance at $\lambda = 284$–288 nm for different media (maximum absorption determined previously).

Bioavailability studies

Two separate studies (Study 1: Test 1 and reference product; Study 2: Test 2 and reference product) were performed under fasting conditions in a contract research organization based in Ahmedabad (India) with prior approval from the local Ethics Committee. The reference product used in both studies had Torasemide N (but tablets from different batches), while both the test products had Torasemide I. Both studies included a third product sourced from certain markets at the time and hence they were designed as 3-way crossover studies. The data obtained from the third product are not given here since they were included only for comparison. The test and reference products were characterized by in-vitro testing before the biostudies.

Both studies had identical protocols and were performed according to ICH guidelines on 18 healthy volunteers. The number of the subjects was calculated on the basis of the coefficient of variation (CV) reported in the literature for the drug to give adequate power of the study. Two tablets (2×10 mg) were administered with 240 mL of water to each volunteer after overnight (at least 10 h) fasting, and blood samples were collected before dosing and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12 and 18 h following administration. For Study 1, one sample was taken after 24 h.

Plasma was separated by centrifugation at 5°C, transferred to clean pre-labelled tubes and frozen at −20°C until analysis by a high-performance liquid chromatography method developed by the contract research organization.

No drink was allowed for 2 h before dosing and 2 h after dosing, but 240 mL of standard drinking water was given to each volunteer at 2 and 4 h after dosing. Two standardized meals were served at 4 h (lunch) and 11 h (dinner) after dosing, and a third meal after about 23–24 h (breakfast). Dietary electrolytes consisting of oral re-hydration supplements were given to all volunteers after the 24-h sampling period. In addition, each volunteer received oral electrolyte supplements enough for the first 2–3 days of the wash-out period between phases of the study.

Pharmacokinetic calculations and statistical analyses

Peak plasma concentration (C_{max}), area under the plasma concentration–time curve (AUC_t), area under the plasma concentration–time curve from zero to infinity (AUC_{inf}), time to reach C_{max} (t_{max}), terminal phase half-life ($t_{\frac{1}{2}}$) and elimination rate constant (k_{el}), were calculated from the plasma concentration values of the drug, as appropriate for testing bioequivalence between test and reference products (according to ICH guidelines).

Statistical analyses were done using SAS software (SAS System for Windows, version 8.2; SAS Institute Inc., Cary, NC, USA). LSMEANS option of the GLM procedure was used for the calculation of least-squares means (LSM), the difference between products LSM, and the standard error associated with this difference.

Two one-sided hypotheses ($P = 0.05$) were tested for AUC_t, AUC_{inf} and C_{max} by expressing a 90% confidence interval (CI) for the ratio of the test to reference geometric means. The 90% CI were derived by exponentiation of the CIs for the difference between the products LSM, obtained from the analysis of variance on the ln-transformed data.

The CI values were expressed as a percentage relative to the reference product, and the bioequivalence criterion was met if the 90% CI for the ratio between the test and reference products fell within the range of 80 to 125%. The values of t_{max} for treatment groups were summarized by descriptive statistics and compared by the non-parametric Wilcoxon's signed rank test, at the significance level of $\alpha = 0.05$.

In-vivo absorption

The plasma levels obtained for Test 1, Test 2 and the reference products were used as input functions to obtain the in-vivo absorption profiles of the drug by deconvolution analysis using the QWERT software program (Version 1.1; SI Computing, Uppsala, Sweden). The bioavailability data reported in the literature following single intravenous administration of torasemide were used as the weighting function (Barr et al 1990a). The absorption profiles obtained by deconvolution for the test and reference products were compared for similarity using analysis of variance (by the SAS GLM procedure). The amounts absorbed at all the sampling points up to 3.5 h were compared and normality of the data was tested with the Kolmogorov test; the assumption of equal group variances was tested with Levene's test. Square root, $\log_{10}$ and inverse transformations of the data were found as optimal with the Box-Cox power transformation family. Outliers were examined by comparing the studentized residuals to a critical value from the *t*-distribution, chosen using a Bonferroni-type adjustment (at $\alpha = 0.05$).

Results

Solubility profile of torasemide

Torasemide I and Torasemide N have very similar solubility profiles in the media studied (Figure 2). The solubility of torasemide is pH-dependent, being greatest at pH 1.2 (within the tested range). As the pH was increased from 1.2 to 2.0, the solubility decreased sharply (from 4.01/5.13 to 0.70/0.85 mg mL^{-1}), to a minimal solubility of 0.16/0.19 mg mL^{-1} at pH 5.0. Thereafter, the solubility increased again to 0.31/0.45 mg mL^{-1} at pH 7.0, with a further increase to 2.30/2.42 mg mL^{-1} at pH 8.5. Considering that the highest dose strength of torasemide (tablets) is 20 mg, the dose/solubility ratios (in pH 5.0 medium) are 62.5 mL and 52.6 mL for Torasemide I and Torasemide N, respectively. Therefore, both these forms of torasemide can be classified as highly soluble according to the BCS.

Characteristics of Torasemide N

Single crystal X-ray diffraction data clearly demonstrate that Torasemide N and Torasemide I are distinct crystallographic forms (Table 1). At pH 5.0, Torasemide N has a higher intrinsic dissolution rate (0.0414 mg min^{-1} cm^{-2}) than Torasemide I (0.02298 mg min^{-1} cm^{-2}). Linear regression analyses of the data (profiles not shown) gave correlation coefficient values of 0.998 and 0.990 for Torasemide I and Torasemide N, the slopes of the regression lines being 0.0131 and 0.0236 mg min^{-1}, respectively.

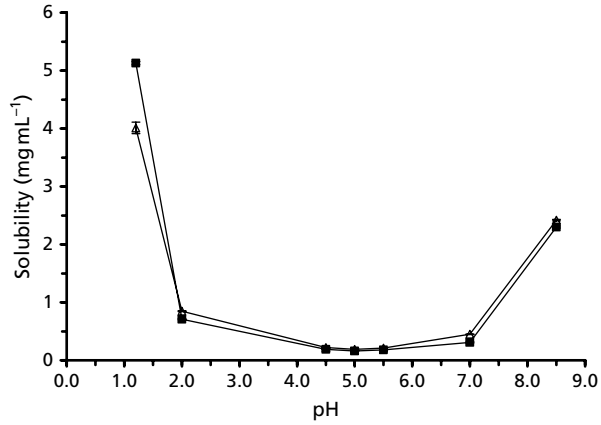

Figure 2 Solubility profiles of Torasemide I (■) and Torasemide N (△) tested in physiologically relevant pH media. Vertical bars indicating s.e.m. (n = 3) are within the points if not visible.

Table 1 Basic crystallographic data obtained from single crystal X-ray diffraction of Torasemide N in comparison with Torasemide I (Dupont et al 1978a, b)

Parameter	Crystal modifications	
	Torasemide I	Torasemide N
Crystal composition	Monoclinic	Monoclinic
Space group	P 2_1/c	P 2_1/c
a (Å)	13.308	11.430(3)
b (Å)	8.223	19.090(6)
c (Å)	31.970	16.695(6)
β (°)	107.01	93.90(2)
V (Å)^3	3345.5	3634.7(2)
Z	4×2	4×2
D_x/gcm^{-3}	1.33	1.274

Dissolution of torasemide from tablets

The comparative dissolution profiles obtained in the FDA recommended media (pH 1.2, 4.5 and 6.8) and in the other two media tested (pH 2.0 and 5.0) for Test 1 and the reference products are presented in Figure 3A and B, respectively. The corresponding dissolution profiles for Test 2 and the reference products are presented in Figure 4A and B, respectively. Both

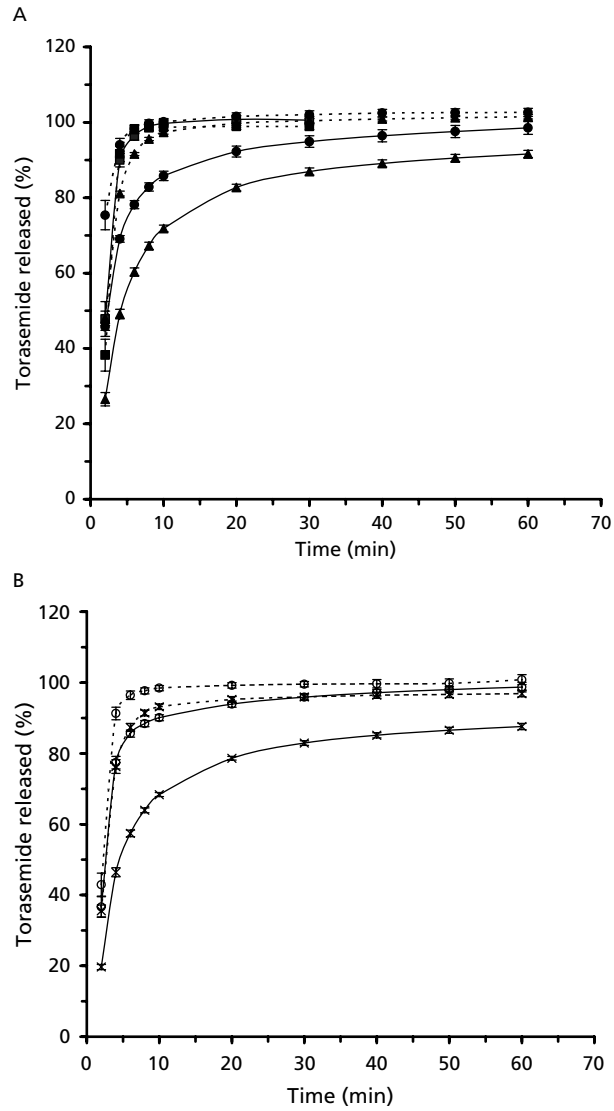

Figure 3 A. Comparative dissolution profiles of the Test 1 (solid line) and the reference (dotted line) products in pH 1.2 (■), pH 4.5 (▲) and pH 6.8 (●) media. The vertical bars indicate s.e.m. (n=6 for pH 1.2 and 6.8; n=12 for pH 4.5). B. Comparative dissolution profiles of the Test 1 (solid line) and the Reference (dotted line) products in pH 2.0 (○) and pH 5.0 (×). The vertical bars indicating s.e.m. (n=6 for pH 2.0 and n=12 for pH 5.0) are within the points if not visible.

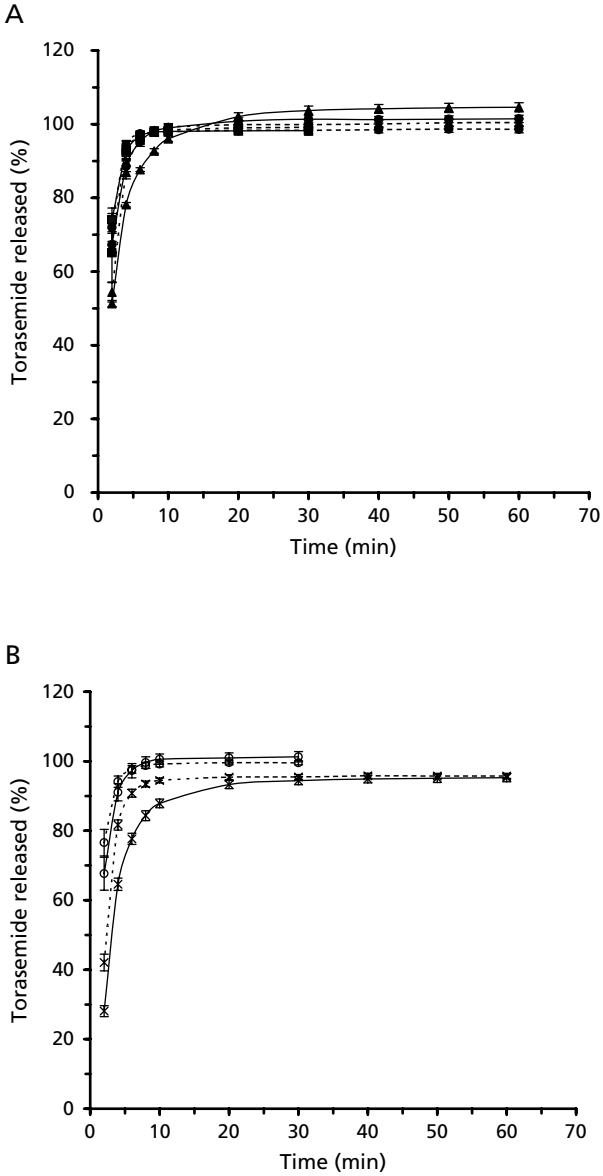

Figure 4 A. Comparative dissolution profiles of the Test 2 (solid line) and the reference (dotted line) products in pH 1.2 (■), pH 4.5 (▲) and pH 6.8 (●). The vertical bars indicate s.e.m. (n = 6). B. Comparative dissolution profiles of the Test 2 (solid line) and the reference (dotted line) products in pH 2.0 (○) and pH 5.0 (×). The vertical bars indicate standard errors of the mean (n = 6).

the Test 1 and the reference products released more than 85% of the drug in 15 min in all the dissolution media, except pH 4.5 and pH 5.0, demonstrating similarity of the dissolution profiles in these media (pH 1.2, 2.0 and 6.8) according to the FDA guidance for biowaiver (CDER 2000) without any statistical evaluation. However, the similarity test performed on the dissolution profiles obtained in pH 4.5 and 5.0 media from Test 1 and the reference products according to the FDA guidance showed non-similarity of these products ($f_2 < 50$).

The Test 1 product released 83% of the drug in 20 min in pH 4.5 medium, whereas the reference product released the same amount of drug in less than 6 min (Figure 3A). The difference between the two products was even greater in pH 5.0 medium, in which the Test 1 product took 40 min to release 85% of the drug, whereas the reference product released the same amount of drug in about 5 min (Figure 3B). In fact, the Test 1 product released only up to 88% of the drug at 60 min (in pH 5.0 medium) when the test was terminated.

The Test 2 product can be considered similar to the reference product without any statistical evaluations as per the FDA guidance for biowaiver (CDER 2000) because both these products released more than 85% of the drug in less than 15 min in all the media tested (Figure 4A and B).

Bioavailability and bioequivalence

Torasemide was quickly absorbed from all the products, with t_{max} values of 54 min for the Test 1 product, and 40–45 min for the Test 2 and reference products. Pharmacokinetic results obtained from Study 1 and Study 2 are presented in Tables 2 and 3, respectively. The Test 1 product gave the lowest C_{max} value and the reference product gave the highest value in Study 1 (Table 2). However, all three products had comparable AUC_t and AUC_{inf} values (Tables 2 and 3).

Geometric means of the main pharmacokinetic parameters, with 90% CIs relevant for the evaluation of bioequivalence of the Test 1 product with the reference product, are shown in Table 2. Although the Test 1 product meets the AUC criterion for bioequivalence, it does not meet the C_{max} criterion for demonstrating bioequivalence to the reference product as the lower limit of the 90% CI obtained for the C_{max} was 78%, which falls outside the set acceptance range of 80–125%.

Geometric means of the main pharmacokinetic parameters, with 90% CIs relevant for the evaluation of bioequivalence of the Test 2 product with the reference product, are shown in Table 3. All pharmacokinetic parameters obtained

Table 2 Comparative bioavailability data (main pharmacokinetic parameters) obtained from Study 1 following oral administration of Test 1 (T1) and the reference (R) products, and summary statistics for determination of bioequivalence

Parameter	Geometric means[a]		Analysis of variance		T1/R ratio (%)	90% Confidence limits	
	T1	R	Intra-subject CV (%)	Power (%)		Lower (%)	Upper (%)
C_{max} (ng mL^{-1})	3455	3947	18.8	93	87.52	78.01	98.19
AUC_t (ng h mL^{-1})	7952	8453	13.7	99	94.07	86.47	102.34
AUC_{inf} (ng h mL^{-1})	8384	8958	13.3	99	93.59	86.26	101.54

[a]Values are back-transformed from the logarithmic scale.

Table 3 Comparative bioavailability data (main pharmacokinetic parameters) obtained from Study 2 following oral administration of Test 2 (T2) and the reference (R) products, and summary statistics for determination of bioequivalence

Parameter	Geometric means[a]		Analysis of variance		T2/R ratio (%)	90% Confidence limits	
	T2	R	Intra-subject CV (%)	Power (%)		Lower (%)	Upper (%)
C_{max} (ng mL^{-1})	3512	3522	23.0	78	99.70	86.65	114.71
AUC_t (ng h mL^{-1})	8398	7835	14.4	99	107.18	98.14	117.06
AUC_{inf} (ng h mL^{-1})	9214	8226	16.4	98	112.01	101.30	123.85

[a]Values are back-transformed from the logarithmic scale.

for the Test 2 product (Table 3) comply with the set criteria for bioequivalence with the reference product.

Absorption in-vivo

The absorption profiles demonstrate that the Test 1 product had a significantly lower rate of absorption compared with the reference product (Figure 5), whereas the Test 2 and reference products have similar absorption profiles (Figure 6). The analysis performed on the absorbed amounts demonstrated significant differences ($P \leq 0.05$) between the two products (Test 1 and reference product) at all the sampling points up to 1.25 h, that is up to and above the t_{max} point (data not shown). At 15 min after administration of the tablets only 14–15% of the drug was absorbed from the Test 1 product, whereas 26–27% was absorbed from the reference product. At about the t_{max} point, only 68% of torasemide was absorbed from the Test 1 product, whereas about 89% of the drug was absorbed from the reference product (Figure 5). For the Test 2 and reference products, differences were not significant at $P \leq 0.05$ (analysis of variance; data not shown).

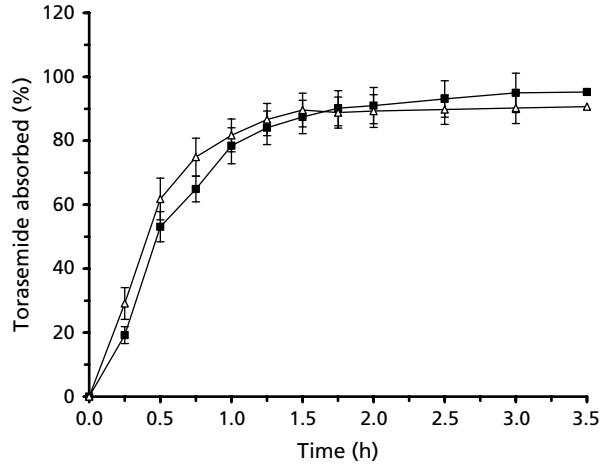

Figure 6 Comparison absorption profiles of torasemide from the Test 2 (■) and the reference (△) products in healthy volunteers (n = 18) following single oral administration of two tablets (each containing 10 mg of torasemide) under fasting conditions. The vertical bars indicate s.e.m. The profiles were obtained by deconvolution of the plasma concentration data.

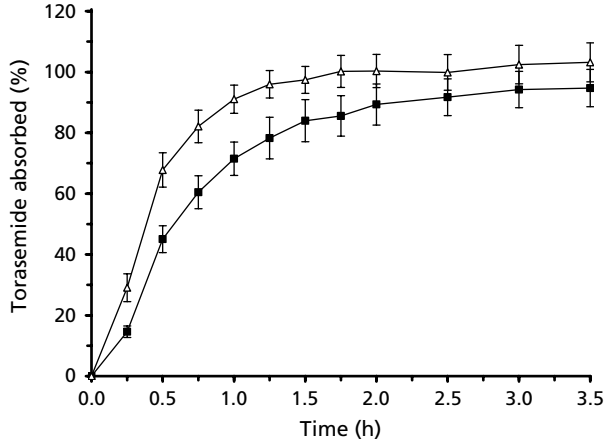

Figure 5 Comparative absorption profiles of torasemide from the Test 1 (■) and the reference (△) products in healthy volunteers (n = 18) following single oral administration of two tablets (each containing 10 mg of torasemide) under fasting conditions. The vertical bars indicate s.e.m. The profiles were obtained by deconvolution of the plasma concentration data.

Discussion

The new crystal modification of torasemide, Torasemide N, has distinctive crystallographic properties compared with Torasemide I, and the intrinsic dissolution data demonstrate its faster solubility rate compared with Torasemide I. Torasemide is known to have dose-linear pharmacokinetics when studied over the range of 2.5–200 mg (Neugebauer et al 1988; Barr et al 1990b; Knauf & Mutschler 1998). The absorption profiles (Figures 5 and 6) obtained by deconvolution of the bioavailability data demonstrate fast (>70% in 1 h) and almost complete absorption of the drug after oral administration of all the products tested. About 95% of the administered dose was absorbed in 3.5 h from both the Test 1 and Test 2 products. This is in line with most literature reports on absolute bioavailability of the drug of more than 90% following oral administration (Lesne et al 1982; Lesne 1988; Kramer et al 1993). The FDA guidance states that drugs with absolute bioavailability greater than 90% can be classified as "highly permeable" drugs (CDER 2000). Therefore, torasemide can be classified as Class I according to the BCS since the solubility profiles of both Torasemide N and Torasemide I meet

the criteria of being "highly soluble" at a maximum dose of about 40 mg, and torasemide is highly permeable given a bioavailability of >90%.

The dissolution data obtained in various media (pH 1.2, 4.5 and 6.8) recommended by the FDA (CDER 2000) as criteria for waiving bioequivalence testing (in humans) for all three products were similar, except in the pH 4.5 medium, where the Test 1 product had a lower dissolution rate than the reference product. The Test 1 product released 83% of the drug in 20 min in pH 4.5 medium, which means that it is marginally outside the criterion (85% release in 15 min) to be similar to the reference product, which released 91% of the drug in less than 6 min. However, the difference was greater at pH 5.0, the medium in which torasemide has the lowest solubility (Figure 2). At pH 5.0, the Test 1 product took 40 min to release 85% of the drug, whereas the reference product released the same amount in less than 6 min.

Considering the marginal differences observed for Test 1 and reference products in their dissolution profiles in pH 4.5, one would not expect the two products to fail to demonstrate bioequivalence since in-vitro conditions are generally considered much more discriminatory than the in-vivo conditions. For example, metoprolol tablets prepared using three different formulations were found to be bioequivalent even though one of the products failed to meet standard USP requirements for in-vitro release (Galia et al 1998). This line of thinking is particularly true when the products are similar (according to the FDA guidance) in the accepted physiologically relevant media, that is under stomach (pH 1.2, pH 2.0) and intestinal (pH 6.8) conditions. In fact, previous reports, including FDA guidance, suggest that dissolution testing in a mild aqueous medium (pH 1.2) would be sufficient for predicting bioavailability and bioequivalence of Class I drugs (CDER 1995; Galia et al 1998). Some reports suggested that a release of not less than 85% of the drug in 30 min (when tested under the conditions described in the FDA guidance for biowaiver) could most likely ensure rapid in-vivo dissolution for Class I drugs (Yu et al 2002). Our findings do not support such a suggestion and demonstrate the importance of selection of appropriate dissolution medium for predicting bioavailability and bioequivalence of Class I products. We suggest that, for drugs like torasemide, with solubility being minimal between stomach and intestinal pH values, the dissolution studies should be performed in the pH medium in which the solubility is the lowest.

Most regulatory authorities, including the FDA, accept statistical comparison (90% CI) of the two main pharmacokinetic parameters, C_{max} and AUC (between test and reference products), as the main criteria for demonstrating bioequivalence for granting marketing authorization. The bioavailability data obtained in Study 1 demonstrate that the Test 1 product fails to meet the bioequivalence criteria with the reference product (Table 2). A comparison of Test 1 product with a marketed product (data not shown) found an even lower 90% CI of 71% (versus 84% for the reference product). These studies were all highly powered (Tables 2 and 3), supporting the conclusion that the difference between Test 1 and the reference products was a real difference and not simply due to an underpowered study. This is not surprising because the Test 1 product had a significantly lower absorption rate than the reference product (Figure 5). At t_{max} of the Test 1 product, only 68% of the drug was absorbed, whereas 89% of the drug was absorbed from the reference product. The fact that the Test 1 and reference products had significantly different absorption profiles and hence were not bioequivalent supports the view that the most discriminatory medium should be used for dissolution testing for the purpose of getting biowaiver for marketing authorization of Class I drug products. For torasemide, we suggest a pH 5.0 medium in which torasemide has minimal solubility.

The results suggest that the criteria set in the FDA guidance (CDER 2000) for waiving bioavailability and bioequivalence studies for Class I drugs needs further consideration, and it is possible that Class I drugs could meet the biowaiver criteria and get marketing authorization without being bioequivalent to the reference product. The relevance of using 0.1 N HCl and pH 6.8 media for testing the similarity between the test and reference products can be understood considering that these media are well accepted in standard Pharmacopoeias due to their supposed physiological relevance. However, the selection of the pH 4.5 medium seems to be arbitrary and without proper justification considering that this particular pH has little physiological relevance (Evans et al 1988). Accordingly, we propose that the regulatory guidance for biowaiver incorporate selection of the most discriminatory medium for in-vitro testing of the products according to the pH solubility profile of the drug.

The criteria for determining the solubility class (i.e. the maximum dose of the drug in 250 mL of the media) seems to be simplistic and somewhat conservative (Yazdanian et al 2004). Solubility determination in media at pH 5.0 (Rinaki et al 2004) and above (Yazdanian et al 2004) was found more appropriate for BCS classification of some non-steroidal anti-inflammatory drugs and for biowaiver than the media range described in the current FDA guidance. BCS also ignores the dynamic character of the in-vivo dissolution/uptake processes by determining solubility under static conditions (Rinaki et al 2004). Furthermore, the apparent solubility/dissolution of some drugs is affected by the amount of the solid used (Kawakami et al 2005), and for some drugs the therapeutic dose ranges can be as high as 10-fold. Therefore, it could happen that the dosage forms with the highest dose of the drug could violate the solubility criterion (for Class I), whereas the lower strengths of the same might be "highly soluble". If so, can a biowaiver be granted to the lower dose products if the other conditions are met, and vice versa? The dose-linear pharmacokinetics of torasemide reported over the range of 2.5–200 mg (Knauf & Mutschler 1998) actually confirms the restrictive nature of the solubility criteria set in the FDA guidance since the drug can be classified as "highly soluble" only up to a maximum dose of 40 mg. Recent studies (Rinaki et al 2003a, b) have demonstrated the usefulness of a dimensionless dose/solubility ratio parameter for the development of a quantitative version of BCS, termed QBCS. The QBCS focuses on the dimensionless parameter, dose/solubility ratio, as a key factor for the absorption phenomena in conjunction with mean time concepts for dissolution, transit and uptake of drugs in the intestine.

The results of the bioequivalence Study 2 support the importance of formulation factors and manipulation of the in-vitro drug release rate in an appropriate medium. The data confirm the importance of in-vitro dissolution profiles to predict

bioavailability for Class I drugs. In this case, both Test 2 and reference products had similar in-vitro release profiles in all the media tested (including pH 5.0), and similar absorption profiles, in agreement with the results of the bioequivalence study of the products being bioequivalent.

Conclusions

The new polymorphic form of torasemide, Torasemide N, was characterized for its physicochemical and biopharmaceutical properties in comparison with the marketed form of the drug, Torasemide I. The data presented here clearly demonstrate the high solubility of torasemide (both crystal modifications) and, given its high bioavailability and hence high permeability, put the drug under Class I according to the BCS up to a maximum dose of 40 mg. One of the two test products failed to demonstrate bioequivalence with the reference product, yet it only marginally failed the criterion to qualify for biowaiver according to the current FDA guidance for in-vitro release (CDER 2000). However, failure to qualify for biowaiver was more pronounced (less marginal) when the in-vitro dissolution medium was selected on the basis of the pH-solubility profiles of the drug. It is concluded that the most discriminatory dissolution medium should be selected to minimize the chance of a biowaiver being granted to a non-bioequivalent product. We suggest that the pH of the medium should be that in which the drug has minimum solubility. We recommend that the FDA (and other interested regulatory authorities) introduce scientifically sound comparative dissolution testing criteria for qualifying Class I drugs for biowaiver.

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