Application of biorelevant dissolution tests to the prediction of in vivo performance of diclofenac sodium from an oral modified-release pellet dosage form

Ekarat Jantratid, a,a, Vincenzo De Maio, b, Emanuela Ronda, b, Valentina Mattavelli, b, Maria Vertzoni, c, Jennifer B. Dressman a

a Institute of Pharmaceutical Technology, Johann Wolfgang Goethe University, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany
b Eurand SpA, Milan, Italy
c Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece

ARTICLE INFO

Article history:
Received 28 January 2009
Received in revised form 23 March 2009
Accepted 27 March 2009
Available online 5 April 2009

Keywords:
Biorelevant dissolution tests
Bio-Dis
Flow-through cell
In vitro–in vivo correlations
Food effects
Diclofenac sodium

ABSTRACT

In vitro biorelevant dissolution tests enabling the prediction of in vivo performance of an oral modified-release (MR) dosage form were developed in this study. In vitro dissolution of MR diclofenac sodium pellets containing 100 mg active ingredient was evaluated under simulated pre- and postprandial conditions using USP Apparatus 3 (reciprocating cylinder, Bio-Dis) and 4 (flow-through cell) and results compared with compendial methods using USP Apparatus 1 (basket) and 2 (paddle). In vivo, the effects of food on the absorption of diclofenac sodium from the pellet dosage form were investigated by administering the product to 16 healthy volunteers pre- and postprandially in a crossover-design study. The in vitro results were compared with the in vivo data by means of Level A in vitro–in vivo correlation (IVIVC) and Weibull distribution analysis. The compendial dissolution tests were not able to predict food effects. The biorelevant dissolution tests predicted correctly that the release (and hence absorption) of diclofenac sodium would be slower in the fed state than in the fasted state. No significant differences in extent of absorption due to changes in extent of release were predicted or observed. The results demonstrate good correlations between in vitro drug release and in vivo drug absorption in both pre- and postprandial states using the biorelevant dissolution test methods.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

For some years attempts have been made to minimize the number of in vivo studies necessary to approve new drug products. One of the approaches currently used is the in vitro–in vivo correlation (IVIVC) (Uppoor, 2001; Emami, 2006; US FDA, 1997b). With respect to the IVIVC concept, in vitro (mainly dissolution) tests are applied as a tool to predict drug product performance in vivo (US FDA, 1997b; USP 30, 2008). The best candidates for IVIVC analysis are products for which dissolution is the overall rate-determining step to drug absorption. There are many studies demonstrating different aspects of IVIVC for products containing BCS Class II compounds housed in immediate-release (IR) dosage forms (Amidon et al., 1995; Dressman and Reppas, 2000; Wei and Löbenberg, 2006; Sunesen et al., 2005) and for modified-release (MR) dosage forms in general (Kortejärvi et al., 2002; Balan et al., 2001; Frick et al., 1998; Takka et al., 2003; Wingstrand et al., 1990; Abrahamsson et al., 1994). Some of these studies employed dissolution media described in the pharmacopeias (so-called “compendial approach”) (Kortejärvi et al., 2002; Balan et al., 2001; Frick et al., 1998; Takka et al., 2003), while others added synthetic surfactants to compendial media (Wingstrand et al., 1990; Abrahamsson et al., 1994; Rossi et al., 2007). As these conditions do not comprehensively represent the gastrointestinal (GI) tract environment, it can be inferred that the results can only be interpreted on an empirical basis.

As a part of a general drive to develop predictive in vitro models, biorelevant media were proposed and have evolved over the last decade as a tool for in vitro biorelevant dissolution tests (Dressman et al., 1998; Galia et al., 1998; Nicolaides et al., 1999; Vertzoni et al., 2004). Recently, the media have been updated to more nearly represent both the pre- and postprandial states in the proximal gut (Dressman et al., 2007; Lue et al., 2008; Jantratid et al., 2008b). The media compositions have been fine-tuned according to the phase of digestion both in the stomach and the upper small intestine, since these factors can dramatically affect drug solubility and dissolution rate. By employing these biorelevant media it should be possible to
predict the effects of food on drug exposure in vivo, which can differ by as much as one or two orders of magnitude.

One limitation of the compendial approaches to simulating the in vivo release profiles of dosage forms with MR properties is that results are typically run in one medium at a time. Only in a limited number of cases (most notably enteric coated products) does the compendial method call for a change in the composition of the medium during the test (USP 30, 2008). This is obviously in contrast to the changing environment to which the dosage forms are generally exposed as they pass through the GI tract. Additionally, for MR drug products, the release of drug may be influenced by the “history” of the dosage form after ingestion. For example, swelling of polymer components under gastric conditions may influence the subsequent release under intestinal conditions or lead to such a reduction in mechanical resistance that the dosage form breaks up upon passage through the ileocecal valve. Thus, it is desirable to set up the in vitro release test conditions for MR dosage forms in such a way (i.e. with a series of media in one experiment) that these effects can be observed and predicted.

The USP Apparatus 3 (reciprocating cylinder, Bio-Dis) and 4 (flow-through cell) offer the advantages of determining release from the dosage form under various, consecutive conditions simulating the GI physiology. The release experiments performed with Bio-Dis and flow-through cell can be set up with a series of dissolution media in one single run, thus making it possible to mimic the “history” of the dosage form as it passes through the GI tract and to generate an IVIVC on an a priori basis.

Diclofenac sodium, a non-steroidal anti-inflammatory drug, was used as a model compound in this study. It was formulated into capsules containing MR pellets consisting of 100 mg active pharmaceutical ingredient (API) per dosage unit using ammonio methacrylate copolymer type A (NF) (USP 30, 2008) as a release-modifying agent. The release of diclofenac sodium from MR pellet dosage forms was compared using Bio-Dis and flow-through cell with USP Apparatus 1 (basket) and 2 (paddle assembly). Food effects on the release of diclofenac sodium, and hence its absorption, were predicted using bioequivalence media simulating the fasted and fed states in the human GI tract (Vertzoni et al., 2005; Fotaki et al., 2005; Dressman et al., 2007; Jantratid et al., 2008b). In vitro results were compared with pharmacokinetic data obtained in the fed and fasted human subjects. Level A IVIVC (USP 30, 2008; Levy and Hollister, 1964; Levy et al., 1965) and RRSBW (Weibull) distribution analysis (Langenbucher, 2005) were applied to assess predictability of the in vitro model.

2. Materials and methods

2.1. Materials

Diclofenac sodium capsules (lot P200750226) were manufactured by Eurand SpA. Acetonitrile and methanol were of gradient grade and obtained from Merck KGaA, Darmstadt, Germany. Egg albumin obtained from Merck KGaA, Darmstadt, Germany. Isopropyl alcohol and n-hexane were purchased from BDH, Milan, Italy. Glacial acetic acid, sodium acetate trihydrate, sodium chloride, sodium dihydrogen phosphate monohydrate and sodium hydroxide pellets were all of analytical grade and purchased from Carlo Erba Reagenti SpA, Milan, Italy. Pancreatin powder (72 USP lipase Units/mg, lot 1279-0057) was obtained from Scientific Protein Laboratories LLC, WI, USA.

2.2. Quantitative analysis of diclofenac sodium

The amounts of diclofenac dissolved in the samples were analyzed using a validated isocratic reversed phase HPLC system. The system consisted of a Waters Alliance 2695 quaternary pump, a Waters Alliance 2695 autosampler, a Waters 996 Photodiode Array Detector (Milford, MA, USA), an integrator (CR5A Shimadzu, Shimadzu, Kyoto, Japan) and an RP-18-e column, 5 μm, 25 mm × 4 mm LiChrospher 100 connected with a RP-18-e guard-column, 5 μm, 4 mm × 4 mm LiChrospher 100. The mobile phase consisted of 63% of 0.05 M ammonium phosphate buffer–pH 5.0, 29.5% acetonitrile and 7.5% tetrahydrofuran (by volume). The flow rate was set at 1.2 mL/min resulting in a run time of 15 min per sample. The injection volume was 20 μL. The detection wavelength was set at 275 nm.

The amounts of diclofenac in the plasma samples were quantified by extracting the acidified (with 0.5N hydrochloric acid solution) plasma with an n-hexane/isopropyl alcohol mixture (95:5). The organic layer was evaporated under nitrogen stream at 50 °C. The dried residues were reconstituted with acetonitrile/water mixture (1:1) and then injected into the HPLC. The HPLC system consisted of a Spectra Physics SP 8800 ternary pump, a Shimatzu SIL-9A autosampler, a Spetra Physics SP 8480 XR scanning spectrophotometer, a PC AX2 (Epson) with Omega 2 Analytical Workstation software integrator (Perkin Elmer) and an HS 5CB column, 5 μm, 15 mm × 4.6 mm I.D. (Perkin Elmer) connected with an Upchurch Perisorb RP-18 guardcolumn, 30–40 μm, 3 cm × 2.1 mm I.D. (Merck). The mobile phase consisted of 47% acetonitrile and 53% of 0.4% ortho-phosphoric acid (by volume). The flow rate was set at 1.0 mL/min resulting in a run time of 10 min per sample. The injection volume was 50 μL. The detection wavelength was set at 275 nm.

2.3. Modified-release formulations

Diclofenac sodium pellets (with a pellet size of approximately 2 mm and the average API content of 100.7%) were manufactured and filled in hard gelatin capsules at a label strength of 100 mg of diclofenac sodium per dosage unit. Ammonio methacrylate copolymer type A (NF) (USP 30, 2008), a pH-independent, permeable polymer, was used as a release-modifying agent.

2.4. Dissolution testing

Quality control (QC) compendial methods using the basket and paddle and bioequivalent methods using the reciprocating cylinder (Bio-Dis III Extended Release Tester, Varian Inc., CA, USA) and the flow-through cell (Sotax S.r.l., Bergamo, Italy) were used to evaluate the dissolution behavior of diclofenac sodium from the MR pellet dosage form.
Table 1
Biorelevant dissolution media simulating the preprandial conditions in the gastrointestinal tract.

<table>
<thead>
<tr>
<th>Medium</th>
<th>FaSSGF</th>
<th>New-FaSSIF</th>
<th>Half-FaSSIF</th>
<th>FaSSIF-sans</th>
<th>SCoF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile secretions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- NaTC</td>
<td>80 µM</td>
<td>3.0 mM</td>
<td>1.5 mM</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Lecithin</td>
<td>20 µM</td>
<td>0.2 mM</td>
<td>0.2 mM</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Enzyme</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Pepsin</td>
<td>0.1 mg/mL</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Pancreatin (lipase Unit/mL)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>1.6</td>
<td>6.5</td>
<td>7.0</td>
<td>7.5</td>
<td>5.8</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>120.7</td>
<td>180</td>
<td>270</td>
<td>270</td>
<td>295</td>
</tr>
<tr>
<td>Buffer capacity (mEq/L ΔpH⁻¹)</td>
<td>-</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>29</td>
</tr>
</tbody>
</table>

Abbreviations: FaSSGF—Fasted State Simulated Gastric Fluid; FaSSIF—Fasted State Simulated Intestinal Fluid; SCoF—Simulated Colonic Fluid; NaTC—sodium taurocholate.

a Vertzoni et al. (2005).
b Dressman et al. (2007) and Jantratid et al. (2008a,b).
c Fotaki et al. (2005).

Table 2
Biorelevant dissolution media simulating the postprandial conditions in the gastrointestinal tract.

<table>
<thead>
<tr>
<th>Medium</th>
<th>FeSSGF</th>
<th>New-FeSSIF</th>
<th>Half-FeSSIF</th>
<th>FaSSIF-sans</th>
<th>SCoF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile secretions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- NaTC</td>
<td>-</td>
<td>7.5 mM</td>
<td>3.0 mM</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Lecithin</td>
<td>-</td>
<td>2.0 mM</td>
<td>1.0 mM</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lipolytic products</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- GMD</td>
<td>-</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Sodium oleate</td>
<td>-</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Enzyme</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Pepsin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Pancreatin (lipase Unit/mL)</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>5.0</td>
<td>5.8</td>
<td>6.5</td>
<td>7.5</td>
<td>5.8</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>400</td>
<td>390</td>
<td>270</td>
<td>270</td>
<td>295</td>
</tr>
<tr>
<td>Buffer capacity (mEq/L ΔpH⁻¹)</td>
<td>25</td>
<td>25</td>
<td>10</td>
<td>10</td>
<td>29</td>
</tr>
</tbody>
</table>

Abbreviations: FeSSGF—Fed State Simulated Gastric Fluid; FeSSIF—Fed State Simulated Intestinal Fluid; GMO—glyceryl monooleate

a Jantratid et al. (2008a,b). This medium contains 50% full fat (3.5%) UHT-milk.
b Dressman et al. (2007).
c The conditions during the late postprandial state in the upper small intestine are similar to the preprandial state. Therefore, FaSSIF-sans was used for simulating both conditions.
d Fotaki et al. (2005).

2.4.1. Quality control method

The QC test conditions for the basket method consisted of 900 mL phosphate buffer, pH 6.8 as a dissolution medium, the basket rotation speed of 50 rpm, and a temperature of 37 °C ± 0.5 °C. The paddle method employed the same conditions as for the basket method, except for the paddle rotational speed, which was set at 75 or 125 rpm. Experiments were conducted in six replicates. The sampling times were 30, 60, 120, 180 and 240 min. Sampling was performed automatically through the sampling device. The volume withdrawn was approximately 5 mL for each sampling time point. The samples were filtered through a 0.45 µm polytetrafluoroethylene (PTFE) filter and then analyzed spectrophotometrically at 275 nm.

2.4.2. Biorelevant methods

2.4.2.1. Bio-Dis method. A biorelevant, pH-gradient method using the Bio-Dis tester was applied to simulate release of diclofenac sodium from the MR pellet dosage form in the GI tract pre- and postprandially. The biorelevant media used in this study and the dissolution test set-up are shown in Tables 1–3. The dissolution experimental design was modified from that proposed previously (Klein et al., 2005, 2008). The detailed compositions and media preparation have mostly been described elsewhere (Vertzoni et al., 2005; Fotaki et al., 2005; Dressman et al., 2007; Jantratid et al., 2008b). The dissolution conditions consisted of a media volume of 220 mL per vessel with a dip rate of 10 dpm. The top and bottom mesh size was 405 µm (40 mesh). The sampling times for the preprandial simulation were 30, 60, 105, 150, 210, 270, 360, 450, 540 and 750 min and for the postprandial simulation 120, 165, 210, 270, 330, 450, 630 and 810 min. The temperature in the vessels was maintained at 37 ± 0.5 °C. Experiments were conducted in six replicates. Sampling was performed automatically using a sampling device (VanKel VK 8000 Dissolution Sampling Station, Varian Inc.,...
CA, USA). The sample volume withdrawn was approximately 5 mL. With the exception of FeSSGF, the samples were filtered through a 0.45 μm PTFE filter and then analyzed by HPLC. The amount of drug released in the FeSSGF was indirectly determined by using the ‘infinity point’ approach (Klein, 2005) by adding one more row of vessels containing phosphate buffer, pH 6.8 to the series of media set-up in the postprandial state. After the release was tested in the first five rows, pellets were exposed to this medium until the release was exhausted (20 dpm, 6 h). By subtracting the cumulative release in all vessels analyzed from the label strength of the dosage form (100 mg), the drug release in the first row can be estimated.

2.4.2.2. Flow-through cell method. The flow-through dissolution tester equipped with 22.6 mm diameter test cells and a piston pump was used to evaluate the release of diclofenac sodium from the pellets during exposure to the bio-relevant, pH-gradient methods. A 5 mm-sized glass bead was placed in the tip of the cell. A total of 1.7 g of 1 mm-sized glass beads were added above the 5 mm glass bead, while a glass fiber (MNGF1, 0.7 μm pore size, 25 mm diameter, Machery-Nagel, Germany) was placed on the top of the cell. During the experiment, the capsule was mounted on a holder. Experiments were performed in triplicate at 37 ± 0.5 °C in dissolution media simulating the compositions of gastric, small intestinal and ascending colonic contents in the fasted and fed states (as for the Bio-Dis experiments). Duration of exposure to the various fasted and fed state simulating media and the corresponding flow rates were adapted from Fotaki et al. (2005). Fluid exiting the flow-cell was collected in a volumetric cylinder. The cylinders were exchanged every 20 min up to 420 min.

The content of diclofenac sodium in each cylinder was analyzed by HPLC. Except for FeSSGF, the samples collected from the experiments were immediately injected into the HPLC system after filtration. In case of FeSSGF, 1 mL of the collected samples was transferred to a tube and 2 mL of acetonitrile was added. After vortexing for 1 min, the sample was centrifuged for 10 min at 4000 rpm. The clear supernatant was then injected into the HPLC system.

2.5. Comparative bioavailability studies

The MR diclofenac sodium pellets were given to 16 healthy volunteers [nine males and seven females, mean age—24.9 years old (range 20–33 years old); mean body weight—65 kg (range 52–83 kg); mean height—173 cm (range 164–185 cm)] in the fasted and fed states on a randomized crossover basis with a wash-out period of one week. The study was conducted following the standard procedure of the US FDA for the assessment of food effects on drug absorption (US FDA, 2002) and the recommendations of the Declaration of Helsinki. The study protocol was approved by the local ethics committee prior to the beginning of the study. Blood samples were collected every hour up to 12 h and the last samples were collected at 24 h. The diclofenac concentrations in plasma were determined using the aforementioned HPLC method.

2.6. Analysis of in vitro dissolution data

Differences in the in vitro dissolution profiles were assessed using the model-independent approach based on the similarity factor (f2) as follows (US FDA, 1997a):

\[
f_2 = 50 \log \left( \left[ 1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right)
\]

where n is the number of time points, R_t is the dissolution value of the reference at time t, and T_t is the dissolution value of the test at time t. The f2 is basically a measurement of the similarity in the percent (%) drug dissolution between the two curves. Values of 50 or above (50–100) ensure similarity (difference ≤ 10%) of the curves.

2.7. Analysis of in vivo pharmacokinetic data

Non-compartmental analysis was applied to the evaluation of pharmacokinetic parameters using WinNonlin® Professional Edition version 4.1 software (Pharsight Corporation, Mountain View, CA, USA) and Microsoft Office Excel® 2003 (Microsoft Corporation, Redmond, WA, USA). Student’s paired t-test was used to statistically interpret the differences between the area under the plasma drug concentration–time curves (AUC0–t) and the maximum plasma drug concentration (Cmax) after administration in the fed and fasted states, while Wilcoxon’s signed-rank test was used for the Tmax comparison. A probability level of 0.01 was applied to all statistical analyses.

2.8. In vitro–in vivo correlations

The plasma drug concentration–time profiles obtained in the pre- and postprandial states were deconvoluted to the fraction drug absorbed (Fa) profiles using WinNonlin® software. The unit impulse response was determined from the literature plasma drug concentration–time data obtained following the intravenous administration of 50 mg diclofenac sodium to seven healthy female volunteers (Willis et al., 1979). The fraction drug dissolved (Fd) profiles were taken directly from the dissolution data.

The Fa vs Fd curve comparisons were determined using the Weibull distribution as described by the following equation:

\[
W_t = W_{\text{max}} \left[ 1 - e^{-\left(\frac{t - \gamma}{\tau_d}\right)^{\beta}} \right]
\]

where W_t is the fraction of drug dissolved/absorbed at time t, W_{\text{max}} is the maximum cumulative fraction dissolved/absorbed, γ is the location parameter (the lag time before the onset of dissolution), τ_d is the time parameter (provides information about the overall rate of the process), and β is the shape parameter. The profiles were fit with the ‘Sigmoidal–Weibull’ distribution with a slight modification using SigmaPlot® software, version 10.0 (Erkrath, Germany).

To generate Level A IVIVC the dissolution results from the QC and bio-relevant methods were plotted against the in vivo drug absorption data, i.e. the relationship between Fa and Fd values, in which the time pairs in vitro (drug dissolution) and in vivo (drug absorption) are correlated (USP 30, 2008; Levy and Hollister, 1964; Levy et al., 1965). No time scale corrections were made for the profile comparison.

3. Results and discussion

3.1. Biorelevant dissolution media

Biorelevant dissolution media have been updated recently (Dressman et al., 2007; Lue et al., 2008; Jantratid et al., 2008b) to better simulate the proximal human GI conditions than their predecessors, i.e. FaSSIF and FeSSIF (Galia et al., 1998). In fact, the present study was performed during the development of the updated biorelevant dissolution media (Jantratid et al., 2008b) and the design of media compositions described herein was based mostly on the recent in vivo data collected from human aspirates in the pre- and postprandial states (Kalantzi et al., 2006). The media compositions, as tabulated in Tables 1 and 2, reflect the physiological changes and the on-going digestive processes along the human GI tract in the fasted and fed states. Crucial parameters affecting drug solubility and dissolution including (i) the levels of bile secretions,
(ii) the presence of lipolytic products and enzymes, and (iii) the pH, buffer capacity and osmolality, were all taken into consideration. In this study, media representing the fasted and fed stomach (FaSSGF and FeSSGF), the small intestine in the fasted (New-FaSSIF, Half-FaSSIF, and FaSSIF-sans) and fed state (New-FeSSIF, Half-FeSSIF, and FaSSIF-sans), and the ascending colon (SCoF) were developed or adopted from the literature (Vertzoni et al., 2005; Fotaki et al., 2005; Dressman et al., 2007; Jantratid et al., 2008b). FaSSIF-sans (pH 7.5) was used for both the pre- and postprandial states to simulate the lower part of the small intestine, where reuptake of the bile components is virtually complete and pH is higher. Likewise, SCoF was used as a dissolution medium to represent the conditions in human colon in both the pre- and postprandial states (Fotaki et al., 2005).

3.2. Quantitative analysis of diclofenac sodium

Validation of the chromatographic method for diclofenac sodium assay from the in vitro tests showed that the limit of detection (LOD) was 0.09 µg/mL and the limit of quantification (LOQ) was 0.3 µg/mL. The mean recovery of 99.0% was observed within the concentration range of 15–150 µg/mL. The accuracy and precision of the assay were less than 5%.

For the analysis of diclofenac sodium concentration in plasma, the LOD was 2 ng/mL and the LOQ was 10 ng/mL. The mean recovery of 99.7% was observed within the concentration range of 10–2000 ng/mL. The accuracy and precision of the assay were less than 10%.

3.3. Dissolution testing

3.3.1. Quality control methods

Fig. 1A shows the dissolution profiles of diclofenac sodium from the MR pellets using the QC methods. Basket and paddle methods gave comparable dissolution results. Additionally, using the paddle method, rotational speeds of 75 and 125 rpm showed no significant differences in the release rate of diclofenac sodium form the pellets ($f_2$ value = 67.3). Approximately 85% drug release was observed in all test conditions within 120 min.

3.3.2. Biorelevant methods

The dissolution profiles of diclofenac sodium from the MR pellets using the Bio-Dis and flow-through methods are shown in Fig. 1B. In this study, the biorelevant dissolution tests were used to simulate not only differences in GI fluid compositions between the pre- and postprandial states, as reflected by the dissolution media compositions, but also differences in the residence times in different regions of the human GI tract in the fasted and fed states using either Bio-Dis or flow-through cell. Owing to the weakly acidic properties of diclofenac (pK_a 3.8) (O’Connor and Corrigan, 2001), the release in the fasted stomach in normal acid secretors is expected to be poor. However, the gastric residence time is short and it can be expected that the release would go up immediately after the dosage form has been emptied into the duodenum. By contrast, after ingestion of the meal, the gastric pH initially increases (Russell et al., 1993; Hardy et al., 1993; Davis et al., 1986; Coupe et al., 1991). For example, gastric emptying and thus arrival at regions in the small intestine with higher pH values is generally quicker in the fasted state than in the fed state. Further, the gastric residence time is usually shorter for multiparticulates than monoliths (Hardy et al., 1993; Davis et al., 1986; Coupe et al., 1991). Based on the literature data, the residence times of the pellets in different regions of the human GI tract in the fasted and fed conditions (Hardy et al., 1993; Davis et al., 1986; Coupe et al., 1991) were applied to the design of the biorelevant dissolution tests, as described in Table 3.

The results in Fig. 1B show that under postprandial conditions the release of diclofenac sodium from the pellets would be slower than in an empty stomach. Weibull analysis ($\tau_a$ and $\beta$ values; Table 4) indicates an apparent difference between the dissolution characteristics in the fasted and fed states using either Bio-Dis or flow-through cell. Weibull parameters derived from the fraction drug absorbed and the fraction drug dissolved profiles.

Table 4

<table>
<thead>
<tr>
<th></th>
<th>Weibull parameters</th>
<th>$F_a$—in vivo</th>
<th>$F_a$—Bio-Dis</th>
<th>$F_a$—Flow-through</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted</td>
<td>$\tau_a$ (min)</td>
<td>243.7</td>
<td>167.3</td>
<td>193.2</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>1.7</td>
<td>2.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Fed</td>
<td>$\tau_a$ (min)</td>
<td>420.5</td>
<td>249</td>
<td>289</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>2.6</td>
<td>4.3</td>
<td>2.7</td>
</tr>
</tbody>
</table>

* $F_a$—in vivo—fraction of drug absorbed deconvoluted from the in vivo plasma profiles.

$F_a$—Bio-Dis—fraction of drug dissolved obtained from Bio-Dis method.

$F_a$—Flow-through—fraction of drug dissolved obtained from flow-through cell method.
Fig. 2. (A) Plasma diclofenac sodium concentration–time profiles obtained following a single oral administration of modified-release diclofenac sodium pellets to 16 healthy volunteers in the fasted and fed states; (B) plasma diclofenac sodium concentration–time profiles obtained following an intravenous administration of 50 mg diclofenac sodium to 7 volunteers (Willis et al., 1979). Each point represents the mean plasma diclofenac sodium concentration.

Dressman et al., 1990; Kalantzi et al., 2006), as reflected by the fed gastric dissolution medium, FeSSGF (pH 5.0) (Jantratid et al., 2008b). At this pH, diclofenac is more soluble than in the acidic conditions in the fasted state and part of the drug can be released. However, the results show that the dissolution rate is not actually faster in the fed gastric conditions. With the delayed gastric emptying induced by food commonly observed in vivo, the dosage form would reach the proximal small intestine, where significant release can occur, after approximately 2–4 h (Hardy et al., 1993; Davis et al., 1986; Coupe et al., 1991). Subsequently, along the small intestine, as the pH increases the release is expected to continue. The dissolution results indicate that after approximately 6 h the release of drug in both fed and fasted states are expected to be complete.

3.4. Comparative bioavailability studies

Fig. 2A shows a slow onset of absorption after meal intake, with significant differences in the plasma drug concentration–time profiles following oral administration of MR diclofenac sodium pellets in the pre- and postprandial states. Both C_{max} and T_{max} were significantly different (p < 0.01, Student’s paired t-test and Wilcoxon’s signed-rank test, respectively). However, the exposure, expressed as AUC, was not significantly different (p > 0.01, Student’s paired t-test). The results are in agreement with previously reported data (Willis et al., 1981; Riad et al., 1995).

3.5. IVIVC analysis and curve comparisons

3.5.1. Deconvolution of pharmacokinetic data

Since no intravenous data was available from this study, they had to be taken from the literature (Fig. 2B) (Willis et al., 1979) to perform numerical deconvolution using the WinNonlin® program. Therefore, the deconvoluted data cannot be interpreted as 100% accurate. By fitting the intravenous plasma drug concentration–time data to the ‘Exponential Decay’ regression using SigmaPlot® program, tri-exponential functions were found to describe the profile appropriately. The unit impulse response was then calculated from the coefficients of tri-exponential equation.

Fig. 3 shows the F_d profiles obtained following the numerical deconvolution of the plasma profiles in the fasted and fed states. Similar patterns to those measured in the in vitro dissolution profiles obtained from the biorelevant methods were observed. By contrast, the QC dissolution methods give dissolution patterns that are obviously different from the in vivo absorption profiles.

3.5.2. Curve comparisons

The Weibull distribution has previously been applied to the evaluation of IVIVC (Nicolaides et al., 2001; Jantratid et al., 2008a). In this study it was used to explain the incremental changes in the F_d and F_a curves over the same time-frame. As demonstrated in Fig. 4, the F_d profiles from both the QC and the biorelevant methods are steeper than the F_a profiles in the fasted and fed states. However, the F_d profile from the QC dissolution methods could be excluded from IVIVC considerations since it runs much ahead of the biorelevant F_d and the F_a profiles, and is exponential in shape. A sigmoidal shape which can be well described by using the Weibull distribution was observed for the F_d profiles from the Bio-Dis and the flow-through cell methods, as well as the F_a profiles. The parameters obtained from Weibull analysis of these profiles are given in Table 4. The τ_d values, which represent the overall rate of dissolution/absorption, confirm that the time-frames required for the dissolution curves are indeed shorter than those required for the absorption curve both in the fasted and fed states. The β values, which describe the shape of the profiles, indicate that although the curve increments in vitro
Fig. 4. Comparison of the fraction drug dissolved \((F_d)\) obtained from the biorelevant dissolution tests vs the fraction drug absorbed \((F_a)\) in the fasted state (A) and fed state (B). The data were fitted to Weibull distribution.

The observed difference between the in vitro dissolution and in vivo absorption, especially in the fed state, can be explained by the variations in the gastric residence time and the variations of the small intestinal pHs. The passage time used for the dissolution setup in this study was set as 2 h for the fed state stomach. However, the in vivo data range from 2 to 4 h (Hardy et al., 1993; Davis et al., 1986; Coupe et al., 1991). It can therefore be expected that the longer fed gastric residence time invoked for the biorelevant methods would lead to in vitro dissolution profiles closer to the in vivo absorption profile. The other reason for the deviation of the in vitro dissolution to the in vivo absorption is that the solubility and dissolution of diclofenac sodium is known to be influenced strongly by pH changes, e.g. conditions in the GI tract, owing to the weakly acidic properties and the \(pK_a\) of the drug itself (Chuasuwan et al., 2009). Therefore the release of drug in vivo, particularly in the postprandial state, can be very sensitive to the conditions in the small intestine. These, in turn, show interindividual variation as well as being affected by the type of meal administered (Clarysse et al., 2009).

3.5.3. In vitro–in vivo correlations

Fig. 5 demonstrates the Level A IVIVC plots. In both fasted and fed states, the least square regressions yield essentially linear patterns \((r^2 > 0.95)\), indicating that the proposed in vitro biorelevant disso-


