ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 46 (1998) 305–311

European Journal of Pharmaceutics and Biopharmaceutics

Research paper

Biopharmaceutical characterization of oral immediate release drug products. In vitro/in vivo comparison of phenoxymethylpenicillin potassium, glimepiride and levofloxacin

Annke Frick, Helga Möller*, Ehrenfried Wirbitzki

Hoechst Marion Roussel Deutschland GmbH, Preclinical Development Analytics, D-65926 Frankfurt am Main, Germany

Received 28 July 1997; accepted 17 March 1998

Abstract

The development of in vitro dissolution tests using the paddle and basket apparatus is described with respect to the qualification/validation of the testing procedure. Three examples of immediate release products containing phenoxymethylpenicillin potassium, glimepiride, and levofloxacin providing different solubility characteristics are evaluated. The solubility was high in the case of phenoxymethylpenicillin potassium and levofloxacin and low for glimepiride according to the biopharmaceutics classification system. The permeability is studied using the human colorectal carcinoma cell line CaCo-2. The permeability (10^{-6}cm/s) of phenoxymethylpenicillin potassium, glimepiride, and levofloxacin was high. The determined permeability data are confirmed by absorption data obtained by means of numerical deconvolution of plasma concentrations. Recommendations are given for the biopharmaceutical characterization of the three immediate release drug products, taking into account in vitro and in vivo comparison as well as the biopharmaceutics drug classification system. The evaluated acceptance criteria are the following: phenoxymethylpenicillin potassium (80% in 30 min), glimepiride (80% in 15 min) and levofloxacin (80% in 30 min). Typically, for immediate release formulations, one limit is specified for the dissolution to ensure the release of the active ingredient within the present time period. Since phenoxymethylpenicillin potassium and levofloxacin belong to Case 1, no in vitro/in vivo correlation is expected, absorption may be gastric emptying dependent. Glimepiride is categorized to Case 2. Nevertheless, a correlation with the in vivo dissolution profile does not exist, because of the pH-dependent low solubility of the drug. Finally, recommendations are made for the batch control of drug products in accordance with the four Cases.

Keywords: Biopharmaceutical characterization; Oral immediate release drug products; Dissolution; Solubility; Permeability; Glimepiride; Levofloxacin; Phenoxymethylpenicillin potassium; In vivo studies

1. Development of dissolution tests

The four dissolution test apparatus of Ph.Eur.2 and USP 23, namely basket and paddle apparatus, Bio-Dis II apparatus and the flow-through cell, either alone, or in combination, serve as adequate tools to characterize the majority of oral drug products. Alternative dissolution devices should not be used unless it is demonstrated that the results obtained with the compendial devices are not satisfactory. In developing a dissolution test for an immediate release (IR) [1,2] drug product, whether for research, quality control or regulatory purposes, it is important to recognize aspects such as the precision of the test procedure (guaranteed by qualification and validation of the apparatus or procedure), the appropriate testing conditions (choice of dissolution medium and agitation) and sink conditions [3]. In certain cases, biopharmaceutically relevant results can be obtained by violating sink conditions [4].

* Corresponding author. Tel.: +49 69 3055424; fax: +49 69 30583467.

0939-6411/98/$19.00
PII S0939-6411(98)00041-1

1.1. Qualification and validation of dissolution tests

Qualification aspects of dissolution test apparatus are summarized in Table 1. With respect to identical dimensions for the apparatus described in Ph.Eur.2, USP 23 and Ph.J.12, progress is already being made in the international harmonisation procedures [4–6]. Therefore, almost comparable monographs now exist. The specification of apparatus recommendations for the critical test characteristics have also already been discussed with respect to the tolerances of temperature (37°C ± 0.5), volume (±1%) and pH (±0.05) of dissolution media. It has been proposed that the speed of stirring devices for paddle and basket be within the tolerance of ±4%, the flow rate for the flow-cell within ±5% and ≤2% for each sampling interval. No detailed description of the filter to be used is available; it may be of use to include a note to the effect that the use of an inert filter of suitable pore size should be defined within the qualification/validation document.

The equipment used for dissolution testing has to be calibrated taking into consideration the specification for geometry and alignment of the dissolution apparatus. Calibration of equipment using USP calibrators or in-house calibrators is recommended [4] but not sufficient to calibrate apparatus dimensions.

Based on practical experience, the purpose of this test is not to check the correct geometry and alignment of the apparatus. In many cases, the value of the high workload of testing the apparatus suitability by dissolution calibrators is questionable, so long as the geometry and adjustment of equipment are well controlled. The USP tablets, described as dissolution calibrators, may be of great importance for collaboration studies, comparing dissolution data generated in different laboratories, which could be subject to different testing conditions e.g. deaeration, vibration, wobble and test performance by the operator.

The validation of analytical procedures is performed in accordance with current ICH recommendations [7].

Appropriate testing conditions include the deaeration procedure of the dissolution media and the use of sinkers. Dissolution results may be influenced by the physical behaviour of the specimen such as floating, adherence to the wall etc. Thus, critical inspection and observation during the tests are required. The appropriate type of sinker has to be described in detail within the qualification document.

Table 1

Qualification of dissolution test apparatus

1.	Check the dimensions of the apparatus in accordance with the compendial monograph
2.	Control the position and speed of the stirring device (paddle and basket)
3.	Control the flow rate (flow-through apparatus)
4.	Control the temperature, volume and pH of the dissolution medium
5.	Define the sampling device
6.	Guarantee a tolerance for the testing time
7.	Calibrate the apparatus using appropriate calibrators

1.2. Dissolution media and agitation

As recommended in the FIP-Guidelines [4], the use of water as a dissolution medium has the disadvantage that test condition details, such as pH and surface tension, can vary depending on the source of water and may be changed during the dissolution test itself due to the influence of the drug products and the absorption of carbon dioxide from air. Therefore, dissolution media with a pH range from 1 to 6.8 are recommended [4].

A higher pH needs to be justified on a case-by-case basis and, in general, should not exceed pH 8. The use of surfactants and enzymes could be considered in specific cases.

Agitation by stirring at 50 to 100 rpm, but, in general, not exceeding 150 rpm, is an important part of justification of the appropriate testing conditions using the paddle and basket methods. The speed of 75 rpm is seen as a reliable agitation speed for the paddle method. For the flow-cell, flow rates should be set to between 8 and 50 ml/min.

1.3. How to develop dissolution testing

During development of a drug product, the final choice of testing conditions and acceptance criteria has to be based on in vivo-in vitro comparison after evaluating whether the rate-limiting step for absorption is dissolution or permeation across the intestinal wall. This information is available after pharmacokinetic studies including bioavailability and information on dose-linearity.

Besides these results which are available after phase I clinical trials, several other aspects are of great importance, such as the description of the drug product (dosage form), composition and manufacturing as well as physical and chemical characteristics of the drug product, in vivo performance considering pharmacokinetics, in vivo/in vitro comparison, definition of the task of dissolution testing (i.e. quality control, prediction of drug absorption), and setting specifications to be used worldwide.

As long as in vivo data are not available, preliminary testing conditions have to be defined taking into account the type of dosage from, for example uncoated tablets, film-coated tablets, hard gelatin and soft gelatin capsules. Composition and the manufacturing process play an important part in the dissolution testing of oral solid dosage forms [3]. The main physical and chemical characteristics of the drug substance which affect dissolution are solubility, particle size distribution and crystallinity, which are determined during preformulation activities. Based on this preliminary information, without knowledge of any in vivo data, preliminary testing conditions are elaborated taking into consideration the state of the art for dissolution testing. In general, the aim is to set preliminary acceptance

Table 2

Biopharmaceutical concept for oral immediate release products

Physical and Chemical Characteristics of the Drug Substance: Solubility, Permeability				
Case	Solubility	Permeability	Absorption	In vitro in vivo correlation (IVIVC) expectations [9]
1	High	High	Gastric emptying controlled	IVIVC is expected if dissolution rate is slower than gastric emptying rate, otherwise limited or no correlation
2	Low	High	Dissolution controlled	IVIVC is expected if in vitro dissolution rate is similar to in vivo dissolution rate, unless dose is very high
3	High	Low	Dissolution independent	Absorption (permeability) is rate determining; limited or no IVIVC with dissolution rate
4	Low	Low	Case-by-case evaluation	Limited or no IVIVC is expected

criteria for oral immediate release drug products, for example, ≥75% (Q) after 30 min. A definition of 'Q' is provided by USP 23 [2]. This preliminary biopharmaceutical concept has to be re-evaluated by in vivo data obtained from phase I studies. In principle, it is of great importance that the composition and the concept of the manufacturing process should mostly remain unchanged during phase I trials.

2. The impact of solubility and permeability on absorption

Solubility and permeability are the fundamental parameters controlling the rate and extent of drug absorption [8]. Drug substances should be categorized worldwide into four biopharmaceutic drug classes based on solubility and permeability characteristics, namely the biopharmaceutics classification system (BCS) [9,10]. This system supports the definition of the role of in vitro dissolution testing (Table 2). Correlations or non-correlations with the in vivo process may be explained by evaluation of these parameters of a drug product (Table 2).

In the following, phenoxymethylpenicillin potassium, glimepiride, and levofloxacin immediate release drug products are described, taking into consideration the biopharmaceutics classification system.

2.1. Solubility

Phenoxymethylpenicillin potassium is a freely soluble (pH-independent) drug substance (≥100 mg/ml water). Glimepiride is a drug with low, pH-dependent solubility. In acidic and neutral aqueous media, glimepiride exhibits very poor solubility at 37°C (<0.004 mg/ml). In media of pH > 7, the solubility of the drug substance is slightly increased (pH 7.8, 0.02 mg/ml). Levofloxacin is a drug with pH-dependent solubility in a range of about 30–300 mg/ml within the range of pH 1–pH 8 (Fig. 1). There is almost no change in solubility from pH 2–5 (200 mg/ml).

2.2. Permeability

A confluent monolayer of an adenocarcinoma cell line (CaCo-2) [11] which demonstrates a well differentiated enterocyte-like phenotype was used to determine the gastrointestinal permeation of the three compounds with different physico-chemical properties. Materials, cell culture, transport studies, assessment of CaCo-2 cell monolayer integrity, methods, applications of the cell model, data treatment and calculation are as described by Frick [12]. The successful prediction of oral absorption could be used as an integral part of drug development. Permeability values (P_{app}) for glimepiride, phenoxymethylpenicillin potassium and levofloxacin examined in this study using CaCo-2 cell monolayers ranged between 23.9×10^{-6} cm/s for phenoxy-

Table 3

Molecular weights (Mw), logarithms of partition coefficients (log P; octanol-water pH 7.4), absorption in humans and concentrations and permeability coefficients (P_{app}) of drug substances generated in CaCo-2 cell monolayers

Drug substance	Mw	Log P	Absorption	Conc. (mM)	P_{app} ($\times 10^{-6}$cm/s)
Glimepiride	491	1.8 [17]	Almost complete (Fig. 2)	0.02	28.8 ± 4.3
			Absolute bioavailability	0.1	32.0 ± 6.3
					mean: 30.4
Levofloxacin	361	–0.4 [17]	Almost complete (Fig. 4)	0.01	28.4 ± 1.8
			Absolute bioavailability	1	25.6 ± 6.3
					mean: 27.0
Phenoxymethylpenicillin potassium	389	–1.7 [18]	Almost complete (Fig. 1)	0.01	23.9 ± 1.2
			Relative bioavailability	1	27.6 ± 9.1
					mean: 25.8
Mannitol	182	–3.1 [19]	<20% [19]	0.009	1.96 ± 0.3

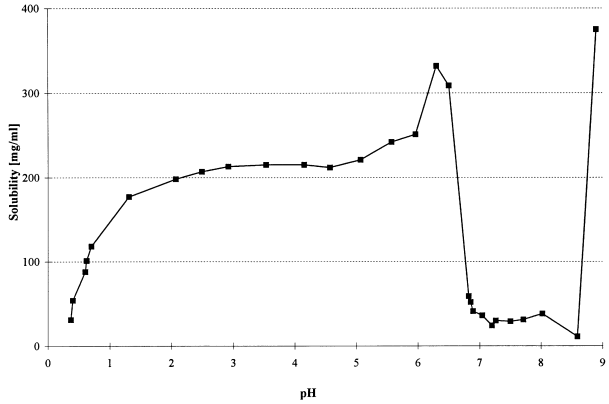

Fig. 1. Solubility of levofloxacin at 20°C.

methylpenicillin potassium and 32.0×10^{-6} cm/s for glimepiride (Table 3). The individual permeability values obtained from this CaCo-2 study were as follows: glimepiride P_{app} 30.4×10^{-6} cm/s, phenoxymethylpenicillin potassium P_{app} 25.8×10^{-6} cm/s, and levofloxacin P_{app} 27.0×10^{-6} cm/s (Table 3).

The measured permeability of glimepiride, levofloxacin and phenoxymethylpenicillin potassium was much greater than mannitol, a passively transported compound (Table 3). Within the tested concentration range (Table 3), this result indicates that the compounds are passively transported across the CaCo-2 monolayer. Phenoxymethylpenicillin potassium, glimepiride, and levofloxacin are almost completely absorbed in humans (Figs. 2, 3 and 4) after oral administration. Less absorbed drugs such as mannitol (low permeability hydrophilic marker molecule) [12] with poor absorption of $< 20\%$ had permeability values $<1.69 \times 10^{-6}$ cm/s (Table 3). A steep increase in compound absorption reflects the switch from mainly paracellular to transcellular transport pathways [13].

Starting out from the P_{app} values, the present study shows that the three compounds involved might have no problems in absorption behaviour in vivo which could be confirmed by the human in vivo studies (Figs. 2, 3 and 4) as well as by Frick [12].

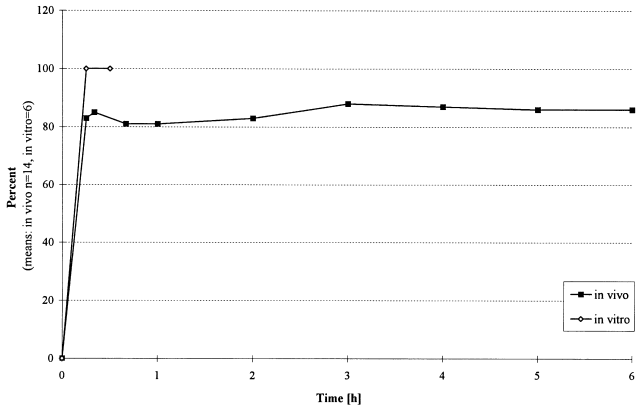

Fig. 2. In vitro dissolution and in vivo dissolution/adsorption of phenoxymethylpenicillin potassium tablets.

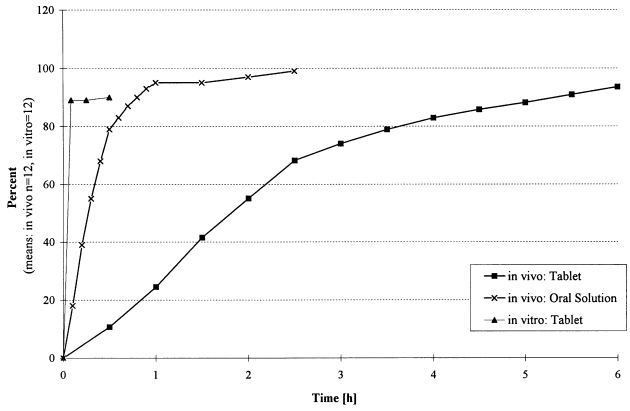

Fig. 3. In vitro and in vivo dissolution of glimepiride tablets. In vivo absorption of glimepiride oral solution.

In contrast, the log P values of 1.8 for glimepiride, –1.7 for phenoxymethylpenicillin potassium, and –0.4 for levofloxacin (Table 3) do not reflect the comparable absorption behaviour of the three compounds, leading to the assumption that log P values are not as predictable as P_{app} values of phenoxymethylpenicillin potassium, glimepiride, and levofloxacin.

It has already been demonstrated by Walter et al. [14] that drug permeabilities across CaCo-2 cell monolayer correlate with the fraction dose absorbed in humans. A good correlation was attained by separating the range of passively absorbed drugs into three groups (i) of well absorbed drugs, (ii) drugs that are 40–70% absorbed and (iii) poorly absorbed drugs. The biopharmaceutics classification system could be useful for the classification of drug permeability values obtained from the CaCo-2 cell model [14]. Further investigations are necessary for setting permeability boundaries to specify high and low permeability and absorption characteristics, respectively. Within the relationship of in vitro permeabilities across CaCo-2 cell monolayer and percentage absorption in humans, further investigations are just now being performed [12] to explicitly characterize P_{app} values which classify boundaries within the CaCo-2 permeabilities which belong to high and low permeability in

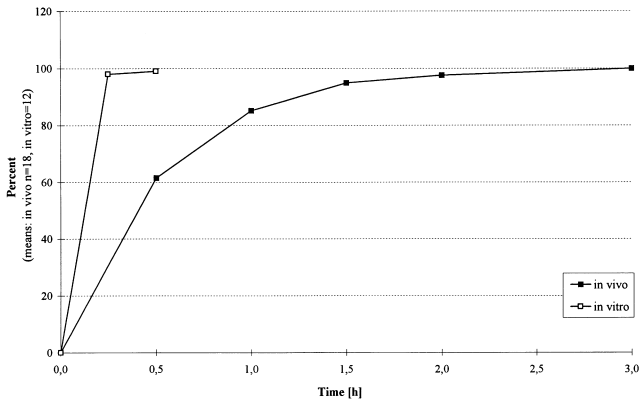

Fig. 4. In vitro dissolution and in vivo absorption of levofloxacin tablets.

humans according to the biopharmaceutics classification system (Table 2).

2.3. In vivo studies

In a relative bioavailability study, a single dose of 1.2 million IU of phenoxymethylpenicillin potassium (780 mg Isocillin®, Hoechst Marion Roussel Deutschland GmbH) was administered to 14 healthy volunteers in the form of a film-coated tablet and reconstituted powder with 100 ml tap water (oral solution) in accordance with a randomisation crossover scheme [15]. The healthy volunteers remained in the recumbent position; a standardised breakfast was served 2 h after dosing. Venous blood samples were drawn before medication and 15, 30, 45, 60, 75, 90, 105 min and 2, 3, 4, 6, 8, and 24 h after medication. Serum concentrations of phenoxymethylpenicillin potassium were analyzed by liquid chromatography (Hoechst Marion Roussel Deutschland GmbH, internal report on human clinical trials).

In a single dose cross-over study, 1 mg of glimepiride (Amaryl®, Hoechst Marion Roussel Deutschland GmbH) was administered as a tablet and an intravenous solution, as described by Badian et al. [16]. The drug substance was micronized with a guaranteed specified particle size distribution. In 12 healthy subjects, a randomised Latin Square design with a washout period of 7 days was used. The tablet was administered with 150 ml of water following a 12-h overnight fast.

Venous blood samples were drawn 5 min before medication and 5, 10, 15, 20, 30, 40, 50 min and 1, 1.15, 1.30, 1.45, 2, 2.30, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 24 h after medication. Serum concentrations of glimepiride were analyzed by liquid chromatography (Hoechst Marion Roussel Deutschland GmbH, internal report on human clinical trials).

Levofloxacin is incorporated into a 500 mg film-coated tablet (Tavanic®, Hoechst Marion Roussel Deutschland GmbH). Single doses of 500 mg of levofloxacin were administered to 18 subjects in the form of a tablet and an intravenous infusion of 500 mg of solution. The trial periods were separated by drug-free periods of 7 days. The tablets were administered with water following an overnight fast. Blood samples were drawn before medication and 15, 30, 45 min and 1, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 48 and 72 h after medication. Serum concentrations of levofloxacin were analyzed by liquid chromatography (Hoechst Marion Roussel Deutschland GmbH, internal report on human clinical trials).

Phenoxymethylpenicillin potassium, glimepiride and levofloxacin are almost completely absorbed (Figs. 2, 3 and 4). Table 3 gives the absolute bioavailability for glimepiride and levofloxacin and the relative bioavailability for phenoxymethylpenicillin potassium. Since the absorption rate of phenoxymethylpenicillin potassium from the tablet is very fast (Fig. 2), the relative and absolute bioavailability should be expected to be comparable, anyhow it is an immediate release solid oral dosage form.

2.4. In vitro dissolution

For in vitro dissolution profiles of phenoxymethylpenicillin potassium (Paddle: 50 rpm, 900 ml of phosphate buffer, pH 6.0) and glimepiride tablets (Paddle: 75 rpm, phosphate buffer pH 7.8) see Figs. 2 and 3, respectively. See Fig. 4 for in vitro dissolution of levofloxacin tablets (Basket: 100 rpm, 900 ml of 0.1 N hydrochloric acid).

Different in vitro dissolution procedures are used, depending on whether dissolution methods already exist [2], i.e. for phenoxymethylpenicillin potassium, as well as considering the individual dissolution characteristics of the drug substances (see Section 2.1 solubility).

3. Results and discussion: in vivo performance and comparison to in vitro

The development of in vivo and in vitro comparisons for immediate release products may be complicated due to the gastrointestinal variability (e.g. gastric emptying and intestinal transit times, intestinal permeability characteristics of the drug in the volunteers/patients, the solubility of the drug substance and dissolution behaviour). Prior to this, analysis of the impact of dissolution on absorption kinetics needs to be performed. With regard to the biopharmaceutics classification system (Case 2) (Table 2) for drugs with high permeability and low solubility, drug dissolution is the rate-limiting step to absorption.

In the development of new chemical compounds several pharmacokinetic studies are performed during phase I clinical trials. Such studies may be used for the biopharmaceutical assessment of drug products, taking into consideration drug absorption (permeability through the intestinal membrane) and in vivo dissolution of the drug product.

Absorption as a function of time may be calculated by means of the numerical deconvolution of plasma concentrations of single-dose cross-over studies (intravenous injection vs. oral solution). The fractions absorbed as a function of time represent the drug permeability through the intestinal membrane. In order to deconvolute plasma concentrations after the oral administration of an immediate release drug product and an oral solution or IV bolus injection, in vivo dissolution or in vivo dissolution/absorption is obtained.

In vivo dissolution calculated by means of the numerical deconvolution of the plasma concentrations of Isocillin® tablets is presented in Fig. 2 and compared to the in vitro dissolution profile. In both cases more than 80% is dissolved in 30 min. Based on these results it is obvious that phenoxymethylpenicillin potassium very rapidly permeates through

the intestinal membrane and absorption is mainly controlled by gastric emptying.

Due to the high solubility and immediate release of the drug product, in vitro dissolution does not control absorption as long as the in vitro dissolution of at least 80% in 30 min is guaranteed.

USP 23 lays down more than 80% in 45 min as an acceptance limit.

In accordance with the biopharmaceutics classification system [8], phenoxymethylpenicillin potassium belongs to Case 1 (absorption is controlled by gastric emptying). In this case, the task of in vitro dissolution is not predictive for absorption. Therefore, the test may be performed periodically for batch control as long as the manufacturing process is validated. In specific cases, in vitro dissolution tests may be replaced by disintegration.

For the development of glimepiride in vitro and in vivo comparison, in vivo dissolution was calculated by means of numerical deconvolution using the glimepiride plasma concentrations of a bioavailability study.

In vivo and in vitro dissolution data for Amaryl® tablets are presented in Fig. 3.

In vivo dissolution shows a prolonged release profile whereas more than 80% is dissolved in approximately 4 h. The prolonged dissolution is caused by the low, and pH-dependent, solubility of the drug substance.

Fig. 3 shows that absorption/permeability is not the rate-limiting step; more than 80% of glimepiride is absorbed in 1 h after administration of an oral solution of the drug substance.

In contrast to in vivo dissolution, glimepiride is dissolved in vitro very rapidly after 15 min (>80%). No correlation exists between in vitro dissolution (>80% after 15 min) and in vivo dissolution (>80% after 4 h) because of the pH-dependent, low solubility of the drug.

Dose linearity is given for the dose range of 1 to 8 mg by the areas under the concentration time profiles and the peak concentrations, which indicates that the acceptance criteria of in vitro dissolution should be identical for all strengths. In addition, the aim is to set identical specifications worldwide.

The design of the in vitro dissolution test takes into consideration the pH-dependent solubility of glimepiride, the strengths of the tablets (1 to 8 mg), dose linearity over the dosing range and, finally, sink conditions (concentration of the dissolved drug substance does not exceed 30% of saturation) for testing the highest strength. With respect to a uniform acceptance criteria for all strengths on the international market, an acceptance limit of >80% in 15 min was set.

Such a acceptance criteria may be seen as a product-specific concept taking into account all biopharmaceutical aspects such as pharmacokinetics and technological issues including specific micronisation processes. This very stringent acceptance limit should not necessarily be used as a general concept for the acceptance criteria of in vitro dissolution.

With regard to the biopharmaceutics classification system, glimepiride may belong to Case 2.

In vivo dissolution/absorption of levofloxacin tablets was calculated by numerical deconvolution from the plasma concentrations measured in an absolute bioavailability study.

In vivo absorption is slightly prolonged when compared with in vitro dissolution (Fig. 4). More than 80% was dissolved in vitro after about 30 min and in vivo after about 1 h. With respect to the prolonged absorption of levofloxacin an acceptance limit of >80% in 30 min was set. The reason for the delayed in vivo absorption has to be seen in the strong hydrophilic character of levofloxacin which may be deduced from the low partition coefficient (Tab. 7). The partitioning of levofloxacin between water and n-octanol tends to the aqueous phase. Maximal partitioning (about 0.4) was determined at pH 7.4 and minimal partitioning (<0.05) was observed at pH 1.4.

Since absorption is almost complete and solubility is high, according to the biopharmaceutics classification system, levofloxacin may be in agreement with Case 1.

4. Acceptance criteria for in vitro dissolution

Dissolution test times and acceptance criteria are usually established on the basis of an evaluation of the dissolution profile data. Typical acceptance criteria for the amount of drug substance dissolved, expressed as a percentage of the labelled content (Q), are in the range of 70% to 80% (Q) dissolved. A Q-value in excess of 80% is not generally used, as allowance needs to be made for assay and content uniformity ranges [2].

Nevertheless, the task of in vitro dissolution should be defined in the light of the biopharmaceutics classification system [8]. As a consequence of the final classification, the testing concept for batch control should be established. The final decision for batch control may be made in accordance with the following classification. If the drug substance belongs to the cases described below, the following recommendations are made for batch control.

Case 1, no batch control (in vitro dissolution) may be necessary, periodical testing is sufficient; disintegration test may be used as a surrogate for dissolution testing. Case 2, in vitro dissolution should be performed routinely for batch control besides other tests (e.g. particle size distribution of the drug substance). Case 3, see Case 1. Case 4, decision for the specific drug substance/drug product is necessary.

Acknowledgements

The authors gratefully thank Dr. K.-K. Hwang for performing the transport experiments and Dr. B. Rosenkranz and Dr. W. Pohler for the critical reading of the manuscript.

References

[1] A. Rudman, L. Lesko, H. Malinowski, S. Roy, R.L. Williams, Immediate release solid oral dosage forms. Scale-up and post-approval changes. Guidance for industry, chemistry, manufacturing, and controls. In vitro dissolution testing, in vivo bioequivalence documentation, Pharm. Technol. 3 (1996) 50–61.

[2] USP 23, General Information, in: In Vitro and In Vivo Evaluation of Dosage Forms.

[3] S.V. Dighe, Development of dissolution tests for immediate release and modified release oral dosage forms, Med. Pharm. Scientific Publishers, Stuttgart, 1995.

[4] FIP Guidelines for Dissolution Testing of Solid Oral Products, Draft. 1997.

[5] International Conference on Harmonisation (ICH 2), Orlando, Florida, 1993.

[6] H. Möller, E. Wirbitzki, Validation of in vitro dissolution, S.T.P. Pharma. Practiques 5 (1993) 348–352.

[7] International Conference on Harmonisation (ICH 3), Guidelines for Validation of Analytical Procedures, Yokohama, Japan, 1995.

[8] G.L. Amidon, H. Lennernas, V.P. Shah, J. Crison, A theoretical basis for a biopharmaceutical drug classification: The correlation of in vitro drug product dissolution and in vitro bioavailability, Pharm. Res. 12 (1995) 413–417.

[9] Amidon et al., AAPS-CRS-FDA-Workshop on Scientific Foundation and Applications for the Biopharmaceutics Classification System and In Vitro-In Vivo Correlations, (April 1997, Arlington VA).

[10] D.M. Oh, R.L. Curl, G.L. Amidon, Estimating the fraction dose absorbed from suspensions of poorly soluble compounds in humans: a mathematical model, Pharm. Res. 10 (1993) 264–268.

[11] G. Wilson, I.F. Hassan, C.J. Dix, I. Williamson, R. Shah, M. Mackay, Transport and permeability properties of human CaCo-2 cells: an in vitro model of intestinal epithelial cell barrier, J. Control. Rel. 11 (1990) 25–32.

[12] A. Frick, Methods to assess intestinal absorption in drug discovery, Doctoral thesis (in preparation).

[13] X. Boulenc, T. Breul, J.C. Gautier, P. Saudemon, H. Joyeux, C. Roques, Y. Berger, G. Fabre, Sodium lauryl sulfate increases tiludronate paracellular transport using human epithelial CaCo-2 monolayers, Int. J. Pharm. 123 (1995) 71–78.

[14] E. Walter, S. Janich, B.J. Roessler, J.M. Hilfinger, G.L. Amidon, HT29-MXT- CaCo-2-cocultures as an in vitro model for the intestinal epithelium: in vitro-in vivo correlation with permeability data from rats and humans, J. Pharm. Sci. 85 (1996) 1070–1077.

[15] B. Bauer, V. Malerczyk, H.J. Roethig, Bioavailability of a new formulation of penicillin V (Isocillin® syrup) and the influence of ethanol on bioavailability of penicillin V, Int. J. Clin. Pharm. Ther. Tox. 27 (1989) 583–587.

[16] M. Badian, A. Korn, K.H. Lehr, V. Malerczyk, W. Waldhäusel, Absolute bioavailability of glimepiride (Amaryl®) after oral administration, Drug Metab. Drug Int. 4 (1994) 331–339.

[17] Internal Report (Hoechst Marion Roussel Deutschland GmbH).

[18] The Pharmaceutical Codex, seventh ed., Pharmaceutical Press, London 1994.

[19] P. Artursson, J. Karlsson, Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (CaCo-2) cells, Biochem. Biophys. Res. Com. 3 (1991) 880–889.