

Comparison of bovine *in vivo* bioavailability of two sulfamethazine oral boluses exhibiting different *in vitro* dissolution profiles

M. N. MARTINEZ*

J. C. KAWALEK[†]

K. D. HOWARD[†]

J. L. WARD[†]

P. MARROUM[‡]

W. MARNANE*

D. BENSLEY*

F. R. PELSOR*

S. HOAG[§]

A. S. TATAVARTI[§]

L. XIE[§] &

R. FAHMY*

*FDA Center for Veterinary Medicine, Office of New Animal Drug Evaluation, Rockville, MD; [†]FDA Center for Veterinary Medicine, Office of Research, Laurel, MD; [‡]FDA Center for Drug Evaluation and Research, Office of Clinical Pharmacology, Silver Spring, MD; [§]University of Maryland School of Pharmacy, Baltimore, MD, USA

Martinez, M. N., Kawalek, J. C., Howard, K. D., Ward, J. L., Marroum, P., Marnane, W., Bensley, D., Pelsor, F. R., Hoag, S., Tatavarti, A. S., Xie, L., Fahmy R. Comparison of bovine *in vivo* bioavailability of two sulfamethazine oral boluses exhibiting different *in vitro* dissolution profiles. *J. vet. Pharmacol. Therap.* 29, 459–467.

The bolus (or oblet) is a dosage form that can be used for the oral administration of pharmaceutical compounds to ruminating species. Unlike traditional tablets, oral boluses may contain quantities of drug on the order of grams rather than milligrams. Due to its size, it is only recently that USP-like *in vitro* dissolution methods have been developed for this dosage form. However, whether or not these dissolution tests can predict product *in vivo* performance has yet to be determined. The importance of this issue is apparent when the U.S. Food and Drug Administration Center for Veterinary Medicine is faced with the decision of whether to require additional *in vivo* bioequivalence study data to support the approval of changes in product chemistry or manufacturing method. The current study was undertaken to determine whether an *in vivo/in vitro* correlation can be established for bovine sulfamethazine oral boluses and to acquire insight into the magnitude of changes in *in vitro* product performance that can occur before corresponding changes are seen in *in vivo* blood level profiles. Based upon the results of this investigation, it is concluded that marked changes in *in vitro* sulfamethazine bolus performance can be tolerated before resulting in altered *in vivo* blood level profiles. However, the data also suggest that rumenal absorption may occur for some compounds. Therefore the degree to which variation in product *in vitro* dissolution profiles can be tolerated may be compound specific.

(Paper received 25 April 2006; accepted for publication 11 July 2006)

Marilyn N. Martinez, FDA Center for Veterinary Medicine, Office of New Animal Drug Evaluation, 7500 Standish Place, HFV-130, Rockville, MD, USA. E-mail: marilyn.martinez@fda.hhs.gov

INTRODUCTION

It is not unusual for manufacturers to modify the composition or manufacturing method of a product over the lifetime of that product. When this occurs, the U.S. Food and Drug Administration (FDA) must determine whether or not that revision alters product safety and effectiveness. In cases of Category II supplements (which includes changes in the active ingredient concentration, composition of the excipients, and changes in the manufacturing process of the new drug substance and/or final dosage form), the FDA Center for Veterinary Medicine (CVM)

may require that the sponsor verify that the product performance is comparable with that of the original approval.

Under certain circumstances, when additional confirmation of *in vivo* performance is needed, *in vitro* dissolution data can be used as a surrogate for demonstrating *in vivo* product bioequivalence. Although a number of FDA guidance documents have been published for these situations as they pertain to human pharmaceuticals (e.g. Scale-Up Post-Approval Changes for Immediate Release Solid Oral Dosage Forms: SUPAC-IR; FDA/CDER Guidance for Industry, 1995), similar guidance documents have not been developed for use in veterinary medicine. While *in vitro* dissolution is employed as a quality control tool by CVM, interspecies differences in gastrointestinal (GI) physiology and the marketing of species-specific dosage forms have impeded its use as a surrogate for confirming veterinary product bioequivalence. Therefore, studies are

The views expressed in this article are those of the authors and do not reflect the official policy of the FDA. No official support or endorsement by the FDA is intended or should be inferred.

needed to determine if and when *in vivo/in vitro* correlations (IVIVC) can be established.

The use of *in vitro* data as a surrogate for *in vivo* bioequivalence is founded upon an understanding of the relationship between the aqueous solubility and intestinal permeability of a molecule, the *in vitro* dissolution characteristics of the formulation, and the rate and extent of *in vivo* drug absorption. With regard to solubility and permeability, a biopharmaceutics classification system (BCS) has been developed for human pharmaceuticals as a tool for predicting formulation effects on the rate and extent of oral drug absorption. According to the BCS, pharmaceutical compounds can be grouped into one of four categories (Amidon *et al.*, 1995):

Class I: High solubility, high permeability: generally very well-absorbed compounds;

Class II: Low solubility, high permeability: exhibit dissolution rate-limited absorption;

Class III: High solubility, low permeability: exhibits permeability-limited absorption;

Class IV: Low solubility, low permeability: very poor oral bioavailability.

For human oral dosage forms, years of research served to validate the accuracy of bioequivalence determinations based upon comparative *in vitro* dissolution data when products contain class I compounds (FDA/CDER Guidance for Industry, 2000). For products containing class I compounds, when products exhibit greater than 85% dissolved in 15 min, the two formulations are declared bioequivalent. For class II compounds, absorption may be dissolution rate-limited. For class IV compounds, absorption may be limited both by dissolution and permeability. Extension of BCS principles to support biowaivers for compounds other than those in class I is still being debated (Polli *et al.*, 2004).

For slow dissolving formulations, it is possible to establish a relationship between the *in vitro* dissolution profile and the *in vivo* blood level profile. In the FDA/CDER Guidance for Industry (1997a), the three categories of IVIVC are described as follows:

- **Level A.** This correlation is usually estimated by a two-stage procedure: deconvolution followed by a comparison of the fraction of drug absorbed to the fraction of drug dissolved. A correlation of this type is generally linear and represents a point-to-point relationship between *in vitro* dissolution and the *in vivo* input rate (e.g. the *in vivo* dissolution of the drug from the dosage form). The level A IVIVC, results in the prediction of the entire *in vivo* time course from the *in vitro* data.
- **Level B.** This correlation uses the principles of statistical moment analysis. The mean *in vitro* dissolution time is compared either to the mean residence time or to the mean *in vivo* dissolution time. The level B correlation, like level A, uses all of the *in vitro* and *in vivo* data, but is not considered to be a point-to-point correlation and does not uniquely reflect the actual *in vivo* plasma level curve, because a number of different *in vivo* curves will produce similar mean residence time values.

- **Level C.** This correlation establishes a single-point relationship between a dissolution parameter, for example, the time for 50% dissolution or the percentage dissolved in X hours and a pharmacokinetic parameter (e.g. area under the curve, AUC). The level C correlation does not reflect the complete shape of the plasma concentration–time curve.

To date, BCS principles have generally not been applied to support biowaivers for solid oral dosage forms in veterinary medicine because the criteria for highly soluble, highly permeable and rapidly dissolving have not been adequately defined for species other than humans. To address this question for dogs, an expert ad hoc panel has been convened by the US Pharmacopoeia (Martinez *et al.*, 2004). Far more challenging, however, is the extrapolation of BCS principles and efforts to establish an IVIVC for solid oral dosage forms intended for administration to ruminants.

The stomach of ruminants is complex, consisting of four distinct chambers, each with its own unique environment and rate of material movement (Austgen *et al.*, 1998). Therefore, oral drug absorption in a ruminant may exhibit markedly different kinetics when compared with those associated with monogastric species. One of the few solid oral dosage forms used in ruminants is the oral bolus. Boluses are large tablets, often containing several grams of the active pharmaceutical ingredient (API). Until recently, because of the size of these boluses and the low aqueous solubility of the API, no discriminative *in vitro* method utilizing traditional US Pharmacopoeia (USP) equipment was available. However, Fahmy *et al.* (2001) published *in vitro* dissolution methods for sulfamethazine boluses that employed USP apparatus II and 900 mL of dissolution medium. The predictive ability of these methods to identify *in vivo* product performance remained a critical but unanswered question. Therefore, CVM initiated a project to compare the *in vitro* performance vs. the oral bioavailability of sulfamethazine 2.5 g oral boluses with different release characteristics.

For this investigation, Fahmy *et al.* (2003) and scientists at the University of Maryland formulated batches with markedly different *in vitro* performance. The batches exhibiting fastest and slowest rates of sulfamethazine release were selected for *in vivo* testing. For the *in vitro* portion of this investigation, *in vitro* dissolution profiles were generated under a variety of conditions. For the *in vivo* component, the oral bioavailabilities of these two sulfamethazine bolus formulations were compared with each other and to an approved sulfamethazine oral solution. Thus, the current study was conducted in an effort to address the following questions:

- 1 Do the complex physiological characteristics of the rumen mask differences observed between the *in vitro* dissolution profiles of products manufactured under very different conditions or can an IVIVC be established?
- 2 Considering the complex nature of the rumen, are conventional bioequivalence criteria (i.e. AUC and C_{max}) able to detect formulation-related differences in drug absorption characteristics?

METHODS

In-life procedures

This study was conducted in accordance with the U.S. Food and Drug Administration (2001) *Good Laboratory Practice Regulations for Nonclinical Laboratory Studies*, along with the National Research Council (1996) *Guide for the Care and Use of Laboratory Animals*, under a protocol approved by FDA/CVM's Office of Research Institutional Animal Care and Use Committee.

Calves were transported by stock trailer to the CVM research facility in Laurel, MD. Thirty-six healthy ruminating calves (119–183 kg at time of dosing) were used in this parallel design three-treatment study (12 animals/treatment group). The parallel design was selected to eliminate potential problems arising from drug carryover between periods and to minimize effects of animal weight gain over the experimental period (during the pilot study animals gained anywhere from 2 to 10 kg over a 10 day period).

Animals were obtained from local farms by the United States Department of Agriculture (USDA). They arrived in groups of 12 at the beginning of each month during the animal phase of the study (June–August, 2004). Animals were all generally higher in weight than originally stipulated in the protocol (~100 kg) and there was usually a rather large range in body weights. To compensate for differences in body weights, the animals were treated in decreasing order of their body weights. Test group assignments were based on the following randomization scheme: first, sort by decreasing body weight and then sort by increasing 'farm #'. The first animal was assigned to the 'A' treatment group, secondly animal was assigned to the 'C' treatment group, and third animal was assigned to the 'Control' group, etc. All animals were dosed with 2.5 g of sulfamethazine, either as a single oblet of the test articles or as 20 mL of the control article. The average calculated dosages for the different treatment groups (slow dissolving bolus, fast dissolving bolus, and oral solution) were 17.0, 17.2 and 17.7 mg sulfamethazine/kg body weight, respectively. ANOVA indicated that there were no significant differences between either of the test groups. Similarly, body weights in the test groups were checked by ANOVA and no significant differences were noted between the different groups.

Calves were identified by attachment of an ear tag at the farm or upon their arrival at the CVM Office of Research. They were housed in individual indoor pens on straw bedding, which was spot cleaned of fecal material daily and strip cleaned twice weekly. Barn temperature was maintained at 18–22 °C with a light cycle of approximately 10:14 (light:dark). All animals had access to fresh water and hay *ad libitum* throughout the study. They received a once daily feeding of a standard grain-based diet at an amount of approximately 1% of feed per unit body weight (w/w).

The components of the boluses are sulfamethazine, corn starch and magnesium stearate. The formulations were wet granulated with a 10% (w/v) starch paste in a high shear granulator and dried at 60 °C in a convection tray dryer. The granulations were prepared and tested by the Department of

Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, MD. The tablets were compressed on a Stokes B2 rotary tablet press running at 30 r.p.m. under GMP conditions by Fort Dodge Laboratories, Princeton, NJ, USA. The two formulations were compressed at two different compression forces (low = 8–9 kN and high = 22–24 kN). The fast and slow dissolving test articles were individually supplied and stored in clear glass scintillation vials that were labeled with the last four digits of the log number and a sequential bolus number, ranging from 1 to 24. The control article was Sulmet Drinking Water Solution® (12.5%, lot no. 031480, expiration, 10/08, manufactured by Fort Dodge Laboratories).

Indwelling jugular catheters were inserted in individual animals prior to the initiation of the study. Nineteen blood samples (~6 mL) were collected from indwelling jugular catheters over a 72-h collection period. During sampling, the animals were restrained by the use of halters. Samples were taken predose and at hours 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 24, 32, 48 and 72 postdose. Whole blood samples were centrifuged at approximately 1000 *g*. The plasma was harvested and stored frozen at –80 °C until analysis. All samples were assayed for concentrations of sulfamethazine using a validated high-performance liquid chromatography (HPLC) method.

HPLC method

Samples and standards were prepared essentially as described by Nouws *et al.* (1985, 1986). Plasma obtained from untreated cows in our dairy herd was used to prepare standard solutions containing 0.25–100 µg sulfamethazine/mL plasma. These stock solutions were subsequently diluted 1:21 using commercially available phosphate-buffered saline to obtain working standard solutions nominally containing 0.010, 0.025, 0.050, 0.10, 0.25, 0.50, 1.00, 2.50, and 5.00 µg sulfamethazine/mL. Triplicate 25-µL aliquots of each sample were diluted with 0.5 mL phosphate-buffered saline. Standards, a plasma blank, and samples were acidified by adding 50 µL of pH adjustment (protein precipitation) buffer (prepared by mixing 24.8 mL 1 M NaOAc, pH 5 buffer + 15.2 mL 0.2 M HOAc.). Ethyl acetate (1 mL) was added to each tube, which was capped and vortex-mixed for 10–20 sec, then centrifuged for 5 min at 16 000 *g* in an Eppendorf Model 5400 microcentrifuge (Eppendorf North America, Westbury, NY). The ethyl acetate layer (0.6 mL) was transferred to glass 12 × 75 mm tubes and dried under nitrogen with a Zymark TurboVap at 50 °C. The residue was redissolved in 0.15 mL 0.02 M sodium acetate buffer, pH 5 and transferred to 96-well microtiter plates vials for LC analysis.

Separations were performed using an Agilent 1100 HPLC (Agilent Technologies, Wilmington, Germany) under control of Agilent's Chemstation (rev. A.10.03) on Zorbax Eclipse XDB C8 columns (150 × 4.6 mm, 5 µm) at 35 °C with a mobile phase containing 10% acetonitrile and 15% methanol in 20 mM sodium acetate, pH 5 buffer, at 2 mL/min with detection at 265 nm. Total run time was 3 min with elution of sulfamethazine at 2.2 min. The detection limit for sulfamethazine was 0.01–0.025 µg/mL.

In vitro dissolution

Work previously completed successfully characterized the *in vitro* behavior of these sulfamethazine boluses (Fahmy *et al.*, 2001). In that article, which reflects an intensive collaborative research effort between CVM and the University of Maryland School of Pharmacy, the basic conditions were established for developing a discriminative *in vitro* method for testing these veterinary boluses using standardized USP equipment (i.e. Apparatus II). On the basis of their findings, additional dissolution testing was conducted in an effort to establish *in vitro* conditions that could reflect *in vivo* differences in product performance. The *in vitro* dissolution profiles were examined under the following conditions:

- 1 USP Apparatus II, 75 r.p.m.
 - (a) 0.1 N HCL + 0.2% sodium dodecyl sulfate (SDS)
 - (b) 0.1 N HCL + 0.5% SDS
 - (c) 0.1 N HCL + 1% SDS
- 2 USP Apparatus II, 125 r.p.m.
 - (a) 0.1 N HCL + 0% SDS
 - (b) 0.1 N HCL + 1% SDS

Although by convention, paddle speeds in excess of 75 r.p.m. are not recommended when testing conventional immediate release tablet formulations, the very rapid speed of 125 r.p.m. was used due to the very large size of these sulfamethazine boluses. Samples of the dissolution medium were taken at 0, 0.25, 0.5, 1, 1.5, 2 and 4 h across all dissolution conditions. A 3-h sample was included for the 75 r.p.m. 1% SDS condition and a 6-, 8-, and 24-h sample was included for the assessment of dissolution of the slow dissolving bolus at 125 r.p.m. 0% SDS. Drug concentrations were measured using a UV spectrophotometer (Spectronic GENESYS 2; Spectronic Instruments, Rochester, NY, USA) at 240 nm.

Due to the limited number of boluses available, only one or two units were evaluated at several of the test conditions. However, tests run at 75 r.p.m. with 1% SDS (the conditions used for assessing the ability to generate an IVIVC) were conducted on 6 units of the fast and slow dissolving boluses.

Pharmacokinetic analysis

Due to the fluctuations in drug concentrations observed in many of the subjects, regardless of treatment group, there was no attempt to fit the data to a specific compartmental model. However, based upon previously published intravenous data in cattle (Bevill *et al.*, 1977), sulfamethazine follows a monoexponential decline, thereby suggesting that without the complexities of oral administration, the drug follows a one-compartment body model.

Two methods of data analysis were employed. For the determination of product bioequivalence, the WinNonLin software (version 4.0.1; Pharsight, Cary, NC, USA) was used to generate noncompartmental parameter values. The estimated pharmacokinetic parameters included the area under the curve

(AUC) from time zero to the last quantifiable concentration (AUC_{0-last}), AUC from time zero and extrapolated to time infinity ($AUC_{0-\infty}$), the observed peak concentration (C_{max}), the time to C_{max} (T_{max}), and the terminal slope (λ_z). Terminal elimination half-life was estimated as $0.693/\lambda_z$, where λ_z included no less than three sequential timepoints. $AUC_{0-\infty}$ was estimated as $AUC_{0-last} + C_{last}/\lambda_z$, where C_{last} = the last concentration at or above the limit of quantification (LOQ) of the analytical method.

In some cases, partial AUC values were estimated. In these cases, the trapezoidal rule was used to estimate areas from time zero to hour 4 postdose (AUC_{0-4}), from hours 5 to 12 postdose (AUC_{5-12}), from hours 14 to 24 postdose (AUC_{14-24}) and from hour 32 to the last sample associated with quantifiable drug concentrations ($AUC_{32-last}$).

Assuming that sulfamethazine follows a one-compartment open body model, the percentage of drug absorbed was estimated using the Wagner–Nelson equation (Wagner & Nelson, 1963). The percentage absorbed was estimated either relative to the total administered dose (a between-subject estimation procedure) or relative to the proportion of the total bioavailable dose within each subject.

Within-subject characterization of drug absorption

Based upon our analysis, we assumed that on average, formulation did not influence the terminal rate of decline. However, there was substantial within-subject variability observed. Therefore, using that subject's own value of $AUC_{0-\infty}$ and k_{el} (which equals λ_z for drugs that follow a one-compartment open body model), the Wagner–Nelson method was used to estimate the proportion of the total bioavailable dose that was absorbed up to any given point in time within that subject.

Evaluation of an IVIVC

As described in the FDA/CDER Guidance for Industry (1997b) a level A correlation is usually estimated by a two-stage procedure: deconvolution followed by comparison of the fraction of drug absorbed to the fraction of drug dissolved. A correlation of this type is generally linear and represents a point-to-point relationship between *in vitro* dissolution and the *in vivo* input rate (e.g. the *in vivo* dissolution of the drug from the dosage form). In a linear correlation, the *in vitro* dissolution and *in vivo* input curves may be directly superimposable or may be made to be superimposable by the use of a scaling factor. For the deconvolution component of this analysis, the Wagner–Nelson method was employed. As our investigation was conducted as a parallel study design, efforts to obtain an IVIVC were based upon the mean concentration–time profiles of the three treatment groups. To execute this mathematical procedure, an estimate of the terminal elimination half-life for the molecule in question is needed. As all animals were randomly assigned to treatment group (oral solution, slow dissolving bolus or fast dissolving bolus), it was assumed that on average, the three groups were physiologically indistinguishable. Therefore, we anticipated no treatment-related differences in the mean values of sulfamethazine clearance and volume of distribution, thereby enabling us to apply the Wagner–Nelson method.

Statistical analysis

Statistically significant differences between treatments were determined using an analysis of variance (ANOVA) procedure where the fixed variable was treatment. The Proc GLM procedure of the SAS statistical software (Version 8.2; SAS Institute Inc., Cary, NC, USA) was employed. To ascertain how any two formulations would compare if this study was conducted to as a tradition bioequivalence trial, the 90% confidence intervals about the ratio of treatment mean values were determined on the basis of the random error associated with pairwise (rather than three-way) comparisons. The confidence intervals were generated using log-transformed values of AUC_{0-last} and C_{max} in accordance with methods described in the FDA/CVM Guidance for Industry (2002).

RESULTS

In vitro dissolution

The rate of *in vitro* dissolution increased both as a function of the percentage SDS included in the dissolution medium and of the paddle speed. The *in vitro* results generated under an array of conditions are provided in Fig. 1.

Without SDS, a very rapid paddle speed (125 r.p.m.) was necessary to achieve 100% dissolution for the slow release bolus. However, as seen in Table 1, when this rapid speed was employed, the dissolution rate was highly variable. Therefore, it was concluded that the *in vitro* dissolution condition of 1% SDS 75 r.p.m. was the optimal method for our effort to establish an IVIVC. This method resulted in a relatively rapid *in vitro* release, but still allowed for the differentiation of the fast and slow releasing formulations.

In vivo bioavailability

There was a high level of intersubject variability in the blood level profiles, regardless of treatment group. Several subjects exhibited multiple peaks and troughs. In a few cases, subjects had a relative flat terminal elimination half-life that suddenly

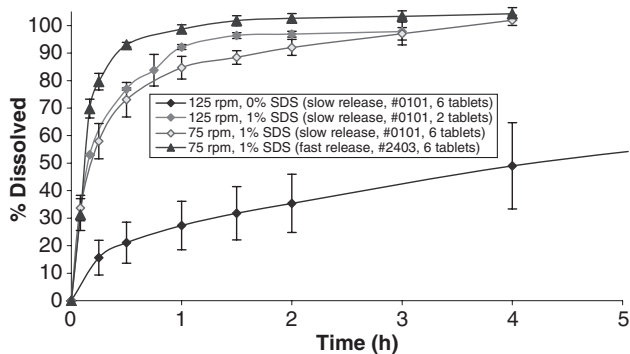


Fig. 1. Dissolution profiles under different dissolution situation (slow release batch, batch no. 0101).

Table 1. Comparison of formulation C release rates with varying levels of sodium dodecyl sulfate (SDS) and paddle speed ($n = 6$ per method)

Time	75 r.p.m. 1% SDS		125 r.p.m. 0% SDS	
	Mean	%CV	Mean	%CV
0	0.00	0.00	0.00	0.00
0.25	57.96	11.08	15.63	40.28
0.5	73.06	8.57	21.09	35.38
1	84.67	4.87	27.30	32.34
1.5	88.43	2.79	31.75	30.50
2	92.03	3.17	35.39	29.88
3	97.01	2.33		
4	101.95	1.83	49.00	31.98
6			58.25	29.88
8			65.72	26.10
24			103.86	2.95

dropped off, resulting in sulfamethazine drug concentrations that dipping below the LOQ of the analytical method. The mean profiles are provided in Fig. 2. Although the rate of absorption appeared to be faster for those subjects receiving the oral solution, there was surprisingly little difference in the profiles associated with the fast vs. slow dissolving bolus.

The mean pharmacokinetic parameter values associated with the three treatment groups are provided in Table 2. As the drug was administered as a uniform milligram dose per subject, the confidence intervals about the ratios of treatment mean values were estimated using both the unadjusted and the dose-normalized values (Table 3). From these calculations, we see that despite differences in the *in vitro* profiles, the C_{max} values were surprisingly similar. Across all comparisons, the C_{max} values succeeded in meeting our traditional bioequivalence criteria based upon the log-transformed values (0.80 to 1.25). The T_{max} of the oral solution occurred somewhat earlier (4.6 h) than did that of either the fast ($T_{max} = 6.2$ h) or the slow dissolving boluses ($T_{max} = 6.4$ h).

A difference in the mean values of AUC_{0-last} was observed between treatments. These differences occurred even when the AUC values were normalized for administered dose. Although these differences were not statistically significant ($P > 0.05$ based upon a three-treatment study design), the high intersubject variability (and consequently, the low statistical power) was likely to have confounded our ability to generate statistical inferences. Therefore, potential reasons for this disparity were explored.

The first question pertained to potential differences in the percentage absorbed. To this end, the partial AUC values (AUC_{0-4} , AUC_{5-12} , AUC_{14-24} and $AUC_{32-last}$) were compared. The results of this analysis are provided in Table 4.

While the AUC_{0-4} of the oral solution was significantly greater than that of the two bolus formulations, the two bolus formulations were indistinguishable. No statistically significant treatment differences were observed for any of the other partial AUC values. When analyzed as a two-treatment parallel design to compare the fast and slow dissolving boluses, the confidence limits were contained within 0.80 to 1.25 only during hours

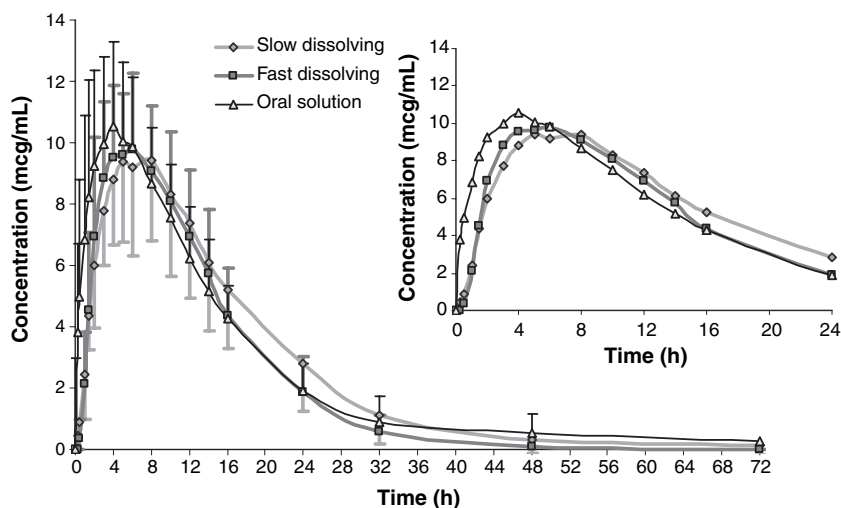


Fig. 2. Plasma sulfamethazine concentration after administration of three formulations with markedly different *in vitro* dissolution profiles. The insert provides an expanded view of differences in mean profiles over the first 24 h postdose.

Table 2. Mean pharmacokinetic parameter values (%CV)

	Solution	Fast bolus	Slow bolus
λ_z (h^{-1})	0.104 (53)	0.138 (34)	0.122 (38)
$T_{1/2}$ (h)	6.66	5.02	5.68
AUC_{0-last} ($\mu g \cdot h/mL$)	176 (29)	161 (34)	172 (29)
$AUC_{0-last}/dose$	9.91	9.02	10.65
$AUC_{0-\infty}$ ($\mu g \cdot h/mL$)	199 (46)	162 (34)	175 (31)
C_{max} ($\mu g/mL$)	11.1 (30)	10.2 (23)	9.9 (27)
$C_{max}/dose$	0.62	0.59	0.58
T_{max}	4.6 (46) ^a	6.2 (29) ^b	6.4 (23) ^b

^{a,b}Values with like letters are not statistically significantly different ($P > 0.05$).

5–12. However, considering the similarity in mean values, the extension beyond the upper equivalence bound of 1.25 most likely reflects random variability.

To compare the respective rates of absorption, the percentage of the bioavailable dose absorbed as a function of time was estimated within each individual subject. In this analysis, the values of k_{el} and $AUC_{0-\infty}$ used in the Wagner–Nelson equation were those values estimated within each subject for that specific treatment. A plot of the results (mean \pm SEM) is provided in Fig. 3 (hours 0–24).

In all cases, although the oral solution was absorbed more rapidly than the two bolus formulations, approximately 100% of the total absorption from each formulation occurred within 24 h

Comparison	AUC_{0-last}		C_{max}		Ratio of treatment mean values	AUC	C_{max}
	Lower CL	Upper CL	Lower CL	Upper CL			
A vs. C – normalized*	0.93	1.41	0.84	1.11	A/C	1.18	0.98
A vs. C – not normalized	0.91	1.41	0.81	1.13	A/C	1.15	0.97
A vs. Solution – normalized	0.84	1.28	0.80	1.08	A/Solution	1.07	0.94
C vs. Solution – normalized	0.76	1.08	0.84	1.09	C/Solution	0.91	0.95

*A = slow dissolving bolus; C = fast dissolving bolus; CL = confidence limit.

Table 4. Mean partial AUC values for each treatment group (%CV)

	Solution	Slow	Fast	Slow vs. fast	
				Lower CL	Upper CL
AUC_{0-4}	51.8 ^a (48)	24.5 ^b (38)	22.2 ^b (30)	0.85	1.34
AUC_{5-12}	68.7 (24)	70.1 (27)	70.7 (26)	0.83	1.17
AUC_{14-24}	45.5 (38)	54.8 (34)	51.3 (45)	0.84	1.50
$AUC_{32-last}$	29.8 (113)	24.4 (71)	21.2 (56)	0.55	1.99

^{a,b}Values with like letters are not statistically significantly different. CL = confidence limit.

postdose. On the basis of this plot, the absorption rates from the two solid oral dosage forms were virtually indistinguishable. These results do not support the possibility that differences in $AUC_{0-\infty}$ values of treatment A vs. C was attributable to a prolonged absorption

In vivo/In vitro correlation

An estimate of the percentage of the dose absorbed is necessary for evaluating whether or not there is a correlation between the percentage of administered dose absorbed vs. the percentage of drug dissolved over time. To estimate bioavailability, a reference standard, the oral solution, provided the estimates of k_{el} and $AUC_{0-\infty}$ used to determine the percentage of dose absorbed from

Table 3. Confidence intervals and ratios for AUC_{0-last} and C_{max} values

Comparison	AUC_{0-last}		C_{max}		Ratio of treatment mean values	AUC	C_{max}
	Lower CL	Upper CL	Lower CL	Upper CL			
A vs. C – normalized*	0.93	1.41	0.84	1.11	A/C	1.18	0.98
A vs. C – not normalized	0.91	1.41	0.81	1.13	A/C	1.15	0.97
A vs. Solution – normalized	0.84	1.28	0.80	1.08	A/Solution	1.07	0.94
C vs. Solution – normalized	0.76	1.08	0.84	1.09	C/Solution	0.91	0.95

*A = slow dissolving bolus; C = fast dissolving bolus; CL = confidence limit.

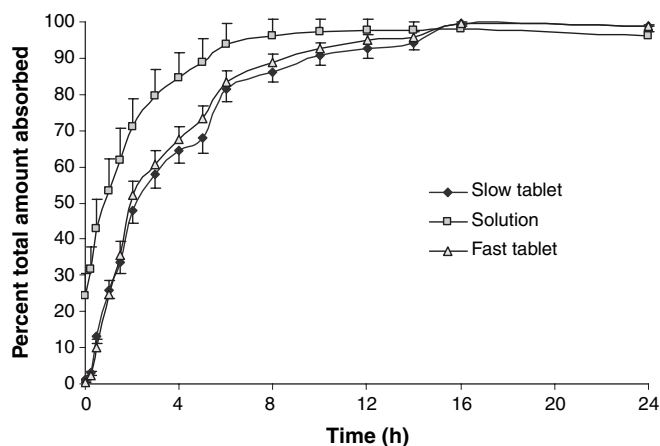


Fig. 3. Comparison of mean values for the percentage of the total amount absorbed as a function of time for each formulation.

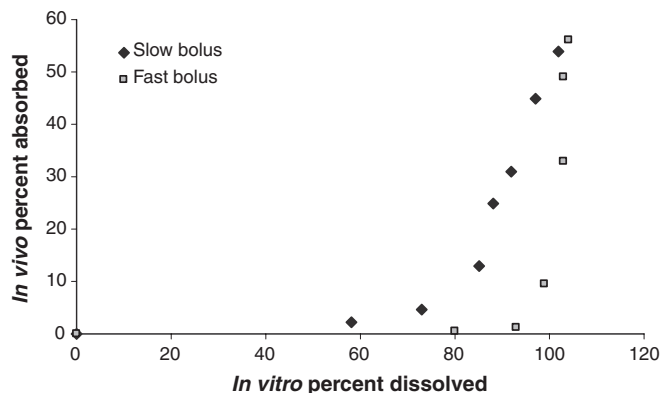


Fig. 4. Relationship between *in vitro* percentage dissolved vs. *in vivo* percentage absorbed for the fast vs. slow dissolving boluses.

the fast and slow dissolving bolus formulations at each sampling time. The percentage absorbed for each treatment mean was then plotted against the *in vitro* percentage dissolved at the corresponding time point. The resulting relationship between percentage absorbed for the fast and slow formulations are provided in Fig. 4. A regression analysis on the values observed at hours 0.5–4 resulted in a slope of 4.63 for the fast tablet and 1.78 for the slowly dissolving tablet. Thus, the two formulations did not result in a singular linear regression of percentage dissolved vs. percentage absorbed. Accordingly, we conclude that a level A IVIVC cannot be established.

DISCUSSION

One of the fundamental questions addressed in this study is whether or not *in vitro* dissolution data can be used to predict the relative bioavailability of formulations intended for use in ruminants. In response to this query, two bolus formulations with markedly different *in vitro* dissolution characteristics were examined. Despite these *in vitro* differences, the C_{max} and T_{max}

values were comparable for the slow and fast dissolving boluses. In fact, the C_{max} of the bolus formulations met the bioequivalence criteria both to each other and to that of the oral solution. This indicates that the traditional bioequivalence parameters were unable to detect differences observed in the rate of sulfamethazine absorption. However, this difference in treatment absorption rate (solution vs. bolus) was detected when examining the individual subject data using either the Wagner–Nelson equation (with each animal serving as its own control) or partial *AUC* values. While these observed differences in absorption rate are clearly present, the pertinent question from a regulatory perspective is whether or not such differences in the rate of absorption have any clinical relevance?

When an IVIVC can be established, *in vitro* dissolution tests provide a valuable prognostic tool for identifying product changes that will influence drug *in vivo* bioavailability. Although modifying the *in vitro* dissolution test identified a substantial difference in product release rate, based upon our assessments, we conclude that there was no singular method that could predict product performance. In other words, the products were different *in vitro* but comparable *in vivo*. It is likely that this disconnection between *in vivo* and *in vitro* performances is attributable to ruminant physiology (gastric transit time) rather than *in vitro* dissolution providing the rate-limiting step in drug absorption. Therefore, it is not surprising that there were marked differences in the slopes of the IVIVC regression lines for the fast and slow dissolving boluses. This suggests that oral bioavailability in ruminants is far more forgiving than would be predicted on the basis of *in vitro* dissolution test results alone. To further explore the validity of this initial conclusion, it would be helpful to repeat this study using compounds that exhibit low solubility and low permeability (i.e. BCS class IV compounds).

This investigation was not the first attempt to examine the ability to predict *in vivo* differences in bovine product bioavailability from *in vitro* dissolution data. The first such study was published by Frazier and Nuessle (1976). However, that investigation did not utilize a standard USP dissolution apparatus, did not have identical *in vivo* and *in vitro* sampling times, and the *in vitro* dissolution tests were terminated before 40% of the drug was released from three of the five formulations examined. Nevertheless, it was interesting to note that unlike the similarity in blood profiles observed across formulations in our investigation, Franzier and Nuessle observed markedly different *in vivo* profiles. Their *in vivo* data are reproduced in Fig. 5. Even more importantly, these differences appeared to correlate with the *in vitro* dissolution profiles (a level C-type correlation). The fundamental difference between their formulations and those used in our study is that while we used immediate release formulations, the boluses used by Franzier and Nuessle were sustained release products. Each of the Franzier and Nuessle boluses contained iron, which increased the weight of the tablets and caused it to remain in the rumeno-reticular sac until disintegration was complete. Therein lays a very important difference and one which needs to be factored into our conclusions. In their study, boluses were prepared with either no disintegrant or with four different disintegrants. Thus, the

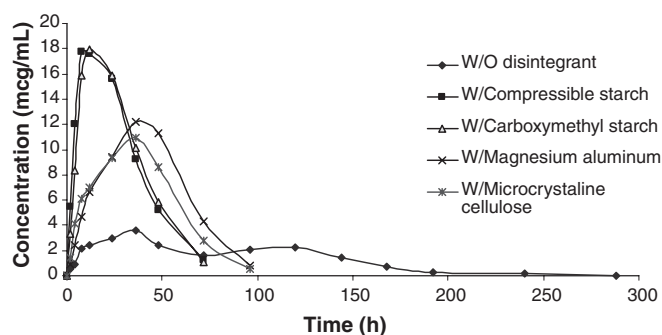


Fig. 5. Relationship between bolus formulation and *in vivo* blood level profile. Based upon the work of Frazier and Nuessle (1976).

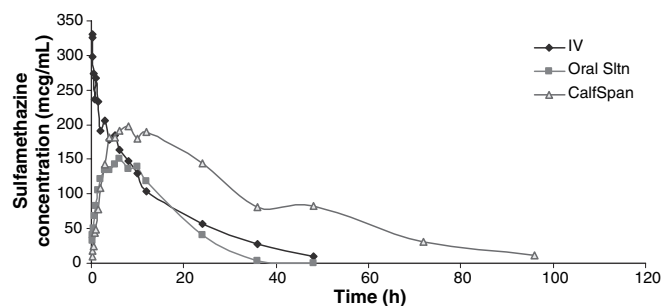


Fig. 6. Profiles of various sulfamethazine preparations in sheep. Based upon work from Bulgin *et al.* (1991).

rate-limiting factor was no longer the transit of dissolved drug or dosage form through the bovine stomach but rather the rate at which the respective products dissolved. In contrast, our formulations were rapidly dissolving relative to the rate of gastric transit (as underscored by the regression equations seen with the slow dissolving bolus). Therefore, in the current investigation, it was the rate of GI transit that was the rate-limiting factor in product bioavailability, and differences in product dissolution were without any substantial *in vivo* effect.

Along a similar line, marked differences in oral bioavailability and elimination $T_{1/2}$ were seen when sulfamethazine was administered as either an i.v. bolus, an oral solution or a sustained release bolus to sheep (Bulgin *et al.*, 1991). The data from the study by Bulgin *et al.* are reproduced in Fig. 6. In this case, the $T_{1/2}$ observed following an i.v. dose was longer than that associated with an oral solution (10.8 h vs. 4.3 h respectively). The sustained release bolus preparation (Calf-Span[®]) had a $T_{1/2}$ of 14.3 h. Reasons for the longer $T_{1/2}$ following i.v. administration could not be determined. Nevertheless, the primary point is that once again, unlike that seen with an immediate release preparation, when a sustained release formulation is involved, the rate-limiting step is no longer the gastric transit time of the ruminant, enabling differences in product dissolution to influence the *in vivo* bioavailability profile.

Interestingly, both in our investigation and in the study of Bulgin *et al.* (1991), quantifiable drug concentrations were seen in the blood within 15 min following the administration of the

oral solution. This was particularly evident in our study following the administration of the oral solution (mean concentration at 15 min postdose = 3.82 $\mu\text{g}/\text{mL}$). Low plasma sulfamethazine concentrations were also observed in many subjects within 15 min following the administration of the fast dissolving (mean concentration at 15 min postdose = 0.08 mg/mL) and slow dissolving boluses (mean = 0.48 $\mu\text{g}/\text{mL}$). Considering that the half-life of fluid transfer in the stomach of the ruminant is on the order of approximately 5–7 h (Faichney & Griffiths, 1978), if sulfamethazine bioavailability depended upon intestinal absorption, we would anticipate the presence of a substantial input lag time. Clearly, this did not occur, suggesting that at least some portion of the dose was capable of being systemically absorbed while still in the rumen.

The rumen is a fermentation vat that can hold up to 60 gallons of material. The temperature (100–108 °F), pH (5.8–6.4) and micro-organisms (bacteria, protozoa and fungi) serve to catabolize the food, releasing nutrients vital to the bovine. The mucosal surface of the rumen is characterized by papillae, which serves as an organ of absorption. The number, size and distribution of papillae are related to the characteristics of the food consumed by the animal. Ruminal retention time can be 20–30 h, depending upon the nature of the feed material (Ishler *et al.*, 1996; Austgen *et al.*, 1998).

Considering rumen physiology vs. the physico-chemical properties of sulfamethazine, we find that it is plausible that some absorption did occur within this gastric compartment. Sulfamethazine contains two pKa values (2.65 and 7.4) and it exhibits optimal solubility under either acidic (below pH 2.65) or alkaline (above pH 7.4) conditions. At pH 7 and 37 °C, its solubility is 192 mg/100 mL. Although its solubility is poor within the pH of the rumen, it is in its most permeable form (neutral charge; Budavari, 1989). Furthermore, although the rumen is not conducive to the dissolution of the sulfamethazine bolus, the detergent-like materials contained in the rumen may facilitate sulfamethazine dissolution within the rumen and may enhance the absorption of any dissolved sulfamethazine. This may explain the very rapid systemic appearance of drug following administration of the oral solution and the small but quantifiable concentrations seen shortly after administration of the two bolus formulations. Thus, the potential for ruminal absorption may need to be considered when evaluating the influence of dissolution rate on the bioavailability of other APIs intended for administration to ruminating species.

Although outside the scope of the current manuscript, it would be interesting to explore the utility of other methods for assessing absorption characteristics in ruminants. Numerical deconvolution methods have been an important tool for acquiring an understanding of drug input processes in humans (e.g. Gillespie & Veng-Pedersen, 1985; Madden *et al.*, 1996; Yeh *et al.*, 2001; Buchwald, 2003). Likewise, it may provide valuable information regarding the oral drug bioavailability process in cattle. Considering the importance of oral drug delivery as a method of dosing in cattle and sheep (e.g. bolus formulations and medicated feeds), it would be valuable to obtain a greater understanding of this dynamic process, particularly as it pertains

to the characteristics of molecules that may be rapidly absorbed (within 15 min postdose).

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support received for this project from Fort Dodge Animal Health for the acquisition of manufacturing operation and equipment. The authors wish to thank William Steber, and Jack Zupan, PhD for their technical assistance.

REFERENCES

- Amidon, G.L., Lennernans, H., Shah, V.P. & Crison, J.R. (1995) Theoretical basis for a biopharmaceutics drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharmaceutical Research*, **12**, 413–420.
- Austgen, L., Bowen, R.A. & Rouge, M. (1998) Ruminant physiology and rumination. In *Hypertext Book Pathophysiology of the Digestive System*. Colorado State University. <http://arbl.cvmbs.colostate.edu/hbooks/pathphys/digestion/index.html> (accessed on 7 December 2005).
- Bevill, R.F., Dittert, L.W. & Bourne, D.W.A. (1977) Disposition of sulfonamides in food-producing animals IV: pharmacokinetics of sulfamethazine in cattle following administration of an intravenous dose and three oral dosage forms. *Journal of Pharmaceutical Sciences*, **66**, 619–623.
- Buchwald, P. (2003) Direct, differential-equation-based in-vitro-in-vivo correlation (IVIVC) method. *Journal of Pharmacy and Pharmacology*, **55**, 495–504.
- Budavari, S. (1989) The Merck Index. In *Encyclopedia of Chemicals, Drugs and Biologicals*, ed. Budavari, S. pp. 1406. Merck and Co., Inc., Rahway, NJ.
- Bulglin, M.S., Lane, V.M., Archer, E., Baggot, J.D. & Craigmill, A.L. (1991) Pharmacokinetics, safety and tissue residues of sustained-release sulfamethazine in sheep. *Journal of Veterinary Pharmacology and Therapeutics*, **14**, 36–45.
- Fahmy, R., Marnane, W., Bensley, D. & Hollenback, R.G. (2001) Dissolution testing of veterinary dosage forms. *Dissolution Technology*, **Feb**, 1–6.
- Fahmy, R.M., Steber, W., Bensley, D.M., Marnane, W.G., Zupan, J.A. & Hollenbeck, R.G. (2003) Effect of equipment and manufacturing process changes on the quality attributes of sulfamethazine boluses. *Pharmaceutical Technology*, **27**, 80–100.
- Faichney, G.J. & Griffiths, D.A. (1978) Behaviour of solute and particle markers in the stomach of sheep given a concentrate diet. *British Journal of Nutrition*, **40**, 71–82.
- FDA/CDER Guidance for Industry (1995) *SUPAC-IR: Immediate-Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*, dated 11/1995. <http://www.fda.gov/cder/guidance/cmc5.pdf> (accessed on 14 February 2006).
- FDA/CDER Guidance for Industry (1997a) *Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations* (Issued 9/1997, Posted 9/26/1997). <http://www.fda.gov/cder/guidance/1306fml.pdf> (accessed on 14 February 2006).
- FDA/CDER Guidance for Industry (1997b) *SUPAC-MR: Modified Release Solid Oral Dosage Forms Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Dissolution Testing and In Vivo Bioequivalence Documentation* (Issued 10/6/1997, Posted 10/6/1997). <http://www.fda.gov/cder/guidance/1214fml.pdf> (accessed on 14 February 2006).
- FDA/CDER Guidance for Industry (2000) *Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System*, dated 08/2000. <http://www.fda.gov/cder/guidance/3618fml.pdf> (accessed on 7 July 2006).
- FDA/CVM Guidance for Industry (2002) *Bioequivalence Guideline (#35)*, dated 10/09/2002. http://www.fda.gov/cvm/Guidance/bioequivalence_Oct02.pdf (accessed on 15 February 2006).
- Frazier, W.F. & Nuessle, N.O. (1976) Correlation of absorption of sulfamethazine boluses with dissolution using a new dissolution apparatus for veterinary tablets. *Journal of Pharmaceutical Sciences*, **65**, 1823–1826.
- Gillespie, W.R. & Veng-Pedersen, P. (1985) A polyexponential deconvolution method. Evaluation of the “gastrointestinal bioavailability” and mean in vivo dissolution time of some ibuprofen dosage forms. *Journal of Pharmacokinetics and Biopharmaceutics*, **13**, 289–307.
- Ishler, V., Heinrichs, V. & Varga, G. (1996) *From Feed To Milk: Understanding Rumen Function*. Penn State University Extension Circular 422, University Park, PA.
- Madden, F.N., Godfrey, K.R., Chappell, M.J., Hovorka, R. & Bates, R.A. (1996) A comparison of six deconvolution techniques. *Journal of Pharmacokinetics and Biopharmaceutics*, **24**, 283–299.
- Martinez, M.N., Papich, M.G. & Riviere, J.E. (2004) Veterinary application of in vitro dissolution data and the biopharmaceutics classification system. *Pharmacoepial Forum*, **30**, 2–10.
- National Research Council. (1996) *Guide for the Care and Use of Laboratory Animals*. National Academy Press, Washington, DC.
- Nouws, J.F., Vree, T.B., Breukink, H.J., Baakman, M., Driessens, F. & Smulders, A. (1985) Dose dependent disposition of sulphadimidine and of its N4-acetyl and hydroxyl metabolites in plasma and milk of dairy cows. *Veterinary Quarterly*, **7**, 177–186.
- Nouws, J.F., Vree, T.B., Baakman, M., Driessens, F., Breukink, H.J. & Mevius, D. (1986) Age and dosage dependency in the plasma disposition and the renal clearance of sulfamethazine and its N4-acetyl and hydroxy metabolites in calves and cows. *American Journal of Veterinary Research*, **47**, 642–649.
- Polli, J.E., Yu, L.X., Cook, J.A., Amidon, G.L., Borchardt, R.T., Burnside, B.A., Burton, P.S., Chen, M.L., Conner, D.P., Faustino, P.J., Hawi, A.A., Hussain, A.S., Joshi, H.N., Kwei, G., Lee, V.H., Lesko, L.J., Lipper, R.A., Loper, A.E., Nerurkar, S.G., Polli, J.W., Sanvordeker, D.R., Taneja, R., Uppoor, R.S., Vattikonda, C.S., Wilding, I. & Zhang, G. (2004) Summary workshop report: biopharmaceutics classification system-implementation challenges and extension opportunities. *Journal of Pharmaceutical Sciences*, **93**, 1375–1381.
- U.S. Food and Drug Administration (2001) *Good Laboratory Practice Regulations for Nonclinical Laboratory Studies*. Code of Federal Regulations (April 1), 229–243.
- Wagner, J.G. & Nelson, E. (1963) Percent absorbed timeplots derived from blood level and/or urinary excretion data. *Journal of Pharmaceutical Sciences*, **52**, 610–611.
- Yeh, K.C., Holder, D.J., Winchell, G.A., Wenning, L.A. & Prueksaranont, T. (2001) An extended point-area deconvolution approach for assessing drug input rates. *Pharmaceutical Research*, **18**, 1426–1434.