07R0800

A biphasic dissolution method to mimic oral absorption from simulated animal gastrointestinal fluids

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2016 AAPS ANNUAL MEETING AND EXPOSITION
NOVEMBER 13–17, 2016
COLORADO CONVENTION CENTER, DENVER
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PURPOSE

A low volume biphasic dissolution method was developed to control a dynamic pH environment in the presence of simulated dog and rat GI media [1]. Experiments were performed with itraconazole suspensions to test the viability of the method for in situ UV analysis of partition rates under biorelevant conditions. The aqueous phases were prepared from bile salts, phospholipids and buffer components as summarized in Table 1. Decanol was used for the organic phase.

Components of fasted state simulated fluid	Concentration in dog gastric fluid (mM)	Concentration in dog intestinal fluid (mM)	Concentration in rat gastric fluid (mM)	Concentration in rat intestinal fluid (mM)
Sodium taurodeoxycholate	0.1	5	-	-
Sodium taurocholate	0.1	5	4	50
Phosphatidylcholine (PC)	0.025	1.25	0.2	2.2
Lysophosphatidylcholine (LPC)	0.025	1.25	-	-
Sodium oleate	0.025	1.25	-	-
Sodium acetate	-	-	92.9	23.22
Sodium dihydrogen phosphate	-	28.65	92.9	23.22
Sodium chloride	14.5	59.63	-	-

Table 1. Compositions of simulated animal GI media [1].

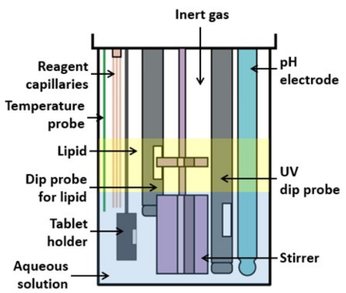

Figure 1. (above) Sirius inForm and (right) the experimental probe set and vessel.

In Sirius inForm, temperature and pH are controlled in situ and media are added automatically. Stirrer paddles agitate both the aqueous and organic layers by immersing one in each layer.

Itraconazole Suspensions

200 mg was found to represent a typical dose to 15 Kg dogs [2] with dose volumes ranging from 7.5 to 75 mL. Hence, a low dose volume concentration of 20 mg/mL and a high dose volume concentration of 2.67 mg/mL were used for the fasted state dog method. 10 mg/Kg was found to represent a typical dose to rats [3], with an estimated average weight of 0.33 Kg, a dose of 3.33 mg was used. Typical dose volumes were found to range from 2 to 10 mL, which led to suspension concentrations of 1.67 mg/mL and 0.33 mg/mL at low and high dose volumes, respectively.

METHOD

The Biphasic Dissolution assays were conducted using the Sirius inForm (Figure 1). Each assay was divided into two time sectors, the first representing gastric conditions of the animal and the second representing intestinal conditions. The pH, volumes of the aqueous and organic layers and the sector durations were specified prior to each experiment. The aqueous layer comprised simulated gastric or intestinal fluid for rat or dog, as specified in Table 1. Suspensions of itraconazole were added at the start of each assay, after which the aqueous layer became too turbid to record UV absorbance from the in situ immersion probe. The organic layer was added at the start of the second sector to provide a vehicle into which drug could partition, thus simulating drug absorption in the intestine. Itraconazole partitioned into the organic layer but the layer remained clear. It was therefore possible to measure UV absorbance in the organic layer, from which the concentration of itraconazole could be determined. Hence, the rates of partition could be compared by determining the area under the curve (AUC) of concentration of drug versus time.

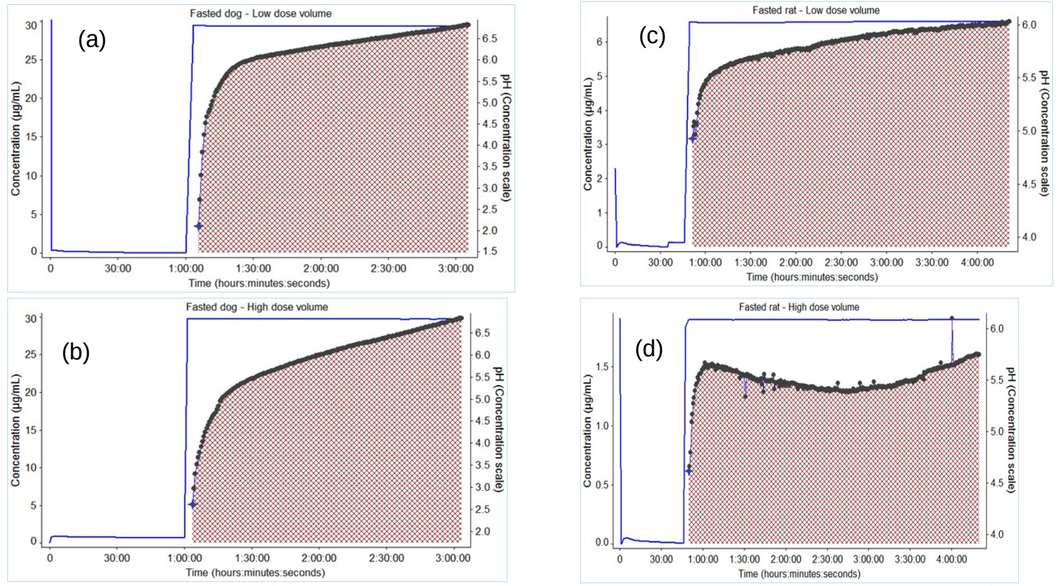

Figure 2. Concentration of itraconazole in the organic layer vs. time (black circles and hashed areas) and pH of the aqueous layer versus time (blue lines).

Fasted state Dog	Low dose volume	High dose volume
Sample volume (mL)	4.5	23.5
Suspension concentration (mg/mL)	26.67	2.67
Sector 1 (gastric)		
pH	1.5	1.5
Duration (min)	60	60
Stirrer speed (RPM)	100	100
Simulated animal fluid concentrate (mL)	0.094	0.049
Diluent (mL)	23.91	12.45
Sector 2 (intestinal)		
pH	6.8	6.8
Duration (min)	120	120
Stirrer speed (RPM)	100	100
Simulated animal fluid concentrate (mL)	10.16	0.51
Diluent (mL)	0.09	4.83
Buffer (mL)	13.75	7.16
Decanol (mL)	30	30
Data		
AUC (µg/mL·hr)	56.02	53.91
Cmax (µg/mL)	29.5	29.84

Fasted state Rat	Low dose volume	High dose volume
Sample volume (mL)	18.5	37.5
Suspension concentration (mg/mL)	1.44	0.33
Sector 1 (gastric)		
pH	3.9	3.9
Duration (min)	45	45
Stirrer speed (RPM)	100	100
Simulated animal fluid concentrate (mL)	0.113	0.053
Buffer (mL)	1.487	0.697
Sector 2 (intestinal)		
pH	6.0	6.0
Duration (min)	210	210
Stirrer speed (RPM)	100	100
Simulated animal fluid concentrate (mL)	24.55	11.37
Buffer (mL)	5.45	2.53
Decanol (mL)	30	30
Data		
AUC (µg/mL·hr)	23.46	5.39
Cmax (µg/mL)	6.59	1.61

Table 2. The experimental parameters of the fasted state dog and rat methods. The areas under the curve (AUC) and maximum concentration (Cmax) represent itraconazole that partitioned into the organic layer

RESULTS

In the fasted dog experiments (Figures 2a and 2b), the AUC and maximum concentration were similar for both dose volumes. This suggested that the vehicle volume did not have much effect on the rate of partitioning.

In the fasted rat experiments, the AUC and maximum concentration for the low dose volume (Figure 2c) were greater than the high dose volume (Figure 2d) by a factor of approximately four. This indicated that the volume of vehicle used was negatively correlated with the rate of partitioning.

CONCLUSION

This work describes the preparation and use of simulated animal GI fluids in biphasic dissolution experiments to study the partition rate of itraconazole suspensions. The organic solvent layer was clear and unobscured by suspended particulates which enabled the use of in situ UV absorbance for detection of dissolved API. Future work will be directed towards further investigation of the partitioning behavior and whether it can be correlated to animal PK data.

FUNDING / GRANTS / ENCORE / REFERENCE or other use

The experiments described in this poster were undertaken during **InViTox**, a collaboration between Sirius, Pfizer and the University of Bath that seeks to develop in vitro methods for toxicokinetic analyses. The authors would like to thank Innovate UK for funding.

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