Bioequivalence prediction based on *in vitro* flux assay through the example of aripiprazole

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PURPOSE

One of the biggest challenges of generic formulation development is to ensure appropriate performance during *in vivo* human correlation studies. *In silico* models supported by various in vitro assays are utilized to yield predictions, but the success rate of bioequivalence studies is still far from 100%. Current mathematical models for the estimation of the fraction absorbed (F_a%) of orally administered drugs base predictions on physicochemical properties. This study introduces a new mathematical model, based on the GUT framework[1], which predicts F_a% versus time profiles using *in vitro* flux profiles measured from formulations as input. The purpose of this study was to predict the *in vivo* performance of the solution drug product and compare it to the original solid dosage form using *in vitro* flux measurement as input for *in silico* modelling. Aripiprazole (ARI) has been selected as the model compound, and both the solid formulation (Abilify, Sample 1) and the oral solution (Abilify Oral Solution, Sample 2) were studied. The obtained flux and the predicted FA data were compared to *in vivo* human data published in the public assessment reports.

METHOD(S)

Small volume flux assay on µFlux

The formulations were tested using MicroFLUX™ apparatus. Concentration in both chambers was monitored using in situ fiber optic dip probes connected to the Rainbow instrument (Pion Inc.). A PVDF membrane impregnated with 25µL n-dodecane was applied to form a lipophilic barrier between the donor and the acceptor chamber.

Figure 1. MicroFLUX™ apparatus

Blank channel

During the blank channel measurement, the flux of the two parabens (without the active ingredient) were determined. This measurement served as a reference and was later taken into account when conducting the flux measurements of the solution.

Multi-component regression

The method is based on a modified classical least squares (CLS) technique, which determines the contribution coefficient of the known spectra (the standard spectra of each component) by minimizing the difference between the calculated spectrum and the measured mixture spectrum.

HPLC method

Chromatographic experiments were conducted on an Agilent 1100 HPLC system with a UV detector. Measurements were performed on a Kinetex column (50x4.6mm, 2.6µm) at 40°C, with a flow rate of 0.9 mL/min. 10 µL samples were injected using a mobile phase comprising water:ACN:acetic acid (70:30:0.1 v/v%) in isocratic mode.

RESULT(S)

Small volume flux results (MicroFLUX)

MicroFLUX assays were performed with both solid and solution formulations. While measurements for the tablet formulation were straightforward (tablet flux =$0.417 \pm 0.04\ \mu g/cm^2 \cdot \min$) the excipients in the solution formulation complicated the UV concentration measurement. Two approaches have been tested to overcome this issue: one is the blank channel correction, and the other is the multi-component analysis (Fig. 2).

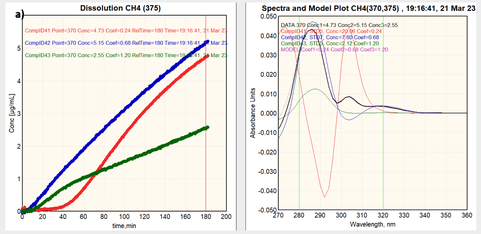

Figure 2. Multi-component regression toolbox in AuPRO™ software (red: aripiprazole; blue: methylparaben; green: propylparaben): concentration of components versus time (a) on their corresponding standard spectra (b). Black spectrum (b) is measured Abilify oral solution sample (superposition of parabens and ARI) at a particular time point.

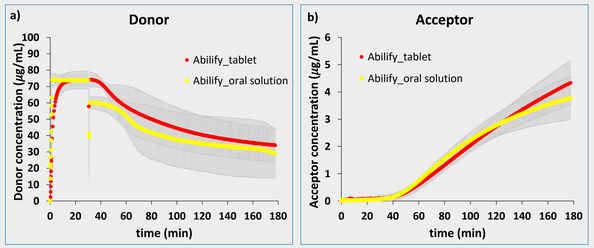

Figure 3. Dissolution (a) and appearance profile (b) of ARI formulations

The average flux value for the Abilify solution, after blank channel correction, was determined to be $0.535 \pm 0.09\ \mu g/cm^2 \cdot \min$, while using linear regression the value was $0.521 \pm 0.07\ \mu g/cm^2 \cdot \min$. A statistical two-sample t-test was performed, indicating that there is no significant difference between the values obtained from the two different UV data evaluations.
To further validate the effectiveness of both blank correction and linear regression, the final concentration on the acceptor side was measured by HPLC for two independent samples.

Sample No.	Concentration with blank correction (µg/mL)	Concentration with regression analysis (µg/mL)	Concentration with HPLC (µg/mL)
1.	2.95	2.90	3.10
2.	4.24	4.25	4.36

Table 1. Determination of ARI concentration using different methods

Determining absolute human fraction absorbed (FA%) from in vitro flux by Predictor™ software

The observed P_{eff} of API was used to identify if the measured flux for each formulation was unstirred water layer or membrane permeability limited. Flux data for the formulations was scaled to *in vivo* rates by calculating the API intestinal surface access from the observed permeability limitation *in vitro*. The scaled flux was used to determine the mass absorbed for a given intestinal surface area and transit time as per equation 1.

$$Mass_{ABS} = J_{in\ vivo} \cdot SA_{GI} \cdot T_{transit} \quad (1)$$

Where $J_{in\ vivo}$ is the *in vivo* scaled flux, SA_{GI} is the intestinal surface area, and $T_{transit}$ is the intestinal transit time. FA% values were calculated relative to the mass absorbed evaluated at the end of the intestinal transit time and the dose administered *in vivo*. Fraction absorbed vs time profiles (Figure 4) were generated by evaluating the sum of the cumulative absorption rate at each time point of the human gastrointestinal residence time.

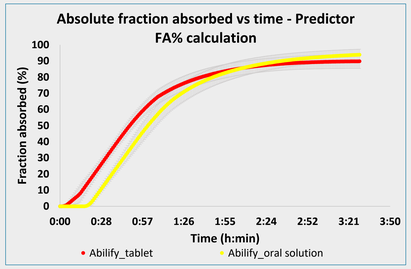

Figure 4. Dissolution (a) and appearance profile (b) of ARI formulations

The results of the FA% calculation at the end of the intestinal transit are presented against human *in vivo* data in Table 2.

Formulation	Calculated FA%	*In vivo* FA%	Deviance
Abilify tablet	93.2	87[2]	6.2
Abilify oral solution	93.9	100[2]	6.1

Table 2. Calculated absolute fraction absorbed values presented relative to *in vivo* fraction absorbed values for fasted-state conditions in humans.

Predictor provided a slight overestimation for the solid dosage form (93%) and slight underprediction for the solution formulation (~94%), but the **prediction accuracy is within the +/- 15% range** that is considered an accurate prediction.

CONCLUSION(S)

The investigation focused on evaluating the suitability of blank correction and linear regression as evaluation methods for in vitro flux measurement of a multi-component drug formulation. Statistical analysis indicated no significant difference between the flux values obtained from these two evaluation approaches, which was also confirmed by HPLC.

FA% prediction by the Predictor software provided an accurate estimation of the in vivo absorption ratio, even though the small difference reported in the bioequivalence study could not be resolved by this estimation.

REFERENCE

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