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DEVELOPMENT AND VALIDATION OF A NOVEL IN-VITRO BLADDER INFECTION MODEL SIMULATING URINARY FOSFOMYCIN PHARMACOKINETICS

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Introduction and purpose

- Urinary tract infections (UTIs) are:
  - among the most commonly encountered bacterial infections
  - a frequent indication for antibiotics
  - a potential breeding-ground for antibiotic resistance

- Fosfomycin remains one of the most active antibiotics against MDR uropathogens, although limited data are available to support current dosing and clinical breakpoints.

- We have developed a novel in-vitro bladder two-compartment infection model simulating urinary fosfomycin pharmacokinetics after oral administration.

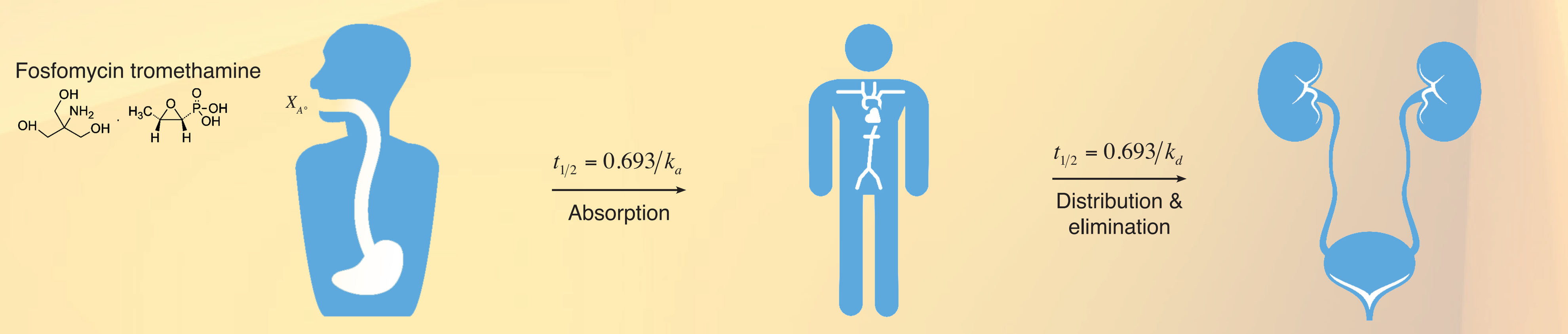
- Establishing supporting evidence for optimal dosing schedules that promote uropathogen kill and suppress emergence of resistance is vital.

Theoretical model

- Modelling oral fosfomycin undergoing first-order absorption in a two-compartment model with first-order elimination.[1]

- Oral fosfomycin does not undergo metabolism and is primarily excreted unchanged in the urine by glomerular filtration, with neither tubular secretion nor re-absorption.

Figure 1. Two-compartment model incorporating oral fosfomycin absorption.



$$X_{intestinal} = X_{A'} \cdot e^{-k_a t}$$

$$X_{circulatory} = \frac{k_a \cdot X_{A'}}{k_d - k_a} \cdot \left(e^{-k_a t} - e^{-k_d t}\right)$$

$$X_{bladder} = X_{A'} - X_{intestinal} - X_{circulatory}$$

- $X_{A'}$ – dose of fosfomycin administered
- $X_{intestinal}$ – amount of fosfomycin in gastrointestinal tract
- $X_{circulatory}$ – amount of fosfomycin in systemic circulation
- $X_{urinary}$ – amount of fosfomycin eliminated into the bladder
- t – time
- $t_{1/2}$ – half-life
- k_a – absorption constant
- k_d – distribution (& elimination) constant

Bioassay

- Fosfomycin concentrations were determined by an *E. coli* bioassay and confirmed by LC-MS.[2]

- Inhibition diameters with standard fosfomycin concentrations were logarithmic.

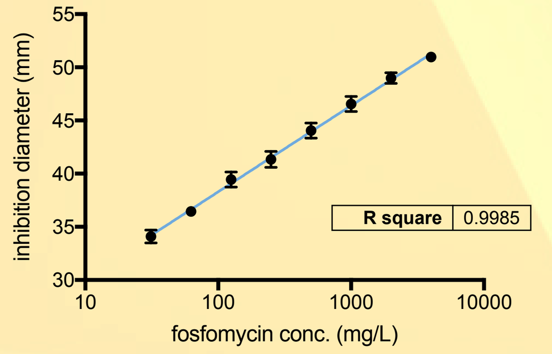


Figure 2. Standard curve (mean, SD)

Mathematical model

- Mathematical equations describing antibiotic concentrations over time were applied to simulate normal urinary pharmacokinetics following a 3 g oral dose of fosfomycin tromethamine.[3]

Figure 3. Model for tandem first-order processes and excretion.

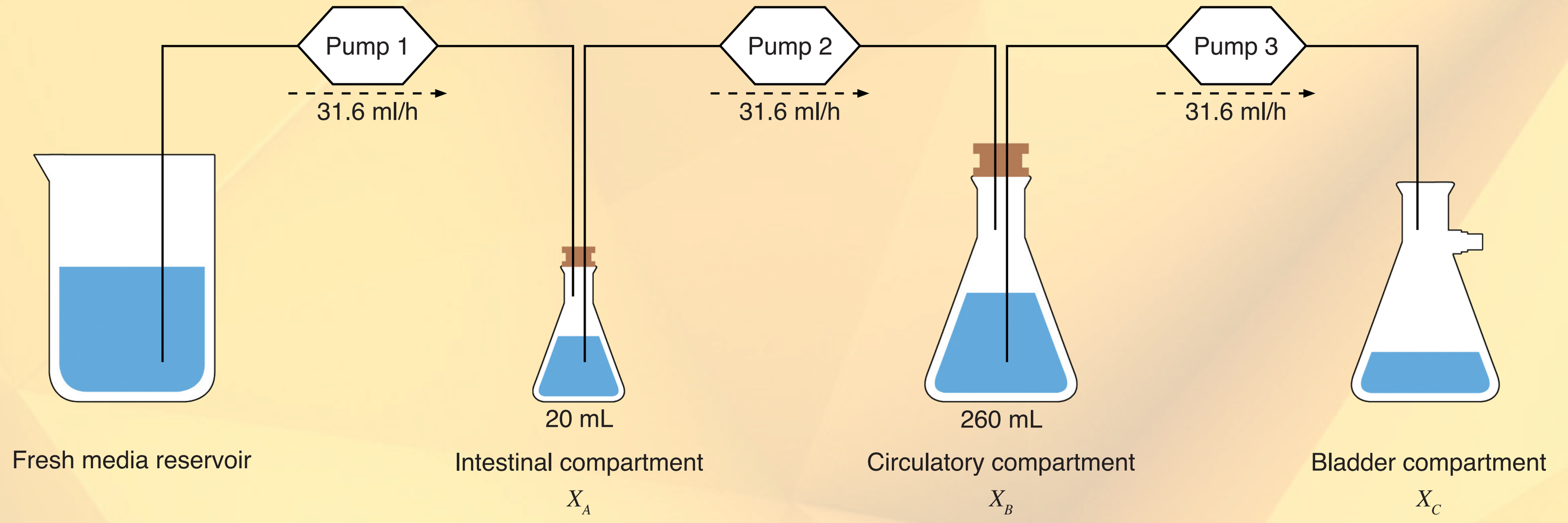
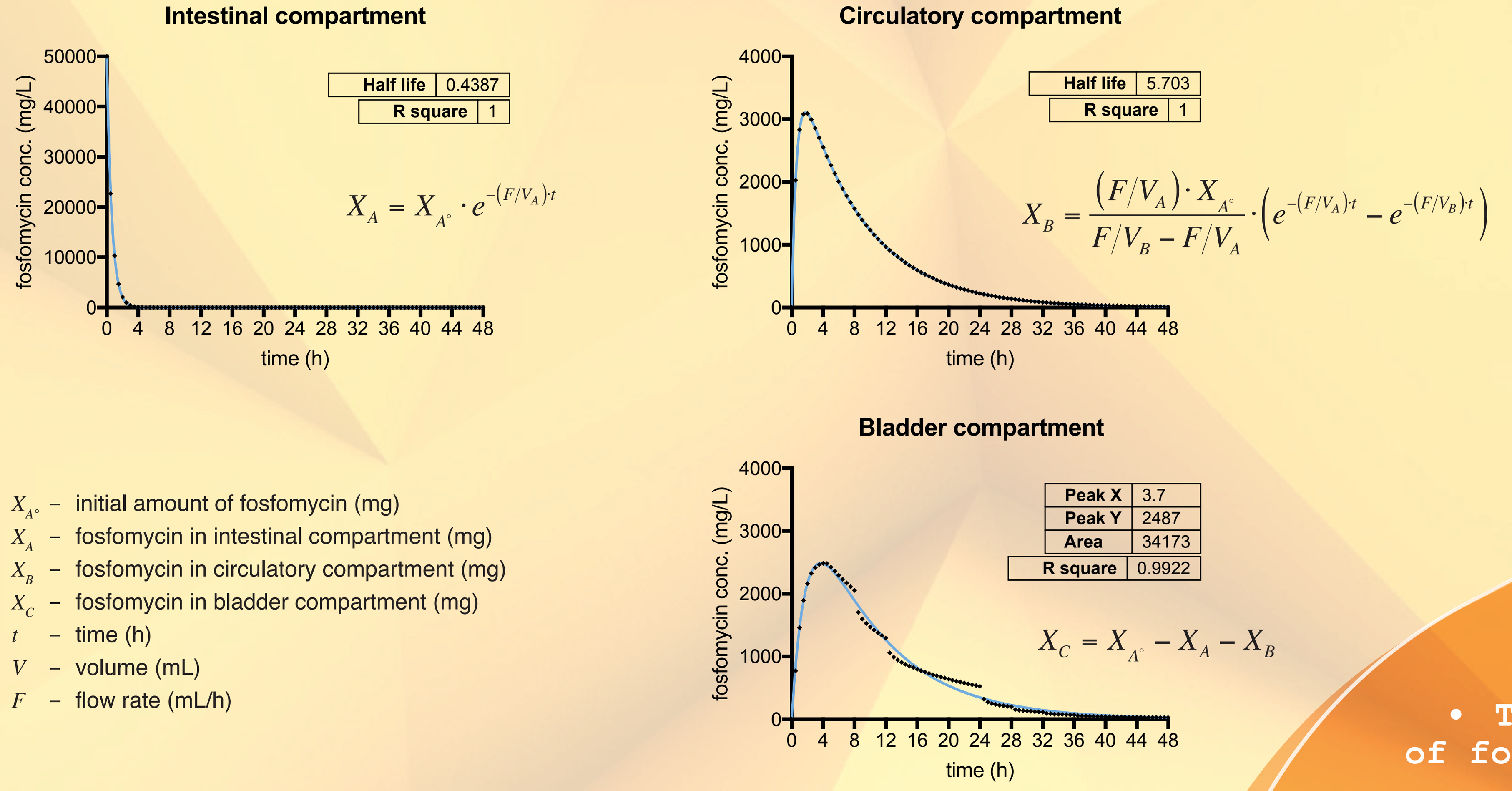


Figure 4. Drug distribution equations.



$$X_A = X_{A'} \cdot e^{-(F/V_A)t}$$

$$X_B = \frac{(F/V_A) \cdot X_{A'}}{F/V_B - F/V_A} \cdot \left(e^{-(F/V_A)t} - e^{-(F/V_B)t}\right)$$

$$X_C = X_{A'} - X_A - X_B$$

- $X_{A'}$ – initial amount of fosfomycin (mg)
- X_A – fosfomycin in intestinal compartment (mg)
- X_B – fosfomycin in circulatory compartment (mg)
- X_C – fosfomycin in bladder compartment (mg)
- t – time (h)
- V – volume (mL)
- F – flow rate (mL/h)

- After a 3 g oral dose, the mathematical model reproduces the expected time course of fosfomycin concentrations.[4]

- Serum elimination half-life was 5.7 hours; peak urinary concentrations of 2486.8 mg/L occurred at 3.7 hours, and remain >128 mg/L for 33.2 hours. 94.1% of total dose excreted by 24 hours.

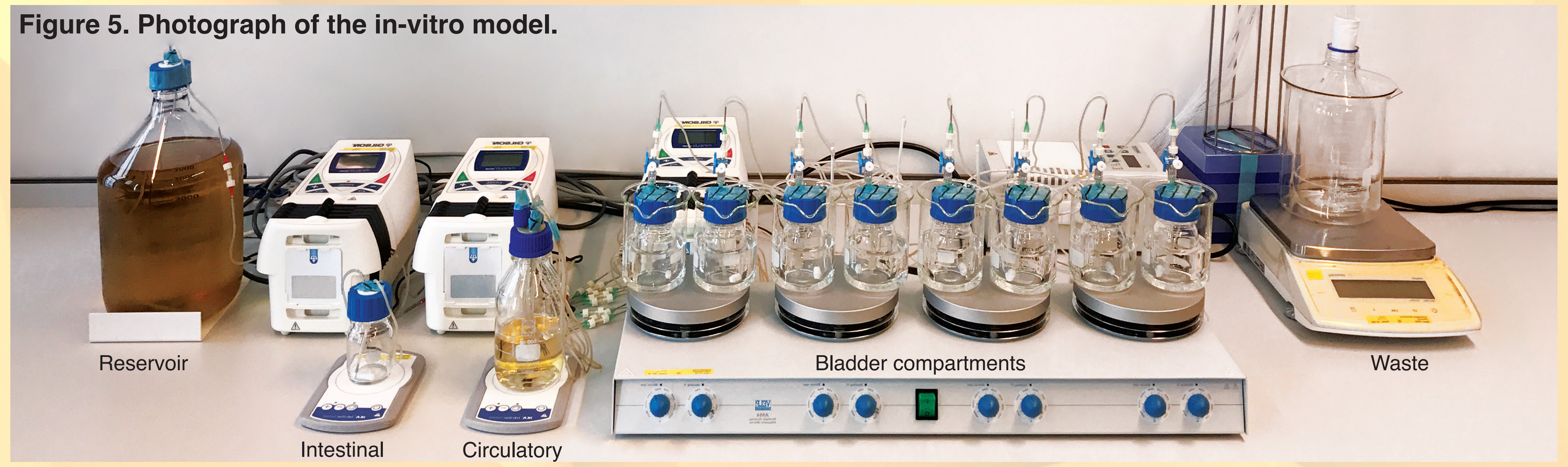
In-vitro model

- Normal human urodynamics was simulated on a 1:15 scale.[5]

- Exponential changes in fosfomycin concentrations undergoing dilution at constant volumes was controlled by three peristaltic pumps and connecting tubing.

- Elimination was into 8 bladder compartments that increased in volume, voided 4-hourly during the day and a 12-hour interval overnight, with >1.5 mL post-void residual.

Figure 5. Photograph of the in-vitro model.

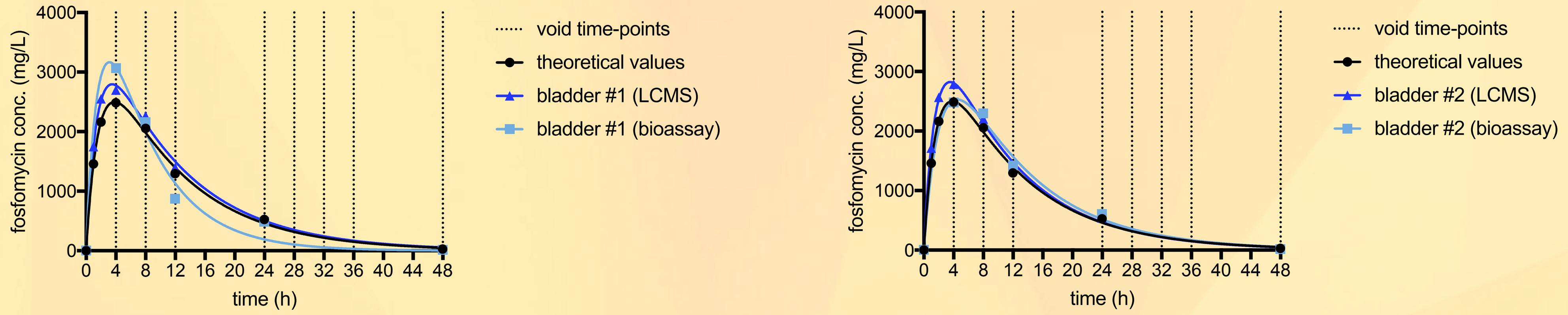


- Fosfomycin was added to the intestinal compartment at a concentration of 50,000 mg/L.

- PK samples were collected at 1 and 2-hours, and then at every simulated void.

- Interpolated fosfomycin concentrations, confirmed by LC-MS, approximate the theoretical concentration-time curve generated by the mathematical model.

Figure 6. Validation of the in-vitro model.



	R square	Peak X	Peak Y	Area
bladder #1 (bioassay)	0.9772	3.171	3166	33952
bladder #1 (LCMS)	0.9956	3.508	2796	41655

	R square	Peak X	Peak Y	Area
bladder #2 (bioassay)	0.9918	4.517	2527	40485
bladder #2 (LCMS)	0.9974	3.556	2822	40761

Conclusions

- **This novel in-vitro bladder infection model simulates a two compartment model that incorporates first-order absorption and bladder elimination.**

- **The model accurately simulates urine pharmacokinetics following an oral dose of fosfomycin tromethamine.**

- **Use of this model will enable the pharmacokinetic and pharmacodynamic assessment of uropathogens exposed to fosfomycin and thereby provide updated evidence for clinical breakpoints and dosing schedules.**

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