Development of An In Vitro Permeation Test Method for Rectal Suppositories

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PURPOSE

Unlike topical dermatological semisolid drug products, characterization-based bioequivalence (BE) approaches for locally-acting rectal suppositories that mitigate the risks associated with potential failure modes for BE have yet to be established. As part of a characterization-based BE approach, a product performance test such as an in vitro permeation test (IVPT) using excised tissues mounted in diffusion cells can facilitate a comparison of the drug permeation between a reference drug product and a prospective generic drug product. However, a reproducible, validated, and discriminatory IVPT study for rectal suppositories needs to be developed. In order to fill the critical knowledge gap, the objective of the present study was to develop a reproducible and discriminatory IVPT method for rectal suppositories using excised porcine rectal tissues as a model membrane.

METHODS

Mesalamine rectal suppositories were chosen as model drug products in the present study. CANASA® mesalamine rectal suppository (1 gram) was selected as the reference product. Three strengths of laboratory-prepared mesalamine rectal suppositories composed of Witepsol® H15 were prepared using a hot melt mixing method. The IVPT studies were conducted using a vertical diffusion cell (VDC) apparatus with a 1.77 cm^2 contact area. Porcine rectal tissue with the fat layer carefully removed was used as the rate-limiting membrane. The selection of study parameters such as study duration and sample loading method were investigated. Following the method development studies, a study duration of 8 hours at 37°C was selected based on the duration over which integrity of the rectal tissue could be maintained. Phosphate buffer (0.2 M, pH 7.5) was utilized as the receptor solution. Receptor solution samples were taken at pre-determined time points and analyzed using a validated high performance liquid chromatography (HPLC) method. Intra- and inter-day reproducibility of the method was evaluated using the reference product (n=3 cells/run, 3 independent runs). The discriminatory ability of the method was evaluated using laboratory-prepared mesalamine suppositories at different strengths (n=3 cells/strength). The observed variability in mesalamine permeation across the tissues from different animals was also assessed. The integrity and thickness of the tissue mucosal layer during studies were monitored.

CONCLUSIONS

An understanding of the IVPT method (i.e., precision, reproducibility, and discriminatory ability) using animal tissues for locally-acting rectal suppositories can facilitate the development of characterization-based BE approaches for locally-acting rectal drug products. The preliminary data demonstrated that an IVPT method developed using porcine rectal tissues was reproducible and sensitive (additional products may need to be evaluated to identify method validation strategies for rectal suppositories) when tissues from the same animal was used. Additional research is planned to evaluate mesalamine permeation through porcine rectal tissues from marketed and laboratory-prepared mesalamine rectal suppositories using the developed IVPT method.

ACKNOWLEDGMENT

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RESULTS

Porcine rectal tissue assessment

Porcine rectal tissues were trimmed by removing the fat layer. The trimmed tissues were tested on the VDC apparatus at 37°C to assess tissue integrity. A histological study (H&E staining) result indicated that the rectal mucosal layer integrity was retained following the tissue trimming process and an 8-hr IVPT study (**Figure 1**).

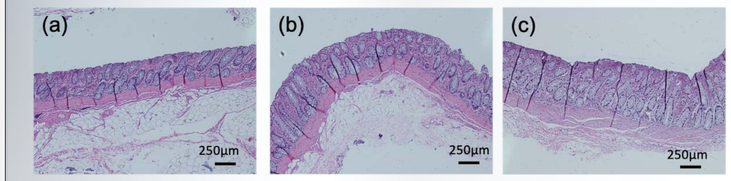
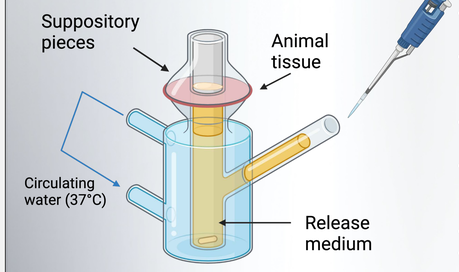


Figure 1. H&E images of porcine rectal membrane (a) with the fat layer and (b) with the fat layer removed. (c) A representative trimmed porcine rectal membrane mounted on the VDC apparatus with the presence of phosphate buffer , 0.2M, pH 7.5) for 8 hours at 37°C (c) (*n*=3).

IVPT VDC method assessment



Scheme 1. Vertical diffusion cell used in IVPT studies (Created with BioRender.com)

Method conditions

- Apparatus: VDC, contact area: 1.77cm^2
- Dose amount: Around 50 mg mesalamine suppository (applied as pieces in the donor chamber)
- Receptor solution: Phosphate buffer (0.2 M, pH 7.5)
- Animal tissue: Porcine rectal membrane with the fat layer removed
- Temperature: 37ºC
- Sampling volume: 0.6 mL
  - Replenish 0.6 mL of release media at each sampling time point

Method assessment (Reproducibility)

The intra- and inter-day coefficient of variation of the developed IVPT method with VDC using rectal tissue from the same animal for the reference product (CANASA®) was below 20%, demonstrating good precision and reproducibility using the same animal tissue (**Figure 2**).

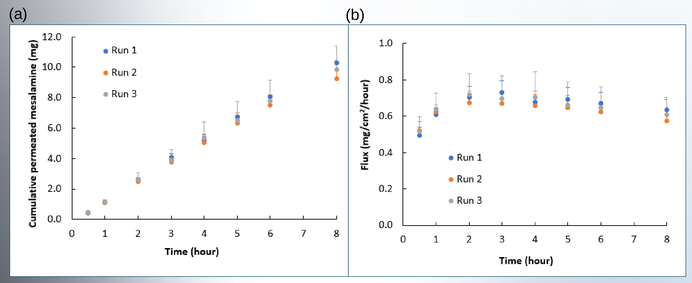


Figure 2. (a) Cumulative in vitro permeation amount and (b) flux profiles of CANASA® obtained from multiple runs using a VDC method at 37°C (*n*=3 cells/run, 3 runs; mean ± SD).

Observed variation – Animal tissue sources

The reference product was evaluated using the IVPT method with VDC using porcine rectal tissues from seven different animals (Animal #1-7). Variations in the in vitro permeation amount and flux were observed for the seven runs, demonstrating the impact of animal tissue sources on IVPT profiles of CANASA® (**Figure 3**).

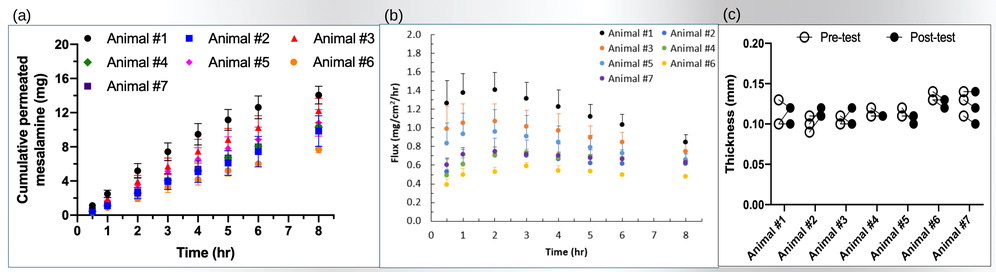


Figure 3. (a) Cumulative in vitro permeation amount and (b) flux profiles of the reference product obtained from multiple runs with different animal tissues using the IVPT method with VDC at 37°C. (c) Animal tissue thickness before and after the 8-hr IVPT studies (*n*=3, mean ± SD).

Method assessment (Discriminatory ability)

The developed method was able to differentiate laboratory-prepared mesalamine suppositories with three different strengths when using tissue from the same animal (**Figure 4**).

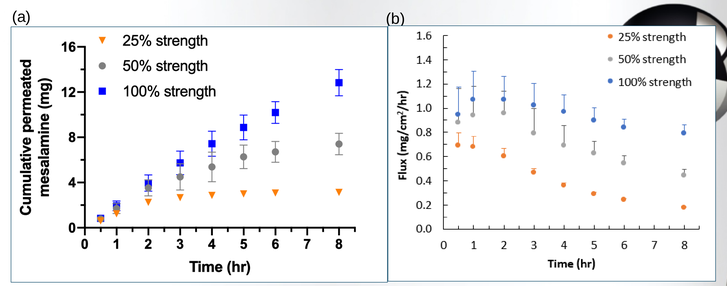


Figure 4. (a) Cumulative in vitro permeation release amount and (b) flux profiles of laboratory-prepared mesalamine suppositories with different strengths using the VDC method at 37°C (*n*=3, mean ± SD).

IVPT of mesalamine suppositories

The reference product (RLD), a generic product (G), and laboratory-prepared mesalamine suppository (LP) were tested using developed IVPT method with VDC (**Figure 5**).

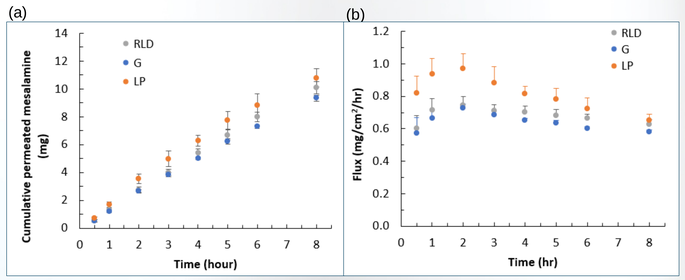


Figure 5. (a) Cumulative in vitro permeation release amount and (b) flux profile of RLD, G, and LP using the VDC method at 37°C (*n*=3, mean ± SD).

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