Use of a Novel Technology, the In Vitro Dissolution Absorption System, to Investigate the Effects of Antioxidants on the Intestinal Permeation of BCS Class III Drugs

Public Workshop:
Mitigation Strategies for Nitrosamine Drug Substance Related Impurities:
Quality and Bioequivalence Considerations for Generics

Session 3:
Impact of Reformulation on the Bioequivalence of Generic Products and FDA Perspectives on Reformulated Generics

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Disclaimer

This presentation reflects the views of the presenter and should not be construed to represent FDA's views or policies

Background

One approach to mitigating the impact of N-nitrosamine drug substance related impurities (NDSRIs) is to reduce their formation by reformulating drug products to include an antioxidant.

A novel technology, the In Vitro Dissolution Absorption System (IDAS), has shown promise in terms of evaluating the impact of excipients on drug dissolution and permeation. Caco-2 cell monolayers in the IDAS are less sensitive to excipients than in conventional Transwell culture.

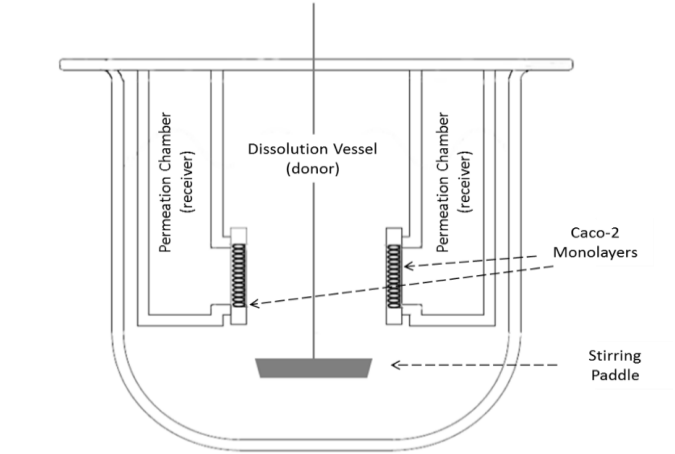
This work is funded by an FDA IDIQ contract.

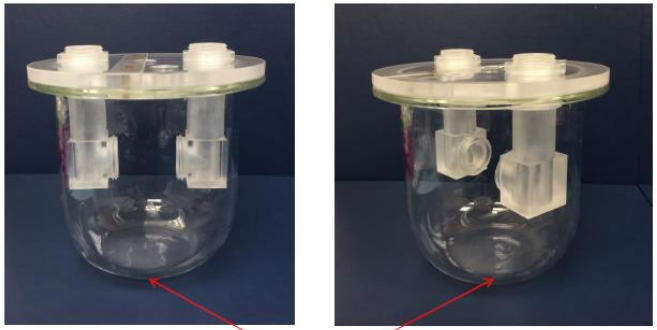
Contractor: Pharmaron (Exton) Lab Services LLC

Objective

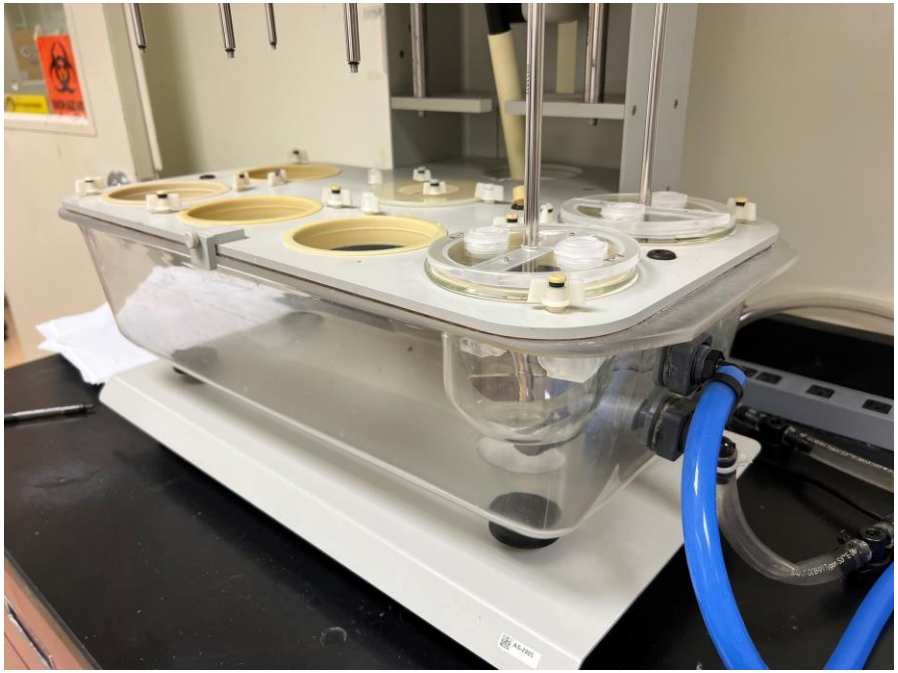
Use the *in-vitro* Dissolution Absorption System (IDAS) to evaluate the impact of antioxidants on drug permeation

IDAS Overview





Dissolution Chamber



Methods

- Validated an LC-MS/MS analytical method for the quantification of four BCS Class III model drugs:
  - Acyclovir
  - Atenolol
  - Cimetidine
  - Ranitidine
- Used IDAS to measure the rate of permeation of the model drugs (pre-dissolved and dosed individually*) in the absence and presence of 4 antioxidants (one at a time), each at three concentrations and with a parallel control (no antioxidant)

Method Validation Tests
Intra-assay accuracy and precision (3 runs)
Inter-assay accuracy and precision
Dilution integrity
Bench-top stability (2 matrices)
Refrigerator stability
Reinjection reproducibility
Autosampler stability
Excipient interference
Stock solution stability

* With the exception of atenolol, which was always co-dosed with each of the other model drugs

Test Antioxidants

Excipient	Concentration (mg/mL)		
	Low	Mid	High
Alpha-Tocopherol	0.01	0.02	0.04
Ascorbic Acid	0.01	0.02	0.04
Cysteine HCl	0.01	0.02	0.04
Propyl Gallate	0.01	0.02	0.04

- It has been reported[1,2] that a small amount (1-2%, w/w) of an antioxidant in a drug product could potentially inhibit the formation of NDSRIs.
- The High test concentration is based on the assumption of a 500 mg dosage unit weight; 2% of 500 mg is 10 mg, and 10 mg in 250 mL (approx. 8 oz. of water for oral administration) is 0.04 mg/mL.
- The amount of antioxidants should be within the maximum daily exposure in the FDA Inactive Ingredients Database.
- The Mid test concentration is 50% of the High; and the Low test concentration is 50% of the Mid.

1. Nanda K, et al., Inhibition of N-nitrosamine formation in drug product: a model study. J Pharm Sci. 2021; 110 (12): 3773-3775
2. Homsak M, et al., Assessment of a diverse array of nitrite scavengers in solution and solid state: a study of inhibitory effect on the formation of alkyl-aryl and dialkyl N-nitrosamine derivatives. Processes. 2022; 10: 2428.

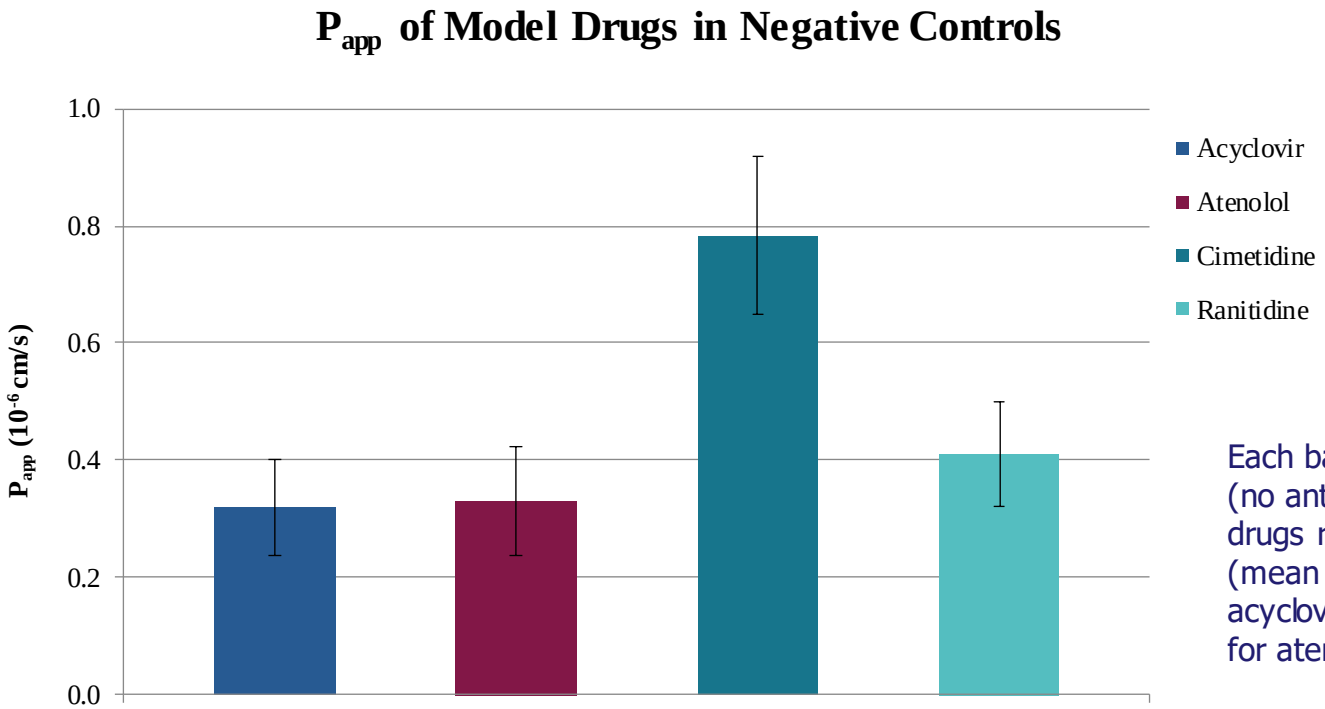
Permeation of Model Drugs +/- Antioxidants

- Treatments performed with n=3/6 replicates per experiment (3 dissolution vessels, 6 permeation chambers)*
- Donor medium: FaSSIF, pH 6.5
- Receiver buffer: HBSSg with added D-glucose, HEPES buffer (pH 7.4) and 4.5% BSA
- Receiver samples collected at 20, 40, 60, 90, and 120 min
- Apparent permeability coefficient (P_{app}) calculated over the duration 20-120 min

Treatments per Antioxidant for Each Model Drug	Replicates
Negative Control	6
0.01 mg/mL	6
0.02 mg/mL	6
0.04 mg/mL	6

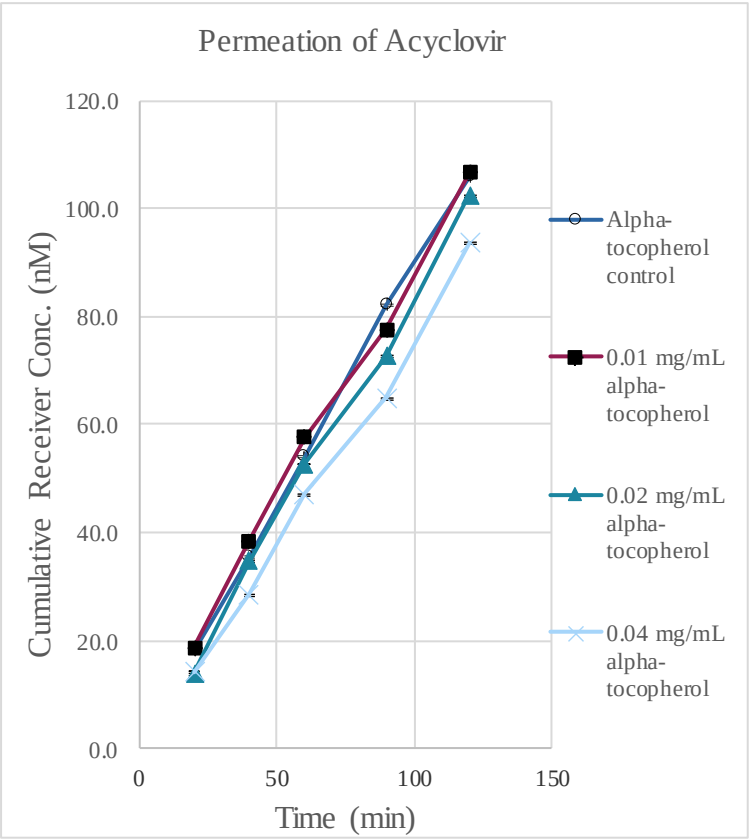
* Note that atenolol was always co-dosed with each of the other model drugs, so there are actually n=18 replicates per antioxidant treatment (n=72 for negative control) for atenolol.

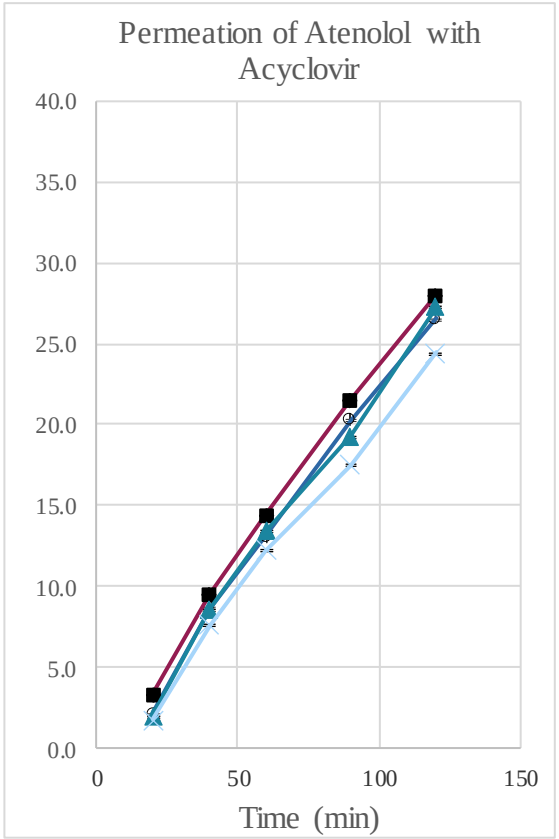
P_{app} of Negative Controls Run in Parallel with Excipients



Each bar represents all negative controls (no antioxidant) for one of the model drugs run in parallel with test antioxidants (mean ± standard deviation, n=24 for acyclovir, cimetidine, and ranitidine; n=72 for atenolol)

Effect of Alpha-Tocopherol on Permeation of Acyclovir and Atenolol





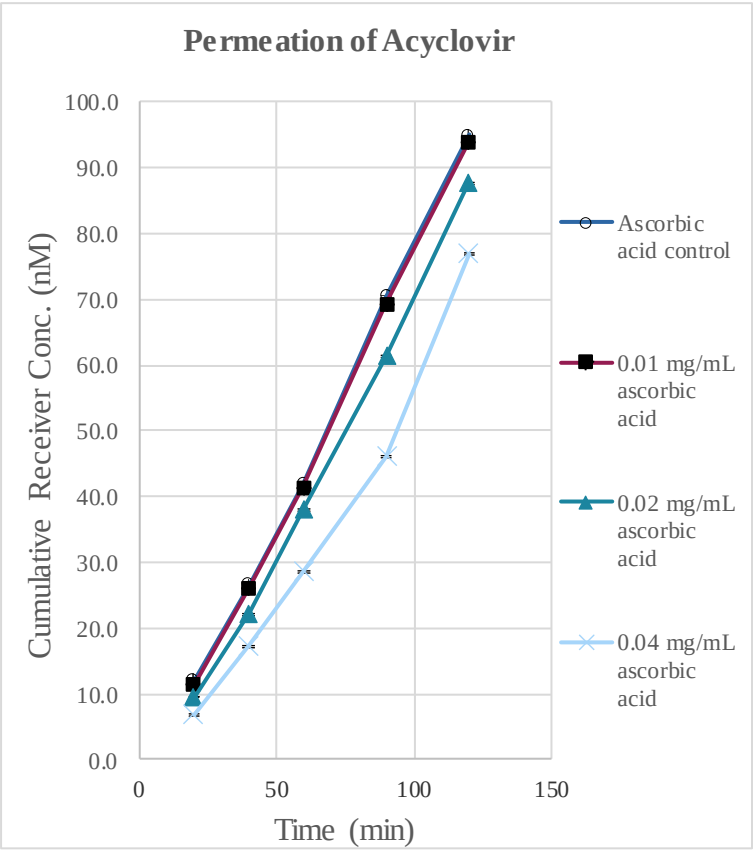
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control*	Acyclovir	0.295	0.023
0.01 mg/mL		0.285	0.080
0.02 mg/mL		0.285	0.071
0.04 mg/mL		0.259	0.041

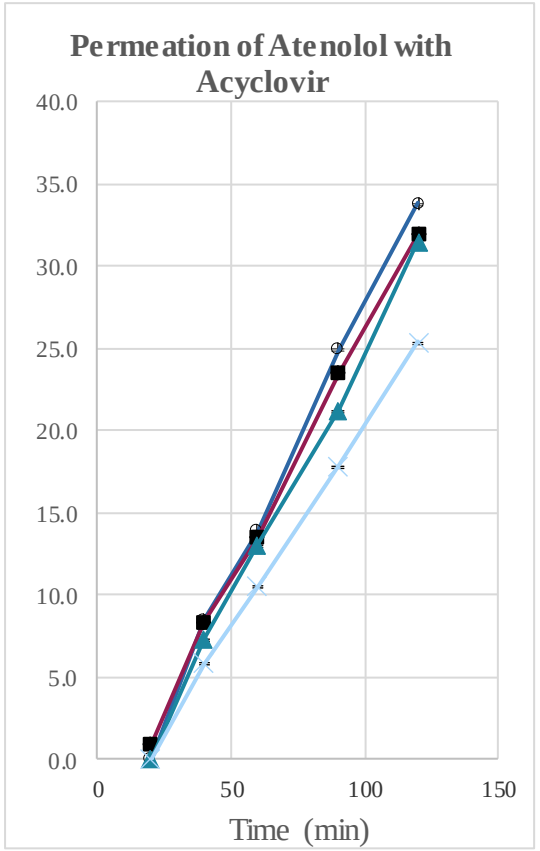
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control*	Atenolol	0.283	0.009
0.01 mg/mL		0.287	0.060
0.02 mg/mL		0.287	0.047
0.04 mg/mL		0.259	0.022

n=6 replicates per treatment, with the exception of:

* One replicate excluded for cell monolayer integrity failure (atenolol $P_{app} > 1 \times 10^{-6}$ cm/s); n=5

Effect of Ascorbic Acid on Permeation of Acyclovir and Atenolol



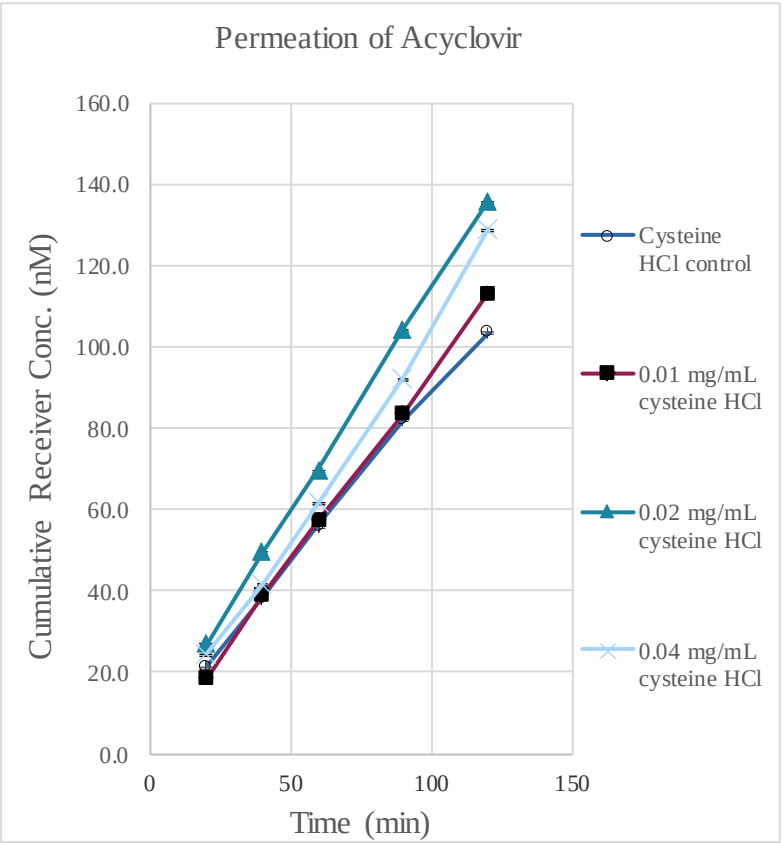


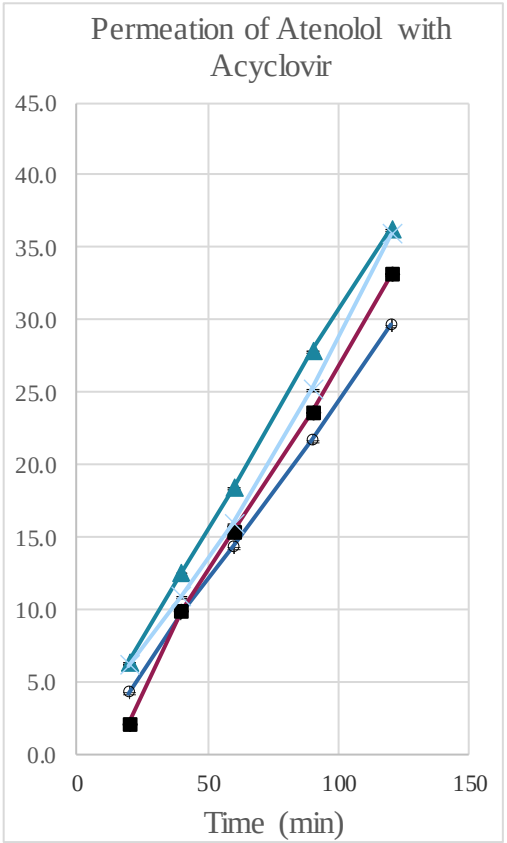
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control	Acyclovir	0.280	0.014
0.01 mg/mL		0.278	0.077
0.02 mg/mL		0.262	0.018
0.04 mg/mL		0.216	0.020

Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control	Atenolol	0.396	0.022
0.01 mg/mL		0.364	0.061
0.02 mg/mL		0.363	0.019
0.04 mg/mL		0.296	0.025

n=6 replicates per treatment

Effect of Cysteine on Permeation of Acyclovir and Atenolol



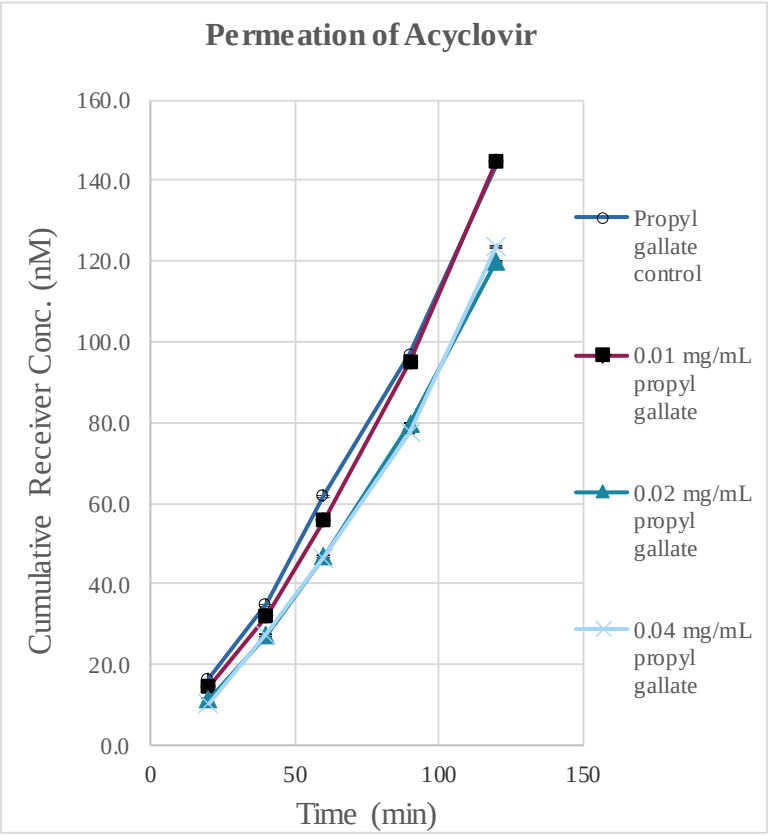


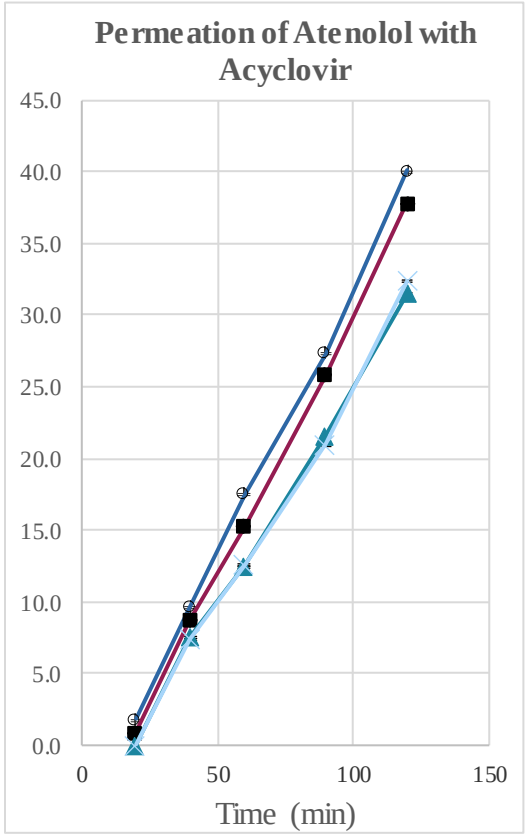
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control	Acyclovir	0.275	0.042
0.01 mg/mL		0.307	0.050
0.02 mg/mL		0.362	0.089
0.04 mg/mL		0.347	0.045

Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control	Atenolol	0.297	0.034
0.01 mg/mL		0.357	0.059
0.02 mg/mL		0.355	0.067
0.04 mg/mL		0.352	0.035

n=6 replicates per treatment

Effect of Propyl Gallate on Permeation of Acyclovir and Atenolol



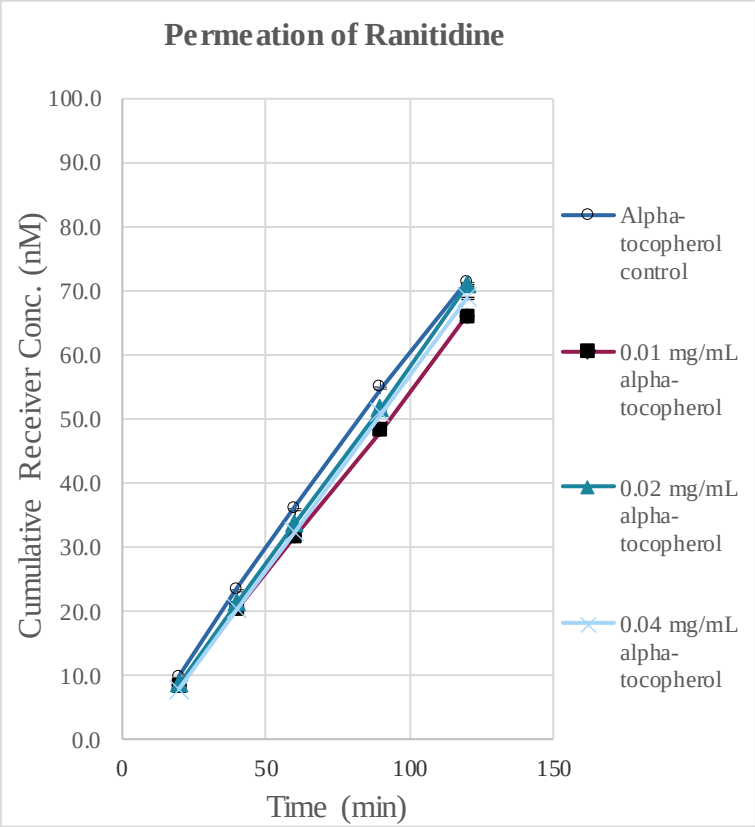


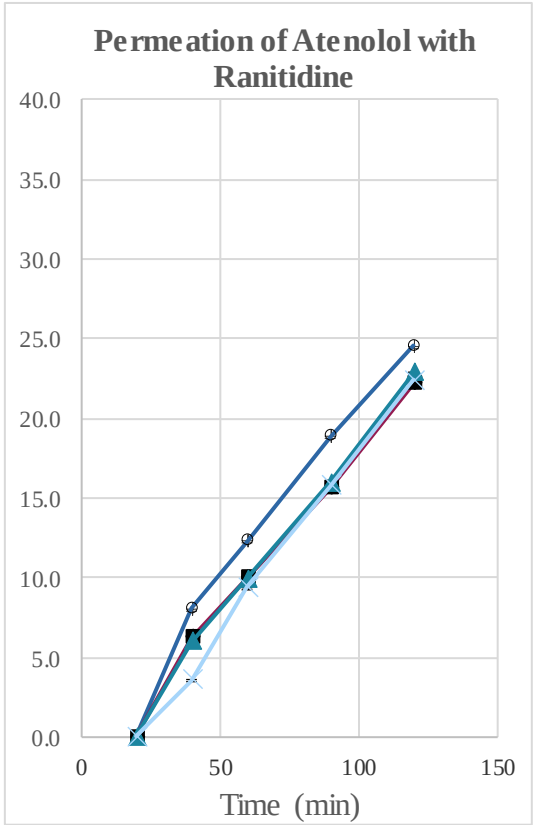
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control	Acyclovir	0.425	0.058
0.01 mg/mL		0.435	0.031
0.02 mg/mL		0.362	0.042
0.04 mg/mL		0.372	0.054

Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control	Atenolol	0.445	0.037
0.01 mg/mL		0.429	0.039
0.02 mg/mL		0.364	0.037
0.04 mg/mL		0.370	0.049

n=6 replicates per treatment

Effect of Alpha-Tocopherol on Permeation of Ranitidine and Atenolol





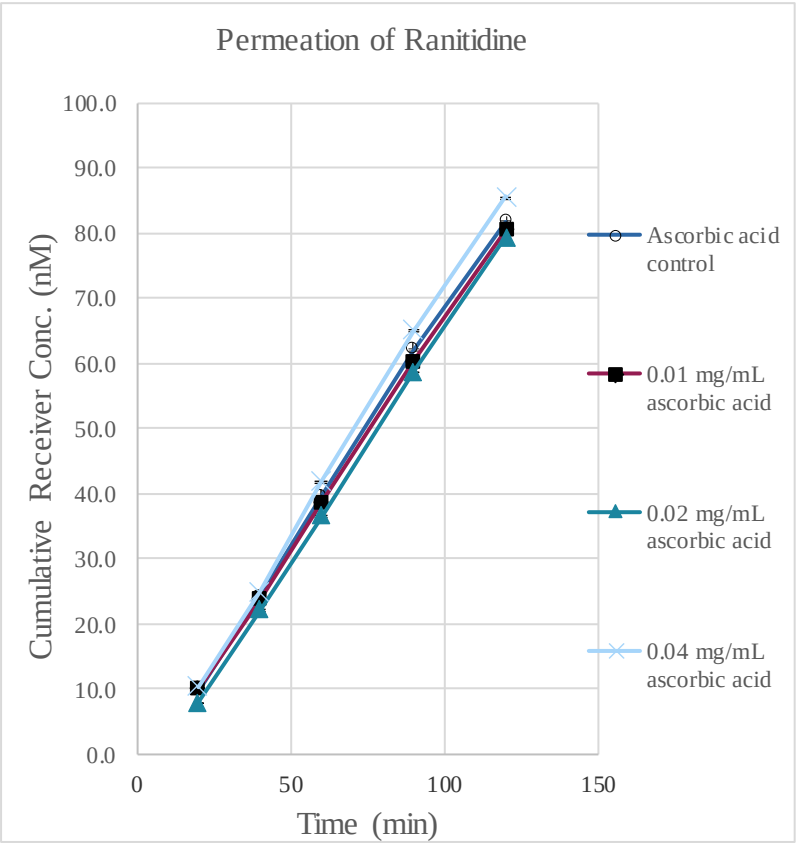
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control[$]	Ranitidine	0.381	0.031
0.01 mg/mL		0.354	0.023
0.02 mg/mL		0.383	0.037
0.04 mg/mL[$]		0.378	0.043

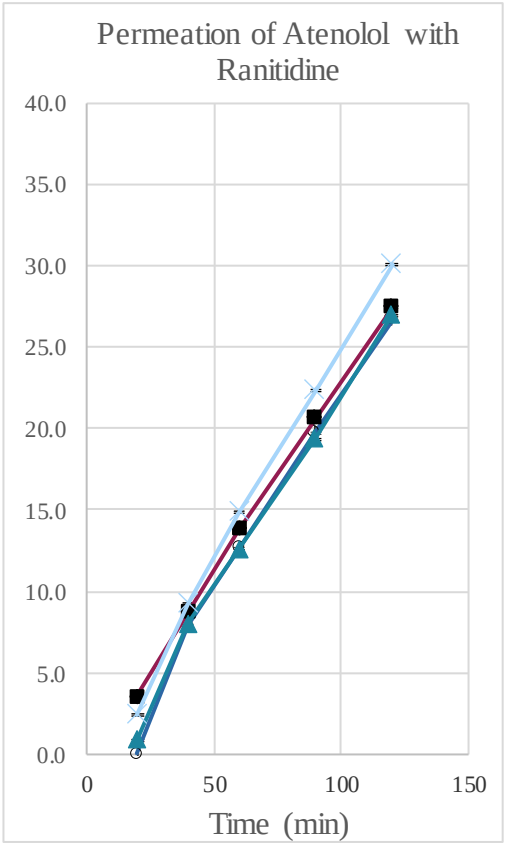
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control[$]	Atenolol	0.279	0.034
0.01 mg/mL		0.252	0.023
0.02 mg/mL		0.262	0.026
0.04 mg/mL[$]		0.269	0.038

n=6 replicates per treatment, with the exception of:

[$] One replicate excluded as a statistical outlier (per Q test at the 90% confidence level); n=5

Effect of Ascorbic Acid on Permeation of Ranitidine and Atenolol





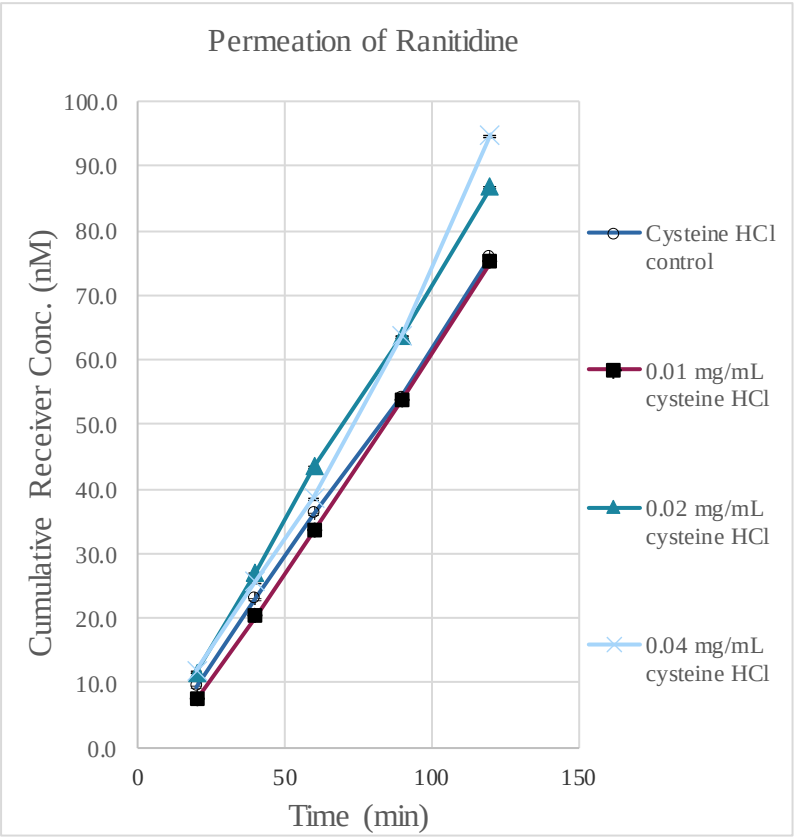
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control*	Ranitidine	0.448	0.045
0.01 mg/mL		0.438	0.049
0.02 mg/mL		0.443	0.048
0.04 mg/mL		0.469	0.042

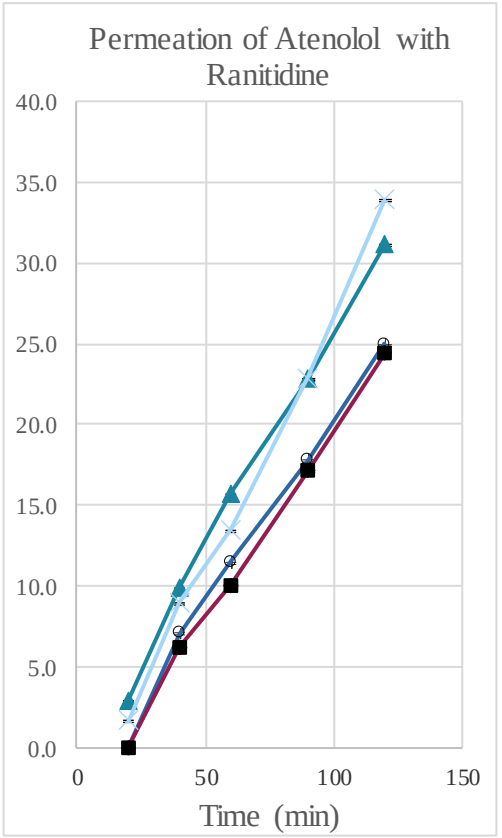
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control*	Atenolol	0.305	0.036
0.01 mg/mL		0.281	0.032
0.02 mg/mL		0.298	0.047
0.04 mg/mL		0.321	0.020

n=6 replicates per treatment, with the exception of:

* One replicate excluded for cell monolayer integrity failure (atenolol $P_{app} > 1 \times 10^{-6}$ cm/s); n=5

Effect of Cysteine on Permeation of Ranitidine and Atenolol



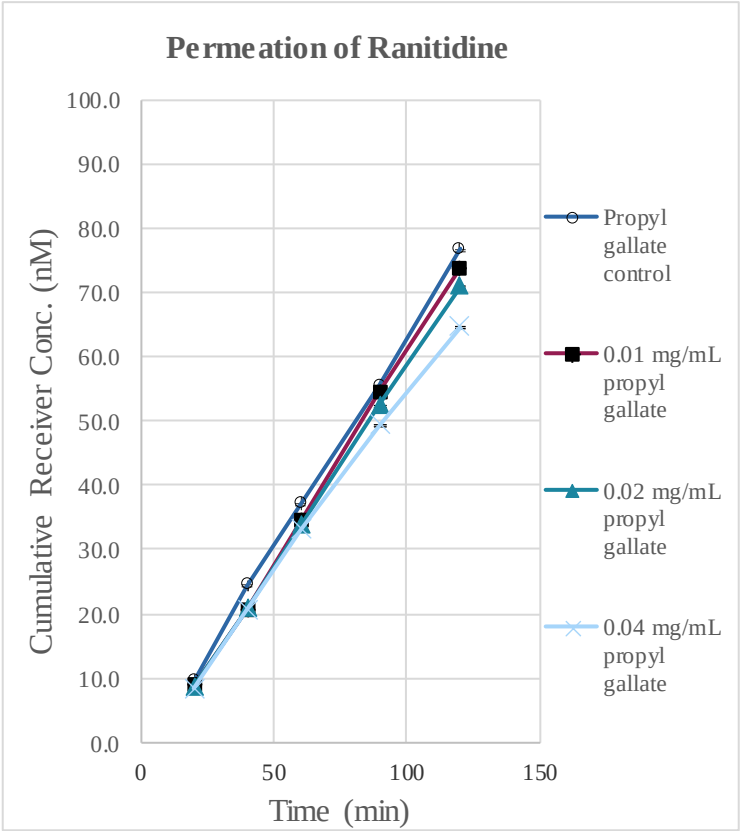


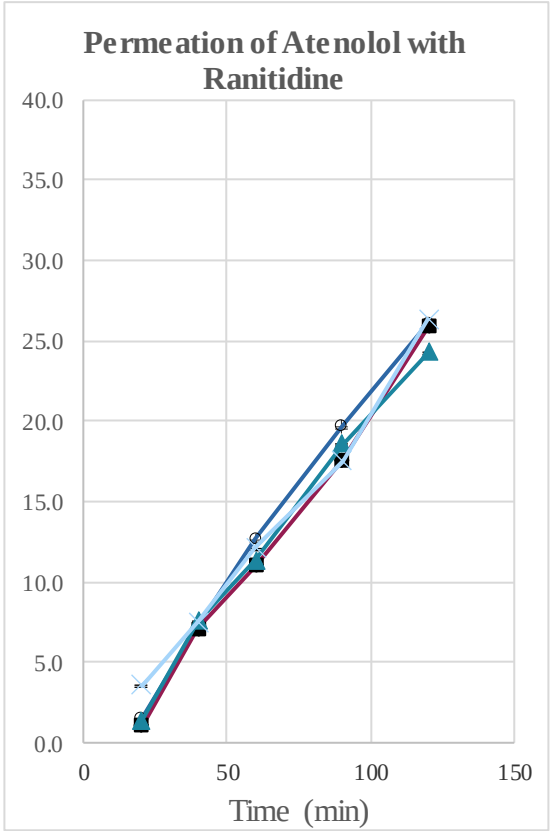
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control	Ranitidine	0.406	0.060
0.01 mg/mL		0.419	0.036
0.02 mg/mL		0.461	0.058
0.04 mg/mL		0.507	0.087

Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control	Atenolol	0.284	0.052
0.01 mg/mL		0.281	0.032
0.02 mg/mL		0.325	0.043
0.04 mg/mL		0.370	0.078

n=6 replicates per treatment

Effect of Propyl Gallate on Permeation of Ranitidine and Atenolol





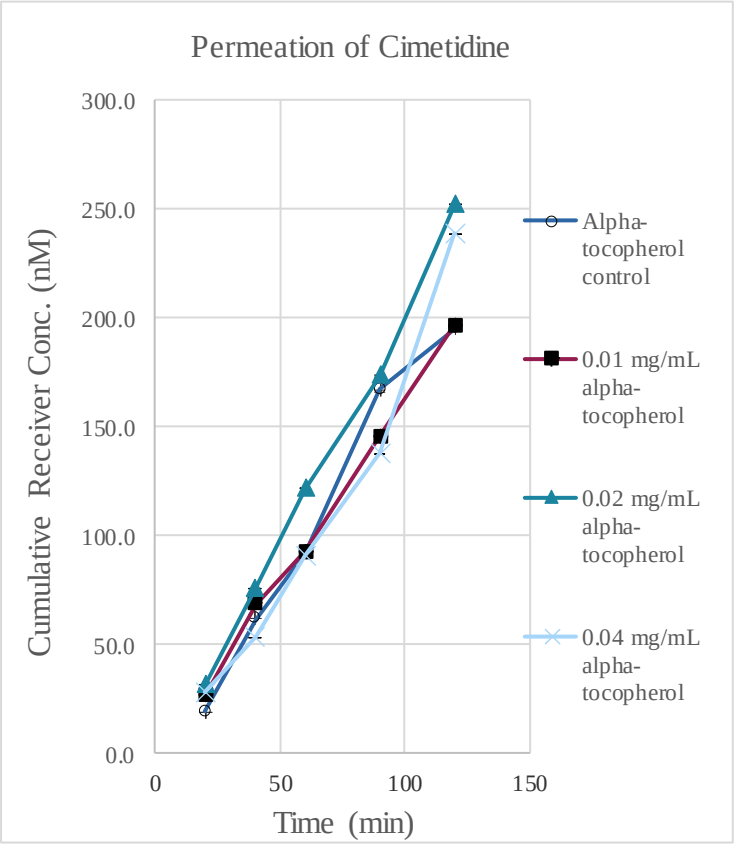
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control*	Ranitidine	0.409	0.040
0.01 mg/mL		0.405	0.077
0.02 mg/mL		0.386	0.070
0.04 mg/mL		0.348	0.038

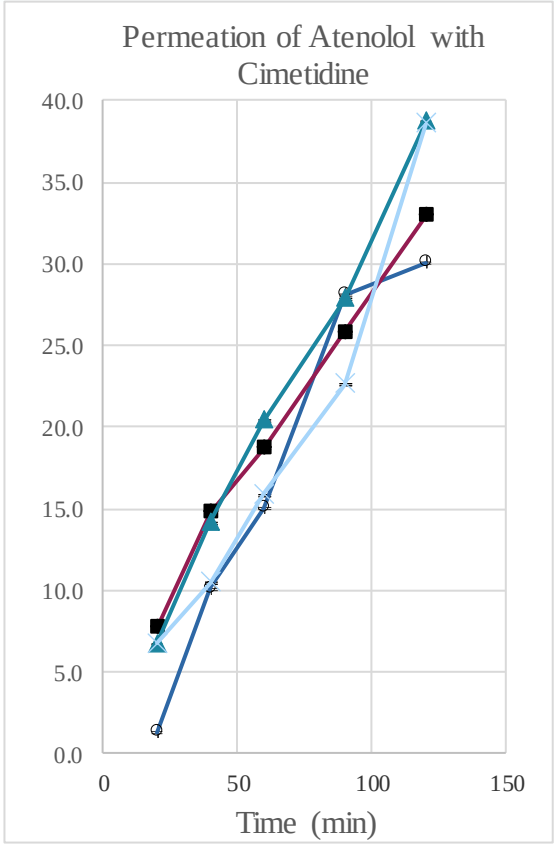
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control*	Atenolol	0.289	0.045
0.01 mg/mL		0.284	0.073
0.02 mg/mL		0.267	0.043
0.04 mg/mL		0.264	0.047

n=6 replicates per treatment, with the exception of:

* One replicate excluded for cell monolayer integrity failure (atenolol $P_{app} > 1 \times 10^{-6}$ cm/s); n=5

Effect of Alpha-Tocopherol on Permeation of Cimetidine and Atenolol





Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control*	Cimetidine	0.0678	0.051
0.01 mg/mL		0.622	0.055
0.02 mg/mL		0.802	0.087
0.04 mg/mL$		0.764	0.065

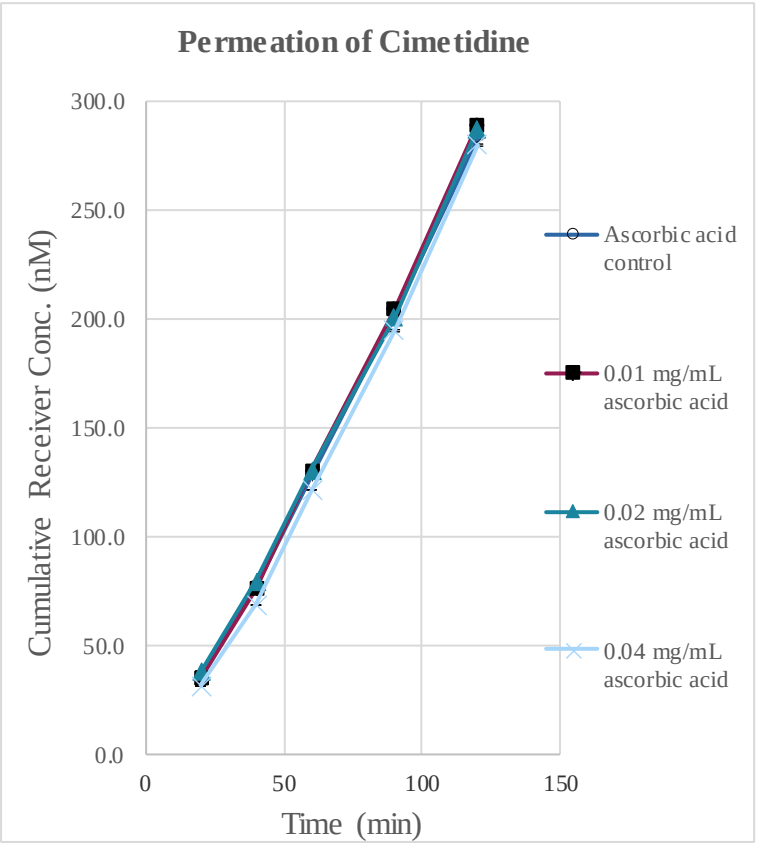
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control*	Atenolol	0.352	0.058
0.01 mg/mL		0.289	0.035
0.02 mg/mL		0.365	0.094
0.04 mg/mL$		0.364	0.046

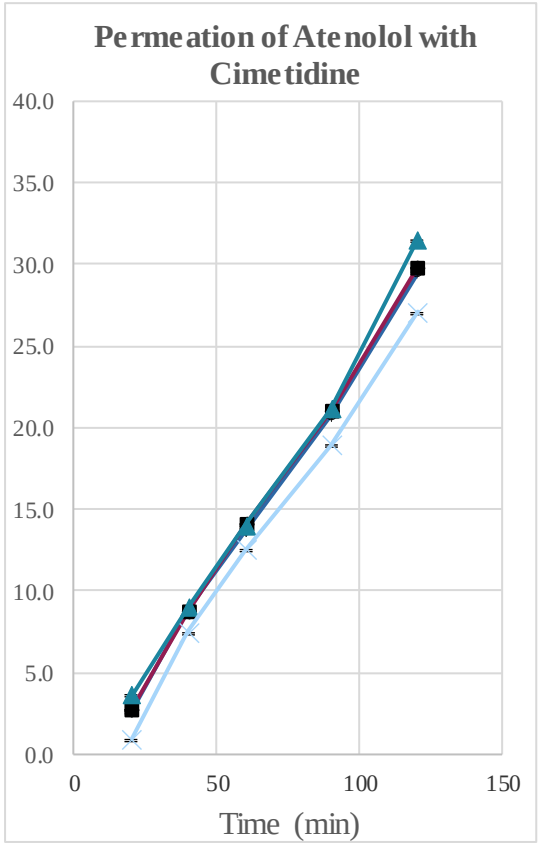
n=6 replicates per treatment, with the exception of:

* One replicate excluded for cell monolayer integrity failure (atenolol $P_{app} > 1 \times 10^{-6}$ cm/s); n=5

$ One replicate excluded as a statistical outlier (per Q test at the 90% confidence level); n=5

Effect of Ascorbic Acid on Permeation of Cimetidine and Atenolol





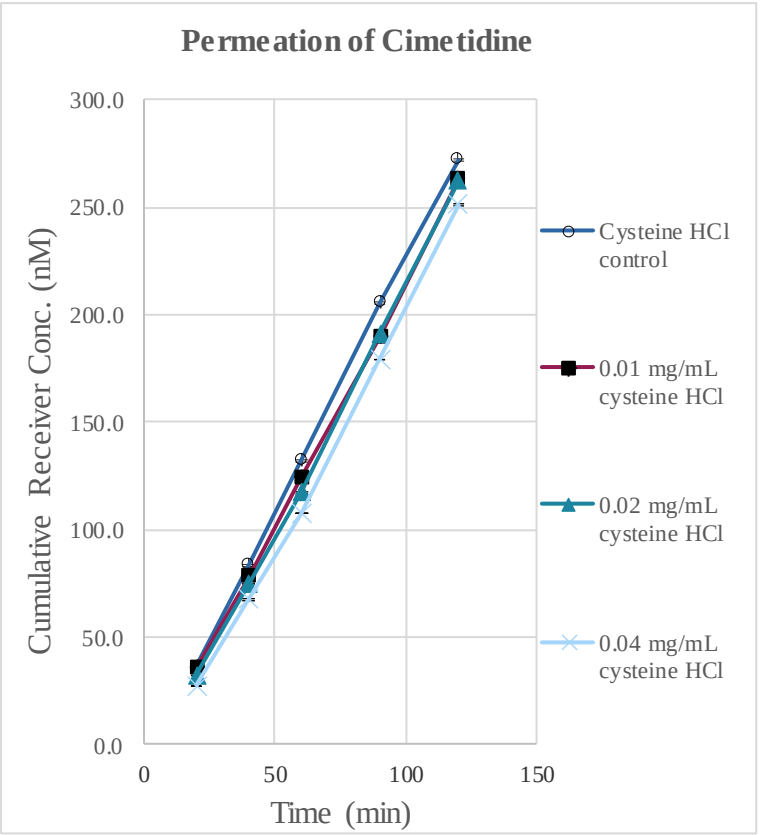
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control	Cimetidine	0.931	0.042
0.01 mg/mL		0.952	0.037
0.02 mg/mL*		0.928	0.081
0.04 mg/mL		0.933	0.050

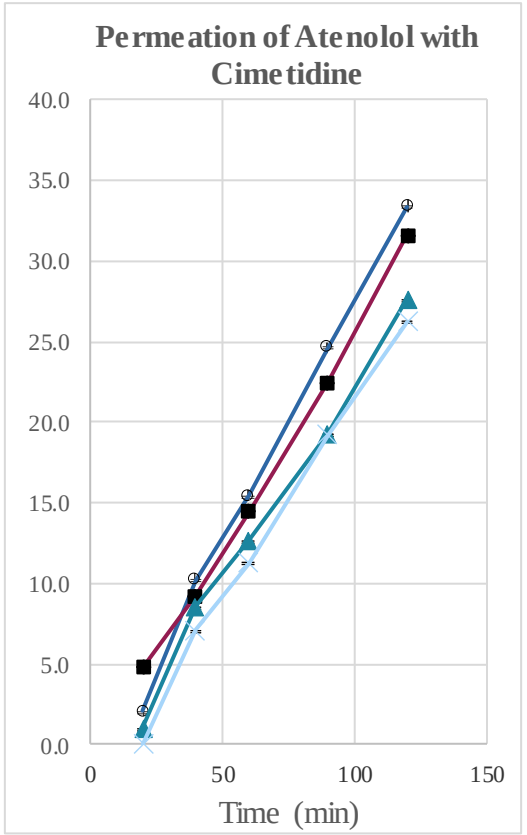
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control	Atenolol	0.310	0.015
0.01 mg/mL		0.313	0.020
0.02 mg/mL*		0.322	0.017
0.04 mg/mL		0.300	0.023

n=6 replicates per treatment, with the exception of:

* One replicate excluded for cell monolayer integrity failure (atenolol $P_{app} > 1 \times 10^{-6}$ cm/s); n=5

Effect of Cysteine on Permeation of Cimetidine and Atenolol



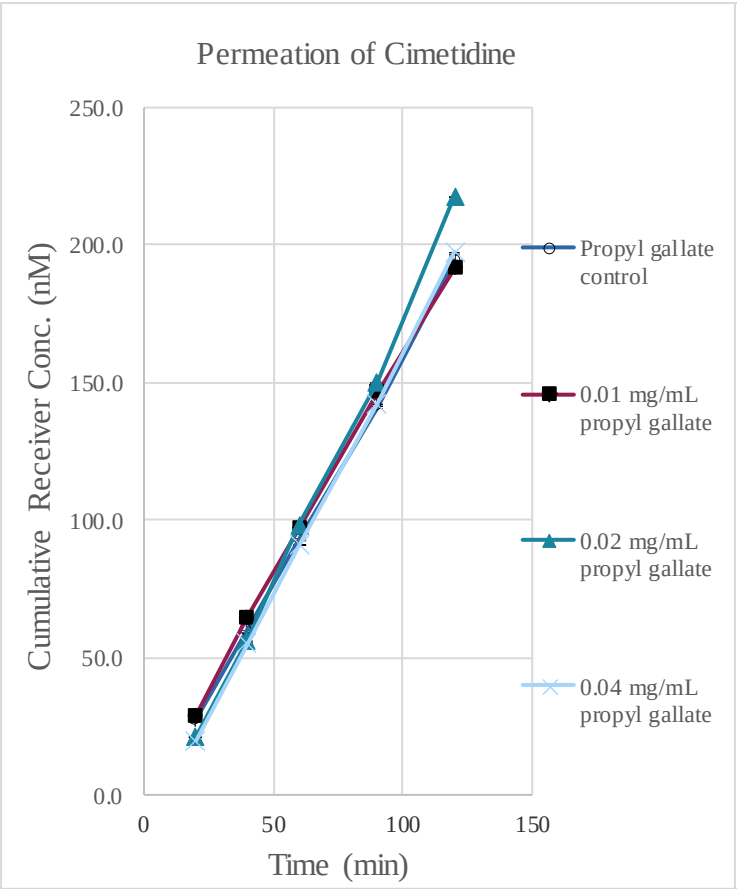


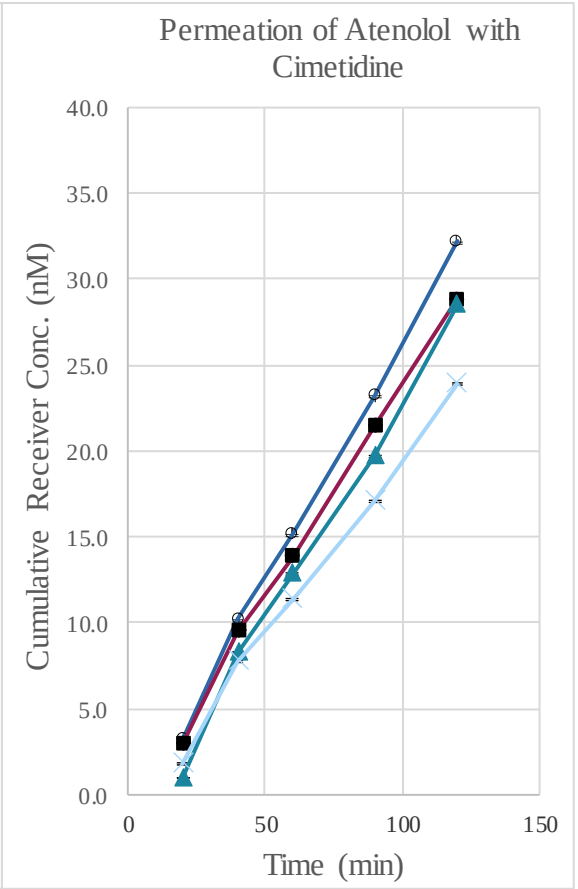
Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control	Cimetidine	0.883	0.111
0.01 mg/mL		0.845	0.047
0.02 mg/mL		0.861	0.045
0.04 mg/mL		0.836	0.070

Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control	Atenolol	0.362	0.083
0.01 mg/mL		0.317	0.062
0.02 mg/mL		0.301	0.036
0.04 mg/mL		0.303	0.043

n=6 replicates per treatment

Effect of Propyl Gallate on Permeation of Cimetidine and Atenolol





Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control	Cimetidine	0.619	0.072
0.01 mg/mL		0.605	0.056
0.02 mg/mL		0.727	0.074
0.04 mg/mL		0.660	0.072

Treatment	Analyte	P_{app} (10^{-6} cm/s)	
		Mean	SD
Control	Atenolol	0.335	0.068
0.01 mg/mL		0.300	0.048
0.02 mg/mL		0.313	0.045
0.04 mg/mL		0.252	0.035

n=6 replicates per treatment

Results with Antioxidants and Class III Model Drugs

Effects	Antioxidant	Model Drugs
No effect on permeation	Alpha-tocopherol	Acyclovir, atenolol, cimetidine, ranitidine
	Ascorbic acid	Acyclovir, atenolol, cimetidine, ranitidine
	Cysteine	Acyclovir, atenolol, cimetidine, ranitidine
	Propyl gallate	Acyclovir, atenolol, cimetidine, ranitidine

Summary of Results and Implications

The antioxidants tested, at the concentrations tested, had little or no effect on the permeation of the four Class III model drugs, suggesting that reformatting drug products to include an antioxidant may be a feasible approach for reducing the formation of NDSRIs.

Next Steps

In vitro data to be used to predict *in vivo* performance in combination with physiologically based pharmacokinetic (PBPK) modeling

康龙化成 PHARMARON

Thank You

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