Nitrosamine Research Studies Inform FDA on Potential Strategies and BE Approaches

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


Everyone deserves
confidence in their *next* dose
of medicine.
Pharmaceutical quality
assures the
availability,
safety,
and efficacy
of *every* dose.

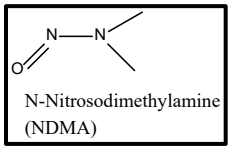
Outline

- Challenges of NDSRIs in drug products
- FDA sponsored research projects to support reformulations of impacted products
- FDA's considerations based on research results

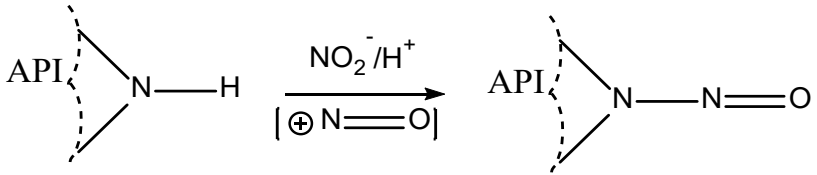
NDSRI: nitrosamine drug substance related impurities

NDSRIs in Drug Products

- Two classes of nitrosamines:
  - Small molecule nitrosamine (e.g., NDMA, NDEA)
  - NDSRIs in drug products (DPs) share structural similarity to the API



- NDSRIs are formed in DP during manufacturing or shelf-life storage



  - APIs (secondary or tertiary amines) are exposed to nitrosating compounds such as nitrite impurities in excipients under acidic conditions
  - Many products are impacted
- Acceptable intake (AI) limit determination for NDSRIs is challenging
  - Unique to each API (or API fragment)
  - Not much data on mutagenicity and carcinogenicity

NDSRI: nitrosamine drug substance related impurities

NDSRI Mitigation Strategies

FDA public announcement 11/18/2021[1]

- Screen excipients for nitrite impurities
- Add Antioxidant
- Add pH modifier
- Other innovative strategies

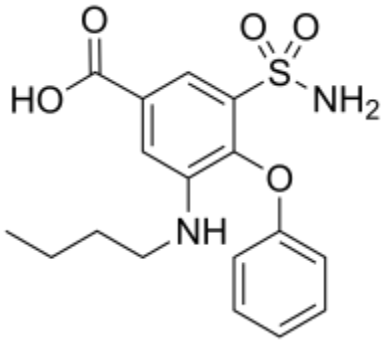
1. https://www.fda.gov/drugs/drug-safety-and-availability/updates-possible-mitigation-strategies-reduce-risk-nitrosamine-drug-substance-related-impurities

FDA Research Projects Related to NDSRI Mitigation

Quality	Bioequivalence	Bioequivalence
• Investigated the risk of NDSRI formation in finished product formulations with excipients in solid state • Studied NDSRI formation inhibition effects of excipients in drug formulations	• Contract project • Studied the impact of antioxidants on in vitro permeability of some BCS III APIs	• CERSI grant • Studied the effects of antioxidants on intestinal drug transporters using in vitro methods

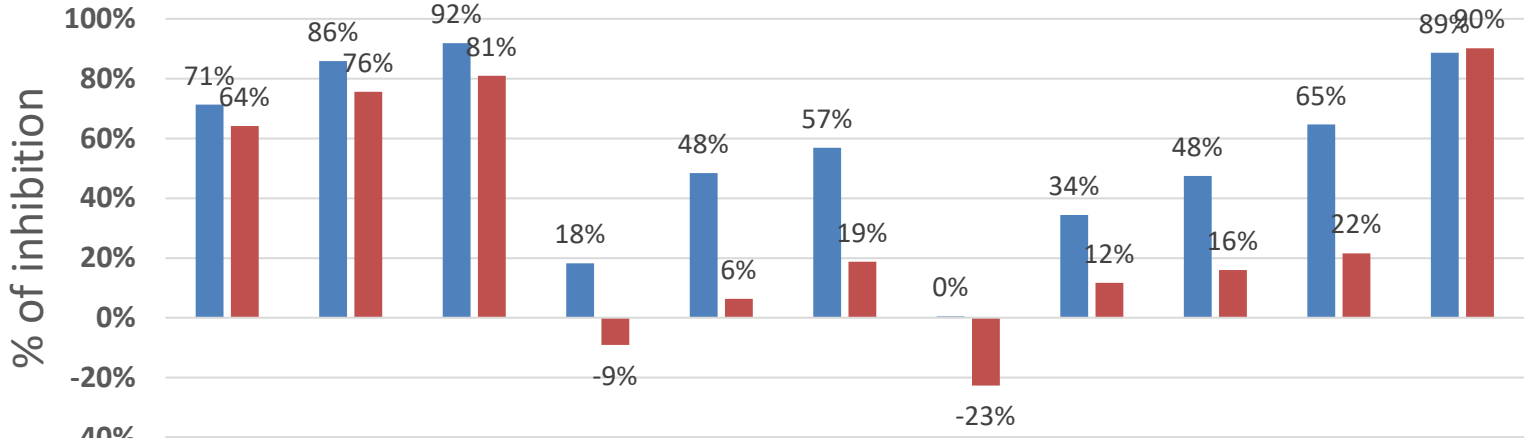
Project 1. Effectiveness of Antioxidants in Selected Model Drugs: Mitigation Strategy and Impact of Reformulation on Stability (quality)[2]

- Bumetanide
  - 3 antioxidants: ascorbic acid, caffeic acid and ferulic acid (0.1, 0.5 and 1%)
  - pH modifiers: acidic pH 3 (0.1N HCl) and basic pH 8 (0.1N $NaHCO_3$)
  - 60°C heated air
- Stability Conditions:
  - 50°C/75%RH 1mo.
  - 40°C/75%RH 1, 2, 3, 6mo.
  - 25°C/60%RH 1, 2, 3, 6mo.



2. J Pharm Sci. https://pubmed.ncbi.nlm.nih.gov/37364772/

% of Inhibition Efficiency by The Antioxidants – 6 Month Stability



	F4 (0.1% Ascorbic acid + 100 ppm nitrite)	F6 (0.5% Ascorbic Acid + 100 ppm Nitrite)	F8 (1% Ascorbic acid + 100 ppm nitrite	F10 (0.1% Caffeic acid + 100 ppm nitrite)	F12 (0.5% Caffeic acid + 100 ppm nitrite)	F14 (1% Caffeic acid + 100 ppm nitrite)	F16 (0.1% Ferulic acid + 100 ppm nitrite)	F18 (0.5% Ferulic acid + 100 ppm nitrite)	F20 (1% Ferulic acid + 100 ppm nitrite)	F22 (0.1N HCl + 100 ppm Nitrite)	F24 (2.75% NaHCO3 + 100 ppm nitrite)
40C-75%RH_6month	71%	86%	92%	18%	48%	57%	0%	34%	48%	65%	89%
25C-60%RH_6month	64%	76%	81%	-9%	6%	19%	-23%	12%	16%	22%	90%

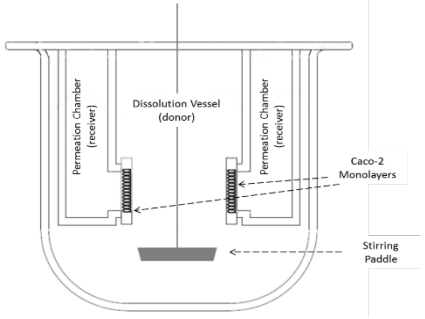
Small amounts of pH modifier or antioxidant can inhibit NDSRI formation

Challenges in Bioequivalence (BE) for Products Reformulated Due to Nitrosamines

- BE considerations
  - Solid oral dosage forms- most cases
  - SUPAC Level 3 changes (IR/MR)- typically in vivo BE study is recommended
  - Cost and time on in vivo BE studies
  - Timeframe to complete supportive studies
- FDA sponsored two research projects related BE
  1. Explored the impact of antioxidants on API permeability
  2. Tested whether antioxidants would inhibit intestinal transporters

Project 2- Effects of Antioxidants on Intestinal Permeability of BCS III Drugs (by Pharmaron)[3]

- BCS III drug substances selected
  - Acyclovir
  - Atenolol
  - Cimetidine
  - Ranitidine
- Four antioxidants
- In Vitro Dissolution Absorption System (IDAS)
- Caco-2 cell monolayers
- Apparent permeability coefficient (P_{app}) tested/calculated up to 120min
- Atenolol as internal marker

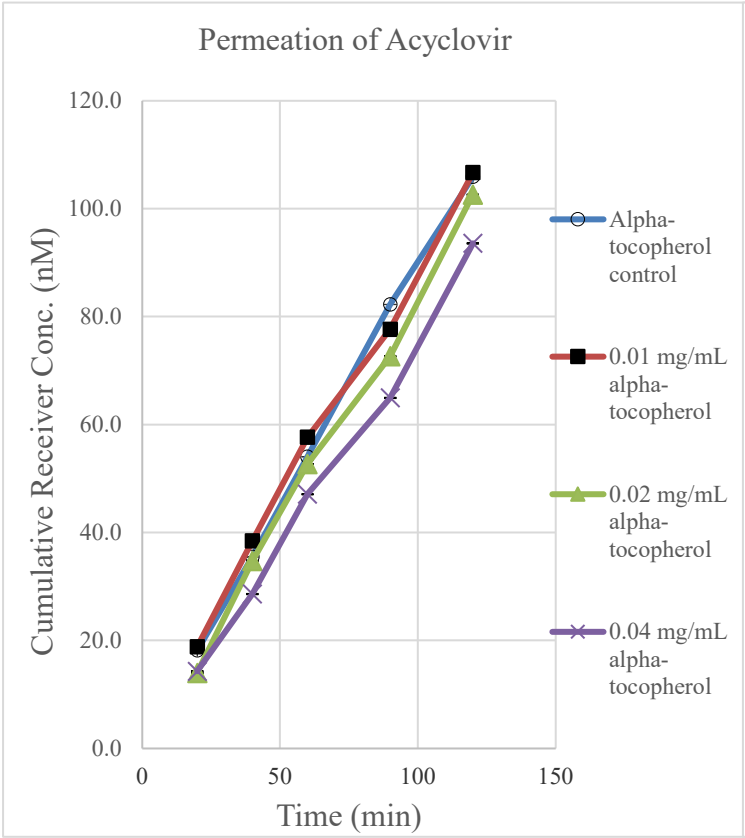


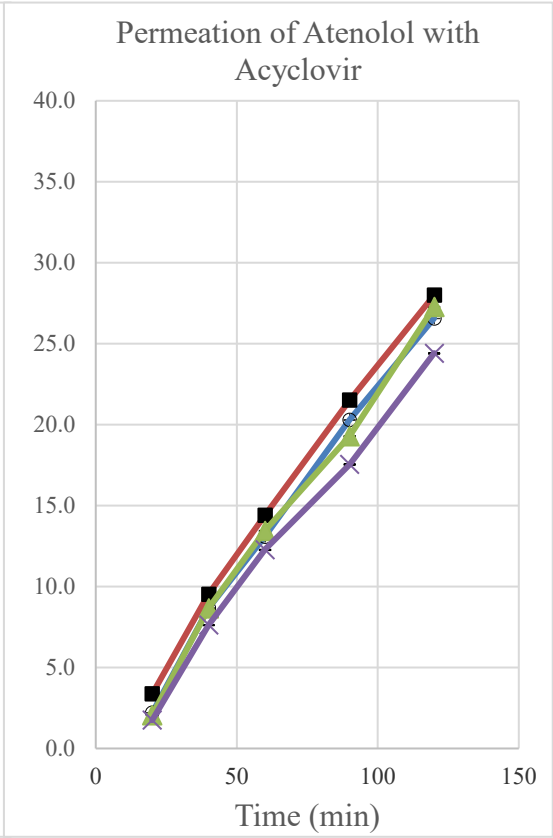
Excipient	Concentration (mg/mL)		
	Low	Mid	High
Alpha-Tocopherol	0.01	0.02	0.04
Ascorbic Acid	0.01	0.02	0.04
Cysteine HCl	0.01	0.02	0.04
Propyl Gallate	0.01	0.02	0.04

max 10 mg (2%) in 500 mg dosage unit, 250 mL water

3. https://www.complexgenerics.org/media/SOP/complexgenerics/pdf/Conference-Slides/NDSRIsmitigationstrategies/3-2_Session3_ChrisBode.pdf

Project 2- Effects of Antioxidants on Intestinal Permeability of BCS III Drugs (by Pharmaron)





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

Project 2- Results

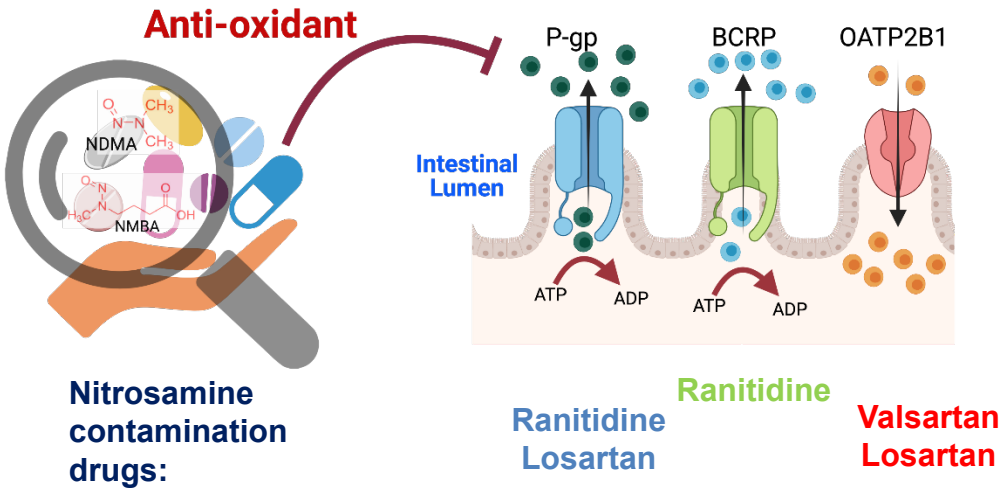
Treatment (n=6)		Mean P_{app} (10^{-6} cm/s) (standard deviation)			
		Acyclovir	Atenolol	Ranitidine	Cimetidine
Ascorbic acid	Control	0.280 (0.014)	0.396 (0.022)	0.448 (0.045)*	0.931 (0.042)
	0.01 mg/mL	0.278 (0.077)	0.364 (0.061)	0.438 (0.049)	0.952 (0.037)
	0.02 mg/mL	0.262 (0.018)	0.363 (0.019)	0.443 (0.048)	0.928 (0.081)*
	0.04 mg/mL	0.216 (0.020)	0.296 (0.025)	0.469 (0.042)	0.933 (0.050)
Cysteine	Control	0.275 (0.042)	0.297 (0.034)	0.406 (0.060)	0.883 (0.111)
	0.01 mg/mL	0.307 (0.050)	0.357 (0.059)	0.419 (0.036)	0.845 (0.047)
	0.02 mg/mL	0.362 (0.089)	0.355 (0.067)	0.461 (0.058)	0.861 (0.045)
	0.04 mg/mL	0.347 (0.045)	0.352 (0.035)	0.507 (0.087)	0.836 (0.070)
Propyl Gallate	Control	0.425 (0.058)	0.445 (0.037)	0.409 (0.040)*	0.619 (0.072)
	0.01 mg/mL	0.435 (0.031)	0.429 (0.039)	0.405 (0.077)	0.605 (0.056)
	0.02 mg/mL	0.362 (0.042)	0.364 (0.037)	0.386 (0.070)	0.727 (0.074)
	0.04 mg/mL	0.372 (0.054)	0.372 (0.054)	0.348 (0.038)	0.660 (0.072)
α-Tocopherol	Control	0.295 (0.023)*	0.283 (0.009)*	0.381 (0.031) #	0.678 (0.051)*
	0.01 mg/mL	0.285 (0.080)	0.287 (0.060)	0.354 (0.023)	0.622 (0.055)
	0.02 mg/mL	0.285 (0.071)	0.287 (0.047)	0.383 (0.037)	0.802 (0.087)
	0.04 mg/mL	0.259 (0.041)	0.259 (0.022)	0.378 (0.043)	0.764 (0.065) #

* One replicate was excluded due to cell monolayer integrity failure (when atenolol Papp > 1×10^{-6} cm/s

One replicate was excluded as a statistical outlier (per Q test at 90% confidence level for 6 replicates)

The tested amount (10 mg) of these tested antioxidants do not have significant impact on model BCS III drug permeability

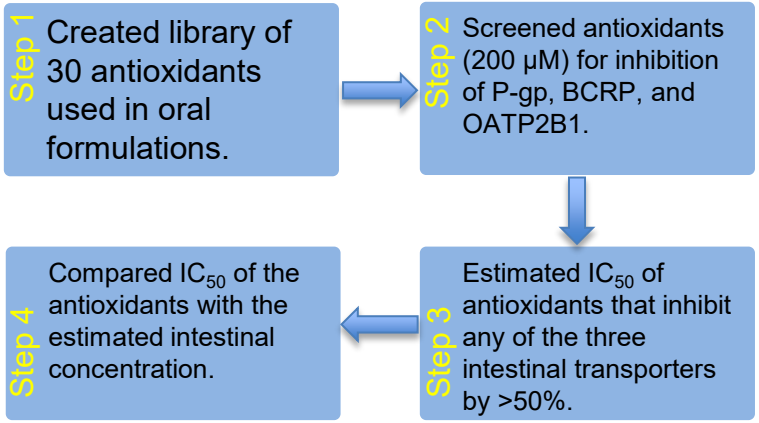
Project 3- Effects of Antioxidants in Drug Products on Intestinal Drug Transporters (by UCSF)[4]



- Some nitrosamine impacted products are substrates of transporters: P-gp, BCRP, OATP2B1
- 30 antioxidants (22 in FDA IID database and 8 natural products) were screened by membrane vesicles overexpressing transporters

4. https://www.complexgenerics.org/media/SOP/complexgenerics/pdf/Conference-Slides/NDSRIsmitigationstrategies/3-3_Session3_SookWahYee.pdf

Project 3- Effects of Antioxidants in Drug Products on Intestinal Drug Transporters (by UCSF)



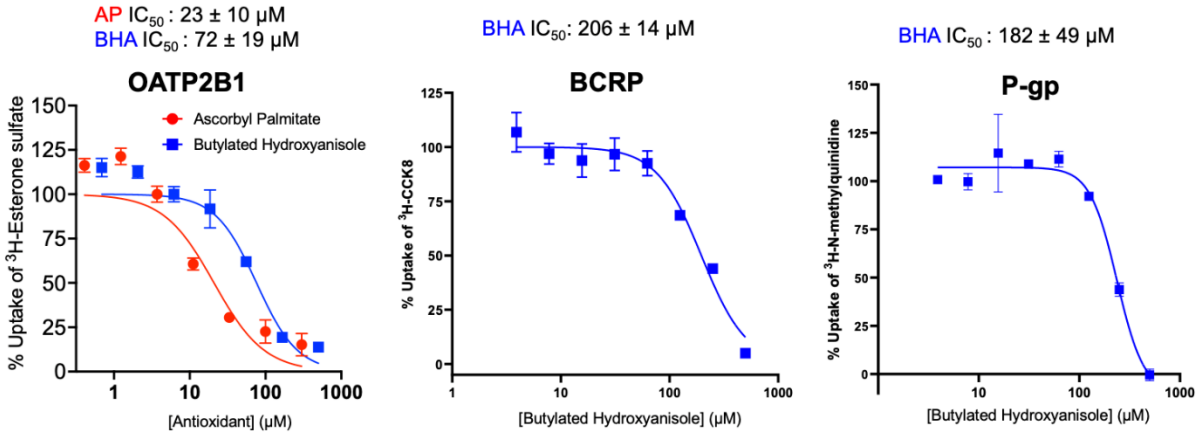
Transporter	Probe substrate
P-gp	^{3}H-N-methylquinidine
BCRP	^{3}H-CCK
OATP2B1	^{3}H-estrone sulfate

Uptake % of substrates when co-dosed with antioxidants

Antioxidants	OATP2B1	BCRP	P-gp
Ascorbic acid	103.6	92.7	113.6
Butylated hydroxytoluene (BHT)	137.1	102.8	98.1
Cysteine hydrochloride	106.5	90	134.8
Propyl gallate	79.3	86.5	149.1
Vitamin E	83.5	81	67.9
Anhydrous citric acid	128.8	93.1	92
EDTA (edetate disodium)	112.8	110.4	69.3
Erythorbic acid	123.3	88.2	81.8
Glycine	125.6	99.7	76.9
Histidine	108.9	92.4	87.6
Methionine	113.2	94.8	86.7
Phosphoric Acid	97.8	83	83.1
Potassium sorbate	98.8	104.8	103.5
Sesamol	103.1	90.3	121.3
Sodium bisulfite	90.1	110.4	96.1
Sodium metabisulfite	118.9	94.5	78.8
Sodium sulfite	92.9	99.7	77.7
Sodium thiosulfate, pentahydrate	104.4	114.2	77.4
Tartaric Acid	101.1	96.9	108

These antioxidants do not impact transporters

Project 3- Effects of Antioxidants in Drug Products on Intestinal Drug Transporters (by UCSF)



Antioxidant	Max intestinal conc (µM) in 250mL fluid
Ascorbyl palmitate (AP)	96.5
Butylated hydroxyanisole (BHA)	178

	I_{gut} / IC_{50}	
	AP	BHA
OATP2B1	96.5 / 23 = 4.2	178 / 72 = 2.5
BCRP	NA	178 / 206 = 0.9
P-gp	NA	178 / 182 = 1.0

I_{gut} / IC_{50} < 10, AP and BHA are unlikely to inhibit OATP2B1, BCRP or P-gp transporters

This project is still ongoing

Results Support Proposed Nitrosamine Mitigation Strategies and Inform Other BE Approaches

- Reformulations with pH modifier and antioxidants
  - Results verified that these two reformulation approaches are valid
  - Small amounts of added excipients can effectively inhibit NDSRI formation
- BE considerations
  - In vitro bridging studies (such as permeability, transporter activity tests) have good potential to demonstrate BE
  - Studied antioxidants at tested ranges did not have impact on in vitro permeability of BCS III drug substances or intestinal transporters (more supportive data are needed for levels beyond those researched)
  - Other approaches can be considered: modeling, IVIVC
  - Risked-based approach: manufacturers may use different approaches for low vs high-risk products
- FDA encourages novel approaches (contact FDA)
- FDA continues to consider approaches for products impacted by NDSRIs

Acknowledgements

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