

# Characterization of Degradation Products of LY518674 from a Drug-Excipient Compatibility Study Using QTOF and FT LC-MS, and Correlation with Those Observed in LY518674 Stressed with AIBN or Formaldehyde

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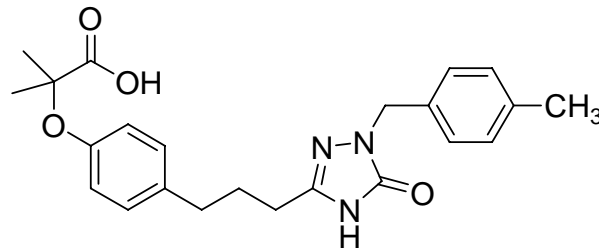
<sup>‡</sup>Eli Lilly-Hamburg, Germany

The Lilly logo is written in a red, cursive script font.

Answers That Matter.

# Introduction

LY518674 is a peroxisome proliferator-activated receptor (PPAR) alpha agonist.



**LY518674**

$C_{23}H_{27}N_3O_4$

Exact Mass: 409.2002

PPAR alpha agonists are intended to be effective in treating atherogenic dyslipidemia, a cholesterol disorder characterized by the elevation of triglycerides and a decrease in high-density lipoprotein ( HDL ) levels in the blood. This lipid disorder is associated with an increased risk of developing cardiovascular disease.

# Introduction

## **Why** drug-excipient compatibility characterization in early Development?

- “More with less” is essential in Pharmaceutical Industry
- Shorten cycle times and speed candidates through milestones by delivering more detailed information earlier in the Drug Development process
- Mitigate risk and increase probability of success
- Detect incompatibilities in pre-clinical stages, prior to prototype formulation development
- Understand chemical interactions and develop control strategies at earlier stages
- For us, this translates to application of deeper structural characterization efforts earlier in Drug Development

# Introduction

## Regulatory Considerations

- FDA “*Guidance for Industry: Q3B (R2) Impurities in New Drug Products*” and EMEA “*Note for Guidance on Impurities in New Drug Products*” for regulatory submissions
- Thresholds for reporting of impurities are at maximum daily doses of less than 1 g drug substance *at the 0.1 % level* (> 1 g drug substance: 0.05 %)
- Does *not apply to excipient compatibility studies* at that stage of development
- FDA draft guidance from November 2004 “*Q8 Pharmaceutical Development*” & EMEA “*Note for Guidance on Pharmaceutical Development*”: Testing of the compatibility of the drug substance with the excipients for the regulatory submission, but not during clinical research stages - BUT the general *principles should be applied*

# Introduction

## GUIDANCE ON PHARMACEUTICAL DEVELOPMENT

(EMA/CHMP/167068/2004 and FDA Q8 Guidance for Industry: Pharmaceutical Development)

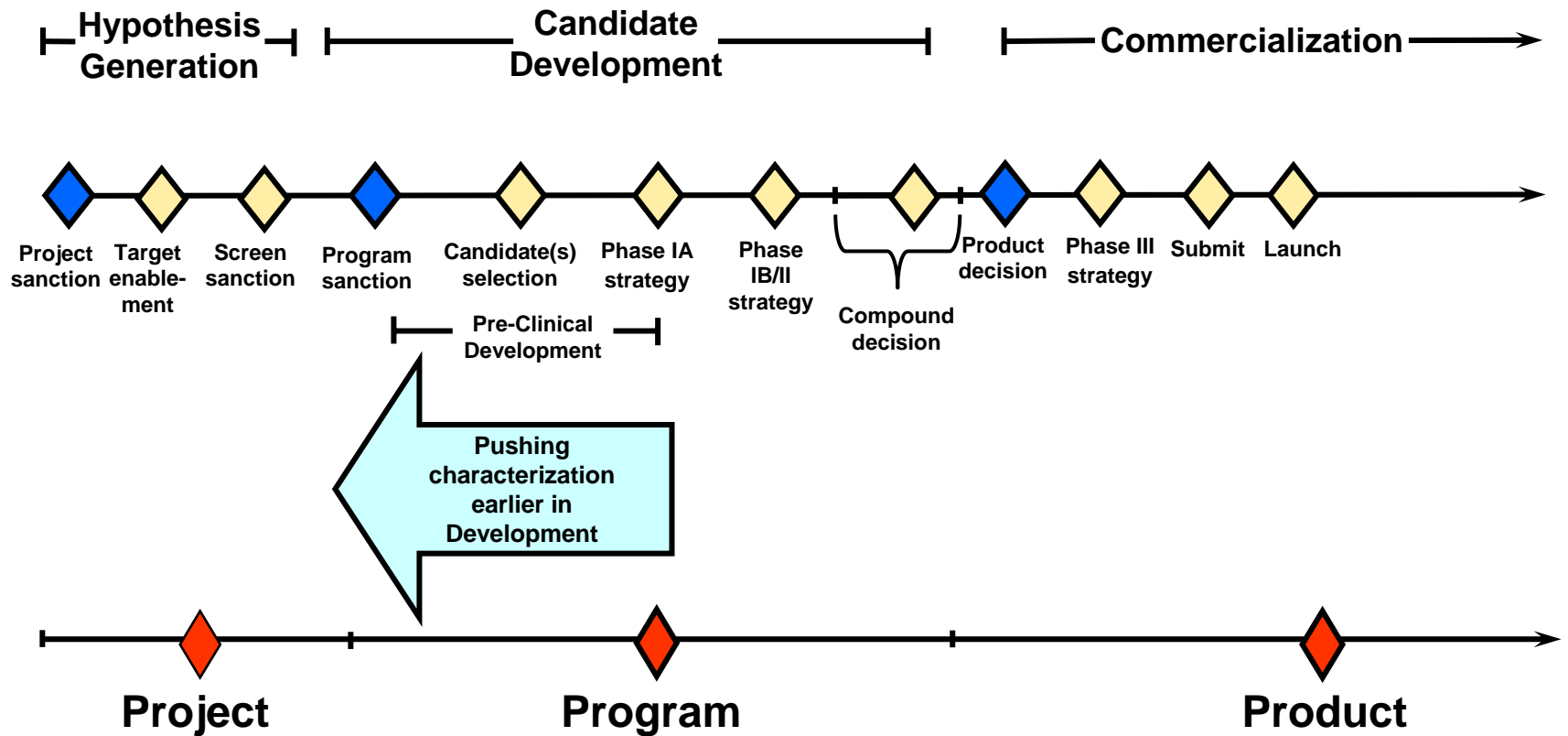
“The guidance does not apply to contents of submissions for drug products during the clinical research stages of drug development. **However the principles in this guideline are important to consider during those stages as well....**”

### 2.1.2 Excipients

*The excipients chosen, their concentration, and the characteristics that can influence the drug product performance (e.g., stability, bioavailability) or manufacturability should be discussed relative to the respective function of each excipient. Compatibility of excipients with other excipients, where relevant (for example combination of preservatives in a dual preservative system), should be established. The ability of excipients (e.g., antioxidants, penetration enhancers, disintegrants, release controlling agents) to provide their intended functionality, and to perform throughout the intended drug product shelf life, should also be demonstrated. **The information on excipient performance can be used, as appropriate, to justify the choice and quality attributes of the excipient, and to support the justification of the drug product specification (3.2.P.5.6)**”.*

# Introduction

## Drug Discovery and Development



# Overview

- A study of the compatibility of LY518674 with 26 excipients typically used in solid state dosage forms was conducted at Eli Lilly-Hamburg, Germany, to support prototype tablet development.
- Primary degradation products in stressed binary and ternary test mixtures containing excipients and LY518674 were screened by HPLC/UV to estimate increases in total impurities.
- Of all excipient mixtures evaluated, the greatest increase in degradation products was observed for a ternary polyethylene glycol (PEG) test mixture, containing LY518674 at 1% (w/w) and PEG 4000 powder at 5% (w/w), which was stressed at 60 °C for four weeks. Interestingly, different degradation product profiles were observed for the PEG 4000 mixture samples with open and closed container conditions.
- Primary degradation products in the PEG test mixtures were characterized at Eli Lilly-Indianapolis by HPLC coupled with QTOF and FT mass spectrometry (FTMS).
- Initial characterization of the major degradation products indicated structures consistent with oxidative degradation mechanisms or formaldehyde adduct formation.
- To support the proposed structures, LY518674 was stressed respectively with a radical initiator, 2,2'-azobisisobutyronitrile (AIBN) or formaldehyde, which generated degradation products consistent with those observed in the compatibility evaluation.

# Excipient Types

- Filler or Diluent: Dilutes drug to tablet or capsule of suitable size
- Binder: Holds tablet together
- Disintegrant: Promotes disintegration and breakup of a tablet in the stomach or intestine
- Glidant: Improves flow and processability of powder during tablet compression
- Lubricant: Lubricates machines to ensure effective operation



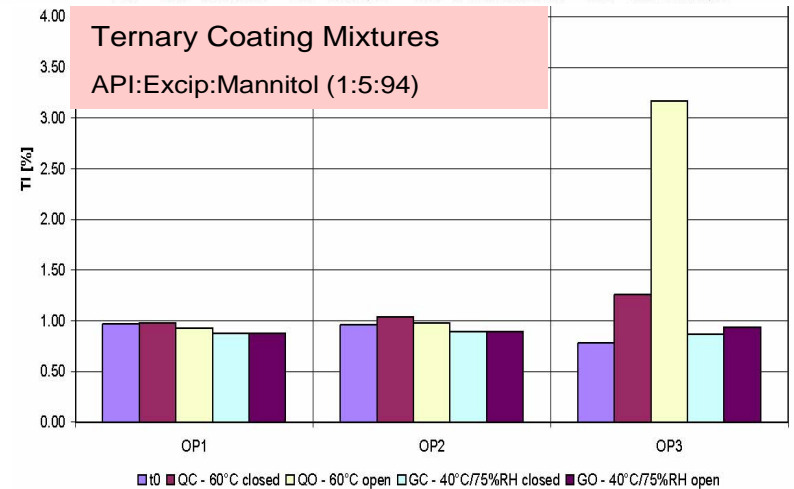
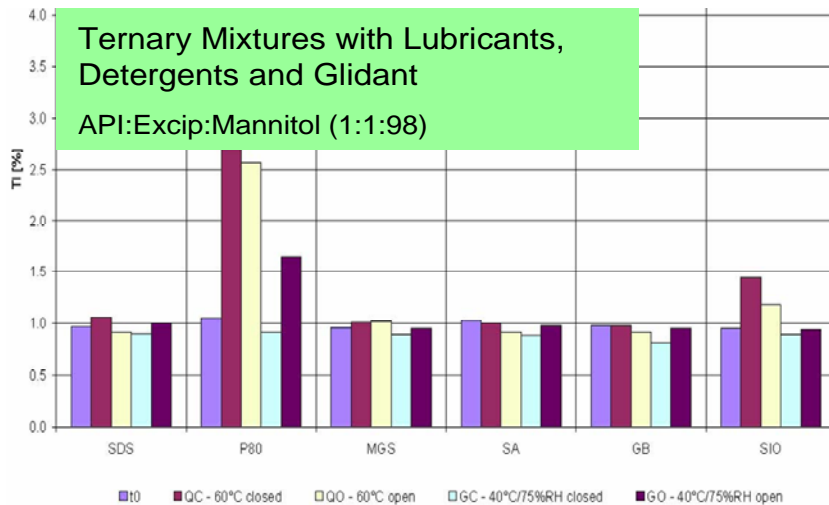
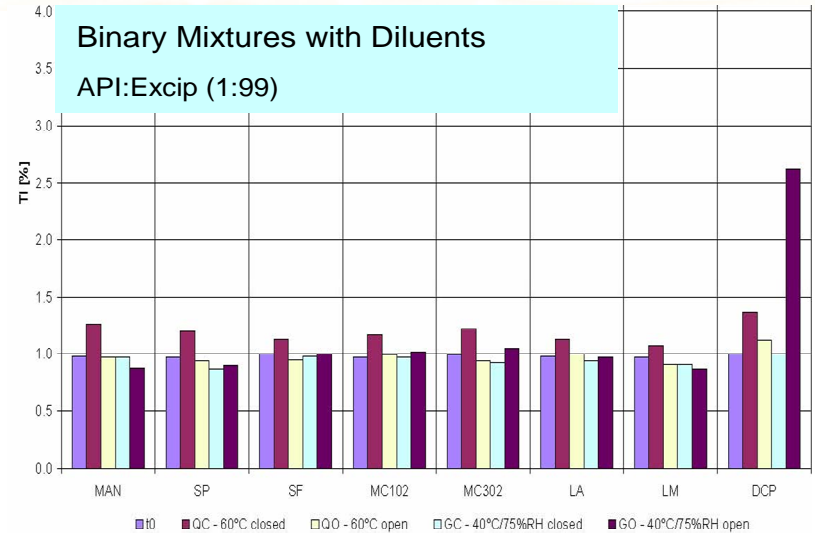
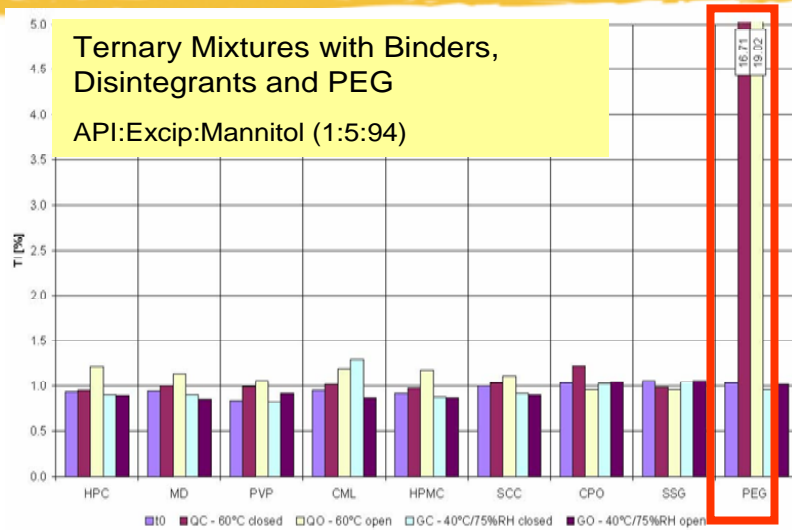
# Excipients Evaluated in Compatibility Study

Category	Excipient	Mixture
Diluent	Mannitol Starch Powder Starch Powder Flowable Microcrystalline Cellulose Granular 102 Microcrystalline Cellulose Granular 302 Lactose Anhydrous Granular Lactose Spray Dried Dibasic Calcium Phosphate Anhydrous	Binary API:Excip (1:99)
Binder	Hydroxypropyl Cellulose Maltodextrin Povidone Hydroxypropyl Methylcellulose	Ternary API:Excip:Mannitol (1:5:94)
Disintegrant	Carmellose Sodium Croscarmellose Sodium Crospovidone Sodium Starch Glycolate	
PEG	Polyethylene Glycol 4000	
Coating	Coating 1 Coating 2 Coating 3	Ternary API:Excip:Mannitol (1:5:94)
Surfactant	Sodium Dodecyl Sulfate Polysorbate 80	Ternary API:Excip:Mannitol (1:1:98)
Lubricant	Magnesium Stearate Stearic Acid Micronized Glyceryl Behenate	
Glidant	Colloidal Silicon Dioxide	

# Excipient Compatibility Study Storage Conditions

Storage Conditions (4 weeks)		
Container	Temperature	RH
Closed	40°C	75%
Open	40°C	75%
Closed	60°C	Ambient
Open	60°C	Ambient

# Excipient Compatibility Study Summary



\*From: Kuehl, P. and Bergemann, C. "Excipient Compatibility Study for PPAR alpha Agonist I", Internal Technical Report (2006)

# Sample Preparation

## ***Drug-Excipient Compatibility Study Samples***

Four PEG test mixtures from the excipient compatibility study were received from Lilly-Hamburg. A 2 mg/mL solution of each mixture sample was prepared in ACN/H<sub>2</sub>O (30/70) for LC-MS analysis.

- PEG/mannitol mixture with LY518674, open and closed container
- PEG/mannitol matrix only, open and closed container

## ***LY518674 Stressed with AIBN***

A solution of ACN/H<sub>2</sub>O (80/20) containing LY518674 at a concentration of 1 mg/mL, and AIBN at 2.8 mg/mL, was held at 40 °C for 24 hours. The solution was diluted 1:5 with ACN/H<sub>2</sub>O (30/70) for LC-MS analysis.

## ***LY518674 Stressed with Formaldehyde***

To a solution of ACN/H<sub>2</sub>O (50/50) containing LY518674 at 1 mg/mL was added 1 mL of formaldehyde solution (37% by wt.). The solution was held in a closed container at ambient temperature for 24 hours, and diluted 1:5 with ACN/H<sub>2</sub>O (30/70) for LC-MS analysis.

# LC-MS Operating Conditions

## HPLC

Instrument: Agilent 1100 HPLC system  
HPLC Column: Zorbax SB-Phenyl, 150 x 4.6 mm, 5.0  $\mu\text{m}$ , 40 °C  
Flow Rate: 1.0 mL/min  
Detection: Photodiode array, 200-400 nm  
Mobile Phase A: 0.1% trifluoroacetic acid in water  
Mobile Phase B: 0.1% trifluoroacetic acid in acetonitrile

Gradient:	Time (min.)	%A	%B	Curve	Flow (mL/min)
	0.00	70	30	-----	1.00
	12.00	50	50	linear	1.00
	20.00	50	50	-----	1.00
	23.00	30	70	linear	1.00
	26.00	30	70	-----	1.00
	27.00	5	95	linear	1.00
	31.00	5	95	-----	1.00

# LC-MS Operating Conditions

## QTOF MS

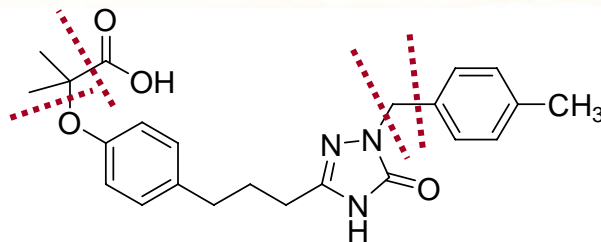
Instrument:	Applied Biosystems/Sciex QSTAR XL tandem quadrupole/time of flight mass spectrometer
Ionization mode:	Positive ion electrospray
Mass Range:	$m/z$ 100-1400 (survey MS)
Nebulizer Gas (N <sub>2</sub> ):	60 arb u
Desolvation Gas (N <sub>2</sub> ):	30 arb u
TurbolonSpray Temperature:	350 °C
IonSpray Voltage:	5.0 kV
MS/MS Operation:	Products of $m/z$ 306.1, 454.2, 440.2, 408.2, 426.2, 424.2, 410.2
MS/MS Collision Energy:	23 V

# LC-MS Operating Conditions

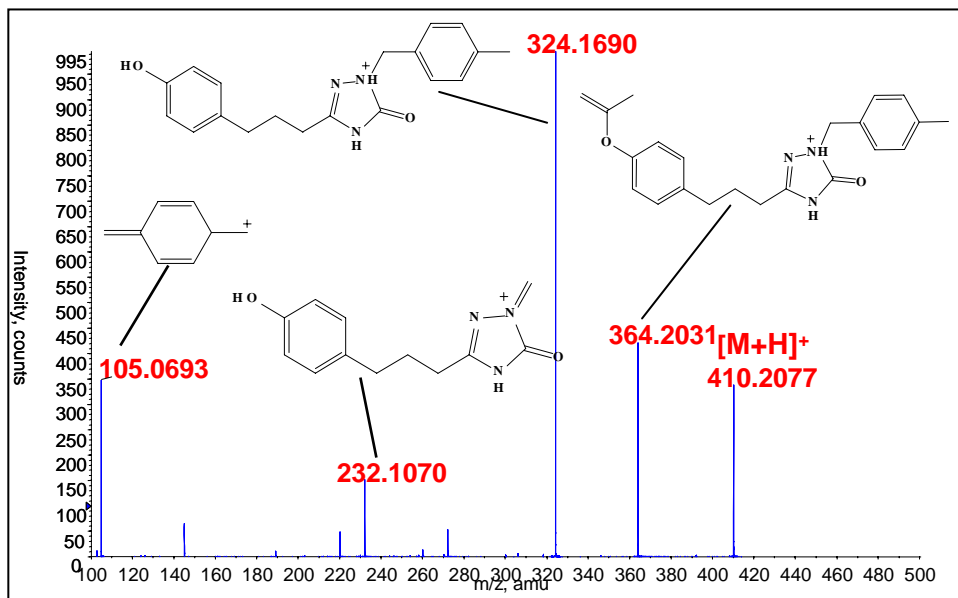
## FTMS

Instrument:	Thermo Electron LTQ FT (6T) Fourier Transform mass spectrometer
Ionization mode:	Positive ion electrospray
Mass Range:	$m/z$ 100-1200 (survey MS)
Sheath Gas (N <sub>2</sub> ):	40 arb u
Capillary Temperature:	300 °C
Capillary Voltage:	49 V
Tube Lens Voltage:	105 V
Source Voltage:	4.0 kV
MS <sup>2</sup> Operating Mode:	Data dependent analysis; products of $m/z$ 306.1, 454.1, 440.1, 408.1, 424.1, 410.1
MS <sup>2</sup> Isolation Width:	3.0
Normalized Collision Energy:	30

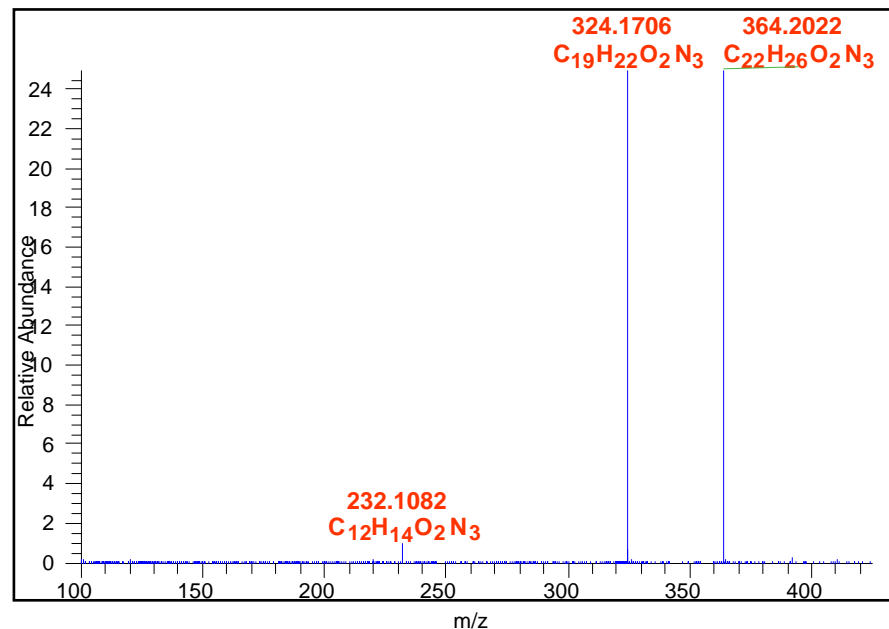
# Primary ESI(+) MS/MS Product Ions of LY518674



**A**

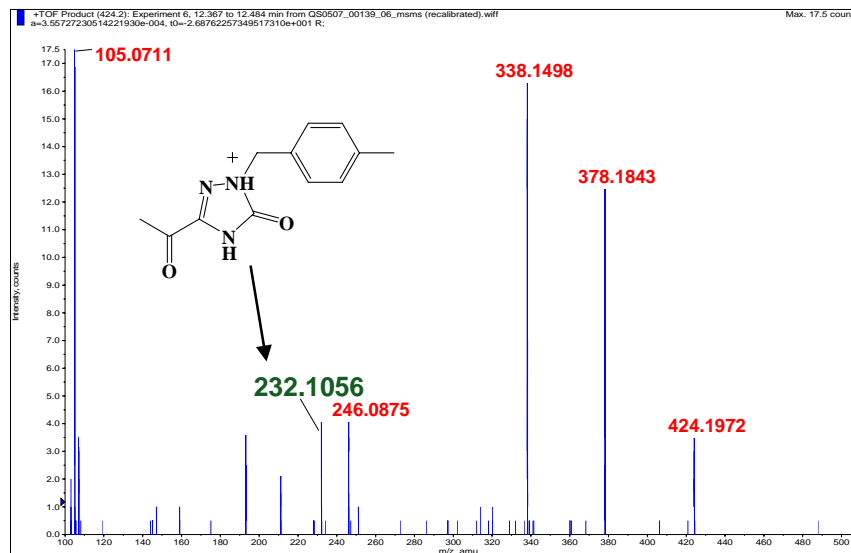
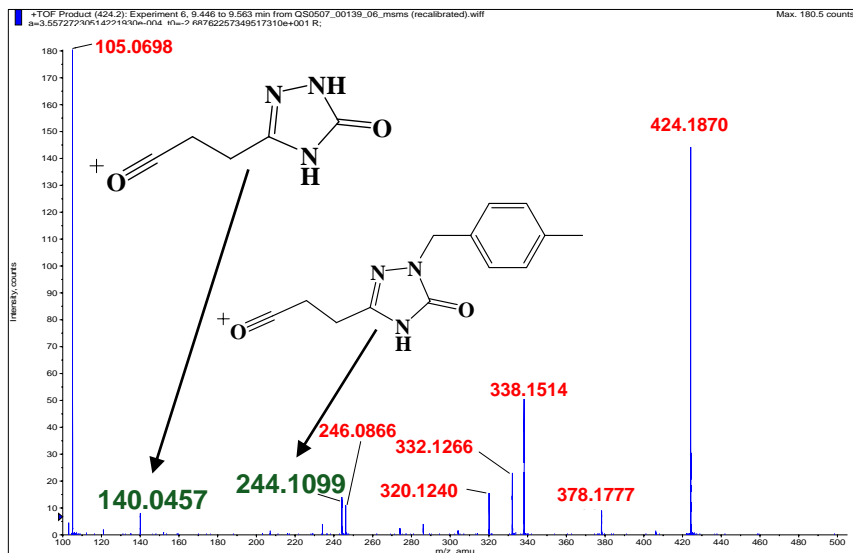
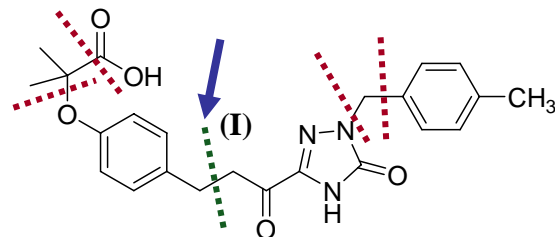
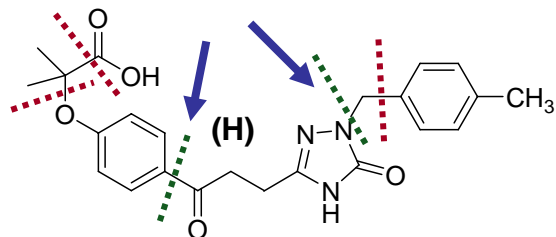


**B**



Comparative QTOF (A) and FT (B) MS/MS product ion spectra of LY518674 illustrating common primary product ions

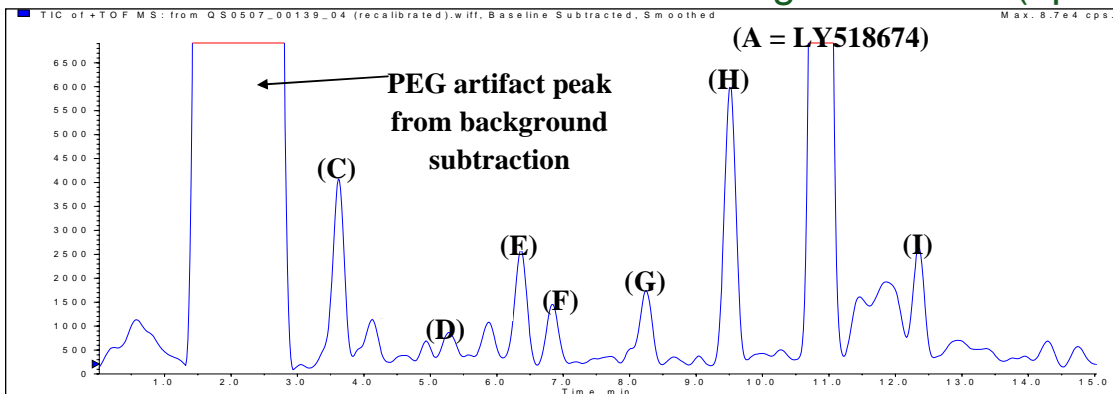
# Primary ESI(+) MS/MS Product Ions of Degradation Products (H) and (I)



Comparative QTOF MS/MS product ion spectra of isomeric degradation products (H) and (I) illustrating unique, diagnostic product ions permitting differentiation based on exact mass and site of modification

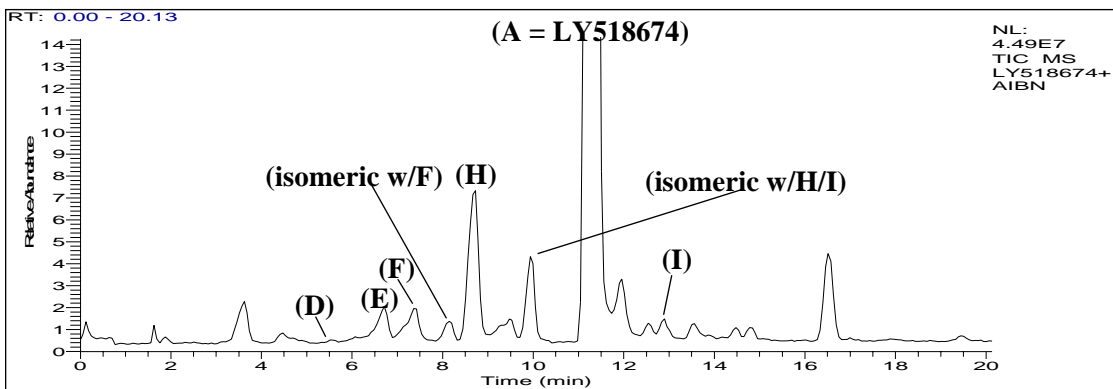
# Total Ion Chromatograms

## QTOF MS TIC of PEG Mixture containing LY518674 (open container)



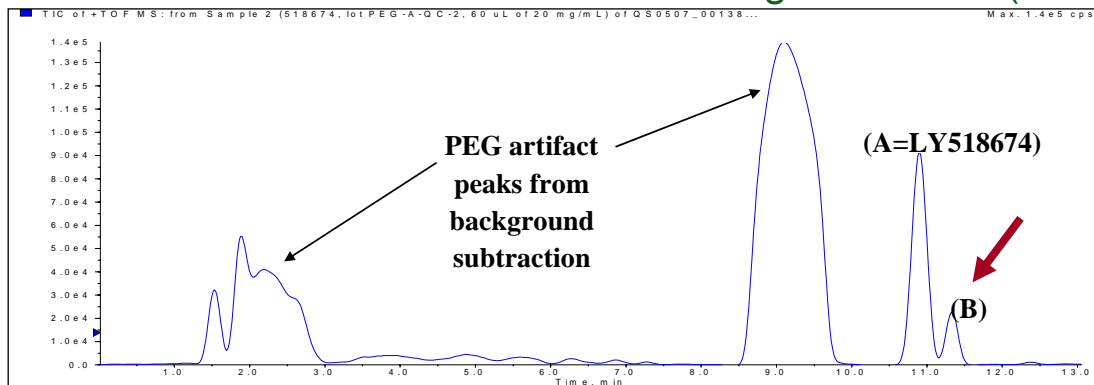
Peak ID	Measured Mass ( $m/z$ ) [M+H] <sup>+</sup>	Calculated Mass ( $m/z$ ) [M+H] <sup>+</sup>	Elemental Composition [M+H] <sup>+</sup>	DBE	Error (mDa)
A	410.2061	410.2074	C <sub>23</sub> H <sub>28</sub> N <sub>3</sub> O <sub>4</sub>	11.5	-1.3
C	306.1453	306.1448	C <sub>15</sub> H <sub>20</sub> N <sub>3</sub> O <sub>4</sub>	7.5	0.5
D	454.1600	454.1608	C <sub>23</sub> H <sub>24</sub> N <sub>3</sub> O <sub>7</sub>	13.5	-0.9
E	440.1822	440.1816	C <sub>23</sub> H <sub>26</sub> N <sub>3</sub> O <sub>6</sub>	12.5	0.6
F	408.1934	408.1917	C <sub>23</sub> H <sub>26</sub> N <sub>3</sub> O <sub>4</sub>	12.5	1.6
G	426.2022	426.2023	C <sub>23</sub> H <sub>28</sub> N <sub>3</sub> O <sub>5</sub>	11.5	-0.1
H	424.1860	424.1866	C <sub>23</sub> H <sub>26</sub> N <sub>3</sub> O <sub>5</sub>	12.5	-0.7
I	424.1863	424.1866	C <sub>23</sub> H <sub>26</sub> N <sub>3</sub> O <sub>5</sub>	12.5	-0.4

## FTMS TIC of LY518674 stressed with AIBN



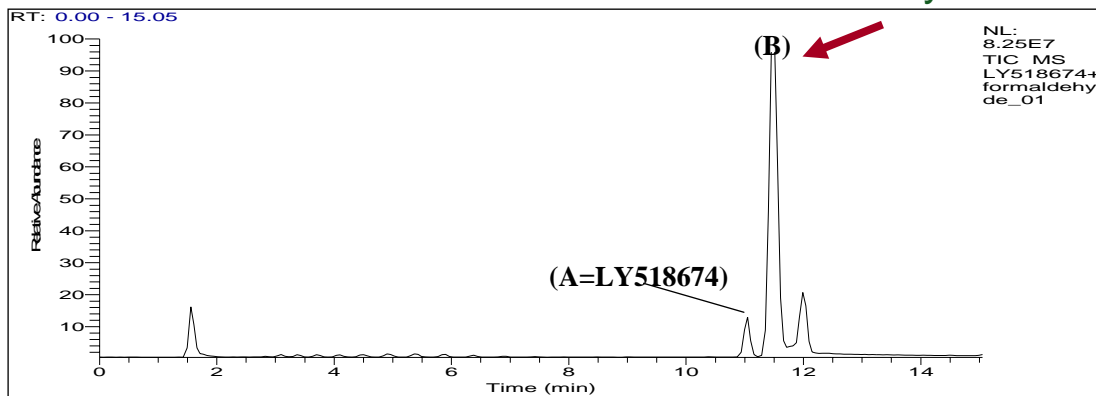
# Total Ion Chromatograms

## QTOF MS TIC of PEG Mixture containing LY518674 (closed container)



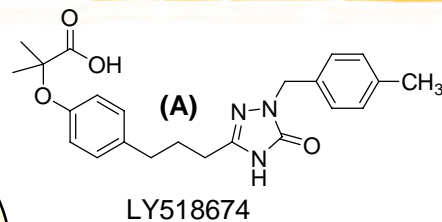
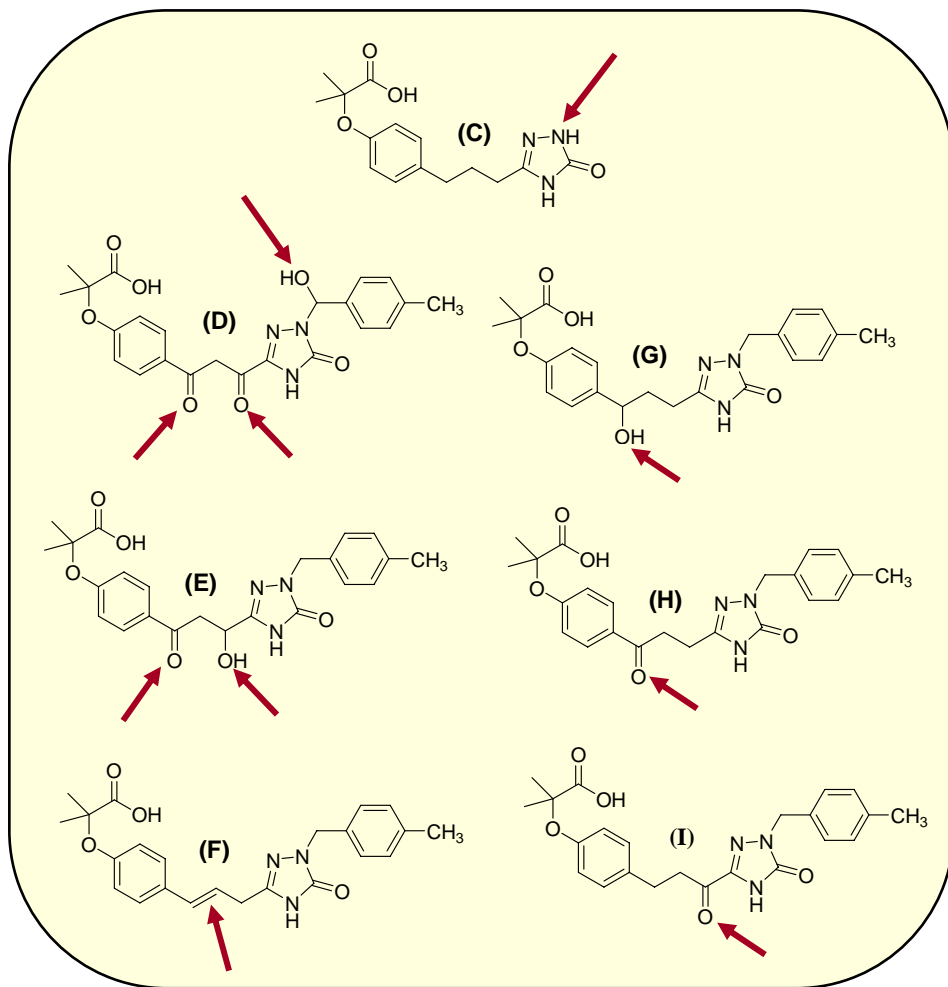
Peak ID	Measured Mass ( $m/z$ ) [M+H] <sup>+</sup>	Calculated Mass ( $m/z$ ) [M+H] <sup>+</sup>	Elemental Composition [M+H] <sup>+</sup>	DBE	Error (mDa)
A	410.2061	410.2074	C <sub>23</sub> H <sub>28</sub> N <sub>3</sub> O <sub>4</sub>	11.5	-1.3
B	440.2179	440.2179	C <sub>24</sub> H <sub>30</sub> N <sub>3</sub> O <sub>5</sub>	11.5	-0.1

## FTMS TIC of LY518674 stressed with formaldehyde

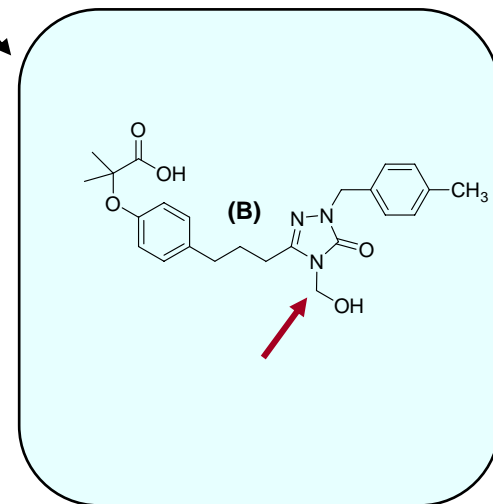


# Proposed Degradation Product Structures

## Open container

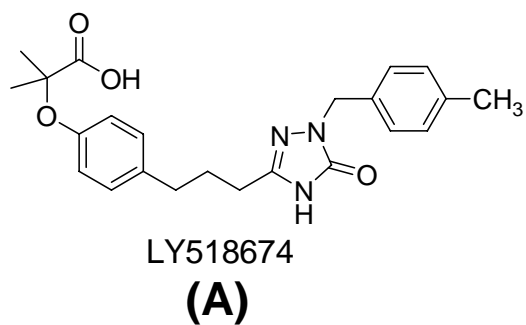


## Closed container

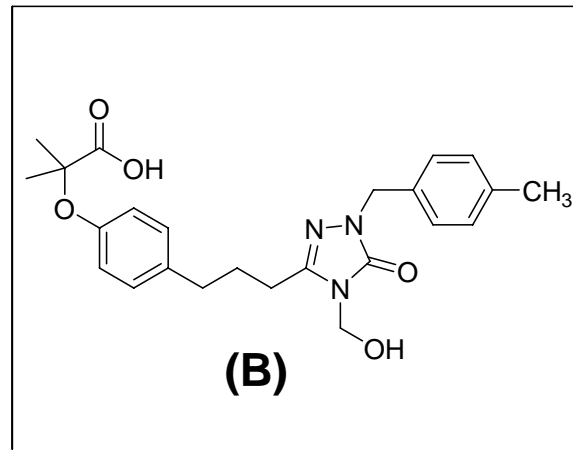


# Proposed Degradation Pathways

Proposed pathway for formation of degradation product (B) in a drug-excipient mixture containing PEG

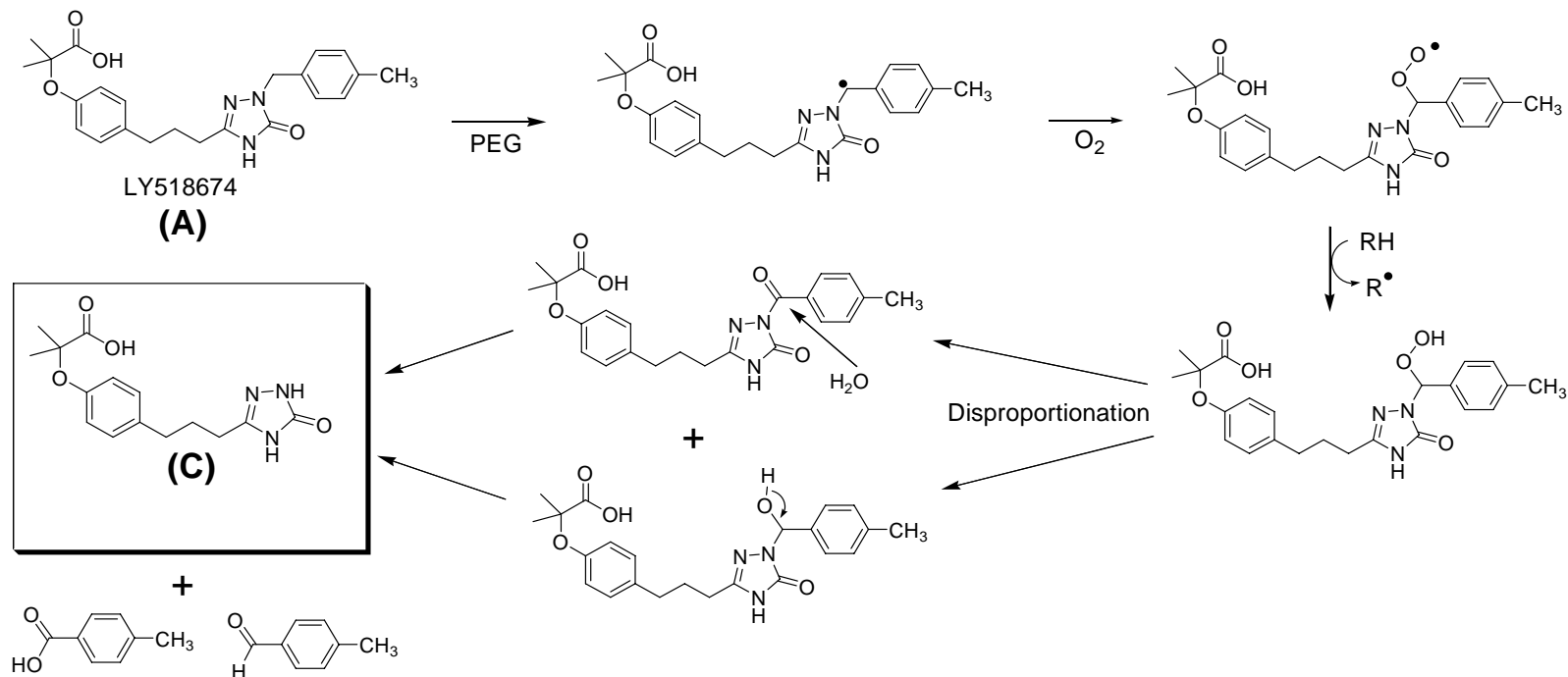


PEG autoxidation  
⇓  
Formaldehyde  
→



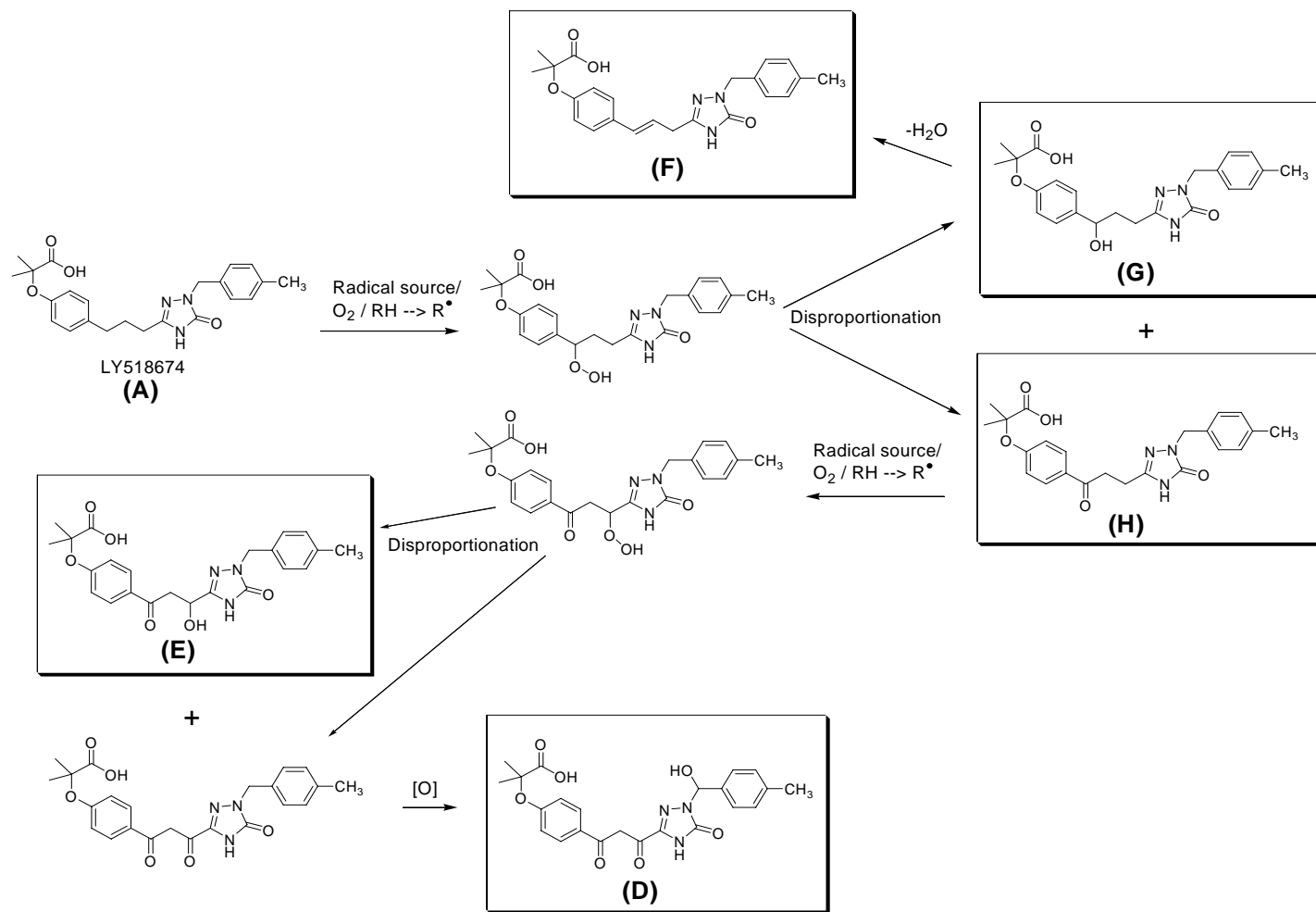
# Proposed Degradation Pathways

Proposed pathway for formation of degradation product (C) in a drug-excipient mixture containing PEG



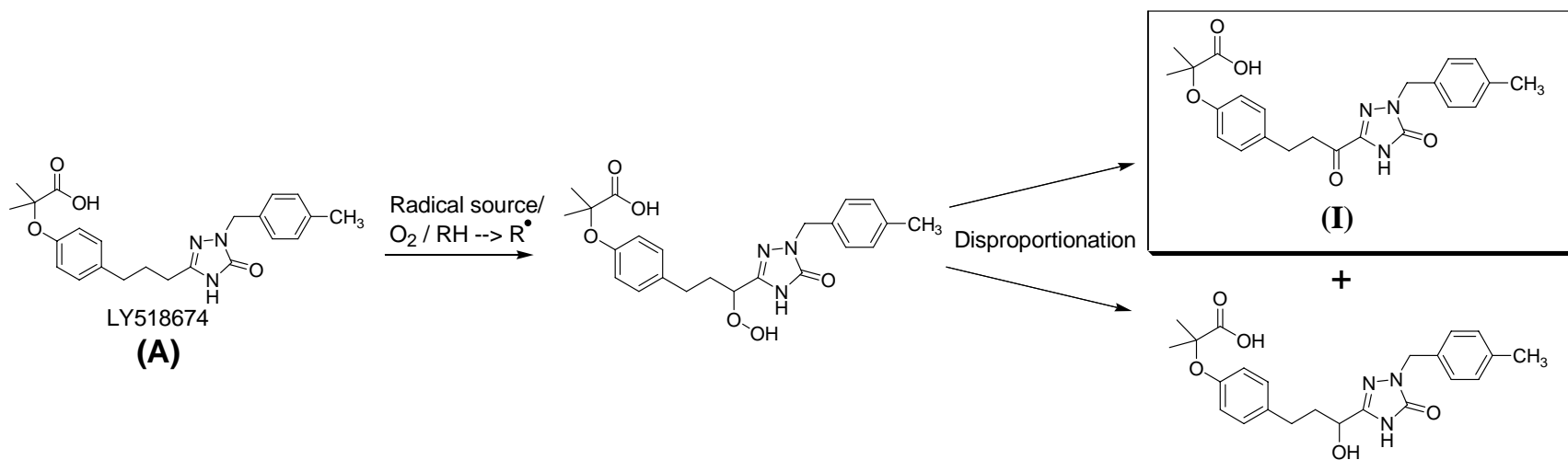
# Proposed Degradation Pathways

Proposed pathway for formation of degradation products (D-H) in a drug-excipient mixture containing PEG



# Proposed Degradation Pathways

Proposed pathway for formation of degradation product (I) in a drug-excipient mixture containing PEG



# Conclusions

- 1) Oxidative degradation is primarily responsible for the observed degradation products of LY518674 in PEG test mixtures stored at 60 °C for four weeks with open container conditions.
- 2) Degradation products of LY518674 observed with open container conditions arise from hydroxylation and ketone formation at benzylic sites, as well as dehydration to form additional sites of unsaturation, and are likely due to the presence of peroxide impurities which are typically present in higher molecular weight PEGs.
- 3) The primary degradation product observed with closed container conditions likely arises from the addition to LY518674 of formaldehyde, a known autoxidation product of PEG.
- 4) Stressing LY518674 with the radical initiator AIBN effectively produced oxidative degradation products consistent with those observed in the PEG test mixtures with open container conditions.
- 5) Solutions of LY518674 stressed with formaldehyde effectively produced a degradation product characterized by N-formyl addition consistent with that observed in the PEG test mixture with closed container conditions.
- 6) There was no evidence of “PEGylation” of LY518674 occurring in the PEG test mixtures.

# Literature Cited

1. Kuehl, P. and Bergemann, C. "Excipient Compatibility Study for PPAR alpha Agonist I", Internal Technical Report (2006)
2. Bindra, D.S., Williams, T.D., and Stella, V.J. "Degradation of O6-Benzylguanine in Aqueous Polyethylene Glycol 400 (PEG 400) Solutions: Concerns with Formaldehyde in PEG 400", *Pharm. Res.*, 11:7, 1060-1064 (1994)
3. Antipas, A.S., Landis, M.S. "Solid-state Excipient Compatibility Testing" *Drugs and the Pharmaceutical Sciences*, 153 (Pharmaceutical Stress Testing), 419-458 (2005)

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