

# Is LC-MS/MS Enough for Characterization of Novel and Regioisomeric Metabolites of Drug Candidates?

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Chandra Prakash, Ph. D.

Pfizer Global Research and Development, Groton, CT

# Presentation Outlines

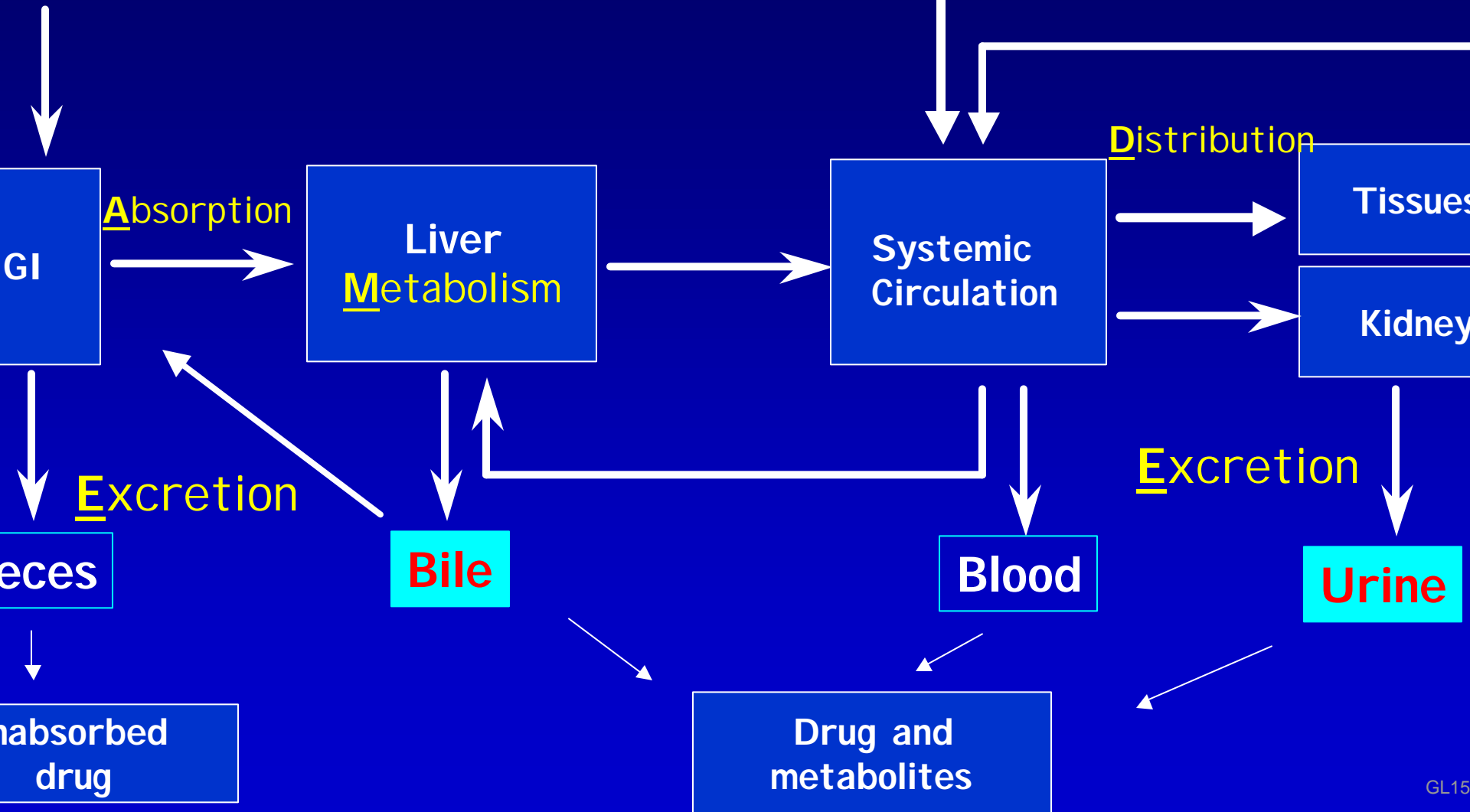
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- Introduction/background
- Need for metabolite identification
- Methods for the identification of metabolites
- Examples
  - Metabolism of a tetrahydroisoquinoline analog
  - Metabolism of of a *tert* butylbenzyl-pyridinylsulfonamide
  - Metabolism of a naphthyridine-3-carboxylic acid benzylamide
- Summary

# Fate Of Drugs In Living Organisms

## Oral Administration

## IV Administration



# Role of Metabolites in Present-Day Drug Development

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## ◆ Discovery

- Identification of metabolic "hot spots"
  - Optimize the metabolic Stability and Intrinsic Clearance
  - Synthesis of more metabolically stable analogs
- Structure-metabolism relationship (new leads)

## ◆ Preclinical Development

- Species comparison
- Pharmacological activity
- Reactive intermediates

## ◆ Clinical Development

- Comparison of human metabolic pathways with those of tox species
- Synthesis of metabolites for toxicology testing
- Metabolite-assay development

# Methods for Identification of Drug Metabolites

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MS (combined with separation techniques, HPLC, GC)

## ◆ Advantages:

- Greater sensitivity
- Greater Selectivity (Through SIM or MS/MS)
- Better resolution (capabilities of monitoring different masses in different channels)

## ◆ Disadvantages

- Does not differentiate isomers
- Does not always provide precise structural identification

# Approaches Combined With Mass Spectrometry for the Identification of Metabolites

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- ◆ Derivatization
- ◆ Hydrogen-deuterium exchange
- ◆ Hydrolysis (enzymatic, acid, base)
- ◆ Isotope Cluster
- ◆ *In vitro* systems (S-9, microsomes, hepatocytes and recombinant enzymes)
- ◆ LC-NMR (Continuous flow or stopped flow)
  - Fast
  - Reportedly sensitive (~200 ng)
  - Amenable to automation
  - Negate the need for isolation

# Example 1

**MS Combined with H/D Exchange and Wet  
Chemistry Techniques**

# Metabolism of a Tetrahydroisoquinoline Analog in HLM: compound A

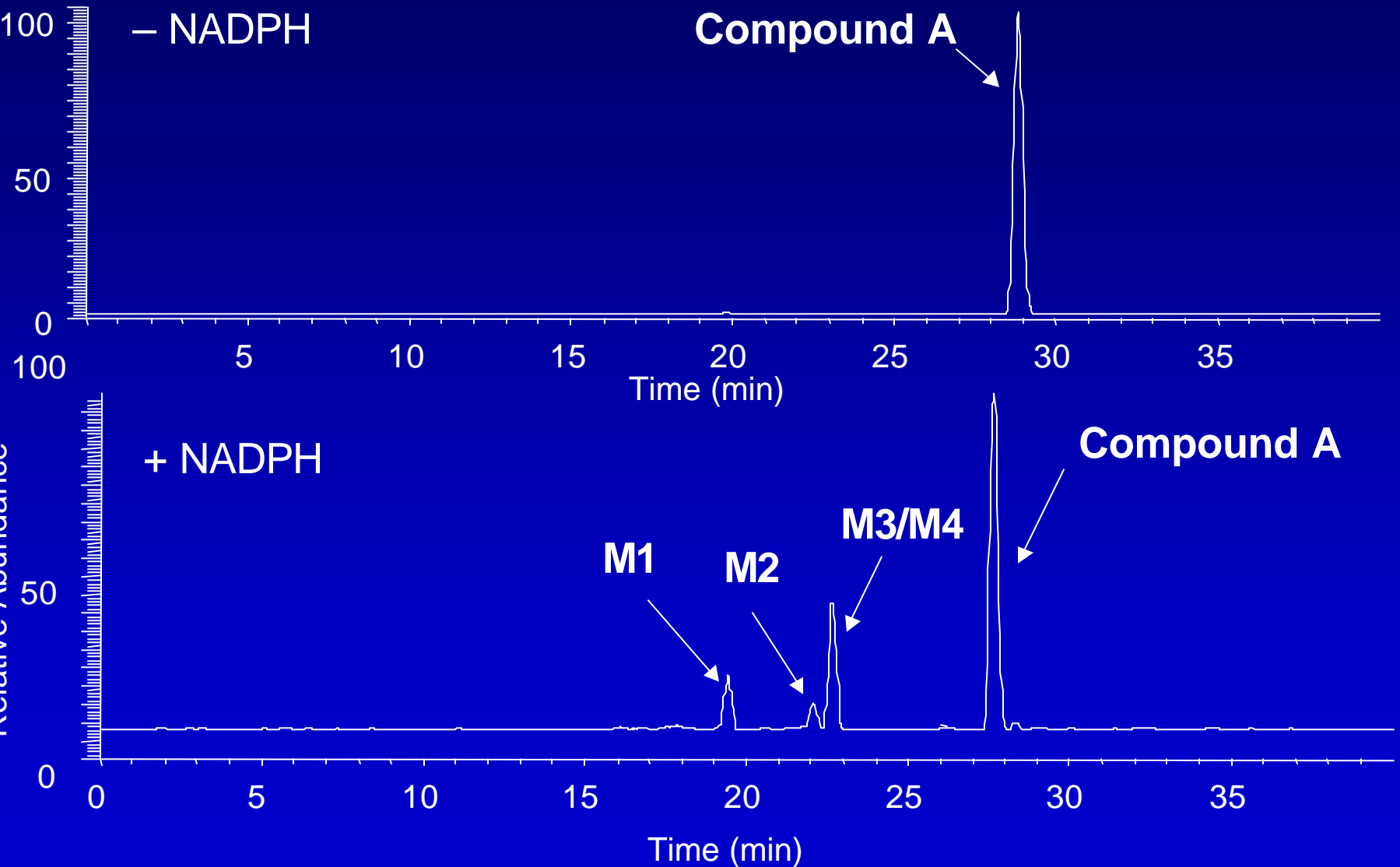
- Compound A, is a potent MTP inhibitor that decrease apo B secretion in human liver cells.
- Preliminary preclinical studies suggested that it is mainly eliminated by Phase I metabolism
- In vitro* metabolism of compound A was investigated in HLM and recombinant CYP isoforms
- Metabolites were identified by electrospray LC/MS/MS and other wet chemistry techniques



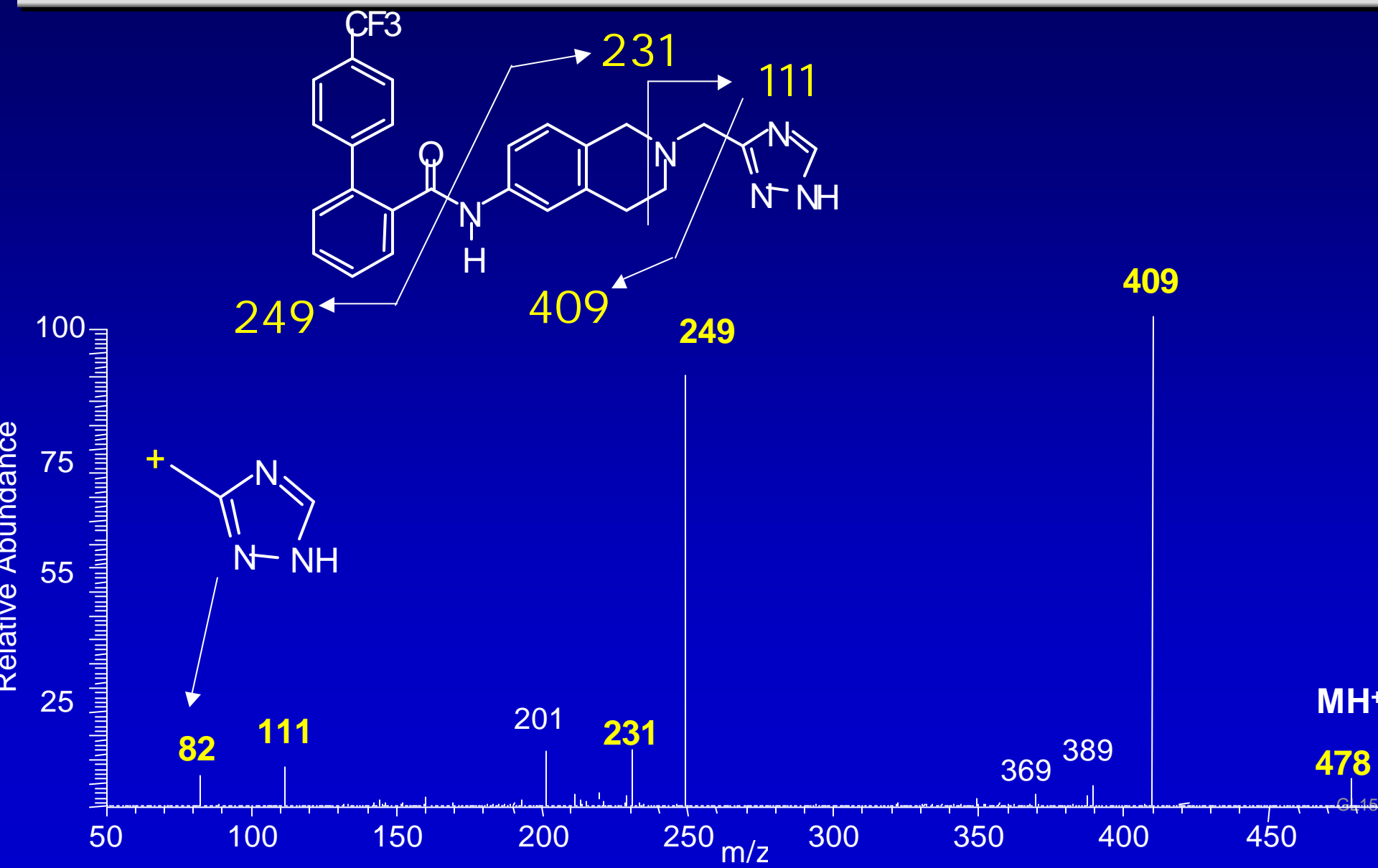
Compound A (MW= 477)

Site of <sup>14</sup>C label

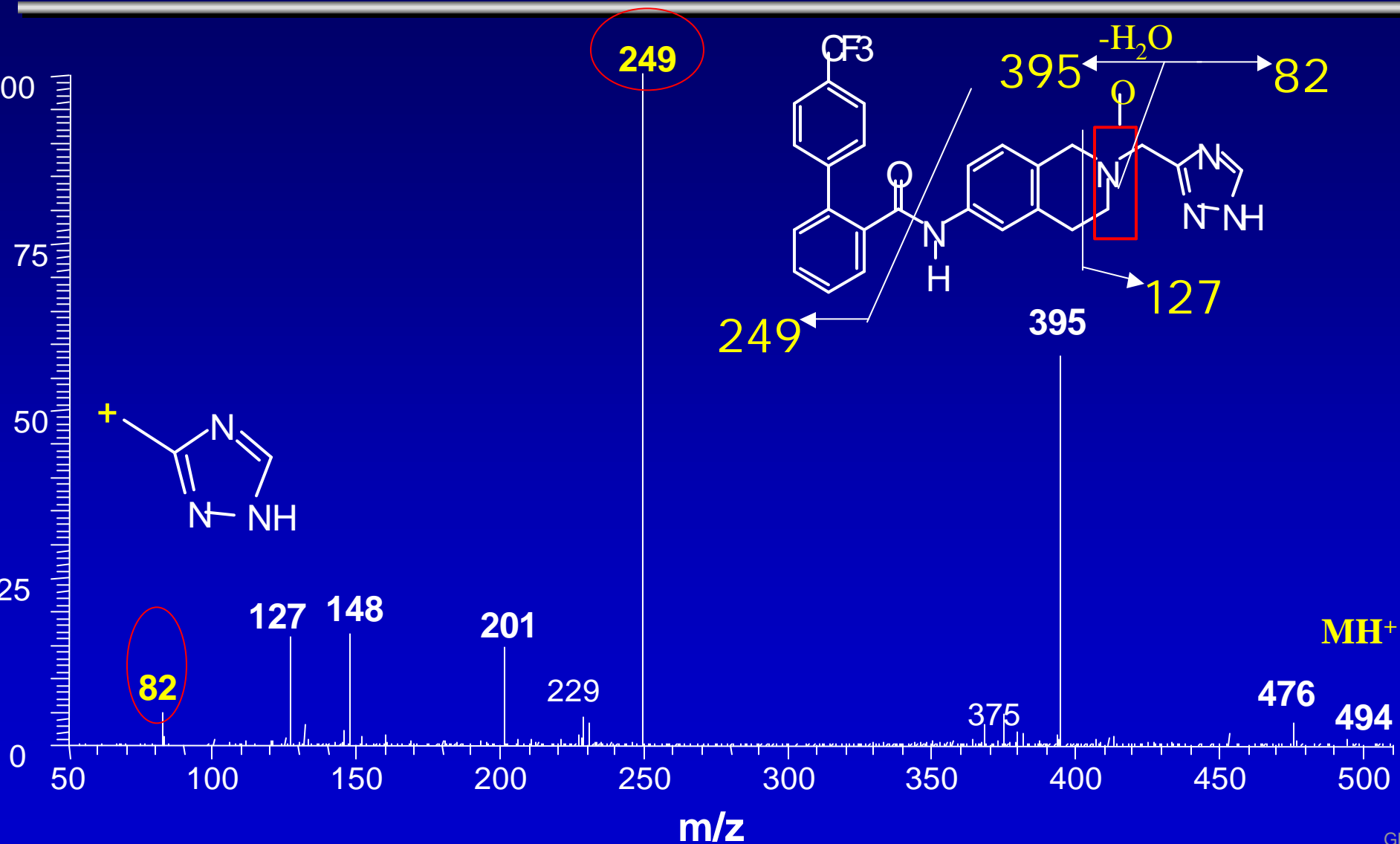
# Metabolites of Compound A in Human Liver Microsomes



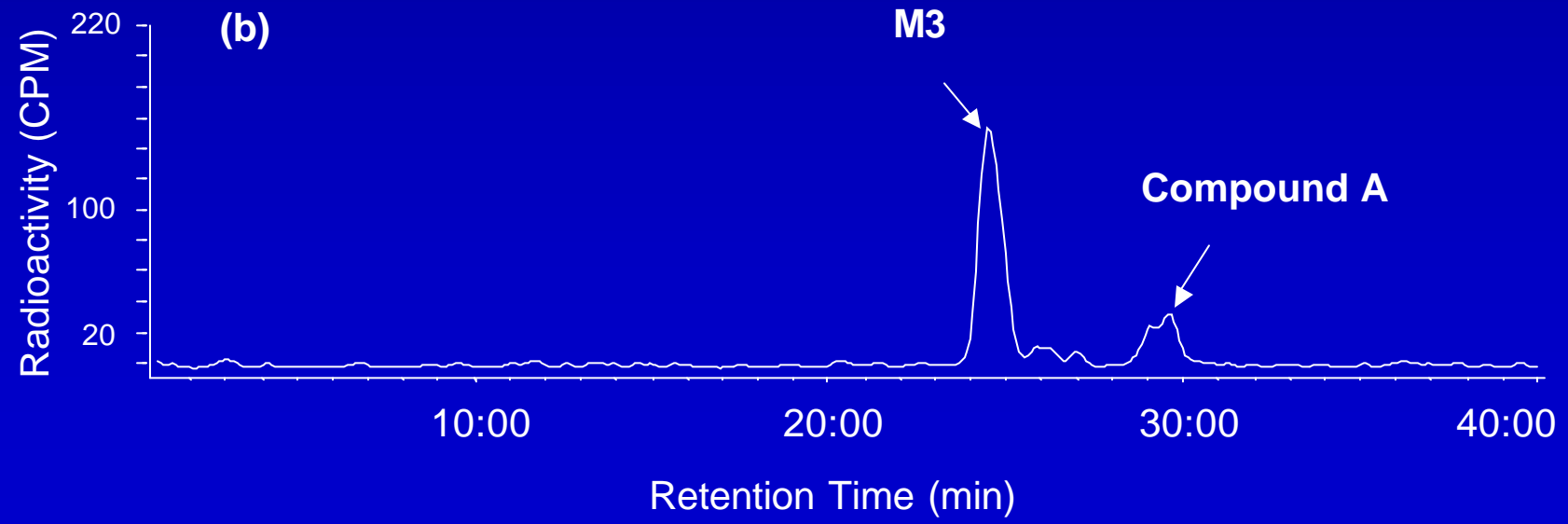
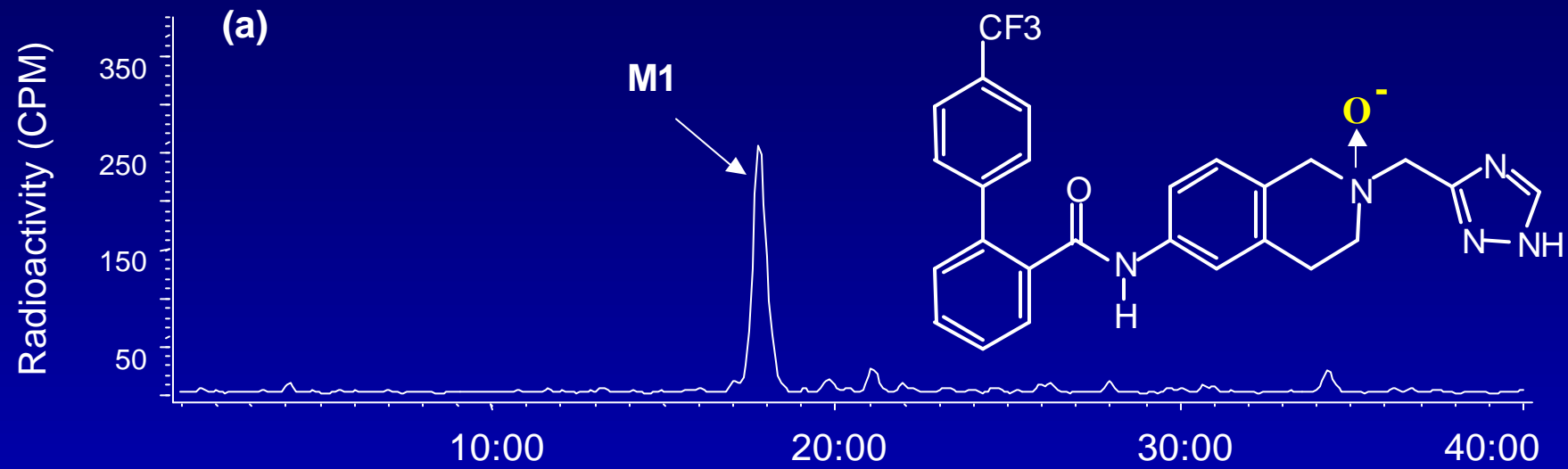
# Product Ion Spectrum of Compound A



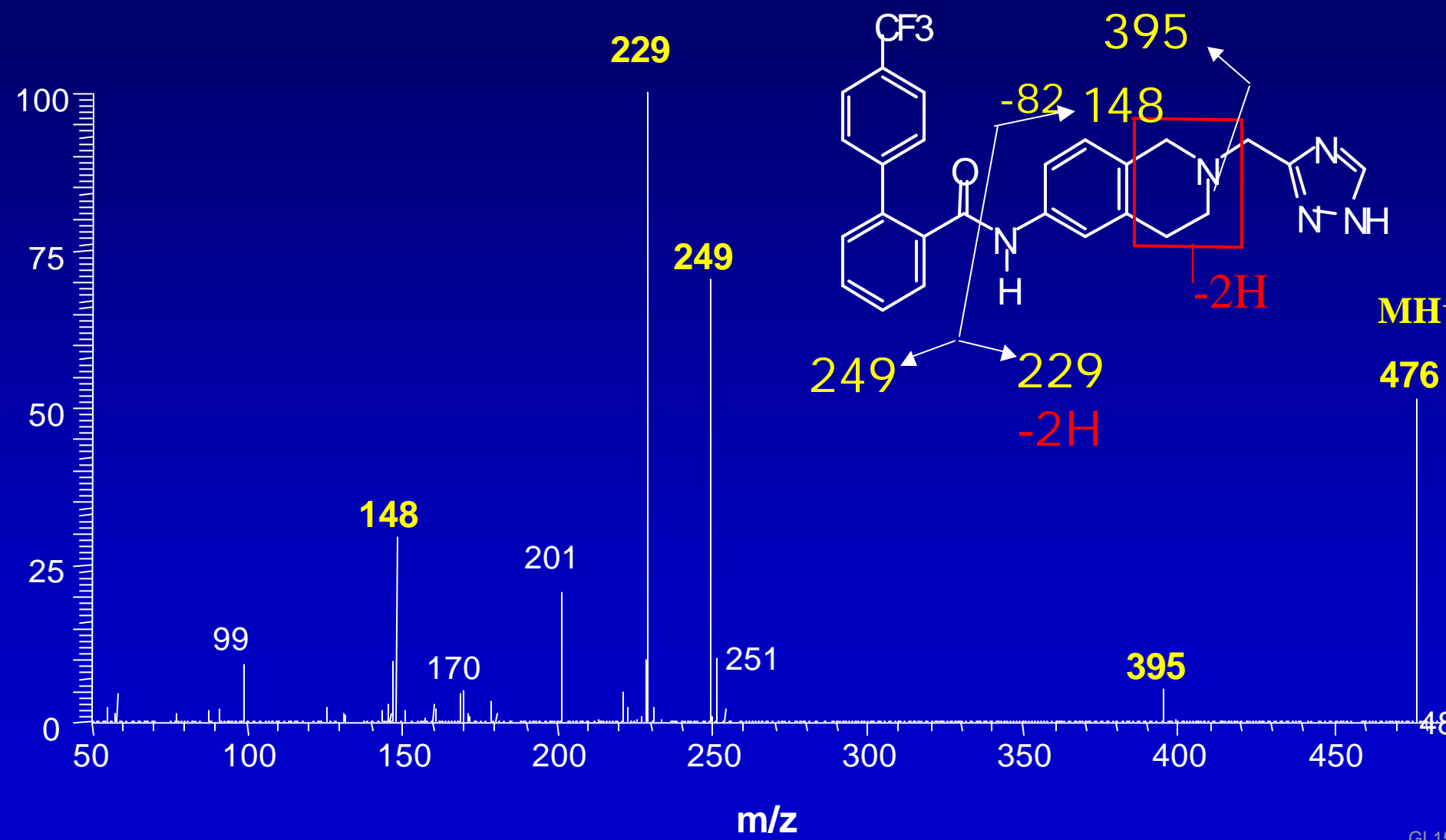
# Product Ion Spectrum of Metabolite M1



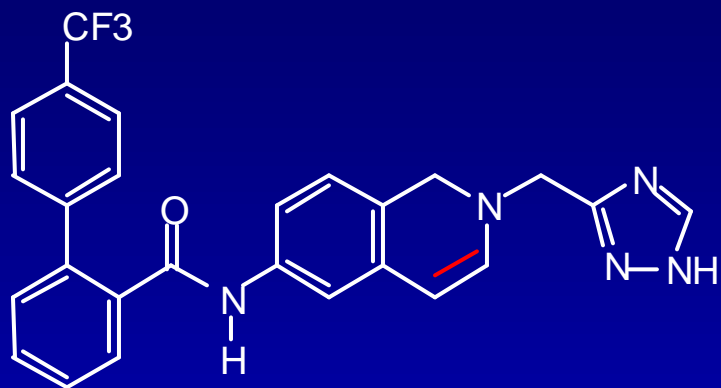
# Radiochromatograms of (a) HPLC Purified M1 and (b) After Treatment with $TiCl_3$



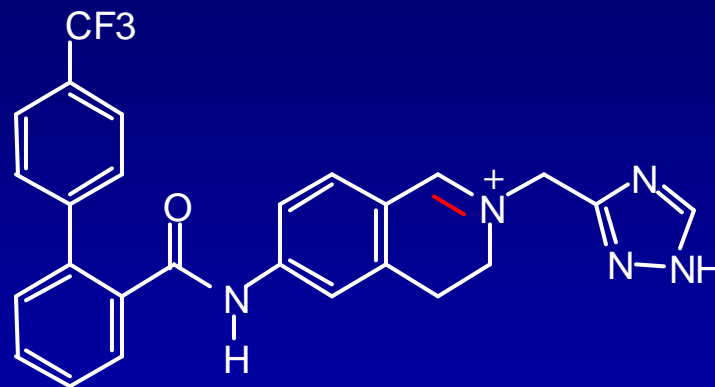
# Product Ion Spectrum of Metabolite M3



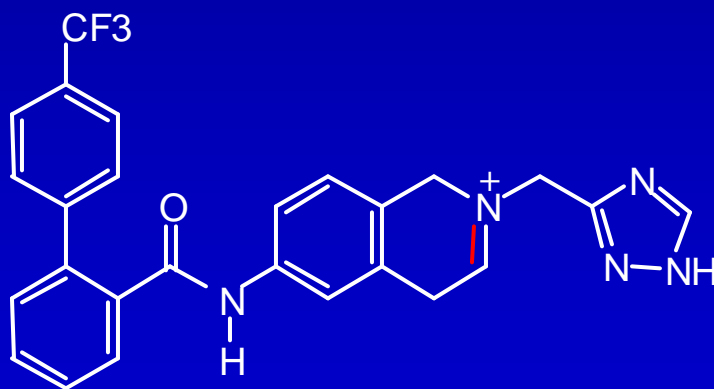
# Possible Structures for M3



**I**

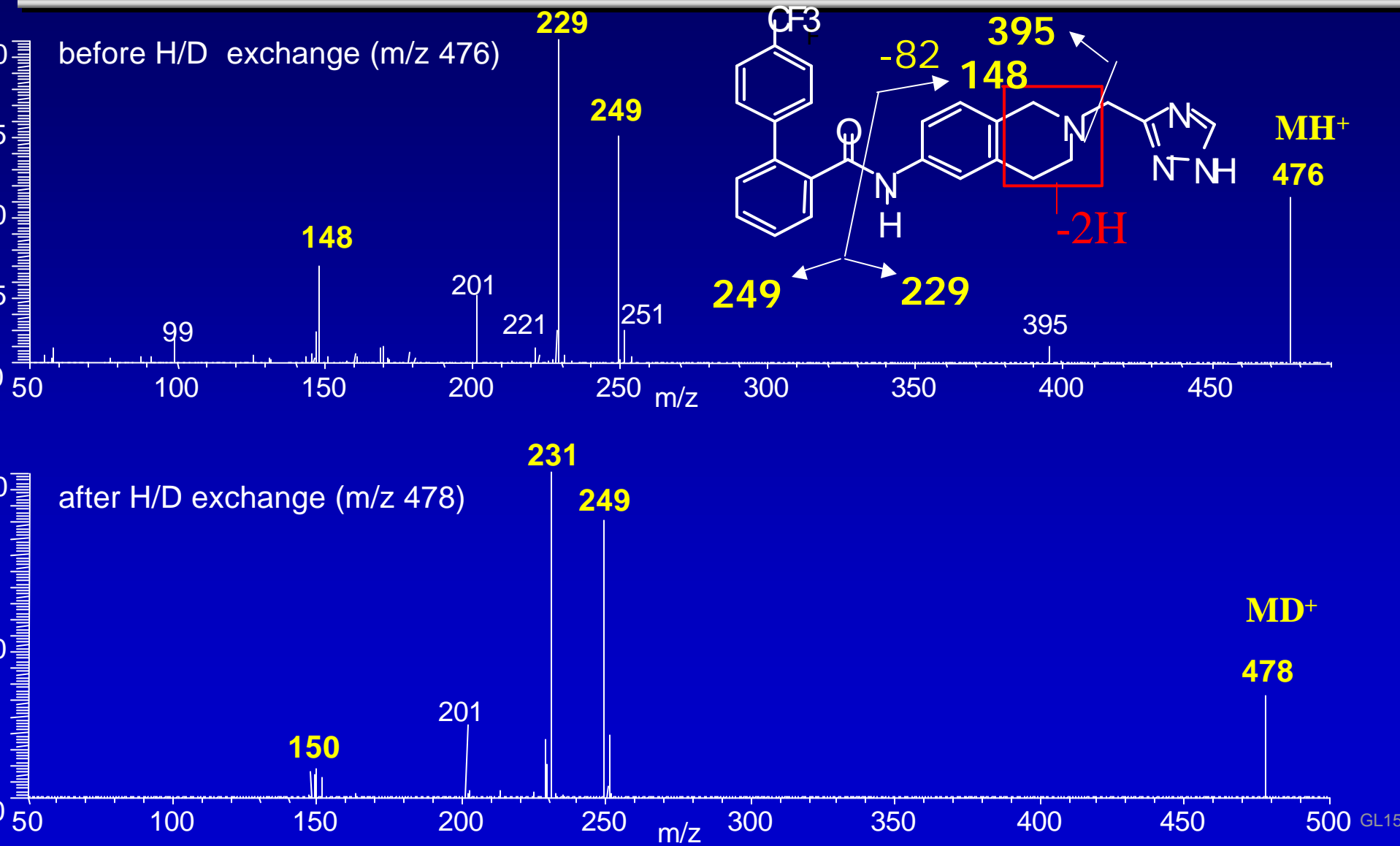


**II**

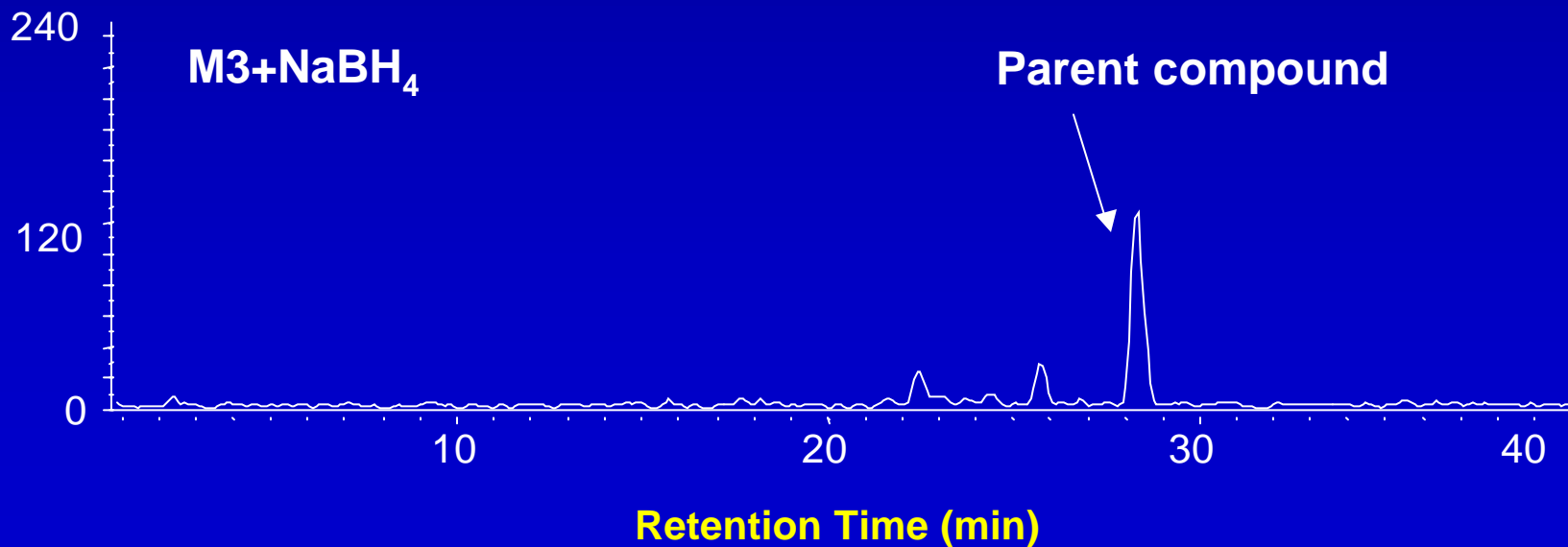
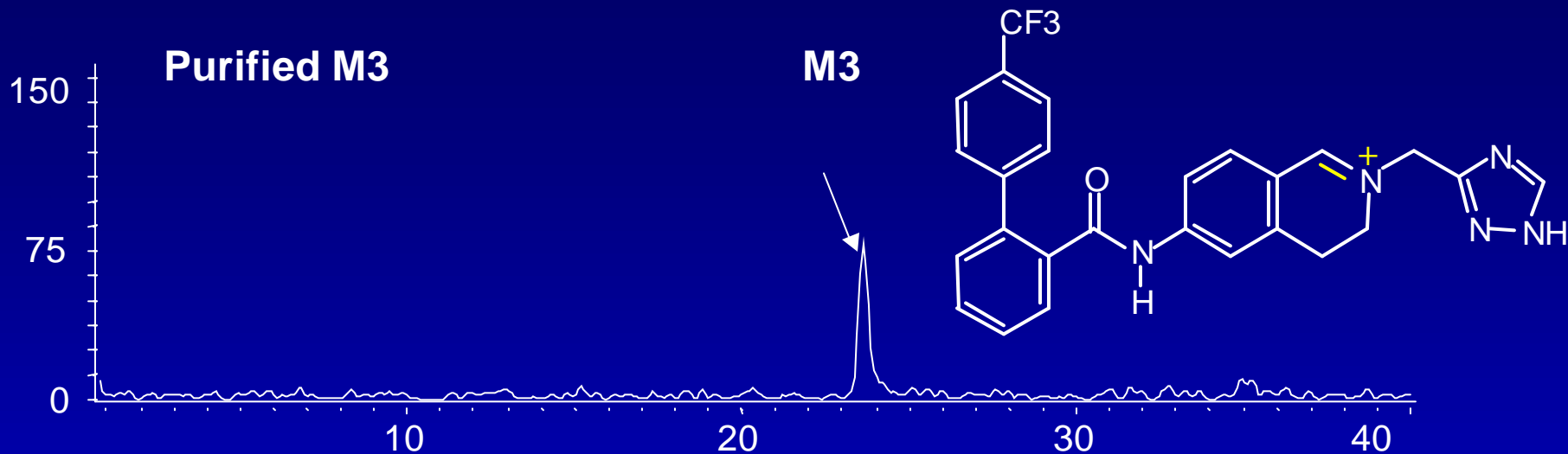


**III**

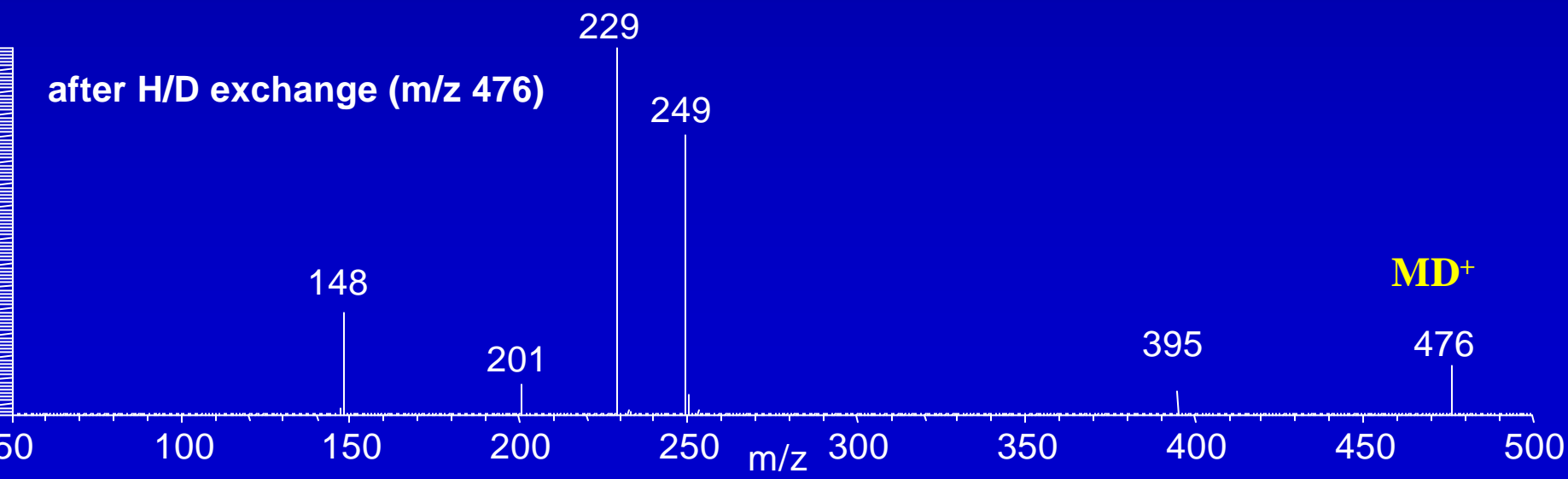
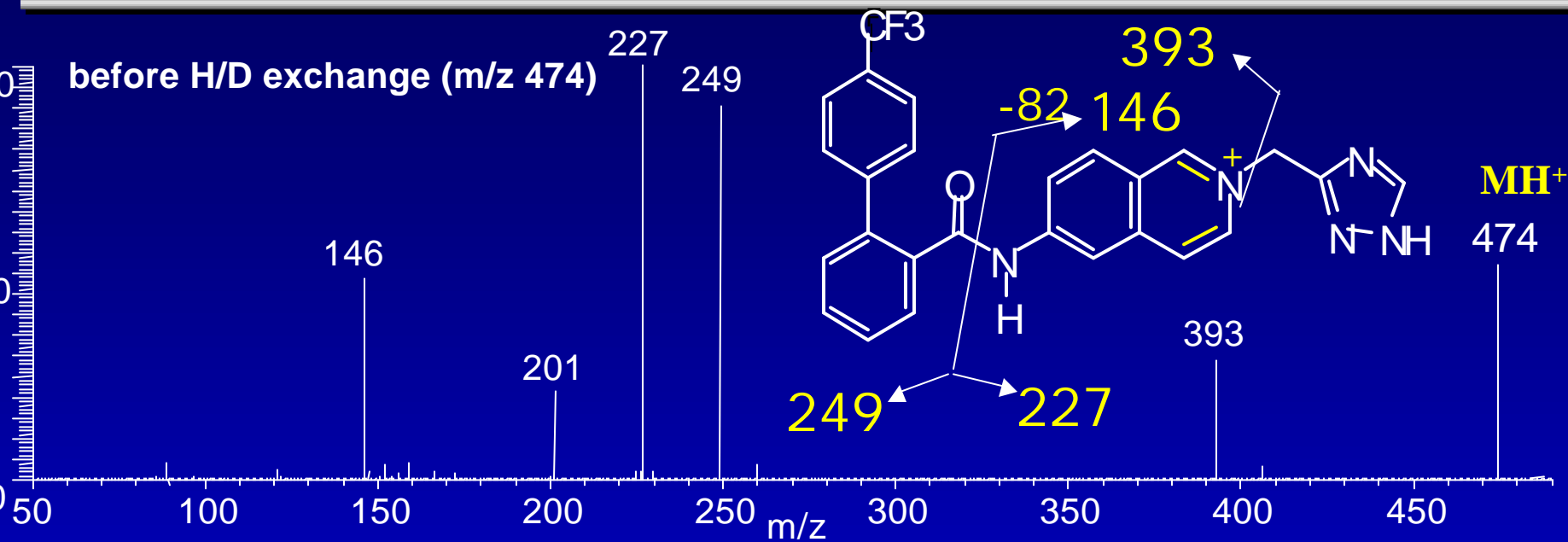
# Product Ion Spectrum of M3 Before and After H/D Exchange



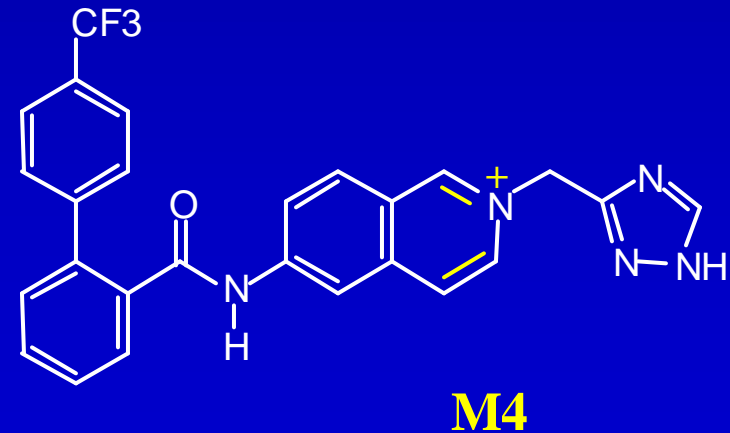
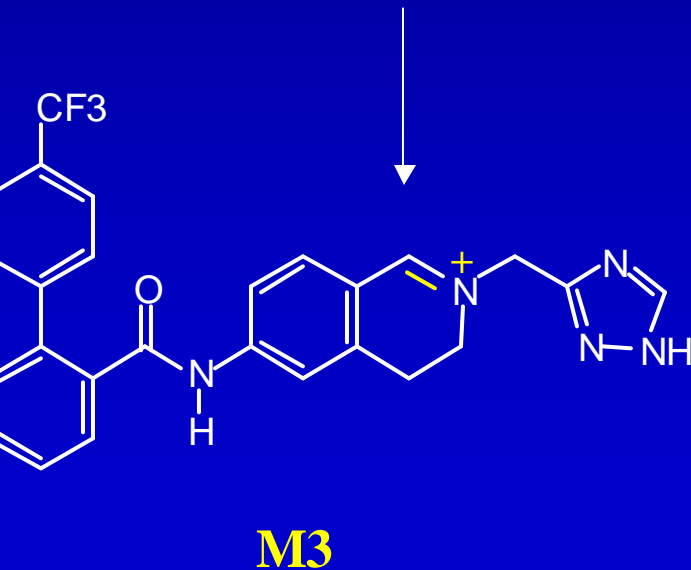
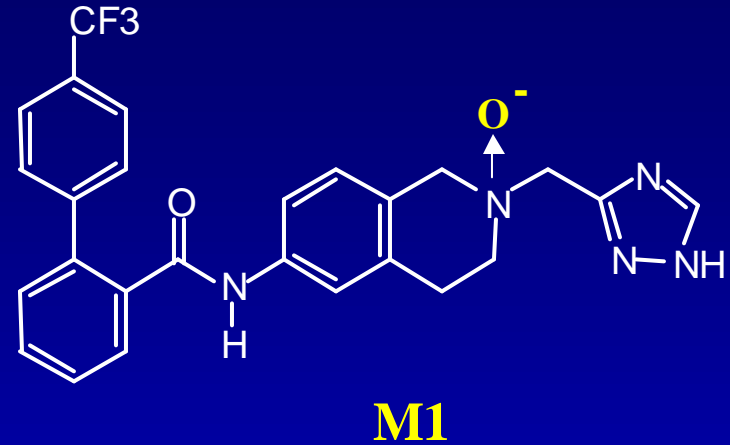
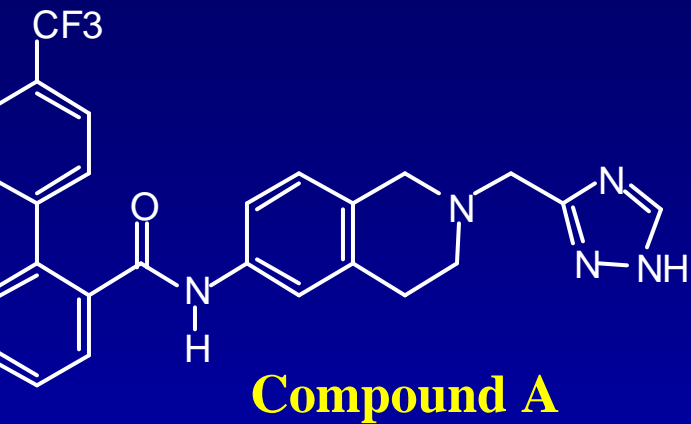
# Radiochromatograms of (a) HPLC purified M3 and (b) M3 treated with NaBH<sub>4</sub>



# Product Ion Spectrum of M4 Before and After H/D Exchange



# In Vitro Metabolic Pathways of Compound A in Human Liver Microsomes



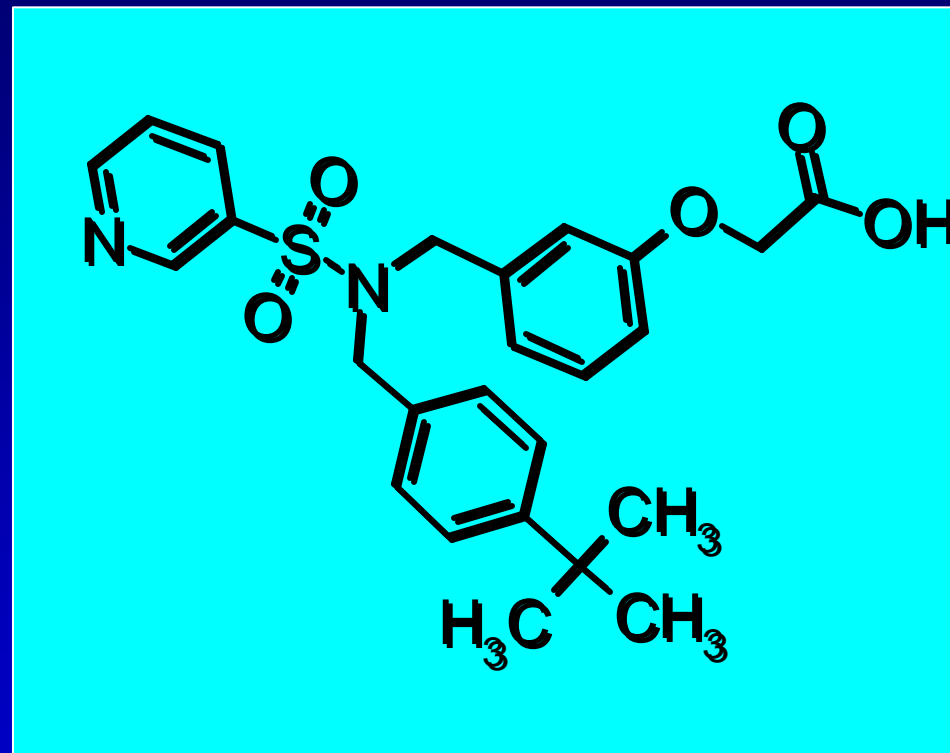
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## Example 2

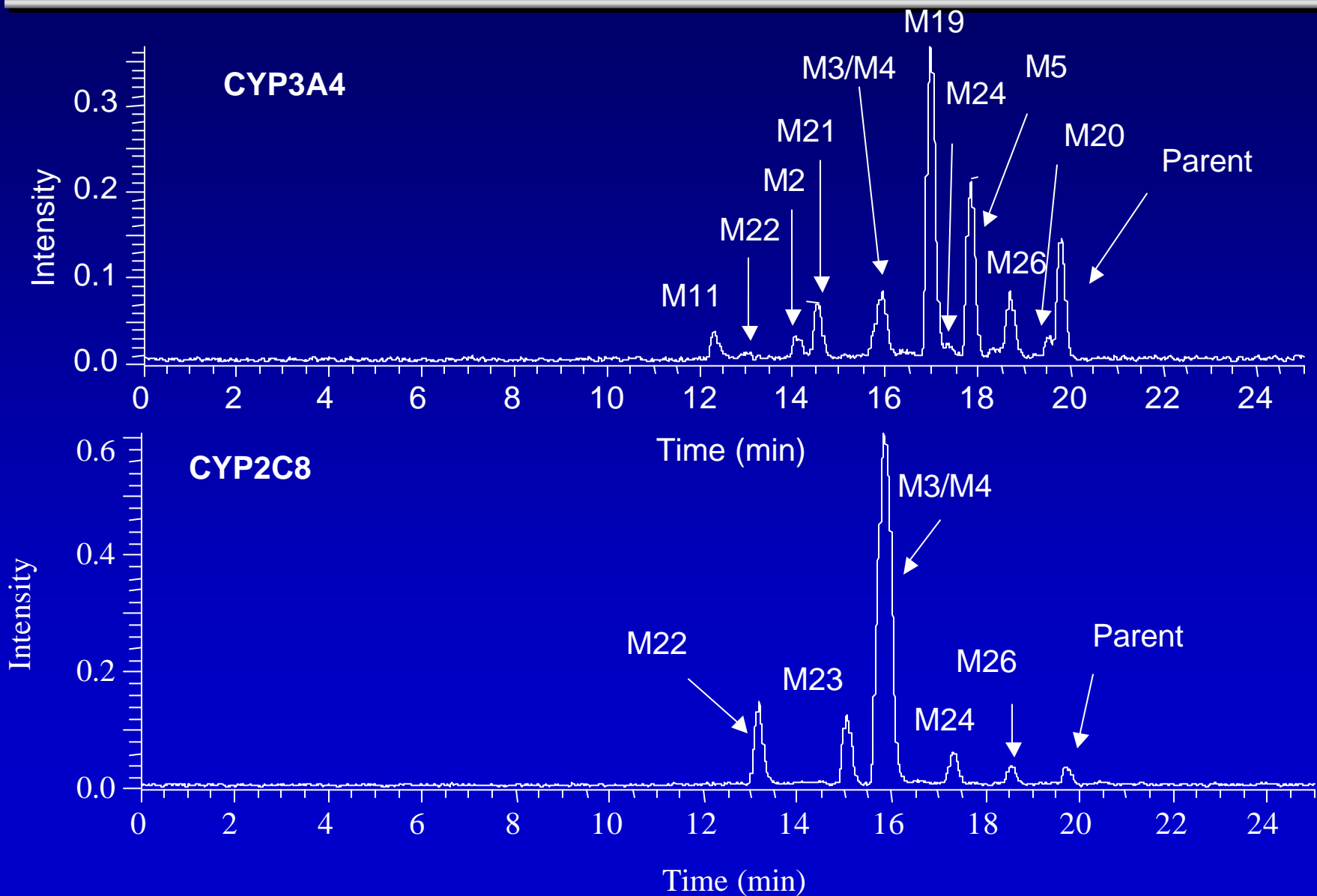
MS Combined with H/D exchange and LC-NMR

# Compound 2: a *tert* butylbenzylpyridinyl-sulfonamide

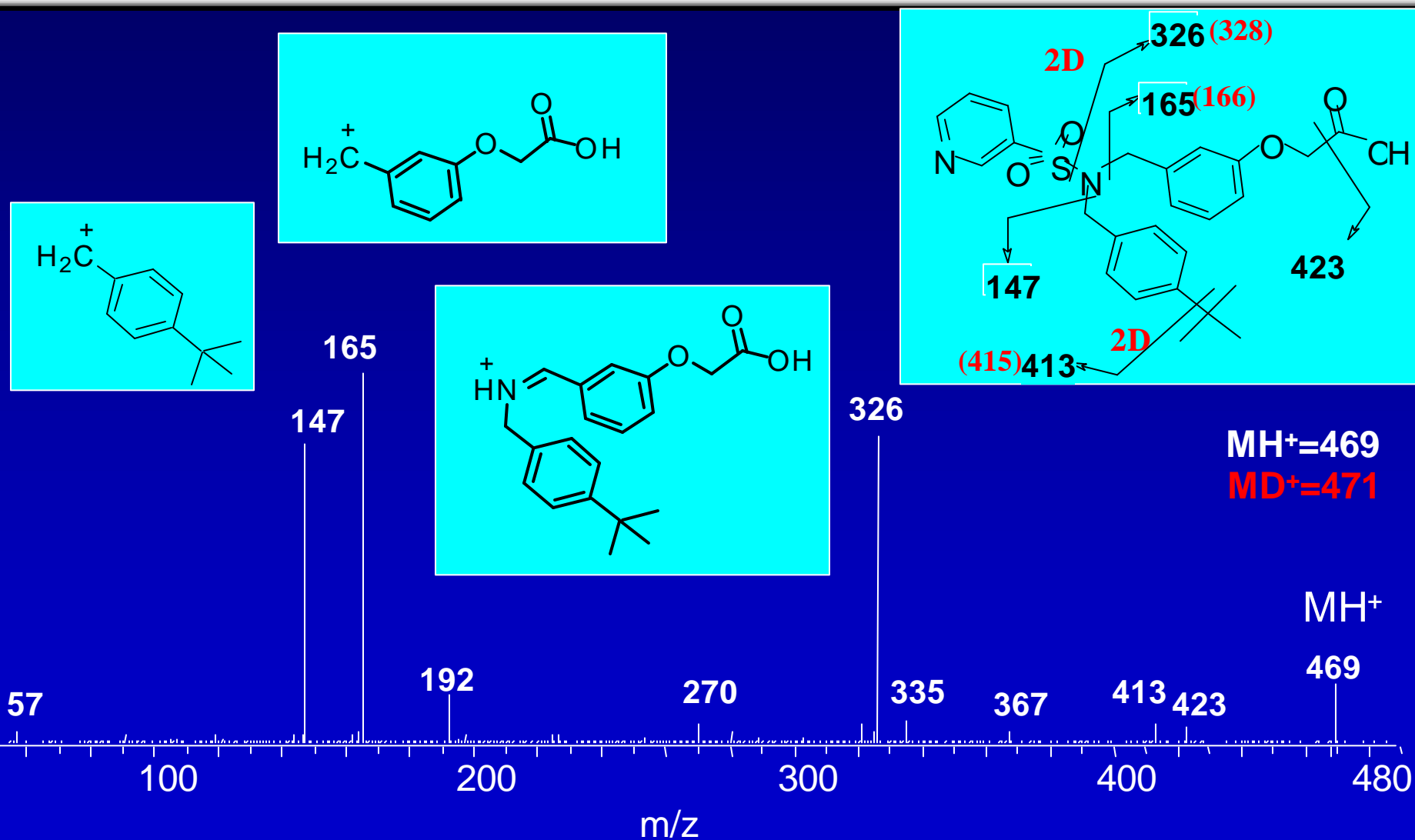
- ▶ Compound 2 is a selective EP<sub>2</sub> agonist that is being developed to aid in the healing of bone fractures.
- ▶ As a drug development process we have studied the in vitro metabolism of CP-533,536 in human recombinant CYP isoforms
- ▶ Several spectroscopic approaches (LC-MS/MS, TOF, LC-NMR and H/D exchange) were used to identify metabolites



# HPLC Radiochromatograms of Metabolites of Compound B in CYP3A4 and 2C8

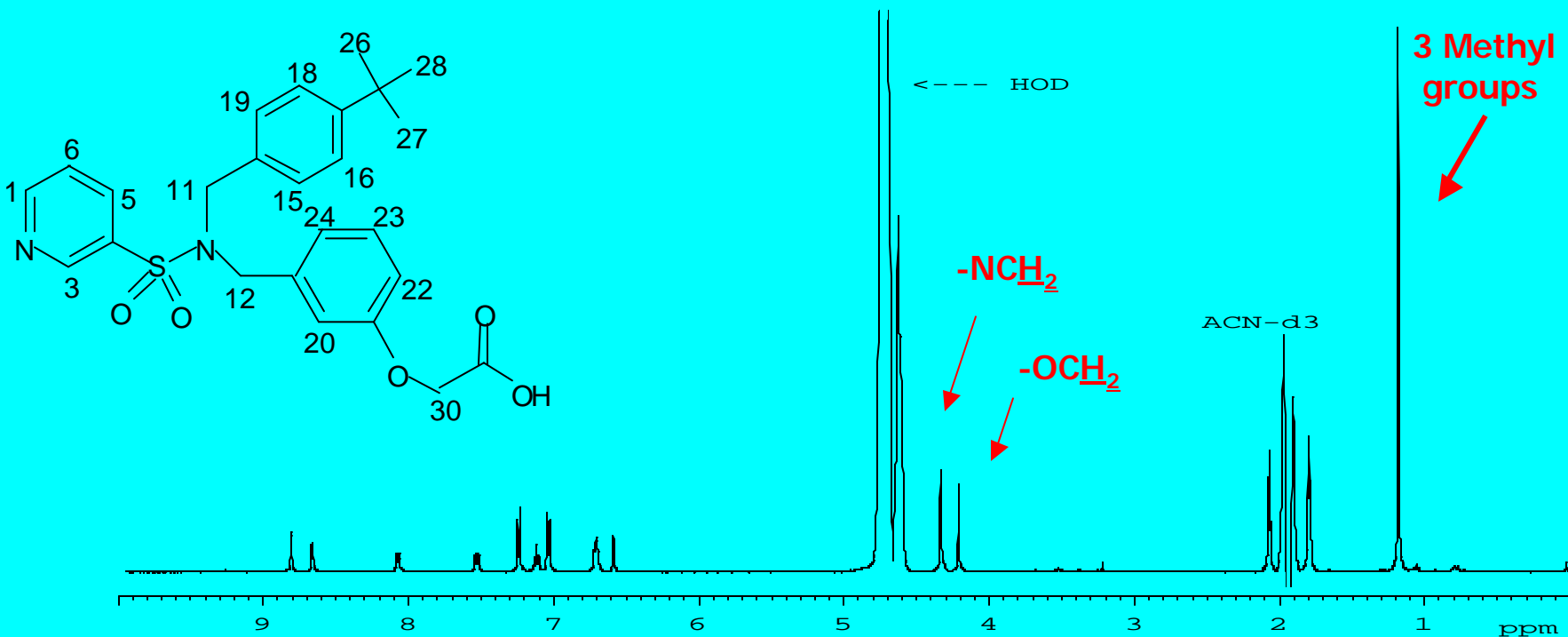


# CID Product Ion Spectrum of Compound B

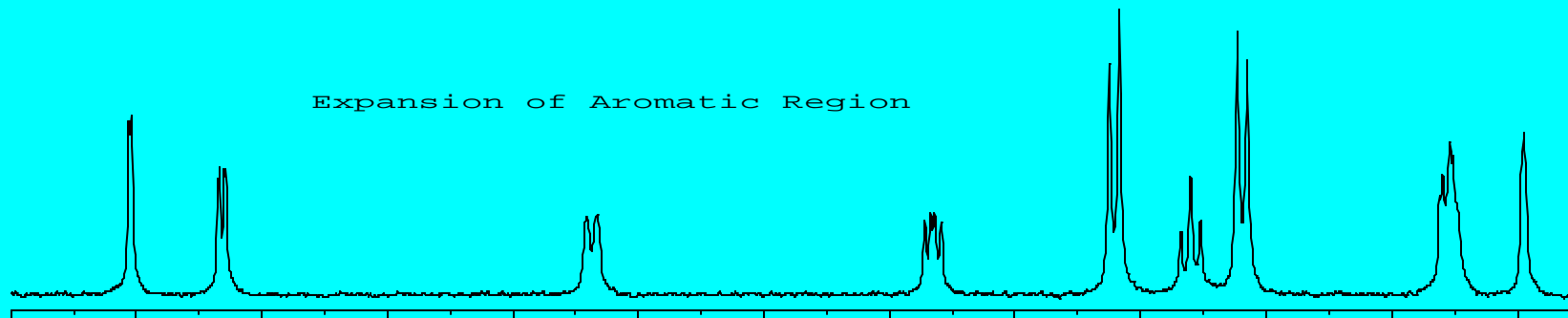


# $^1\text{H}$ NMR of Compound 2

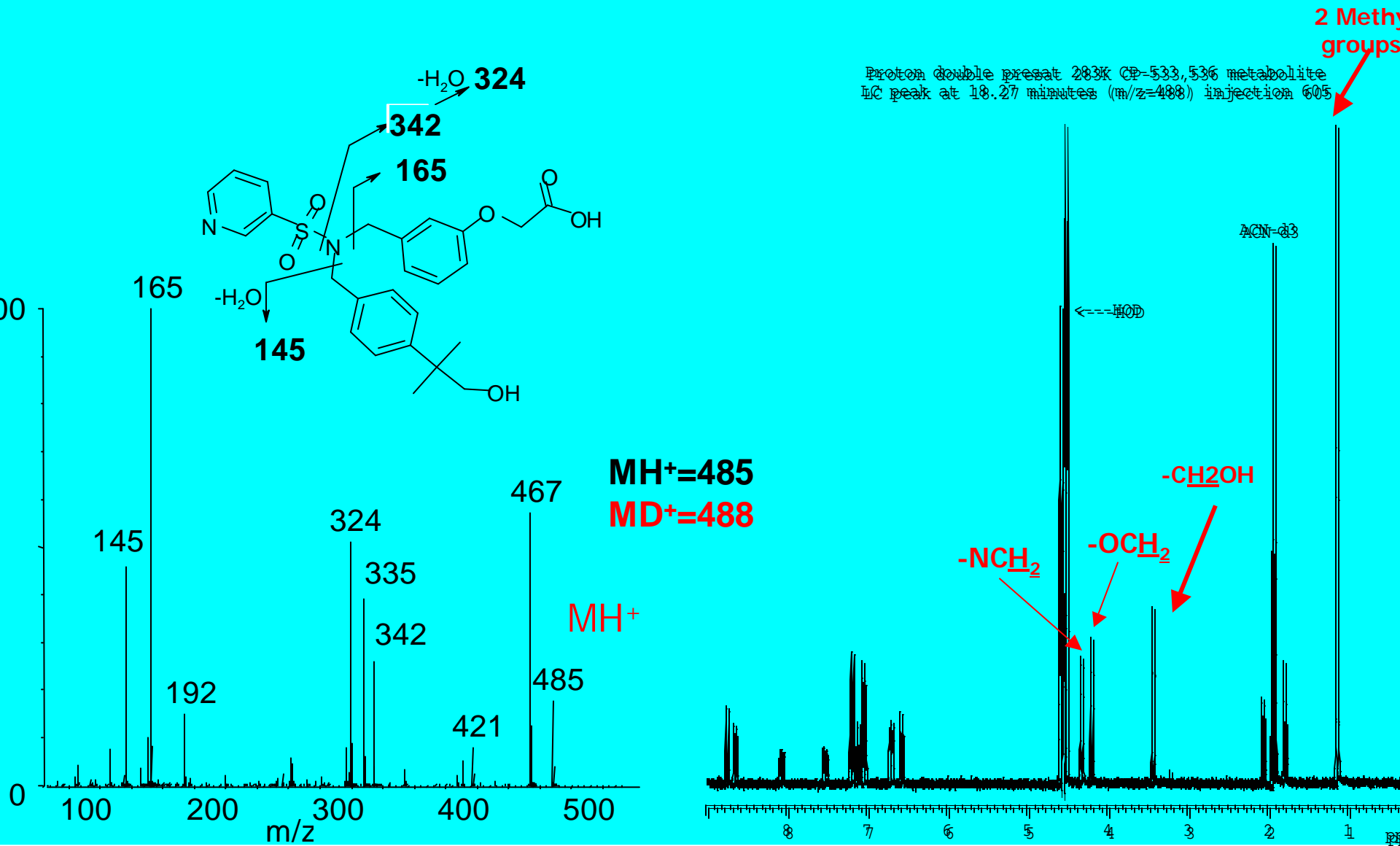
Proton double presat 278K d1=10sec CP-533,536  
LC peak at 57.41 minutes (m/z=471) injection # 636



Expansion of Aromatic Region

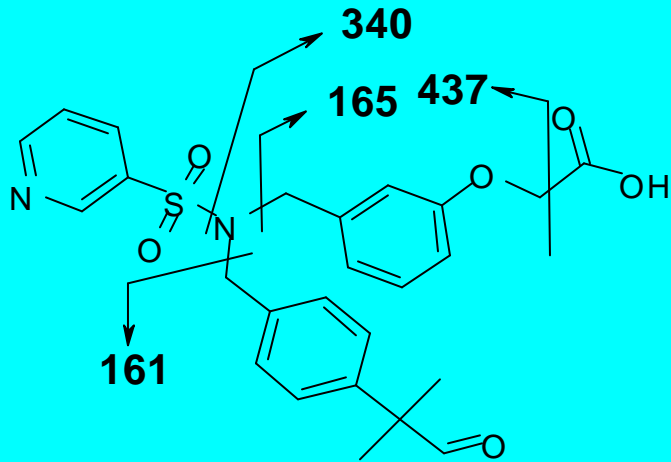


# CID Mass and NMR Spectra of M4



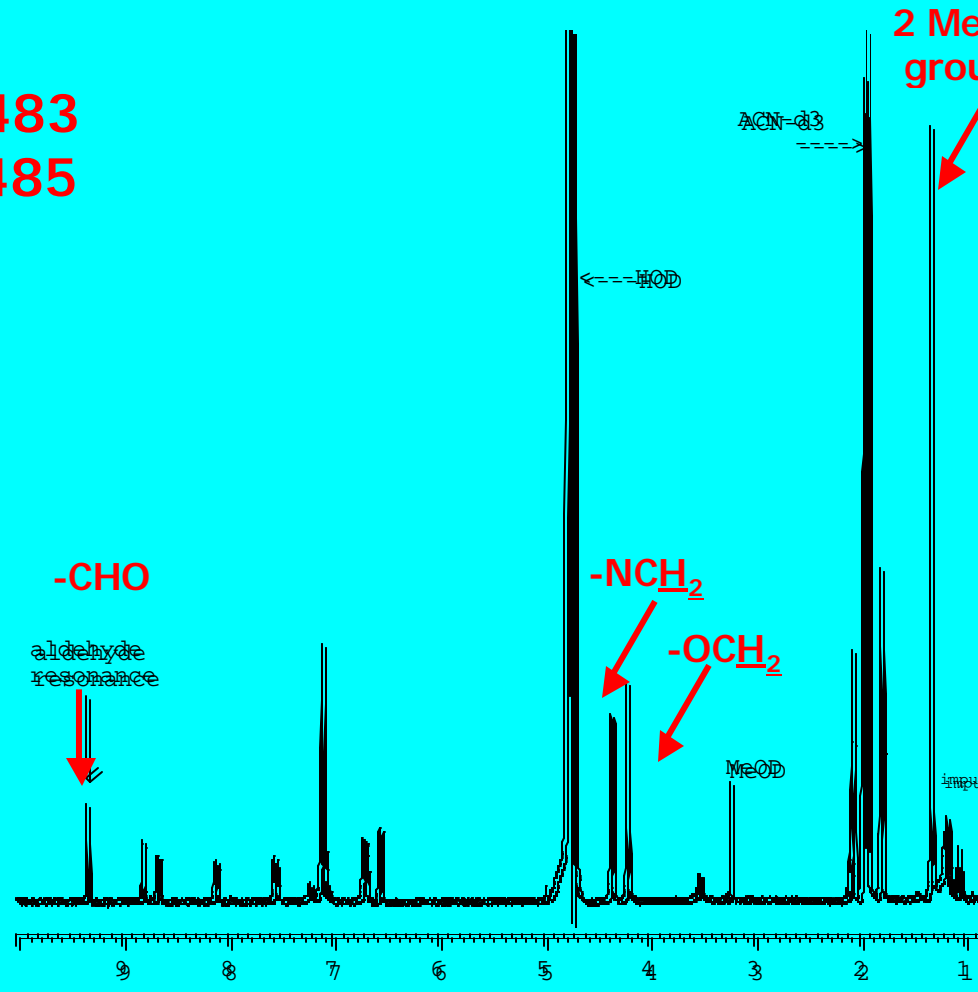
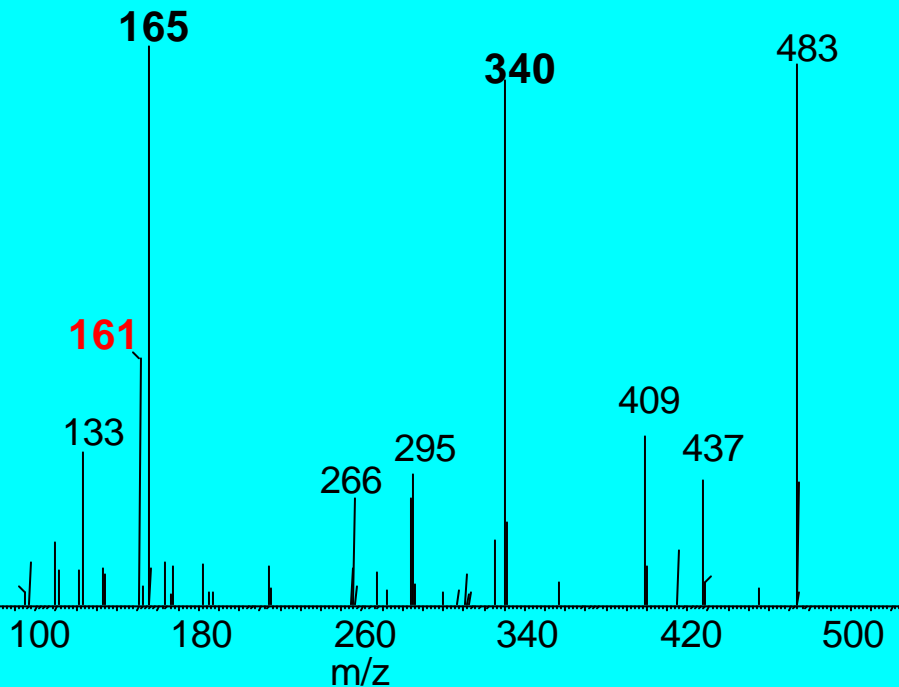
# CID Mass and NMR Spectra of M24

Proton double presat 278K CP-533,536 metabolite  
 LC peak at 45.30 minutes (m/z=485) injection # 622



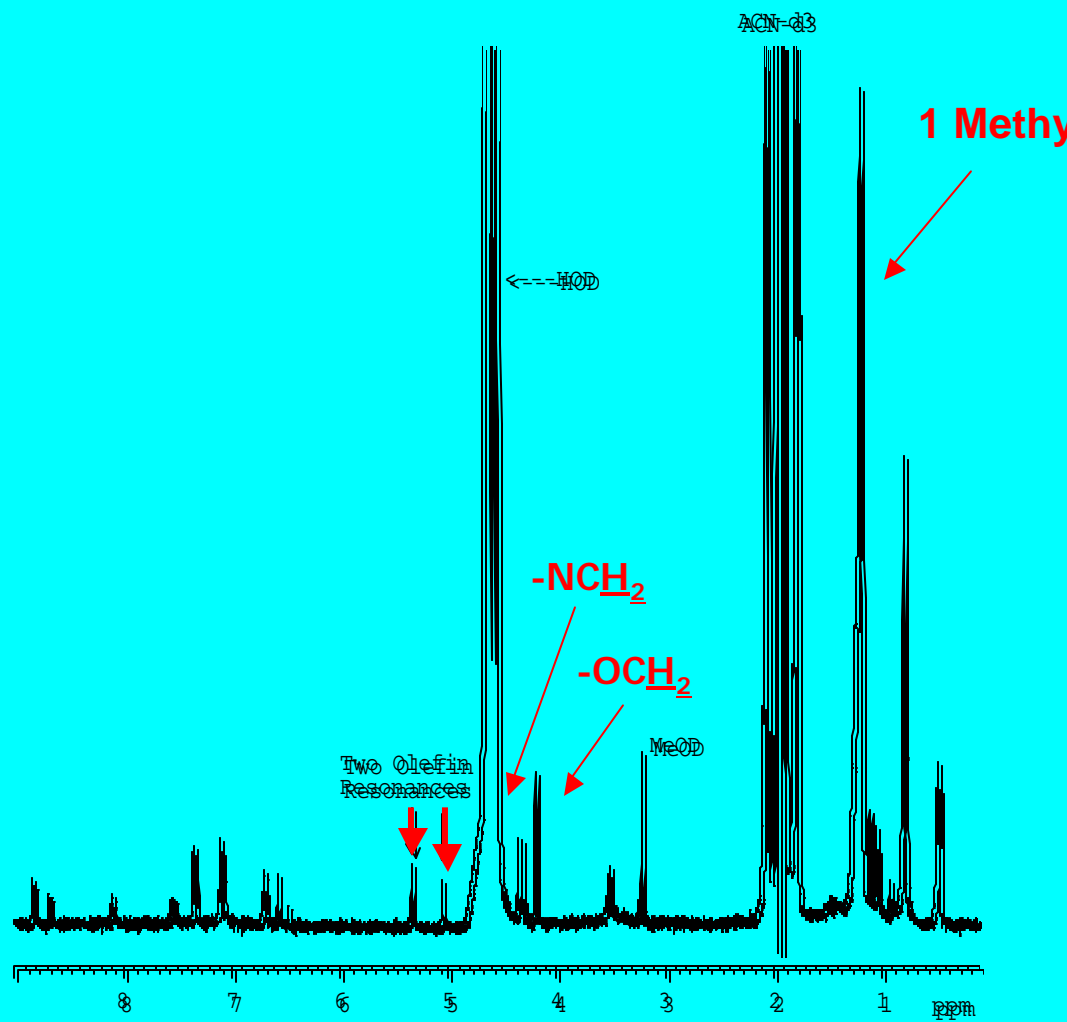
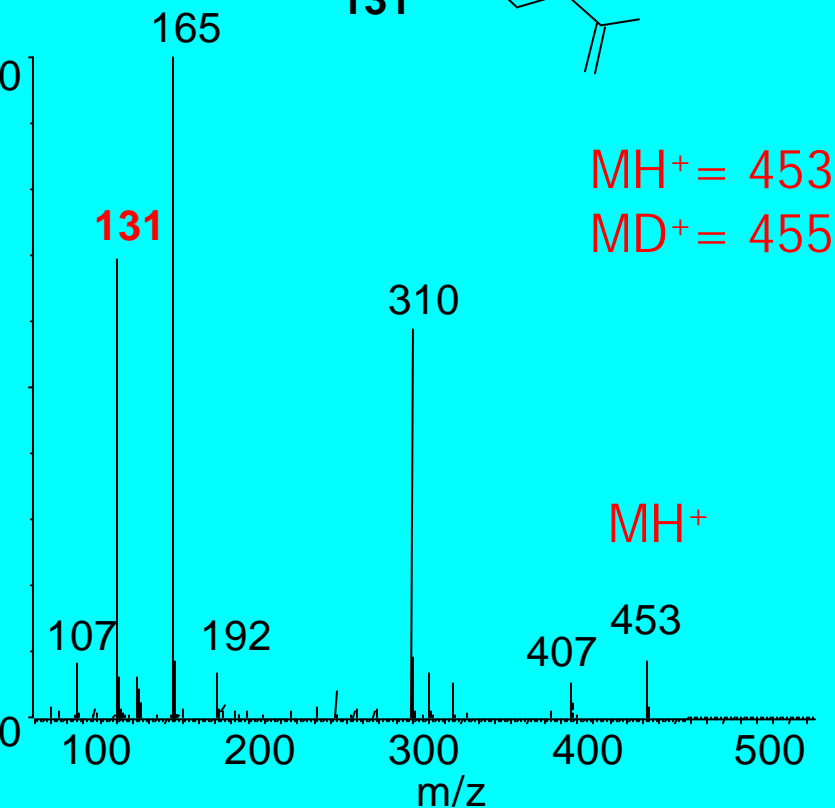
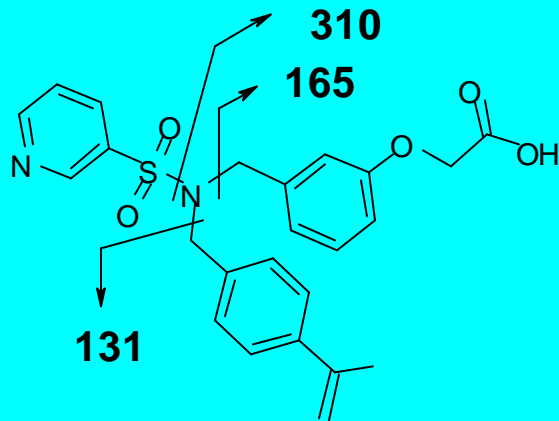
**MH<sup>+</sup> = 483**  
**MD<sup>+</sup> = 485**

MH<sup>+</sup>

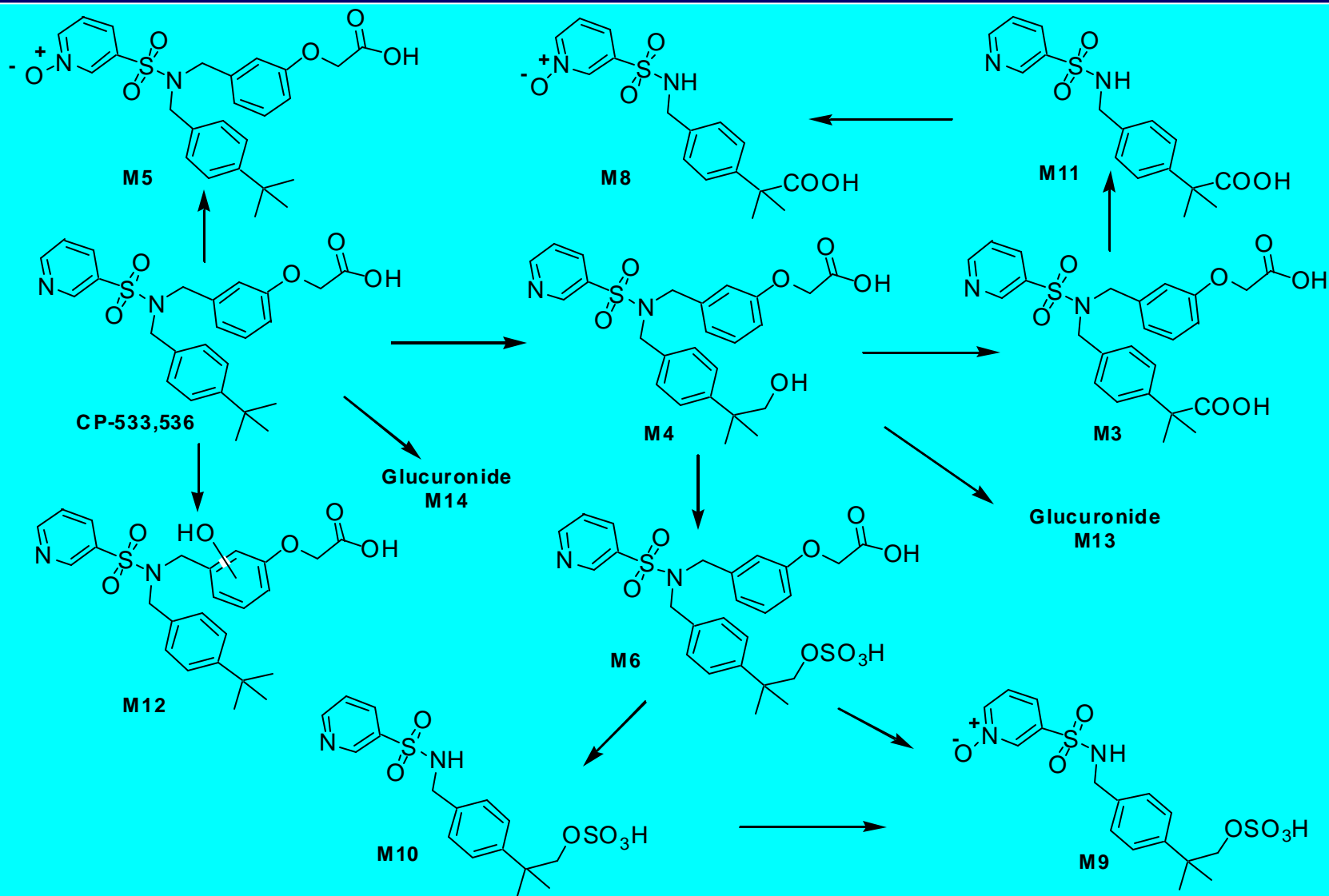


# CID Mass and NMR Spectra of M26

Proton double peak at 278K CP-533,536 metabolite  
 LC peak at 52.16 minutes (m/z=455) injection # 622



# Proposed Metabolic Pathways of Compound B

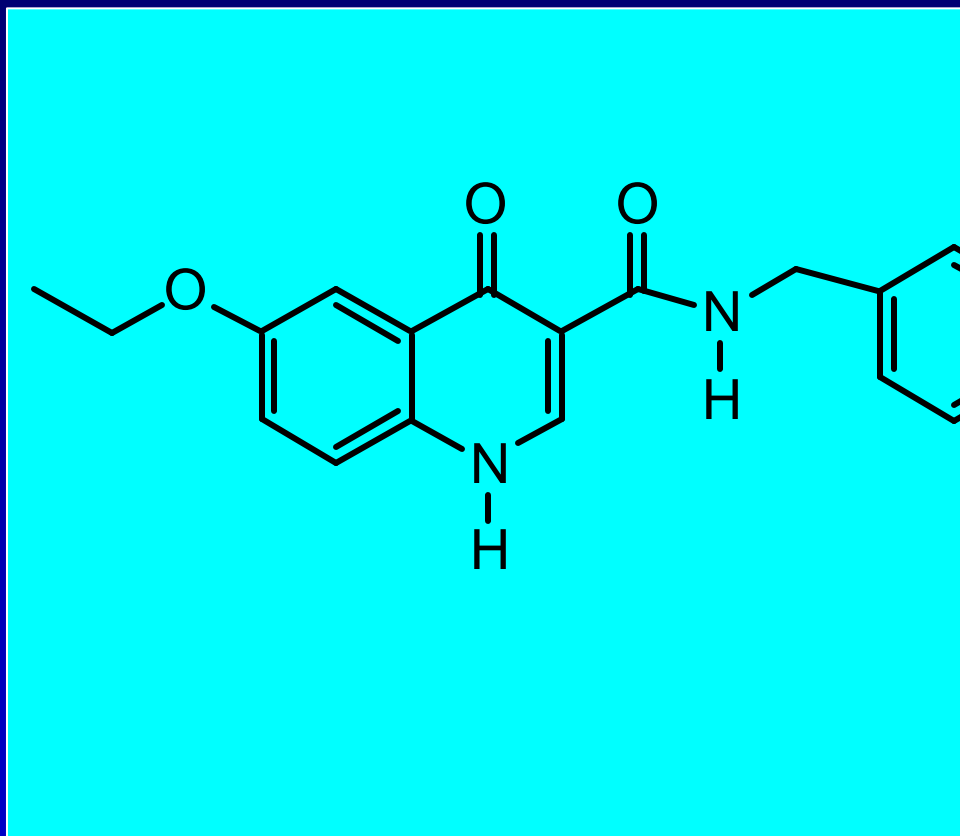


## Example-3

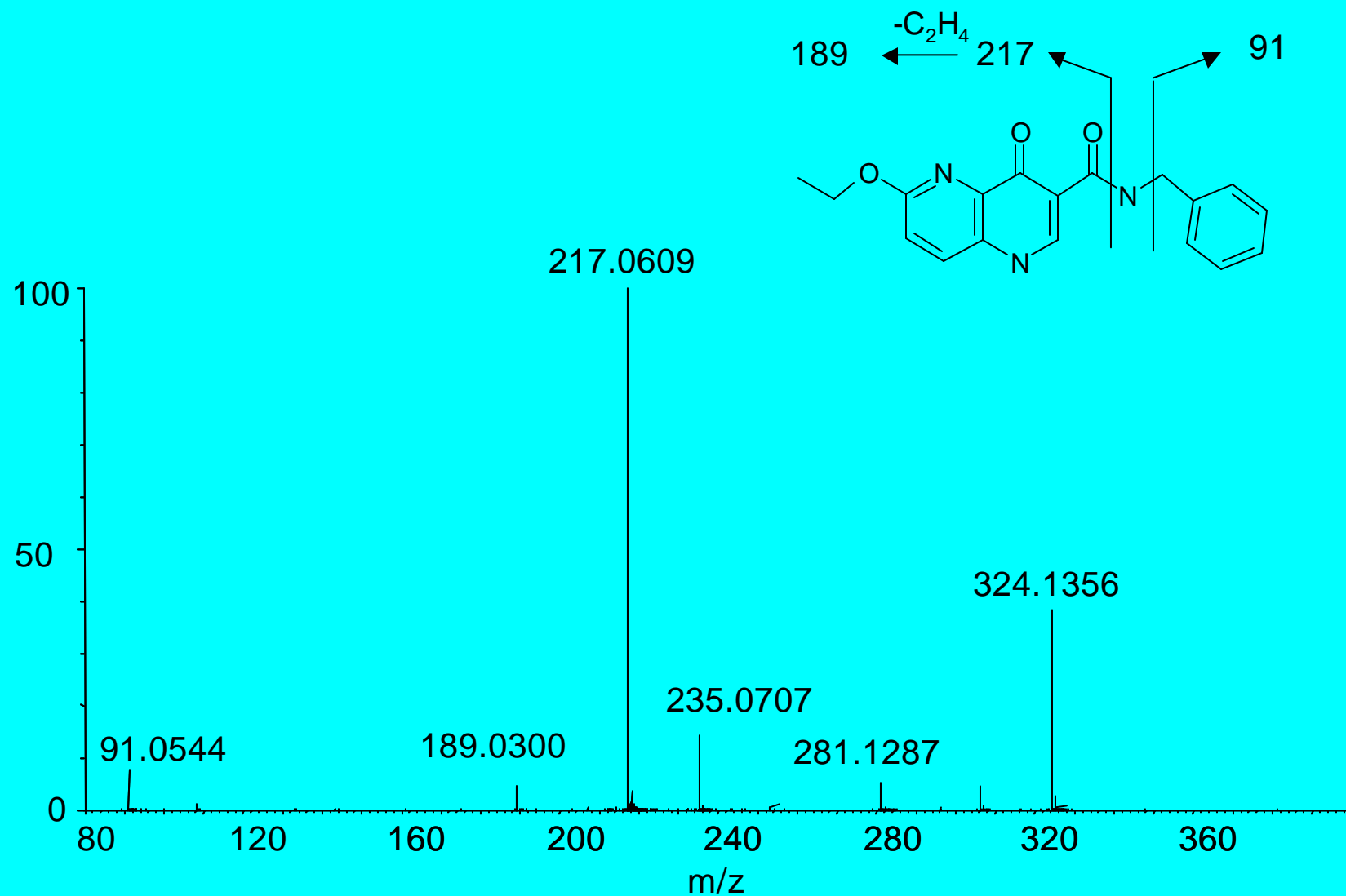
S Combined with H/D exchange and LC-NM

# Compound C: A Naphthyridine-3-Carboxylic acid Benzylamide

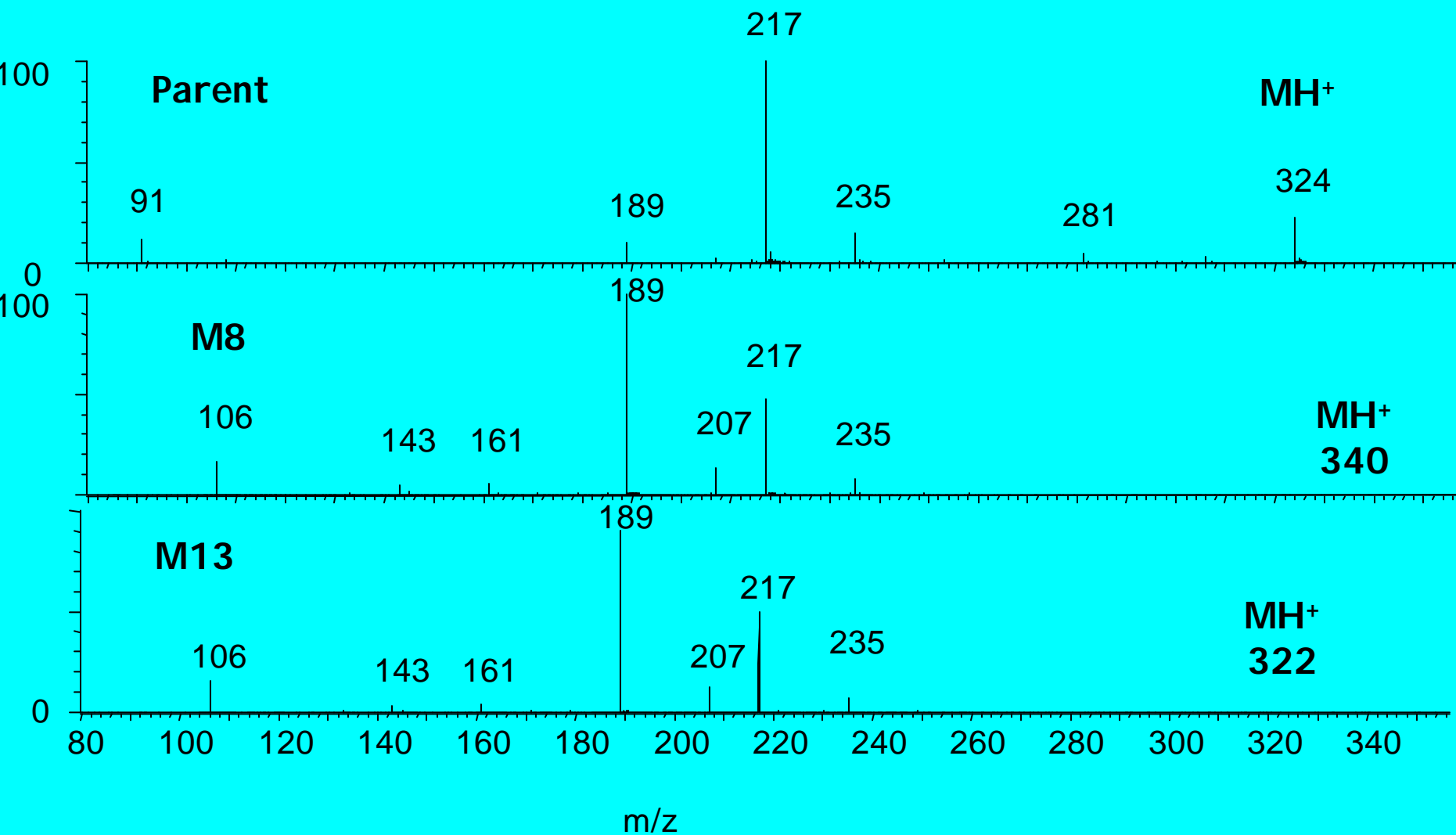
- It is a partial agonist and partial inverse agonist of the GABA receptor
- Lacks anxiogenic or motor impairing properties
- Low to moderate clearance and low volume of distribution
- In vitro metabolism was investigated in HLM



# CID Spectrum of Compound C



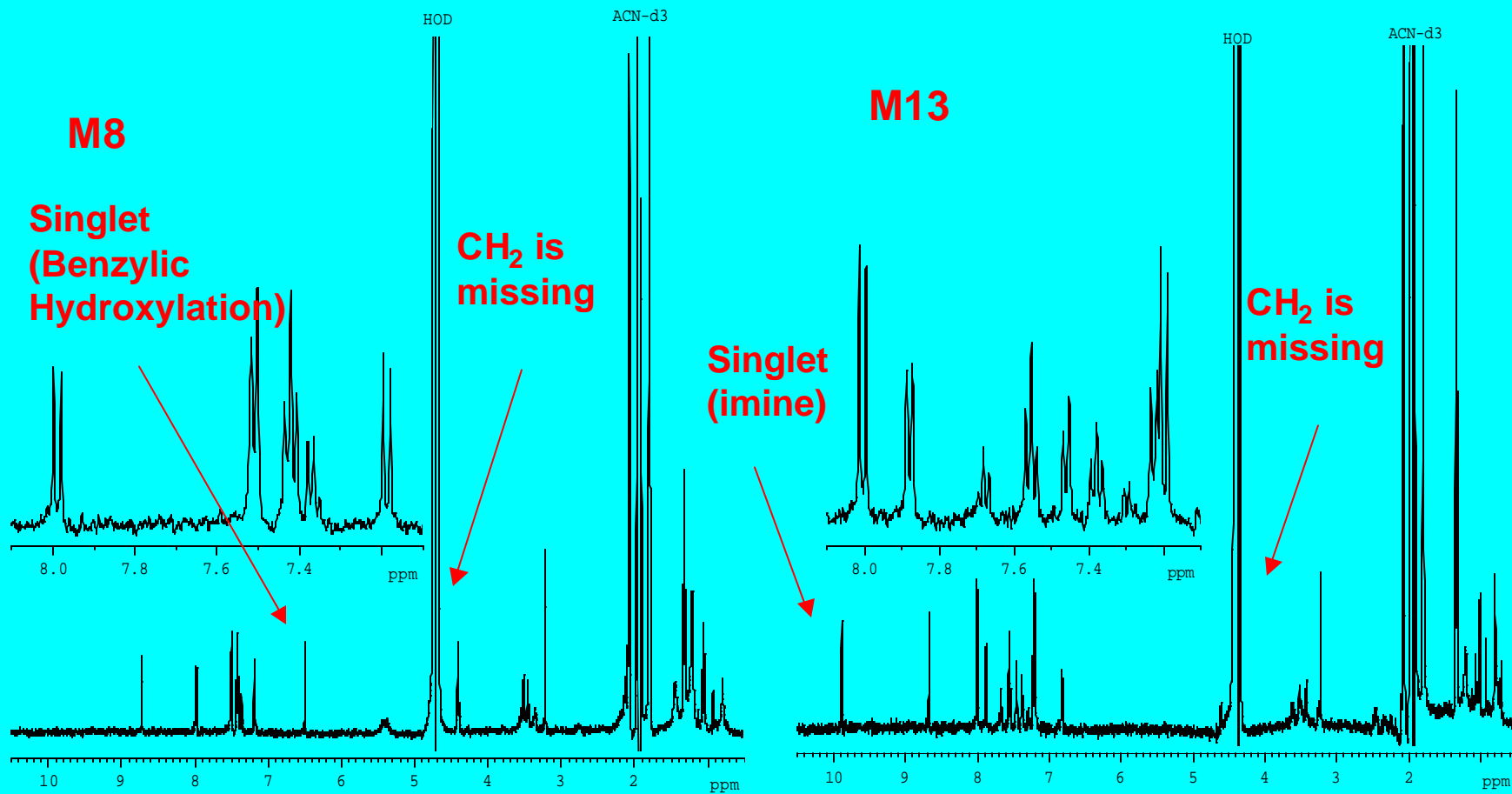
# CID Mass Spectra for Parent and M8 and M13



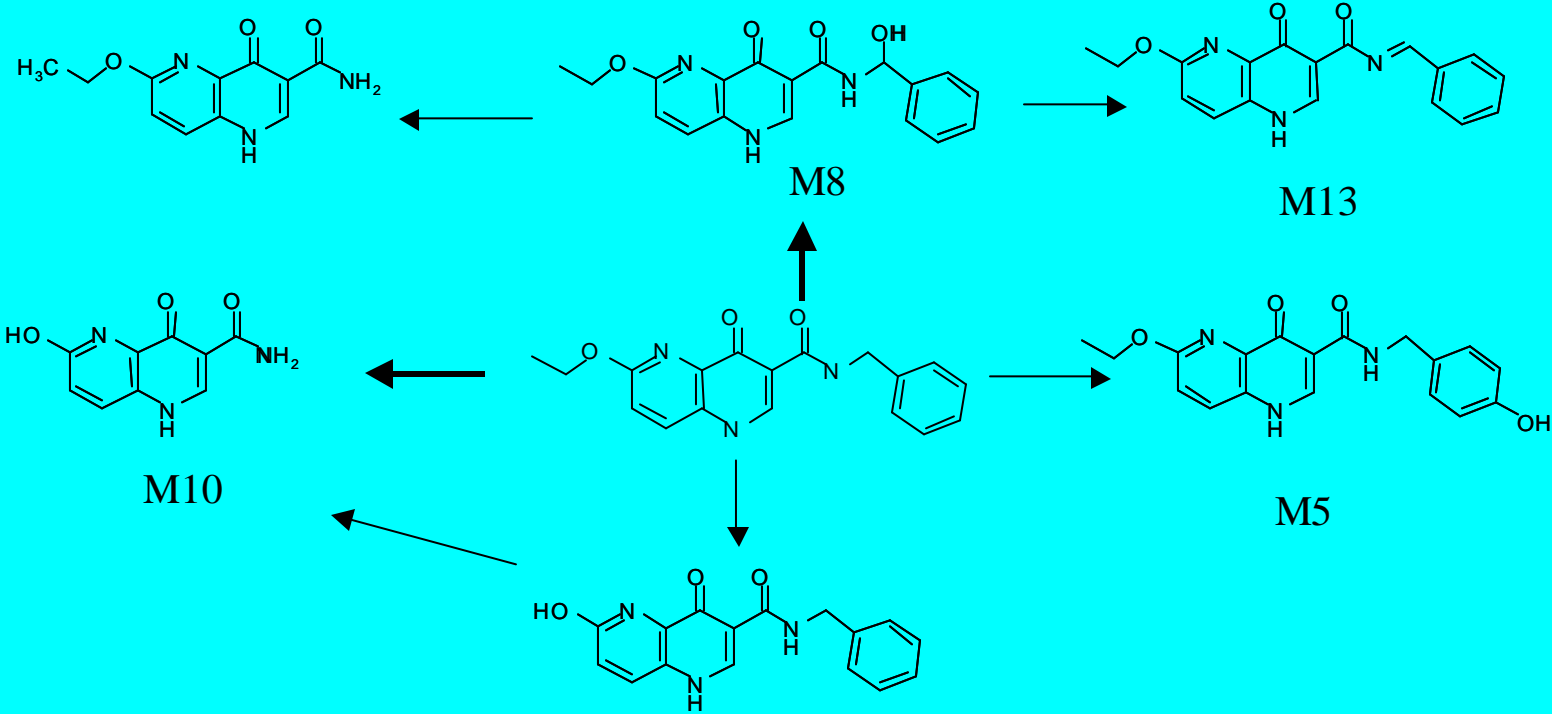
# LC-NMR [<sup>1</sup>H] Spectra of Metabolites M8 and M13

Proton double presat 273K CP-457,920 metabolite  
LC peak at 29.36 minutes (m/z=324) injection # 632

Proton double presat 298K CP-457,920 metabolite  
LC peak at 28.65 minutes (m/z=324) injection # 631



# Proposed Biotransformation Pathways of Compound 3



# Summary

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- ◆ Demonstrated the use of LC/MS for the identification of metabolites of three structurally different drugs.
- ◆ The Q-TOF allows on-line exact mass measurement of precursor and product ions
- ◆ Wet chemistry and *in vitro* techniques were found to be very useful for differentiation of regioisomeric and unusual structures
- ◆ Combination of LC/MS/MS with other analytical approaches (LC-NMR, H/D exchange, derivatization) is a powerful tool for solving difficult problems encountered in the analysis of drug metabolites.
- ◆ LC-NMR was found to be very useful for structural characterization of unstable metabolites

# Acknowledgments

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- ◆ Kim Johnson
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- ◆ David Plowchalk
- ◆ Sandra Miller
- ◆ Kathy Zandi
- ◆ Klass Schildknecht
- ◆ Carry Wager
- ◆ Larry Tremaine