Identification of Drug Metabolites via Mass Spectrometry

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Outline

- *Introduction*
- *What are Drug Metabolites*
- *What is Mass Spec*
- *Identification of Drug Metabolites*
- *Example*
- *Conclusion & Questions*

Introduction

- The study of how the drug is:
  - Absorbed
  - Distributed
  - Metabolized
  - Eliminated

→ *It is VITAL, however costly and time-consuming in the drug discovery process.*

Introduction

- The uptake of almost all organic compounds is followed by metabolism (biotransformation) reactions
- Among all compounds, drugs and pesticides are the most important as the biotransformation does not always lead to inactivation (detoxification) of the agent but in some instances, may lead to more active (bioactivation) or even more toxic compounds (biotoxification)

  - these sometimes toxic compounds are known as

-Metabolites-

- *-Pharmaceutical industries are mandated by regulatory agencies to identify ALL metabolites*

What is the importance of Metabolites

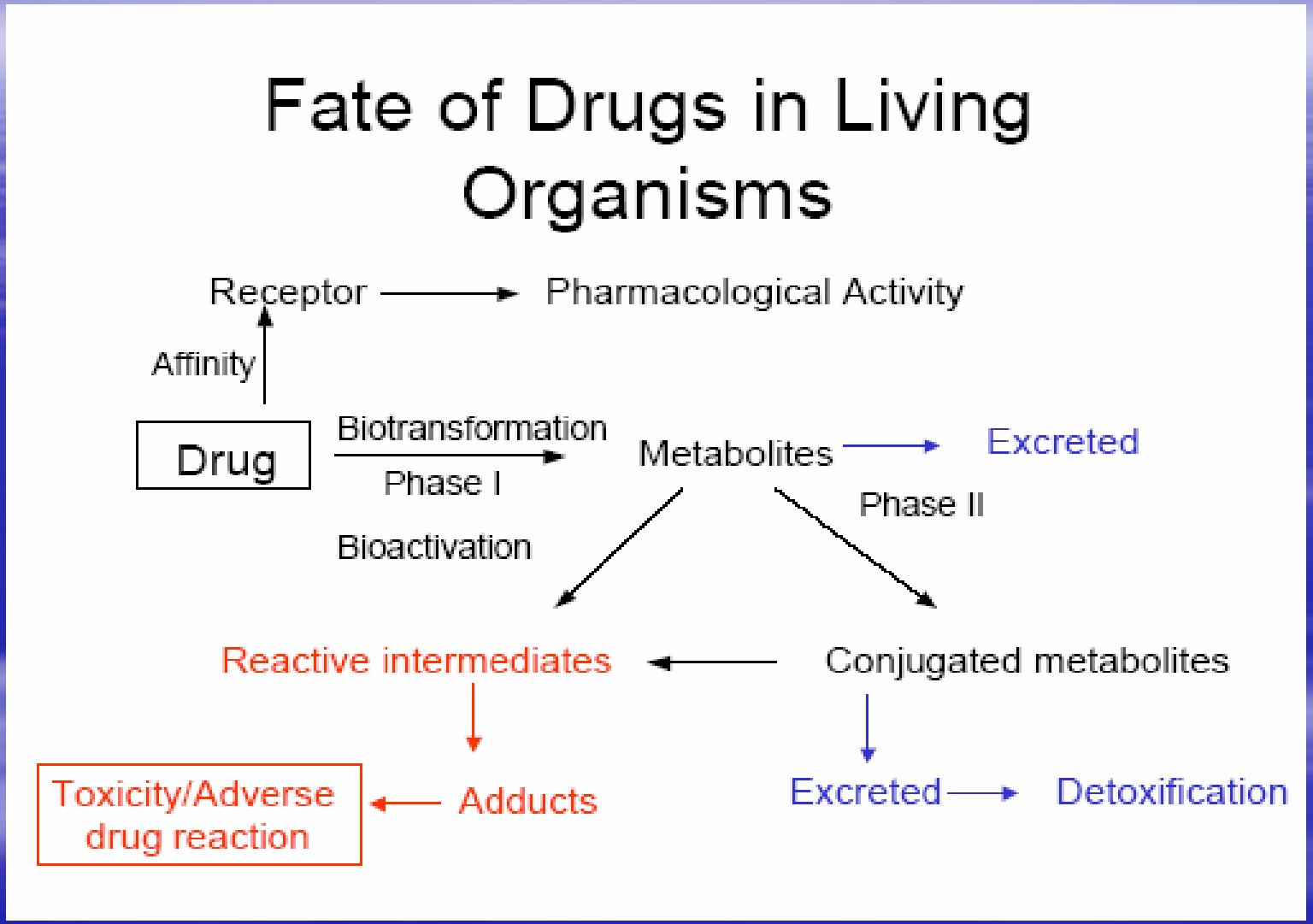
- Most of the drugs are eliminated from the body by metabolism: Detoxification process.
- The metabolites modulate the efficacy of drugs in the treatment of disease.
- The metabolites may possess pharmacological activity.
- The metabolites may be toxic: Bioactivation- *BAD*.
- Metabolites may provide leads to new and more sophisticated drugs.

…..continuing

- Until recently, Metabolite identification only took place once the compound had been chosen for drug developlent

- Due to the toxicity of drug metabolites,
  - Drug Metabolite identification is a lot more serious and closely monitored
  - Metabolite identification studies are done in the early phases of drug selection

continuing…..



Adopted from: *Metabolite Identification and Characterization* by Chandra Prakash, Ph. D.

Metabolism

- Two phases:
- **Phase I: (activation/detoxification)**
  - Biotransformation reactions: oxidation, reduction and hydrolysis
  - Polar groups introduced, more water soluble, less lipophilic
- **Phase II: (detoxification)**
  - Conjugation reactions
  - Reactions most often abolish biological activity and add more polarity
  - Very water soluble

Techniques for Identification of Metabolites

- LC –MS
  - Single Stage Quadrupole (SSQ) LC/MS
  - Triple Stage Quadrupole (TSQ) LC/MS/MS
  - Ion Traps (LCQ and LTQ)
  - QTOF
- Analytical Techniques combined with MS
  - Derivatization
  - Enzymatic hydrolysis
  - H/D exchange
  - LC/NMR

What is Mass Spectrometry (MS)

- MS does not measure the mass of a compound
- Mass spectrometers use the difference in mass-to-charge ratio (m/z) of ionized compounds to separate them from each other.
- Compounds have distinctive fragmentation patterns that provide structural information to specifically detect each compound very precisely.

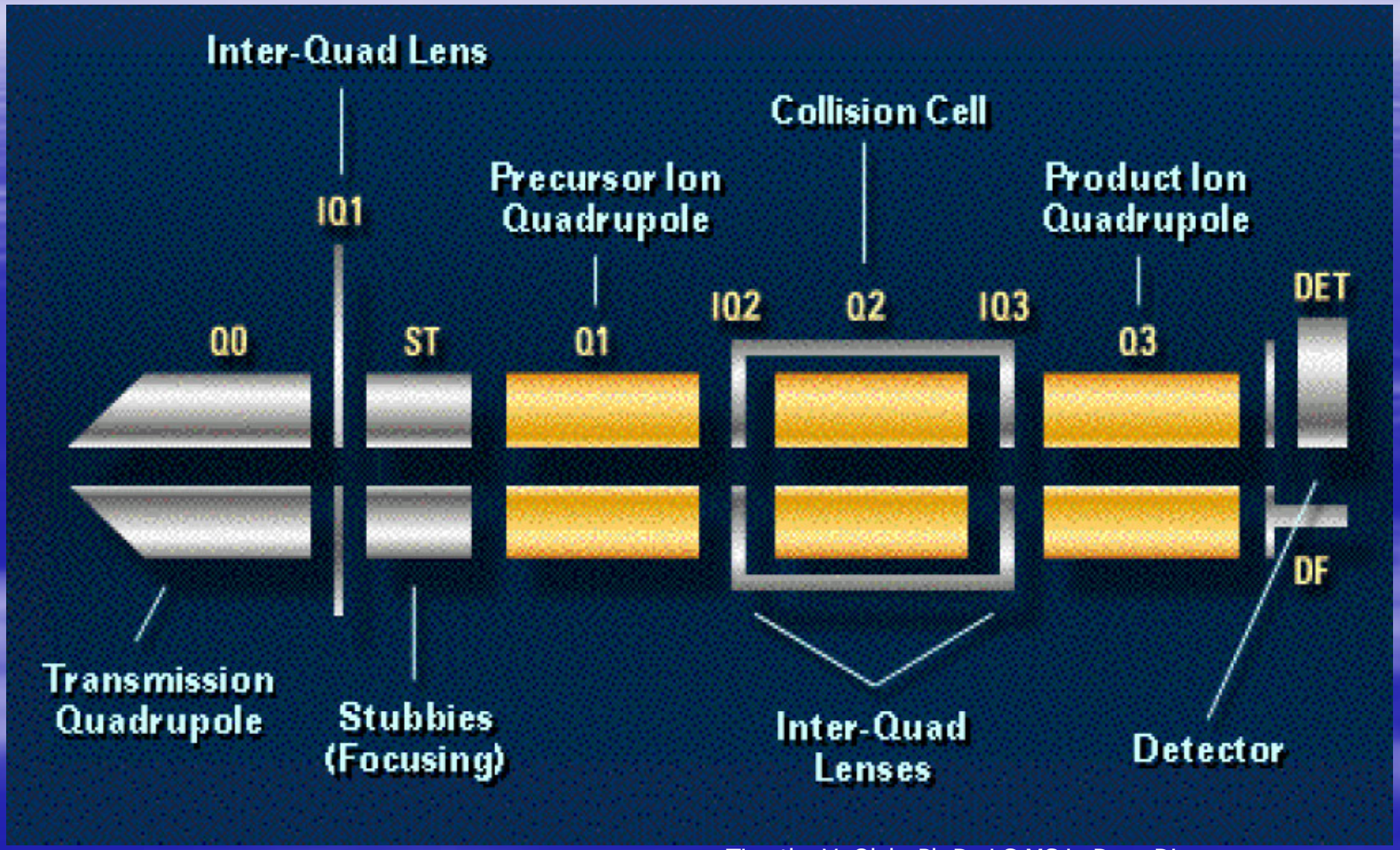
Continuing….

- MS – has emerged as an ideal technique for the identification of almost all structurally diverse metabolites.
- MS/MS data provides tremendous structural information for any drug metabolites
- Due to its superb speed, high selectivity and high sensitivity, MS has become the method of choice in drug discovery and development

Types of Mass Spec.

- **LC-MS** (Single quadrupole)
- **LC-MS/MS** (Triple quadrupoles)
- **LC-TOF-MS** (Time-of-flight)
- **Q-TOF-MS** (Quadrupoletime-of-flight)
- **LC-Q** (Ion traps, linear ion traps)
- **LC-Q-TRAPS** (Quadrupolelinear ion trap)
- **MALDI-TOF-MS**
- **FT-MS** (Fourier Transform)

LC-MS/MS (Triple quadrupoles)



Timothy V. Olah, Ph.D. LC-MS in Drug Discovery

Chemistry Dept, UCONN

Illustration

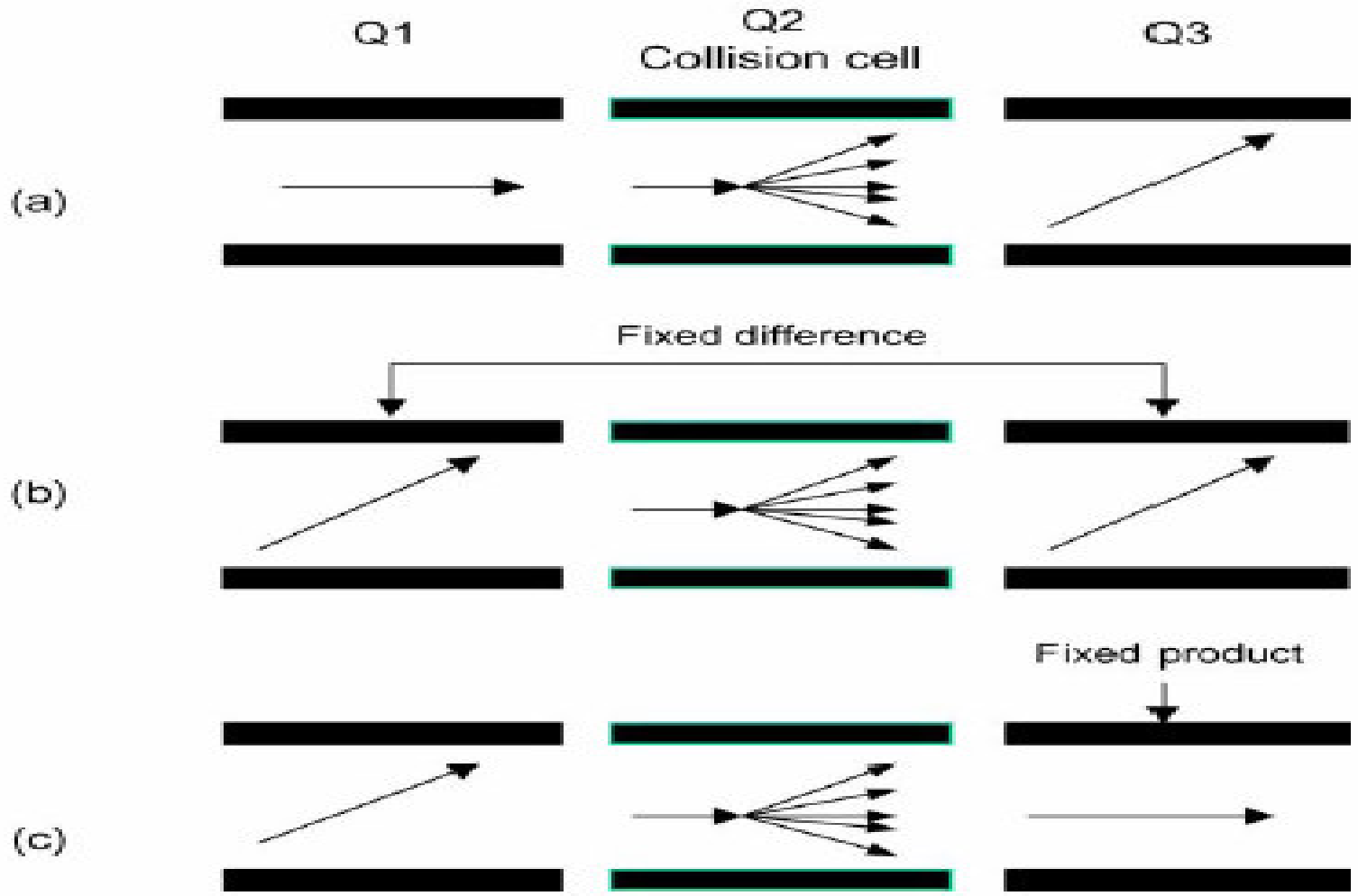


Fig. 1. Schematic illustrations of: (a) product ion scan; (b) neutral loss scan; and (c) precursor ion scan detection modes on a triple quadrupole mass spectrometer. Single ion transition (——►); CID of a selected ion (——◄); Scanning from low to high masses (——↗).

D.Q. Liu, C.E.C.A. Hop / Journal of Pharmaceutical and Biomedical Analysis 37(2005) 1–18

MS provides info about:

- The elemental composition of samples of matter
- The structures of organic, inorganic and biological molecules
- The qualitative and quantitative composition of complex mixtures
- Isotopic ratios of atoms and samples
- Structure and composition of solid surfaces

Preparation and Identification of Drug Metabolites

- *In vitro*
- Prepare a buffer solution, or a film
- Add the enzyme (ex. CYP450)
- Add Styrene (1%sln)
- Incubate for given amount of time
- Some metabolites form (such as Styrene Oxide)
- Identify suspected metabolites via MS

Example

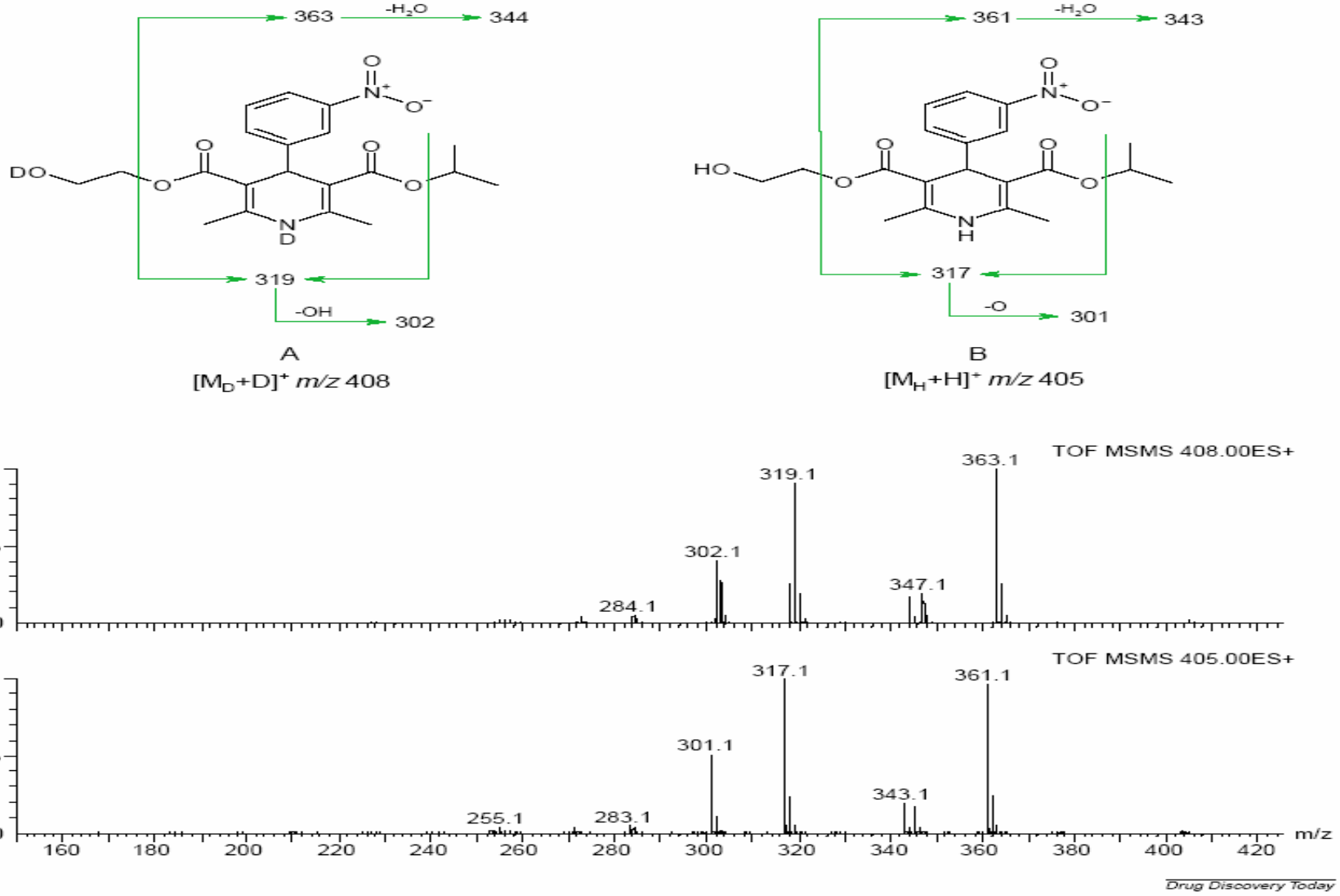


Figure 4. Representative TOF- MS- MS spectra of Nimodipine-metabolite in **(a)** D_2O *m/z* 408 and **(b)** H_2O *m/z* 405 following incubation of Nimodipine [Nimotop® (Bayer; http://www.bayer-pharmaceuticals.com)] with human liver microsomes. Each arrow indicates a possible site of fragmentation, with the corresponding ion. Abbreviation: TOF- MS- MS, time of flight- mass spectrometry- mass spectrometry.

Importance

- Every time a drug is administered metabolites will form
- Sometimes toxic metabolites form
- Toxic metabolites harmful to the body
  - Cause DNA damage => cancer
  - May damage different body organs such as
    - Liver, stomach, intestines,
    - Some even the nervous system

-Due to these potential risks it is VITAL to identify all drug metabolites

Today's Research

- Formulate and develop drugs with least toxicity and high efficiency
- Use minimal amount of drug
- Identify all possible metabolites for a given drug in the early stages of drug formulation
- Be able to identify very small traces of metabolites, or identify the disease in its early stages (such as cancer)
- Develop faster, more accurate and more precise methods for drug metabolite identification

Conclusion

- Metabolite identification is very important in new drug formulation and development
- Different methods being used to identify drug metabolites
- Mass Spectrometry methods
  - Most commonly used
  - Most sophisticated and enhanced methods
  - Fairly fast and accurate

  Research is focused in developing faster more accurate methods to identify and separate even smaller traces of metabolites

References:

- *D.Q. Liu, C.E.C.A. Hop / Journal of Pharmaceutical and Biomedical Analysis 37(2005) 1–18*
- Timothy V. Olah, Ph.D: **LC-MS in Drug Discovery**
- Alaa-Eldin F. Nassar and Rasmy E. Talaat: Strategies for dealing with metabolite elucidation in drug discovery and development DDT Vol. 9, No. 7 April 2004
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- Holler F.J., Nieman A.T., Skoog A.D.: *Principles of Instrumental Analysis Philadelphia 1998*

QUESTIONS???