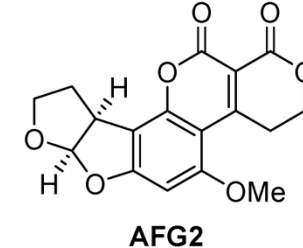
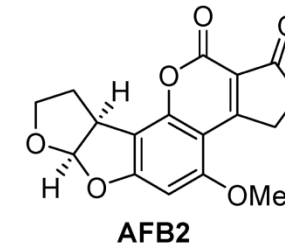
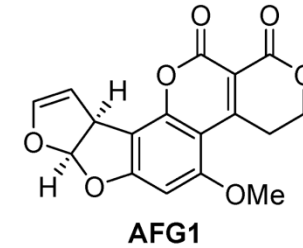
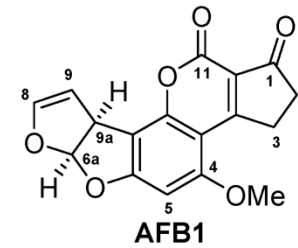


# What are Aflatoxins?

- Toxins produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*
  - Aflatoxins are secondary fungal metabolites.
  - Aflatoxin types include B1, B2, G1, G2.
  - B1 is most prevalent and toxic aflatoxin.



Microscopic view : spore formation of *Aspergillus*



### **Influence of aflatoxins on human health**

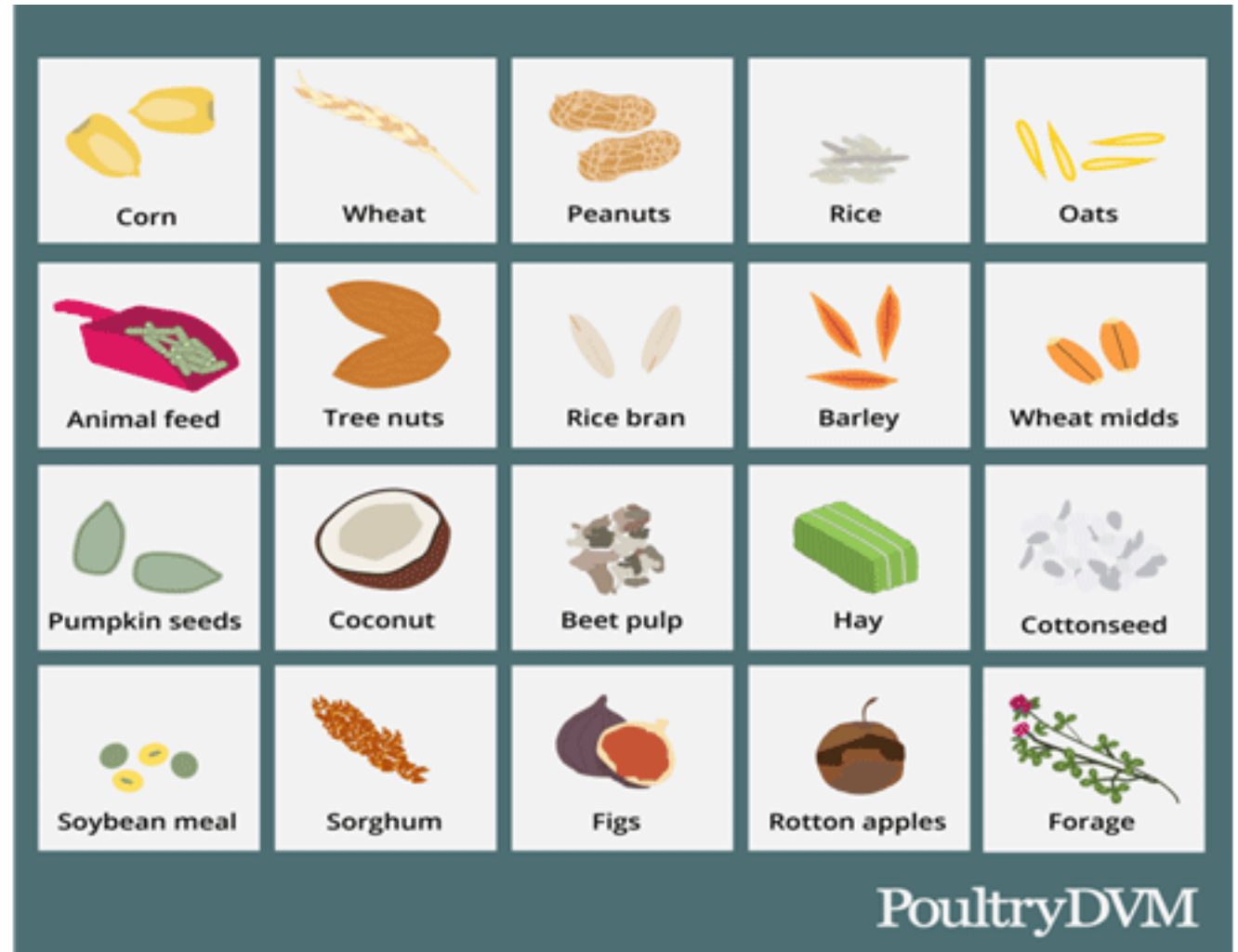
Acute aflatoxicosis can be **fatal**.  
Presenting symptoms are determined by amount  
of toxin consumed.

Clinical symptoms in humans include:

- Abdominal pain
- Vomiting
- Pulmonary edema
- Liver necrosis

Mycotoxins have carcinogenic, mutagenic action,  
suppress the immune system, affect the kidneys, liver,  
nervous and circulatory system.

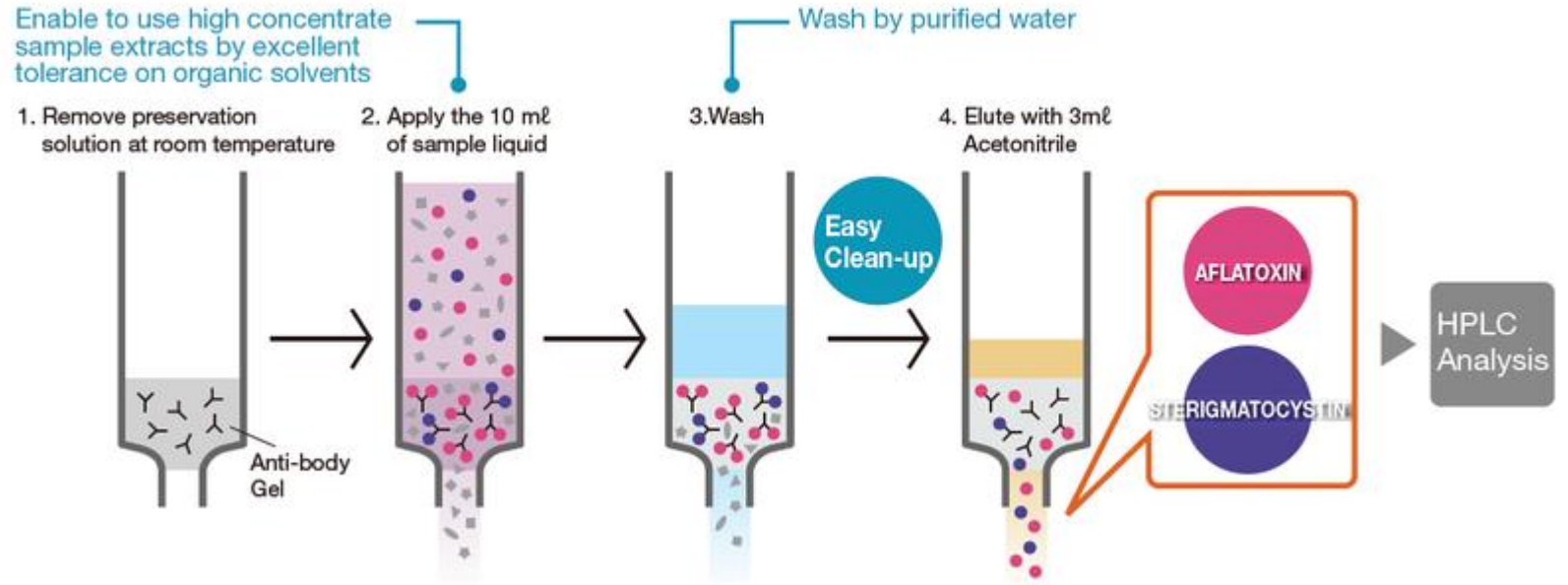
# AFLATOXINS IN FOOD



# Classical Extraction



Immunoaffinity Columns



LONG PROCEDURE  
HIGH COSTS





**LAB-EXPERIENCE  
INTRODUCTION  
28 April**



## A) Sample Treatment



FOOD SUPPLEMENTS



## B) Sample Weight

2.5 g of each  
sample

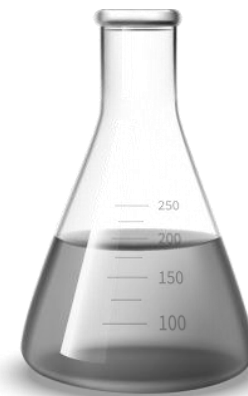


## C)



Vortex

[C]=???  
mg/mL



**Add Solvent: 5mL MeOH/H<sub>2</sub>O  
(80:20)**

# Sample extraction: Solid Phase Extraction (SPE)

**Immunoaffinity columns (ALFAPREP)** consists of a solid phase extraction based in the interaction between specific antibodies that are chemically selective for aflatoxins (in this case for AFLA B1, AFLA B2, AFLA G1 and AFLA G2)

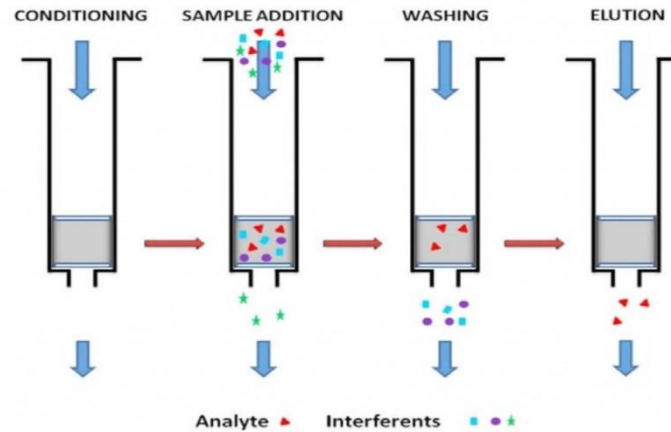


## Samples



**MIP-SPE** consists of a solid phase extraction with a sorbent phase composed of Molecular Imprinted Polymers MIPs.

MIP was synthesized with a dummy template as , naftoic acid that presents specif chemical affinity with the structure of aflatoxins. The Mip is selective with the molecule template.



Schematic representation of SPE clean-up procedure

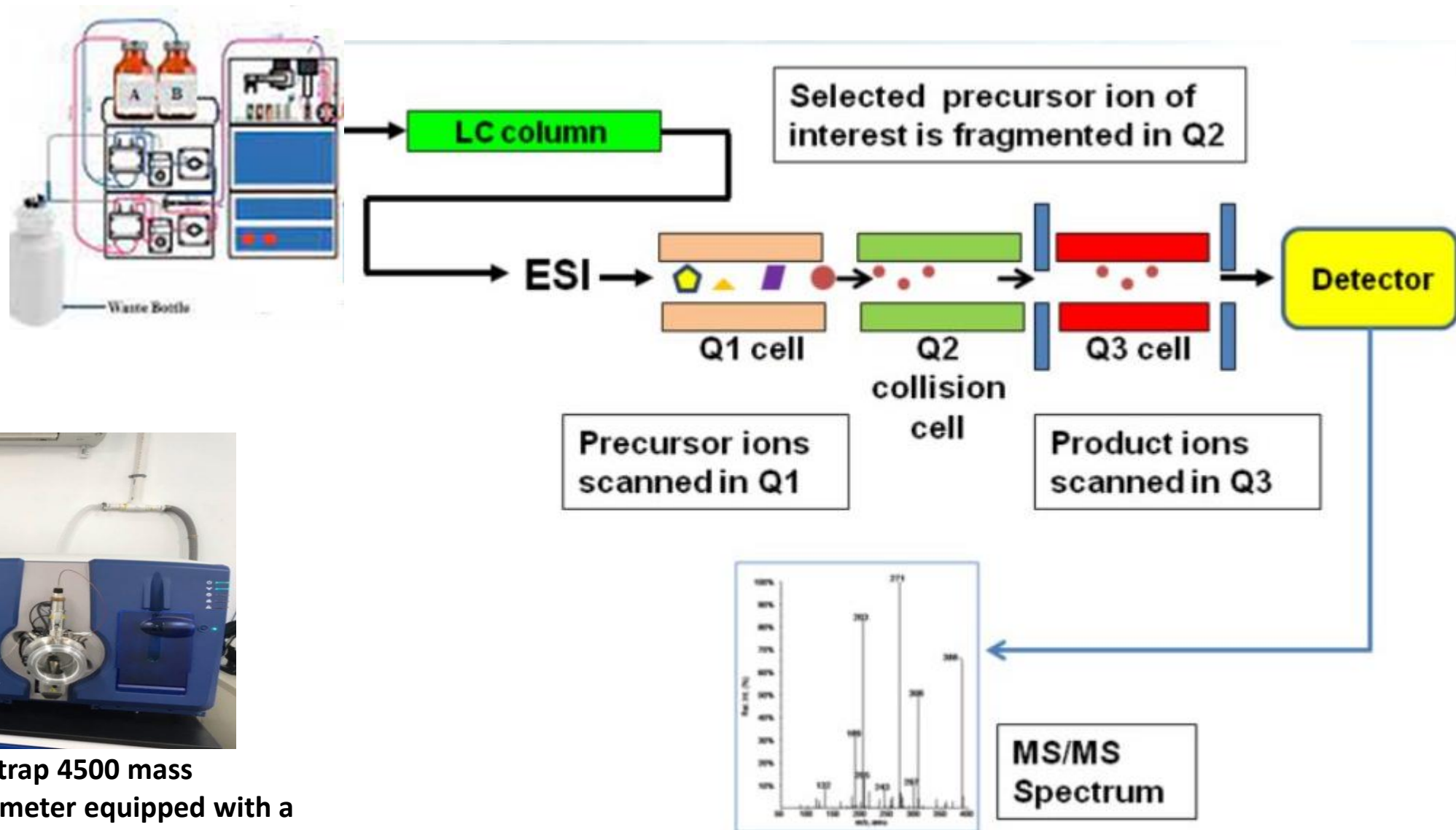


MIP (POWDER)

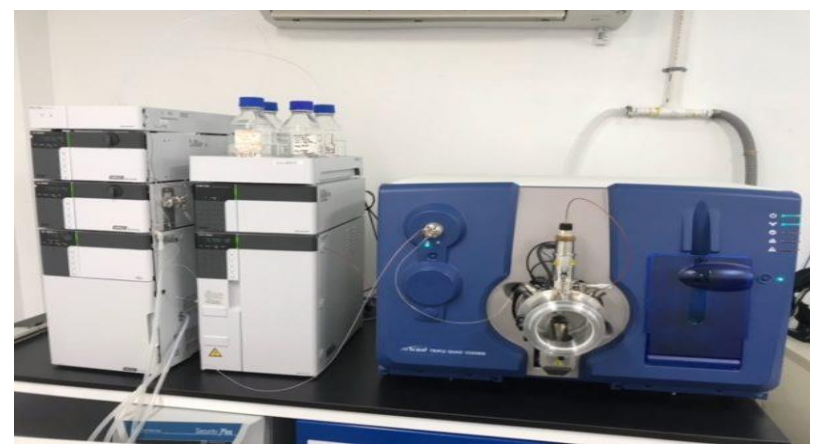




# UHPLC-MS/MS Analysis



UHPLC Shimadzu Nexera



Sciex Qtrap 4500 mass spectrometer equipped with a V turbo source, which works in ESI positive mode.

# UHPLC-MS/MS Analysis: Parameter

Column: C18 2.6  $\mu\text{m}$  50x2.1mm



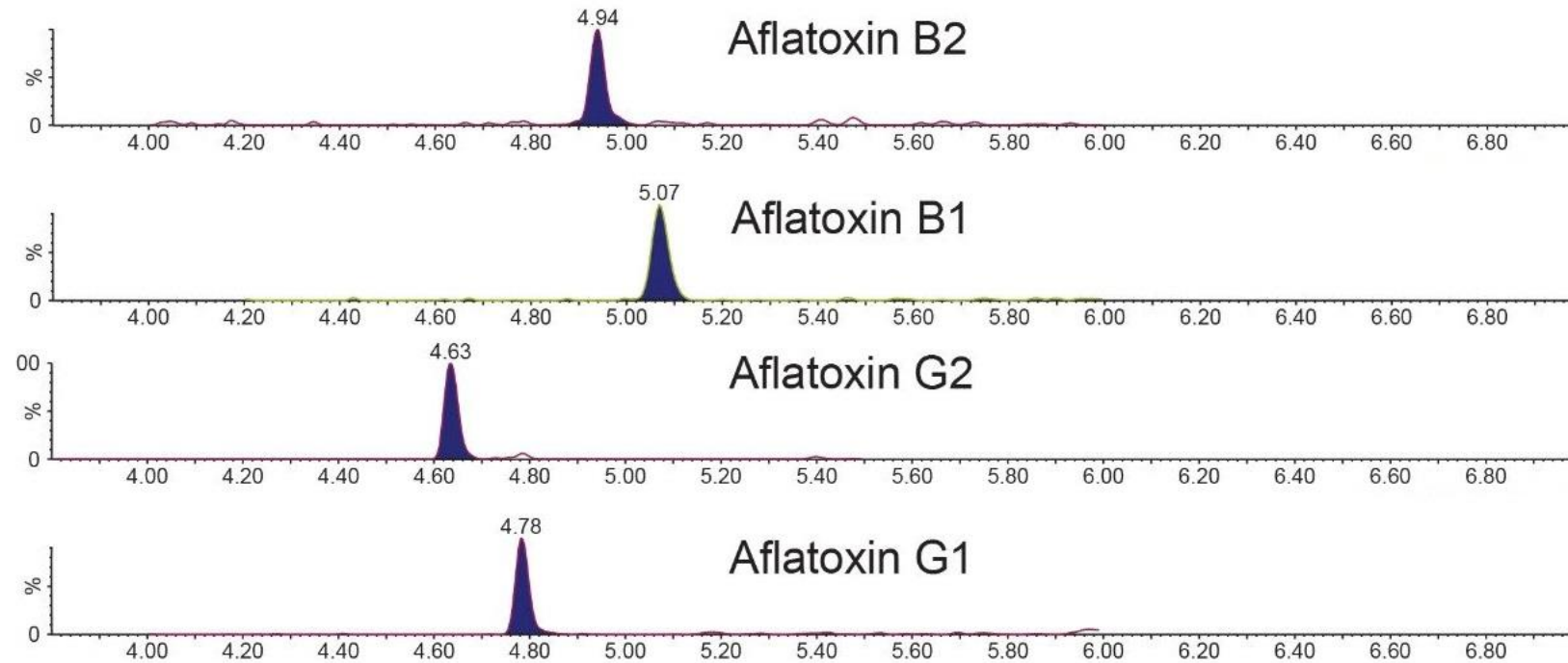
Mobile Phases:

-Water Phase : Ammonio Formiate 5mM

-Organic Phase: MeOH/ACN (50:50) 5 mM Formic acid

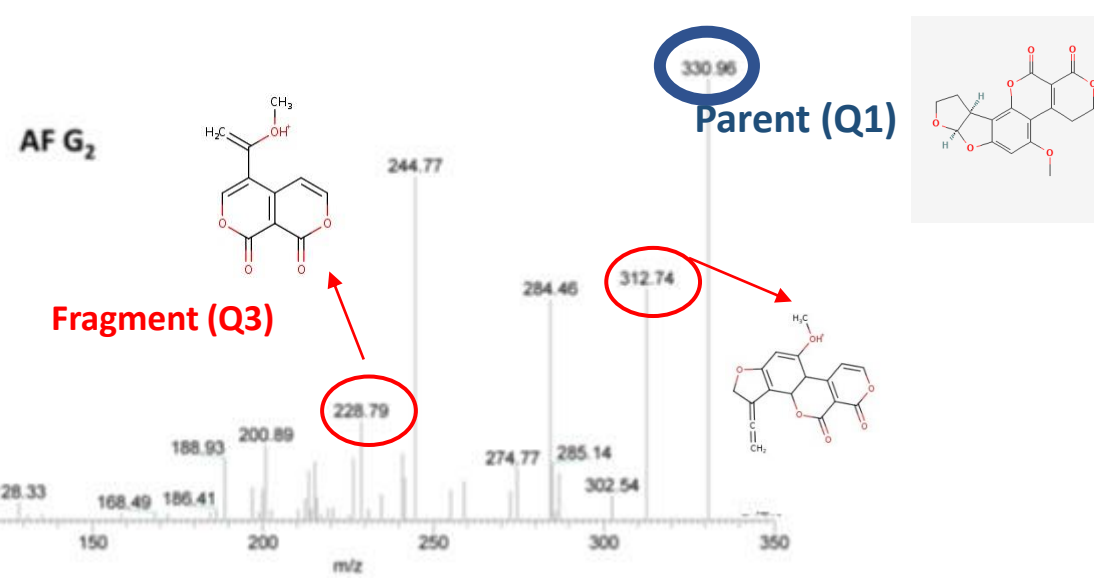
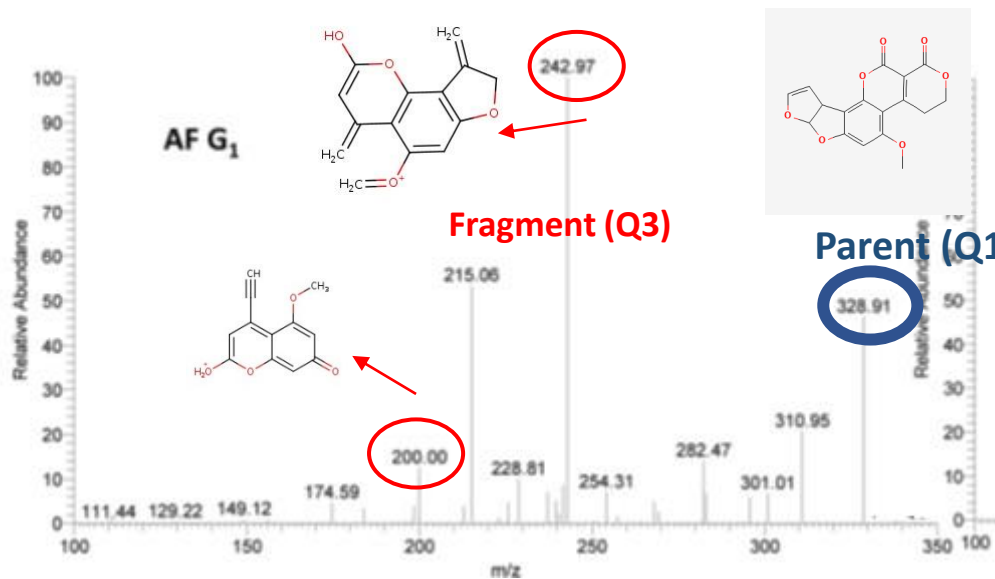
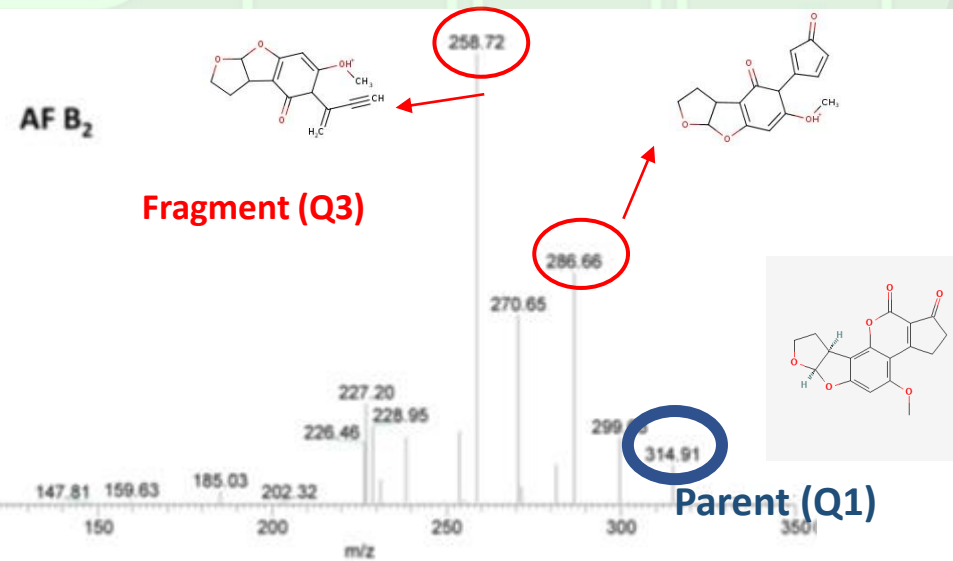
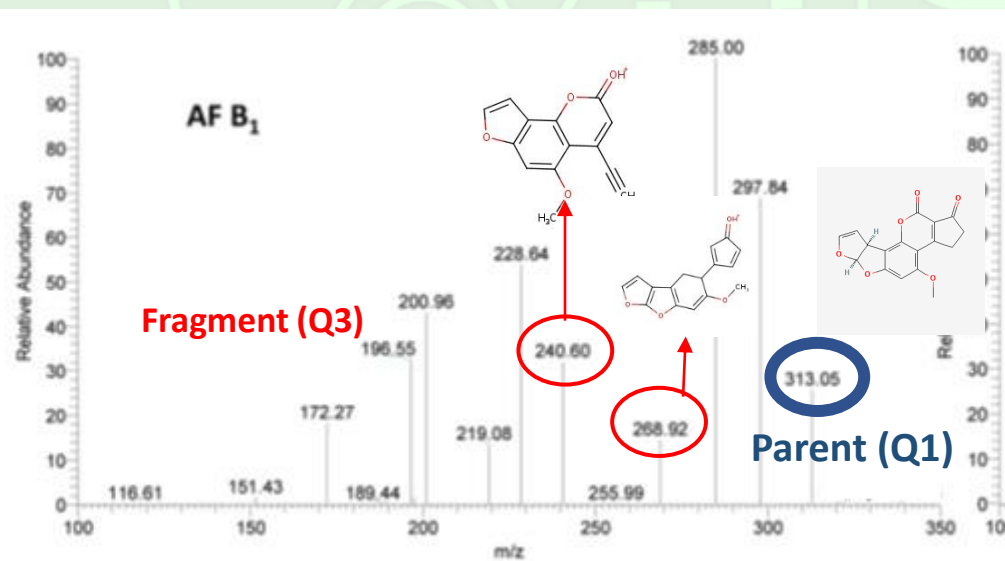
|         | Q1<br>(amu) | DP<br>(V) | DwT<br>(ms) | EP<br>(V) | Q3<br>(amu) | CE<br>(V) | CXP<br>(V) |
|---------|-------------|-----------|-------------|-----------|-------------|-----------|------------|
| ANALITA |             |           |             |           |             |           |            |
| AFLA B1 | 313.1       | 107       | 50          | 7         | 241.1       | 50        | 9          |
|         |             |           |             |           | 269.0       | 40        | 10         |
|         |             |           |             |           | 285.2       | 32        | 11         |
| AFLA B2 | 315.1       | 100       | 50          | 9         | 287.1       | 36        | 9          |
|         |             |           |             |           | 243.0       | 53        | 15         |
|         |             |           |             |           | 258.8       | 40        | 11         |
| AFLA G1 | 329.1       | 110       | 50          | 10        | 199.9       | 53        | 9          |
|         |             |           |             |           | 242.9       | 36        | 9          |
|         |             |           |             |           | 310.9       | 31        | 10         |
| AFLA G2 | 331.0       | 77        | 50          | 10        | 313.1       | 13        | 11         |
|         |             |           |             |           | 228.0       | 16        | 10         |
|         |             |           |             |           | 95.0        | 25        | 8          |

(Q1: molecular ion mass; DP: declustering potential; DwT: dwell time; EP: entrance potential; Q3: fragment ion mass; CE: collision energy; CXP: collision exit potential.)



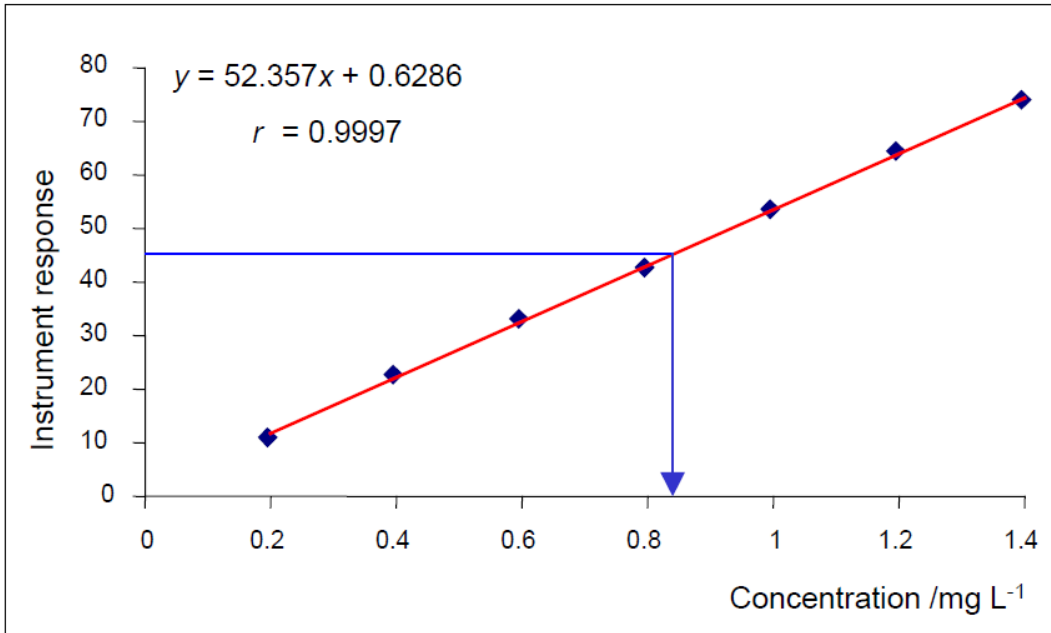
**UHPLC-MS/MS**

# UHPLC-MS/MS Analysis



## Calibration curve construction and sample quantification

Calibration curve  
Area vs. [Standard Concentration]



● Unknown sample

