

Rupture Technologies Workshop

Metabolomics and Molecular Interactions
for Biology and Health | ITQB | Oeiras | 13-15 Nov 2013



**Mass Spectrometry tutorial -
*a case study***



Introduction

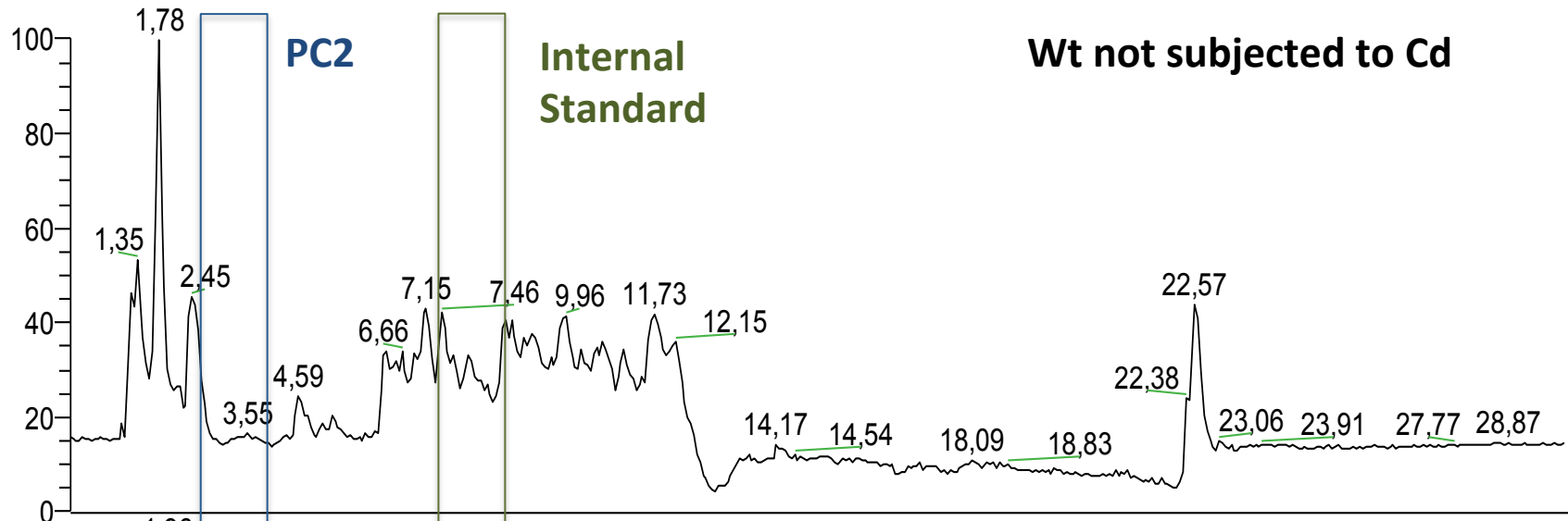
- **Phytochelatins (PCs)** are compounds produced by a wide range of higher plants, as well as algae and some fungi.
- These compounds act as **chelators** as they can bind heavy metals.
- Phytochelatins sequester free metal ions from the cytoplasm into the central **vacuole** where PC–metal complexes are stored to spatially **prevent oxidative damage** by the metals.

Objective of this study

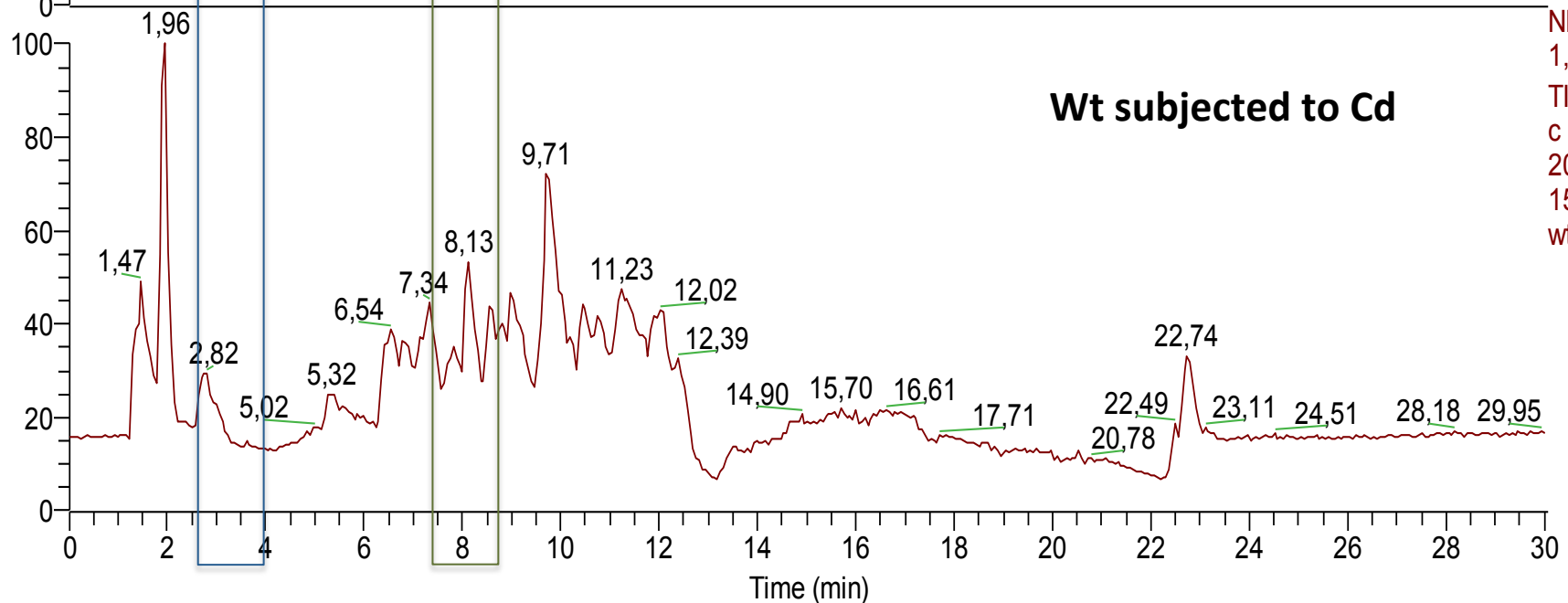
- A plant line (**Wt**) from *Nicotiana tabacum* was used and subjected to one stress level (concentration level – 50 mM) imposed by **Cadmium (Cd)**.
- The plant extracts enriched in PC were submitted to **LC-ESI-MS/MS assays**
- We proposed to identify and relative quantify phytochelatins (namely **PC2, m/z=540**) to an internal standard (peptide X, **m/z=768**)
- Our objective is to infer what is the involvement of phytochelatins in the plant defense mechanism against toxic levels of trace elements.

Total Ion Current (TIC) chromatograms

RT: 0,00 - 30,06



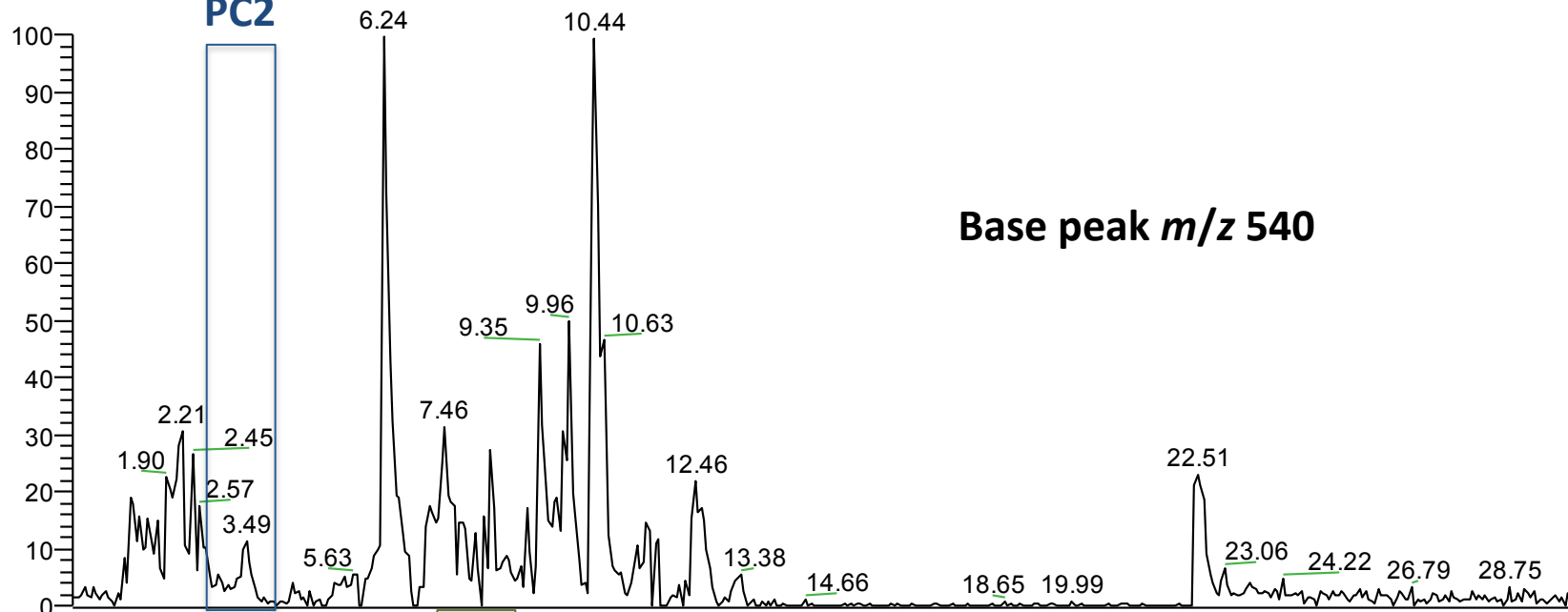
NL:
1,49E7
TIC F: ITMS +
c ESI Full ms [
200,00-
1500,00] MS
wt_1



NL:
1,41E7
TIC F: ITMS +
c ESI Full ms [
200,00-
1500,00] MS
wt_cd_1

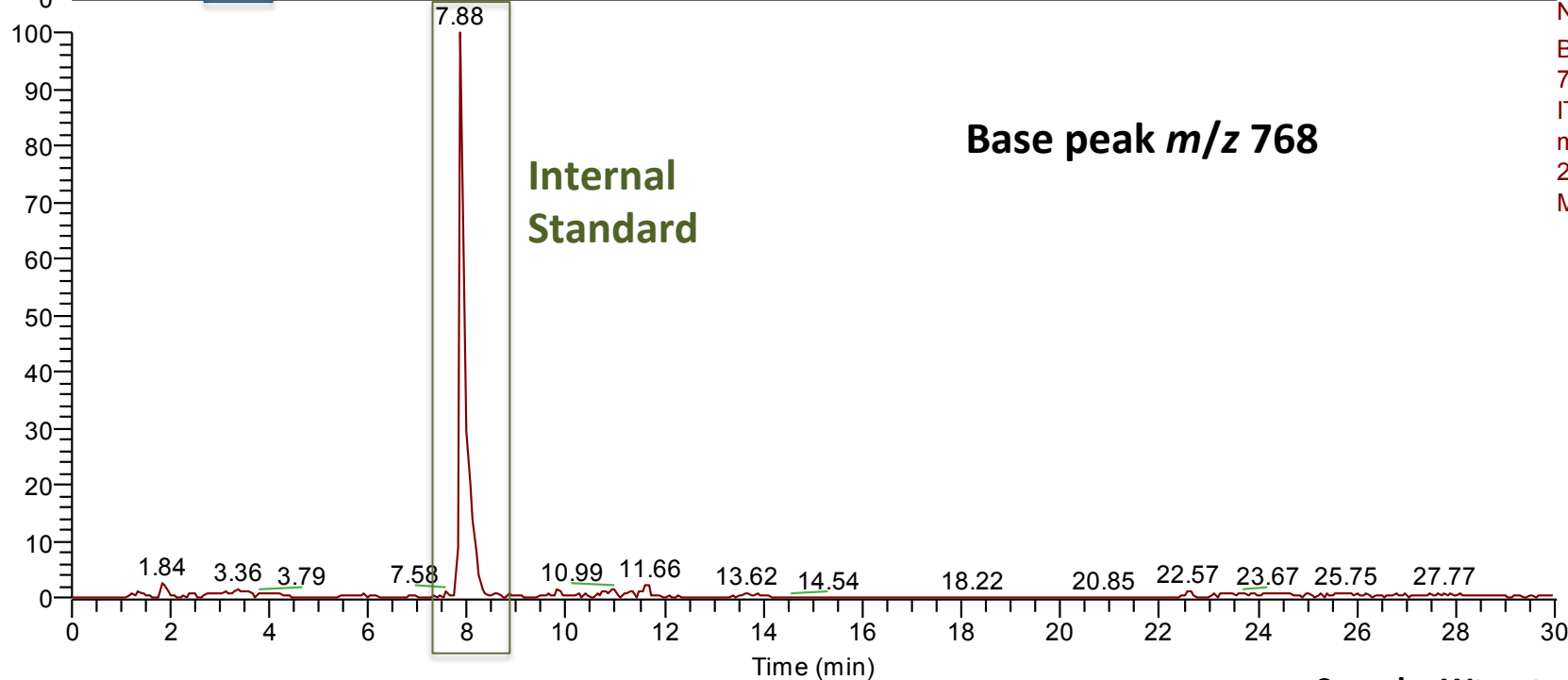
RT: 0.00 - 30.01

PC2



NL: 1.63E4

Base Peak m/z =
539.50-540.50 F:
ITMS + c ESI Full
ms [
200.00-1500.00]
MS wt_1

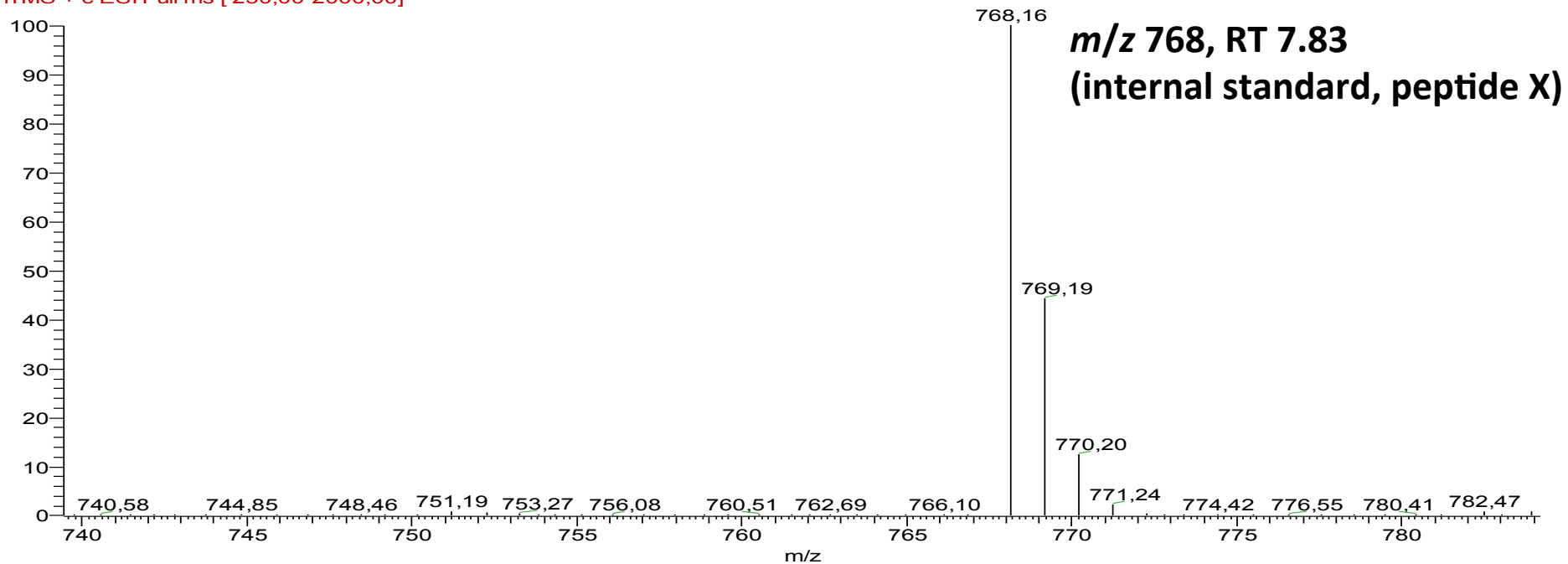


NL: 5.74E5

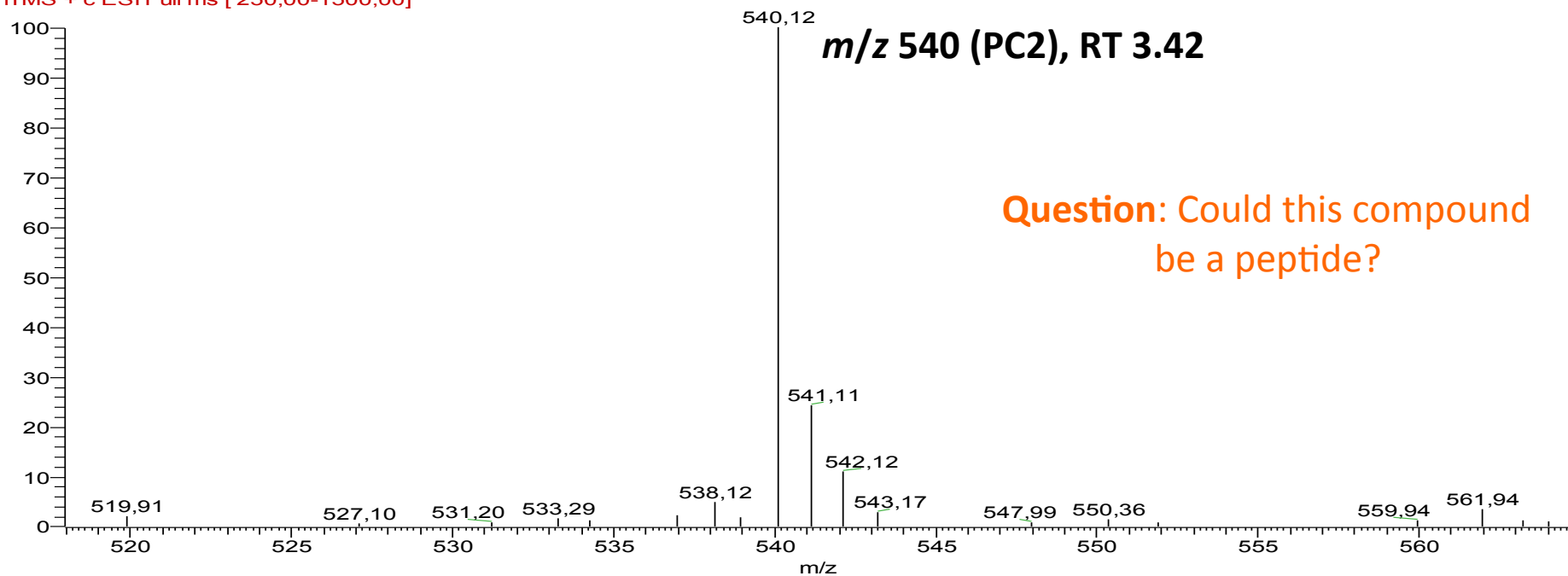
Base Peak m/z =
767.50-768.50 F:
ITMS + c ESI Full
ms [
200.00-1500.00]
MS wt_1

Sample: Wt not subjected to Cd

768_ms_pos #2-116 RT: 0,01-1,00 AV: 115 NL 4
F: ITMS + c ESI Full ms [250,00-2000,00]



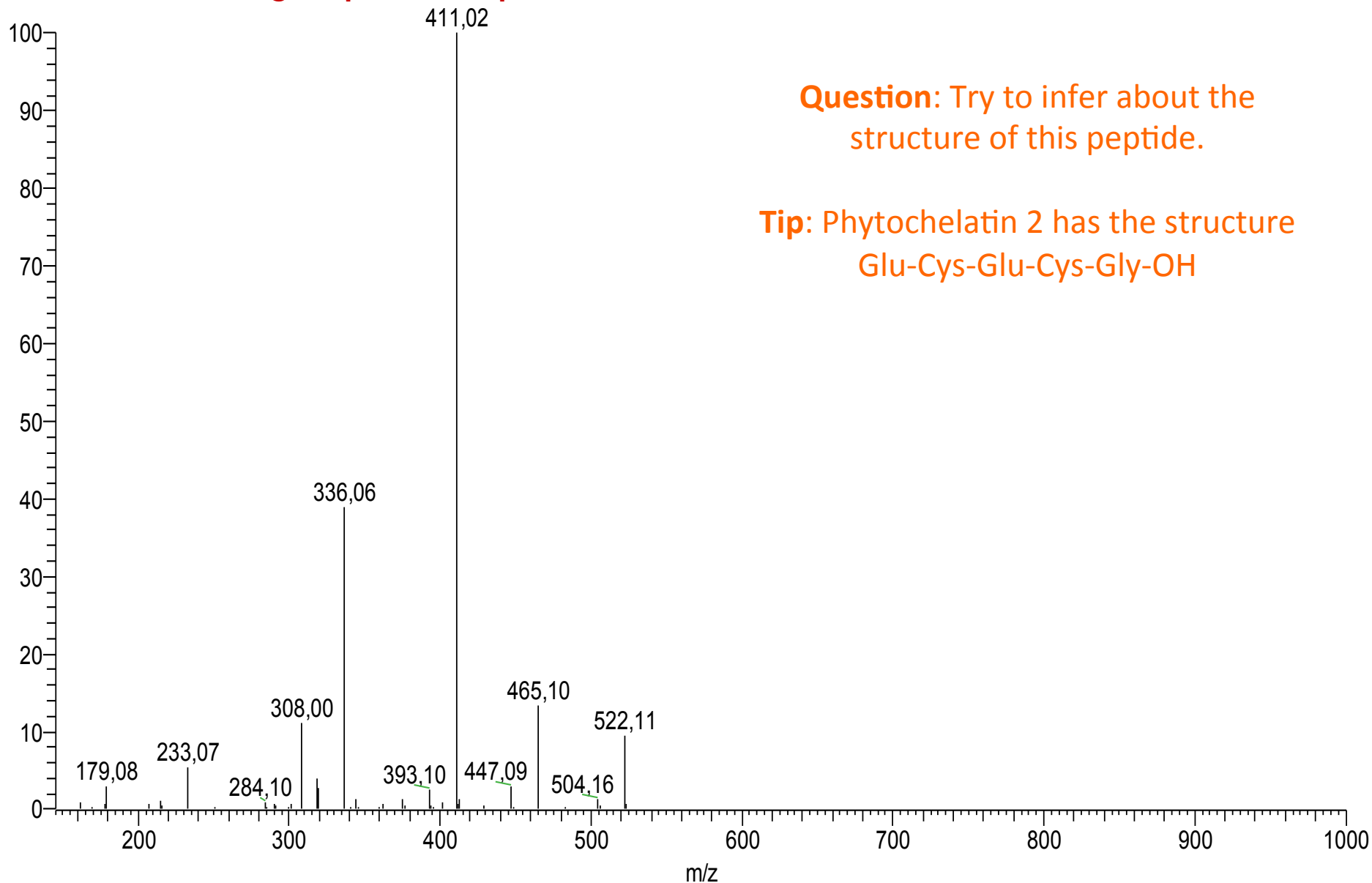
wt_cd_1 #271 RT: 3,42 AV: 1 NL: 3,99E3
F: ITMS + c ESI Full ms [250,00-1500,00]



MS/MS spectrum of PC2

wt_cd_1 #267 RT: 3,37 AV: 1 NL: 1,28E3

F: ITMS + c ESI Full ms2 540,00@35,00 [145,00-1000,00]



Question: Try to infer about the structure of this peptide.

Tip: Phytochelatin 2 has the structure
Glu-Cys-Glu-Cys-Gly-OH

Relative Quantification

Consider the following data:

		Wt not subjected to Cd		Wt subjected Cd	
Compound	<i>m/z</i>	Intensity	Area of the peak	Intensity	Area of the peak
Internal standard	768	7.90×10^2	30506	6.12×10^2	22173
PC2	540	1.58×10^2	10693	2.81×10^3	32597

Question: If the internal standard had a final concentration of 1 mg/L (recovery factor:60%), what is the concentration of PC2 in both experiences?