

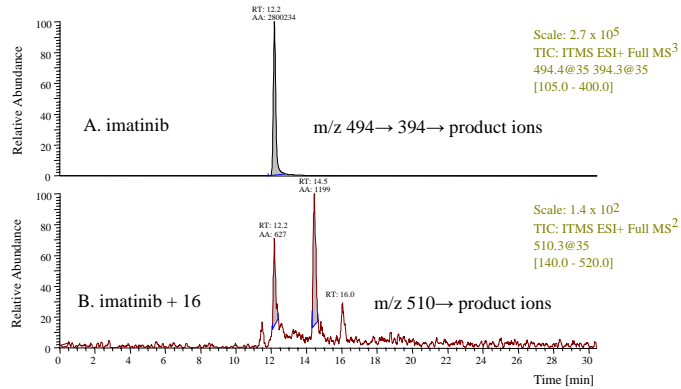
**SOME EXAMPLES OF QUANTITATIVE
AND QUALITATIVE ANALYSES**

PERFORMED AT

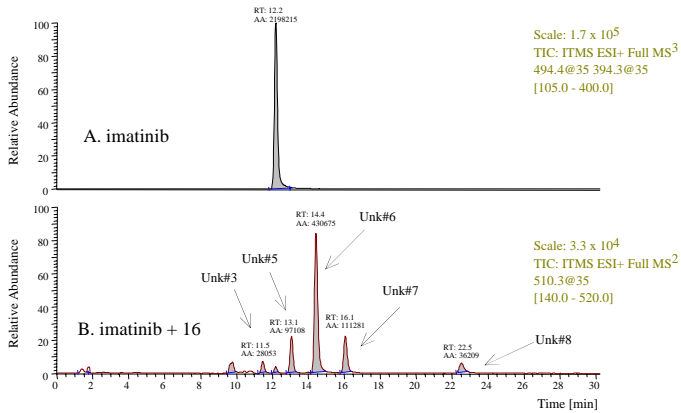
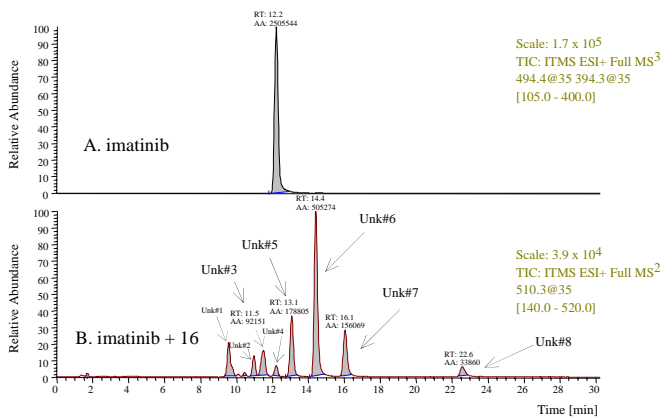


***q* MSF**

AN EXAMPLE OF *IN VITRO*
DRUG METABOLISM

I.

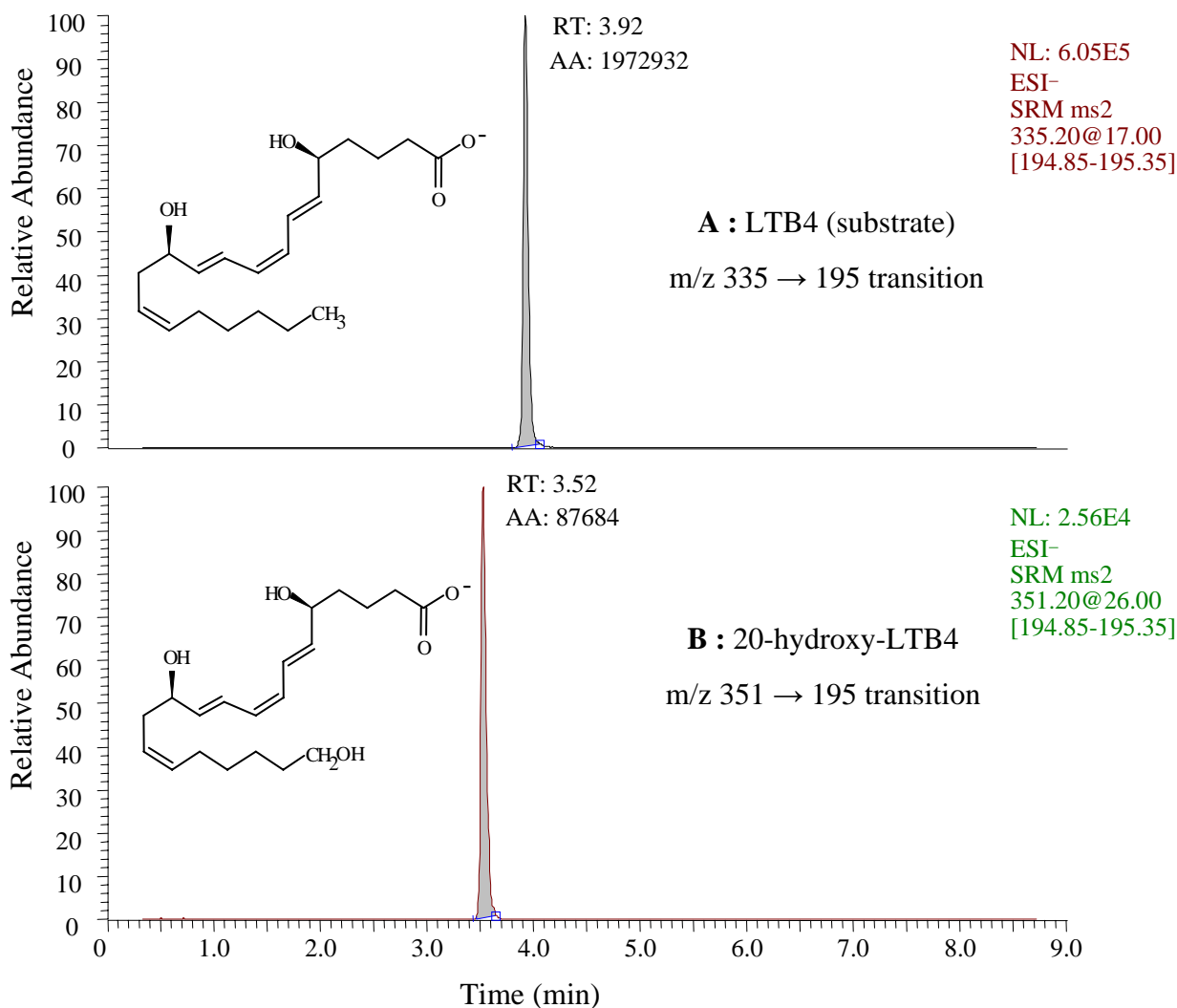
LC-MS2 chromatograms of microsome incubations in presence of an anticancer agent, imatinib, and cDNA expressed cytochrome P-450.

II.**III.**

LC-MS2 total ion chromatograms of m/z 494, 510 product ion scans corresponding to imatinib ions and N-oxide / hydroxy metabolite ions (numbered Unk#3 to #8), respectively. The 3 samples injected were the incubation extracts of control microsomes (no CYP activity; I.), human liver microsomes (HLM; III.) or cDNA expressed CYP3A4 microsomes (III.). Imatinib (A.) and N-oxide / hydroxy metabolites (imatinib + 16) were resolved using hydrophobic interaction liquid-chromatography coupled to ESI+ - ion trap mass spectrometer (LC- MSn). RT: retention time (min). AA: chromatographic peak areas.

**AN EXAMPLE OF QUANTITATION OF
ENDOGENOUS COMPOUNDS**

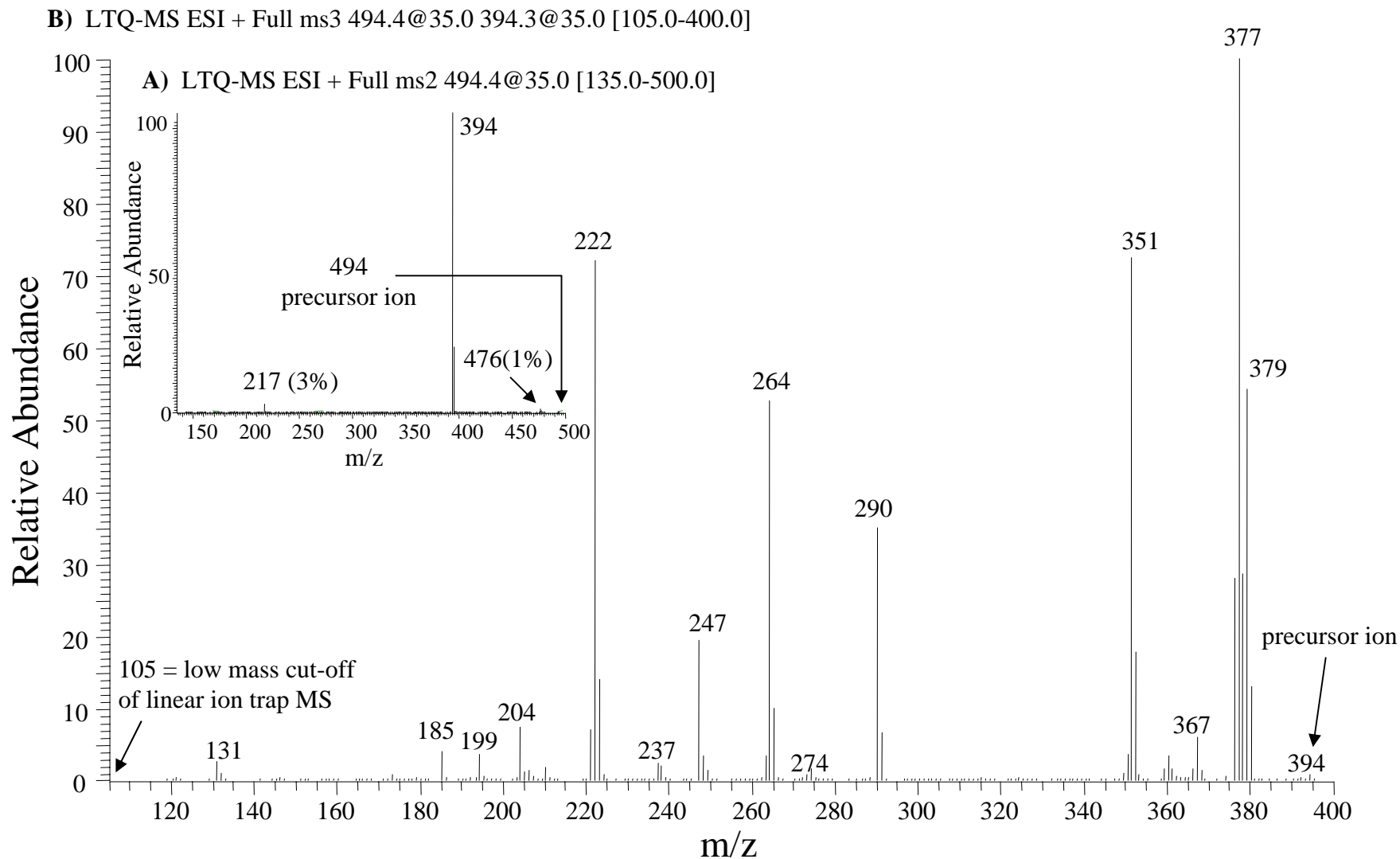
ESI-LC-MS/MS chromatogram of leukotriene B4 (LTB4) and 20-hydroxyl-LTB4, after microsomal incubation with cDNA expressed CYP4F2



Chromatogram of a deproteinized incubation extract injected onto a LC-triple quadrupole MS. Incubation was performed with CYP4F2 microsomes in presence of 10 μ M leukotriene B4 (LTB4) but in absence of imatinib added as potential inhibitor. LTB4 and its hydroxylation product, 20-hydroxyl-LTB4, were recorded in negative electrospray ionization by their specific parent-product ion transitions A and B, respectively. Compounds were resolved using a 2.1 mm x 150 mm C8 column. Formation rate of 20-hydroxyl-LTB4 was determined by its peak area. RT: retention time (min). AA: chromatographic peak areas.

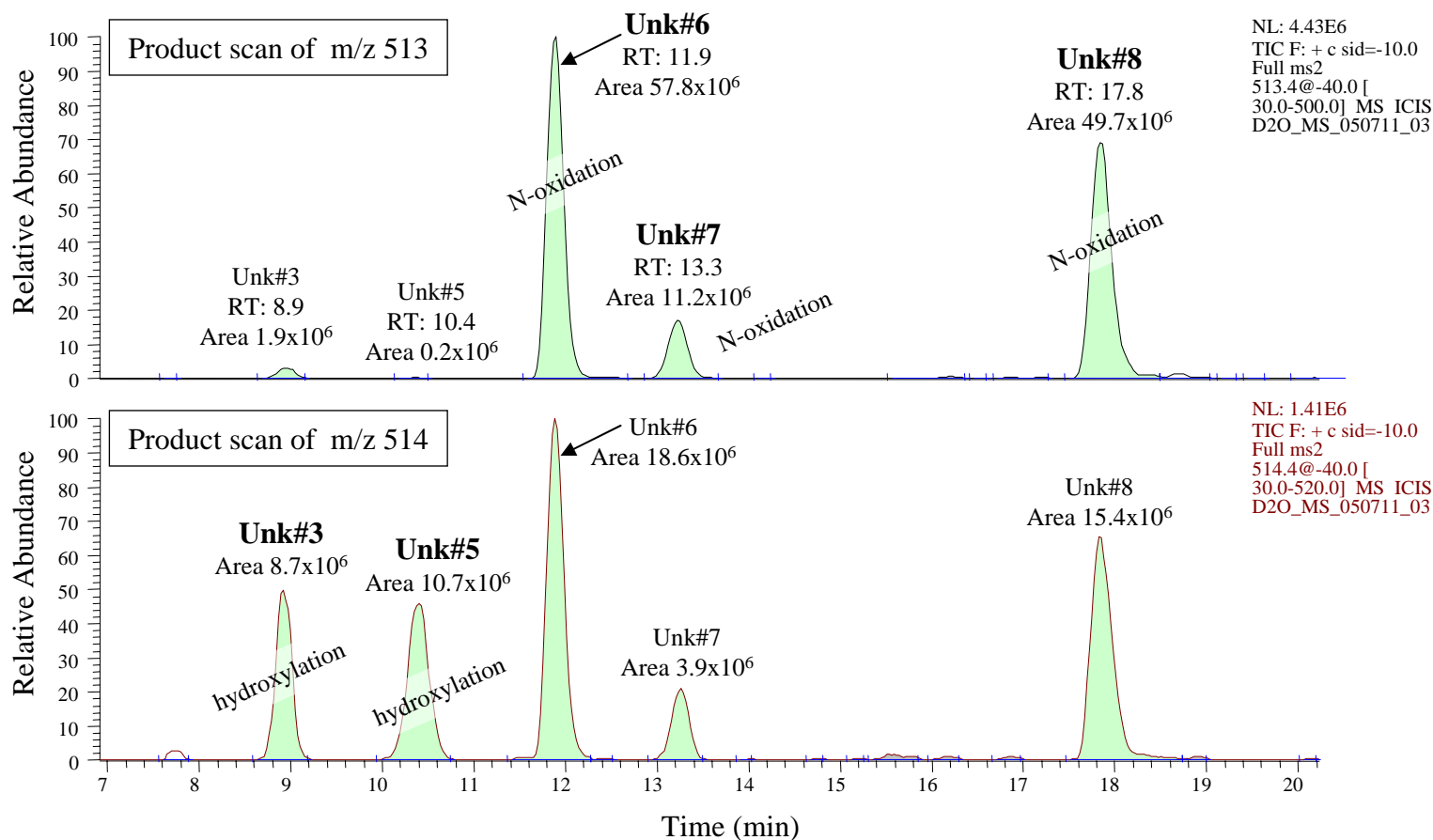
**AN EXAMPLE OF FRAGMENTATION
OF IMATINIB, AN ANTICANCER AGENT**

ESI+-LTQ-MS2 (A) and MS3 (B) product ion spectra of $[M+H]^+$ imatinib ion:
 m/z 494 \rightarrow product ions and m/z 494 \rightarrow 394 \rightarrow product ions, respectively.



**AN EXAMPLE OF IDENTIFICATION OF
N-OXIDE AND HYDROXY METABOLITES
WITH D₂O-CONTAINING MOBILE PHASE**

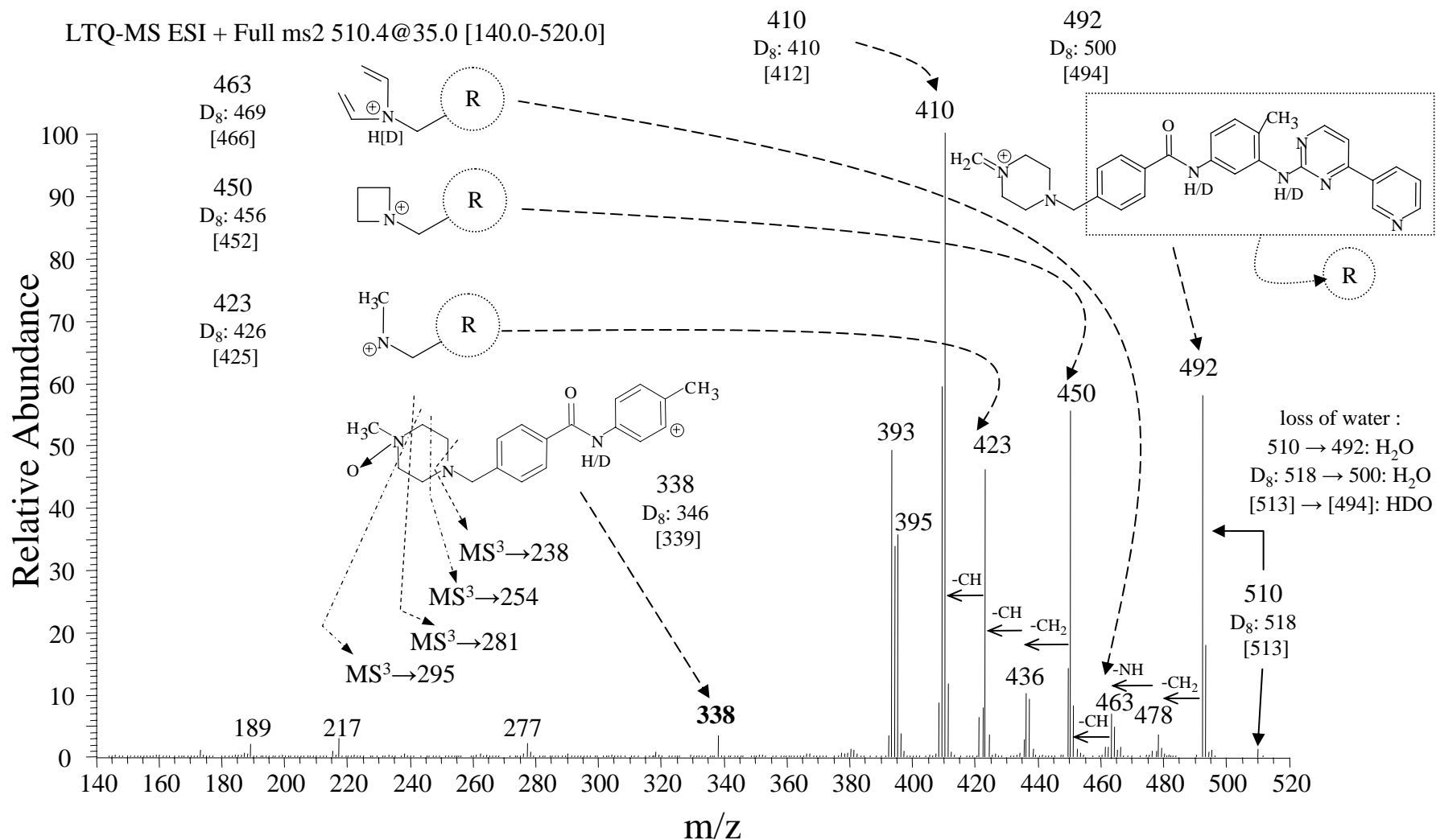
LC-LTQ-MS chromatograms of imatinib product scans at m/z 513 and 514 of a microsomal incubation extract injected on using D2O-containing mobile phase



Total ion chromatograms of m/z 513 and 514 product scans of a CYP3A4 microsomal incubation extract injected on LC-LTQ-MS using D2O-containing mobile phase. N-oxide and hydroxy metabolites show [MD+D]⁺ parent ion of m/z 513 and m/z 514, respectively. Relative abundance (base peaks) revealed that Unk#3 and #5 are hydroxyl and Unk#6,#7 and #8 are N-oxide metabolites of imatinib. RT: retention time (min).

**AN EXAMPLE OF STRUCTURE
ELUCIDATION OF A N-OXIDE
METABOLITE OF IMATINIB**

LC-ESI+-ion trap-MS2 product ion spectra of a imatinib metabolite at m/z 510



MS2 product ion spectra of Unk#6 m/z 510 on the LC-ESI+-LTQ-MS. Fragmentation of the methylpiperazine is elucidated by comparing MS2 fragment ions of N-oxide imatinib-D8 (m/z 518; D8) with N-oxide imatinib (m/z 510) and by MS2 fragment ions of Unk#6 in D₂O-containing mobile phase (m/z 513; values given in brackets). Product ion at m/z 338 indicates that N-oxidation is located in methylpiperazine. Further fragmentation of m/z 338 (MS³) is given and suggests that N-oxidation is located at N#4

**AN EXAMPLE OF PEPTIDE
IDENTIFICATION BY ION TRAP,
BIOWORKS-SEQUEST AND SWISSPROT**

ESI+-LTQ-MS2 product ion spectra of digested gliadine peptide :

Identification of peptide by-products

Altergen_0511107_03.raw - BioworksBrowser

File Edit View Display Options Actions Tools Help

Peptmatch Parameters:
Sequence... Modifications...

Fragmentation Ions:
 A A* Ao
 B B* Bo
 Y Y* Yo
 C D' W' X Z'
 H Internal P Internal

General Parameters
Threshold: 0.1
Charge: 1
Mass type: Average Monoisotopic
Labeling mode: Overwrite Append
Label color: Color...
Reset Color
Update... Help

RT: 3.83 - 5.14

Peptide retention time : 4.4 min

NL: 2.65E5
Base Peak F: ITMS + c
ESI Full ms2
834.20@25.00 [225.00-2000.00] MS
Altergen_0511107_03

Number of traces: 1

Modifications: C=57.02150
Mass type: Monoisotopic Charge: 1

	AA	A	A*	Ao	B	B*	Bo	C	X	Y	Y*	Yo	Z'	W'	D'	H int	P int
1	P				98.06004	81.03349	80.04948										
2	Q				226.11862	209.09207	208.10805			1568.81076	1551.78421	1550.80019					
3	P				323.17138	306.14483	305.16082			1440.75218	1423.72563	1422.74161					
4	Q				451.22996	434.20341	433.21939			1343.69941	1326.67286	1325.68885					
5	L				564.31402	547.28747	546.30346			1215.64064	1198.61429	1197.63027					
6	P				661.36679	644.34024	643.35622			1102.55677	1085.53022	1084.54621					
7	Y				824.43012	807.40357	806.41955			1005.50401	988.47746	987.49344					
8	P				921.48288	904.45633	903.47231			842.44068	825.41413	824.43012					
9	Q				1049.54146	1032.51491	1031.53089			745.38792	728.36137	727.37735					
10	P				1146.59422	1129.56767	1128.58366			617.32934	600.30279	599.31877					
11	Q				1274.65280	1257.62625	1256.64223			520.27657	503.25003	502.26601					
12	L				1387.73686	1370.71031	1369.72630			392.21800	375.19145	374.20743					
13	P				1484.78963	1467.76308	1466.77906			279.13393	262.10738	261.12337					
14	Y									182.08117	165.05462	164.07060					
15																	
16																	
17																	
18																	
19																	

Determination of ion series

Altergen_0511107_03 #866 RT: 4.43 NL: 2.65E5
F: ITMS + c ESI Full ms2 834.20@25.00 [225.00-2000.00]

Unknown peptide product scan

Sequence Editor

Amino Acids: A C D E F G H I K L M N O P

Sequence: MH+: 1665.86352 Length: 14 pl: 5.55
PQPQLPYPQPQLPY

Peptide identification with match score

Ready

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