



UE Metabolomics



Lecture: GC-MS in Metabolomics

Lena Fragner

*Molecular Systems Biology,
University of Vienna*



Mosys

**Department
Molecular Systems Biology**



**universität
wien**

University of Vienna | Faculty of Life Sciences | Althanstrasse 14 | 1090 Vienna | Austria

Overview

- ◉ Introduction
- ◉ Technical aspects
 - ◉ Chromatography
 - ◉ Ionisation techniques
 - ◉ Mass analyzer
- ◉ Data validation

Introduction

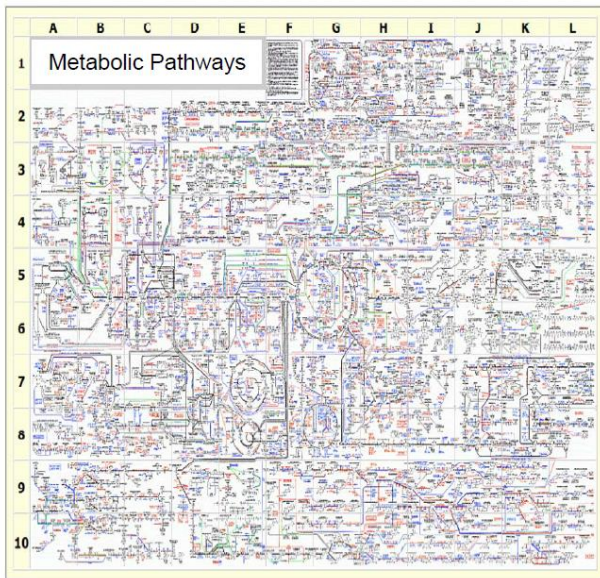


metabolomics



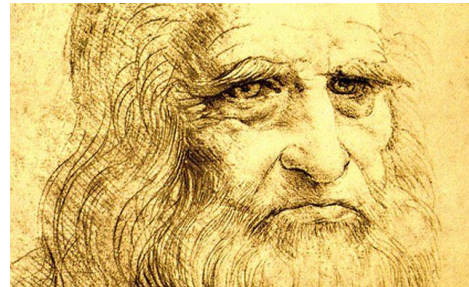
highly complex biological systems

**physico-chemical
diversity of metabolites**



> 200.000 - 1 million
putative structures
occurring in
plant kingdom

**variable biological
matrix**



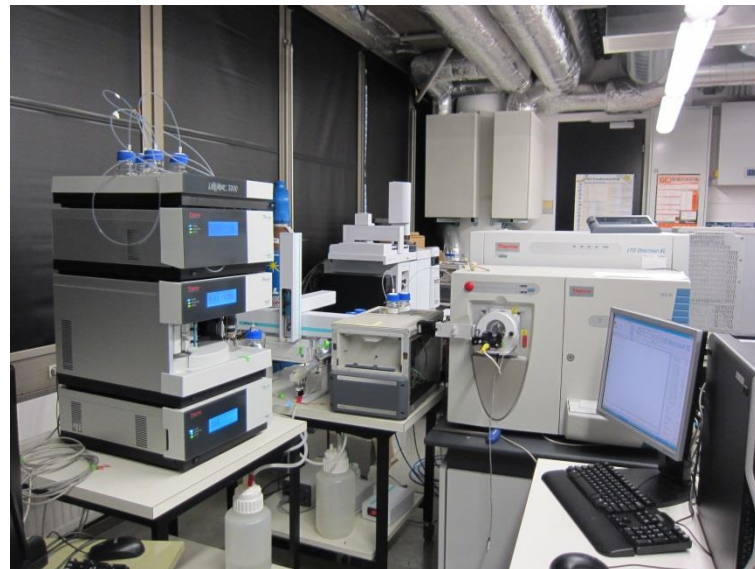
**high dynamic
range of analytes**



GC-MS



LC-MS



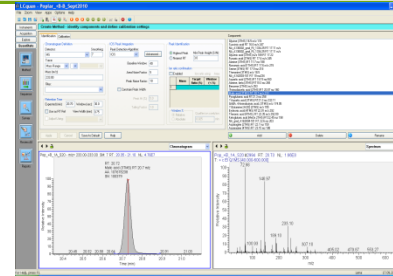
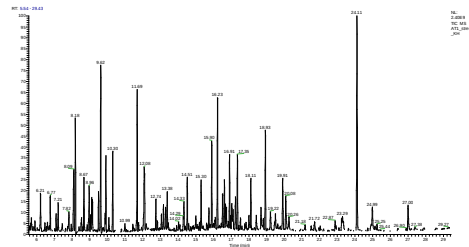
IC-(MS)



sample pathway

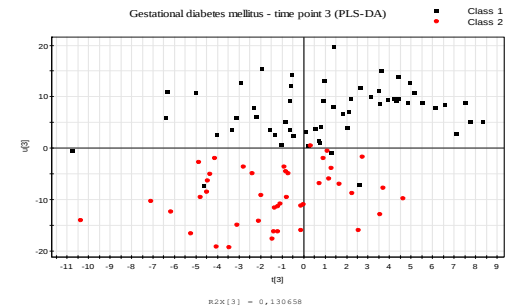
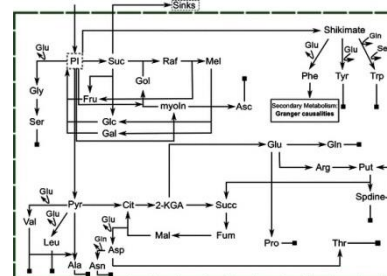
from biological material to interpretable data

sample pathway



sample preparation & extraction

Experimental design



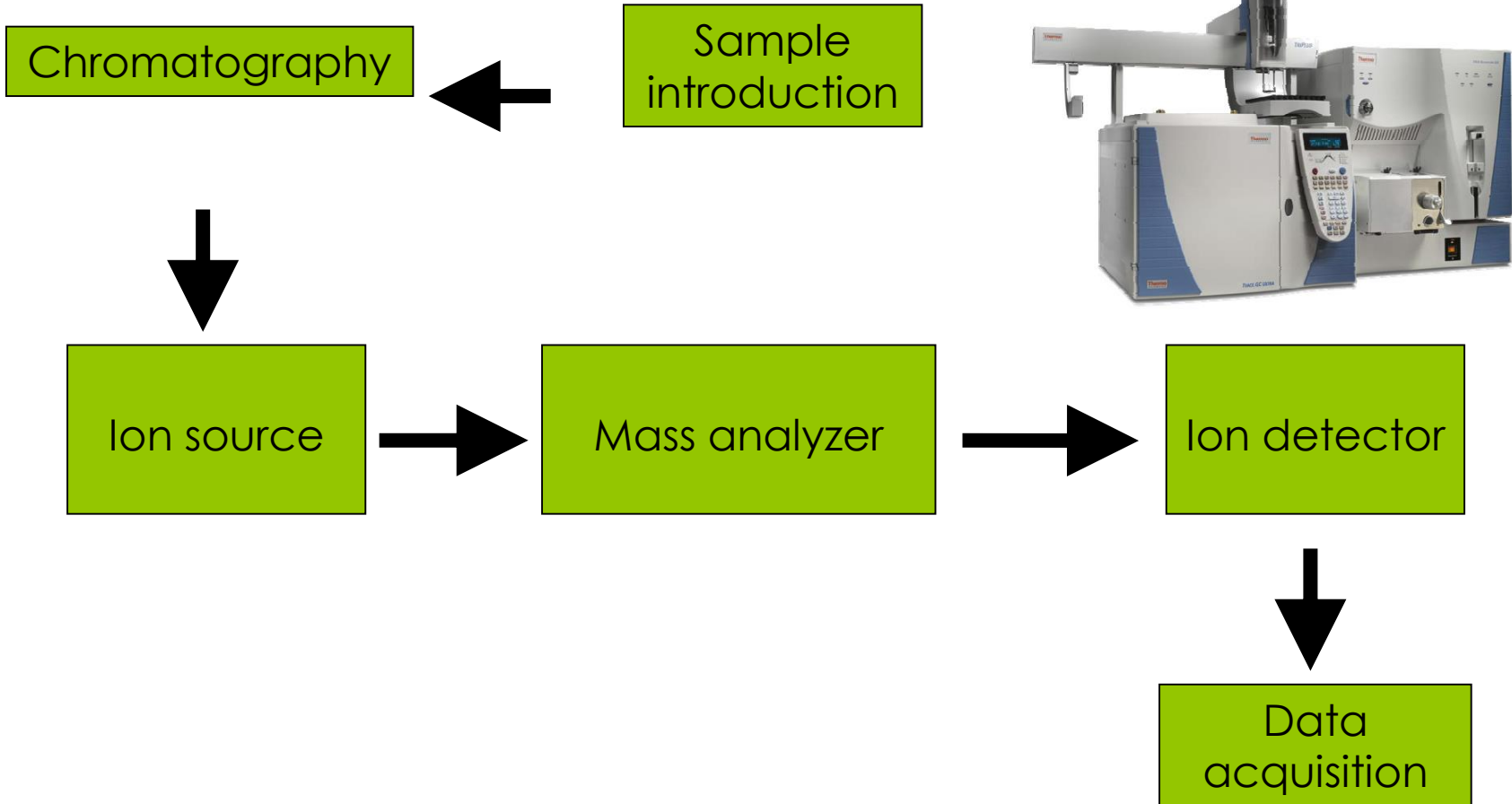
MS analysis

derivatisation

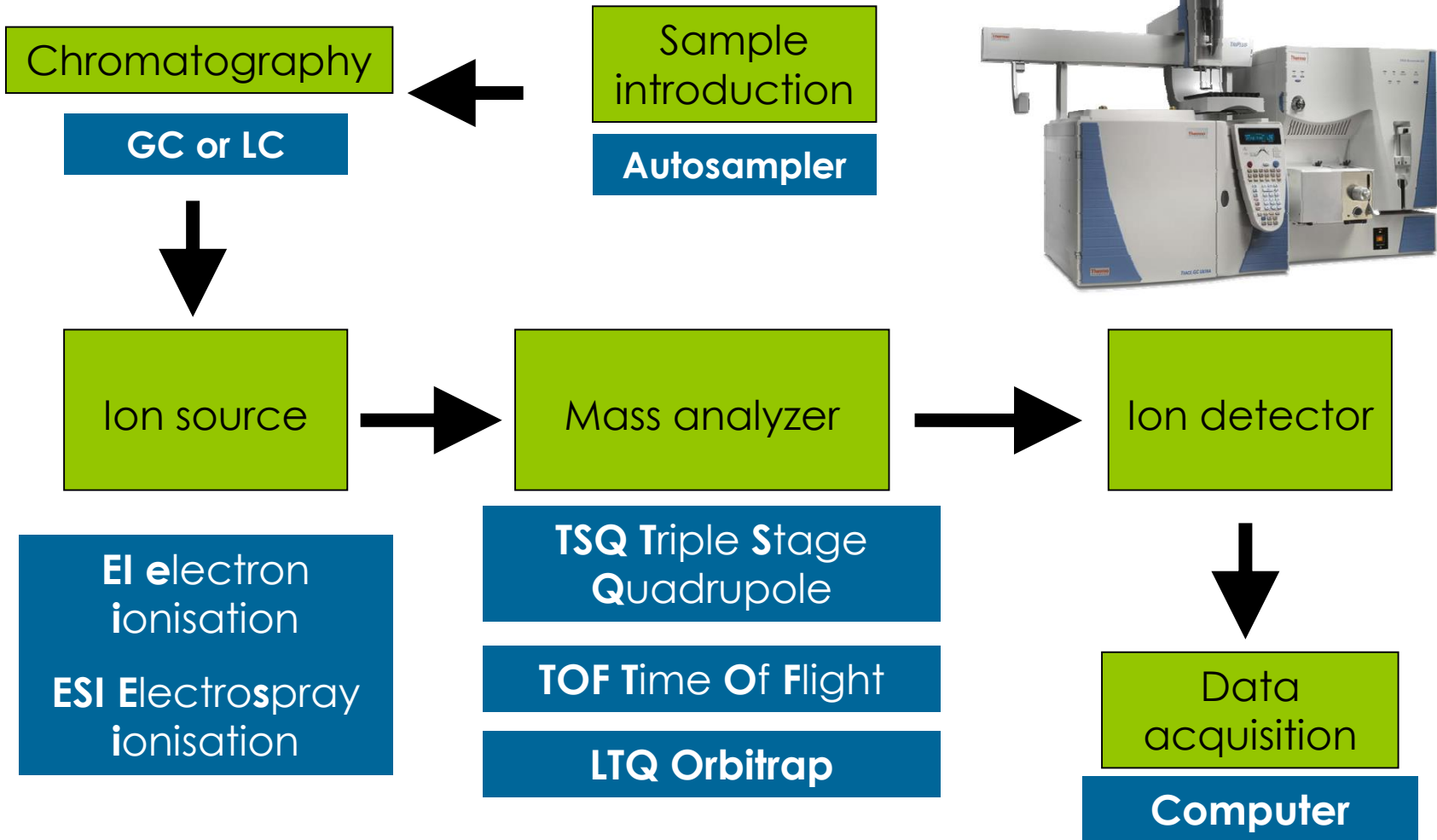
data processing & interpretation

Technical aspects

Components of a mass spectrometer

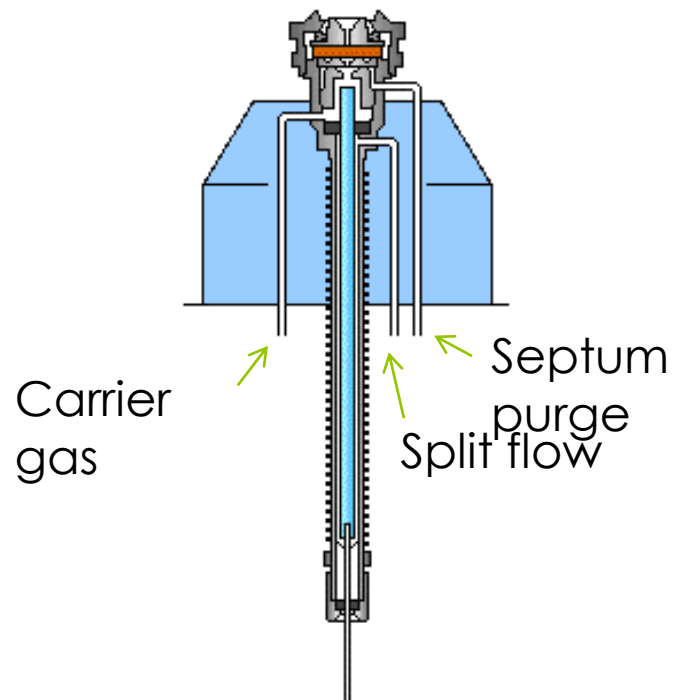


Components of a mass spectrometer



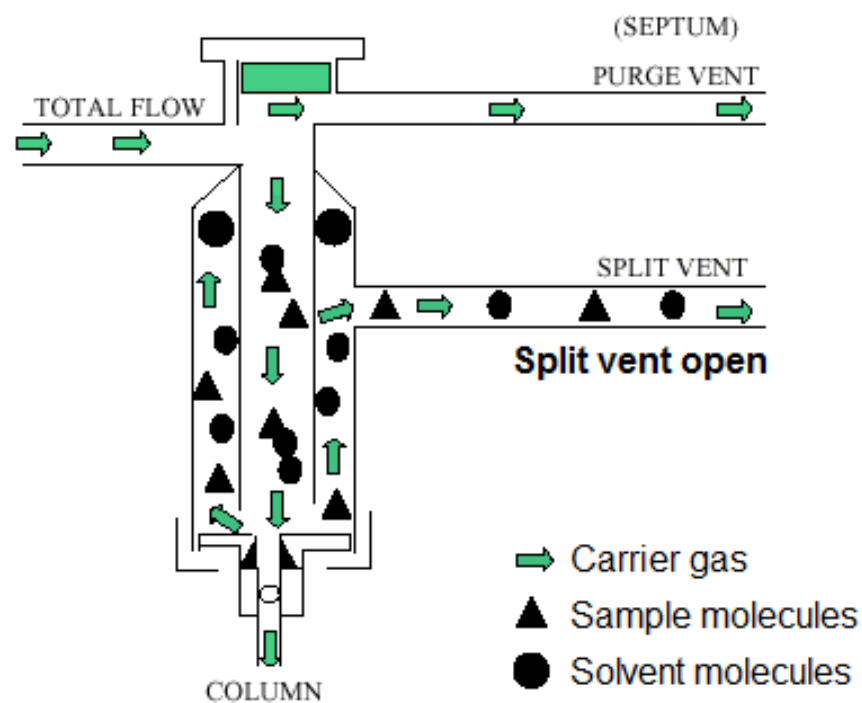


Injector (GC) -



- Liquid sample introduction
- Vaporisation in liner
- S/SL
- PTV

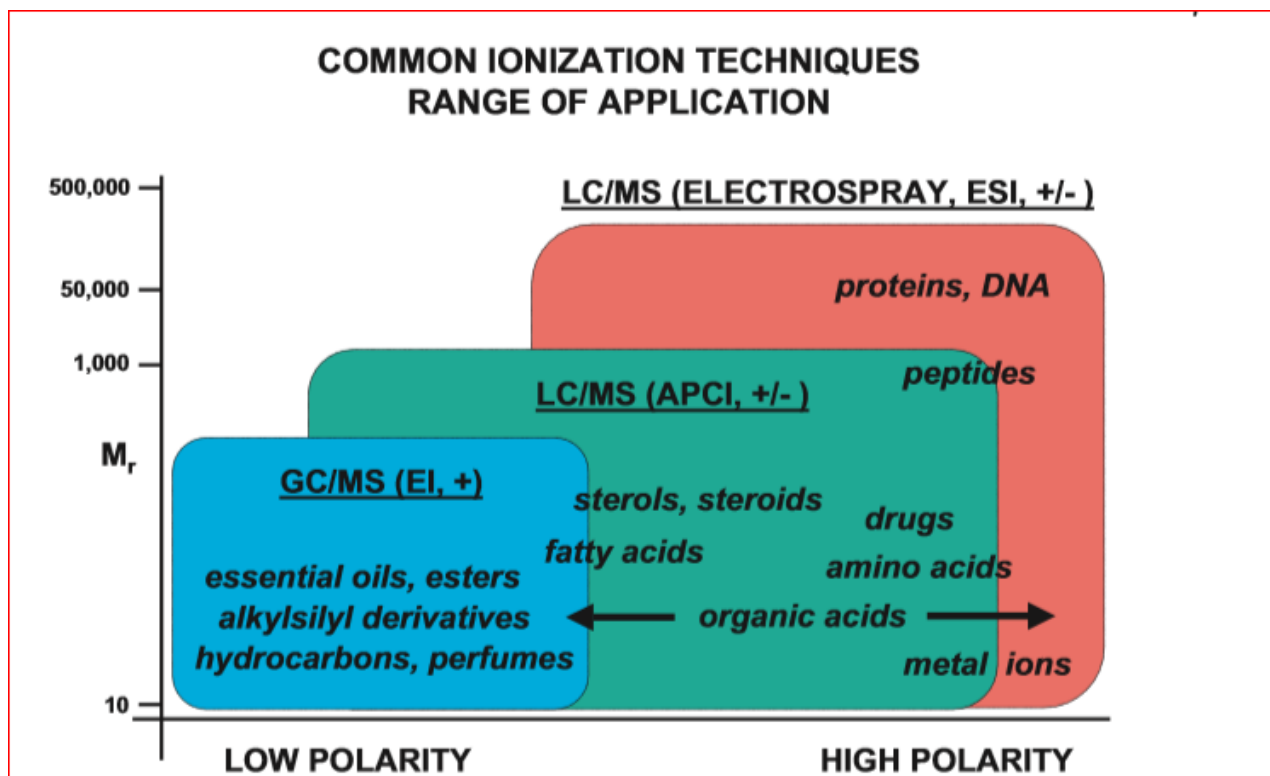
Split injection



<https://www.trajanscimed.com/pages/training-gc-injection>

Chemical derivatisation

- increase of volatility
- Decrease polarity
- Reduce formed peaks in chromatogram

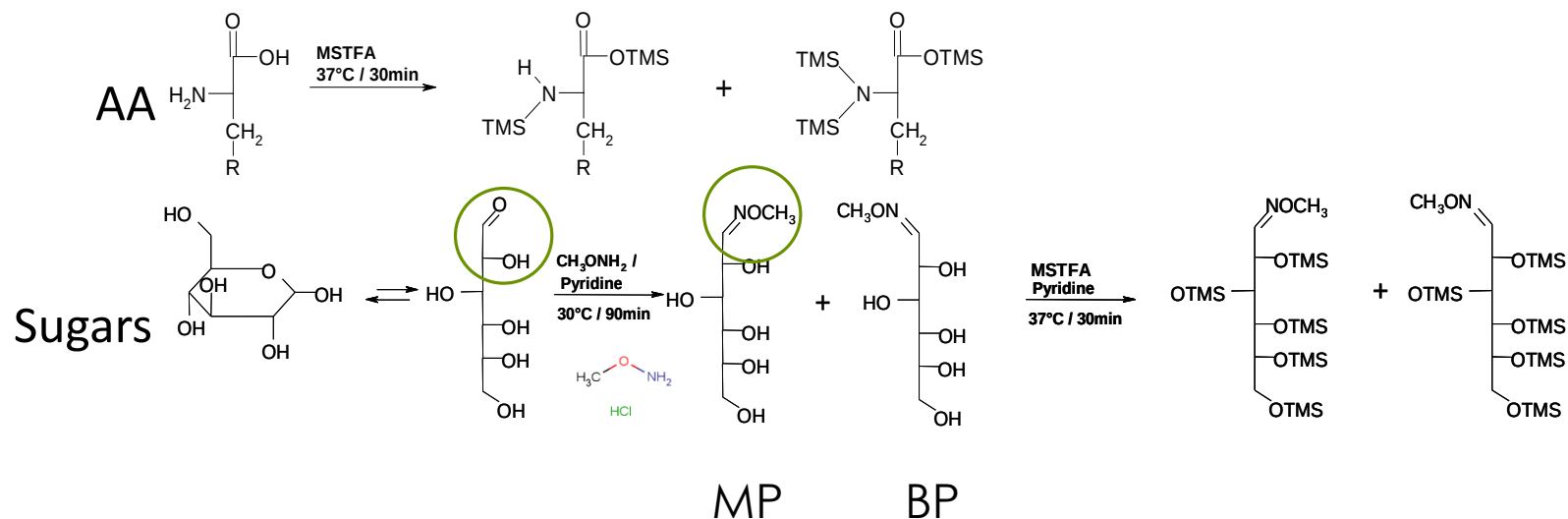


Halket et. al., 2005, J. of. Exp. Bot.

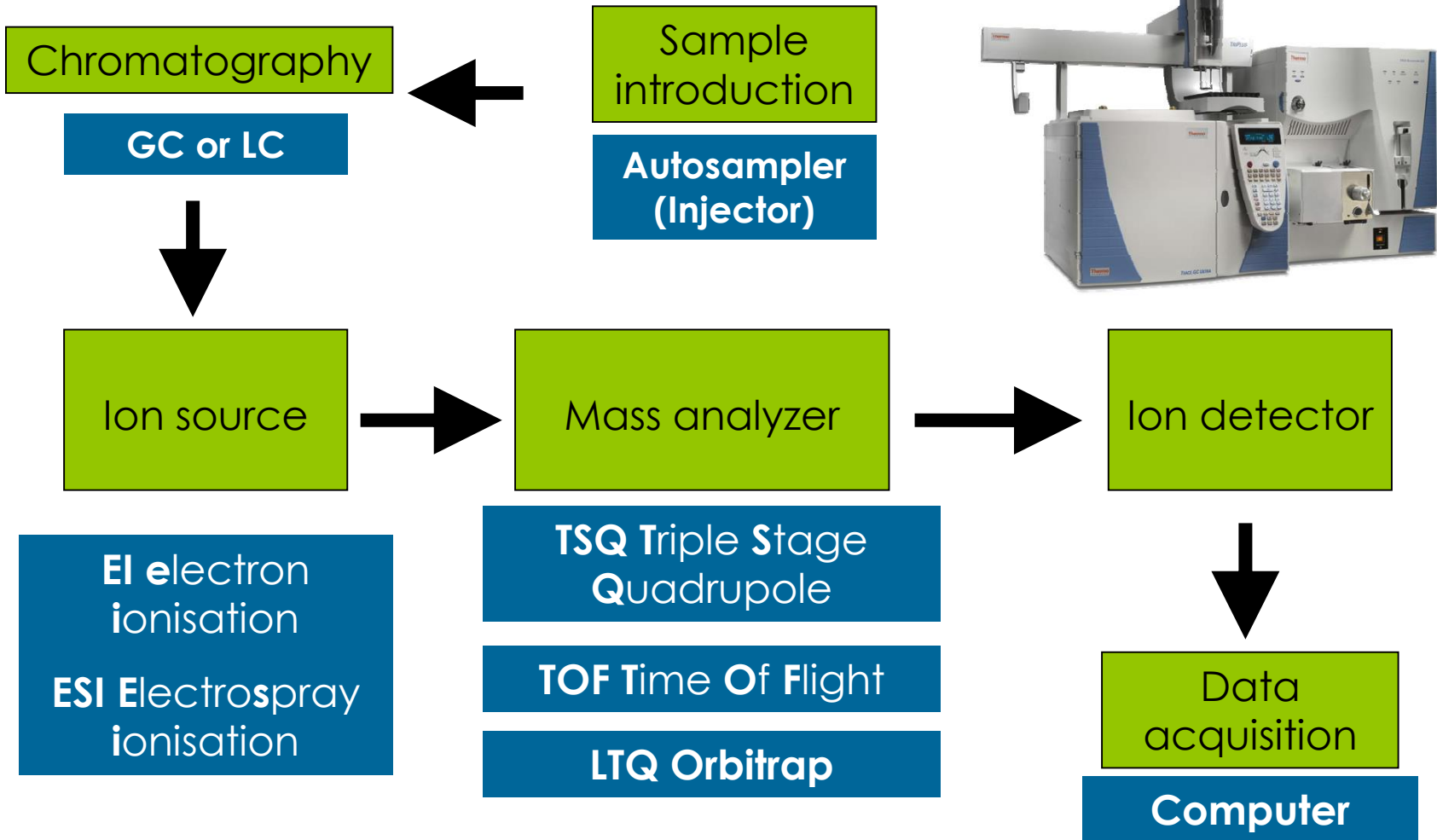
Chemical derivatisation

Two steps

1. Methoxymation
2. Silylation



Components of a mass spectrometer

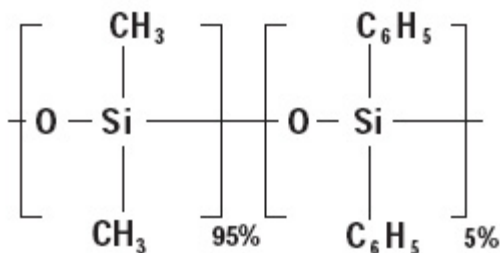
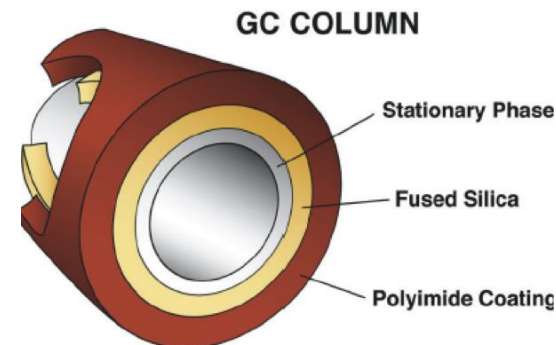


Chromatography

- **LC** → **H**igh-**P**erformance **L**iquid **C**hromatography (HPLC)
mobile phase: liquid (solvents), stationary phase: hydrophobic (RP) or hydrophilic (NP) columns
- **GC** → **G**as **C**hromatography (Helium mobile phase, hydrophobic-coated capillary column)
- **CE** → **C**apillary **e**lectrophoresis (ions migrate in an electrical field)
- **IC** → **I**on **C**hromatography
(ions are separated according to the affinity to the ion exchanger)

Chromatography - GC

- Optional uncoated precolumn
- Analytical column
 - e.g. HP-5ms (siloxanes substituted with 5% phenyl and 95% methyl groups)
 - Separation according to boiling point (volatility) (adsorption and desorption)
 - And interaction with active groups of column (polarity)
 - Apolar analytes: Van-der-Waals-forces
 - Polar analytes: hydrogen bonds



5% phenyl (HP-5, DB-5, etc.)

35% phenyl (HP-35, DB-35, etc.)

50% phenyl (HP-50+, DB-17, etc.)

Nonpolar

Mid-polar

Mid-polar

Chromatography - LC

HPLC

Normal phase column

- stationary phase: high polar rigid silica, or silica-based compositions
- mobile phases: relatively nonpolar solvent, hexane, methylene chloride, or mixtures of these
- more polar solvent has higher eluent strength
- the least polar component is eluted first

HPLC

Reverse phase column

- stationary phases: nonpolar hydrocarbons, waxy liquids, or bonded hydrocarbons (such as C18, C8, etc.)
- mobile phase: polar solvents or mixtures such as methanol-water or acetonitrile-water
- the most polar component is eluted first
- less polar solvent has higher eluent strength
- less sensitive to polar impurities

▽ Avoid to measure a sample that pH value is greater than 7.5 in a reversed –phase column, because of hydrolysis of the siloxane.

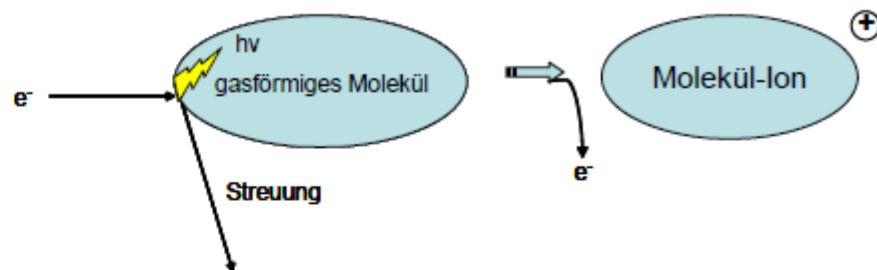
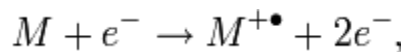
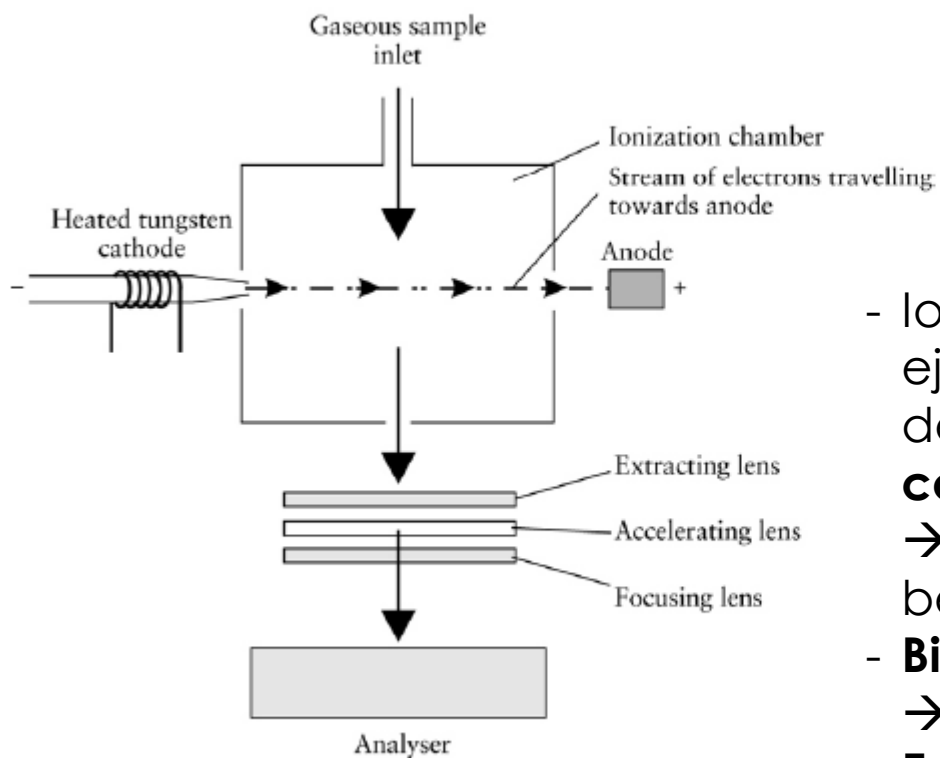
Ion Source

Ionisation

Ionisation

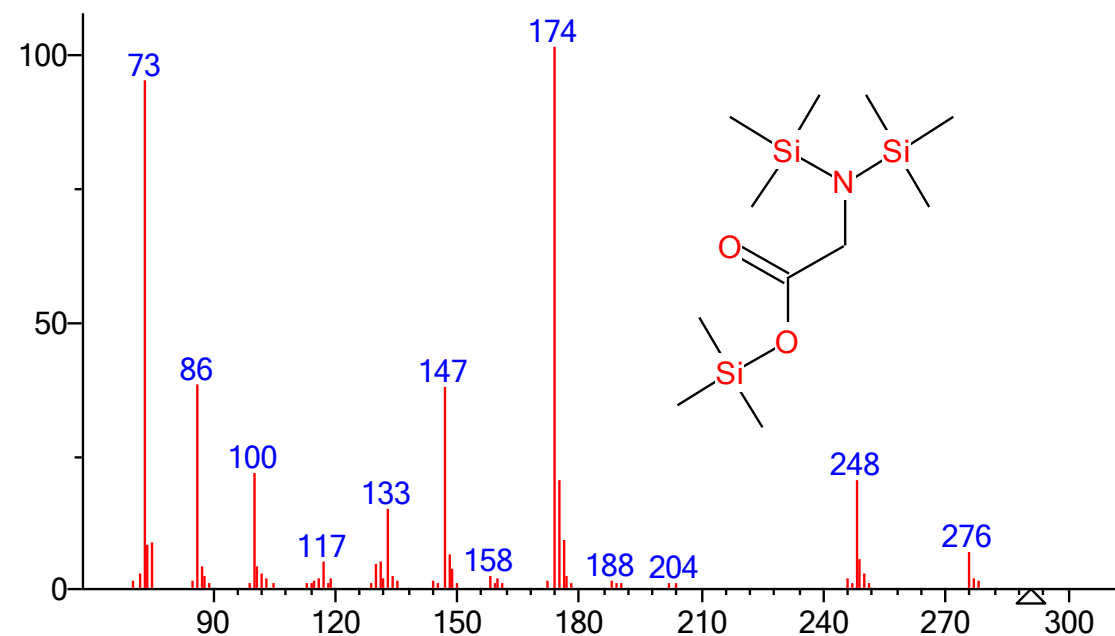
- Ionisation essential („hard“ and „soft“)
- EI = electron ionisation (electron impact)
- CI = chemical ionization
- API (atmospheric pressure ionization): ESI, APCI
- MALDI = Matrix-assisted Laser Desorption Ionisation

EI - Electron Impact

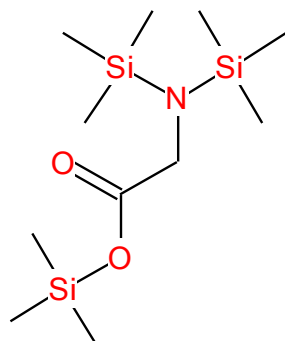


- Ionisation is caused by electron ejection from analyte or by analyte decomposition, **not by direct collision**
 → energy transfer from electron beam to analyte
- **Binding energy** of organic molecules
 → 2-8eV/mol
- **Excitation energy of 70eV** gets converted into Oscillation energy
 → leading to fragmentation

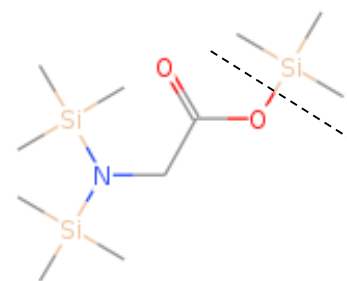
El spectrum



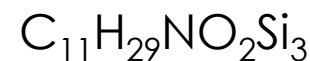
(gmd_pred_mosys-ri) Glycine (3TMS)_M000031_A133001-101-xxx_NA_1302,5_



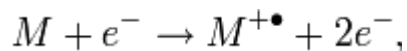
Glycine (3TMS)



Sum formular:



$M^{+\bullet}$ 291.15061



Single charged fragment ions

ESI – Electrospray Ionisation

John Benne
Fenn



photo: Allen Jones, VCU



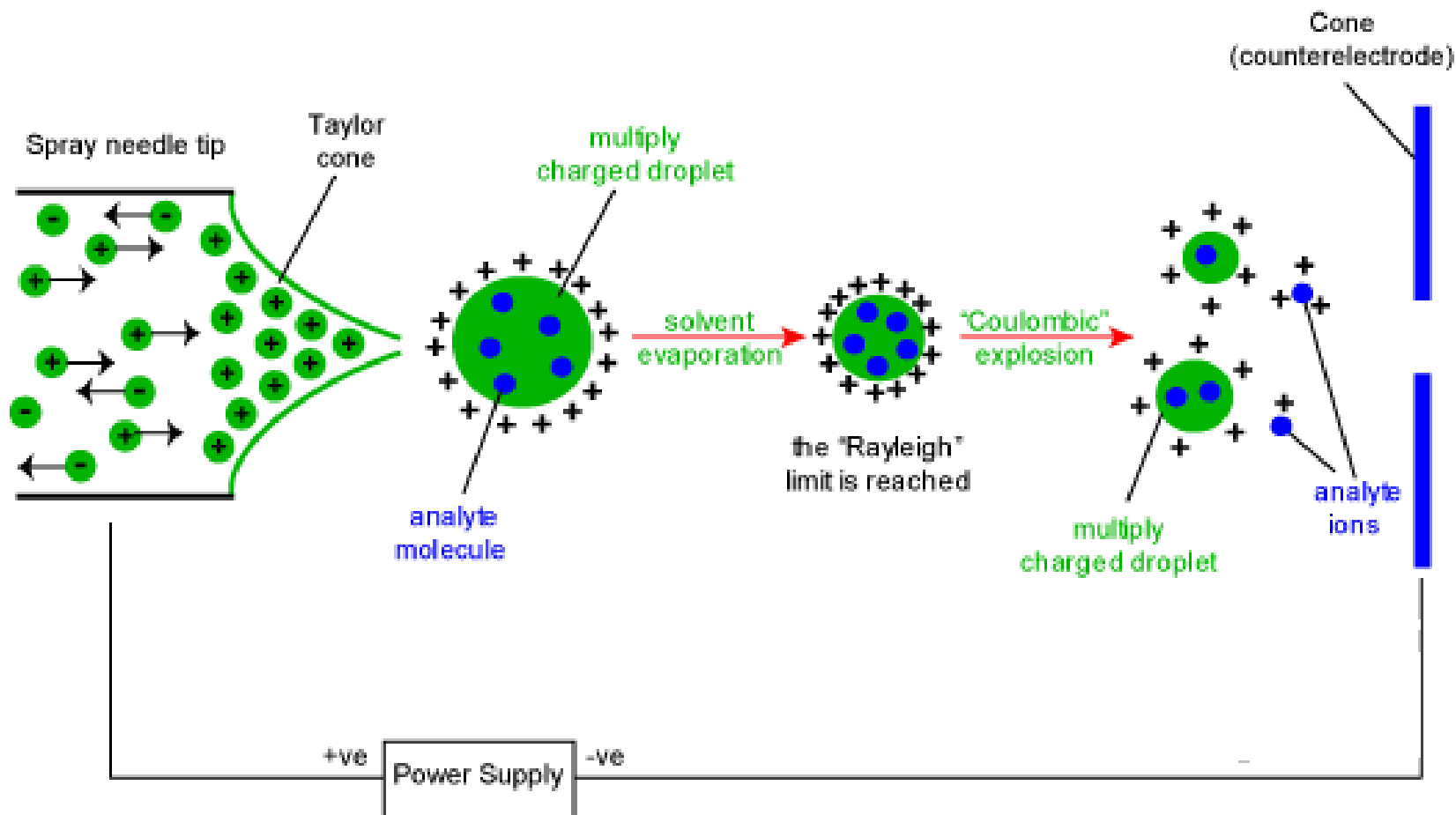
Koichi Tanabe

Nobelprice for chemistry
2002



„Of course, proteins can fly!“

ESI – Electrospray Ionisation



<http://www.bris.ac.uk/nerclsmf/techniques/hplcms.html>

ESI – spectrum



Exact mass of molecular ion -->
mass accuracy <5ppm
→ sum formular calculation

Still structure = ??????

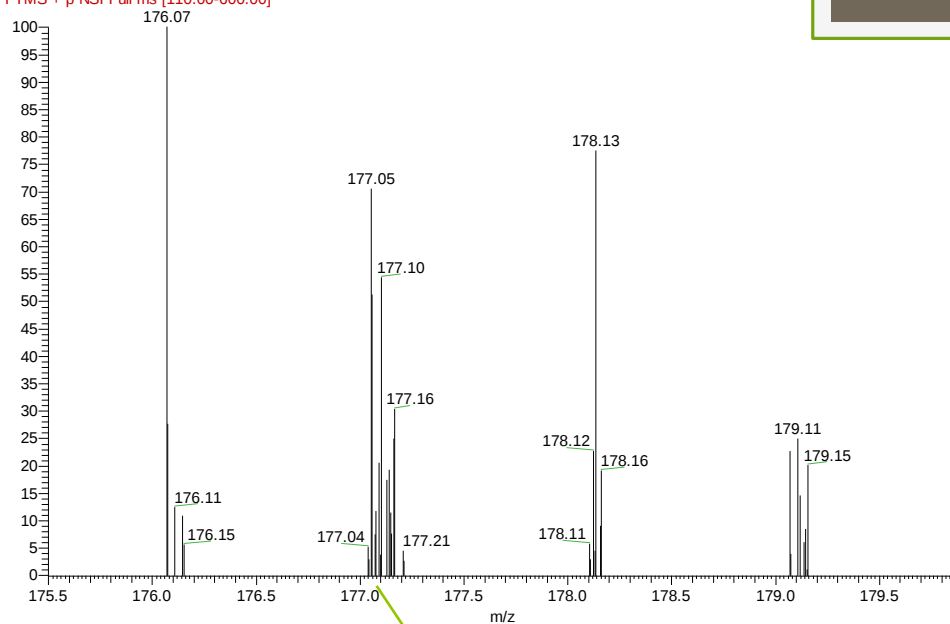
→ MSⁿ fragmentation pattern
→ Comparison with standards

Monoisotopic mass C₁₀H₁₂N₂O: 176.094955 Da

Monoisotopic mass H: 1.007276 Da

[M+H]⁺ 177.102231 Da

4_2 #817 RT: 7.52 AV: 1 NL: 1.35E5
F: FTMS + p NSI Full ms [110.00-600.00]

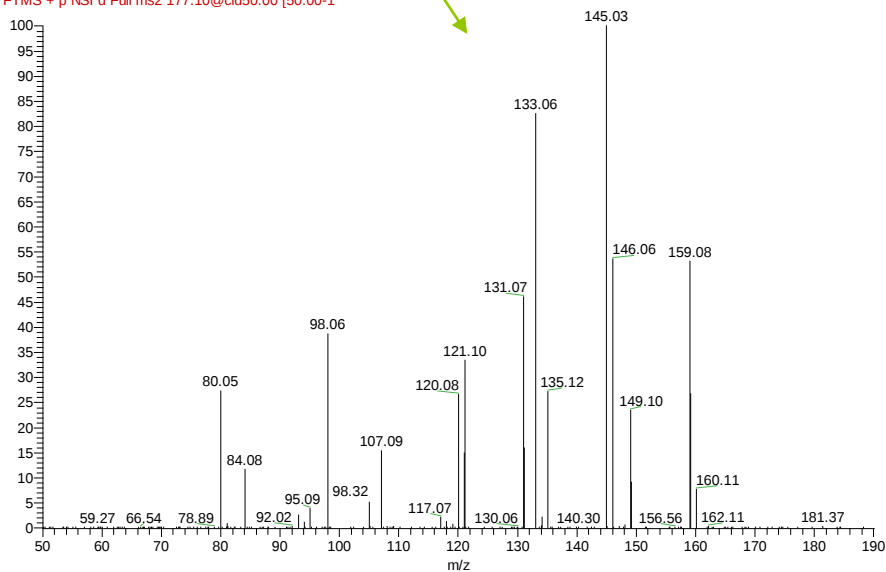


Full Scan MS1

Precursor:
[M+H]⁺ 177.102231

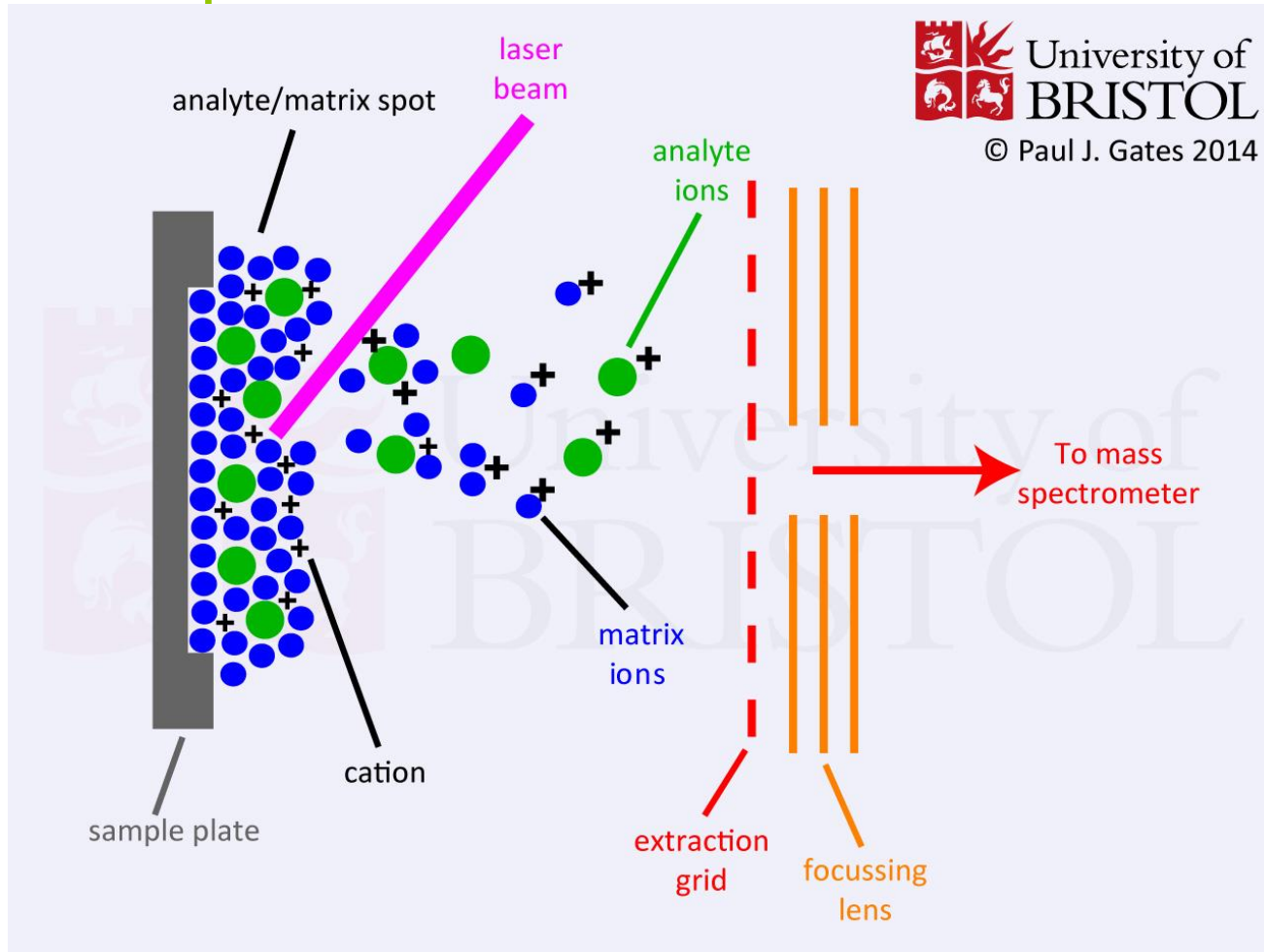
Multiple charges possible

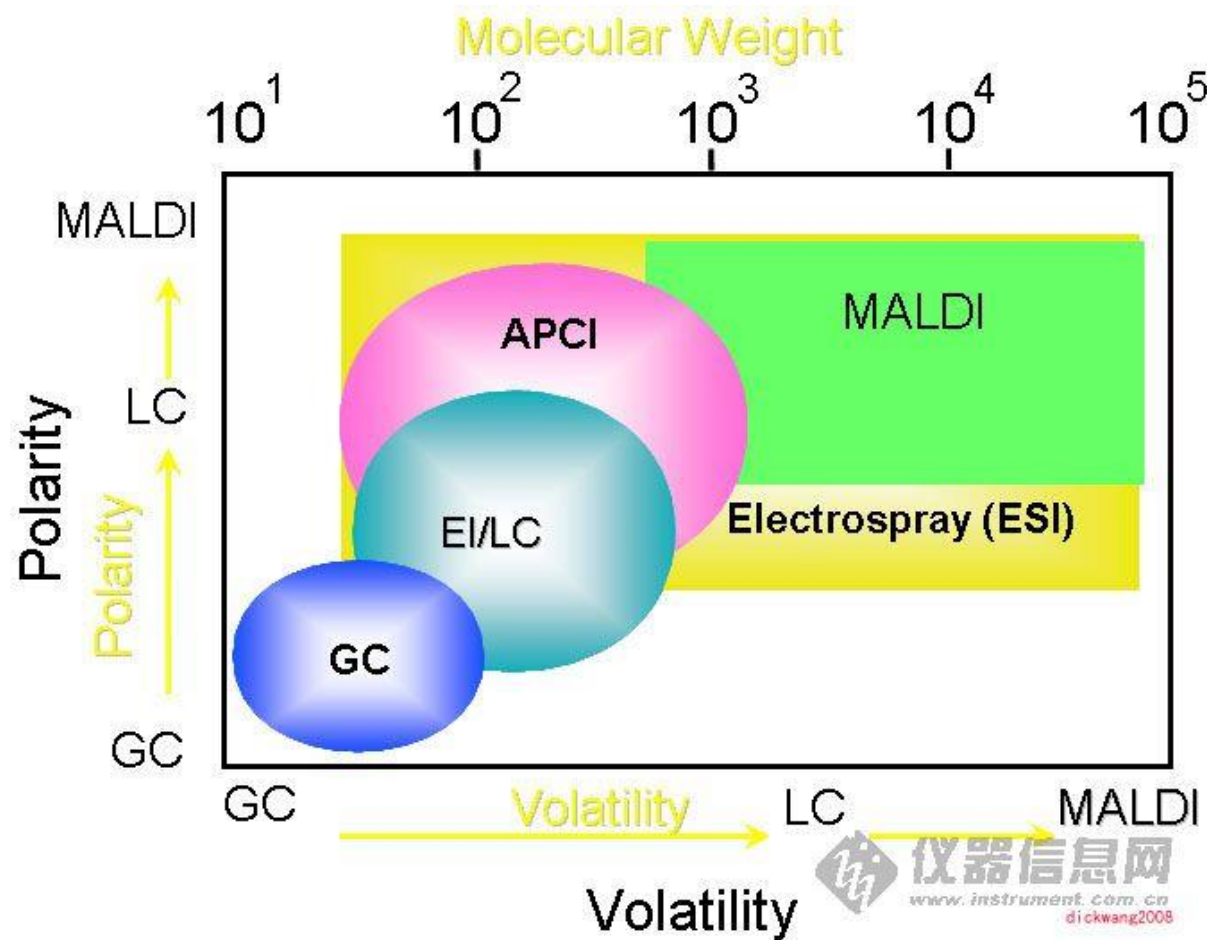
4_2 #419-997 RT: 4.57-8.15 AV: 9 NL: 4.37E3
F: FTMS + p NSI d Full ms2 177.10@cid50.00 [50.00-1]



Full Scan MS2

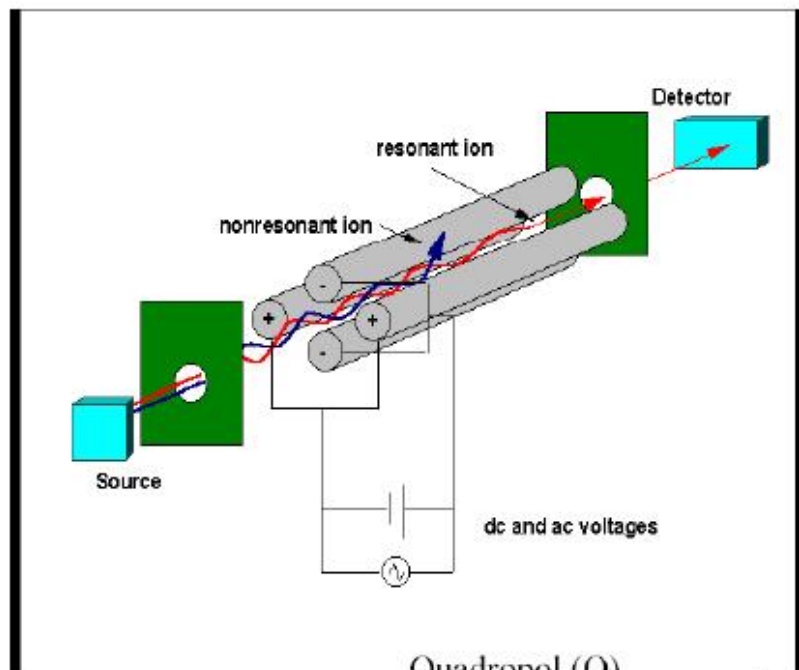
MALDI – Matrix-assisted Laser Desorption Ionisation





Mass analyzer

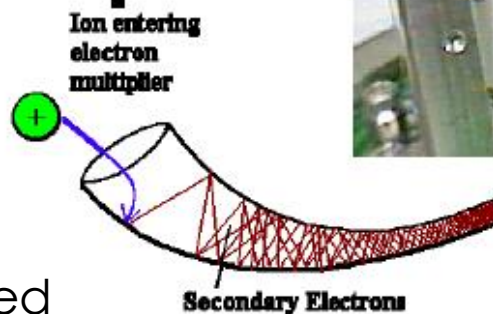
Quadrupole



$$m/z = 5.7U/\omega^2 r^2$$

ω : Frequenzanteil, r : $\frac{1}{2}$ Stababstand

U : Beschleunigungsspannung (10-20 V)



oscillating electric fields are used
to select or scan specific ions

TOF – Time-of-flight

Figure 2.

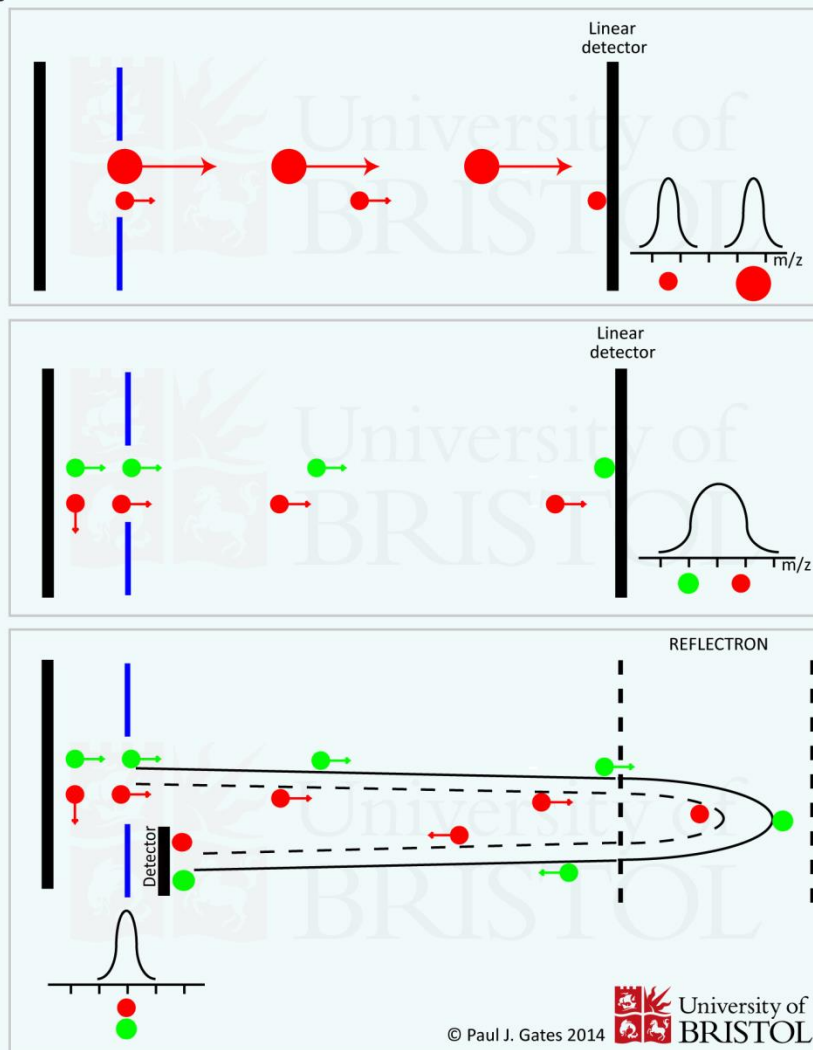
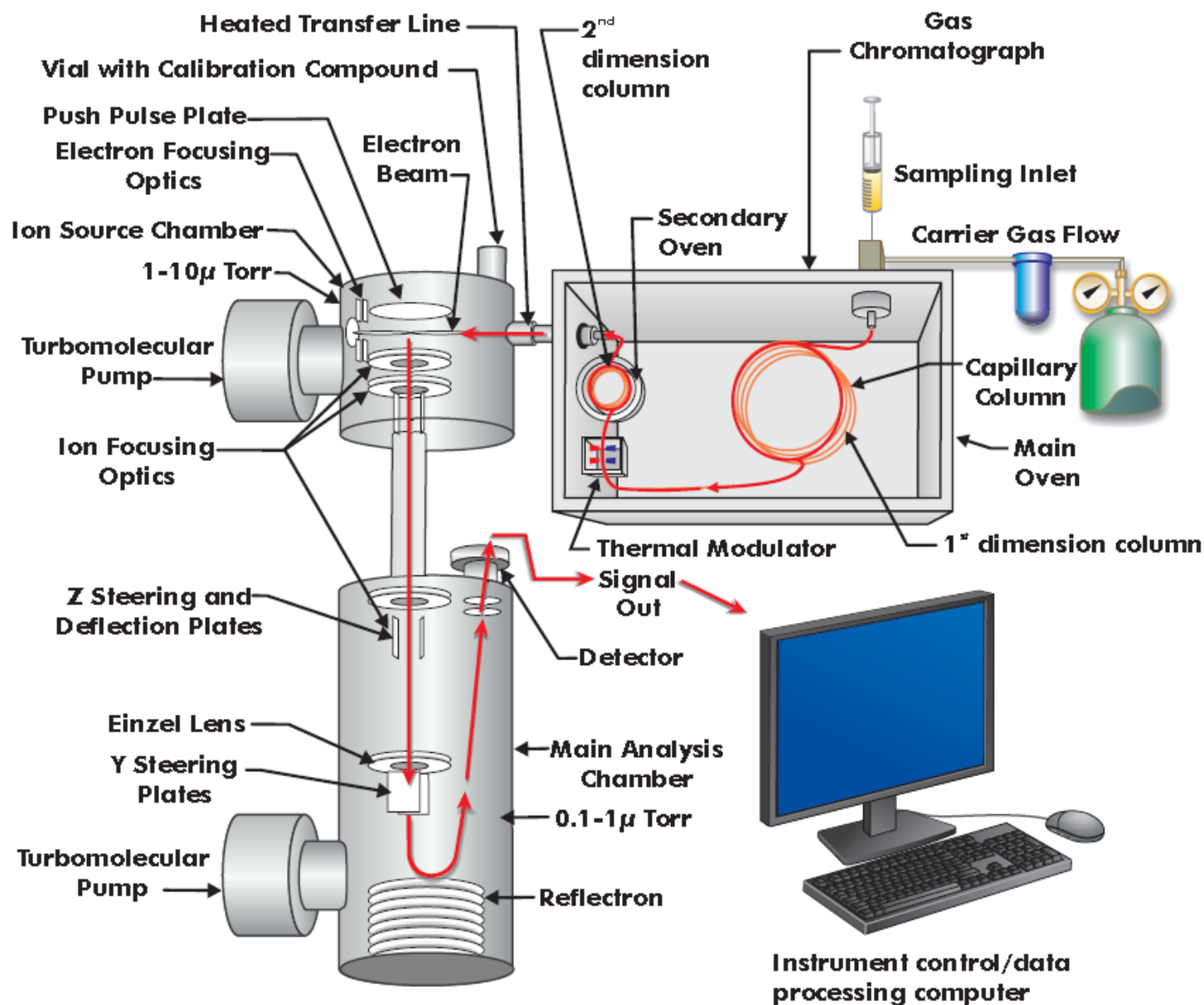


Diagram of GCxGC-TOFMS Instrument



Ion Trap

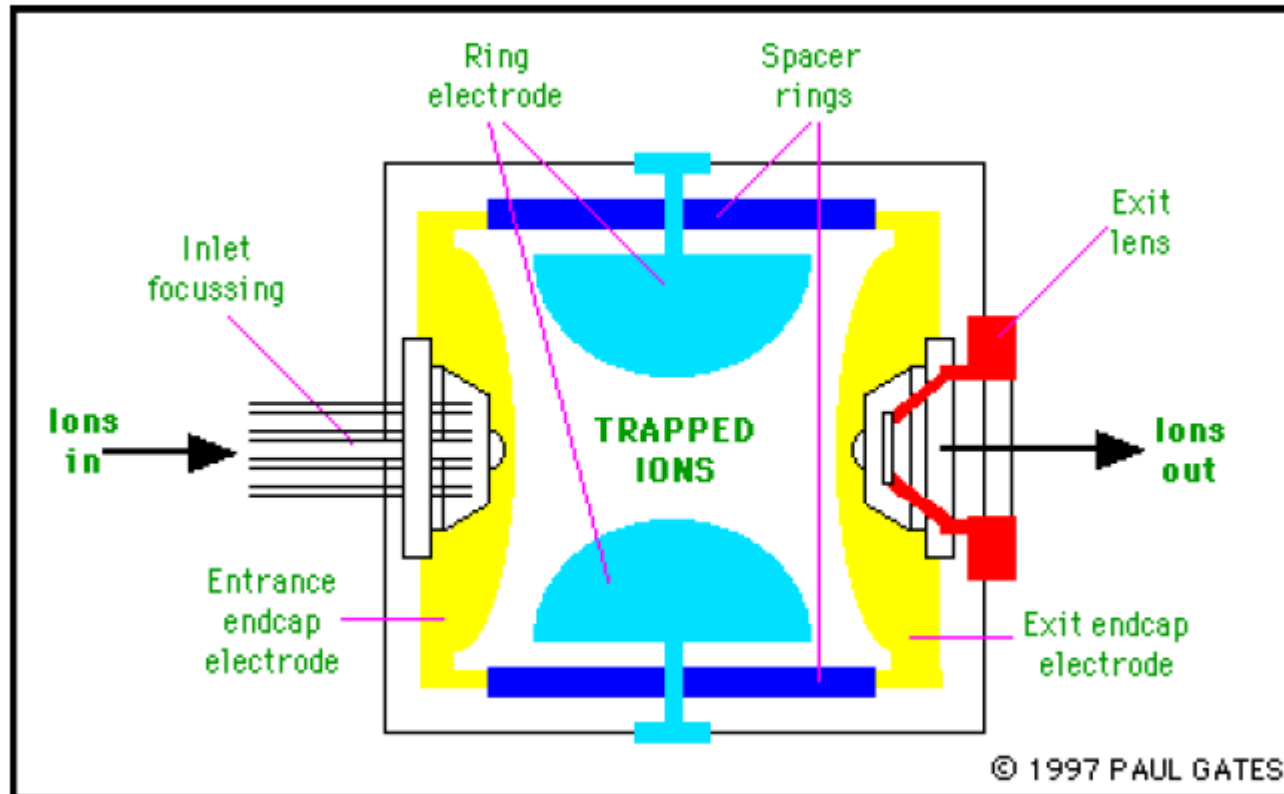


Prof. Wolfgang Paul
Nobel price in physics 1989

1950ies: Quadrupole Ion Trap
(QIT) was developed
together
with Quadrupole

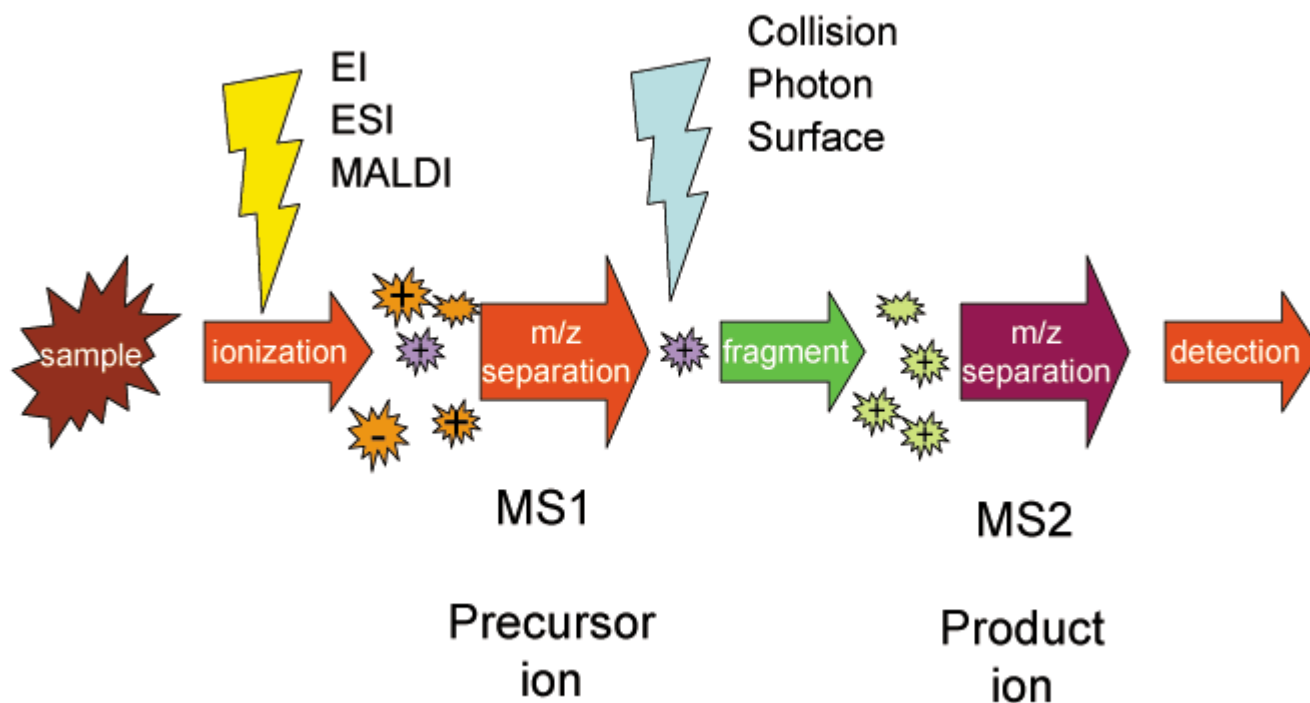
Ion Trap

e.g. Quadrupole Ion Trap (Paul trap)



Trapping of ions by
static direct current and (DC)
radio frequency oscillating electric fields (AC)

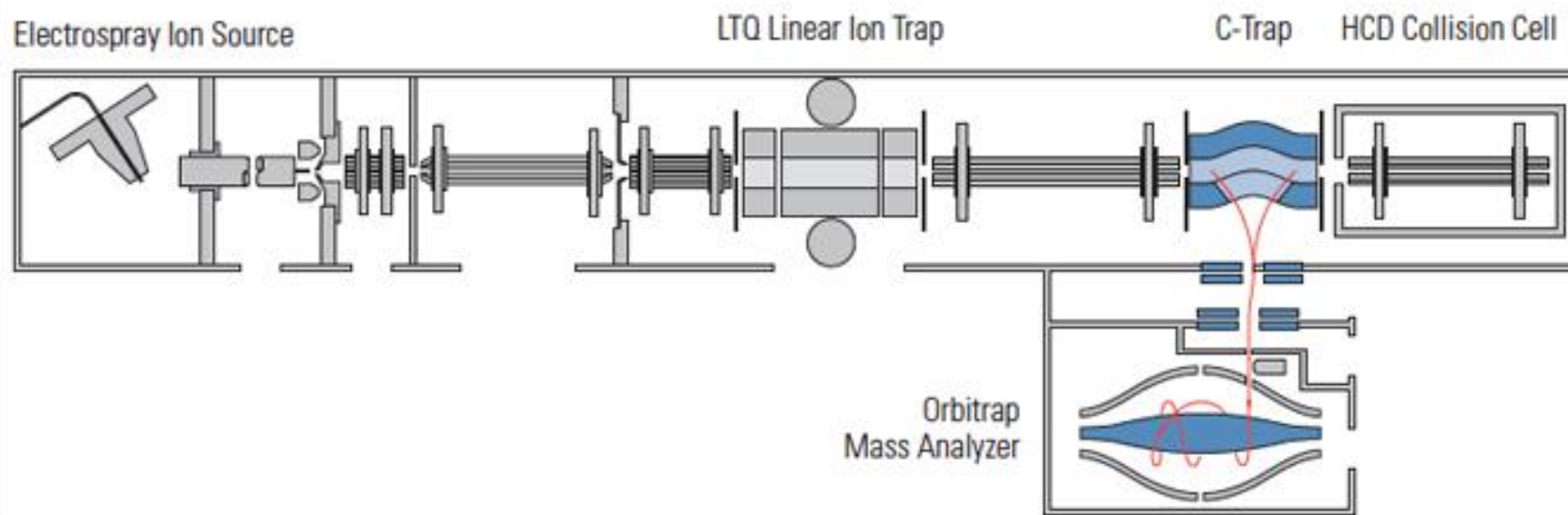
Tandem mass spectrometry (MS/MS)



By K. Murray (Kkmurray) - Own work, CC BY-SA 3.0,
<https://commons.wikimedia.org/w/index.php?curid=1943319>

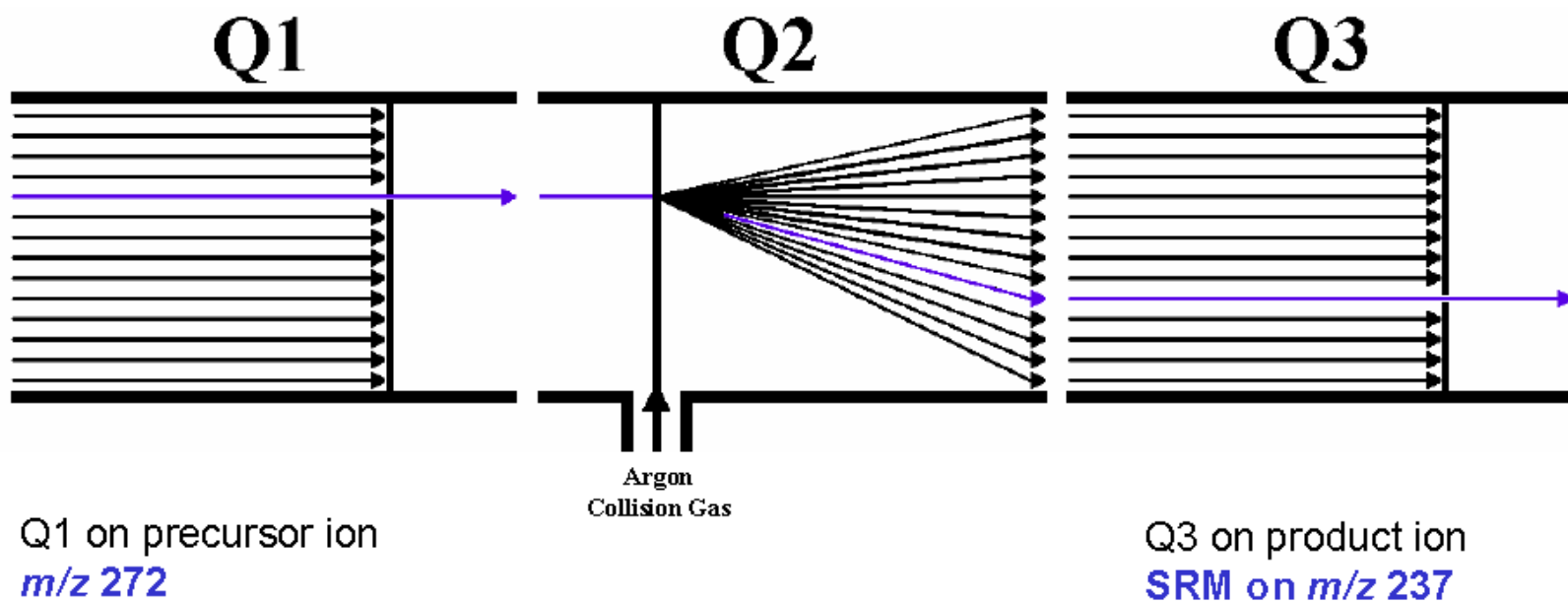
Tandem mass spectrometry

LTQ Orbitrap



<http://planetorbitrap.com/ltq-orbitrap-xl#.VrYYEfnhDq4>

“Monitor a Transition”
from *Precursor in Q1* to *Product Ion in Q3*



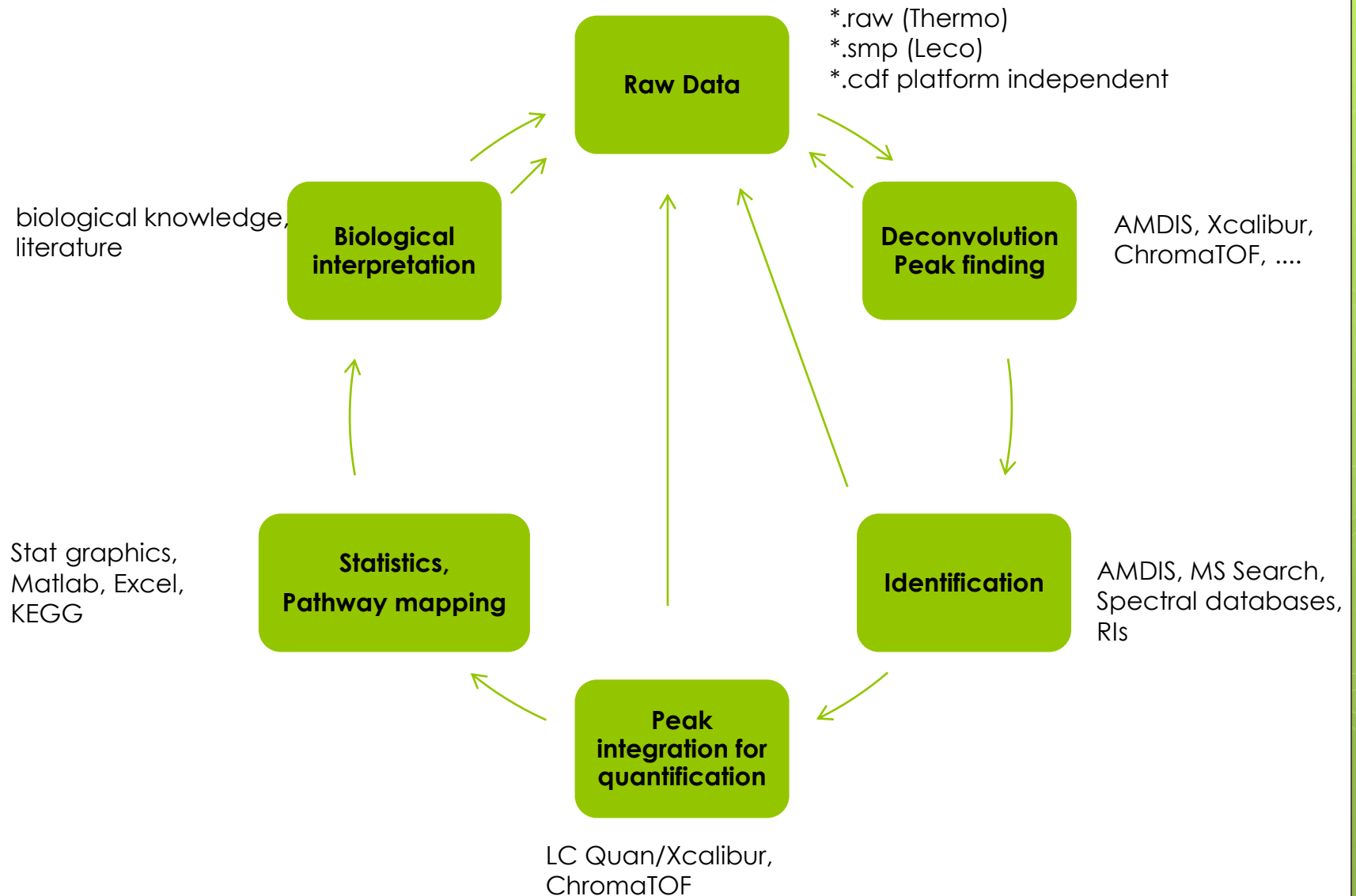
Q1
mass filter

Q2
collision cell
2nd fragmentation

Q3
scanning of selected
product ion

Data validation

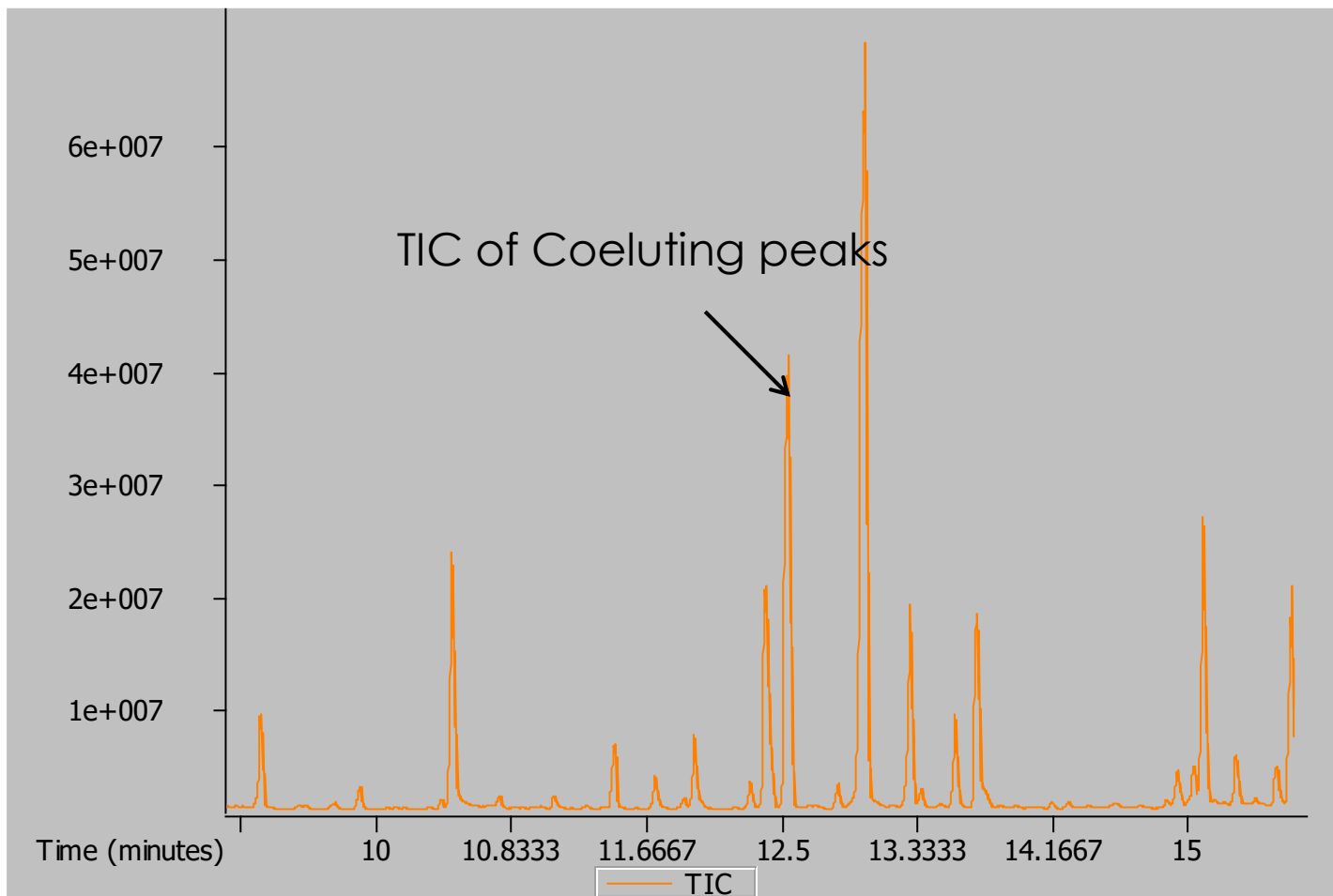
workflow



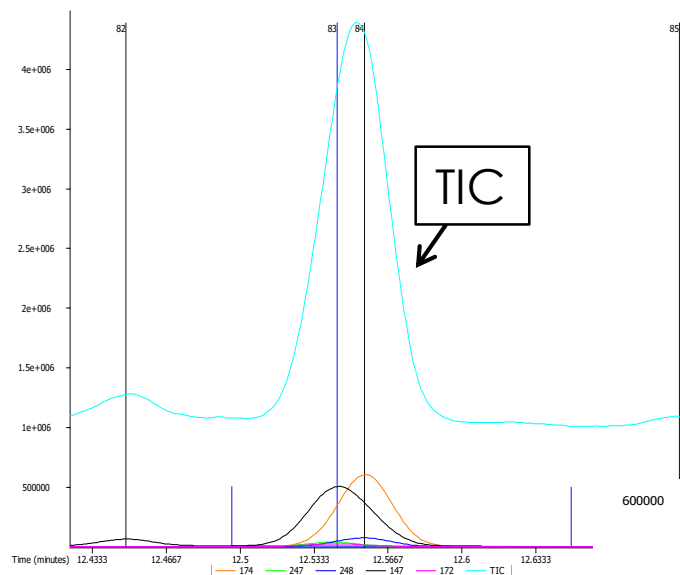
Spectrum and Chromatogram



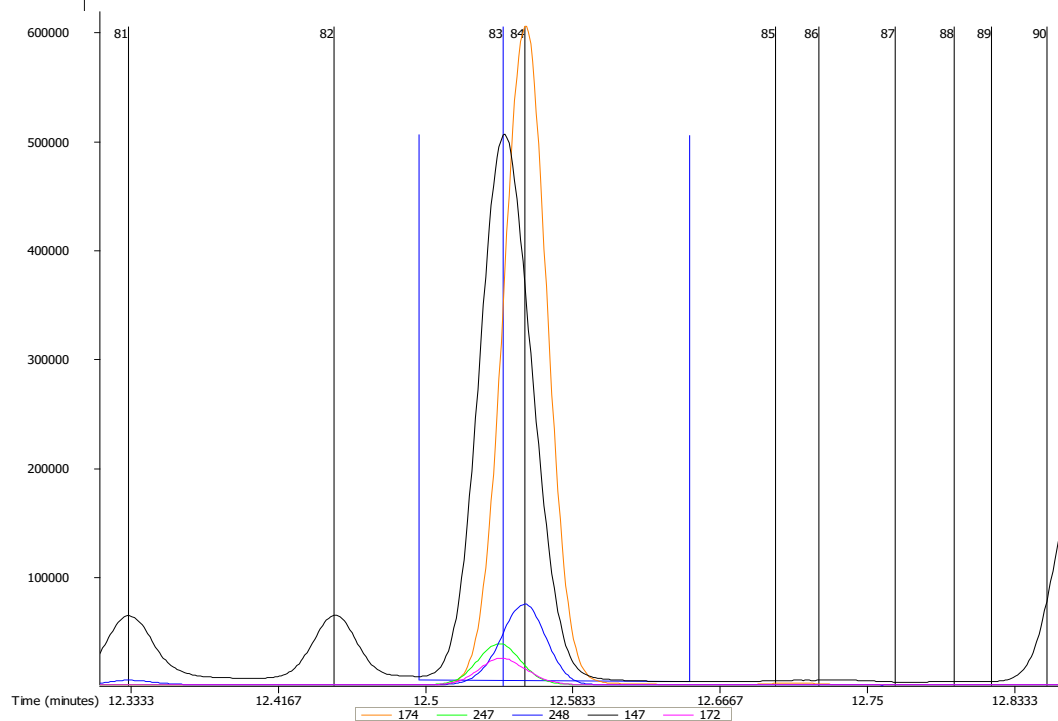
chromatographic and mass spectral separation



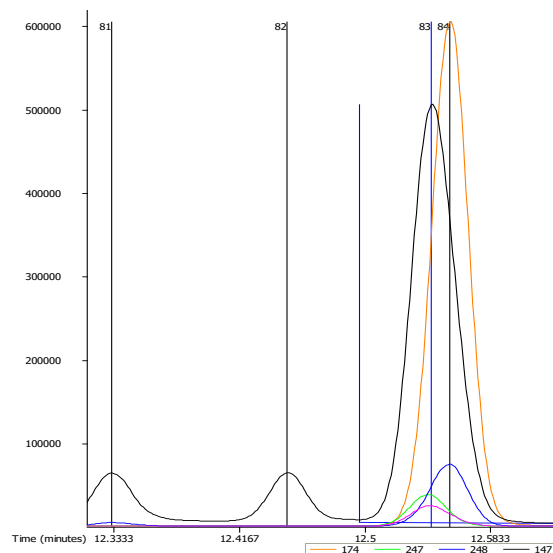
deconvolution



EIC m/z 174, 247, 248, 147, 172

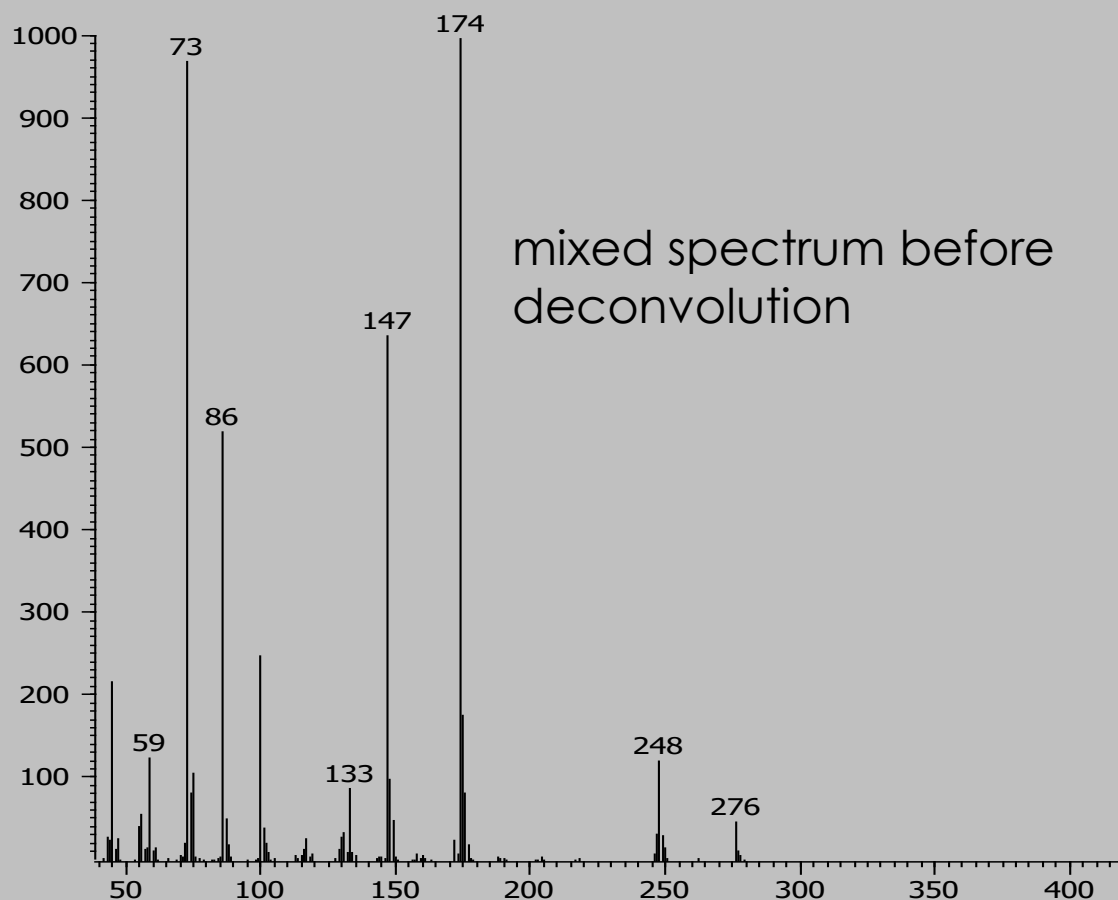


deconvolution



m/z 174, 247, 248, 147, 172

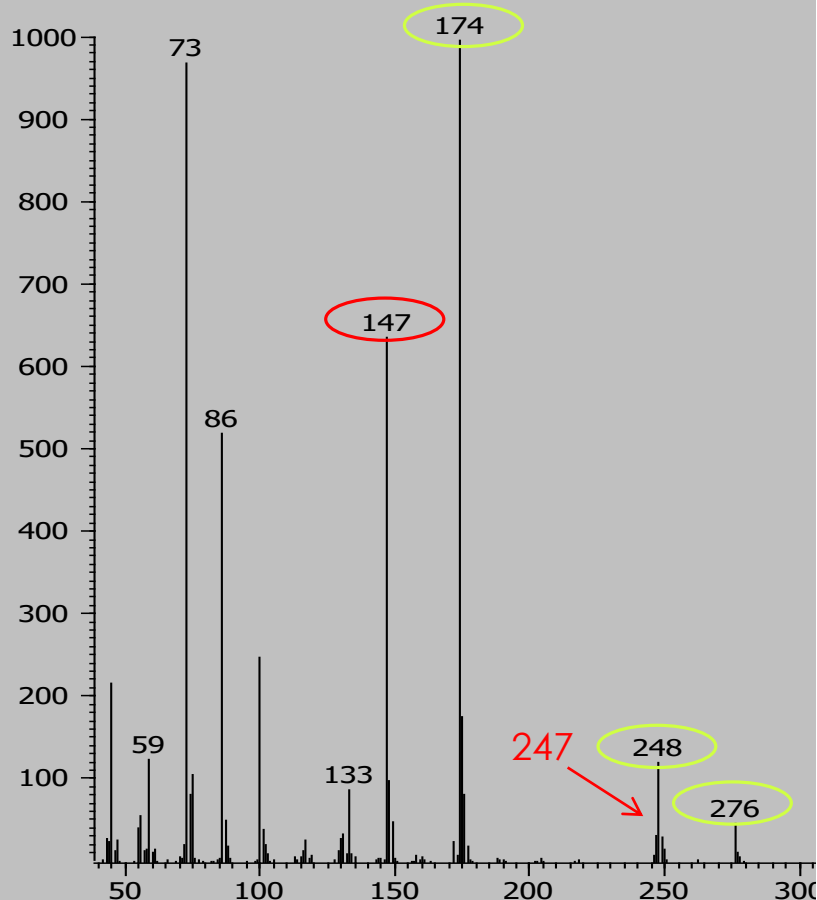
Caliper - sample "Ara_50_1D_SL:1", 12.5545 minutes to 12.5545 minutes - 12.6655



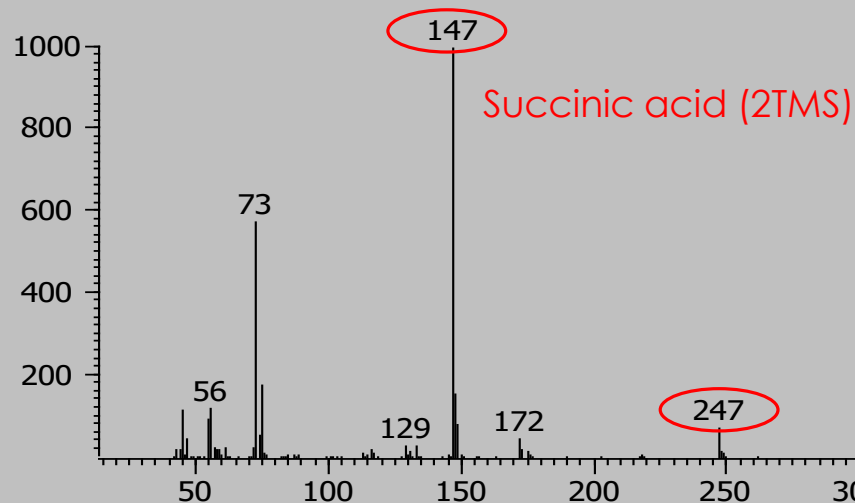
mixed spectrum before
deconvolution

mixed spectrum before deconvolution

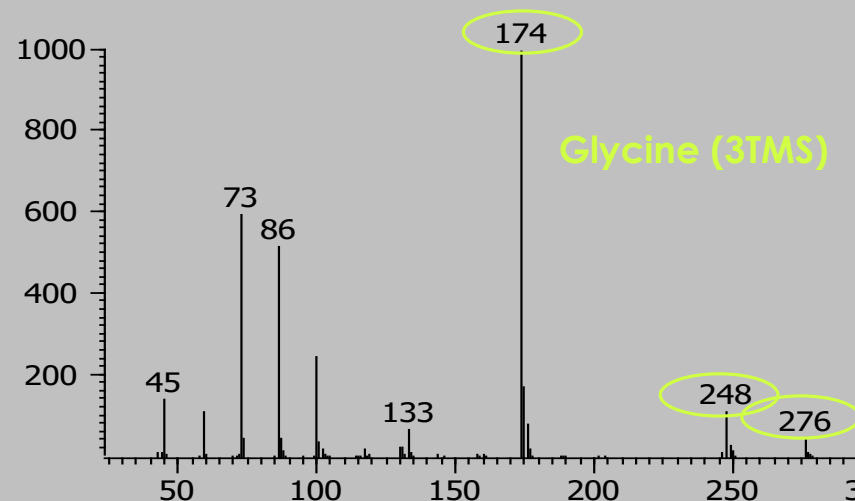
Caliper - sample "Ara_50_1D_SL:1", 12.5545 minutes to 12.5



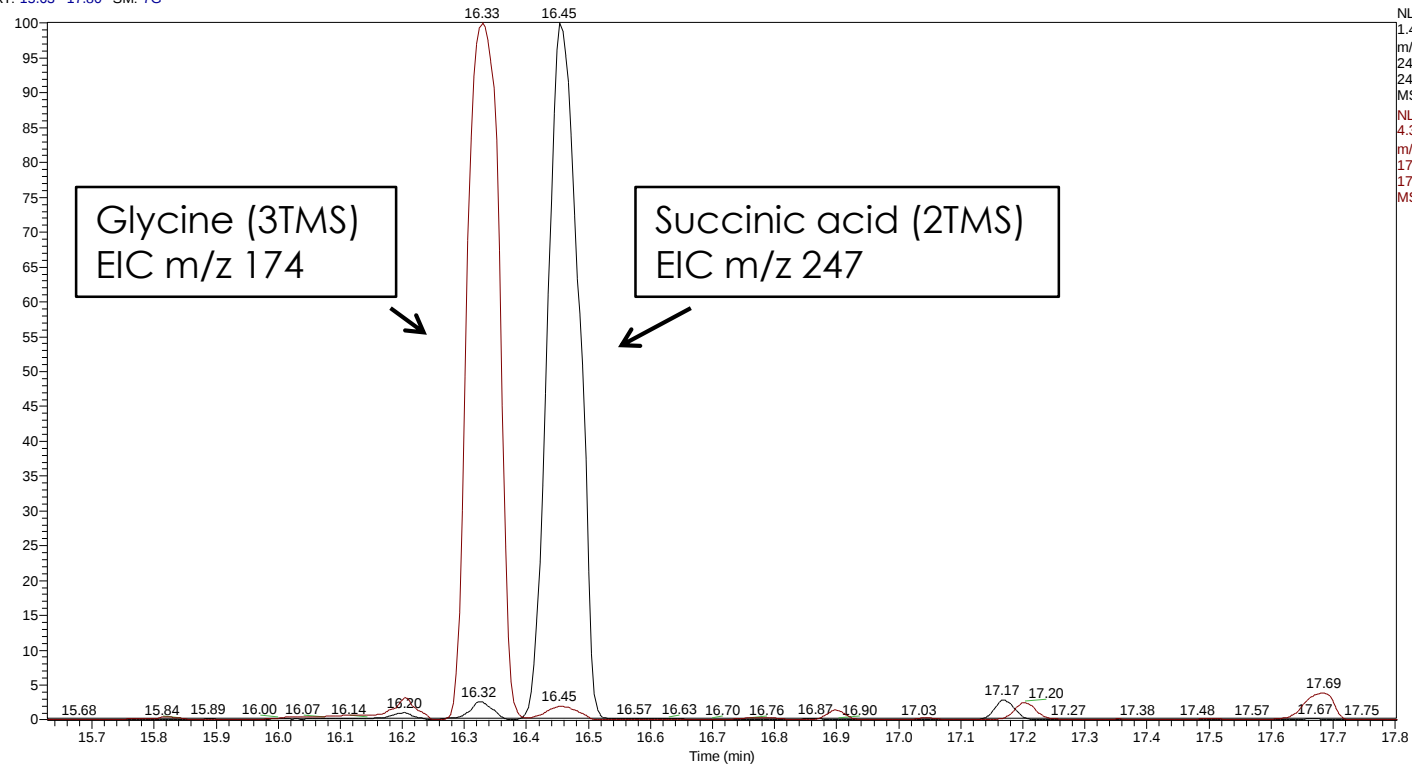
Peak True - sample "Ara_50_1D_SL:1", peak 83, at 12.5438



Peak True - sample "Ara_50_1D_SL:1", peak 84, at 12.556

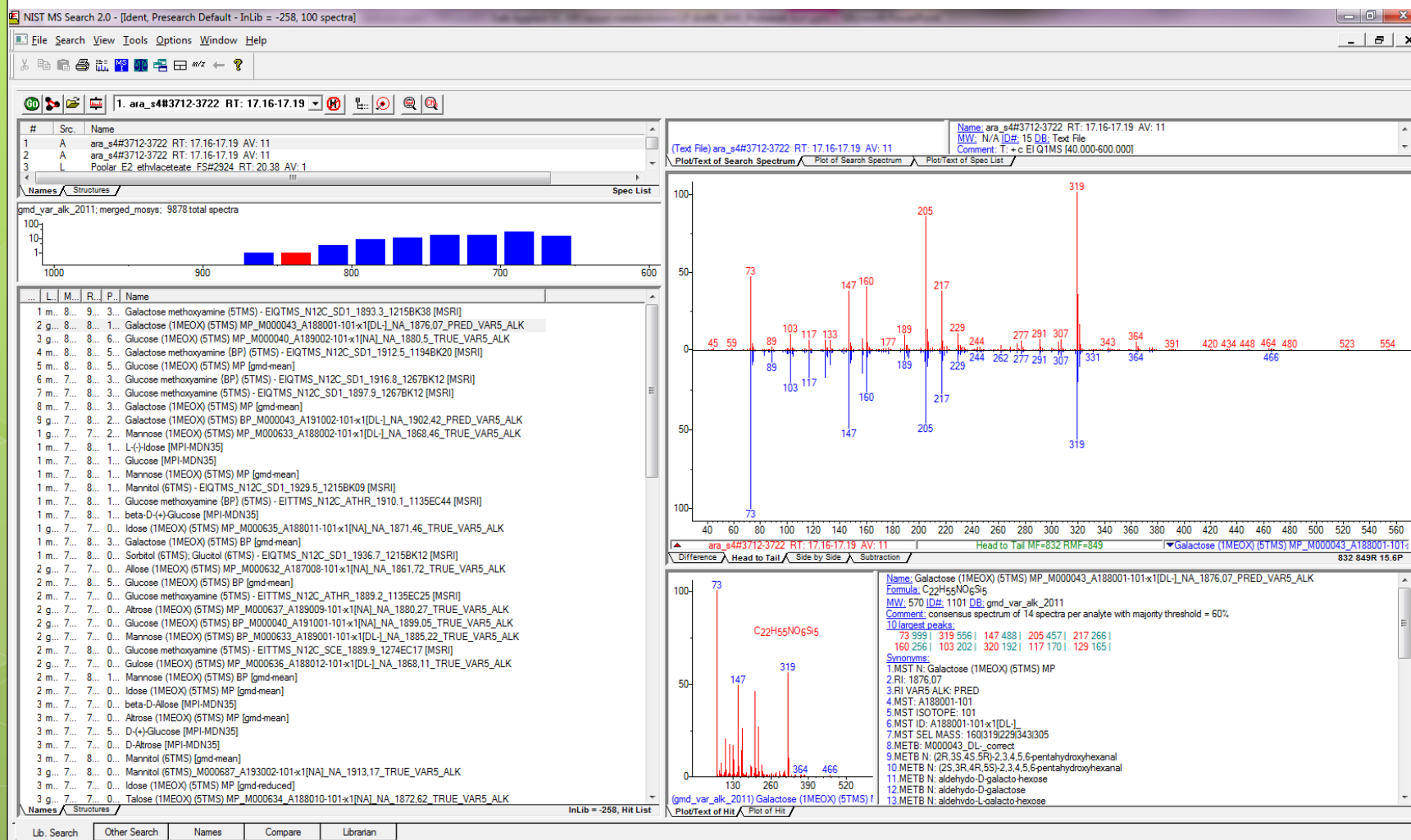


RT: 15.63 - 17.80 SM: 7G

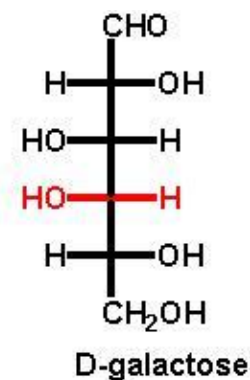
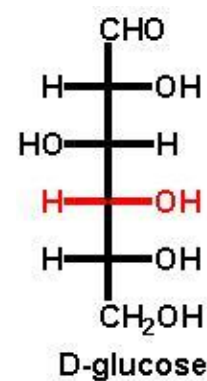
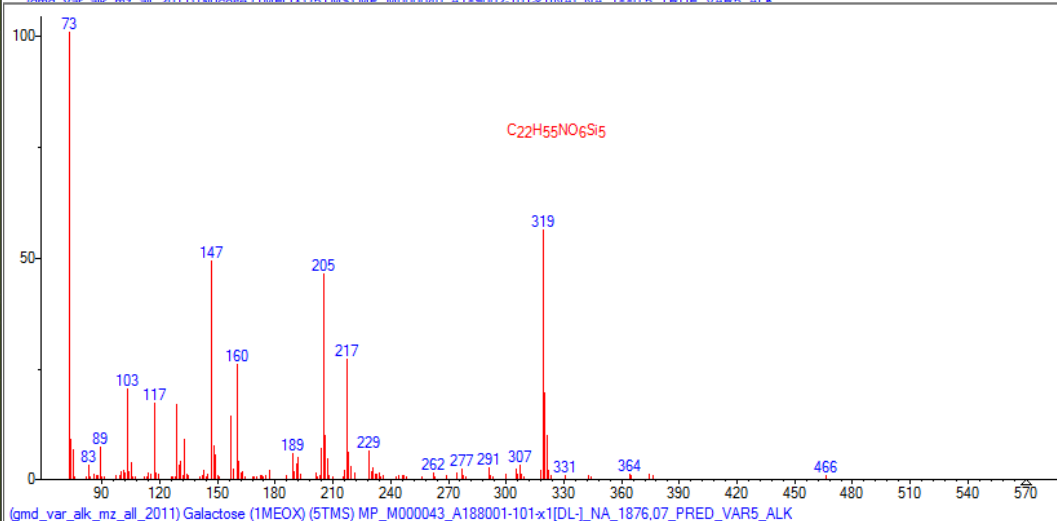
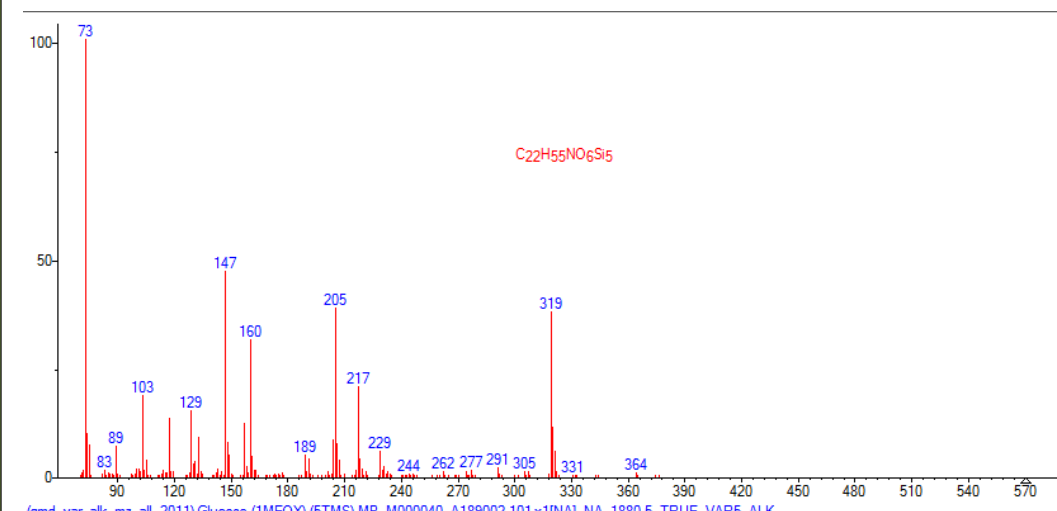


baseline separation
using a longer
temperature gradient

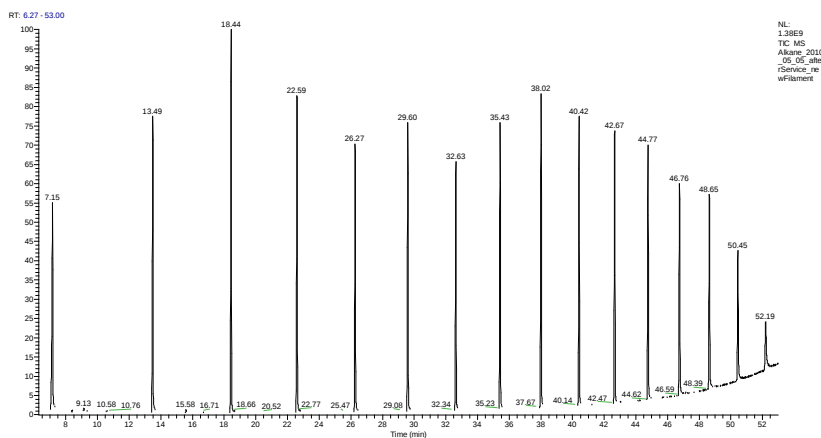
identification – spectral match



identification – spectral match

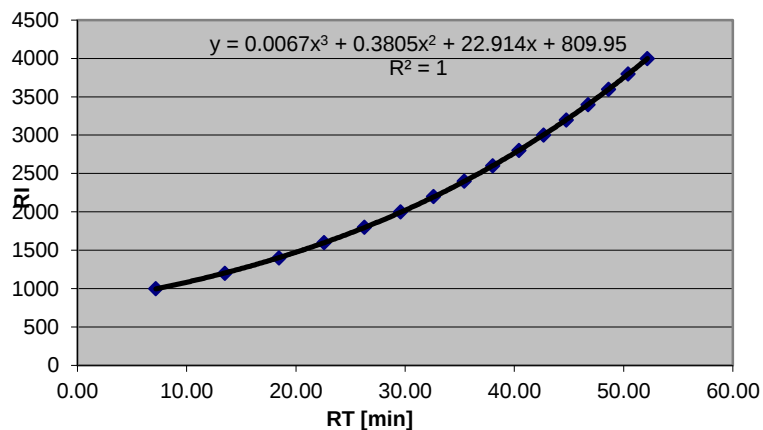


identification – retention index



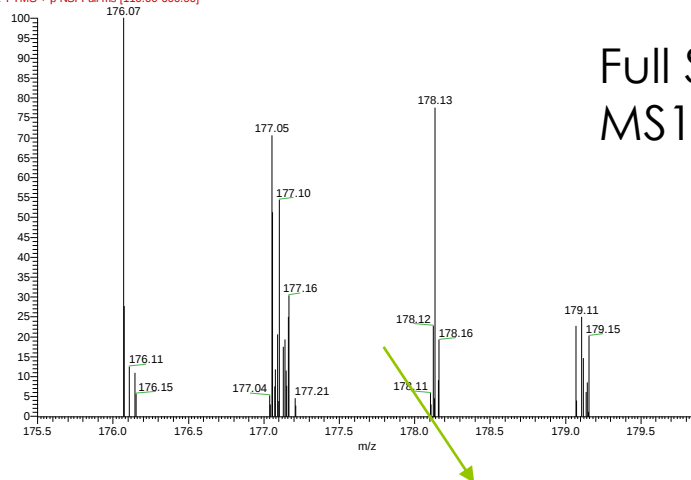
	RT	RI
Decane (C ₁₀ H ₂₂)	7.31	1000
Dodecane (C ₁₂ H ₂₆)	12.96	1200
Tetradecane (C ₁₄ H ₃₀)	17.74	1400
...	...	...

Calculation RI Polynomial regression 3rd order



„identification“ – exact mass

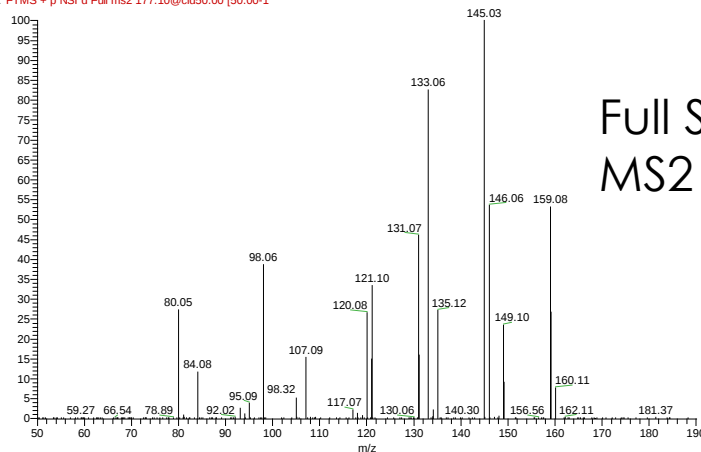
4.2 #817 RT: 7.52 AV: 1 NL: 1.35E5
F: FTMS + p NSI Full ms [110.00-600.00]



Sum formula annotation by exact mass

Precursor:
[M+H]⁺ 177.102231

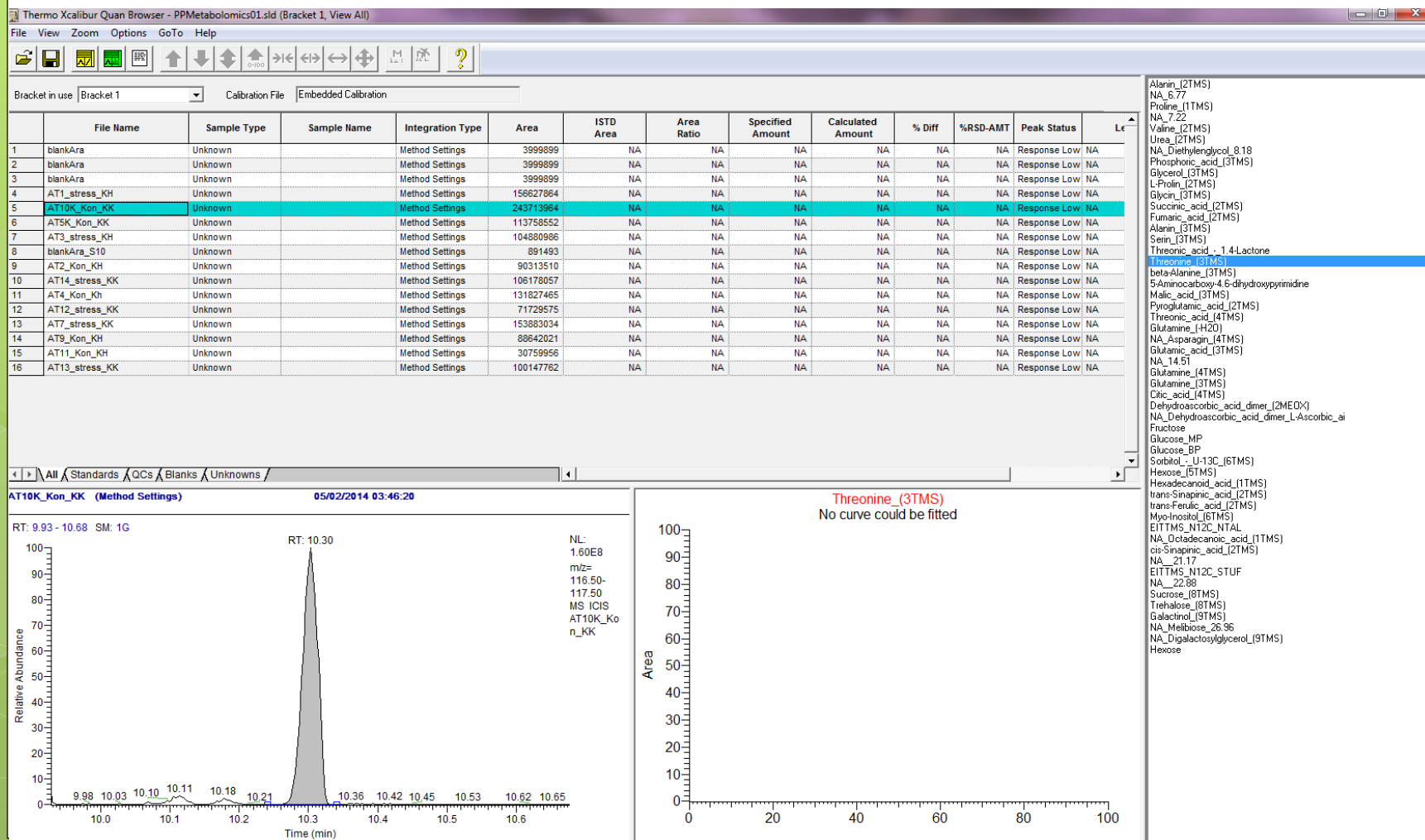
4.2 #419-997 RT: 4.57-8.15 AV: 9 NL: 4.37E3
F: FTMS + p NSI d Full ms2 177.10@cid50.00 [50.00-1



Multiple charges possible

Structure information by comparing MS_n spectra to libraries

alignment & peak integration



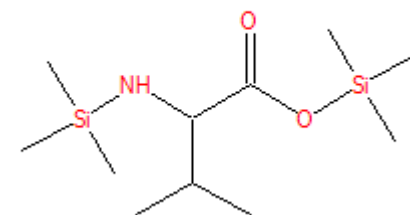
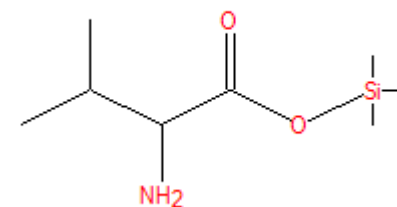
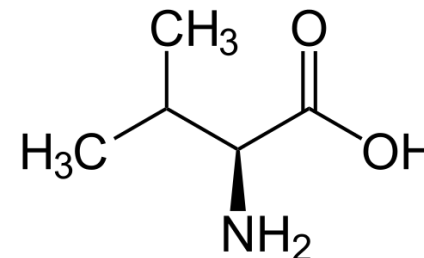
raw data matrix

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000
---	---	---	---	---	---	---	---	---	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	------

	S1	S2	S3
M1_D1	680518	22042	5913
M1_D2	2218715500	1177147637	114881670
M2	12090493	1674566	2551986
M3	38668521	16650636	4641401
....	...		

curated data matrix

	S1	S2	S3
Valine (1+2TMS)	56856630	3066789	2316542
Malic acid	1010149883	232986113	999818170
Glucose (MP)	3230720402	2316640517	1818410005
....		...	...



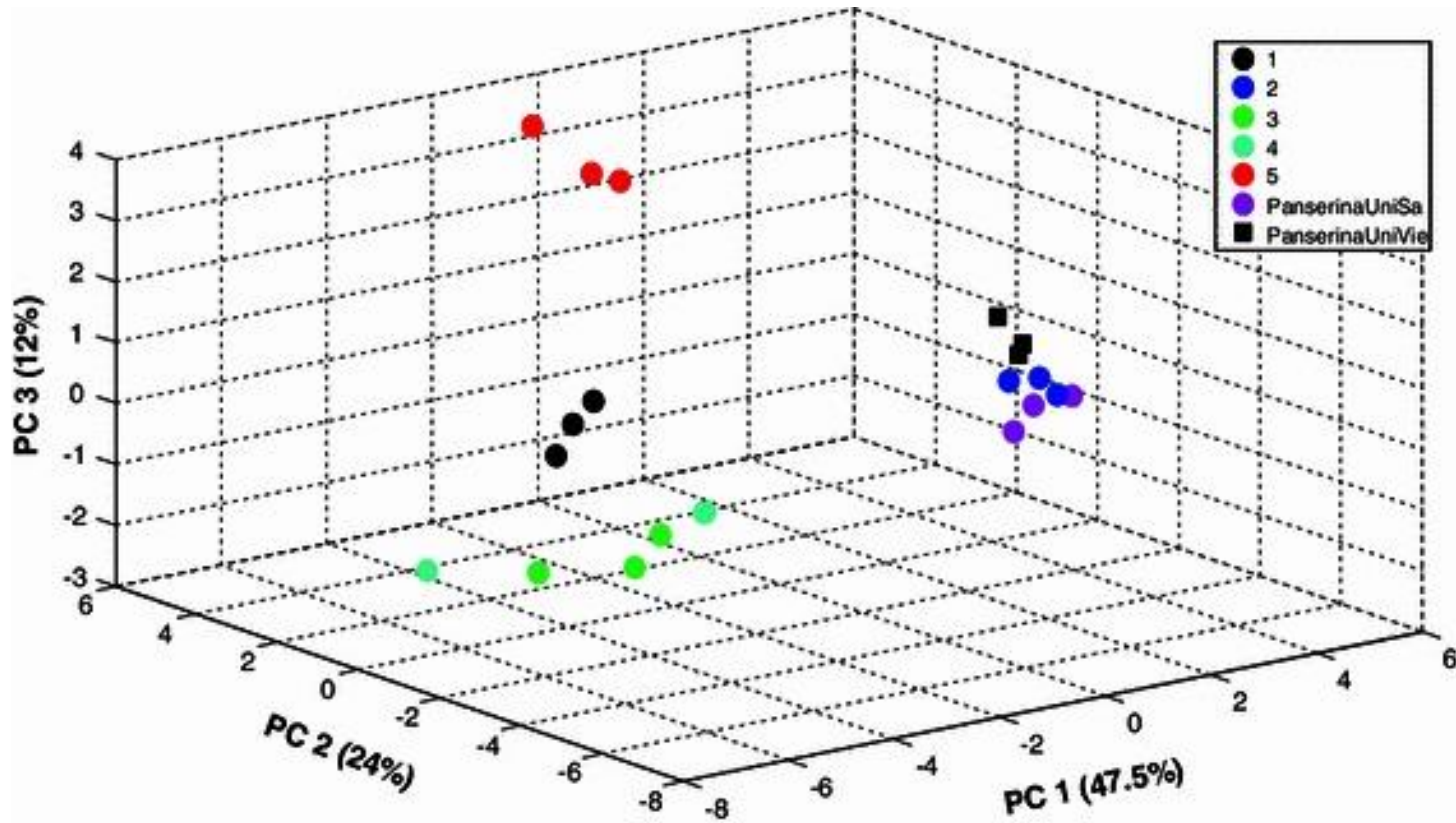
summed derivatives

normalised to sample amount (weight, volume, protein content)

checked for 0-values, contaminants,

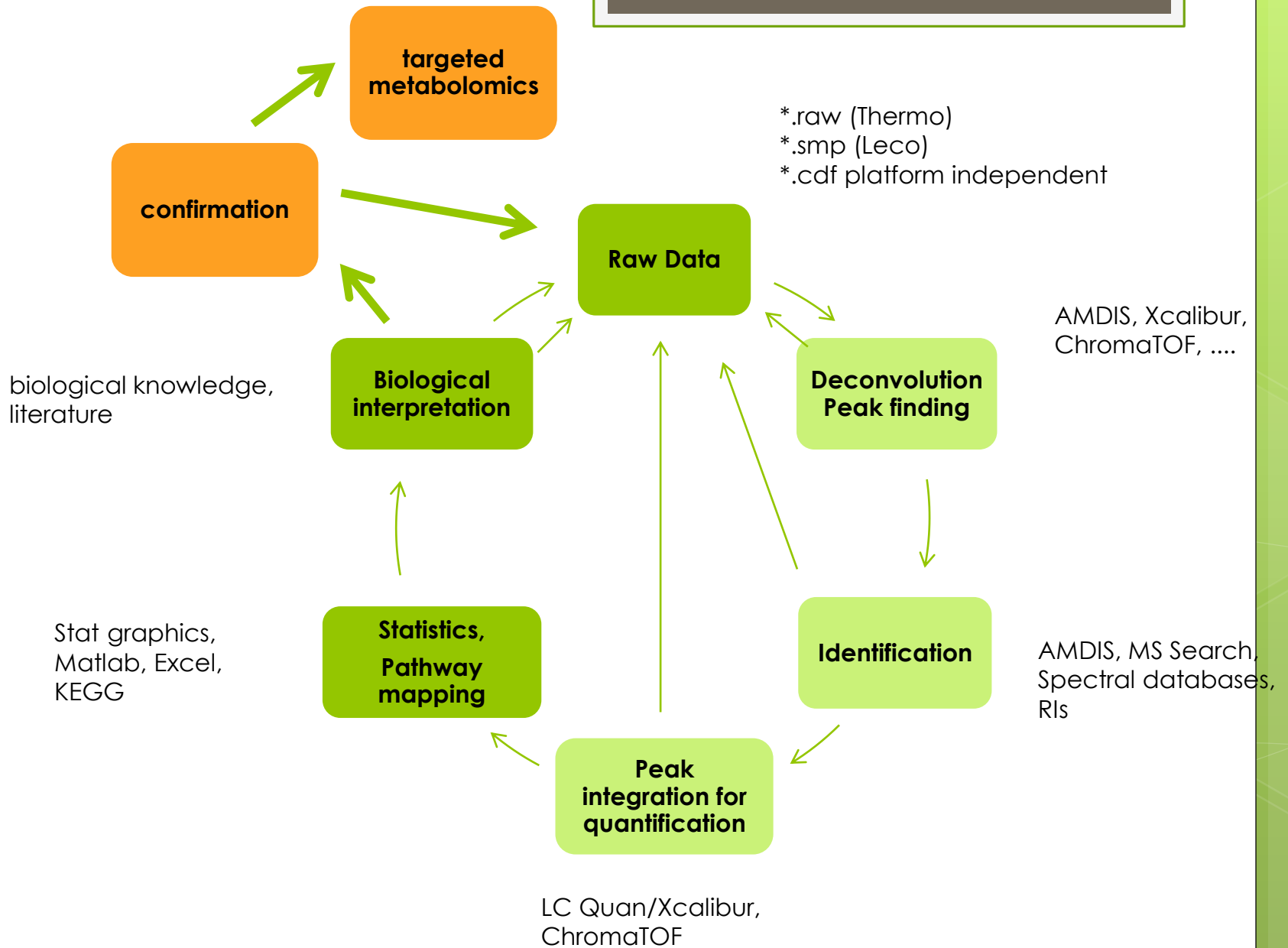
Absolute quantification using calibration curves of standard mix

statistics - PCA



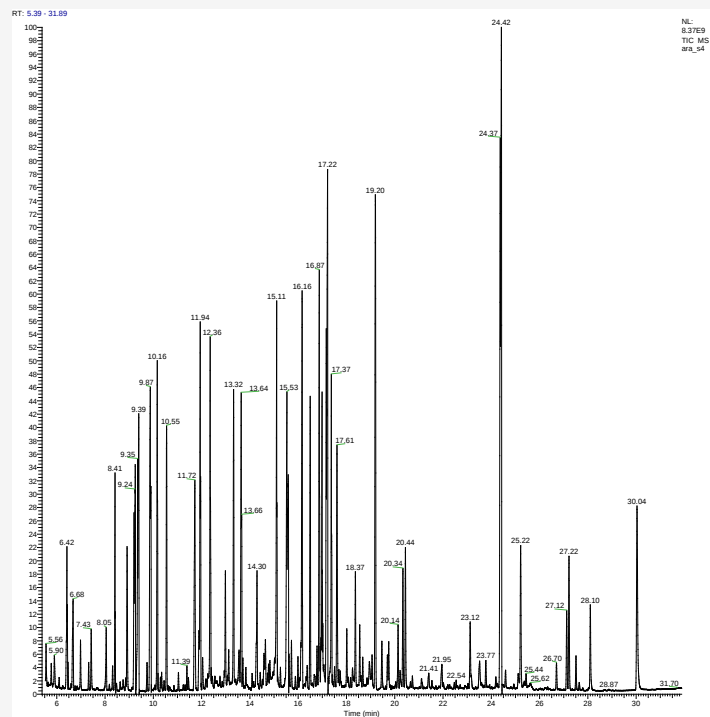
Mari et al. (2013),
Metabolomics 9(3): 564–574

data processing





Questions?



Mosys

Department
Molecular Systems Biology



universität
wien

University of Vienna | Faculty of Life Sciences | Althanstrasse 14 | 1090 Vienna | Austria

Nachtrag

ESI – spectrum



Exact mass of molecular ion -->
mass accuracy <5ppm
→ sum formular calculation

Still structure = ??????

→ MSⁿ fragmentation pattern
→ Comparison with standards

Monoisotopic mass C₁₀H₁₂N₂O: 176.094955 Da

Monoisotopic mass H: 1.007276 Da

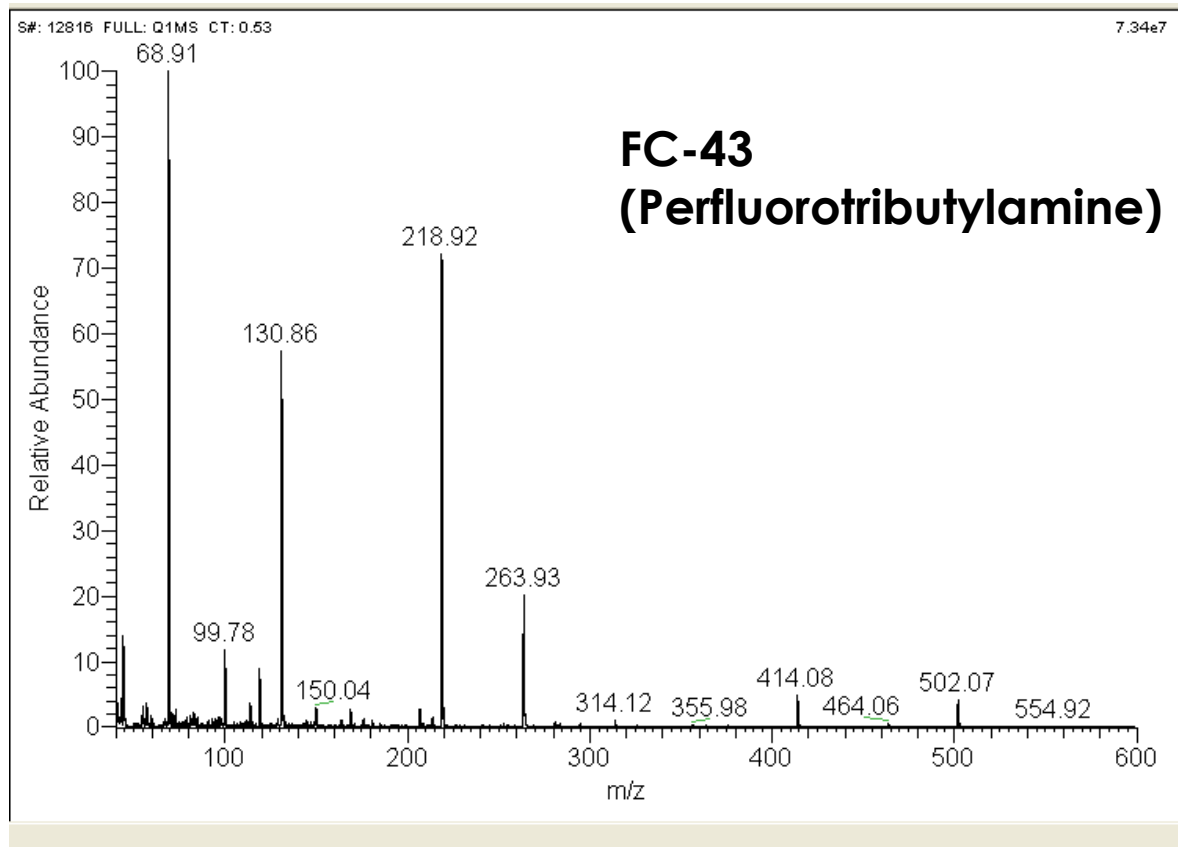
$$[M+H]^+ = 177.102231 \text{ Da}$$

Mass error calculation [ppm]:

(theoretical mass-experimental m/z)/

Theoretical mass * 10⁶

calibration gas



Thank you!

