Application of HR-LC-MS in screening methods for pesticides in cereals

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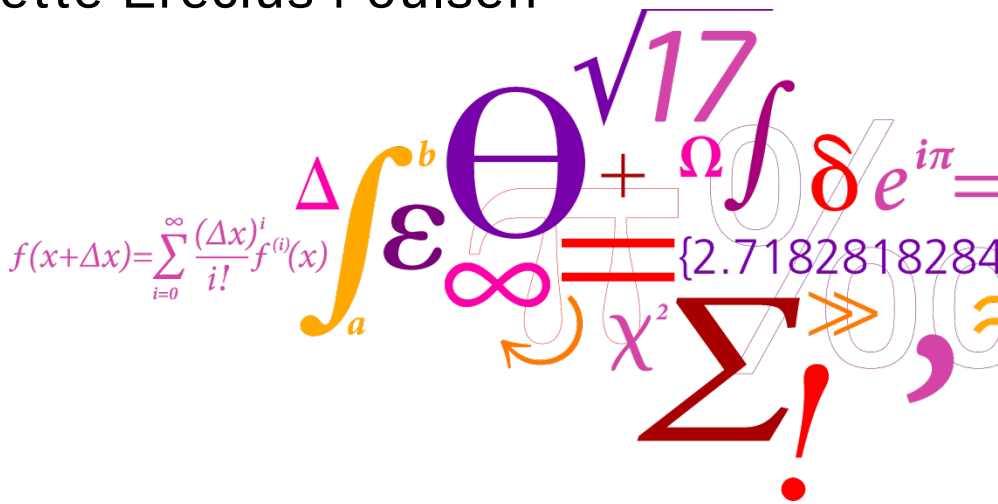
Almeria, 12 June 2014



DTU Food
National Food Institute

Disposition

- Analysis at the instrument
- Standard mixtures
- Validation plan
- Validation
- Virtual computers – for data treatment
- Sample preparation of cereals – QuEChERS

LC-QTOF instruments

Disposition

- Analysis at the instrument
- Standard mixtures
- Validation plan
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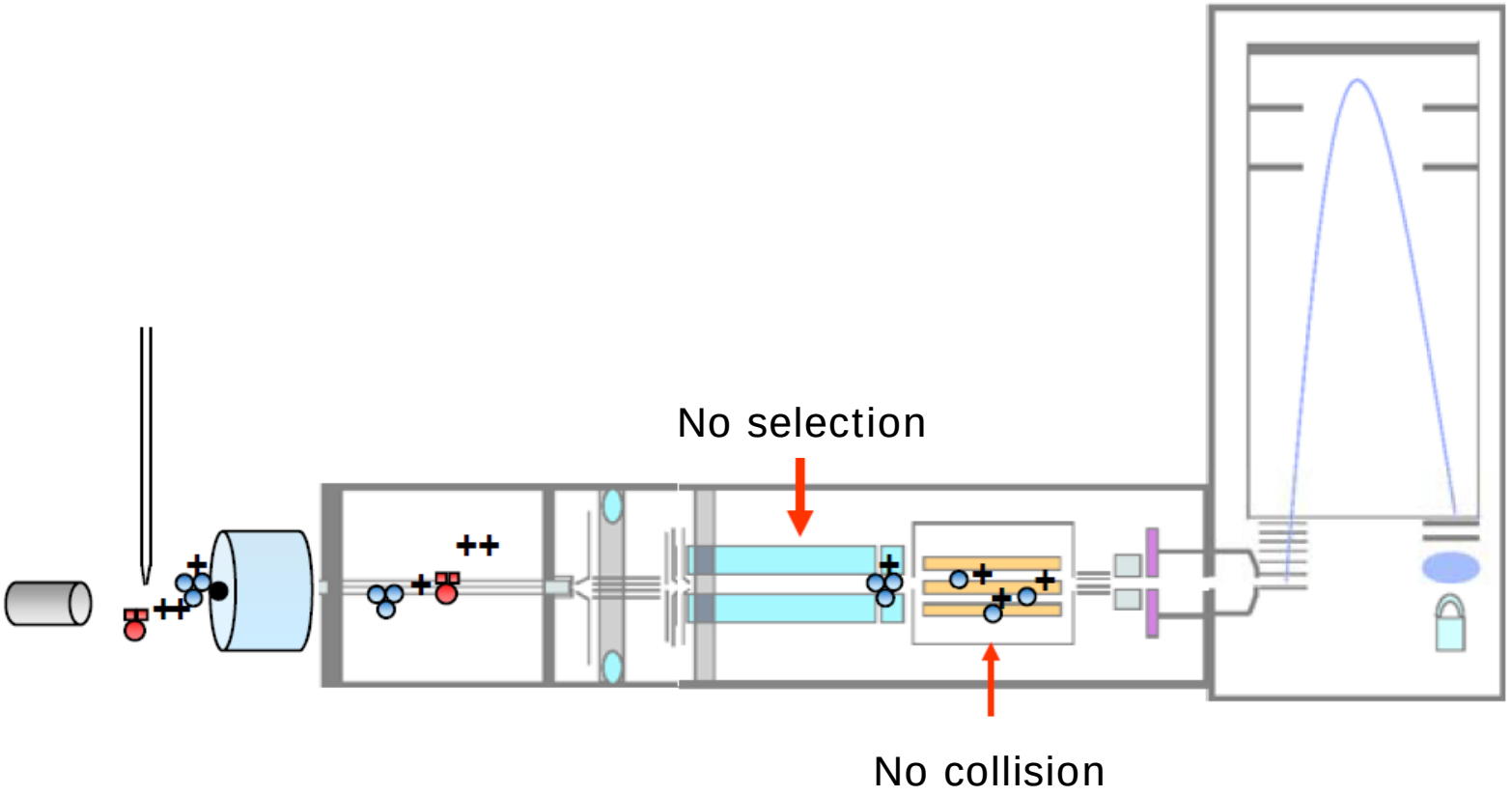
PCD – CSV file

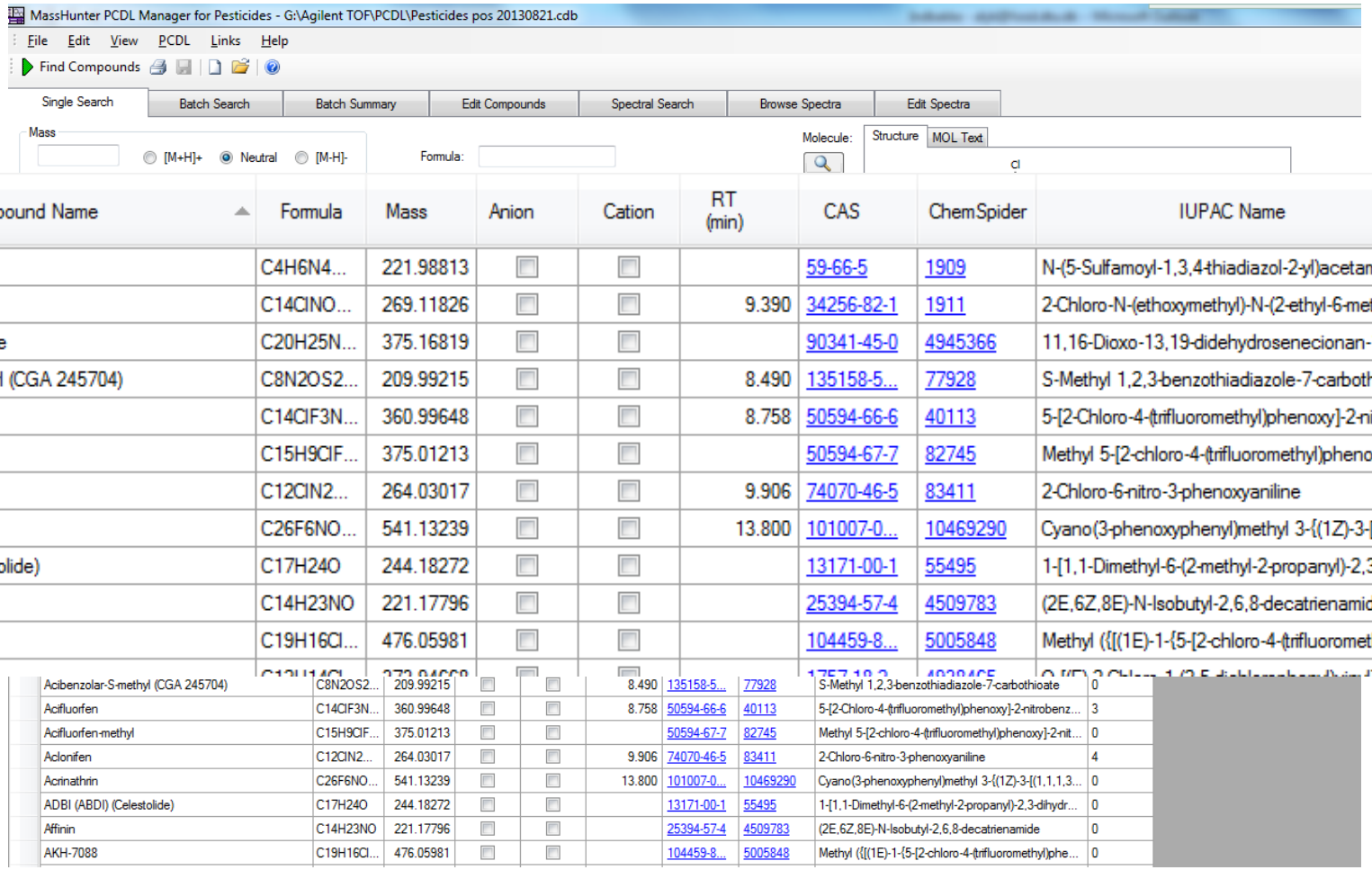
	A	B	C	D	E	F
22	# First line is 'Agilent TOF Formula data store'					
23	# Second line is 'Version:' followed by a version number					
24	#					
25	# Additionally	comments (such as these) may be inserted on individual				
26	# lines by specifying a '#' character at the beginning of the line					
27	#					
28	#					
29	### Formula	Retention Time	Mass	Compound name	Description	
30	# Formula	RT	Mass	Cpd	Comments	
31	C17H22O7	4.51	338.1365531	15 Acetyl DON		
32	C17H22O7		338.1365531	3 Acetyl DON		
33	C45H57N3O9	13	783.4094804	Beuvercin		
34	C19H26O7	5.46	366.1678532	DAS		
35	C15H20O6	3.47	296.1259884	Deoxynivalenol		
36	C36H63N3O9	13.83	681.4564306	Enniatin A		
37	C35H61N3O9	13.32	667.4407806	Enniatin A1		
38	C33H57N3O9	12.17	639.4094804	Enniatin B		
39	C34H59N3O9	12.75	653.4251305	Enniatin B1		
40	C34H59NO14	13.32	705.3935556	Fumonisin B2		

QTOF-analysis – MS scan

- Used to determine retention time

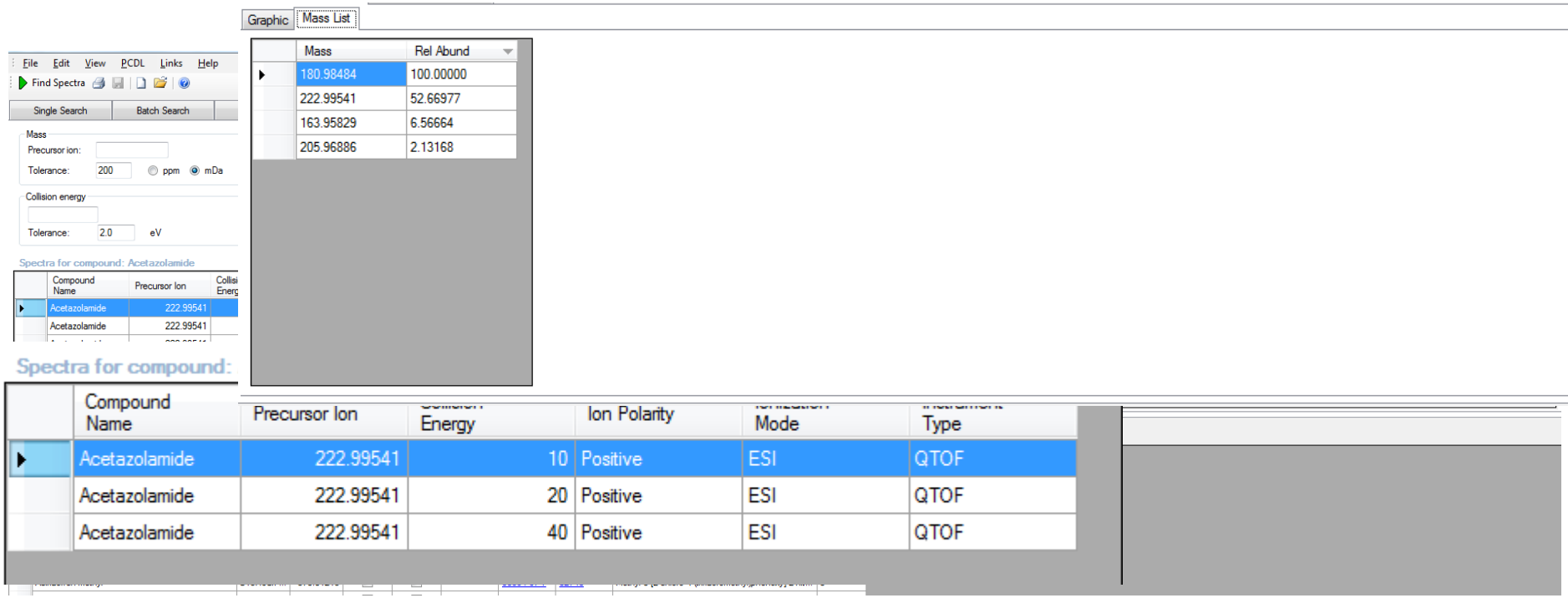


PCDL - library



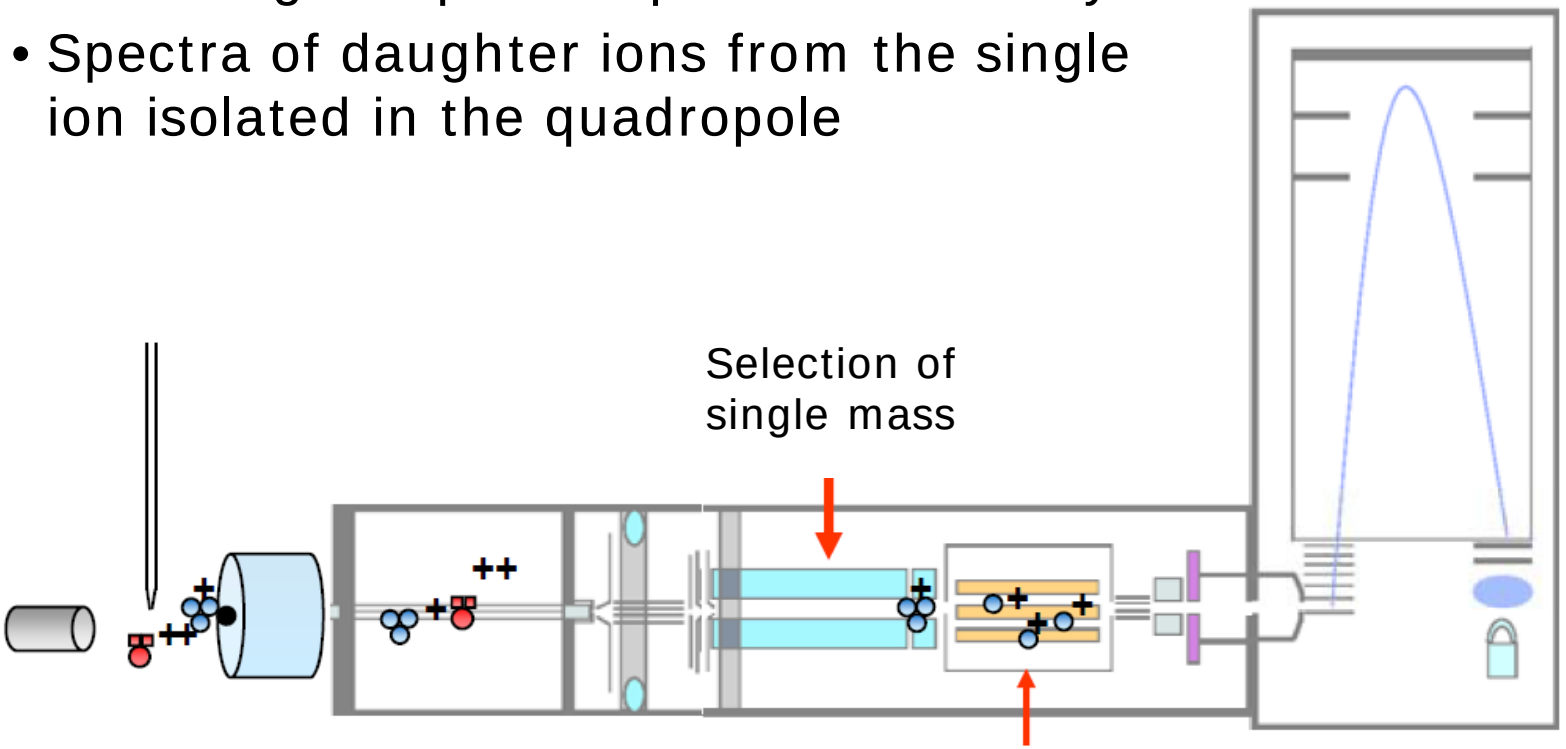
Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSpider	IUPAC Name	Num Spectra
Acetazolamide	C4H6N4...	221.98813				59-66-5	1909	N-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide	3
Acetochlor	C14ClNO...	269.11826			9.390	34256-82-1	1911	2-Chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphe...	3
Acetyl-Seneciphylline	C20H25N...	375.16819				90341-45-0	4945366	11,16-Dioxo-13,19-didehydrosenecionan-12-yl ac...	0
Acibenzolar-S-methyl (CGA 245704)	C8N2OS2...	209.99215			8.490	135158-5...	77928	S-Methyl 1,2,3-benzothiadiazole-7-carbothioate	0
Acifluorfen	C14ClF3N...	360.99648			8.758	50594-66-6	40113	5-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenz...	3
Acifluorfen-methyl	C15H9ClF...	375.01213				50594-67-7	82745	Methyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nit...	0
Aclonifen	C12ClN2...	264.03017			9.906	74070-46-5	83411	2-Chloro-6-nitro-3-phenoxyaniline	4
Acrinathrin	C26F6NO...	541.13239			13.800	101007-0...	10469290	Cyano(3-phenoxyphenyl)methyl 3-{(1Z)-3-[(1,1,1,3...	0
ADBI (ABDI) (Celestolide)	C17H24O	244.18272				13171-00-1	55495	1-[1,1-Dimethyl-6-(2-methyl-2-propanyl)-2,3-dihydr...	0
Affinin	C14H23NO	221.17796				25394-57-4	4509783	(2E,6Z,8E)-N-Isobutyl-2,6,8-decatrienamide	0
AKH-7088	C19H16Cl...	476.05981				104459-8...	5005848	Methyl ({[(1E)-1-{5-[2-chloro-4-(trifluoromethyl)phe...	0

PCDL – library - spectra



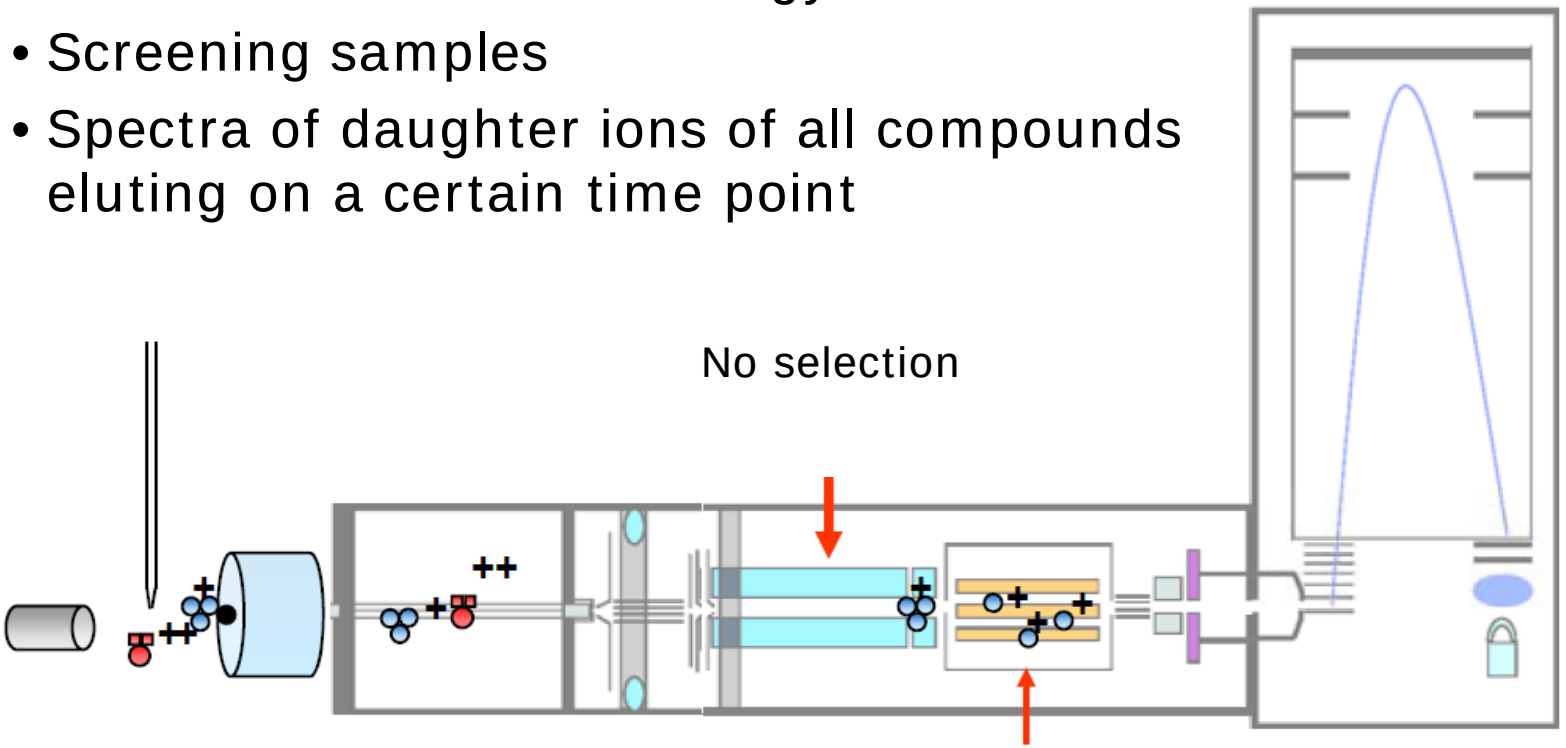
QTOF-analysis – Targeted MS/MS

- Obtaining compound spectra for Library
- Spectra of daughter ions from the single ion isolated in the quadropole

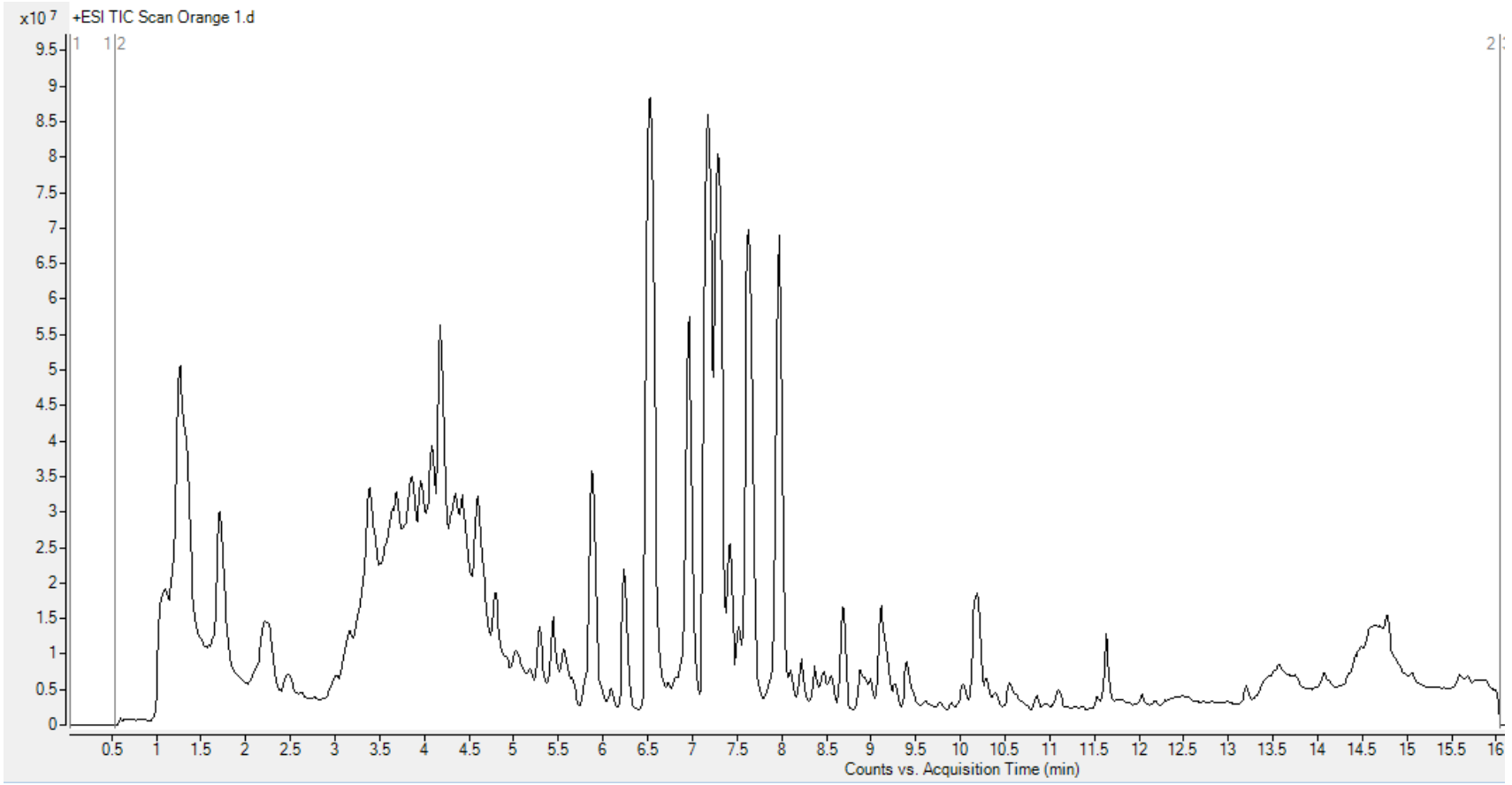


QTOF-analysis – All ion MS

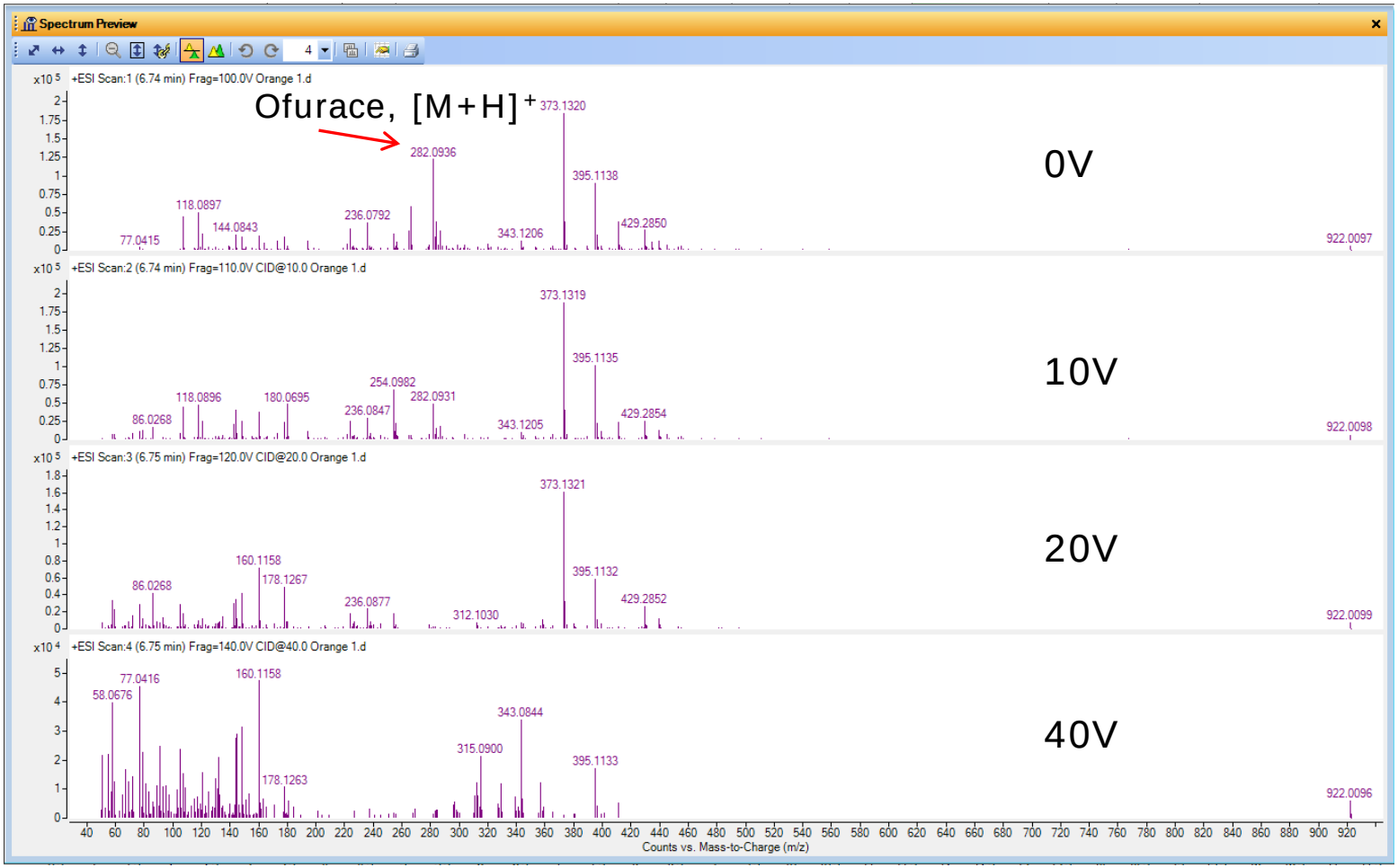
- MS scan with collision energy
- Screening samples
- Spectra of daughter ions of all compounds eluting on a certain time point



Chromatogram - TIC



Spectra – MSscan incl. collision



Disposition

- Analysis at the instrument
- **Standard mixtures**
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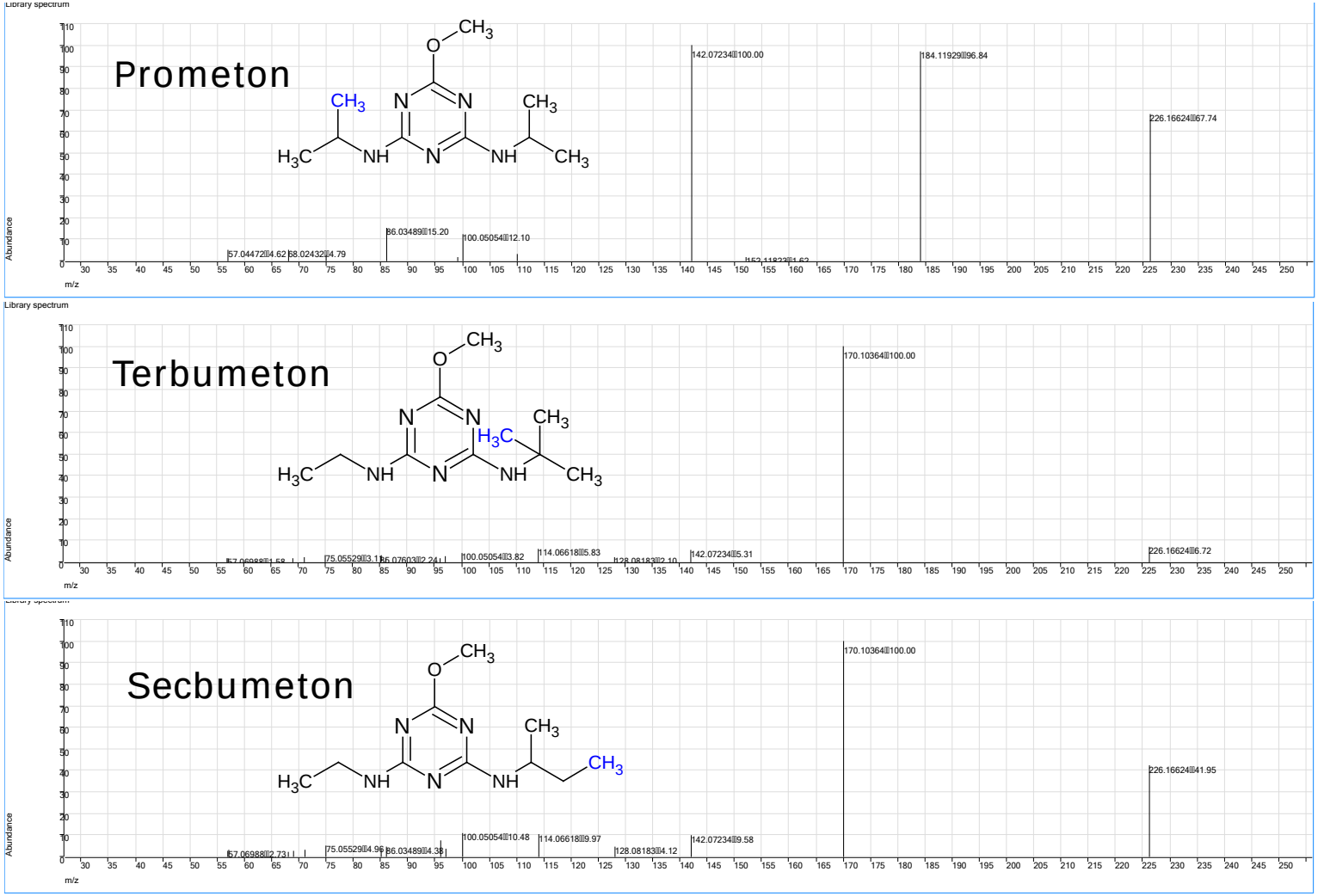
- Sample preparation of cereals – QuEChERS

Standard mixtures

Mixtures	50-100 pesticides
Isomers	Avoiding isomers in the mixtures
Validation	2-6 mixtures together

Spectra of isomers in Agilents library



Disposition

- Analysis at the instrument
- Standard mixtures
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Validation

- My alternative validation plan
- 20-25 samples – spiked at 0.1 mg/kg
- 10-20 blank samples

	Spiked – 0.1 mg/kg	Blank
Rice	5	5
Oat	5	5
Barley	5	5
Wheat	5	5
Rye	5	5

Dilution of spiked samples

Rye	0.1 mg/kg	Blank
0.10 mg/kg	1000 µl	-
0.02 mg/kg	200 µl	800 µl
0.01 mg/kg	100 µl	900 µl
Blank	-	1000 µl

Spiked rye is diluted with Blank rye …

Matrix is the same

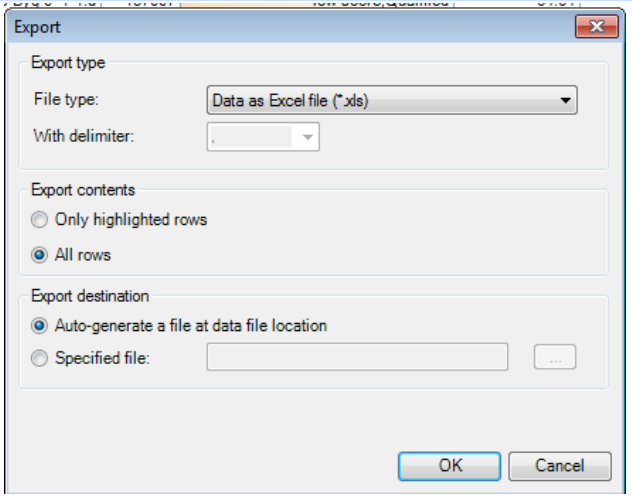
Recovery is assumed to be the same at the different concentration levels

Disposition

- Analysis at the instrument
- Standard mixtures
- Validation plan
- **Validation**
- Virtual computers – for data treatment
- Sample preparation of cereals – QuEChERS

Compound list – exported to Excel

Name	File	Area	Flags (Tgt)	Score (Tgt)	RT Diff (Tgt)	Diff (Tgt, ppm)	RT	Fls Co	Label
Aldicarb	Pos Byg 0_1-1.d	137207	No H adduct;Qualified	92.1	-0.059	-1.46	5.337	80	Cpd 11: Ald
Aldicarb-sulfone (Aldoxycarb)	Pos Byg 0_1-1.d	1459914	low score;Not qualified	73.93	-0.05	-2.92	4.681	0	Cpd 36: Aldicarb-sulfone (Aldoxy
Aldicarb-sulfoxide	Pos Byg 0_1-1.d	17148	multiple IDs;Not qualified	75.48	0.009	1.08	3.229	0	Cpd 18: Aldicarb-sulf
Amidosulfuron	Pos Byg 0_1-1.d	299303	Qualified	77.56	-0.122	-0.8	6.273	80	Cpd 194: Amidosul
Aminocarb	Pos Byg 0_1-1.d	88888	Qualified	82.26	-0.036	1.77	1.484	20	Cpd 22: Amin
Amitraz	Pos Byg 0_1-1.d	187007	low score;Qualified	64.64	0.291	-2.59	13.031	60	Cpd 98: An
Anthraquinone	Pos Byg 0_1-1.d	182161	low score; No H adduct;Not qualified	63.47	0.127	4.02	7.837	0	Cpd 21: Anthraqu
Atrazine	Pos Byg 0_1-1.d	2655497	Qualified	89.22	-0.072	-0.33	6.608	100	Cpd 29: Atr
Azaconazole(II)	Pos Byg 0_1-1.d	63278	low score; No H adduct;Not qualified	35.65	-0.203	-8.81	6.441	0	Cpd 105: Azaconaz
Azimsulfuron (IN A8947)	Pos Byg 0_1-1.d	144789	Qualified	89.01	-0.068	-1.24	6.51	40	Cpd 228: Azimsulfuron (IN A
Azinphos-ethyl (Guthion ethyl)	Pos Byg 0_1-1.d	120726	Qualified	75.62	-0.127	-0.64	9.373	60	Cpd 171: Azinphos-ethyl (Guthion
Azoxystrobin	Pos Byg 0_1-1.d	3200956	Qualified	83.17	-0.098	0.72	8.228	100	Cpd 219: Azoxyst
Benalaxyl	Pos Byg 0_1-1.d	3780302	low score;Qualified	74.98	-0.141	0.79	9.819	100	Cpd 146: Bena
Bendiocarb	Pos Byg 0_1-1.d	280916	Qualified	84.75	-0.083	1.21	6.147	100	Cpd 38: Bendi
Benfuracarb	Pos Byg 0_1-1.d	272873	Qualified	80.34	-0.107	-0.43	11.663	60	Cpd 224: Benfur
Bensulfuron-methyl	Pos Byg 0_1-1.d	658684	Qualified	94.14	-0.049	-0.44	7.111	60	Cpd 222: Bensulfuron-m



All file gathered in one Excel-file

	N	O	P	Q	R	S	T	U	V	AW	AX	AY	AZ	BA	BB	BC	BD	BE	
1																			
2																			
3	Pred M	Sequer	Seq Na	Seq Typ	Tgt Seq	AGILEN	CAS	Compo	Name	Score D	Abund	Algorit	Area	Avg Ma	Base Pe	Z Count	CE	MS/MS	File
4							30560-19-1		Acephate(I)		19138	Find By Fc	153900		189.1598	1			Pos
5							135410-20-7		Acetamiprid		114050	Find By Fc	1385340		190.0434	1			Pos
6							50594-66-6		Acifluorfen		18863	Find By Fc	82018		121.0508	1			Pos
7							2701-86-2		Akton oxon		67015	Find By Fc	1147525		121.0509	1			Pos
8							116-06-3		Aldicarb		15656	Find By Fc	115981		121.0509	1			Pos
9							1646-88-4		Aldicarb-sulfone (Al		114050	Find By Fc	1055620		190.0434	1			Pos
10							120923-37-7		Amidosulfuron		12554	Find By Fc	314108		304.264	1			Pos
11							33089-61-1		Amitraz		120052	Find By Fc	758480		121.0507	1			Pos
12							1912-24-9		Atrazine		243062	Find By Fc	2260854		207.1496	1			Pos
13							1007-28-9		Atrazine-desisoprop		3631	Find By Fc	32746		207.1496	1			Pos
14							120162-55-2		Azimsulfuron (IN A8		5492	Find By Fc	121926		121.0508	1			Pos
15							2642-71-9		Azinphos-ethyl (Gut		21377	Find By Fc	176158		121.0509	1			Pos
16							131860-33-8		Azoxystrobin		55627	Find By Fc	2119022		404.1245	1			Pos
17							71626-11-4		Benalaxyl		215368	Find By Fc	3107846		121.0508	1			Pos
18							22781-23-3		Bendiocarb		27395	Find By Fc	204079		274.2537	1			Pos
19							82560-54-1		Benfuracarb		263928	Find By Fc	2615067		356.2437	1			Pos
20							83055-99-6		Bensulfuron-methyl		30913	Find By Fc	421688		121.0509	1			Pos
21							55179-31-2		Bitertanol		10747	Find By Fc	65937		224.1193	1			Pos
22							188425-85-6		Boscalid (Nicobifen)		10513	Find By Fc	110547		121.0509	1			Pos
23							116255-48-2		Bromuconazole(I)		23265	Find By Fc	289466		121.0508	1			Pos
24							116255-48-2		Bromuconazole(II)		16231	Find By Fc	209939		121.0509	1			Pos
25							1563-66-2		Carbofuran		9774	Find By Fc	1257818		274.2533	1			Pos
26							16655-82-6		Carbofuran, - 3 hydr		34797	Find By Fc	921386		203.0928	1			Pos
27							55285-14-8		Carbosulfan		235551	Find By Fc	1847735		680.5103	1			Pos
28							5234-68-4		Carboxin		159397	Find By Fc	1144864		121.0509	1			Pos
29							143807-66-3		Chromafenozide		90344	Find By Fc	919310		226.1346	1			Pos
30							94593-91-6		Cinosulfuron		38738	Find By Fc	435723		121.0509	1			Pos
31							99129-21-2		Clethodim(I)		5171	Find By Fc	51484		121.0508	1			Pos
32							105512-06-9		Clodinafop-propargy		45525	Find By Fc	330422		121.0508	1			Pos

Fragment 0.01 | Fragment 0.02 | Fragment 0.1 | PPM blank | PPM 0.01 | PPM 0.02 | PPM 0.1 | Pos 20140117 samstik ABCD-1 | Pos 201

Area of all samples spiked at 10 ppb

	A	B	C	D	E	F	G	H	I
1	*Skabelon: jhia 27/2-2013 (NB: Celler er ikke låste, så ændringer skal kontrolleres)*								
2									
3	***Kolonne med stofnavn***	***V***	***V***	***V***	***V***	***V***	***V***	***V***	***V***
4	***Kolonne med data***	***AZ***	***AZ***	***AZ***	***AZ***	***AZ***	***AZ***	***AZ***	***AZ***
5	***Nedeste række med data***	***200***	***200***	***200***	***200***	***200***	***200***	***200***	***200***
6									
7	**Data**	**Area**	**Area**	**Area**	**Area**	**Area**	**Area**	**Area**	**Area**
8	**Faneblad**	**nvede 0_01-1**	**Hvede 0_01-2**	**de 0_01-3**	**de 0_01-4**	**Hvede 0_01-5**	**yg 0_01-1**	**yg 0_01-2**	**yg 0_01-3**
53	Chlormephos	-----	-----	-----	-----	-----	-----	-----	-----
54	Chlorobenzilate	-----	-----	-----	-----	-----	-----	-----	-----
55	Chlorpropham	-----	-----	-----	-----	-----	-----	-----	-----
56	Chlorothalonil	-----	-----	-----	-----	-----	-----	-----	-----
57	Chlorpropylate	-----	-----	-----	-----	-----	-----	-----	-----
58	Chlorpyrifos	-----	-----	-----	-----	-----	-----	-----	-----
59	Chlorpyrifos-methyl	-----	-----	-----	-----	-----	-----	-----	-----
60	Chlorthal-dimethyl	-----	-----	-----	-----	-----	-----	-----	-----
61	Chromafenozide	141344	142148	112840	122602	140363	132370	138340	142966
62	Cinidon-ethyl	-----	-----	-----	-----	-----	-----	-----	-----
63	Cinosulfuron	88804	75673	59014	67722	74279	69385	63358	65773
64	Clethodim(II)	-----	-----	-----	-----	-----	-----	-----	-----
65	Clodinafop-propargyl	40158	30783	40762	-----	36048	37315	33719	78682
66	Clofentezin	-----	-----	-----	-----	-----	-----	-----	-----
67	Clomazone	52924	49092	52854	46925	52430	43874	45868	53189
68	Clothianidin	-----	-----	-----	-----	-----	-----	-----	-----

Comparison of areas in spiked samples with blank

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	*Skabelon: jhia 27/2-2013 (NB: Celler er ikke låste, så ændringer skal kontrolleres)*												
2													
3	***Kolonne med stofnavn***	***A***	***A***	***A***	***A***	***A***	***A***	***A***	***A***	***A***	***A***	***A***	***A***
4	***Kolonne med data***	***Y***	***Y***	***Y***	***X***	***AA***	***AA***	***AA***	***Y***	***AA***	***AA***	***AA***	***Y***
5	***Nedeste række med data***	***400***	***400***	***400***	***400***	***400***	***400***	***400***	***400***	***400***	***400***	***400***	***400***
6		***MIN***	***MIN***	***MIN***	***Max***								
7	**Data**	**Areal**	**Min**	**Min**	**Max**	**N**	**N**	**N**	**N**	**Med mindst et fragment**			
8	**Faneblad**	**pos0.01**	**pos0.02**	**pos0.1**	**pos blank**	**pos0.01**	**pos0.02**	**pos0.1**	**pos blank**	**ment 0.01**	**ment 0.02**	**gment 0.1**	**ent blank**
201	Mesotrione	0	0	0	0	0	0	0	0	0	0	0	0
202	Metaflumizone	0	0	0	0	0	0	0	0	0	0	0	0
203	Metalaxyl(I)	315804	724716	231227	64922	20	20	17	2	20	18	14	0
204	Metalaxyl(II)	0	58865	81263	0	0	1	15	0	0	0	0	0
205	Metamitron	164622	329042	92575	0	19	18	15	0	16	14	12	0
206	Metconazole	75837	122612	113384	80481	20	20	20	3	0	0	4	0
207	Methacrifos	262153	494818	2276131	0	1	3	3	0	0	0	0	0
208	Methamidophos(I)	0	0	0	0	0	0	0	0	0	0	0	0
209	Methamidophos(II) (Metamidopho	0	0	37388	0	0	0	10	0	0	0	3	0
210	Methidathion	0	36634	167709	0	0	20	17	0	0	18	15	0
211	Methiocarb (Mercaptodimethur)	0	31632	42811	0	0	11	7	0	0	0	0	0
212	Methiocarb sulfone	32456	66421	383397	0	13	13	13	0	0	0	0	0
213	Methiocarb sulfoxide	89617	487522	101424	0	19	17	15	0	12	12	10	0
214	Methomyl	0	32143	31662	0	0	1	7	0	0	0	5	0
215	Methoprene	0	0	0	0	0	0	0	0	0	0	0	0
216	Methoxychlor	0	0	0	0	0	0	0	0	0	0	0	0

Disposition

- Analysis at the instrument
- Standard mixtures
- Validation plan
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- Virtual computers – for data treatment
- Sample preparation of cereals – QuEChERS

Virtual computers for data-analysis

Challenges

- The Agilent software demands Windows Professional 64 bit – english version
- Back up of all laboratory computer on LAB-domain
- Laboratory domain, with no access from personal computers

Solution

- Virtual computers from Citrix
  - On the Laboratory domain
  - Windows Professional 64 bit
  - Access to all data – back up early in the morning
  - Few licenses are necessary
  - Access from all computers with internet

Disposition

- Analysis at the instrument
- Standard mixtures
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FVII cocktail IV – project

- Aim is to develop a cereal-method to analyze many analytes in one method.

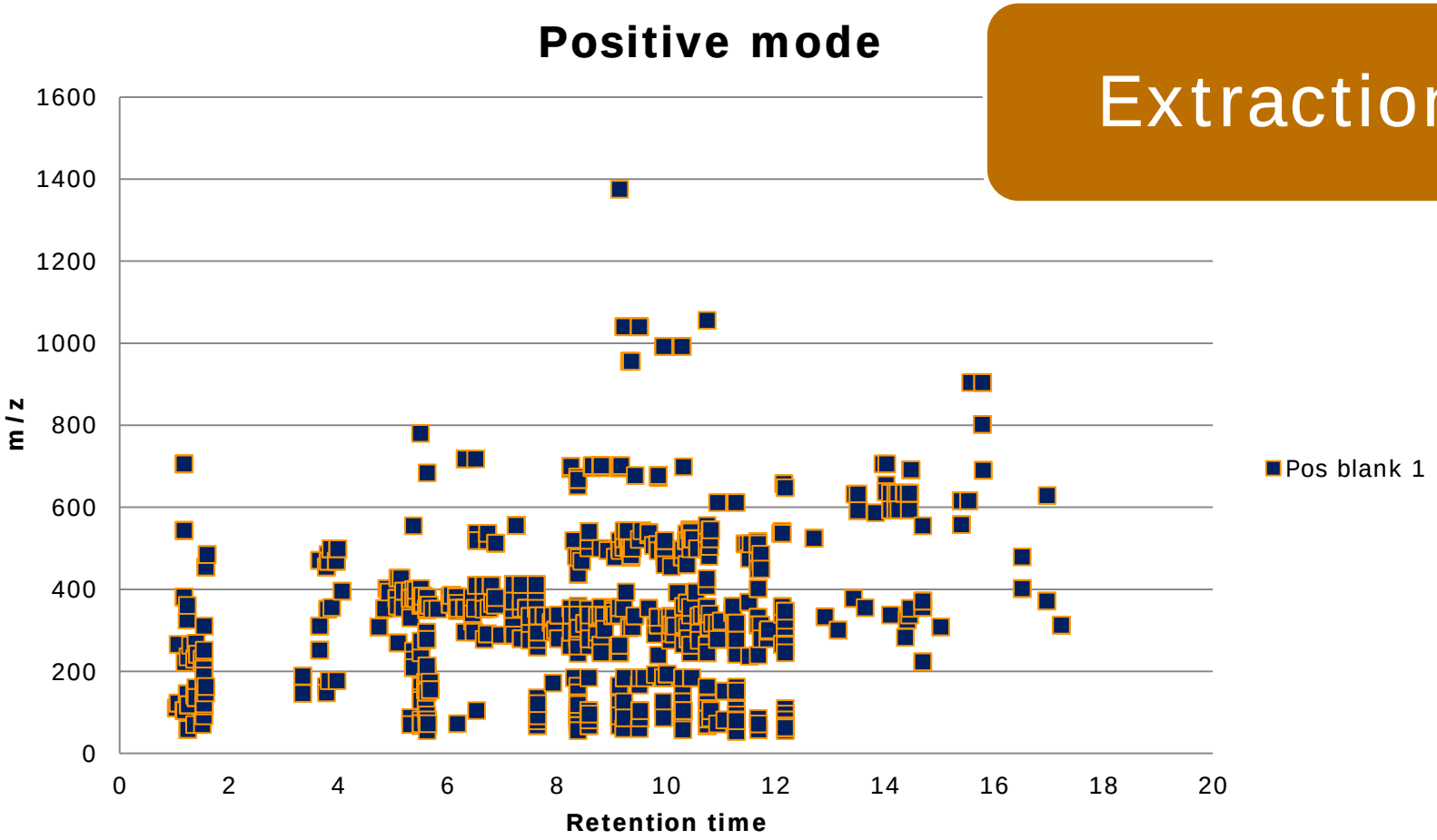
- Analytes:
  - Pesticides (The pesticides most present in cereal – in practice the standard mixtures which we use for our quantitative methods).

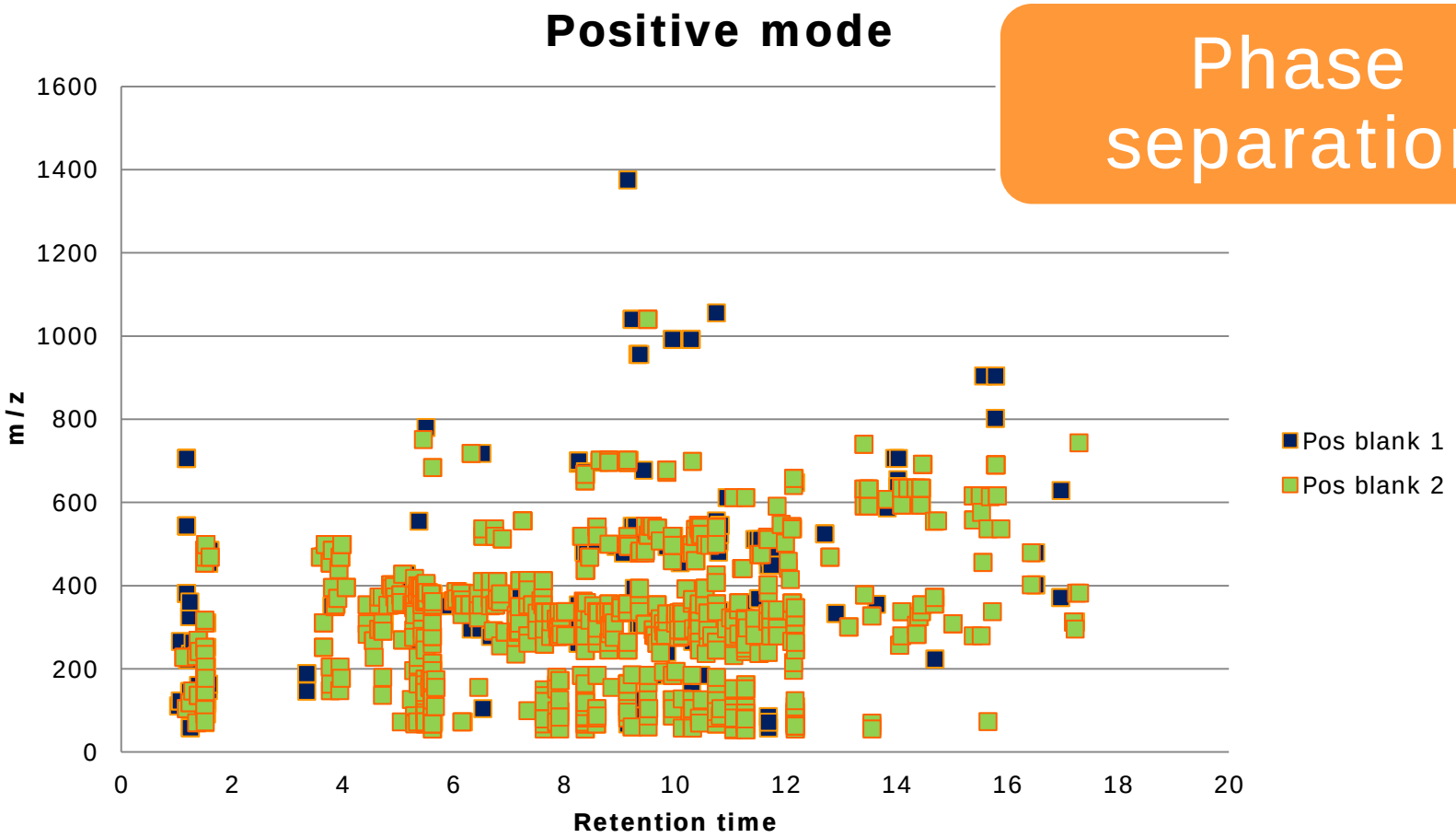
  - Mycotoxins: Most important mycotoxins in cereals.

Method development - QuEChERS

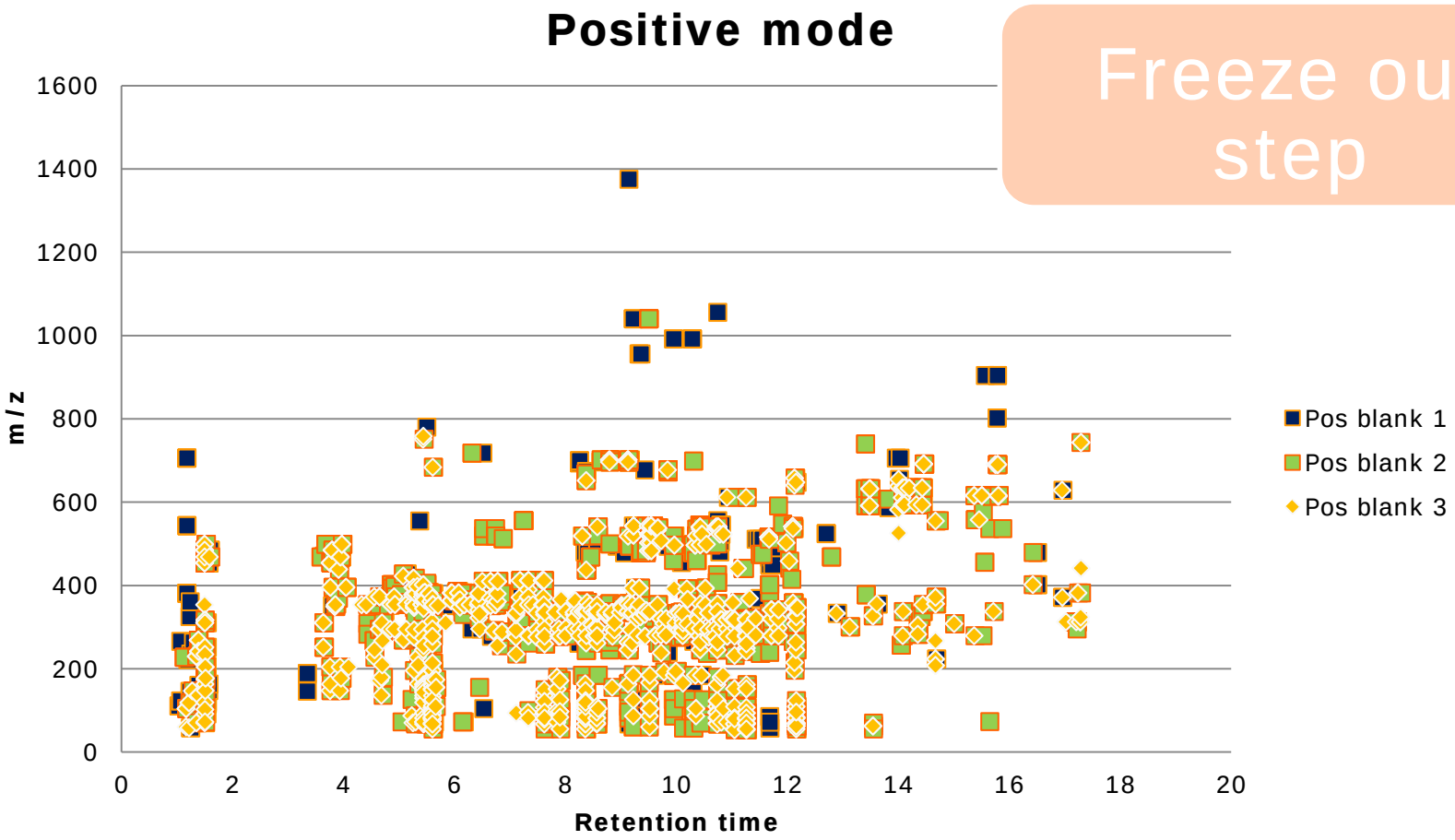
Extraction	• Water • Acetonitrile
Phase separation	• Magnesium sulphate • NaCl, Citrate buffer
Freeze out step	• 1 h at -80 ºC • Centrifugation
PSA clean up	• PSA • Magnesium sulphate

Extraction


Positive mode
m/z
1600
1400
1200
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
16
18
20
Retention time
Pos blank 1

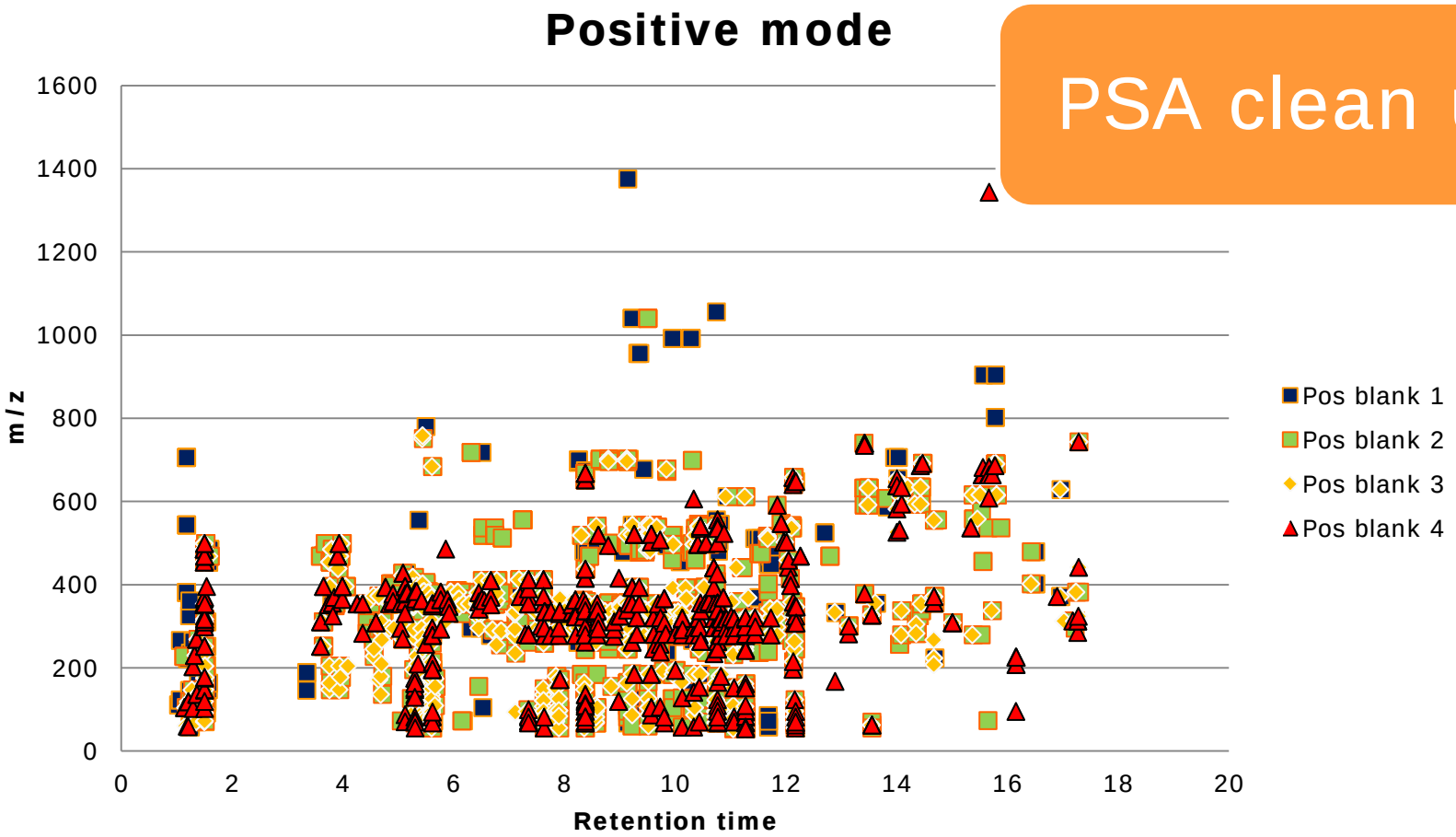


Phase separation

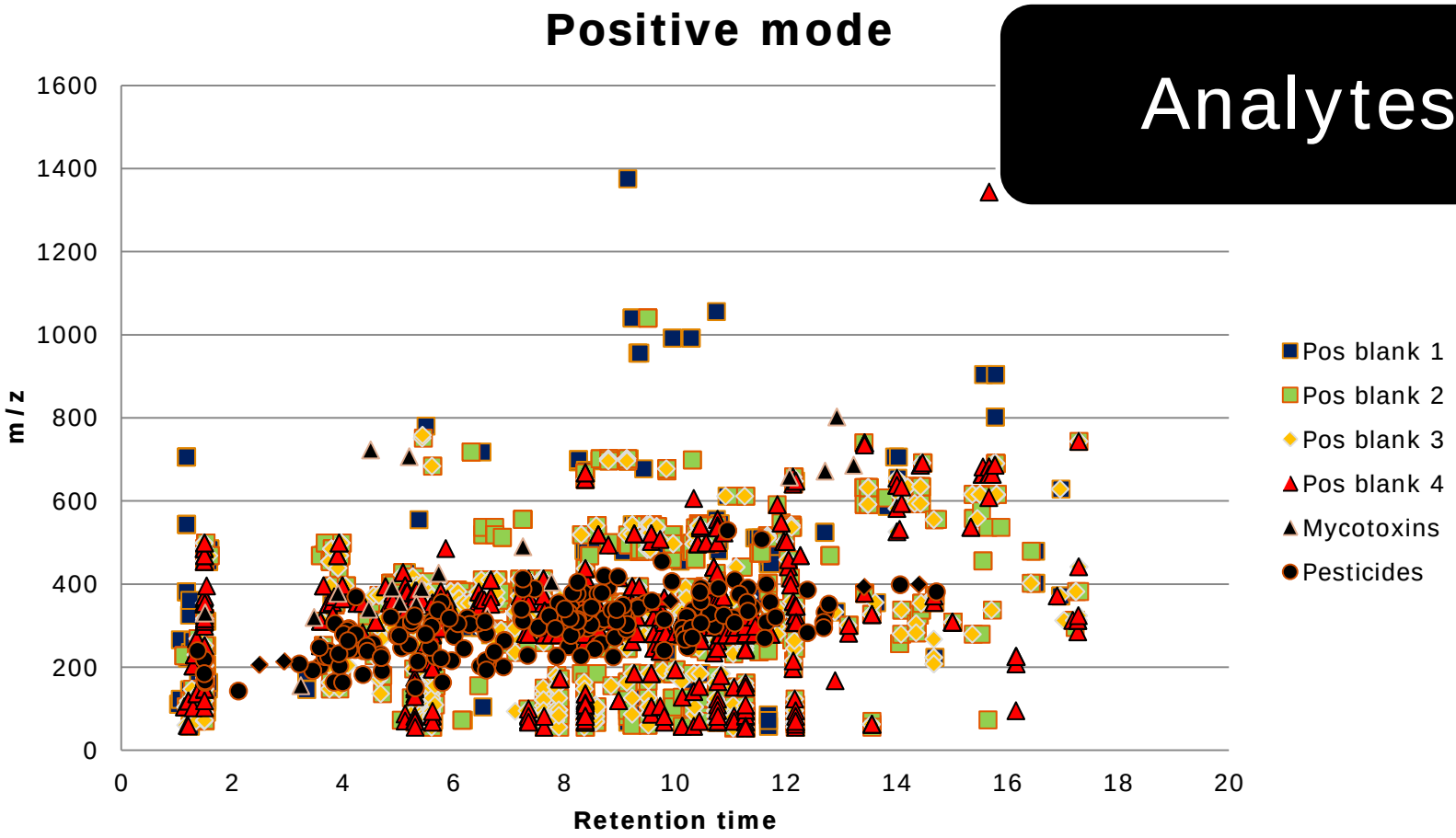


Freeze out step

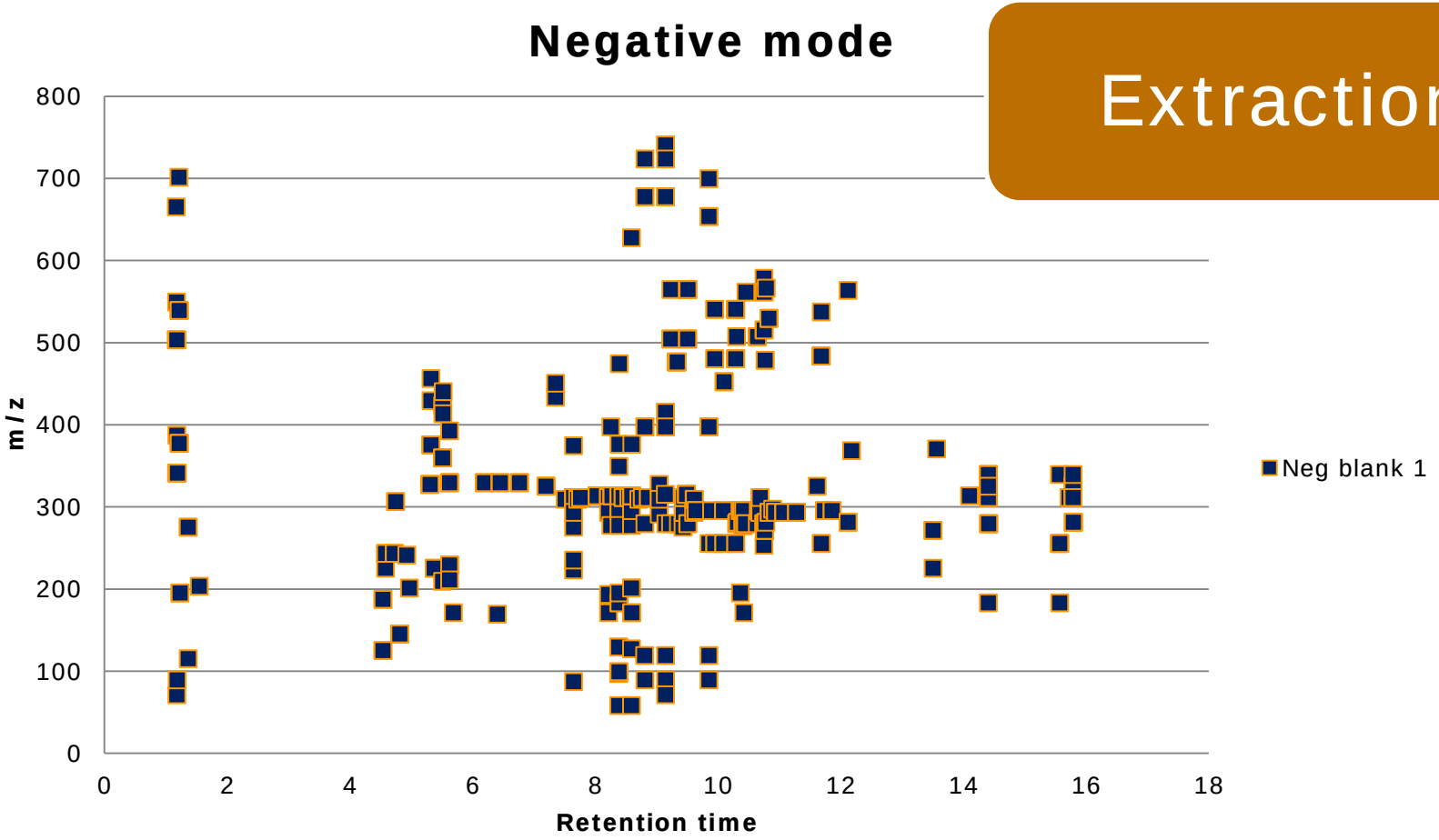
PSA clean up

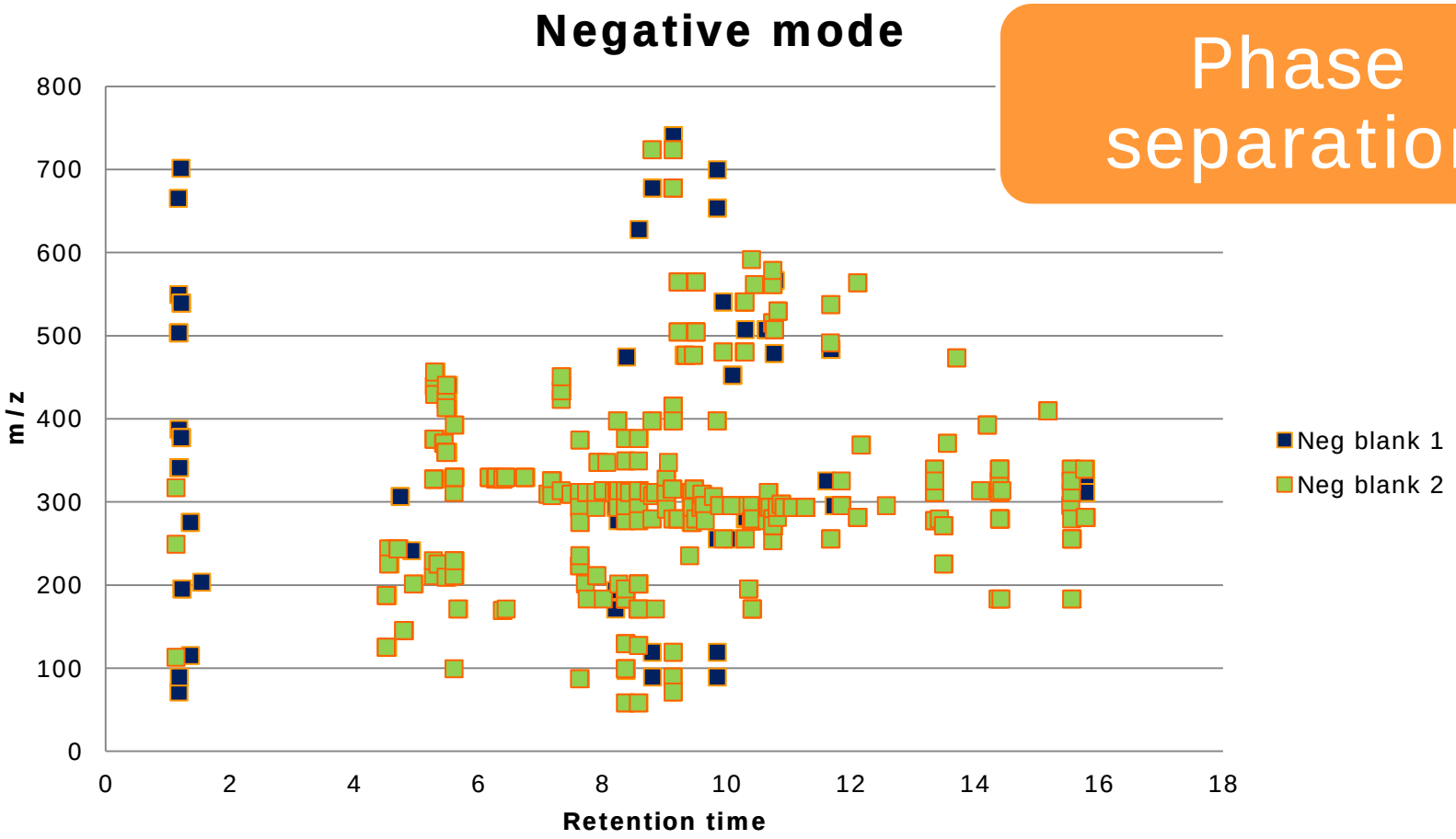

Positive mode
m/z
1600
1400
1200
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
16
18
20
Retention time
Pos blank 1
Pos blank 2
Pos blank 3
Pos blank 4

Analytes

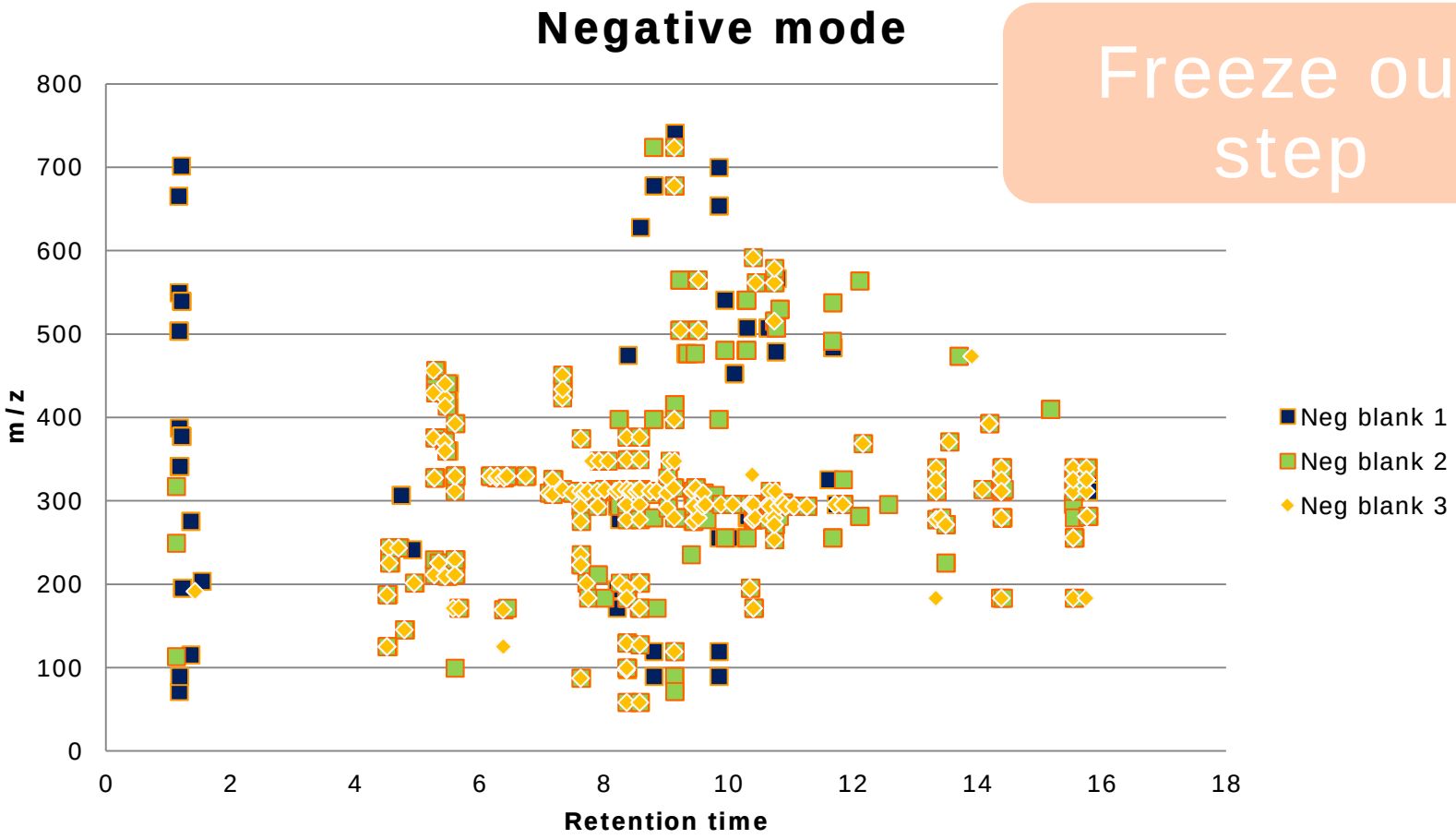

Positive mode
m/z
Retention time
0
200
400
600
800
1000
1200
1400
1600
0
2
4
6
8
10
12
14
16
18
20
Pos blank 1
Pos blank 2
Pos blank 3
Pos blank 4
Mycotoxins
Pesticides

Extraction

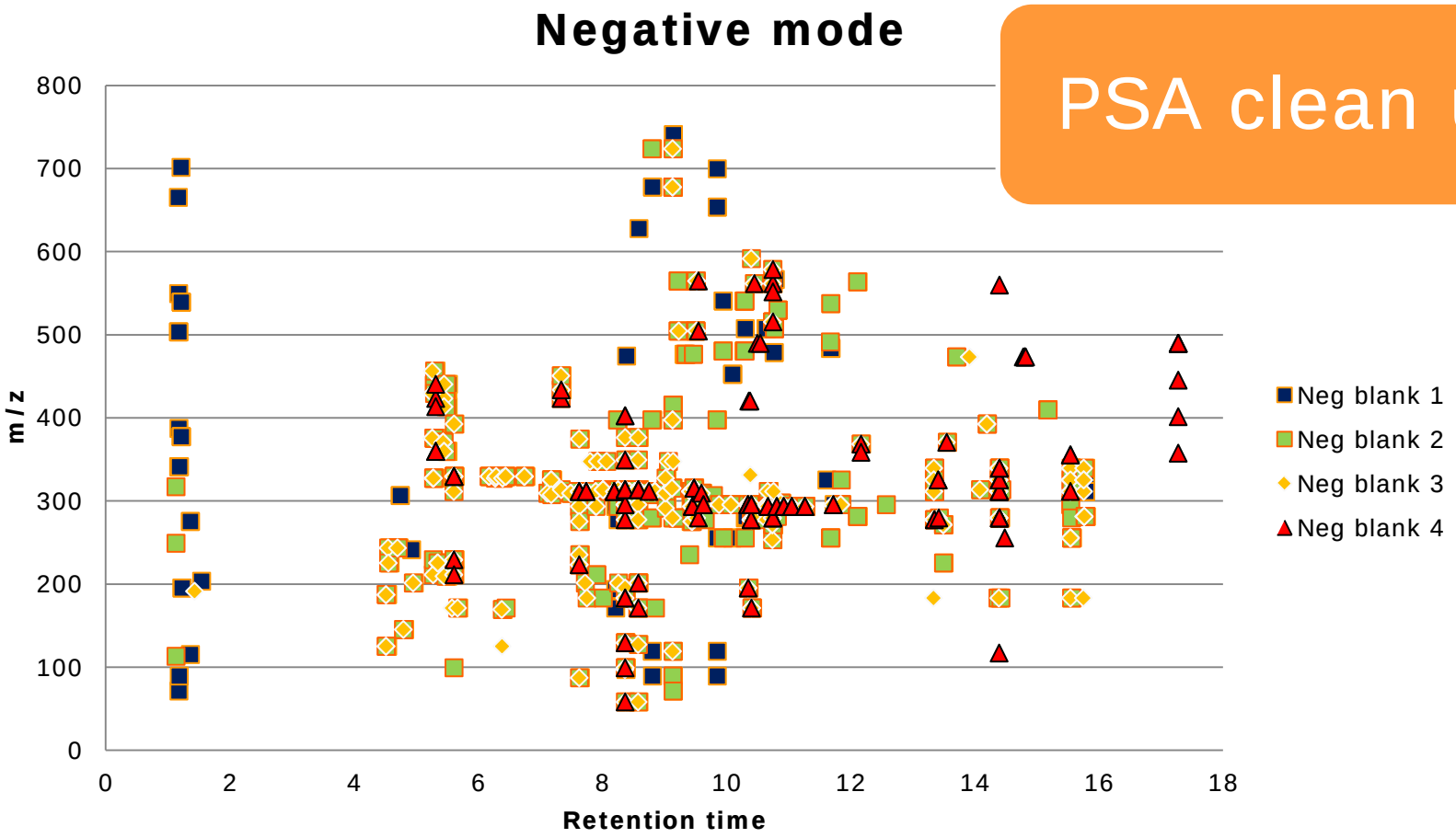

Negative mode
800
700
600
500
400
300
200
100
0
m/z
0
2
4
6
8
10
12
14
16
18
Retention time
Neg blank 1



Phase separation

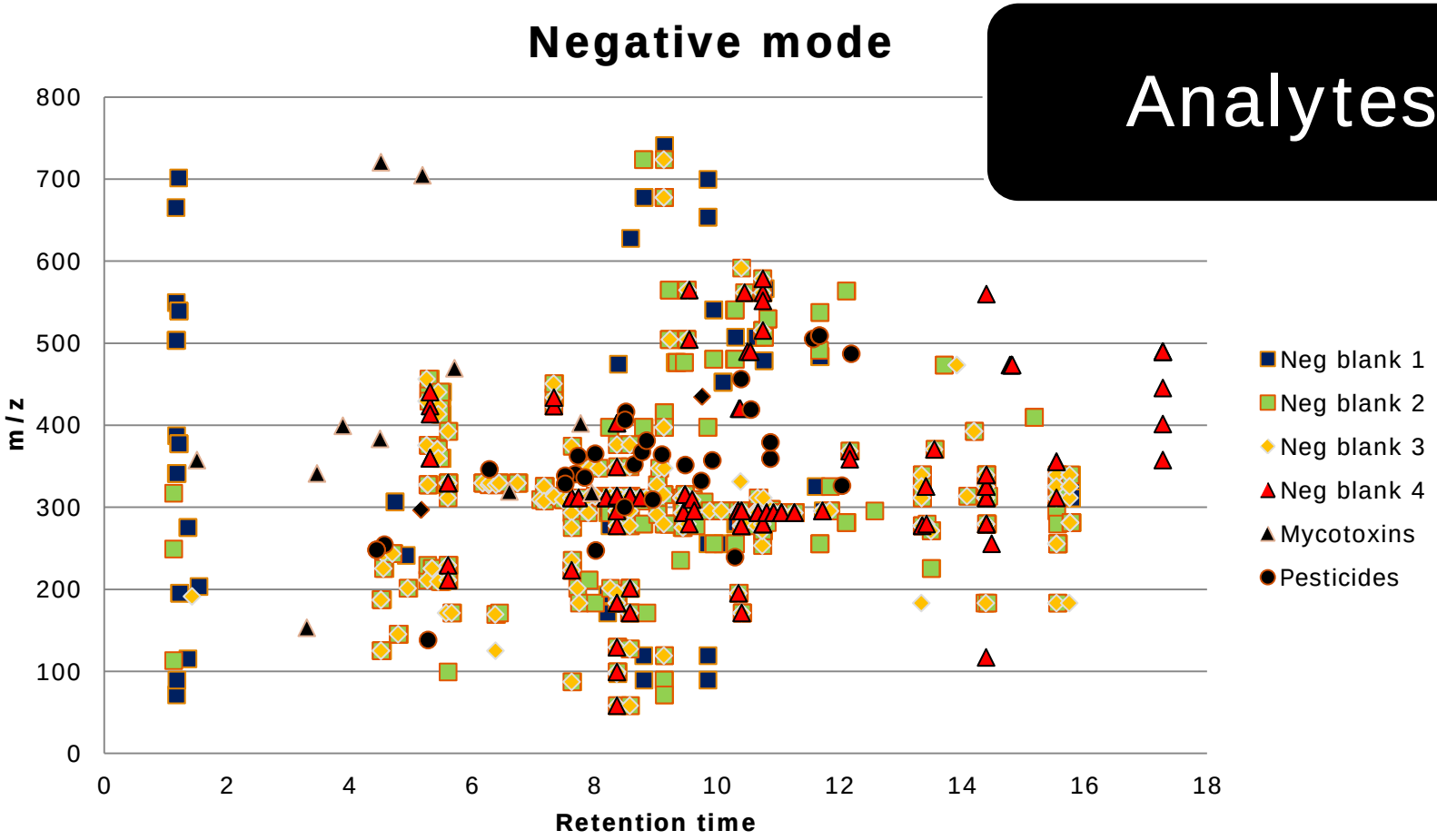


Freeze out step

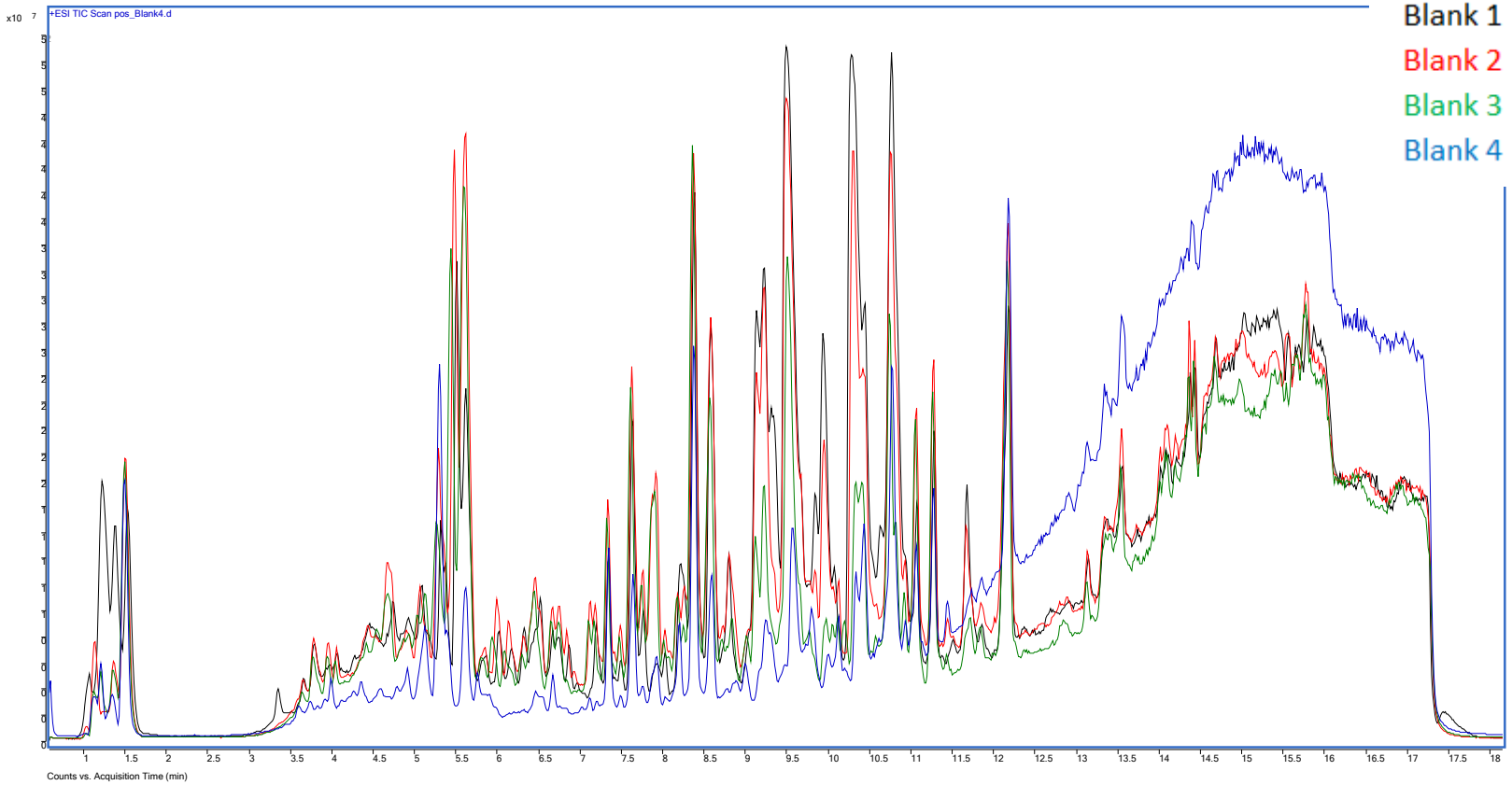

Negative mode
m/z
800
700
600
500
400
300
200
100
0
0
2
4
6
8
10
12
14
16
18
Retention time
Neg blank 1
Neg blank 2
Neg blank 3
Neg blank 4

PSA clean up

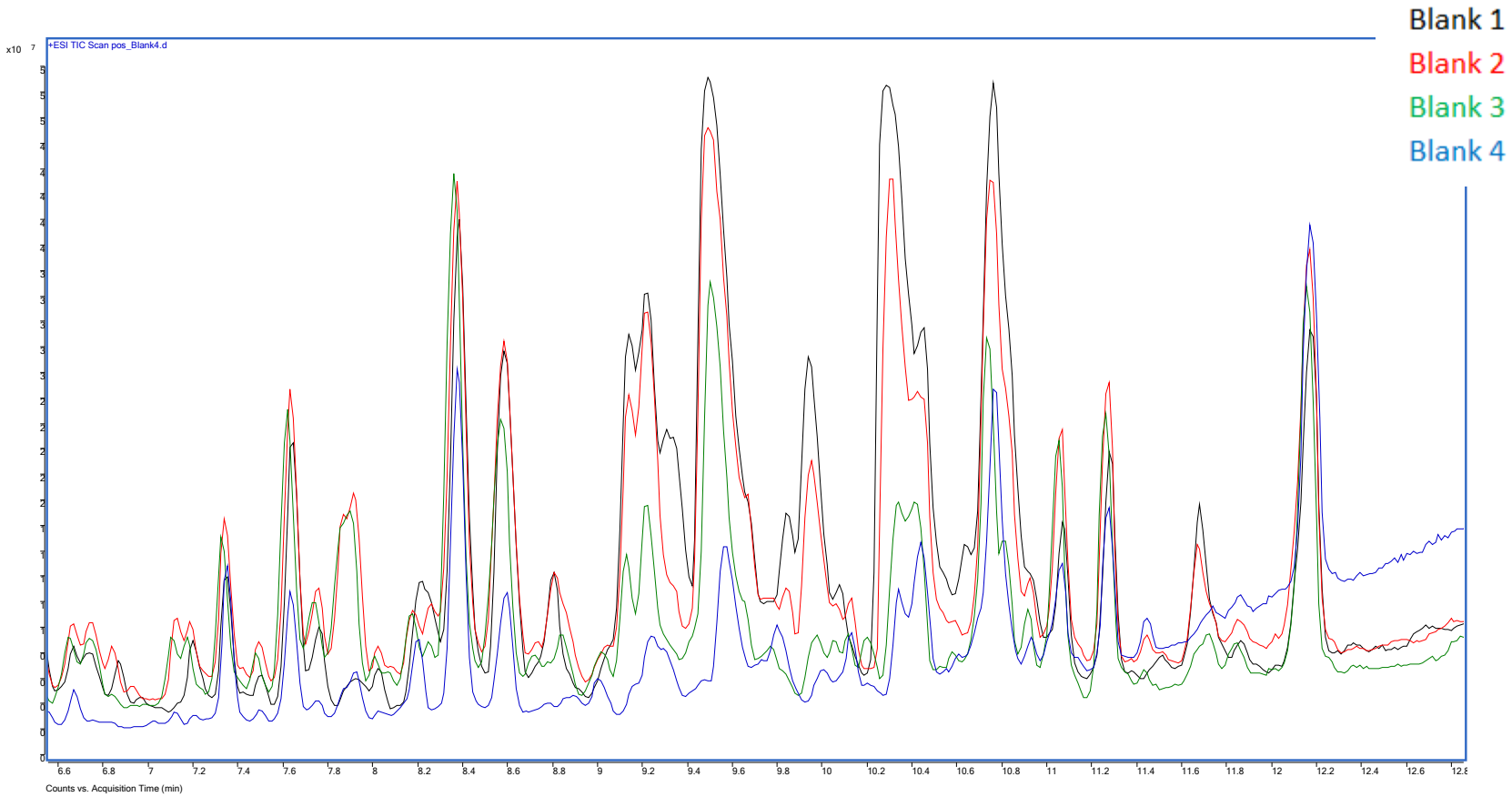
Analytes


Negative mode
m/z
Retention time
0
100
200
300
400
500
600
700
800
0
2
4
6
8
10
12
14
16
18
Neg blank 1
Neg blank 2
Neg blank 3
Neg blank 4
Mycotoxins
Pesticides

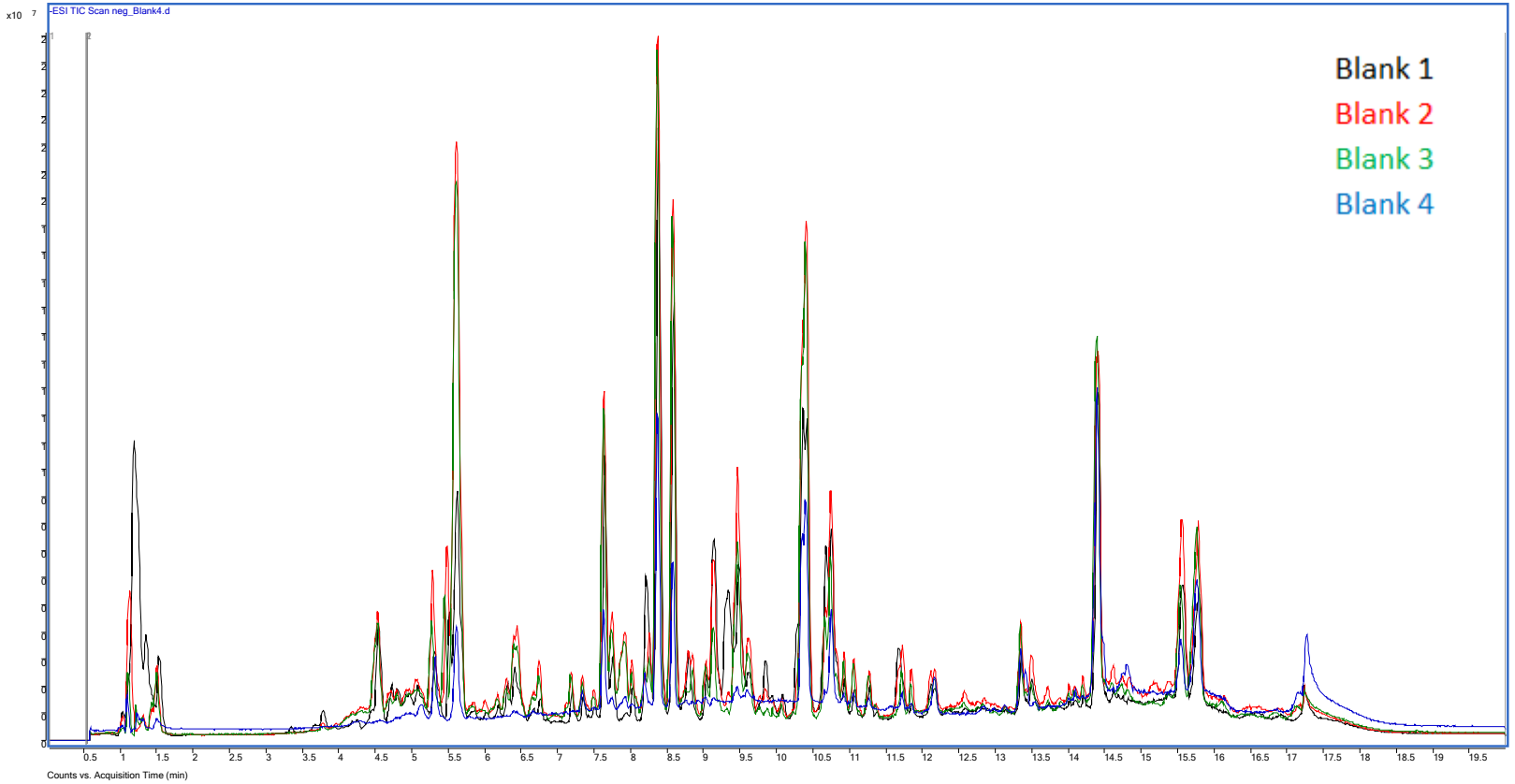
TIC af blank i positiv mode



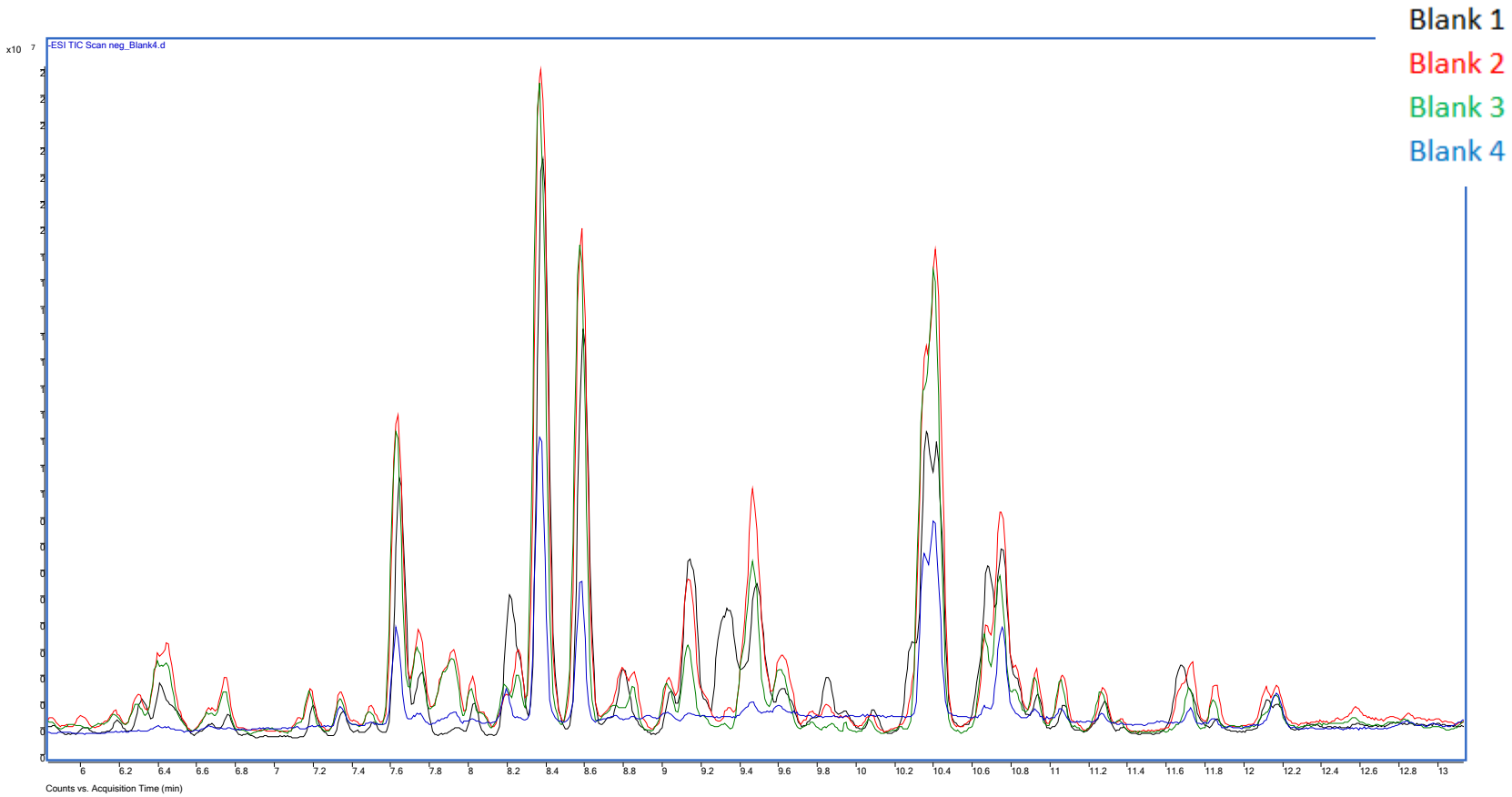
TIC af blank i positiv mode



TIC af blank i negativ mode

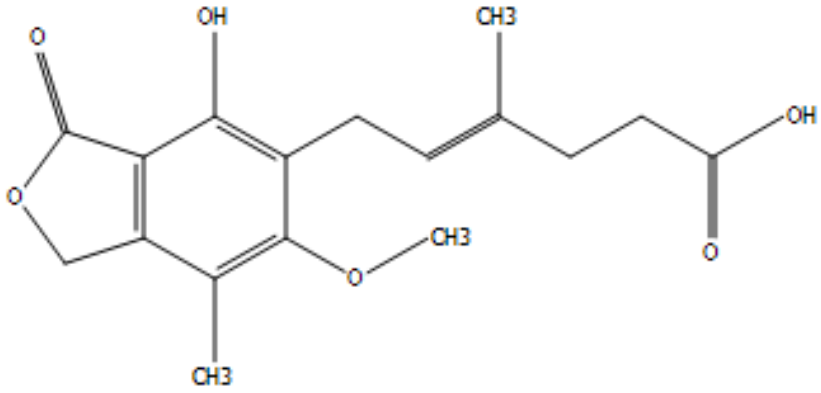


TIC af blank i negativ mode



Matrix effect & recovery – Mycophenol acid

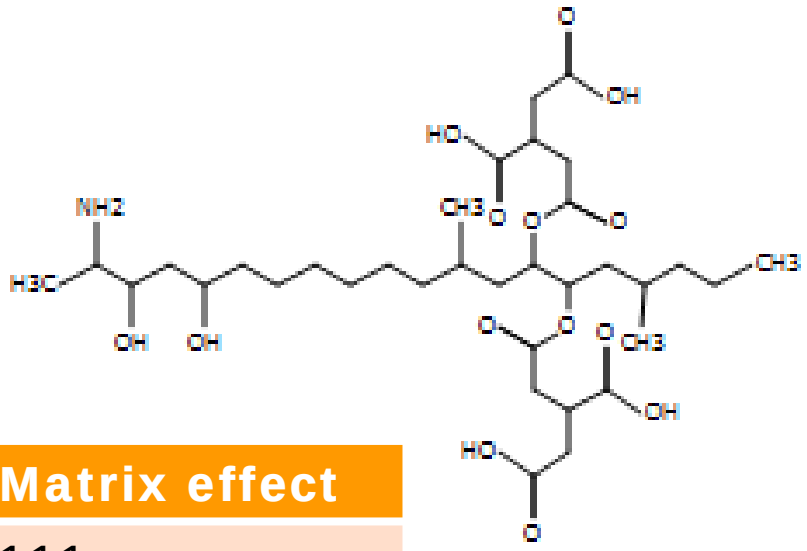
- Mycophenol acid
- 6.63 min
- Mass: 320.1260



	Recovery	Matrix effect
PSA	7	106
Freeze out	114	153
Phase separation	105	149
Extraction	123	159

Matrix effect & recovery – Fumonisin B2

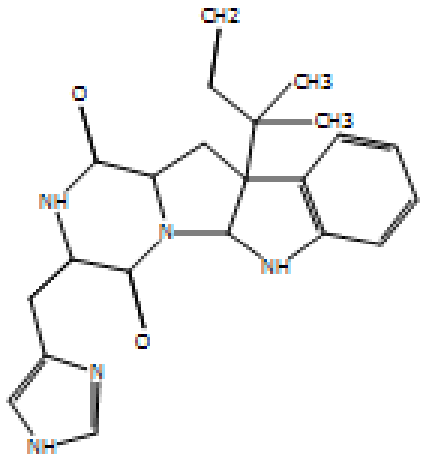
- Fumonisin B2
- 5.07 min
- Mass: 705.3936



	Recovery	Matrix effect
PSA	6	111
Freeze out	36	151
Phase separation	73	166
Extraction	76	153

Matrix effect & recovery – Roquefortin C

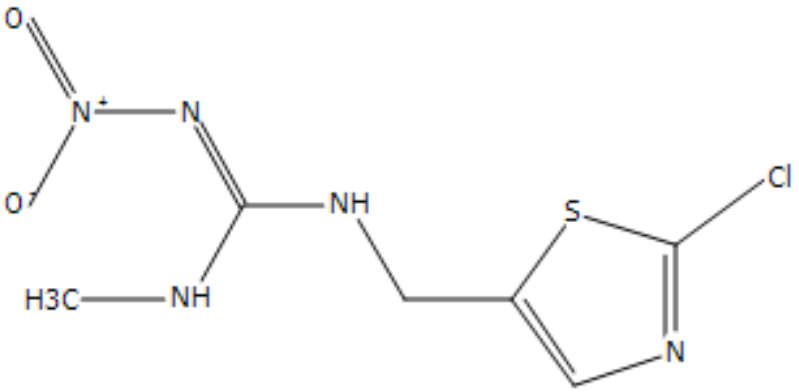
- Roquefortin C
- 5.15 min
- Mass: 389.1852



	Recovery	Matrix effect
PSA	92	75
Freeze out	92	41
Phase separation	101	38
Extraction	153	31

Matrix effect & recovery – Clothianidin

- Clothianidin
- 4.46 min
- Mass: 249.0087



	Recovery	Matrix effect
PSA	89	83
Freeze out	95	61
Phase separation	76	57
Extraction	153	32