Chemical Warfare Agents by LC-MS/MS

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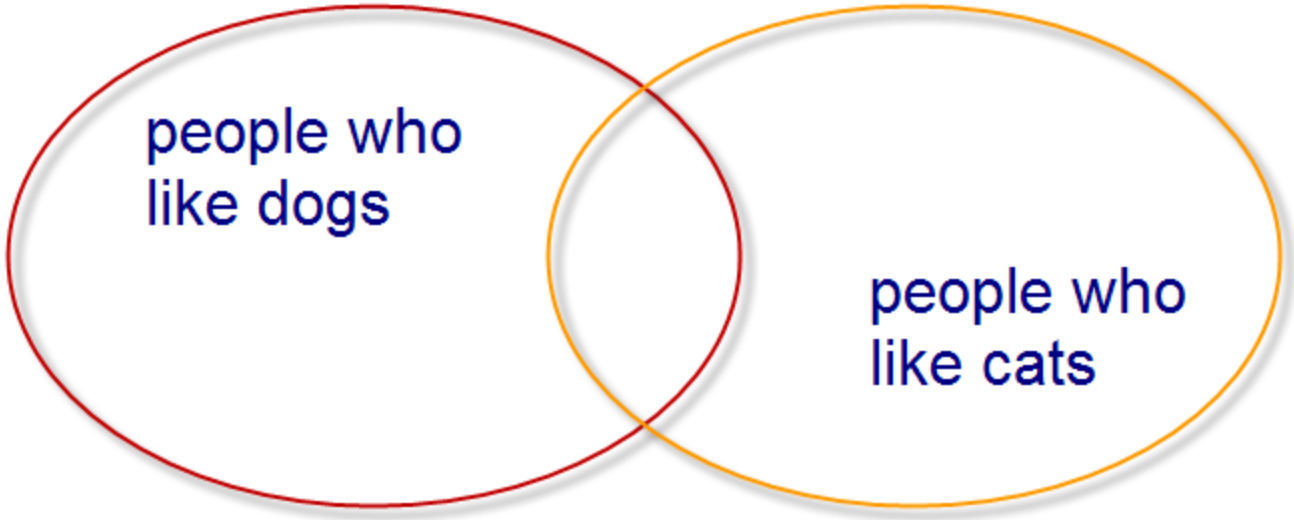
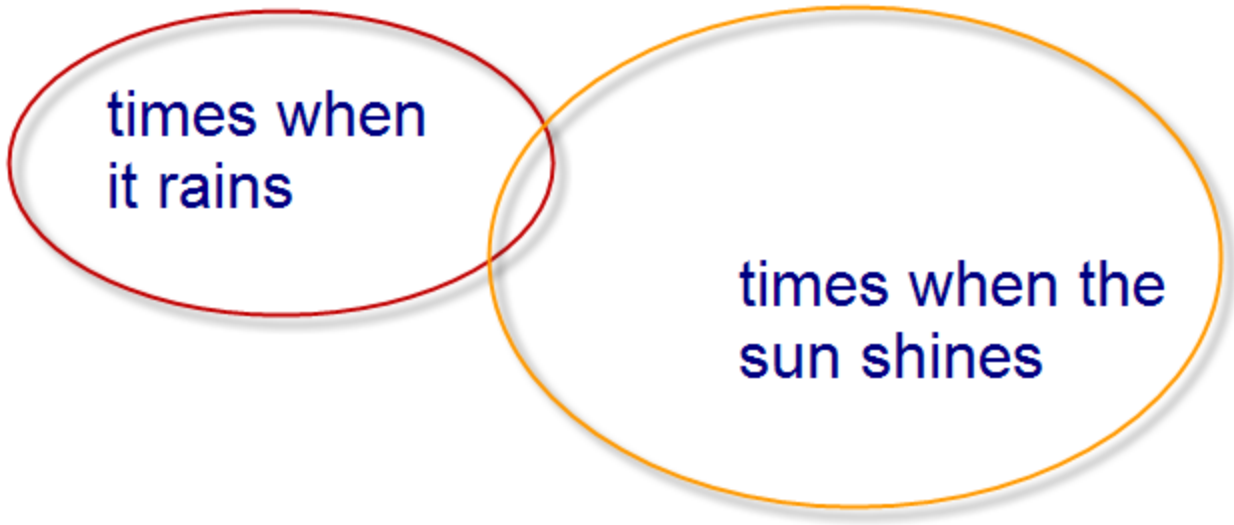
Working on the base in Suffield

- Tried to get a picture on the base...

Venn Diagram #1

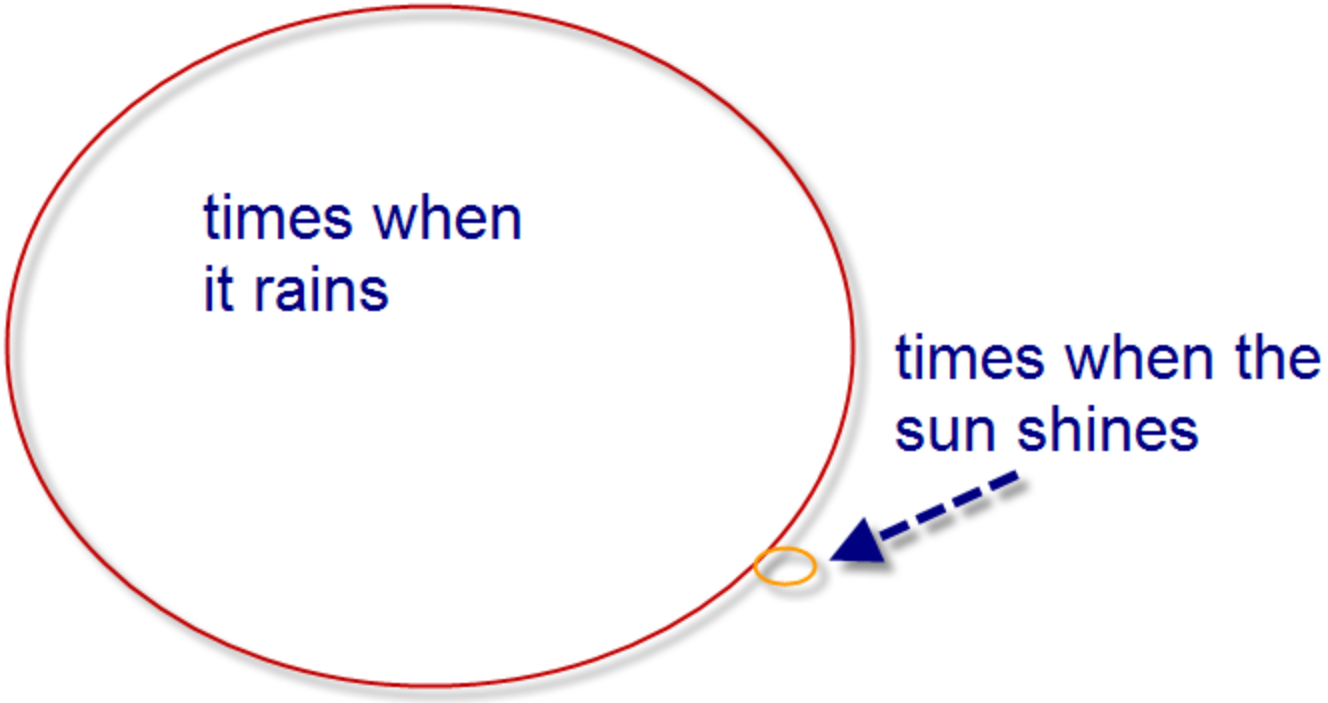


Venn Diagram #2

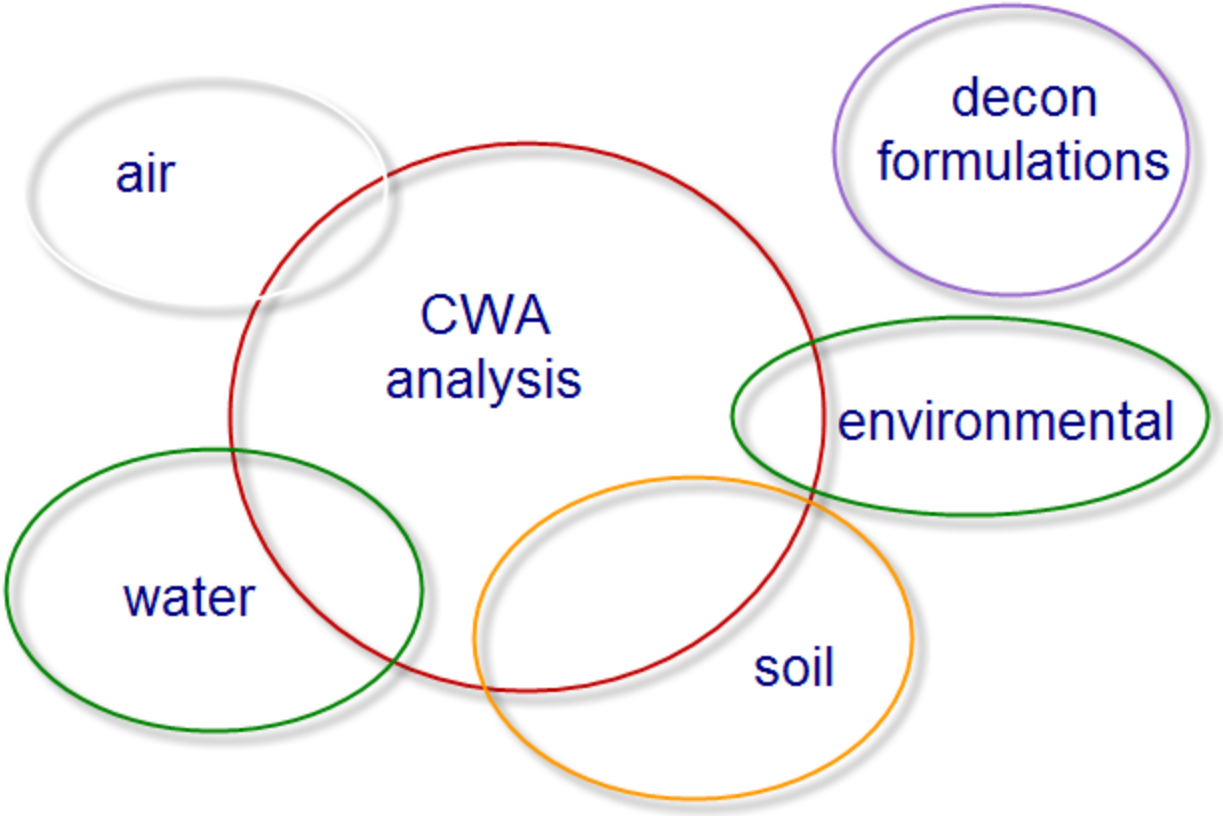


Venn Diagram #2

Vancouver version



CWA Venn Diagram



CWA Method of Analysis

- many methods for CWA
- historical methods using GC-MS, derivatization required for breakdown products
- more recently, LC-MS methods allowing detection without derivatization

CWA Method of Analysis - 2

- existing methods for
  - environmental samples (water, soil, extracts of surfaces)
  - biological fluids
- needed a method for CWAs in decontamination formulations

Decontamination Formulations

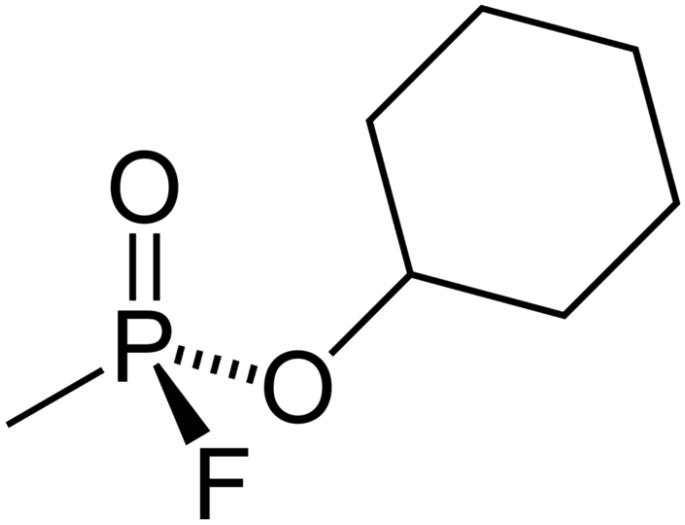
- the problem – “real time” analysis of agent in complex matrix
- the matrix – solvent of some type to dissolve agent; reactive ingredient; possible counter ion (e.g. Na)

Decontamination Formulations - 2

- need to deactivate the active ingredient quickly to capture a agent concentration in solution
  → stop the reaction
- direct analysis by “dilute and shoot”
  LC-MS/MS should be perfect

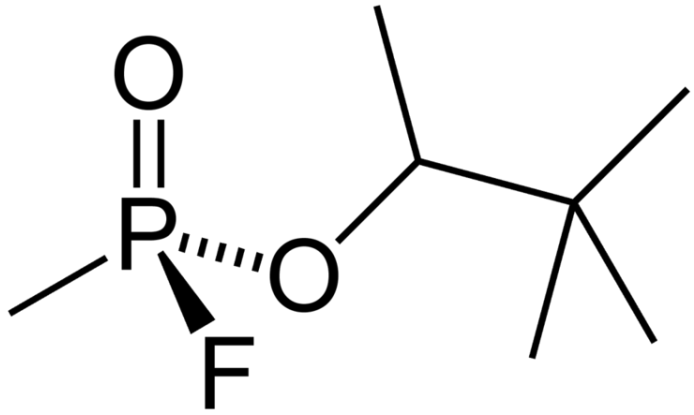
G Agents - GF

- Cyclohexyl sarin
- Cyclohexyl methylphosphonofluoridate



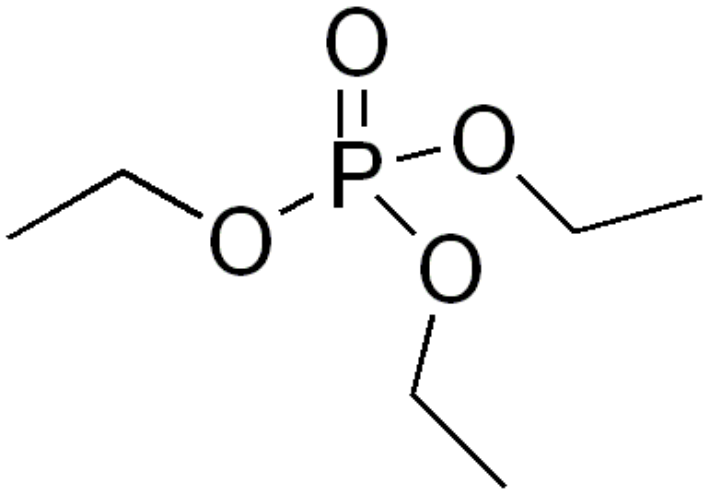
G Agents - GD

- Soman
- o-Pinacolyl methylphosphonofluoridate



ISTD

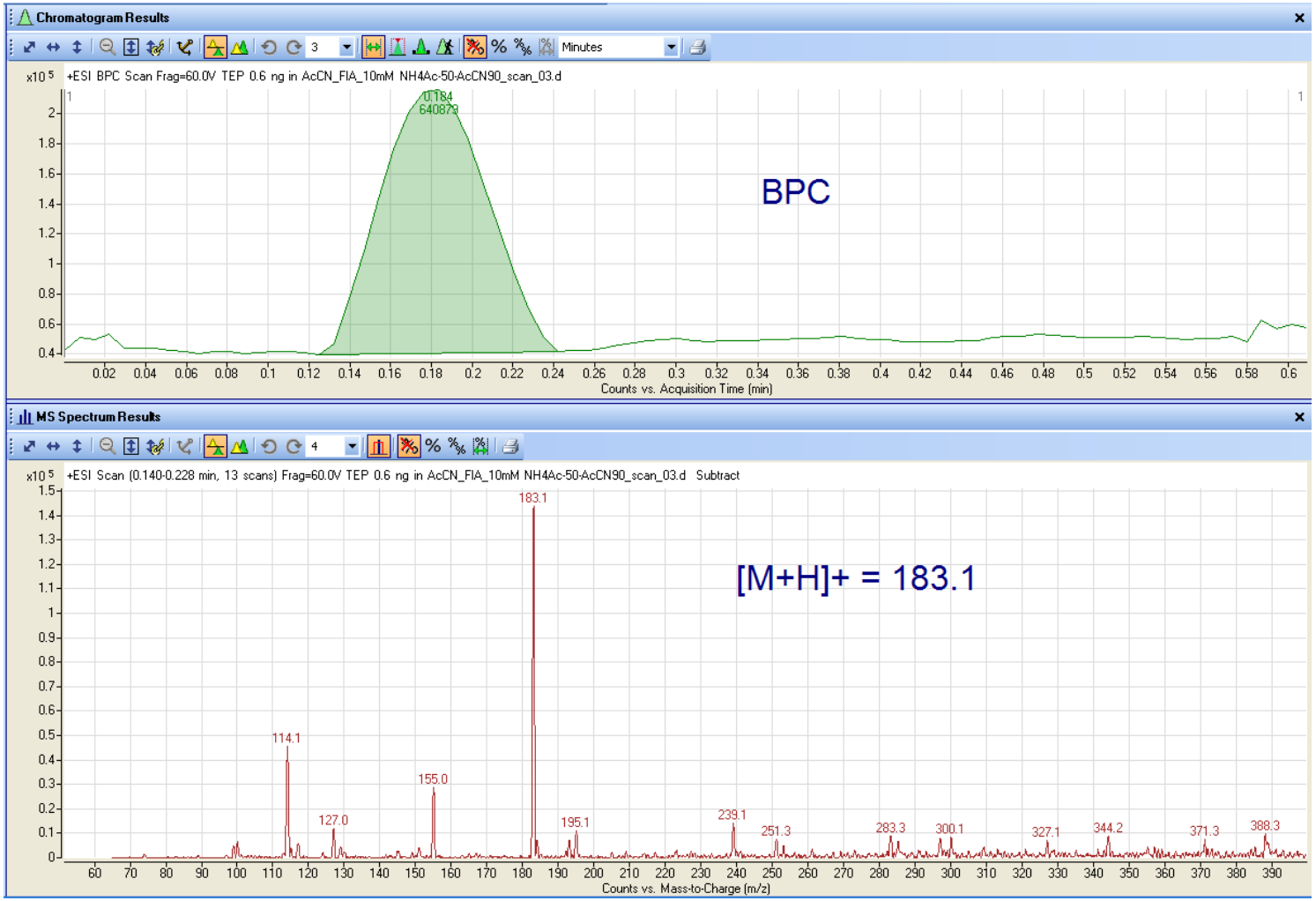
- tough to get isotopically labeled CW agents!
- TEP, Triethyl phosphate
- resistant to hydrolysis



MS/MS Optimization

- 3rd step in working with new compound is optimization of MS/MS parameters
- 2nd step: run full scan
  → do I have what I think I have in the vial?
  - also run a blank!
- 1st step?

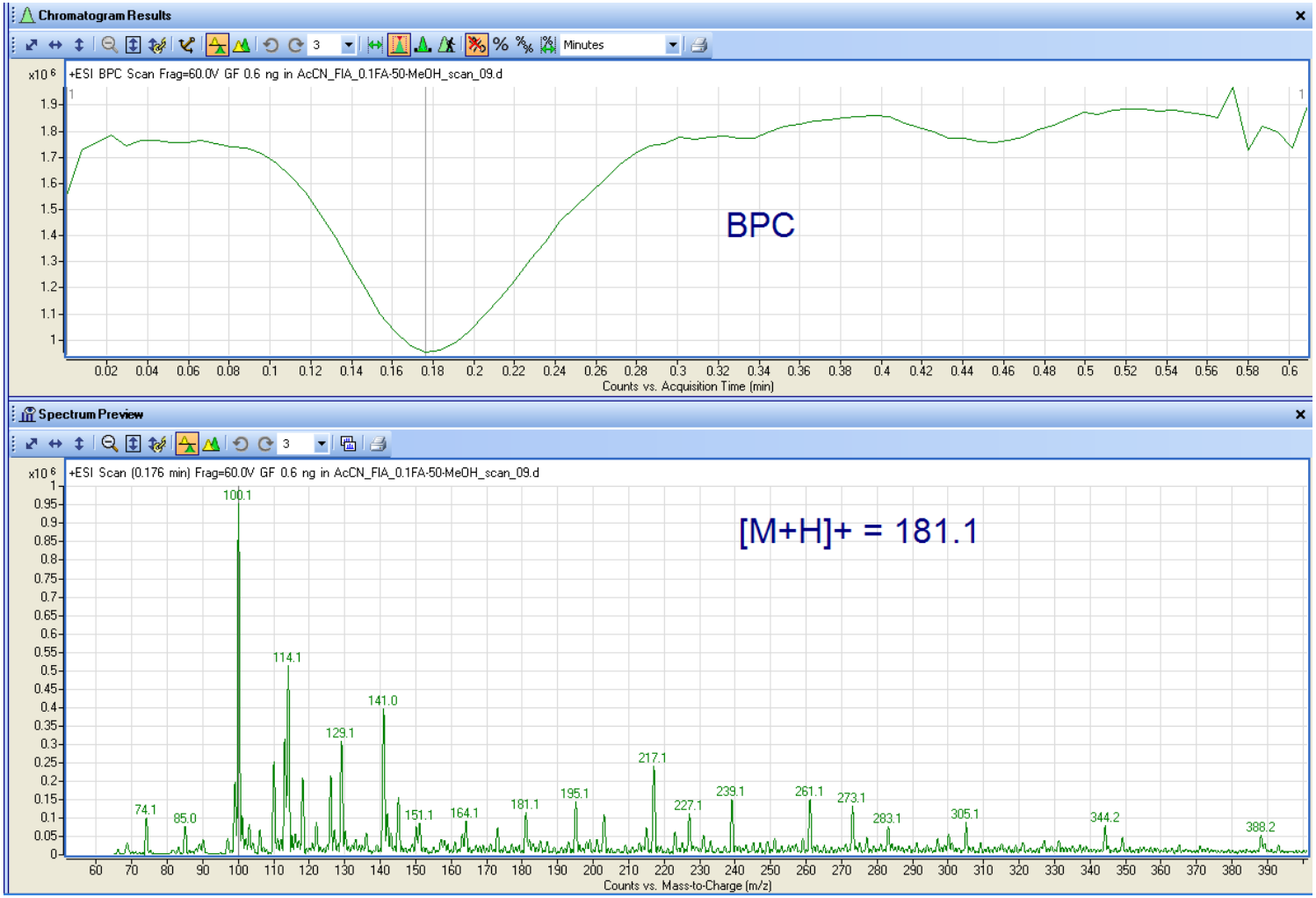
TEP in ACN - FIA



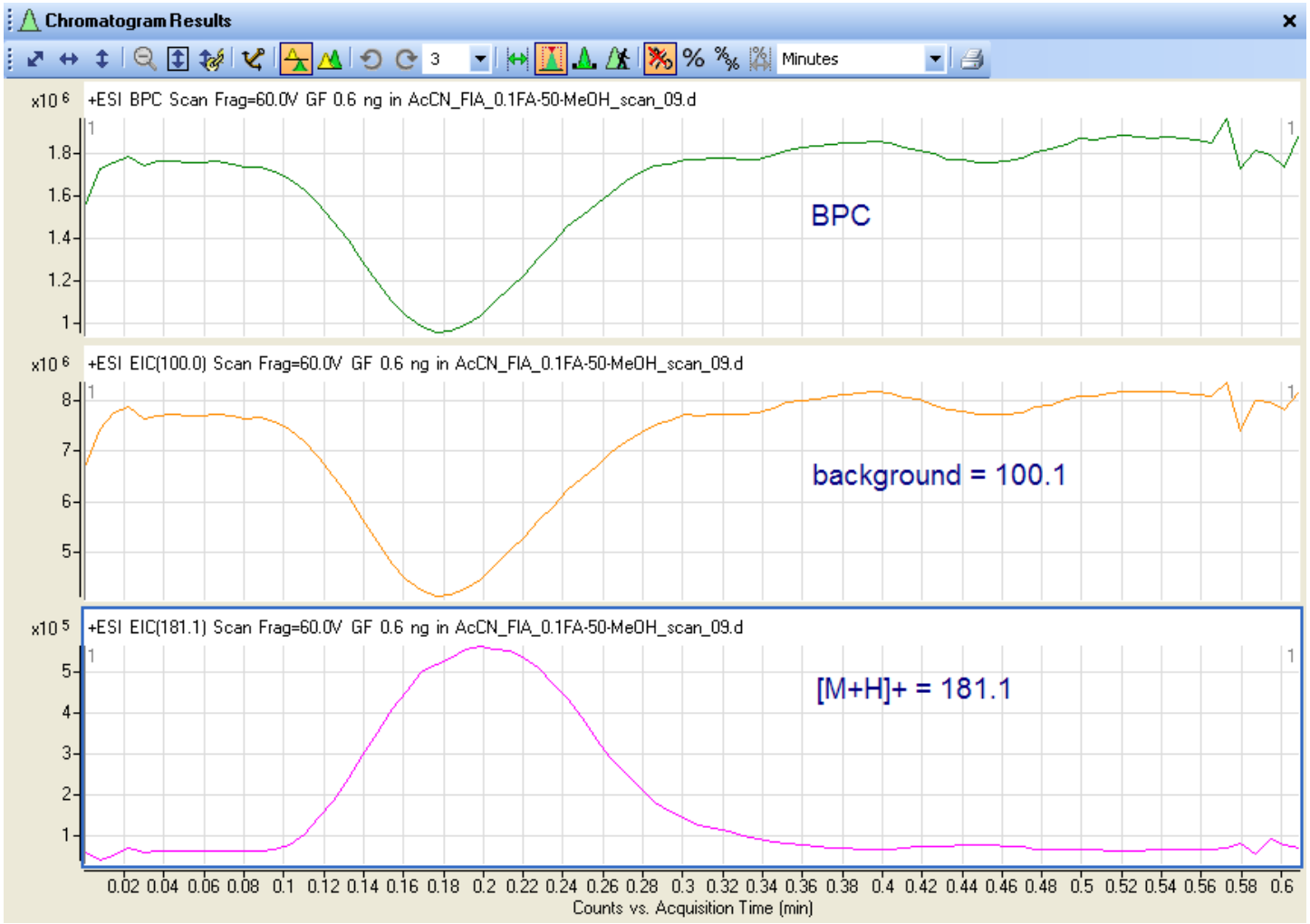
MS/MS Optimization - GF

- GF by FIA – didn't look like I had GF in the vial
  - very small $[M+H]^+$
  - lots of other ions

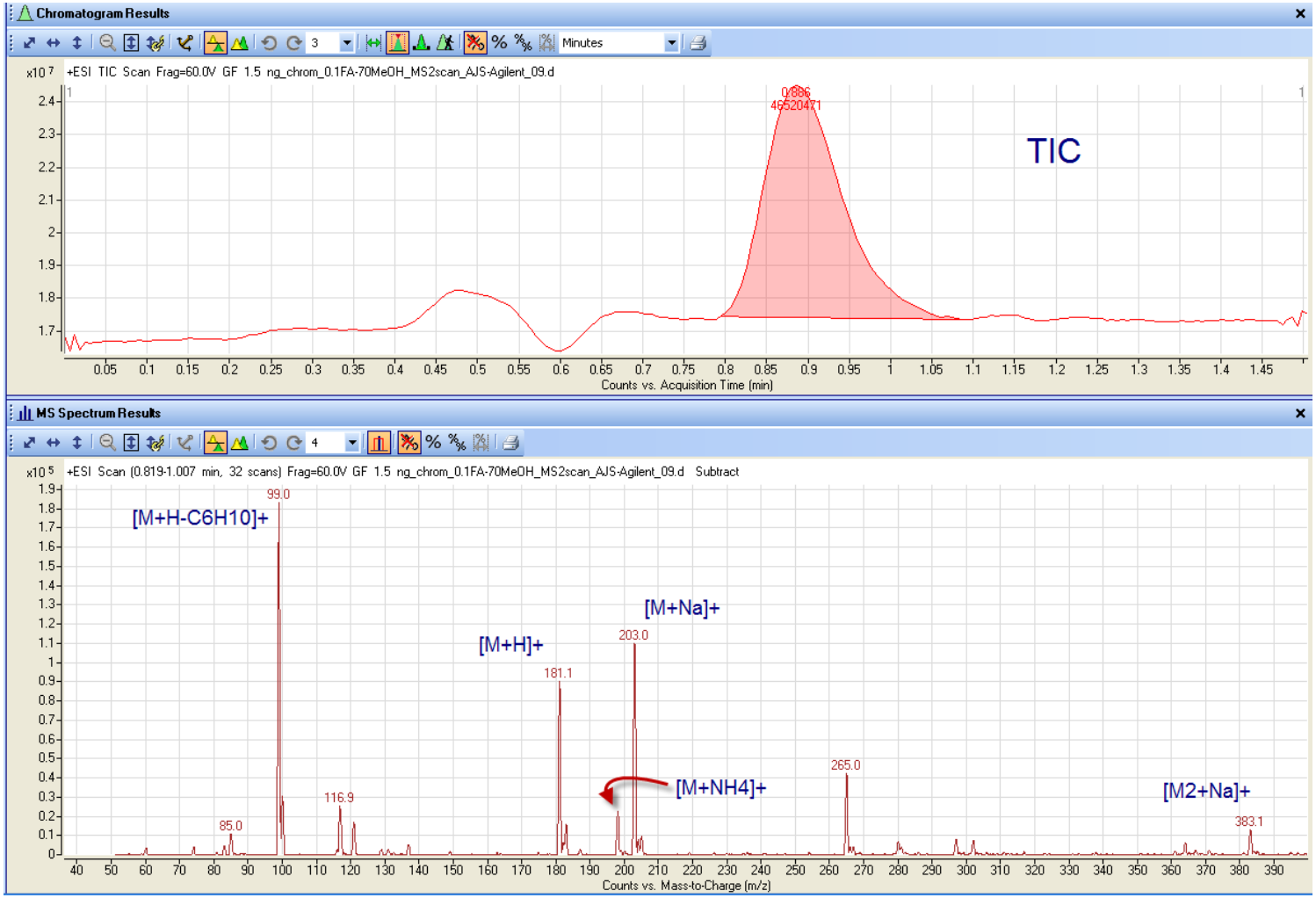
GF in ACN – flow injection analysis



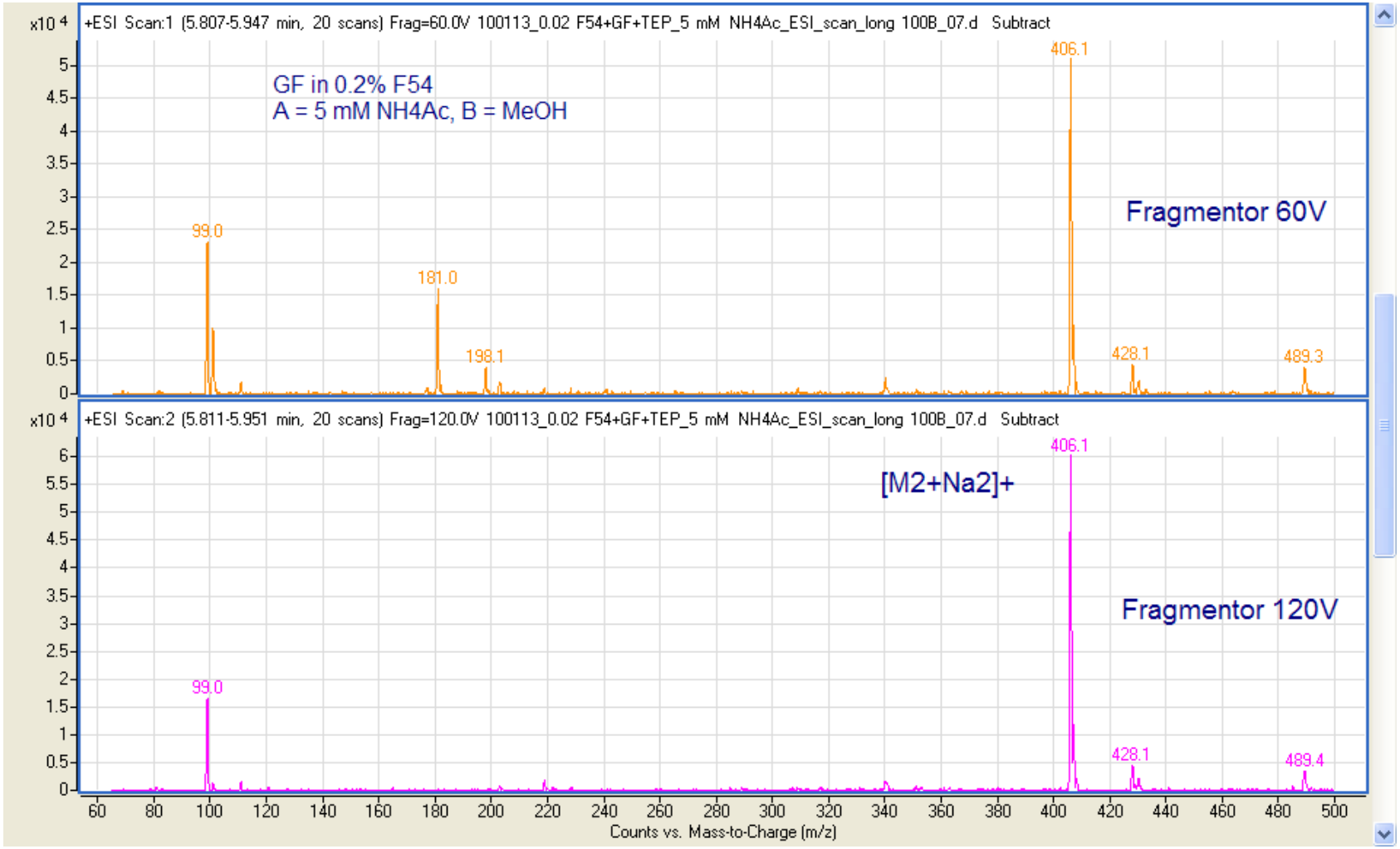
FIA – negative peaks?



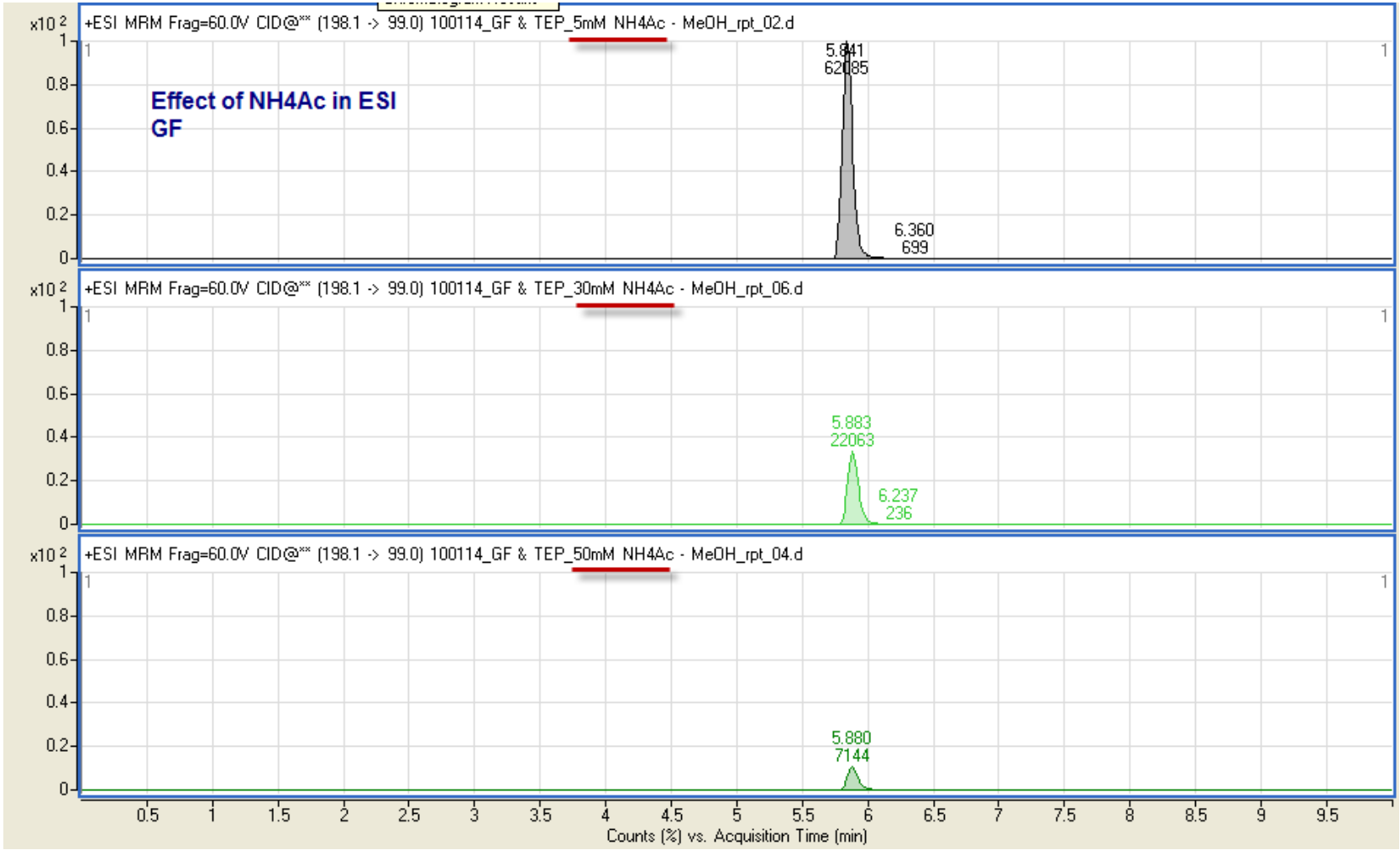
GF in ACN – fast chromatography



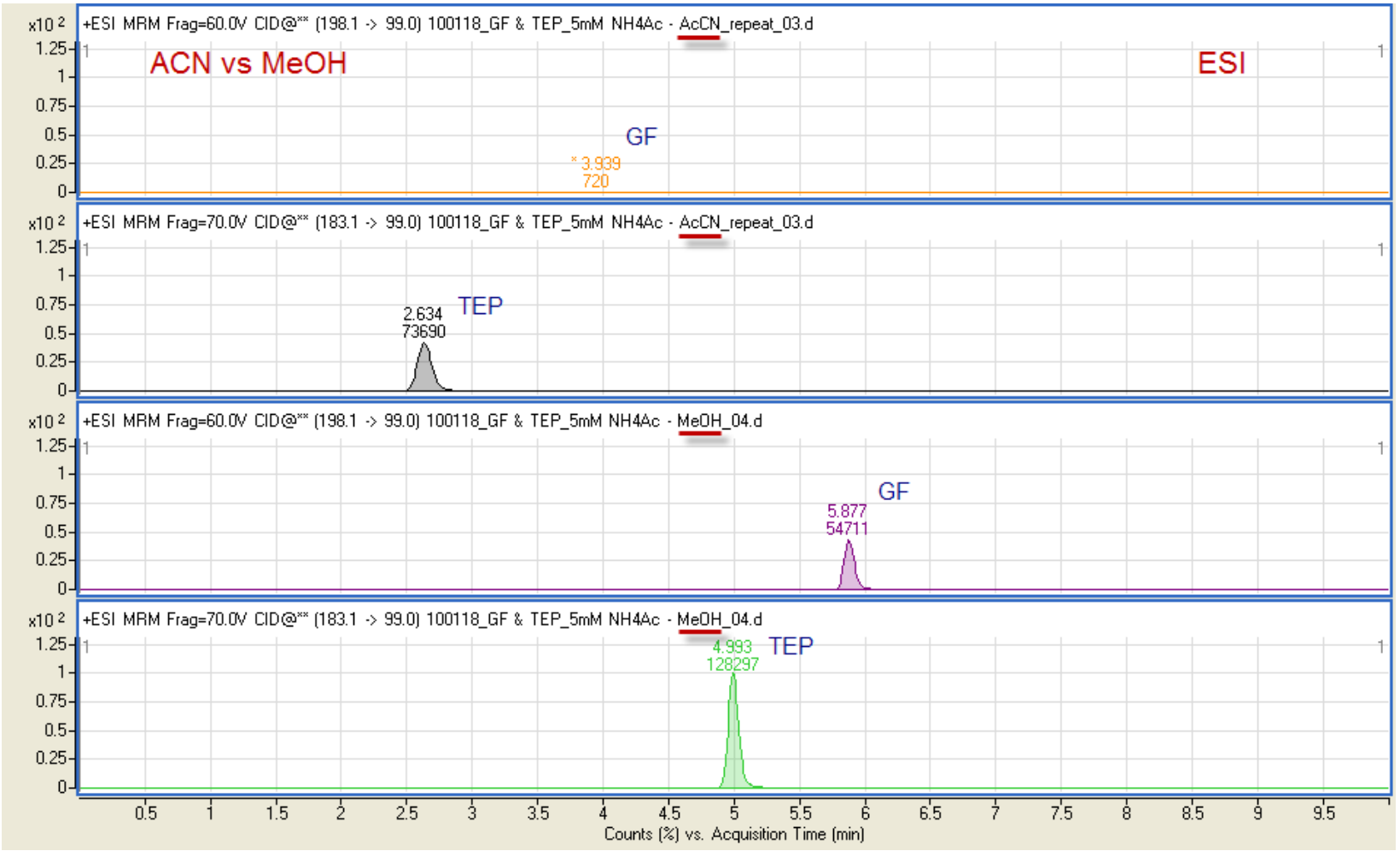
GF in decon formulation



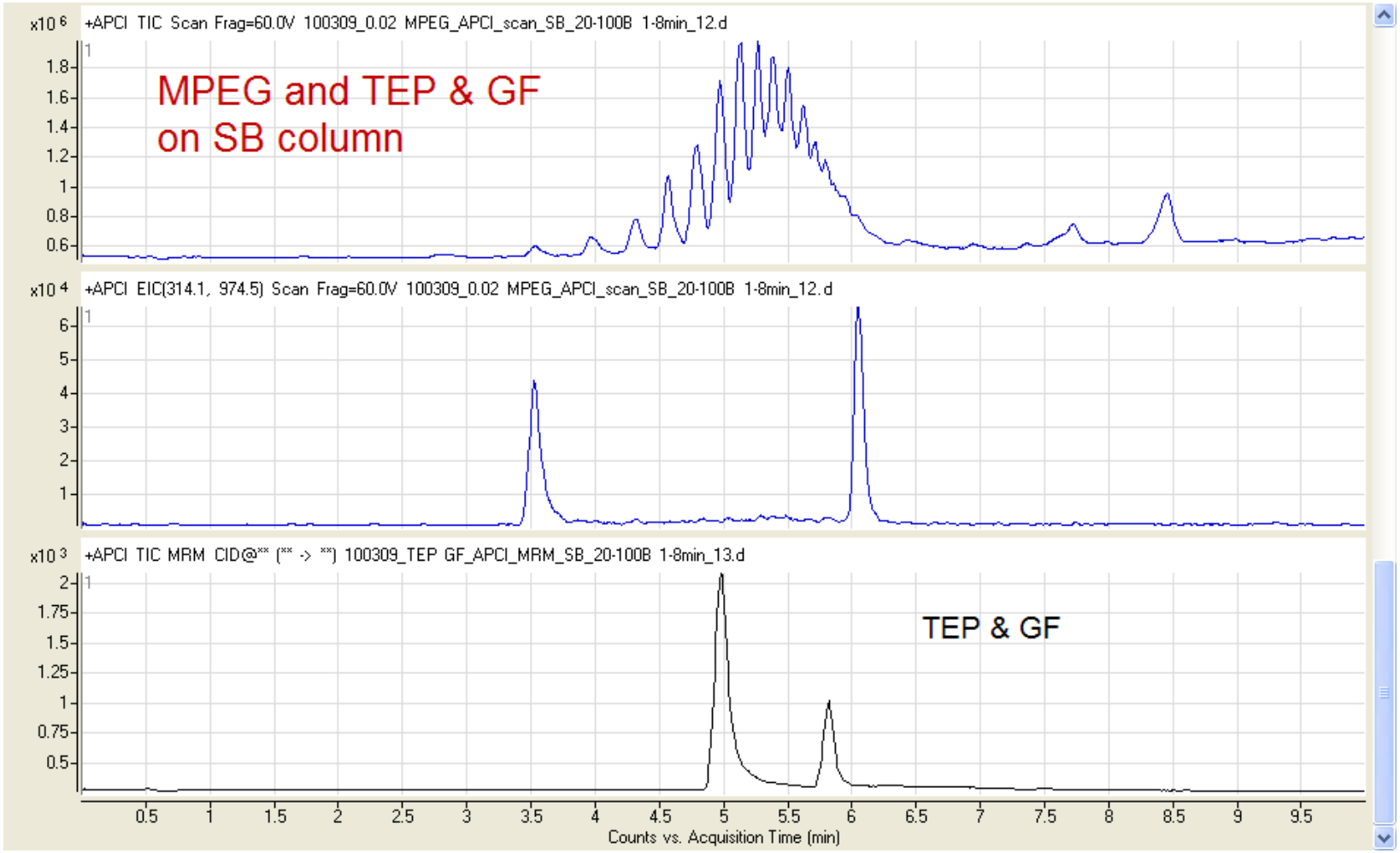
GF - Effect of NH_4Ac in ESI



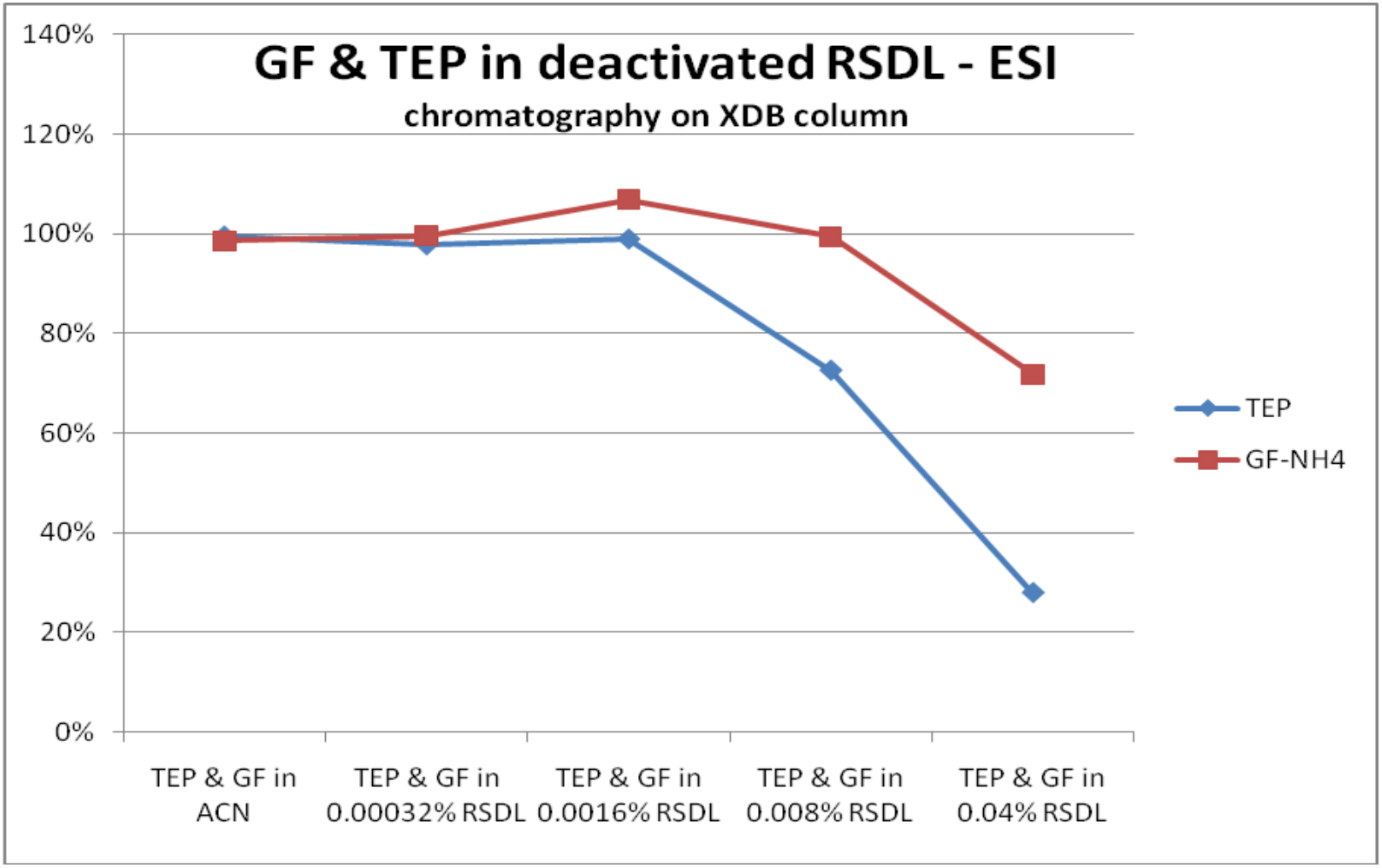
GF & TEP – Effect of MeOH vs ACN



GF & TEP in MPEG matrix



TEP – good indicator of ion suppression



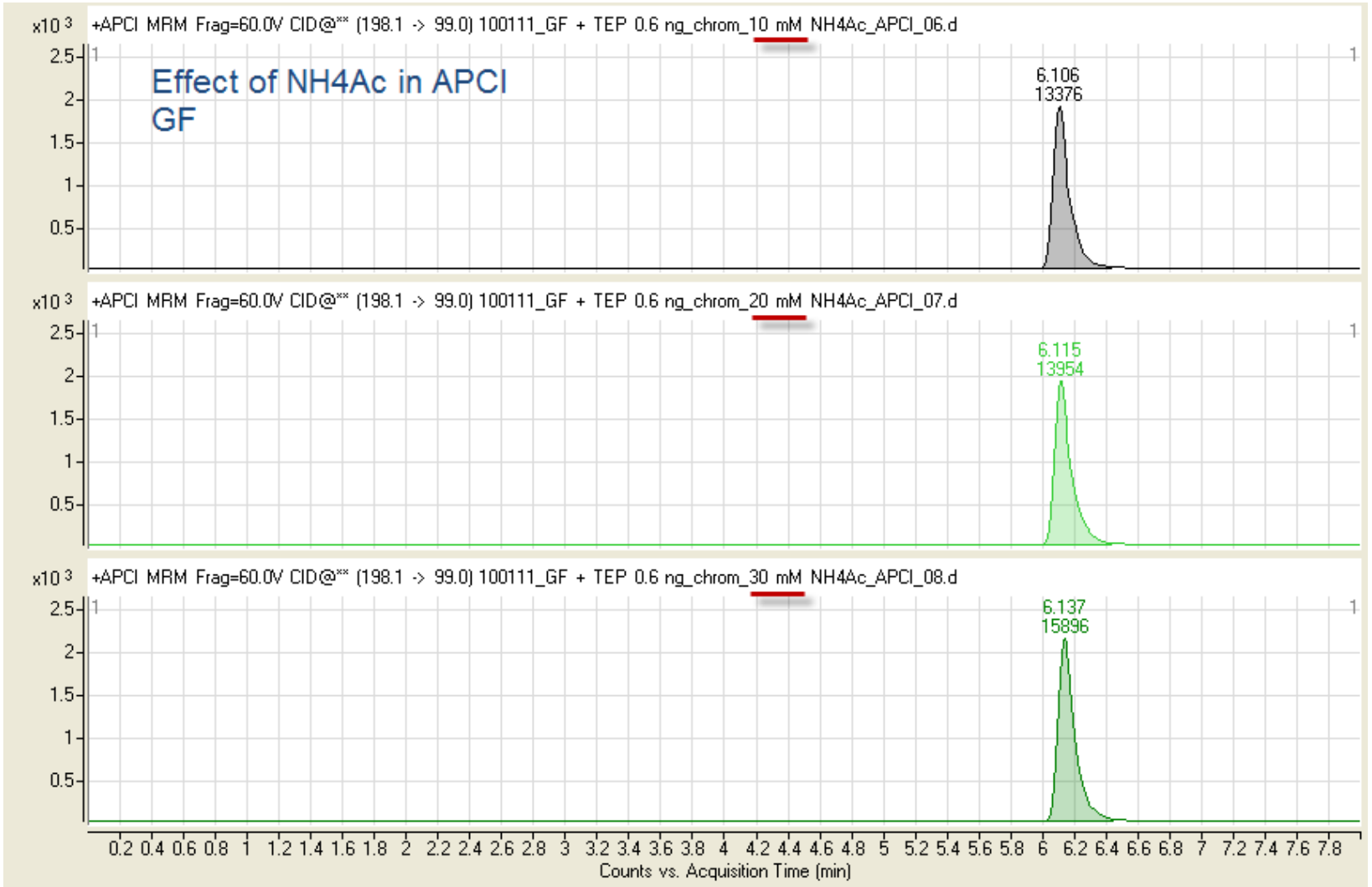
APCI vs ESI

- ESI shows greater sensitivity (AJS)
- APCI less susceptible to chromatographic additives and matrix

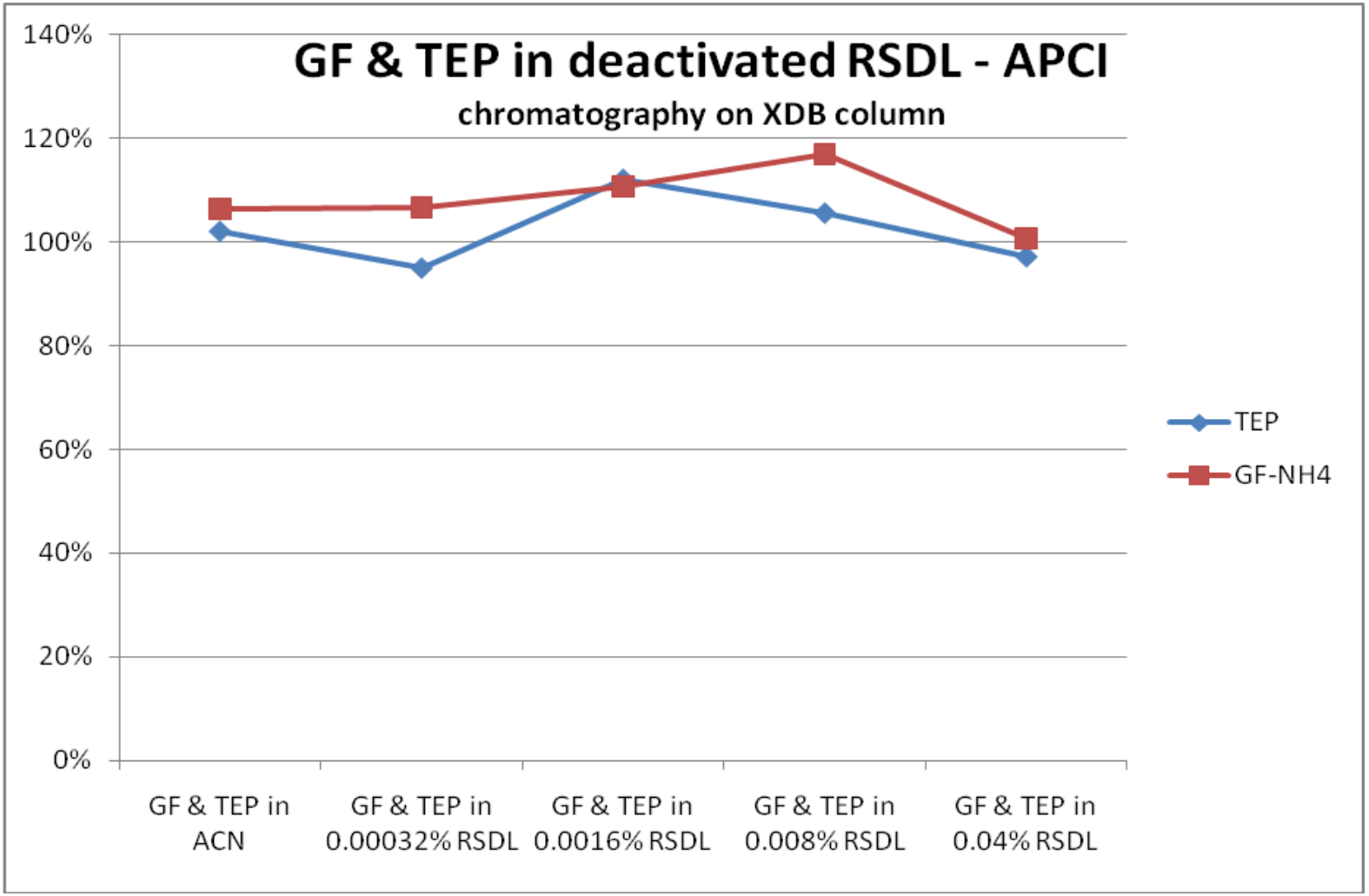
Injected	[M+NH4]+ to [M+H]+ Area Ratio	
	ESI	APCI
GF 0.0046 ng	15.7	
GF 0.023 ng	17.2	20.6
GF 0.12 ng	17.4	16.2
GF 0.6 ng	17.5	16.5
GF 3 ng	17.1	14.9
average	*17.0*	*17.0*

Relative intensity of [M+NH4]+ to [M+H]+ for GF by ESI and APCI

GF - Effect of NH_4Ac in APCI



GF - Matrix Effects in APCI



ESI vs APCI

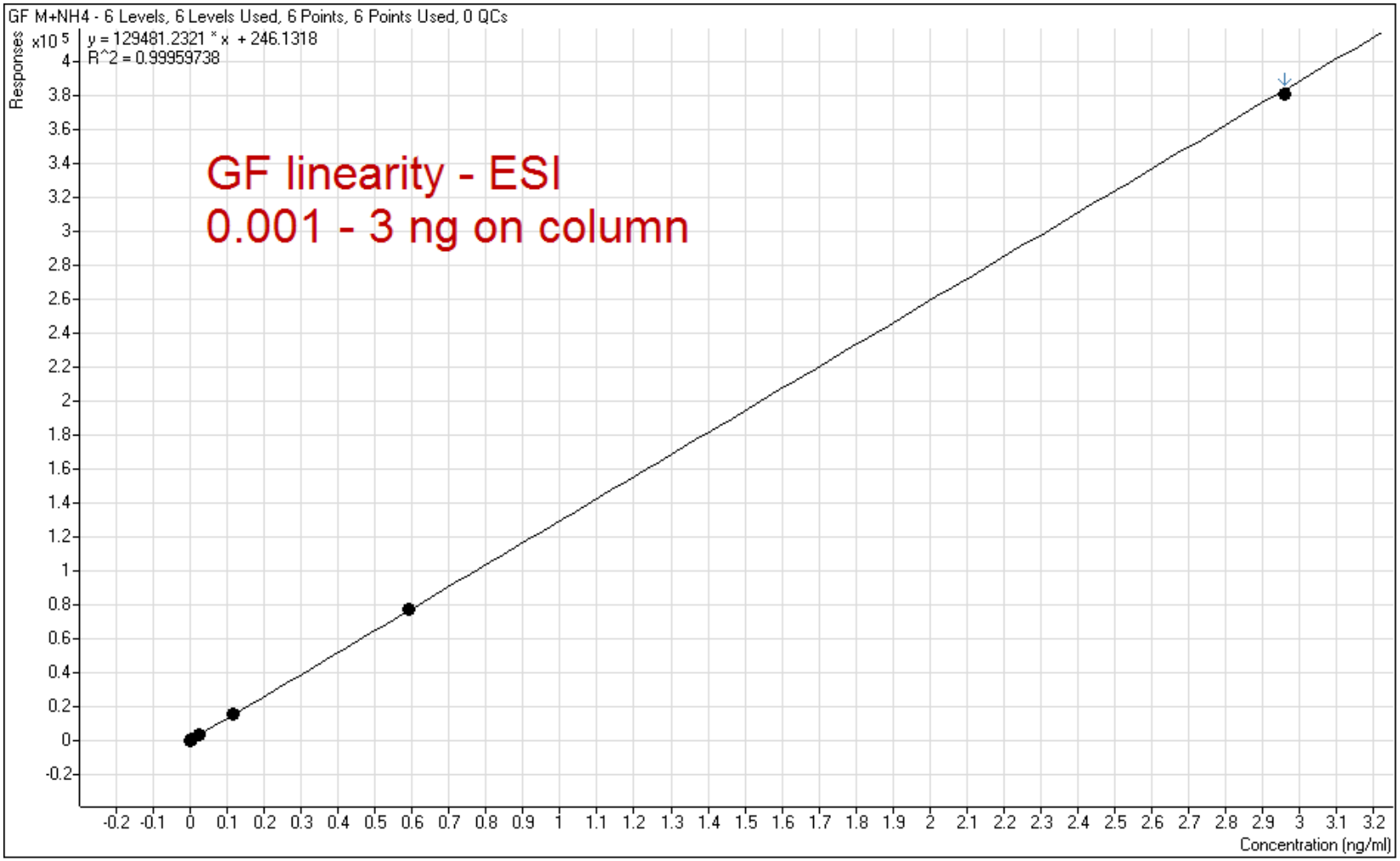
- final method chosen was ESI
  - why, if APCI shows less variability from matrix and effects chromatographic conditions?
- ESI-AJS was more precise
- dilute matrix out to level where no ion suppression is also good for decon reaction quenching
- use TEP as indicator of ion suppression – increased confidence in results

ESI vs APCI - precision

Name	Acq. Date-Time	TEP Area	GF-NH4 Area
GF & TEP Std	2010/1/12	54266	23248
GF & TEP Std	2010/1/12	54343	22844
GF & TEP Std	2010/1/12	54955	22845
GF & TEP Std	2010/1/12	54686	22897
GF & TEP Std	2010/1/12	54587	22689
GF & TEP Std	2010/1/12	54733	22513
GF & TEP Std	2010/1/12	54840	22611
GF & TEP Std	2010/1/12	54419	22563
	average	54603.6	22776.2
	std dev	244.9	237.9
	%RSD	0.4%	1.0%

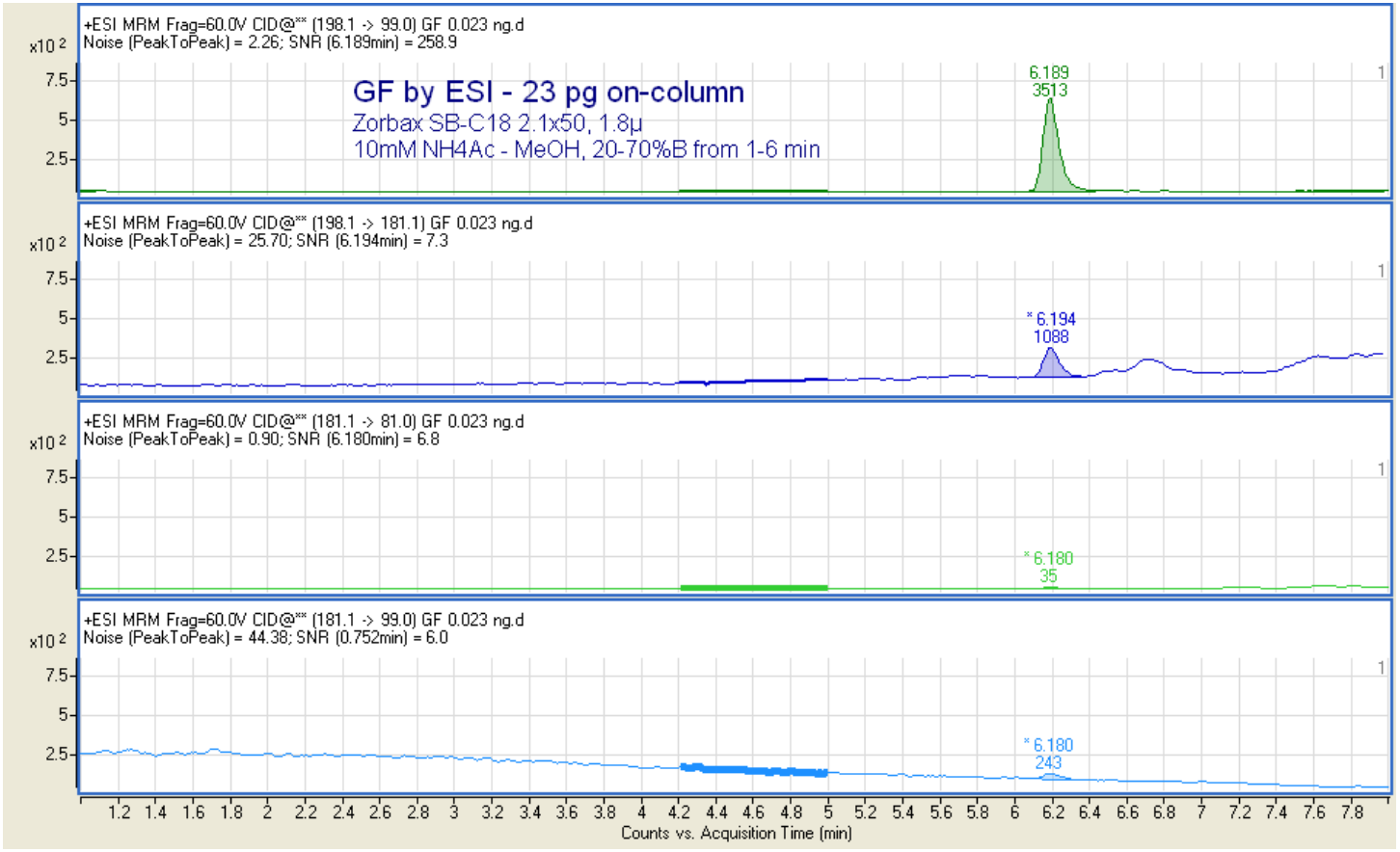
Name	Acq. Date-Time	TEP Area	GF-NH4 Area
TEP & GF in AcCN	2010/3/8	7184	3281
TEP & GF in AcCN	2010/3/8	7036	3272
TEP & GF in AcCN	2010/3/8	7246	3331
TEP & GF in AcCN	2010/3/8	7282	3479
TEP & GF in AcCN	2010/3/8	7624	3578
TEP & GF in AcCN	2010/3/8	8033	3703
TEP & GF in AcCN	2010/3/8	7600	3585
TEP & GF in AcCN	2010/3/8	7634	3867
TEP & GF in AcCN	2010/3/8	7989	3641
TEP & GF in AcCN	2010/3/8	7901	3622
	average	7552.9	3535.7
	std dev	353.8	194.2
	%RSD	4.7%	5.5%

GF linearity using ESI-AJS



GF - Instrument Detection Limit

- approximately 8 pg on-column



Decon Formulation Experiment

Sample	TEP Results		GF-NH4 Results		TPP Results	
Name	RT	Resp.	RT	Resp.	RT	Resp.
GF TEP TPP in ACN	4.931	82854	5.776	34107	7.023	33447
RSDL decon_GF_93MM11-1a	4.933	82661	5.777		7.022	34401
RSDL decon_GF_93MM11-1b	4.936	79185			7.026	34445
RSDL decon_GF_93MM11-1c	4.932	81374	5.788		7.031	35064
RSDL decon_GF_93MM11-1d	4.932	84794			7.027	33699
GF TEP TPP in ACN	4.940	87920	5.780	35513	7.024	32225
GF TEP TPP in ACN	4.933	85502	5.777	34692	7.019	31084
RSDL decon_GF_93MM11-1a	4.929	81623	5.780		7.022	31936
RSDL decon_GF_93MM11-1b	4.929	78733			7.022	31666
RSDL decon_GF_93MM11-1c	4.930	80343			7.019	32207
RSDL decon_GF_93MM11-1d	4.930	83079			7.009	31705
GF TEP TPP in ACN	4.931	87051	5.771	34806	7.019	31481
n	12	12	7	4	12	12
average	4.932	82926	5.778	34779	7.022	32780
std dev	0.0033	2932	0.0052	577	0.0054	1356
%RSD	0.07%	3.54%	0.09%	1.66%	0.08%	4.14%

Conclusions

- dilute and shoot LC-MS/MS method is appropriate for CWA studies in decontamination formulations
  - quench decon reaction
  - dilute out matrix
- use ESTD calculation
  - TEP works well as indicator of ion suppression
  - TPP works well as surrogate


Thanks for your attention!