

High Recovery Method of HybridSPE®-Phospholipid for Cleanup of Biological Samples Prior to LC-MS Analysis

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Outline:

Brief Overview of HybridSPE

Case studies of low-rec compounds:

1. Strong Bases
2. Strong Acid
3. Neutral hydrophobic
4. Acid-labile compound

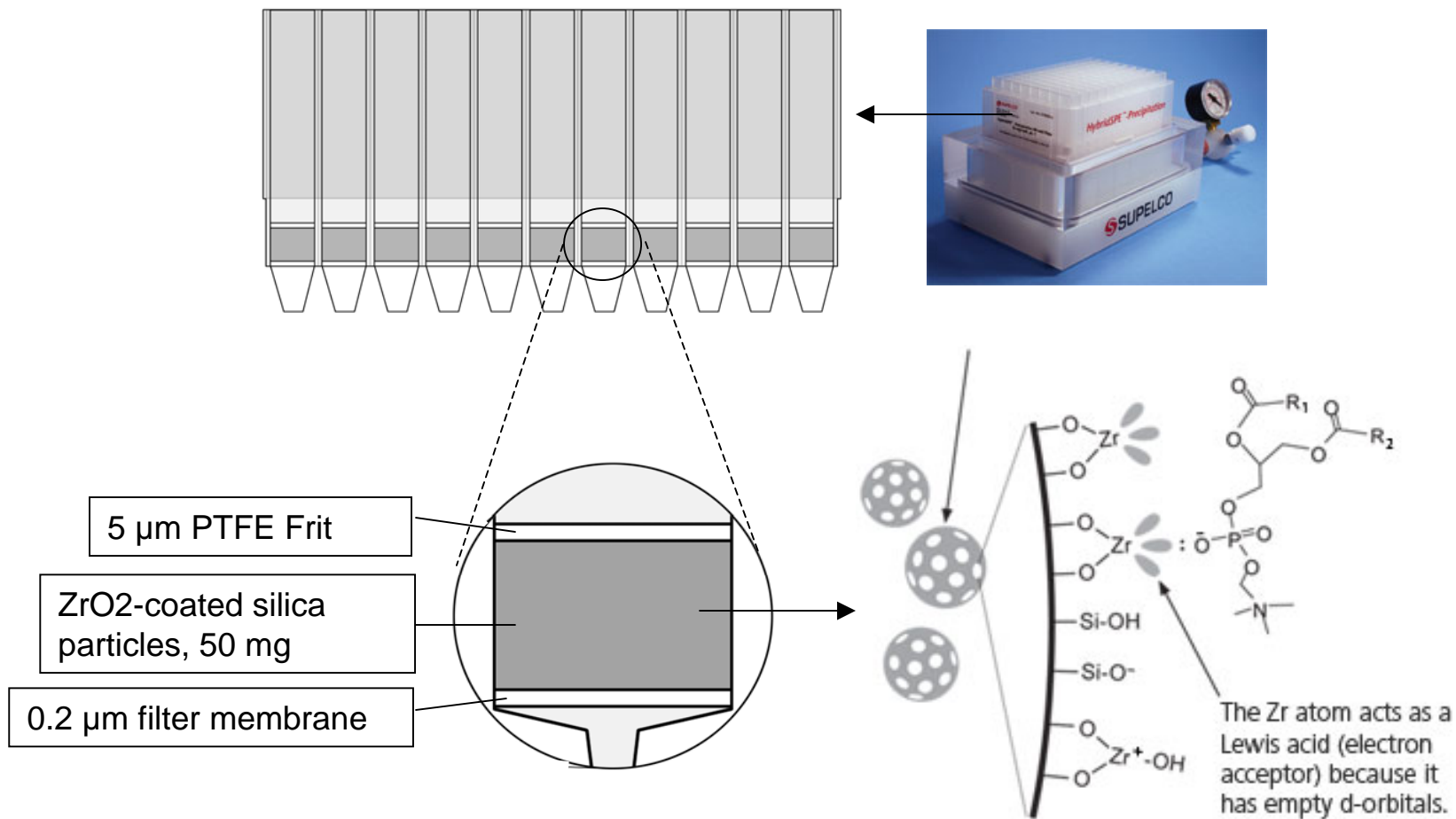
Summary

Overview of HybridSPE-Phospholipid

Functions: Protein precipitation and Phospholipid removal

- ✓ In-well protein precipitation via the addition of organic solvents, e.g. Acetonitrile and MeOH.
- ✓ PLs removal by proprietary zirconia-modified silica particles.
- ✓ The operation is both simple and fast.

How Are Proteins and Phospholipids Removed?



Critical Conditions: Protein Crashing Solvent and Additives

Protein precipitation efficiency

Phospholipid removal

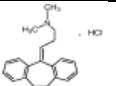
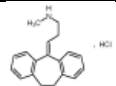
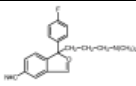
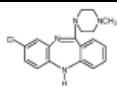
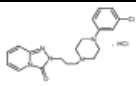
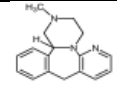
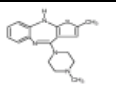
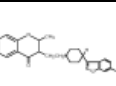
Compound recovery

Primary method: ACN w/ 1% formic acid

One of the common methods used in bioanalyses. The recommended method when the product was launched.

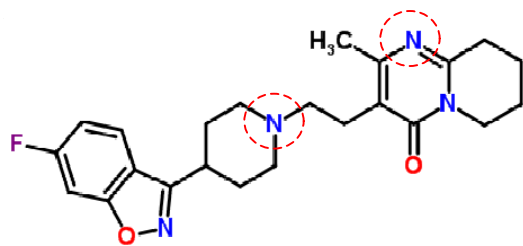
Good recovery from HybridSPE for many of our tested compounds.

Case 1: Strong Bases

Eluate 8,3 ng/mL	Structure Formula												
	composition %			Recovery [%]									
Sample Name	ACN	MeOH	Formic Acid	Amitriptylin	Nortriptylin	Citalopram	Clozapin	Trazodon	Mirtazapin	Desmethyl mirtazapin	Olanzapin	Risperidon	Hydroxy-risperidon
Standard				100	100	100	100	100	100	100	100	100	100
Probe F8	100	0	1.0	106	106	95	88	93	38	65	13	42	84
Probe F9				101	111	95	91	97	42	70	13	45	85
Probe F10				105	113	99	88	97	41	66	17	45	81
Probe F11			110	112	101	96	101	45	72	17	52	85	
Probe F12			2.0	89	95	86	57	85	20	45	7	18	63
Probe G1				110	115	106	104	93	50	56	39	68	92
Probe G2	90	10	1.0	77	82	74	68	73	33	51	13	38	60
Probe G3				95	102	90	87	88	45	64	15	53	75
Probe G4				77	85	73	57	76	24	48	7	25	60
Probe G5			1.5	94	102	90	77	93	40	61	24	48	74
Probe G6				53	60	52	21	53	7	20	3	7	30
Probe G7			2.0	92	97	86	71	86	33	61	16	34	72
Probe G9			70	30	1.0	73	81	70	58	75	30	47	12
Probe G10	92	100				84	71	90	33	60	15	34	70
Probe G11	1.5	75			82	67	57	75	33	46	15	39	59
Probe G12		72			80	68	37	71	16	37	5	16	48
Probe H1	2.0	76			85	71	66	78	42	49	29	52	60
Probe H2	50	50			1.0	91	97	80	80	90	40	66	32
Probe H4			57	65		51	35	61	15	30	6	16	36
Probe H5			1.5	63	71	58	53	65	35	42	24	45	51
Probe H6				65	75	63	47	68	25	36	19	32	47
Probe H7			2.0	62	69	56	40	65	23	33	16	29	42

Case 1: Strong Bases

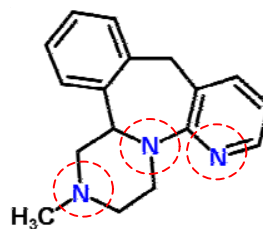
NR3(+) Vs Recovery:



Risperidone

42%

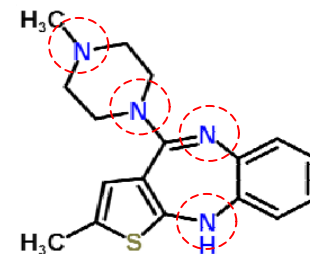
2 -NR3 groups



Mirtazapine

38%

3 -NR3 groups

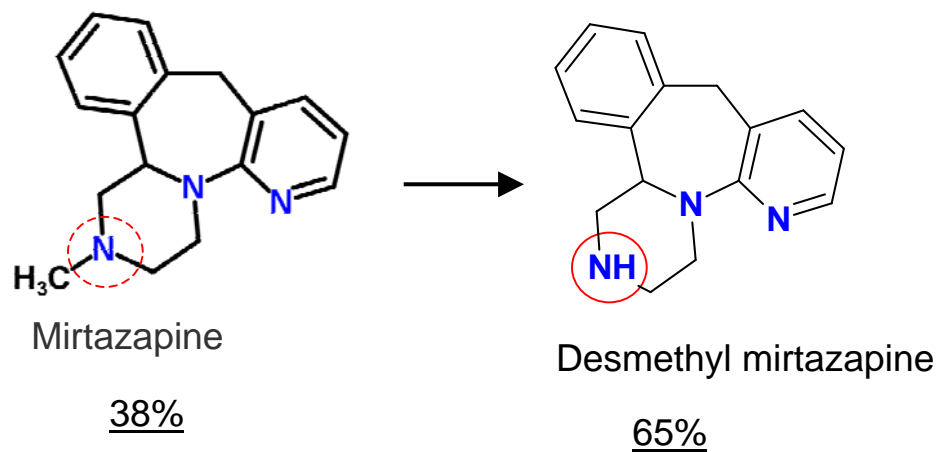


Olanzapine,

13%

3 -NR3 groups

Improved Recovery with Less – NR3 Group



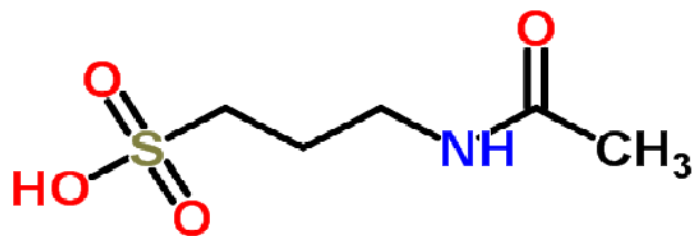
Improved Recovery with Salts

Recovery of Standard Without Matrix Plasma					
Analyte	MeCN/1% FA	MeOH	MeOH/1% NH ₄ FA	MeOH/1% NH ₄ Cl	MeOH/150 mM NaCl
Mirtazapine (266/195)	0.0	13.2	96.5	38.2	99.0
Risperidone (411/191)	0.0	10.4	99.1	111.6	64.0
Olanzapine (313/256)	0.0	13.6	89.4	NO experiment	74.0

Learning from Case 1- Strong Bases:

- ❖ The low recovery bases typically have at least one $-NR_3$ groups.
- ❖ The low recovery very likely due to cation-exchange interactions with the Silica support.
- ❖ The ion-exchange interactions were effectively suppressed by the addition of salts such as NH_4FA , NH_4Cl , and $NaCl$.
- ❖ Methanol is a better solvent for the salt additives than $MeCN$.

Case 2: Strong Acid

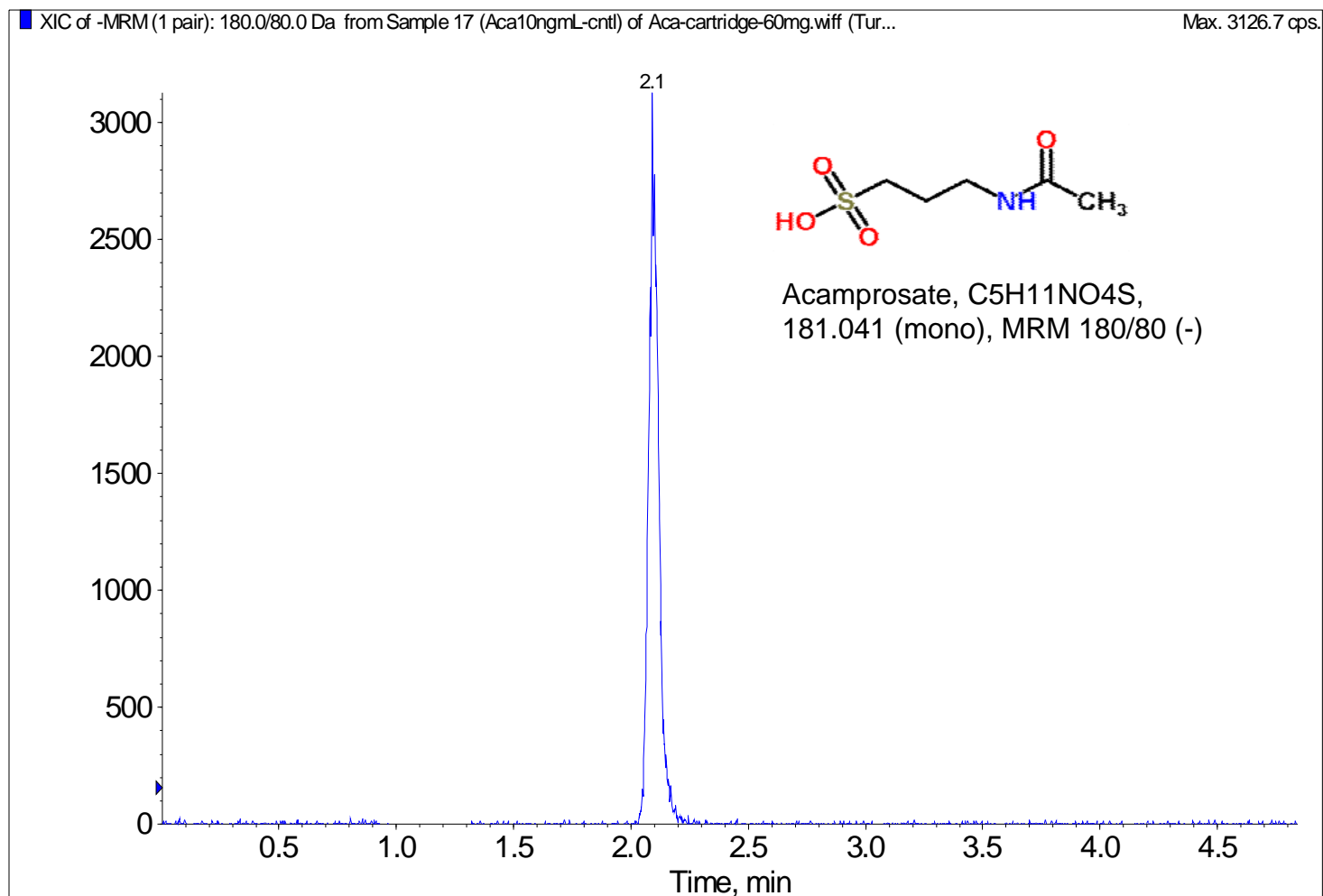


Acamprosate, Zero recovery from
HybridSPE plates or cartridges !

High Recovery with the Addition of NH_4ClO_4

Recovery of Acamprosate std from HybridSPE 96-well plate		
Conditions	Recovery (%)	Standard Deviation (%)
ACN/1% formic acid	19.0	1.7
MeOH/1% ammonium formate	43.3	5.2
MeOH/2% ammonium formate	54.2	3.3
MeOH/5% ammonium formate	6.5	0.9
MeOH/1% ammonium malate	39.1	3.5
MeOH/100mM ammonium perchlorate	84.2	8.1
MeOH/200mM ammonium perchlorate	100.7	8.8

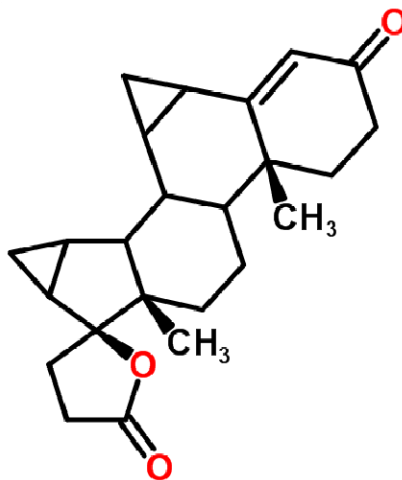
High Recovery with Fast Separation of Strong Acid



Case 3: Neutral Hydrophobic

Drospirenon problems:

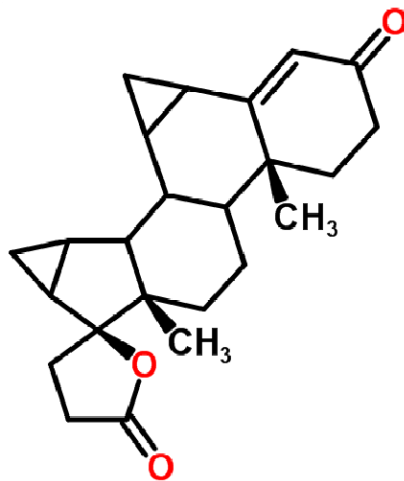
1. Low Rec
2. Variation of recovery 15-30%



Drospirenone, C₂₄H₃₀O₃, MW 366.227 (mono),
ACD/LogD (pH 7.4): 3.16
MRM: 367.2→97.1 or 91

Possible Causes:

1. Low solubility in MeCN
2. Strong affinity to transporter proteins

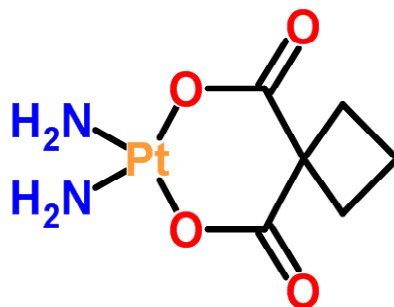


Drospirenone, C₂₄H₃₀O₃, MW 366.227 (mono),
ACD/LogD (pH 7.4): 3.16
MRM: 367.2→97.1 or 91

Nice Recovery and Reproducibility with MeOH/1%NH₄FA

Replicate	Recovery of Drospirenone Spiked in Rat Plasma	
	10 ng/mL spike	60 ng/mL spike
1	89.9	91.9
2	87.0	93.7
3	91.8	88.4
4	96.9	93.5
5	91.9	90.4
6	91.0	87.4
7	94.2	87.5
8	97.0	88.0
9	86.1	87.5
10	87.0	87.9
11	84.0	87.8
12	86.9	87.7
Avg	90.3	89.3
STD	4.3	2.4
%CV	4.7	2.7

Case 4: Acid-labile Compounds



Carboplatin:

<u>Recovery</u>	<u>Protein Precipitation Conditions</u>
20%	ACN/1% formic acid
85%	MeOH/1% NH ₄ FA

Summary of Learning:

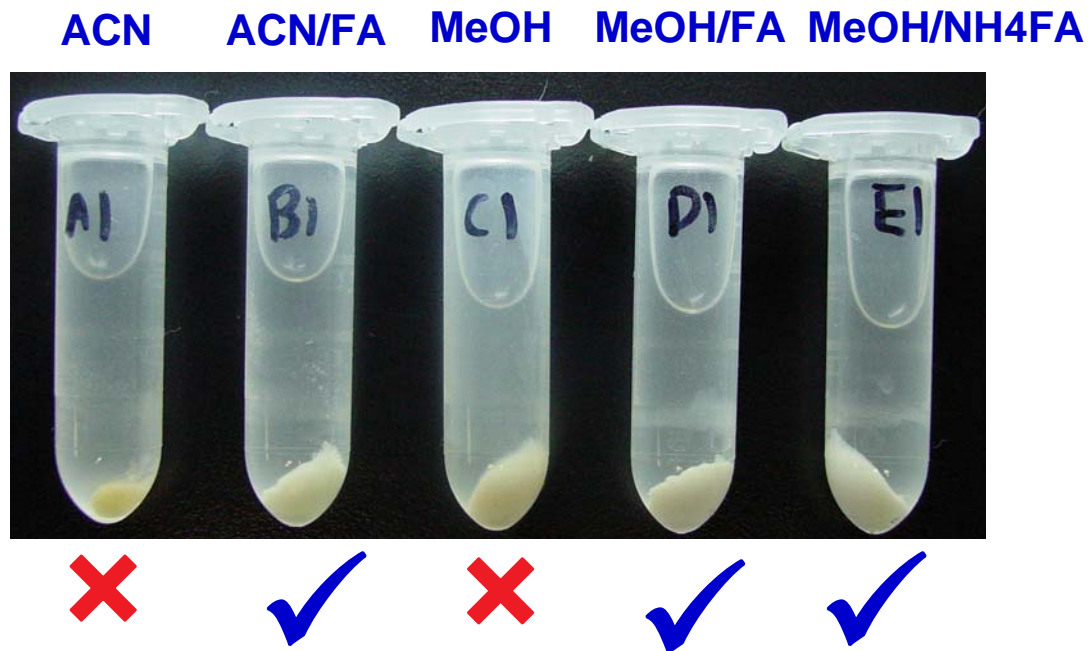
Addition of salt additives, e.g. NH_4FA and NH_4ClO_4 , is necessary to reduce the non-specific bindings, and therefore improve the recoveries of both strong acids and bases.

Many of the salt additives are not soluble in MeCN, but soluble in MeOH.



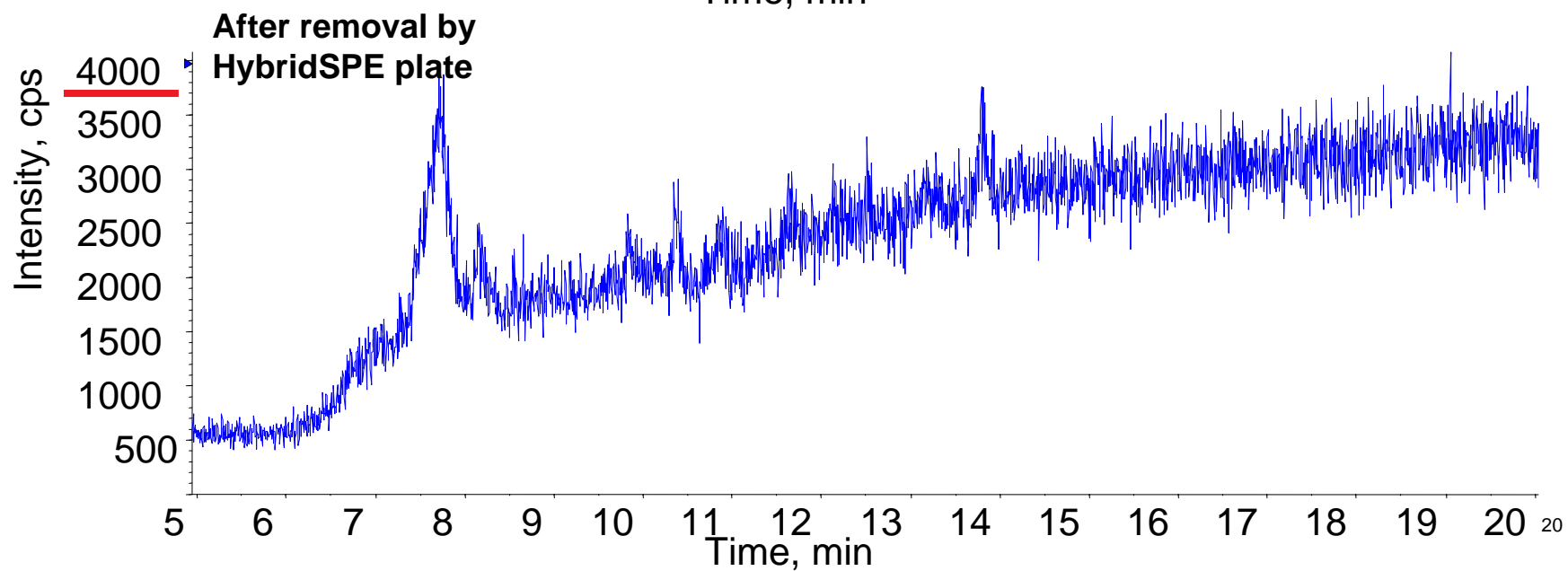
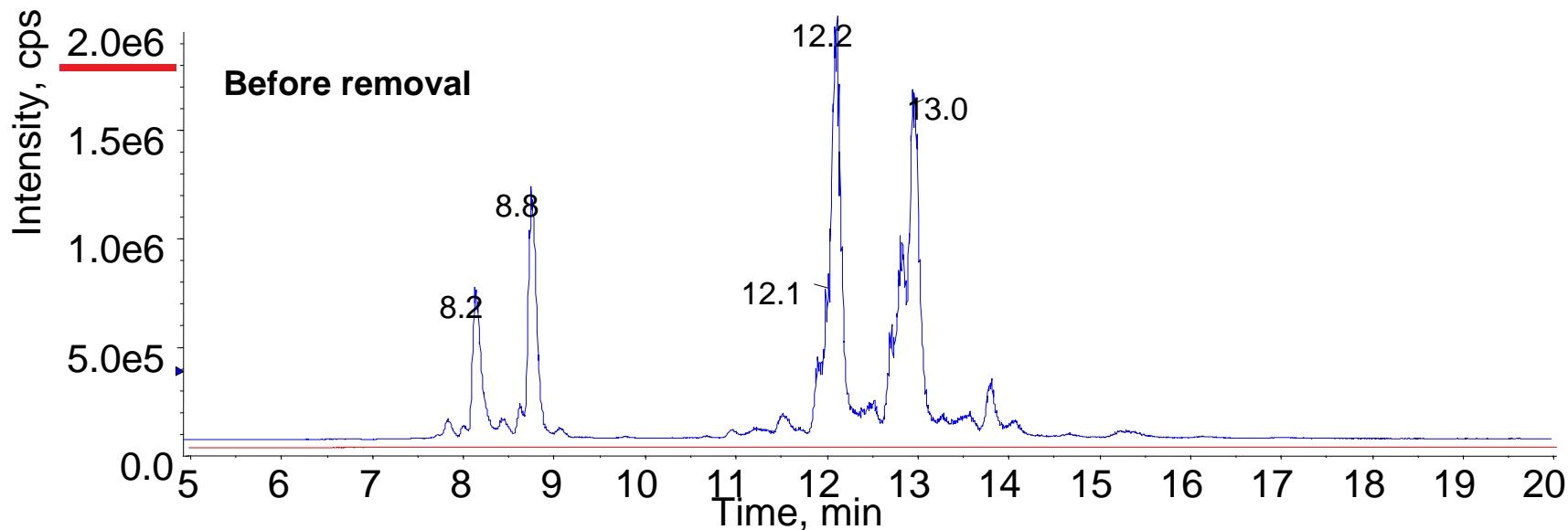
MeOH/salts may be a better protein crashing condition.

Protein Precipitation in ACN and Methanol



Comparable Results with Methanol.

Phospholipid Removal – PCs at 184 and 104



Conclusion:

MeOH/Salt additive is an alternative protein crashing method for HybridSPE

- ❖ **Comparable efficiency in protein precipitation and phospholipid removal.**
- ❖ **Improved recovery for strong bases and acids.**
- ❖ **Improved reproducibility for low soluble compounds in MeCN**
- ❖ **Improved recovery for acid-labile solutes.**
- ❖ **Alternative solvent if MeCN shortage is a concern.**

Acknowledgement

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Covance

Thanks!