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-liable compound

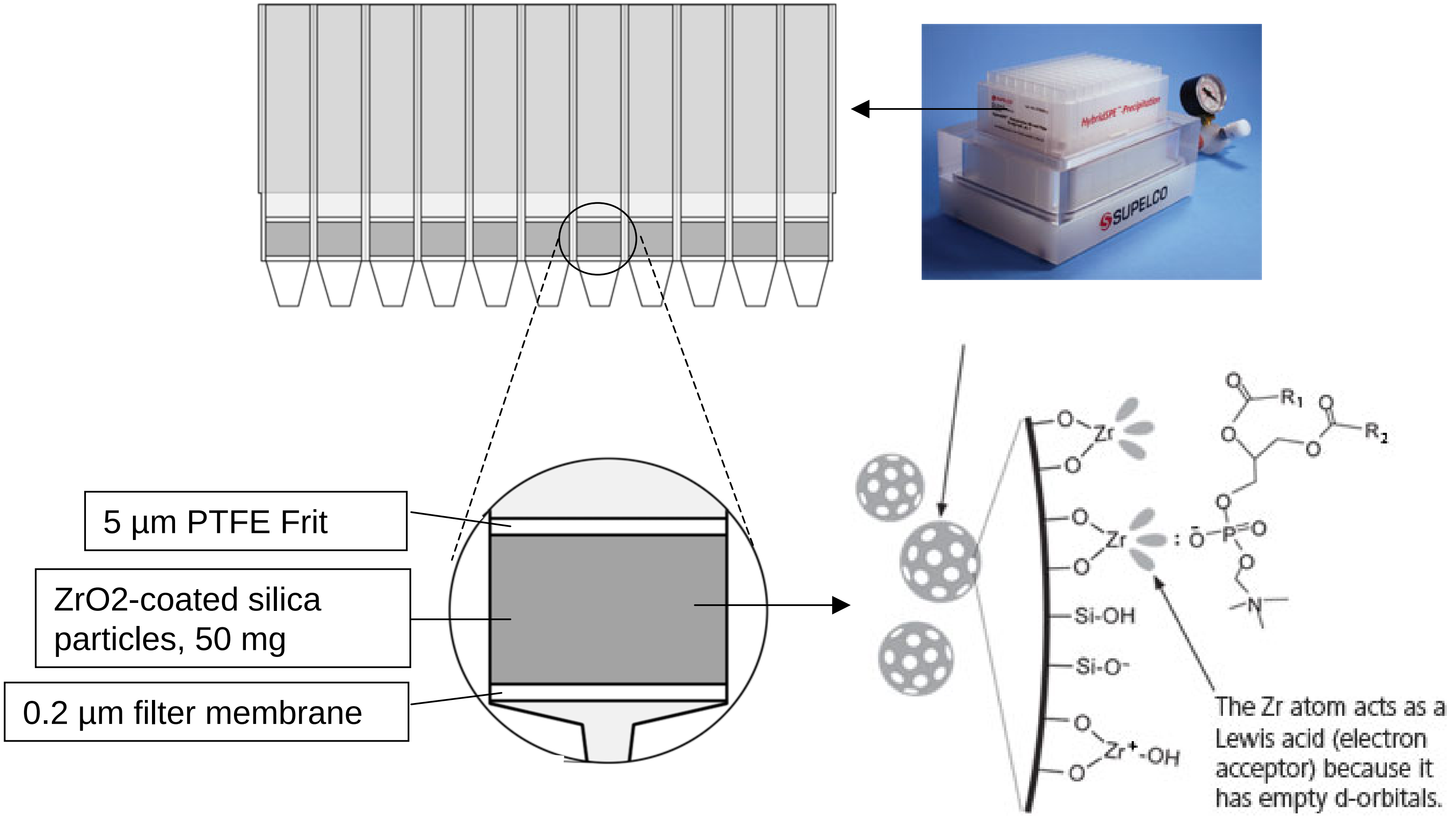
Summary

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?



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Critical Conditions: Protein Crashing Solvent and Additives

Protein precipitation efficiency

Phospholipid removal

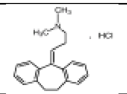
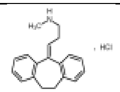
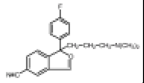
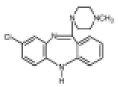
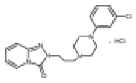
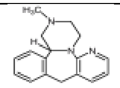
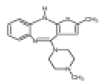
Compound recovery

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			1.5	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			1.5	77	85	73	57	76	24	48	7	25	60
Probe G5				94	102	90	77	93	40	61	24	48	74
Probe G6			2.0	53	60	52	21	53	7	20	3	7	30
Probe G7				92	97	86	71	86	33	61	16	34	72
Probe G9	70	30	1.0	73	81	70	58	75	30	47	12	35	55
Probe G10			1.5	92	100	84	71	90	33	60	15	34	70
Probe G11				75	82	67	57	75	33	46	15	39	59
Probe G12			2.0	72	80	68	37	71	16	37	5	16	48
Probe H1				76	85	71	66	78	42	49	29	52	60
Probe H2	50	50	1.0	91	97	80	80	90	40	66	32	46	69
Probe H4			1.5	57	65	51	35	61	15	30	6	16	36
Probe H5				63	71	58	53	65	35	42	24	45	51
Probe H6			2.0	65	75	63	47	68	25	36	19	32	47
Probe H7				62	69	56	40	65	23	33	16	29	42

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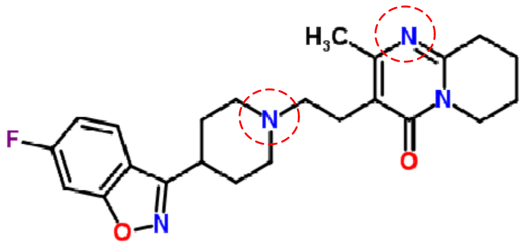
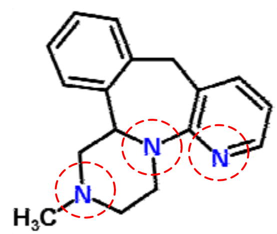
Case 1: Strong Bases

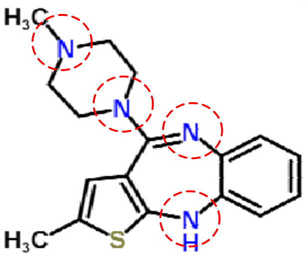
NR3(+) Vs Recovery:



Risperidone



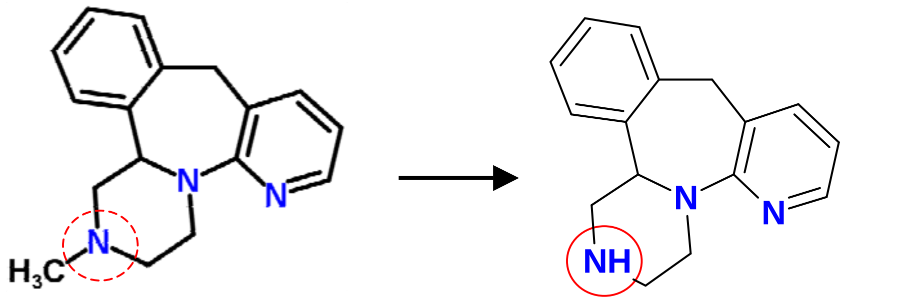
Mirtazapine



Olanzapine,

Risperidone	Mirtazapine	Olanzapine
42%	38%	13%
2 -NR3 groups	3 -NR3 groups	3 -NR3 groups

Improved Recovery with Less – NR3 Group



Mirtazapine

38%

Desmethyl mirtazapine

65%

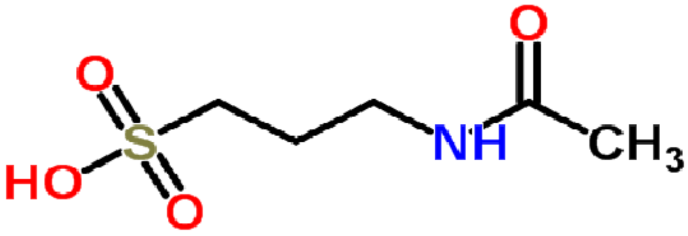
Improved Recovery with Salts

Recovery of Standard Without Matrix Plasma					
Analyte	**MeCN/1% FA**	**MeOH**	**MeOH/1% NH4FA**	**MeOH/1% NH4Cl**	**MeOH/150 mM NaCl**
Mirtazapine (266/195)	0.0	13.2	96.[illegible]	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 –NR3 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 NH4FA, NH4Cl, 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**

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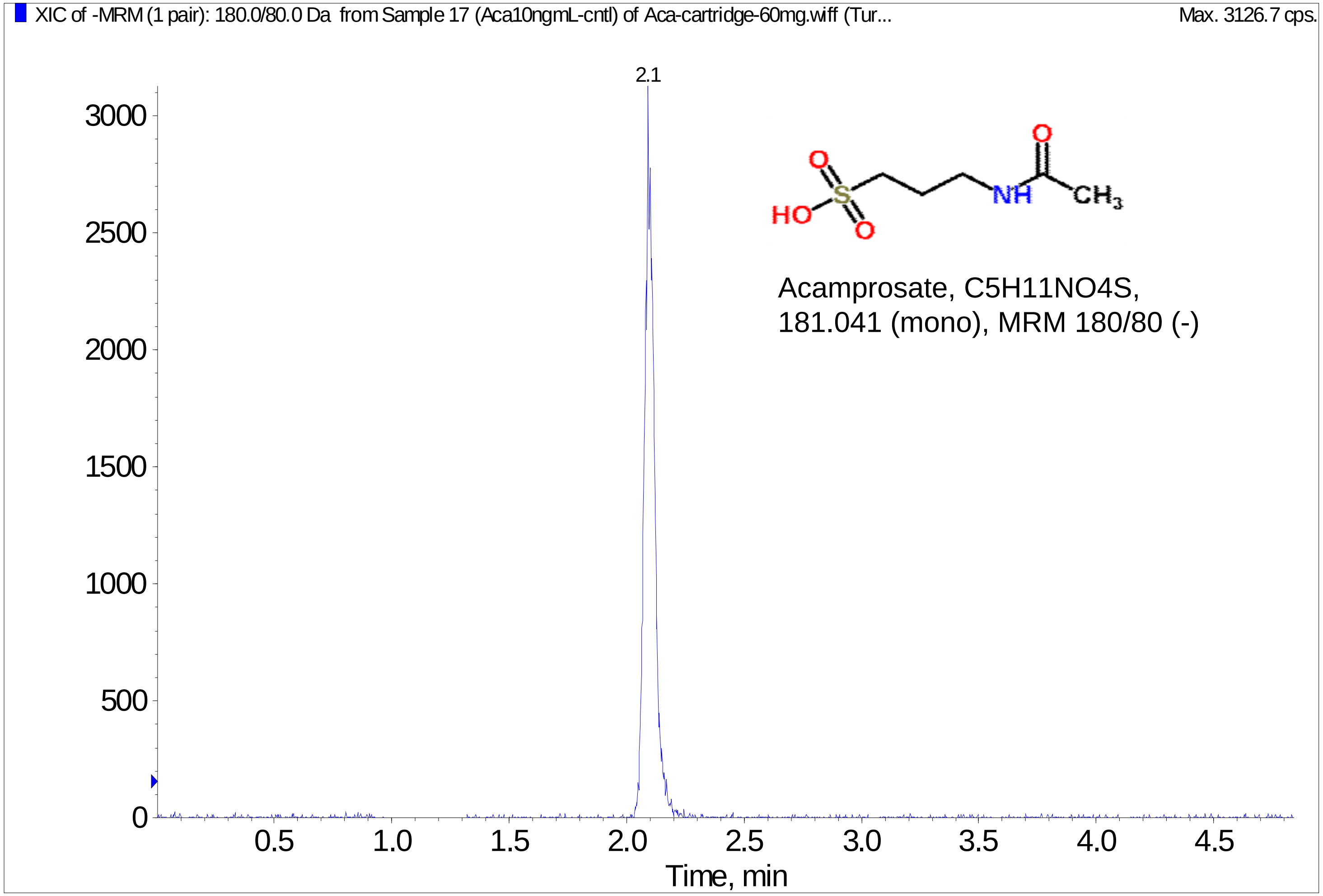
High Recovery with Fast Separation of Strong Acid



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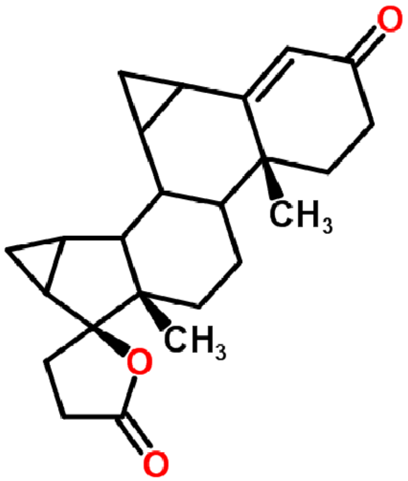
Case 3: Neutral Hydrophobic

Drospirenon problems:

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



Drospirenone, $C_{24}H_{30}O_3$, MW 366.227 (mono),
ACD/LogD (pH 7.4): 3.16
MRM: 367.2→97.1 or 91

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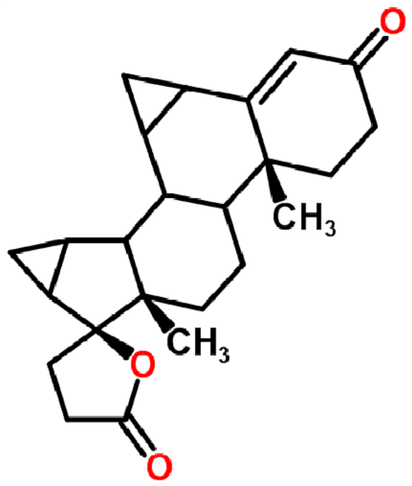
Possible Causes:

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



Drospirenone, $C_{24}H_{30}O_3$, MW 366.227 (mono),
ACD/LogD (pH 7.4): 3.16
MRM: 367.2→97.1 or 91

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Nice Recovery and Reproducibility with MeOH/1%NH_4FA

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

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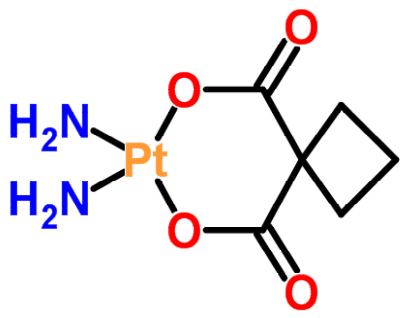
Case 4: Acid-liable Compounds



Carboplatin:

Recovery	Protein Precipitation Conditions
20%	ACN/1% formic acid
85%	MeOH/1% NH_4FA

Summary of Learning:

Addition of salt additives, e.g. NH4FA and NH4ClO4, 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

ACN | ACN/FA | MeOH | MeOH/FA | MeOH/NH4FA

Comparable Results with Methanol.

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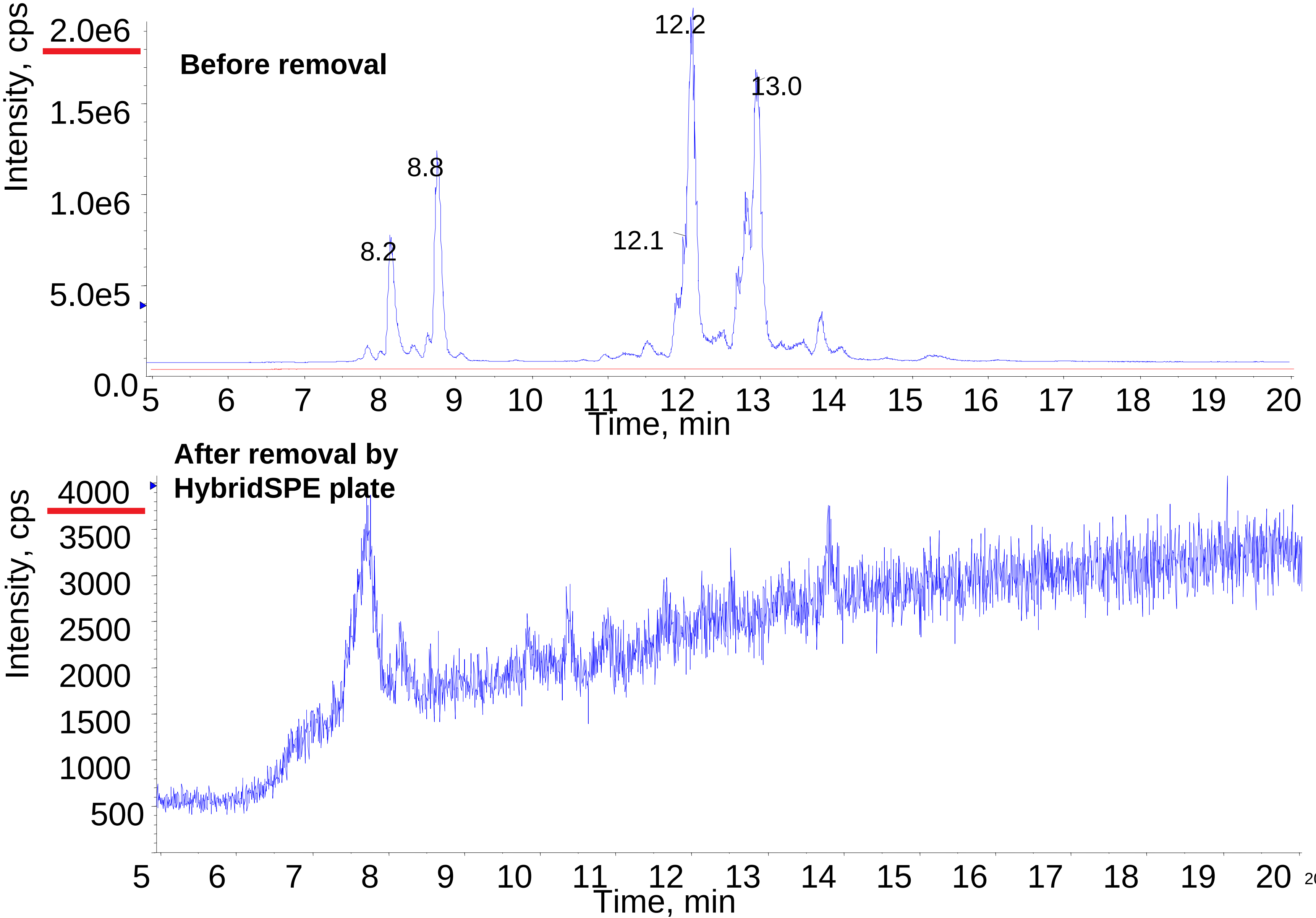
Phospholipid Removal – PCs at 184 and 104



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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-liable solutes.
- ❖ Alternative solvent if MeCN shortage is a concern.

Acknowledgement

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Covance

Thanks!

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