Alternative Method of Hybrid SPE Sample Preparation for High Recovery LC-MS Analysis of Pharmaceutical Compounds in Plasma

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Outline:

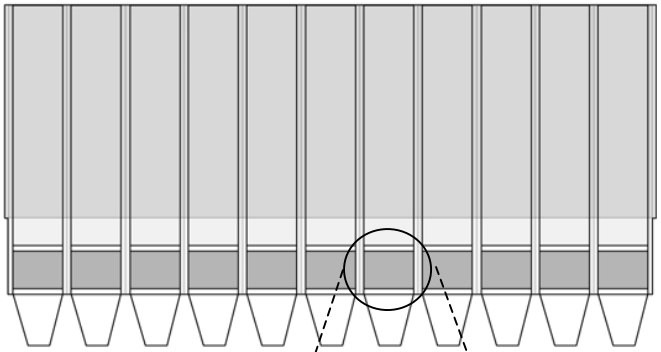
- **Introduction to HybridSPE-PPT**
- **Alternative method – Methanol-based method, two examples.**
- **Summary**

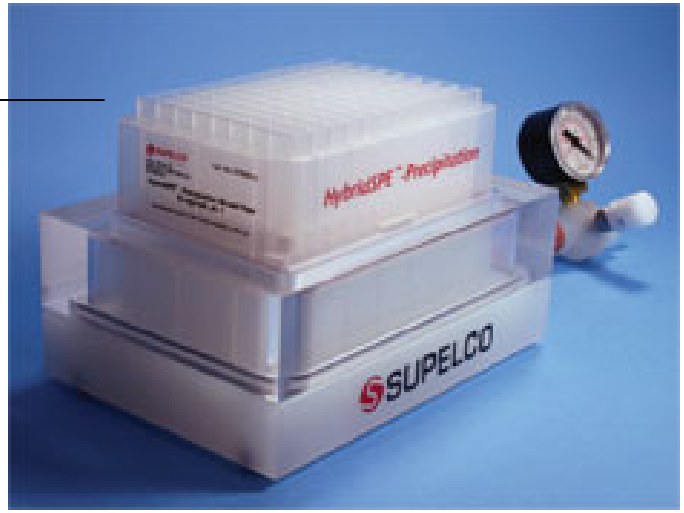
Introduction of HybridSPE PPT Technique

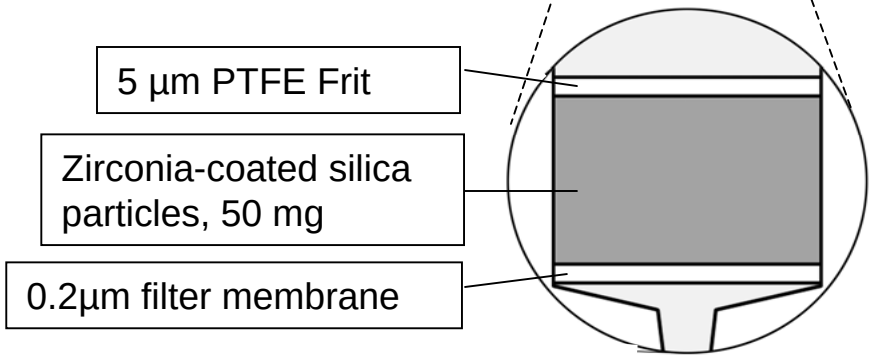
A **sample cleanup** method that removes both **proteins** and **phospholipids** in a simple platform.

- ✓ Protein are removed by precipitation with addition of organic solvents (e.g. acetonitrile, methanol).
- ✓ Phospholipids are removed by proprietary zirconia particles.
- ✓ The operation is both simple and fast, and is amenable to high throughput.

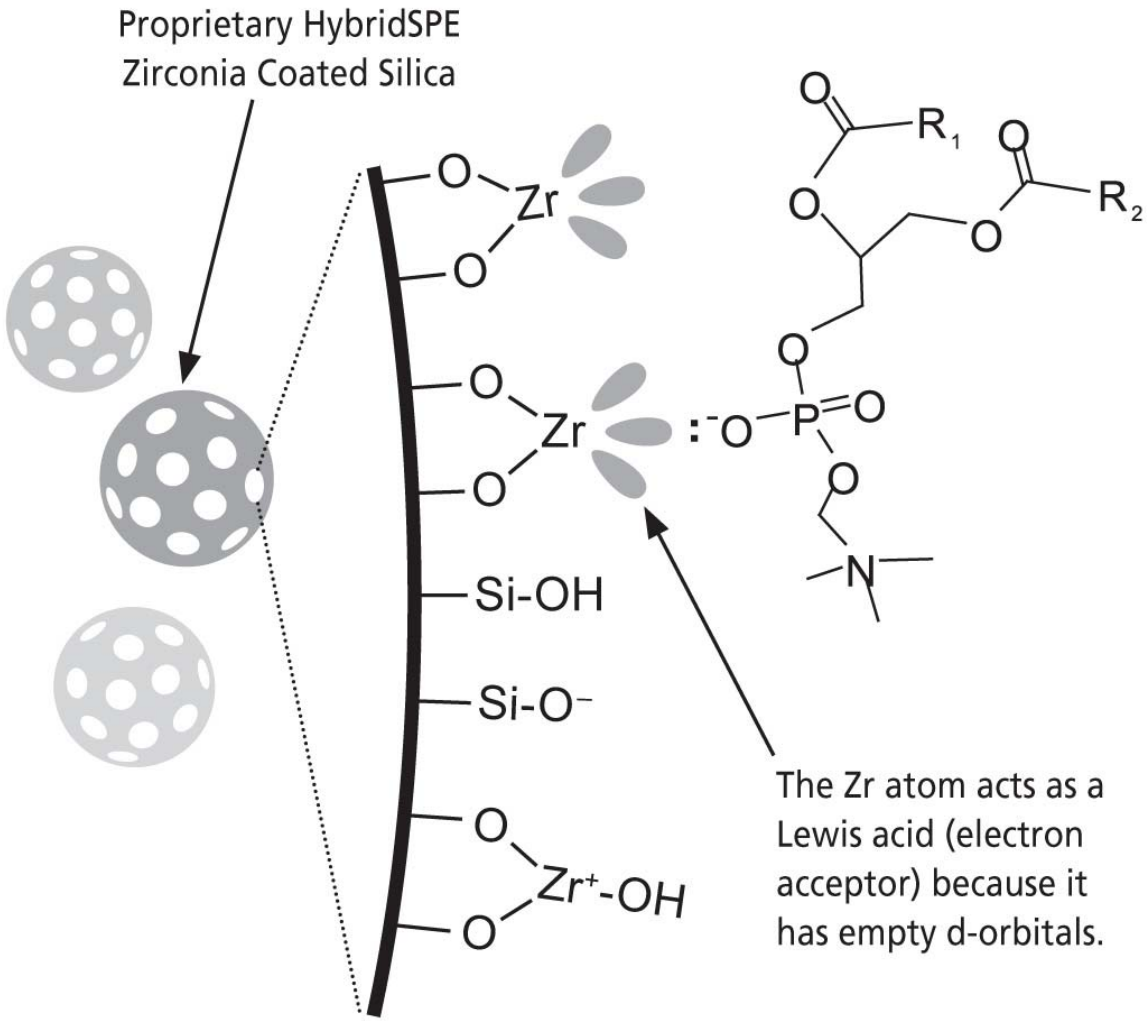
How Are Proteins and Phospholipids Removed?







Interaction of Phospholipids with Zirconia coated Silica Surface

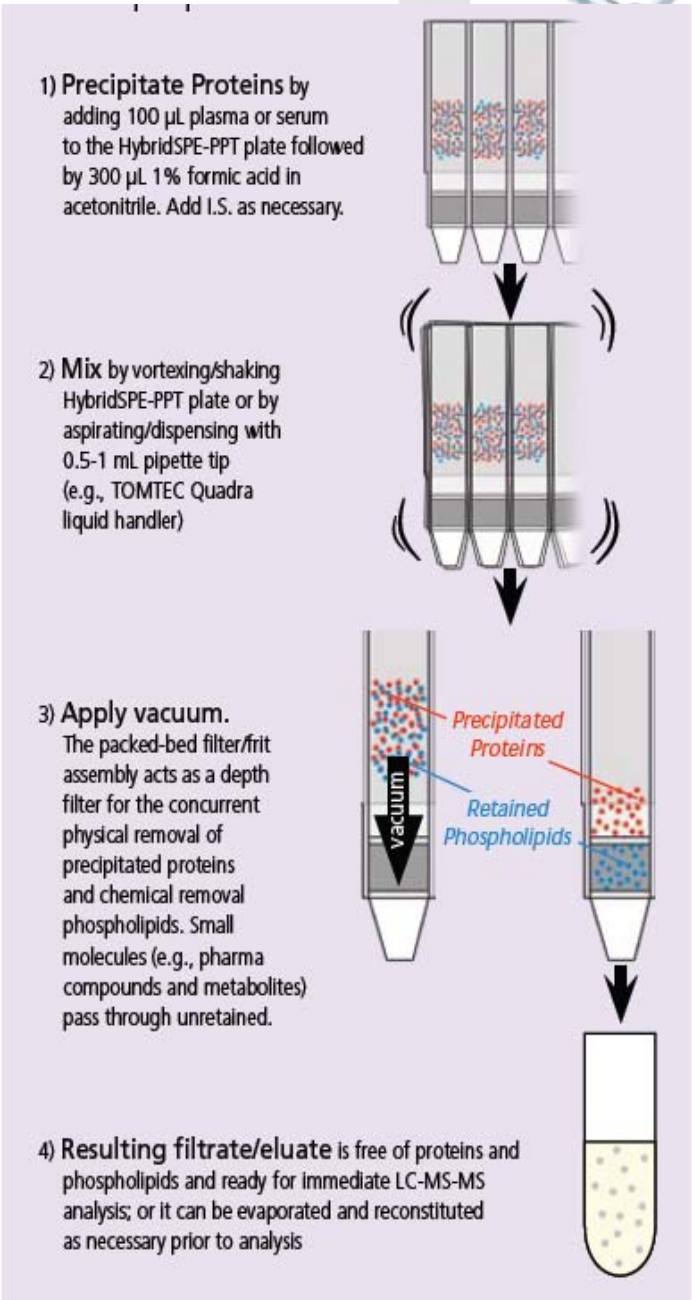


Standard Protocol:

Protein precipitation agents including organic solvent and additives

1% formic acid acetonitrile

- ❑ **primary recommended procedure.**
- ❑ **exhibits high recovery for broad range of test compounds in terms of recovery and protein PPT efficiency.**



1. Load sample and protein precipitation agent

2. Mix

3. Apply Vacuum

4. Samples ready for analysis

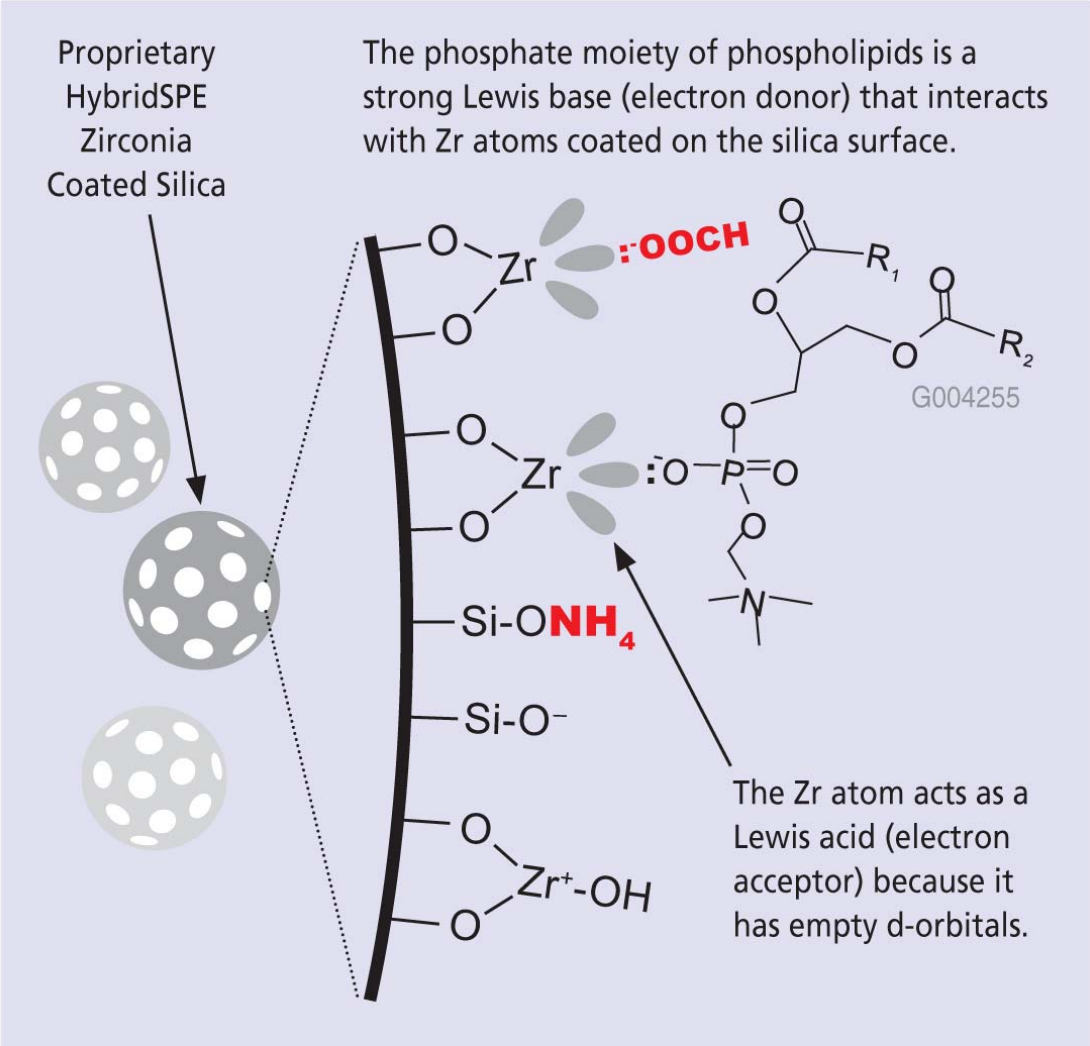
Standard Protocol:
Acetonitrile -Based Protein Precipitation

- In some cases, strongly basic compounds can exhibit lower recovery from the HybridSPE-PPT.
- Secondary ion-exchange interactions with Zirconia coated Silica surface and strongly basic compounds can cause lower recovery
- In some cases, limited solubility of analyte in acetonitrile can be cause of lower recovery
- Investigate alternative method for these types of compounds

Alternative Method:
Methanol-Based Protein Precipitation

- 1% ammonium formate (NH4FA) in methanol
- Milder condition for unstable metabolites when compared to 1% formic acid acetonitrile
- In cases, can exhibit higher recovery for basic compounds.
- Methanol can exhibits increased solubility for some pharmaceutical compounds.
- Methanol can disrupt secondary hydrogen bonding of analytes with silica surface

Alternative Method:
Methanol-Based Protein Precipitation

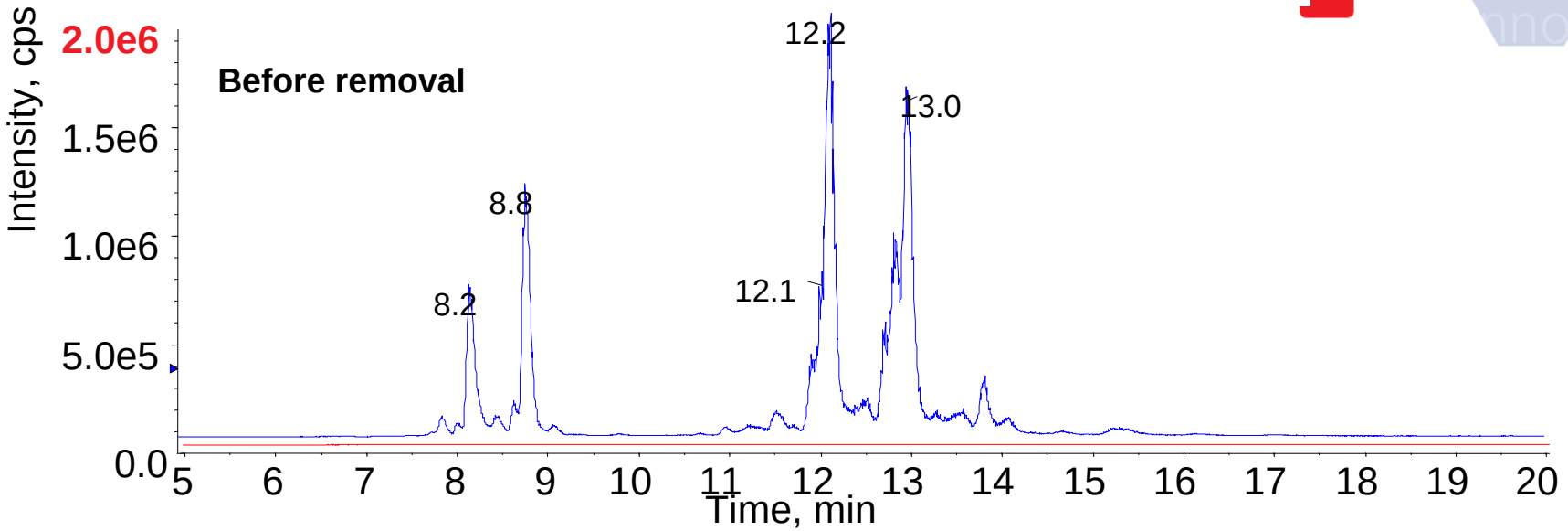


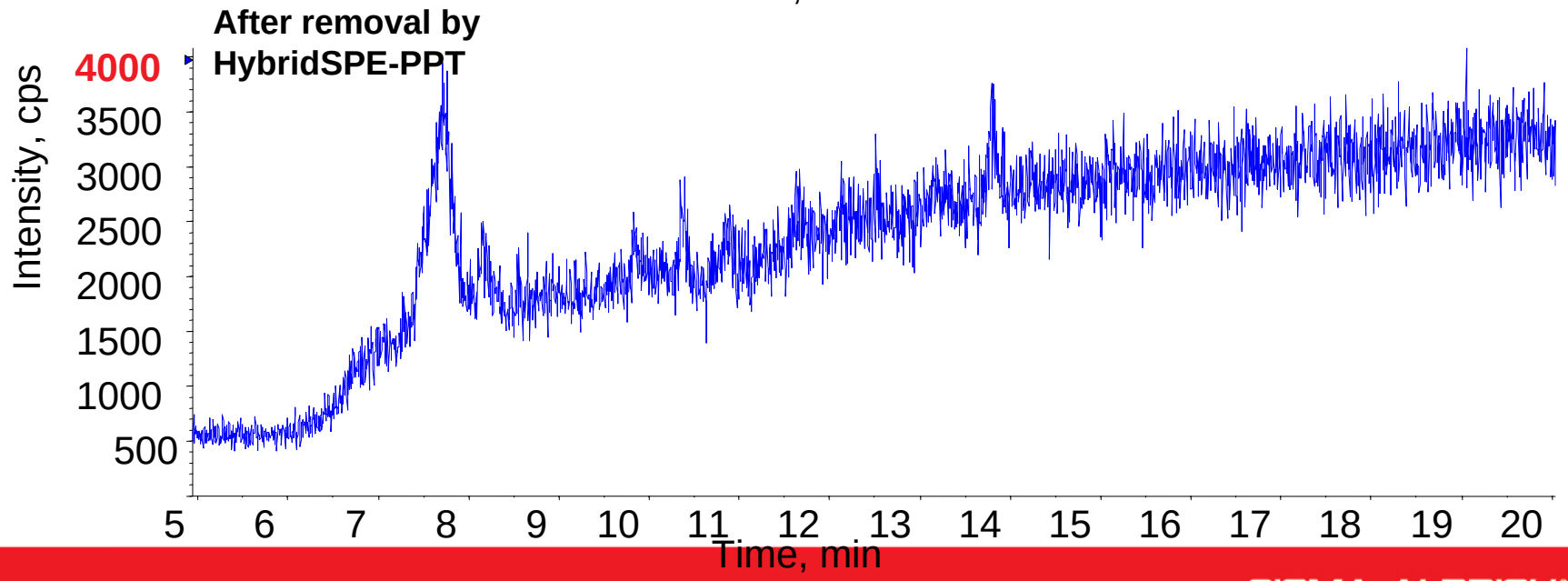
Protein Precipitation Comparison between Acetonitrile and Methanol

acetonitrile	1% formic acetonitrile	methanol	1% formic acid methanol	1% ammonium formate methanol

Comparable or better protein precipitation with Methanol.

Methanol Method – Complete Phospholipid Removal from 100 µL of Rabbit Plasma





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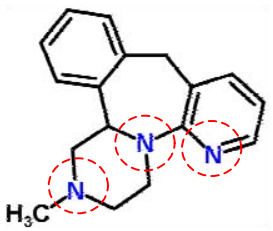
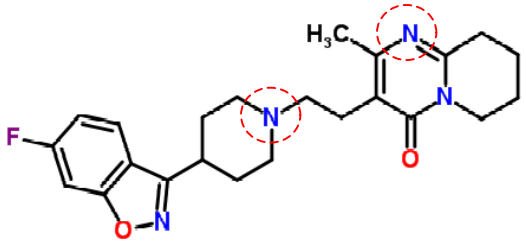
Examples of Improved Recovery of Basic Compounds

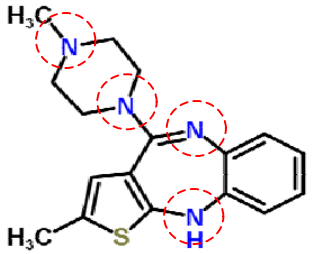
Recovery of Standard without matrix plasma					
Analyte	**1% formic acetonitrle**	**methanol**	**1% ammonium formate methanol**	**1% ammonium chloride methanol**	**150mM NaCl methanol**
Mirtazapine (266/195)	0.0	13.2	96.0	38.2	99.0
Risperidone (411/191)	0.0	10.4	99.1	111.6	64.0
Olanzapine (313/256)	0.0	13.6	89.4	NO experiment	74.0



Mirtazapine



Risperidone



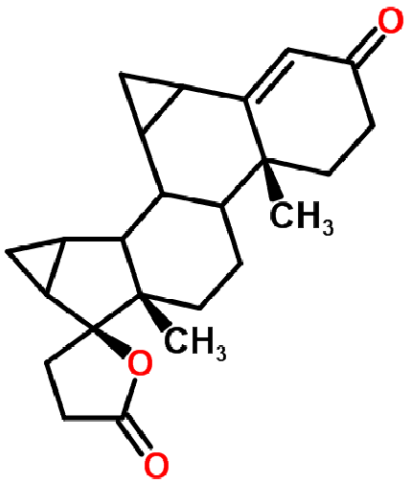
Olanzapine,

Potential Interactions between basic compounds and the Zirconia coated Silica

- Ion-exchange interaction is significant for basic compounds having at least one tertiary amine group.
- The ion-exchange interactions were effectively suppressed by the addition of salts such as ammonium formate, ammonium chloride and NaCl.
- Additions of additive raise effective pH of sample, decreasing ionization of analytes
- Ammonia ions bind with silanol surface thus decreasing surface activity
- The salt additives are readily dissolved in methanol, limited in acetonitrile.

Improved Recovery and Reproducibility of Neutral Hydrophobic Compounds

- Customer experienced low an irregular recovery using 1% formic acid acetonitrile protocol
- Variation of recovery 15-30%
- Analyte had limited solubility in acetonitrile

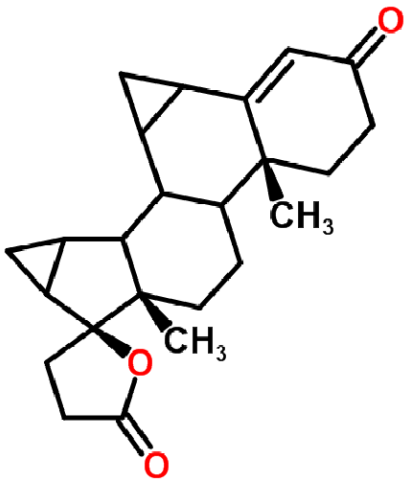


Drospirenone, C24H30O3, MW 366.227 (mono),
ACD/LogD (pH 7.4): 3.16
MRM: 367.2→97.1 or 91

Recovery and Reproducibility using 1% ammonium formate methanol

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

Why better recovery and reproducibility?



Drospirenone has a limited solubility in acetonitrile causing increase interaction with precipitated proteins. This resulting precipitate caused fluctuation in analyte recovery

Summary

- An alternative and improved method using 1% ammonium formate in methanol
- Demonstrate improved recovery of model basic compounds over standard acetonitrile method
- Can improve recovery and reproducibility for analytes with low solubility in acetonitrile.
- The more basic conditions of the 1% ammonium formate in methanol is less aggressive towards unstable metabolites.