Comparison of Sample Preparation Techniques for Reduction of Matrix Interference in Biological Analysis by LC-MS-MS

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Abstract

Three different sample preparation techniques are evaluated in this study for effective removal of phospholipids matrix interference. Sample preparation techniques include protein precipitation (PPT), solid phase extraction (SPE) and HybridSPE technique.

The degree of phospholipids interference varied greatly between the three sample preparation techniques. HybridSPE technique efficiently removed phospholipids and protein resulting in the least matrix interference. SPE removed minimal phospholipids and protein resulting moderate matrix interference. Protein precipitation removed only gross of levels of proteins from biological with no removal of phospholipids resulting in greatest matrix interference.

Introduction

Matrix effects in biological samples have been shown to be a source of variability and inaccuracy in liquid chromatography mass spectrometry (LC-MS). Co-elution of endogenous phospholipids with analyte can cause matrix effect ion-suppression or enhancement that dramatically impact quantitative LC-MS-MS. In this study, three different sample preparation techniques are evaluated for effective removal of phospholipids matrix interference. HybridSPE method are optimized in this study and SPE and PPT methods both are generic methods without further modification.

Sample Preparation Methods

HybridSPE Techniques:	Protein Precipitation (PPT):
• Load 100 µL rat or dog plasma • Add 300 µL acetonitrile with 1% FA • Vortex 1 min. before vacuum • Analyze the eluent via LC-MS	• Load 100 µL rat or dog plasma • Add 300 µL acetonitrile with 1% FA • Mix for 1 min. and centrifuge at 5K RPM for 3 min. • Analyze the sup via LC-MS

Generic polymeric SPE (60 mg/3 mL):

- Condition with 1 mL methanol and 1 mL water
- Load 500 µL rat or dog plasma
- Wash with 5% methanol in water
- Elute with 1 mL methanol
- Evaporate and recondition with 2 mL of water/acetonitrile with 1% FA (1:3)

Experiment 1

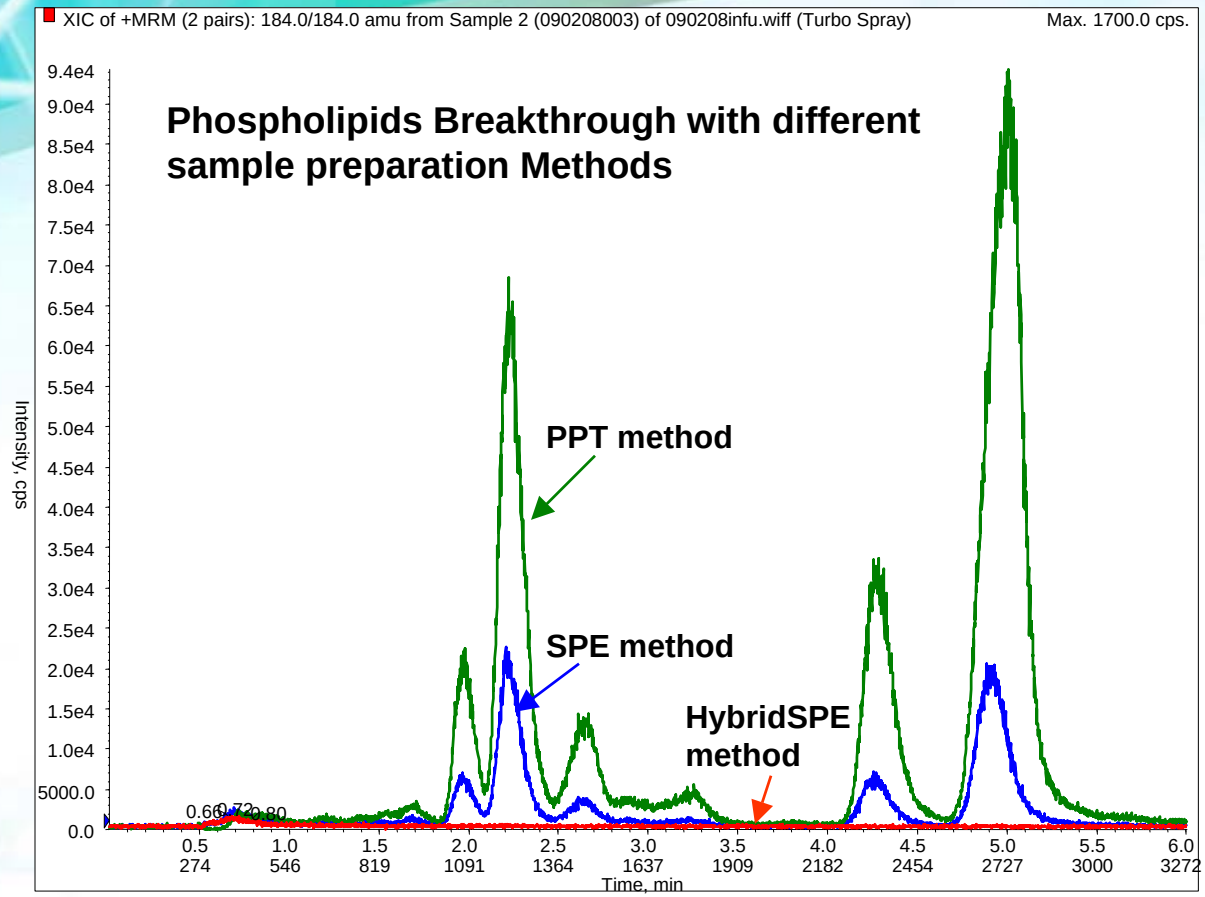
Study Phospholipids Breakthrough

LC-MS:	Agilent 1100/ABI Q-trap 3200, Turbo Ion Spray ESI+
column:	Ascentis® Express C18, 5 cm x 2.1 mm I.D.
mobile phase A:	65% acetonitrile with 0.1% ammonium formate
mobile phase B:	35% water with 0.1% ammonium formate
flow rate:	200 µL/min.
temp.:	30 °C
injection:	5 µL dog plasma sample prepared by different sample preparation methods

Mass Parameters:

CUR	IS	TEM	GS1	GS2	ihe	CAD
20	3500	500	40	55	ON	Medium

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	DP	EP	CEP	CE	CXP
184	184	50	100	10	10	29	4



Almost 100% phospholipids in dog plasma were removed by HybridSPE technique, moderate phospholipids were removed by generic polymeric SPE method, and no phospholipids were removed by traditional PPT.

Experiment 2

Study Drug Compounds Recovery

LC-MS: Agilent 1100/ABI Q-trap 3200, Turbo Ion Spray ESI+
column: Discovery® HS F5, 10 cm x 2.1 mm I.D.
mobile phase A: acetonitrile with 10 mM ammonium formate
mobile phase B: water with 10 mM ammonium formate
flow rate: 200 µL/min.
temp.: 30 °C
injection: 5 µL of the rat plasma samples cleanup with different sample preparation methods

Step	Total Time (min.)	Flow Rate (µL/min.)	A (%)	B (%)
0	0.0	200	75	25
1	2.0	200	95	5
2	4.5	200	95	5
3	5.0	200	75	25
4	7.0	200	75	25

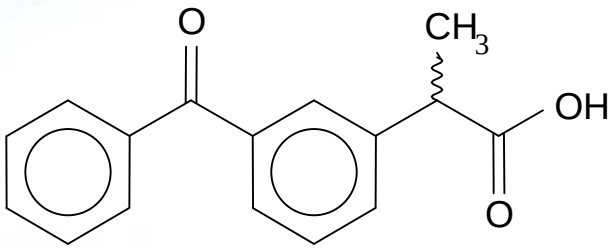
Mass Parameters

CUR	IS	TEM	GS1	GS2	ihe	CAD
25	4500	450	35	20	ON	Medium

Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	DP	EP	CEP	CE	CXP
255.2	209.10	150	36	12	18	17	4
260.30	116.10	150	41	9.5	14	25	4

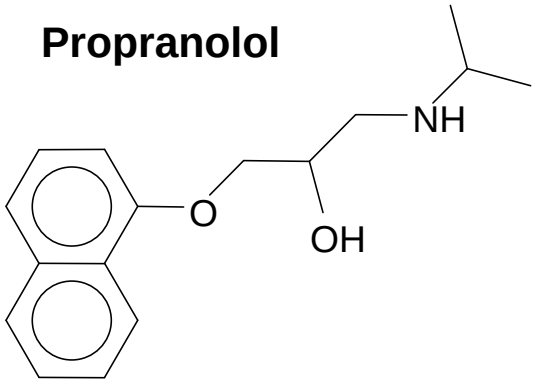
Structures of Compounds in this Study

Ketoprofen



+MRM: 255.20/209.10 amu

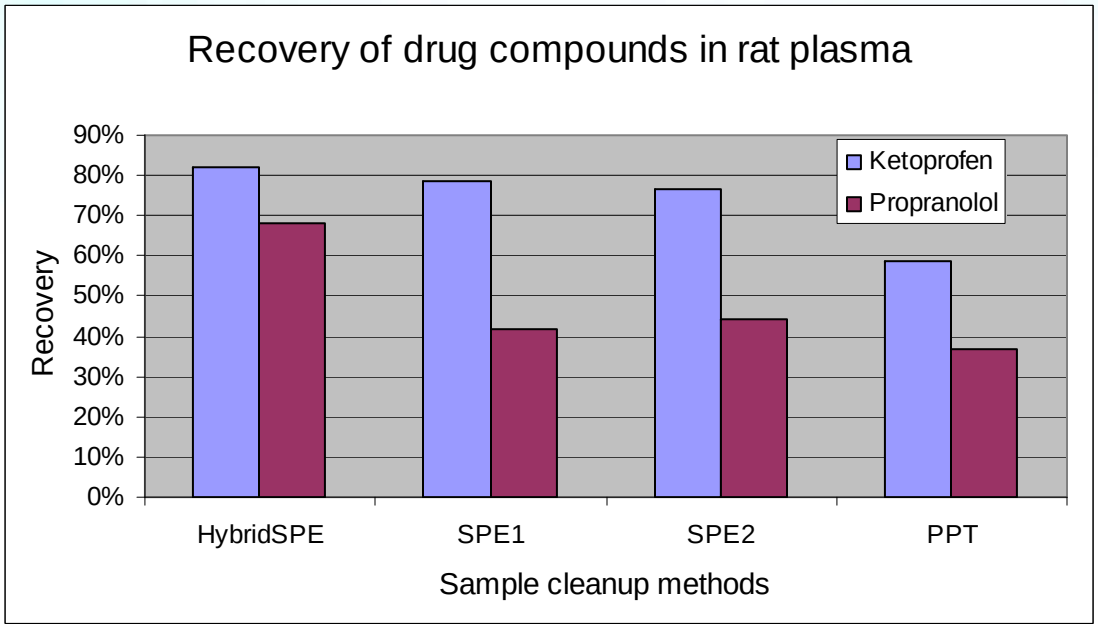
Propranolol



+MRM: 260.30/116.10 amu

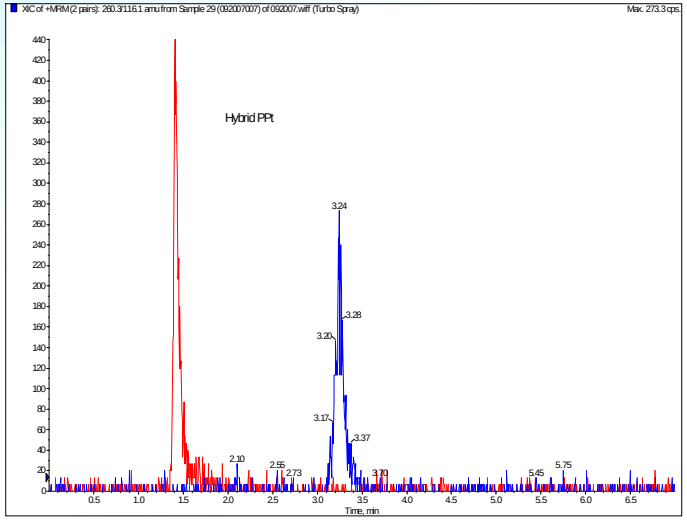
Recovery of Drug Compounds in Rat Plasma by different Sample Preparation Methods

Sample Preparation Method	Ketoprofen	Propranolol
HybridSPE	82.0%	68.0%
Traditional PPT	58.8%	37.0%
Generic polymeric SPE1	78.4%	42.0%
Generic polymeric SPE2	76.4%	44.4%

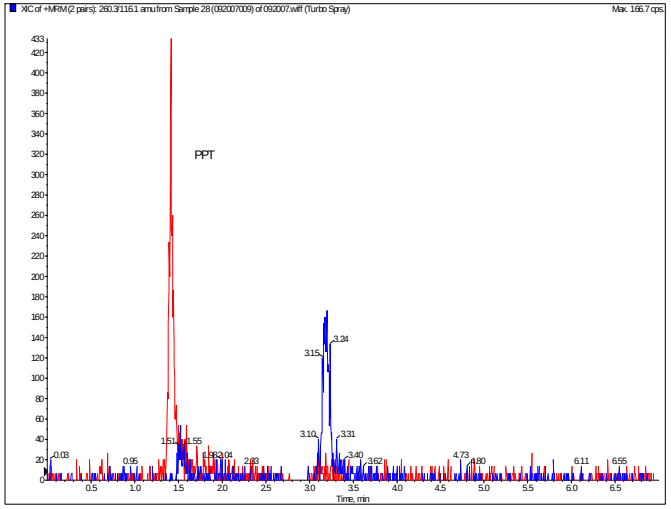


HybridSPE method provided highest recovery for both compounds and generic polymeric SPE method provided moderate recovery of both compounds while PPT method provided least recovery of both compounds.

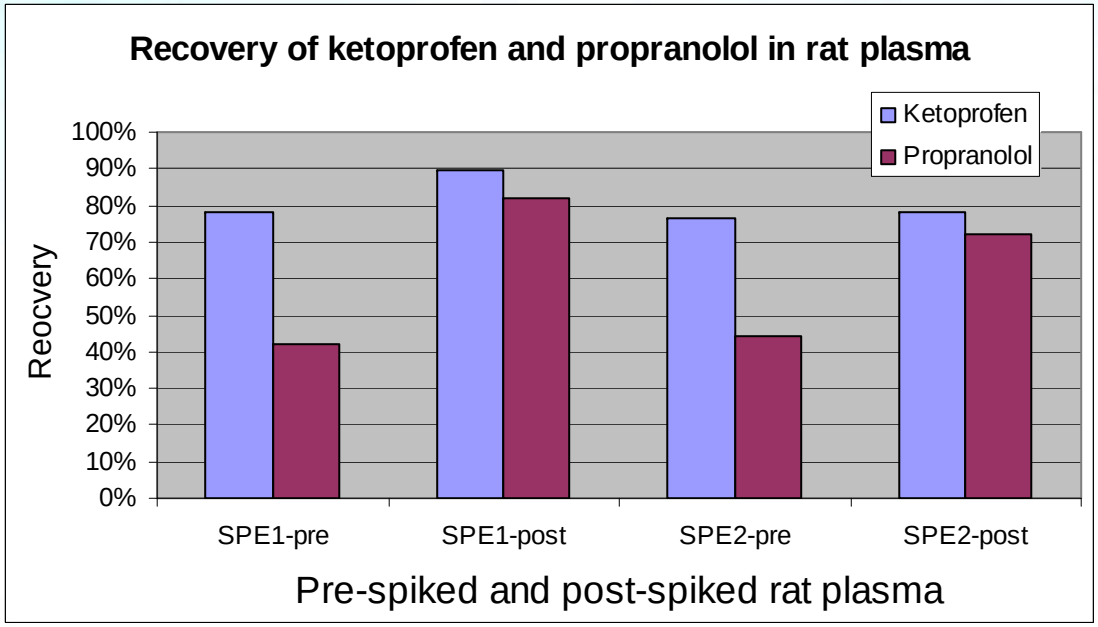
Ketoprofen and Propranolol in Rat Plasma cleanup by HybridSPE



Ketoprofen and Propranolol in Rat Plasma cleanup by PPT

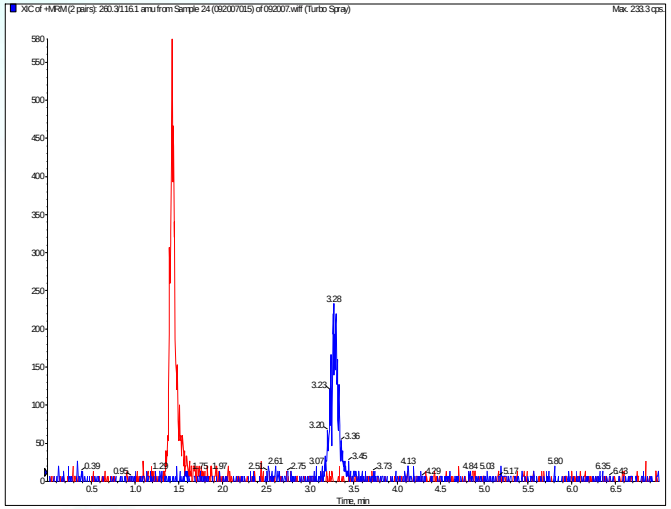


Recovery of propranolol in rat plasma by PPT method are much lower than the recovery of propranolol by HybridSPE method because of biological sample matrix interference in PPT sample.

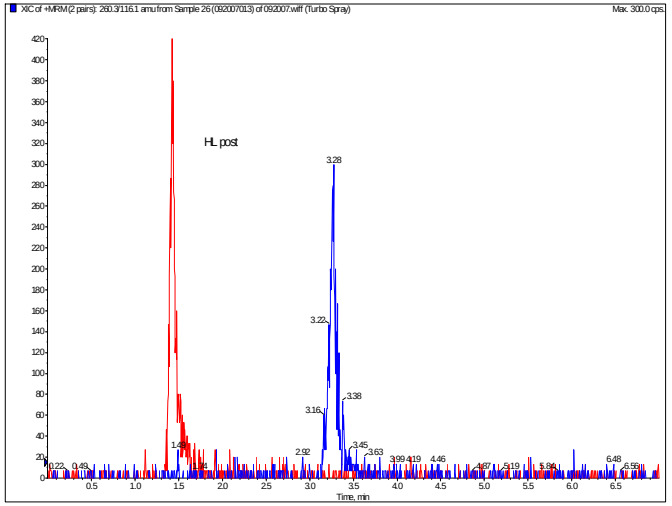


However, lower recovery of propranolol in SPE samples was also found. Some propranolol were found adsorbing on polymeric SPE stationary phase. Therefore, stronger organic solvent may require to elute the rest of propranolol from polymeric SPE phase to improve its recovery.

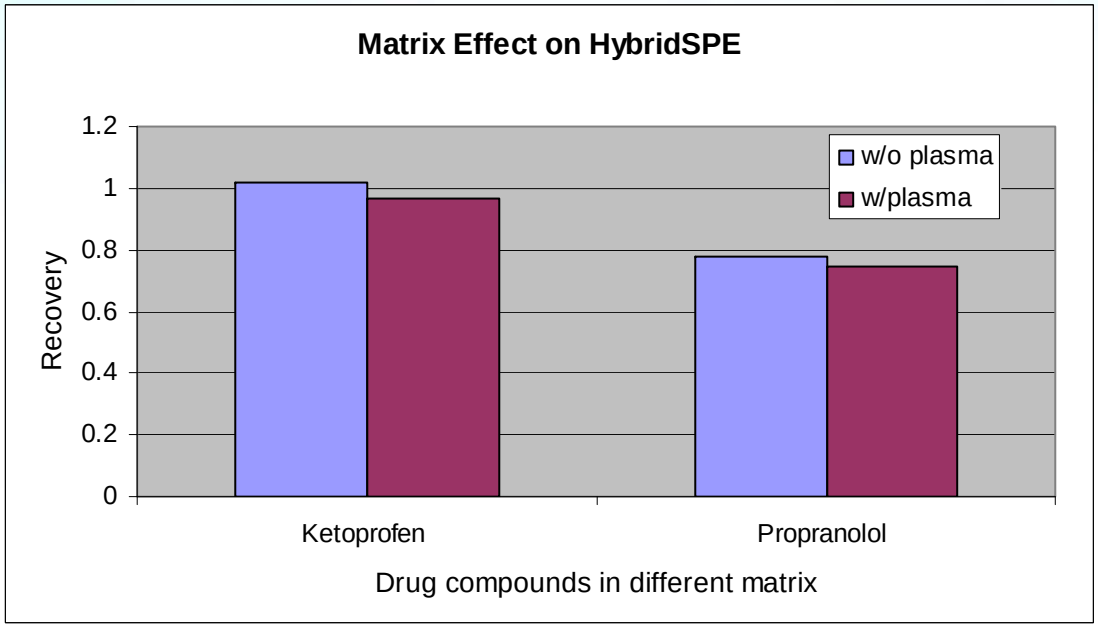
Pre-spiked Ketoprofen and Propranolol in Rat Plasma cleanup by SPE



Post-spiked Ketoprofen and Propranolol in Rat Plasma cleanup by SPE



Recovery of pre-spiked propranolol in rat plasma by SPE method are low and recovery of post-spiked propranolol in rat plasma by SPE method are higher.



Two compounds with or without rat plasma were studied by HybridSPE method. There is no big difference in their recovery by using this method. That means there is no or less matrix effect on these compounds by HybridSPE technique, but some propranolol may adsorb on the HybridSPE under current condition.

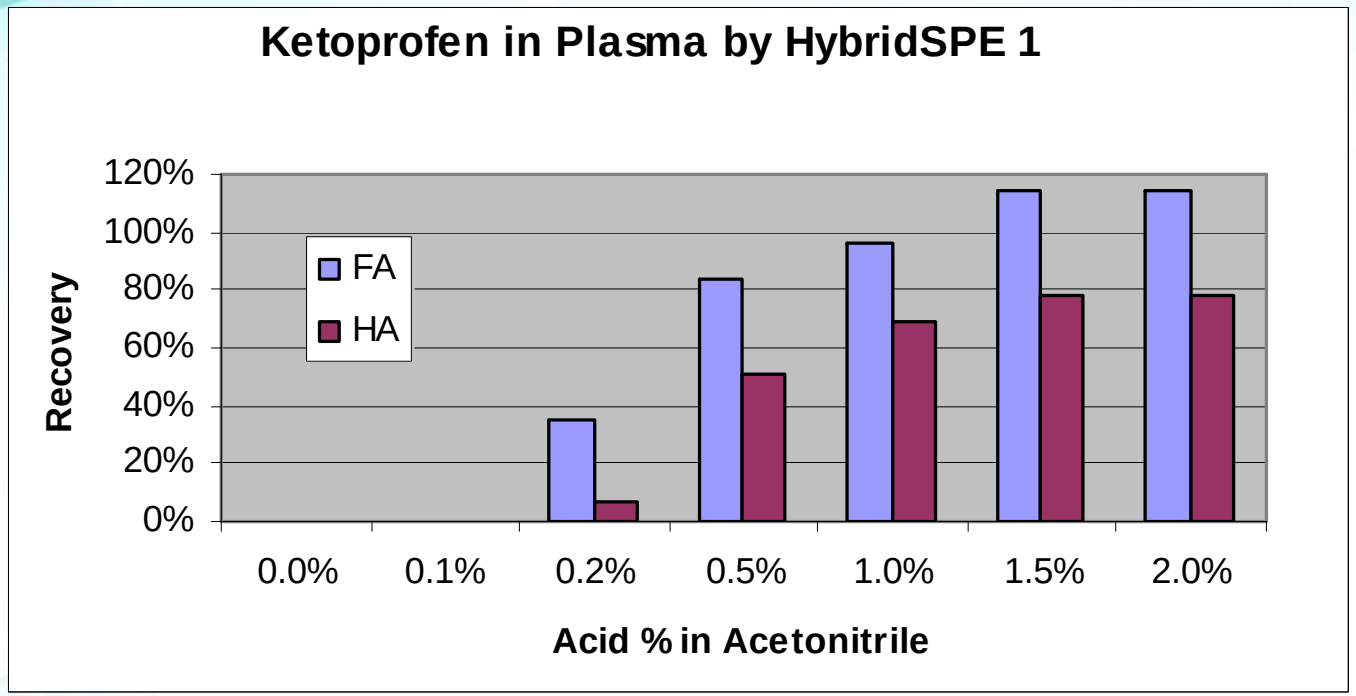
Experiment 3

Optimization of HybridSPE Method

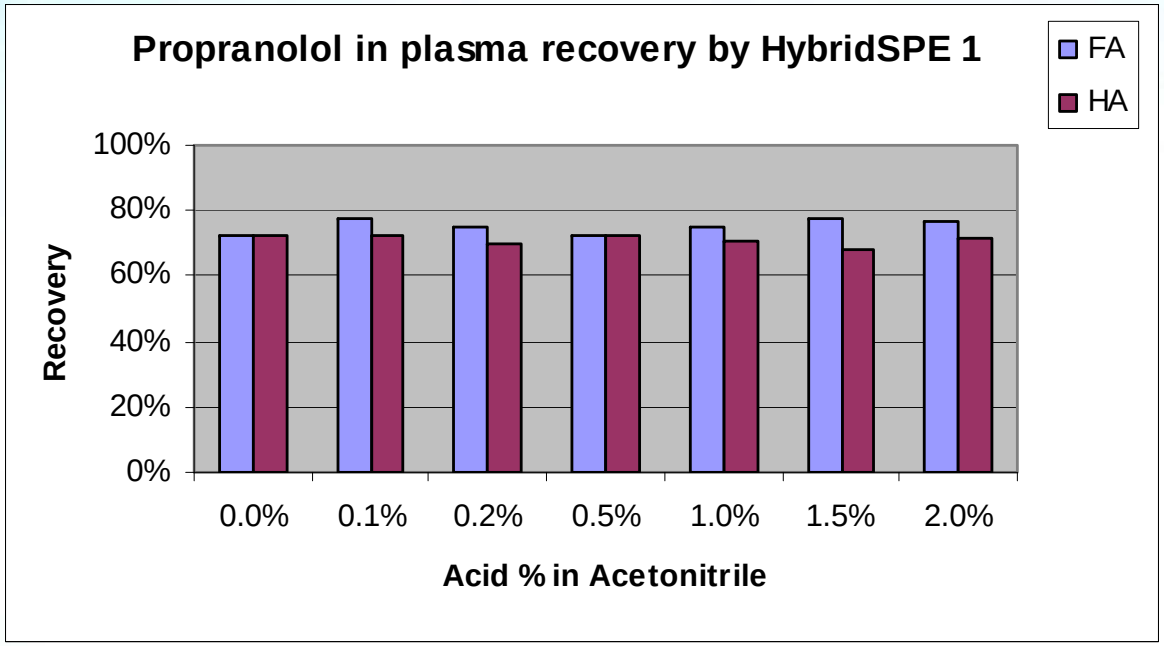
Two different acid modifiers were compared in this study. The acids tested were formic acid (FA) and acetic acid (HA). The acid concentration was systematically adjusted and measured against recovery using a representative acidic compound (ketoprofen) and basic compound (propanolol) diluted in rat plasma (100 ng/mL).

Ratio of plasma and crashing solvent volume with two different acid modifiers were also studied. The ratio was adjusted to 1:2, 1:3 and 1:5, respectively.

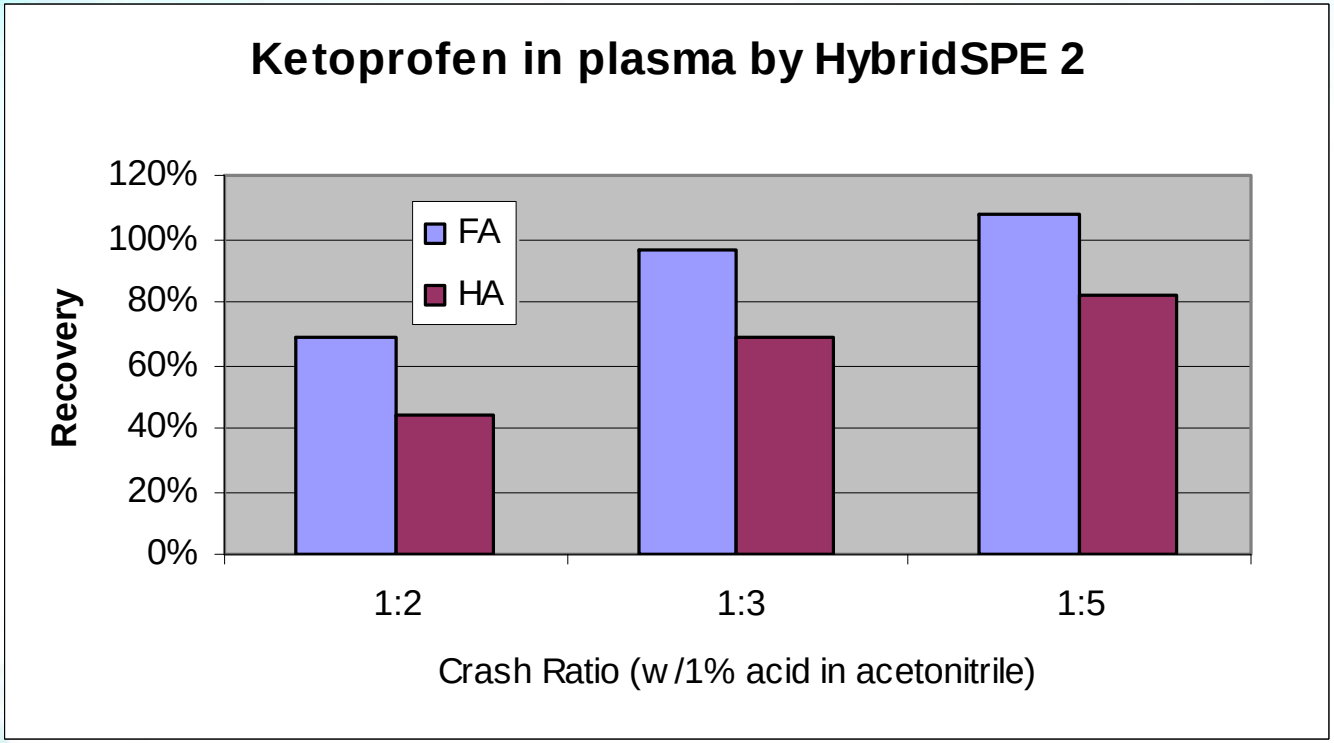
HPLC and MS condition are the same as for Experiment 2.



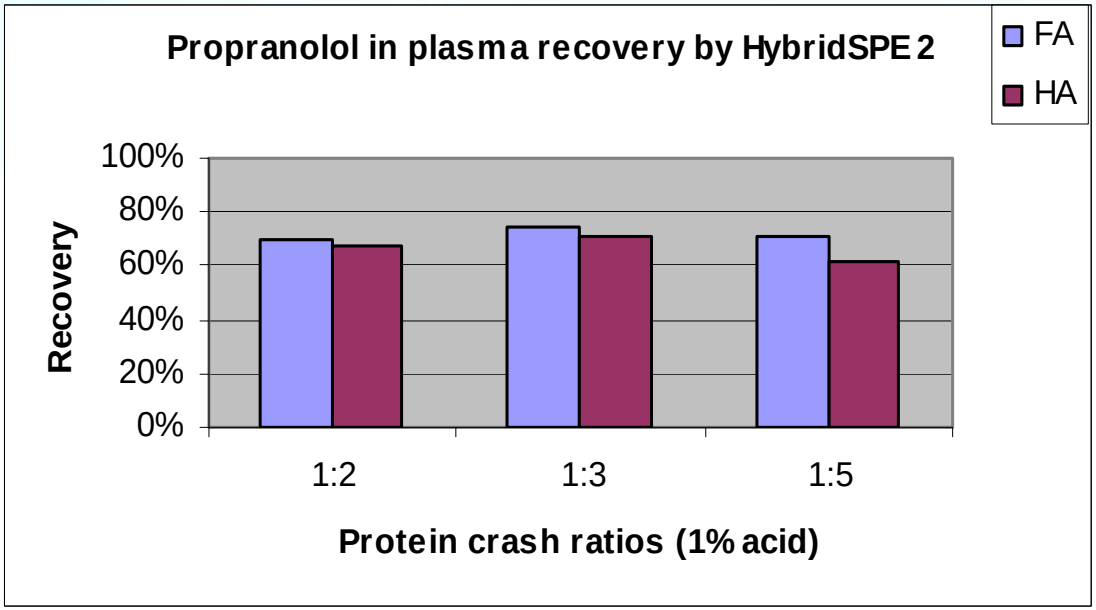
Acid type and amount had great affect on the recovery of ketoprofen (acidic compounds). 1% acid modifier are optimized for giving an acceptable recovery of acidic compounds.



Acid type and amount had little affect on the recovery of propanolol (basic compounds).



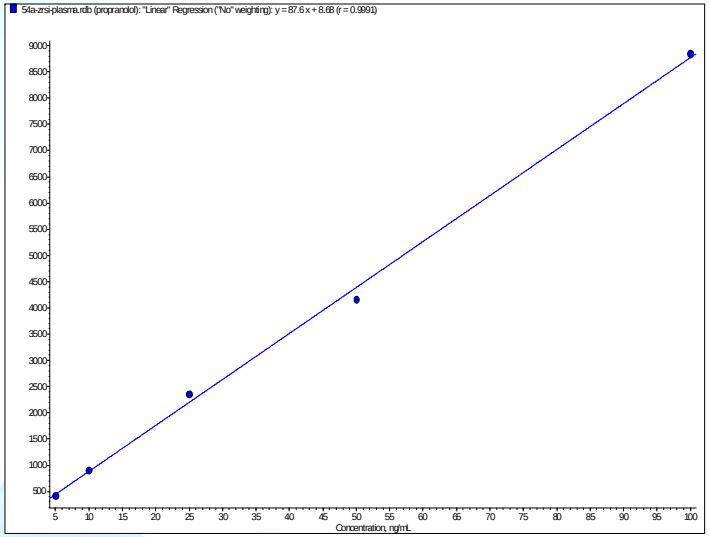
Ratio of plasma and crashing solvent with different acid modifiers had some affect on the recovery of ketoprofen (acidic compounds).



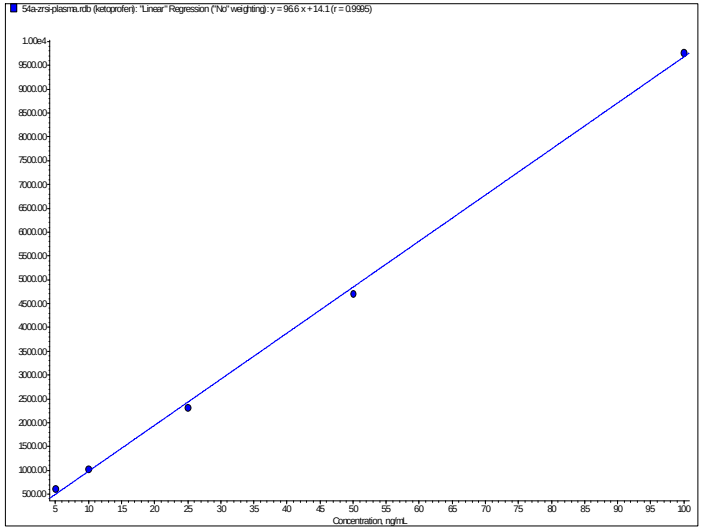
Ratio of plasma and crashing solvent with different acid modifiers had little affect on the recovery of propranolol (basic compounds). 1:3 protein crash ratio is optimized for acidic and basic compounds in plasma samples.

Calibration Curves of Ketoprofen and Propranolol in Rat Plasma cleanup by HybridSPE

Propranolol Calibration (R = 0.9991)



Ketoprofen Calibration (R = 0.9995)



Conclusions

The degree of phospholipids interference varied greatly between the three sample preparation techniques. HybridSPE technology efficiently removed phospholipids and protein resulting in the least matrix interference. SPE removed minimal phospholipids and protein resulting moderate matrix interference. Protein precipitation removed only gross levels of proteins from biological with no removal of phospholipids resulting in greatest matrix interference.

1:3 ratio of plasma and acetonitrile with 1% formic acid is the optimized HybridSPE sample preparation condition.