

Comparison of Two Clean-up Techniques and a Quick Analysis of 39 Pain Management Drugs from Serum

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Overview

In this application, serum was cleaned up and compared with both a single protein precipitation protocol and also by the addition of a phospholipid removal product. The analysis was analyzed with a Kinetex 2.6 µm Biphenyl LC column for both in **Figure 1 and 2** to show the separation similarities. The phospholipid traces in Figure 3 show the differences between the two clean-up techniques and displays why Phree is a necessary addition to the clean-up steps along with the comparative recoveries for the two techniques in **Table 1**.

Sample Preparation Protocol

Sample Pretreatment: 200 µL of serum was aliquoted into a tube and 600 µL of chilled (0 to -20°C) Acetonitrile/Methanol (95:5) was added and vortex/mixed for 5-10 seconds. The tube was centrifuged at 3000 rpm for 10 minutes and the supernatant was collected and 25 µL of 1% formic acid was added.

Load: Pre-treated sample onto the Phree 96-Well Plate (Part No.: [8E-S133-TGB](#))

Vacuum: 4-5 psi to collect supernatant

Dry Down: Sample under a gentle stream of Nitrogen at 40-45°C

Reconstitute: In 200 µL initial mobile phase

LC Conditions

(for representative chromatogram)

Column: Kinetex® 2.6 µm Biphenyl

Dimensions: 50 x 3.0 mm

Part No.: [00B-4622-YO](#)

Mobile Phase: **A:** 0.1% Formic acid in Water

B: 0.1% Formic acid in Methanol

Gradient: Time	%B
0	15
3.5	95
5	95
5.01	15
7	15

Flow Rate: 0.5 mL/min

Temperature: Ambient

Injection: 5 µL

Detection: SCIEX 4500 MS/MS (ESI+)

System: Agilent® Technologies 1200 Series

(for phospholipid comparison)

Column: Kinetex® 2.6 µm C18

Dimensions: 50 x 2.1 mm

Part No.: [00B-4462-AN](#)

Mobile Phase: **A:** 0.1% Formic acid in Water

B: 0.1% Formic acid in Methanol

Gradient: Time	%B
0	40
0.5	95
11.5	95
11.51	40
13.5	40

Flow Rate: 0.4 mL/min

Temperature: 40°C

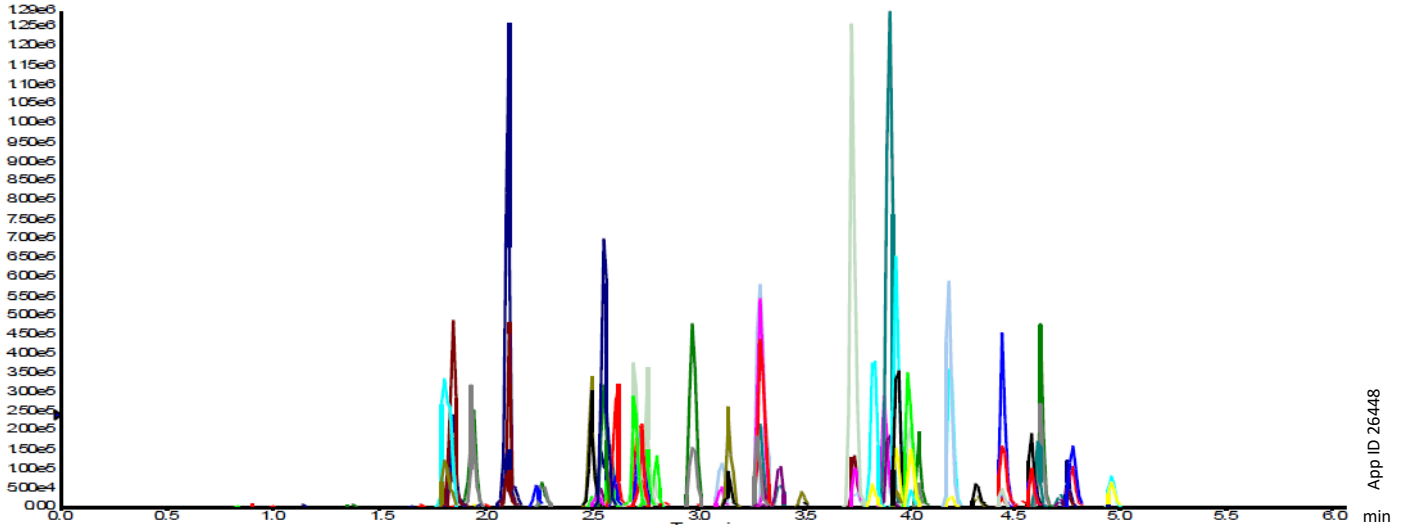
Injection: 1 µL

Detection: SCIEX 4500 MS/MS (ESI+)

System: Agilent® Technologies 1200 Series

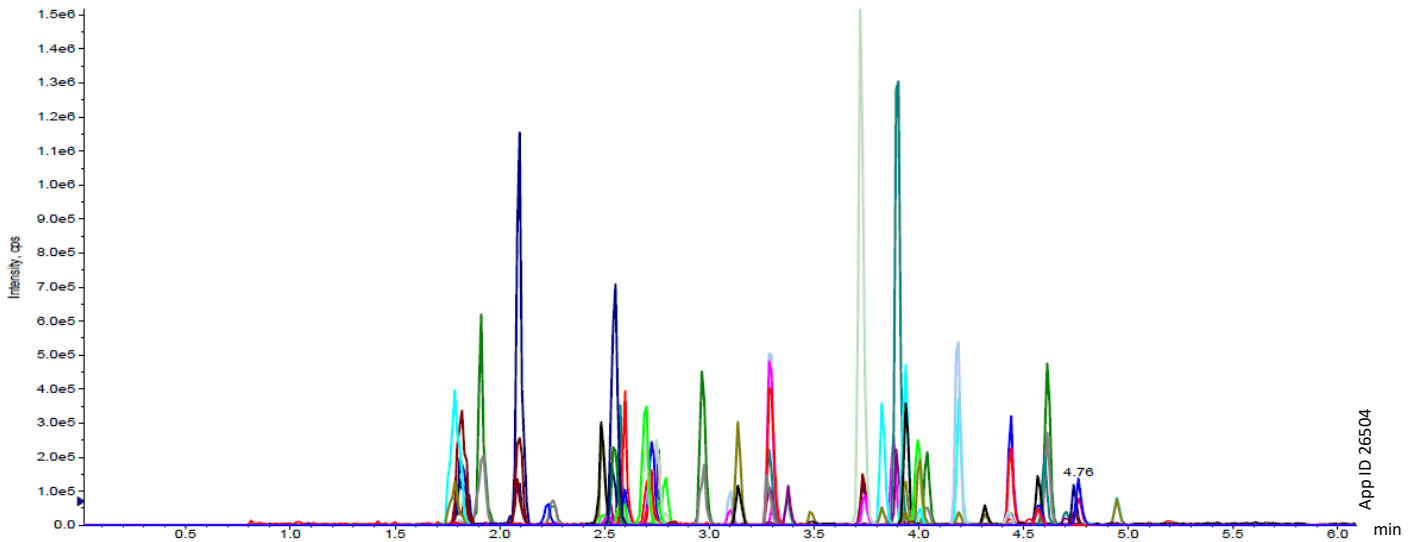


Figure 1. Separation of 39 Pain Panel Analytes using Protein Precipitation



App ID 26448

Figure 2. Separation of 39 Pain Panel Analytes Using Protein Precipitation and Phree™ Phospholipid Removal



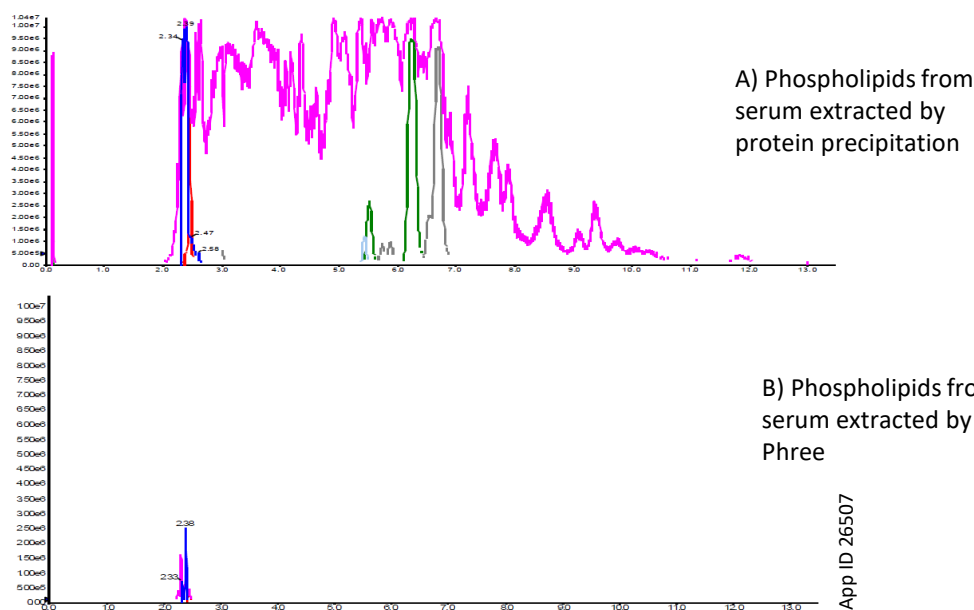
App ID 26504



Table 1. Comparison of Protein Precipitation and Phospholipid Removal Recoveries and CVs for 39 Analytes

Peak No.	Analyte Name	RT (min)	Q1	Q3	Protein Precipitation		Phree PLR	
					% Rec.	%CV (N=4)	% Rec.	%CV (N=4)
1	Alprazolam	4.8	309.1	281.1	93	4.3	107	5.6
2	Amphetamine	2.3	136.1	91.1	88	9.6	81	8.8
3	Benzoylcegonine	3.3	290.1	168.1	87	3.9	91	13.8
4	Codeine	2.6	300.2	152.1	84	7.5	91	15.2
5	Diazepam	4.9	285	193.2	85	9.9	91	12
6	MDMA	2.9	194.1	105.1	75	0.9	95	12.4
7	Methamphetamine	2.6	150.1	91	78	13.5	85	15
8	Norbuprenorphine	3.6	414.3	83.2	95	10.3	93	13.4
9	Oxazepam	4.4	287	241	93	4.1	100	5.1
10	Oxymorphone	2	302.1	227	72	11.7	73	4.8
11	PCP	4	244.3	91	80	7.4	95	10.3
12	Propoxyphene	4	340.3	266.3	93	12.5	100	10.6
13	Sufentanil	4.1	387.2	238.1	92	14.3	90	3.2
14	6MAM	2.57	328.1	165.1	71	2.9	80	13.5
15	Buprenorphine	3.9	468.3	55.2	93	12.5	97	10.6
16	Carisoprodol	3.9	261.1	176.2	103	11.9	98	11
17	Clonazepam	4.4	316.1	270.1	94	2	96	6.5
18	EDDP	4.2	278.2	234.2	84	4.1	88	4.8
19	Fentanyl	3.9	337.3	105.1	103	9.3	105	11.6
20	Flunitrazepam	4.7	314.1	268.2	86	1.9	97	3.3
21	Flurazepam	4	388.2	315.2	112	13.3	104	3.8
22	Hydrocodone	2.8	300.2	199	85	8.6	94	14.2
23	Hydromorphone	2.1	286.1	185.1	78	7.2	82	12.6
24	Lorazepam	4.3	321	275	100	4.1	109	12.3
25	MDA	2.7	180.1	133	78	7	93	8.8
26	MDEA	3	208.2	163	81	9.7	91	15.3
27	Meperidine	3.4	248.2	220.2	98	4.9	95	7.2
28	Methadone	4.4	310	265	99	14.9	99	7.1
29	Midazolam	4.1	326.1	291.1	107	11.1	93	8.6
30	Morphine	1.9	286.1	152.1	92	15.7	94	5.4
31	Naloxone	2.56	328.2	212	76	15.1	104	8.5
32	Naltrexone	2.8	342.2	267.1	94	14.1	92	10.5
33	Nordiazepam	4.64	271	140	98	5.3	98	8.9
34	Norfentanyl	3.2	233.2	84.1	99	6.5	94	11.2
35	Normeperidine	3.4	234.1	160.1	93	8.5	86	13.1
36	Norpropoxyphene	4.1	308.2	100.1	99	15	97	4.4
37	Oxycodone	2.8	316.1	241.2	95	2.4	110	7.6
38	Temazepam	4.7	301.1	255.1	90	4.6	89	5.8
39	Tramadol	3.2	264.1	58.1	87	10.2	89	13.9
% Recovery range for 39 analytes					71-112%		73-110%	

Figure 3. Clean Up of Phospholipids Using Phree™ Phospholipids Removal Product



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