

# Fast Clean-Up of Whole Blood Using Phree™ Phospholipid Removal and Analysis of 39 Pain Management Drugs by Kinetex® 2.6 µm Biphenyl

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## Overview

Whole blood is a common matrix for toxicology testing, yet it can be very complicated to clean up. Using Phree Phospholipid Removal as a pass through with an easy protein precipitation, the whole blood matrix is efficiently cleaned up while still yielding high recoveries and low variation before analysis on LC-MS/MS using a Kinetex Biphenyl LC column.

## 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) along with 50 µL 5% ZnSO<sub>4</sub> (w/v) was added to it.

Vortex/mix the tube for 5-10 secs. The tube was centrifuged at 3000 rpm for 10 minutes and the supernatant was collected and 25 µL of 1% formic acid was added.

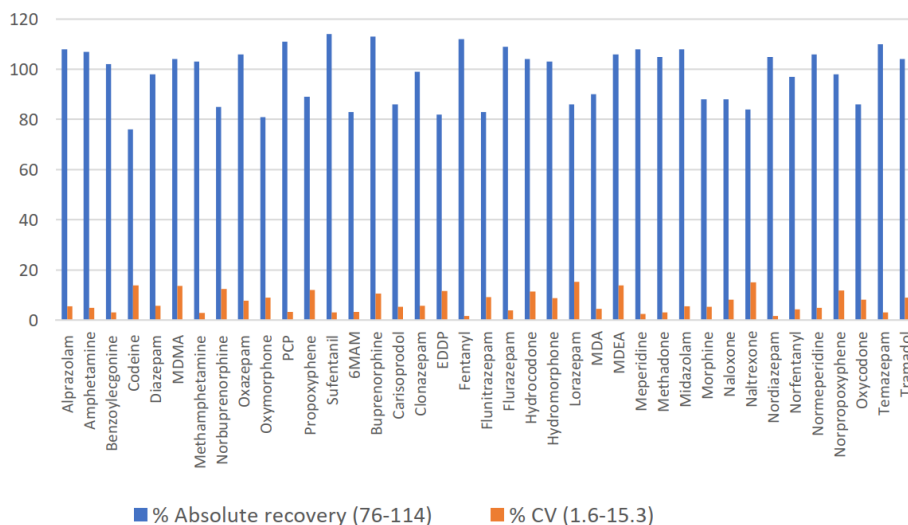
**Load:** Pre-treated sample onto the Phree 96-Well Plate (Part No.: [8E-S133-TGB](#))

**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

**Table 1. Absolute Recoveries and %CVs for 39 Drugs Extracted from Whole Blood Using Phree Phospholipid Removal Product**



## LC Conditions

(for representative chromatogram)

**Column:** Kinetex 2.6 µm Biphenyl

**Dimensions:** 50 x 3.0 mm

**Part No.:** [00B-4622-Y0](#)

**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
	.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

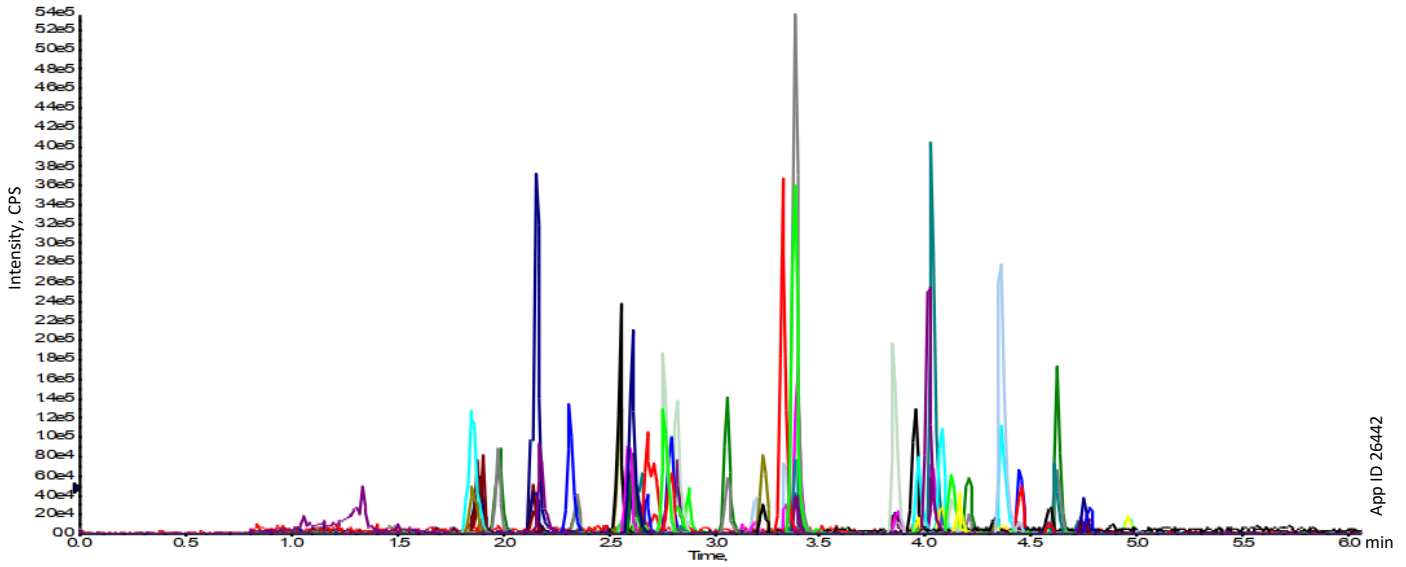


**Table 1. Analyte Details for 39 Pain Management Drugs Extracted from Whole Blood**

Peak ID	Analyte Name	RT (min)	Q1	Q3	% Rec.	%CV (N=4)
1	Alprazolam	4.8	309.1	281.1	108	5.5
2	Amphetamine	2.3	136.1	91.1	107	4.9
3	Benzoylcegonine	3.3	290.1	168.1	102	3.1
4	Codeine	2.6	300.2	152.1	76	13.9
5	Diazepam	4.9	285	193.2	98	5.7
6	MDMA	2.9	194.1	105.1	104	13.7
7	Methamphetamine	2.6	150.1	91	103	2.8
8	Norbuprenorphine	3.6	414.3	83.2	85	12.4
9	Oxazepam	4.4	287	241	106	7.8
10	Oxymorphone	2	302.1	227	81	8.9
11	PCP	4	244.3	91	111	3.3
12	Propoxyphene	4	340.3	266.3	89	12
13	Sufentanil	4.1	387.2	238.1	114	3.1
14	6MAM	2.57	328.1	165.1	83	3.3
15	Buprenorphine	3.9	468.3	55.2	113	10.5
16	Carisoprodol	3.9	261.1	176.2	86	5.3
17	Clonazepam	4.4	316.1	270.1	99	5.7
18	EDDP	4.2	278.2	234.2	82	11.6
19	Fentanyl	3.9	337.3	105.1	112	1.6
20	Flunitrazepam	4.7	314.1	268.2	83	9.1
21	Flurazepam	4	388.2	315.2	109	3.9
22	Hydrocodone	2.8	300.2	199	104	11.3
23	Hydromorphone	2.1	286.1	185.1	103	8.7
24	Lorazepam	4.3	321	275	86	15.3
25	MDA	2.7	180.1	133	90	4.4
26	MDEA	3	208.2	163	106	13.8
27	Meperidine	3.4	248.2	220.2	108	2.5
28	Methadone	4.4	310	265	105	3.1
29	Midazolam	4.1	326.1	291.1	108	5.5
30	Morphine	1.9	286.1	152.1	88	5.2
31	Naloxone	2.56	328.2	212	88	8.2
32	Naltrexone	2.8	342.2	267.1	84	15
33	Nordiazepam	4.64	271	140	105	1.6
34	Norfentanyl	3.2	233.2	84.1	97	4.3
35	Normeperidine	3.4	234.1	160.1	106	4.9
36	Norpropoxyphene	4.1	308.2	100.1	98	11.7
37	Oxycodone	2.8	316.1	241.2	86	8.2
38	Temazepam	4.7	301.1	255.1	110	3.1
39	Tramadol	3.2	264.1	58.1	104	9

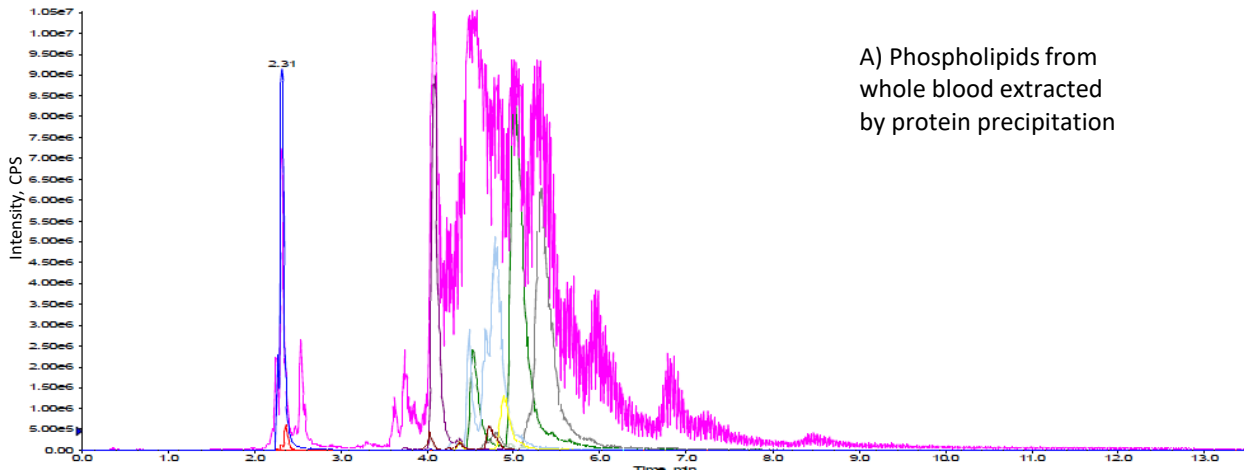


Figure 1. Separation of 39 Pain Panel Analytes



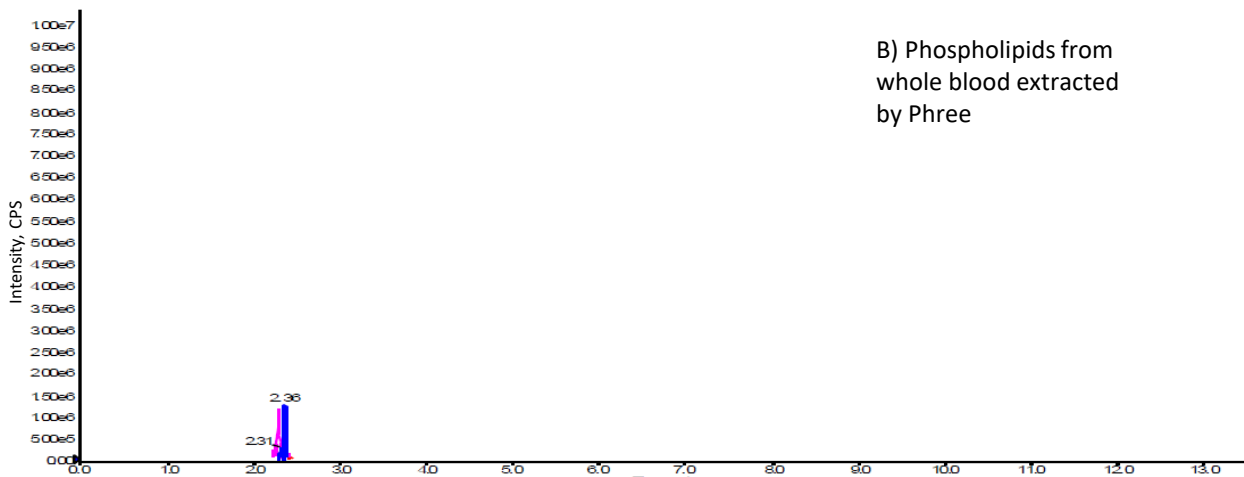
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Figure 2. Clean Up of Phospholipids Using Phree™ Phospholipids Removal Product



A) Phospholipids from whole blood extracted by protein precipitation

App ID 26561



B) Phospholipids from whole blood extracted by Phree

App ID 26507



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