Reduction of Phospholipid Matrix Effects in Electrospray Ionization by Adding Colloidal Silica and Manganese(II) Chloride

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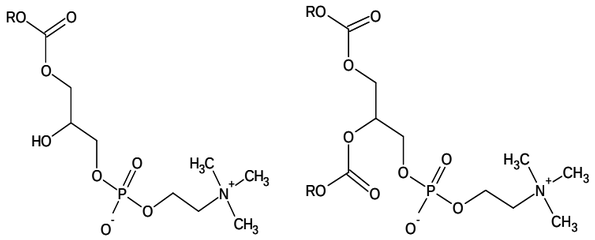
INTRODUCTION

Phospholipids are surface components of human plasma lipoproteins and comprise between 7 and 33% by weight of the various lipoprotein classes (chylomicrons, VLDL, IDL, LDL, and HDL). They are present in plasma at total concentrations of 1-3 mg/mL and have been shown to significantly contribute to ionization/matrix effects when using electrospray ionization.

It is well known that polyanions such as dextran sulfate and heparin react with positively charged groups on lipoproteins, which is facilitated with divalent cations that interact with negatively charged groups, which results in lipoprotein precipitation. We found a robust polyanion/divalent cation combination in LUDOX AS-40, a 40 wt. % suspension of colloidal silica in water at pH 9.1 with ammonium counter ion, and $MnCl_2$ to precipitate choline phospholipids and significantly reduce matrix effects.

LYSO-PHOSPHATIDYL CHOLINE **PHOSPHATIDYL CHOLINE**



R = LONG ALKYL CHAIN, SATURATED OR UNSATURATED

METHODS
A. Colloidal Silica and $MnCl_2$ added directly to human plasma

Add human plasma (K_3EDTA) to a centrifuge tube.
↓
Add LUDOX AS-40 and $MnCl_2$
↓ Vortex / Centrifuge
Pour supernatant into another centrifuge tube, add acetonitrile (ACN)
↓ Vortex / Centrifuge
Supernatant dried, reconstituted, and assayed

METHODS
B. Colloidal Silica and $MnCl_2$ added to a reconstituted extract

Add human plasma (K_3EDTA) to a centrifuge tube.
↓
Add acetonitrile (ACN)
↓ Vortex / Centrifuge
Supernatant dried, reconstituted
↓ Transfer to a centrifuge tube
Add LUDOX AS-40 and $MnCl_2$
↓ Vortex / Centrifuge
Assay Supernatant

METHODS
C. Colloidal Silica and $MnCl_2$ added to an MTBE extact

Mix human plasma with MTBE for Liq/Liq extraction
↓ Freeze / Decant
Add LUDOX AS-40 and $MnCl_2$
↓ Vortex / Centrifuge
↓ Freeze / Decant
MTBE layer dried, reconstituted, and assayed

LC/MS/MS

Instrument: API 365/Ionics EP10+ or API-3000 with the TIS source operated in positive ion mode

Column: Phenomenex Luna phenyl-hexyl, 5 µ, 2 x 50 mm

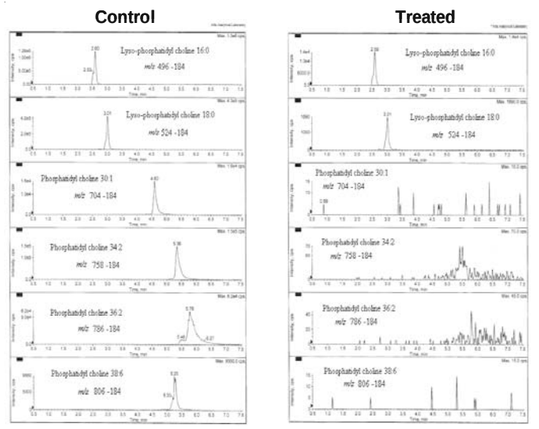
Mobile phases: A: 10 mM NH_4OAc B: ACN

Flow rate: 400 µL/min

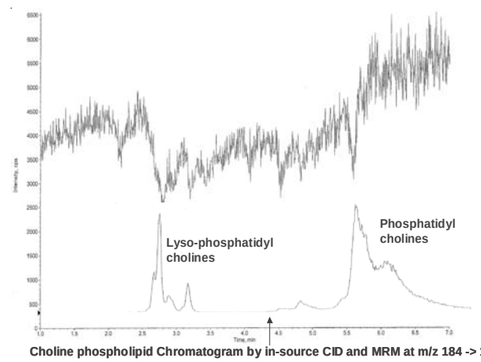
Gradient: 30%B to 90% B over 5 minutes

Analyte	SRM transition (*m/z*)
Lyso-phosphatidyl choline 16:0	496 → 184
Lyso-phosphatidyl choline 18:0	524 → 184
Phosphatidyl choline 30:1	704 → 184
Phosphatidyl choline 34:2	758 → 184
Phosphatidyl choline 36:2	786 → 184
Phosphatidyl choline 38:6	806 → 184

RESULTS
Figure 1: Chromatograms of Choline Phospholipids in Control Plasma and Plasma Treated with Ludox AS-40 and $MnCl_2$



RESULTS
Figure 2a: Matrix Effect Chromatogram of Extracted Blank Plasma Post-Column Infused with a Caffeine Solution



Choline phospholipid Chromatogram by in-source CID and MRM at m/z 184 -> 184

RESULTS
Figure 2b: Matrix Effect Chromatogram of Blank Plasma Treated with LUDOX AS-40 and 2 M $MnCl_2$ and Post-Column Infused with a Caffeine Solution

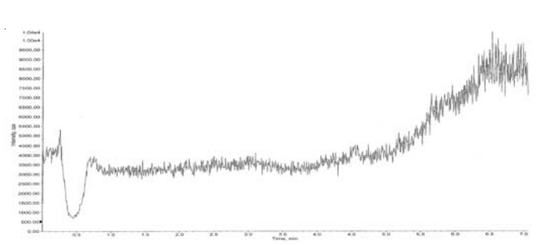


Table 1: Percent Reduction of Choline Phospholipids in Plasma Treated with LUDOX AS-40 and $MnCl_2$

Volume of plasma (µL)	Volume of Ludox AS-40 (µL)	Volume of 2 M $MnCl_2$ (µL)	lyso-PC 16:0	lyso-PC 18:0	PC 30:1	PC 34:2	PC 36:2	PC 38:6
100	30	0	8	5	36	13	20	18
100	10	5	25	43	94	80	86	84
100	30	5	37	49	90	63	71	69
100	30	15	82	91	> 99	> 99	> 99	> 99
100	30	30	76	88	> 99	> 99	> 99	> 99
100	30	80	83	91	> 99	> 99	> 99	> 99
100	40	30	89	95	> 99	> 99	> 99	> 99
100	50	30	92	97	> 99	> 99	> 99	> 99
100	60	5	23	30	79	59	67	63
100	60	10	77	88	> 99	> 99	> 99	> 99
100	60	30	99	> 99	> 99	> 99	> 99	> 99
100	70	30	> 99	> 99	> 99	> 99	> 99	> 99
100	80	30	> 99	> 99	> 99	> 99	> 99	> 99

Table 2: Percent Reduction of Choline Phospholipids in Reconstituted Extract Treated with LUDOX AS-40 and $MnCl_2$

Volume of reconstituted extract (µL)	Volume of Ludox AS-40 (µL)	Volume of 2 M $MnCl_2$ (µL)	lyso-PC 16:0	lyso-PC 18:0	PC 30:1	PC 34:2	PC 36:2	PC 38:6
300	50	10	> 99	> 99	> 99	> 99	> 99	> 99
300	100	10	> 99	> 99	> 99	> 99	> 99	> 99
300	100	20	> 99	> 99	> 99	> 99	> 99	> 99
300	150	20	> 99	> 99	> 99	> 99	> 99	> 99
300	150	30	> 99	> 99	> 99	> 99	> 99	> 99

Table 3: Percent Reduction of Choline Phospholipids in MTBE Extract Treated with LUDOX AS-40 and $MnCl_2$

Volume of MTBE extract (mL)	Volume of Ludox AS40 (µL)	Volume of 2 M $MnCl_2$ (µL)	lyso-PC 16:0	lyso-PC 18:0	PC 38:6
10	1000	50	98	98	>99
10	1000	100	98	98	99
10	1000	200	99	99	>99
10	1000	500	98	99	>99

CONCLUSIONS

- LUDOX AS-40 colloidal silica and MnCl2 can effectively (>99%) precipitate out choline phospholipids/
- Matrix effect on caffeine post-column infused chromatogram can be eliminated as shown in Figures 2a and 2b.
- These reagents can be easily added to existing bioanalytical extraction methods at various steps and automated using workstations like the Tomtec Quadra.