



***A Source for Imprecision Resulting
from Ionization Suppression from
Strongly Retained Phospholipids
and Dioctyl Phthalate***

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Introduction

Endogenous phospholipids are present in extremely high concentrations in biological matrices such as plasma. Phospholipid concentrations can vary greatly between subject samples and experimental timepoints in pharmaceutical studies. Our experiments using 10 different plasma lots demonstrated that the %RSD of measured phospholipids range from 20 to 50%. These phospholipids are present in almost all extraction techniques typically used in bioanalysis and result in ion suppression and matrix effects. With each injection, these phospholipids and other material can accumulate on HPLC columns and elute at various times and with varying intensities during gradient or isocratic elution resulting in inconsistent retention times, divergent curves or imprecise results. This research describes how the primary phospholipids can create assay imprecision through their ability to cause ion suppression.

Instrumentation and Equipment

Mass Spectrometer:	MDS Sciex API 4000
Ionization source:	TurboIonSpray™
HPLC:	Shimadzu 10 ADvP gradient system
Autosampler:	Perkin-Elmer PE200 Series
HPLC Columns:	ThermoHypersil Betasil C8 2mm x 50mm, 5 µm

Analytical Conditions

HPLC conditions:

Flow rate:	500 microliter/minute
"A" solvent:	2mM ammonium formate, pH 3.3
"B" solvent:	acetonitrile
Linear gradient:	– 0-1 Min. 30% "B" – 1-3 Min. 30% "B" to 70% "B" – 3-5 Min. 70% "B"
Injection:	No Injections; just gradient cycling

Experimental conditions:

- No Samples Extracted.
- HPLC columns previously used for GLP plasma analysis.
- For Column 2 experiments – continuous infusion of Buspirone (100 ng/ml) post-column at 10 microliter/minute¹

MS Conditions:

Analyte	SRM Transitions (m/z)
Buspirone (infused)	386:122
Diethyl Phthalate	391:149
Lyso-Phosphatidylcholine; 16:0	496:184
Lyso-Phosphatidylcholine; 18:0	524:184
Phosphatidylcholine; 30:1	704:184
Phosphatidylcholine; 34:2	758:184
Phosphatidylcholine; 36:2	786:184
Phosphatidylcholine; 38:6	806:184

Typical for phospholipids and lysophospholipids.²

Experiment/File Name (Sample Name)	Results	Figure
Column 1; No infusion • Initial Gradient run	<ul style="list-style-type: none"> • Elution of significant lyso-phosphotidylcholine (m/z 496 and 524) 	Figure 1a
Column 1; No infusion • Six Gradient cycles • 90% "B" Wash out at 42 minutes	<ul style="list-style-type: none"> • Elution of lyso-phosphotidylcholine (m/z 496) after first cycle • Consistent increase of higher mass phosphotidylcholines • Unpredictable increase in Dioctyl phthalate elution • Wash out – inconsistent elution of PL's and dioctyl phthalate; can see peaks from previous sample injections elute. 	Figure 1b
Column 2; w/ Buspirone infusion2 • First of 10 gradient cycles	<ul style="list-style-type: none"> • Strong elution of lyso-phosphotidylcholines and dioctyl phthalate • Constant elution of larger phosphotidylcholines during gradient • Suppression of Buspirone correlates with phospholipid and dioctyl phthalate elution • Dioctyl phthalate suppressed during elution (negative peak) 	Figure 2a
Column 2; w/ Buspirone infusion • Third of 10 gradient cycles	<ul style="list-style-type: none"> • Elution of lyso-phosphotidylcholines completed (no elution) • Increased and constant elution of larger phosphotidylcholines during gradient • Increased and constant elution of dioctyl phthalate • Suppression of Buspirone correlates with phospholipid and dioctyl phthalate elution 	Figure 2b
Column 2; w/ Buspirone infusion • Fifth of 10 gradient cycles	<ul style="list-style-type: none"> • Elution of lyso-phosphotidylcholines completed (no elution) • Significantly reduced and inconsistent elution of larger phosphotidylcholines during gradient • Significantly reduced, but constant elution of dioctyl phthalate • Suppression of Buspirone correlates with phospholipid and dioctyl phthalate elution 	Figure 2c
Column 2; w/ Buspirone infusion • Seventh of 10 gradient cycles	<ul style="list-style-type: none"> • Elution of lyso-phosphotidylcholines completed (no elution) • Significantly reduced and inconsistent elution of larger phosphotidylcholines during gradient • Constant elution of dioctyl phthalate • Suppression of Buspirone correlates with phospholipid and dioctyl phthalate elution 	Figure 2d
Column 2; w/ Buspirone Infusion • Washout w/90% "B"; no gradient	<ul style="list-style-type: none"> • Significantly reduced and inconsistent elution of larger phosphotidylcholines during gradient • Significantly reduced, but constant elution of dioctyl phthalate • Suppression of Buspirone correlates with phospholipid and dioctyl phthalate elution • Buspirone increase in Buspirone correlates to elution of PL's off of column 	Figure 3
No column; w/ Buspirone Infusion	<ul style="list-style-type: none"> • No suppression of Buspirone during gradient • Signal actually increases with the increase in organic 	Figure 4a
Column 2; w/ Buspirone Infusion • Post wash out gradient cycle	<ul style="list-style-type: none"> • Buspirone results similar to no column – no suppression when matrix components eluted from column 	Figure 4b

Figure 1a.

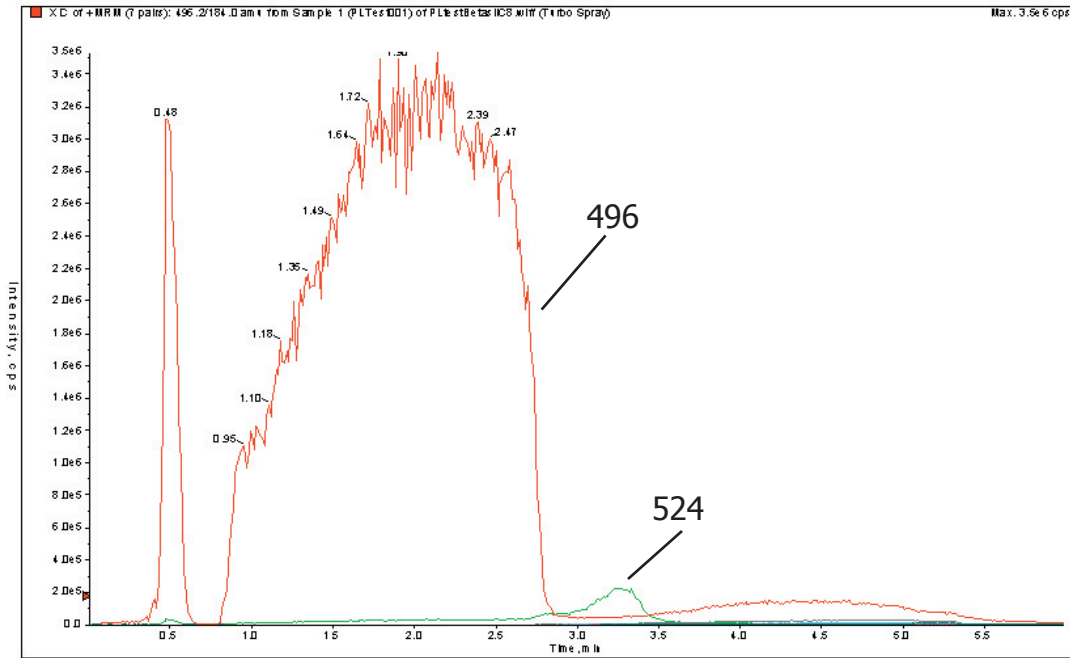


Figure 1b.

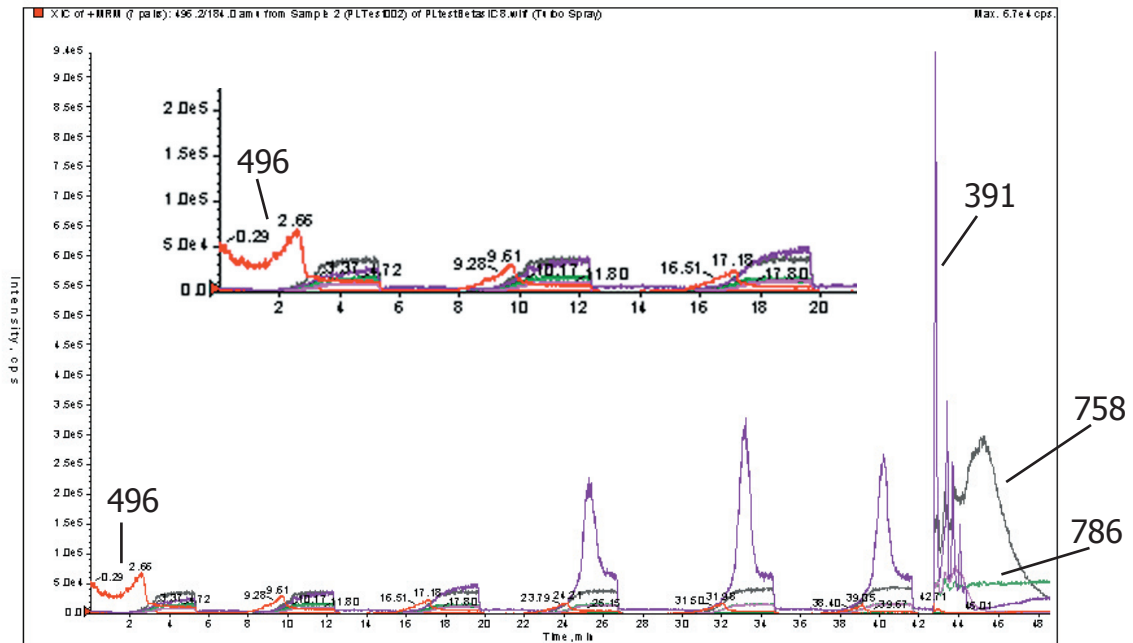


Figure 2a.

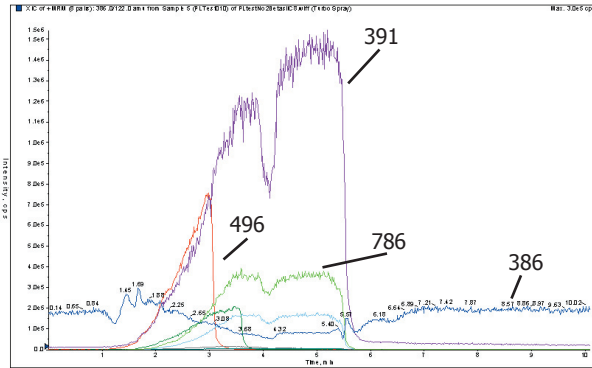


Figure 2b.

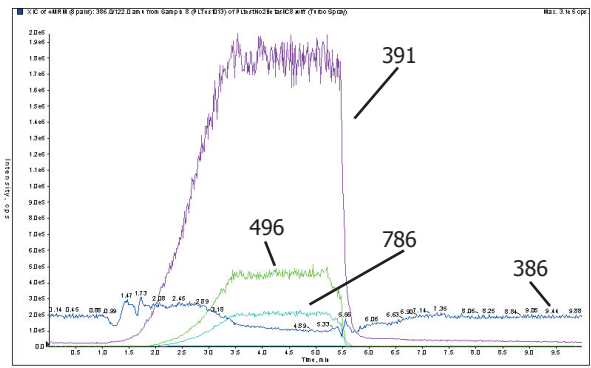


Figure 2c.

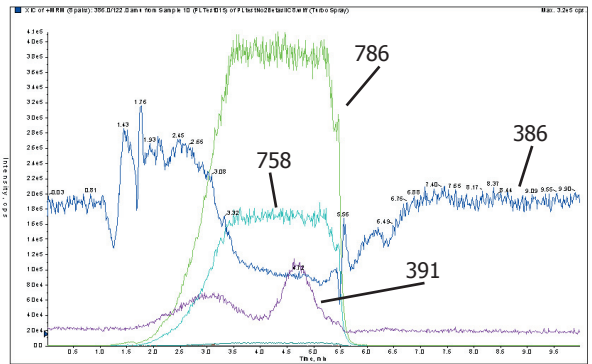


Figure 2d.

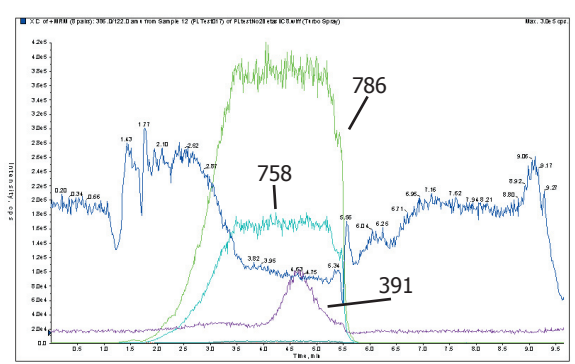


Figure 3.

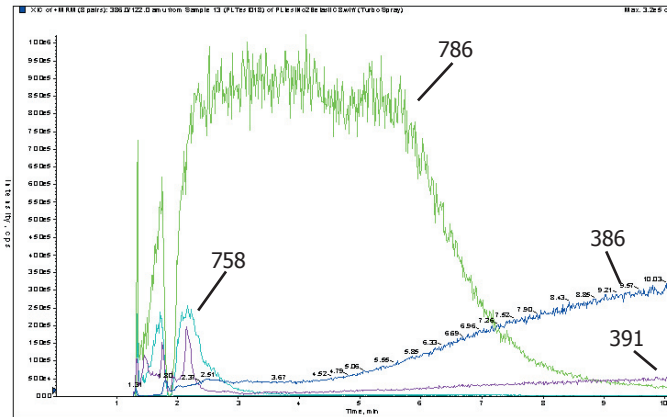


Figure 4a.

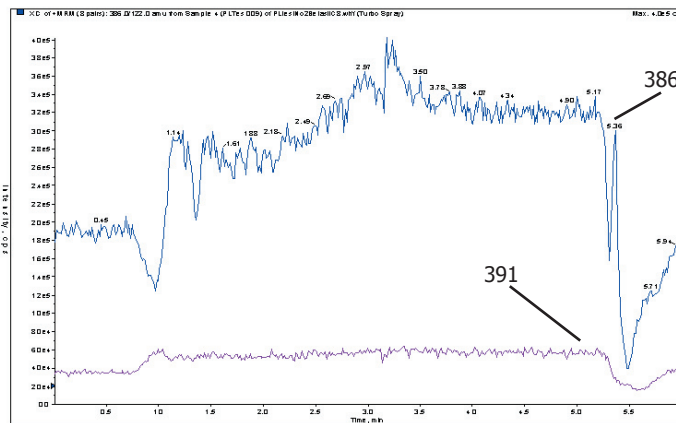
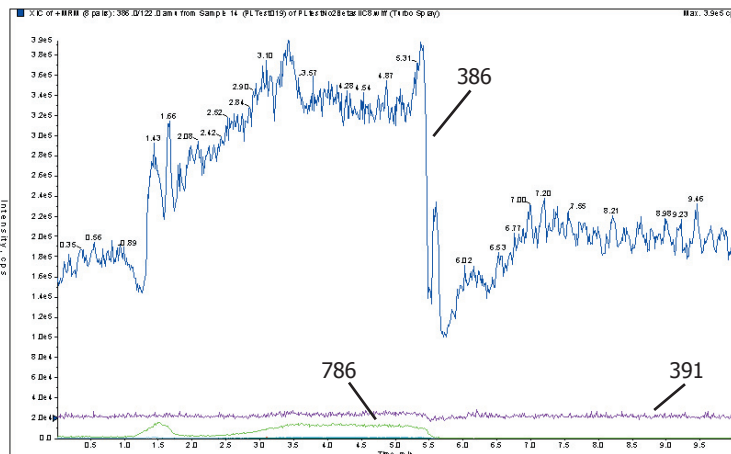


Figure 4b.



Discussion

Buspirone signal:

- Approximately 1.8e5 cps. at baseline for all experiments
- 3.8e5 cps. during peak gradient for "no column" and "post-wash out" column
- Gradual decrease to approximately 1.0e5 cps. when phospholipids and dioctyl phthalate elute during gradient

Conclusions

1. A major source of ionization suppression and matrix effects in positive ion mode result from endogenous phospholipids³⁻⁵ and potentially environmentally persistent dioctyl phthalate.
2. The presence of these compounds may result in divergent calibration curves and assay imprecision.
3. Matrix effects can sometimes be avoided by resolving these components from analytes chromatographically⁶, but not the continuous or unpredictable elution from an HPLC column.
4. Because of their variable chromatographic retention and instability, removal of these compounds prior to injection is optimal.

References

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