

Is Your "Good-Enough" Sample Preparation Really Good-Enough?

Lipids and the hidden dangers of suboptimal matrix removal

Introduction

Over the years, you have refined the workflow in your lab to be as manageable and efficient as possible. The popularity of protein precipitation (PPT) for biological sample preparation and QuEChERS for food sample preparation are great examples of how complicated sample preparation can be performed with a simplified approach. If asked to describe your workflow today, more than likely, you will say that your workflow is good-enough to get the job done. But are those good-enough sample preparation tactics really good-enough?

Your laboratory workflow faces many challenges, and you are constantly being asked to improve the efficiency or throughput metrics. A typical workflow comprises sample collection, sample preparation, introduction to the instrument, analysis, data review, and finally, data reporting, where sample preparation has always been considered the bottleneck and rate-limiting step. Current advanced instrumentation greatly increase your capability to analyze various samples, considering the required sensitivity, selectivity, reliability, and feasibility of complex sample testing. Therefore, the sample preparation step has been minimized to reduce time, cost, and complexity, improve the universal applicability, and it has been reduced to be just good-enough.

The most important workflow is yours. There are several hidden dangers in suboptimal sample preparation, such as wasted time, instrument maintenance/failure issues, and inaccurate data, that can arise and affect your overall success. These hidden dangers can be eliminated or greatly minimized by proper sample preparation workflow strategies. This improvement can be realized without having to retrain your scientist, making large capital expenditures, or having to rewrite your standard operating procedures (SOPs).

This White Paper outlines some of the known sample interferences that you are challenged with daily, and identifies some lesser-known hidden obstacles that you may encounter if you do not optimize matrix removal in your sample preparation workflow. In addition learning about the known and unknown consequences, you will also be introduced to a novel solution to improve your productivity, enhance technician efficiency, and put your analytical instrumentation at less risk of contamination. This can improve recovery and reproducibility without having to dramatically change your SOP, sacrificing throughput, or adding complexity to your workflow. Now, that should be your definition of good-enough sample preparation!

Why does anyone perform sample cleanup?

The two main reasons to do sample preparation are:

- Remove unwanted matrix
- Concentrate the analytes of interest

This White Paper looks at the obvious and less obvious pitfalls of not performing the matrix removal tasks as efficiently as possible.

Unwanted matrix components, if left undealt with or not sufficiently removed, can contaminate your flowpath. For GC, GC/MS, and GC/MS/MS analysis, the nonvolatile matrix co-extractives can have a detrimental impact on GC flow components such as the inlet, gold seal,

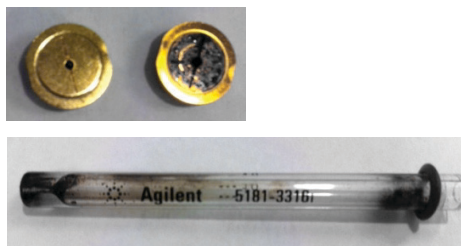


Figure 1. GC system component contamination from a "good-enough" sample prep routine.

and column (Figure 1). For LC, LC/MS, and LC/MS/MS analysis, the matrix particulates and salts can damage the valve, online filter, and LC column. Matrix co-extractives can result in significant ion suppression on MS source (Figure 2), and sequentially negative impacts on method reliability. Although most analytical chemists filter their samples before injecting into flow systems to avoid the above known danger, and filtration is a great way to minimize particulate matrix effect, what about dissolved unwanted matrix? Where does that end up if you only filter?

Figure 2 shows that not removing dissolved unwanted matrix co-extractives, which can be dissolved salts, pigments, proteins, lipids and so forth, can have detrimental effects on your analytical hardware.

Due to its high sensitivity and selectivity, mass spectrometry is the preferred analytical method for a wide range of applications. However, ion suppression or ion enhancement caused by matrix components has a negative impact on accuracy, precision, and robustness (as indicated by high Relative Standard Deviations, RSDs). Post column infusion is a technique that identifies ion suppression or ion enhancement caused by matrix, and Figure 3 is a typical setup for such an experiment (see Application Note 5991-8007EN[®]).

If you were to run this comparison study, you may reveal that your "good-enough" sample preparation might not be as efficient as needed. The Agilent sample preparation solution can help make your "good-enough" sample preparation more efficient and reliable.

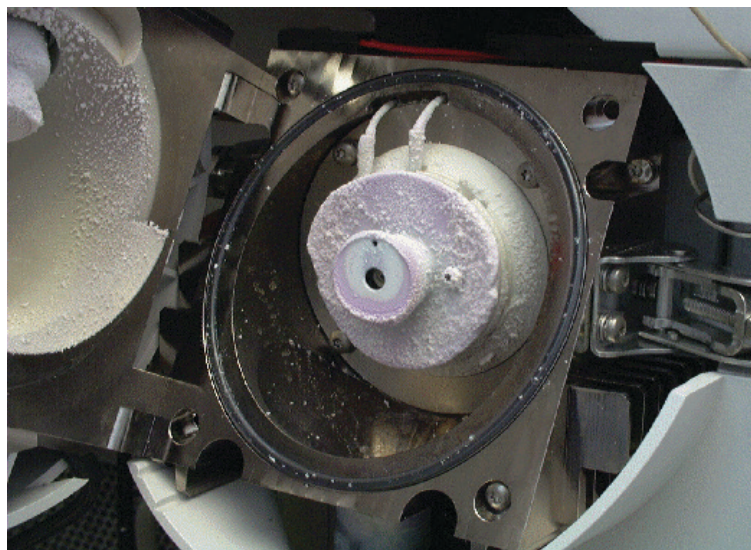


Figure 2. Salt buildup in an LC/MS ion source from nonremoved salts in a "good-enough" sample preparation routine.

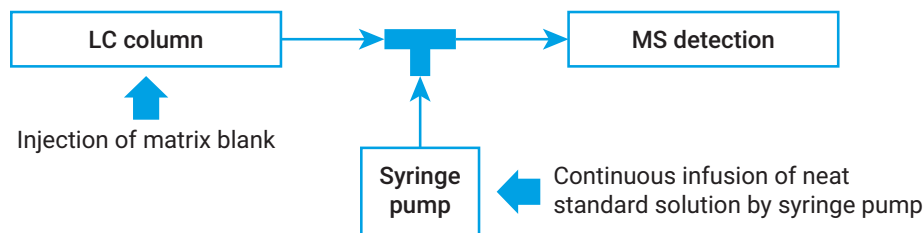


Figure 3. Standard post column infusion setup diagram for a matrix ion-suppression evaluation and comparison study.

How severe is the impact of lipids?

Of all sample matrix co-extractives, the most challenging but often overseen component could be lipids, due to the difficulty in their efficient removal and physical invisibility of its accumulation. Using a post column infusion experiment (as shown in Figure 3), matrix effects on the MS signal, for example, serum, or plasma, can be assessed by continuous infusion of matrix blank. Any variation of signal intensity could indicate the presence of substances from the matrix that cause ion suppression or enhancement.

The examples (zones 2 and 3 in Figure 4) show how dramatic the impact of lipids can be on the quality of your data.

Protein precipitation, although efficient at removing abundant proteins in biological matrix, does not remove phospholipids, which may cause significant ion suppression. (see Application Note 5991-8007EN³).

The consequence of poor data is a significant reduction in overall lab productivity.

The following is a list of some of the effects that poor matrix removal on your LC/MS/MS or GC/MS/MS analysis can have on your productivity:

Method reliability and data quality

- Unacceptable method sensitivity and selectivity
- Unacceptable calibration linearity
- Poor method accuracy and precision
- Difficult peak identification and integration

Method applicability

- Longer method development and optimization time
- More method troubleshooting
- Variable detection depending on matrix
- More sample reruns

Mass spectrometer cleanliness

- More frequent MS ion source cleaning
- More frequent capillary cleaning or replacement
- Loss of ability to establish or maintain vacuum levels
- Additional troubleshooting

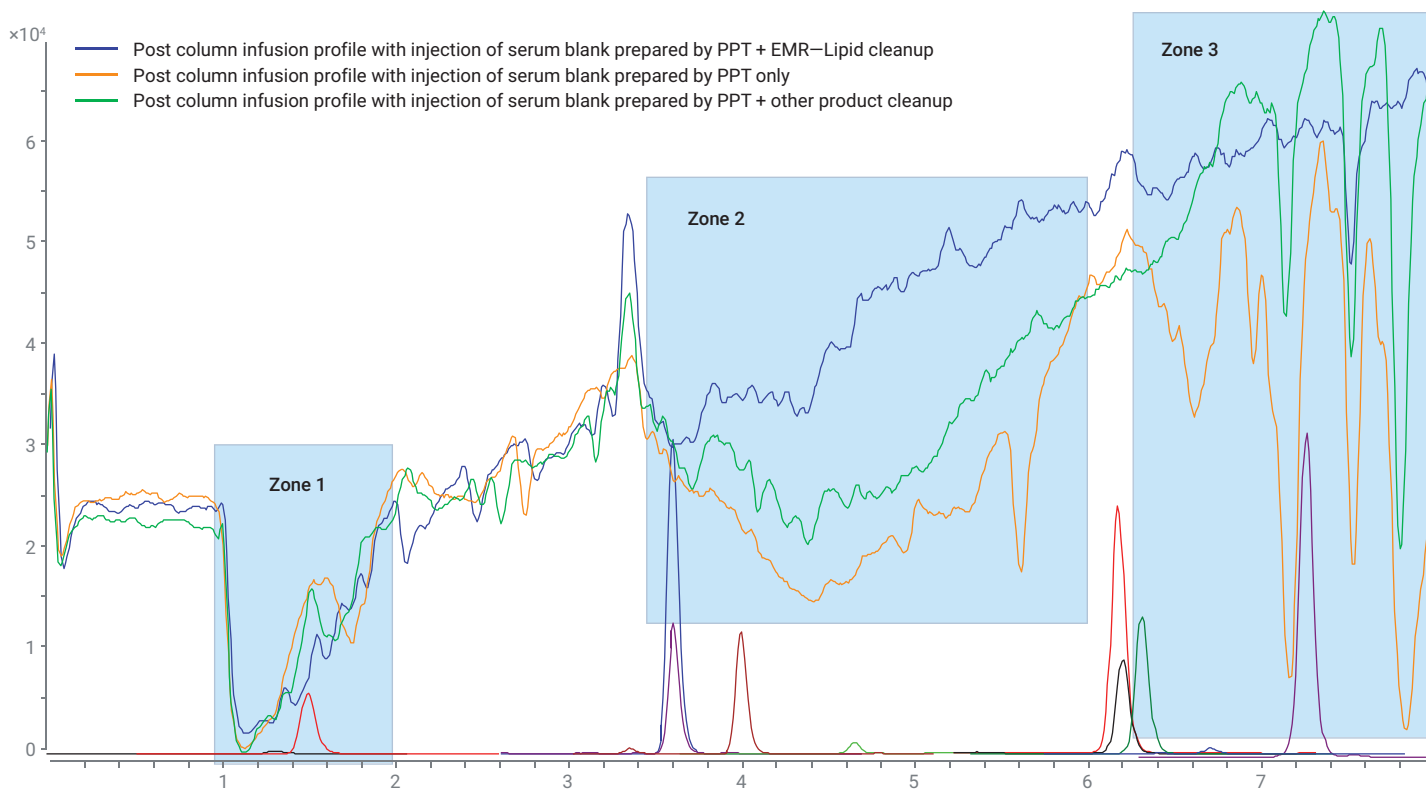


Figure 4. Standard post column infusion profiles comparison and demonstration of matrix ion suppression on target analytes.

Lipid buildup on column

- Shortened LC and GC column testing lifetime
- More frequent GC inlet maintenance
- More frequent GC column clipping
- LC column back pressure
- More frequent system flushing or equilibration
- Longer run times

Although workflows for lipid removal exist, current sample cleanup methods often sacrifice analyte recovery, removing some of the target analytes along with the lipids. Many analysts have learned to live with this situation by doing more maintenance, changing consumables more frequently, or by implementing more comprehensive sample preparation techniques. The latter succeeds in improving data quality, but adds significantly more cost, time, and expertise, which in turn reduces productivity.

Why is simply performing "good-enough" sample preparation is not a viable solution?

To generate better quality data, and significantly improve productivity, a better and simpler methodology is desirable for lipid and general matrix removal. To achieve these objectives, Agilent has developed Captiva EMR—Lipid, which cleans samples effectively with a simplified workflow. The Captiva EMR—Lipid approach is simple and universally applicable for the reduction of lipid-based matrix effects with good analyte recovery.

When faced with challenging complex samples such as food or biological matrices, you need to decide which sample preparation techniques will work best for your target analytes. Many techniques are difficult, laborious, or time-consuming, and may still have issues with compound recovery and matrix interference. A common requirement in sample preparation is easier protocols with fewer steps. Sample cleanup must effectively remove matrix interferences, and minimize impact on recoveries for a large diversity of analytes.

Table 1. Comparison of centrifugation-based traditional PPT (96-well plate) on plate versus filtration-based PPT with Agilent Captiva EMR—Lipid cleanup (96-well plate).

Traditional PPT (96-well plate) (Centrifugation)			PPT + Captiva EMR—Lipid cleanup (96-well plate) (Filtration)		
Step	Time needed (min)	Consumables needed	Step	Time needed (min)	Consumables needed
Plate labeling and sample aliquot	30	96 tips (small size) 1 Collection plate	Plate labeling and sample aliquot	30	96 tips (small size) 1 Collection plate 1 EMR—Lipid plate
Internal standard addition	5	1 Repeater tip	Internal standard addition	5	1 Repeater tip
Sample mixing	2	Plate cover	Sample mixing	2	Plate cover
Crashing solvent addition	5	1 Repeater tip	Crashing solvent addition	5	1 Repeater tip
Sample mixing	5	Plate cover	Sample mixing	5	Plate cover needed only for active mixing
Centrifugation	10		Filtration	10	
Supernatant transfer, collection plate labeling	30	96 tips (medium size) 1 Collection plate		Various	1 Plate mat
Sample post treatment	Various	1 Plate mat	Sample post treatment		
Total sample prep time needed	87 minutes by post treatment		Total sample prep time needed	57 minutes by post treatment (~30 % time savings)	
Instrument running time and solvent use	100 %		Instrument running time and solvent use	<90 % (save at least 10 % on time and solvent use)	
Matrix removal	Proteins only		Matrix removal	Proteins and lipids	

Imagine trying to identify your compounds of interest amidst the overwhelming matrix components, as shown in the Figure 5 GC full scan

chromatogram of avocado extract with no post treatment (poor), C18 cleanup (not good-enough), and EMR-Lipid cleanup (optimal).

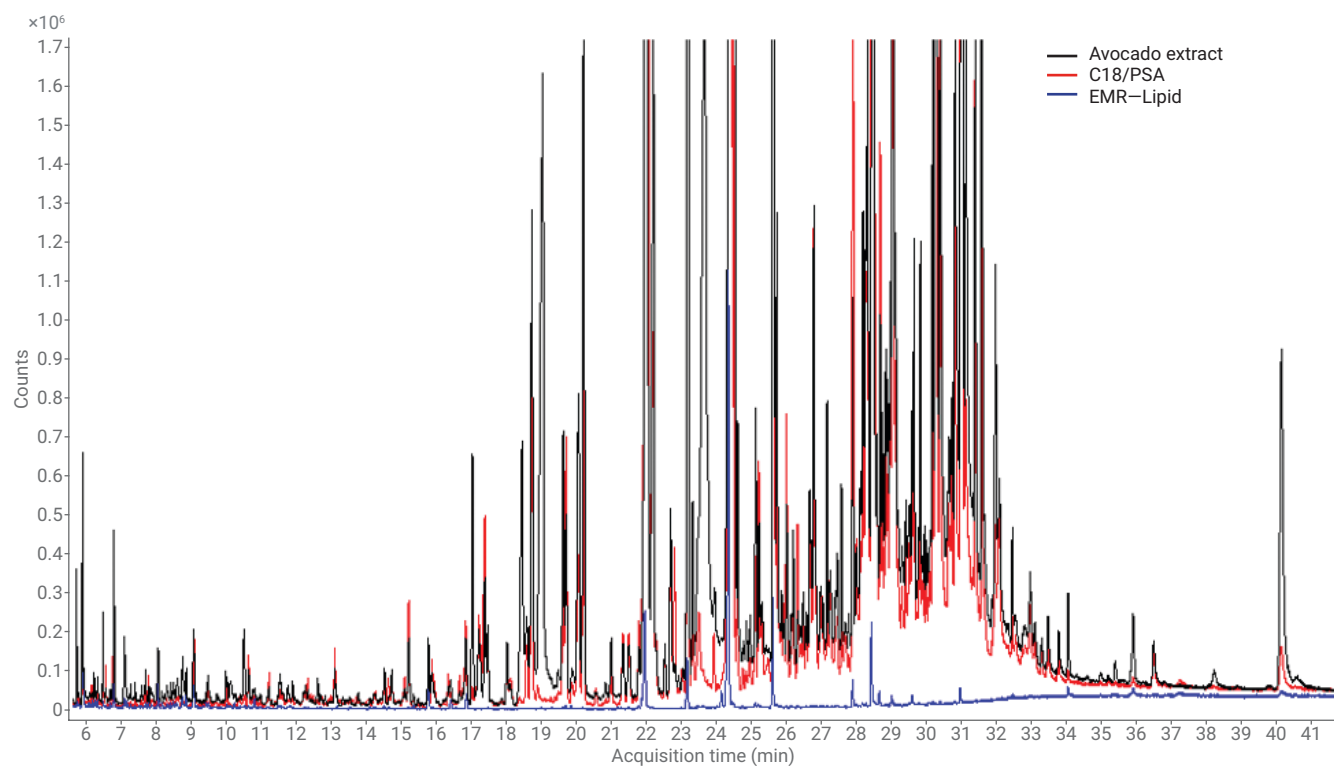


Figure 5. GC/MS full-scan chromatogram overlay of an untreated QuEChERS avocado extract (black), compared to traditional C18/PSA cleanup (red) and an extract treated with Agilent EMR-Lipid (blue).

Lipids can cause significant matrix ion suppression and result in lower detection sensitivity and poor method reproducibility. Phospholipids in biological matrices are always troublesome, due to the dramatic matrix effect they cause. Figure 6 shows that a sample with Captiva EMR–Lipid cleanup has significantly higher analyte response and better RSDs than a sample with PPT alone in the analysis of vitamin D metabolites.

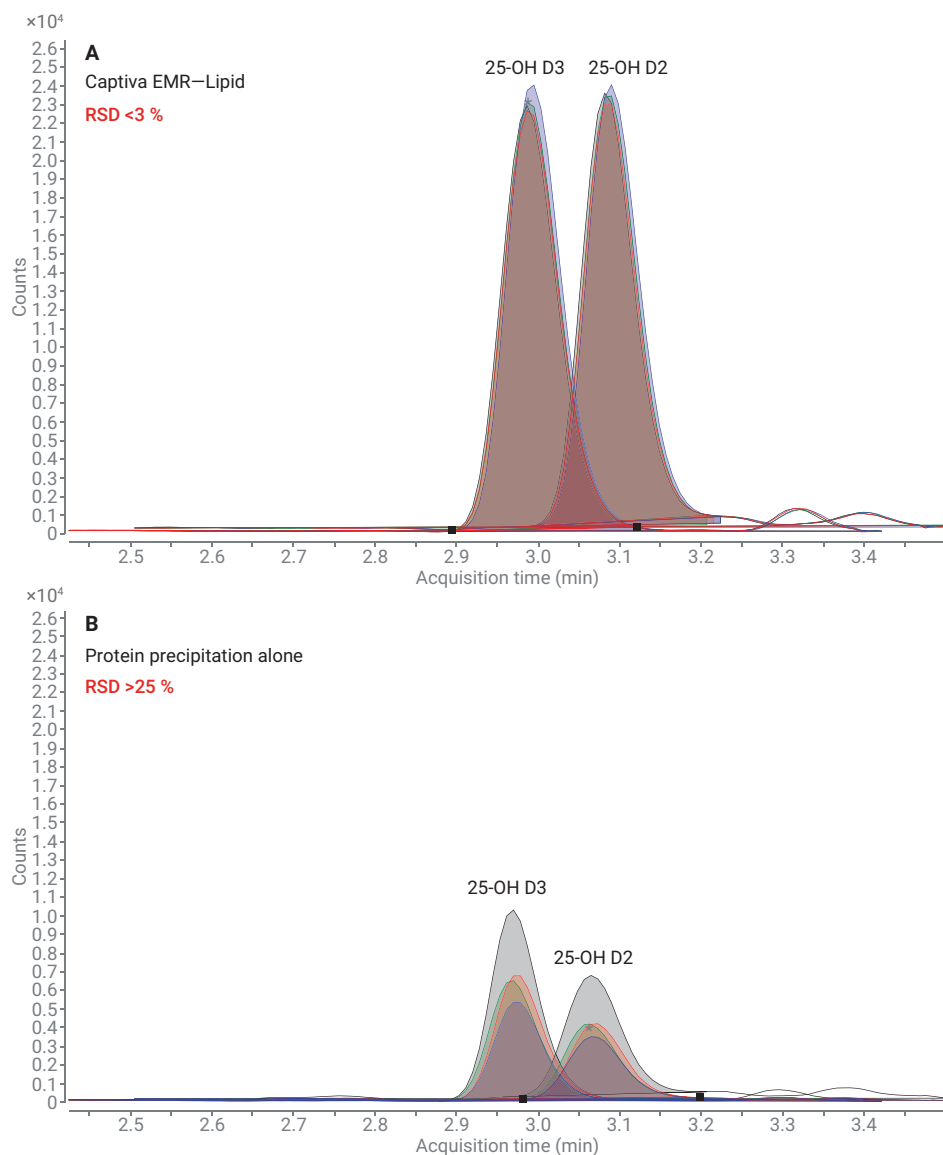


Figure 6. Analyte response and RSD value comparison between PPT alone and EMR–Lipid with PPT.

Now that we can effectively remove lipids, what can be done to speed up analysis?

With Captiva EMR—Lipid, not only will you be able to improve analytical method reliability and data quality, you can also reduce analytical run time, and

improve your laboratory productivity. The graph in Figure 7 shows that because the phospholipids were sufficiently removed prior to sample introduction, you do not need to wait for these hydrophobic compounds to elute in the reversed-phase chromatography, thus saving you precious instrument time.

Thanks to a cleaner sample, your analysis can be run faster, and you will

not have to do system flushing nearly as often. A cleaner sample also means that you will run more samples between scheduled preventative maintenance times. That means higher sample throughput given your current lab capacity. That means higher sample throughput and a better economic outcome for your laboratory.

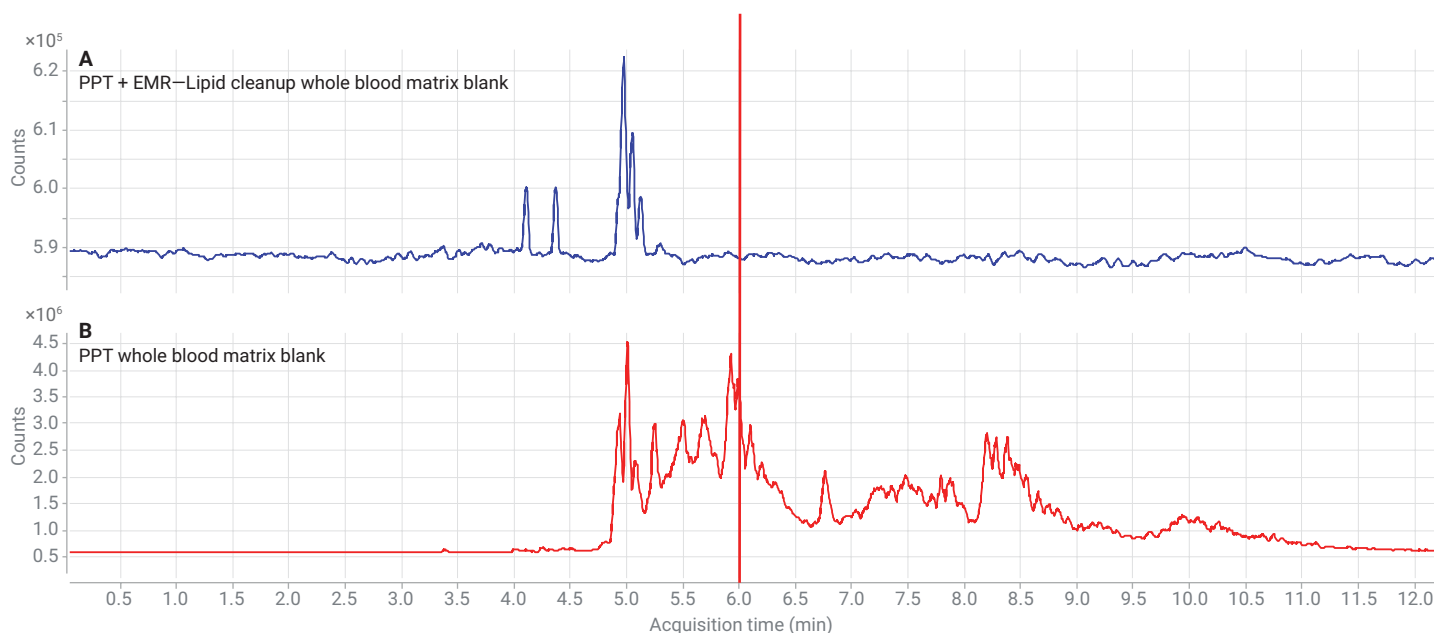


Figure 7. Matrix blank phospholipids profile comparison to demonstrate the feasibility of shorter cycle time.

Conclusions

Why is sample cleanup so important? Because complex samples contain many problematic matrix components, including lipids, proteins, salts, pigments, and so on. Lipids are one of the most problematic matrix component for several reasons.

The benefits of using a better sample preparation workflow compared to a good-enough sample preparation workflow are that you will create additional hours of instrument uptime, cut down analysts' bench work time in lab, and potentially reduce chromatography run time, enabling more samples to be run every day. The ultimate cause of having to perform timely maintenance is due to matrix contamination. If you remove more matrix, you will extend the time between

maintenance activities. This can be achieved without changing your SOP or adding more time to your workflow. Without sample preparation, you may not see the peaks of interest clearly enough to be able to quantify them accurately. In fact, you may even not see the peaks of interest at all. All these effects can increase the amount of time it takes for data processing, and can lead to increased numbers of rerun samples and batches due to poor data quality.

Learn more about Agilent Captiva EMR—Lipid

www.agilent.com/chem/Captiva-EMR-Lipid



Agilent Captiva EMR—Lipid cleanup

Learn more about productivity gains with Agilent Sample Preparation

www.agilent.com/chem/did-you-know

Recommended Application Notes

1. Quantitative Determination of Drugs of Abuse in Human Whole Blood by LC/MS/MS, *Agilent Technologies Application Note*, publication number 5991-9251EN.
2. Protein Precipitation for Biological Fluid Samples Using Captiva EMR—Lipid 96-Well Plates, *Agilent Technologies Application Note*, publication number 5991-9222EN.
3. Quantitative LC/MS/MS Analysis of Drugs in Human Serum with Captiva EMR—Lipid Cleanup, *Agilent Technologies Application Note*, publication number 5991-8007EN.
4. Vitamin D Metabolite Analysis in Biological Samples Using Agilent Captiva EMR—Lipid, *Agilent Technologies Application Note*, publication number 5991-7956EN.
5. Multiclass Multiresidue Veterinary Drug Analysis in Beef, *Agilent Technologies Application Note*, publication number 5991-8598EN.

Interested in veterinary drug analysis? You can also reference the journal article in the *Journal of Chromatography A*.

Multiclass multiresidue analysis of veterinary drugs in meat using enhanced matrix removal lipid cleanup and liquid chromatography-tandem mass spectrometry, *Journal of Chromatography A*, Volume 1549, 14-24.

www.agilent.com/chem/sampleprep

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