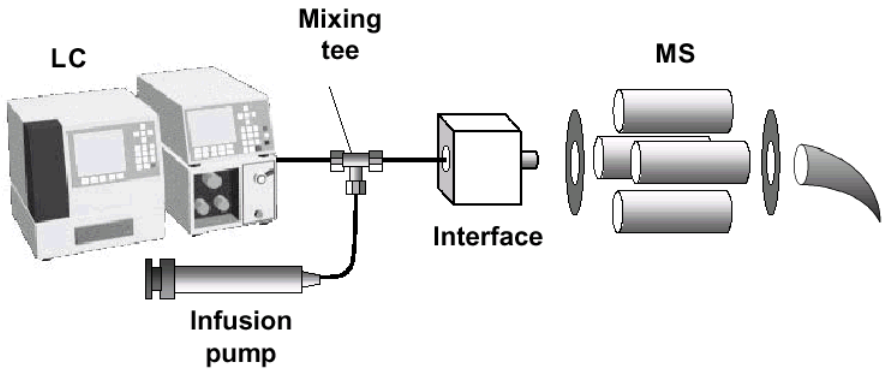
SECTION 10 Ion Suppression

10-1

LC Resources

071115

Some Interferences Reduce the Signal: Ion Suppression



- **Run proposed LC conditions**
- **Infuse analyte**
- **Infuse internal standard**
- **Inject blank extracted matrix**

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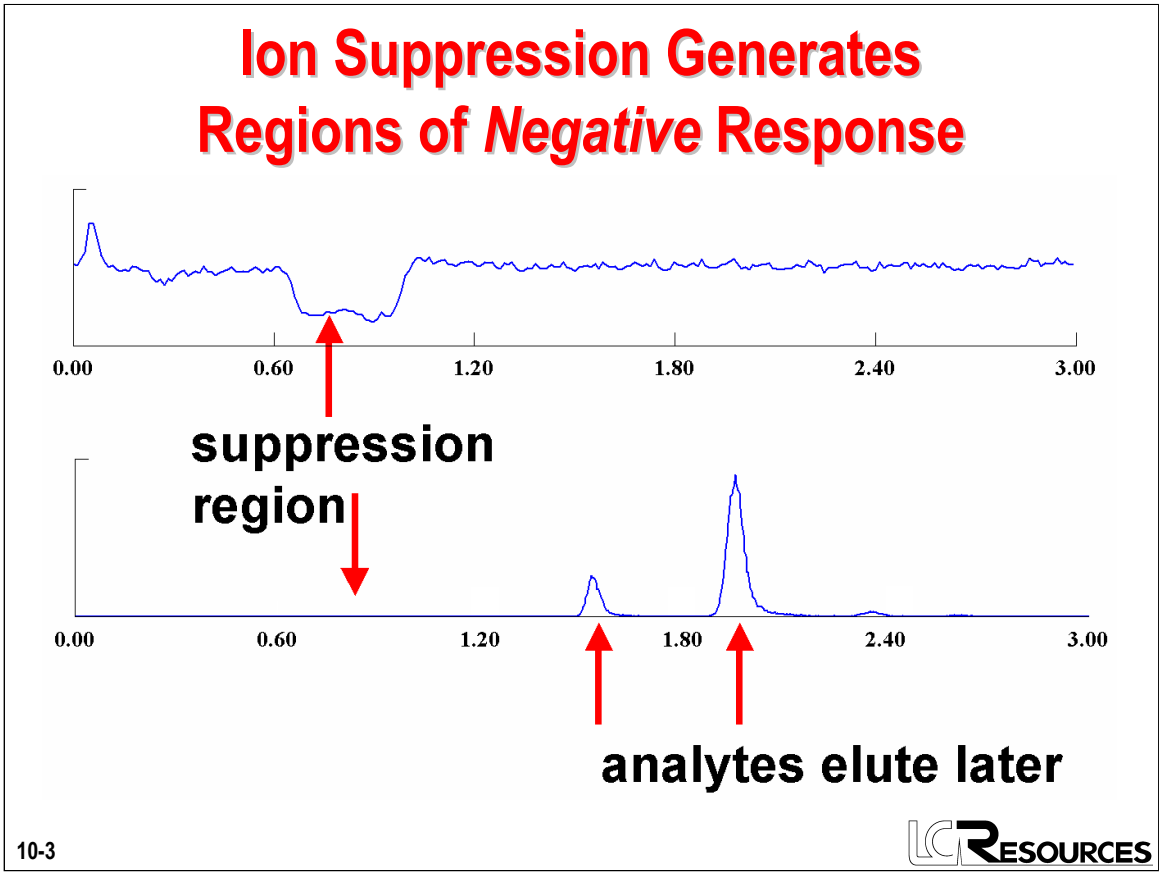
LC Resources

One way that accuracy can be compromised is if the signal is suppressed by a co-eluting material in the sample.

With LC methods, one usually wants sufficient retention so that the garbage at the solvent front doesn't interfere with the analyte. This usually means k > 1. With LC-MS, this early-eluting material can suppress ionization of analytes. Suppressed ionization can lead to non-linearity and inaccurate quantification.

One easy way to check for suppression is shown here. A constant concentration of a standard is infused into the mobile phase stream after the column. Once the baseline stabilizes, an injection of an extracted matrix blank is made. At the solvent front a negative dip will be seen as ionization suppressing materials elute and reduce the baseline signal. When the baseline returns to normal, all these suppressing agents have passed through the detector.

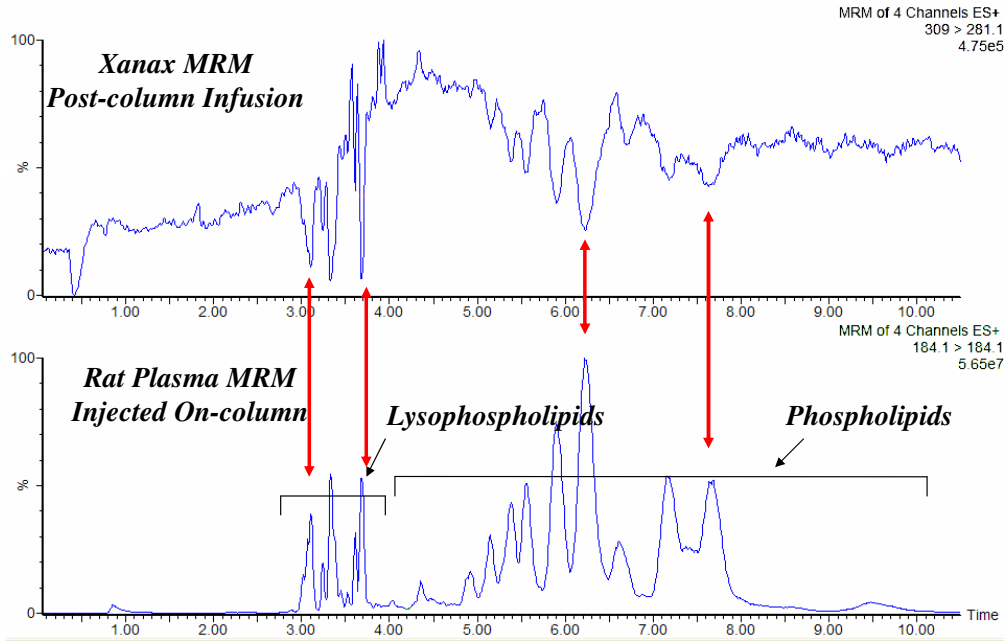


In this example, a dilute solution of paclitaxel was infused into the LC-MS/MS and blank extracted plasma was injected to obtain the top plot. The steady-state signal for paclitaxel dropped when ionization was suppressed. By adjusting retention so that the analytes of interest elute after this suppression region, the risk of signal loss due to ionization suppression is greatly reduced.

Just as analyte peaks can elute anywhere in the run, ion suppression can occur anywhere in the run, so it is very important to run the ion suppression experiment to be sure that ion suppression will not compromise the method.

This check of ion suppression is delayed until the sample prep, LC, and MS conditions have been selected. If problems occur, you may need to adjust some of the parameters. With the DryLab dataset, often the chromatography can be adjusted with little effort. Sometimes a persistent suppression region will require additional sample preparation to minimize the problem.

Matrix Suppression - Phospholipids



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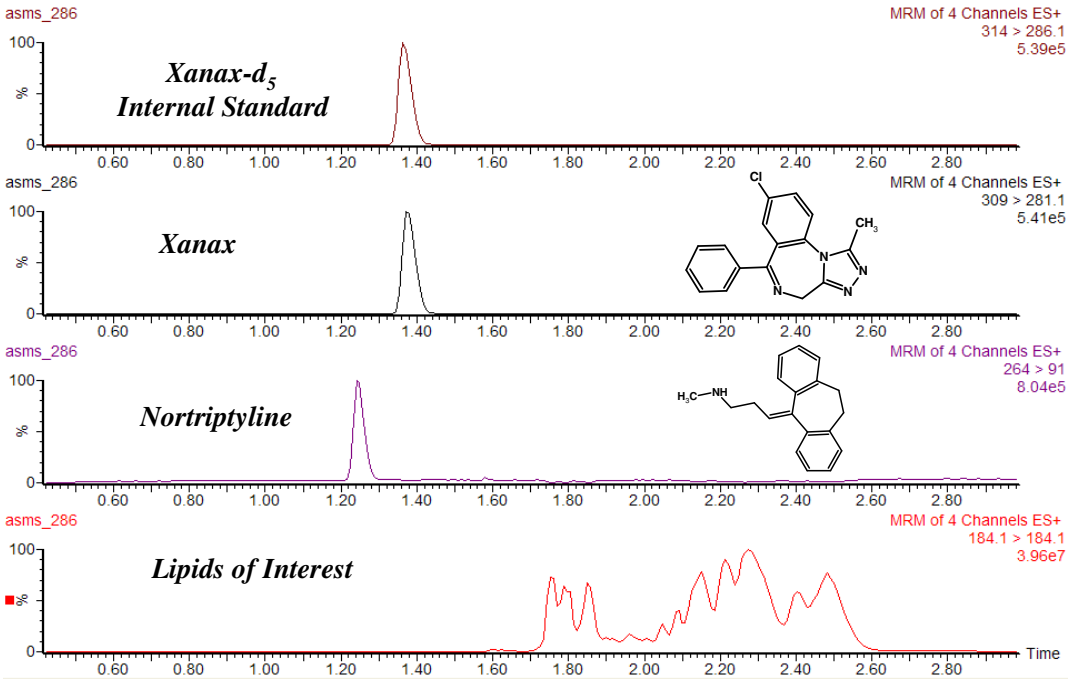
LC Resources

Matrix Suppression Shown by Post-Column Infusion of 180 ng/ml Solution of Xanax and Injection of Rat Plasma Sample. Note how phospholipid peaks correspond with dips in Xanax baseline – these are ion suppression regions greatly limiting the region in which Xanax can elute without suppression.

Courtesy of James Little, Eastman Kodak. J Chromatogr. B (2006) submitted for publication.

Retention Adjusted for Ion Suppression



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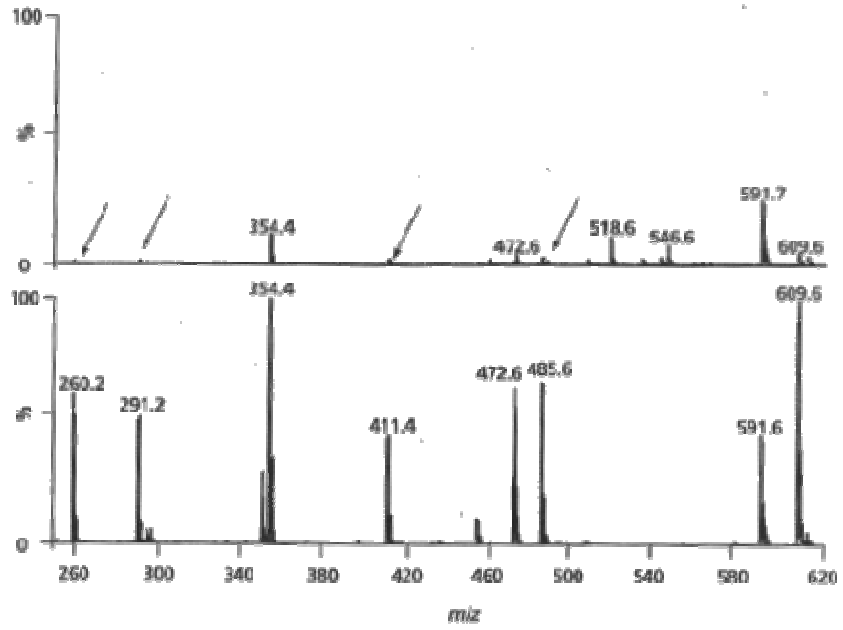
HPLC conditions adjusted from previous slide.

Courtesy of James Little, Eastman Chemical. J. Chromatogr. B (2006) submitted for publication.

Ion Suppression: Rat Plasma Precipitate vs. Standards



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LC Resources

Here is an example of how suppression can appear during sample analysis.

Plasma precipitation is a popular and simple method to clean up samples prior to injection. This example shows that although plasma proteins may be removed to make the sample look visibly clear, remaining materials can cause severe ion suppression.

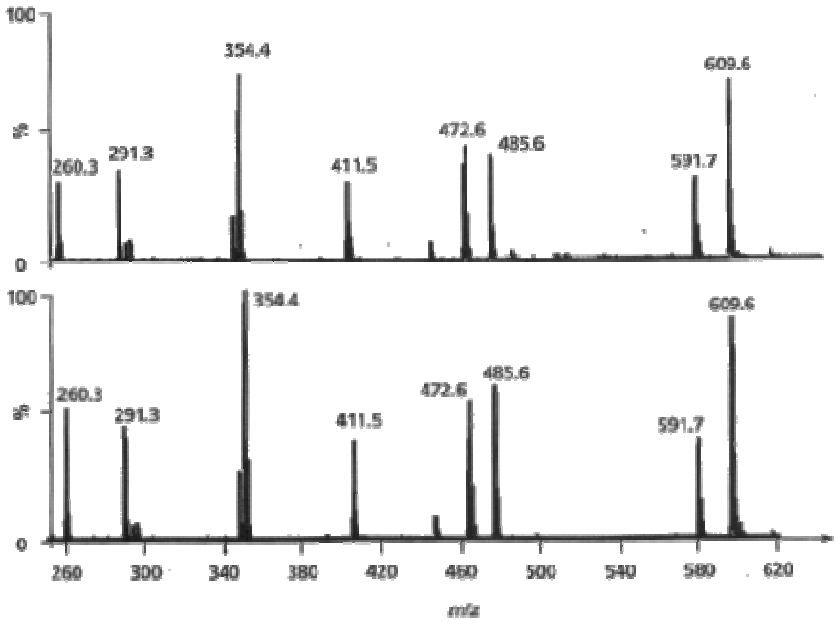
Top: rat plasma precipitate containing prednisolone, diphenhydramine, betamethasone, amytriptiline, naproxin, and ibuprofin. Bottom: standards in solvent.

C.R. Mallet, Z. Lu, and J.R. Mazzio, Rapid Commun. Mass Spectrom., 18 (2004) 49-58.

and

D.M. Diehl and M.P. Balogh, LC/GC, 22 (2004) 344-352.

Ion Suppression: Rat Plasma SPE Extract vs. Standards



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LC Resources

The same rat plasma sample was subjected to SPE cleanup using a mixed-mode resin. As can be seen here, this cleanup technique is much more effective for this sample than simple plasma precipitation.

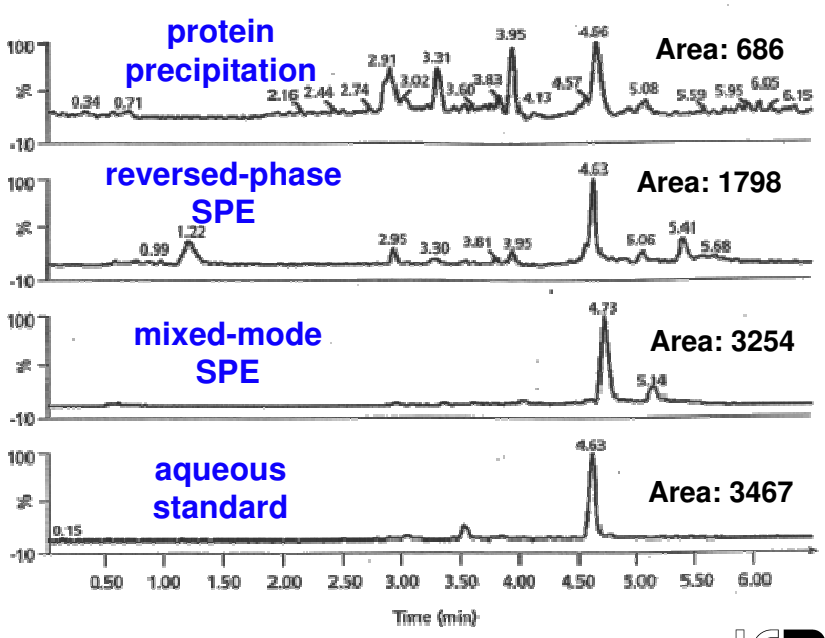
Top: rat plasma containing prednisolone, diphenhydramine, betamethasone, amytriptiline, naproxin, and ibuprofin following solid phase extraction with mixed-mode cartridge. Bottom: standards in solvent.

C.R. Mallet, Z. Lu, and J.R. Mazzio, Rapid Commun. Mass Spectrom., 18 (2004) 49-58.

and

D.M. Diehl and M.P. Balogh, LC/GC, 22 (2004) 344-352.

Ion Suppression: Rat Plasma Extracts vs. Standards



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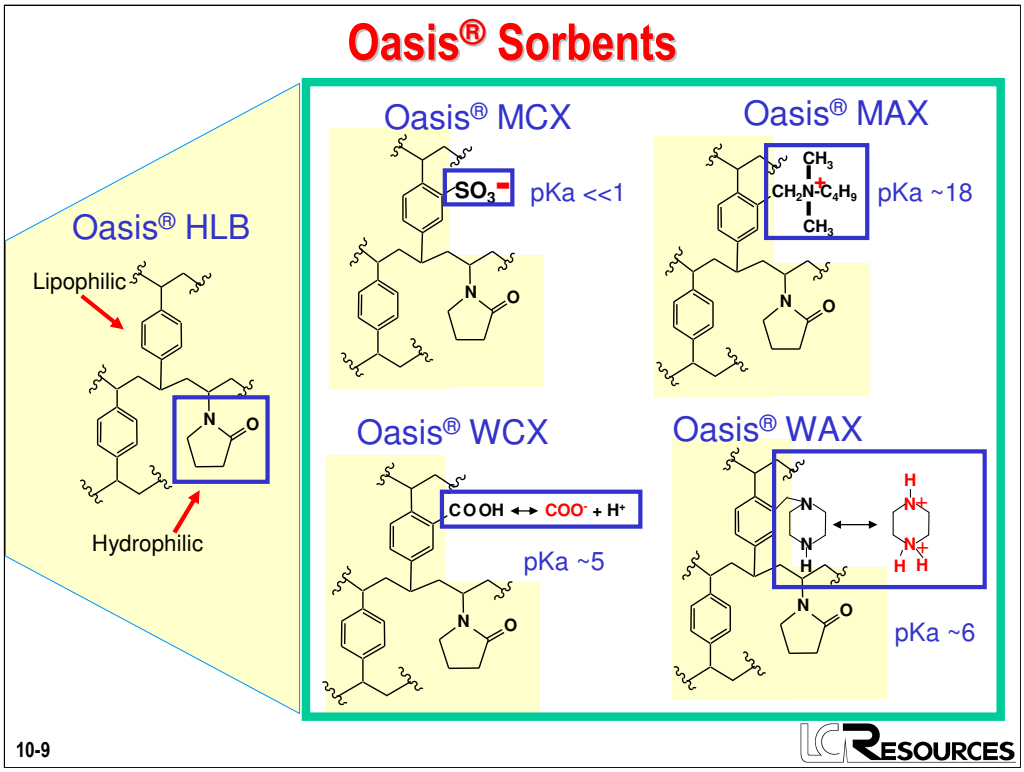
LC Resources

Here is a comparison of the response of the MS to rat plasma containing 0.1 ng/mL amitryptiline that has been prepared with various extraction schemes. It usually is best to use a cleanup technique that works on a different principle than the LC separation. We can see that the mixed-mode cleanup was more effective than reversed-phase cleanup when a reversed-phase column was used for the analytical separation.

M. Gerdes and H. Waldmann, J. Comb. Chem., 5 (2003) 814 – 820.

and

D.M. Diehl and M.P. Balogh, LC/GC, 22 (2004) 344-352.



One line of SPE materials are those from Waters. Many other manufacturers also offer SPE products. For those that maybe are not familiar with Oasis, these are the structures of the sorbents

Oasis HLB – hydrophilic-lipophilic balanced co-polymer – reversed-phase retention

Oasis MCX (Mixed-mode Cation eXchanger)

Strong sulfonate (-SO3H) groups bonded to Oasis® HLB co-polymer (1 meq/g)

Oasis MAX (Mixed-mode Anion eXchanger)

Quaternary amine bonded to Oasis HLB co-polymer (0.25 meq/g)

Oasis WCX (mixed-mode weak cation exchanger)

Carboxylic acid bonded to Oasis HLB co-polymer (0.7 meq/g, pKa ~5)

Oasis WAX (mixed-mode weak anion exchanger)

Piperazine bonded to Oasis HLB (0.6 meq/g, pKa ~6)

Courtesy of Waters Corp.

Sample Preparation Methods

Protein Precipitation (PPT)
3:1 ACN to plasma

Liquid-Liquid Extraction (LLE)
3:1 MTBE to plasma

SPE: Oasis® HLB or Sep-Pak® tC_{18} (Reversed-Phase)
Wash: 5% MeOH in H_2O
Elute: MeOH

SPE: Oasis® MCX (Mixed-mode cation exchanger)
Wash 1: 0.1 N HCl
Wash 2: MeOH
Elute: 5% NH_4OH in MeOH

SPE: Oasis® HLB – 2D Optimized Method
Wash 1: 5% MeOH in H_2O
Wash 2: 40% MeOH with 2% NH_4OH in H_2O
Wash 3: H_2O
Elute: 70% MeOH with 2% FA

SPE: Oasis® WCX (Mixed-mode weak cation exchanger)
Wash 1: 25 mM phosphate buffer, pH 7.0
Wash 2: MeOH
Elute: 2% FA in MeOH

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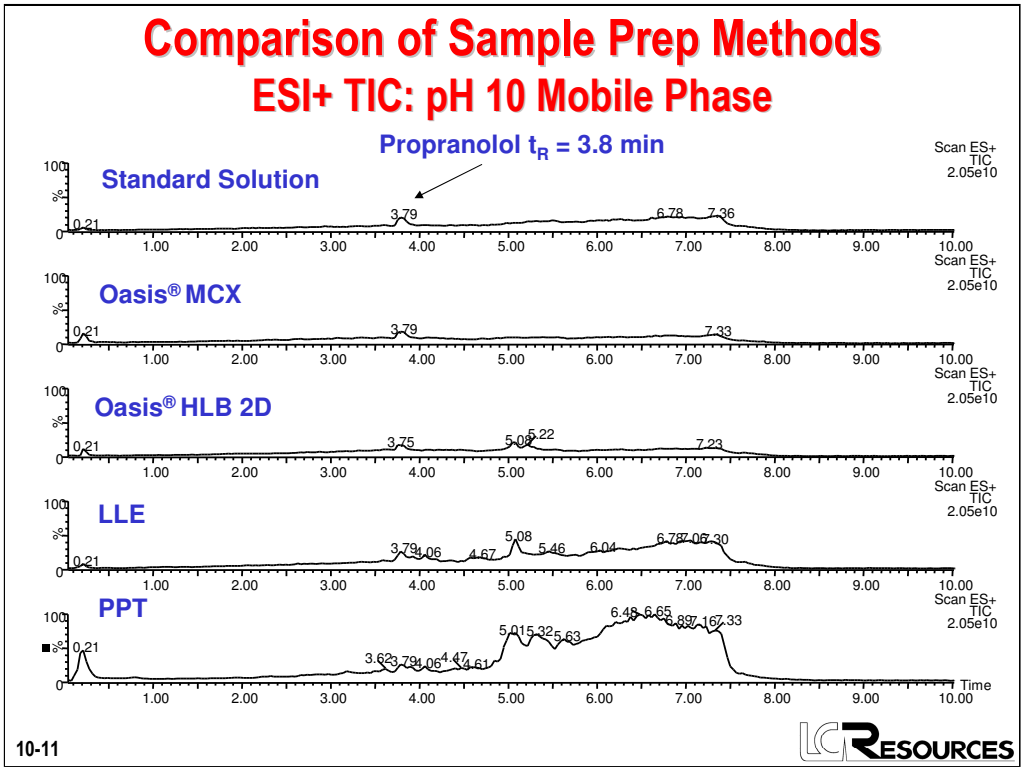
LC Resources

Sample preparation conditions for following experiments.

Courtesy of Waters Corp.

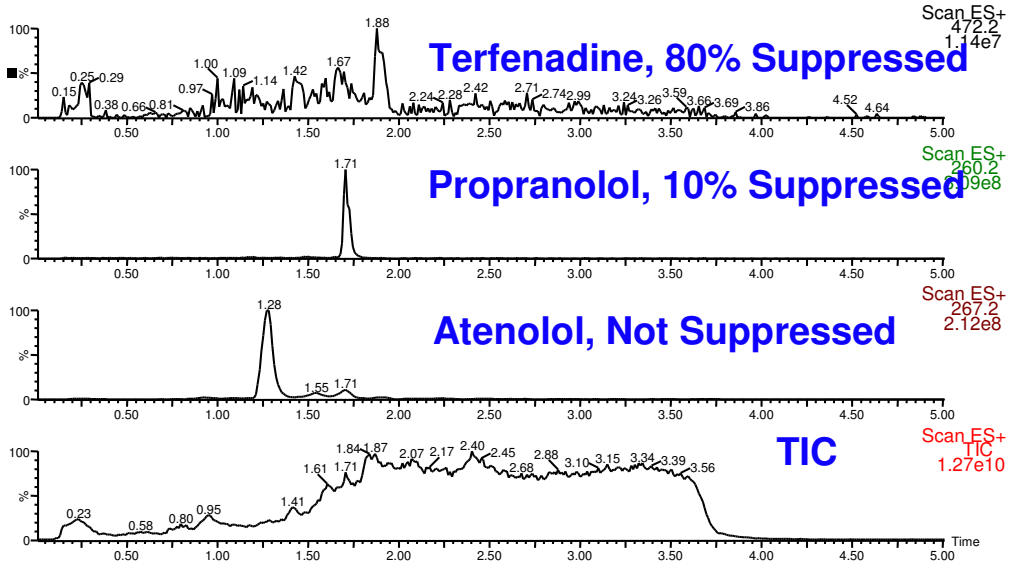


Full scan from 100 to 1000 m/z. This is the TIC which is the sum off all ions from 100 to 1000 m/z. Note the increasing level of residual matrix interferences as you move from top to bottom. Although propranolol does not elute in the region where most of the matrix components are eluting, other analytes may elute, thereby opening up the possibility of ion suppression. Clearly, with MCX the extract is the cleanest.

Note, this scale is normalized to the PPT extract.

Courtesy of Waters Corp.

Protein Precipitation



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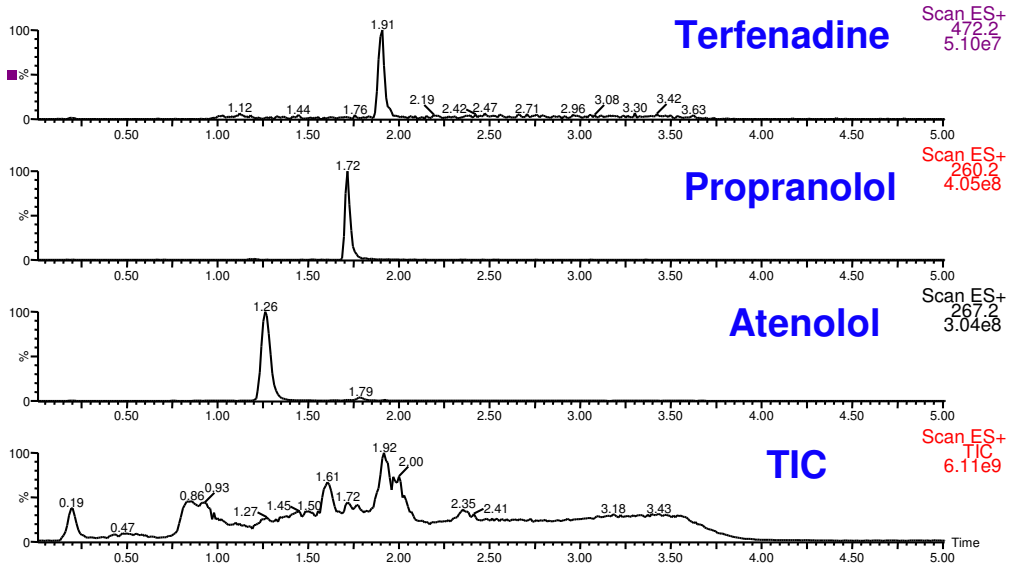
LC Resources

Protein precipitation alone may or may not compromise the sample due to ion suppression.

Courtesy of Waters Corp.

Oasis® MCX Results



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LC Resources

Here are the same samples with the MCX preparation method. No ion suppression is observed with the MCX method.

Courtesy of Waters Corp.

MRM Transitions

Analyte:

Propranolol m/z 259.9 → 183.1

Phospholipid Interferences from rat plasma*:

Lysophospholipids

m/z 496.4 → 184.3

m/z 524.4 → 184.3

Phospholipids

m/z 704.4 → 184.3

m/z 758.4 → 184.3

m/z 806.4 → 184.3

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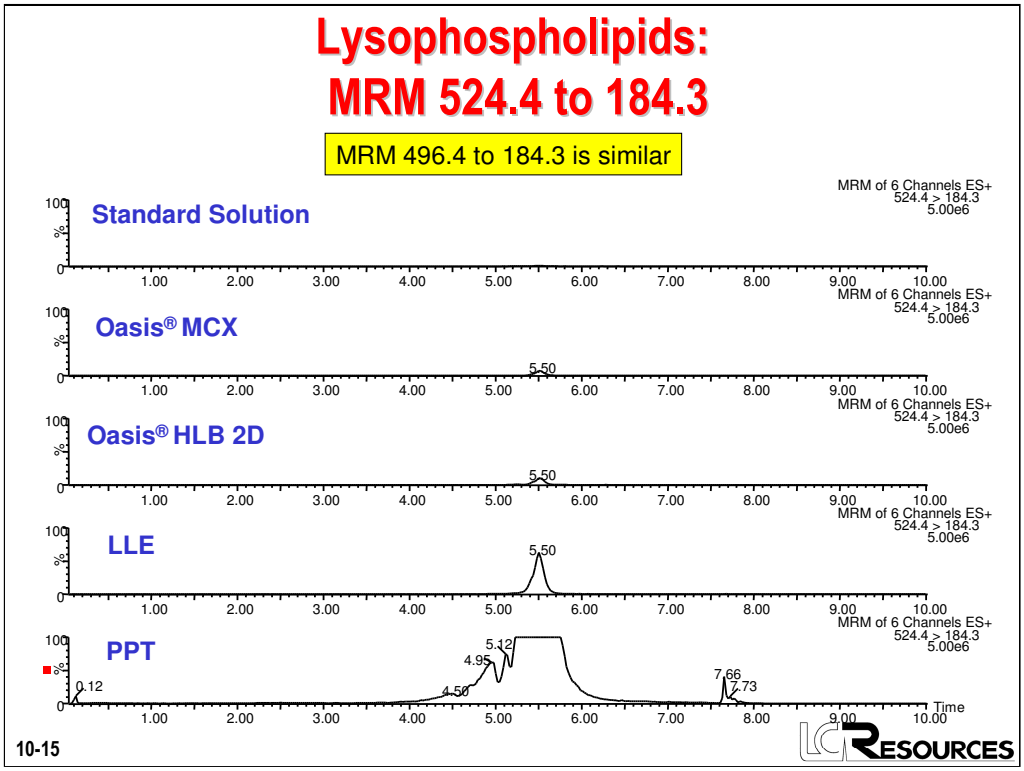
LC Resources

Phospholipids and lysophospholipids have been implicated as general sources of ion suppression. Note that all the phospholipids have a 184.3 product ion which can be monitored to determine the presence of these compounds.

*Van Horne, K.C.; Bennett, P. K. Matrix Effects Prevention by Using New Sorbents to Remove Phospholipids from Biological Samples, Poster, AAPS 2003.

Courtesy of Waters Corp.



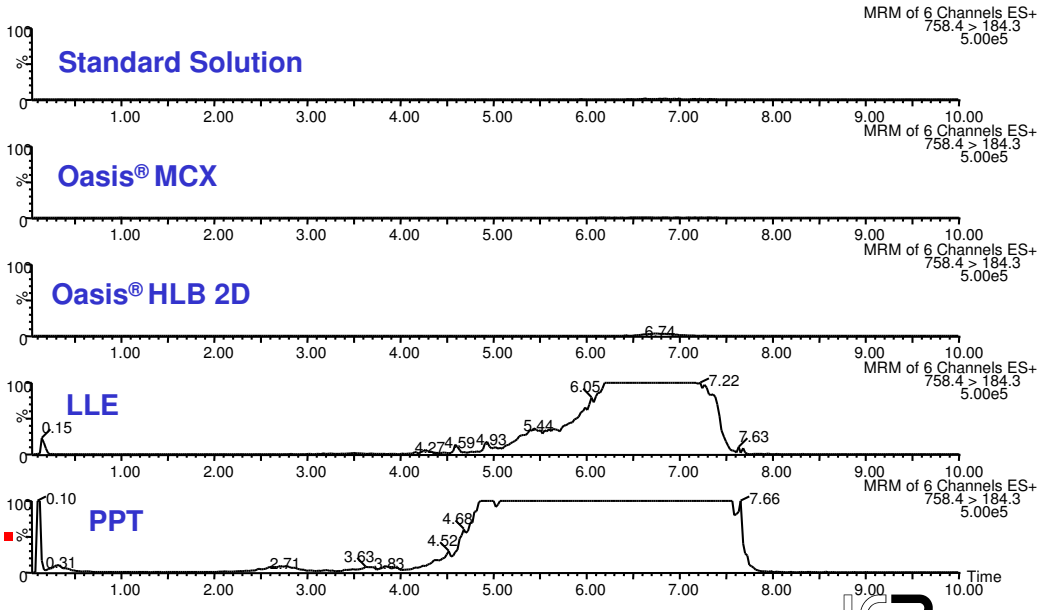
Here is the MRM monitoring the presence of lysophospholipids in various cleanup schemes.

Note the amount of this phospholipid in the PPT. This is collecting on your column and in the MS.

Courtesy of Waters Corp.

Phospholipids: MRM 758.4 to 184.3

MRM's 806.4 to 184.3 and 704.4 to 184.4 look similar



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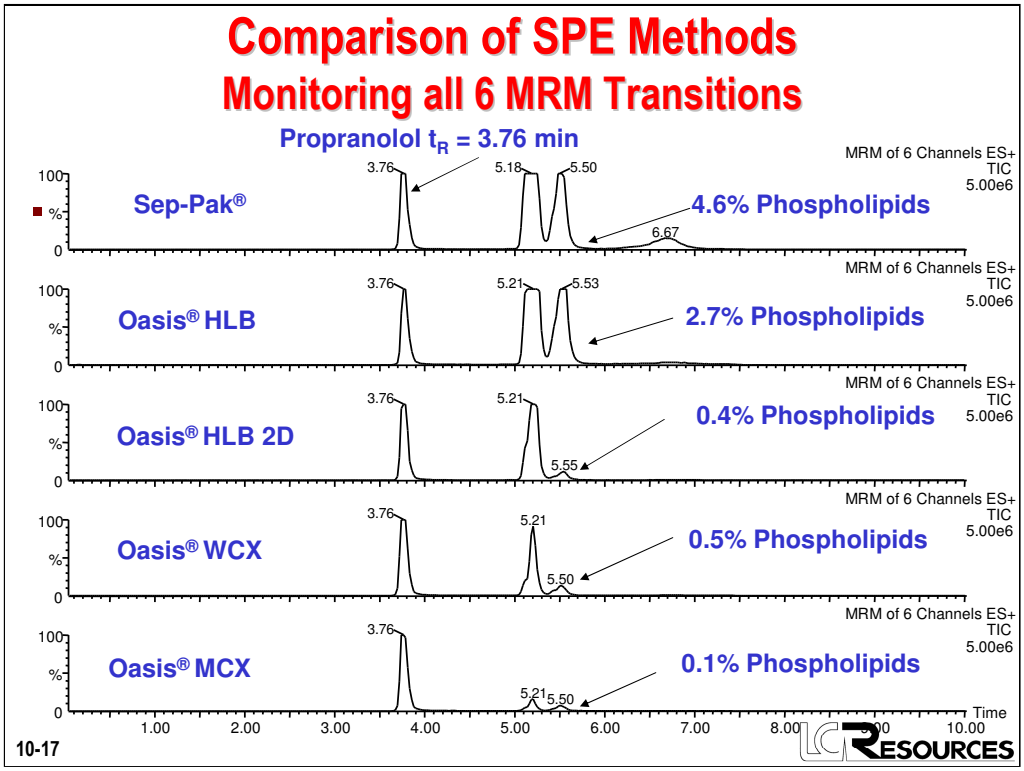
Monitoring the phospholipid transitions yields similar results.

Courtesy of Waters Corp.



Here the propanolol signal is shown with all 6 of the lipid transitions. The %-phospholipid is relative to precipitation as 100%.

Area Counts:

PPT: 37057320

Sep-Pak: 957194

HLB: 1029210

HLB 2D: 88236

WCX: 99194

MCX: 51958

Courtesy of Waters Corp.

Checking Ion Suppression

- Infuse analyte
  - Inject blank extracted matrix
  - Inject blank spiked with IS (R_s<1)
- Infuse internal standard
  - Inject blank extracted matrix
  - Inject blank spiked with analyte

10-18

LC Resources

In addition to checking the matrix for materials that may suppress ionization of the sample, you also should make the same test for ion suppression of the internal standard. If a stable-label internal standard is used or any time the resolution between the analyte and IS is less than 1, you should check to be sure the analyte and IS do not suppress each other.