



An Efficient Approach for Solid Phase Extraction for Reducing Ion Suppression in Liquid Chromatography /Mass Spectrometry (LC/MS)

Keith J. Duff, Carmen T. Santasania,
David S. Bell, and Yuhui Yang

Supelco, 595 North Harrison Road,
Bellefonte, PA 16823, USA

T403108
FWJ

1



Abstract

The use of high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) has become the standard for analyzing many classes of compounds from biological samples. The combination of the superior separating power of HPLC along with the sensitive and selective properties of the MS make this the technique of choice for analysis of compounds from complex matrices. In many cases, sample clean-up is required to remove endogenous compounds from the analyte of interest before LC/MS analysis. Matrix effects in the form of ion suppression or ion enhancement are often associated with the coelution of the target analyte with endogenous species giving rise to poor quantitation. Given the affect of ion suppression and enhancement, there has been a renewed interest in sample preparation for LC/MS analyses. It is well known that procedures such as protein precipitation and liquid-liquid extraction are non-selective methods of sample preparation. The general theme of this work is to show how the analyst can more efficiently perform sample preparation to eliminate or reduce matrix effects in LC/MS analyses.

We describe a procedure using pharmaceutically-relevant analytes for the efficient screening of solid phase extraction (SPE) media chemistries based on analyte structure and complementary media interactions. Additionally, we describe a targeted procedure to efficiently identify the most appropriate solvents and pH levels to be used for a given analyte and sample matrix. The obtained data, in turn, is evaluated based on the resulting matrix effects against standard generic SPE protocols.

In addition to SPE, we explore the use of HPLC stationary phases that can reduce matrix effects and speed up LC/MS analyses. Final SPE eluants are often elevated in organic content and thus require time-consuming solvent evaporation/reconstitution steps using traditional alkyl stationary phases. Where applicable, a new pentafluorophenylpropyl HPLC phase was used, which often allows the direct analysis of analytes in highly organic sample solvents.



Introduction

- **Accuracy, reproducibility and ruggedness of LC/MS methods requires proper sample preparation**
- **Coelutions, especially with variable endogenous species, often cause ionization enhancement or suppression which can lead to irreproducible results**
- **Solid phase extraction (SPE) is often used to:**
 - Strip the analyte(s) away from endogenous interferences.
 - Concentrate analytes(s) for better sensitivity.
 - Exchange sample environments for better chromatography



Introduction

- **SPE protocols are often generic, consisting of standard load, wash and elute solvents**
- **Although this approach is less time-consuming, it often results in less than optimal recovery and selectivity**
- **For LC/MS methods intended for extensive use (ie. clinical studies), extra effort in SPE method development can save costly reanalysis, redevelopment and revalidation time**
- **Systematic solid phase extraction development as described in this study can be used to determine optimal solid phase extraction procedures**



Approach

- **Systematic Solid Phase Extraction**
- **Step 1:**
 - Screen several SPE phases for loadability
 - Spiked matrix sample is loaded onto plate and fully eluted
 - Eluent is analyzed for breakthrough
- **Step 2:**
 - Establish optimal wash and elution solvents
 - Load samples according to Step 1
 - Wash/elute with solvents varying in pH and percent organic
 - Analyze for recovery and plot results
 - Determine optimal wash and elution solvents from plot
- **Step 3:**
 - Combine load, wash and elute procedures



Approach

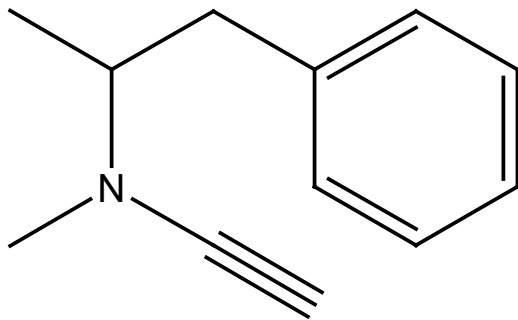
- In this study 12 SPE phases were evaluated for the extraction of the anti-parkinsonian drug selegiline and a major metabolite methamphetamine from urine using the SSPE approach
- Method development was conducted using 96-well plates loaded with 100mg SPE phase/well
- Loading study was performed at 3 pH levels (pH 3, pH 7, and pH 10)
- Wash/elute studies were performed on the appropriate phases from 0 to 100% methanol in 3 buffers at pH levels of 3, 7, and 10
- Recoveries for the wash/elute study were monitored by both UV and MS detection
- Results were compared to determine the most appropriate wash and elute conditions

Experimental

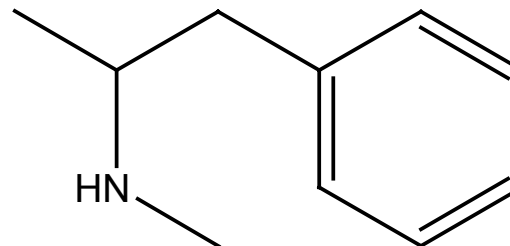
- **Phases Investigated**

- **Discovery DSC phenyl, Discovery DSC C18LT, Discovery DSC C8, Discovery DSC C18, Discovery DSC Cyano, Discovery Silica, Discovery SCX, Discovery WCX, Discovery Diol, Discovery C18**

- **Analytes**



Selegiline



Methamphetamine



Experimental

Conditions (MS):

- Column: Discovery F5, 5cm x 2.1mm ID, 3 μ m particles
- Mobile Phase: (5:95) 5mM Ammonium Acetate, pH 4.0 with Acetic Acid:CH₃CN
- Flow Rate: 200 μ L/min
- Det.: MS-ESI (+) ion mode, scan from m/z 120 to m/z 500
- Temp.: 40°C
- Inj.: 10 μ L
- Sample: 500ng/mL of selegiline and methamphetamine in mobile phase

Conditions (UV):

- Column: Discovery F5, 10cm x 4.6mm ID, 5 μ m particles
- Mobile Phase:(35:65) 10mM Ammonium Acetate, pH 4.0 with Acetic Acid:CH₃CN
- Flow Rate: 2mL/min
- Det.: UV,210nm
- Temp.: 40°C
- Inj.: 10 μ L
- Sample: 500ng/mL of selegiline and methamphetamine in mobile phase

- Note: retention for uv analysis was intentionally increased due to matrix interferences



Results – Loading Study

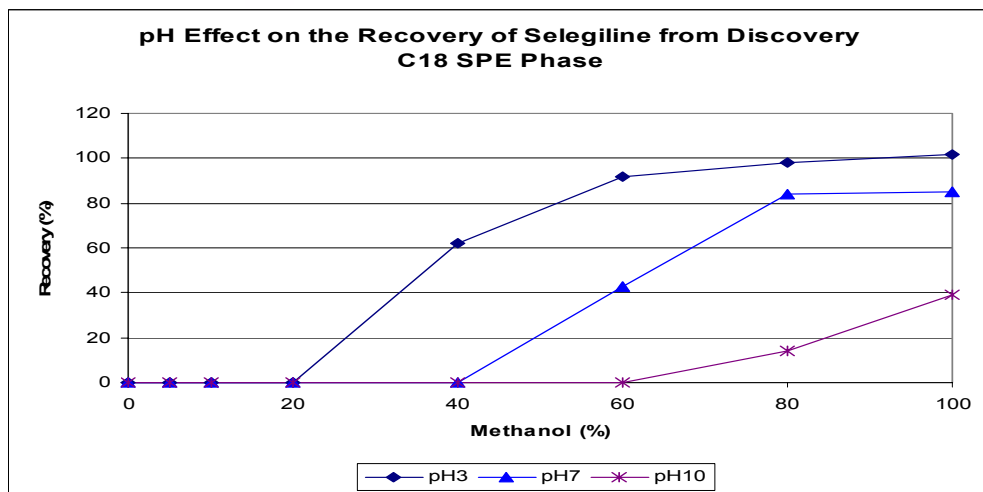
- Retention of the analytes was not observed under any of the pH conditions for Discovery Diol
- At pH 3 and pH 7, the Discovery CN phase also showed poor retention
- As a result, these phases/conditions were eliminated



Results, Wash/Elute

- **Wash/elute studies showed that under the conditions studied, the analytes were not fully recovered from the silica, WCX or SCX SPE phases. These were thus eliminated.**
- **Figure 1 shows the wash/elute profiles obtained for each analyte using Discovery DSC-18**
- **For contrast Figure 2 shows the wash/elute profiles obtained for each analyte using Discovery DSC-8**
- **Similar profiles were obtained for the remaining phases studied.**

Wash/Elute Study- C18 Phase [Figure 1]



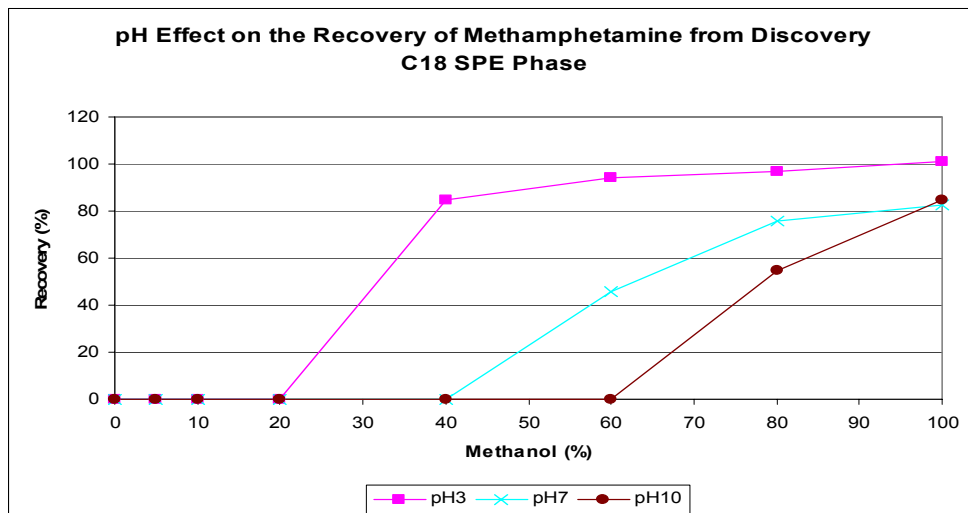
The data clearly shows that both selegiline and methamphetamine can be fully eluted at pH 3 but not at higher pH

The profile indicates that wash solvents may contain up to 20% organic and that an elution solvent containing down to 80% organic will likely provide excellent recovery

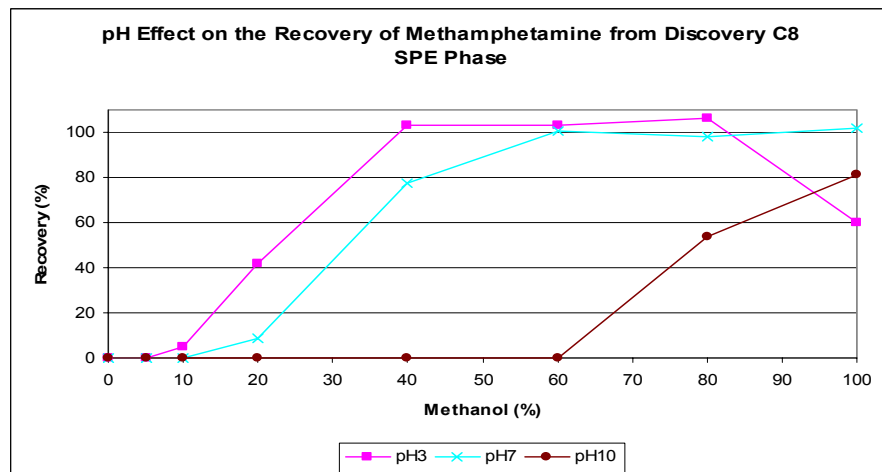
A generic approach usually would call for a 100% aqueous wash and 100% methanol elution.

In this case a stronger solvent could be used for the wash procedure. This would likely lead to improved selectivity for the target analytes

Additionally, a lower organic elution solvent is likely to leave behind more hydrophobic contaminants



Wash/Elute Study- C8 Phase [Figure 2]

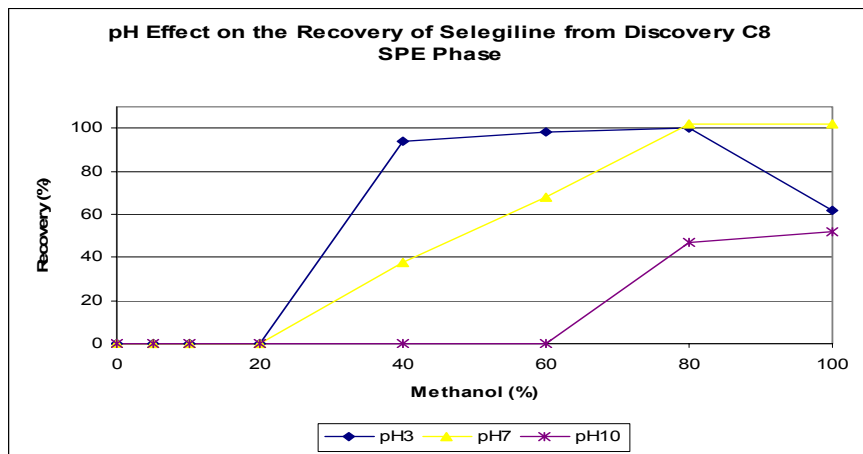


As with the C18 results, full recovery is not observed under the pH 10 conditions, however both pH 3 and pH 7 show promising results

An advantage of using the C8 vs. the C18 may lie in the reduced organic percentage required to elute the analytes. This may lead to greater selectivity

The major disadvantage, however is that lower strength wash solvents must be utilized due to methamphetamine breakthrough

It is also noted that at the pH 3 level 100% methanol as usually called for in generic methods would likely result in less than optimal recoveries



Results, Cont.

- **Samples providing near 100% recovery via UV analysis were further examined using MS detection.**

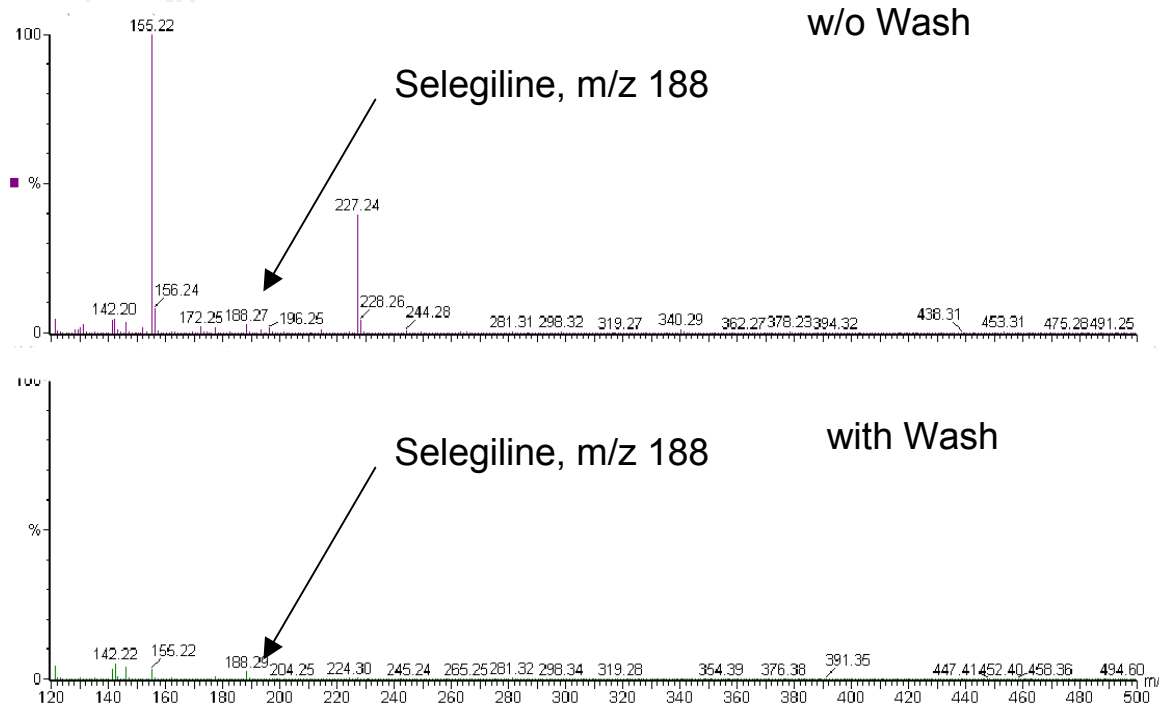
SPE phases	seleg	meth
DSC phenyl, washed by 40% methanol, pH3	107	95
DSC phenyl, washed by 60% methanol, pH3	116	97
DSC phenyl, washed by 80% methanol, pH3	101	91
DSC C18LT, washed by 60% methanol, pH3	109	92
DSC C18LT, washed by 80% methanol, pH3	99	88
DSC C18LT, washed by 100% methanol, pH3	107	87
DSC C8, washed by 40% methanol, pH3	98	79
DSC C8, washed by 60% methanol, pH3	81	79
DSC C8, washed by 80% methanol, pH3	78	66
DSC C18, washed by 80% methanol, pH3	84	77
DSC C18, washed by 100% methanol, pH3	97	88
DSC C8, washed by 80% methanol, pH7	82	86
DSC C8, washed by 100% methanol, pH7	81	78

Reasonable correlations were made between the UV and MS recoveries.

In cases where there appeared to be an enhancement or suppression of ionization, spectral data were investigated.

Results, cont.

Mass Spectrum Obtained for Selegiline Response Using Phenyl System



Several mass responses were observed for the elution profile samples, which could likely effect the ionization process.

Following incorporation of all steps (load, wash and elute) interferences are observed to be minimized.



Conclusions

- **Systematic solid phase extraction development has been shown to be a valuable tool for LC/MS method development**
- **Profiling how analytes behave on a given phase allows the method developer to:**
 - Choose the best phase chemistry for a given analysis
 - Understand the interactions of an analyte with a phase under various pH and %organic conditions
 - Optimize extraction conditions to provide the desired recovery and minimize interferences
- **For the extraction performed in this study, several SPE phases were shown to perform well**



Conclusions

- **Generic approaches to SPE can lead to poor recovery and less than optimal selectivity**
- **For methods intended for extensive use, such as clinical studies, time spent early in method development will likely avert costly reanalysis, redevelopment, and revalidation efforts over the lifetime of the method**