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Retention Mechanisms of Mixed-Mode Cation-Exchange SPE Sorbents

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Introduction

Mixed-mode SPE sorbents combine an effective reversed-phase chemistry with ion-exchange sites on a single particle. Mixed-mode cation-exchange sorbents are often employed for the extraction of organic bases from complex matrices. For example, these sorbents are utilized for the isolation of basic drugs from urine or other biological fluids or for the extraction of basic pesticides from plant or animal tissue. This type of analysis demonstrates one of the great advantages obtained using mixed-mode cation-exchange; the ability to effectively recover basic drugs from sample matrices that have been acidified to release protein-bound residues. However, the benefits of mixed mode SPE extend well beyond the ability to recover drug residues under acidic conditions. For example, the highly polar aminotriazole cannot be effectively extracted using reversed-phase sorbents at any pH, but are effectively recovered using mixed-mode cation-exchange. Another compelling advantage of the mixed-mode sorbents is the ability to effectively retain both acids and bases in the same experiment.

In this presentation, we will then look at some applications that demonstrate each of three analytical categories that mixed-mode cation-exchange provides a clear advantage; 1. extraction of organic bases from acidified matrices, 2. extraction of highly polar bases from aqueous samples, and 3. multi-residue extraction of acids and bases from environmental samples. Finally, we will demonstrate benefit of mixed-mode sorbent chemistries containing weak cation-exchange sites for reterntion of strong bases and quaternary ammonium compounds.

Retention Behavior

Figure 1. Reversed-Phase

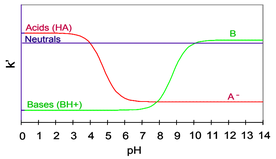
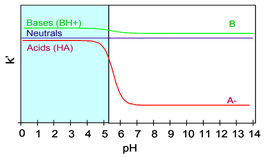


Figure 2. Mixed-Mode Cation-Exchange



Reversed-phase sorbents are the most commonly used SPE materials. Among the sorbents used for reversed-phase SPE are the alkyl bonded silicas (C8, C18 etc.) and polymeric materials such as polystyrene/divinylbenzene. Oasis HLB is a reversed-phase sorbent produced by co-polymerization of hydrophobic (divinylbenzene) and hydrophilic (vinyl pyrrolidone) monomers. SPE devices are typically utilized for extraction of drugs, pesticides, pollutants, metabolites and other analytes of interest, from various matrices. Figure 1 (above left) shows theoretical retention of acids, bases and neutrals on of a reversed-phase sorbent. At low pH, acids and neutrals are retained, but the bases in cationic form, are not. At high pH, bases and neutrals are retained, but the acids in anionic form, are not. Now, consider Figure 2 (above right) that shows the theoretical retention of the compounds on a mixed-mode cation-exchange sorbent. This diagram indicates that good retention of all classes of compounds is possible at low pH.

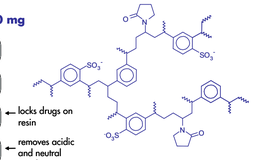
Example 1. Extraction of Organic Bases From Acidified Matrices

This type of analysis is appropriate for drug or drug metabolite residue analysis in biological fluids such as plasma, urine or saliva. Most often the compounds of interest are bases and the sample is acidified to release the residues from protein binding. Figure 1 shows that there is minimal reversed-phase retention of basic compounds at low pH. However, Figure 2 shows that mixed-mode retention mechanism is *favored* under such conditions. This example shows the determination of MDMA (ecstasy) and metabolites in urine. This example and examples 2 and 3 utilize Oasis MCX, a mixed-mode strong cation-exchange sorbent.

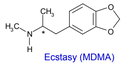
SPE Protocol
Oasis® MCX Cartridge 1 cc, 30 mg

- Condition: 1mL methanol/ 1 mL water
- Load: 0.5 mL sample
- Wash #1: 1 mL 0.1 N HCl ← locks drugs on resin
- Wash #2: 1 mLmethanol ← removes acidic and neutral interferences
- Elute: 1 mLmethanol (10%NH_4OH) ← releases drugs as free bases

Oasis® MCX



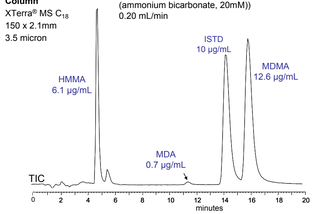
The LC-MS chromatogram below is from a urine sample obtained from an individual suspected of driving under the influence of illicit drugs (HMMA and MDA are metabolites of MDMA).



Ecstasy (MDMA)

Column
XTerra® MS C_{18}
150 x 2.1mm
3.5 micron

Mobile Phase
30% Methanol
70% pH 9 buffer
(ammonium bicarbonate, 20mM))
0.20 mL/min

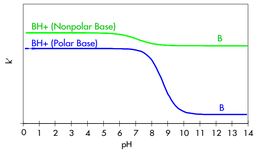


LC/MS System
Waters™ Alliance 2695 Separations Module
Waters/Micromass ZQ™ mass spectometer

Example 2. Extraction of Highly Polar Bases

Figure 3 shows that the retention mechanism of highly polar bases on mixed-mode cation-exchange sorbents is mostly cation-exchange (assume pKa of 8-9 for each base). This type of analyte is poorly retained on reversed-phase sorbents, but is retained, under acidic conditions, using the mixed-mode sorbent.

Figure 3. Mixed-Mode Cation-Exchange Polar Base Retention



Amitrole (aminotriazole) is a common herbicide that is a highly polar base. This example shows the determination of amitrole at sub ppb levels in river water of.high humic content.

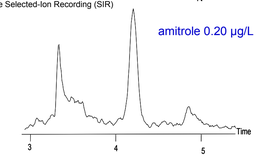
SPE Protocol
Oasis® MCX Cartridge 6 cc, 150 mg

- Prepare Sample: acidify to pH 2.7
- Condition: 5 mLmethanol/1 mL water
- Load: 250 mLsample (5 mL/min)
- Wash: 2 mL5 % MeOH/water
- Elute: 5 mLMeOH/MTBE/NH_4OH (22:75:3)
- Evaporate andReconstitute: 250 mL mobile phase

LC
Waters™ 2690 Separations Module
Waters Resolve™ Silica, 3.9 x 150 mm (5µm)
Gradient: A: acetonitrile
B: 0.1 % formic acid
0 % B to 100 % B in 8 min,
400 µL/min flow 20 µL injection

MS
Waters/Micromass ZQ™
Positive Electrospray (ESI+)
Multiple Selected-Ion Recording (SIR)

amitrole 0.20 µg/L



Example 3. Multi-Residue Extraction of Acids and Bases

To effectively extract acidic and basic compounds using standard liquid extraction methods or traditional SPE methods, two separate extractions must be performed on the same sample. Consider the retention diagram presented in Figure 2. At low pH, mixed-mode cation-exchange sorbents will retain acidic and neutral compounds by the reversed-phase mechanism and basic compounds by the mixed-mode mechanism. Therefore, the mixed-mode sorbent can be used to simultaneously extract acids, bases and neutrals in one SPE experiment. We have applied this technology to the GC-MS analysis of a test mixture containing 33 compounds (acids, bases and neutrals) listed in EPA method 8270C. Structures for a representative acid, base and neutral compound from this list are shown below.

SPE Protocol
Oasis® MCX Cartridge 6 cc, 150 mg

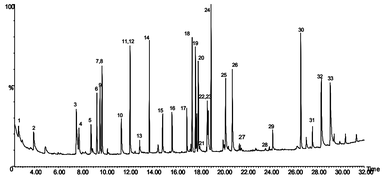
- Prepare Sample: acidify below pH 2 (HCl)
- Condition: 3 mLDCM/ 3 mL methanol/1 mL water
- Load: 250 mL sample (5 mL/min)
- Wash: 2 mL 5 % MeOH/water
- Elute: 5 mL MeOH in DCM (0.7 M NH_4)
- Evaporate to ca. 300 µL Bring to 500 L Final Volume

p-phenylenediamine (base)

dinoseb (acid)

4-nitroquinoline-N-oxide (neutral)

Mass Spectrometer: Waters/Micromass GCT™ with Agilent 6890 GC
RTX 5-MS, 30 m x 0.25 mm (ID)
Helium carrier gas @ 1 mL/min
35°C for 4 minutes, 8°/min, to 300°



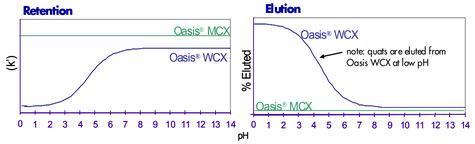
1. pyridine
2. picoline
3. aniline
4. phenol
5. benzyl alcohol
6. *o*-cresol
7,8. *m,p*-cresol
9. *o*-toluidine
10. phentermine
11. chloroaniline
12. dichlorophenol
13. phenylenediamine
14. 2-methylnaphthalene
15. trichlorophenol
16. 2-nitroaniline
17. 3-nitroaniline
18. dibenzofuran
19. 1-aminonaphthalene
20. 2-aminonaphthalene
21. tetrachlorophenol
22. 2-methyl-5-nitroaniline
23. 4-nitroaniline
24. diphenylamine
25. phenacetin
26. aminobiphenyl
27. dinoseb
28. nitroquinoline oxide
29. methapyrilene
30. dimethylaminoazobenzene
31. dimethylbenzidine
32. acetamidofluorene
33. dichlorobenzidine

acids bases neutrals

A Novel Mixed-Mode SPE Sorbent for Quaternary Ammonium Compounds and Strong Bases

These types of analytes are well retained by the mixed-mode mechanism on a strong cation-exchange based sorbent (Oasis® MCX). However, elution must be accomplished at high ionic strength using highly concentrated salt solutions or strong acids. Such an eluant cannot be evaporated and reconstituted in mobile phase and the high ionic strength is not compatible with LC-MS analysis. However, consider the retention and elution behavior shown in Figure 4. Cationic species retained on the weak ion-exchanger, Oasis® WCX, are easily eluted using a mildly acidic eluent.

Figure 4. Retention and Elution of Quats on Mixed-Mode Sorbents

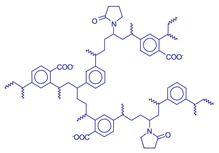


An SPE protocol and the structure of the sorbent are shown below. Also shown are LC-MS analysis of river water samples for paraquat and diquat using the Oasis® WCX Protocol.

SPE Protocol
Oasis® WCX Cartridge 3 cc, 60 mg

- Prepare Sample: adjust to pH 7
- Condition: 1mL methanol/ 1 mL water
- Load: up to 25 mL sample
- Wash: 1 mL pH 7 buffer/1mL methanol
- Elute: 1.5 mL ACN/water/TFA 84:14:2
- Evaporate and Reconstitute: 0.5 mL mobile phase

Oasis® WCX
weak ion-exchange



MS Conditions
Instrument: Waters/Micromass QuattroMicro™
Paraquat: MRM 171 → 155
Diquat: MRM 183 → 168

LC Conditions
Column: YMC™ PVA-Sil S-5 2.0 x 150 mm
Flow: 0.4 mL/min
Mobile Phase: 62 % acetonitrile
38 % aqueous buffer pH 4
(250 mM ammonium formate)
Column Temperature: 30 °C
Sample Temperature: 5 °C
Injection volume: 10 µL

0.20 µg/L River Water Sample