

Controlling SPE Selectivity through pH and Organic Modifier Manipulation

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Introduction

Solid phase extraction (SPE) methods are frequently developed by copying/modifying an existing application or choosing a generic method. Although these approaches are less time-consuming, it can often be very difficult to determine and troubleshoot the root cause(s) of problems associated with an SPE method when they arise. For example, if poor recovery is observed, is it due to: 1) poor analyte retention during sample load; 2) pre-mature analyte elution during the wash step (b/w sample load and elution); or 3) analyte over retention during elution?

Like HPLC, SPE is a form of chromatography, and as such, basic chromatographic principles should be used when developing, optimizing, and troubleshooting a given SPE method. In this report, we demonstrate the use of pH and organic modifier manipulation during SPE wash/elution to control the retention and elution of three different compounds (neutral, basic, and acidic) on three different reversed-phase SPE chemistries of decreasing hydrophobicity (C18, C8, and cyanopropyl-CN).

The Role of pH and Organic Modifiers in SPE

Most reversed-phase SPE protocols follow the general procedure in which the phase is first conditioned and equilibrated with aqueous miscible solvent (e.g., methanol or acetonitrile) followed by sample load. The sample must be aqueous because a polar mobile phase environment is necessary to drive reversed-phase retention. To elute compounds of interest, reversed-phase interactions are disrupted by decreasing polarity of the mobile phase environment. Common elution solvents include methanol and acetonitrile. Prior to elution, a wash step of intermediate solvent strength is typically employed to remove any endogenous interferences that may have co-retained with the analytes of interest (e.g., 5-20% methanol).

Most analytes contain ionizable functional groups, and a compounds ionization state can drastically change its retention and elution characteristics on a given SPE sorbent. When an analyte is in its neutral form, it becomes more hydrophobic and retention strengthens under reversed-phase conditions. This may allow for stronger wash solvents to remove co-retained interferences prior to elution. In contrast, in the ionized form, compounds become more

polar weakening the interaction strength between analytes of interest and reversed-phase functional groups. As a result, one may be able to elute with weaker solvent conditions (e.g. 50% methanol as opposed to 100% methanol) which could possibly eliminate the evaporation/reconstitution step common in SPE protocols. Figure 1 describes the role of pH in SPE.

Figure 1. The Role of pH in Reversed-Phase SPE

Acids (e.g. Carboxylic Acids): $R-COOH \leftrightarrow R-COO^-$

HA (neutral)	\leftrightarrow	H ⁺ + A ⁻ (ionized)
50%	@ pKa	50%
100%	2 pH units below pKa	0%
0%	2 pH units above pKa	100%

Bases (e.g. Amines): $R-NH_3^+ \leftrightarrow R-NH_2$

BH ⁺ + OH ⁻ (ionized)	\leftrightarrow	B (neutral)
50%	@ pKa	50%
0%	2 pH units below pKa	100%
100%	2 pH units above pKa	0%

Neutral State (Blue) = Strengthens reversed-phase interaction
 Ionized State (Red) = Weakens reversed-phase interaction

Method

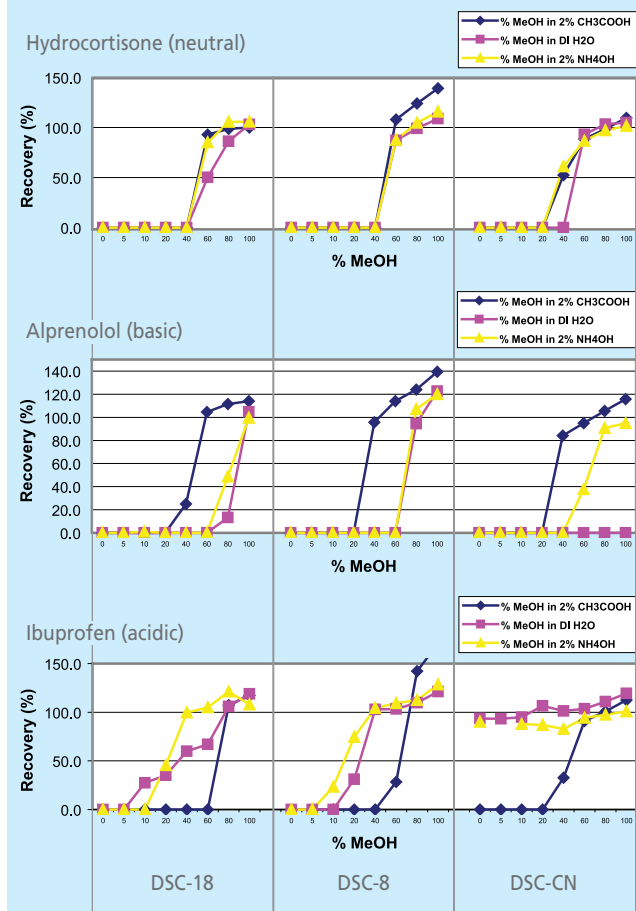
1 mL standards of 20 µg/mL ibuprofen (acidic), hydrocortisone (neutral), and alprenolol (basic) in 20 mM potassium phosphate, pH 7 were loaded on to three different 96-well SPE plates conditioned and equilibrated with 1 mL methanol and DI water per well. The SPE phase chemistries tested were Discovery DSC-18 (C18), DSC-8 (C8), and DSC-CN (cyanopropyl), 100 mg/well.

Respective wells were washed/eluted with 1 mL test solvents ranging from 0-100% methanol in 2% NH₄OH, pH 11 (high pH), DI H₂O (neutral pH), and 2% CH₃COOH, pH 3 (low pH). The wash/elute eluate was collected for each well, and analyzed for compound breakthrough via HPLC-UV.

Retention-Elution Profile for Hydrocortisone (neutral)

Figure 2 represents a retention-elution profile of the three compounds tested in which % recovery was measured against changing extraction conditions (pH vs. % organic modifier vs. phase chemistry). Hydrocortisone is a neutral compound that contains no ionizable functional groups. In Figure 2, we see that changes in pH across all three SPE chemistries had very little effect in manipulating elution

Figure 2. Retention-Elution Profile Hydrocortisone, Alprenolol, and Ibuprofen on DSC-18, DSC-8, and DSC-CN SPE



selectivity. Up to 40% methanol can be used as a possible wash solvent for both DSC-18 and DSC-8. DSC-CN is much more polar reversed-phase SPE chemistry. As a result, analyte breakthrough occurs between 20-40% methanol. 100% methanol is required to completely recover this moderately polar to non-polar compound.

Retention-Elution Profile for Alprenolol (basic)

Alprenolol is a basic compound with a pKa of ~9.5. At higher pH levels, it deprotonates into its neutral form. At low pH levels, it is in its ionized form. In contrast with hydrocortisone, pH modification has a great affect in controlling selectivity. At neutral and high pH conditions, alprenolol can withstand up to 60% methanol on DSC-18 and DSC-8 SPE before compound breakthrough occurs. At low pH conditions, compound breakthrough initiates at greater than 20% methanol. On DSC-CN, the short alkyl functional groups allow greater compound access to silanol groups which act as a secondary weak cation exchanger. As a result, at neutral pH conditions compounds are retained by both cation exchange and reversed-phase, and compounds remain retained from 0-

100% methanol. At high pH conditions, alprenolol is in its neutral form disrupting secondary ionic interactions allowing for elution from 40-100% methanol. At low pH, alprenolol is ionized but silanol groups are protonated and neutral resulting in elution between 20-100% methanol.

Retention-Elution Profile for Ibuprofen (Acidics)

Ibuprofen is an acidic compound with a pKa of ~4.2. In contrast to alprenolol, the compound is neutralized at low pH and ionized at high pH environments. On DSC-18 and DSC-8, up to 60 and 40% methanol in 2% acetic acid can be used as a possible wash solvent. At neutral and high pH levels where the compounds are ionic and thereby more polar, the retention limit is 5-10% methanol before compound breakthrough occurs. On DSC-CN, retention is very weak at high and neutral pH, and buffer alone will elute the compound. At low pH levels, wash solvents of up to 20% methanol can be employed.

Note on High Recoveries

Note that >100% recovery was often observed. When injecting a sample of greater solvent strength than the HPLC mobile phase, fluctuations in retention time and peak shape are often observed (data not shown) which can result in erroneously high signals. We observed this trend in our data because the SPE eluate was directly analyzed, and a high level of % methanol was used during SPE elution in part of the study. Although recovery data was not accurate, the purpose of the data was to describe general recovery trends observed by systematically changing elution conditions.

Conclusion

Both pH and % organic modifier play a critical role in determining retention and elution of ionizable compounds in reversed-phase SPE. By controlling the pH of the SPE mobile phase, one can control the relative hydrophobicity of an ionizable compound allowing for stronger wash solvents resulting in improved sample cleanup. pH manipulation may also allow for weaker elution solvents possibly minimizing processing time by negating the eluate evaporation/reconstitution step common in most reversed-phase procedures. By understanding how a compound interacts with the SPE phase under changing extraction conditions, one can manipulate the conditions to offer the most selective procedure.

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