Applications *Update...*

PolyAspartic Acid WCX (PolyCAT A™)

Uncharging a Weak Cation Exchange Column with Acetic Acid to Elute Peptides

In cation exchange HPLC, peptides and proteins are usually eluted with a gradient of increasing salt and/or pH. However, a gradient of *decreasing* pH protonates the functional groups of a weak cation exchange stationary phase, leading to elution of retained peptides. A convenient solvent system is a gradient from 10 mM ammonium acetate to 20% acetic acid. These solvents have the advantage of being volatile but the disadvantage of absorbing below 230 nm; thus, peptides must have a least one aromatic residue in order to permit UV/VIS detection. A material for weak cation exchange (WCX) is preferable to one for strong cation exchange (SCX), since much less acetic acid is required to uncharge the column. Loading capacity in this mode is quite high. This method is particularly convenient for very basic synthetic peptides, which may be difficult to purify another way, and it affords good selectivity.

Reversed-phase chromatography (RPC) is the most commonly-used general-purpose method for peptide HPLC. However, RPC fails in some cases: some peptides are not retained and some co-elute. A good alternative is cation exchange HPLC. At pH < 4, the carboxyl- groups in peptides lose their (-) charge, and peptides have a net (+) charge. They are retained by a strong cation exchange (SCX) material and can be eluted by an increasing salt gradient, in order of increasing absolute number of basic residues[1,2]. This is *displacement* chromatography; the ions of the salt out compete the peptides for the binding sites of the stationary phase. The capacity is approx. 4x greater than with RPC.

Michael Selsted (Univ. of Calif.-Irvine) had used a PolySULFOETHYL Aspartamide™ (an SCX material) for preliminary purification of crude synthetic peptides. The peptides were adsorbed in 10 mM ammonium acetate (NH4OAc) or 10mM acetic acid (HOAc) and after deprotection fragments had eluted, the peptides were eluted with a gradient to 15% HOAc. HOAc is a weak acid and is only 1% dissociated in aqueous solution. This suggested that it could uncharge the stationary phase of a weak cation exchange material. The volatility of the solvent made this method appealing, and the following chromatogram illustrates its utility for a variety of peptide applications. We feel that for volatile solvent applications, the use of acetic acid and the PolyCAT A WCX column is a superb choice.

Advantages

1) *General-purpose mode*: selectivity complements RPC's.
2) *Volatile solvent:* useful for mass spec, bioassays, sequencing.
3) *Prep-scale advantages:*
   - High capacity (@ 4x RPC)
   - HOAc safer to handle than TFA.
   - Cheaper and easier solvent disposal than RPC .
4) *Good Selectivity.*
5) Peptides recovered in the acetate salt form: better for bioassays and pharmaceutical applications than the trifluoroacetate form.
6) Good solvent for peptides.
7) Elutes very basic solvents easier than SCX.

Disadvantages

1) *Detection:* Can not monitor < 235 nm.
   - Limited to peptides containing Phe-, Tyr- or Trp- (@ 254, 265, or 280 nm, resp.) or use some other detector.(ELSD, MS, etc.)
2) Denatures large proteins.
   - Not a problem if the objective is isolation for sequencing.

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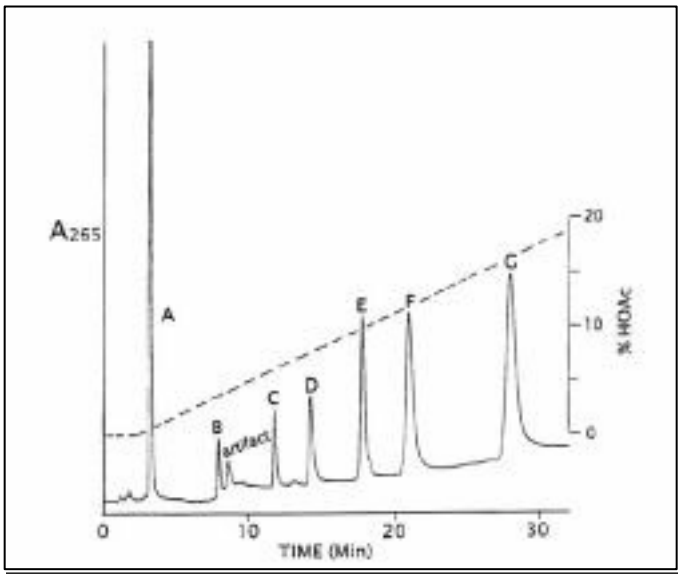
Cation-Exchange of Peptide Standards on PolyCAT A (Acetic Acid Gradient)

KEY (net charge pH 3.0)

A) Gly-Tyr (+1)
B) Oxytocin (+1)
C) [Arg^8]-Vasopressin (+2)
D) Tyr-Somatostatin-14 (+3)
E) Lys-Arg-Pro-Ser(PO_4)-Gln-Arg-His-Gly-Ser-Lys-Tyr-NH_2 (+6-1)
F) Lys-Arg-Pro-Ser-Gln-Arg-His-Gly-Ser-Lys-Tyr-NH_2 (+6)
G) ACTH (1-39)[human] (+9)



Elution of Peptides from PolyCAT A with an HOAc Gradient.

Gradient: 0-100% B in 80'. A) 10 mM NH4OAc, pH 5.5. B) 50% HOAc.
Flow Rate: 1.0 ml/min. A_{265} = 0.1 AUFS.

A pH of 5.5 was chosen for Mobile Phase A to insure that the stationary phase was charged initially. Under these conditions, peptides now elute in order of increasing absolute number of basic residues. 18% (3 M) HOAc suffices to elute all peptides.

Ordering Information

Description	*Particle/Pore*	*Part #*
PolyCAT A WCX column, 200 x 4.6mm	(5µ; 300Å)	P204CT0503
PolyCAT A WCX column, 200 x 2.1mm	(5µ; 300Å)	P202CT0503
PolyCAT A WCX column, 100 x 4.6mm	(5µ; 300Å)	P104CT0503
PolyCAT A WCX column, 100 x 2.1mm	(5µ; 300Å)	P102CT0503

Other column dimensions are available from 0.5 to 21.5mmID and 12µ particle size.

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