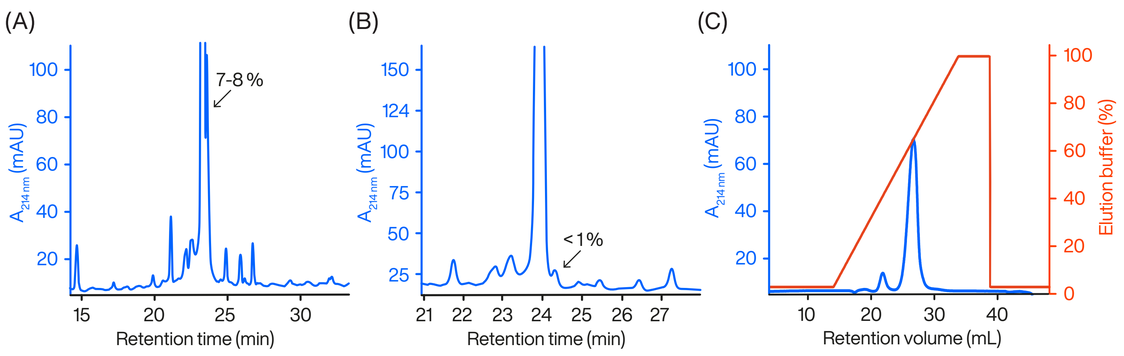
Optimizing the buffer conditions for a two-step purification of a glycopeptide conjugate API

Jenny Thorsén and Cecilia Unoson
Bio-Works, Uppsala, Sweden

Implementation of cation exchange chromatography (CIEX)

A glycopeptide conjugate API was difficult to purify with reverse phase chromatography (RPC) due to a main impurity eluting close to the target resulting in column performance decay. To investigate if the implementation of CIEX as an orthogonal technique could mitigate these issues, a screening purification using generic mobile phases was performed. Here, consistent buffer conditions were used in both IEX and RPC (0.1 % TFA, pH 2). Pre- and post CIEX purification analysis of crude peptide are shown in Figures 1 A-B. Figure 1C shows the screening purification using IEX where the main impurity eluted prior to the target peptide peak with baseline separation, facilitating easy collection of target.

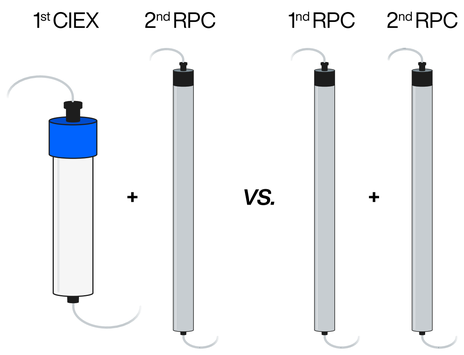


The peptide target

- Small cyclic peptide (<2 kDa)
- Isoelectric point >8
- Target purity ~78 %
- Difficult impurity associated to the conjugate coupling reaction constitutes of ~7-8 %

Figure 1. Impurity profile from analytical RPC of the crude sample (A) or post IEX (B). IEX purification using WorkBeads™ 40S (C).

Post screening where CIEX was shown to remove the main impurity, the buffer conditions were optimized to obtain a robust process scale setup. Purities and yields were evaluated and we compared CIEX + RPC *vs.* the more common RPC + RPC approach.



Method

Glycopeptide conjugate was purified using

- CIEX + RPC or
- RPC + RPC

Analytical RPC was used for the assessments.

Figure 2. CIEX + RPC *vs.* RPC + RPC purifications.

Table 1. Columns used in study.

Preparative RPC	Chromatorex™ C18 SPS150-10, 10 µm, 4.6 × 250 mm
Analytical RPC	Nanologica SVEA™ Gold C18, 5 µm, 4.6 × 250 mm
CIEX	WorkBeads™ 40S, 45 µm, (BabyBio S 1 mL)

Buffers

The same generic RPC buffers were used during the screening phase in both the CIEX and the RPC steps with the difference that mobile phase B contained 1 M NaCl for the CIEX step and 80% acetonitrile for the RPC step. After optimization for preparative conditions different buffer systems were used for CIEX and RPC, respectively. However, the same buffer was used in the second RPC step in both approaches (Table 2).

Table 2. Buffer conditions.

	Mobile phase A	Mobile phase B
Screening buffers		
CIEX	0.1% TFA, 5% ACN, pH 2.0	0.1% TFA, 5% ACN, 1 M NaCl, pH 2.0
RPC	0.1% TFA, 5% ACN, pH 2.0	0.1% TFA, 80% ACN, pH 2.0
Optimized prep buffers		
CIEX	20 mM $NH_4CH_3CO_2$, 10% ACN, pH 5.2	20 mM $NH_4CH_3CO_2$, 10% ACN, 1 M NaCl, pH 5.2
1st RPC	20 mM NaH_2PO_4, pH 6.5	20 mM NaH_2PO_4, 50% ACN, pH 6.5
2nd RPC	20 mM NaH_2PO_4, pH 2.2	20 mM NaH_2PO_4, 50% ACN, pH 2.2

Mass overloading conditions: CIEX + RPC *vs.* RPC + RPC

Mass overloading conditions were applied with a load of 25 mg crude peptide/mL resin for the CIEX purification and 10.8 mg crude peptide/mL resin for the preparative RPC purification.

Depending on the required purity, the yield will be affected, see the black trace in figure 3A and 3C. Due to the goal here being to remove the main impurity, the target peak was collected with the fractions having low abundance of the difficult impurity (requirement: target purity > 87%). These pooled fractions were further loaded onto the second RPC polishing steps (Figure 3B and 3D). All collected pools (as visualized as purity profile with green bars in elution profiles) were analyzed for purity, yield and abundance of the main impurity. Fractions were pooled to obtain a theoretical yield of 90%, the purity of each fraction was above 87%.

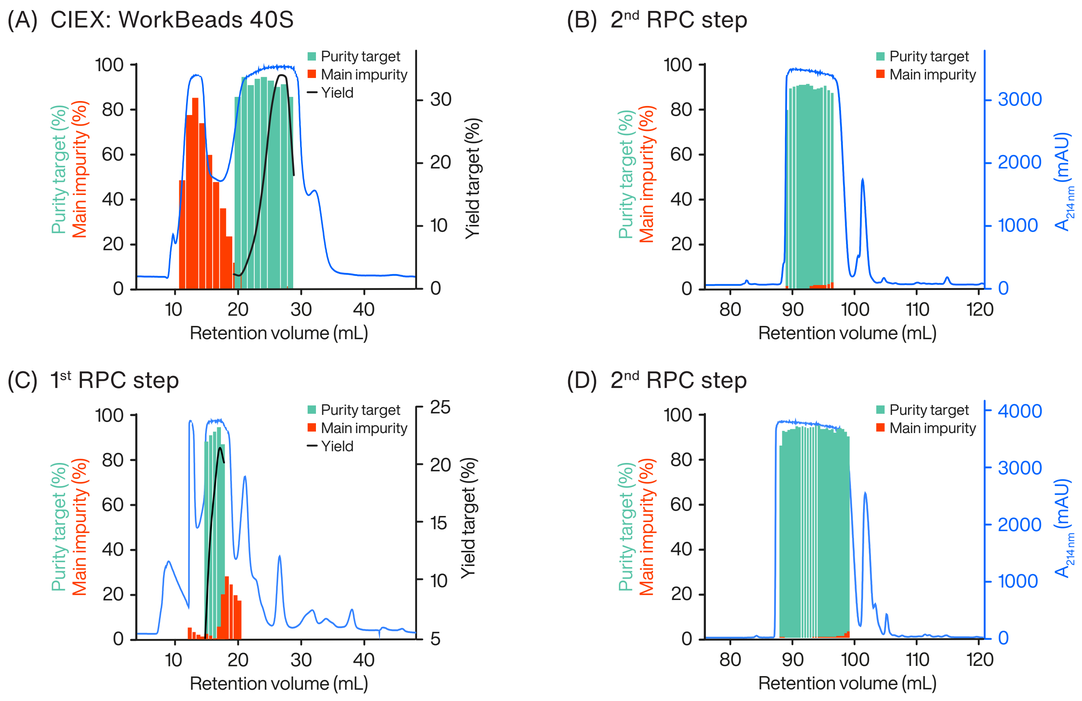


Figure 3. Purity profiles for the chromatographic steps. (A) CIEX elution profile. (B) Polishing RPC elution profile (post CIEX). (C) Prep RPC elution profile. (D) Polishing RPC elution profile (post prep RPC). Blue trace corresponds to absorbance at 214 nm, green bars is the purity of target, orange bars is the purity of the main impurity, and the black trace corresponds to the target yield. The flow rate applied was 150 cm/h (0.9 mL/min) for the IEX step and 300 cm/h (1 mL/min) for the RPC steps.

Figure 3A shows that the main impurity is resolved on the CIEX column, where the target peak could easily be collected. However, the main impurity did not resolve as well on the RPC column (Fig. 3C), but the overall purity was still improved. Figures 3B and 3D are the respective polishing steps, showing the target purity in each eluted fraction compared to the main impurity. By combining the orthogonal techniques CIEX and RPC a purity of 96% at a yield of 89% was reach as compared to a purity of 95% at a significantly lower yield of 78% for RPC + RPC.

Table 3. Target purity, yield and main impurity.

	Target purity	Yield	Main impurity
Crude peptide	78%	100%	7.5%
CIEX	92%	90%	<1%
CIEX + RPC	**96%**	**89%**	**0.6%**
1st RPC	92%	79%	2.5%
RPC + RPC	95%	78%	0.9%

CIEX → 92% target purity, <1% main impurity, 90% yield
CIEX + RPC → 96% target purity, <1% main impurity, 89% yield

Conclusions

For challenging peptide purifications like this one, the use of RPC is not always enough to remove problematic impurities. Here we have shown the advantage of employing orthogonality into a difficult purification by adding CIEX prior to RPC. Initial CIEX runs are easily set up using RPC-compatible buffers for screening purposes. Purification setup can later be optimized using more CIEX-specific buffers. For this glycopeptide conjugate API the difficult main impurity could be decreased to below 1 % at a yield of 89%, and a total purity of 96%.

bio-works.com

PS 45 20 00 01 AB

Bio-Works