

Two-Step Purification of a Glucagon-Like Peptide-1 (GLP-1) Receptor Agonist Using DuPont™ AmberChrom™ Chromatography Resins

Highlighting the importance of high-quality peptide purification resins in GLP-1 therapeutics

Introduction

The increasing demand for glucagon-like peptide-1 (GLP-1) receptor agonists in recent years has emphasized the need for high-quality peptide purification resins. GLP-1 therapeutics mimic the natural GLP-1, which is a peptide hormone produced by the human body. GLP-1 promotes insulin secretion and decreases glucagon, thus reducing glucose levels and enhancing satiety. However, its activity is hindered by its fast degradation and clearance in the body. Through various synthetic design strategies, such as sequence modification and covalently-bound fatty acids, GLP-1 receptor agonists exhibit an extended half-life in the body which enhances their effects and increases their duration of action.¹

Liraglutide is an example of a modified GLP-1 where a 16-carbon fatty acid is conjugated to lysine at position 26 through a glutamic acid, and lysine-34 is substituted with arginine (Figure 1). Compared to the native GLP-1 half-life of two minutes, liraglutide's modifications increase its *in vivo* stability and provide a half-life of approximately 9 - 13 hours.² This study showcases the purification of liraglutide using DuPont™ AmberChrom™ XT20 chromatography resin (Table 1), a versatile polymeric resin with high chemical resistance.

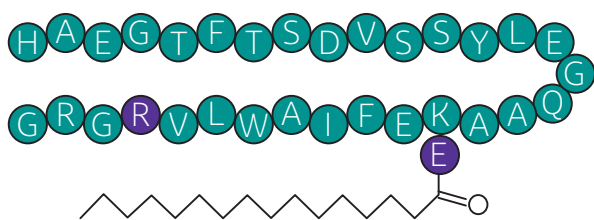


Figure 1: Liraglutide structure showing modifications (16-C fatty acid and an arginine instead of lysine), which extend its half-life.

Table 1: Properties of the resin used in this study.

Property	DuPont™ AmberChrom™ XT20 chromatography resin
Particle size, mean	20 µm
Pore size, average	300 Å
Chemistry	Crosslinked divinylbenzene (DVB)
Pressure rating	up to 60 bars
pH and temperature stability	pH 1 – 14 60 °C

Experimental Conditions

RP-HPLC Purification Method: DuPont™ AmberChrom™ Profile™ XT20 column, 4.6 x 250 mm, 4.15 mL, was utilized in RP purification; flow rate 1 mL/min. UV Detection was at 220nm.

Basic Conditions: Mobile phase A: 0.1 M ammonium acetate pH 8.2; mobile phase B: acetonitrile. Gradient elution of product over 25-50% B.

Acidic Conditions: Mobile phase A: 0.1% trifluoroacetic acid (TFA) in water; mobile phase B: 0.1% TFA in acetonitrile, pH ~2. Gradient elution of product over 40 to 60% B. Loading: 10 mg crude liraglutide per mL resin or 1 mL from pooled fractions.

Analytical Method: Waters™ XSelect™ Premier CSH C18 HPLC Column, 60 °C, 10 µL injection, flow rate 0.5 mL/min. Mobile phase A: 0.1% TFA in Water; Mobile phase B: 0.1% TFA in acetonitrile. Gradient elution over 40-80%. UV detection at 214nm.

All runs and fractionations were performed on ÄKTA pure™ 150 M at room temperature. Analytical runs were performed on an Agilent™ 1260 Infinity II HPLC system. Salt and solvents were purchased from Fisher Scientific™.

Results

Two-step purification

In a separate study, we explored the purification of liraglutide through a first step with DuPont™ AmberChrom™ TQ1 resin, a hydrophilic anion exchange resin, followed by a reverse phase polishing step with DuPont™ AmberChrom™ XT20 resin. Various processes involving ion exchange and reverse phase purifications can be used in an orthogonal fashion to purify GLP-1 agonists. Here, we explore the widely employed two-step reverse-phase high-performance liquid chromatography (RP-HPLC) process with the polymeric DuPont™ AmberChrom™ XT20 resin.

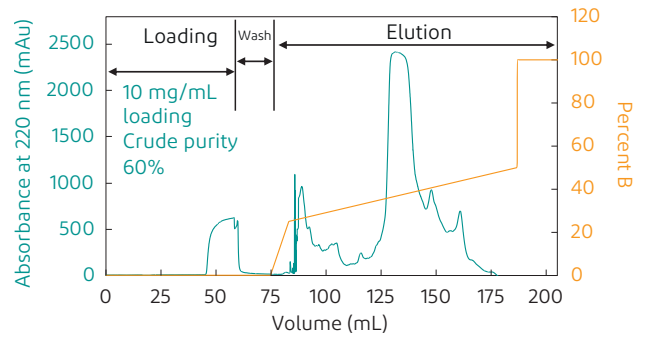
The two steps in this reverse phase process typically utilize the same resin, but the properties of the mobile phases used to elute the peptide in each step vary. Here, we explore elution in various pH conditions with step 1 starting in acidic or basic conditions and a subsequent step 2, following each step 1, that utilizes acidic or basic conditions.

Step 1 Purification: In each of these purifications, the column was first loaded with 10 mg of crude liraglutide per mL of resin in basic conditions (0.1 M ammonium acetate pH 8.2) or in acidic conditions (0.1% trifluoroacetic acid pH 2). Elution was performed in acetonitrile (Figure 2A shows an example of step 1 basic purification).

Step 2 Purification: After analyzing the results of step 1, the highest purity fractions were pooled and 1 mL was reinjected onto the column utilizing mobile phases at a similar or varying pH. Elution was also performed in acetonitrile (Figure 2B shows an example of the basic-to-acidic purification).

In each of the first purifications, purity increased from 60% to approximately 90% independent of mobile phase pH (Figure 3). In the second purification, where both steps utilize the same mobile phase pH, the highest purity achieved was approximately 95% (Figure 3). In the second purification where the pH was modified from the first step to utilize an orthogonal approach, the highest purity achieved was 98 – 99% (Figure 3).

A) Basic Purification 1



B) Acidic Purification 1

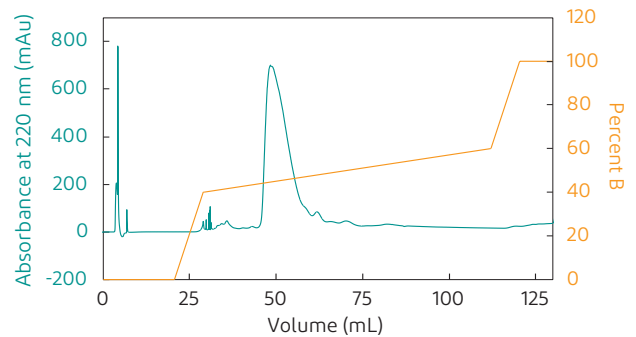


Figure 2: Example two-step purification showing **A)** purification 1 in basic conditions and **B)** purification 2 in acidic conditions.

Yield-Purity Profiles

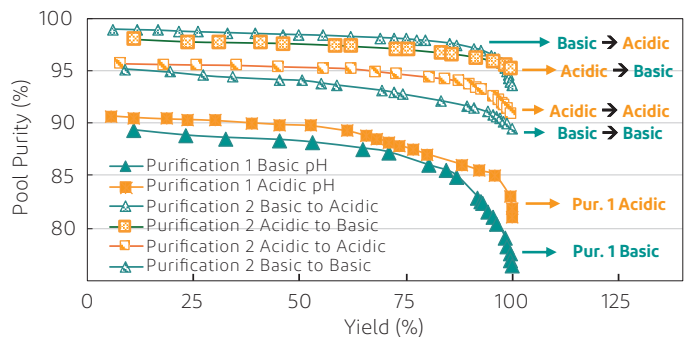


Figure 3: Yield-purity curves for each of the purification 1 runs and the subsequent purification 2 runs in various conditions.

Table 2 summarizes the results of each run with various conditions, showing that alternating the conditions between purification 1 and purification 2 delivered fractions with the highest purity. Also, the table shows that the first purification delivered a high-yield for fractions with a pool purity of 85%, emphasizing the efficient performance of this resin in separating product from impurities in these runs.

Table 2 shows that while alternating conditions result in higher maximum purities of 98–99%, when a 98% pool purity threshold is chosen for step 2, the basic-to-acidic purification delivers a higher overall yield. In scaled-up processes, obtaining a high-yielding and high-purity pool is important to assess the overall performance of the resin.

Table 2: Summary of yields and purities from the two-step purification runs of liraglutide on DuPont™ AmberChrom™ XT20 resin.

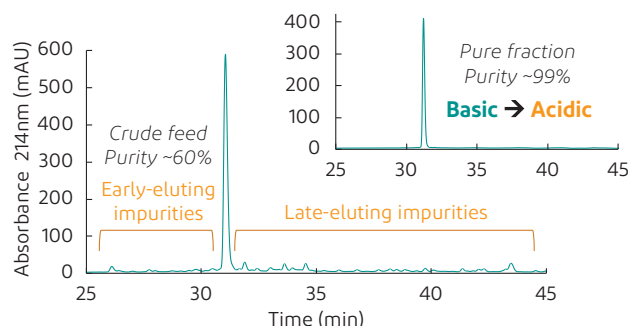
1 st Purification	Highest Purity Fraction (%)	Yield at 85% Pooled Purity (%)
Pur. 1 Basic	89.3	85
Pur. 1 Acidic	90.7	95
2 nd Purification	Highest Purity Fraction (%)	Yield at 85% Pooled Purity (%)
Basic → Basic	95.2	0
Acidic → Acidic	95.6	0
Acidic → Basic	98.0	12
Basic → Acidic	99.0	77

Results

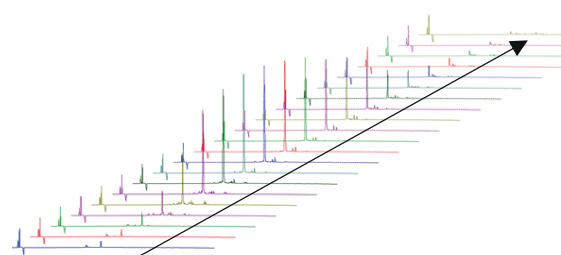
Understanding the yield-purity results of the two-step process

To understand the reason behind these results, the analytical chromatograms were investigated (Figure 4A). When examining these chromatograms, it appeared that elution in basic conditions removes more early-eluting impurities and leaves behind late-eluting impurities (Figure 4B). The opposite effect is seen in acidic conditions, which seem to remove more late-eluting impurities (Figure 4C). The combination of these conditions help reach the higher purity observed in Table 2.

A) Analytical chromatograms of crude feed and pure fraction

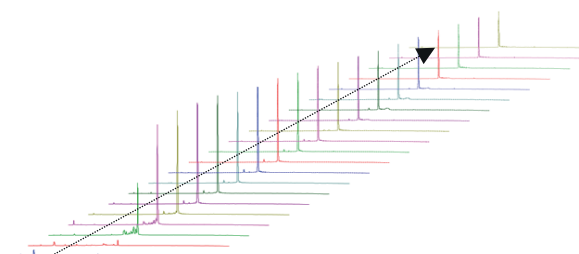


B) Fractions eluted in basic conditions



In fractions obtained after elution in basic conditions, more **late-eluting impurities remain**

C) Fractions eluted in acidic conditions



In fractions obtained after elution in acidic conditions, more **early-eluting impurities remain**

Figure 4:

A) Example analytical chromatograms of the crude feed and a pure fraction. Analytical chromatograms of fractions collected from elution in **B)** basic conditions and **C)** acidic conditions.

Conclusions

Purification of liraglutide using DuPont™ AmberChrom™ XT20 chromatography resin achieves high-purity and yield when taking an orthogonal approach to the pH conditions utilized in a two-step process. Compared to most RP-HPLC silica resins widely used for GLP-1 purification, polymeric resins are compatible with the entire pH range, allowing their use with various mobile phases and with high-pH clean-in-place (CIP) conditions.

References

- 1) Bjerre Knudsen & Lau, *Frontiers in Endocrinology* 2019, DOI: 10.3389/fendo.2019.00155
- 2) Yu et al., *Adv. Drug. Deliv. Rev.* 2018, DOI: 10.1016/j.addr.2018.07.009

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