

Immobilization of Chondroitin Sulfate A into Monolithic Epoxy Column for Chiral Separation

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Abstract

Chondroitin sulfate A was successfully immobilized into epoxy monolithic column at a concentration of 3% (w/v) in the presence of ethylene diamine. The epoxy group of monolithic column was first converted to aldehyde group by successive hydrolyzation and oxidation. A Schiff base reaction at pH 8.0 was used to attach the diamine-spacer to aldehyde group. The chondroitin sulfate A was introduced into the monolithic column by circulating the solution at a flow rate of 0.1 mL/min for 24 hours. The chondroitin sulfate A-immobilized epoxy column was evaluated for chiral separation of verapamil enantiomers under optimized HPLC conditions at a wavelength of 230 nm. As a mobile phase, 20 mM Na₂HPO₄ (pH 2.9) was used. A resolution (R_s) of about 1.5 was achieved for the separation of verapamil enantiomers. A good repeatability of the retention time at two concentration levels ($n=8$) with RSD < 1% was obtained. The linear responses of verapamil enantiomers were in the range of 1.0-3.0 ppm with R^2 of about 0.994.

Overview

The high recognition capacity and enantioselectivity of polysaccharides make them ideal chiral selectors [1-3]. Several polysaccharide-based chiral HPLC columns are currently commercially available [4]. Chondroitin sulfate A belongs to mucopolysaccharides. It has shown an initial promising chiral recognition ability on capillary electrophoresis [5], but has not been tried on HPLC yet. Macroporous epoxy-based columns establish low backpressure on HPLC system and have good separation performance [6]. Having porous backbone makes the monolithic stationary phase a good candidate for the immobilization process [6, 7]. In this study, the immobilization of chondroitin sulfate A into the monolithic epoxy column and the evaluation of its chiral recognition ability were carried out.

Experimental

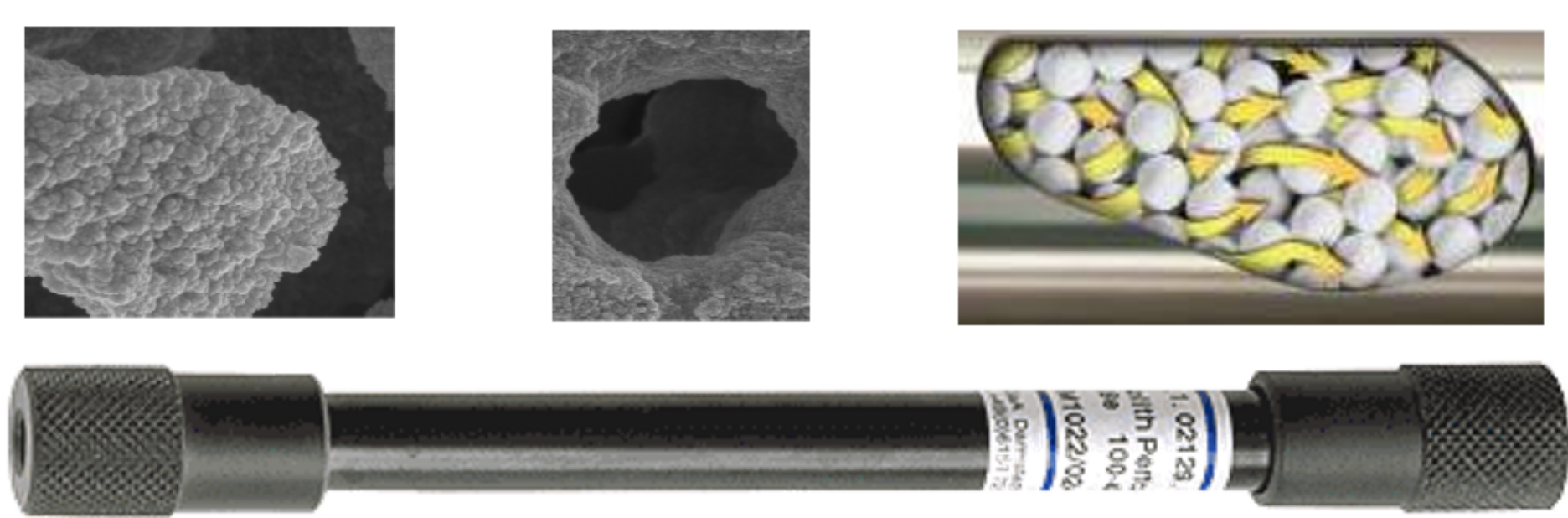


Figure 1. Monolithic silica column.

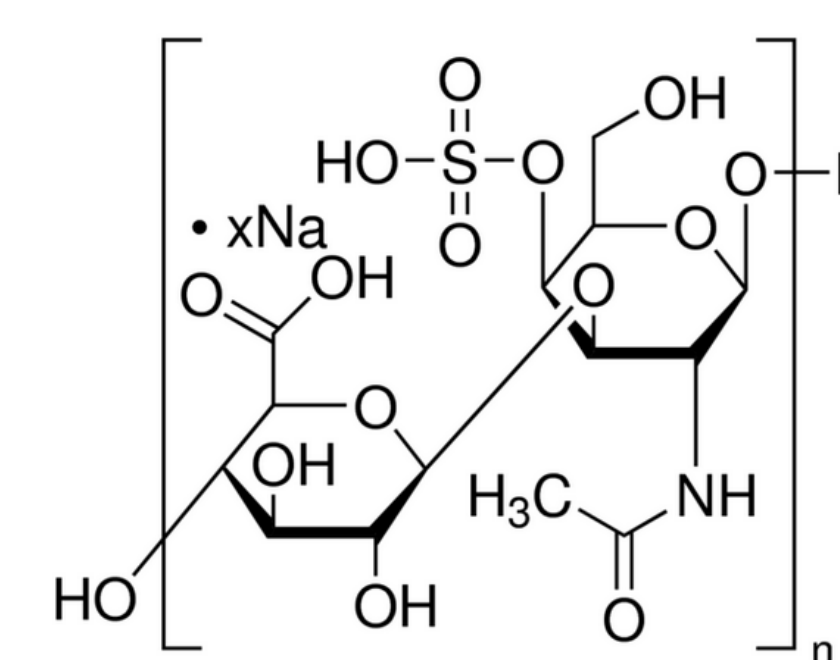


Figure 2. Structure of chondroitin sulfate A sodium salt.

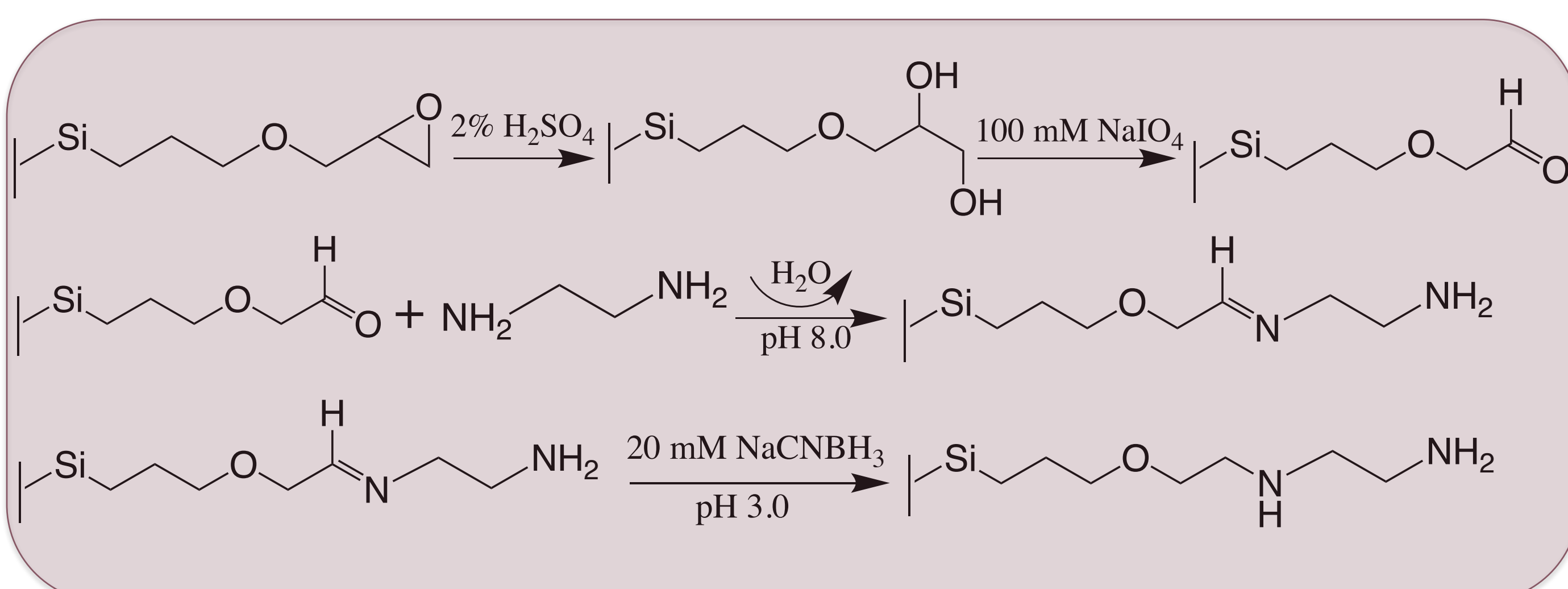


Figure 3. Hydrolysis of epoxy groups (monolithic epoxy silica column) and addition of diamine-spacer by Schiff base reaction.

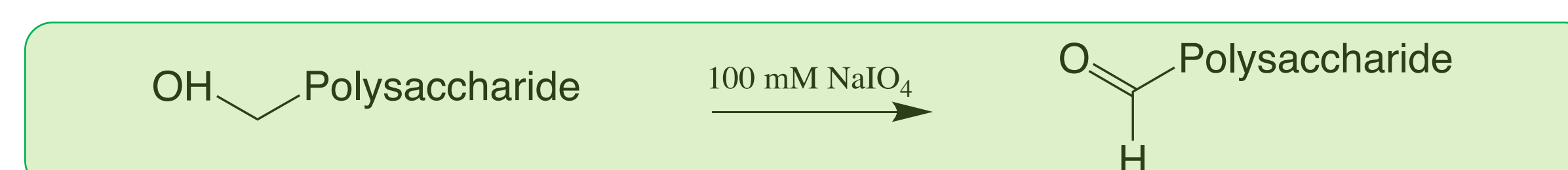


Figure 4. Oxidation of hydroxyl groups to aldehyde group of the polysaccharide

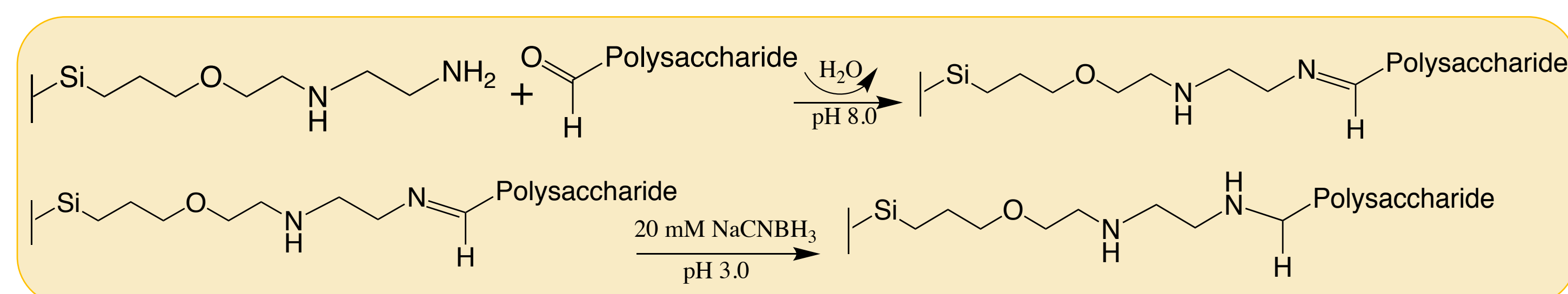


Figure 5. Immobilization of polysaccharide into monolithic column.

Results

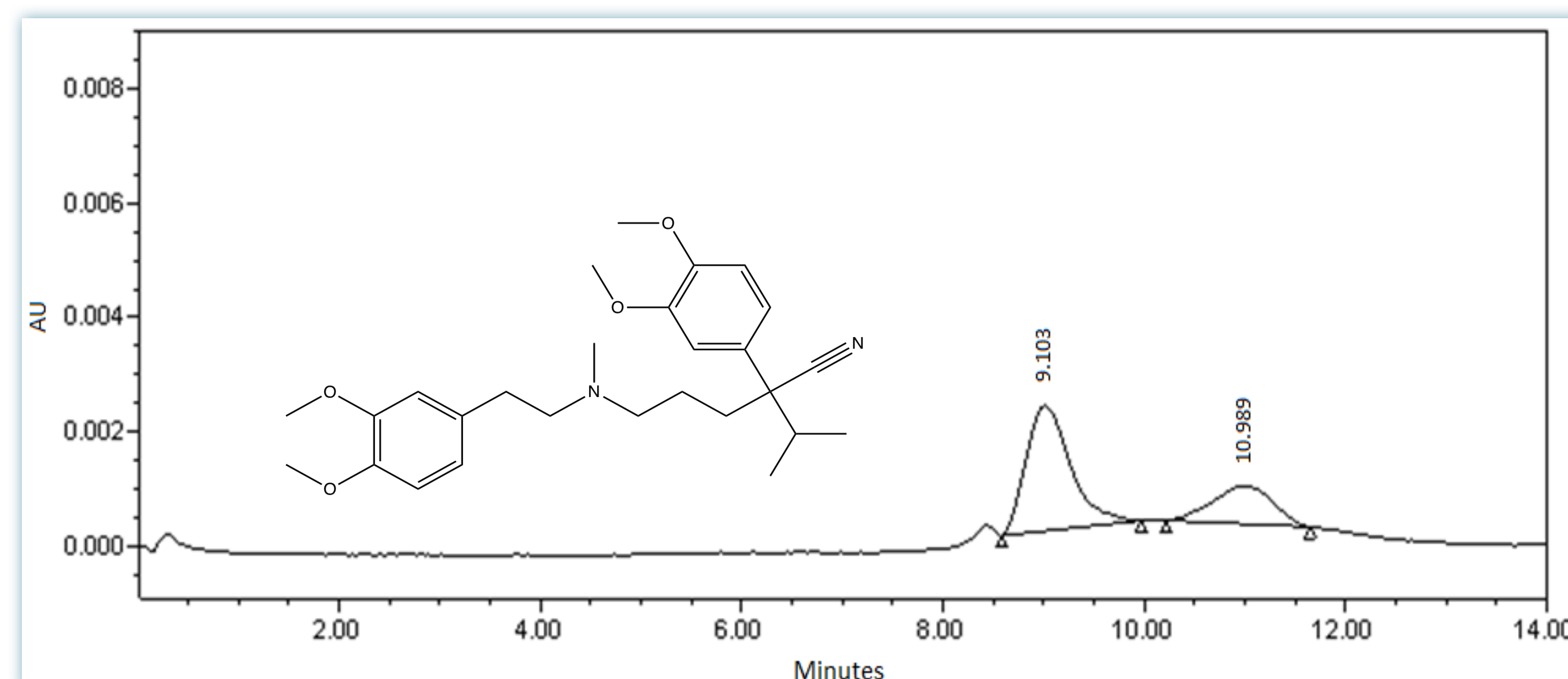


Figure 6. Chromatographic separation of racemic verapamil; mobile phase: Na₂HPO₄ 20 mM pH 2.9; UV detection at 230 nm; R_s 1.52.

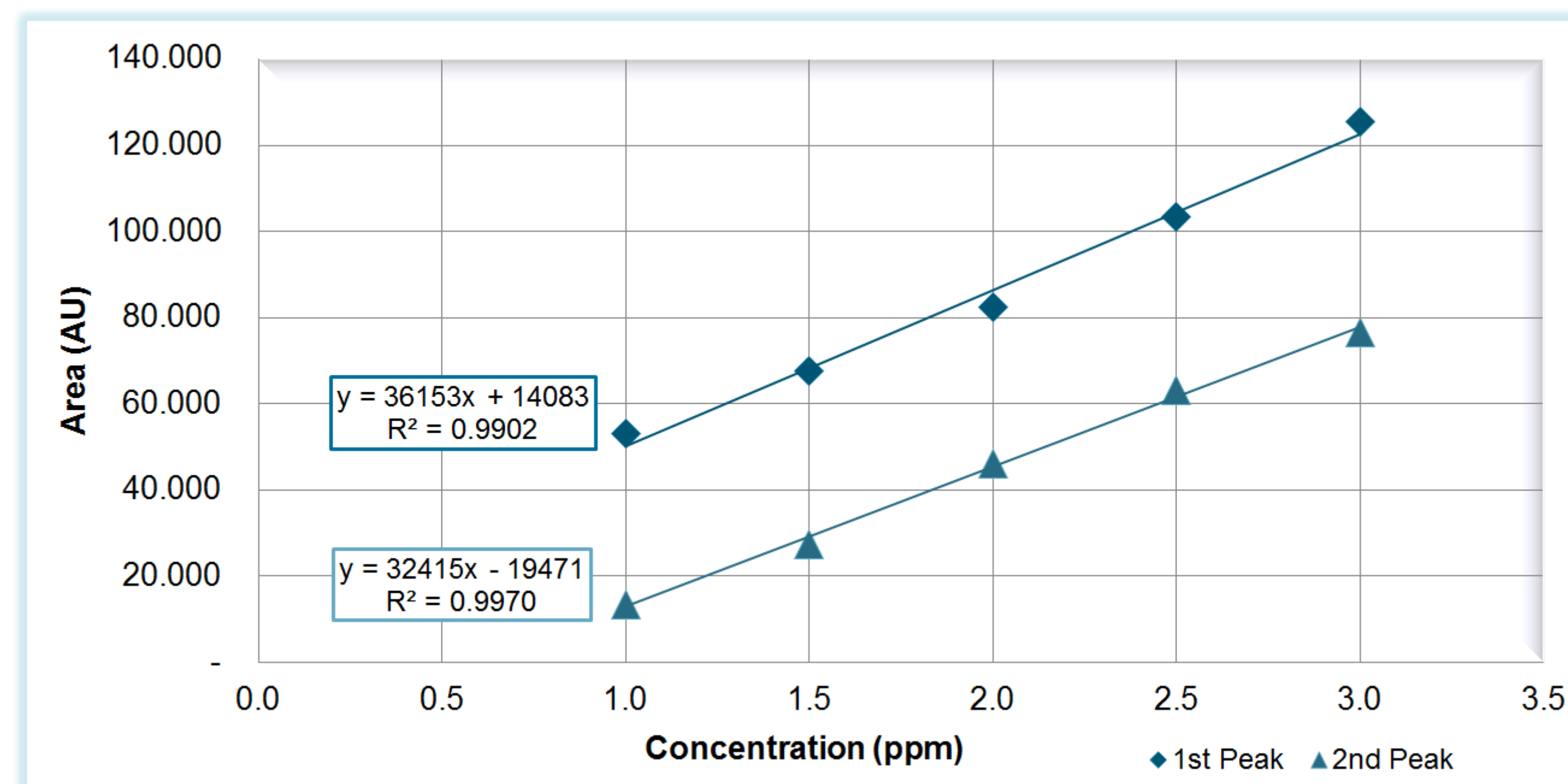


Figure 7. Calibration curve for verapamil enantiomers.

Conclusion

- Immobilization of chondroitin sulfate A 3% (w/v) in monolithic epoxy column (Chromolith® widepore epoxy 100-4.6 mm) was successful in the presence of ethylene diamine through a Schiff base reaction.
- The immobilized chondroitin sulfate A has shown initial chiral separation ability for verapamil enantiomers.

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