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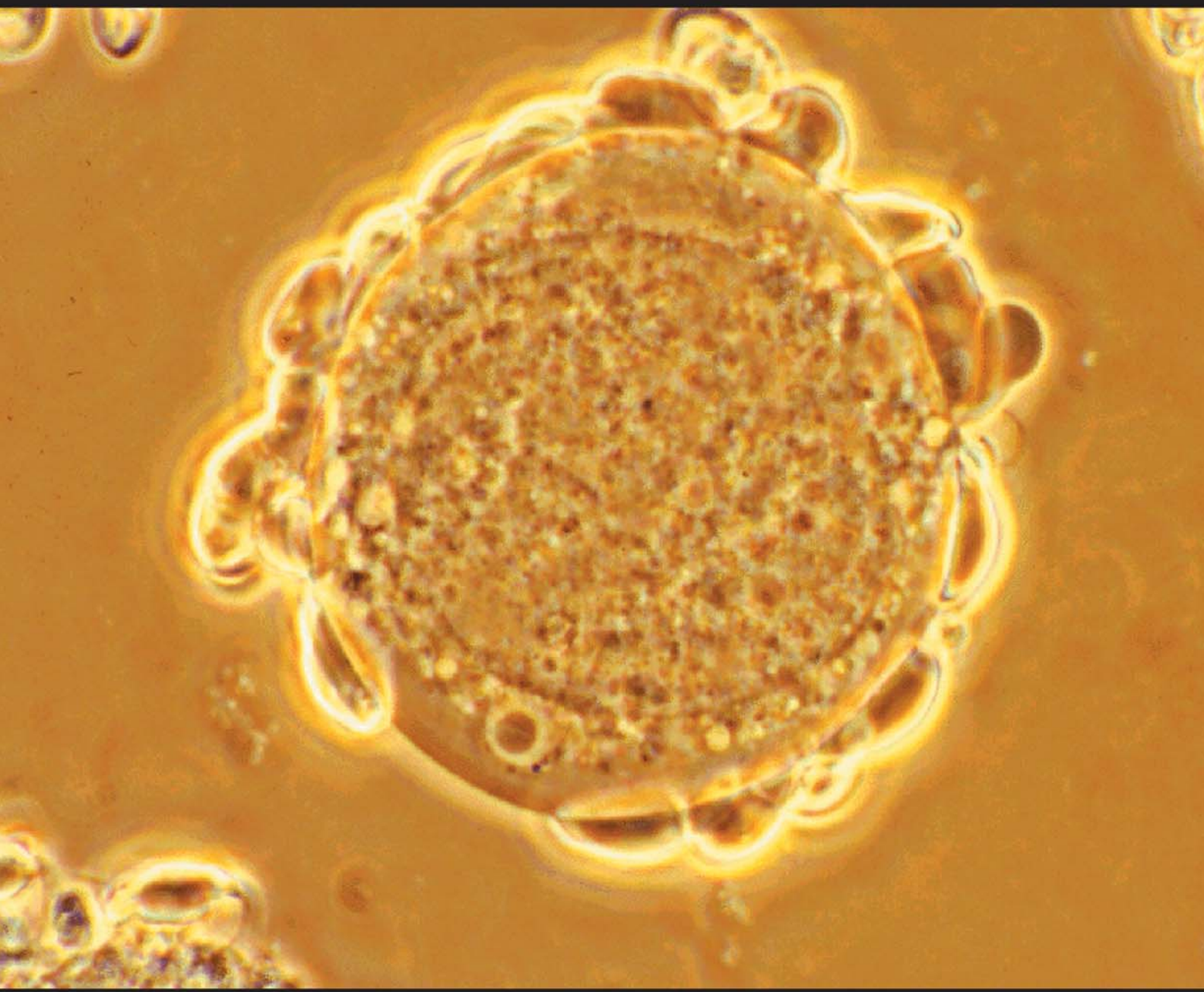

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Adenovirus Type 5 (Ad5) Chromatographic Purification Process at the 20 L Scale

BY NORMAND ARCAND, ALICE BERNIER, JULIA TRANSFIGURACION, DANIELLE JACOB, HELENE COELHO, AND AMINE KAMEN

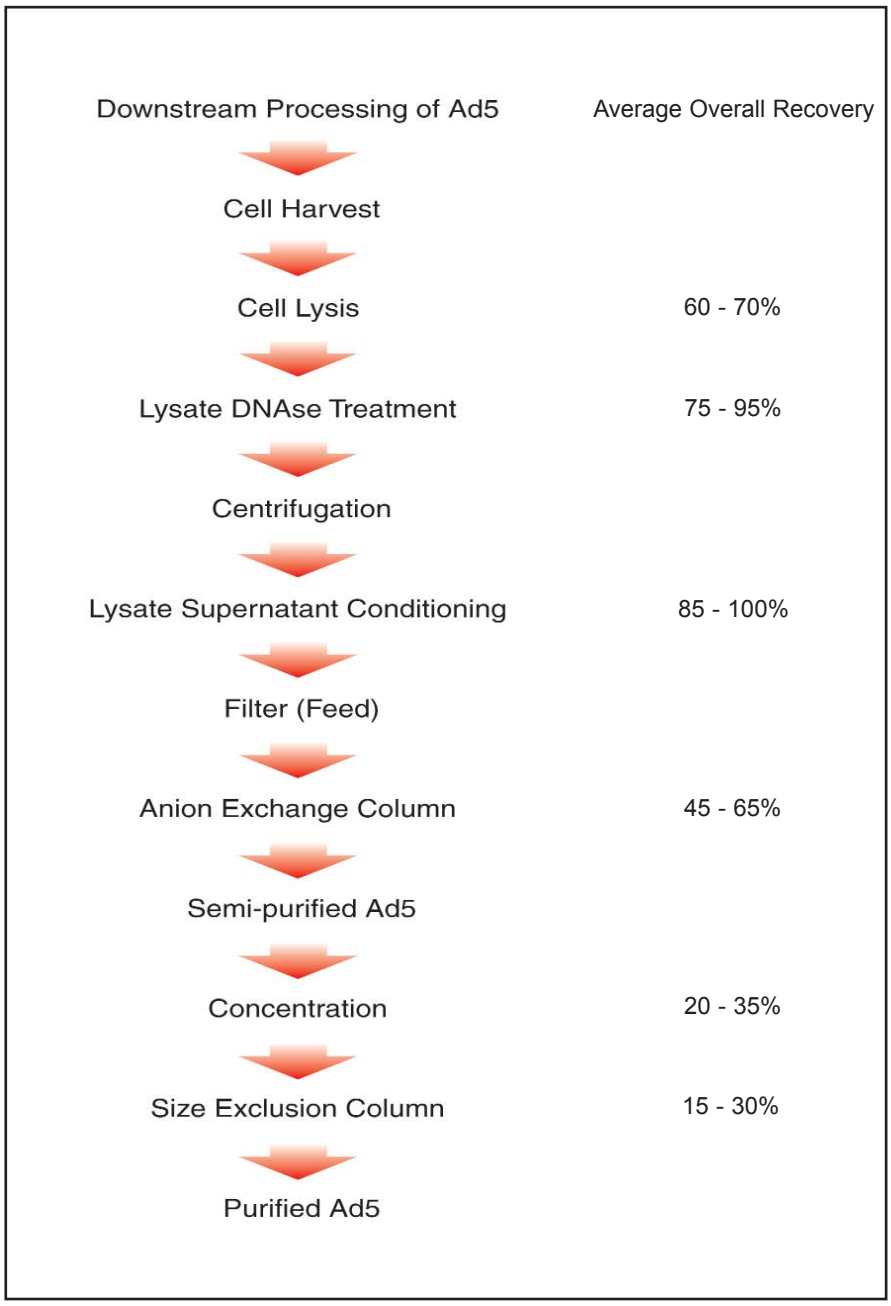

Figure 1.

Recombinant Adenovirus are attractive as vectors for Gene Therapy because:

- They exhibit wide tissue tropism and high transduction efficiency,
- Adenovirus cultures can reach high specific titers (10^{10} VP/mL), and
- Their use in the treatment of cancer and other serious diseases is valuable.

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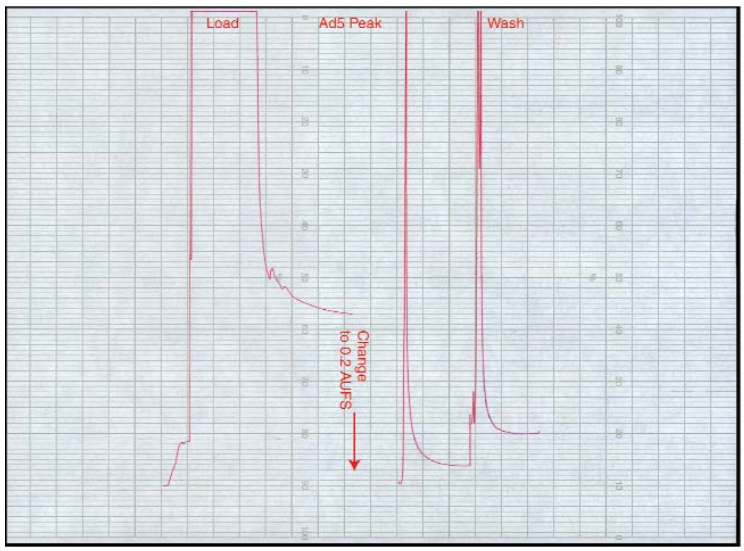
A primary mode of Adenovirus purification continues to be CsCl density gradient centrifugation. Our scalable chromatographic purification process for Ad5 at the 20 L scale is shown in Figure 1.

After 42 hours post infection (hpi), 293 SF cells, infected with Ad5 in NSFM-13 media, are harvested. A Centritech is used to harvest the cells from the bioreactor, and a centrifugation step is done to pellet the cells containing Ad5. The cells are lysed by osmotic shock with a low ionic strength buffer. To avoid the entrapment of the viral particles by the exogenous dsDNA, Benzonase® is added to the lysate. This step also decreases the viscosity of the lysate. Afterwards, a centrifugation step is performed to get rid of the cellular debris, and the lysate supernatant is conditioned with NaCl to match the ionic strength of the equilibration buffer of the first column. Adding salt also decreases the ionic interaction with proteins and decreases

1st Column: Anion Exchange

Diameter Column:	7 cm
Area:	38.5 cm^2
Bed Height:	12 cm
Bed Volume:	460 mL
Linear Flow Rate:	150 cm/h

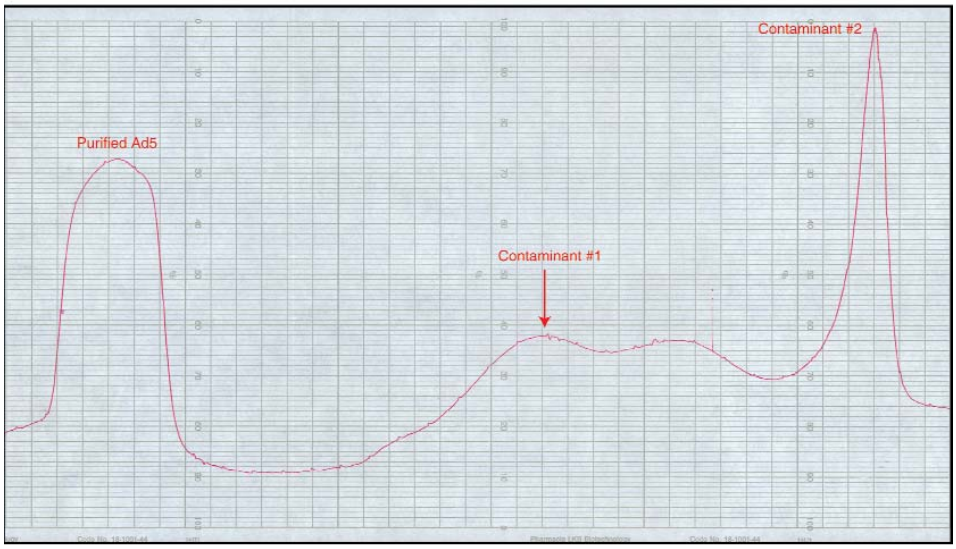


Feed: 2197 mL, 10X conc. conditioned lysate evaluated at 9.5 x 10^{10} VP/mL
(2.1 x 10^{14} Total VP)

Figure 2.

2nd Column: Size Exclusion Chromatography

Diameter Column:	5 cm
Area:	19.6 cm^2
Bed Height:	56 cm
Bed Volume:	1100 mL
Linear Flow Rate:	30 cm/h



Feed: 10 % bed volume of concentrated semi-purified Ad5 evaluated at 2.9 x 10^{11} VP/mL
(2.9 x 10^{13} Total VP)

Figure 3.

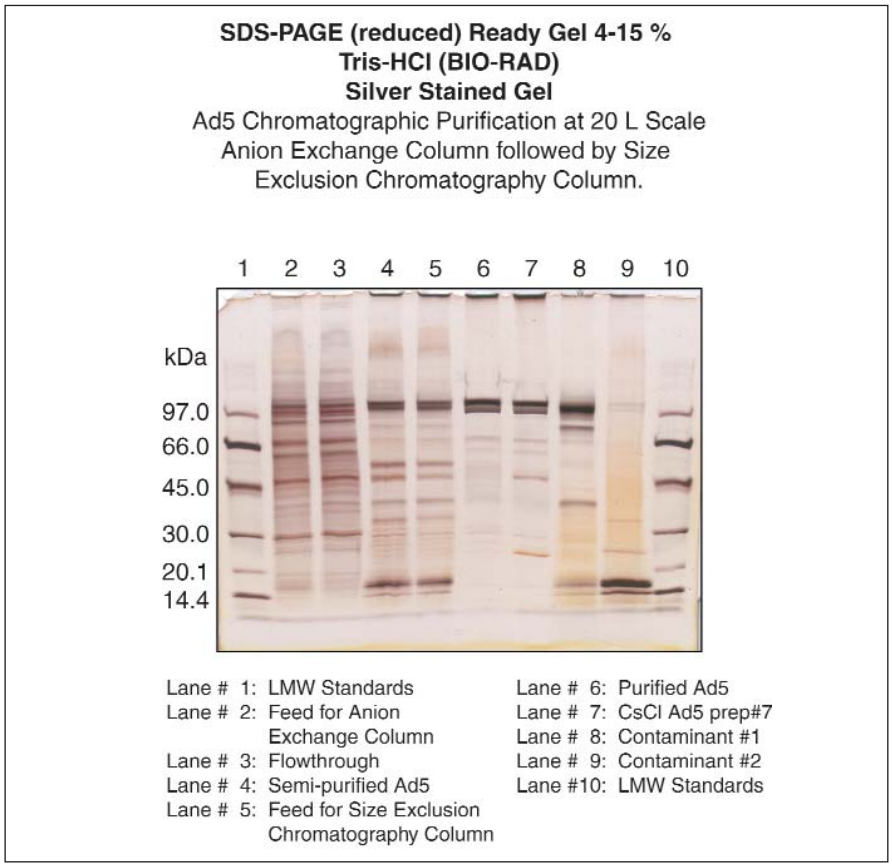


Figure 4.

aggregation. Benzonase® treatment and conditioning with NaCl allow an average increase in Ad5 recovery of 30-40%. The conditioned lysate is filtered through a 0.8/0.2 µm dead end filter, and is then loaded on the first column, a Fractogel® DEAE (anion exchanger), to capture the Adenovirus (Fig. 2). The elution of Ad5 is accomplished by increasing the NaCl concentration, and the recovery for this step is between 50 and 65%.

Before going to the second column, the semi-purified Ad5 has to be concentrated approximately 5-10X using a Stirred Cell with a 300K MWCO ultrafiltration membrane. During this step, 50% of the total virus particles are lost. The polishing step is the final purification of Ad5 on a Size Exclusion column containing Sephacryl® S-400 HR (Fig. 3). The diameter of Ad5 is 80 nm and the pore size of the Sephacryl® is 31 nm. The Ad5 passes through the column in the void volume while the

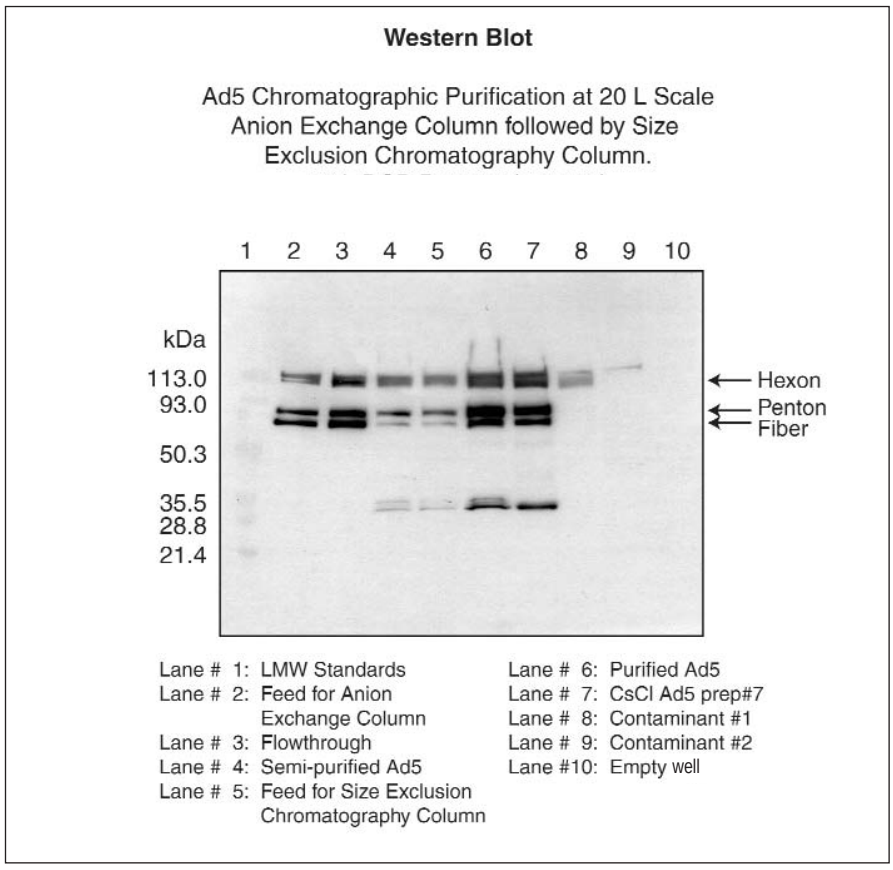


Figure 5.

protein contaminants and DNA enter inside the beads and are eluted later. This process gives a very high purity of Ad5 as compared to CsCl purified material, and it is also scalable. The SDS-PAGE and Western Blot for this material are shown in Figures 4 and 5, respectively.

Moreover, the purity profile by HPLC, and the 260/280 nm ratio of Ad5 purified by column chromatography, is comparable to Ad5 purified by CsCl (Figures 6 and 7). Ad5 purified by column chromatography is free of residual dsDNA as confirmed by the PicoGreen dsDNA Assay (data not shown here).

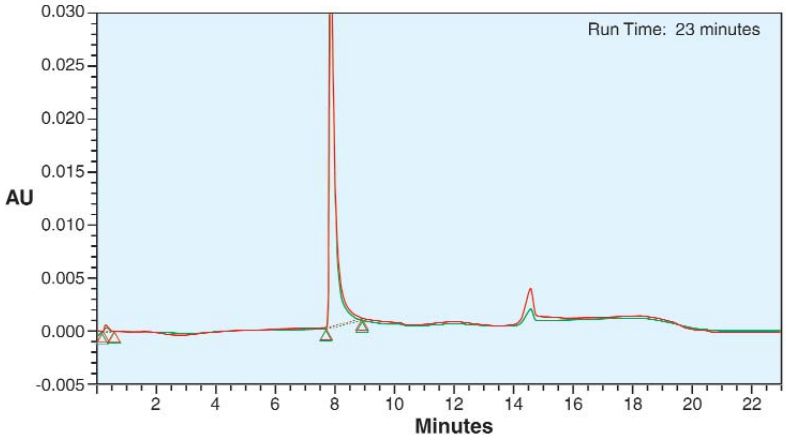
Conclusion

We have developed a chromatographic purification process for Ad5, at the 20 L scale, which gives the same high purity of Ad5 purified with CsCl. The first column (Anion Exchange) is relatively effective in capturing Ad5, and results in a purity of approximately 85% and a recovery of between 45 and 65%. However, during the concentration step, 50% of the total VP are lost. This step must be improved in order to increase the overall recovery of Ad5. The second chromatographic step, a Size Exclusion Column, is very efficient in removing the residual dsDNA, as well as increasing the final purity to greater than 98%. HPLC analysis of the purified material shows only one peak corresponding to Ad5, which has a 260/280 nm ratio of 1.21 corresponds to the expected value.

Further development work continues in order to reduce the loss of Ad5 during the concentration step, and to validate our chromatographic purification process at the 100 L scale.

Purity Profile of Purified Ad5 determined by HPLC

RT	Area	Concentration	Units	Channel name	Ratio
0.281	110			260 nm	0.45
0.276	243			280 nm	
0.407	5676			260 nm	1.27
0.434	4465			280 nm	
7.950	547762	1.7 x 1011	VP/mL	260 nm	1.21
7.951	452919			280 nm	

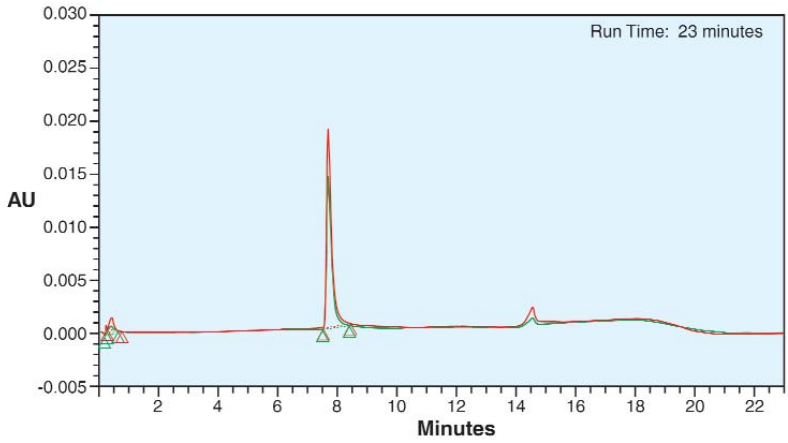


$$\text{Purity at 280 nm} = \frac{452\,919}{243 + 4465 + 452\,919} \times 100 = 99\ \%$$

Figure 6.

Purity Profile of CsCl Ad5 determined by HPLC

RT	Area	Concentration	Units	Channel name	Ratio
0.369	2181			260 nm	1.19
0.366	1834			280 nm	
0.542	10979			260 nm	2.15
0.540	5106			280 nm	
7.777	224004	6.9 x 1010	VP/mL	260 nm	1.29
7.777	173083			280 nm	



$$\text{Purity at 280 nm} = \frac{173\,083}{1834 + 5106 + 173\,083} \times 100 = 96\ \%$$

Figure 7.