Guidelines to an effective purification method development

Nowadays, chromatographic purification on a lab scale is indispensable in many areas such as academia and pharmaceutical research. However, this is very time consuming. Therefore, effective development of the purification method is essential. These guidelines demonstrate the main steps for an effective purification method development.

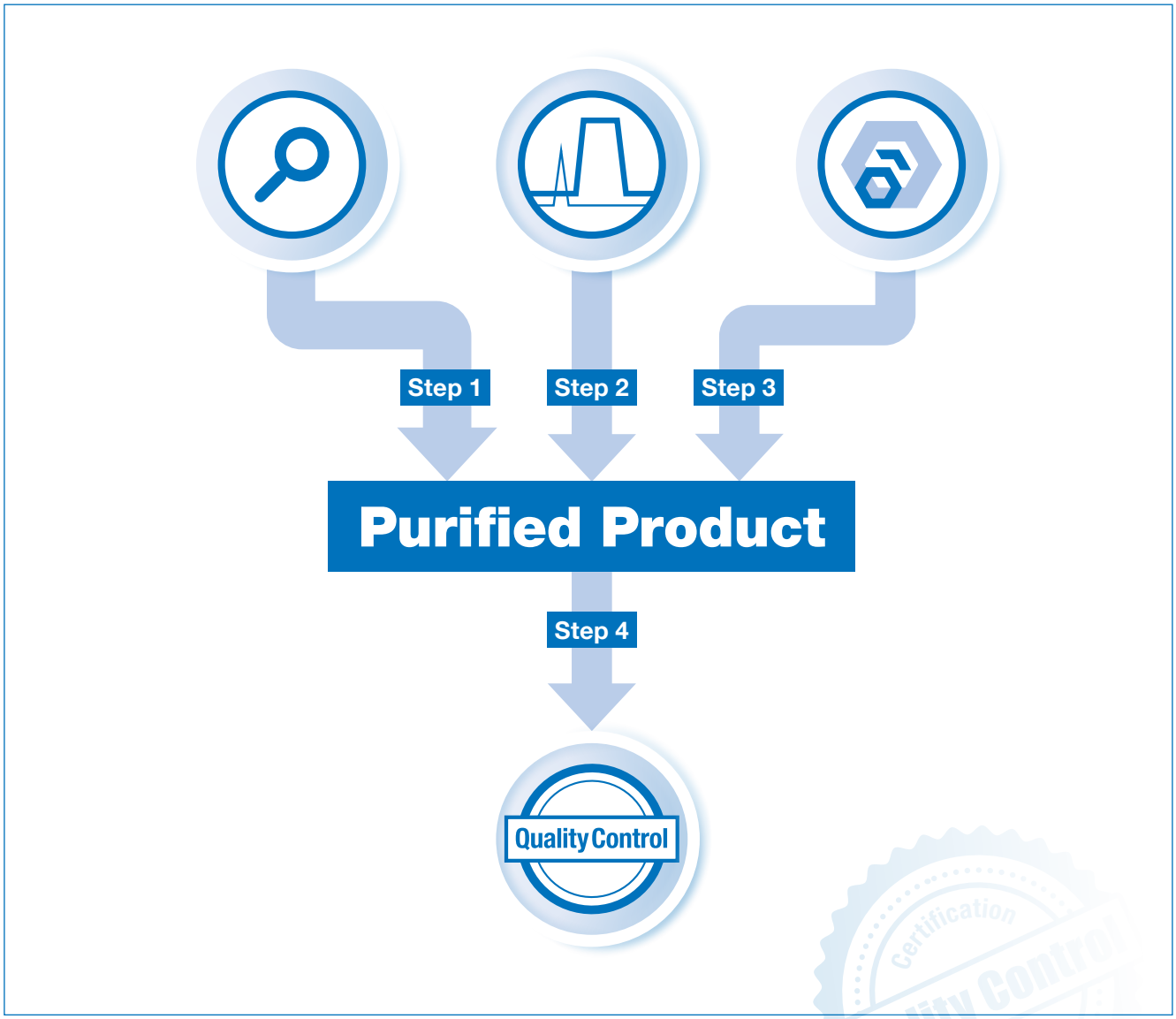

Figure 1: Overview of the main steps for effective purification method development.

Step 1 Developing a well optimised separation on an analytical scale

Step 2 Loading study on an analytical scale

Step 3 Scale-up to semi-preparative column dimensions

Step 4 Quality control/proof of concept

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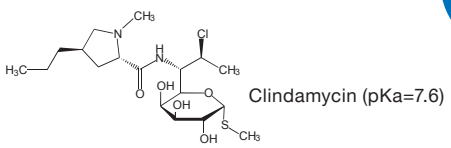
Step 1 Developing a well optimised separation on an analytical scale

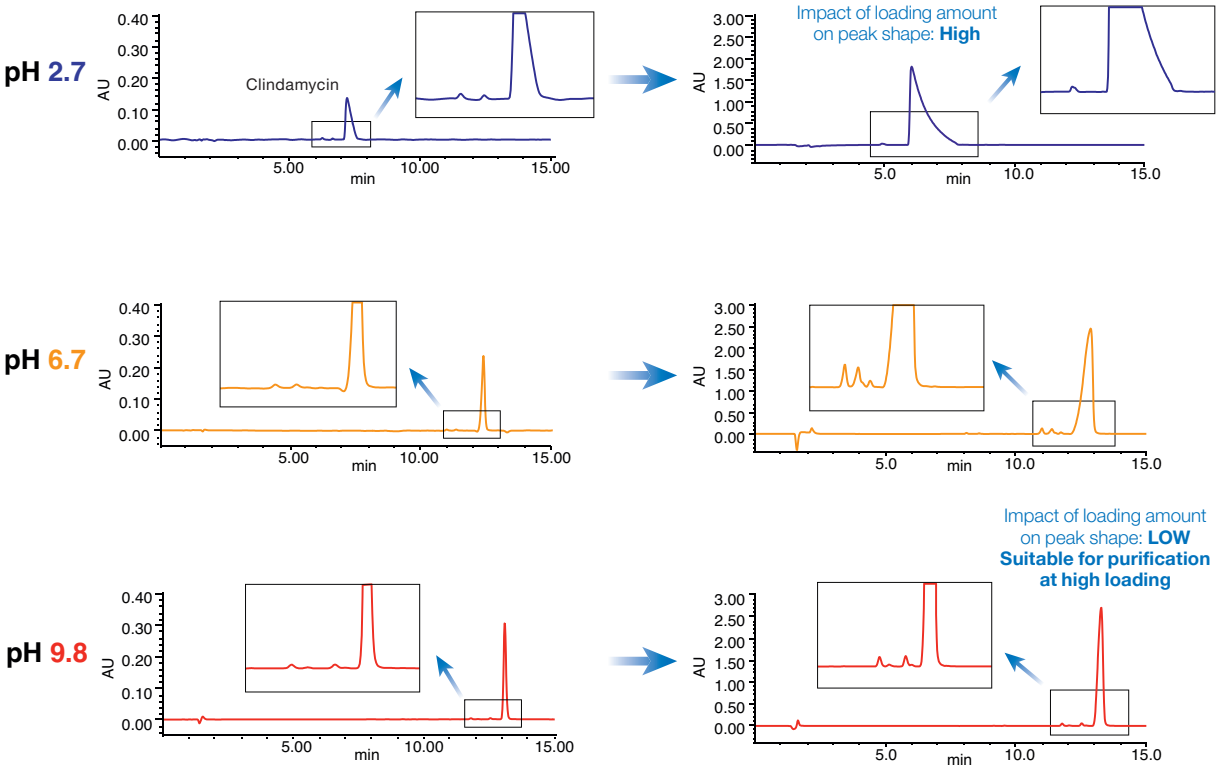
The development of an effective preparative method starts with a well-optimised analytical method. The optimal separation conditions are determined by testing different chromatographic conditions such as stationary phase, mobile phase or temperature. Ideally, a particle size that is suitable for the preparative scale is already used here in order to simplify the later scale-up. It is also advantageous to use the same column length for the analytical and preparative methods. Figure 2 shows a method development for clindamycin by varying the pH of the mobile phase from acidic to neutral to basic.



YMC-Triart C18 (5 µm) 150 x 4.6 mm ID

0.01 mg Loading
10 µl injection, 1.0 mg/mL in water

0.5 mg Loading
10 µl injection, 50 mg/mL in water



Part No.:	TA12S05-1546PTH
Eluent:	A) 20 mM HCOOH (pH 2.7) 20 mM $HCOONH_4$ (pH 6.7) 20 mM $HCOONH_4$-NH_3 (pH 9.8) B) acetonitrile
Gradient:	10–75%B (0–15 min)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV at 210 nm
Pressure:	7.0 MPa

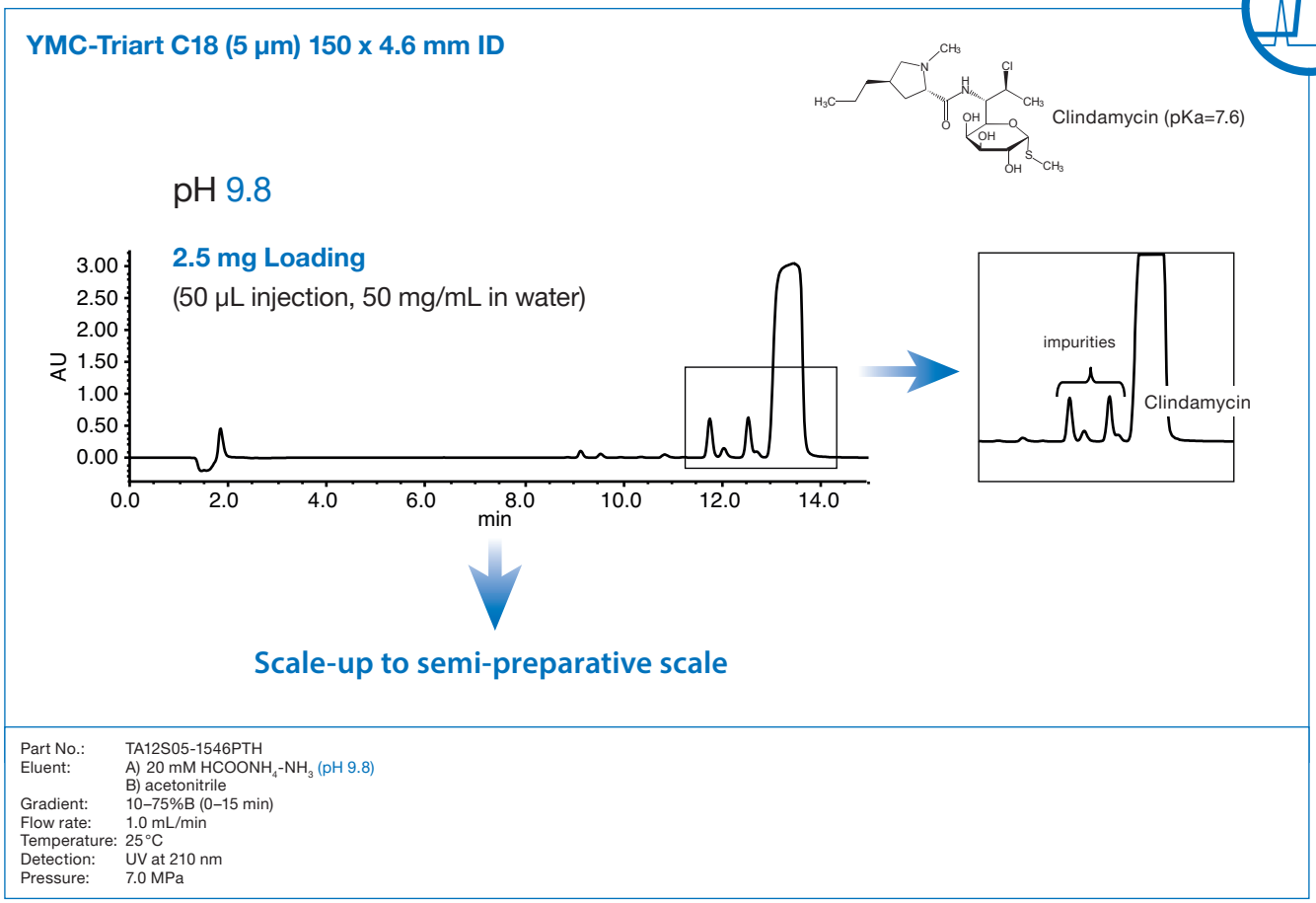
Figure 2: Analytical method development of clindamycin.

Step 2 Loading study on an analytical scale

Once an optimum analytical method has been developed, loadability studies must be performed. Here too, analytical column dimensions are beneficial for this evaluation. Figure 3 shows the maximum loading of crude clindamycin on a YMC-Triart C18 column with a particle size of 5 µm, a length of 150 mm and an internal diameter of 4.6 mm.

The same applies here: optimise the method beforehand so that the study is then carried out with preparative parameters. The particle size of the stationary phase and the column length are particularly important. Both should match the later preparative scale. Also, use a 4.6 or 6.0 mm inner diameter for these studies.

If the dedicated preparative column is larger than 100 mm ID, it is advisable to insert another step with a scout column of 20 mm ID. This will allow the prediction of loadability and calculations of the running costs to be determined more accurately.



Part No.: TA12S05-1546PTH
Eluent: A) 20 mM $HCOONH_4$-NH_3 (pH 9.8)
B) acetonitrile
Gradient: 10–75%B (0–15 min)
Flow rate: 1.0 mL/min
Temperature: 25 °C
Detection: UV at 210 nm
Pressure: 7.0 MPa

Figure 3: Loading study of crude clindamycin.

Step 3 Scale-up to semi-preparative column dimensions

Proceed with the preparative separation by scaling up the chromatographic parameters such as flow rate, column ID and sample load by the required factor. Of all the steps in this process, the most demanding one will be the scale-up of the chromatographic parameters in order to meet the preparative demands.

There are several scalable parameters to consider including: flow rate, column ID, sample load, tubing ID, sample injection concentration, volume of sample loop, consumption of solvent, dead volume, fraction mass, size of the detector cell.

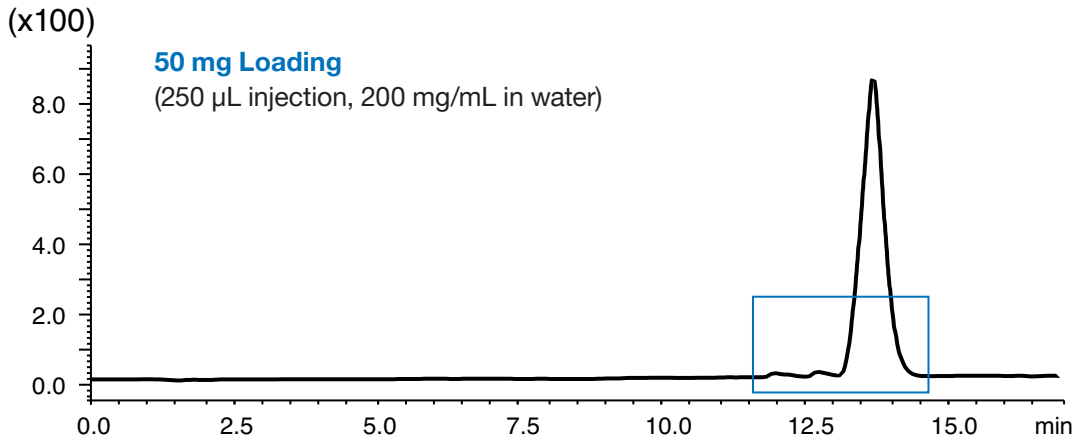
The easiest way is to perform a linear scale-up. This is shown in figure 4 for clindamycin. The column dimensions are changed from 150 x 4.6 mm ID to 150 x 20 mm ID, while the particle size of 5 µm was maintained. The flow rate was adjusted according to the linear scale-up from 1.0 mL/min to 18.9 mL/min. The column load was adjusted from 2.5 mg to 50 mg.

Linear scale-up

For a linear scale-up the column length and the packing material remain the same for the analytical and the semi-preparative column. The only dimension that is varied is the internal diameter of the column e.g., from 4.6 mm to 20 mm. In this case the scale-up factor SF is calculated as follows:

$$SF = \frac{ID^2_{prep}}{ID^2_{anal}} \qquad Eq.\ 1$$

The chromatographic parameters such as flow rate and column load have to be adjusted by the calculated SF.



Part No.: TA12S05-1520WX
Eluent: A) 20 mM $HCOONH_4$-NH_3 (pH 9.8)
B) acetonitrile
Gradient: 10–75%B (0–15 min)
Flow rate: 18.9 mL/min
Temperature: ambient
Detection: UV at 210 nm
Pressure: 8.4 MPa

Figure 4: Scale-up to semi-preparative column dimensions.

Step 4 Quality control/proof of concept

The last step is the collecting and analysis of the fractions from the purification method, to check the purity of the target. In this case 5 fractions were taken, 1–4 from the impurity peaks and fraction 5 is the collected target peak (see figure 5). In figure 6 the analyses of the crude mixture and the fractions are shown. Clindamycin could be obtained with a purity of 100% and a recovery of 86 % after the purification.

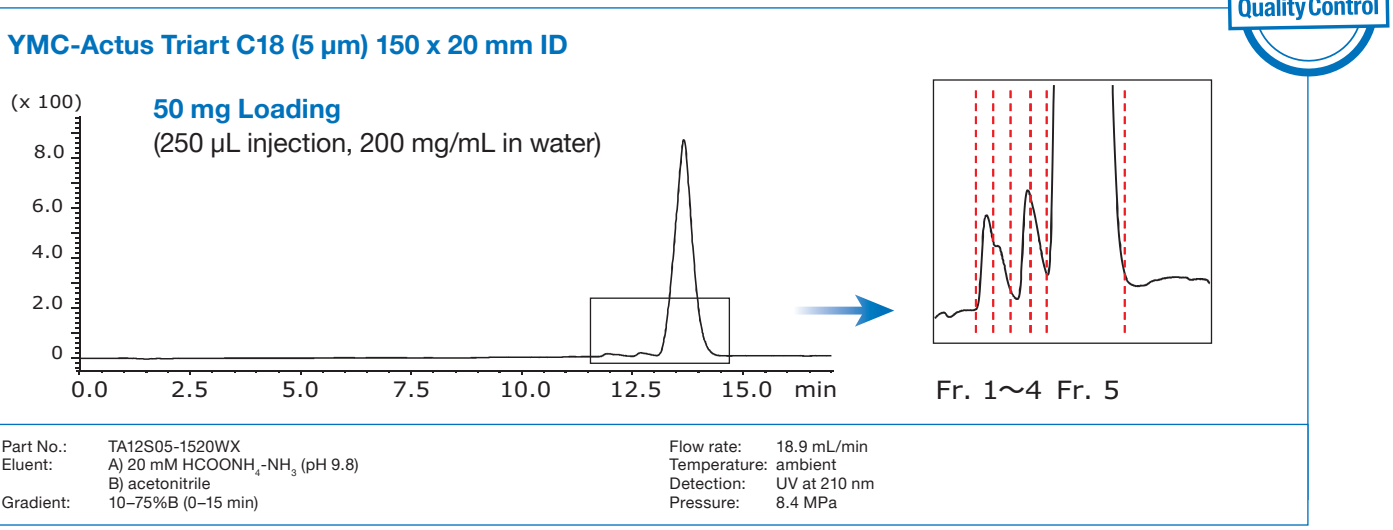


Figure 5: Semi-preparative purification and fraction collection of clindamycin.

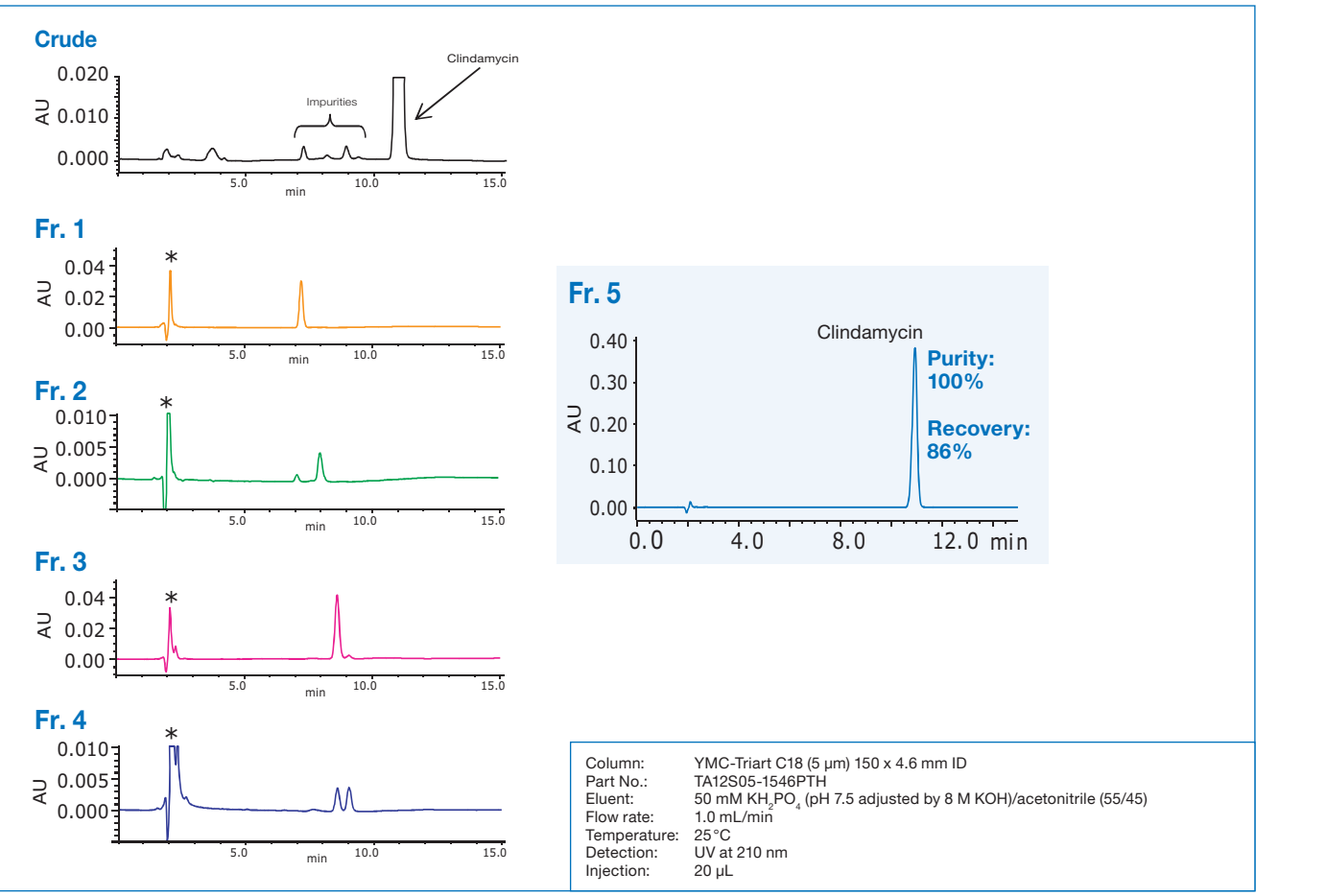


Figure 6: Analysis of the crude mixture and the collected fractions.