



## Efficient analysis of sgRNA and its impurities by IP-RP using YMC Accura Triart Bio C18

Single guide RNA (sgRNA) is a central component of CRISPR-Cas genome editing systems. Its integrity, purity, and sequence fidelity are critical to ensure efficient and specific gene editing performance. High-performance liquid

chromatography (HPLC) is therefore an essential analytical tool for monitoring sgRNA quality, with ion-pair reversed-phase (IP-RP) HPLC being the most commonly applied technique.



However, the analysis of sgRNA presents several analytical challenges. During synthesis, closely related by-products may be generated that differ only slightly in length (e.g.,  $n-1$  or  $n+1$  species). In combination with the comparatively large size of sgRNA (~100mer), this makes the separation of structurally similar impurities particularly demanding. Consequently, high-resolution chromatographic separation is required, making the appropriate choice of stationary phase and optimised method conditions critical. In addition, due to its length, sgRNA readily adopts complex secondary structures. The formation and stability of these structures

strongly depend on analytical conditions such as temperature, salt concentration, and pH, which can significantly influence retention behaviour and overall chromatographic performance. Another important consideration is the potential interaction of RNA with metal surfaces in the column hardware, making the use of bioinert hardware essential to minimise nonspecific adsorption and ensure reliable results.

In this Application Note, the effective separation of sgRNA and closely related impurities is demonstrated using a bioinert YMC Accura Triart Bio C18 column.

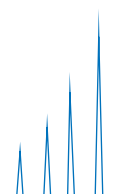




Table 1: Method conditions.

Column:	YMC Accura Triart Bio C18 (1.9 µm, 30 nm) 50 x 2.1 mm ID
Eluents*:	A) 100 mM TEAA* in water B) 100 mM TEAA* in water/acetonitrile (75/25)
Gradient:	0-1 min 30%B, 1-10 min 80%B, 10-10.1 min 100%B, 10.1-11 min 100%B, 11-11.1 min 30%B, 11.1-14 min 30%B
Flow rate:	0.2 mL/min
Temperature:	80 °C
Injection:	1 µL (0.02 mg/mL, 0.5 mg/mL or 2 mg/mL)
Detection:	UV at 260 nm
Sample:	Modified sgRNA PSMD7 (obtained from Kaneka Eurogentec, Seraing, Belgium)

\*Triethylammonium acetate

Table 2: Modified sgRNA sequences and length.

sgRNA	Length	Sequence
Modified sgRNA PSMD7	100	mG*mA*mU*AUCAAGACACGACGGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU
3' Modified sgRNA PSMD7 3' n-1	99	mG*mA*mU*AUCAAGACACGACGGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU
3' Modified sgRNA PSMD7 3' n-2	98	mG*mA*mU*AUCAAGACACGACGGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU
3' Modified sgRNA PSMD7 3' n-5	95	mG*mA*mU*AUCAAGACACGACGGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUG

\*phosphorothioate linkage

mX: 2'-O-methyl modification at base X

## High resolution with a widepore YMC Accura Triart Bio C18 column

The sgRNA PSMD7 used in this study contains terminal 2'-O-methyl nucleotides and phosphorothioate backbone modifications at both the 5' and 3' ends. These modifications enhance resistance to exonuclease degradation, improve overall molecular stability, and reduce susceptibility to enzymatic cleavage. For the analysis of the modified sgRNA PSMD7, a YMC Accura Triart Bio C18 column was used. Its widepore

stationary phase is specifically suited for the separation of large oligonucleotides, ensuring efficient mass transfer and optimal peak shape. In addition, the bioinert coated YMC Accura column hardware minimises unwanted interactions with metal surfaces, thereby reducing analyte loss and improving reproducibility. The analysis of the sgRNA (2 mg/mL) demonstrates high recovery and good resolution (Figure 1).

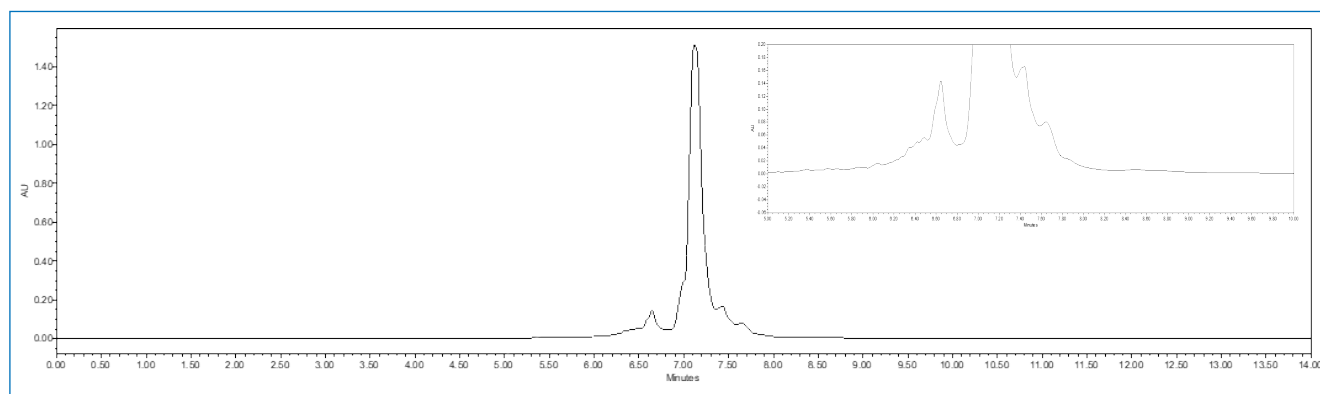
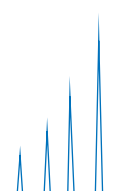


Figure 1: Total elution profile and zoom-in of the sgRNA using the widepore YMC Accura Triart Bio C18 column.





## Consistent resolution even at low concentrations

To test the sensitivity of the sgRNA analysis, the analysis was also conducted with a low concentration of 0.5 mg/mL (Figure 2). Consistent resolution was demonstrated even at low concentrations.

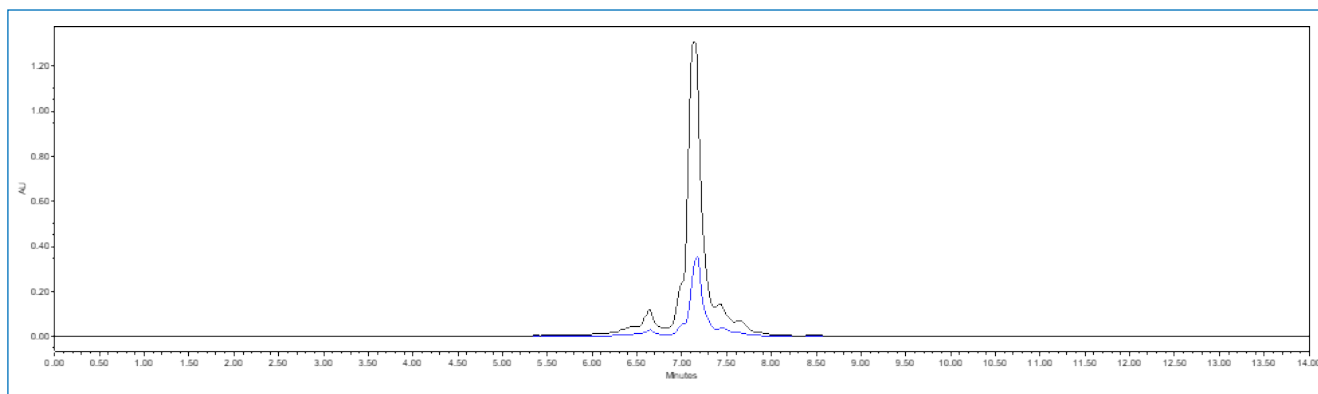


Figure 2: Comparison of sgRNA analysis at different concentrations. Black: 2 mg/mL, blue: 0.5 mg/mL.

## Minimised carry-over with bioinert hardware and inert stationary phase

Carry-over is common in HPLC analysis of sgRNA because RNA molecules tend to adsorb to metal surfaces and active sites within the column and flow path, especially under ion-pair reversed-phase conditions. Therefore, a blank run using water was performed to assess potential carry-over and to confirm that no interfering

peaks were present prior to the subsequent sample injection. No carry-over was detected in the blank run, demonstrating that the YMC Accura Triart Bio C18 column effectively minimises nonspecific interactions with both the bioinert coated column hardware and inert stationary phase (Figure 3).

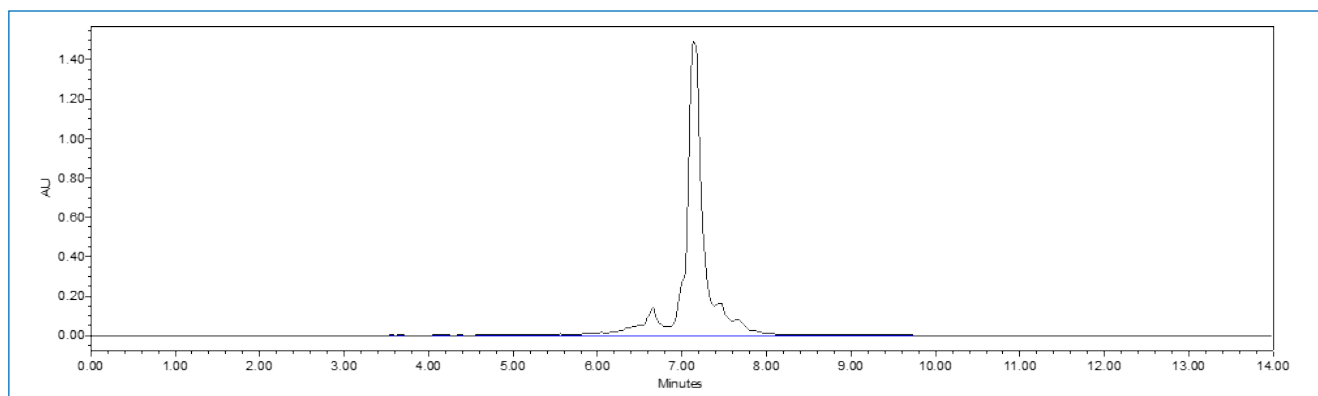
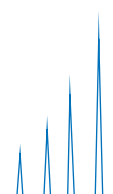


Figure 3: Comparison of a blank run using water (blue) and a standard injection (black) demonstrating the absence of carry-over.

## Successful sgRNA impurity separation

To evaluate the sensitivity and separation efficiency of the method for sgRNA impurity analysis, two mixtures containing truncation impurities that differ only slightly in length were prepared and analysed at a low concentration of 0.02 mg/mL (Figure 4). A mixture containing all sgRNA impurities (n-1, n-2, n-5; black) and a mix-

ture containing the full-length sgRNA and only the n-2 impurity (red) were compared. In the first mixture, all impurities were successfully resolved. Particularly high resolution was achieved in the second mixture between the full-length sgRNA and the n-2 impurity.



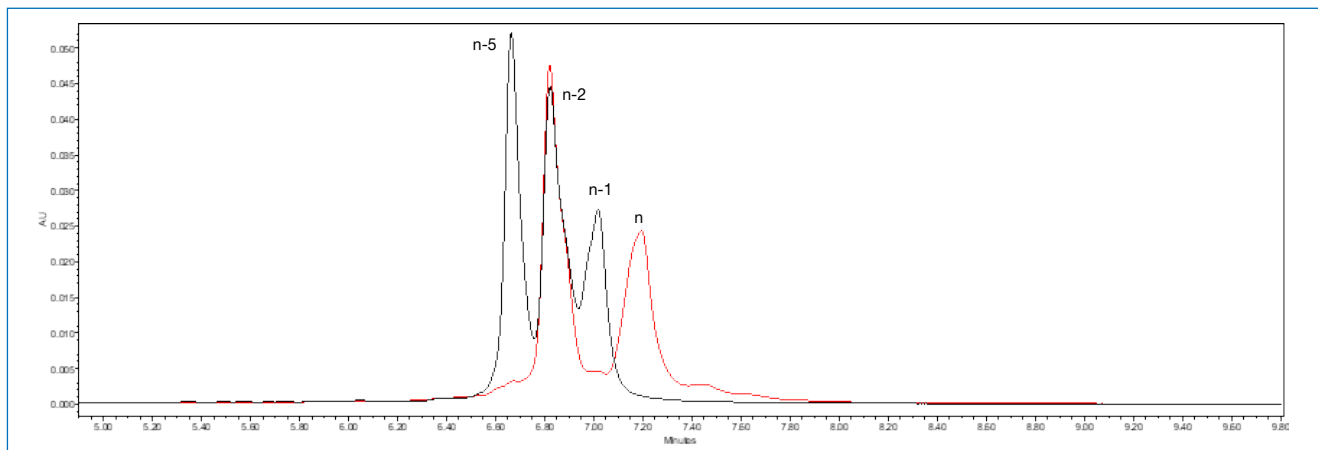


Figure 4: sgRNA impurity analysis at low concentration (0.02 mg/mL). Black: sgRNA mix of n-1, n-2 and n-5 impurities; Red: sgRNA mix of full-length sgRNA and n-2 impurity.

## Conclusion

The developed method using the YMC Accura Triart Bio C18 column demonstrates:

- High-resolution separation of full-length sgRNA and closely related truncation impurities
- Reliable performance and consistent peak shape, even at low concentrations
- Minimised carry-over due to bioinert YMC Accura hardware and inert YMC-Triart stationary phase
- Robust and reproducible sgRNA purity assessment under IP-RP conditions.

Overall, the YMC Accura Triart Bio C18 column, featuring bioinert hardware and a widepore stationary phase, provides a robust and reliable solution for the analysis of sgRNA and its closely related impurities.

Application data by courtesy of Quality Assistance SA, Thuin, Belgium

 Quality Assistance

