SARTORIUS

CIMac pDNA-0.3 Analytical Column

Optimising Its Chromatographic Reproducibility

Technical Note

Executive Summary

CIMac pDNA-0.3 Analytical Columns (Pores 1.4 µm) were introduced by BIA Separations d.o.o. in 2010. In the following years they were recognised by the biotechnological and analytical community as excellent choice for fast and exact plasmid DNA (pDNA) monitoring and quantification.

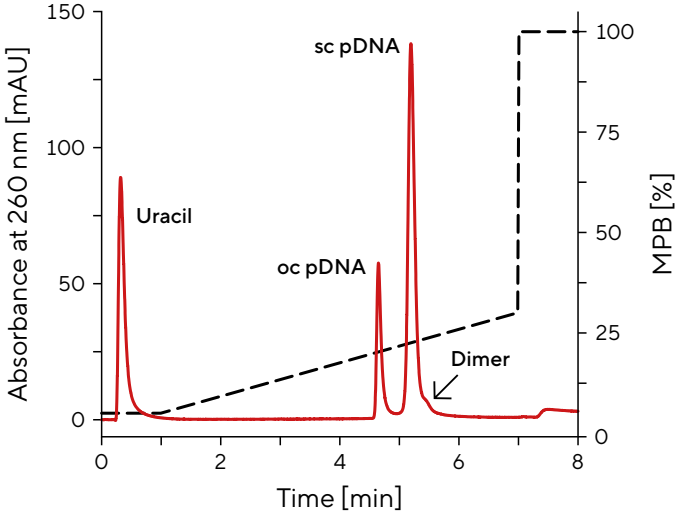
Due to high sensitivity of the column to small changes in chromatographic conditions we publish this technical note, how to exploit the column's excellent chromatographic characteristics to the highest possible extent.

Method

A standard testing protocol for the columns is analytical separations of open circular (oc) and supercoiled (sc) pDNA isoforms of a 4.7 kbp large plasmid DNA molecule. The concentration of the pDNA sample used was 23 µg/mL. The sample contained between 21 and 22% of the oc pDNA isoform and an exact amount of uracil as unbound tracer. The separation of the isoforms was performed in a linear gradient of NaCl with the temperature of the whole system controlled to 15.0±0.5 °C. The typical experimental conditions together with an example of chromatogram are shown in Figure 1.

Column	CIMac pDNA-0.3 Analytical Column (Pores 1.4 µm)
Load	50 µL of standard pDNA sample dissolved in 100 mM TRIS-HCl, 0.6 M NaCl, pH 8.0
Flow rate	1.0 mL/min
Mobile phases	Mobile phase A: 100 mM TRIS-HCl, 0.6 M NaCl, pH 8.0 Mobile phase B: 100 mM TRIS-HCl, 1.0 M NaCl, pH 8.0
Gradient elution method	95% MPA (1min), linear gradient to 70% MPA (6 min), 0% MPA (1 min)
Wash	95% MPA (3 min)

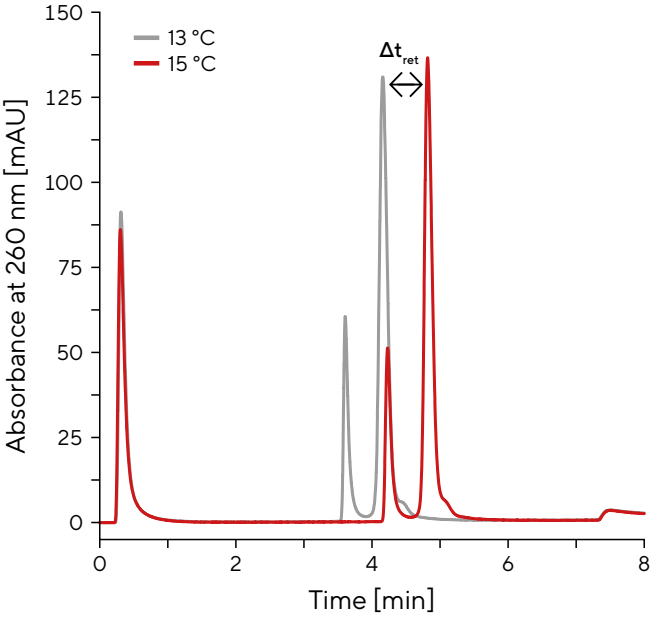
Figure 1: *Typical Separation of 4.7 kbp Large pDNA Molecule in Linear Gradient of NaCl*



The influence of temperature on pDNA separation

Retention times of pDNA molecules on CIMac pDNA analytical columns are extremely sensitive to the temperature of the chromatographic system. It is therefore of great importance to keep temperature fluctuations within 1 °C. The temperature change affects only retention times of pDNA, while the resolution between the isoforms is retained. An example of the effect of temperature is shown in Figure 2, where a column was tested under the same experimental conditions, at different temperatures. The analysis at 13 °C corresponds to a shift of approximately 0.5 min towards shorter time in comparison to 15 °C, while the resolution between the pDNA isoforms remains unchanged. Important: It is strongly recommended to control the temperature of the column and buffers within 1 °C interval to obtain reproducible chromatograms.

Figure 2: *Shift of pDNA Retention Times Due to Small Changes in Experimental Temperature*



The influence of buffer preparation on pDNA separation

Deviations in buffer preparation have a similar effect on pDNA separation as temperature fluctuations. As an example, two mobile phases A were prepared with slightly different conductivity – one with 57.5 mS/cm at 24.3 °C, the other with 56.7 mS/cm at 24.3 °C. Both buffers were tested with the same column at exactly the same conditions and injecting the same pDNA sample. The 1 mS/cm decrease in conductivity of buffer A resulted in 0.5 min shorter retention time for pDNA. The conductivity difference of buffer A only influences the retention time of pDNA, but not the resolution between pDNA isoforms (check Table 1).

Table 1: *Changing Chromatographic Properties With Small Differences in Mobile Phase A Preparation*

Mobile phase A characteristics	$t_{ret\ sc\ pDNA}$	oc-sc pDNA resolution
57.5 mS/cm, pH 8.00	4.85 min	3.43
56.7 mS/cm, pH 8.00	5.22 min	3.34
56.8 mS/cm, pH 7.92	5.45 min	3.29

In the second example, two different mobile phases A were prepared with the same conductivity, but one with pH value of 8.00 and the other 7.92. Both buffers were tested with the same column and injecting the same pDNA sample. The 0.1 pH unit change resulted in 0.2 min different retention time for pDNA.

Important: Buffers have to be prepared extremely precisely. Any small deviation in buffer preparation will influence the chromatographic characteristics of the column. It is advisable that the buffers are always used freshly prepared. Storage of buffer over days or weeks can change their characteristics sufficiently to affect pDNA retention on the column.

The influence of capillary diameter on pDNA separation

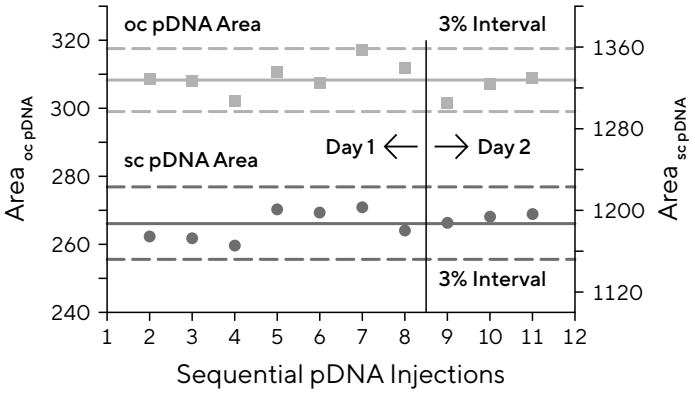
HPLC capillaries with internal diameter (ID) of 0.13 mm and total length of 120 cm were replaced with 0.25 mm ID capillaries. Replacing the tubing changed the dead volume of the chromatographic system by 42 µL which resulted in 10% resolution decrease between two pDNA isoforms. We strongly recommend to customer to minimize the dead volume of the systems as much as possible in order to exploit the CIMac pDNA characteristics to the highest possible extent.

Influence of sequential pDNA injections

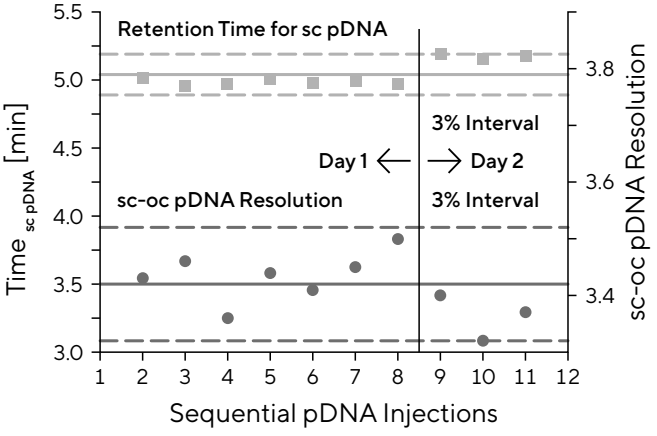
The first injection of the pDNA sample usually results in 5 to 10% lower area for oc pDNA isoform. From the second injection on the separation is stabilised and the relative standard deviation is below 3% for the following factors: pDNA retention times, oc-sc pDNA resolution, sc pDNA area, oc pDNA area (see Figures 3A and 3B). Additionally, inter-day reproducibility was confirmed to be below 3% as well (see the last three injections on Figures 3A and 3B). The statistically different retention times for pDNA on day 2 are consequence of small differences in buffer composition used on day 2.

Figure 3: *Repeatability (Sequential pDNA Injections on the Same Column in One Day) And Reproducibility (The Same Column and Method, but Different Buffers and Time of Analysis) Of CIMac pDNA Column.*

A Repeatability (day 1) and reproducibility (day 1 vs. day 2) of pDNA injections on single column



B Repeatability (day 1) and reproducibility (day 1 vs. day 2) of pDNA injections on single column



A) Area on chromatograms for sc pDNA (dark gray) and oc pDNA (light gray) versus the number of injection. All values are within 3% interval around the average value.

B) Retention time for sc pDNA (light gray) and sc-oc pDNA resolution (dark gray) from the same analyses as on Figure 3A. All values are within 3% interval around the average value.

Our quality control testing method for CIMac pDNA is based on analytical pDNA separation and the main criteria for column approval are oc-sc pDNA resolution and pDNA retention time. The resolution between two pDNA isoforms as well as retention time for sc pDNA have to be in the 5% interval around prescribed values, which allows reproducible analyses even when the a column is replaced with a new one from different batch.

Conclusions

CIMac pDNA column is an excellent choice for implementing Process Analytical Technology (PAT) in pDNA downstream processing. To achieve the performance and reproducibility required for quick and accurate HPLC analyses of pDNA samples, it is important to maintain working conditions (buffer composition, dead volume of the system and temperature of the system and mobile phases constant.

References

B. Gabor, U. Černigoj, M. Barut, A. Štrancar.
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J. Chromatogr. A 1311 (2013) 106–114

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

Slovenia
Sartorius BIA Separations d.o.o.
Mirce 21
5270 Ajdovščina
Phone +386 59 699 500

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For more information, visit
www.sartorius.com

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