

High Physiological Thermal Triplex Stability

-Optimization of Twisted Intercalating Nucleic Acids (TINA)

Niels Bomholt,¹ Amany M. A. Osman¹ and Erik B. Pedersen*¹

¹ Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M (Denmark)

Electronic Supplementary Information

Melting curves

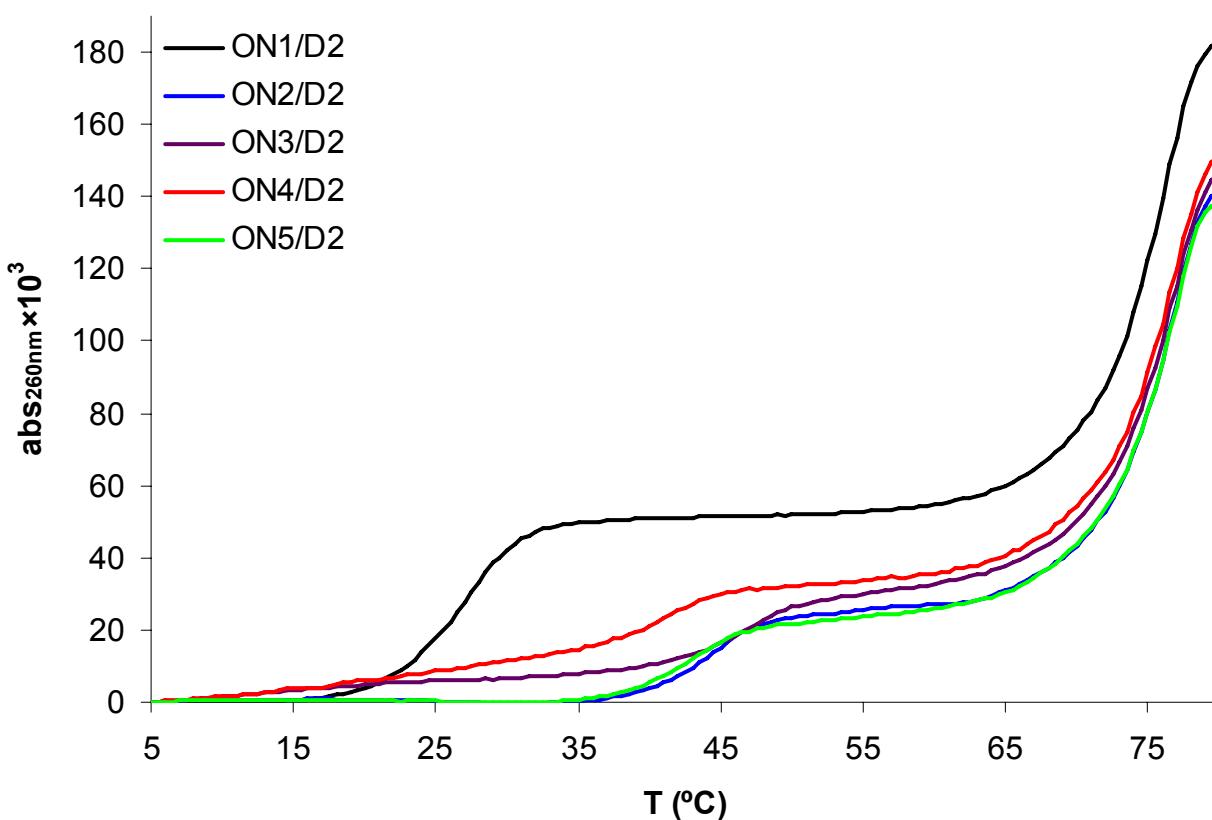


Figure 1. Melting curves of thermal denaturation experiments of triplexes (ON1-ON5/D2) recorded in 20 mM sodium cacodylate, 100 mM NaCl, 10 mM MgCl₂, pH 6.0, at 260nm versus temperature, with a heating of 1.0 °C/min.

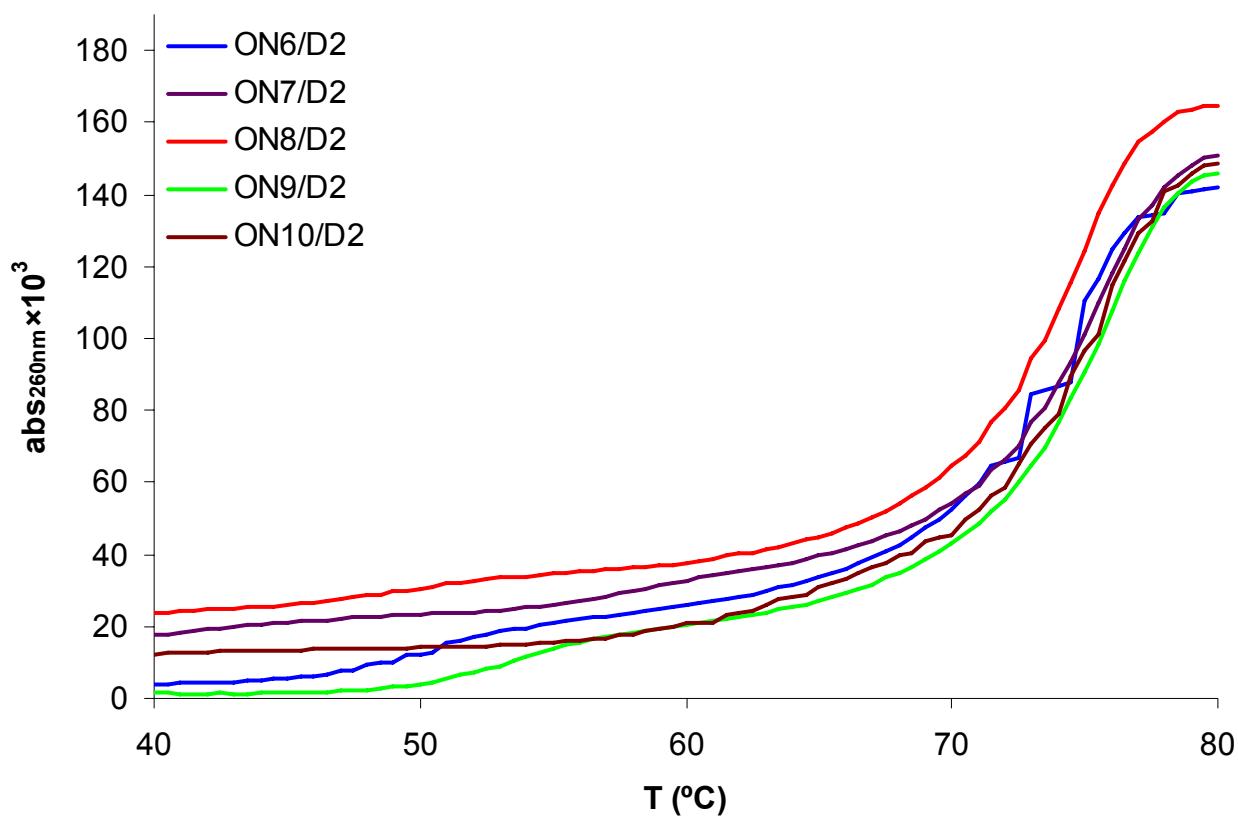


Figure 2. Melting curves of thermal denaturation experiments of triplexes (**ON6-ON10/D2**) recorded in 20 mM sodium cacodylate, 100 mM NaCl, 10 mM MgCl₂, pH 6.0, at 260nm versus temperature, with a heating of 1.0 °C/min.

First derivative plots

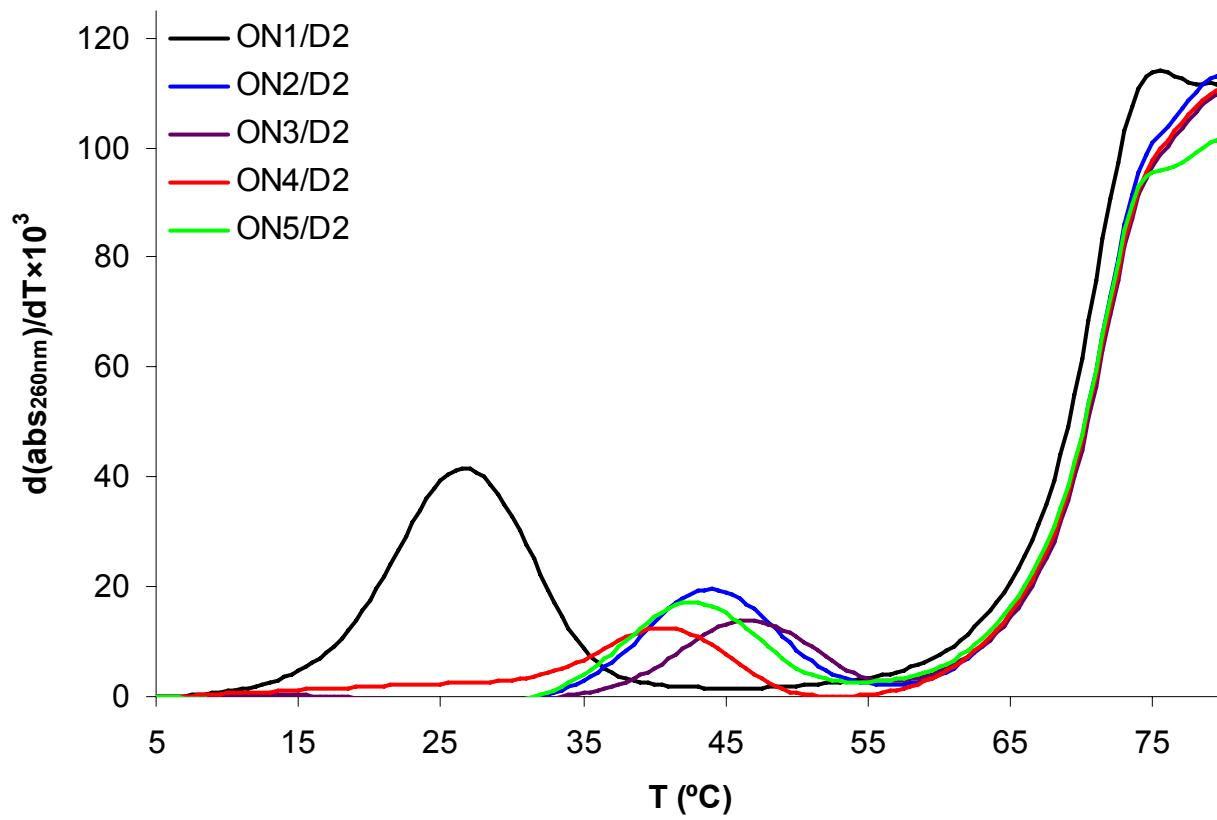


Figure 3. First derivate plots of thermal denaturation experiments of triplexes (**ON1-ON5/D2**) recorded in 20 mM sodium cacodylate, 100 mM NaCl, 10 mM MgCl₂, pH 6.0, at 260nm versus temperature, with a heating of 1.0 °C/min.

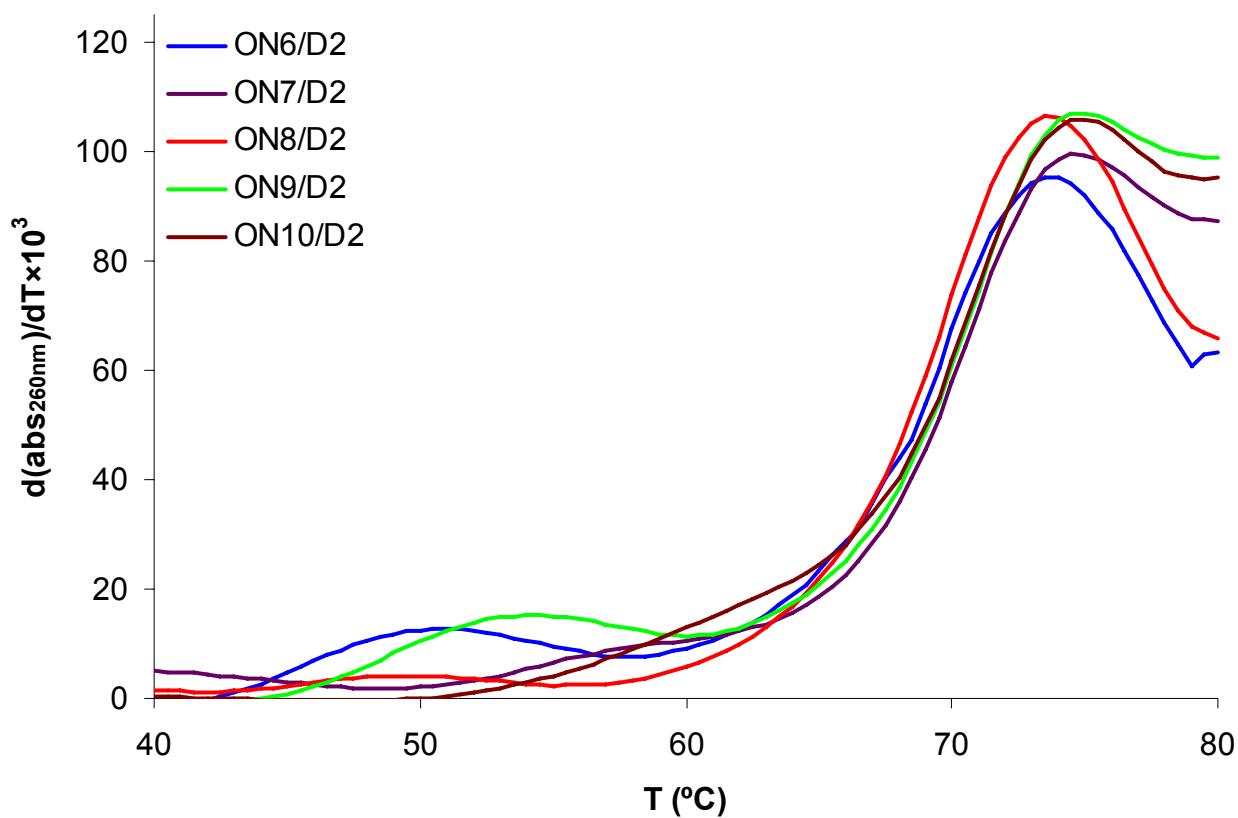


Figure 4. First derivate plots of thermal denaturation experiments of triplexes (**ON6-ON10/D2**) recorded in 20 mM sodium cacodylate, 100 mM NaCl, 10 mM MgCl₂, pH 6.0, at 260nm versus temperature, with a heating of 1.0 °C/min.

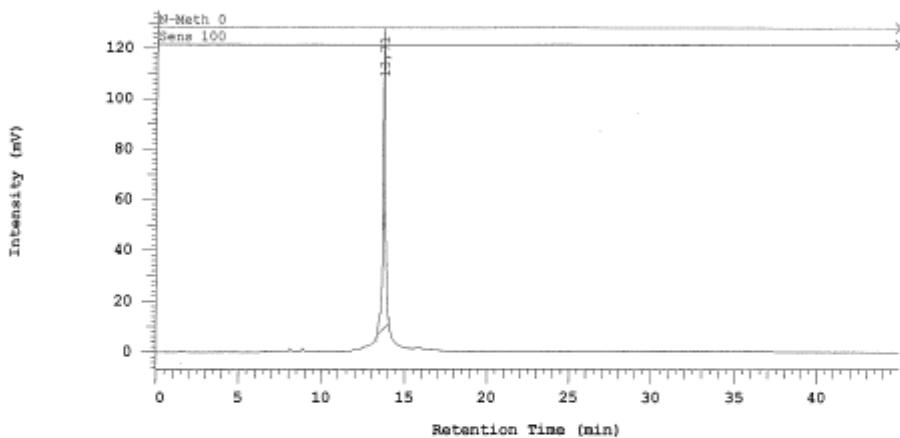
HPLC ion-exchange chromatography

D-7000 HSM: Ionchromatografi Series: 3652 Report: original System: Sys 1

D-7000 HPLC System Manager Report

Analyzed: 25-04-07 05:07 Reported: 27-04-07 09:43
Data Path: C:\WIN32APP\HSM\Ionchromatografi\DATA\3652\
Processing Method: DNA Pac_40-700mM_pH8_45min
System(acquisition): Sys 1 Series: 3652
Application: Ionchromatografi Vial Number: 13
Sample Name: BOM-T01 Vial Type: UNK
Injection from this vial: 1 of 1 Volume: 30,0 ul
Sample Description:

Chrom Type: HPLC Channel : 1



Acquisition Method: DNA Pac_40-700mM_pH8_45min
Column Type: Developed by: Suzy Lena
Pump A Type: L-7100
Solvent A: H₂O Solvent B: 0.2M NaOH
Solvent C: 0.25M Tris-Cl Solvent D: 2M NaCl
Method Description: Metode til ionchromatografi, kolonne DNA Pac-100

Chrom Type: HPLC Channel : 1

Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1
1	13,71	1210880	100,000
		1210880	100,000

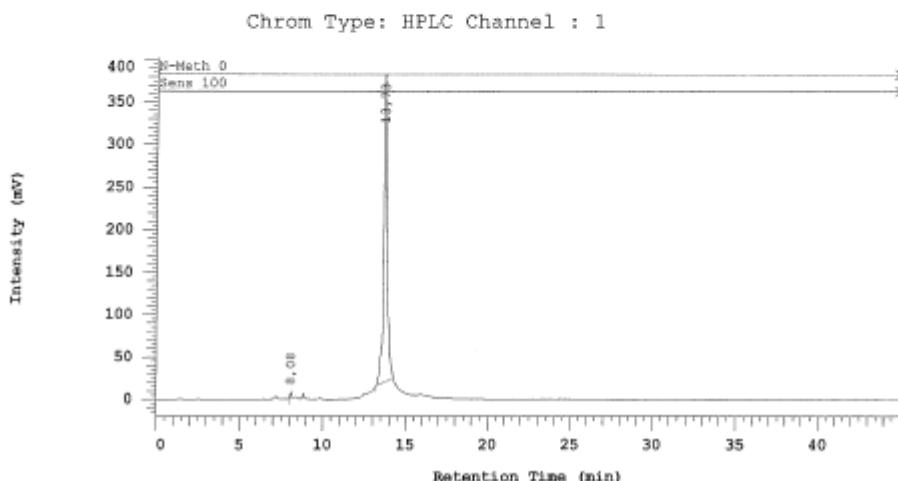
Peak rejection level: 20000

Figure 5. HPLC ion-exchange chromatography, purity determination of **ON3**

D-7000 HSM: Ionchromatografi Series: 3652 Report: original System: Sys 1

D-7000 HPLC System Manager Report

Analyzed: 25-04-07 03:34 Reported: 27-04-07 09:43
Data Path: C:\WIN32APP\HSM\Ionchromatografi\DATA\3652\
Processed: 25-04-07 04:19
Processing Method: DNAPac_40-700mM_pH8_45min
System(acquisition): Sys 1 Series:3652
Application: Ionchromatografi Vial Number: 11
Sample Name: BOM-T02 Vial Type: UNK
Injection from this vial: 1 of 1 Volume: 30,0 ul
Sample Description:



Acquisition Method: DNAPac_40-700mM_pH8_45min
Column Type: Developed by: Suzy Lena
Pump A Type: L-7100
Solvent A: H₂O Solvent B: 0.2M NaOH
Solvent C: 0.25M Tris-Cl Solvent D: 2M NaCl
Method Description: Metode til ionchromatografi, kolonne DNAPac-100

Chrom Type: HPLC Channel : 1

Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1
1	8,08	23499	0,494
2	13,73	4730406	99,506
		4753905	100,000

Peak rejection level: 20000

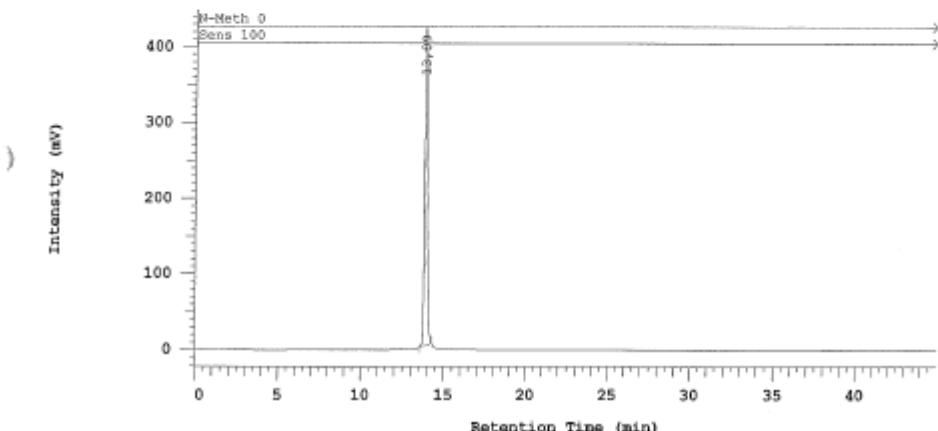
Figure 6. HPLC ion-exchange chromatography, purity determination of ON4

D-7000 HSM: Ionchromatografi Series: 3749 Report: original System: Sys 1

D-7000 HPLC System Manager Report

Analyzed: 14-06-07 20:36 Reported: 15-06-07 10:39
Data Path: C:\WIN32APP\HSM\Ionchromatografi\DATA\3749\
Processing Method: DNA Pac_40-700mM_pH8_45min
System(acquisition): Sys 1 Series: 3749
Application: Ionchromatografi Vial Number: 21
Sample Name: AAO-011 Vial Type: UNK
Injection from this vial: 1 of 1 Volume: 30,0 ul
Sample Description:

Chrom Type: HPLC Channel : 1



Acquisition Method: DNA Pac_40-700mM_pH8_45min
Column Type: Developed by: Suzy Lena
Pump A Type: L-7100
Solvent A: H₂O Solvent B: 0.2M NaOH
Solvent C: 0.25M Tris-Cl Solvent D: 2M NaCl
Method Description: Metode til ionchromatografi, kolonne DNA Pac-100

) Chrom Type: HPLC Channel : 1

Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1
1	13,89	4384217	100,000
		4384217	100,000

Peak rejection level: 20000

Figure 7. HPLC ion-exchange chromatography, purity determination of **ON5**

D-7000 HSM: Ionchromatografi Series: 3743 Report: original System: Sys I

D-7000 HPLC System Manager Report

Analyzed: 12-06-07 15:43

Reported: 13-06-07 11:10

Processed: 12-06-07 16:29

Data Path: C:\WIN32APP\HSM\Ionchromatografi\DATA\3743\

Processing Method: DNAPac_40-700mM_pH8_45min

System(acquisition): Sys 1 Series:3743

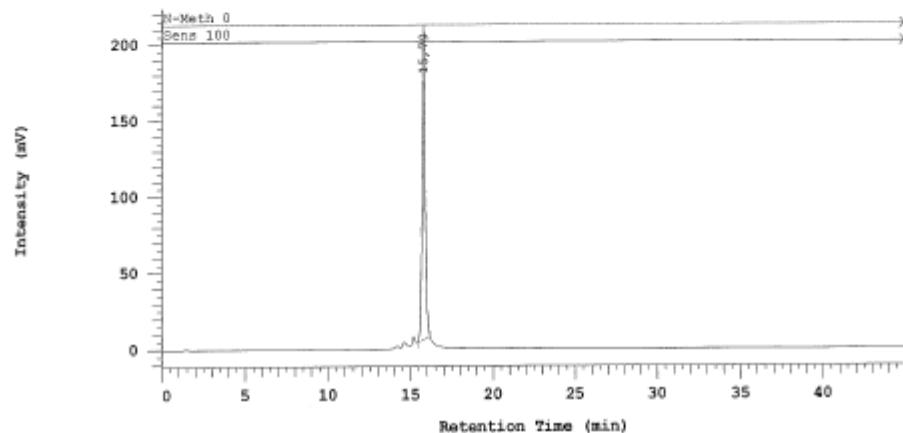
Application: Ionchromatografi Vial Number: 28

Sample Name: 07.06.07 gl. 5 *Bom-Tos* Vial Type: UNK

Injection from this vial: 1 of 1 Volume: 30,0 ul

Sample Description:

Chrom Type: HPLC Channel : 1



Acquisition Method: DNAPac_40-700mM_pH8_45min

Column Type: Developed by: Suzy Lena

Pump A Type: L-7100

Solvent A: H₂O

Solvent B: 0.2M NaOH

Solvent C: 0.25M Tris-Cl

Solvent D: 2M NaCl

Method Description: Metode til ionchromatografi, kolonne DNAPac-100

Chrom Type: HPLC Channel : 1

Peak Quantitation: AREA

Calculation Method: AREA%

No.	RT	Area	Conc 1
1	15,79	2467618	100,000
		2467618	100,000

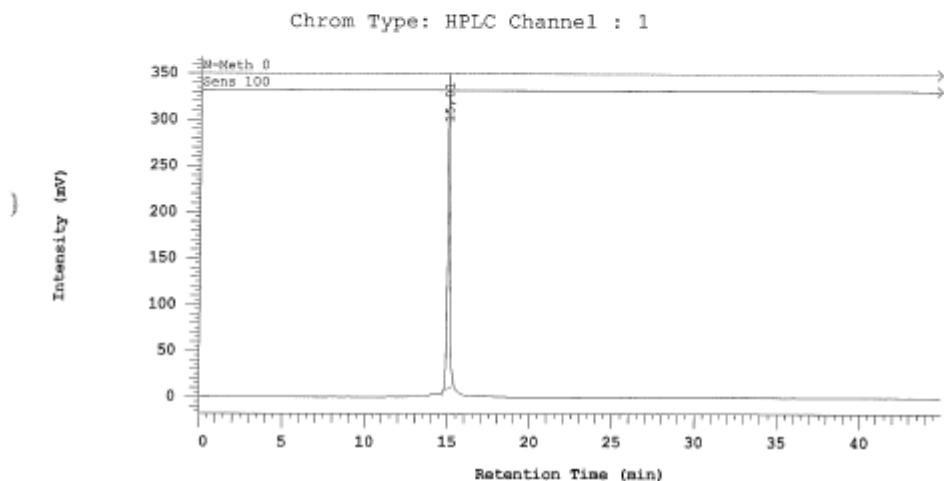
Peak rejection level: 20000

Figure 8. HPLC ion-exchange chromatography, purity determination of ON7

D-7000 HSM: Ionchromatografi Series: 3749 Report: original System: Sys 1

D-7000 HPLC System Manager Report

Analyzed: 14-06-07 22:09 Reported: 15-06-07 10:39
Data Path: C:\WIN32APP\HSM\Ionchromatografi\DATA\3749\
Processing Method: DNAPac_40-700mM_pH8_45min
System(acquisition): Sys 1 Series:3749
Application: Ionchromatografi Vial Number: 23
Sample Name: AAO-014 Vial Type: UNK
Injection from this vial: 1 of 1 Volume: 30,0 ul
Sample Description:



Acquisition Method: DNAPac_40-700mM_pH8_45min
Column Type: Developed by: Suzy Lena
Pump A Type: L-7100
Solvent A: H₂O Solvent B: 0.2M NaOH
Solvent C: 0.25M Tris-Cl Solvent D: 2M NaCl
Method Description: Metode til ionchromatografi, kolonne DNAPac-100

Chrom Type: HPLC Channel : 1

Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1
1	15,01	3305150	100,000
		3305150	100,000

Peak rejection level: 20000

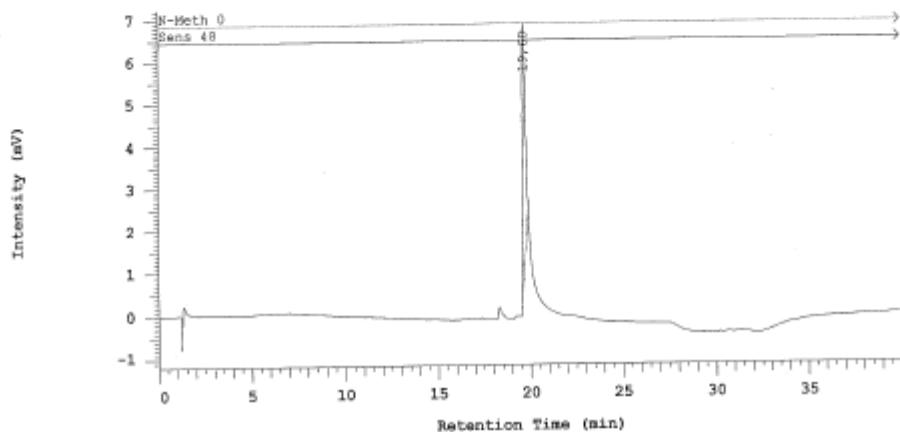
Figure 10. HPLC ion-exchange chromatography, purity determination of **ON9**

D-7000 HSM: Ionchromatografi Series: 4276 Report: original System: Sys 1

D-7000 HPLC System Manager Report

Analyzed: 24-01-08 00:11 Reported: 24-01-08 10:21
Processed: 24-01-08 00:51
Data Path: C:\WIN32APP\HSM\Ionchromatografi\DATA\4276\
Processing Method: DNAPac_pH8_700-800mM_40min
System(acquisition): Sys 1 Series: 4276
Application: Ionchromatografi Vial Number: 15
Sample Name: BOM T09 1 Vial Type: UNK
Injection from this vial: 1 of 1 Volume: 20,0 ul
Sample Description:

Chrom Type: HPLC Channel : 1



Acquisition Method: DNAPac_pH8_700-800mM_40min Developed by: Suzy Lena
Column Type: Pump A Type: L-7100
Solvent A: H₂O Solvent B: 0.05M NaOH
Solvent C: 0.25M Tris-Cl + 0.001M EDTA Solvent D: 2M NaCl
Method Description: Metode til ionchromatografi, kolonne DNAPac-100

Chrom Type: HPLC Channel : 1

Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1
1	19,68	59427	100,000
		59427	100,000

Peak rejection level: 20000

Figure 11. HPLC ion-exchange chromatography, purity determination of **ON10**