

告别色谱残留 | 科诺美 Frontier 生物惰性液相

攻克寡核苷酸 ASO 分析瓶颈

前言：寡核苷酸药物（Oligonucleotide Drugs）是一类以寡核苷酸为基础的治疗药物，主要用于调节基因表达或干扰与疾病相关的 RNA 分子功能。其中，反义寡核苷酸（ASO）作为技术成熟、应用广泛的核酸药物，全球年销售额就达到约 32 亿美元，成为基因治疗领域的重要支柱。因此，对寡核苷酸进行高灵敏度和稳定的定性定量分析变得越来越重要。LC-MS/MS 由于其优异的选择性和高灵敏度可为寡核苷酸定量分析提供有效帮助。然而 ASO 硫代骨架是强负电，大分子易金属螯合，容易产生强非特异性吸附，导致 LC-MS/MS 残留大，高浓度进样后，后续空白持续出峰。针对寡核苷酸药物在色谱端残留大的问题，科诺美推出 Frontier 生物惰性超高效液相色谱系统（Frontier Bio Inert LC），该系统与质谱仪联用，可以大大减少乃至消除非特异性吸附，有效提高检测精准度；无需钝化处理系统，提高仪器使用效率，为寡核苷酸药物测定提供优质解决方案。

一、仪器

Frontier Bio Inert LC 联用 SCIEX 5500+ MS/MS



二、色谱条件

液相方法:

色谱柱: KINETEX 2.6 μ m EVO C18 100Å 50*2.1mm

流动相: A: 水(0.15%DIEA+0.7%HFIP); B: 乙腈(0.15%DIEA+0.7%HFIP)

梯度洗脱: 0.01-3.0min, 5%B-30%B; 3.0-5.0min, 30%B-100%B; 5.0-5.9min, 100%B。

流速: 0.3mL/min

柱温: 40°C

进样量: 5 μ L

质谱方法:

数据采集方法: MRM (多反应监测)

离子源: ESI 负模式

气帘气 CUR: 35psi IS 电压: 5500V

源温度 TEM: 550°C 雾化气 GS1: 50psi

辅助气 GS2: 50psi 碰撞气 CAD: high

表 1 寡核苷酸 (ASO) 的 MRM 离子对信息

Compound ID	Q1 mass	Q3 mass	DP	CE
ID-1	741.5	78.9	-120.0	-50.0
ID-2	834.3	78.9	-120.0	-50.0
ID-3	741.5	94.9	-120.0	-50.0
ID-4	834.3	94.9	-120.0	-50.0



三、实验结果

1. Carryover 测试结果对比

按照空白-500ng/mL-空白 1-空白 2 的进样顺序编辑序列，运行结束后根据目标峰的峰面积计算 500ng/mL 浓度之后的第一针和第二针空白的残留水平，结果如表 2 所示。

Bio inert 系统新增多种自动进样器清洗方式，通过增加针清洗和阀清洗等方式残留可降至 0.1% 以内。

表 2 生物惰性 Frontier Bio Inert 不同进样器清洗程序仪器残留对比表（以峰面积计算）

洗针程序	默认方法				方法优化后（增加针清洗与阀清洗）			
化合物	ID-1	ID-2	ID-3	ID-4	ID-1	ID-2	ID-3	ID-4
500ng/mL 后 空白 1 残留 %	0.91%	0.83%	0.95%	0.97%	0.09%	0.13%	0.12%	0.11%
500ng/mL 后 空白 2 残留 %	0.23%	N/A	0.23%	0.24%	N/A	N/A	0.04%	0.03%

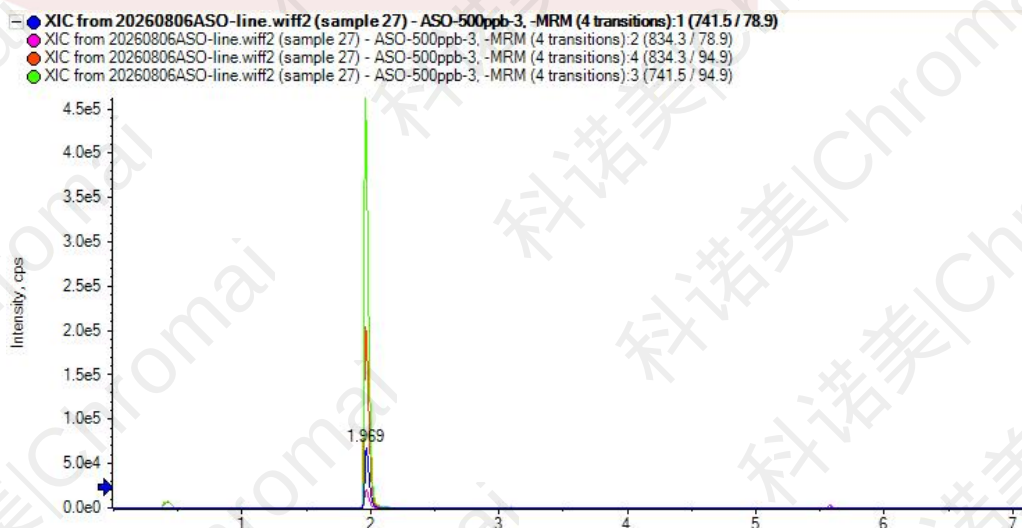


图 1 寡核苷酸的提取离子流图（XIC）



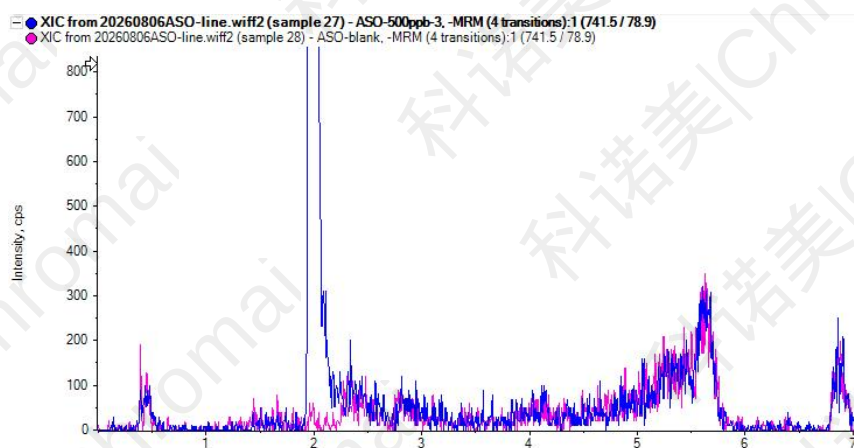


图2 寡核苷酸 (ID-1) 500ng/mL 以及空白 1 残留图

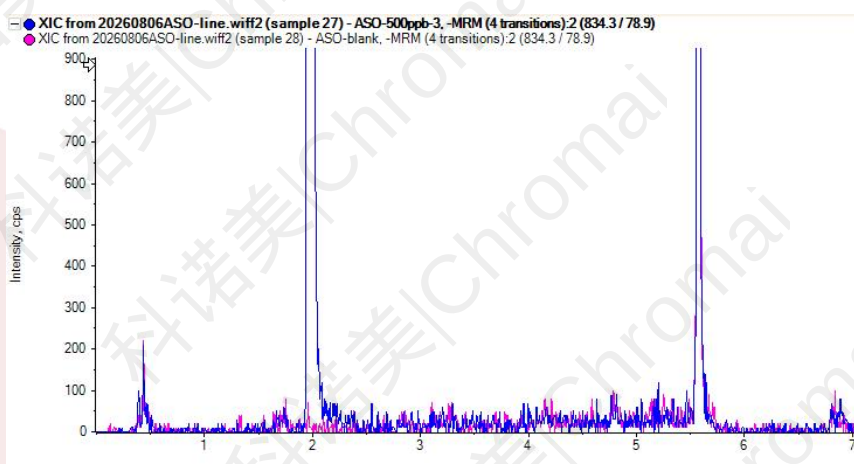


图3 寡核苷酸 (ID-2) 500ng/mL 以及空白 1 残留图

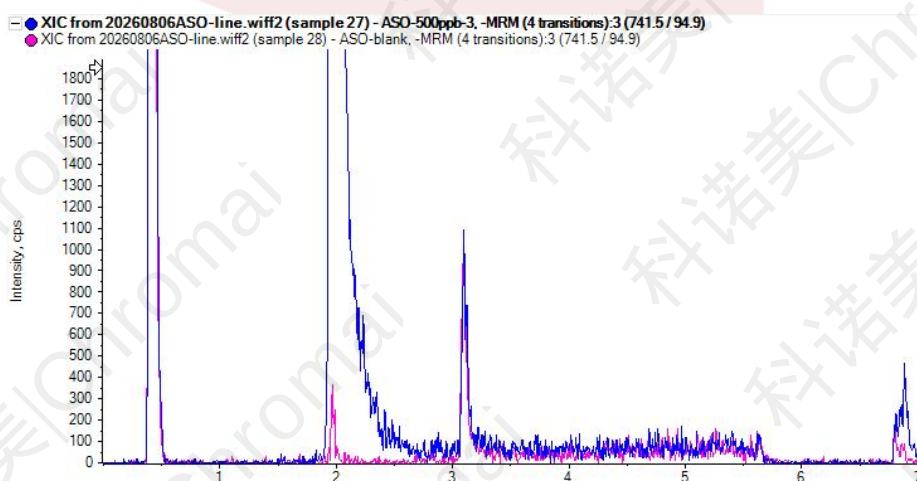


图4 寡核苷酸 (ID-3) 500ng/mL 以及空白 1 残留图



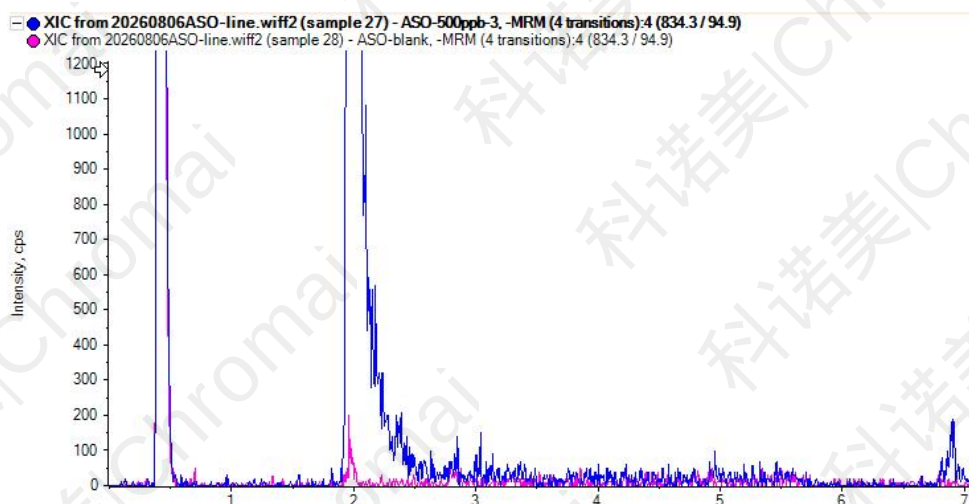


图 5 寡核苷酸 (ID-4) 500ng/mL 以及空白 1 残留图

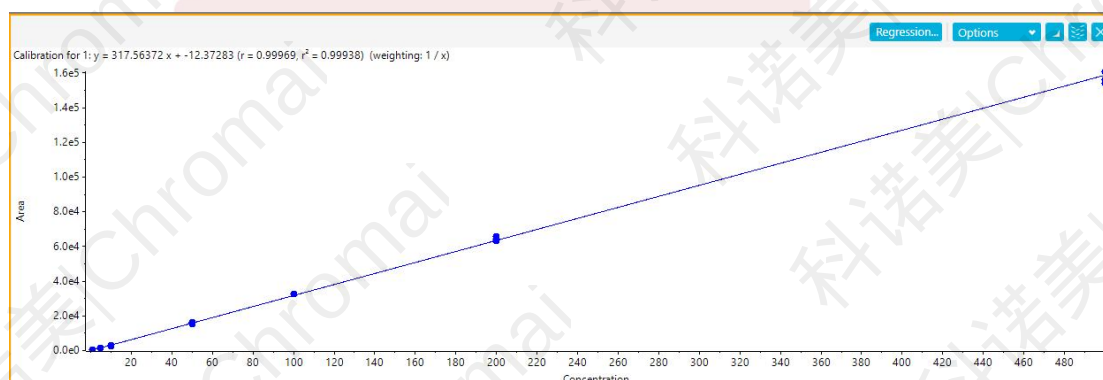
2.线性

将适量寡核苷酸 (ASO) 用处理后的大鼠血清上清液稀释成浓度为 1.0ng/mL,

5.0ng/mL, 10.0ng/mL, 50.0ng/mL, 100.0ng/mL, 200ng/mL, 500ng/mL 的标准

工作液分别进样分析。寡核苷酸线性结果如图 4 所示, 在 1.0ng/mL~500ng/mL 范围内

线性良好, r^2 均大于 0.999。



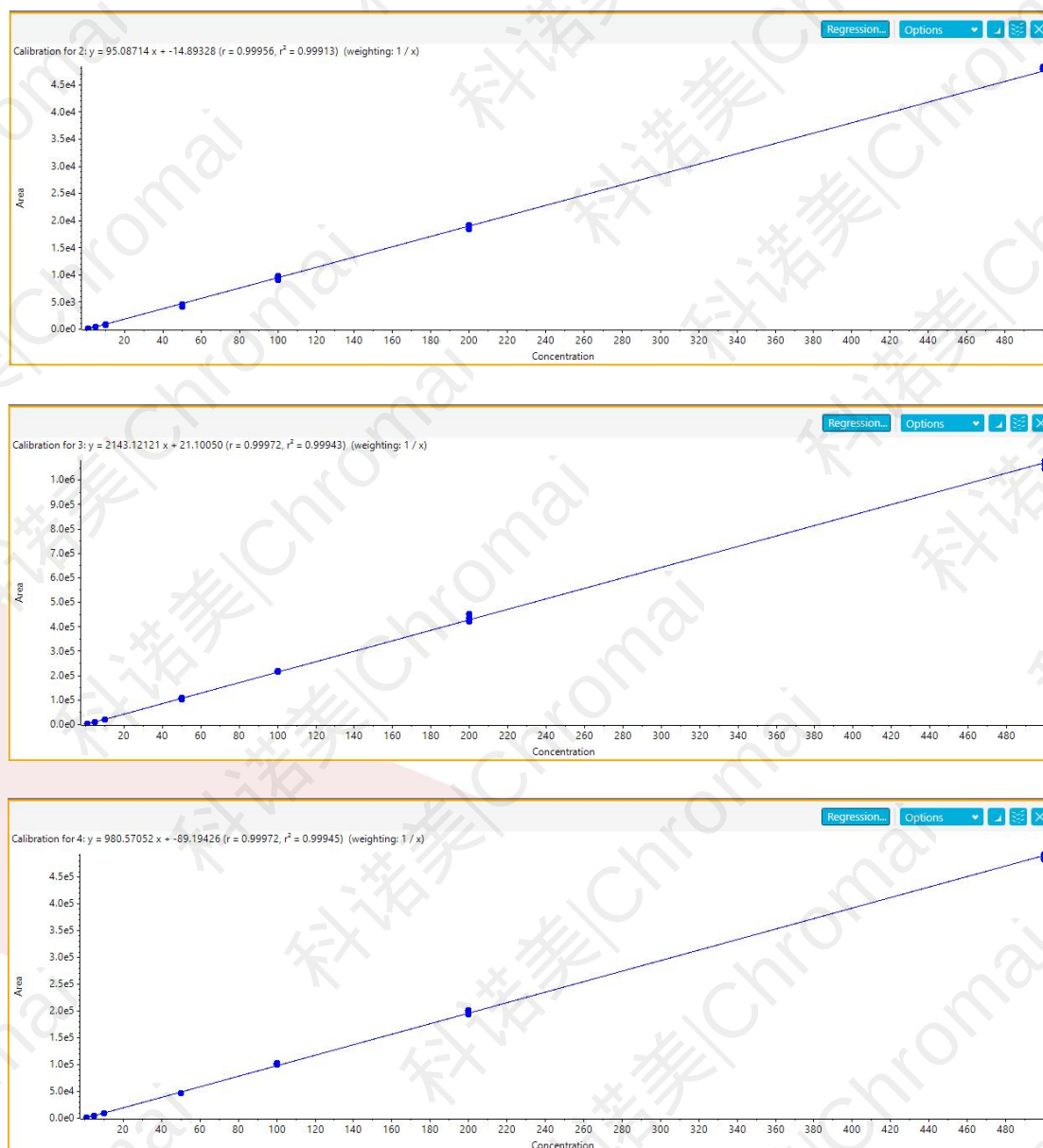


图 6 寡核苷酸 (ASO) 的标准曲线结果

3. 重现性

将 100ng/mL 的寡核苷酸 (ASO) 样品连续进样 6 次进行重复性考察。保留时间重复性在 0.24%-0.26%之间，峰面积重复性在 0.84%-1.30%之间。(图 7-图 10)。



Index	Sample Name	Sample Type	Component Name	Component Type	Component Group Name	Actual Concentr...	Expected RT	Area	Height	Retent... Time	Retenti... Time D...	U...	Calculated Concentrat...
137	ASO-100ppb-CV-1	Quality Control	1	Quantifiers	Group 1	100.00	2.01	6.068e4	2.940e4	1.97	0.04	☒	8.969e1
141	ASO-100ppb-CV-2	Quality Control	1	Quantifiers	Group 1	100.00	2.01	6.137e4	2.946e4	1.96	0.05	☒	9.071e1
145	ASO-100ppb-CV-3	Quality Control	1	Quantifiers	Group 1	100.00	2.01	6.130e4	2.877e4	1.97	0.04	☒	9.060e1
149	ASO-100ppb-CV-4	Quality Control	1	Quantifiers	Group 1	100.00	2.01	6.175e4	2.841e4	1.98	0.03	☒	9.128e1
153	ASO-100ppb-CV-5	Quality Control	1	Quantifiers	Group 1	100.00	2.01	6.198e4	2.848e4	1.98	0.03	☒	9.161e1
157	ASO-100ppb-CV-6	Quality Control	1	Quantifiers	Group 1	100.00	2.01	6.045e4	2.846e4	1.97	0.04	☒	8.935e1

Group by	Sample Name prefix	Sample Type	Quality Control	Metric type	Retention Time	Save Results and Export...
----------	--------------------	-------------	-----------------	-------------	----------------	----------------------------

Row	Component ...	Sample Name	Num. Values	Mean	Standard Deviation	Percent CV	Value #1	Value #2	Value #3	Value #4	Value #5	Value #6
1	1	ASO-100ppb-CV	6 of 6	1.97	0.01	0.26	1.97	1.97	1.97	1.98	1.98	1.97

Group by	Sample Name prefix	Sample Type	Quality Control	Metric type	Area	Save Results and Export...
----------	--------------------	-------------	-----------------	-------------	------	----------------------------

Row	Component ...	Sample Name	Num. Values	Mean	Standard Deviation	Percent CV	Value #1	Value #2	Value #3	Value #4	Value #5	Value #6
1	1	ASO-100ppb-CV	6 of 6	6.126e4	5.937e2	0.97	6.068e4	6.137e4	6.130e4	6.175e4	6.198e4	6.045e4

图 7 寡核苷酸 ASO (ID-1) 100ng/mL 连续 6 针重复性结果表

Index	Sample Name	Sample Type	Component Name	Component Type	Component Group Name	Actual Concentr...	Expected RT	Area	Height	Retent... Time	Retenti... Time D...	U...	Calculated Concentrat...
138	ASO-100ppb-CV-1	Quality Control	2	Qualifiers	Group 1	100.00	2.00	1.691e4	7.756e3	1.97	0.03	☒	8.905e1
142	ASO-100ppb-CV-2	Quality Control	2	Qualifiers	Group 1	100.00	2.00	1.738e4	8.550e3	1.96	0.04	☒	9.148e1
146	ASO-100ppb-CV-3	Quality Control	2	Qualifiers	Group 1	100.00	2.00	1.730e4	8.142e3	1.97	0.03	☒	9.110e1
150	ASO-100ppb-CV-4	Quality Control	2	Qualifiers	Group 1	100.00	2.00	1.740e4	7.671e3	1.97	0.03	☒	9.162e1
154	ASO-100ppb-CV-5	Quality Control	2	Qualifiers	Group 1	100.00	2.00	1.756e4	7.939e3	1.97	0.03	☒	9.245e1
158	ASO-100ppb-CV-6	Quality Control	2	Qualifiers	Group 1	100.00	2.00	1.716e4	8.005e3	1.96	0.04	☒	9.033e1

Group by	Sample Name prefix	Sample Type	Quality Control	Metric type	Retention Time	Save Results and Export...
----------	--------------------	-------------	-----------------	-------------	----------------	----------------------------

Row	Component ...	Sample Name	Num. Values	Mean	Standard Deviation	Percent CV	Value #1	Value #2	Value #3	Value #4	Value #5	Value #6
1	2	ASO-100ppb-CV	6 of 6	1.97	0.00	0.25	1.97	1.97	1.97	1.97	1.97	1.96

Group by	Sample Name prefix	Sample Type	Quality Control	Metric type	Area	Save Results and Export...
----------	--------------------	-------------	-----------------	-------------	------	----------------------------

Row	Component ...	Sample Name	Num. Values	Mean	Standard Deviation	Percent CV	Value #1	Value #2	Value #3	Value #4	Value #5	Value #6
1	2	ASO-100ppb-CV	6 of 6	1.729e4	2.247e2	1.30	1.691e4	1.738e4	1.730e4	1.740e4	1.756e4	1.716e4

图 8 寡核苷酸 ASO (ID-2) 100ng/mL 连续 6 针重复性结果表

Index	Sample Name	Sample Type	Component Name	Component Type	Component Group Name	Actual Concentr...	Expected RT	Area	Height	Retent... Time	Retenti... Time D...	U...	Calculated Concentrat...
140	ASO-100ppb-CV-1	Quality Control	3	Qualifiers	Group 1	100.00	2.00	4.301e5	1.988e5	1.96	0.04	☒	9.259e1
144	ASO-100ppb-CV-2	Quality Control	3	Qualifiers	Group 1	100.00	2.00	4.268e5	1.941e5	1.96	0.04	☒	9.187e1
148	ASO-100ppb-CV-3	Quality Control	3	Qualifiers	Group 1	100.00	2.00	4.259e5	1.915e5	1.97	0.04	☒	9.168e1
152	ASO-100ppb-CV-4	Quality Control	3	Qualifiers	Group 1	100.00	2.00	4.202e5	1.967e5	1.97	0.03	☒	9.045e1
156	ASO-100ppb-CV-5	Quality Control	3	Qualifiers	Group 1	100.00	2.00	4.257e5	1.929e5	1.97	0.03	☒	9.162e1
160	ASO-100ppb-CV-6	Quality Control	3	Qualifiers	Group 1	100.00	2.00	4.188e5	1.964e5	1.96	0.04	☒	9.014e1

Group by	Sample Name prefix	Sample Type	Quality Control	Metric type	Retention Time	Save Results and Export...
----------	--------------------	-------------	-----------------	-------------	----------------	----------------------------

Row	Component ...	Sample Name	Num. Values	Mean	Standard Deviation	Percent CV	Value #1	Value #2	Value #3	Value #4	Value #5	Value #6
1	3	ASO-100ppb-CV	6 of 6	1.96	0.00	0.24	1.96	1.96	1.97	1.97	1.97	1.96

Group by	Sample Name prefix	Sample Type	Quality Control	Metric type	Area	Save Results and Export...
----------	--------------------	-------------	-----------------	-------------	------	----------------------------

Row	Component ...	Sample Name	Num. Values	Mean	Standard Deviation	Percent CV	Value #1	Value #2	Value #3	Value #4	Value #5	Value #6
1	3	ASO-100ppb-CV	6 of 6	4.246e5	4.275e3	1.01	4.301e5	4.268e5	4.259e5	4.202e5	4.257e5	4.188e5

图 9 寡核苷酸 ASO (ID-3) 100ng/mL 连续 6 针重复性结果表



Index	Sample Name	Sample Type	Component Name	Component Type	Component Group Name	Actual Concentr...	Expected RT	Area	Height	Retent... Time	Retenti... Time D...	U...	Calculated Concentrat...
139	ASO-100ppb-CV-1	Quality Control	4	Qualifiers	Group 1	100.00	2.00	1.855e5	8.391e4	1.97	0.04	☒	9.308e1
143	ASO-100ppb-CV-2	Quality Control	4	Qualifiers	Group 1	100.00	2.00	1.849e5	8.620e4	1.96	0.04	☒	9.277e1
147	ASO-100ppb-CV-3	Quality Control	4	Qualifiers	Group 1	100.00	2.00	1.829e5	8.087e4	1.97	0.03	☒	9.181e1
151	ASO-100ppb-CV-4	Quality Control	4	Qualifiers	Group 1	100.00	2.00	1.865e5	8.879e4	1.97	0.03	☒	9.359e1
155	ASO-100ppb-CV-5	Quality Control	4	Qualifiers	Group 1	100.00	2.00	1.836e5	8.293e4	1.97	0.03	☒	9.214e1
159	ASO-100ppb-CV-6	Quality Control	4	Qualifiers	Group 1	100.00	2.00	1.825e5	8.447e4	1.96	0.04	☒	9.161e1

Group by

Sample Name prefix

Sample Type

Quality Control

Metric type

Retention Time

Save Results and Export...

Row	Component ...	Sample Name	Num. Values	Mean	Standard Deviation	Percent CV	Value #1	Value #2	Value #3	Value #4	Value #5	Value #6
1	4	ASO-100ppb-CV	6 of 6	1.97	0.00	0.24	1.97	1.96	1.97	1.97	1.97	1.96

Group by

Sample Name prefix

Sample Type

Quality Control

Metric type

Area

Save Results and Export...

Row	Component ...	Sample Name	Num. Values	Mean	Standard Deviation	Percent CV	Value #1	Value #2	Value #3	Value #4	Value #5	Value #6
1	4	ASO-100ppb-CV	6 of 6	1.843e5	1.549e3	0.84	1.855e5	1.839e5	1.829e5	1.865e5	1.836e5	1.825e5

图 10 寡核苷酸 ASO (ID-4) 100ng/mL 连续 6 针重复性结果表

结论：本方案采用科诺美生物惰性超高效液相色谱系统 Frontier Bio Inert LC，该系统与质谱仪联用，采用新的自动进样器清洗方式，有效降低寡核苷酸非特异性吸附，线性最高点 500ng/mL 后空白残留在 0.1% 以内，在浓度范围 1ng/mL-500ng/mL 内线性良好，线性相关系数大于 0.999，重复性好，满足寡核苷酸药物的精准测定。

