U.S. FOOD & DRUG ADMINISTRATION

Quality Considerations for Generic Oligonucleotides

Likan Liang, Ph.D.

Division of Product Quality Assessment X
Office of Product Quality Assessment II, OPQ, CDER

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FDA
Everyone deserves confidence in their *next* dose of medicine.
Pharmaceutical quality assures the availability, safety, and efficacy of *every* dose.

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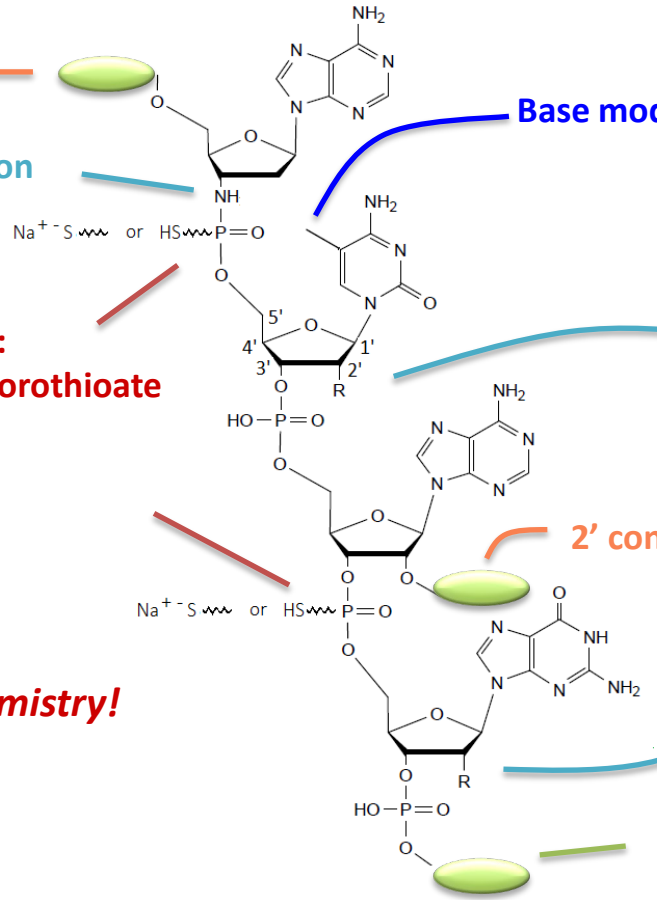
The author declares no competing financial interest.

Oligonucleotide (ON) Drug Products

- Short strings of synthetic or semisynthetic nucleic acids with therapeutic effects
- Nucleic acid structures
  - Unmodified (e.g., Defibrotide)
  - Modified (most CDER-approved ONs)
- Single stranded (e.g., ASO), double stranded (e.g., siRNA), etc.

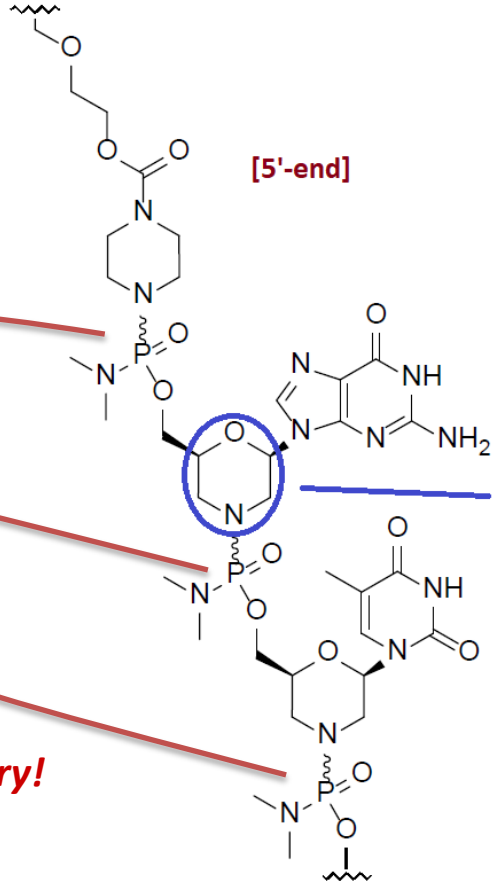
ON Modifications and Diastereomers



Nucleotide linkage modification:
(e.g., non-stereospecific phosphorothioate (PS):
***R* and *S* ratio @ each PS.**
n x PS: PS diastereomers = 2^n

Complexity of ON Stereochemistry!

ON Modifications and Diastereomers (cont.)



Nucleotide linkage modification:
(e.g., non-stereospecific phosphorodiamidate (PDA):
***R* and *S* ratio @ each PDA**
n x PDA: PDA diastereomers = 2^n

Morpholino replacement of ribose

Complexity of ON Stereochemistry!

Diastereomers in CDER-Approved Oligonucleotide Drug Products

Type of ON	CDER-Approved ONs [a]	Length of ONs [c]	Modifications [c]	Possible Diastereomers [d]
Antisense Oligonucleotides (ASO)	Fomivirsen (Vitravene)[b]	21 mer	**20*PS**	> 1 million PS diast.
	Mipomersen (Kynamro)[b]	20 mer	**19*PS**, 10*2'-MOE, 9*Me-C	> Half million PS diast.
	Eteplirsen (Exondys 51)	30 mer	**30*PDA**, 30*morpholino	> 1 billion PDA diast.
	Nusinersen (Spinraza)	18 mer	**17*PS**, 4*Me-C	> 130,000 PS diast.
	Inotersen (Tegsedi)[b]	20 mer	**19*PS**, 10*2'-MOE, 5*Me-C	> Half million PS diast.
	Golodirsen (Vyondys 53)	25 mer	**25*PDA**, 25*morpholino	> 33 million PDA diast.
	Viltolarsen (Viltepso)	21 mer	**20*PDA**, 21*morpholino	> 1 million PDA diast.
	Casimersen (Amondys 45)	22 mer	**22*PDA**, 22*morpholino	> 4 million PDA diast.
	Tofersen (Qalsody)	20 mer	**15*PSH**, 10*2'-MOE, 5*Me-C	> 32,000 PSH diast.
	Eplontersen (Wainua)	20 mer	**13*PS**, 10*2'-MOE, 5*Me-C, GalNAc	> 8,000 PS diast.
	Olezarsen (Tryngolza)	20 mer	**19*PS**, 10*2'-MOE, 5*Me-C, GalNAc	> Half million PS diast.
	Donidalorsen (Dawnzera)	20 mer	**15*PS**, 10*2'-OME, 5*Me-C, GalNAc	> 32,000 PS diast.
siRNA (sense || antisense)	Patisiran (Onpattro)	21 mer || 21 mer	9*2'-M||2*2'-M	N/A
	Givosiran (Givlaari)	21 mer || 23 mer	**2*PS**, 16*2'-M, 5*2'-F, GalNAc || **4*PS**, 12*2'-M, 11*2'-F	4 PS diast. || 16 PS diast.
	Lumasiran (Oxlumo)	21 mer || 23 mer	**2*PS**, 17*2'-M, 4*2'-F, GalNAc || **4*PS**, 17*2'-M, 6*2'-F	4 PS diast. || 16 PS diast.
	Inclisiran (Leqvio)	21 mer || 23 mer	**2*PS**, 18*2'-M, 2*2'-F, GalNAc || **4*PS**, 13*2'-M, 10*2'-F	4 PS diast. || 16 PS diast.
	Vutrisiran (Amvuttra)	21 mer || 23 mer	**2*PS**,17*2'-M,4*2'-F,GalNAc || **4*PS**, 18*2'-M, 5*2'-F	4 PS diast. || 16 PS diast.
	Nedosiran (Rivfloza)	36 mer || 22 mer	**1*PS**,23*2'-M,9*2'-F,4*GalNAc || **5*PS**, 12*2'-M, 10*2'-F	2 PS diast. || 32 PS diast.
	Fitusiran (Qfitlia)	21 mer || 23 mer	**2*PS**,9*2'-M,12*2'-F,GalNAc || **4*PS**, 14*2'-M, 9*2'-F	4 PS diast. || 16 PS diast. 8
	Plozasiran (Redemplo)	21 mer || 21 mer	**3*PS**,18*2'-M,3*2'-F,GalNAc || **4*PS**, 13*2'-M, 8*2'-F	PS diast. || 16 PS diast.

[a] As of 3/1/2026 [b] Discontinued. [c] Based on product labeling; 2'-F = 2'-fluoro; 2'-M = 2'-O-methyl; 2'-MOE = 2'-O-methoxyethyl; Me-C = methyl cytosine; PDA = phosphorodiamidate; PS = phosphorothioate; PSH = phosphorothioate, free acid form; S-PA = thio-phosphoroamidate; aG = aminoglyceryl. [d] Derived from product labeling; diast. = diastereomers

Diastereomers in CDER-Approved Oligonucleotide Drug Products (cont.)

Type of ON	CDER-Approved ONs [a]	Length of ONs [c]	Modifications [c]	Possible Diastereomers [d]
Aptamers, Other Inhibitors	Pegaptanib (Macugen)[b] Avacincaptad pegol (Izervay) Imetelstat (Rytelo)	28 mer 39 mer 13 mer	PEG, 12*2'-M, 13*2'-F PEG, 14*2'-M, 21*2'-F **12*S-PA**,**1*PS**,13*3'-amino,1*fatty acid linked via chiral aminoglyceryl (**1*aG**)	N/A N/A > 16,000 S-PA* PS*aG diast.
Polydisperse Oligonucleotide	Defibrotide (Defitelio)	Mixture (~4 mer to ~100 mer)	(Predominantly single-stranded polydeoxyribonucleotide, derived from porcine intestinal tissue)	N/A

[a] As of 3/1/2026 [b] Discontinued. [c] Based on product labeling; 2'-F = 2'-fluoro; 2'-M = 2'-O-methyl; 2'-MOE = 2'-O-methoxyethyl; Me-C = methyl cytosine; PDA = phosphorodiamidate; PS = phosphorothioate; PSH = phosphorothioate, free acid form; S-PA = thio-phosphoroamidate; aG = aminoglyceryl. [d] Derived from product labeling; diast. = diastereomers

Generic Oligonucleotides: Sameness and Quality Consideration

- Active ingredient sameness
  - Sequence, chemical structure, composition (e.g., diastereomers, duplex, double strand), potency/activity, etc.
- Physicochemical characteristics
  - Higher order structures/aggregation in drug product
- Safety/quality considerations
  - Impurity profiles, levels, and control strategy
  - Risks in immunogenicity, inflammation, off-target effects, general toxicity, etc.

Generic Oligonucleotides: Active Ingredient Sameness

- Implementing the Hatch-Waxman Amendments: *Active ingredient in this context means the active ingredient in the finished drug product prior to its administration*[1]
- If changes are possible from drug substance to drug product
  - Obtain thorough understanding, ensure consistency – e.g., double strand content, duplex.
- Active ingredient sameness studies
  - On drug substance or drug product, depending on the test methods and purpose of the tests. Justify your choices.
  - At least three batches of RLD drug product, as available
  - Diastereomeric composition analysis - sample processing should not change composition

[1] 54 FR 28872, at 28881 (July 10, 1989)

Diastereomeric Composition Sameness and Manufacturing Consistency - siRNAs

Current siRNAs – small number of diastereomers

- Quantitative RLD-comparison at individual diastereomer level possible with adequate diastereomer resolution [1]
- Consider using multiple methods (complementary/ orthogonal separation principles), if one method cannot separate all diastereomers of a single strand [2]
- Assess influence from co-eluting impurities

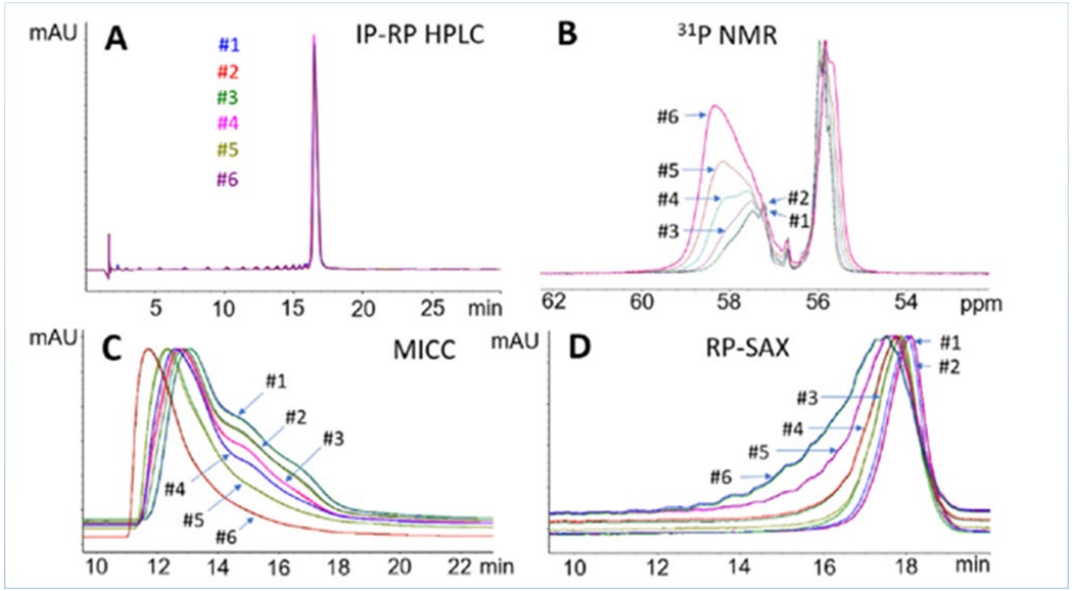
[1] A. Goyon *et al*, Journal of Chromatography A 1761 (2025) 466308
[2] F. Li *et al*, Journal of Chromatography A 1730 (2024) 465076

Generic ONs with High Number of Diastereomers

High diastereomer numbers and potential coeluting impurities vs limited resolving power / sensitivity / ability of a single method

#	DCI/ETT
1	19:0
2	18:1
3	14:5
4	9:10
5	4:15
6	0:19

> half million diastereomers in each sample



Graphs adapted with permission from Roussis, S. G.; Cedillo, I.; Rentel, C. Analytical Chemistry 2021, 93 (48), 16035-16042. Copyright 2021 American Chemical Society.

Diastereomeric Composition Sameness and Manufacturing Consistency – High Number of Diastereomers

- Use multiple methods (orthogonal and complementary separation) – multiple distribution profiles at maximized resolution
- Demonstrate suitability/sensitivity of the methods towards composition changes
  - Use multiple suitability test standards with intentional small perturbations to the composition (e.g. altering R/S ratios at various chiral internucleotide linkages)
- Measure R/S ratios at each chiral internucleotide linkage following respective elongation cycle – facilitate strategy development
- Refer to Product Specific Guidance (PSG) for additional recommended studies

ON Synthetic Routes and Impurities

(A hypothetical 23 mer) **5'-CAATGCCATCTTGGAAGTTCCTG-3'**

- Via linear synthesis:

  Cycles: (1) solid support + C, (2) SS-C + A, … (23) SS-CAATGCCATCTTGGAAGTTCCT + G

- Possible impurities:
  - Some chain length-related impurities (sequences also altered)
    - n-1/n-2: all impurities missing any one/two nucleotide/nucleotides from full length
      - n-C: all impurities missing one C.
    - n+1/n+2: all impurities with one/two extra nucleotide/nucleotides to full length
  - Many other possible oligonucleotide-related impurities
  - Any apparent immunomodulating motifs? e.g., TCGTT, TCGTA, TCGTCG, GTCGTT, TTAGGG
- Innovator ON product: various safety evaluations qualified its impurities
- Generic ONs: Same synthetic approach as RLD? Thorough comparative impurity profile assessment.

ON Synthetic Routes and Impurities

(Same hypothetical 23 mer) **5'-CAATGCCATCTTGGAAGTTCCTG-3'**

- Via hypothetical convergent synthesis – fragments/blockmers (for illustration purpose)

  CAATG (blockmer 1) + CCATC (blockmer 2), then + TTGGAA (blockmer 3), then + GTTCCTG (blockmer 4)

- Very different impurity profile than the one from linear synthesis:
  - Any apparent immunomodulating motifs? e.g., TCGTT, TCGTA, TCGTCG, GTCGTT, TTAGGG
  - New impurity (missing block 3): **5'-CAATGCCATCGTTCCTG-3'**
- Generic ONs: Potentially much higher risks in introducing new impurities / new sequences when using synthetic approach different from RLD's
  - Immunogenicity/inflammatory risks?
  - Off-target effect?
  - General toxicity risks?

Generic ON Challenges: Impurities

- Limitation of current analytical tools in resolving ON impurities → co-eluting impurities (particularly isobaric and isomeric impurities)
  - Use orthogonal and complementary methods, coupled with high resolution MS or other advanced technologies
- Control strategy: Retention time region-based approach or similar grouping approaches not adequate for generic ON without further controls at specific impurity levels or proper and adequate justification.
- RLD comparison is the rule of thumb.

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- PSG development oligonucleotide SME triage team
- OPQ/OPQR oligonucleotide research teams