

Testing type: Group Test

Name	RT12 RE-TEST	Batch/CAS no	green cap/purple crimp
Formula	C223H343F3N46O70	Origin	Nexaph
Lot No		Test Date	December 4, 2024
Storage	2-8C vacuum sealed container		
Test Items	Acceptance Criteria	Test results	
Optical Rotation		-11.6°	
Solubility	Free soluble in water	Conforms	
Identification			
MS identification	Monoisotopic mass: 4731.33 +/- 1		
Amino Acid Sequence	Aromatic regions (6.66-7.19) multiple signals		aromatic protons from Tyr and Phe residues
	alpha-proton region (4.0-4.8): complex		Conforms
	Aliphatic region (0.05-3.93): complex side chains		Conforms
	methyl groups: Ile and Leu		Conforms (0.71-0.87)
	Carbonyl region (170-178): multiple peaks indicating peptide bonds		Conforms
	Aliphatic region: alpha-carbon (53-63), side chain carbon (10-40 ppm)		Conforms
	Clear spin systems for Tyr and Phe residues		Conforms
			Leu/Ile side chain
			Pro residue spin system
			PEG2 modifier signals
Sequence verification: Aib residues, alpha-MeuLeu, PEG2-y-Glu modification		present but with potential degradation MeuLeu region	
Stoichiometry	Integration rations: 348 total protons, side chain methyl groups, aromatic protons match Tyr-Phe. Stdev +/- 5% max.		Conforms
	Expected amino acid types		Conforms
Impurity profiling			
Carbon backbone structure	225 carbon signals: 40 carbonyl carbons (165-175 ppm), backbone Ca signals (45-65 ppm), side chain carbons match AA.		Conforms

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Impurity identification C13	Maximum unidentified signals <2%	Conforms
	Deletion products: incomplete coupling	5-7% Pro-rich C-terminal
	Oxidation products: potential Tyr/Phe oxidation	2-3%
	Racemization products: expected	1-2% Conforms
	NMR Purity assessment: main product : by integration of signals 1H, 13C, TOCSY, 19F, 31P	89%-92% No unexpected major impurities detected
Elemental Analysis	Carbon 57.21%	N/A
	Hydrogen 7.41%	N/A
	Nitrogen 14.21%	N/A
Other fluorine-containing impurities	NMT 0.1%	Conforms
Sodium	0.5% to 2.0%	Does not conform
Trifluoroacetic acid	NMT 0.1%	Conforms
Fungal Testing	<10 CFU/100 mL	Conforms
Bacterial Endotoxin	<0.25 EU/mL	Conforms

Conforms:

No significant unusual shifts in the amide or aromatic regions suggesting aggregate formation. Backbone shows clear resolution. NMR alone sees a clear peak/line and no broadening which suggests no aggregation during synthesis or PTM. Chemical shifts conform.

Potential Risks: Med-High

High sodium adduct present in M/S confirmed by NMR potentially from salt form issues and stability. Compound is most likely Na form.

Mass accuracy outside of specifications <5 ppm

Slightly higher level of oxidation at oxidation-sensitive residues:

- 3 Tyr
- 2 Phe
- PEG2-y-Glu modification

Might suggest: exposure to oxidizing conditions during synthesis, purification or storage.

Possibly insufficient antioxidant protection during processing.

TOCSY 2D NMR Data disclaimer: all exchangeable NH proton information not accounted for and secondary structure is limited.

Conclusion: some exposure to oxidizing conditions is the major concern causing stability issues and affecting oxidation-sensitive residues.