Determination of Oligomer Content in Benzonatate Drug Substance by HPLC

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Abstract. A reverse-phase liquid chromatographic method has developed and validated to determine oligomer content in Benzonatate Drug substances. This method has a separation of relatively few monomer units, which constitutes an oligomer with a minimum adequate chromatographic resolution of 1.0 from each of the subject component peaks. The separation achieved using Phenomenex Luna C18 (250 X 4.6 mm) 5 µm column at a flow rate of 1.0 ml/min with an isocratic elution method. The mobile phase consisting of 0.25% Ammonium formate buffer and methanol in the ratio 350:650 (*v/v*), respectively. The Oligomer compounds detection carried out at UV 310 nm, and the LC method validated as per the current ICH Q2 guidelines. The method is effectively validated and proved to be precise, specific, linear, robust, and rugged to quantitate oligomer content in Benzonatate drug substance.

Key words: benzonatate (BNZ), oligomers, HPLC, method validation.

Introduction

Benzonatate (BNZ) is an oral antitussive drug used to relieve and suppress cough in patients older than ten years of age (Oligomer Wikipedia, 2020). Currently, BNZ is the only non-narcotic antitussive available as a prescription drug. The chemical structure resembles that of the anesthetic agents in the para-amino-benzoic acid class (such as procaine and tetracaine), BNZ exhibits anesthetic or numbing action. BNZ also inhibits the transmission of impulses of the cough reflex in the medulla's vagal nuclei (Tessalon, 2020). There are several proposed mechanisms of BNZ; it is also a potent voltage-gated sodium channel inhibitor.BNZ, a non-narcotic antitussive agent, is 2, 5, 8, 11, 14, 17, 20, 23, 26-nonaoxaoctacosan-28-yl p-(butylamino) benzoate. The appearance of the drug is a clear, pale yellow, viscous liquid with slight hygroscopic nature. The drug has a pka of 3.03 and is freely soluble in water, methanol, acetone, ethanol, and tetrahydrofuran. Oligomerization is a chemical process that transform monomers into macromolecular complexes through a finite degree of polymerization. Oligomers are low molecular weight polymers containing a small number of repeat units whose physical attributes are significantly dependent on the chain's length. Oligomers are essential intermediates of the polymerization reaction that find wide, direct applications in material science. Oligomers grouped into addition oligomers and condensation oligomers, based on the repeating unit (Tessalon, 2020).

There are various other publications found in the literature in terms of its synthesis of polymerization (Fache et al., 2015: 526-535; Goodbody et al., 2008: 414-423; Fallas et al., 2017: 353-360; Leguizamon and Scott, 2020: 784; FDA, 2010; Yang, 2018a: 2986; Yang, 2018b: 516). The reported literature evaluated the oligomer content using the various analytical techniques that were not suitable for quantitative determination. None of the available methods found suitable. Hence a novel, specific, and selective analytical

method in HPLC with UV detection is developed and validated for determination (ICH, 2005; ICH, 2005; USP, 2017) of oligomers content in BNZ drug substance.

Materials and Methods

Chemicals and Reagents

BNZ (purity-99.5%) primary and/or united standard reference standard procured from Siegfried AG Pharma, an Active Pharmaceutical Ingredient supplier. All other chemicals and solvents used as Analytical / HPLC grade. The analysis carried out on Waters Alliance HPLC systems 2695 separation module connected to 2996 Photodiode array detector and the data acquisition carried out through Empower 3 software.

Experimental Chromatographic conditions

The separation of oligomers achieved using 0.25% of Ammonium formate buffer and methanol as a mobile phase in the ratio of 350: 650 (v/v) with Phenomenex Luna C18, 250 X 4.6 mm 5-micron column. Methanol used as a sample diluent, and the isocratic elution of the method has a flow rate, 1.0 ml/min with an injection volume of 20 µL at UV detection of 310 nm. The column oven temperature is 40 °C and the total chromatographic run time established is about 80 minutes.

Dilute Standard Preparation

Note: BNZ Standard compound is a semisolid, and appropriate care should be taken before weighing

The dilute standard solution for BNZ is prepared with diluent to obtain a concentration of 0.5 mg/ml. Representative chromatograms of the blank and dilute standard preparation are shown in Fig. 1.

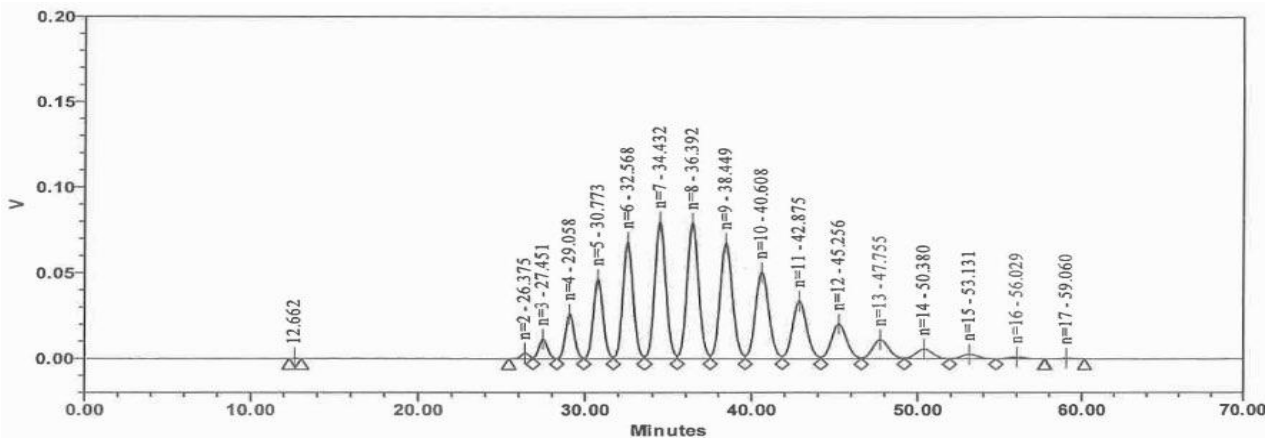

Fig. 1. Dilute standard solution

The system suitability and chromatographic evaluation are presented in Table 1.

Table 1. System suitability results

n#	Average Peak area	Retention time	Average %Area	Theoretical Plates	Resolution
3	404445	27.40	1.62	13506	1.1
4	989791	29.00	3.96	13306	1.6
5	1888038	30.72	7.55	13084	1.6
6	2951612	32.51	11.81	12885	1.6
7	3697257	34.37	14.79	12687	1.5
8	3896971	36.32	15.59	12497	1.5

9	3538814	38.38	14.15	12316	1.5
10	2816910	40.53	11.27	12158	1.5
11	1994687	42.79	7.98	12026	1.5
12	1271173	45.17	5.08	11921	1.5
13	741581	47.67	2.97	11811	1.4
14	394737	50.28	1.58	11658	1.4
15	189222	53.03	0.76	11798	1.4
16	82775	55.92	0.33	12174	1.4
17	28418	58.94	0.11	14001	1.5
Mean	1562298	40.59	6.25	NA	NA
SD	1415107	10.4	5.7		
%RSD	90.6	25.7	90.6		

Sample Preparation

Note: Shake well before removing the oral solution.

Accurately weigh the BNZ drug substance sample is prepared to obtain a concentration of 0.5 mg/ml. Representative chromatograms of the sample preparation are shown in Fig. 2.

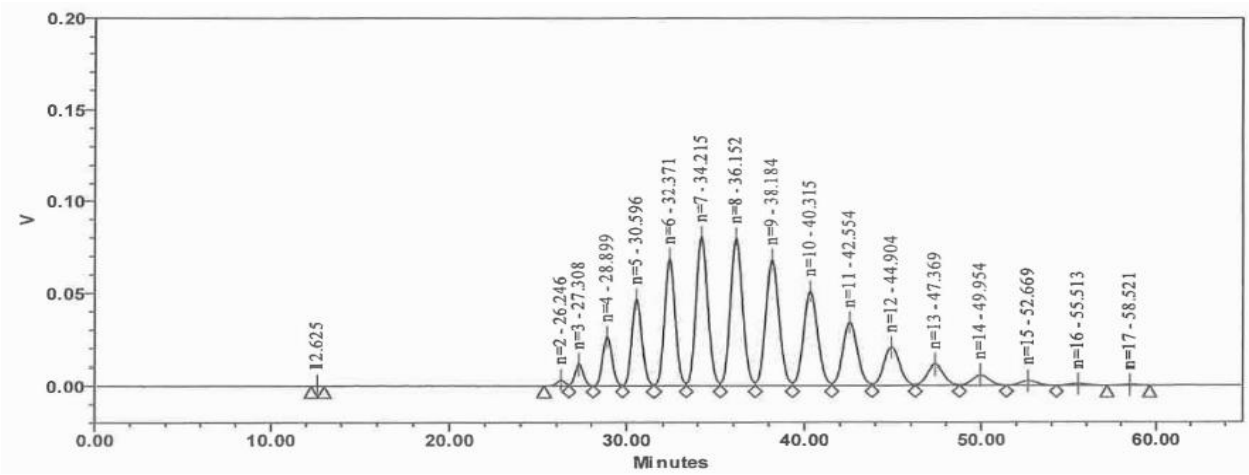

Fig. 2. Sample Chromatogram

Method Development

The analytical method's initial development is to achieve the separation of all Oligomer along with unspecified oligomers with a minimum adequate chromatographic resolution of 1.0 from each of the subject component peaks under common chromatographic conditions. Initially, the separation of all the peaks was studied using a Phenomenex Luna C18 (250 X 4.6 mm) 5-micron column with isocratic elution, which was used in the HPLC, equipped with a photodiode array detector. The detection wavelength maximum observed at 310 nm. The mobile phase consisted of water and acetonitrile in the ratio of 30:70, respectively, at a flow rate, 1.0 ml/min and the column temperature maintained at 30°C. Co-elution of all oligomer peak shapes was not well separated among n=3 to n=17. Chromatography achieved using the mixture of 0.25% of Ammonium formate buffer and methanol in the ratio of 35:65 (v/v), respectively, at a flow rate of 1.0 ml/min with a column temperature at 40°C. Using the above-optimized

conditions, all Oligomer were well-separated from each other. Hence, the optimized method validated as per current ICH Q2 guidelines (Dongala et al., 2020).

Results

Analytical method Validation

Specificity

Specificity is one of HPLC's significant features, and it refers to the analytical method's ability to unequivocally between the analyte and the other components in the complex mixture (Katakam et al., 2020a,2020b, 2020c, 2020d).

Repeatability and Intermediate Precision

The analytical method's repeatability was demonstrated by analyzing six preparation; each of the oligomer samples was performed by two different analysts in two different columns and LC systems. The average area of the twelve preparations of repeatability and intermediate precision data is summarized in Table 2.

Table 2. Method Precision and Intermediate Precision results

Preparation#	n#	% Area	
		Method Precision	Intermediate Precision
1	8	15.16	15.11
2	8	15.18	15.18
3	8	15.14	15.13
4	8	15.16	15.14
5	8	15.16	15.16
6	8	15.16	15.17
Mean		15.16	15.15
SD		0.0	0.03
%RSD		0.09	0.18
Overall Mean		NA	15.15
Overall SD			0.02

Linearity

Oligomer's peak area response versus concentrations is linear in the range between 0.8 to 1.2 mg/ml (representing 80-120% of the Oligomer concentration). The difference for peak area % of each oligomer peak at each concentration compare with nominal concentration. The results are given in Table 3.

Table 3. Linearity 80-120% results

n#	80%	90%	100%	110%	120%
3	1.21	1.22	1.21	1.22	1.21
4	3.07	3.08	3.08	3.08	3.07
5	6.18	6.18	6.17	6.17	6.16
6	10.24	10.23	10.23	10.23	10.21
7	13.60	13.58	13.57	13.57	13.55
8	15.18	15.16	15.16	15.16	15.14
9	14.60	14.58	14.59	14.58	14.57

10	12.30	12.30	12.30	12.31	12.29
11	9.22	9.22	9.24	9.23	9.23
12	6.22	6.23	6.24	6.23	6.24
13	3.83	3.83	3.85	3.84	3.85
14	2.15	2.15	2.16	2.16	2.18
15	1.10	1.10	1.10	1.10	1.13
16	0.51	0.51	0.51	0.51	0.53
17	0.19	0.20	0.20	0.20	0.21

Stability of Sample Solutions

The solution stability and mobile phase stability were established for the sample and dilute standard solutions of assay determination level at room temperature condition (Palakurthi et al., 2020). The solutions' stability is evaluated using fresh dilute standard solution, and % recoveries are compared from the initial (T_0) to T_t hours and found to be stable for 72 hours at room temperature storage condition.

Robustness

The deliberate variations in the optimized method parameters (effect of change in flow rate, temperature, and methanol composition in the mobile phase) were performed and evaluated the system suitability parameters in terms of retention time, %RSD, and resolution n=7, n=8, and n=8 & n=9 were studied. The method was unaffected with deliberate changes, and the results are summarized in Table 4.

Table 4. Robustness results

Parameter		Peak Area % RSD	Retention Time	Resolution between n=7 & n=8	Resolution between n=8 & n=9
Flow variation (± 0.05 mL/min)	Normal Conditions	0.30	0.04	1.5	1.5
	0.95 mL/min	0.12	0.25	1.5	1.5
	1.05 mL/min	0.12	0.05	1.5	1.5
Temperature Variation (+5°C)	35°C	0.17	0.10	1.5	1.5
	45°C	0.26	0.19	1.6	1.5
Mobile phase variation (± 10% v/v) (Organic Composition)	350:585 v/v	0.04	0.08	1.6	1.6
	350:715 v/v	0.28	0.03	1.6	1.5

Representative chromatograms are shown in Figs. 3-8.

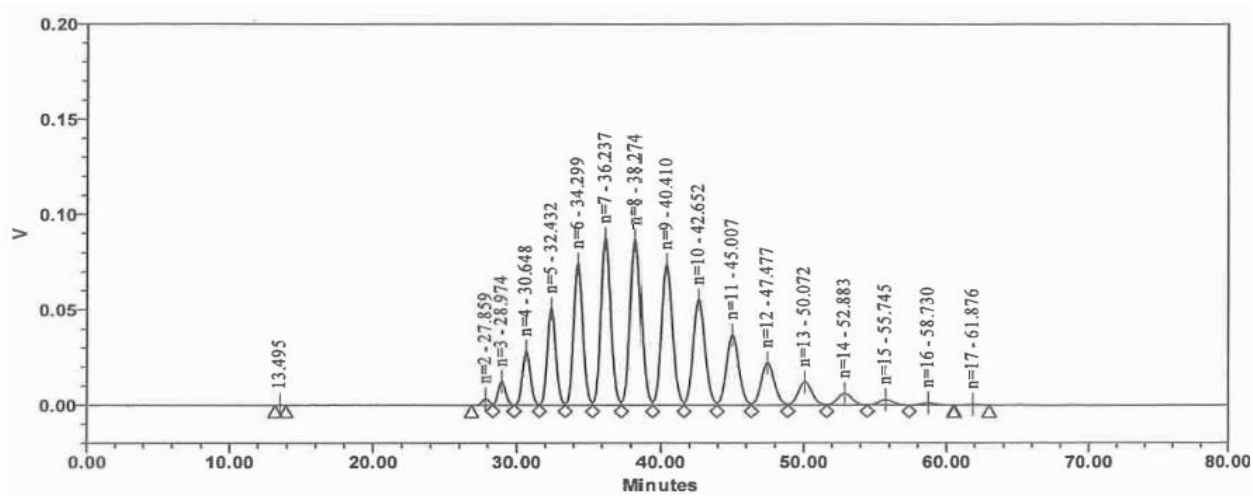

Fig. 3. Robustness Low-flow rate Chromatogram

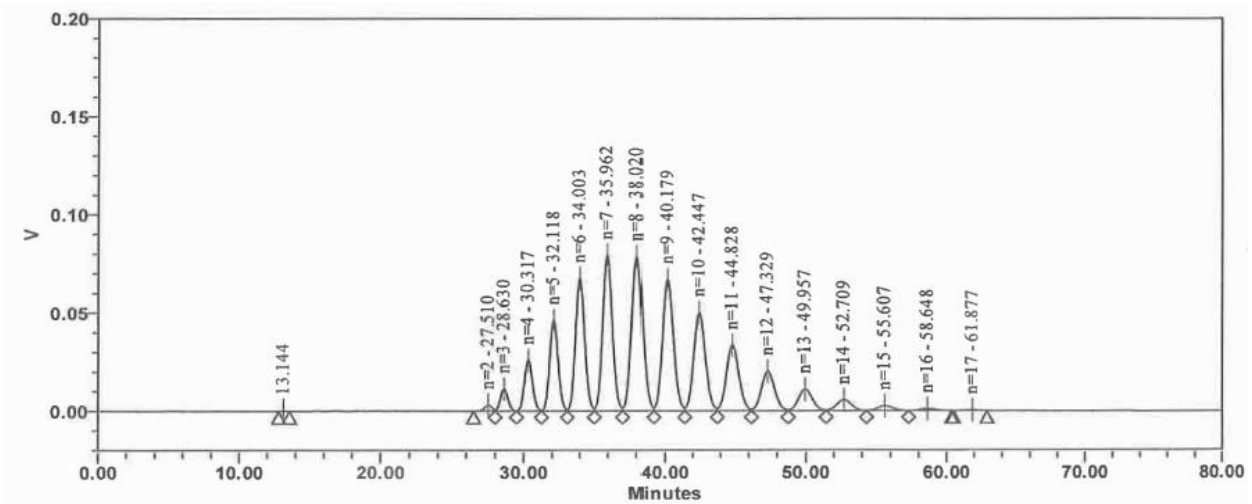

Fig. 4. Robustness High- flow rate Chromatogram

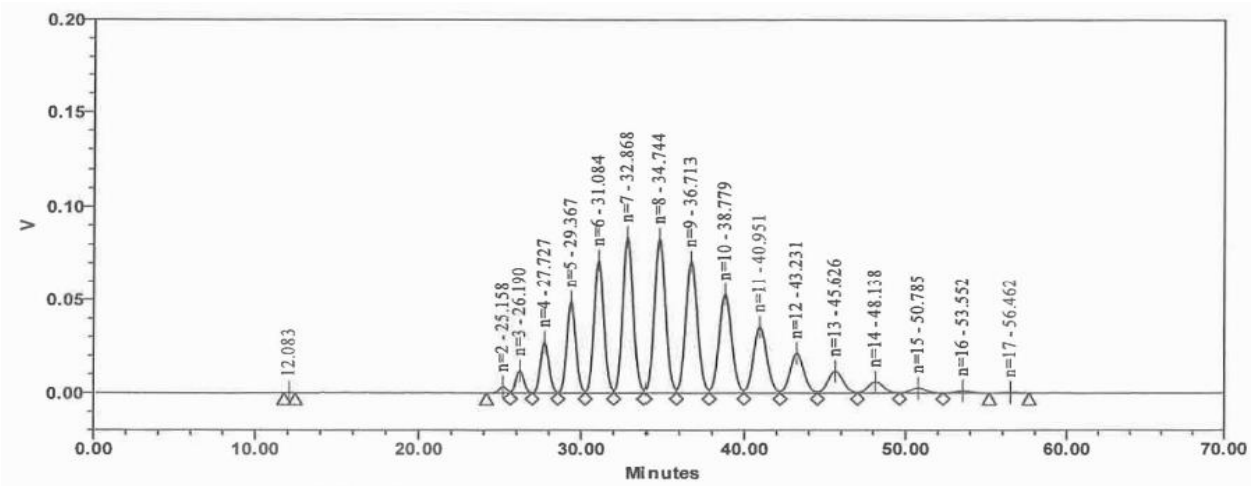

Fig. 5. Robustness Low-Column Temperature Chromatogram

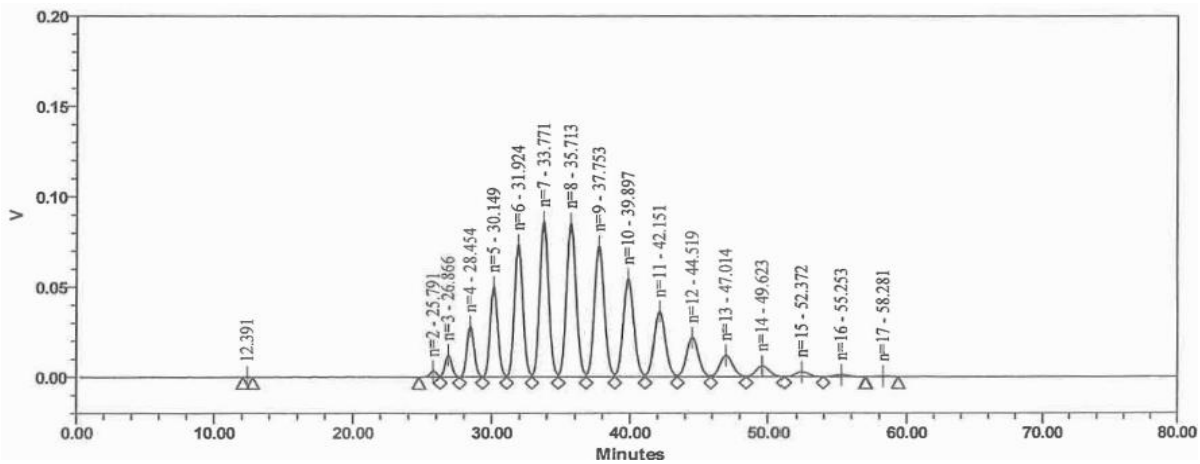

Fig. 6. Robustness High- Column Temperature Chromatogram

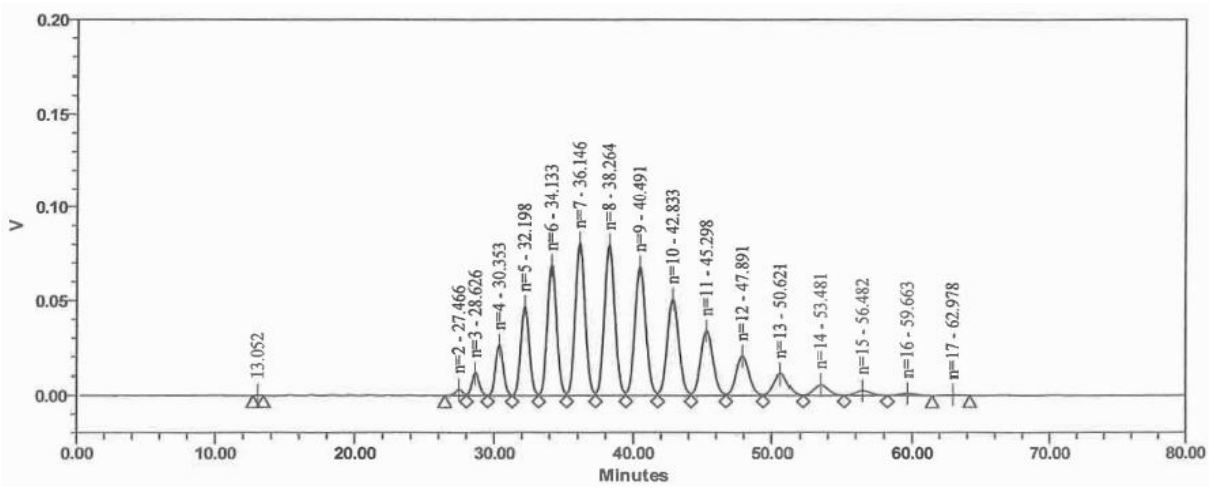

Fig. 7. Robustness Low-Organic Composition Chromatogram

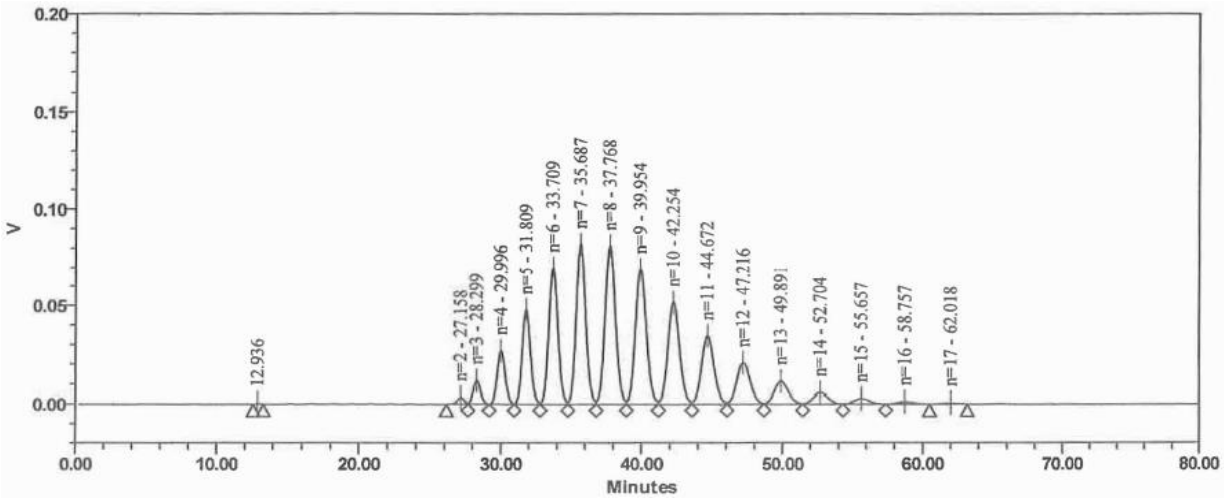

Fig. 8. Robustness High-Organic Composition Chromatogram

Sample Analysis

The commercial drug substances of three different lots were tested using this validated method, and the oligomer content for n=3 to n=17 was determined through the

% area normalization method. The obtained results and specifications are presented in Table 5.

Table 5. Sample Analysis result

n#	% Area			Specification
	Batch# 1	Batch# 2	Batch# 3	
3	1.62	1.51	1.45	NMT 2%
4	3.96	3.66	3.68	NMT 4%
5	7.55	7.43	7.49	NMT 8%
6	11.81	11.67	11.43	6% -12%
7	14.79	14.43	14.56	9% - 17%
8	15.59	15.46	15.38	10% - 20%
9	14.15	14.56	14.99	10% - 20%
10	11.27	11.76	11.59	9% -17%
11	7.98	7.56	7.34	7% - 13%
12	5.08	5.76	5.54	5% - 9%
13	2.97	2.54	2.12	NMT 6%
14	1.58	1.32	1.17	NMT 4%
15	0.76	0.89	0.55	NMT 2%
16	0.33	0.78	0.69	NMT 2%
17	0.11	0.64	0.46	NMT 1%

Conclusion

The method provides selective quantification of oligomers without the interference of blank. Method validation experiments proved to be precise, linear, specific, robust, and rugged. This validated analytical method can determine oligomer content in BNZ Drug substances in the quality control laboratory.

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