

Certificate of Analysis

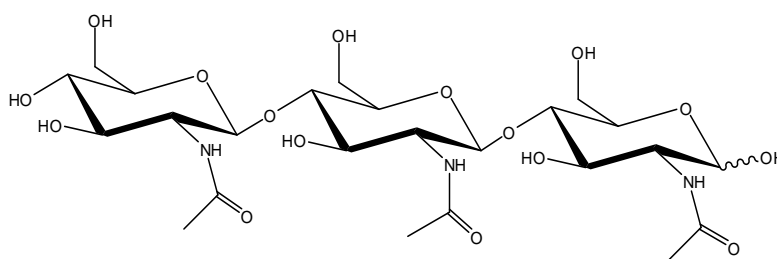
Chitotriose Standard

Cat. # : BQ-CHITOTRIOSE-01

Batch : B29D-01

Size : 5 ± 0.30 nmol by qNMR

Structure



Chemical Formula: $C_{24}H_{41}N_3O_{16}$
 Exact Mass: 627.25
 Molecular Weight: 627.59
 m/z: 627.25 (100.0%), 628.25 (28.1%), 629.25 (3.6%), 629.26 (3.5%), 630.26 (1.2%)
 Elemental Analysis: C, 45.93; H, 6.58; N, 6.70; O, 40.79



Oxford Notation



CFG Notation



Text Notation

Purity: 94% pure as assessed by HPLC of 2-AB label chitotriose (see Fig 3)

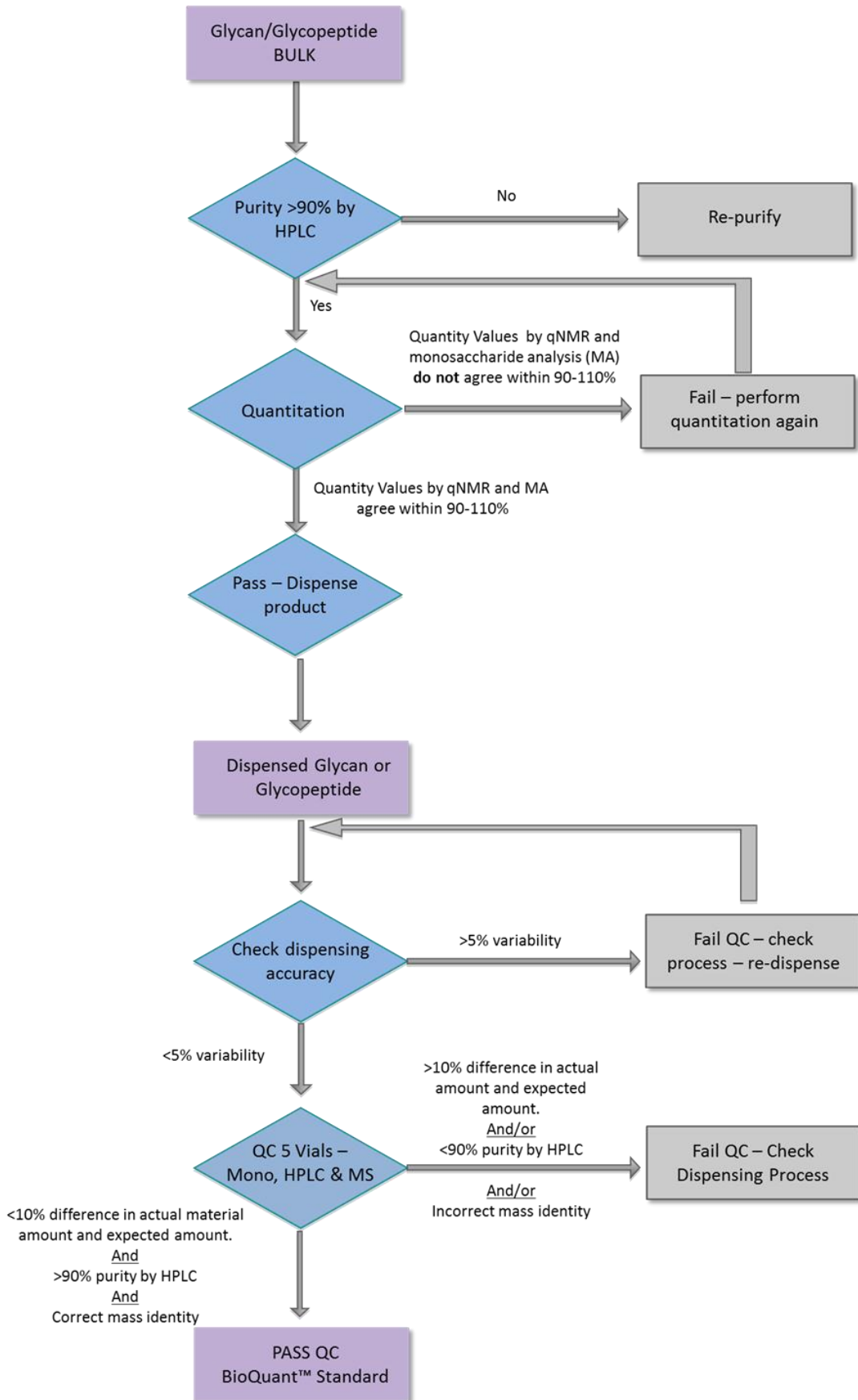
BQ-CHITOTRIOSE-01 Quantity Summary

The amount of chitotriose to be dispensed per vial is determined by quantitative Nuclear Magnetic Resonance (qNMR) of the bulk chitotriose stock.

Once dispensed the amount of chitotriose per vial is determined by monosaccharide analysis.

These determinations are detailed on the following pages, but a summary is provided below:

	BQ-Chitotriose-01
qNMR based determination: derived from chitotriose bulk stock	= 5 ± 0.30 nmol
<i>5% dispensing error (determined by proprietary method)</i>	
Monosaccharide based determination	= 4.79 ± 0.14 nmol



Quantitative Nuclear Magnetic Resonance (qNMR)

The concentration of the bulk chitotriose was calculated by qNMR by comparison to a certified quantitative standard (Table 1).

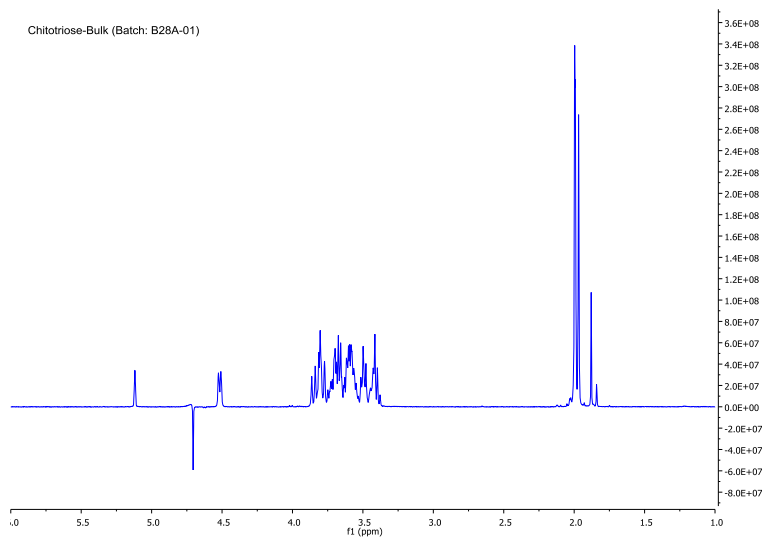


Figure 1: 500MHz ¹H-NMR of Chitotriose-Bulk (BQ-Chitotriose-Bulk, Batch B28A-04)

Sample	Batch number	qNMR determined concentration (mM)	V Eu Chloride added (μl)	V total (μl)	Concentration re-calculated after adding Eu (mM)
BQ-CHITOTRIOSE-BULK	B28A-04	0.162 ± 0.05	23	3523	0.161 ± 0.05

Table 1. Concentration of BQ-Chitotriose-Bulk (Batch B28A-04) calculated by qNMR

The bulk chitotriose concentration value was used to determine the amount of sample to be dispensed per vial to obtain 5 nmol of chitotriose per vial. The dispensing error is predicted to be than less 5%.

Randomly selected vials were then chosen for monosaccharide analysis in order to verify the amount of chitotriose dispensed per vial.

Monosaccharide analysis of BQ-Chitotriose-01

The analysis was performed on 4 replicates using hydrochloric acid (HCl) hydrolysis to obtain N-acetylglucosamine (GlcNAc – acid hydrolysed to glucosamine GlcN) values using the LudgerTag 2-AA Monosaccharide Release and Labelling Kit.

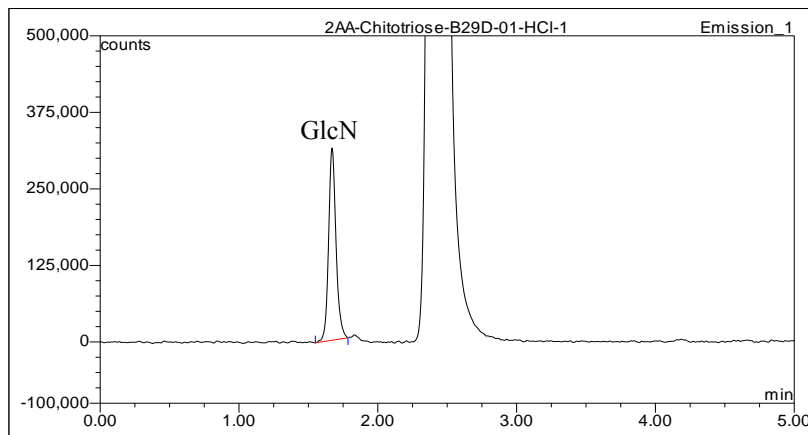


Figure 2: LudgerSep-uR2 HPLC profile of 2-aminobenzoic acid (2-AA) labeled GlcN of HCl hydrolysed BQ-Chitotriose-01 (Batch B29D-01).

BQ-Chitotriose-B29D-01 nmol of GlcN found in each vial			
Sample 1	13.74		
Sample 2	14.69		
Sample 3	14.54		
Sample 4	14.54	Spec 90-100% of expected value of 15nmol	
Average	14.38	nmol	
SD	0.43	95.84	
CV	3.01	PASS	
nmol of Chitotriose found in each vial			
Sample 1	4.58		
Sample 2	4.90		
Sample 3	4.85		
Sample 4	4.85		
Average	4.79		
SD	0.14		
CV	3.01		

Table 2. Amount of BQ-Chitotriose-01 (Batch B29D-01) per vial calculated by monosaccharide analysis

The chitotriose quantity per vial was calculated using the amount of GlcN per vial. By averaging the results from 4 separate vials, we get a quantity of 4.74 ± 0.14 per vial.

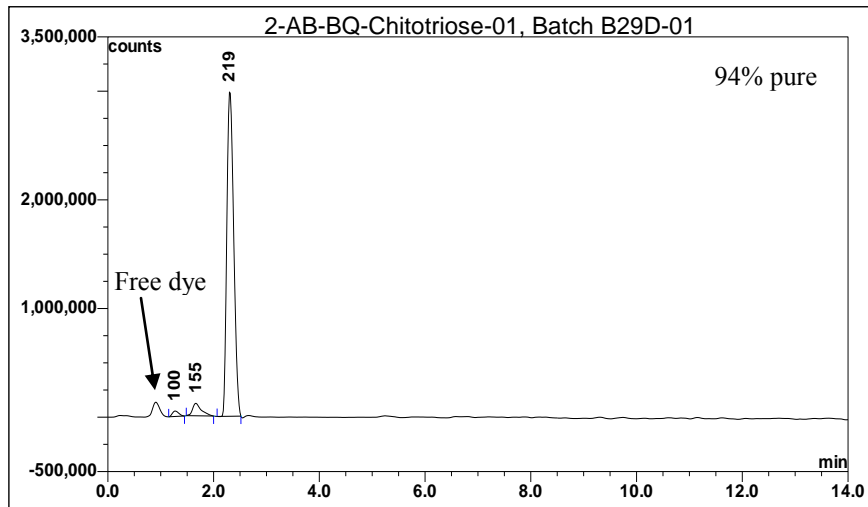


Figure 3: LudgerSep-BEH HPLC profile of 2AB labelled chitotriose (BQ-Chitotriose-01, Batch B29D-01)

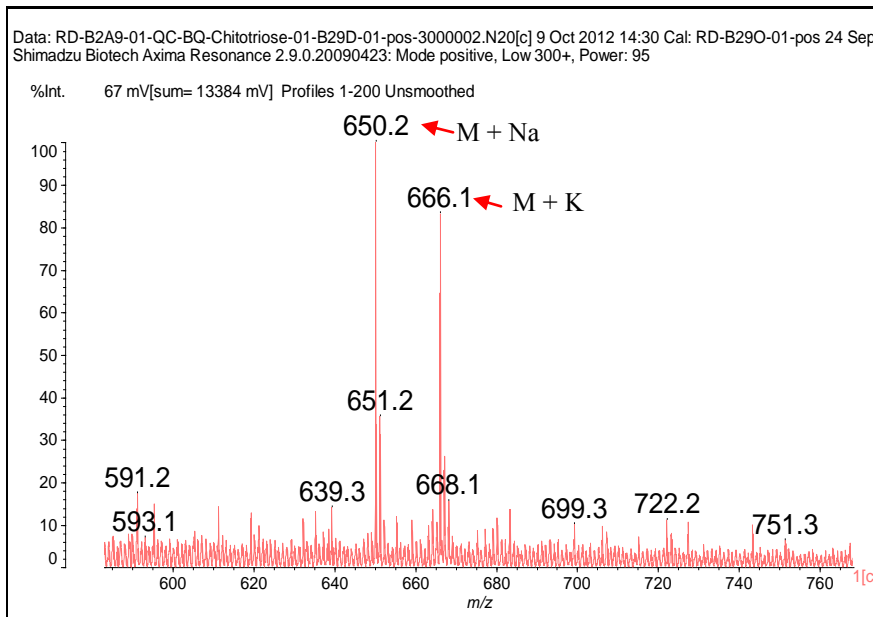


Figure 4: Positive ion MALDI mass spectrum of Chitotriose-Bulk (BQ-Chitotriose-01, Batch B29D-01)