



<b>Owner:</b> [REDACTED]	<b>Number:</b> Chem-Form-62
<b>Author:</b> [REDACTED]	<b>Version:</b> 1
<b>Active:</b> 15/06/2021	<b>Review:</b> <QPulse_DocReviewDate>
<b>Title:</b> Assay for Lipids in Vaccines by HPLC CAD (Bio-BPC-Method-24 Pfizer)	

TGA SAMPLE No. 2111004115

SUBSTANCES ASSAYED: ALC-0159, Cholesterol,

Lot number: FL5333

ALC-0315, DSPC

METHOD REFERENCE: TM100010322 (Pfizer)/BIO-BPC-Method-24

INSTRUMENT OR SYSTEM No. 20 with CAD detector... Method Modifications approved by:

SYSTEM SUITABILITY REQUIREMENTS MET (Y/N) ?..... ASSAY REQUIREMENTS MET (Y/N)?.....

## RESULTS

TGA Sample number	Preparation	Lipid content (mg/mL)			
		ALC-0159	Cholesterol	ALC-0315	DSPC
2111004115	1	0.792	2.776	6.161	1.395
	2	0.800	2.776	6.166	1.393
	3	0.804	2.804	6.213	1.405
Average	-	0.80	2.79	6.18	1.40
% RSD [REDACTED]	-	0.8	0.6	0.5	0.5

**ATTACHMENTS:** Analysts Notes worksheets  Result summary report  System suitability report   
 Example chromatograms  Run and method summary  Calibration plots   
 MP preparation details  or refer to sample Other .....

## REQUIREMENTS (Manuf.) [REDACTED]

Identification RT requirements met ? (RT  $\pm$  5% compared to SST) (Y/N)

IDENTIFIED AS ALC-0159, Cholesterol, ALC-0315 and DSPC

RESULT

PASS FAIL 

Signature of Analyst Electronically signed by [REDACTED]

Date 17/11/2021

Checked by Official Analyst... Electronically signed by [REDACTED]

Date... 19/11/21.....

## WORKING STANDARD CURVE PREPARATION DETAILS

Date Stock solution prepared: 01/11/2021 Prepared by: [REDACTED] Within expiry Y/N

Attached  or Refer to (TRIM Record number/Sample number) [D21-3283855](#)

	ALC-0159	Cholesterol	ALC-0315	DSPC5
Concentration in stock solution (mg/mL)	0.29830	1.00776	2.29626	0.55186

Prepared by: [REDACTED] Date prepared 16/11/2021 POVA LIMS: 33271

Level	Mixed Lipid Soln (uL)	MeOH (uL)	DF	Final Target conc (mg/mL)		Actual conc (mg/mL)
1	50	950	20	ALC-0159	0.015	0.01492
				Cholesterol	0.050	0.05039
				ALC-0315	0.120	0.11481
				DSPC	0.028	0.02759
2	100	900	10	ALC-0159	0.030	0.02983
				Cholesterol	0.100	0.10078
				ALC-0315	0.240	0.22963
				DSPC	0.056	0.05519
3	100	300	4	ALC-0159	0.075	0.07458
				Cholesterol	0.250	0.25194
				ALC-0315	0.600	0.57407
				DSPC	0.140	0.13797
4	200	200	2	ALC-0159	0.150	0.14915
				Cholesterol	0.500	0.50388
				ALC-0315	1.200	1.14813
				DSPC	0.280	0.27593
5	225	75	1.33	ALC-0159	0.225	0.22373
				Cholesterol	0.750	0.75582
				ALC-0315	1.800	1.72220
				DSPC	0.420	0.41390

## ASSAY CONTROL PREPARATION DETAILS

Date control solution prepared: 27 Sep 2021 Within expiry Y/N

Attached  or Refer to (TRIM/ Record number/Sample number) [D21-3147439](#)

Note: Assay control solution out of expiry. Results accepted as still within range and recovery for Drug Product reference material acceptable.

## DP REFERENCE MATERIAL PREPARED Y/N

LIMS number	Lot number/Expiry	Sample solution (uL)	MeOH (uL)	DF
2103001141	EE8493 – 05/02/2022	50	950	20

**SAMPLE PREPARATION DETAILS****Date prepared:** 16/11/2021 **Prepared by:** [REDACTED] **POVA LIMS:** 33271

Preparation	Sample solution (uL)	MeOH (uL)	DF
1	50	950	20
2	50	950	20
3	50	950	20

Methanol LCMS grade; Supplier: Fisher

Lot No. 205074

**METHOD MODIFICATIONS**

Additional parameters required for eSATIN module

Environment	50 Hz
Sampling rate	2
Scale factor	1000

The range on the CAD detector was set to 500

Level 3 dilution for standard curve ~~200uL~~ (100uL) mixed lipid stock solution added to ~~200uL~~ (300uL) methanol instead of 100uL stock solution added to 100uL methanol. (to increase amount of solution, concentration not changed)

Level 4 dilution for standard curve ~~400uL~~ (200uL) mixed lipid stock solution added to ~~300uL~~ (200uL) methanol instead of 50uL stock solution added to 150uL methanol (to increase amount of solution, concentration not changed)

Above script amended by [REDACTED] on 17/11/2021

*Expected results for drug product reference material ALC0159: 0.86; Cholesterol: 2.87; ALC-0315: 6.78; DSPC: 1.43 mg/mL. Recovery ALC0159: 95.3; Cholesterol: 100.3; ALC0315: 96.3; DSPC: 97.9 %.*