Appendix 5 AEL QA/QC

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Pigments STD Calibration Levels used for Developing the Calibration Curves

		level1	level2	level3	level4	level5	level6	level7	Original, ug/L	RT
#1	**Chlc2**	8.3	16.7	41.7	83.4	166.8	417.0	X	**834**	**3.48**
#2	**Fuco**	9.0	18.0	36.0	71.0	143.0	286.0	571.0	**1141**	**4.94**
#3	**Ddx**	11.9	23.9	47.9	95.7	191.4	478.5	X	**957**	**6.89**
#4	**Sud**	25.0	50.0	100.0	180.0	350.0	760.0	1600.0	**3200**	**7.43**
#5	**Allo**	9.0	18.0	36.0	72.0	144.0	287.0	574.0	**1147**	**7.71**
#7	**Zea**	8.0	16.0	32.0	65.0	120.0	240.0	475.0	**950**	**7.95**
#8	**Lut**	11.0	26.0	44.0	88.0	175.1	320.0	700.5	**1401**	**8.27**
#9	**Cant**	9.0	18.0	35.0	70.0	140.0	280.0	560.0	**1118**	**9.79**
#10	**Chlb**	19.0	38.0	76.0	151.0	303.0	605.0	X	**1210**	**10.80**
#11	**Chla**	14.2	28.5	57.0	120.0	228.0	441.0	911.0	**1822**	**12.31**
#12	**DVCh-a**	10.7	21.4	53.6	107.1	214.2	535.5	X	**1071**	**12.55**
#13	**Echi**	8.0	16.0	31.0	62.0	120.0	220.0	408.5	**817**	**13.18**
#15	**Phta**	14.0	28.0	55.0	108.0	220.0	440.0	879.0	**3517**	**19.43**
#16	**Acar**	11.0	22.0	44.0	88.0	176.0	352.0	X	**703**	**20.30**
#17	**Beta**	14.5	29.0	58.0	72.7	145.4	363.5	X	**727**	**21.11**

Calibration Standard (CAL) – A solution prepared from dilution of a stock standard solution.
The CAL solution is used to calibrate the instrument response with respect to analyte concentration or mass.
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The calibration created for each pigment individually was detector response versus concentration of a pigment in CAL solution (ug/L).
A calibration curve constructed for each individual pigment is a linear regrassion with a typical coefficient of dtermination >0.9.

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.

RT -- Retention Time of an analytes on a chromatogram (min).

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HPLC Performance-verification using CALCHK

	Analyte	CALCHK ug/L	Low WL -15%	High WL +15%	Batch#1_1-27 CALCHK,%
#2	**Fuco**	700.0	595.0	805.0	106.5%
#4	**Sud**	1200.0	1020.0	1380.0	99.4%
#9	**Cant**	150.0	127.5	172.5	112.5%
#11	**Chla**	100.0	85.0	115.0	106.8%

na- not applicable

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.
Warning Limit (WL) ***for Sud is +/-15%***

Calibration Check Standard (CALCHK) – A mid-point calibration solution that is analyzed periodically in a sample set to verify that the instrument response to the analyte has not changed during the course of analysis.
CALCHK is analyzed for every batch os samples. It is analyzed first, before the sample batch, so to verify calibration. If CAKCHK is not +/-15% of the expected for any analyte, then the instrument must be recalibrated. CALCHK is calculated and presented in % Recovery compared to the expected concentration.
Warning Limit (WL) ***for CALCHK is +/-15% for each individual Pigment Analyte***

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HPLC Performance-verification using sample duplicate

		RT, min	Batch#1_1-27 Dup.Reproduce., %
#1	**Chlc2**	**3.48**	114.8
#2	**Fuco**	**4.94**	108.6
#3	**Ddx**	**6.89**	111.6
#4	**Sud**	7.43	107.1
#5	**Allo**	**7.71**	112.7
#7	**Zea**	**7.95**	114.0
#8	**Lut**	**8.27**	97.4
#9	**Cant**	**9.79**	105.0
#10	**Chlb**	**10.80**	106.8
#11	**Chla**	**12.31**	109.3
#12	**DVCh-a**	**12.55**	116.5
#13	**Echi**	**13.18**	103.8
#15	**Phta**	**19.43**	104.0
#16	**Acar**	**20.30**	107.6
#17	**Beta**	**21.11**	na

RT -- Retention Time of an analytes on a chromatogram (min).

Duplicate Reproducibility- ***is verified using a sample that is treated exactly the same throughout field and laboratory procedures.***
Analyses of duplicate sample give a measure of the precision associated with the sample preparation procedures, as well as analytical determination.

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HPLC Performance-verification using Pigments STD spiked into a sample matrix.

#			SPK-level ug/L	RT	Low WL, -30%	High WL, +30%	Batch#1_1-27 %
#2	1	**Fuco**	400.0		280.0	520.0	100.74
#9	2	**Cant**	640.0		448.0	832.0	111.2
#11	3	**Chla**	400.0		280.0	520.0	90.2

Matrix Spike Recovery -a sample with pre-determined levels of analytes (analyzed in duplicate) is used. Known amounts of 3 Pigments are added prior to extraction and analytical procedures. This QA/QC is used as a measure of both sample extraction and analytical determination procedures.

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HPLC Performance-verification using Pigments STD as surrogates spiked into a MBSR sample followed by SamplePrep procedure.

		SPK-level	RT	Batch#1_1-27
		ug/L		Surrogate Recov., %
#2	**Fuco**	120.0	**4.94**	102.5
#5	**Sud**	450.0	**7.43**	94.8
#9	**Cant**	120.0	**9.79**	114.3
#11	**Chla**	80.0	**12.31**	85.2
#1	**Chlc2**	0.0	**3.48**	ND
#3	**Ddx**	0.0	**6.89**	ND
#5	**Allo**	0.0	**7.71**	ND
#7	**Zea**	0.0	**7.95**	ND
#8	**Lut**	0.0	**8.27**	ND
#9	**Cant**	0.0	**9.79**	ND
#10	**Chlb**	0.0	**10.80**	ND
#12	**DVCh-a**	0.0	**12.55**	ND
#13	**Echi**	0.0	**13.18**	ND
#15	**Phta**	0.0	**19.43**	ND
#16	**Acar**	0.0	**20.30**	ND
#17	**Beta**	0.0	**21.11**	ND

ND--Non detectable.

Method Blank-Spike Recovery (MBSR) – A blank sample (empty glass tube) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal reference standards (Sud at 400-800 ug/L), and surrogates (Fuco, Cant, Chla at the concentrations 120, 120, 80 ug/L, respectively). The MBSR is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus. If the MBSR value of the analytes other than Fuco, Sud, Cant and Chla constitute 10% or more of the analyte level determined in a sample, the contamination must be corrected and fresh samples must be analyzed when acceptable MBSR values have been obtained. The % Recovery of the surrogates (Fuco, Cant, Chla) must be within +/-20%. At least one MBSR is analyzed with each sample batch.

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Pigments STD Calibration Levels used for Developing the Calibration Curves

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#13	**Echi**	8.0	16.0	31.0	62.0	120.0	220.0	408.5	**817**	**13.18**
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HPLC Performance-verification using CALCHK

	Analyte	CALCHK ug/L	Low WL -15%	High WL +15%	ch#2_28-45,61,62 CALCHK,%
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#4	**Sud**	1200.0	1020.0	1380.0	92.5%
#9	**Cant**	150.0	127.5	172.5	105.0%
#11	**Chla**	100.0	85.0	115.0	108.1%

na- not applicable

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HPLC Performance-verification using sample duplicate

		RT, min	Batch#2_28-45,61,62 Dup.Reproduce., %
#1	**Chlc2**	**3.48**	102.2
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#3	**Ddx**	**6.89**	107.5
#4	**Sud**	7.43	98.4
#5	**Allo**	**7.71**	96.8
#7	**Zea**	**7.95**	98.3
#8	**Lut**	**8.27**	106.6
#9	**Cant**	**9.79**	96.5
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HPLC Performance-verification using Pigments STD spiked into a sample matrix.

#			SPK-level ug/L	RT	Low WL, -30%	High WL, +30%	Batch#2_28-45,61,62 %
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#9	2	**Cant**	120.0		84.0	156.0	101.9
#11	3	**Chla**	80.0		56.0	104.0	109.1

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HPLC Performance-verification using Pigments STD as surrogates spiked into a MBSR sample followed by SamplePrep procedure.

		SPK-level ug/L	RT	Batch#2_28-45,61,62 Surrogate Recov., %
#2	**Fuco**	560.0	**4.94**	83.6
#5	**Sud**	1200.0	**7.43**	100.5
#9	**Cant**	120.0	**9.79**	113.7
#11	**Chla**	80.0	**12.31**	83.4
#1	**Chlc2**	0.0	**3.48**	ND
#3	**Ddx**	0.0	**6.89**	ND
#5	**Allo**	0.0	**7.71**	ND
#7	**Zea**	0.0	**7.95**	ND
#8	**Lut**	0.0	**8.27**	ND
#9	**Cant**	0.0	**9.79**	ND
#10	**Chlb**	0.0	**10.80**	ND
#12	**DVCh-a**	0.0	**12.55**	ND
#13	**Echi**	0.0	**13.18**	ND
#15	**Phta**	0.0	**19.43**	ND
#16	**Acar**	0.0	**20.30**	ND
#17	**Beta**	0.0	**21.11**	ND

ND--Non detectable.

Method Blank-Spike Recovery (MBSR) – A blank sample (empty glass tube) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal reference standards (Sud at 400-800 ug/L), and surrogates (Fuco, Cant, Chla at the concentrations 560, 120, 80 ug/L, respectively). The MBSR is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus. If the MBSR value of the analytes other than Fuco, Sud, Cant and Chla constitute 10% or more of the analyte level determined in a sample, the contamination must be corrected and fresh samples must be analyzed when acceptable MBSR values have been obtained. The % Recovery of the surrogates (Fuco, Cant, Chla) must be within +/-20%. At least one MBSR is analyzed with each sample batch.

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Pigments STD Calibration Levels used for Developing the Calibration Curves

		level1	level2	level3	level4	level5	level6	level7	Original, ug/L	RT
#1	**Chlc2**	8.3	16.7	41.7	83.4	166.8	417.0	X	**834**	**3.48**
#2	**Fuco**	9.0	18.0	36.0	71.0	143.0	286.0	571.0	**1141**	**4.94**
#3	**Ddx**	11.9	23.9	47.9	95.7	191.4	478.5	X	**957**	**6.89**
#4	**Sud**	25.0	50.0	100.0	180.0	350.0	760.0	1600.0	**3200**	**7.43**
#5	**Allo**	9.0	18.0	36.0	72.0	144.0	287.0	574.0	**1147**	**7.71**
#7	**Zea**	8.0	16.0	32.0	65.0	120.0	240.0	475.0	**950**	**7.95**
#8	**Lut**	11.0	26.0	44.0	88.0	175.1	320.0	700.5	**1401**	**8.27**
#9	**Cant**	9.0	18.0	35.0	70.0	140.0	280.0	560.0	**1118**	**9.79**
#10	**Chlb**	19.0	38.0	76.0	151.0	303.0	605.0	X	**1210**	**10.80**
#11	**Chla**	14.2	28.5	57.0	120.0	228.0	441.0	911.0	**1822**	**12.31**
#12	**DVCh-a**	10.7	21.4	53.6	107.1	214.2	535.5	X	**1071**	**12.55**
#13	**Echi**	8.0	16.0	31.0	62.0	120.0	220.0	408.5	**817**	**13.18**
#15	**Phta**	14.0	28.0	55.0	108.0	220.0	440.0	879.0	**3517**	**19.43**
#16	**Acar**	11.0	22.0	44.0	88.0	176.0	352.0	X	**703**	**20.30**
#17	**Beta**	14.5	29.0	58.0	72.7	145.4	363.5	X	**727**	**21.11**

Calibration Standard (CAL) – A solution prepared from dilution of a stock standard solution.
The CAL solution is used to calibrate the instrument response with respect to analyte concentration or mass.
All CAL were prepared using Original STD individual pigment solutions.
Seven concentration levels were prepared using serial dilutions of Original STD solution of each pigment.
The dilution factor of each sequential dilution is 2, the solution used for dilution is Acetone/Methanol at 90/10 ratio.
The calibration created for each pigment individually was detector response versus concentration of a pigment in CAL solution (ug/L).
A calibration curve constructed for each individual pigment is a linear regrassion with a typical coefficient of dtermination >0.9.

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.

RT -- Retention Time of an analytes on a chromatogram (min).

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HPLC Performance-verification using CALCHK

	Analyte	CALCHK ug/L	Low WL -15%	High WL +15%	Batch#3_46-60 CALCHK,%
#2	**Fuco**	700.0	595.0	805.0	101.7%
#4	**Sud**	1200.0	1020.0	1380.0	92.5%
#9	**Cant**	150.0	127.5	172.5	105.0%
#11	**Chla**	100.0	85.0	115.0	100.1%

na- not applicable

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.
Warning Limit (WL) ***for Sud is +/-15%***

Calibration Check Standard (CALCHK) – A mid-point calibration solution that is analyzed periodically in a sample set to verify that the instrument response to the analyte has not changed during the course of analysis.
CALCHK is analyzed for every batch os samples. It is analyzed first, before the sample batch, so to verify calibration. If CAKCHK is not +/-15% of the expected c for any analyte, then the instrument must be recalibrated. CALCHK is calculated and presented in % Recovery compared to the expected concentration.
Warning Limit (WL) ***for CALCHK is +/-15% for each individual Pigment Analyte***

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HPLC Performance-verification using sample duplicate

		RT, min	Batch#3_46-60 Dup.Reproduce., %
#1	**Chlc2**	**3.48**	108.1
#2	**Fuco**	**4.94**	99.9
#3	**Ddx**	**6.89**	108.4
#4	**Sud**	7.43	101.3
#5	**Allo**	**7.71**	na
#7	**Zea**	**7.95**	103.7
#8	**Lut**	**8.27**	115.2
#9	**Cant**	**9.79**	97.5
#10	**Chlb**	**10.80**	na
#11	**Chla**	**12.31**	103.3
#12	**DVCh-a**	**12.55**	91.9
#13	**Echi**	**13.18**	101.9
#15	**Phta**	**19.43**	90.5
#16	**Acar**	**20.30**	86.4
#17	**Beta**	**21.11**	na

RT -- Retention Time of an analytes on a chromatogram (min).

Duplicate Reproducibility- ***is verified using a sample that is treated exactly the same throughout field and laboratory procedures.***
Analyses of duplicate sample give a measure of the precision associated with the sample preparation procedures, as well as analytical determination.

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HPLC Performance-verification using Pigments STD spiked into a sample matrix.

#			SPK-level	RT	Low WL, -30%	High WL, +30%	Batch#3_46-60
			ug/L				%
#2	1	**Fuco**	560.0		392.0	728.0	80.2
#9	2	**Cant**	120.0		84.0	156.0	98.2
#11	3	**Chla**	800.0		560.0	1040.0	115.2

Matrix Spike Recovery -a sample with pre-determined levels of analytes (analyzed in duplicate) is used. Known amounts of 3 Pigments are added prior to extraction and analytical procedures. This QA/QC is used as a measure of both sample extraction and analytical determination procedures.

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HPLC Performance-verification using Pigments STD as surrogates spiked into a MBSR sample followed by SamplePrep procedure.

		SPK-level ug/L	RT	Batch#3_46-60 Surrogate Recov., %
#2	**Fuco**	560.0	**4.94**	109.3
#5	**Sud**	1200.0	**7.43**	94.4
#9	**Cant**	120.0	**9.79**	95.3
#11	**Chla**	80.0	**12.31**	114.5
#1	**Chlc2**	0.0	**3.48**	ND
#3	**Ddx**	0.0	**6.89**	ND
#5	**Allo**	0.0	**7.71**	ND
#7	**Zea**	0.0	**7.95**	ND
#8	**Lut**	0.0	**8.27**	ND
#9	**Cant**	0.0	**9.79**	ND
#10	**Chlb**	0.0	**10.80**	ND
#12	**DVCh-a**	0.0	**12.55**	ND
#13	**Echi**	0.0	**13.18**	ND
#15	**Phta**	0.0	**19.43**	ND
#16	**Acar**	0.0	**20.30**	ND
#17	**Beta**	0.0	**21.11**	ND

ND--Non detectable.

Method Blank-Spike Recovery (MBSR) – A blank sample (empty glass tube) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal reference standards (Sud at 400-1200 ug/L), and surrogates (Fuco, Cant, Chla at the concentrations 560, 120, 80 ug/L, respectively). The MBSR is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus. If the MBSR value of the analytes other than Fuco, Sud, Cant and Chla constitute 10% or more of the analyte level determined in a sample, the contamination must be corrected and fresh samples must be analyzed when acceptable MBSR values have been obtained. The % Recovery of the surrogates (Fuco, Cant, Chla) must be within +/-20%. At least one MBSR is analyzed with each sample batch.

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Pigments STD Calibration Levels used for Developing the Calibration Curves

		level1	level2	level3	level4	level5	level6	level7	Original, ug/L	RT
#1	**Chlc2**	8.3	16.7	41.7	83.4	166.8	417.0	X	**834**	**3.48**
#2	**Fuco**	9.0	18.0	36.0	71.0	143.0	286.0	571.0	**1141**	**4.94**
#3	**Ddx**	11.9	23.9	47.9	95.7	191.4	478.5	X	**957**	**6.89**
#4	**Sud**	25.0	50.0	100.0	180.0	350.0	760.0	1600.0	**3200**	**7.43**
#5	**Allo**	9.0	18.0	36.0	72.0	144.0	287.0	574.0	**1147**	**7.71**
#7	**Zea**	8.0	16.0	32.0	65.0	120.0	240.0	475.0	**950**	**7.95**
#8	**Lut**	11.0	26.0	44.0	88.0	175.1	320.0	700.5	**1401**	**8.27**
#9	**Cant**	9.0	18.0	35.0	70.0	140.0	280.0	560.0	**1118**	**9.79**
#10	**Chlb**	19.0	38.0	76.0	151.0	303.0	605.0	X	**1210**	**10.80**
#11	**Chla**	14.2	28.5	57.0	120.0	228.0	441.0	911.0	**1822**	**12.31**
#12	**DVCh-a**	10.7	21.4	53.6	107.1	214.2	535.5	X	**1071**	**12.55**
#13	**Echi**	8.0	16.0	31.0	62.0	120.0	220.0	408.5	**817**	**13.18**
#15	**Phta**	14.0	28.0	55.0	108.0	220.0	440.0	879.0	**3517**	**19.43**
#16	**Acar**	11.0	22.0	44.0	88.0	176.0	352.0	X	**703**	**20.30**
#17	**Beta**	14.5	29.0	58.0	72.7	145.4	363.5	X	**727**	**21.11**

Calibration Standard (CAL) – A solution prepared from dilution of a stock standard solution.
The CAL solution is used to calibrate the instrument response with respect to analyte concentration or mass.
All CAL were prepared using Original STD individual pigment solutions.
Seven concentration levels were prepared using serial dilutions of Original STD solution of each pigment.
The dilution factor of each sequential dilution is 2, the solution used for dilution is Acetone/Methanol at 90/10 ratio.
The calibration created for each pigment individually was detector response versus concentration of a pigment in CAL solution (ug/L).
A calibration curve constructed for each individual pigment is a linear regrassion with a typical coefficient of dtermination >0.9.

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.

RT -- Retention Time of an analytes on a chromatogram (min).

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HPLC Performance-verification using CALCHK

	Analyte	CALCHK ug/L	Low WL -15%	High WL +15%	Ann-Var_41-70 CALCHK,%
#2	**Fuco**	2500.0	2125.0	2875.0	96.9%
#4	**Sud**	1200.0	1020.0	1380.0	99.5%
#9	**Cant**	500.0	425.0	575.0	112.9%
#11	**Chla**	500.0	425.0	575.0	99.3%

na- not applicable

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.
Warning Limit (WL) for Sud is +/-15%

Calibration Check Standard (CALCHK) – A mid-point calibration solution that is analyzed periodically in a sample set to verify that the instrument response to the analyte has not changed during the course of analysis.
CALCHK is analyzed for every batch os samples. It is analyzed first, before the sample batch, so to verify calibration. If CAKCHK is not +/-15% of the expected concentration for any analyte, then the instrument must be recalibrated. CALCHK is calculated and presented in % Recovery compared to the expected concentration.
Warning Limit (WL) for CALCHK is +/-15% for each individual Pigment Analyte

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HPLC Performance-verification using sample duplicate

		RT, min	Ann-Var_41-70 Dup.Reproduce., %
#1	**Chlc2**	**3.48**	102.8
#2	**Fuco**	**4.94**	94.8
#3	**Ddx**	**6.89**	94.9
#4	**Sud**	7.43	100.2
#5	**Allo**	**7.71**	93.0
#7	**Zea**	**7.95**	99.0
#8	**Lut**	**8.27**	97.2
#9	**Cant**	**9.79**	96.1
#10	**Chlb**	**10.80**	95.9
#11	**Chla**	**12.31**	101.7
#12	**DVCh-a**	**12.55**	99.3
#13	**Echi**	**13.18**	101.6
#15	**Phta**	**19.43**	94.9
#16	**Acar**	**20.30**	101.2
#17	**Beta**	**21.11**	102.41

RT -- Retention Time of an analytes on a chromatogram (min).

Duplicate Reproducibility- *is verified using a sample that is treated exactly the same throughout field and laboratory procedures.*
Analyses of duplicate sample give a measure of the precision associated with the sample preparation procedures, as well as analytical determination.

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HPLC Performance-verification using Pigments STD spiked into a sample matrix.

#			SPK-level ug/L	RT	Low WL, -30%	High WL, +30%	Ann-Var_41-70 %
#2	1	**Fuco**	2000.0		1400.0	2600.0	73.66
#9	2	**Cant**	400.0		280.0	520.0	74.0
#11	3	**Chla**	400.0		280.0	520.0	106.4

Matrix Spike Recovery -a sample with pre-determined levels of analytes (analyzed in duplicate) is used. Known amounts of 3 Pigments are added prior to extraction and analytical procedures. This QA/QC is used as a measure of both sample extraction and analytical determination procedures.

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HPLC Performance-verification using Pigments STD as surrogates spiked into a MBSR sample followed by SamplePrep procedure.

		SPK-level ug/L	RT	Ann-Var_41-70 Surrogate Recov., %
#2	**Fuco**	2000.0	**4.94**	98.7
#5	**Sud**	1200.0	**7.43**	98.0
#9	**Cant**	400.0	**9.79**	97.3
#11	**Chla**	400.0	**12.31**	88.0
#1	**Chlc2**	0.0	**3.48**	ND
#3	**Ddx**	0.0	**6.89**	ND
#5	**Allo**	0.0	**7.71**	ND
#7	**Zea**	0.0	**7.95**	ND
#8	**Lut**	0.0	**8.27**	ND
#9	**Cant**	0.0	**9.79**	ND
#10	**Chlb**	0.0	**10.80**	ND
#12	**DVCh-a**	0.0	**12.55**	ND
#13	**Echi**	0.0	**13.18**	ND
#15	**Phta**	0.0	**19.43**	ND
#16	**Acar**	0.0	**20.30**	ND
#17	**Beta**	0.0	**21.11**	ND

ND--Non detectable.

Method Blank-Spike Recovery (MBSR) – A blank sample (empty glass tube) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal reference standards (Sud at 400-1200 ug/L), and surrogates (Fuco, Cant, Chla at the concentrations 2000, 400, 400 ug/L, respectively). The MBSR is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus. If the MBSR value of the analytes other than Fuco, Sud, Cant and Chla constitute 10% or more of the analyte level determined in a sample, the contamination must be corrected and fresh samples must be analyzed when acceptable MBSR values have been obtained. The % Recovery of the surrogates (Fuco, Cant, Chla) must be within +/-20%. At least one MBSR is analyzed with each sample batch.

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Pigments STD Calibration Levels used for Developing the Calibration Curves

		level1	level2	level3	level4	level5	level6	level7	Original, ug/L	RT
#1	**Chlc2**	8.3	16.7	41.7	83.4	166.8	417.0	X	**834**	**3.48**
#2	**Fuco**	9.0	18.0	36.0	71.0	143.0	286.0	571.0	**1141**	**4.94**
#3	**Ddx**	11.9	23.9	47.9	95.7	191.4	478.5	X	**957**	**6.89**
#4	**Sud**	25.0	50.0	100.0	180.0	350.0	760.0	1600.0	**3200**	**7.43**
#5	**Allo**	9.0	18.0	36.0	72.0	144.0	287.0	574.0	**1147**	**7.71**
#7	**Zea**	8.0	16.0	32.0	65.0	120.0	240.0	475.0	**950**	**7.95**
#8	**Lut**	11.0	26.0	44.0	88.0	175.1	320.0	700.5	**1401**	**8.27**
#9	**Cant**	9.0	18.0	35.0	70.0	140.0	280.0	560.0	**1118**	**9.79**
#10	**Chlb**	19.0	38.0	76.0	151.0	303.0	605.0	X	**1210**	**10.80**
#11	**Chla**	14.2	28.5	57.0	120.0	228.0	441.0	911.0	**1822**	**12.31**
#12	**DVCh-a**	10.7	21.4	53.6	107.1	214.2	535.5	X	**1071**	**12.55**
#13	**Echi**	8.0	16.0	31.0	62.0	120.0	220.0	408.5	**817**	**13.18**
#15	**Phta**	14.0	28.0	55.0	108.0	220.0	440.0	879.0	**3517**	**19.43**
#16	**Acar**	11.0	22.0	44.0	88.0	176.0	352.0	X	**703**	**20.30**
#17	**Beta**	14.5	29.0	58.0	72.7	145.4	363.5	X	**727**	**21.11**

Calibration Standard (CAL) – A solution prepared from dilution of a stock standard solution.
The CAL solution is used to calibrate the instrument response with respect to analyte concentration or mass.
All CAL were prepared using Original STD individual pigment solutions.
Seven concentration levels were prepared using serial dilutions of Original STD solution of each pigment.
The dilution factor of each sequential dilution is 2, the solution used for dilution is Acetone/Methanol at 90/10 ratio.
The calibration created for each pigment individually was detector response versus concentration of a pigment in CAL solution (ug/L).
A calibration curve constructed for each individual pigment is a linear regrassion with a typical coefficient of dtermination >0.9.

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.

RT -- Retention Time of an analytes on a chromatogram (min).

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HPLC Performance-verification using sample duplicate

		RT, min	Ann-Var_71-80 Dup.Reproduce., %
#1	**Chlc2**	**3.48**	95.9
#2	**Fuco**	**4.94**	97.1
#3	**Ddx**	**6.89**	98.3
#4	**Sud**	7.43	103.6
#5	**Allo**	**7.71**	na
#7	**Zea**	**7.95**	95.8
#8	**Lut**	**8.27**	104.6
#9	**Cant**	**9.79**	95.8
#10	**Chlb**	**10.80**	na
#11	**Chla**	**12.31**	92.4
#12	**DVCh-a**	**12.55**	81.6
#13	**Echi**	**13.18**	89.3
#15	**Phta**	**19.43**	na
#16	**Acar**	**20.30**	95.3
#17	**Beta**	**21.11**	na

RT -- Retention Time of an analytes on a chromatogram (min).

Duplicate Reproducibility- *is verified using a sample that is treated exactly the same throughout field and laboratory procedures.*
Analyses of duplicate sample give a measure of the precision associated with the sample preparation procedures, as well as analytical determination.

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HPLC Performance-verification using Pigments STD spiked into a sample matrix.

#			SPK-level ug/L	RT	Low WL, -30%	High WL, +30%	Ann-Var_71-80 %
#2	1	**Fuco**	400.0		280.0	520.0	95.03
#9	2	**Cant**	80.0		56.0	104.0	92.7
#11	3	**Chla**	400.0		280.0	520.0	106.0

Matrix Spike Recovery -a sample with pre-determined levels of analytes (analyzed in duplicate) is used. Known amounts of 3 Pigments are added prior to extraction and analytical procedures. This QA/QC is used as a measure of both sample extraction and analytical determination procedures.

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HPLC Performance-verification using CALCHK

	Analyte	CALCHK ug/L	Low WL -15%	High WL +15%	Ann-Var_71-80 CALCHK,%
#2	**Fuco**	500.0	425.0	575.0	113.6%
#4	**Sud**	1200.0	1020.0	1380.0	94.0%
#9	**Cant**	100.0	85.0	115.0	115.4%
#11	**Chla**	500.0	425.0	575.0	95.7%

na- not applicable

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.
Warning Limit (WL) for Sud is +/-15%

Calibration Check Standard (CALCHK) – A mid-point calibration solution that is analyzed periodically in a sample set to verify that the instrument response to the analyte has not changed during the course of analysis.
CALCHK is analyzed for every batch os samples. It is analyzed first, before the sample batch, so to verify calibration. If CAKCHK is not +/-15% of the expected concentration for any analyte, then the instrument must be recalibrated. CALCHK is calculated and presented in % Recovery compared to the expected concentration.
Warning Limit (WL) for CALCHK is +/-15% for each individual Pigment Analyte

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HPLC Performance-verification using Pigments STD as surrogates spiked into a MBSR sample followed by SamplePrep procedure.

		SPK-level ug/L	RT	Ann-Var_71-80 Surrogate Recov., %
#2	**Fuco**	400.0	**4.94**	116.5
#5	**Sud**	1200.0	**7.43**	94.0
#9	**Cant**	80.0	**9.79**	115.4
#11	**Chla**	400.0	**12.31**	86.6
#1	**Chlc2**	0.0	**3.48**	ND
#3	**Ddx**	0.0	**6.89**	ND
#5	**Allo**	0.0	**7.71**	ND
#7	**Zea**	0.0	**7.95**	ND
#8	**Lut**	0.0	**8.27**	ND
#9	**Cant**	0.0	**9.79**	ND
#10	**Chlb**	0.0	**10.80**	ND
#12	**DVCh-a**	0.0	**12.55**	ND
#13	**Echi**	0.0	**13.18**	ND
#15	**Phta**	0.0	**19.43**	ND
#16	**Acar**	0.0	**20.30**	ND
#17	**Beta**	0.0	**21.11**	ND

ND--Non detectable.

Method Blank-Spike Recovery (MBSR) – A blank sample (empty glass tube) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal reference standards (Sud at 400-1200 ug/L), and surrogates (Fuco, Cant, Chla at the concentrations 400, 80, 400 ug/L, respectively). The MBSR is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus. If the MBSR value of the analytes other than Fuco, Sud, Cant and Chla constitute 10% or more of the analyte level determined in a sample, the contamination must be corrected and fresh samples must be analyzed when acceptable MBSR values have been obtained. The % Recovery of the surrogates (Fuco, Cant, Chla) must be within +/-20%. At least one MBSR is analyzed with each sample batch.

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Pigments STD Calibration Levels used for Developing the Calibration Curves

		level1	level2	level3	level4	level5	level6	level7	Original, ug/L	RT
#1	**Chlc2**	8.3	16.7	41.7	83.4	166.8	417.0	X	**834**	**3.48**
#2	**Fuco**	9.0	18.0	36.0	71.0	143.0	286.0	571.0	**1141**	**4.94**
#3	**Ddx**	11.9	23.9	47.9	95.7	191.4	478.5	X	**957**	**6.89**
#4	**Sud**	25.0	50.0	100.0	180.0	350.0	760.0	1600.0	**3200**	**7.43**
#5	**Allo**	9.0	18.0	36.0	72.0	144.0	287.0	574.0	**1147**	**7.71**
#7	**Zea**	8.0	16.0	32.0	65.0	120.0	240.0	475.0	**950**	**7.95**
#8	**Lut**	11.0	26.0	44.0	88.0	175.1	320.0	700.5	**1401**	**8.27**
#9	**Cant**	9.0	18.0	35.0	70.0	140.0	280.0	560.0	**1118**	**9.79**
#10	**Chlb**	19.0	38.0	76.0	151.0	303.0	605.0	X	**1210**	**10.80**
#11	**Chla**	14.2	28.5	57.0	120.0	228.0	441.0	911.0	**1822**	**12.31**
#12	**DVCh-a**	10.7	21.4	53.6	107.1	214.2	535.5	X	**1071**	**12.55**
#13	**Echi**	8.0	16.0	31.0	62.0	120.0	220.0	408.5	**817**	**13.18**
#15	**Phta**	14.0	28.0	55.0	108.0	220.0	440.0	879.0	**3517**	**19.43**
#16	**Acar**	11.0	22.0	44.0	88.0	176.0	352.0	X	**703**	**20.30**
#17	**Beta**	14.5	29.0	58.0	72.7	145.4	363.5	X	**727**	**21.11**

Calibration Standard (CAL) – A solution prepared from dilution of a stock standard solution.
The CAL solution is used to calibrate the instrument response with respect to analyte concentration or mass.
All CAL were prepared using Original STD individual pigment solutions.
Seven concentration levels were prepared using serial dilutions of Original STD solution of each pigment.
The dilution factor of each sequential dilution is 2, the solution used for dilution is Acetone/Methanol at 90/10 ratio.
The calibration created for each pigment individually was detector response versus concentration of a pigment in CAL solution (ug/L).
A calibration curve constructed for each individual pigment is a linear regrassion with a typical coefficient of dtermination >0.9.

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.

RT -- Retention Time of an analytes on a chromatogram (min).

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HPLC Performance-verification using CALCHK

	Analyte	CALCHK ug/L	Low WL -15%	High WL +15%	Ann-Var_81-91 CALCHK,%
#2	**Fuco**	500.0	425.0	575.0	105.9%
#4	**Sud**	1200.0	1020.0	1380.0	85.0%
#9	**Cant**	100.0	85.0	115.0	99.3%
#11	**Chla**	500.0	425.0	575.0	85.1%

na- not applicable

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.
Warning Limit (WL) for Sud is +/-15%

Calibration Check Standard (CALCHK) – A mid-point calibration solution that is analyzed periodically in a sample set to verify that the instrument response to the analyte has not changed during the course of analysis.
CALCHK is analyzed for every batch os samples. It is analyzed first, before the sample batch, so to verify calibration. If CAKCHK is not +/-15% of the expected concentration for any analyte, then the instrument must be recalibrated. CALCHK is calculated and presented in % Recovery compared to the expected concentration.
Warning Limit (WL) for CALCHK is +/-15% for each individual Pigment Analyte

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HPLC Performance-verification using sample duplicate

		RT, min	Ann-Var_81-91 Dup.Reproduce., %
#1	**Chlc2**	**3.48**	103.3
#2	**Fuco**	**4.94**	103.7
#3	**Ddx**	**6.89**	101.8
#4	**Sud**	7.43	99.0
#5	**Allo**	**7.71**	na
#7	**Zea**	**7.95**	97.2
#8	**Lut**	**8.27**	102.2
#9	**Cant**	**9.79**	101.8
#10	**Chlb**	**10.80**	102.1
#11	**Chla**	**12.31**	105.9
#12	**DVCh-a**	**12.55**	90.3
#13	**Echi**	**13.18**	106.3
#15	**Phta**	**19.43**	102.5
#16	**Acar**	**20.30**	101.7
#17	**Beta**	**21.11**	na

RT -- Retention Time of an analytes on a chromatogram (min).

Duplicate Reproducibility- *is verified using a sample that is treated exactly the same throughout field and laboratory procedures.*
Analyses of duplicate sample give a measure of the precision associated with the sample preparation procedures, as well as analytical determination.

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HPLC Performance-verification using Pigments STD spiked into a sample matrix.

#			SPK-level ug/L	RT	Low WL, -30%	High WL, +30%	Ann-Var_81-91 %
#2	1	**Fuco**	400.0		280.0	520.0	112.46
#9	2	**Cant**	80.0		56.0	104.0	80.5
#11	3	**Chla**	400.0		280.0	520.0	112.3

Matrix Spike Recovery -a sample with pre-determined levels of analytes (analyzed in duplicate) is used. Known amounts of 3 Pigments are added prior to extraction and analytical procedures. This QA/QC is used as a measure of both sample extraction and analytical determination procedures.

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HPLC Performance-verification using Pigments STD as surrogates spiked into a MBSR sample followed by SamplePrep procedure.

		SPK-level ug/L	RT	Ann-Var_81-91 Surrogate Recov., %
#2	**Fuco**	400.0	**4.94**	116.5
#5	**Sud**	1200.0	**7.43**	94.0
#9	**Cant**	80.0	**9.79**	115.4
#11	**Chla**	400.0	**12.31**	86.6
#1	**Chlc2**	0.0	**3.48**	ND
#3	**Ddx**	0.0	**6.89**	ND
#5	**Allo**	0.0	**7.71**	ND
#7	**Zea**	0.0	**7.95**	ND
#8	**Lut**	0.0	**8.27**	ND
#9	**Cant**	0.0	**9.79**	ND
#10	**Chlb**	0.0	**10.80**	ND
#12	**DVCh-a**	0.0	**12.55**	ND
#13	**Echi**	0.0	**13.18**	ND
#15	**Phta**	0.0	**19.43**	ND
#16	**Acar**	0.0	**20.30**	ND
#17	**Beta**	0.0	**21.11**	ND

ND--Non detectable.

Method Blank-Spike Recovery (MBSR) – A blank sample (empty glass tube) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal reference standards (Sud at 400-1200 ug/L), and surrogates (Fuco, Cant, Chla at the concentrations 400, 80, 400 ug/L, respectively). The MBSR is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus. If the MBSR value of the analytes other than Fuco, Sud, Cant and Chla constitute 10% or more of the analyte level determined in a sample, the contamination must be corrected and fresh samples must be analyzed when acceptable MBSR values have been obtained. The % Recovery of the surrogates (Fuco, Cant, Chla) must be within +/-20%. At least one MBSR is analyzed with each sample batch.

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Pigments STD Calibration Levels used for Developing the Calibration Curves

		level1	level2	level3	level4	level5	level6	level7	Original, ug/L	RT
#1	**Chlc2**	8.3	16.7	41.7	83.4	166.8	417.0	X	**834**	**3.48**
#2	**Fuco**	9.0	18.0	36.0	71.0	143.0	286.0	571.0	**1141**	**4.94**
#3	**Ddx**	11.9	23.9	47.9	95.7	191.4	478.5	X	**957**	**6.89**
#4	**Sud**	25.0	50.0	100.0	180.0	350.0	760.0	1600.0	**3200**	**7.43**
#5	**Allo**	9.0	18.0	36.0	72.0	144.0	287.0	574.0	**1147**	**7.71**
#7	**Zea**	8.0	16.0	32.0	65.0	120.0	240.0	475.0	**950**	**7.95**
#8	**Lut**	11.0	26.0	44.0	88.0	175.1	320.0	700.5	**1401**	**8.27**
#9	**Cant**	9.0	18.0	35.0	70.0	140.0	280.0	560.0	**1118**	**9.79**
#10	**Chlb**	19.0	38.0	76.0	151.0	303.0	605.0	X	**1210**	**10.80**
#11	**Chla**	14.2	28.5	57.0	120.0	228.0	441.0	911.0	**1822**	**12.31**
#12	**DVCh-a**	10.7	21.4	53.6	107.1	214.2	535.5	X	**1071**	**12.55**
#13	**Echi**	8.0	16.0	31.0	62.0	120.0	220.0	408.5	**817**	**13.18**
#15	**Phta**	14.0	28.0	55.0	108.0	220.0	440.0	879.0	**3517**	**19.43**
#16	**Acar**	11.0	22.0	44.0	88.0	176.0	352.0	X	**703**	**20.30**
#17	**Beta**	14.5	29.0	58.0	72.7	145.4	363.5	X	**727**	**21.11**

Calibration Standard (CAL) – A solution prepared from dilution of a stock standard solution.
The CAL solution is used to calibrate the instrument response with respect to analyte concentration or mass.
All CAL were prepared using Original STD individual pigment solutions.
Seven concentration levels were prepared using serial dilutions of Original STD solution of each pigment.
The dilution factor of each sequential dilution is 2, the solution used for dilution is Acetone/Methanol at 90/10 ratio.
The calibration created for each pigment individually was detector response versus concentration of a pigment in CAL solution (ug/L).
A calibration curve constructed for each individual pigment is a linear regrassion with a typical coefficient of dtermination >0.9.

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.

RT -- Retention Time of an analytes on a chromatogram (min).

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HPLC Performance-verification using CALCHK

	Analyte	CALCHK ug/L	Low WL -15%	High WL +15%	Ann-Var_AV1-7 CALCHK,%
#2	**Fuco**	600.0	510.0	690.0	104.6%
#4	**Sud**	700.0	595.0	805.0	98.9%
#9	**Cant**	150.0	127.5	172.5	110.7%
#11	**Chla**	500.0	425.0	575.0	97.0%

na- not applicable

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.
Warning Limit (WL) for Sud is +/-15%

Calibration Check Standard (CALCHK) – A mid-point calibration solution that is analyzed periodically in a sample set to verify that the instrument response to the analyte has not changed during the course of analysis.
CALCHK is analyzed for every batch os samples. It is analyzed first, before the sample batch, so to verify calibration. If CAKCHK is not +/-15% of the expected concentration for any analyte, then the instrument must be recalibrated. CALCHK is calculated and presented in % Recovery compared to the expected concentration.
Warning Limit (WL) for CALCHK is +/-15% for each individual Pigment Analyte

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HPLC Performance-verification using sample duplicate

		RT, min	Ann-Var_AV1-7 Dup.Reproduce., %
#1	**Chlc2**	**3.48**	86.5
#2	**Fuco**	**4.94**	97.6
#3	**Ddx**	**6.89**	98.9
#4	**Sud**	7.43	102.6
#5	**Allo**	**7.71**	101.0
#7	**Zea**	**7.95**	99.8
#8	**Lut**	**8.27**	98.6
#9	**Cant**	**9.79**	105.8
#10	**Chlb**	**10.80**	99.3
#11	**Chla**	**12.31**	102.0
#12	**DVCh-a**	**12.55**	81.0
#13	**Echi**	**13.18**	104.3
#15	**Phta**	**19.43**	89.6
#16	**Acar**	**20.30**	90.0
#17	**Beta**	**21.11**	na

RT -- Retention Time of an analytes on a chromatogram (min).

Duplicate Reproducibility- *is verified using a sample that is treated exactly the same throughout field and laboratory procedures.*
Analyses of duplicate sample give a measure of the precision associated with the sample preparation procedures, as well as analytical determination.

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HPLC Performance-verification using Pigments STD spiked into a sample matrix.

	#		SPK-level ug/L	RT	Low WL, -30%	High WL, +30%	Ann-Var_AV1-7 %
#2	1	**Fuco**	600.0	**4.94**	420.0	780.0	100.33
#9	2	**Cant**	120.0	**9.79**	84.0	156.0	90.1
#11	3	**Chla**	400.0	**12.31**	280.0	520.0	124.2

Matrix Spike Recovery -a sample with pre-determined levels of analytes (analyzed in duplicate) is used. Known amounts of 3 Pigments are added prior to extraction and analytical procedures. This QA/QC is used as a measure of both sample extraction and analytical determination procedures.

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HPLC Performance-verification using Pigments STD as surrogates spiked into a MBSR sample followed by SamplePrep procedure.

		SPK-level ug/L	RT	Ann-Var_AV1-7 Surrogate Recov., %
#2	**Fuco**	2000.0	**4.94**	110.7
#5	**Sud**	1200.0	**7.43**	100.1
#9	**Cant**	400.0	**9.79**	80.3
#11	**Chla**	400.0	**12.31**	83.8
#1	**Chlc2**	0.0	**3.48**	ND
#3	**Ddx**	0.0	**6.89**	ND
#5	**Allo**	0.0	**7.71**	ND
#7	**Zea**	0.0	**7.95**	ND
#8	**Lut**	0.0	**8.27**	ND
#9	**Cant**	0.0	**9.79**	ND
#10	**Chlb**	0.0	**10.80**	ND
#12	**DVCh-a**	0.0	**12.55**	ND
#13	**Echi**	0.0	**13.18**	ND
#15	**Phta**	0.0	**19.43**	ND
#16	**Acar**	0.0	**20.30**	ND
#17	**Beta**	0.0	**21.11**	ND

ND--Non detectable.

Method Blank-Spike Recovery (MBSR) – A blank sample (empty glass tube) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal reference standards (Sud at 400-1200 ug/L), and surrogates (Fuco, Cant, Chla at the concentrations 480, 120, 400 ug/L, respectively). The MBSR is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus. If the MBSR value of the analytes other than Fuco, Sud, Cant and Chla constitute 10% or more of the analyte level determined in a sample, the contamination must be corrected and fresh samples must be analyzed when acceptable MBSR values have been obtained. The % Recovery of the surrogates (Fuco, Cant, Chla) must be within +/-20%. At least one MBSR is analyzed with each sample batch.

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Pigments STD Calibration Levels used for Developing the Calibration Curves

		level1	level2	level3	level4	level5	level6	level7	Original, ug/L	RT
#1	**Chlc2**	8.3	16.7	41.7	83.4	166.8	417.0	X	**834**	**3.48**
#2	**Fuco**	9.0	18.0	36.0	71.0	143.0	286.0	571.0	**1141**	**4.94**
#3	**Ddx**	11.9	23.9	47.9	95.7	191.4	478.5	X	**957**	**6.89**
#4	**Sud**	25.0	50.0	100.0	180.0	350.0	760.0	1600.0	**3200**	**7.43**
#5	**Allo**	9.0	18.0	36.0	72.0	144.0	287.0	574.0	**1147**	**7.71**
#7	**Zea**	8.0	16.0	32.0	65.0	120.0	240.0	475.0	**950**	**7.95**
#8	**Lut**	11.0	26.0	44.0	88.0	175.1	320.0	700.5	**1401**	**8.27**
#9	**Cant**	9.0	18.0	35.0	70.0	140.0	280.0	560.0	**1118**	**9.79**
#10	**Chlb**	19.0	38.0	76.0	151.0	303.0	605.0	X	**1210**	**10.80**
#11	**Chla**	14.2	28.5	57.0	120.0	228.0	441.0	911.0	**1822**	**12.31**
#12	**DVCh-a**	10.7	21.4	53.6	107.1	214.2	535.5	X	**1071**	**12.55**
#13	**Echi**	8.0	16.0	31.0	62.0	120.0	220.0	408.5	**817**	**13.18**
#15	**Phta**	14.0	28.0	55.0	108.0	220.0	440.0	879.0	**3517**	**19.43**
#16	**Acar**	11.0	22.0	44.0	88.0	176.0	352.0	X	**703**	**20.30**
#17	**Beta**	14.5	29.0	58.0	72.7	145.4	363.5	X	**727**	**21.11**

Calibration Standard (CAL) – A solution prepared from dilution of a stock standard solution.
The CAL solution is used to calibrate the instrument response with respect to analyte concentration or mass.
All CAL were prepared using Original STD individual pigment solutions.
Seven concentration levels were prepared using serial dilutions of Original STD solution of each pigment.
The dilution factor of each sequential dilution is 2, the solution used for dilution is Acetone/Methanol at 90/10 ratio.
The calibration created for each pigment individually was detector response versus concentration of a pigment in CAL solution (ug/L).
A calibration curve constructed for each individual pigment is a linear regrassion with a typical coefficient of dtermination >0.9.

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.

RT -- Retention Time of an analytes on a chromatogram (min).

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HPLC Performance-verification using CALCHK

	Analyte	CALCHK ug/L	Low WL -15%	High WL +15%	Ann-Var_AV26-42 CALCHK,%
#2	**Fuco**	600.0	510.0	690.0	96.8%
#4	**Sud**	700.0	595.0	805.0	103.5%
#9	**Cant**	150.0	127.5	172.5	102.6%
#11	**Chla**	500.0	425.0	575.0	86.1%

na- not applicable

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.
Warning Limit (WL) for Sud is +/-15%

Calibration Check Standard (CALCHK) – A mid-point calibration solution that is analyzed periodically in a sample set to verify that the instrument response to the analyte has not changed during the course of analysis.
CALCHK is analyzed for every batch os samples. It is analyzed first, before the sample batch, so to verify calibration. If CAKCHK is not +/-15% of the expected concentration for any analyte, then the instrument must be recalibrated. CALCHK is calculated and presented in % Recovery compared to the expected concentration.
Warning Limit (WL) for CALCHK is +/-15% for each individual Pigment Analyte

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HPLC Performance-verification using sample duplicate

		RT, min	Ann-Var_AV26-42 Dup.Reproduce., %
#1	**Chlc2**	**3.48**	91.4
#2	**Fuco**	**4.94**	92.2
#3	**Ddx**	**6.89**	94.0
#4	**Sud**	7.43	99.6
#5	**Allo**	**7.71**	95.7
#7	**Zea**	**7.95**	100.6
#8	**Lut**	**8.27**	100.5
#9	**Cant**	**9.79**	104.7
#10	**Chlb**	**10.80**	92.8
#11	**Chla**	**12.31**	89.9
#12	**DVCh-a**	**12.55**	94.1
#13	**Echi**	**13.18**	107.1
#15	**Phta**	**19.43**	100.0
#16	**Acar**	**20.30**	83.1
#17	**Beta**	**21.11**	na

RT -- Retention Time of an analytes on a chromatogram (min).

Duplicate Reproducibility- *is verified using a sample that is treated exactly the same throughout field and laboratory procedures.*
Analyses of duplicate sample give a measure of the precision associated with the sample preparation procedures, as well as analytical determination.

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HPLC Performance-verification using Pigments STD spiked into a sample matrix.

	#		SPK-level	RT	Low WL, -30%	High WL, +30%	Ann-Var_AV26-42
			ug/L				%
#2	1	**Fuco**	600.0	**4.94**	420.0	780.0	113.03
#9	2	**Cant**	120.0	**9.79**	84.0	156.0	123.7
#11	3	**Chla**	400.0	**12.31**	280.0	520.0	73.5

Matrix Spike Recovery -a sample with pre-determined levels of analytes (analyzed in duplicate) is used. Known amounts of 3 Pigments are added prior to extraction and analytical procedures. This QA/QC is used as a measure of both sample extraction and analytical determination procedures.

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HPLC Performance-verification using Pigments STD as surrogates spiked into a MBSR sample followed by SamplePrep procedure.

		SPK-level ug/L	RT	Ann-Var_AV26-42 Surrogate Recov., %
#2	**Fuco**	2000.0	**4.94**	103.1
#5	**Sud**	1200.0	**7.43**	97.7
#9	**Cant**	400.0	**9.79**	110.4
#11	**Chla**	400.0	**12.31**	85.5
#1	**Chlc2**	0.0	**3.48**	ND
#3	**Ddx**	0.0	**6.89**	ND
#5	**Allo**	0.0	**7.71**	ND
#7	**Zea**	0.0	**7.95**	ND
#8	**Lut**	0.0	**8.27**	ND
#9	**Cant**	0.0	**9.79**	ND
#10	**Chlb**	0.0	**10.80**	ND
#12	**DVCh-a**	0.0	**12.55**	ND
#13	**Echi**	0.0	**13.18**	ND
#15	**Phta**	0.0	**19.43**	ND
#16	**Acar**	0.0	**20.30**	ND
#17	**Beta**	0.0	**21.11**	ND

ND--Non detectable.

Method Blank-Spike Recovery (MBSR) – A blank sample (empty glass tube) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal reference standards (Sud at 400-1200 ug/L), and surrogates (Fuco, Cant, Chla at the concentrations 480, 120, 400 ug/L, respectively). The MBSR is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus. If the MBSR value of the analytes other than Fuco, Sud, Cant and Chla constitute 10% or more of the analyte level determined in a sample, the contamination must be corrected and fresh samples must be analyzed when acceptable MBSR values have been obtained. The % Recovery of the surrogates (Fuco, Cant, Chla) must be within +/-20%. At least one MBSR is analyzed with each sample batch.

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Pigments STD Calibration Levels used for Developing the Calibration Curves

		level1	level2	level3	level4	level5	level6	level7	Original, ug/L	RT
#1	**Chlc2**	8.3	16.7	41.7	83.4	166.8	417.0	X	**834**	**3.48**
#2	**Fuco**	9.0	18.0	36.0	71.0	143.0	286.0	571.0	**1141**	**4.94**
#3	**Ddx**	11.9	23.9	47.9	95.7	191.4	478.5	X	**957**	**6.89**
#4	**Sud**	25.0	50.0	100.0	180.0	350.0	760.0	1600.0	**3200**	**7.43**
#5	**Allo**	9.0	18.0	36.0	72.0	144.0	287.0	574.0	**1147**	**7.71**
#7	**Zea**	8.0	16.0	32.0	65.0	120.0	240.0	475.0	**950**	**7.95**
#8	**Lut**	11.0	26.0	44.0	88.0	175.1	320.0	700.5	**1401**	**8.27**
#9	**Cant**	9.0	18.0	35.0	70.0	140.0	280.0	560.0	**1118**	**9.79**
#10	**Chlb**	19.0	38.0	76.0	151.0	303.0	605.0	X	**1210**	**10.80**
#11	**Chla**	14.2	28.5	57.0	120.0	228.0	441.0	911.0	**1822**	**12.31**
#12	**DVCh-a**	10.7	21.4	53.6	107.1	214.2	535.5	X	**1071**	**12.55**
#13	**Echi**	8.0	16.0	31.0	62.0	120.0	220.0	408.5	**817**	**13.18**
#15	**Phta**	14.0	28.0	55.0	108.0	220.0	440.0	879.0	**3517**	**19.43**
#16	**Acar**	11.0	22.0	44.0	88.0	176.0	352.0	X	**703**	**20.30**
#17	**Beta**	14.5	29.0	58.0	72.7	145.4	363.5	X	**727**	**21.11**

Calibration Standard (CAL) – A solution prepared from dilution of a stock standard solution.
The CAL solution is used to calibrate the instrument response with respect to analyte concentration or mass.
All CAL were prepared using Original STD individual pigment solutions.
Seven concentration levels were prepared using serial dilutions of Original STD solution of each pigment.
The dilution factor of each sequential dilution is 2, the solution used for dilution is Acetone/Methanol at 90/10 ratio.
The calibration created for each pigment individually was detector response versus concentration of a pigment in CAL solution (ug/L).
A calibration curve constructed for each individual pigment is a linear regrassion with a typical coefficient of dtermination >0.9.

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.

RT -- Retention Time of an analytes on a chromatogram (min).

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HPLC Performance-verification using CALCHK

	Analyte	CALCHK ug/L	Low WL -15%	High WL +15%	Ann-Var_AV43-51 CALCHK,%
#2	**Fuco**	600.0	510.0	690.0	102.6%
#4	**Sud**	700.0	595.0	805.0	100.8%
#9	**Cant**	150.0	127.5	172.5	102.6%
#11	**Chla**	500.0	425.0	575.0	86.7%

na- not applicable

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.
Warning Limit (WL) for Sud is +/-15%

Calibration Check Standard (CALCHK) – A mid-point calibration solution that is analyzed periodically in a sample set to verify that the instrument response to the analyte has not changed during the course of analysis.
CALCHK is analyzed for every batch os samples. It is analyzed first, before the sample batch, so to verify calibration. If CAKCHK is not +/-15% of the expected concentration for any analyte, then the instrument must be recalibrated. CALCHK is calculated and presented in % Recovery compared to the expected concentration.
Warning Limit (WL) for CALCHK is +/-15% for each individual Pigment Analyte

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HPLC Performance-verification using sample duplicate

		RT, min	Ann-Var_AV43-51 Dup.Reproduce., %
#1	**Chlc2**	**3.48**	104.1
#2	**Fuco**	**4.94**	96.0
#3	**Ddx**	**6.89**	103.5
#4	**Sud**	7.43	98.1
#5	**Allo**	**7.71**	na
#7	**Zea**	**7.95**	82.9
#8	**Lut**	**8.27**	106.8
#9	**Cant**	**9.79**	92.8
#10	**Chlb**	**10.80**	96.0
#11	**Chla**	**12.31**	81.9
#12	**DVCh-a**	**12.55**	95.4
#13	**Echi**	**13.18**	na
#15	**Phta**	**19.43**	85.8
#16	**Acar**	**20.30**	100.8
#17	**Beta**	**21.11**	na

RT -- Retention Time of an analytes on a chromatogram (min).

Duplicate Reproducibility- *is verified using a sample that is treated exactly the same throughout field and laboratory procedures.*
Analyses of duplicate sample give a measure of the precision associated with the sample preparation procedures, as well as analytical determination.

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HPLC Performance-verification using Pigments STD spiked into a sample matrix.

	#		SPK-level	RT	Low WL, -30%	High WL, +30%	Ann-Var_AV43-51
			ug/L				%
#2	1	**Fuco**	600.0	**4.94**	420.0	780.0	103.9
#9	2	**Cant**	120.0	**9.79**	84.0	156.0	75.1
#11	3	**Chla**	400.0	**12.31**	280.0	520.0	100.2

Matrix Spike Recovery -a sample with pre-determined levels of analytes (analyzed in duplicate) is used. Known amounts of 3 Pigments are added prior to extraction and analytical procedures. This QA/QC is used as a measure of both sample extraction and analytical determination procedures.

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HPLC Performance-verification using Pigments STD as surrogates spiked into a MBSR sample followed by SamplePrep procedure.

		SPK-level ug/L	RT	Ann-Var_AV43-51 Surrogate Recov., %
#2	**Fuco**	2000.0	**4.94**	112.9
#5	**Sud**	1200.0	**7.43**	108.0
#9	**Cant**	400.0	**9.79**	94.5
#11	**Chla**	400.0	**12.31**	90.2
#1	**Chlc2**	0.0	**3.48**	ND
#3	**Ddx**	0.0	**6.89**	ND
#5	**Allo**	0.0	**7.71**	ND
#7	**Zea**	0.0	**7.95**	ND
#8	**Lut**	0.0	**8.27**	ND
#9	**Cant**	0.0	**9.79**	ND
#10	**Chlb**	0.0	**10.80**	ND
#12	**DVCh-a**	0.0	**12.55**	ND
#13	**Echi**	0.0	**13.18**	ND
#15	**Phta**	0.0	**19.43**	ND
#16	**Acar**	0.0	**20.30**	ND
#17	**Beta**	0.0	**21.11**	ND

ND--Non detectable.

Method Blank-Spike Recovery (MBSR) – A blank sample (empty glass tube) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal reference standards (Sud at 400-1200 ug/L), and surrogates (Fuco, Cant, Chla at the concentrations 480, 120, 400 ug/L, respectively). The MBSR is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus. If the MBSR value of the analytes other than Fuco, Sud, Cant and Chla constitute 10% or more of the analyte level determined in a sample, the contamination must be corrected and fresh samples must be analyzed when acceptable MBSR values have been obtained. The % Recovery of the surrogates (Fuco, Cant, Chla) must be within +/-20%. At least one MBSR is analyzed with each sample batch.

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Pigments STD Calibration Levels used for Developing the Calibration Curves

		level1	level2	level3	level4	level5	level6	level7	Original, ug/L	RT
#1	**Chlc2**	8.3	16.7	41.7	83.4	166.8	417.0	X	**834**	**3.48**
#2	**Fuco**	9.0	18.0	36.0	71.0	143.0	286.0	571.0	**1141**	**4.94**
#3	**Ddx**	11.9	23.9	47.9	95.7	191.4	478.5	X	**957**	**6.89**
#4	**Sud**	25.0	50.0	100.0	180.0	350.0	760.0	1600.0	**3200**	**7.43**
#5	**Allo**	9.0	18.0	36.0	72.0	144.0	287.0	574.0	**1147**	**7.71**
#7	**Zea**	8.0	16.0	32.0	65.0	120.0	240.0	475.0	**950**	**7.95**
#8	**Lut**	11.0	26.0	44.0	88.0	175.1	320.0	700.5	**1401**	**8.27**
#9	**Cant**	9.0	18.0	35.0	70.0	140.0	280.0	560.0	**1118**	**9.79**
#10	**Chlb**	19.0	38.0	76.0	151.0	303.0	605.0	X	**1210**	**10.80**
#11	**Chla**	14.2	28.5	57.0	120.0	228.0	441.0	911.0	**1822**	**12.31**
#12	**DVCh-a**	10.7	21.4	53.6	107.1	214.2	535.5	X	**1071**	**12.55**
#13	**Echi**	8.0	16.0	31.0	62.0	120.0	220.0	408.5	**817**	**13.18**
#15	**Phta**	14.0	28.0	55.0	108.0	220.0	440.0	879.0	**3517**	**19.43**
#16	**Acar**	11.0	22.0	44.0	88.0	176.0	352.0	X	**703**	**20.30**
#17	**Beta**	14.5	29.0	58.0	72.7	145.4	363.5	X	**727**	**21.11**

Calibration Standard (CAL) – A solution prepared from dilution of a stock standard solution.
The CAL solution is used to calibrate the instrument response with respect to analyte concentration or mass.
All CAL were prepared using Original STD individual pigment solutions.
Seven concentration levels were prepared using serial dilutions of Original STD solution of each pigment.
The dilution factor of each sequential dilution is 2, the solution used for dilution is Acetone/Methanol at 90/10 ratio.
The calibration created for each pigment individually was detector response versus concentration of a pigment in CAL solution (ug/L).
A calibration curve constructed for each individual pigment is a linear regrassion with a typical coefficient of dtermination >0.9.

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.

RT -- Retention Time of an analytes on a chromatogram (min).

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HPLC Performance-verification using CALCHK

	Analyte	CALCHK ug/L	Low WL -15%	High WL +15%	Ann-Var_AV8-25 CALCHK,%
#2	**Fuco**	600.0	510.0	690.0	110.3%
#4	**Sud**	700.0	595.0	805.0	100.6%
#9	**Cant**	150.0	127.5	172.5	107.6%
#11	**Chla**	500.0	425.0	575.0	97.6%

na- not applicable

Sud- Sudan is used as Internal Refence STD added to a sample extract at a known amount (typically in a range between 400 to 1200 ug/L) just prior to HPLC analysis to varify the accuracy of the injection volume.
Warning Limit (WL) for Sud is +/-15%

Calibration Check Standard (CALCHK) – A mid-point calibration solution that is analyzed periodically in a sample set to verify that the instrument response to the analyte has not changed during the course of analysis.
CALCHK is analyzed for every batch os samples. It is analyzed first, before the sample batch, so to verify calibration. If CAKCHK is not +/-15% of the expected concentration for any analyte, then the instrument must be recalibrated. CALCHK is calculated and presented in % Recovery compared to the expected concentration.
Warning Limit (WL) for CALCHK is +/-15% for each individual Pigment Analyte

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HPLC Performance-verification using sample duplicate

		RT, min	Ann-Var_AV8-25 Dup.Reproduce., %
#1	**Chlc2**	**3.48**	93.1
#2	**Fuco**	**4.94**	97.9
#3	**Ddx**	**6.89**	101.5
#4	**Sud**	7.43	103.6
#5	**Allo**	**7.71**	114.1
#7	**Zea**	**7.95**	107.6
#8	**Lut**	**8.27**	102.2
#9	**Cant**	**9.79**	96.1
#10	**Chlb**	**10.80**	93.9
#11	**Chla**	**12.31**	101.4
#12	**DVCh-a**	**12.55**	102.5
#13	**Echi**	**13.18**	92.5
#15	**Phta**	**19.43**	97.4
#16	**Acar**	**20.30**	95.4
#17	**Beta**	**21.11**	na

RT -- Retention Time of an analytes on a chromatogram (min).

Duplicate Reproducibility- *is verified using a sample that is treated exactly the same throughout field and laboratory procedures.*
Analyses of duplicate sample give a measure of the precision associated with the sample preparation procedures, as well as analytical determination.

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HPLC Performance-verification using Pigments STD spiked into a sample matrix.

	#		SPK-level	RT	Low WL, -30%	High WL, +30%	Ann-Var_AV8-25
			ug/L				%
#2	1	**Fuco**	600.0	**4.94**	420.0	780.0	74.3
#9	2	**Cant**	120.0	**9.79**	84.0	156.0	125.1
#11	3	**Chla**	400.0	**12.31**	280.0	520.0	121.1

Matrix Spike Recovery -a sample with pre-determined levels of analytes (analyzed in duplicate) is used. Known amounts of 3 Pigments are added prior to extraction and analytical procedures. This QA/QC is used as a measure of both sample extraction and analytical determination procedures.

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HPLC Performance-verification using Pigments STD as surrogates spiked into a MBSR sample followed by SamplePrep procedure.

		SPK-level ug/L	RT	Ann-Var_AV8-25 Surrogate Recov., %
#2	**Fuco**	2000.0	**4.94**	106.2
#5	**Sud**	1200.0	**7.43**	86.1
#9	**Cant**	400.0	**9.79**	82.4
#11	**Chla**	400.0	**12.31**	88.3
#1	**Chlc2**	0.0	**3.48**	ND
#3	**Ddx**	0.0	**6.89**	ND
#5	**Allo**	0.0	**7.71**	ND
#7	**Zea**	0.0	**7.95**	ND
#8	**Lut**	0.0	**8.27**	ND
#9	**Cant**	0.0	**9.79**	ND
#10	**Chlb**	0.0	**10.80**	ND
#12	**DVCh-a**	0.0	**12.55**	ND
#13	**Echi**	0.0	**13.18**	ND
#15	**Phta**	0.0	**19.43**	ND
#16	**Acar**	0.0	**20.30**	ND
#17	**Beta**	0.0	**21.11**	ND

ND--Non detectable.

Method Blank-Spike Recovery (MBSR) – A blank sample (empty glass tube) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal reference standards (Sud at 400-1200 ug/L), and surrogates (Fuco, Cant, Chla at the concentrations 480, 120, 400 ug/L, respectively). The MBSR is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus. If the MBSR value of the analytes other than Fuco, Sud, Cant and Chla constitute 10% or more of the analyte level determined in a sample, the contamination must be corrected and fresh samples must be analyzed when acceptable MBSR values have been obtained. The % Recovery of the surrogates (Fuco, Cant, Chla) must be within +/-20%. At least one MBSR is analyzed with each sample batch.