

Appendix 10 HPLC Photopigment QA/QC Reports (supplied by AEL)

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20-Aug-12 Page: 1 of 1

Contract: 307071-00614

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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	Pha	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 regression with a typical coefficient of determination >0.9.

Sud- Sudan is used as Internal Reference STD added to a sample extract at a known amount (typically in a range between 400 to 800 ug/L) just prior to HPLC analysis to verify 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	Low WL	High WL	Batch#157-179 CALCHK,%
		ug/L	-15%	+15%	
#2	Fuco	170.0	144.5	195.5	99.5%
#4	Sud	700.0	595.0	805.0	105.2%
#9	Cant	120.0	102.0	138.0	93.9%
#11	Chla	100.0	85.0	115.0	112.0%

na- not applicable

Sud- Sudan is used as Internal Reference STD added to a sample extract at a known amount (typically in a range between 400 to 800 ug/L) just prior to HPLC analysis to verify 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 of samples. It is analyzed first, before the sample batch, so to verify calibration. If CALCHK 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	Batch#157- 179, %
#1	Chlc2	3.48	103.2
#2	Fuco	4.94	107.8
#3	Ddx	6.89	101.9
#4	Sud	7.43	98.2
#5	Allo	7.71	112.9
#7	Zea	7.95	110.3
#8	Lut	8.27	98.2
#9	Cant	9.79	82.5
#10	Chlb	10.80	79
#11	Chla	12.31	107.1
#12	DVCh-a	12.55	104.9
#13	Echi	13.18	109.9
#15	Phta	19.43	111.8
#16	Acar	20.30	114.8
#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 as surrogates spiked into a MBSR sample followed by SamplePrep procedure.

		SPK-level	RT	Batch#157-179
		ug/L		Surrogate Recov., %
#2	Fuco	136.0	4.94	96.1
#5	Sud	600.0	7.43	98.1
#9	Cant	120.0	9.79	105.8
#11	Chla	80.0	12.31	86.5
#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 400-800 ug/L), and surrogates (Fuco, Cant, Chla at the concentrations 136, 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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HPLC Performance-verification using Pigments STD spiked into a sample matrix.

#		SPK-level ug/L	RT	Batch#157-179		
				Low WL, -30%	High WL, +30%	%
#2	1	Fuco	136.0	95.2	176.8	91.9
#9	2	Cant	120.0	84.0	156.0	87.0
#11	3	Chla	500.0	350.0	650.0	93.9

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.

Note: Since the expected concentration of Chla in the samples of this batch is in the range of 2000 ug/L, the level of Chla spiked into the MatrixSpike sample was increased to 500ug/L.



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#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.

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A calibration curve constructed for each individual pigment is a linear regression with a typical coefficient of determination >0.9.

Sud- Sudan is used as Internal Reference STD added to a sample extract at a known amount (typically in a range between 400 to 800 ug/L) just prior to HPLC analysis to verify 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	Low WL -15%	High WL +15%	Batch#180a-201a CALCHK,%
		ug/L			
#2	Fuco	170.0	144.5	195.5	107.4%
#4	Sud	500.0	425.0	575.0	108.4%
#9	Cant	120.0	102.0	138.0	109.9%
#11	Chla	100.0	85.0	115.0	113.5%

na- not applicable

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Warning Limit (WL) for Sud is +/-15%

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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#180a-201a
#1	Chlc2	3.48	102.8
#2	Fuco	4.94	102.1
#3	Ddx	6.89	101.4
#4	Sud	7.43	103.2
#5	Allo	7.71	103.2
#7	Zea	7.95	101.2
#8	Lut	8.27	98.4
#9	Cant	9.79	114.8
#10	Chlb	10.80	99.0
#11	Chla	12.31	94.0
#12	DVCh-a	12.55	96.5
#13	Echi	13.18	113.6
#15	Phta	19.43	101.0
#16	Acar	20.30	93.8
#17	Beta	21.11	na

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

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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 as surrogates spiked into a MBSR sample followed by SamplePrep procedure.

		SPK-level	RT	Batch#180a-201a
		ug/L		Surrogate Recov., %
#2	Fuco	136.0	4.94	86.3
#5	Sud	500.0	7.43	95.4
#9	Cant	120.0	9.79	109.5
#11	Chla	80.0	12.31	85.4
#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 added at the concentrations 136, 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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#9	2	Cant	120.0	84.0	156.0	112.7
#11	3	Chla	500.0	350.0	650.0	113.7

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.

Fuco, Cant and Chla added at the concentrations of 136, 120 and 500ug/L, respectively



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10-Oct-12

Page: 1 of 1

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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 regression with a typical coefficient of determination >0.9.

Sud- Sudan is used as Internal Reference STD added to a sample extract at a known amount (typically in a range between 400 to 800 ug/L) just prior to HPLC analysis to verify the accuracy of the injection volume.

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



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Page: 1 of 1

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HPLC Performance-verification using CALCHK

	Analyte	CALCHK	Low WL -15%	High WL +15%	Batch#278-288 CALCHK,%
		ug/L			
#2	Fuco	170.0	144.5	195.5	107.4%
#4	Sud	500.0	425.0	575.0	108.4%
#9	Cant	120.0	102.0	138.0	109.9%
#11	Chla	100.0	85.0	115.0	113.5%

na- not applicable

Sud- Sudan is used as Internal Reference STD added to a sample extract at a known amount (typically in a range between 400 to 800 ug/L) just prior to HPLC analysis to verify 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 of samples. It is analyzed first, before the sample batch, so to verify calibration. If CALCHK 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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Page: 1 of 1

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HPLC Performance-verification using sample duplicate

		RT, min	Batch#278-288 Dup.Reproduc., %
#1	Chlc2	3.48	96.1
#2	Fuco	4.94	97.8
#3	Ddx	6.89	101.5
#4	Sud	7.43	98.7
#5	Allo	7.71	102.2
#7	Zea	7.95	97.3
#8	Lut	8.27	100.7
#9	Cant	9.79	106.3
#10	Chlb	10.80	98.5
#11	Chla	12.31	93.4
#12	DVCh-a	12.55	87.5
#13	Echi	13.18	107.7
#15	Phta	19.43	98.5
#16	Acar	20.30	102.9
#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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10-Oct-12 Page: 1 of 1

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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#278-288
		ug/L		Surrogate Recov., %
#2	Fuco	136.0	4.94	88.6
#5	Sud	500.0	7.43	93.8
#9	Cant	120.0	9.79	114.1
#11	Chla	80.0	12.31	90.1
#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 added at the concentrations 136, 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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HPLC Performance-verification using Pigments STD spiked into a sample matrix.						
#		SPK-level	RT	Low WL, -30%	High WL, +30%	Batch#278-288
		ug/L				
#2	1	Fuco	136.0	95.2	176.8	91.3
#9	2	Cant	120.0	84.0	156.0	112.7
#11	3	Chla	500.0	350.0	650.0	113.7

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.

Fuco, Cant and Chla added at the concentrations of 136, 120 and 500ug/L, respectively

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