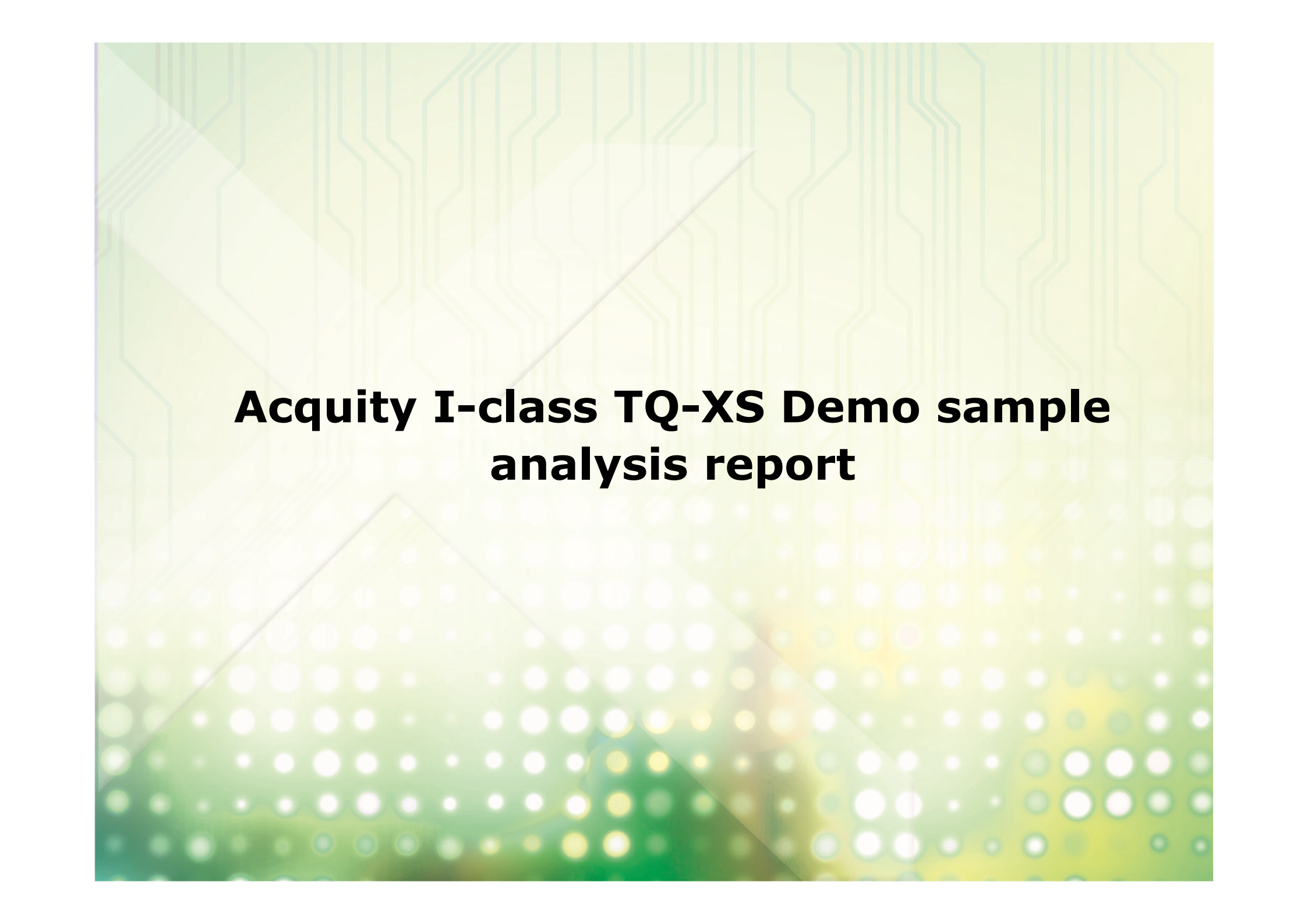


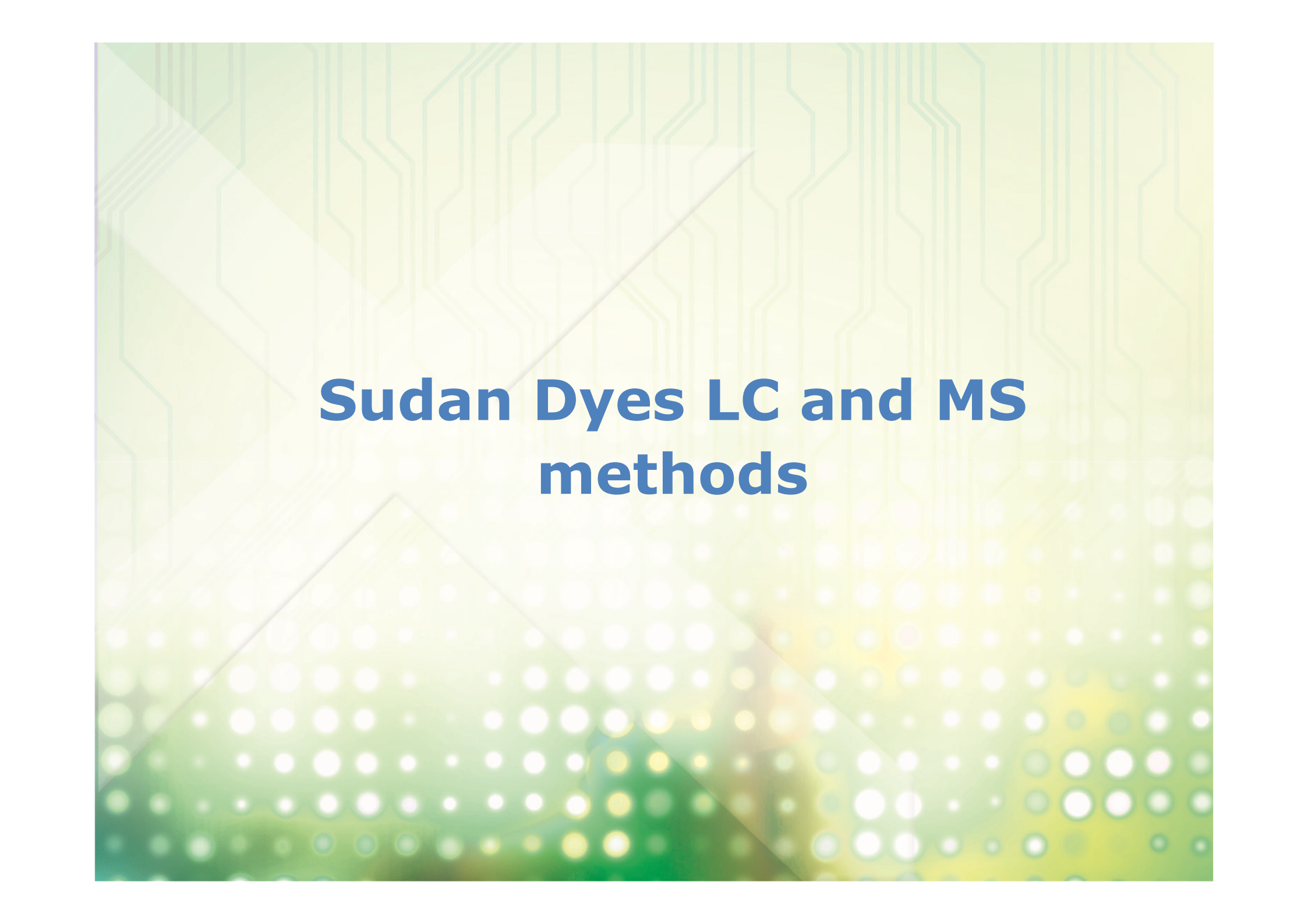


Acquity I-class TQ-XS Demo sample analysis report

Introduction

AVA has provided us with four groups of samples.

- Group -1 : Sudan Dyes
 - Group -2 : Sweeteners
 - Group -3 : Marine Biotoxins List A
 - Group -4 : Marine Biotoxins List B
-
- This system must be able to run a variety of applications, including a sensitive assay for all the group of compounds mentioned in the above samples.
 - This report demonstrates the potential for the I-Class FTN/XEVO TQ-S to provide robust and sensitive results for the above mentioned compounds.
 -



Sudan Dyes LC and MS methods

Samples Provided

Samples :

- Three samples: 1 blank and 2 spiked extracts Y(S1) and Y(S2) were provided in acetonitrile.
- One Sudan dye standard 100ppb in Acetonitrile (Std Y) was given for the preparation of the calibration curve.

Samples were ran on the XEVO TQ-XS in order to assess the systems in the most efficient way, with sample stability not influencing results

Experimental

Sample Analysis:

- Calibration standards were prepared at 0.05, 0.125, 0.25, 0.5 and 1ppb levels in neat solvent
- For robust chromatography, the diluent of the samples was adjusted to 30% aqueous prior to injection. (See appendix for comparison of elution profiles at different diluent strength)
- MRM transitions and MS acquisition conditions were optimized using available Sudan dye standards in Waters lab.

Quantification

- Y (Blank), Y(S1) and Y(S2) were analyzed on the calibration curve.
- Concentration was back-calculated for the unknown sample from the calibration curve.

LC Conditions

LC Conditions	
Column	Acquity UPLC BEH C8 2.1x100 mm, 1.7µm
Solvent A	Water (0.1%FA)
Solvent B	Acetonitrile
Injection volume	10 µL

Time	Flow rate	% B
0	0.4	40
4	0.4	99
6	0.4	99
6.3	0.4	40
8	0.4	40

MS Method: 11 Sudan Dyes

Analyte	Parent	Daughter	Dwell	Cone Voltage	Collision energy	Mode of Ionization
Sudan I	248.90	92.95	0.010	20	22	ES+
	248.90	127.98	0.010	20	22	ES+
	248.90	156.0	0.010	20	20	ES+
Sudan II	277.03	106.01	0.010	20	36	ES+
	277.03	120.96	0.010	20	20	ES+
	277.03	155.99	0.010	20	20	ES+
Sudan III	353.03	76.99	0.010	28	26	ES+
	353.03	91.88	0.010	28	34	ES+
	353.03	196.74	0.010	28	18	ES+
Sudan IV	381.10	90.96	0.010	20	24	ES+
	381.10	105.92	0.010	20	34	ES+
	381.10	156.00	0.010	20	28	ES+
Sudan Black B	457.16	182.23	0.010	31	40	ES+
	457.16	194.00	0.010	31	36	ES+
	457.16	211.09	0.010	31	24	ES+
Sudan Orange G	214.78	77.10	0.010	20	20	ES+
	214.78	93.07	0.010	20	20	ES+
	214.78	122.00	0.010	20	18	ES+

MS Method: 11 Sudan Dyes

Analyte	Parent	Daughter	Dwell	Cone Voltage	Collision energy	Mode of Ionization
Sudan red B	381.2	209.00	0.011	32	32	ES+
	381.2	224.00	0.011	32	32	ES+
	381.2	134.10	0.011	32	32	ES+
Sudan red G	278.97	107.97	0.010	20	34	ES+
	278.97	122.99	0.010	20	18	ES+
	278.97	156.01	0.010	20	14	ES+
Sudan red 7B	380.16	114.98	0.010	25	48	ES+
	380.16	168.99	0.010	25	30	ES+
	380.16	183.09	0.010	25	14	ES+
Rhodamine B	443.22	282.07	0.012	10	72	ES+
	443.22	341.08	0.012	10	64	ES+
	443.22	355.05	0.012	10	56	ES+
Para red	293.90	127.99	0.010	26	30	ES+
	293.90	143.00	0.010	26	28	ES+
	293.90	156.00	0.010	26	16	ES+

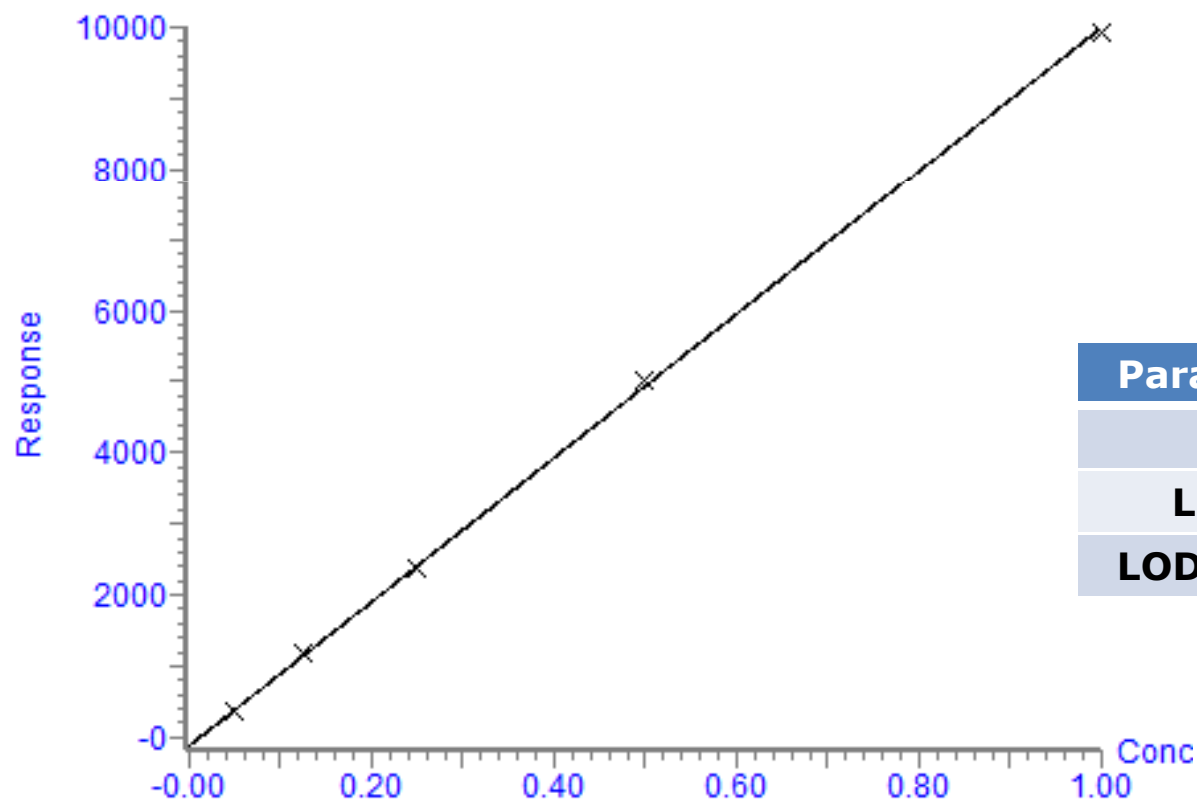
*Sudan red B standard not available for tuning, MRM transitions were obtained from Quanpedia and published literature.



Results

Calibration Curve – Para Red

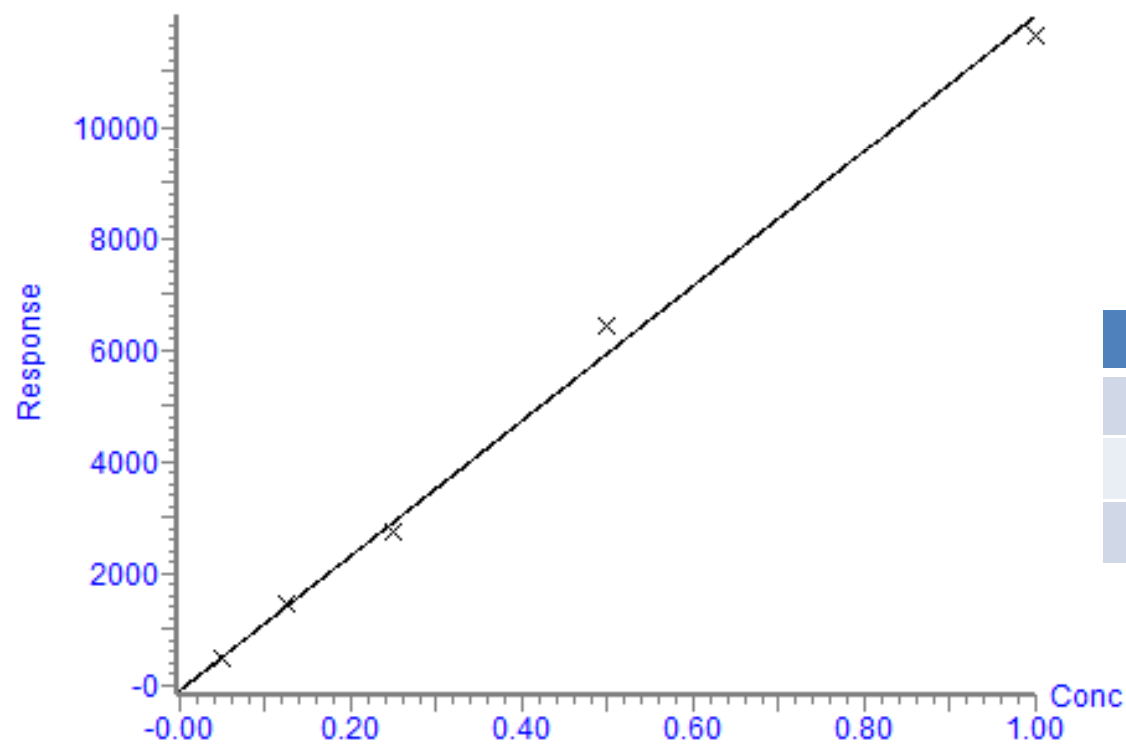
Compound name: Para Red
Correlation coefficient: $r = 0.999872$, $r^2 = 0.999743$
Calibration curve: $10120.9 * x + -116.718$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None



Parameter	Result
R²	0.999
LLOQ	0.05 ppb
LOD (calc)	0.002ppb

Calibration Curve – Sudan I

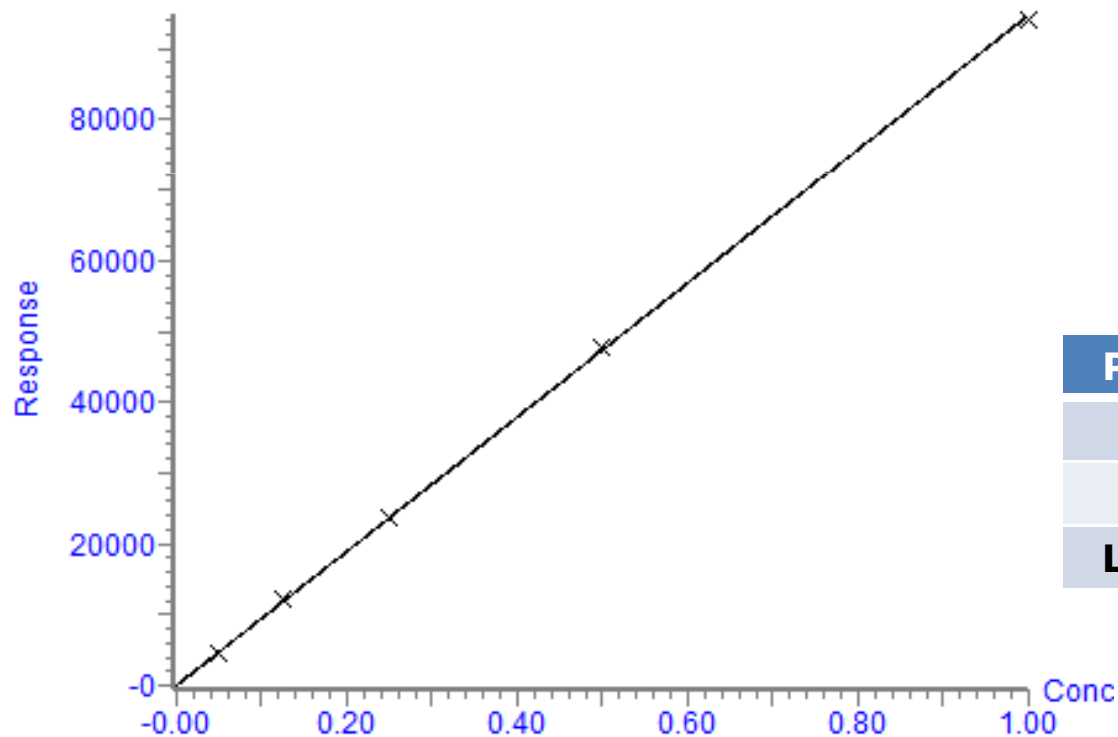
Compound name: Sudan I
Correlation coefficient: $r = 0.997805$, $r^2 = 0.995616$
Calibration curve: $12080.8 * x + -93.4036$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None



Parameter	Result
R ²	0.996
LLOQ	0.05 ppb
LOD (calc)	0.001ppb

Calibration Curve – Sudan II

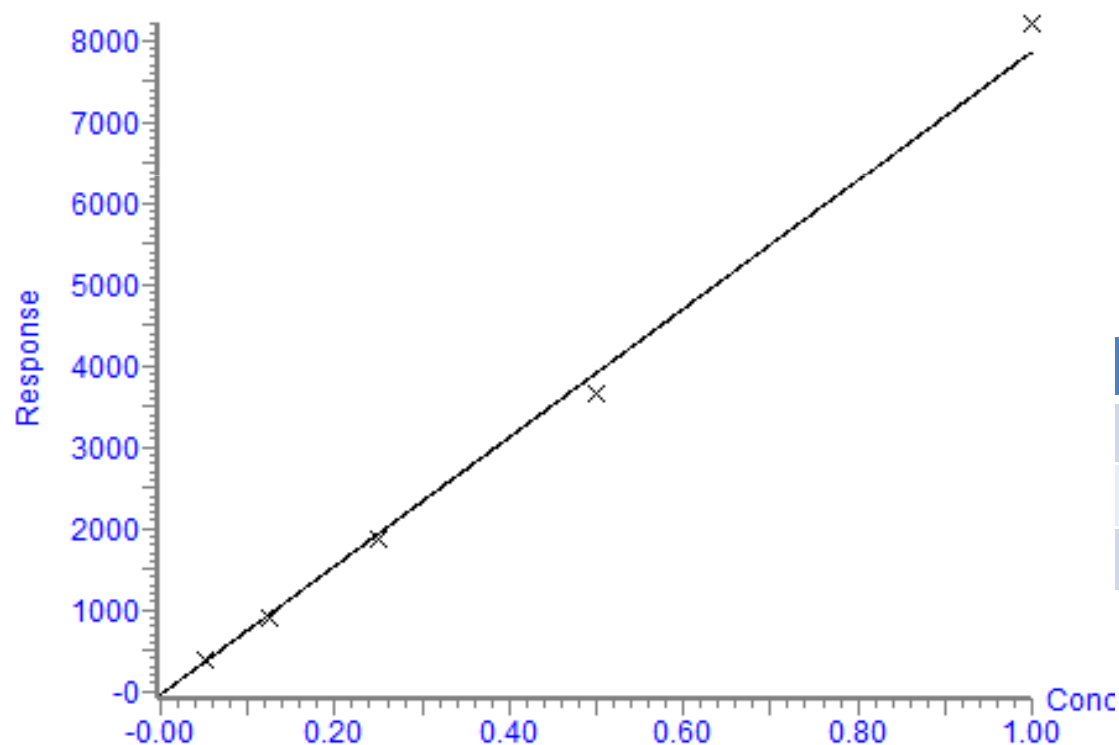
Compound name: Sudan II
Correlation coefficient: $r = 0.999878$, $r^2 = 0.999756$
Calibration curve: $94795.4 * x + -4.38286$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Parameter	Result
R ²	0.999
LLOQ	0.05 ppb
LOD (calc)	0.004ppb

Calibration Curve Sudan III

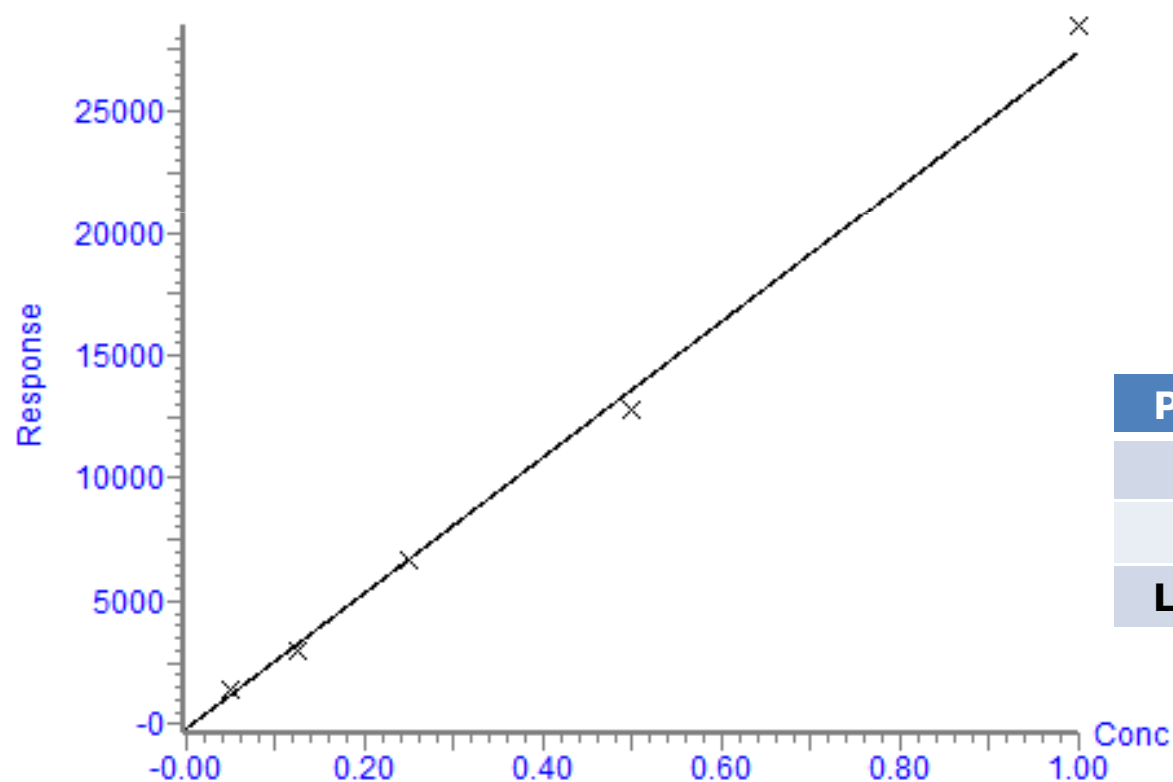
Compound name: Sudan III
Correlation coefficient: $r = 0.997776$, $r^2 = 0.995557$
Calibration curve: $7906.02 * x + -40.6786$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Parameter	Result
R ²	0.996
LLOQ	0.05 ppb
LOD (calc)	0.003ppb

Calibration Curve – Sudan IV

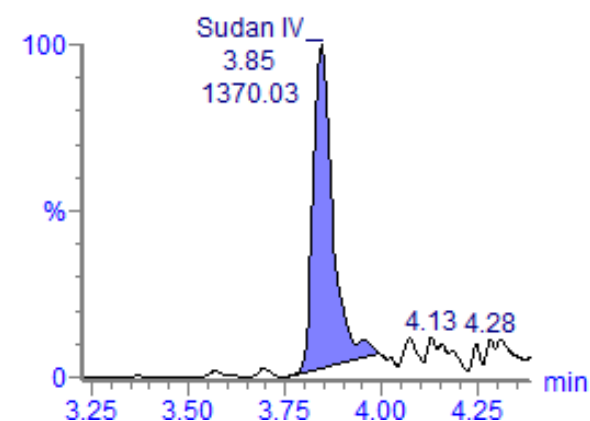
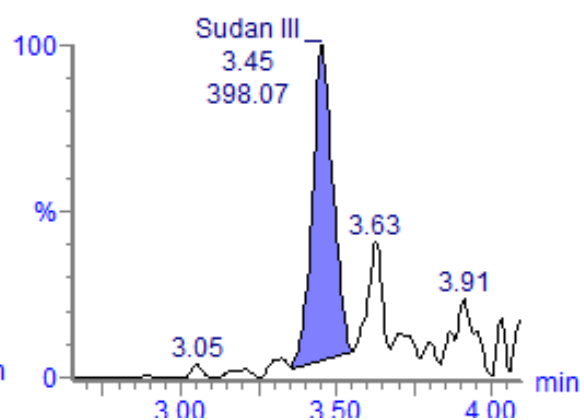
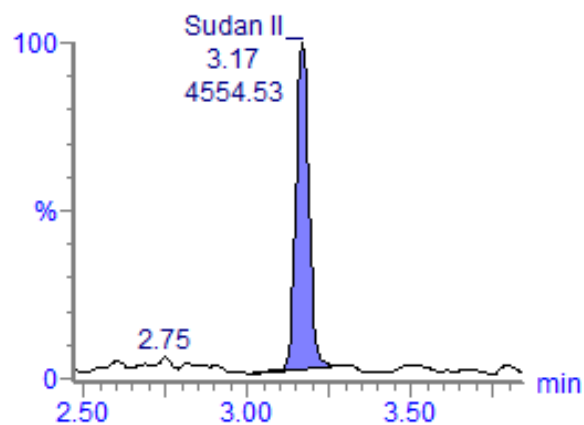
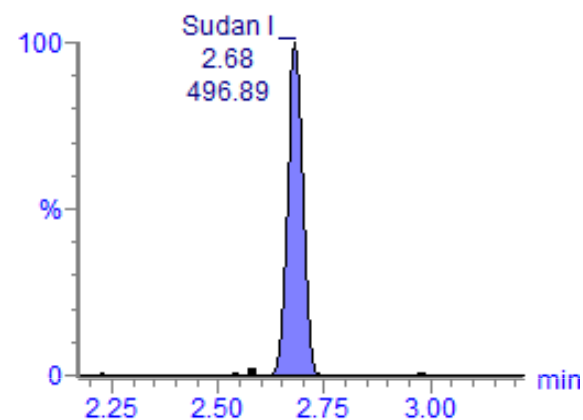
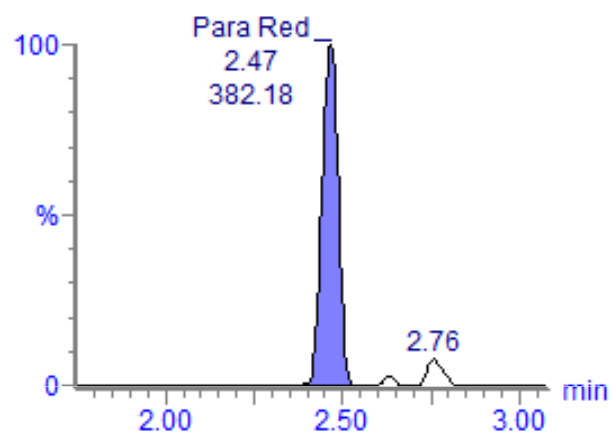
Compound name: Sudan IV
Correlation coefficient: $r = 0.997889$, $r^2 = 0.995783$
Calibration curve: $27617.5 * x + -175.11$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Parameter	Result
R²	0.996
LLOQ	0.05 ppb
LOD (calc)	0.001ppb

Lower Concentration Chromatograms

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®



Signal to Noise ratio for LLOQ

ANALYTE	SIGNAL TO NOISE RATIO
PARA RED	84
SUDAN I	138
SUDAN II	40
SUDAN III	58
SUDAN IV	144

Quantitation Results

Sudan Dyes	Std Y 100ppb	Y (Blank) ppb	Y (S1) ppb	Y (S2) ppb
Para Red	Detected	<LOQ	0.188	0.102
Sudan I	Detected	<LOQ	0.203	0.106
Sudan II	Detected	0.433	0.697	0.612
Sudan III	Detected	<LOQ	0.332	0.144
Sudan IV*	Detected	0.109	0.333	0.050

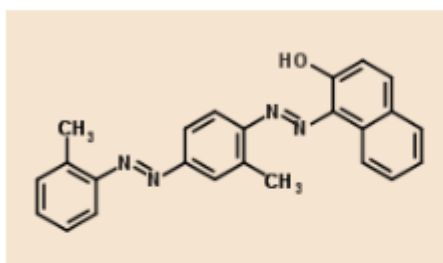
*High background noted (Sudan IV)

Quantitation Results

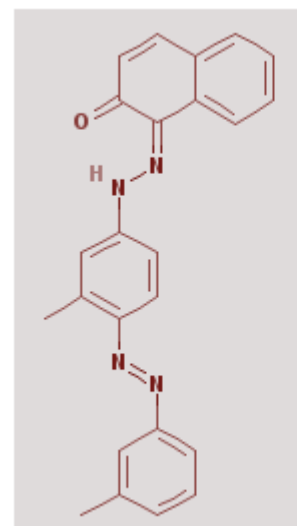
Sudan Dyes	Std Y (100ppb)	Remarks
Sudan Orange G	Not detected	
Sudan red G	Not detected	
Sudan red 7B	Not detected	
Rhodamine B	Not detected	

Quantitation Results

Sudan Dyes	Std Y (100ppb)	Remarks
Sudan red B	Detected but not quantified	Structural isomer of Sudan IV, shares same mass transitions. Close RT, not distinguishable from Sudan IV on the system



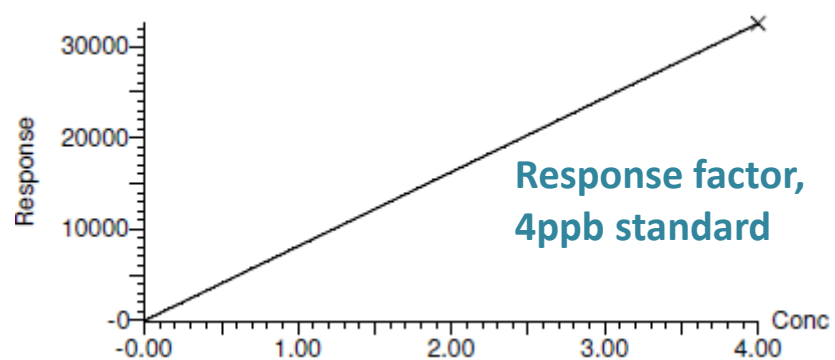
Sudan IV
C₂₄H₂₀N₄O



**Sudan
Red B**
C₂₄H₂₀N₄O

Quantitation Results

Sudan Dyes	Std Y (100ppb)	Remarks
Sudan Black B	RT shifts	Detected in sample



Based on Area Response

Y blank ppb	YS1 ppb	YS2 ppb
0	1.81	0.612

Conclusion

- A LC-MS/MS method under 9 minutes was used to analyse ppb levels of Sudan dyes. 3 MRM transitions per compound were specified in the MS method.
- In the semi-blind demo analysis, 6 sudan dyes Para Red, Sudan I, Sudan II, Sudan III, Sudan IV and Sudan Black B were detected in spiked samples.
- The samples were found to contain the following concentrations against the Standard Y:

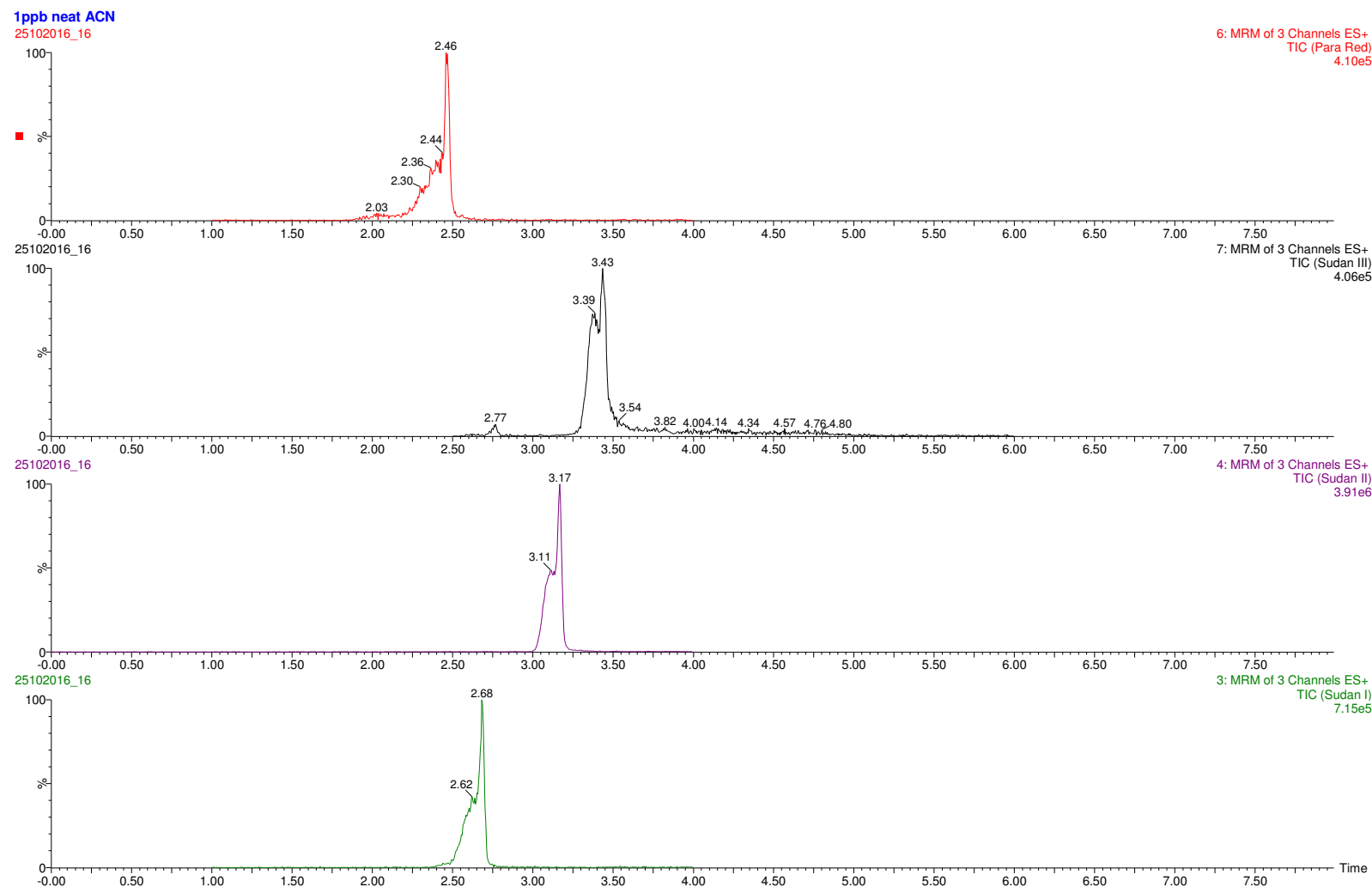
Sudan Dyes	Y (Blank) ppb	Y (S1) ppb	Y (S2) ppb
Para Red	<LOQ	0.188	0.102
Sudan I	<LOQ	0.203	0.106
Sudan II	0.433	0.697	0.612
Sudan III	<LOQ	0.332	0.144
Sudan IV	0.109	0.333	0.050

- In summary, the analysis has shown that the Xevo TQ-XS demonstrates good potential to provide high quality data in terms of linearity and sensitivity for detection and quantitation of Sudan dyes.

Appendix

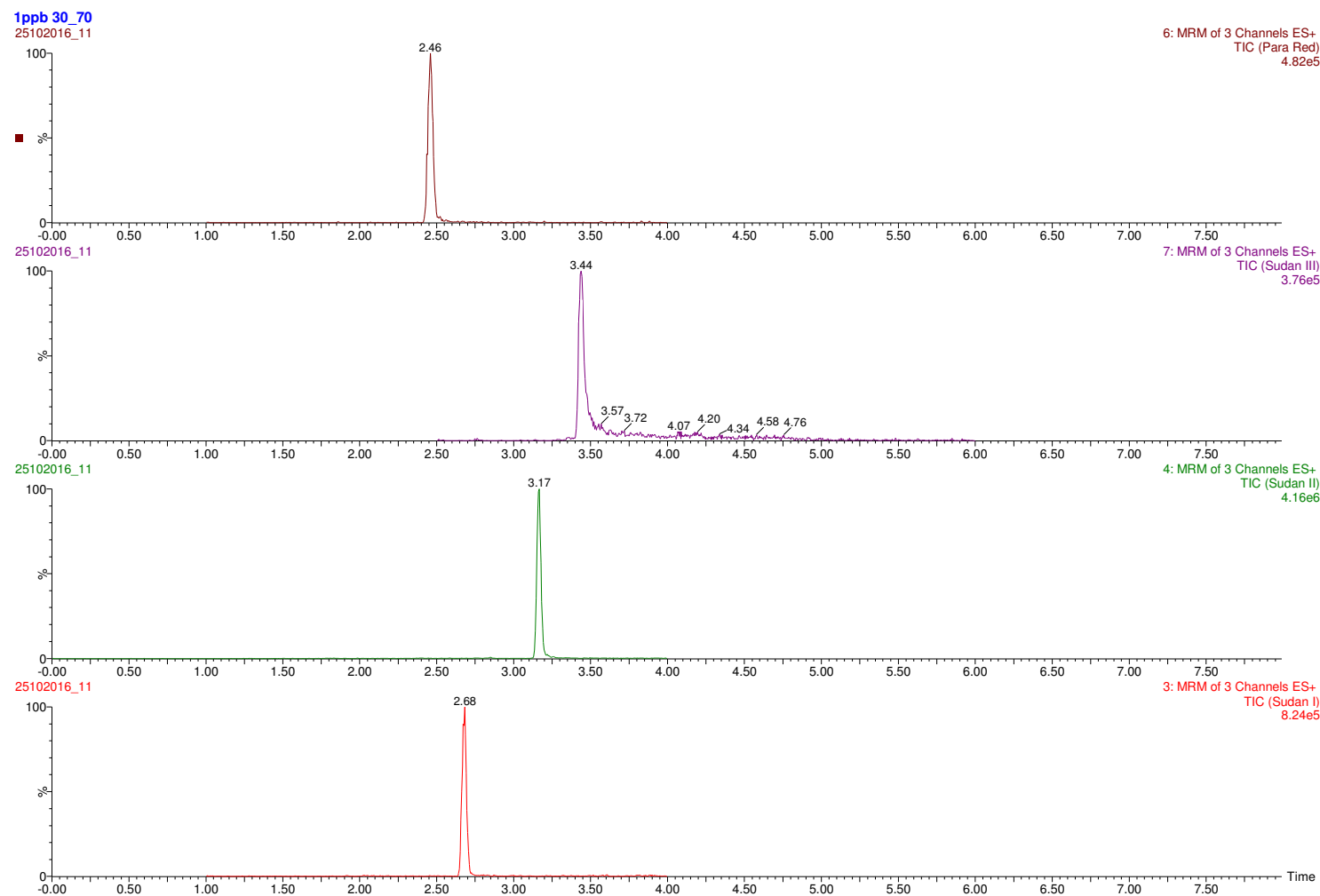
Appendix

■ Peak fronting with diluent as 100% organic



Appendix

- Better peak with diluent with 30% aqueous



Appendix

- Pure Sudan IV standard – shows up in Sudan Red B mass transitions

