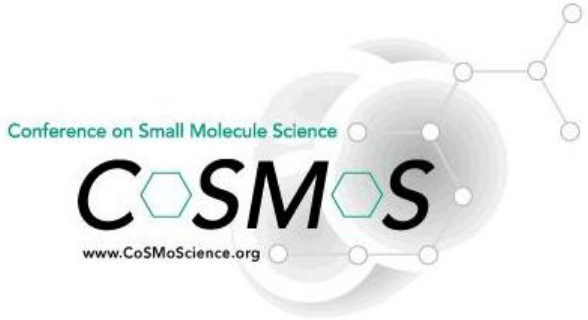


AN INVESTIGATION INTO THE COMPLEXITIES OF LIBRARY QUALITY

Cynthia Jeffries

Group Leader, Analytical Chemistry and High Throughput Purification

Chembridge Research Labs

San Diego, CA

Conference on Small Molecule Science
CoSMoS
www.CoSMoScience.org

Company Overview

I. Chembridge Corporation, Moscow
   - Started in 1993
   - Employs 300+ chemists
   - Provides Screening Compounds, Small molecule Libraries, and Building Blocks to pharmaceutical research companies

II. Chembridge Research Labs, San Diego
   - Started in 2000
   - Employs ~30 Chemists
   - Offers: Library Synthesis, Purification, and QC
     - BioAssayScreening
     - Medicinal Chemistry Support
   - In-House Drug Discovery Program

Conference on Small Molecule Science

CoSMoS

www.CoSMoScience.org

“QUALITY”

Why is it important?

Conference on Small Molecule Science
COSMOS
www.CoSMoScience.org

Successful Drug Discovery Depends on Accurate and complete SAR results:

Large number of compounds through screening
Pure compounds required to prevent interferences.
Absolute requirement for proper identity as given.

Lack of Quality results in:

Waste of Time and Resources
Incluclisive SAR Results
Frustration
Delay in the progression of a project

Conference on Small Molecule Science
COSMOS
www.CoSMoScience.org

QUALITY vs QUANTITY

Greater Quality Requirements:

Less Compounds
Higher Cost
Greater Turnaround Time
Larger Quantity and Complexity of Data

Lower Quality Can Produce:

False Hits
Missed Hits
Lack of confidence in Results

Conference on Small Molecule Science

COSMOS

www.CoSMoScience.org

Library Quality:

ANALYTICAL DEFINITION

Purity

Identity

Quantity

Conference on Small Molecule Science
COSMOS
www.CoSMoScience.org

Perceived Quality:

Color of powder or clarity of solution

Stability of compound in shipped format

Number and strength of hits obtained with the library.

Conference on Small Molecule Science
COSMOS
www.CoSMoScience.org

Quality:

As defined By Our Customers:

Minimum Purity:

UV
Evaporative Scattering Light Detection (ELSD)
Total Ion Chromatograph (TIC)
[Chemiciluminescence Nitrogen Detection (CLND)]

May require HPLC purification regardless of crude purity results

Identity:

NMR
Mass Spectrometry

Minimum Amount Accepted

Success Rate

Purification Method: (Acceptable Salt Forms)

Conference on Small Molecule Science
COSMOS
www.CoSMoScience.org

Additional Considerations Affecting Quality:

Point in process that final QC aliquot is removed for analysis.
(From Mobile Phase or After Drying)

Solvent Used in Final QC
Interference in analysis in UV and MS
Stability of compounds

Conference on Small Molecule Science

COSMOS

www.CoSMoScience.org

Final Form of Compounds for Shipment:

- Dry or In Solution
- Concentration if in solution
- Dry Ice or Ambient

Data Format and Fields to Accompany Compounds

QC/purification methods requested?

How will diastereomers be handled?

Conference on Small Molecule Science
CoSMoS
www.CoSMoScience.org

PURITY DEFINITIONS WE HAVE USED

Purity Determined by Following Detectors, Singly or in Various Combinations:

UV: Wavelength at:

210 nm

215 nm

254 nm

260 nm

Total Wavelength Chromatogram A chromatographic display created by summing all of the absorbance values in the acquired wavelength range and then plotting the values against time.

ELSD:

Total Ion Chromatogram from APCI (Atmospheric Pressure Chemical Ionization) **Source**

Conference on Small Molecule Science
COSMOS
www.CoSMoScience.org

I. Purity must be = or > 90% pure by ELSD

II. Purity must be = or >85% pure by UV at 260 nm

III. Purity must be = or > 85% pure by UV at 254 nm and ELSD.

IV. Purity must be = or > 80% pure by UV at 210 nm and = or > than 85% by ELSD.

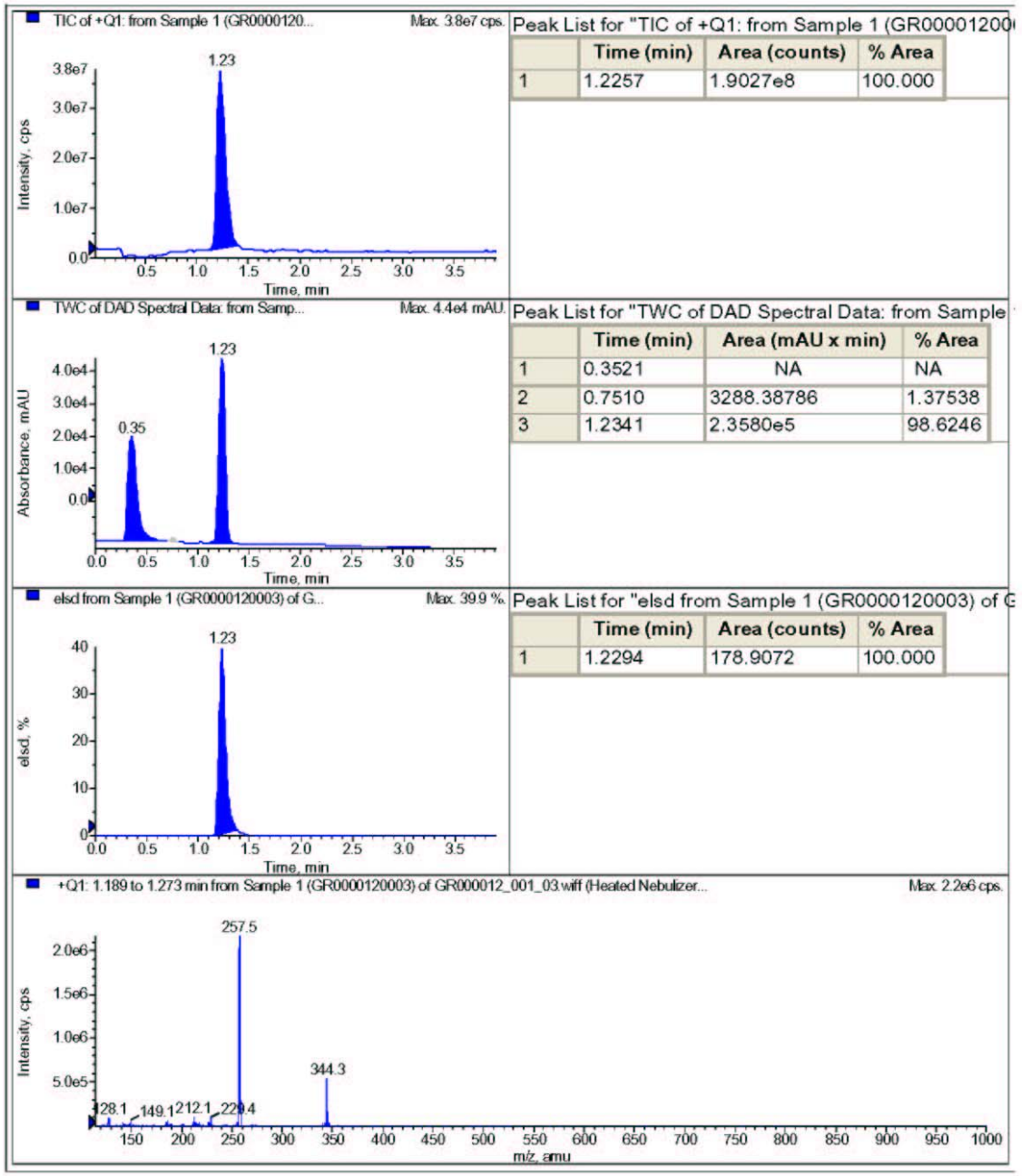
V. Purity must be = or >85% pure by 2 of the following detectors:
UV at 260 nm or TWC (Total Wavelength Chromatogram)
ELSD
TIC (Total Ion Chromatogram) using APCI source

Conference on Small Molecule Science

CoSMoS

www.CoSMoScience.org

Custom Annotation: MW: 343.2372; Formula: C19H29NSO;



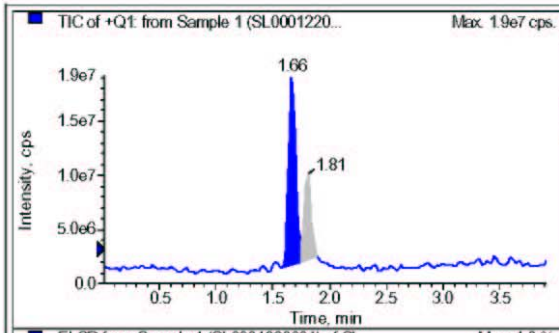
CA

Conference on Small Molecule Science

COSMOS

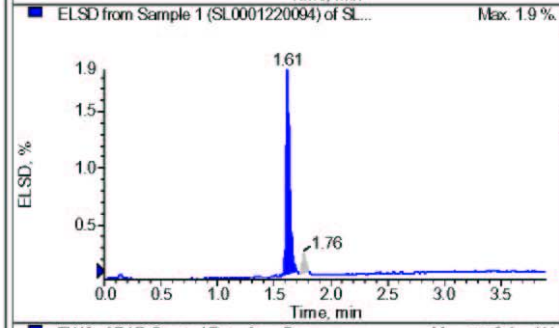
www.CoSMoScience.org

Custom Annotation: MW: 384.15; Formula: C21H24N2O3S;



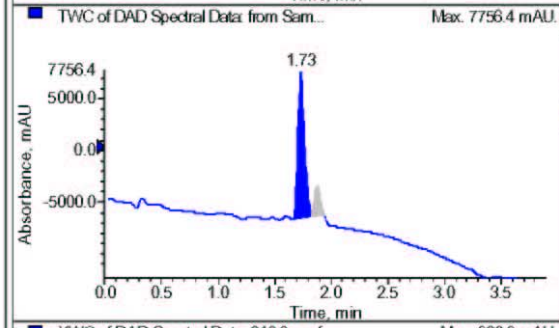
Peak List for "TIC of +Q1: from Sample 1 (SL00012200

	Time (min)	Area (counts)	% Area
1	1.6640	7.6597e7	69.4498
2	1.8094	3.3694e7	30.5502



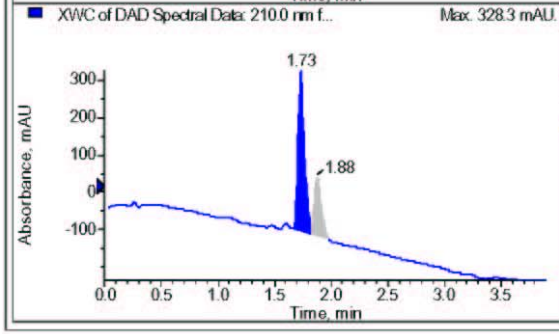
Peak List for "ELSD from Sample 1 (SL0001220094) of

	Time (min)	Area (counts)	% Area
1	1.6148	4.4836	92.2035
2	1.7592	0.3791	7.7965



Peak List for "TWC of DAD Spectral Data: from Sample

	Time (min)	Area (mAU x min)	% Area
1	1.7302	5.6224e4	85.9543
2	1.8763	9187.5471	14.0457



Peak List for "XWC of DAD Spectral Data: 210.0 nm fron

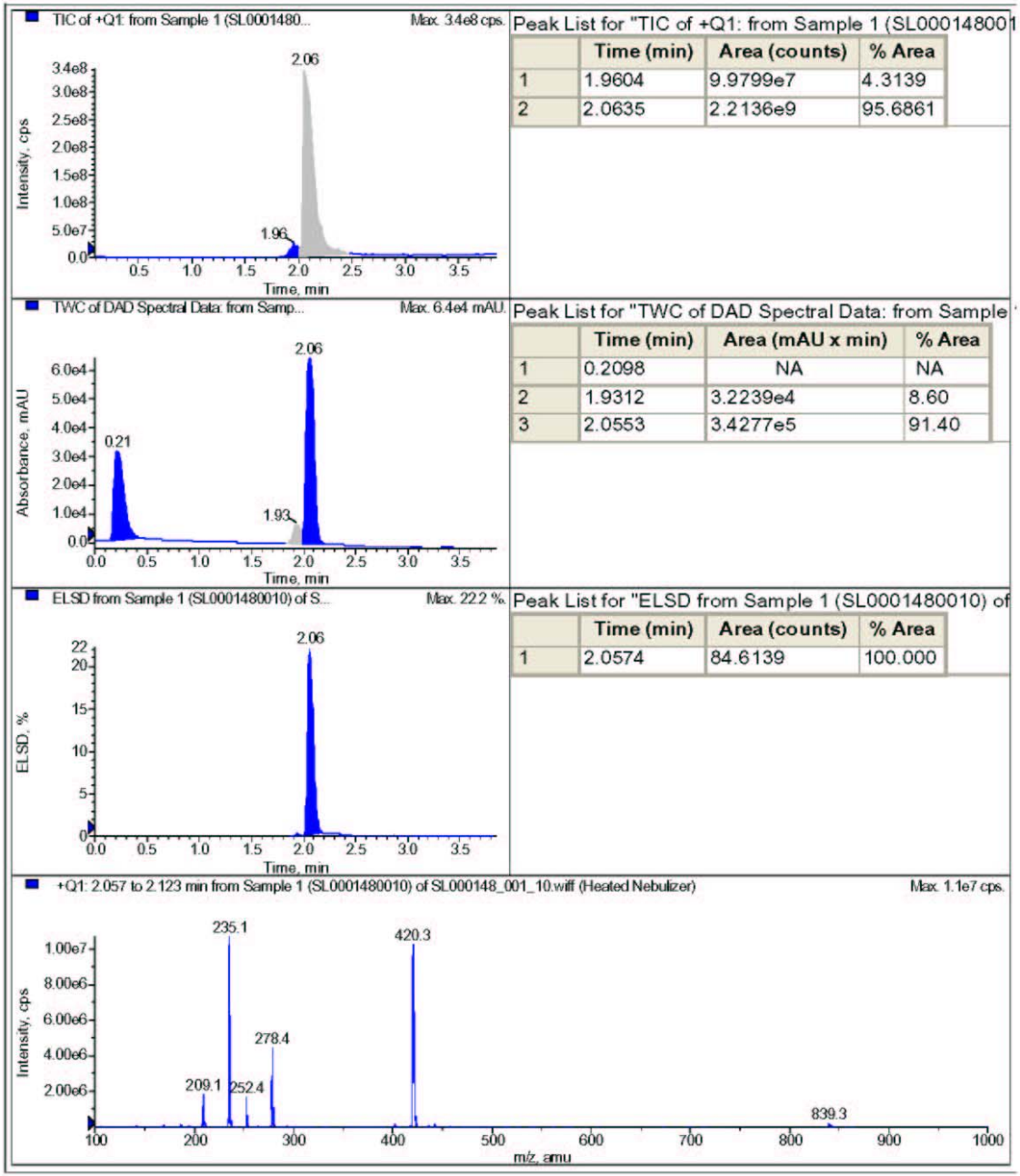
	Time (min)	Area (mAU x min)	% Area
1	1.7298	1667.6640	72.2482
2	1.8762	640.5788	27.7518

Conference on Small Molecule Science

COSMOS

www.CoSMoScience.org

Custom Annotation: MW: 419.13036; Formula: C23H21N3O3S;



Peak List for "TIC of +Q1: from Sample 1 (SL00014800

	Time (min)	Area (counts)	% Area
1	1.9604	9.9799e7	4.3139
2	2.0635	2.2136e9	95.6861

Peak List for "TWC of DAD Spectral Data: from Sample

	Time (min)	Area (mAU x min)	% Area
1	0.2098	NA	NA
2	1.9312	3.2239e4	8.60
3	2.0553	3.4277e5	91.40

Peak List for "ELSD from Sample 1 (SL0001480010) of

	Time (min)	Area (counts)	% Area
1	2.0574	84.6139	100.000

Conference on Small Molecule Science
COSMOS
www.CoSMoScience.org

OBSERVATIONS:

1. Many compounds with excellent purity results by TIC and ELSD have purities below 80% in UV at 210 nm.

2. Compounds and impurities have varying detection biases which result in recorded purities that do not reflect reality.

3. Often compounds have consistent purities in 2 of the 3 detectors (UV, ELSD, TIC) with 1 outlier.

Conference on Small Molecule Science
COSMOS
www.CoSMoScience.org

IDENTITY:

Mass Spec: fast and reliable.

NMR Not conducive to High Throughput. Can be used for compounds that do not ionize or which fragment readily.

Acceptable Spectra:

M + 1 in positive mode
M – 1 in negative mode
M + 23 in positive mode
Logical fragments

Quantity

Various Agreements for Acceptable Shipped Quantities:

1. Specified umole amount (5 – 50)

2. Specified mg amount (25 mg)

3. Range of Quantity Accepted, but Pay is Bilevel:
   Above a certain minimum, full pay is received
   Levels below this amount given a percentage of full pay.

4. Range of quantities allowed within specified limits:

Ex:

5 – 15 uMoles	25%
>15 uMoles	75%

Conference on Small Molecule Science

COSMOS

www.CoSMoScience.org

Purification Method:

HPLC

Flash Chromatography

Prep TIC

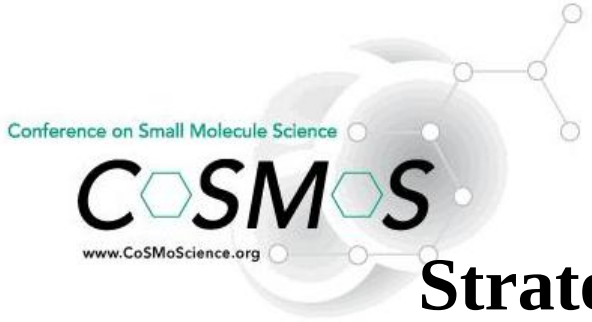
Analytical Method:

Column Properties (Size, Efficiency, Packing Material)

Gradient Time

Mobile Phase Requirements

Mass Spec Requirements (Source, Polarity)



Strategies for Setting Specs for Libraries:

Determine The Minimum Requirements Needed for Your Project

Does Your Screening Assay Demand Low UV Interference at Specific Wavelengths?

Are Anticipated Synthetic Side Products/Catalysts/Starting Materials Expected to Give Erroneous Screening Results?

WHAT PURITY SPECIFICATIONS WILL BE USED?

1. **ELSD is Generally Considered the most quantitative of the 3 detectors.**
   Problem: Volatile compounds/impurities
2. **UV using TWC is generally more representative of quantities than extracted wavelengths.**
   Problem: UV transparent compounds/impurities
3. **TIC: APCI positive mode picks up a large number of compounds/impurities**
   Problem: Variations in ionization capacities of compounds/ impurities.
   Compounds/impurities that only ionize in negative mode.

Conference on Small Molecule Science
COSMOS
www.CoSMoScience.org

Personal Observations:

A combination of all 3 detectors will give the most accurate, representative picture of a compound's purity.

Simplicity is best when dealing with the large numbers of library compounds.

UV AND ELSD transparent compounds should have a mechanism to quickly determine acceptance.

Non-ionizing compounds should also have an alternative method for determining identity.

Conference on Small Molecule Science
COSMOS
www.CoSMoScience.org

Example:

Purity:

I. 80% purity in UV using TWC.
85% purity in ELSD.
80% purity in TIC

Problem: UV/ELSD transparent compounds.

II. 85% purity required in 2 of the 3 detectors – UV using TWC unless screening assay requires low interference at low wavelengths.

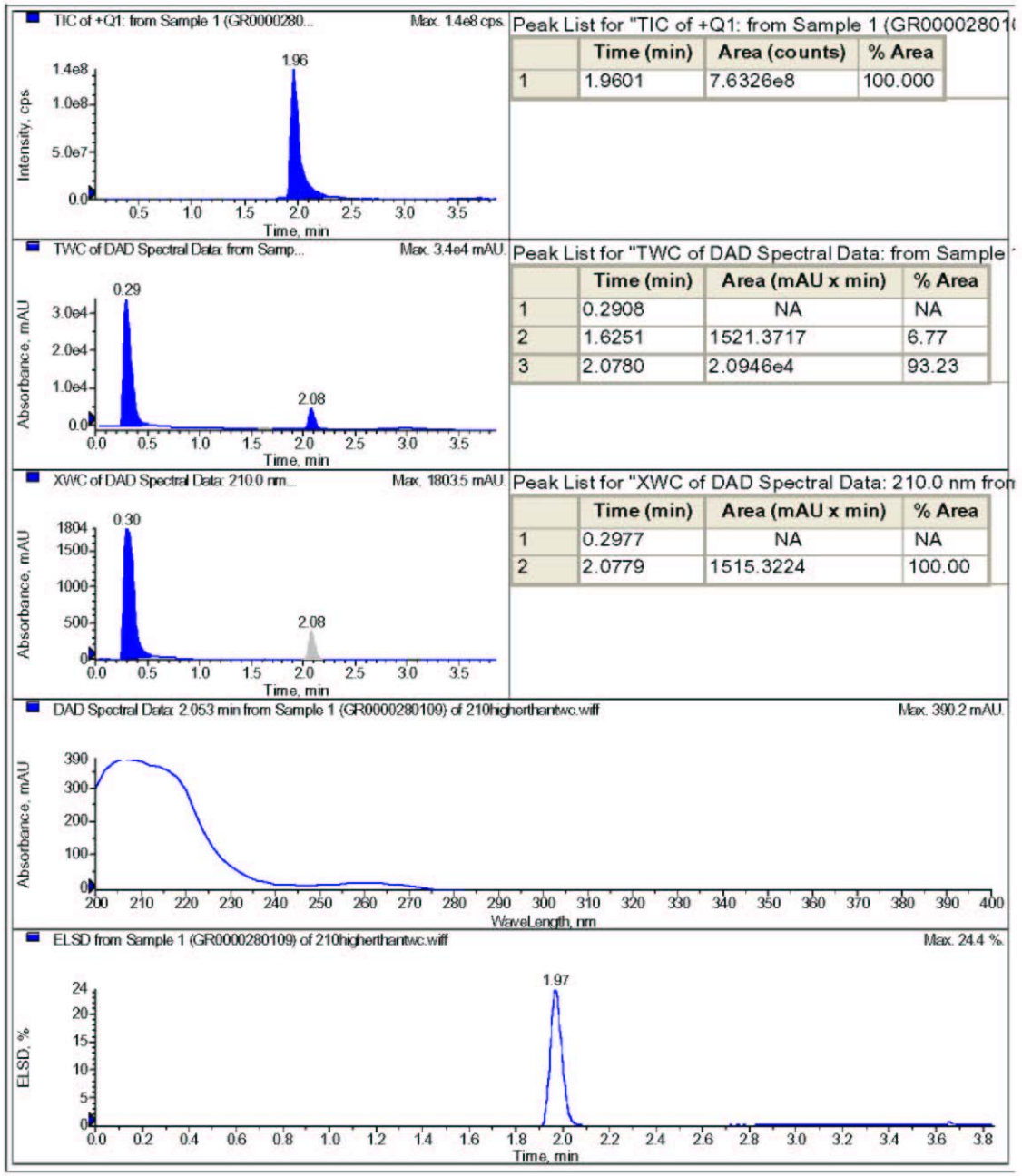
Identity: Accept logical fragments for identity of non-ionizable compounds or NMR confirmation

Conference on Small Molecule Science

COSMOS

www.CoSMoScience.org

Sample Name: GR0000280109 Custom Annotation: MW: 428.22867; Formula: C22H31F3N2O8;



Peak List for "TIC of +Q1: from Sample 1 (GR00002801

	Time (min)	Area (counts)	% Area
1	1.9601	7.6326e8	100.000

Peak List for "TWC of DAD Spectral Data: from Sample

	Time (min)	Area (mAU x min)	% Area
1	0.2908	NA	NA
2	1.6251	1521.3717	6.77
3	2.0780	2.0946e4	93.23

Peak List for "XWC of DAD Spectral Data: 210.0 nm fron

	Time (min)	Area (mAU x min)	% Area
1	0.2977	NA	NA
2	2.0779	1515.3224	100.00

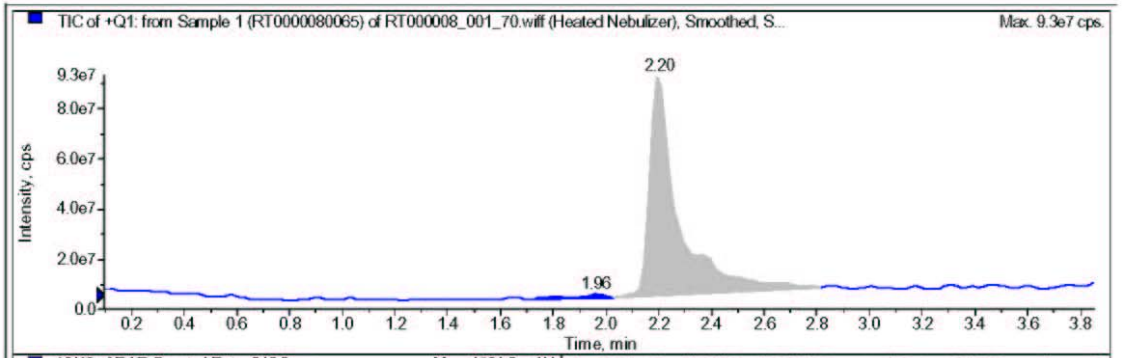
CA

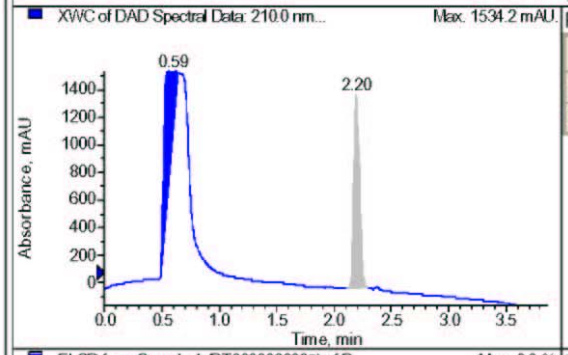
Conference on Small Molecule Science

COSMOS

www.CoSMoScience.org

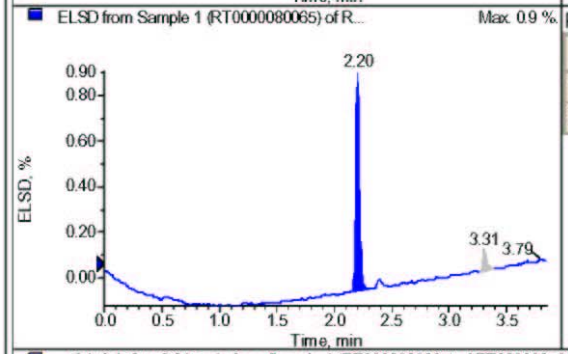
Sample Name: RT0000080065 Custom Annotation: MW: 218.08554; Formula: C12H11FN2O;





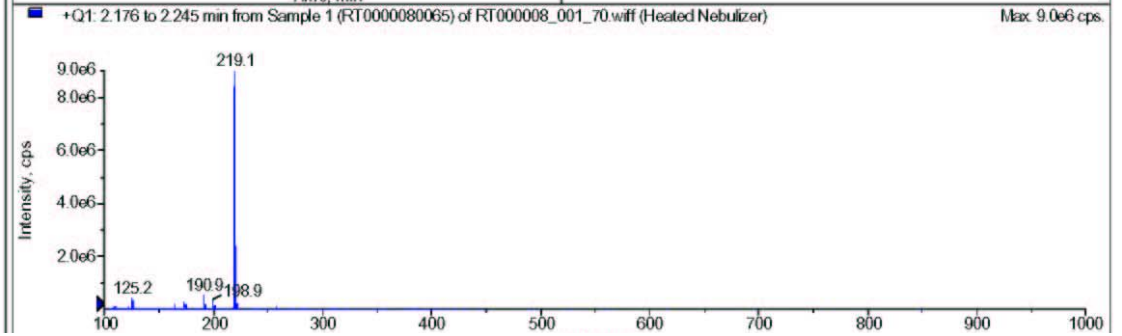
Peak List for "XWC of DAD Spectral Data: 210.0 nm from Sa

	Time (min)	Area (mAU x min)	% Area
1	0.5914	NA	NA
2	2.1963	4730.9431	100.000



Peak List for "ELSD from Sample 1 (RT0000080065) of RTC

	Time (min)	Area (counts)	% Area
1	2.2006	2.1582	90.9576
2	3.3061	0.2146	9.0424

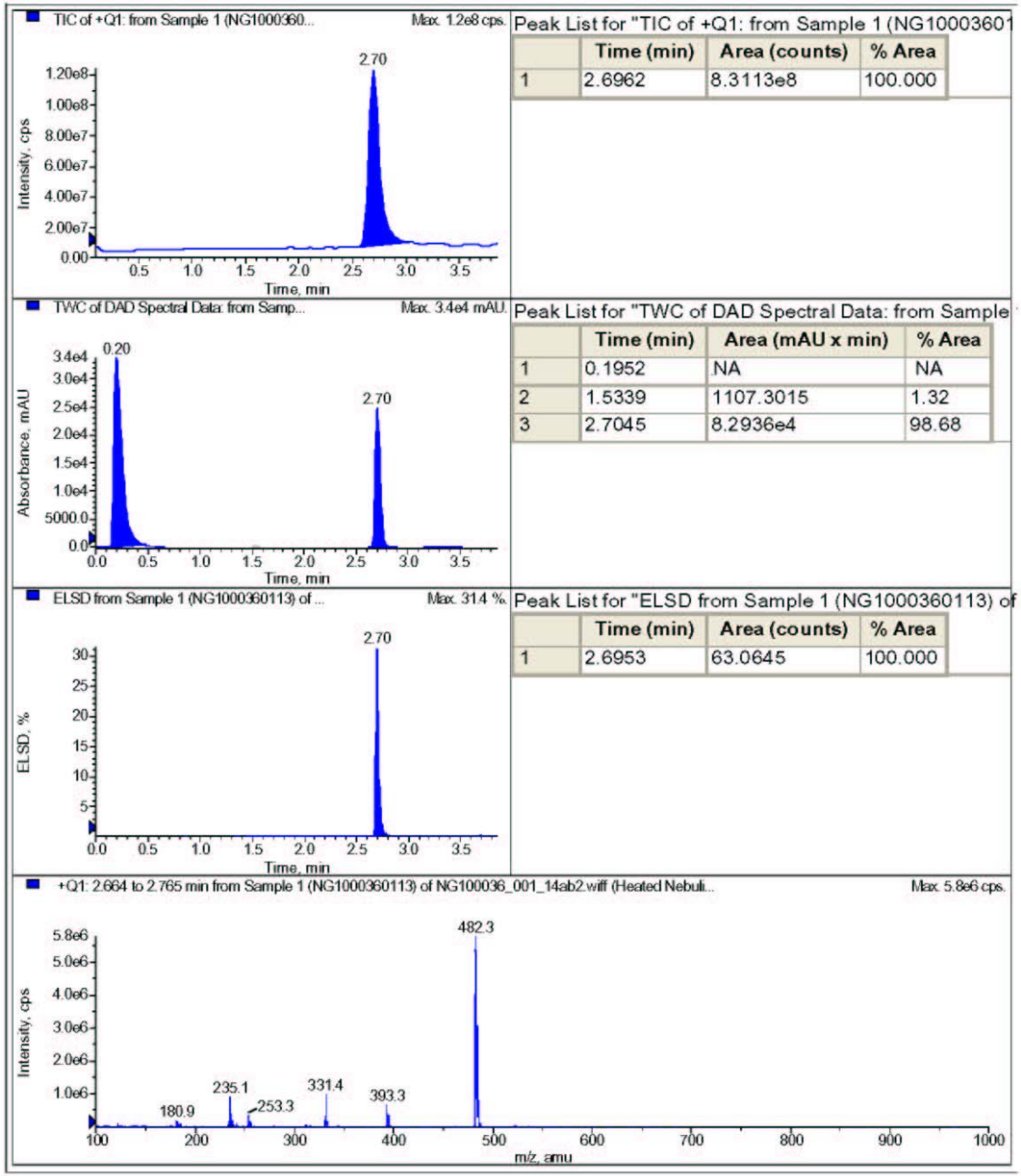


Conference on Small Molecule Science

CoSMoS

www.CoSMoScience.org

Custom Annotation: MW: 481.13685; Formula: C26H22ClF2N3O2;

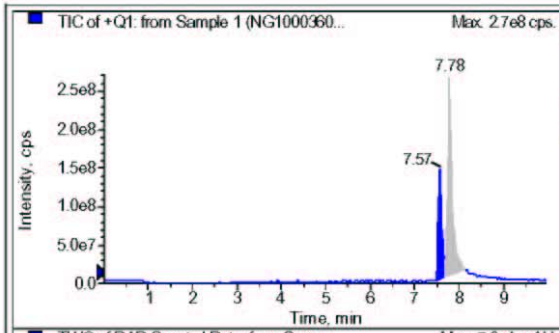


Conference on Small Molecule Science

COSMOS

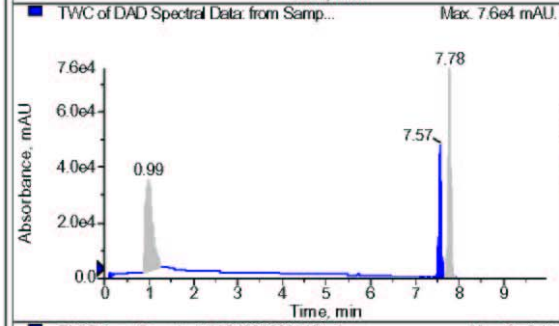
www.CoSMoScience.org

Custom Annotation: MW: 481.13685; Formula: C26H22ClF2N3O2;



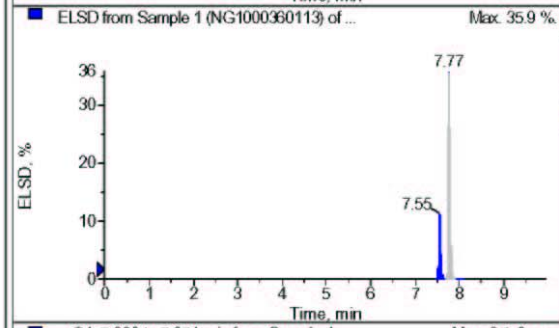
Peak List for "TIC of +Q1: from Sample 1 (NG1000360

	Time (min)	Area (counts)	% Area
1	7.5665	8.2989e8	33.3135
2	7.7790	1.6613e9	66.6865



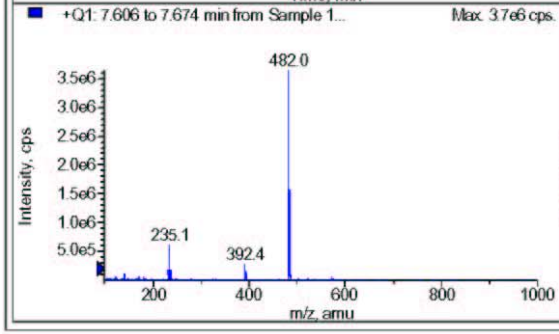
Peak List for "TWC of DAD Spectral Data: from Sample

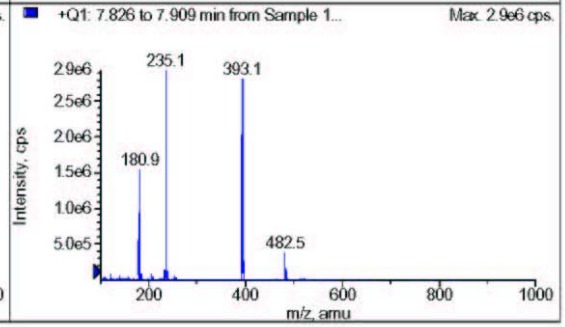
	Time (min)	Area (mAU x min)	% Area
1	0.1196	NA	NA
2	0.9916	NA	NA
3	7.5654	1.9079e5	37.49
4	7.7791	3.1813e5	62.51



Peak List for "ELSD from Sample 1 (NG1000360113) of

	Time (min)	Area (counts)	% Area
1	7.5549	36.1468	23.9452
2	7.7661	114.8097	76.0548

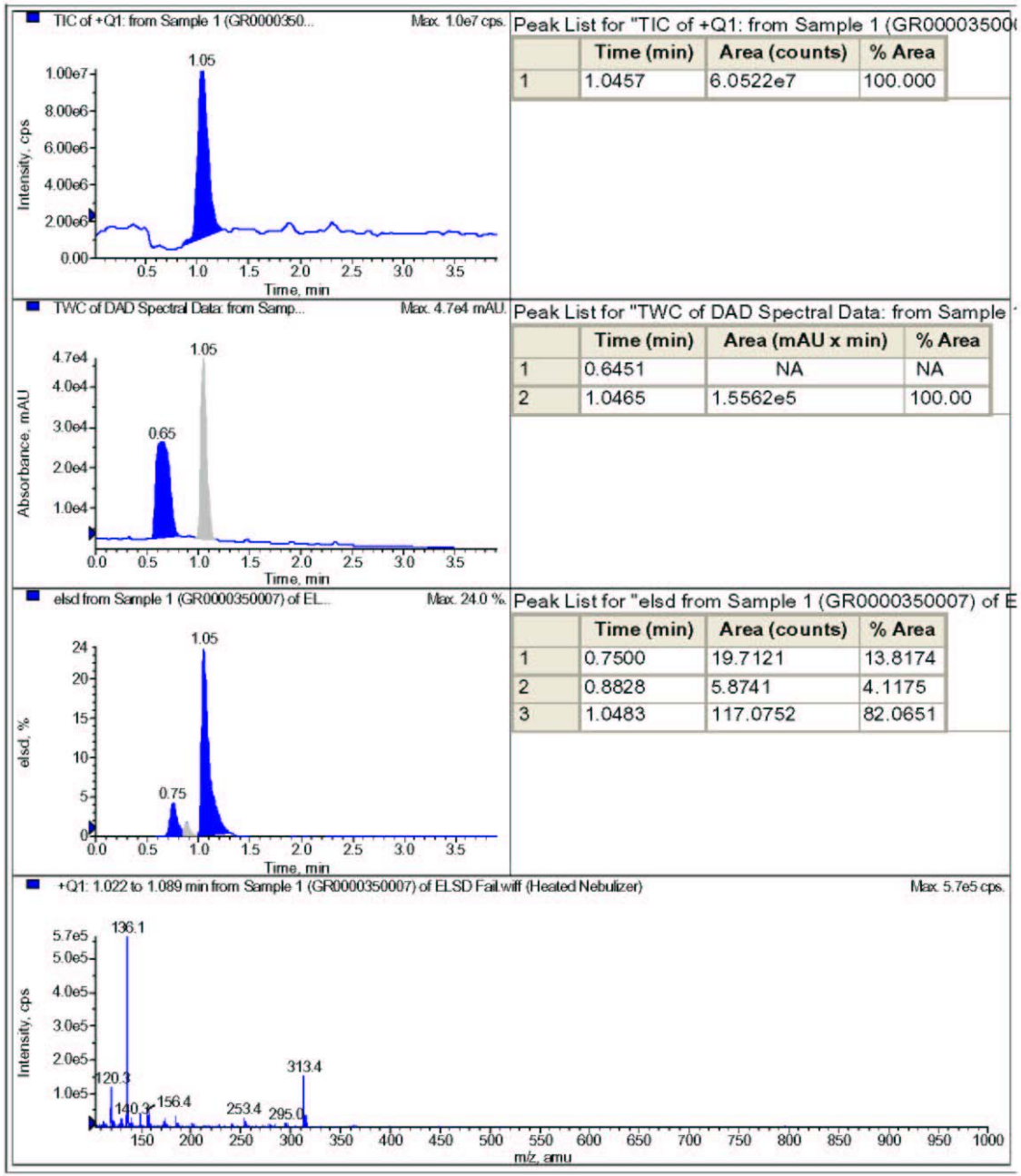




Conference on Small Molecule Science

COSMOS

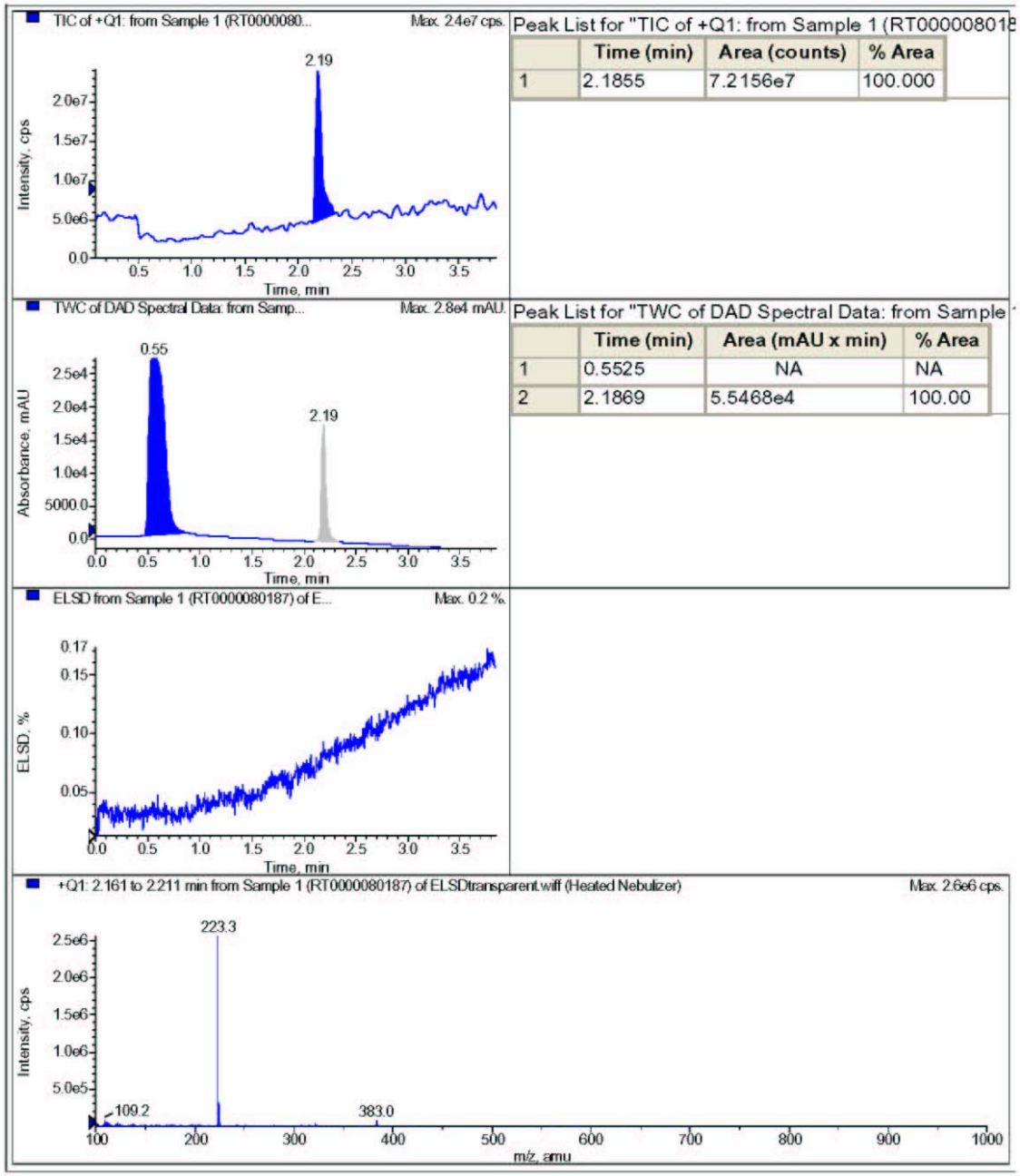
www.CoSMoScience.org



Conference on Small Molecule Science

COSMOS

www.CoSMoScience.org



Conference on Small Molecule Science

COSMOS

www.CoSMoScience.org

ACKNOWLEDGEMENTS

CRL Analytical Department

Marina Abdurahkmonova
Simone Ferguson
Alex Abdurakhmonov
James Kurdziel

Aleksey Lapin

Conference on Small Molecule Science

COSMOS

www.CoSMoScience.org

Million Dollar Question:

Which Detectors and Settings or Combination of Detectors and Settings will most accurately reflect the reality of the compound purity?