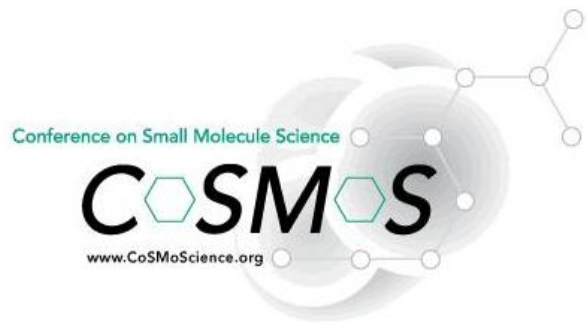


Comparison of Chemiluminescent Nitrogen Detection, Evaporative Light Scattering, and NMR for High Throughput Quantification

Kenneth Lewis, Ph.D.
OpAns, LLC
RTP, NC



Acknowledgements

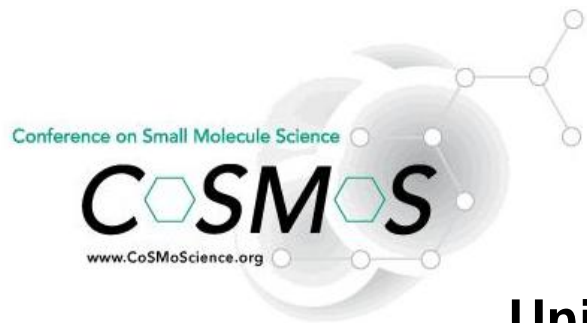
Coauthors on original presentation

- Kathryn Lawrence, Thomas Mitchell, Sandeep Kalelkar
Previously at Eli Lilly & Co, Sphinx Laboratories, RTP, NC 27709
- Andrea Sefler
GlaxoSmithKline, RTP, NC 27709

Jean-Francois Borny and Mark Homan of Antek Instruments.

Bill Fitch at Affymax

Dean Phelps at GlaxoSmithKline, RTP



Why care about quantification without reference standards?

Universal questions for compound analysis

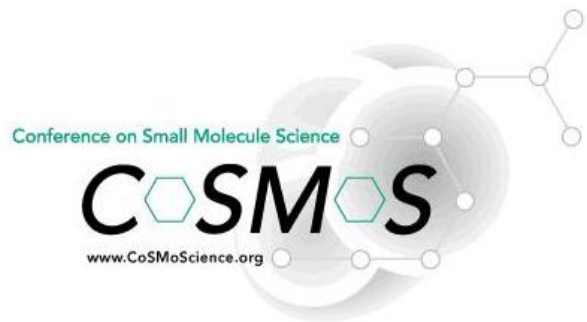
- Is the expected compound present?
- How pure is the sample?
- How much is there?

Wide Applicability

- Chemistry Support
- Compound Management QA/QC
- ADME Applications
- Manufacturing QC

Why do we need a solution?

- No generic solution currently available
- Reference Material is not available
- Not enough material to weigh
- Weighing is cumbersome
- Samples are impure



Options for High Throughput Quantification

NMR

Advantages

- Universal
- Very accurate

Drawbacks

- Requires assignment of the spectrum
- Not currently automated
- Best with pure samples
- Expensive instrumentation
- Poor sensitivity

ELSD

Advantages

- Universal
- Inexpensive
- Easy to use
- Compatible with LC

Drawbacks

- Compound dependent response

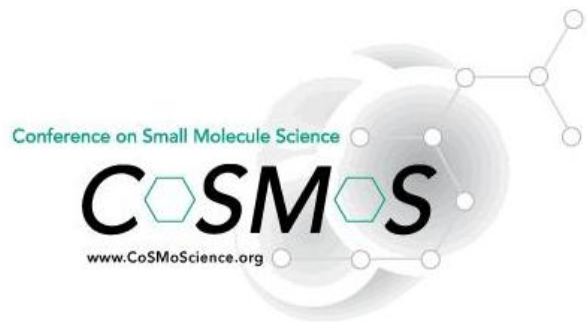
CLND

Advantages

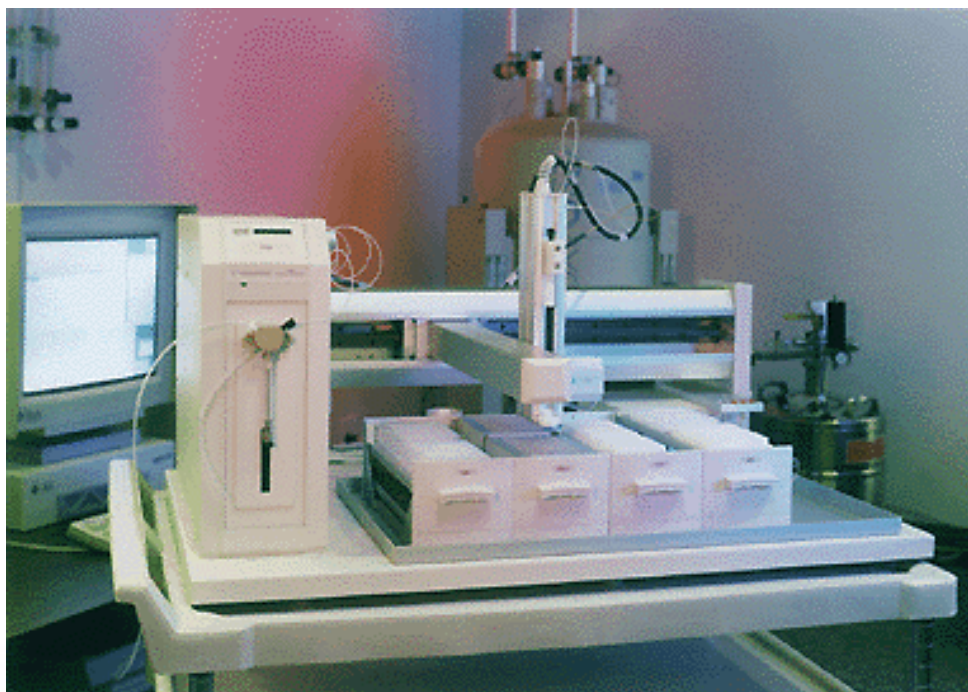
- Accurate without reference standards
- Linear response
- Compatible with LC

Drawbacks

- Compound must contain Nitrogen
- No acetonitrile
- Robustness?



Direct Inject NMR

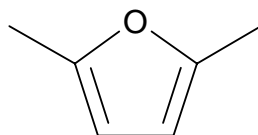


Varian (VAST)

Bruker (BEST)

^1H NMR Quantification

- Use 2,5-dimethylfuran as the internal standard

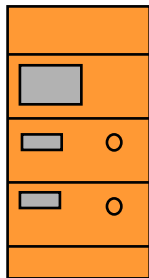


- Assign spectra and ratio integrated peak areas
- Dimethylfuran can be removed by vacuum concentration (bp 92 °C)
- Newer techniques reference internal probe signals.

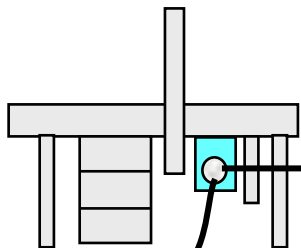
Samuel Gerritz and Andrea Seifler, *J. Comb. Chem.* **2000**, 2, 39-41

Instrument Schematic

HPLC Pump



Autosampler



Column Oven



CLND
150 μ L/min

ELSD
700 μ L/min

MS
150 μ L/min

Column: 2.1 x 50 mm Luna C18

Mobile Phase: Water (0.1% Formic Acid) / Methanol (0.08% Formic Acid)

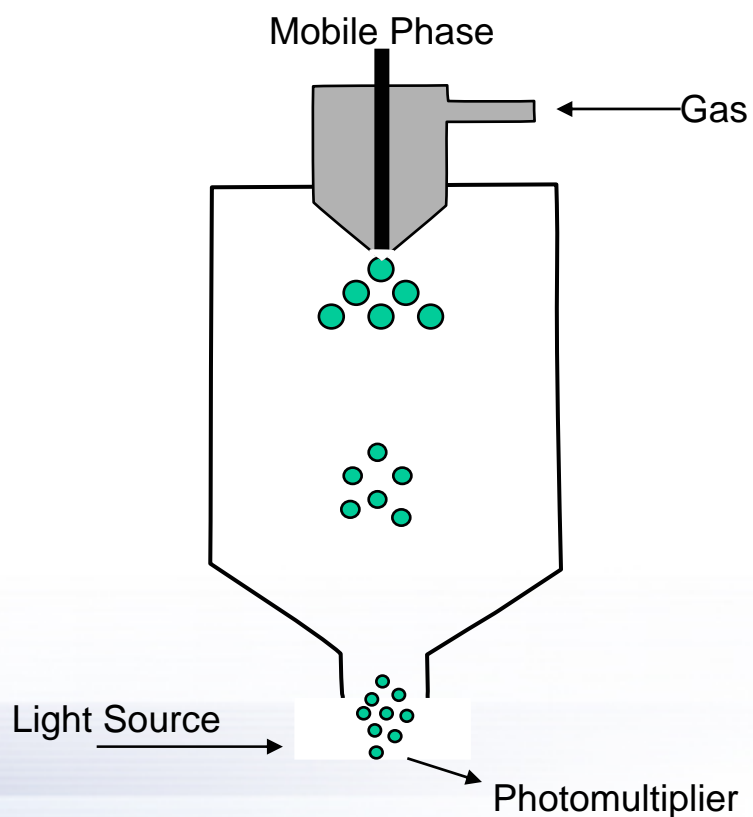
Gradient: 5 to 100% Methanol in 7.5 minutes, 30 second hold, 30 second recovery

Flow: 1.0 mL/min

Instruments: Sedex 75 ELSD, Antek 8060 CLND, Micromass ZQ MS

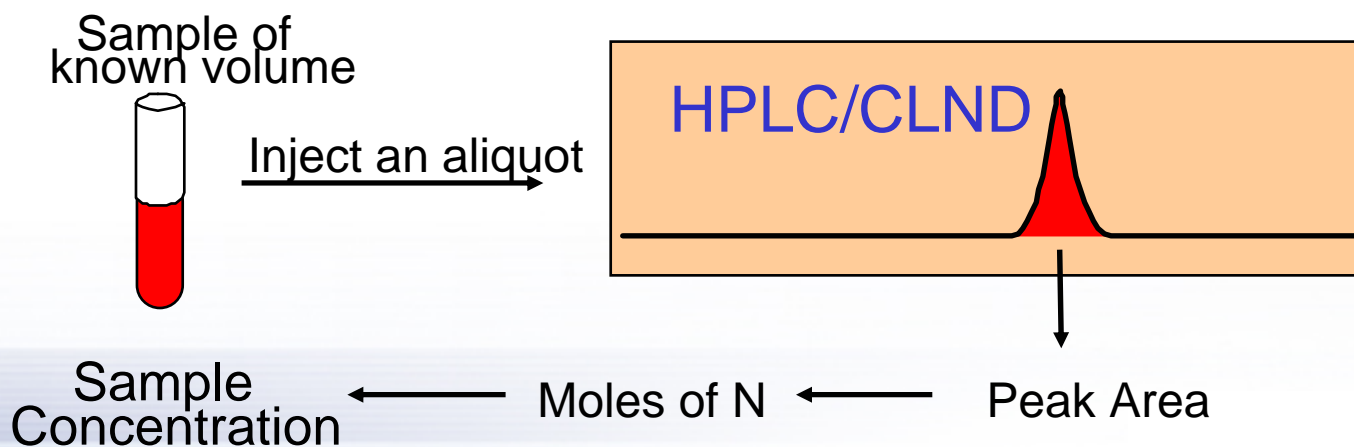
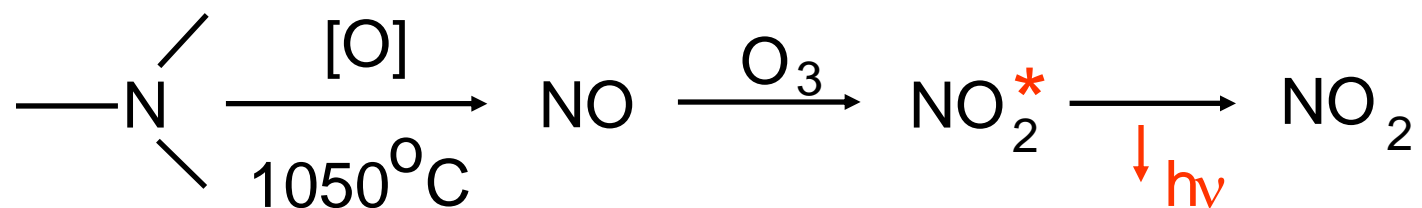
Evaporative Light Scattering Detection

A destructive detector which detects the non-volatile residue content of a chromatographic peak.

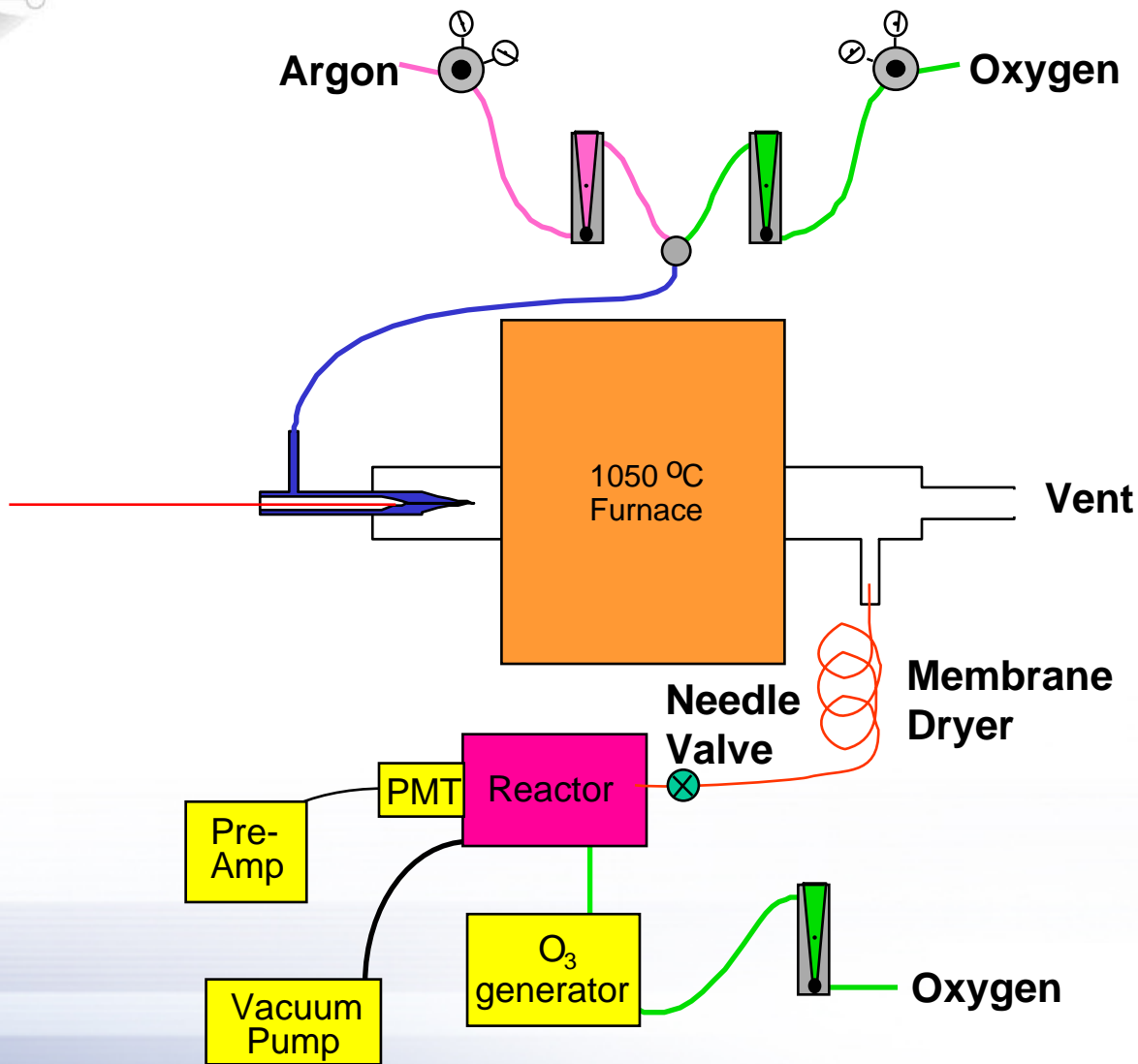


Chemiluminescent Nitrogen Detector

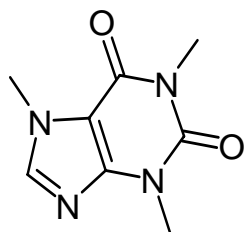
A destructive detector which detects the total nitrogen content of a chromatographic peak.



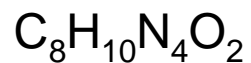
Antek CLND Schematic



Calibration Compounds

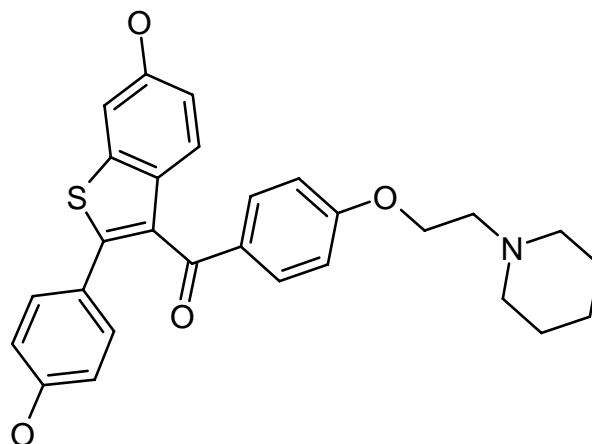


Caffeine

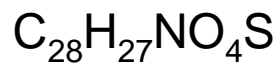


MW=194.2

Elutes at 24% MeOH

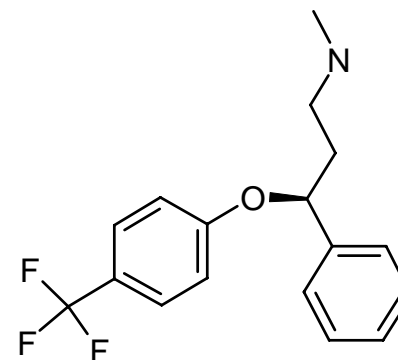


Raloxifene

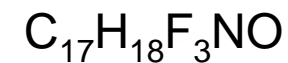


MW = 473.4

Elutes at 40% MeOH



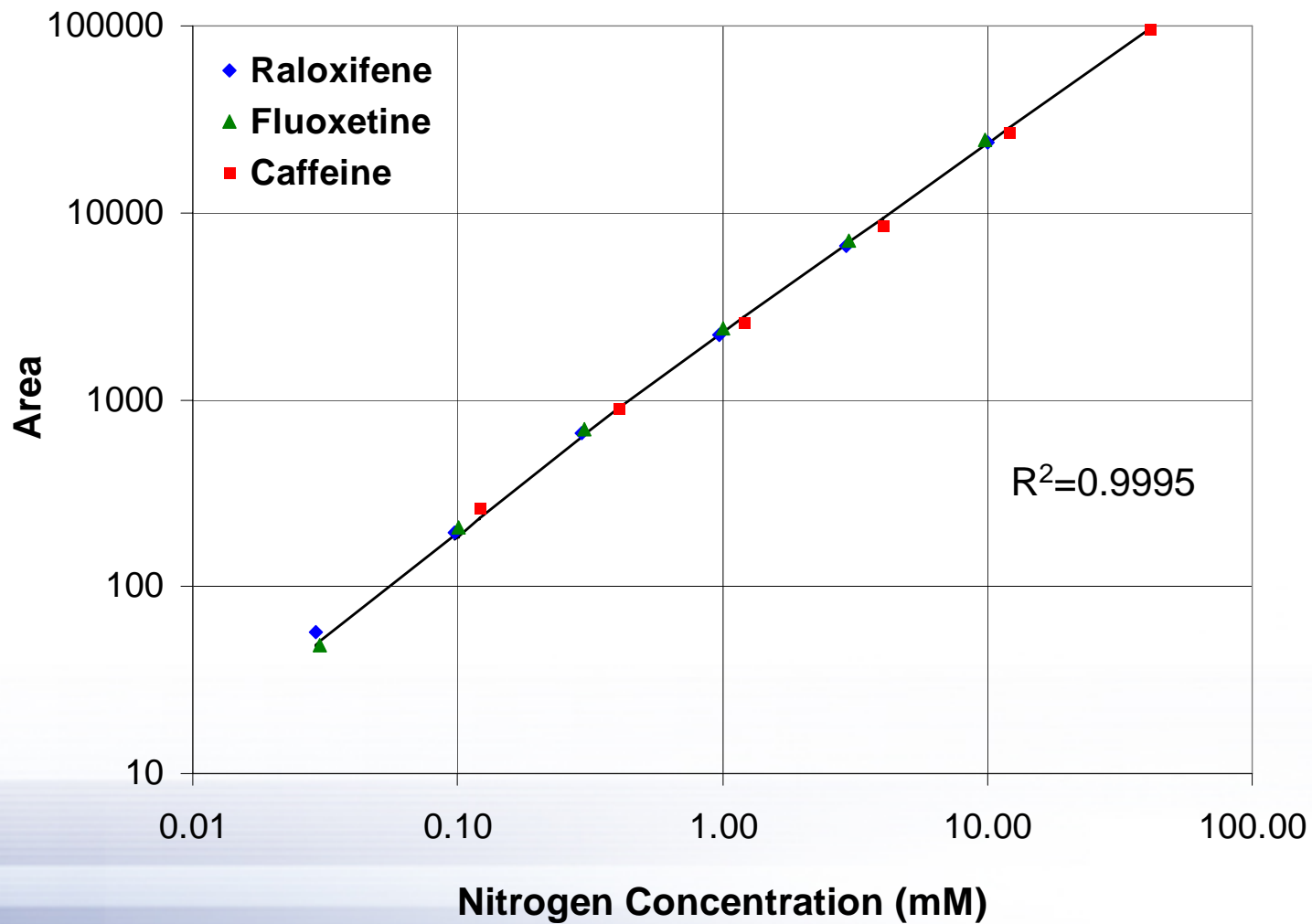
Fluoxetine



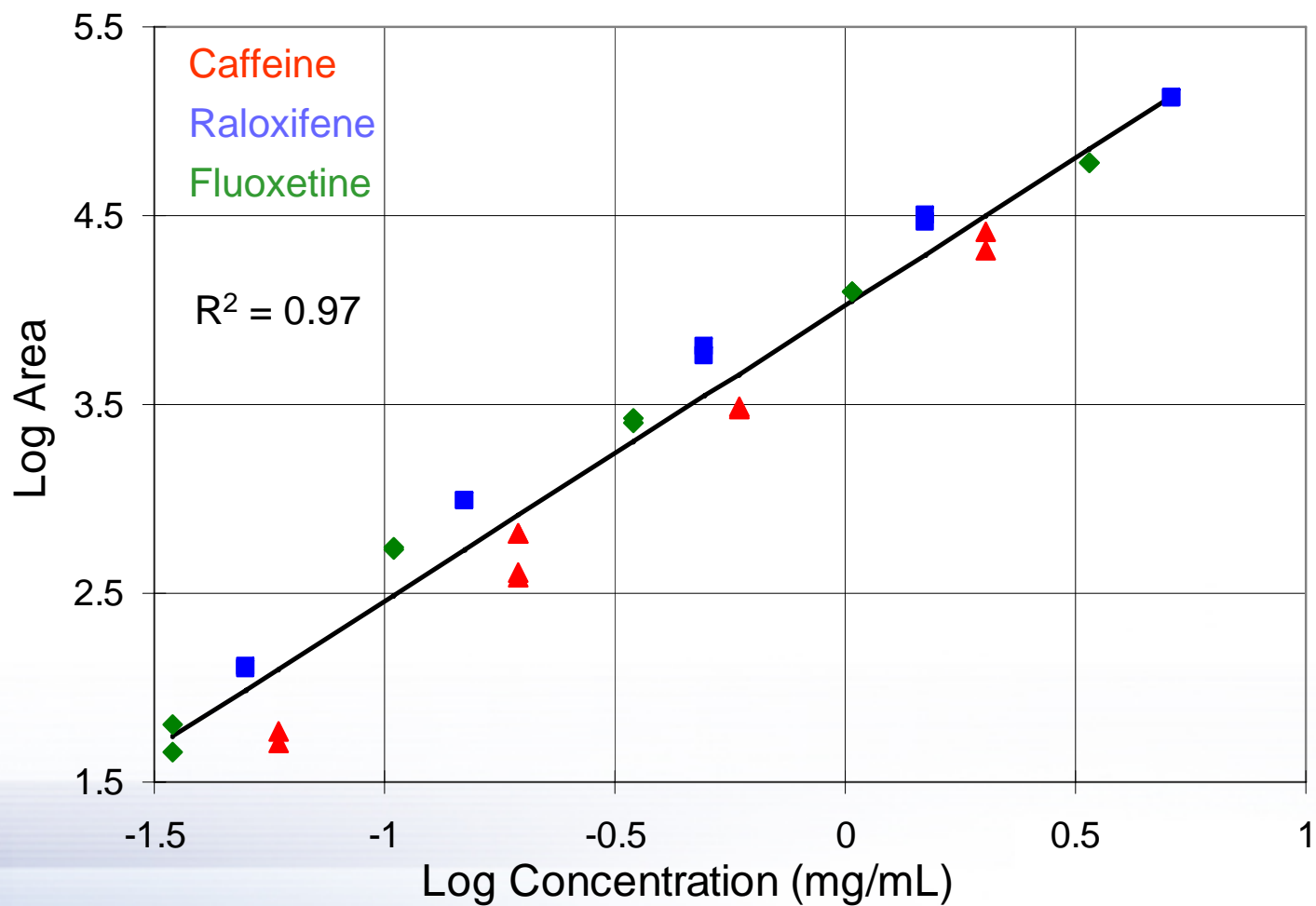
MW = 309.3

Elutes at 47% MeOH

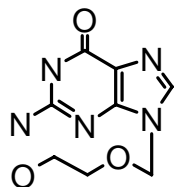
CLND Calibration Curve



ELSD Calibration Curve

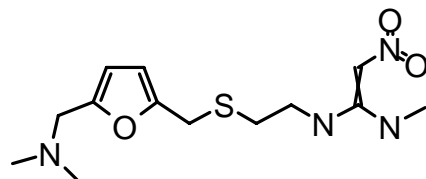


% Error - CLND ELSD CHN NMR



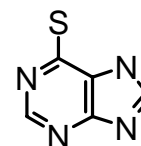
Acyclovir (Zovirax)

0 -27 1 3



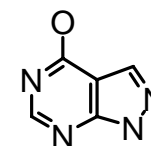
Ranitidine (Zantac)

-1 53 2 2



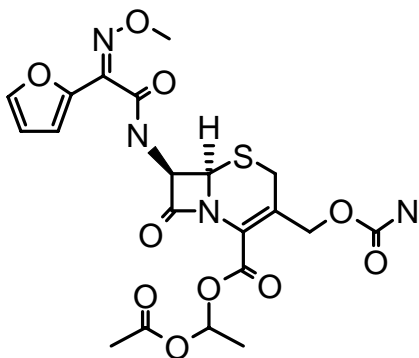
Mercaptopurine (Purinethol)

-2 -65 0 3



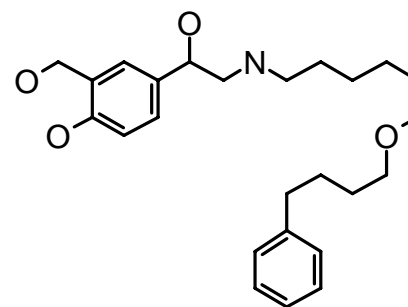
Allopurinol (Zyloprim)

-2 -30 0



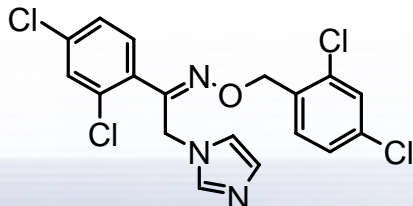
Cefuroxime axetil (Ceftin)

1 -42 1



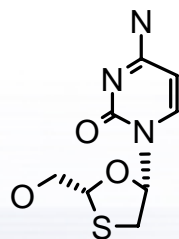
Salmeterol (Serevent)

-1 30 3 3



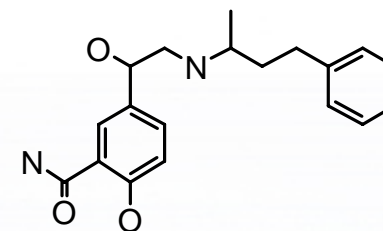
Oxiconazole (Oxistat)

2 24 -1 3



Lamivudine (Epivir)

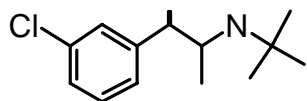
0 42 2



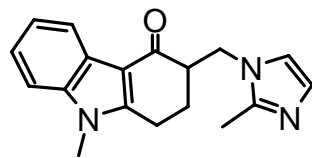
Labetalol (Trandate)

-2 13 2 1

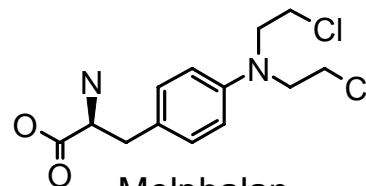
% Error - CLND ELSD CHN NMR



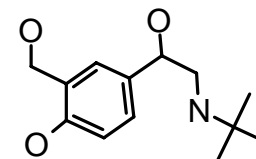
Bupropion
(Wellbutrin)
3 N/D 1 2



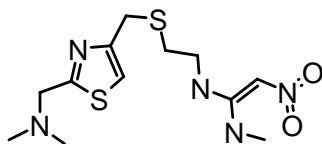
Ondansetron
(Zofran)
-1 -28 -1 3



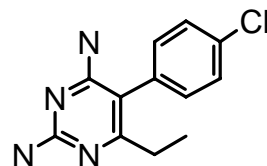
Melphalan
(Alkeran)
1 -6 4 9



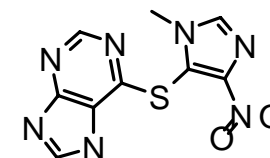
Salbutamol
(Ventolin)
0 46 3



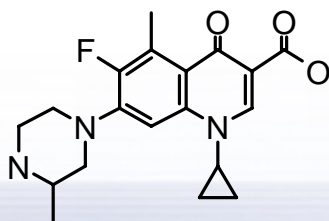
Nizatidine
(Axid)
2 9 1



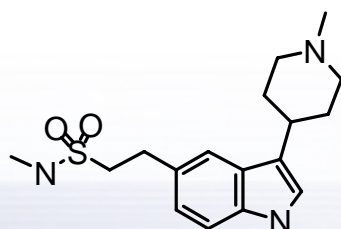
Pyrimethamine
(Daraprim)
-1 -34 1 -2



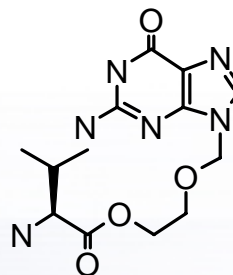
Azathioprine
(Imuran)
2 -31 -2 1



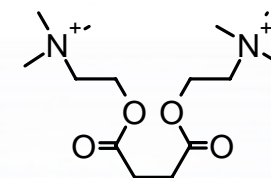
Grepafloxacin
(Raxar)
-2 -3 1 8



Naratriptan
(Amerge)
1 -3 2 -1

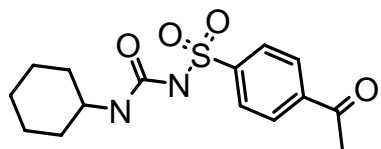


Valacyclovir
(Valtrex)
1 -35 2 2

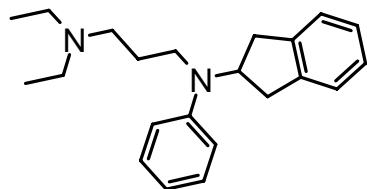


Succinylcholine
(Anectine)
0 -11 -8

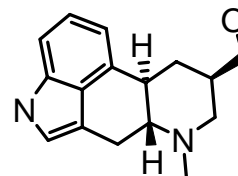
% Error - CLND ELSD CHN



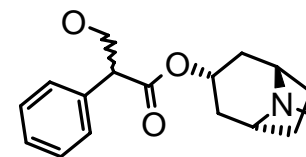
Acetohexamide
(Dymelor)
-3 -9 2



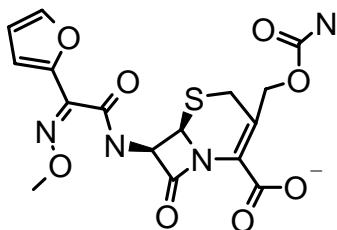
Aprindine
2 5 3



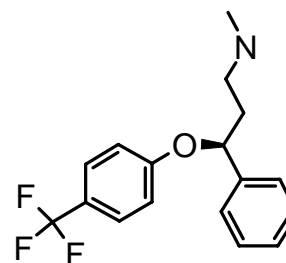
Dihydroelymoclavine
-3 -4 -2



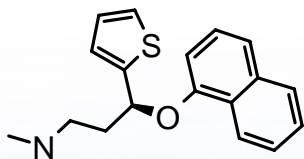
Atropine
0 -27 12



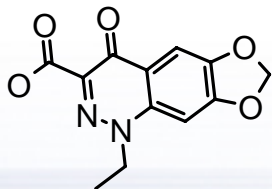
Cefuroxime
(Kefurox)
-6 -31 4



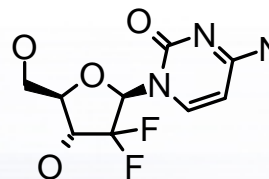
Fluoxetine
(Prozac)
1 3 -1



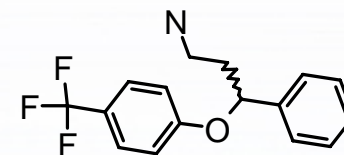
Duloxetine
1 20 0



Cinoxacin
(Cinobac)
-7 -31 -2



Gemcitabine
(Gemzar)
0 -17 0

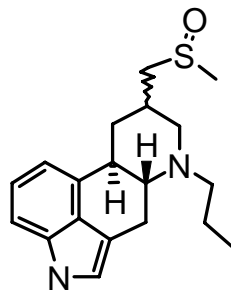


Norfluoxetine
-10 -4 -1

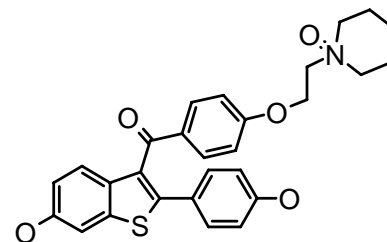
% Error - CLND ELSD CHN



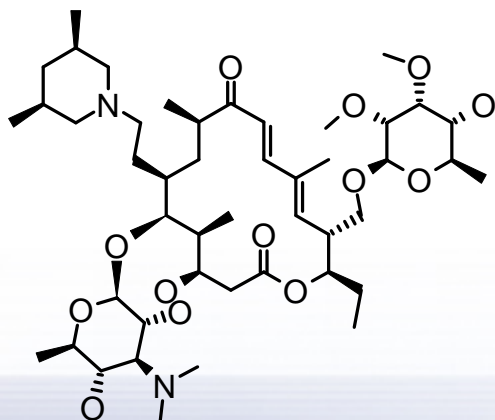
Pergolide mesylate
(Permax)
-2 16 2



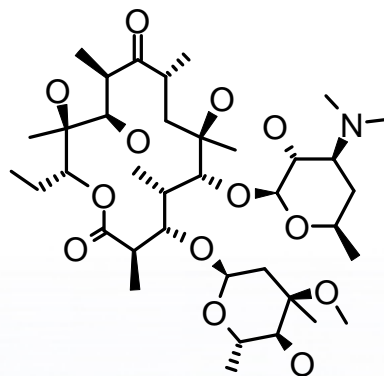
Pergolide sulfoxide
-1 -8 1



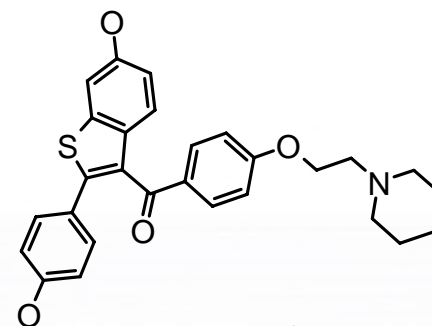
Raloxifene N-Oxide
1 16



Tilmicosin
(Micotil)
-5 -14 -4

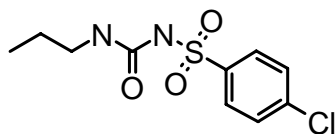


Erythromycin
(Ilotycin)
-8 -19 -6

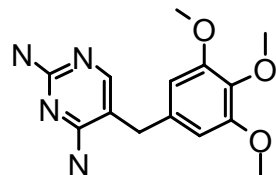


Raloxifene
(Evista)
-7 32 0

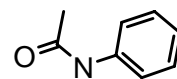
% Error - CLND ELSD CHN



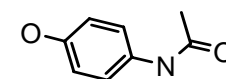
Chlorpropamide
0 4 1



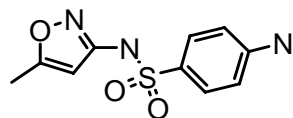
Trimethoprim
-3 -30 0



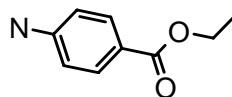
Acetanilide
3 -85 1



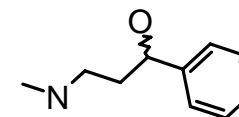
Acetaminophen
2 -27 1



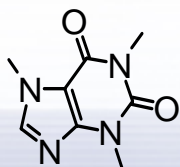
Sulfamethoxazole
2 -3 0



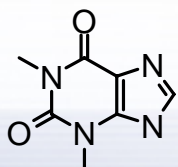
Benzocaine
7 -76



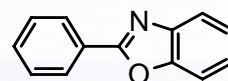
Phenylmethlamino propanol
-2 28 -2



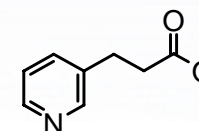
Caffeine
-2 -37 1



Theophylline
-4 -59 0



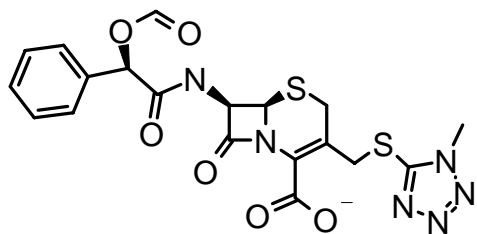
2-Phenylbenzoxazole
-2 -98 0



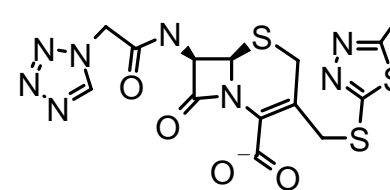
3-Pyridinepropionic acid
-1 14 3

Problem Structures for CLND

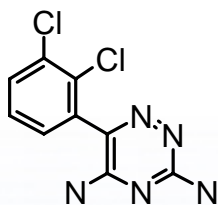
% Error - CLND **ELSD** CHN



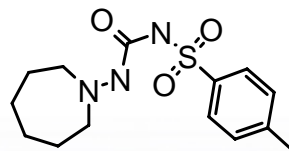
Cefamandole
(Mandol)
-37 -36 0



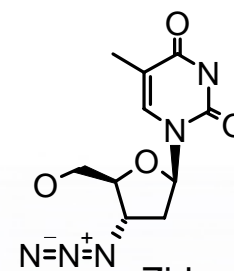
Cefazolin
(Kefzol)
-39 -42 11



Lamotrigine
(Lamictal)
-19



Tolazamide
-30 -16 1



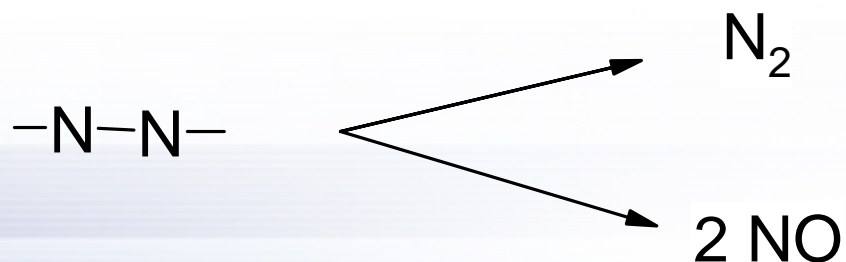
Zidovudine
(Retrovir)
-42 -61

Response Rules

1. Azo groups are NOT detected

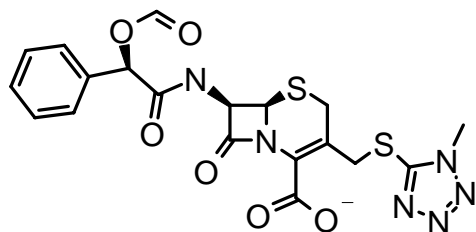


2. N-N bonds produce ~50% response



Problem Structures for CLND

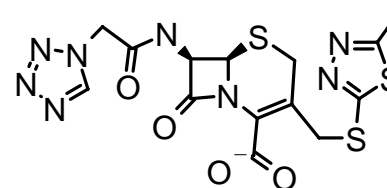
N and % CLND Error Before Rule
N and CLND Error After Rule



Cefamandole
(Mandol)

6 -37%

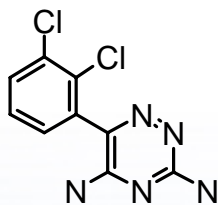
4 -5%



Cefazolin
(Kefzol)

8 -39%

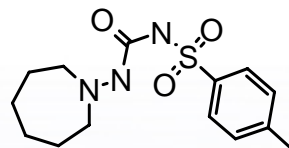
5 3%



Lamotrigine
(Lamictal)

5 -19%

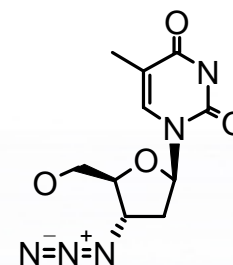
4 1%



Tolazamide

3 -30%

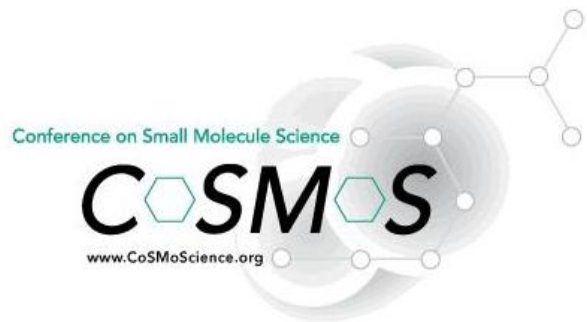
2 5%



Zidovudine
(Retrovir)

5 -42%

3 -3%

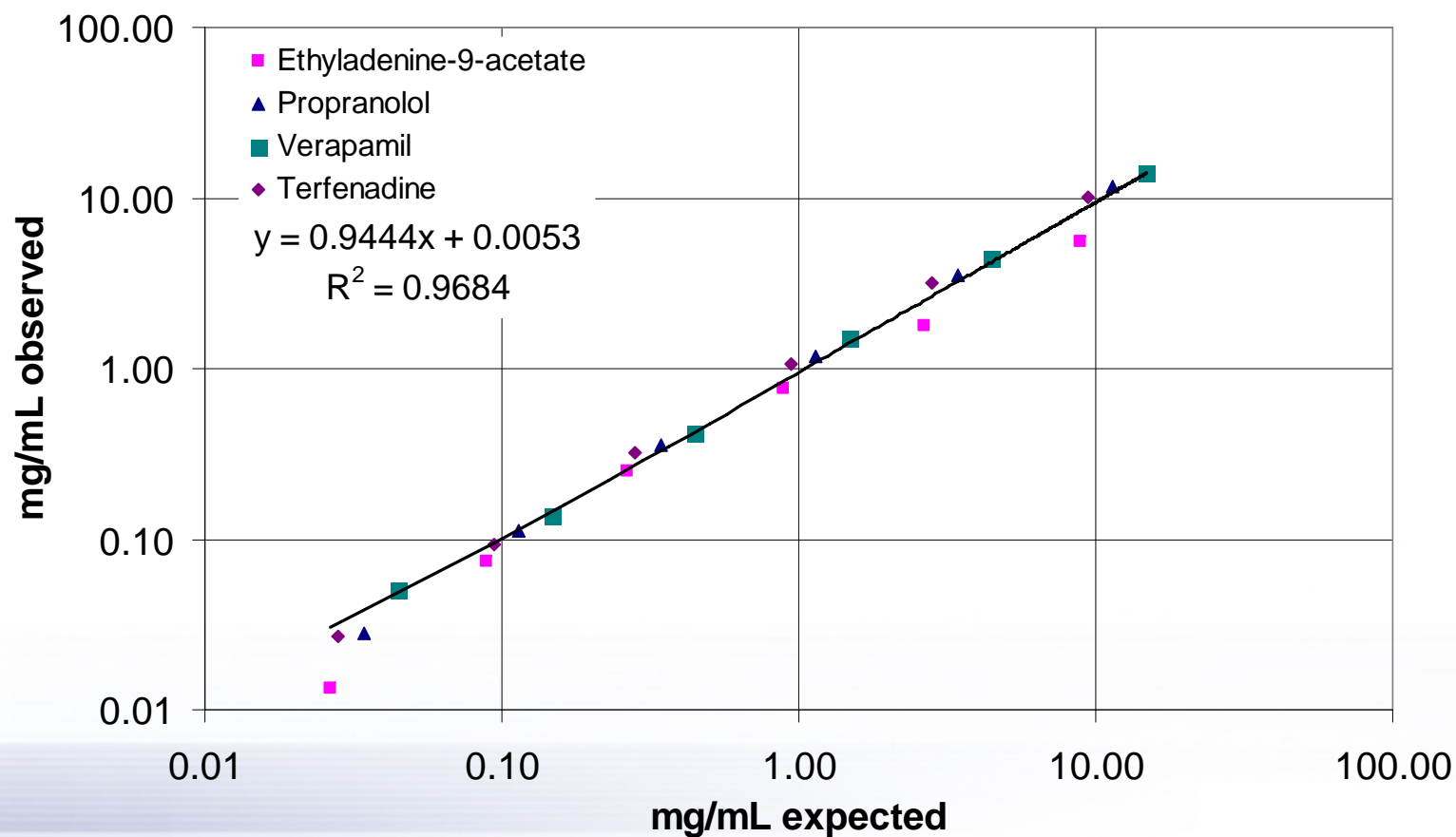


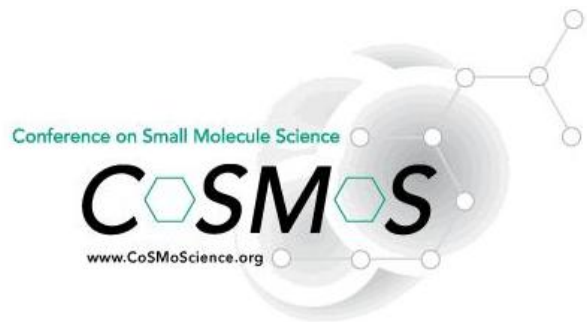
What about the Corona Detector?

- ESA licensed technology from TSI (Stan Kaufman).
- Similar to ELSD except with detection based on charge/current instead of light scattering.
- Same limitations (volatility, etc) as ELSD
- Lower detection limit than ELSD.

- Significantly affected by solvent composition
- Signal must be squared, not cubed.

Signal Squared, Gradient Adjusted CAD Quantification Accuracy





Conclusions

- No method is perfect all of the time.
- NMR requires pure samples and internal standards for accurate quantification.
- CLND and ELSD rely on good chromatography.
- CLND is the best technique when little sample is available and it is of questionable quality.
- ELSD is easy and robust for ballpark quantification.

A bright sun is positioned in the upper right quadrant of the image, casting a wide, soft glow across the sky. The sun's rays are visible as a hazy, golden-white area. The background is a clear, light blue sky. The word "Applications" is centered in the middle of the image in a bold, green, sans-serif font.

Applications

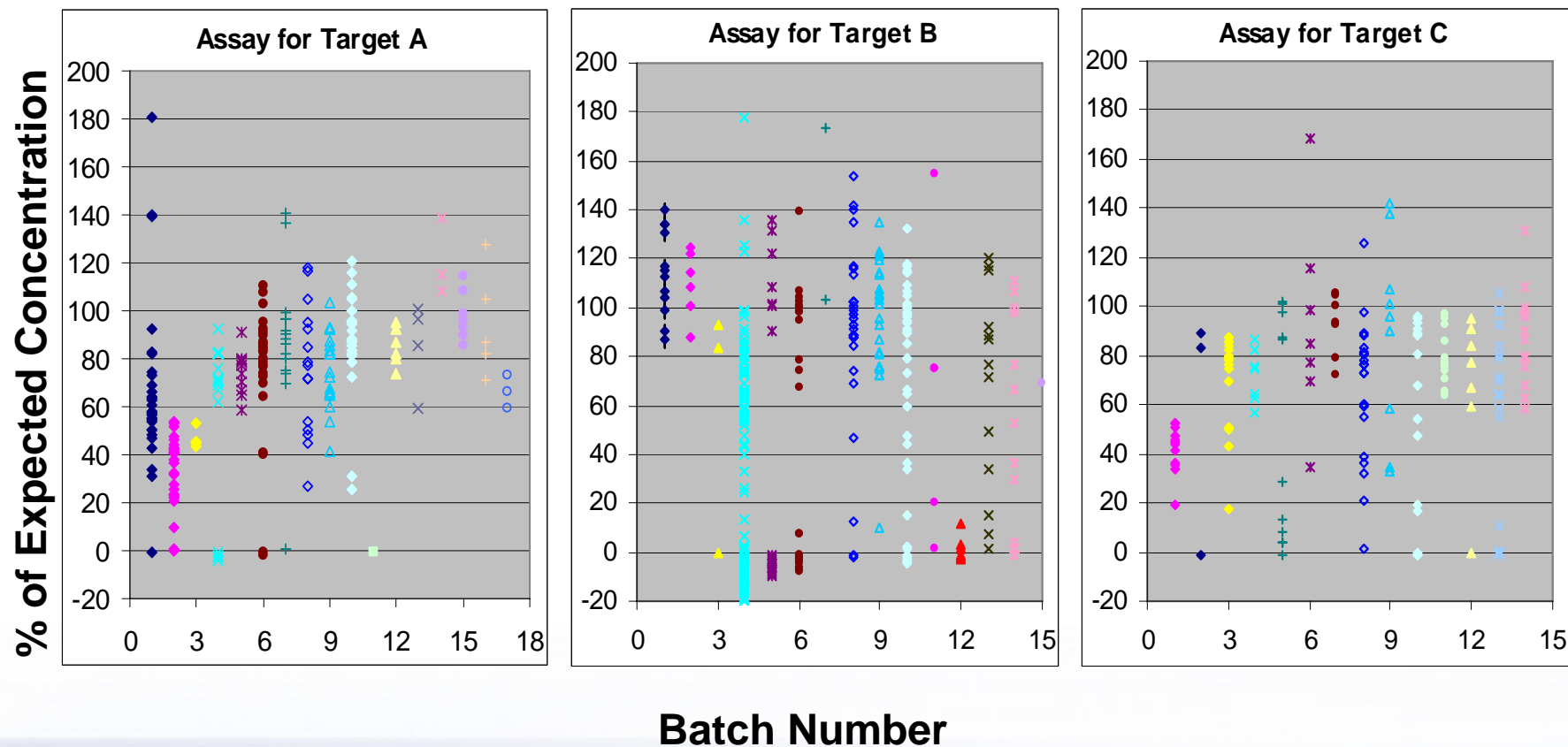
The LC/DAD/CLND/ELSD/MS System @ RsDqv

- 1100 Binary HPLC stack
- 1956 SL single quad MS
- MultiMode ionization source
- MSD nebulizer is mounted directly underneath DAD cell
- ELSD and CLND inlets close to MSD nebulizer
- Recently replaced 1100 HPLC with 1200SL RR Stack



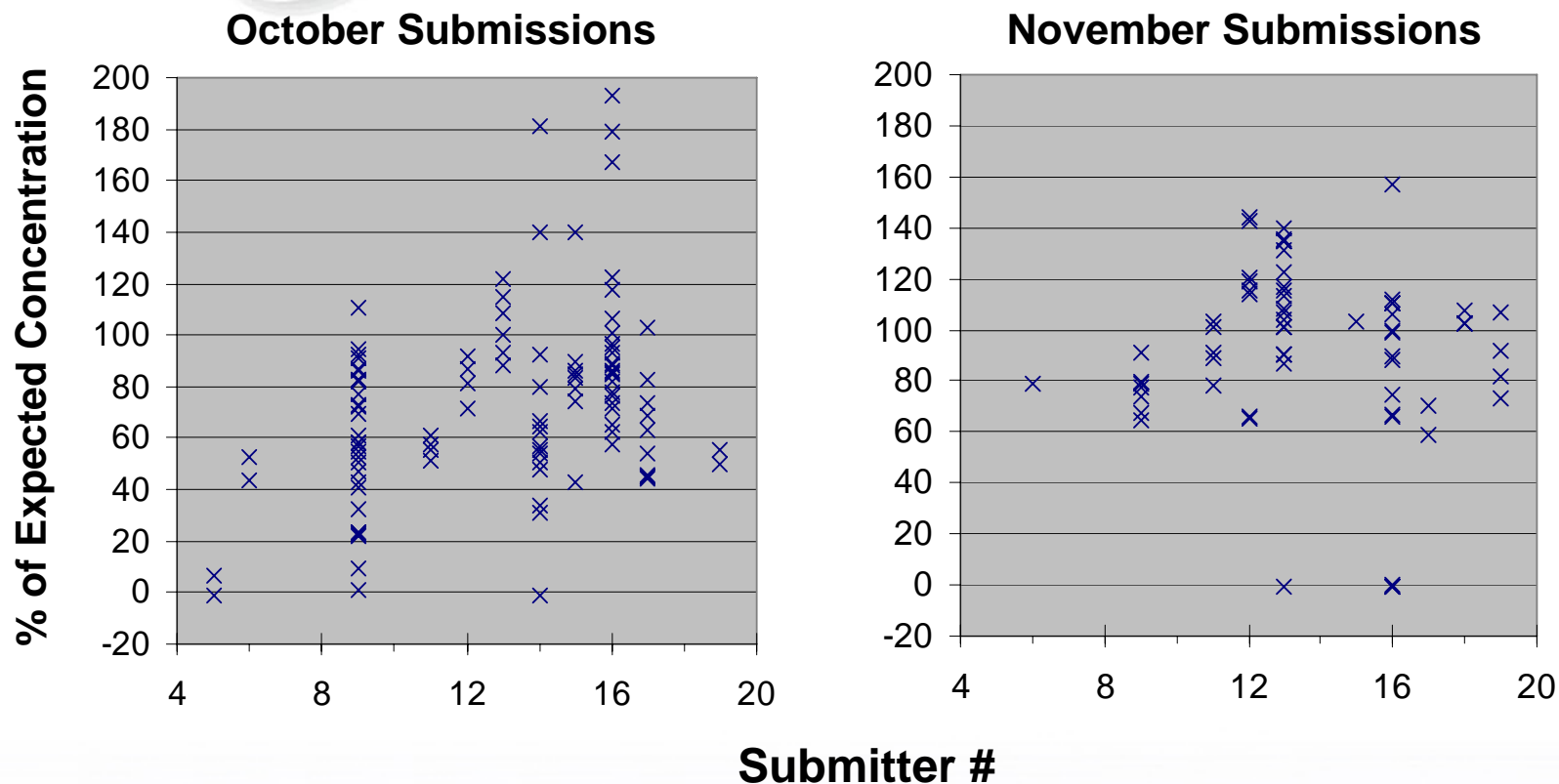
Example QC Results

Lead Validation Samples



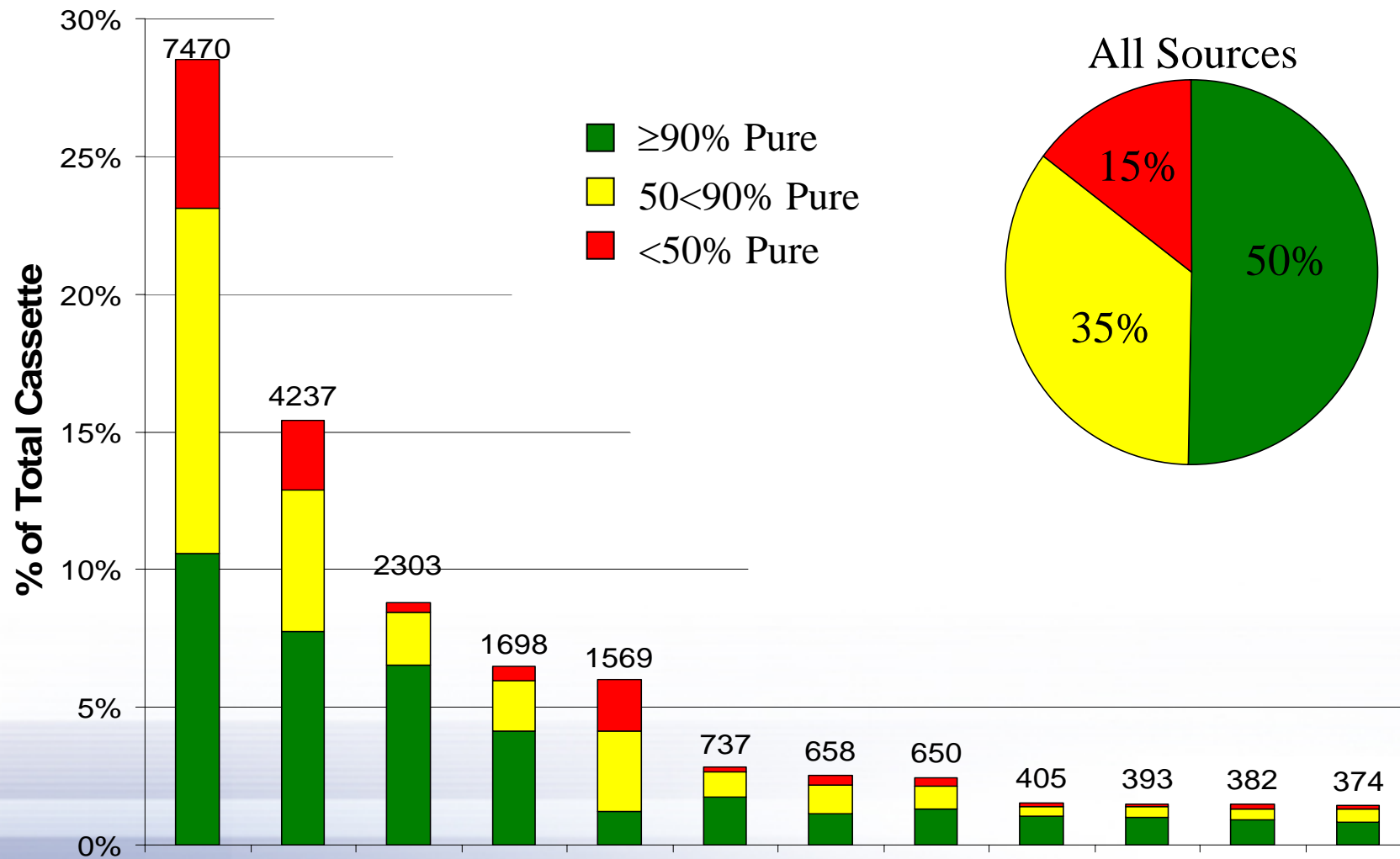
- Samples were synthesized specifically for each SAR project.
- Samples were weighed by the chemist and dissolved by hand.

The Effect of QC on Quantity



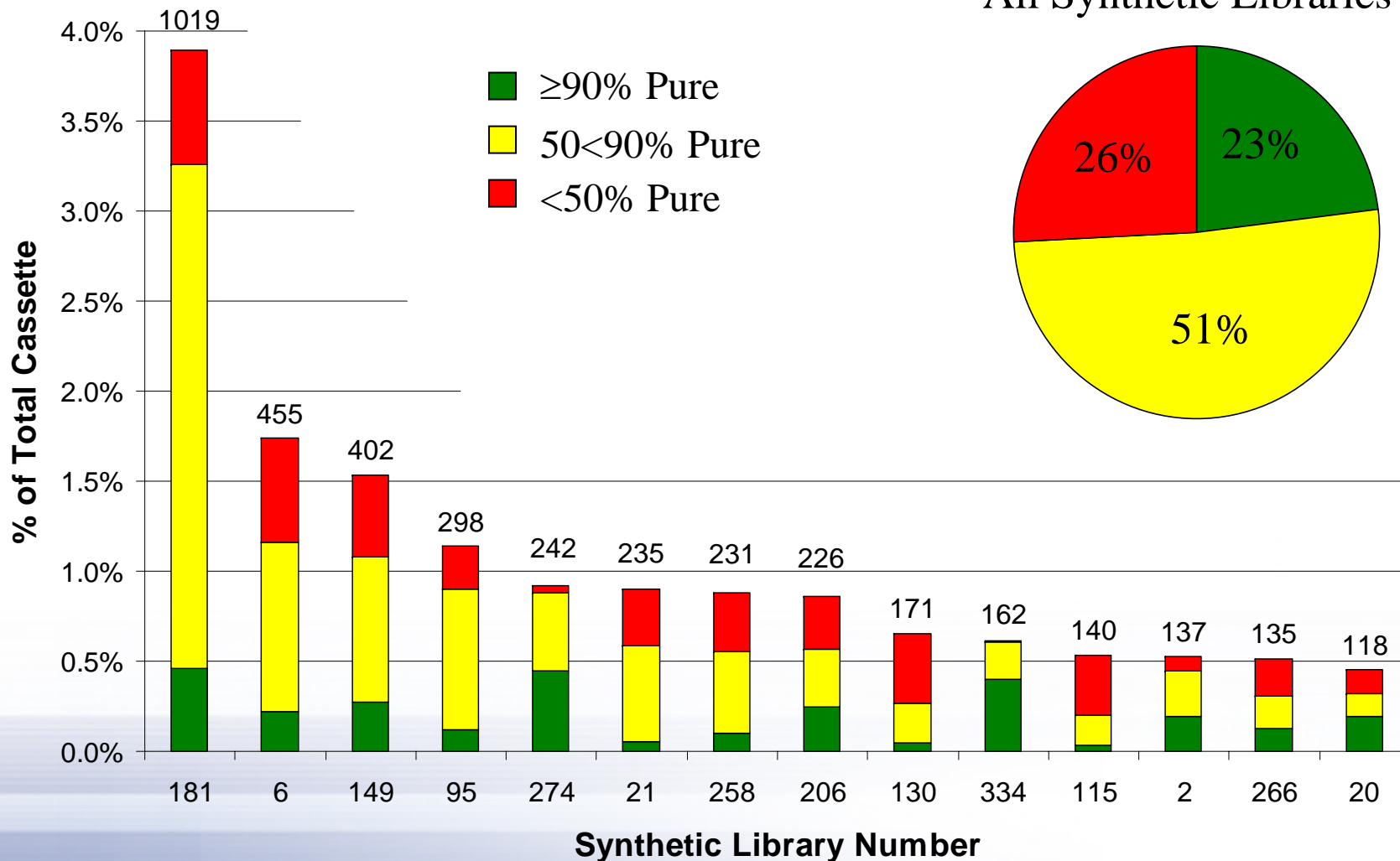
- All samples were for SAR projects.
- Samples were weighed by the chemist and dissolved by hand.
- Only process change was the implementation of QC in October.

Compound Quality by Source

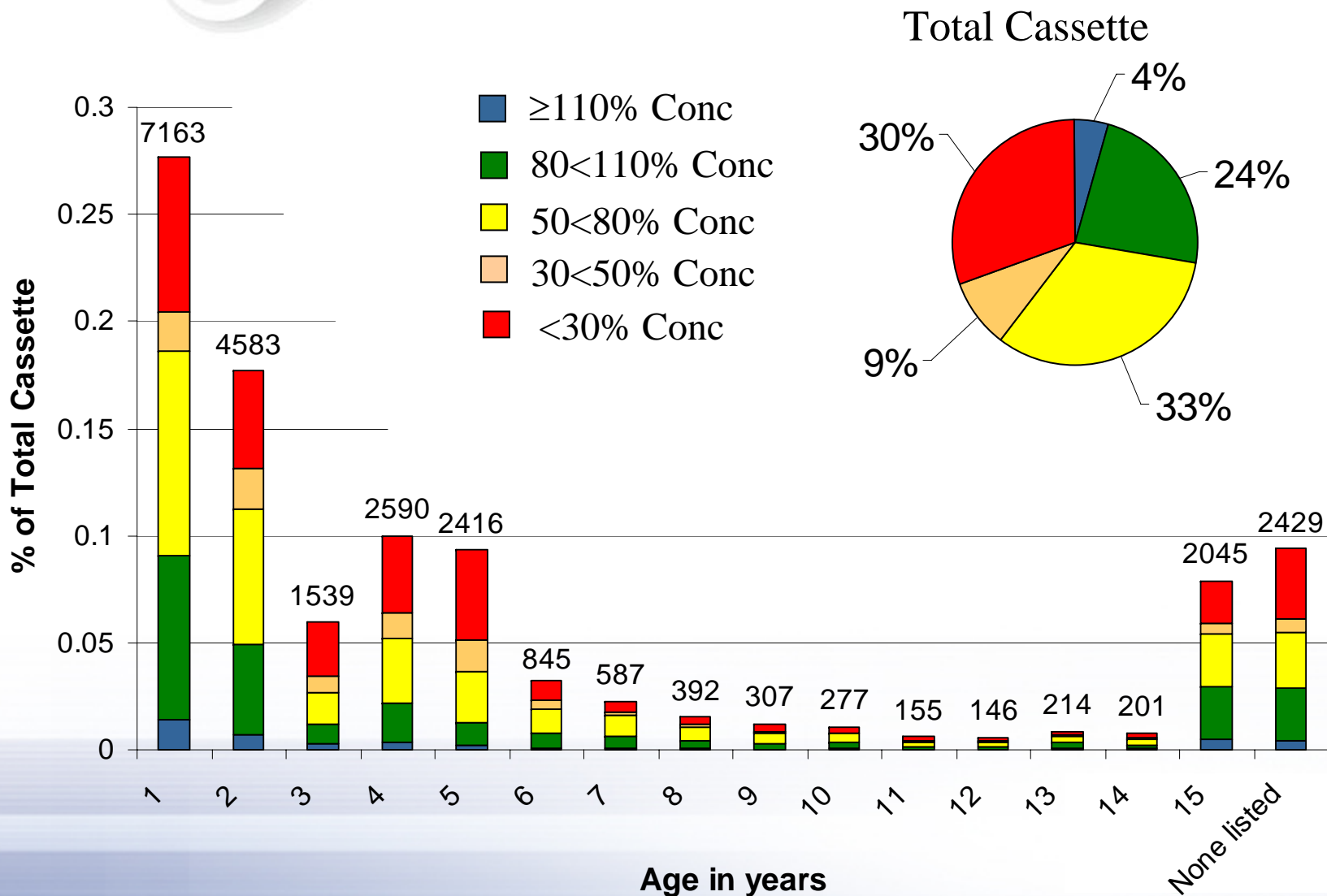


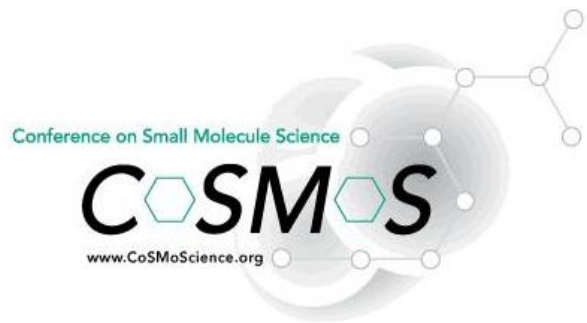
Compound Quality by Synthetic Library

All Synthetic Libraries



Quantity for Samples >90% Pure





Comparison of Chemiluminescent Nitrogen Detection, Evaporative Light Scattering, and NMR for High Throughput Quantification

Kenneth Lewis, Ph.D.
OpAns, LLC
RTP, NC