

# Method validation for NO (10 ppm to 1000 ppm) in nitrogen using the Fischer Rosemount chemiluminescence analyser

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# Presentation Outline

- Introduction
- Principle of operation
- Precision
- Linearity
- Limit of detection and limit of quantification
- Measurement uncertainty
- Conclusions

# Introduction

- What is method validation?
  - Process of proving a method is acceptable for its intended purpose.
  - It ensures the quality of the results obtained.
  - Provides confidence in the measurement system.
  
- It can't be separated from method development.
  
- A criteria is set for all the parameters to be measured.

# Introduction continues....

- Nitric oxide is highly reactive.
- It's an air pollutant produced by cigarette smoke, automobile exhausts and power plants.
- NO is an important messenger molecule involved in the physiological and pathological process within mammalian bodies.
- A concentration of NO can be determined using a chemiluminescence analyser.

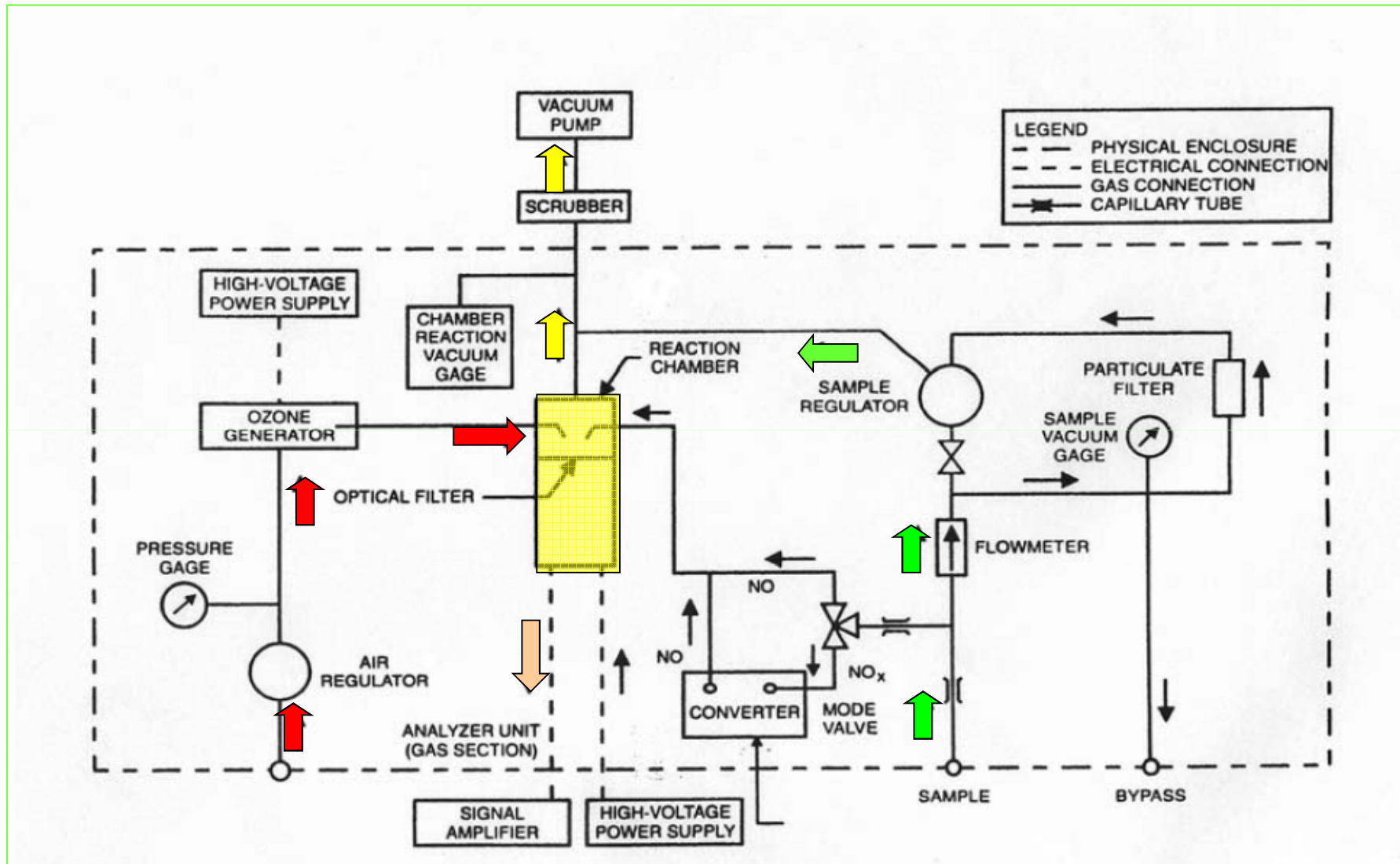
# Principle of Operation

- Nitric oxide can be determined using the chemiluminescent reaction involving ozone.



- Light produced can be measured with a photo detector.

# Chemiluminescence Analyser Flow Diagram



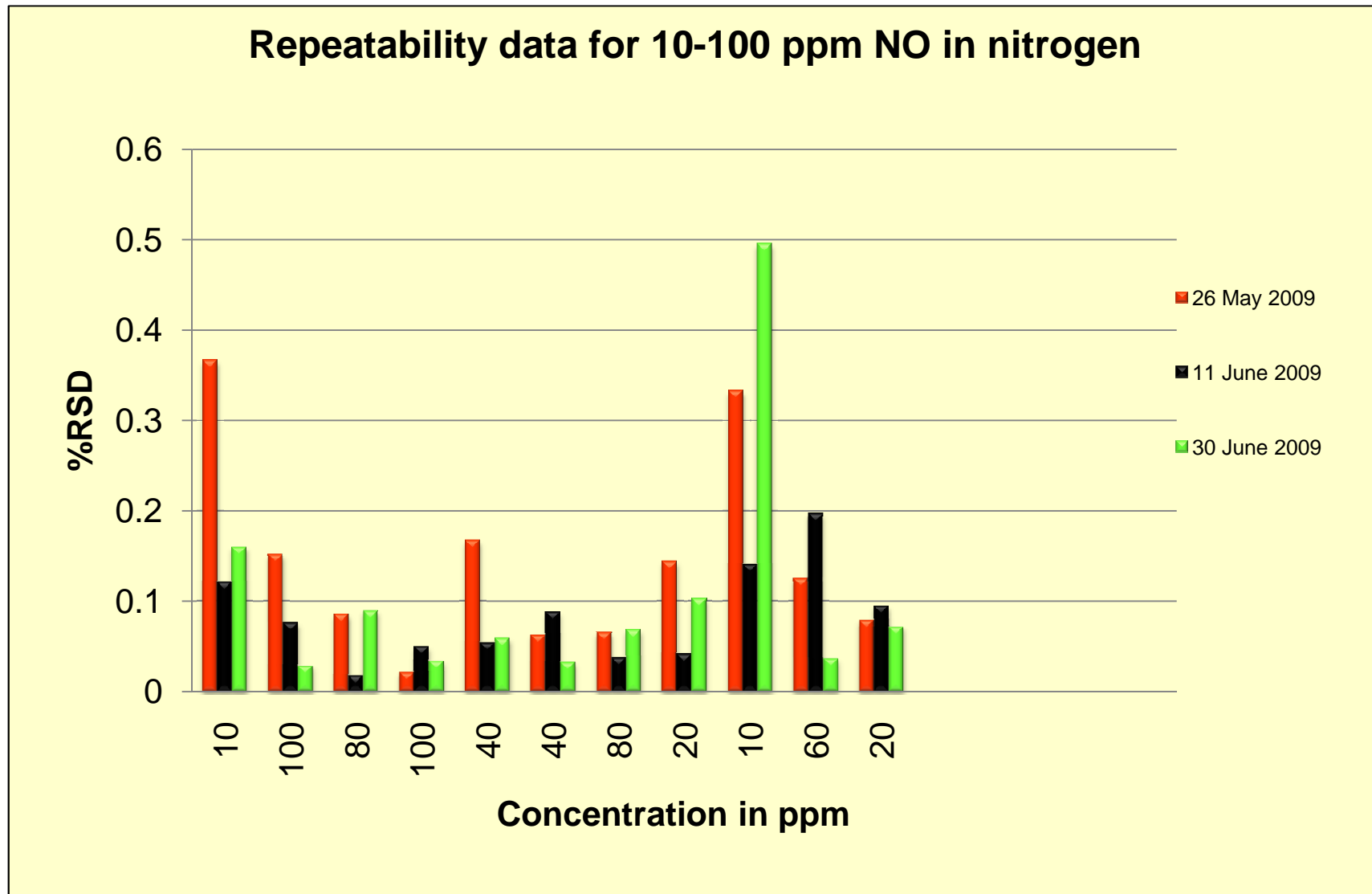
# Precision

- The closeness of agreement between independent test results obtained under stipulated conditions.
- Precision study is divided into repeatability and reproducibility.
  - Repeatability of a measurement method is the closeness of the agreement between the results of successive measurements of the same measurand carried out under the same conditions of measurement.
  - Reproducibility is the closeness of the agreement between the results of the measurements of the same measurand carried out under changed conditions of measurement.

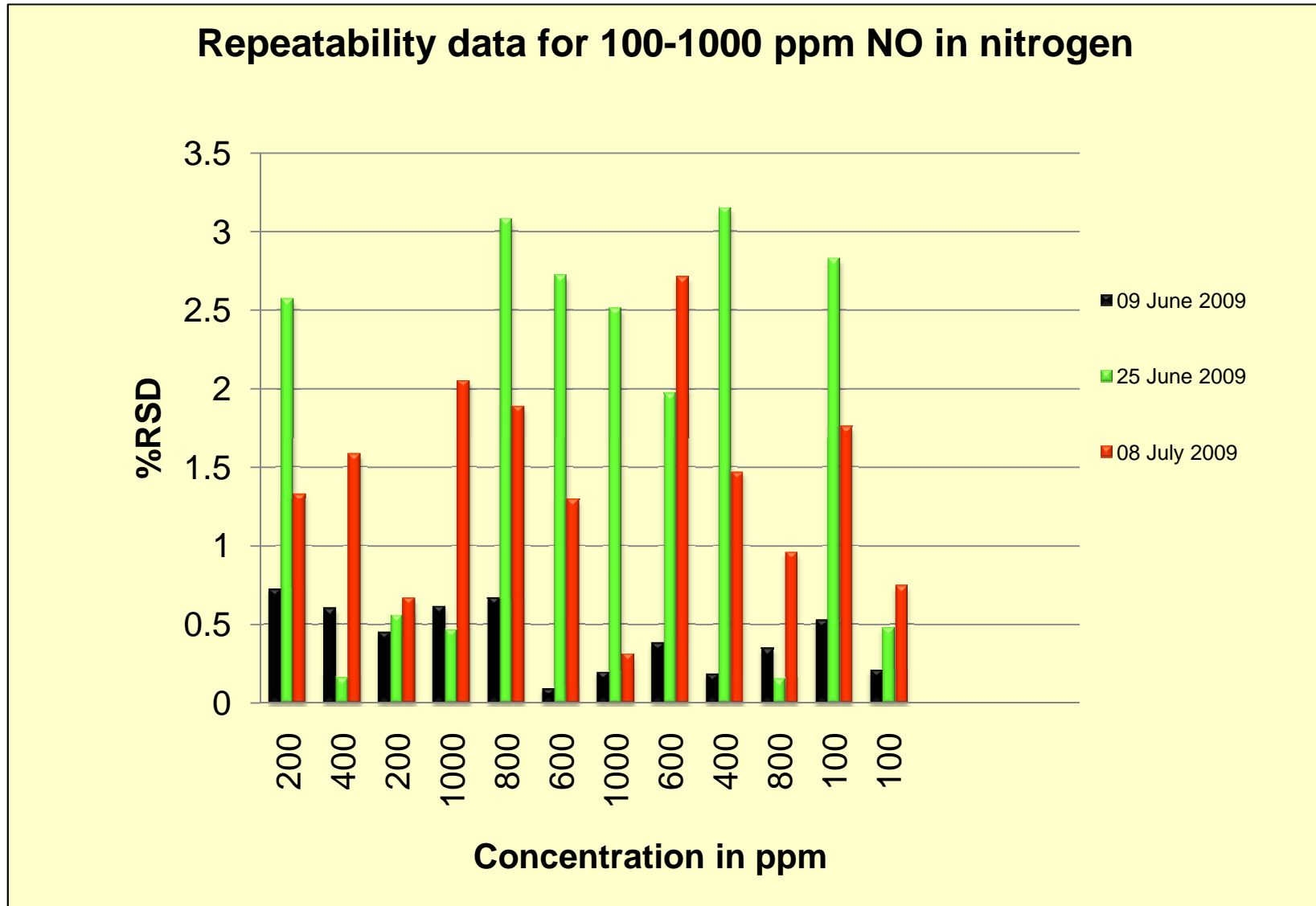
# PRECISION CONT.....

- Precision is normally expressed as % RSD (Relative Standard Deviation).
- A lower % RSD value indicates a higher precision.
- Criteria chosen for the precision for this method was a % RSD  $\leq 0.5$ .
- Criteria for linearity, correlation coefficient value to be close or equals to 1 ( shows good linearity).
- The units used are parts per million (ppm)

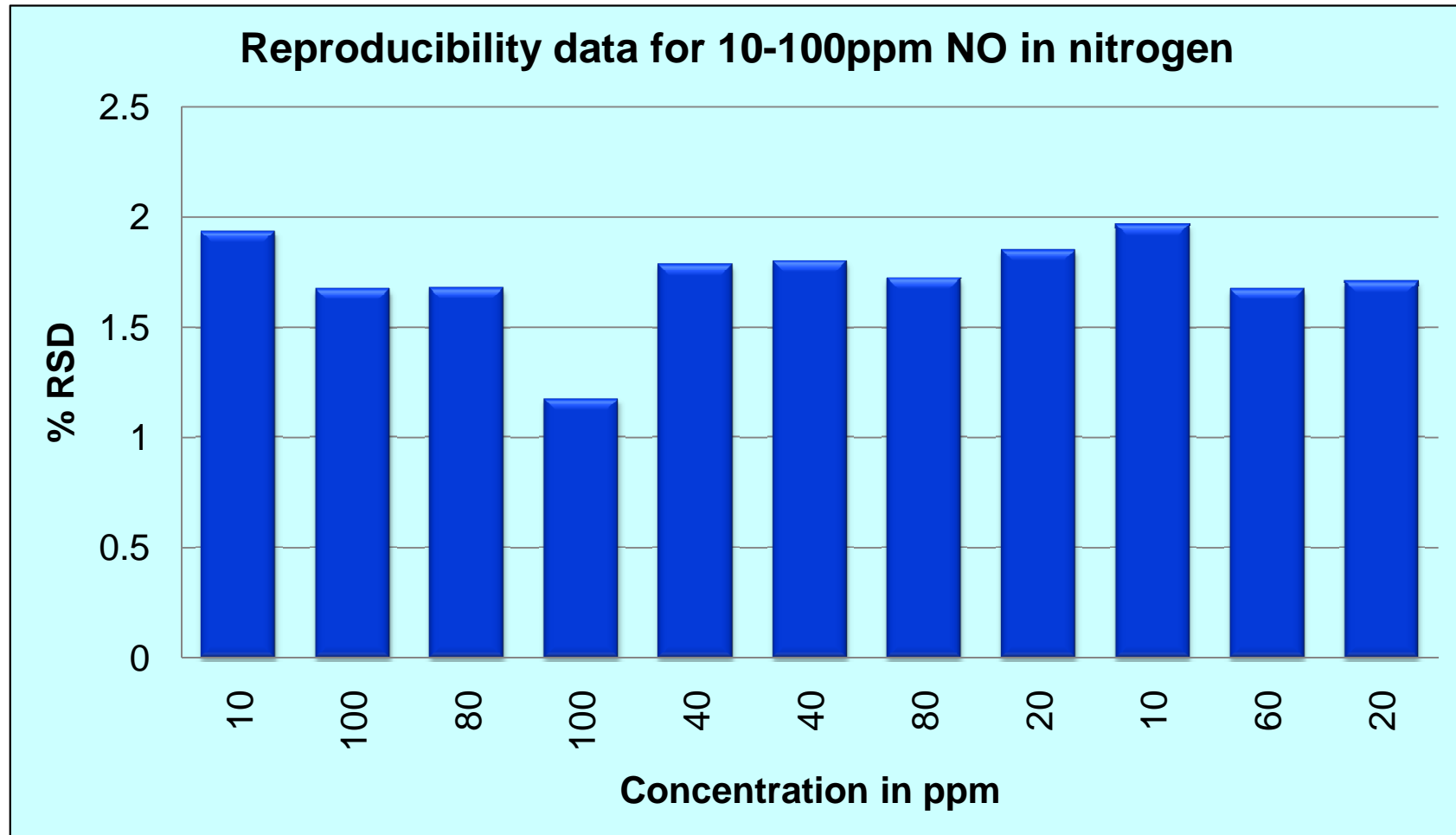
# Repeatability



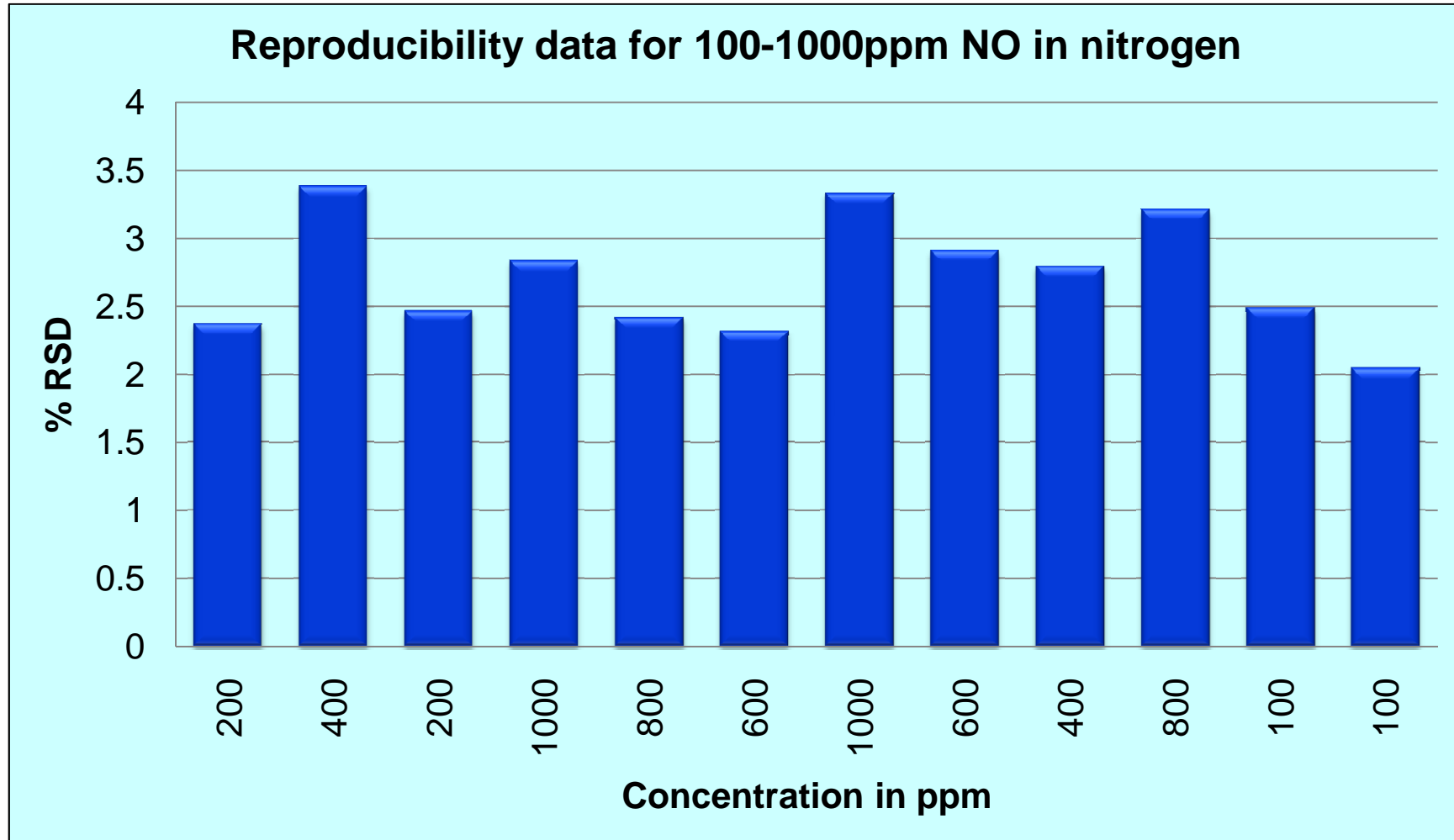
# Repeatability cont....



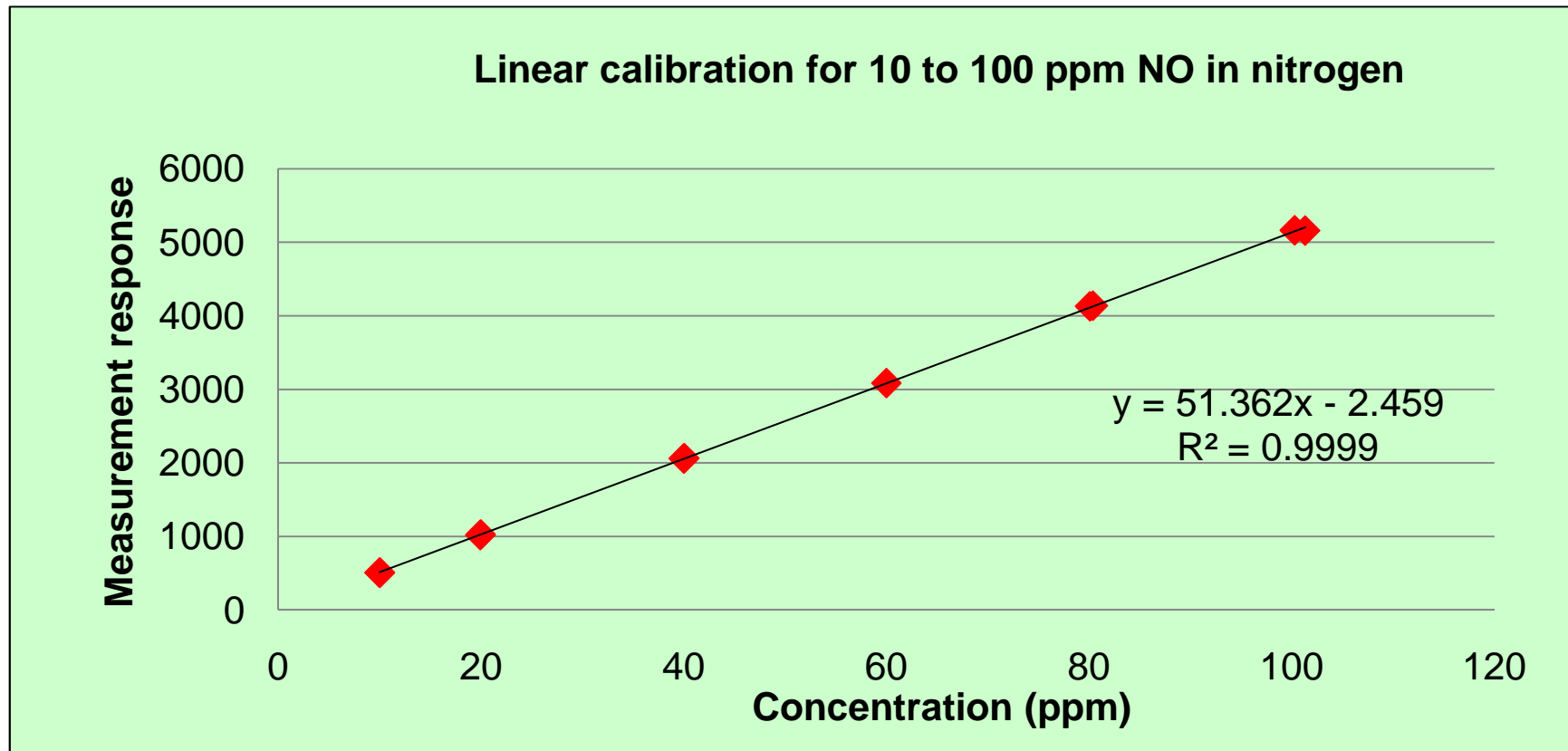
# Reproducibility



# Reproducibility cont.....

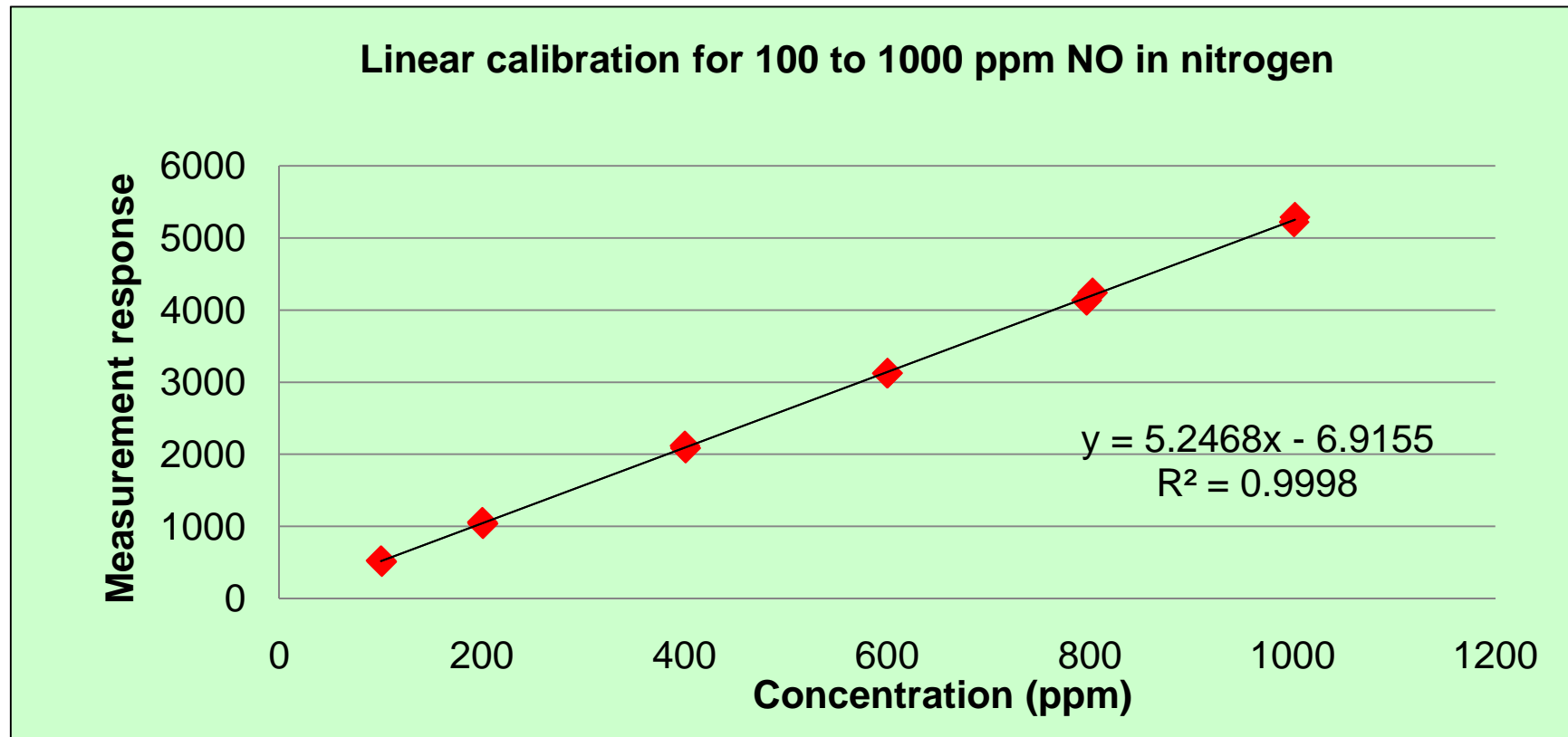


# Linearity



Calibration curves for NO(10-100ppm),  $R^2$  (Correlation coefficient) = 0.9999, which is indicative of good linearity.

## Linearity Cont....



Calibration curves for NO(100-1000ppm),  $R^2$  (Correlation coefficient) = 0.9998, which is indicative of good linearity.

## Limit of Detection

- The detection limit of a method is the lowest signal that can be detectable above the noise level of the system.
- The detection limit is determined only to establish the lower end of the practical operating range of a method.

$$LOD = 3 \times \frac{s_x}{m}$$

- CLD Low (10 to 100ppm)

Dates	Slope (m)	Std deviation of blank	LOD
26/05/2009	51.36	0.59	0.03
11/06/2009	50.30	2.41	0.14
30/06/2009	49.97	0.72	0.04
Mean LOD			0.074 ppm

## Limit of Detection cont.....

- CLD High (100 to 1000ppm)

Dates	Slope (m)	Std deviation of blank	LOD
09/06/2009	5.08	0.19	0.109
25/06/2009	5.25	0.18	0.104
08/07/2009	5.40	0.11	0.060
Mean LOD			0.091 ppm

# Limit of Quantification

- The limit of quantification is the lowest level of measurand that can be quantified.
- The limit of quantification can be 5, 6 or 10 standard deviations of the blank.
- The limit of quantification for this method was calculated using 10 standard deviations of the blank.

$$LOQ = 10 \times \frac{s_x}{m}$$

- The value of **LOQ** for 10 to 100 ppm was calculated to be 0.25 ppm.
- The value of **LOQ** for 100 to 1000 ppm was calculated to be 0.30 ppm.

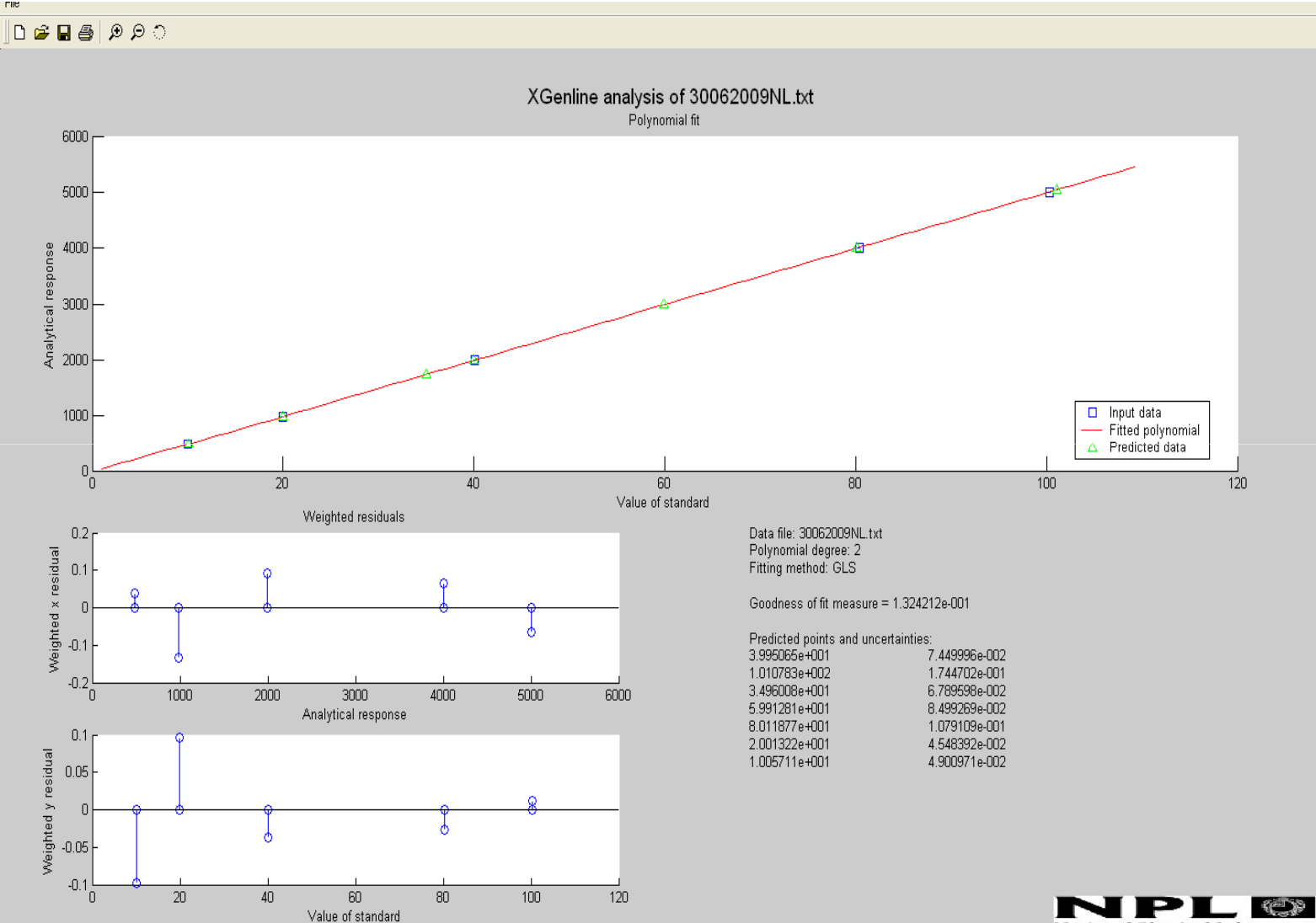
# Measurement Uncertainty

- A non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used
- All uncertainty contributors are written as standard uncertainties.
- All standard uncertainties can be combined and called combined standard uncertainties.
- For commercial purposes, expanded uncertainty,  $U$ , is reported rather than the combined standard uncertainty.
- Expanded uncertainty is obtained by multiplying the combined standard uncertainty with a coverage factor,  $k$ .

# Data for the regression analysis for 10 to 100 ppm NO in nitrogen on 26 May 2009, for input into XGENLINE

Cylinder Numbers	x(Gravimetric Concentration)	u(x) (Standard uncertainty gravimetry)	y(Analyser response)	u(y) (Standard uncertainty for Analyser response)
4869	100.2823	0.1906	5162.16	6.20
4884	40.0733	0.0756	2062.93	2.94
543835	19.9302	0.0379	1017.52	1.74
543845	80.0964	0.1518	4127.74	2.60
4889	10.0410	0.0191	506.43	1.93
4911	10.0123	0.0175	504.94	1.72
4849	100.3943	0.1910	5159.04	1.74
4864	80.3723	0.1523	4137.88	3.16
4879	19.9980	0.0380	1024.00	1.64
543925	40.0215	0.0758	2059.27	1.67
543879	59.9988	0.1132	3083.25	3.32

# Regression analysis (30/06/2009) using XGENLINE



## Data for 10 to 100 ppm NO in nitrogen over three days

		26/05/2009		11/06/2009		30/06/2009	
Cylinder no.	Gravimetric conc	Predicted conc	Standard uncert	Predicted conc	Standard uncert	Predicted conc	Standard uncert
4889	10.0410	10.0315	0.0509	9.9954	0.0374	10.0571	0.0490
4869	100.2823	100.3708	0.2052	99.7241	0.1744	100.2515	0.2963
4864	80.3723	80.3877	0.1137	80.0601	0.0982	80.3821	0.1551
4859	100.3943	10.0287	0.1854	101.2748	0.1777	101.0783	0.1745
4884	40.0733	40.1028	0.0871	40.0567	0.0711	40.1136	0.0671
54 3925	40.0215	40.0339	0.0694	40.1038	0.0688	39.9507	0.0745
54 3845	80.0964	80.1867	0.1079	80.0756	0.0963	80.1188	0.1079
4879	19.9980	20.0295	0.0460	20.0047	0.0421	20.0132	0.0455
4911	10.0123	10.0247	0.0509	10.0464	0.0385	9.9850	0.0535
54 3879	59.9988	59.8795	0.1019	59.7561	0.1212	59.9128	0.0850
54 3835	19.9302	19.8930	0.0466	19.9536	0.0421	19.9474	0.0429

Note: All units are x 10<sup>-6</sup> mol/mol

## % expanded uncertainty for 10 to 100 ppm NO in Nitrogen

Cylinder no	Gravimetric conc	Std dev	Grav uncert	Standard uncertainty over 3 days	Combined uncert	Expanded uncert (U)	% expanded uncert (U)
4889	10.0410	0.0310	0.0191	0.0458	0.0585	0.1170	1.1651
4869	100.2823	0.3441	0.1906	0.2253	0.4533	0.9067	0.9041
4864	80.3723	0.1875	0.1523	0.1223	0.2708	0.5415	0.6738
4849	100.3943	0.6699	0.1910	0.1792	0.7193	1.4386	1.4330
4884	40.0733	0.0302	0.0756	0.0751	0.1108	0.2216	0.5529
54 3925	40.0215	0.0767	0.0758	0.0709	0.1291	0.2581	0.6449
54 3845	80.0964	0.0560	0.1518	0.1040	0.1924	0.3847	0.4803
4879	19.9980	0.0126	0.0380	0.0445	0.0599	0.1198	0.5991
4911	10.0123	0.0312	0.0175	0.0476	0.0595	0.1190	1.1889
54 3879	59.9988	0.0825	0.1132	0.1027	0.1737	0.3475	0.5791
54 3835	19.9302	0.0333	0.0379	0.0438	0.0669	0.1337	0.6710

## % expanded uncertainty for 100 to 1000 ppm NO in Nitrogen

Cylinder no	Gravimetric conc	Std dev	Gravimetric uncert	Standard uncertainty over 3 days	Combined uncert	Expanded uncert (U)	% expanded uncert (U)
4859	101.3098	2.9573	0.1929	1.3859	3.2717	6.5433	6.4587
4843	200.4101	2.1989	0.3811	1.7884	2.8599	5.7198	2.8541
4873	400.3145	3.1913	0.7572	4.4530	5.5306	11.0611	2.7631
4848	600.1379	4.8407	1.1327	6.7992	8.4229	16.8458	2.8070
4853	802.4005	6.9944	1.5200	6.9831	9.9998	19.9997	2.4925
4844	1001.2223	13.9030	1.9057	12.6078	18.8648	37.7296	3.7684
4849	100.3943	1.7663	0.1910	1.0797	2.0789	4.1579	4.1416
4917	200.9847	2.8560	0.3826	2.4424	3.7774	7.5547	3.7588
4852	400.9210	3.6642	0.7591	5.1300	6.3497	12.6995	3.1676
4862	599.8588	1.4938	1.1316	4.7623	5.1178	10.2356	1.7063
4858	796.5851	7.4567	1.5110	11.2842	13.6095	27.2190	3.4170
4854	1002.1219	10.8212	1.9058	12.4812	16.6286	33.2572	3.3187

## Conclusion

- The criterion of 0.5% expanded uncertainty for the precision study for 10 to 1000 ppm was not met.
- The %RSD seems to increase as the NO concentrations became higher.
- Method development for the purity analysis of high purity NO is in progress (FTIR).
- New NO primary standard mixtures (PSM) will be prepared.
- It is expected that the new NO PSMs will be more stable in the cylinders for a longer period of time.
- The new NO PSMs will be used to check this optimized method again.

THANK YOU