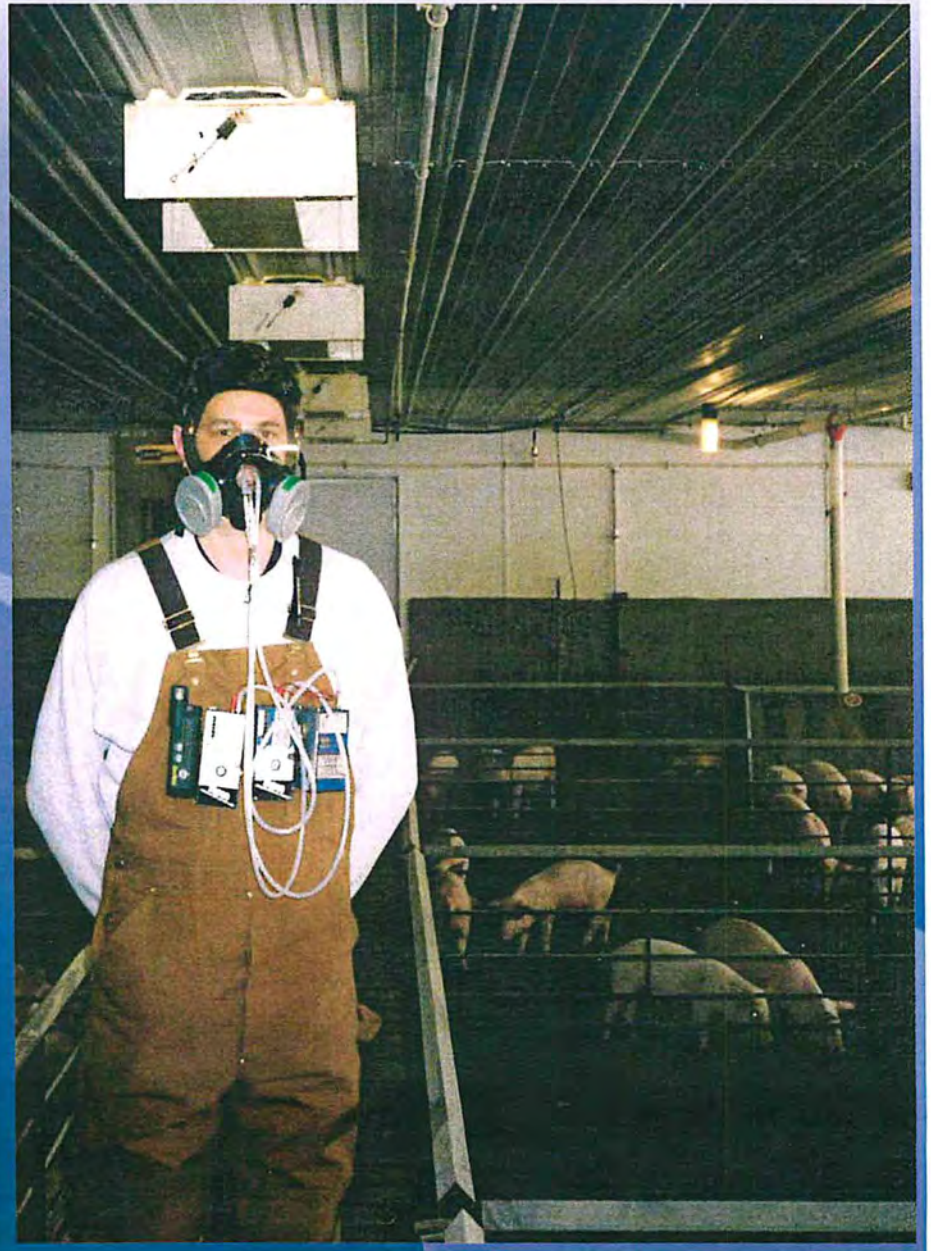
Evaluation of a Fluorometric Method for Analysis of Ammonia in Ambient Air

W. Groves, D. Agarwal, M. Chandra,
Penn State University, University Park, PA;
S. Reynolds, Colorado State University,
Fort Collins, CO

Introduction – NH_3 Analysis

- Specialized applications require analytical methods with greater sensitivity
  - Studies of environmental / atmospheric concentrations of ammonia
  - Studies examining short-term fluctuations of ammonia concentrations
  - Measurement of respirator workplace protection factors (WPFs)



Application - WPF Studies

- In-mask concentrations expected to be at least 10-50 times lower than environment concentrations
  - May actually be lower by several orders of magnitude
  - Sample times limited (1-2 hrs)
- Existing methods not adequate

Comparison of NH_3 Methods

Method	Type	Media	LOQ(ug)	Sample Times [1] 0.1xTLV	0.01xTLV
NIOSH 6015 [2]	VIS	acid treated silica gel	2	5.7 min	57 min
NIOSH 6016	IC	acid treated silica gel	7	20 min	200 min
NIOSH S347	ISE	acid treated silica gel	20	57 min	570 min
OSHA ID188	IC	acid treated carbon beads	30	86 min	860 min

[1] assuming 200 mL/min sampling rate

[2] Instrument specific method

Fluorometric Method

- "A Simple and Precise Method for Measuring Ammonium in Marine and Freshwater Ecosystems", Holmes et al, *Can J. Fish. Aquat. Sci.*, 56:1801-1808 (1999)
- Excellent sensitivity, relatively simple sample processing / analysis
- Aqueous samples – method not designed for air samples, acidified collection media

Specific Aims for Project

- Develop sampling and analytical protocol for fluorometric analysis of NH_3
  - Acid treated solid granular sorbent tube
  - Digital filter fluorometer
- Evaluate performance of the method
  - LOQ, stability/recovery, working range
  - Compare results for new method to reference laboratory / methods

Experimental Approach

- **Phase I:** Reproduction of Holmes method in laboratory
- **Phase II:** Introduction of acid treated sorbent to analytical protocol
- **Phase III:** Identification of key analytical parameters and optimization
- **Phase IV:** Method Evaluation

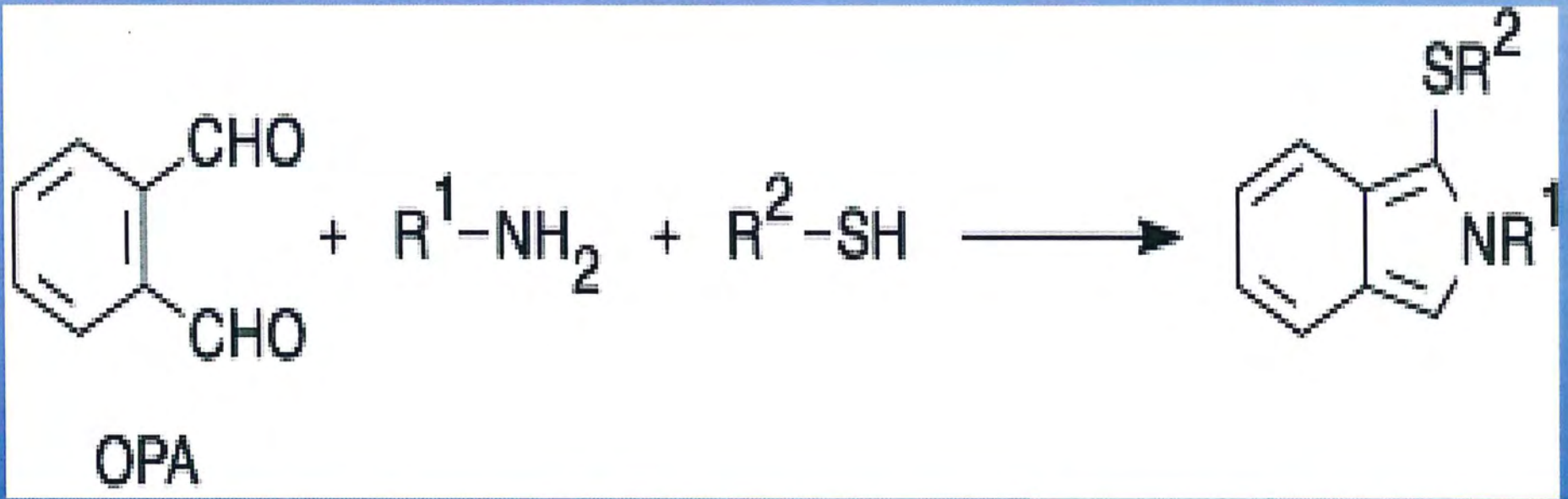
Phase I Experimental Procedure

- Fluorometric method reproduced in laboratory using liquid standards
- Standard solutions prepared by serial dilution from certified 1000 ug/mL ammonium stock solution
- Standards representing sample loadings of 0.1–2 ug NH_3 examined

Fluorometric Method

- Working Reagent
  - o-phthaldialdehyde (OPA)
  - Sodium sulfite
  - Sodium borate
- Ammonium reacts with OPA and sulfite to form fluorescent isoindole
  - Two hour room temperature incubation

Generalized OPA Reaction



o-phthaldialdehyde (OPA)

Molecular Formula: $C_8H_6O_2$

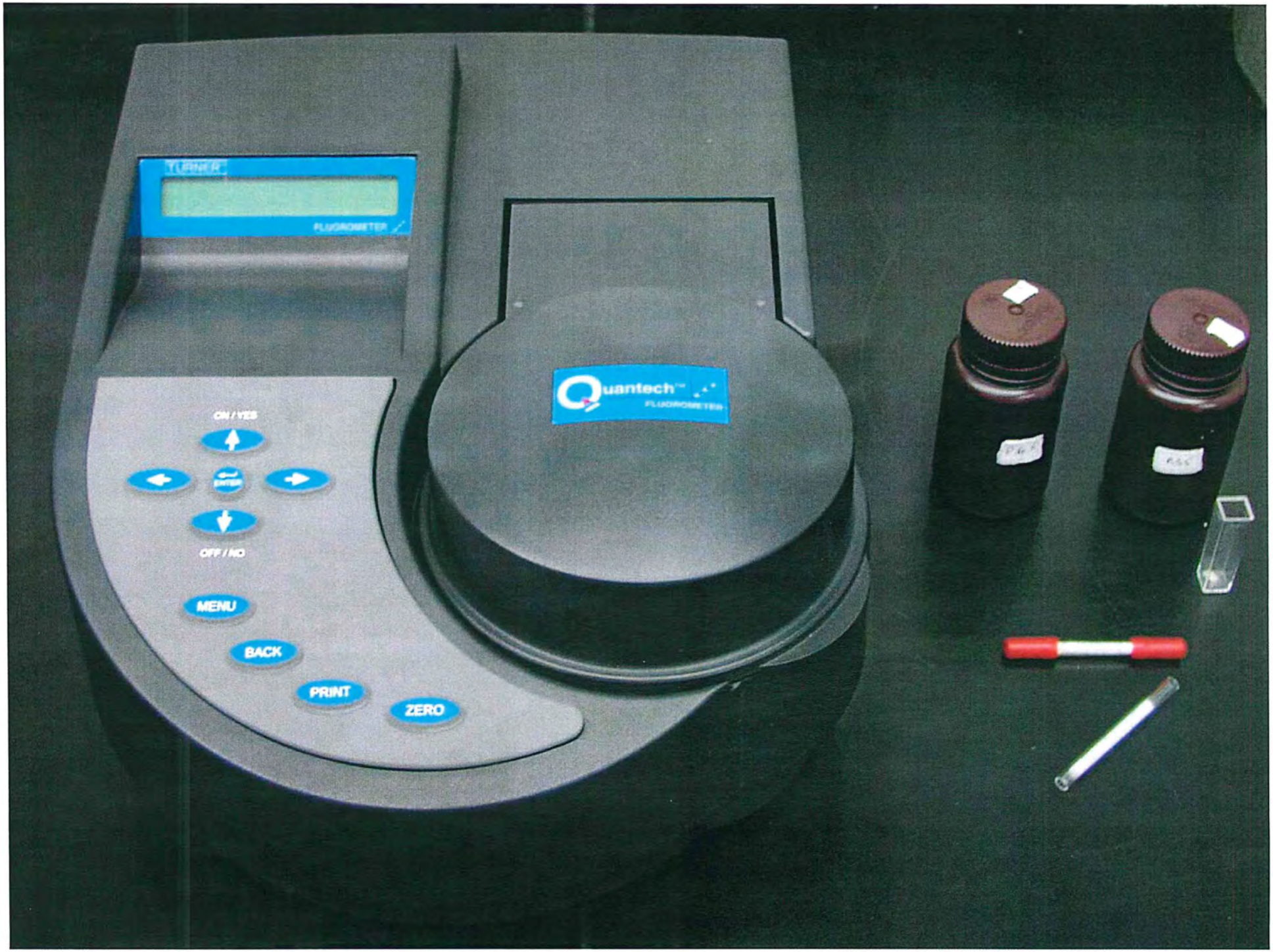
Molecular Weight: 134.13

λ **Max Excitation = 365 nm**

λ **Max Emission = 425 nm**

Instrumentation - Fluorometer

- Turner®-Quantech Digital Filter Fluorometer Model FM109515
- Quartz halogen lamp (340-650 nm)
- 365 nm narrow band excitation filter
- 420 nm narrow band emission
- 12.5x12.5x45mm sq. cuvettes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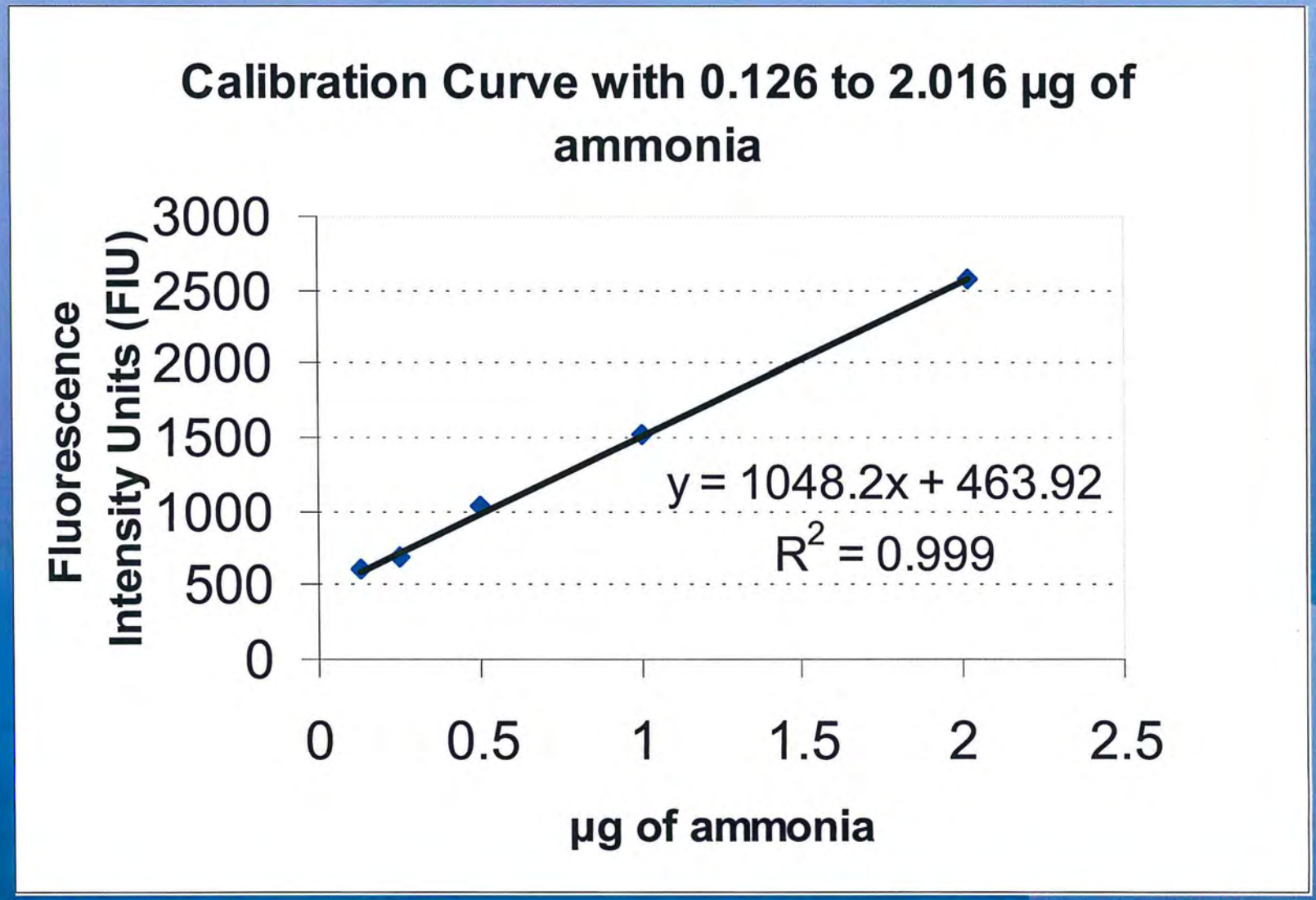
Phase I Results - Liquid Standards



Phase II Experimental Procedure

- Added acid treated sample media to protocol
  - Silica gel (NIOSH)
  - Carbon beads (OSHA)
- Analysis of spiked sorbent tubes
  - Sample desorption using DI water

Phase II Results

- Good results for some sorbent tubes
- Magnitude of blank response variable for different tube types / quantities
  - Carbon bead sorbent tubes eliminated from further consideration
  - Possible effect of different manufacturers and sorbent lots for silica gel tubes

Phase III Results - Optimal Protocol

- SKC Sorbent Tube (Cat. No. 226-10-6)
  - 100 mg (backup section)
- Protocol
  - Sample desorbed in 80 mL D.I. (1 Hr)
  - 20 mL working reagent added
  - 2 hr room temperature incubation
- Sample transferred to cuvette and read with fluorometer

Phase IV

- Method Evaluation
  - Chamber studies examining method performance for different sampling flow rates and NH_3 concentrations
  - Comparison of new method to reference laboratory
  - Sorbent tube fortification using span gas
  - Evaluation of LOQ

Phase IV Experimental Procedure

Chamber Studies

- Span gas used to prepare NH_3 test atmospheres ranging from 0.1-4 PPM
- Samples collected at flow rates ranging from 50-200 mL/min
- Mass of ammonia determined using fluorometric method and compared with expected

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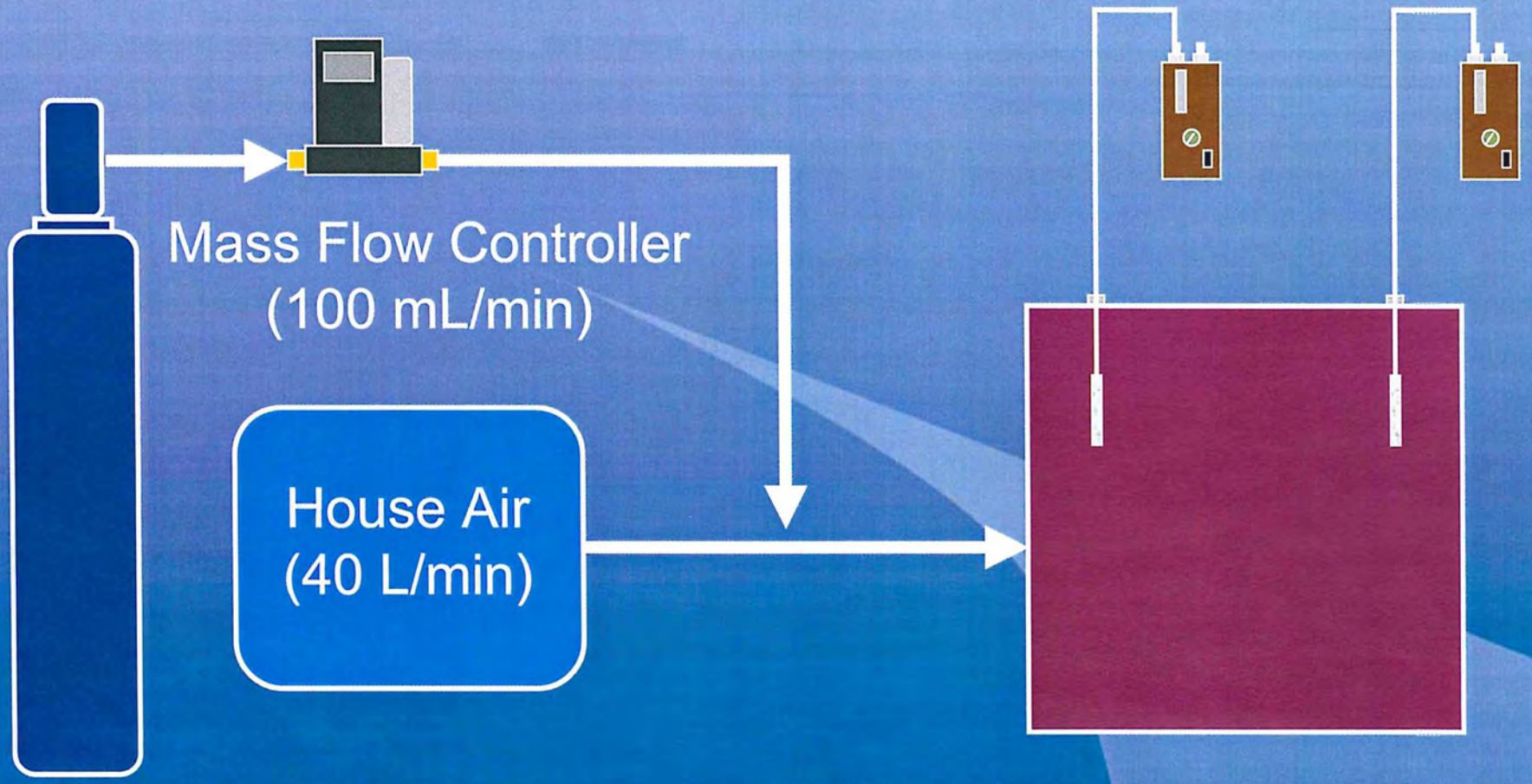
Phase IV - Test Atmosphere Generation

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Phase IV Chamber Study Results

Ammonia Concentration[1] (ppm)	Sample Time (min)	Sample Flow Rate (mL / min)	Predicted Sample Mass[2] (µg)	Experimental Sample Mass (µg)	% Difference[3]
4.00	8	102	2.28	2.72	19.2
	8	101	2.27	2.61	14.9
	8	101	2.28	2.61	14.5
	8	101	2.26	2.55	12.8
1.00	15	201	2.10	2.05	-2.5
	15	101	1.06	1.00	-5.6
	15	50.6	0.530	0.546	3.0
0.500	15	201	1.06	1.03	-2.7
	15	99.9	0.524	0.511	-2.5
	15	50.7	0.266	0.215	-19.2
0.250	15	201	0.530	0.493	-6.9
	15	101	0.265	0.261	-1.5
	15	50.8	0.134	0.131	-2.2
0.126	15	201	0.265	0.264	-0.4
	15	103	0.136	0.165	21.3
	15	50.8	0.067	0.113	68.6*

Phase IV Experimental Procedure

Reference Laboratory Comparison

- 1.01 PPM test atmosphere generated
- Side-by-side samples collected
  - Fluorometric method samples collected for 120 min at 50 mL/min (n=26)
  - Reference lab samples collected for 240 min at 200 mL/min (n=13) (NIOSH S347)
- Results compared

Phase IV Results-Reference Lab

Result	Reference Laboratory	Fluorometric Method
Ave. Conc. (PPM)	0.740	1.06
SD	0.069	0.027
CV	9.4%	2.5%
n	12	26
Error Relative to Reference Lab		43%
Error Relative to Expected Concentration	-24%	5%

Phase IV Results-Reference Lab

- Fluorometric method biased relative to reference lab (43%)
- Within 5% of expected concentration (1.01 PPM)
- Reference lab results for seven QC samples average 75% of expected

Phase IV Results-Reference Lab

Result	Reference Laboratory (adjusted)	Fluorometric Method
Ave. Conc. (PPM)	0.919	1.06
SD	0.086	0.027
CV	9.4%	2.5%
n	12	26
Error Relative to Reference Lab		15%
Error Relative to Expected Concentration	-9%	5%

Phase IV Results-Reference Lab

- Still significant bias even after adjusting reference lab reported concentrations using QC sample results
- Worked with reference lab to investigate
  - Samples were close to reporting limit for lab (20 ug)
  - Tried larger sample loadings (500 ug)
  - Exchanged spiked samples
- Problems never resolved

Phase IV Experimental Procedure

Analysis of fortified sorbent tubes

- Certified calibration gas used to fortify sample tubes with known amount of NH_3
- Fluorometric method results compared to expected mass of NH_3 (analyzed blind)
- Loadings ranged from 150-550ug NH_3
  - Three tubes at each of five loadings

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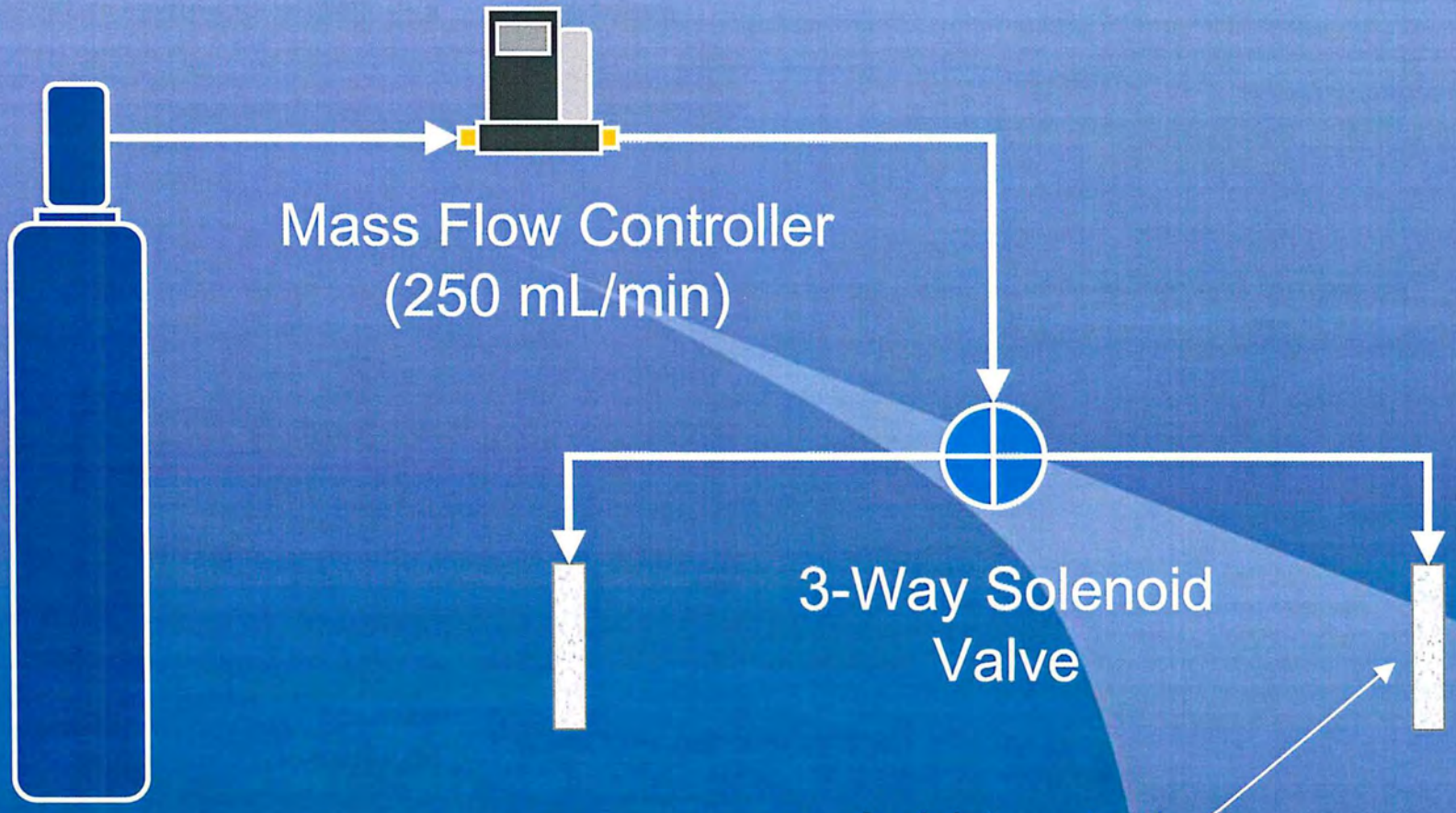
Phase IV - Sorbent Tube Fortification



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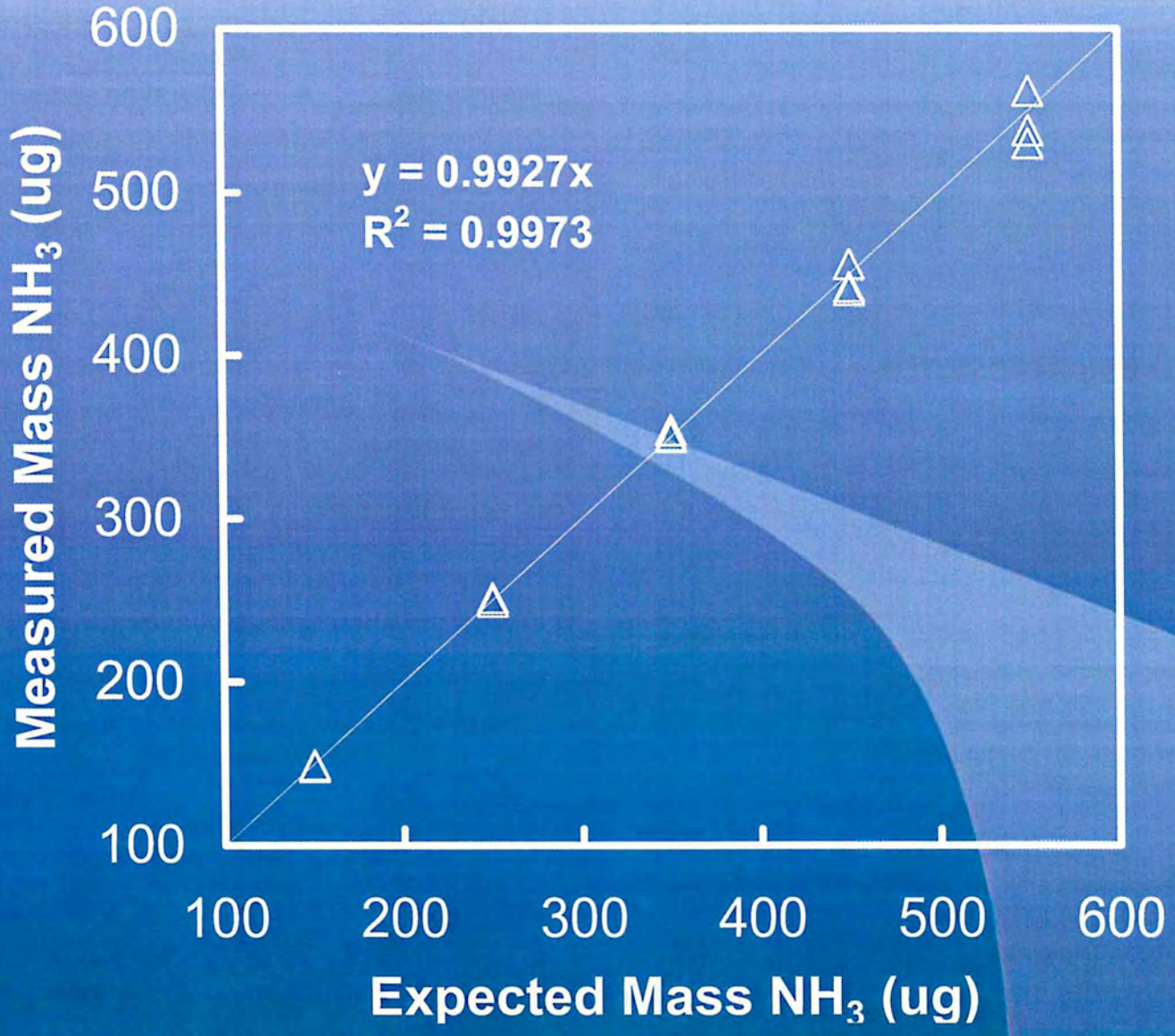
Phase IV Results - Fortified Samples



Phase IV Results - Fortified Samples

- No significant difference between experimental results and calculated levels of fortification
- All errors less than 5%

Average error = **-0.57%**

Range of error = **-3.6 – 2.2%**

Ave. Abs Error = **1.3%**

Phase IV Results (cont.)

- LOQ estimated to be 0.08 ug
  - Based on 10 x SD of the blank response
  - Represents a 20-300 fold improvement in sensitivity compared to existing methods
- Stability studies showed an average recovery of 98% after seven day storage at room temp (0.25, 0.50, 1.0 ug)

Conclusions

- A fluorometric method for analysis of NH_3 in ambient air has been developed and evaluated in the laboratory
  - Results demonstrate excellent sensitivity, accuracy, and precision
  - Relatively simple sample processing and analysis
- Additional work needed to field validate method and to compare results with other reference methods

Acknowledgement

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  - National Institute for Occupational Safety and Health SERCA grant (5K01 OH00177)
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