A SENSITIVE METHOD FOR QUANTITATION OF HYDRAZINE IN MAINSTREAM TOBACCO SMOKE

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Objective

To develop a sensitive method for quantitative analysis of trace levels of hydrazine in mainstream tobacco smoke.

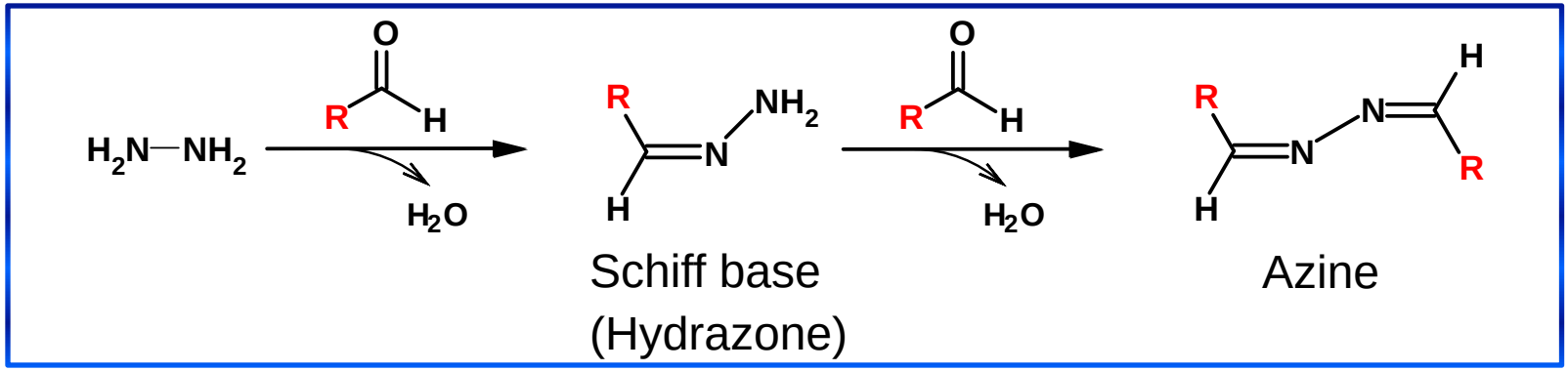
Challenges:

- sensitivity
- selectivity
- robustness

Analytical Background

- Hydrazine is a small, polar, basic and strongly reducing agent.

- Conventional methodology for analysis of hydrazine: “Schiff base” formation



Analytical Challenges

Previous approaches for analysis of hydrazine in mainstream tobacco smoke:

Ref.	Sample Generation/Extraction	Sample Preparation	Analysis	Detection Limit	[Hydrazine] (ng/cig.)
Hoffmann (1974)	. 20 non filter cig. / "ISO" smoking . trapping solution (aqu. buffer/PFBA) . no filter pad	. Silica gel plate (2X) . TLC (Al_2O_3 plate) . Extract with ether	GC/ECD	0.1 (ng/ cig.)	31
Plunkett (2002)	. puff-by-puff measuring of a flowing stream of smoke	none	IR-TDL	0.4 (ppmv)	ND

Hoffmann et al., *Anal. Chem.*, 46(7), 885-889, 1974
Plunkett et al. *Spectrochimica Acta Part* A, 58, 2505-2517, 2002

Analysis of hydrazine in tobacco smoke requires a quantitative isolation procedure to prevent its loss due to:

- its oxidation with other reactive smoke constituents
- poor stability of hydrazine derivatized products

Analytical Approach

Derivatization Reagent

- Compare derivatization efficiencies of benzaldehyde derivatives

Internal Standard

- Investigate and Select the Internal Standard

Analysis

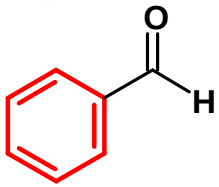
- Separation and Detection Methods

Trapping Mechanism

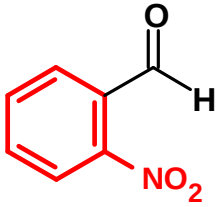
- Establish a quantitative trapping /extraction procedure from smoke (*i.e.* effect of glass fibre filter pad, Impinging volume etc…)

Method Development - Derivatization Reagent

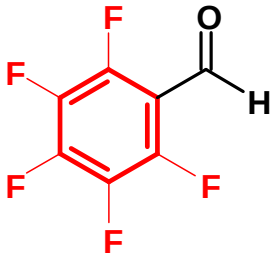
Benzaldehyde

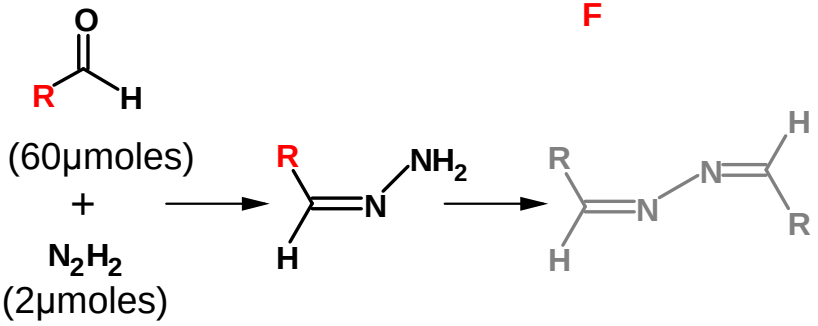


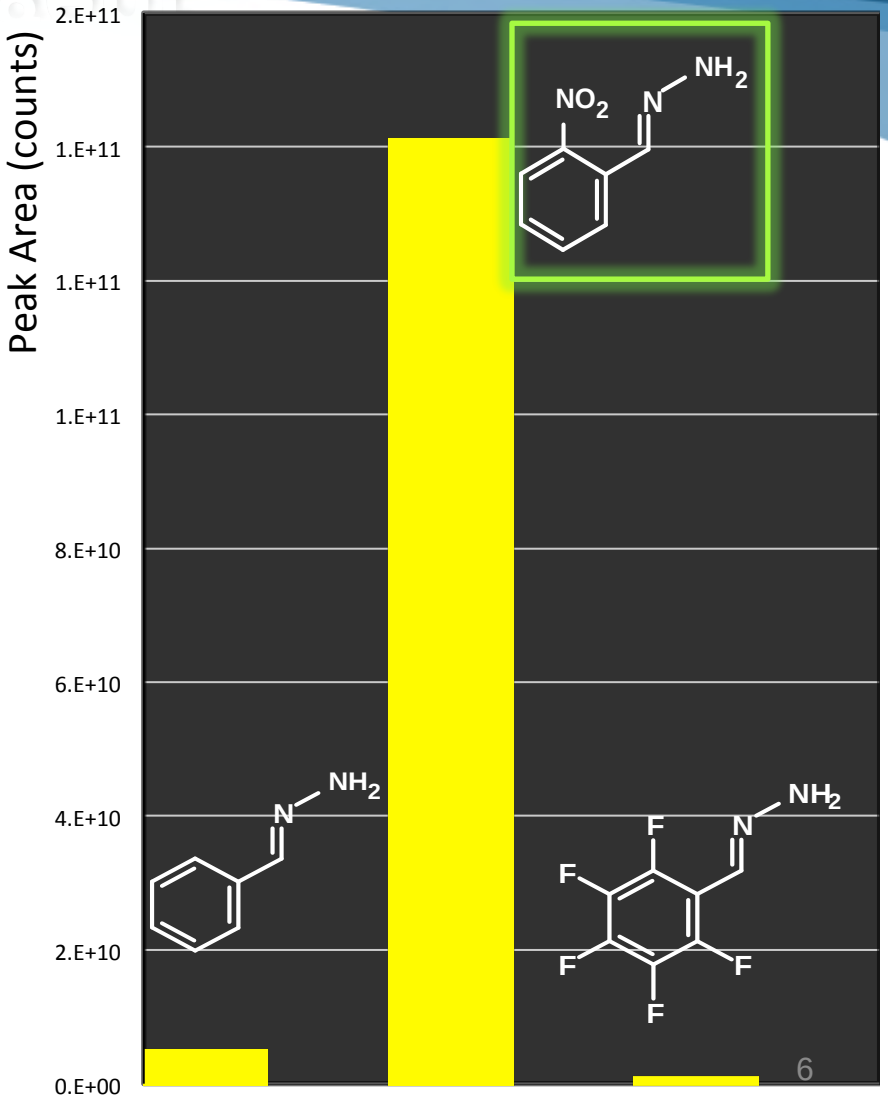
2-Nitrobenzaldehyde



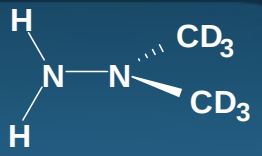
Pentafluorobenzaldehyde



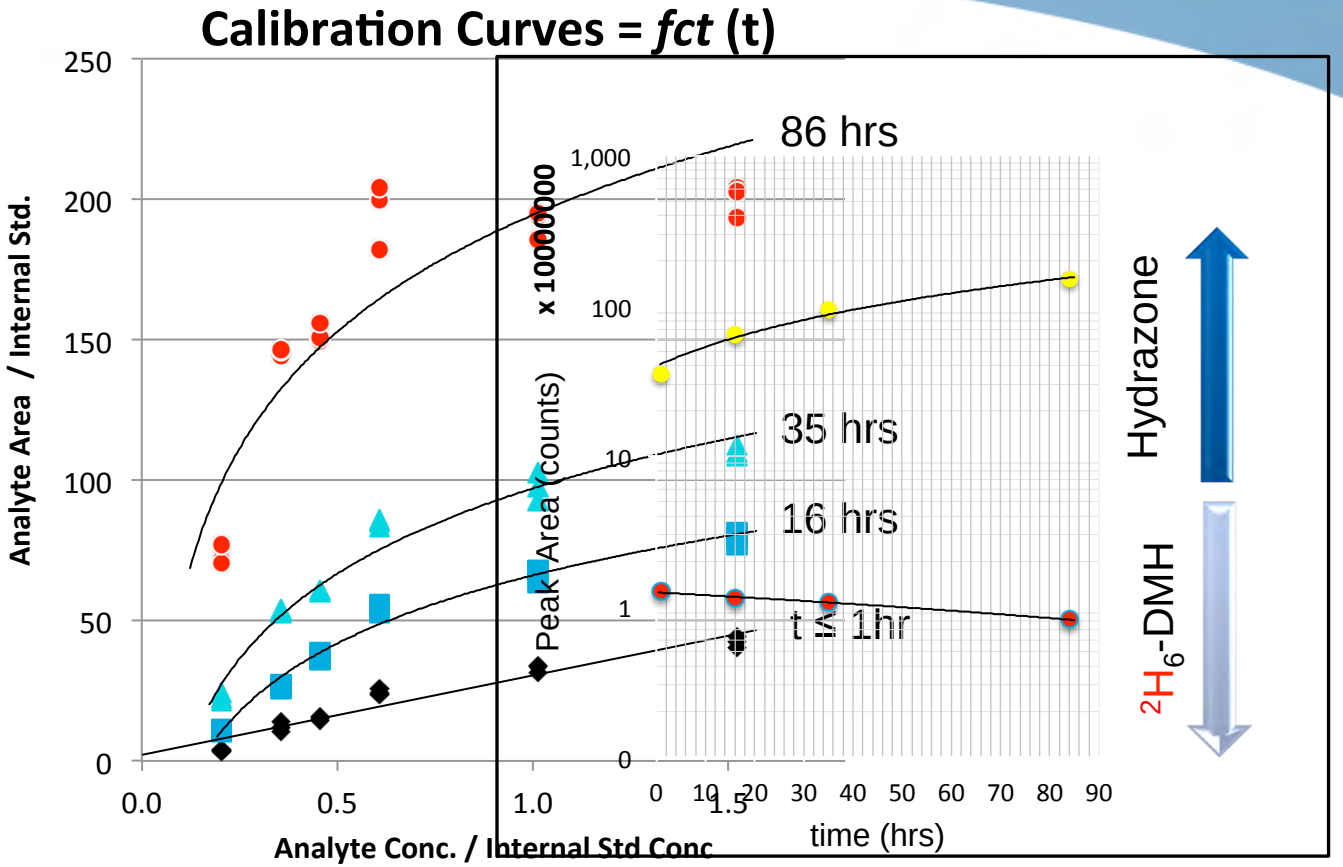




Method Development



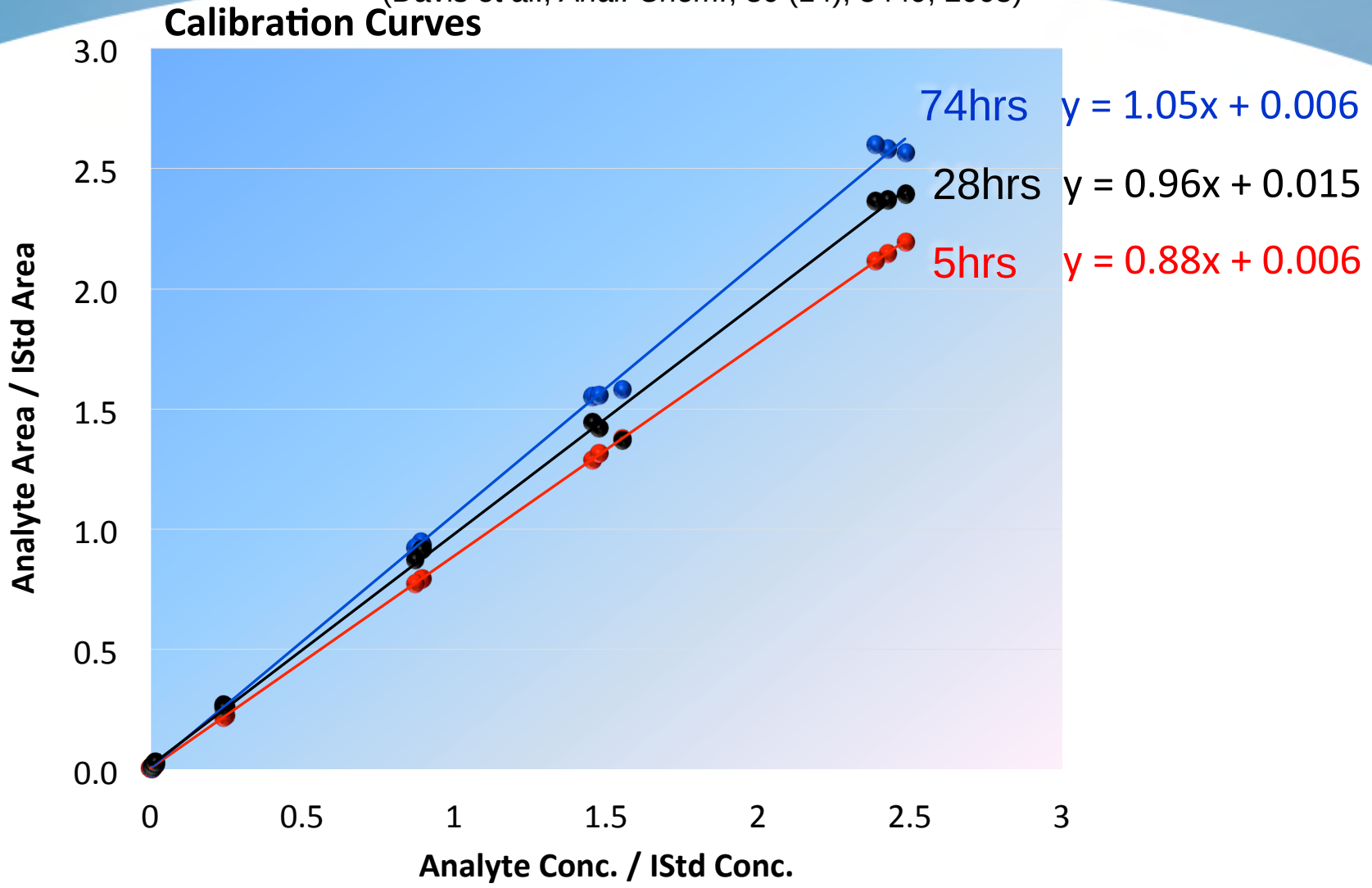
- Selection of Internal Standard: $^2H_6$-Dimethylhydrazine



Internal Std (DMH) signal decreased over time probably due to its degradation in methanol (solvent).

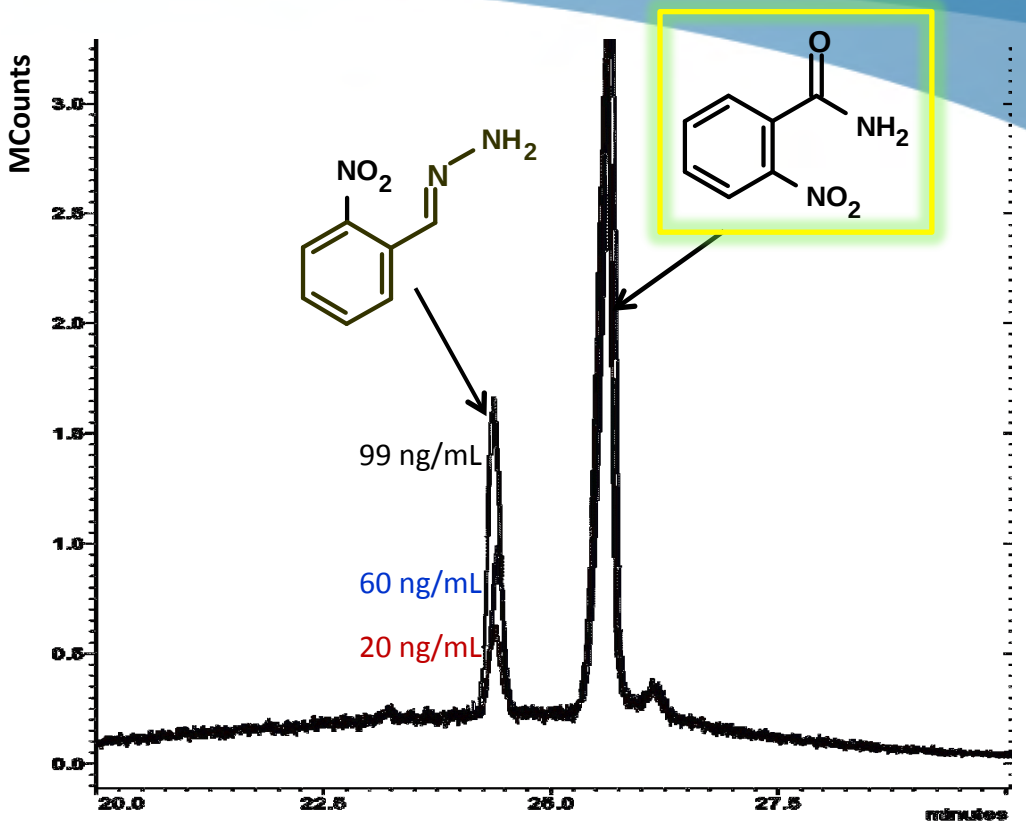
Method Development - Internal Standard: $^{15}N_2$-Hydrazine

(Davis et al., *Anal. Chem.*, 80 (14), 5449, 2008)



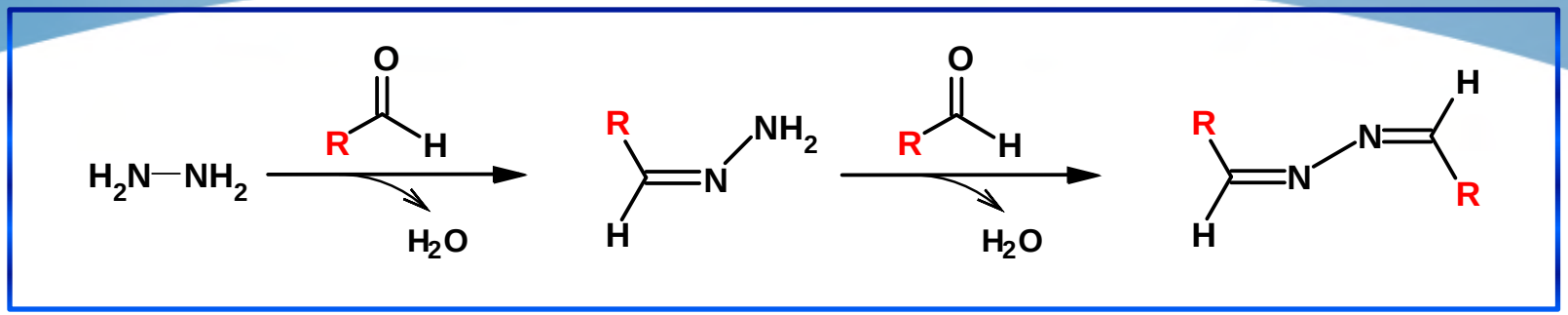
Method Development - Analysis Method: GC-MS

GC column	Wax 30 m x 0.25 mm x 0.25 µm
Injector Temp.	260°C
Column Temp.	55°C, hold for 2 minutes
	15°C / minute to 150°C, hold for 25 minutes
	10°C / minute to 240°C, hold for 2 minutes
Constant Flow	1.4 mL/min



2-NBA undergoes an adverse reaction to form 2-nitrobenzylamide which generates an ion at *m/z* 166 (isobaric product ions) in the mass spec. source.

Method Development
- Analysis Method: LC-MS/MS



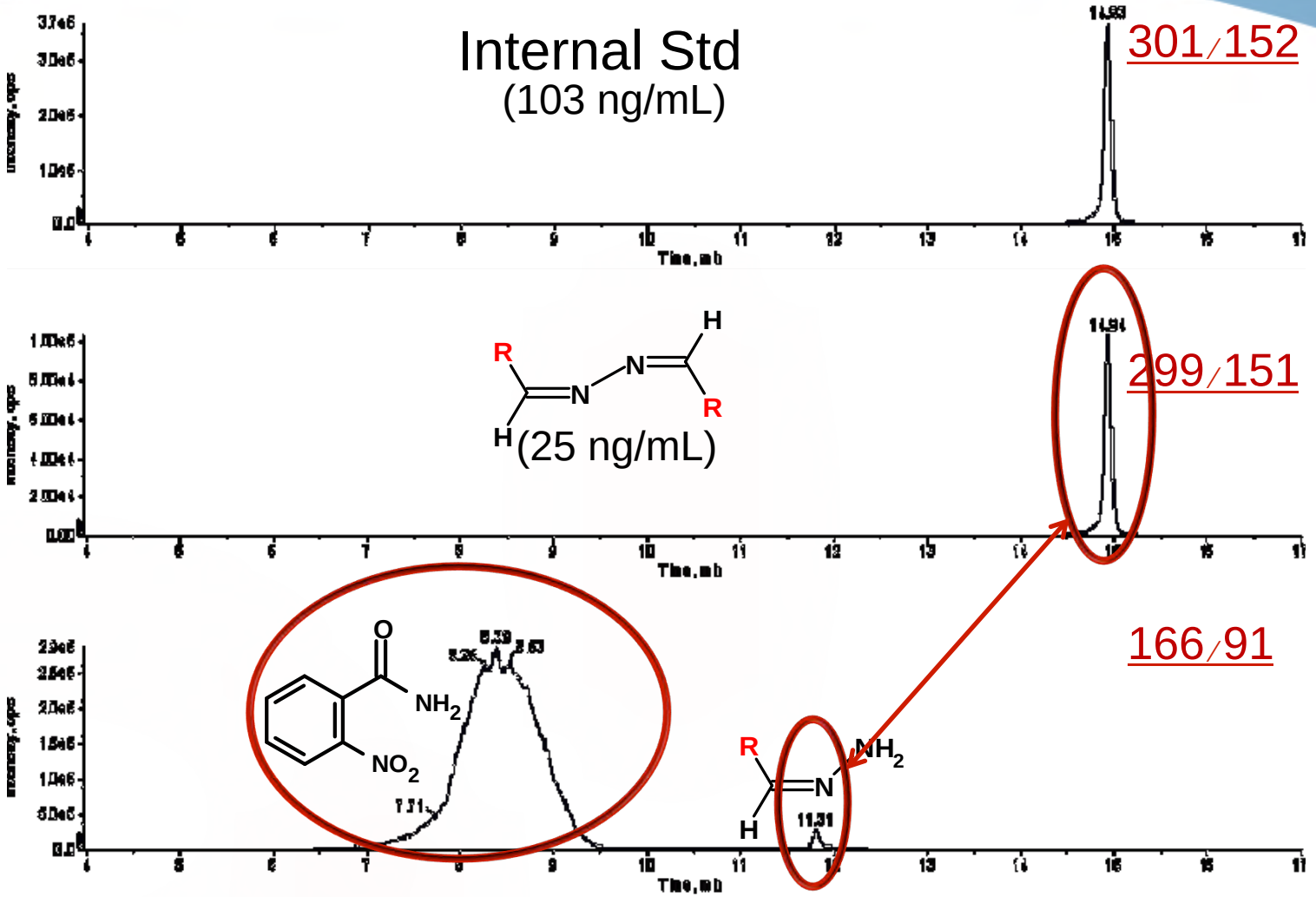
MRM Transition (*m/z*): **166 / 91**

299 / 151
299 / 78
301 / 152 (ISTD)
301 / 105 (ISTD)

Ionization / Mode:	ESI/MRM
Polarity:	Positive
IonSpray Voltage:	5500 V
Source Temperature:	400°C
Curtain Gas Flow:	12 mL/minute
CID Gas Flow:	12 mL/minute
Nebulizing Gas:	9 mL/minute

Method Development
- Analysis Method: LC-MS (Chromatogram)

Derivatization Reaction in MeOH

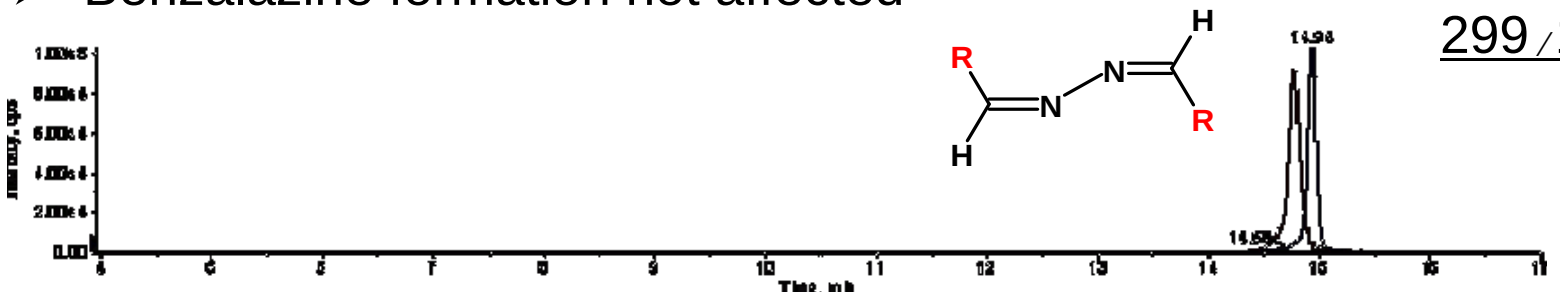


Method Development - Analysis Method: LC-MS

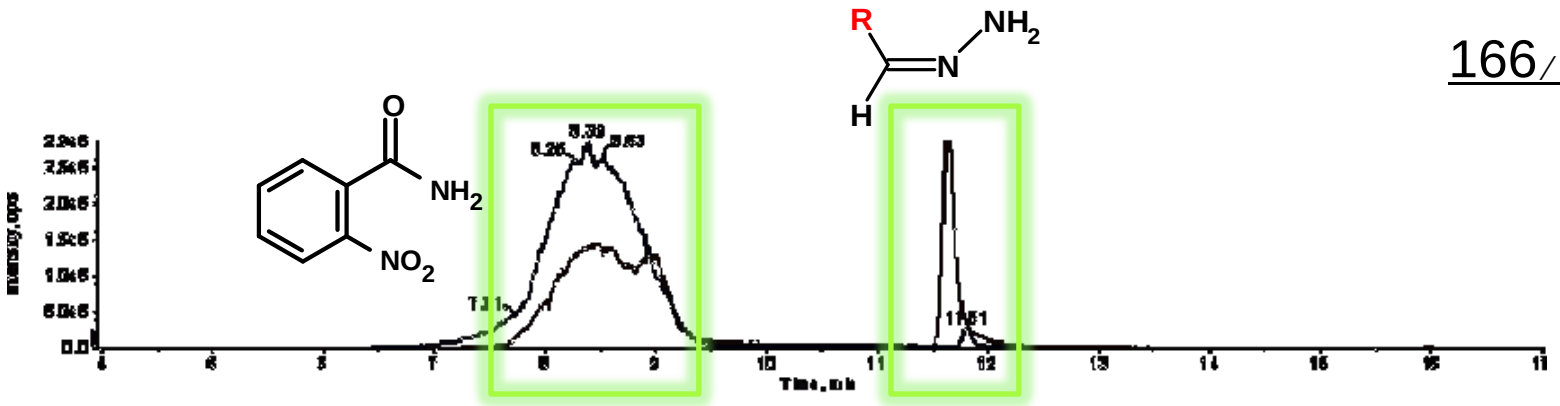
MeOH *vs.* Na_2HPO_4:MeOH (55:45, v/v) ✓

- Benzalazine formation not affected

299 / 151



166 / 91



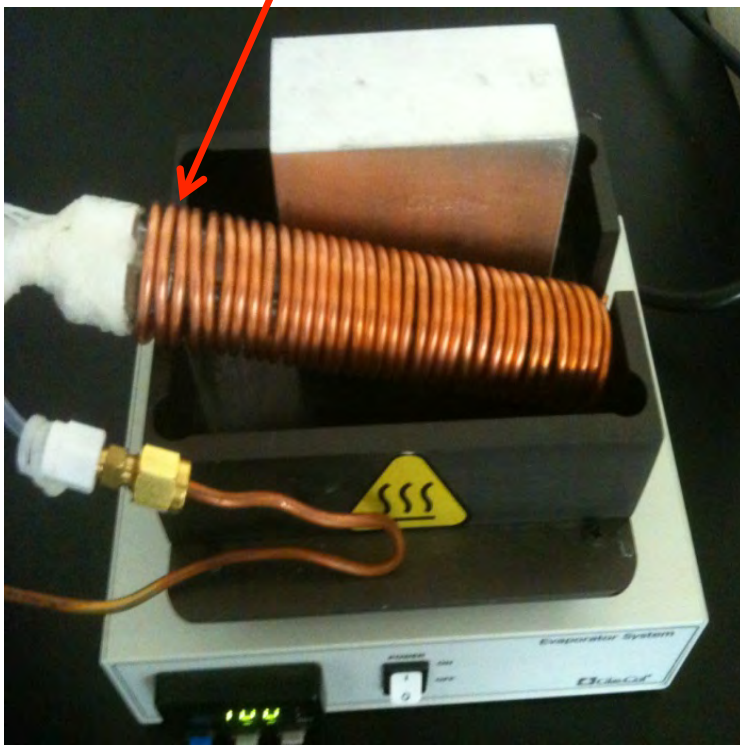
- Hydrazone formation significantly favoured in acidified solvent

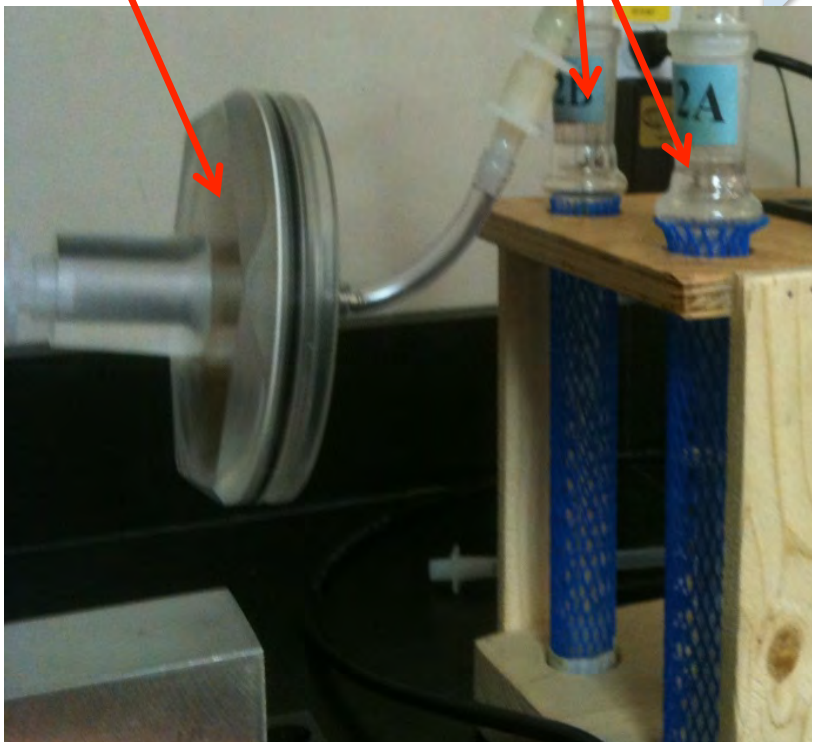
Method Development
- Evaluating Trapping Efficiency (Apparatus)

Permeation Tube

Pad Holder

Trapping Solution

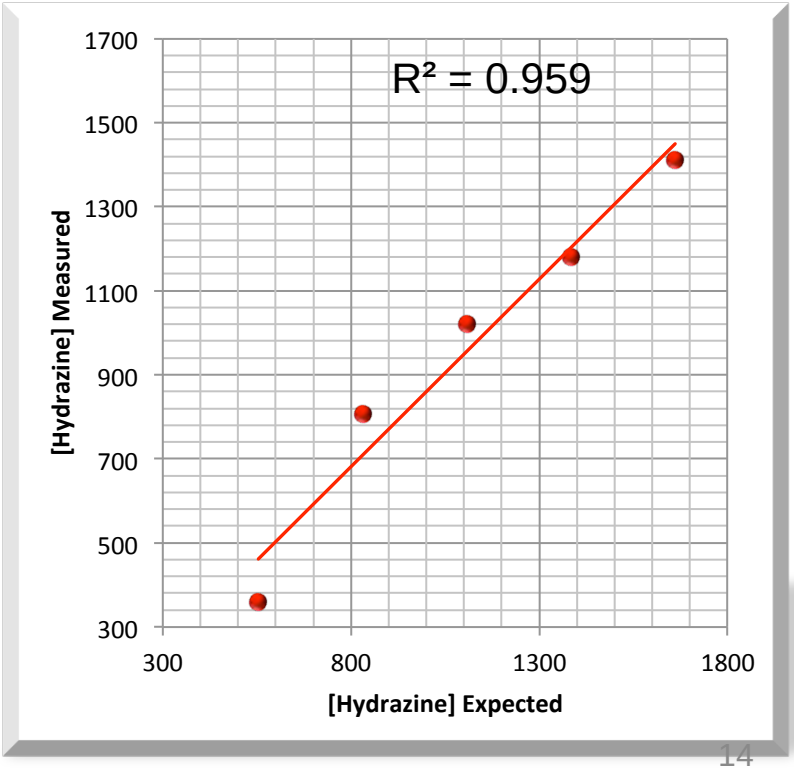




Method Development
- Evaluating Trapping Efficiency (Permeation)

$$[\text{Hydrazine}] = \frac{P \times K_0}{F \times V} \times t$$

- P permeation rate (Temp. dependant)
- K_0 molar constant (0.699)
- F Carrier gas (N_2) flow rate (mL/minute)
- V trapping volume (mL)
- t sampling time (min)

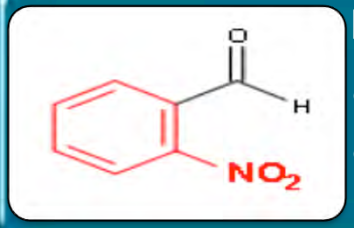


Method Development
- Trapping Efficiency of Hydrazine Vapour

Parameters	Values	Comment
Temperature (Permeation Tube)	~ 70°C	Measurement after equilibration period (4 hrs)
Trap solution Volume (V)	2 X 40 mL	Wash bottles connected in series
Sampling Period (t)	15 min.	Hydrazine flow is drawn through the system
Recovery % Calculation		$\frac{[Azine]_{measured}}{[Azine]_{ref.(trap)}} \times 100$

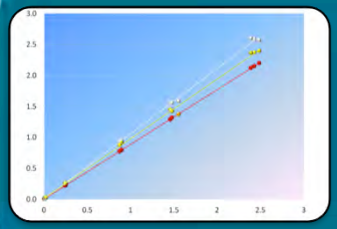
Trials Description	[Azine] Measured			Recovery	
	Filter Pad (ng/mL)	Trap A (ng/mL)	Trap B (ng/mL)	Filter Pad	Trap A
Reference (no Pad)	-	*637 (ref.)*	*nd*	-	100 %
Blank Pad	546	96	nd	86%	15%
Smoked Pad (TPM)	621	14	nd	98%	2%
DNPH-treated Pad	766	6	nd	120%	1%

Summary of Findings



Reagent / Trapping Solvent

- 2-Nitrobenzaldehyde (10g/L)
- Aqueous Phosphate Buffer Solution (PBS:MeOH, 55/45)

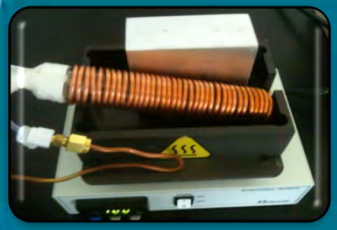


Internal Std / Solvent

- Isotope dilution Calibration using $^{15}N_2$-Hydrazine
- Calibration Stds prepared in 0.1 % CH_3COOH solution

Detection

- LC : Core-Shell PFP phase; acidified buffer 0.1% CH_3COOH
- ESI^+-MS/MS (MRM)



Trapping Mechanism (Hydrazine vapour)

- Volume of Trapping solution : 40mL
- Hydrazine mostly present in PP

Methodology

Sample Generation

- 10 cigarettes smoked
- 92mm glass fiber filter disc
- 40mL of impinging solution (PO^{4-}: MeOH; 2-NB; ISTD)

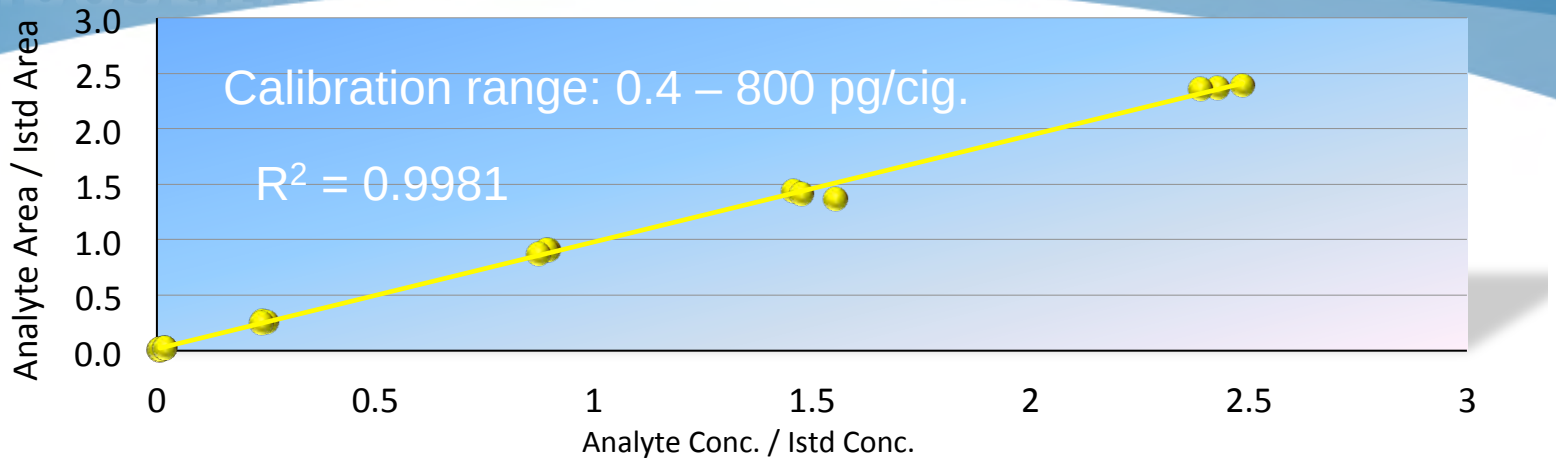
Sample Extraction

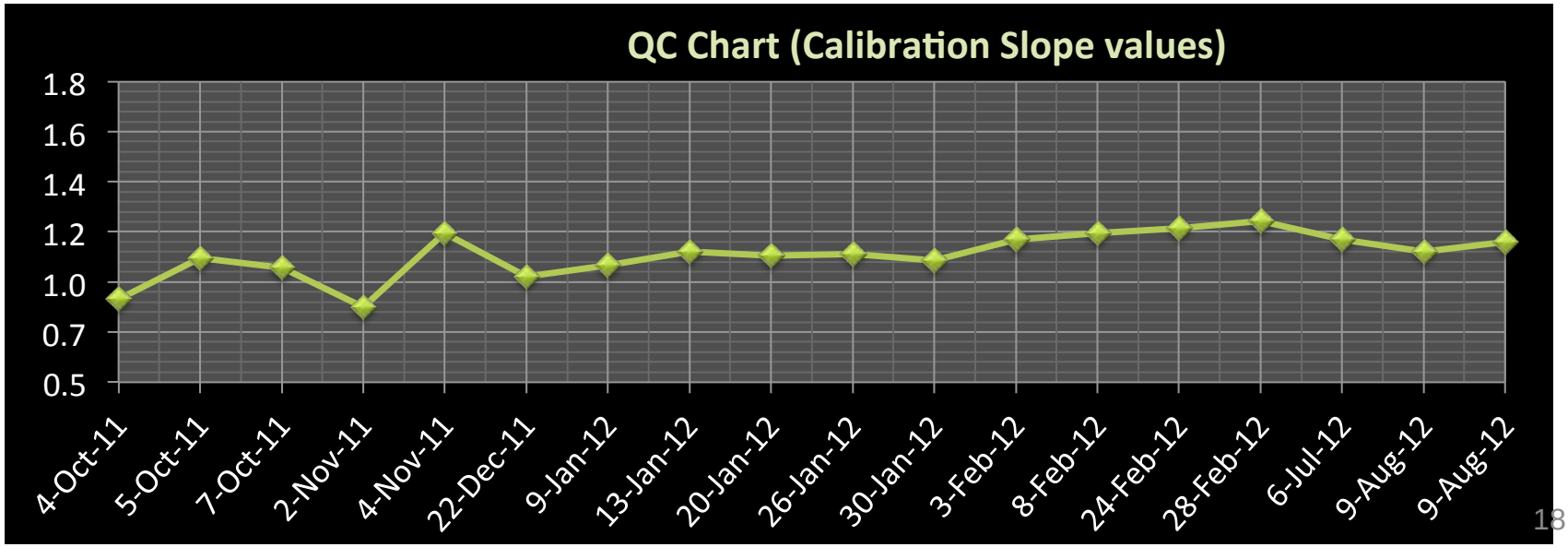
- Extract filter pad with impinger solution (40 mL)
- Dilute with fresh derivatization solution (40 mL)
- Incubate extract for 30 minutes at 35°C
- Shake extract for 30 minutes
- Centrifuge at 10,000g for 5 minutes

Sample Analysis

- LC-ESI-MS/MS operating under MRM mode

Analytical Performances - Linearity





Analytical Performances - Sensitivity (Instrument)

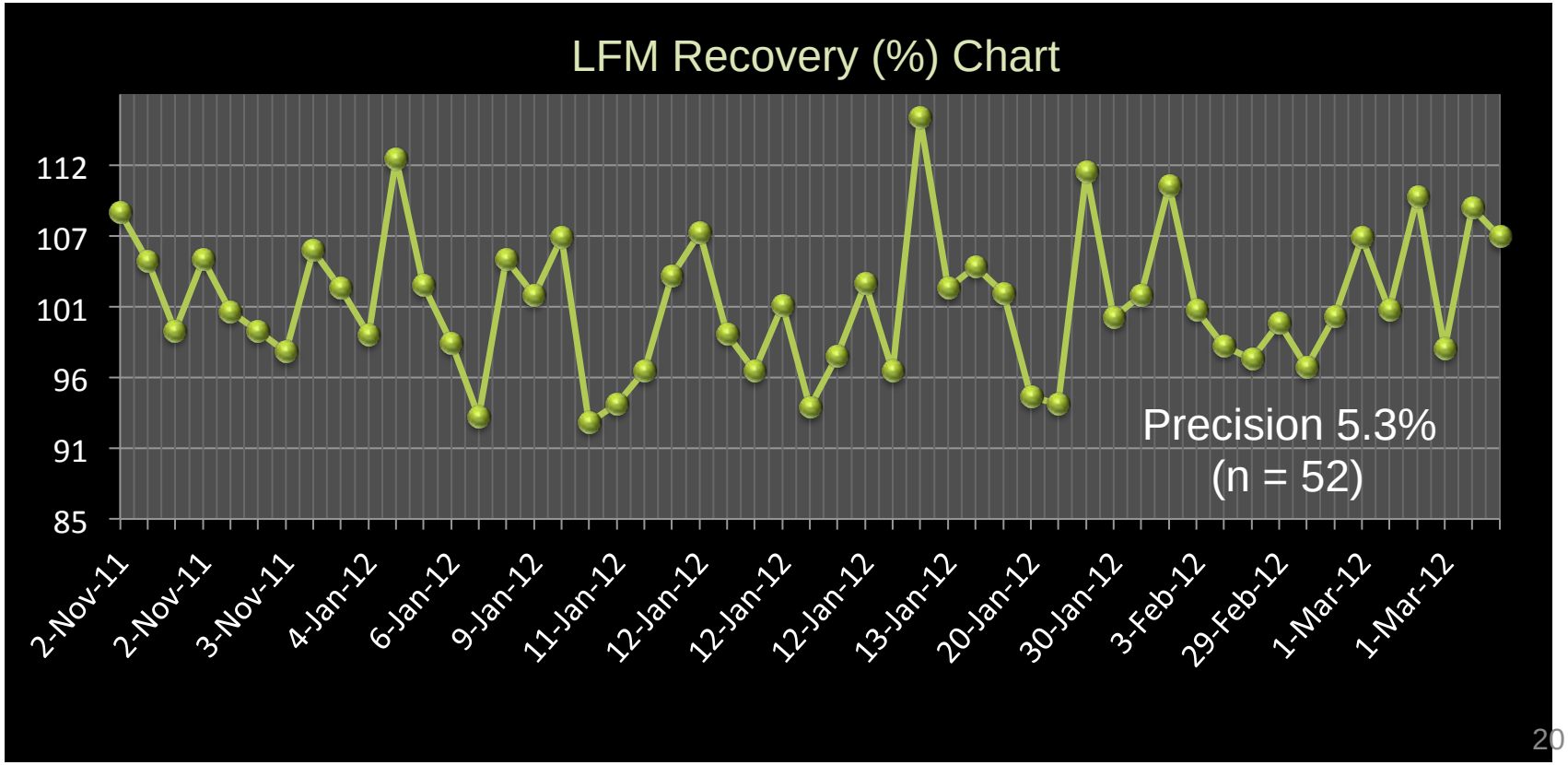
Injection Date/Time	[Hydrazine] (pg/cig.)	Recovered as % of Expected
Day 1	603	96%
Day 1	578	92%
Day 1	594	94%
Day 1	628	99%
Day 2	647	102%
Day 2	628	99%
Day 2	603	95%
Day 2	686	109%
Day 2	651	103%
Day 3	681	108%
Day 3	694	110%
Day 3	669	106%
Average	639	101
Std Dev	39	6
RSD (%)	6.1	6.2

Limit of Detection **0.116 ng/cig.**

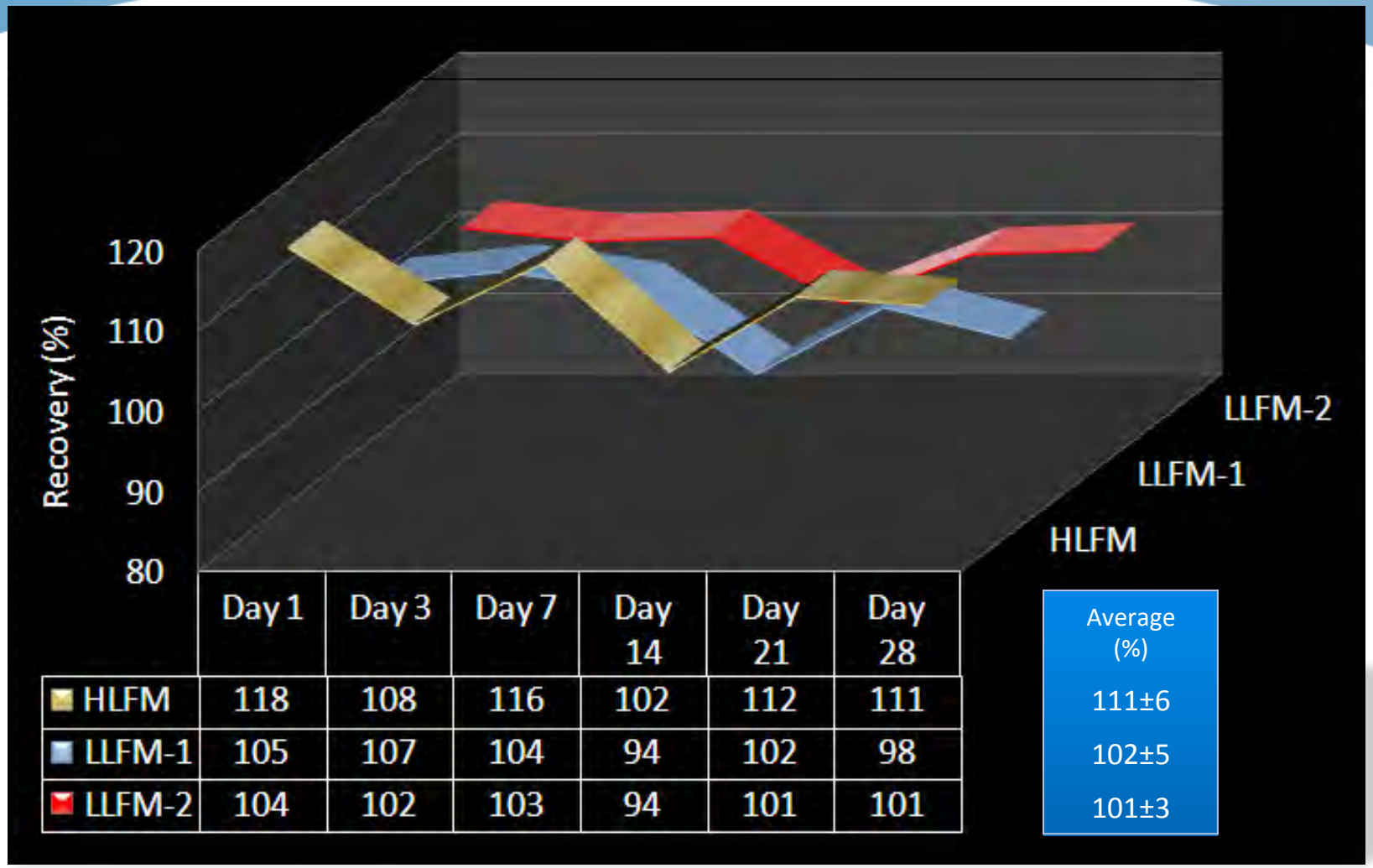
Limit of Quantitation **0.388 ng/cig.**

Analytical Performances - Method Recovery

Fortification Range (ng/mL)	Recovery (%)	Precision (n = 16)
60 - 120	85 - 110	6.2 %



Analytical Performances - Stability of Extracted Samples



	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28	Average (%)
HLFM	118	108	116	102	112	111	111±6
LLFM-1	105	107	104	94	102	98	102±5
LLFM-2	104	102	103	94	101	101	101±3

Conclusion

- A quantitative, sensitive and reliable method for analysis of hydrazine in tobacco smoke was developed.

- Scope of application: Quantitation of hydrazine in tobacco smoke (i.e. MS/SS) generated under both “ISO” and “intense” smoking regimens.

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