Development, validation and routine use of a method for the determination of carbonyl compounds in cigar smoke

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Method Considerations

- CRM 74 has been shown to be fit for purpose for the analysis of carbonyls in mainstream cigarette smoke
- Smoking qualities (yield and puff count) and smoking regime (CRM 64) differ between cigar and cigarettes
- Total yield for carbonyl compounds in cigar smoke are expected to be much greater than found in cigarette smoke
- Machine made cigars are available with a wide range of flavors (US market)

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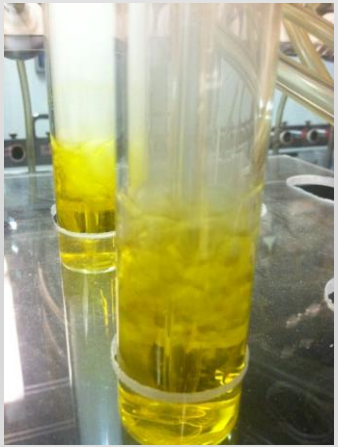
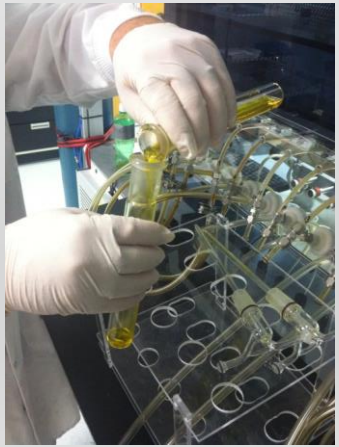
CRM 74-Carbonyls in Mainstream Cigarette Smoke

1. Smoke 2 cigarettes into 2 impingers connected in series-
   -Collection time ~20 to 30 minutes
1. Mix the contents of both impingers
2. Add base to stabilize the hydrazones
3. Inject, separate, and quantize DNPH derivatives using HPLC-UV/Vis





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Method Validation

- Accuracy
  - Stability of the compounds in the trapping system
  - Collection by the trapping system
  - Recovery in the presence of matrix
- Specificity
  - Lack of interfering peaks from other native and flavor compounds in cigar smoke
- Precision
  - Short term instrument and method precision
  - Long term process monitor

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Some DNPH Derivatives are Unstable in Acid

"*(neutralization)… needs to be carried out immediately after smoking as some of the DNPH derivatives are unstable."* – UK smoke constituents study

> *"Impinger methods using DNPH have historically given low recovery for acrolein. The acidic solution is believed to be responsible for the loss of the hydrazone formed in the reaction of DNPH and the aldehyde.."* – Ashland Specialty Chemical Company

Formaldehyde and acrolein decrease in linear fashion when cigarette smoke samples are not neutralized. Enthalpy Analytical TSRC 2011, 65, abstr. 32. Slides 8 and 19

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Hydrazone Stability

- Samples
  - Machine made cigars were used for the initial validation work.
- Smoking
  - Collected under CRM-64. Smoking time ~45 minutes (total)
  - Impingers placed at 0^{o}C
- Trapping Solution
  - Three DNPH solutions: 0:100, 25:75 and 50:50 water to acetonitrile ratio

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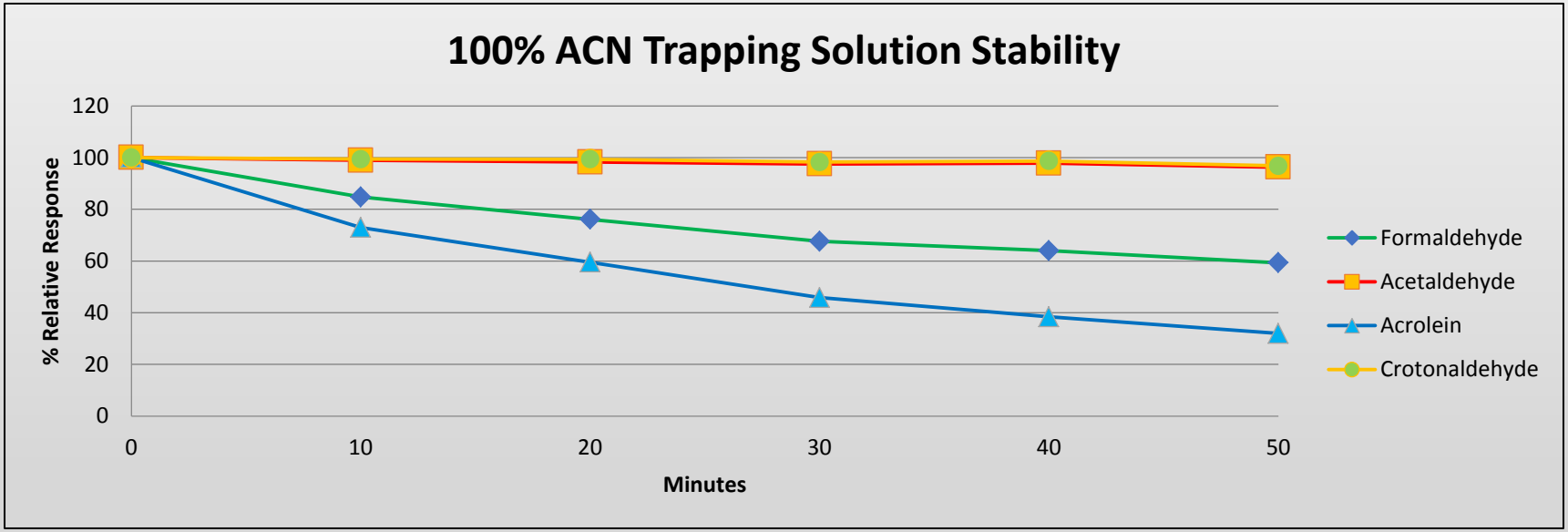
Stability after Smoking



50 minutes after completion of smoking, ~40% decrease in formaldehyde and 65% decrease in acrolein. Note: Samples were collected at 0°C

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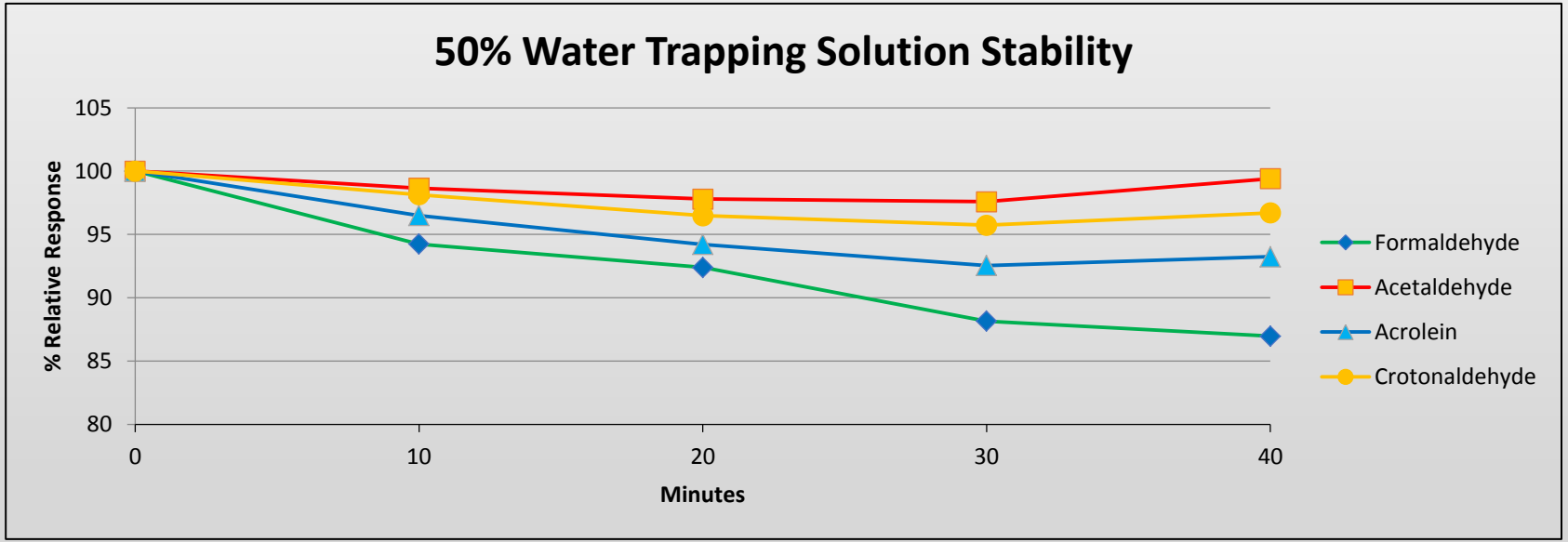
Stability after Smoking



40 minutes after completion of smoking, ~13% decrease in formaldehyde and ~7% decrease in acrolein. Note: Samples were collected at 0°C

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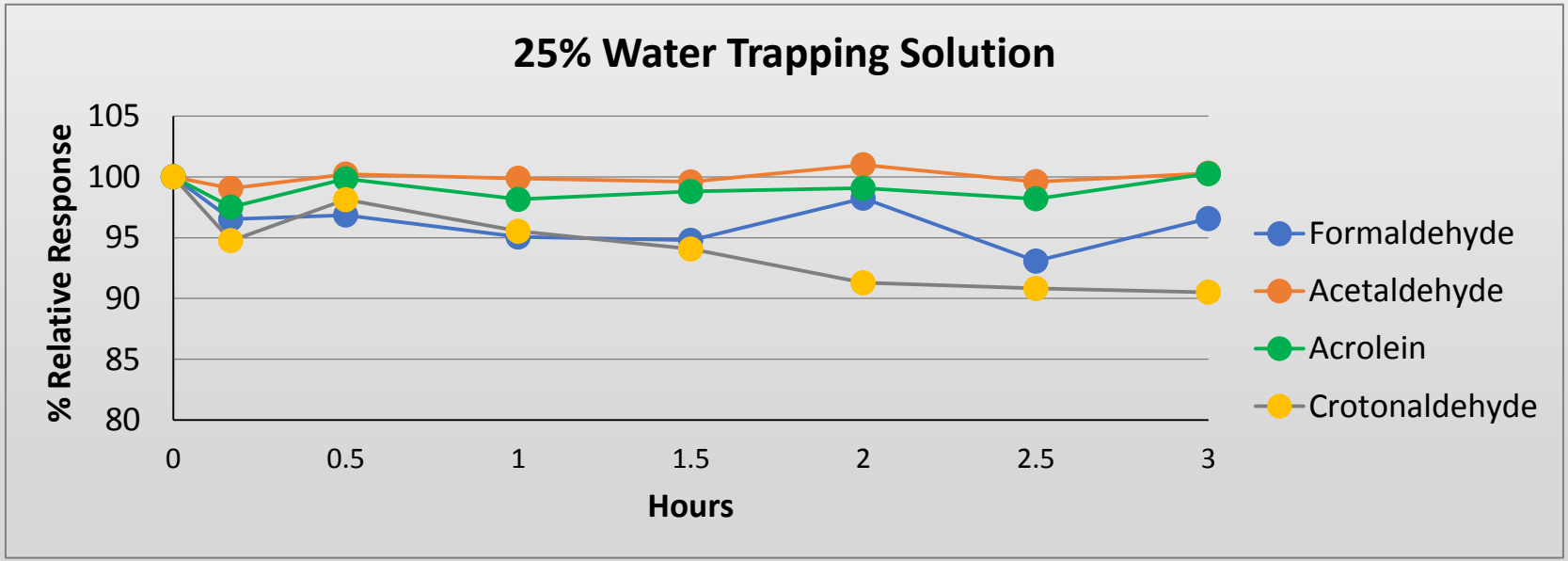
Stability after Smoking



2 hours after completion of smoking, <2% decrease in formaldehyde and acrolein. Note: Samples were collected at 0°C

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Parameters

- Trapping Solution
  - DNPH in 25% water to 75% acetonitrile was found to give the best balance between trapping capacity and stability
- Validation Samples
  - Three machine made cigars were evaluated during the validation
    - Pipe Tobacco 1- with added flavor
    - Pipe Tobacco 2- with added flavor
    - Fermented Dark Air Cured- with added flavor
- Labyrinth Seal Holders
  - Altria design, see STPOST-06 for details

Instrumental conditions: See supporting information at the end

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Validation Samples

	Length (mm)	Average Puff Count (per cigar)	Average TPM (mg/cigar)	Average CO (mg/cigar)	Average Water (mg/cigar)	Average Nicotine (mg/cigar)	Average NFDPM (mg/cigar)
Pipe Tobacco- 1	103	34.7	67.0	70.1	6.83	1.70	58.5
Pipe Tobacco- 2	103	33.4	72.1	71.8	8.40	1.54	62.1
Dark Air Cured	106	41.7	72.1	103.3	5.67	2.58	63.9

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Breakthrough Studies

	Formaldehyde	Acetaldehyde	Acrolein	Crotonaldehyde
Run 1	4.7%	ND	ND	ND
Run2	2.8%	ND	ND	0.2%
Run 3	5.6%	ND	ND	ND

Less then 6 % breakthrough was found for any compounds. Breakthrough was determined by adding a third impinger to the trapping system. The third impinger was analyzed as a discrete sample.

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Recovery from Matrix

- Samples were spiked with neat carbonyl compounds after sample collection

	Formaldehyde (ug/mL)	Acetaldehyde (ug/mL)	Acrolein (ug/mL)	Crotonaldehyde (ug/mL)
Native Concentration	0.643	38.1	1.61	0.806
Low Spike Amount	0.470	17.5	1.74	0.871
% Recovery	**94.9**	**93.3**	**82.0**	**89.8**
High Spike Amount	1.20	44.7	4.46	2.23
% Recovery	**100.7**	**92.2**	**83.3**	**90.9**

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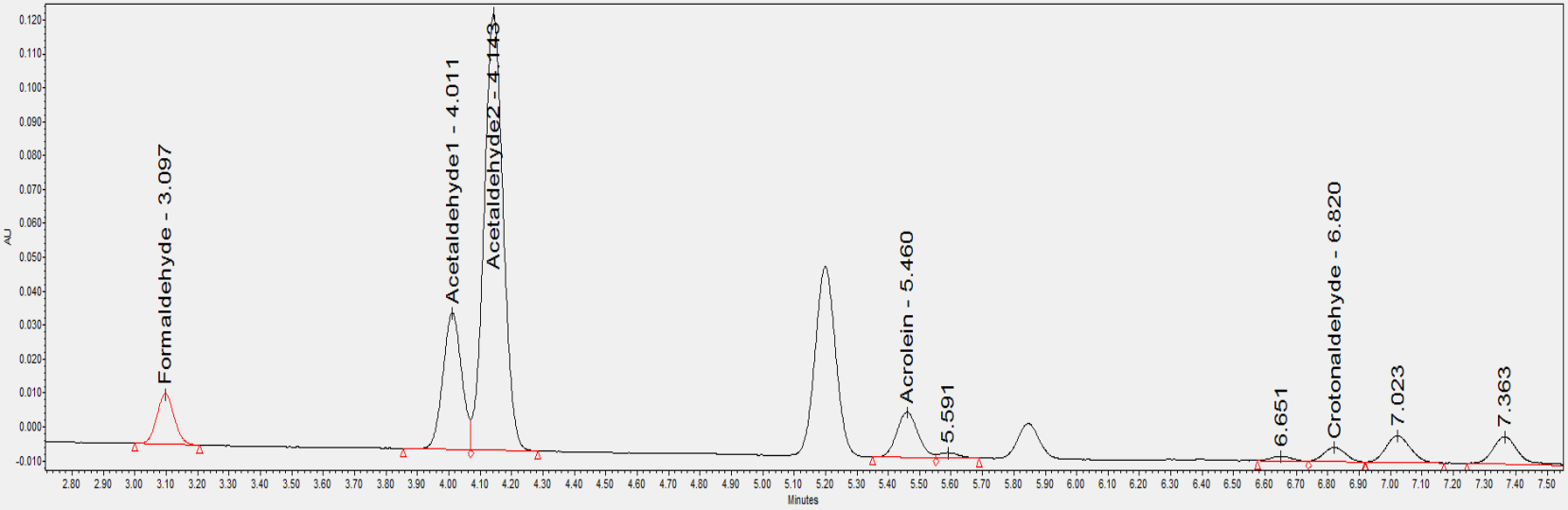
Specificity



Analytical Standard-Mid point of the calibration curve

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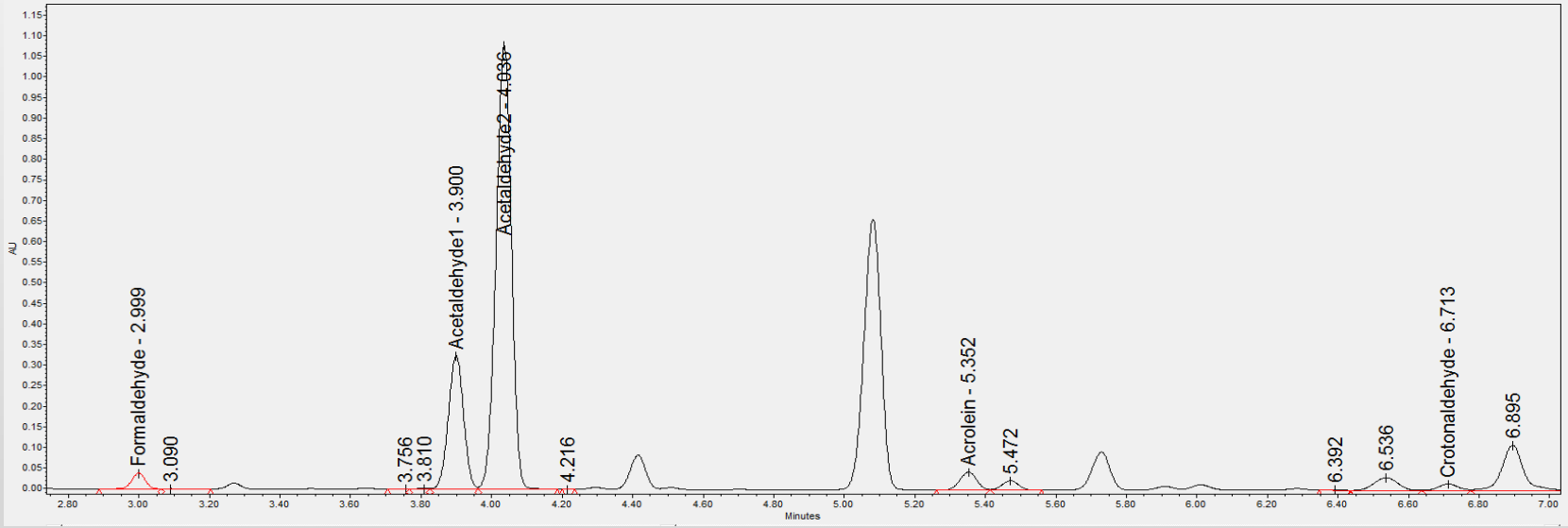
Specificity



Pipe Tobacco 1: No interfering peaks observed

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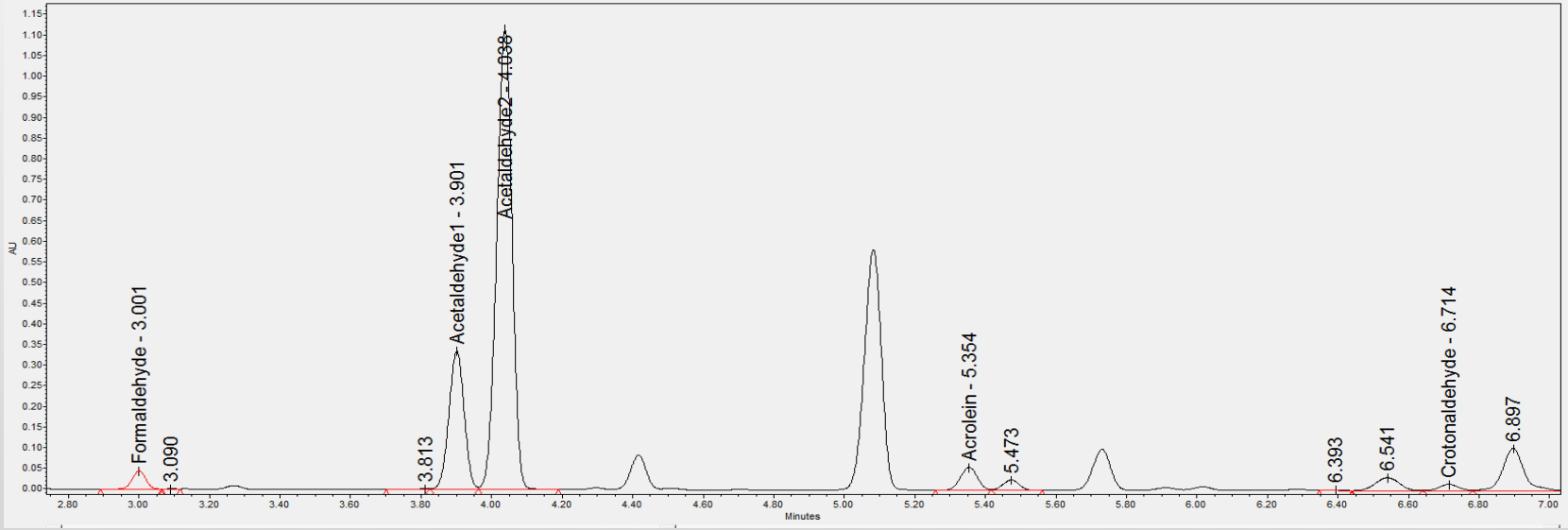
Specificity



Pipe Tobacco 2: No interfering peaks observed

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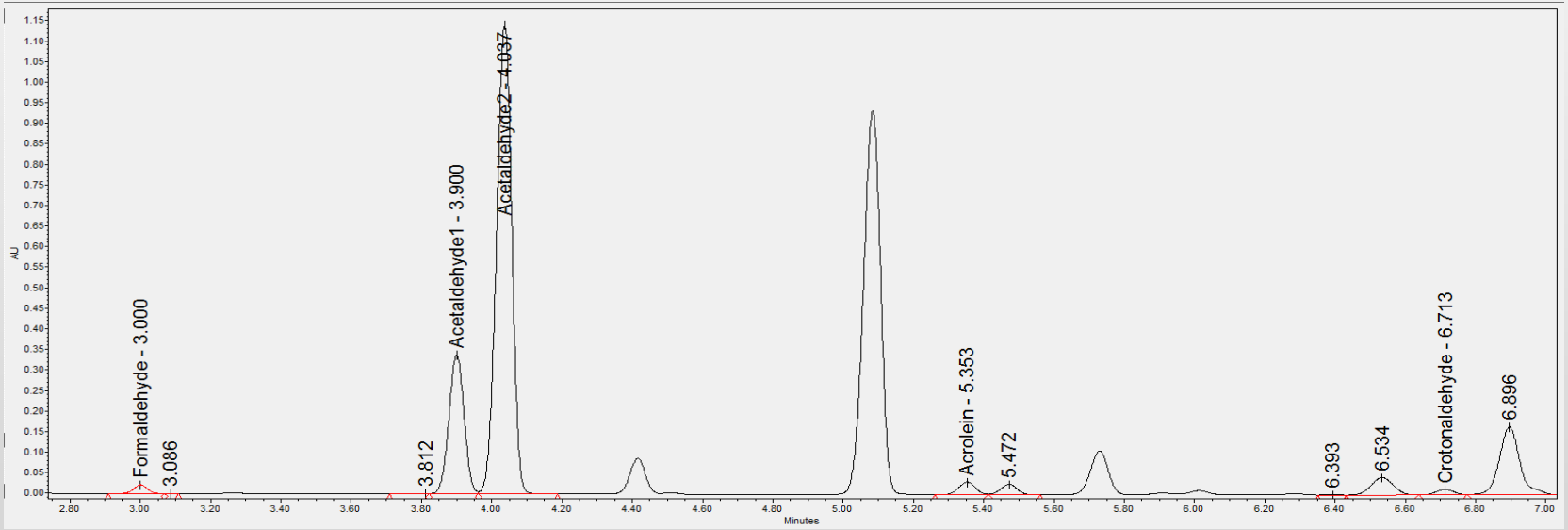
Specificity



Fermented Dark Air Cured Tobacco: No interfering peaks observed

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Three day method precision

	Formaldehyde ug/cigar	Acetaldehyde ug/cigar	Acrolein ug/cigar	Crotonaldehyde ug/cigar
Pipe Tobacco 1				
Average (n=14)	33.3	2362	93.5	44.5
STD	7.0	333	11.2	6.45
%RSD	21.1	14.1	12.0	14.5
Pipe Tobacco 2				
Average (n=14)	36.3	2507	114.0	45.0
STD	10.6	229	16.6	6.22
%RSD	29.3	9.1	14.6	13.8
Dark Air Cured Tobacco				
Average (n=14)	13.7	2480	60.0	38.1
STD	3.07	315	5.70	4.17
%RSD	22.4	12.7	9.5	10.9

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Long-term Method Precision

- Standard cigarette monitors were found to not be suitable as a process monitor controls
  - ISO 3308 smoking versus smoking under CRM 64
  - Number of puffs per sample
  - Total smoke yield differences
- Cigar process monitor
  - Uniform as possible
    - Machine made product, 10 mm diameter
    - Single production batch
    - Long term storage at -20^{o}C
    - Selected for pressure drop, 85 to 180

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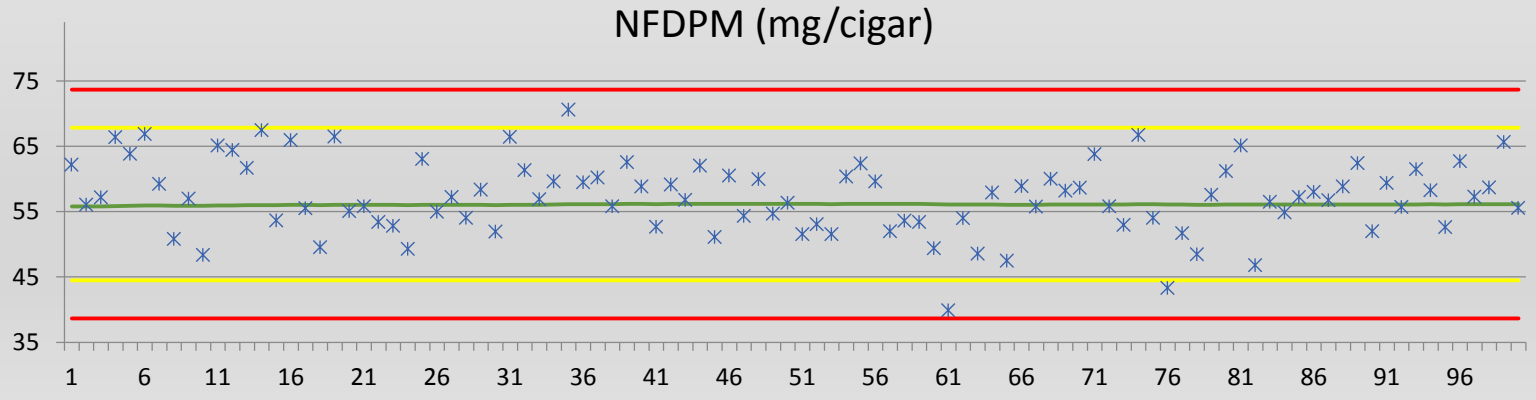
Process Monitor-Nicotine and NFDPM

	Nicotine mg/cigar	NFDPM mg/cigar
Process Control		
Average (n=100)	2.1	56.1
STD	0.3	5.8
%RSD	15.1	10.4



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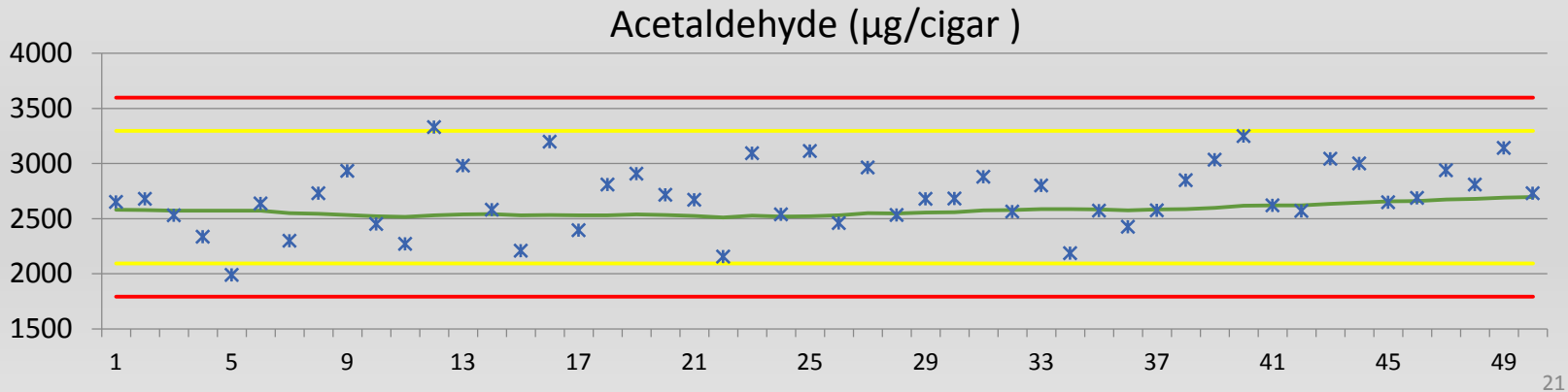
Process Monitor-Carbonyls Compounds

	Formaldehyde ug/cigar	Acetaldehyde ug/cigar	Acrolein ug/cigar	Crotonaldehyde ug/cigar
Process Control				
Average (n=50)	14.8	2696	64.8	38.8
STD	3.6	301	7.8	4.9
%RSD	24.4	11.2	12.0	12.7



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Large-Handmade Cigars

- Large handmade cigars are suitable for use with the method
  - >2 hours of hydrazone stability, cigar smoking time ~2 hours
  - Samples collected with custom Enthalpy holders (16 mm to 25 mm)

Average Puff Count (per/cigar)	Average TPM (mg/cigar)	Average NFDPM (mg/cigar)	Average Nicotine (mg/cigar)	Average CO (mg/cigar)	Average Water (mg/cigar)
155	269	170	9.9	404	89

- Typical observed concentrations:

Formaldehyde (ug/cigar)	Acetaldehyde (ug/cigar)	Acrolein (ug/cigar)	Crotonaldehyde (ug/cigar)
9.02	3892	27.7	20.2

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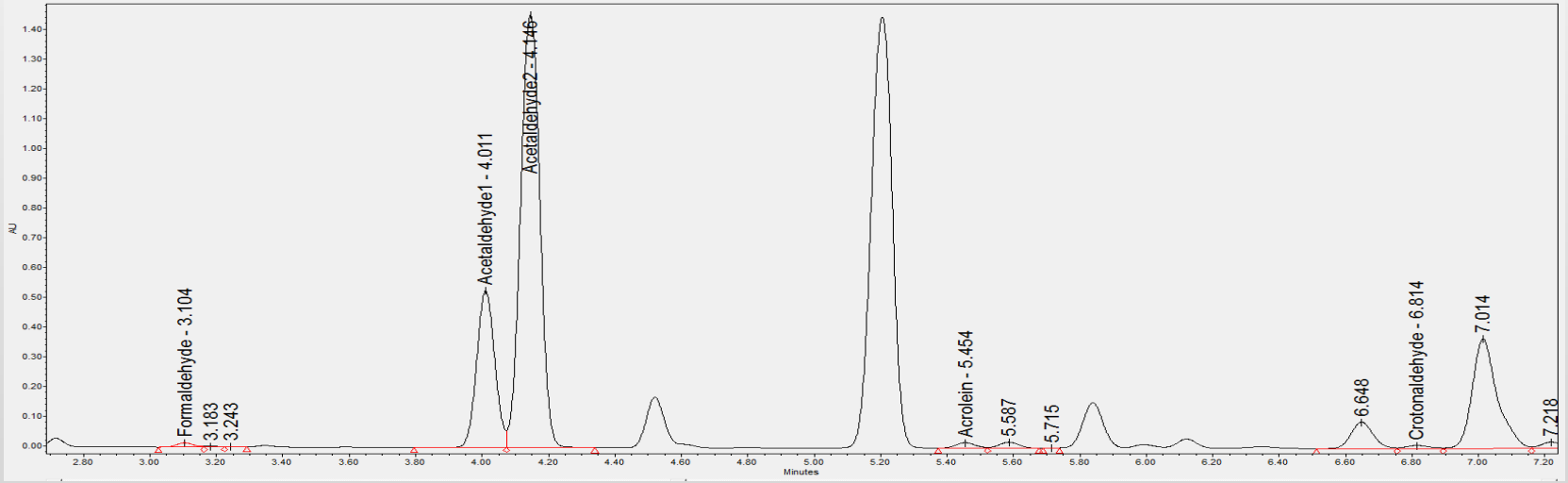
Specificity-Premium Products



No interfering peaks observed in 20 mm Premium handmade cigars

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Conclusion

- A method for the determination of carbonyl compounds in cigar smoke was shown to be fit for purpose
  - Method is based on CRM 74
    - modified trapping solution
    - sample collection at 0^{o}C
  - Method is suitable for the analysis of a wide range of cigar types
    - Small machine made to large premium cigars
- Long-term method variability
  - Comparable to nicotine yield variability

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Thank you for your attention

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