

Are Available Test Methods for the Determination of Carbonyls in Mainstream Cigarette Smoke Fit for the Analysis of Cigars?



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CORESTA SSPT 2017, October 8-12, 2017, Kitzbühel, Austria

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Introduction & Objectives

Introduction

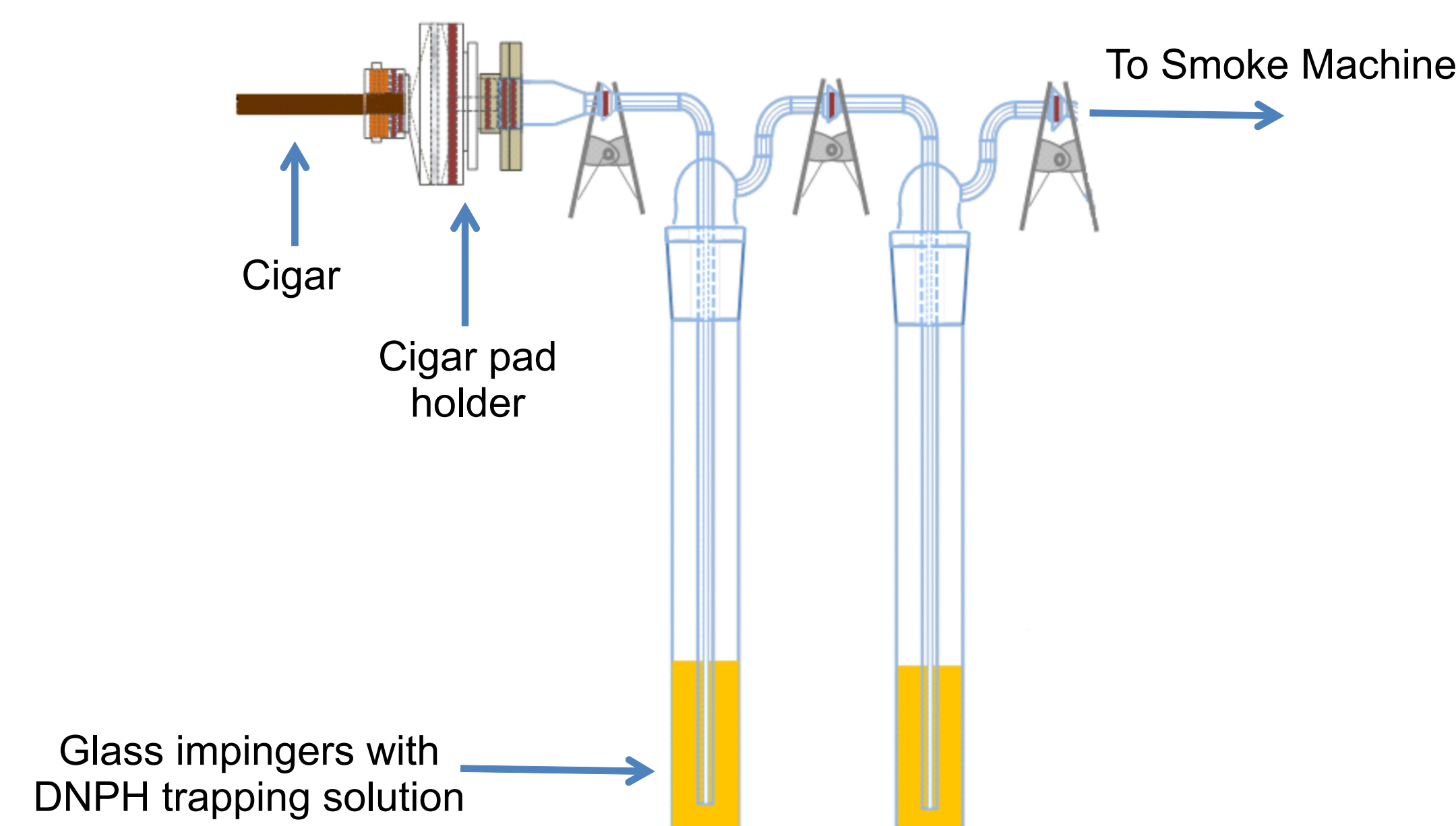
In May 2016, the U.S. Food and Drug Administration (FDA) issued a final rule to deem cigars to be subject to the Federal Food, Drug, and Cosmetic Act, as amended by the Family Smoking Prevention and Tobacco Control Act. As part of this regulation, FDA will require manufacturers to report the quantities of Harmful and Potentially Harmful Constituents (HPHCs) in cigar filler and smoke. Standardized methods do exist for the analysis of carbonyls in cigarette smoke such as CORESTA Recommended Method No. 74 (CRM 74), which was based on Health Canada method T-104 and is the basis of ISO/CD 21160:2017, Determination of selected carbonyls in the mainstream smoke of cigarettes -- Method using High Performance Liquid Chromatography; however, these methods have not been shown to be fit for purpose for cigar smoke analysis.

Objectives

- Determine if the carbonyl-2,4-dinitrophenylhydrazine derivative (prepared according to CRM 74, T-104, and ISO/CD 21160:2017) is stable for the duration of cigar smoke collection.
- Determine if the instrumental analysis procedure described in CRM 74 is suitable for cigar smoke analysis.

DNPH Stability Experiment (Cont.)

Smoke Collection Configuration



DNPH Stability Experiment

Smoke Machine Parameters

- Smoke regime: 55 mL puff volume, 5 s duration, 30 s interval
- Impinger setup*: Two tandem impingers with 35 mL each of 2,4-dinitrophenylhydrazine (DNPH) trapping solution

Analytical Method

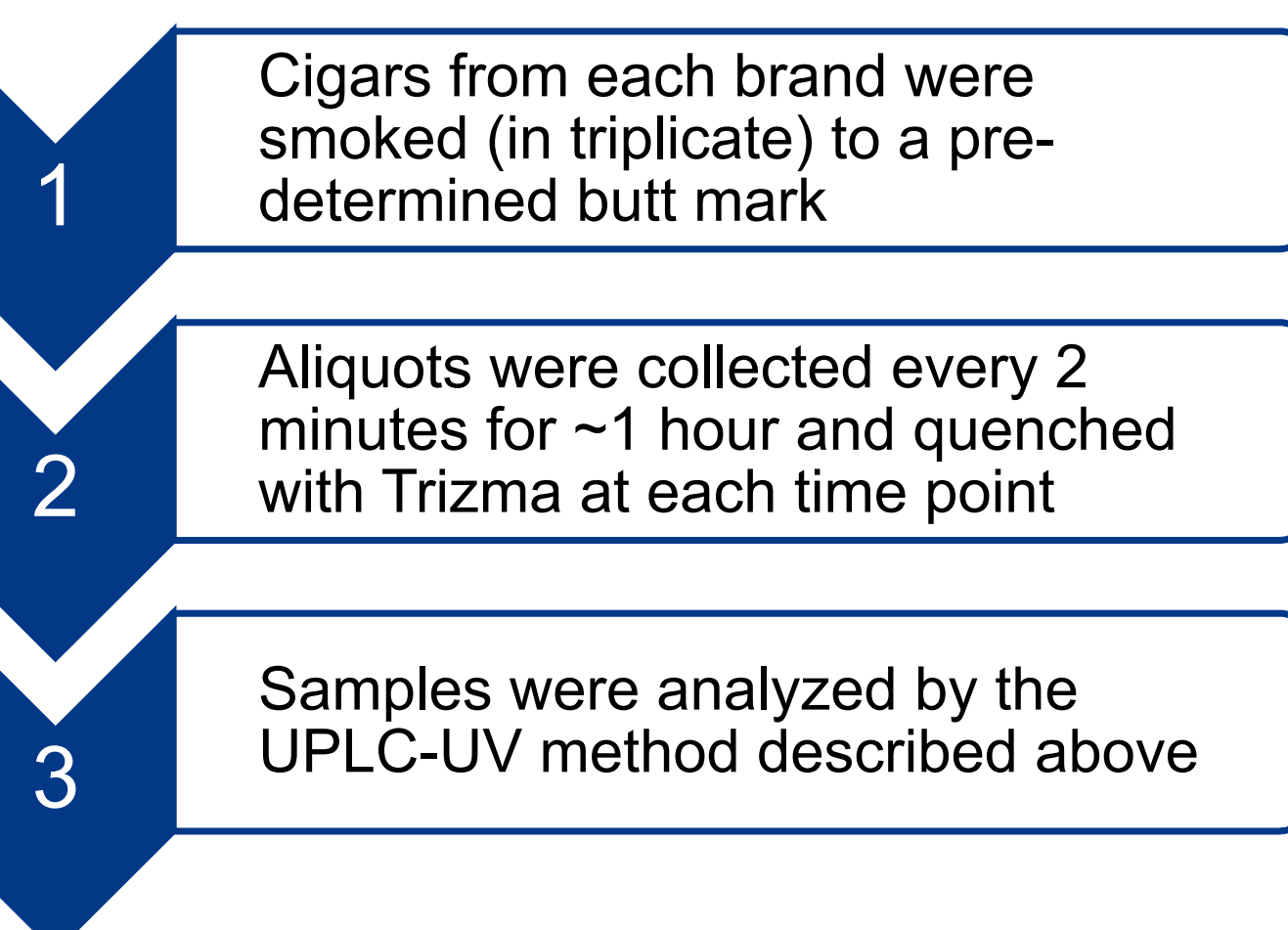
- Instrument: Waters Acquity UPLC
- Column: Acquity UPLC BEH Shield RP18, 2.1 mm x 100 mm, 1.7 µm particle
- Mobile Phase A: Water (18 MΩ)
- Mobile Phase B: Acetonitrile
- Injection volume: 2 µL
- UV Detector: 365 nm

Time (min)	Flow (mL/min)	A (%)	B (%)	Curve
0:00	0.60	75	25	6
3:20	0.60	70	30	6
5:50	0.60	60	40	6
9:00	0.60	47	53	6
9:01	0.60	20	80	6
10:01	0.60	20	80	6
10:02	0.60	75	25	6
11:50	0.60	75	25	6

Experimental Design

Two types of cigars were chosen for the study:

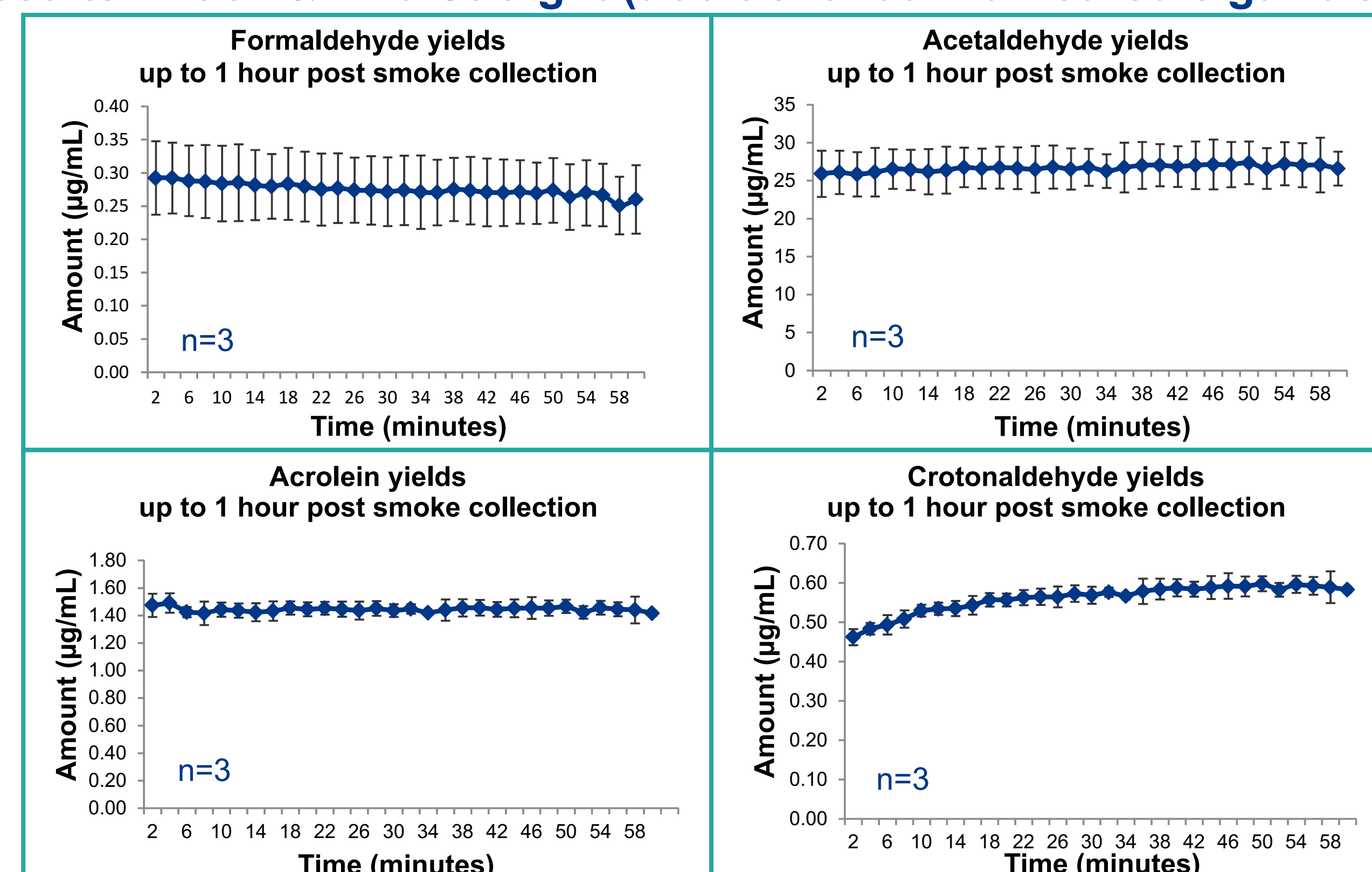
1. Black & Mild Straight (traditional dark air cured cigar blend)
2. Black & Mild Wine Plastic tip (flavored cigar blend)



*All impingers were kept at ambient temperature for the duration of the experiment.

DNPH Stability Results

Results: Black & Mild Straight (traditional dark air cured cigar blend)

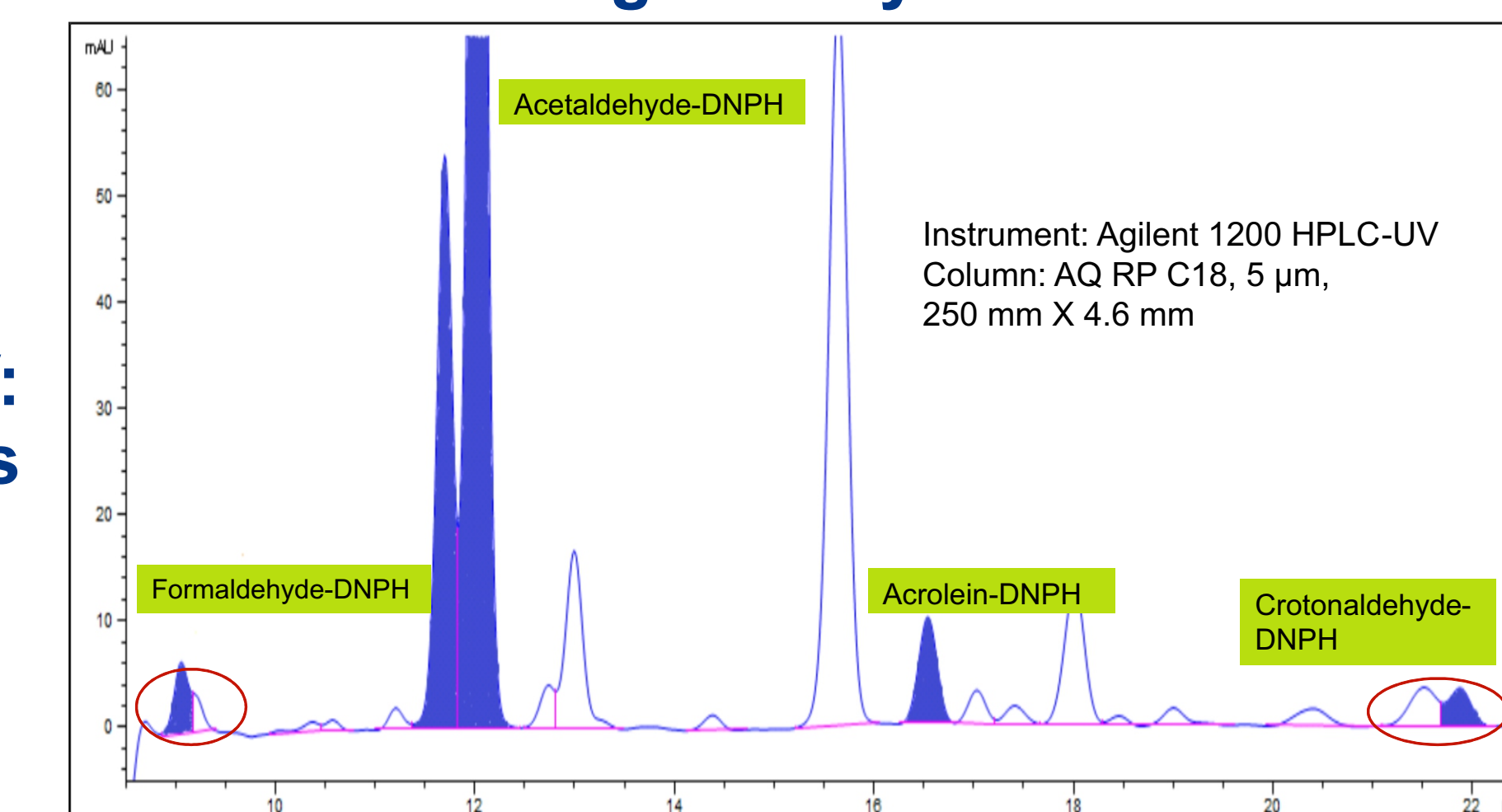


- No degradation of the DNPH derivatives was observed up to one hour at ambient temperature. This differs from preliminary data used to write the abstract.
- Similar results observed for analysis of the flavored cigar blend (Black & Mild Wine Plastic tip).

Comparison of Detection Techniques

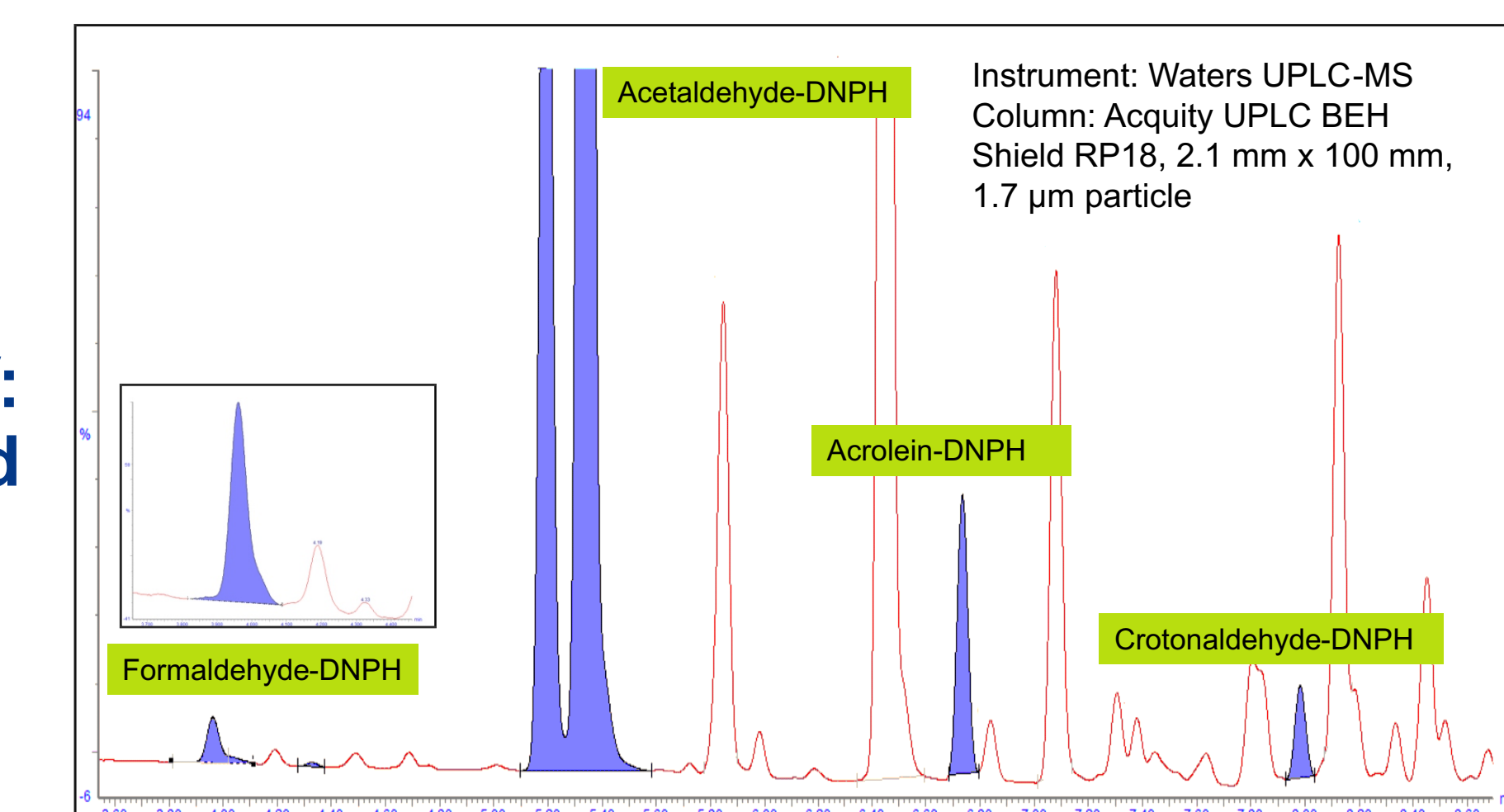
Cigar smoke was analyzed by HPLC-UV, UPLC-UV, and UPLC-MS to determine which was most suitable for cigar analysis.

HPLC-UV: CRM-74 Conditions



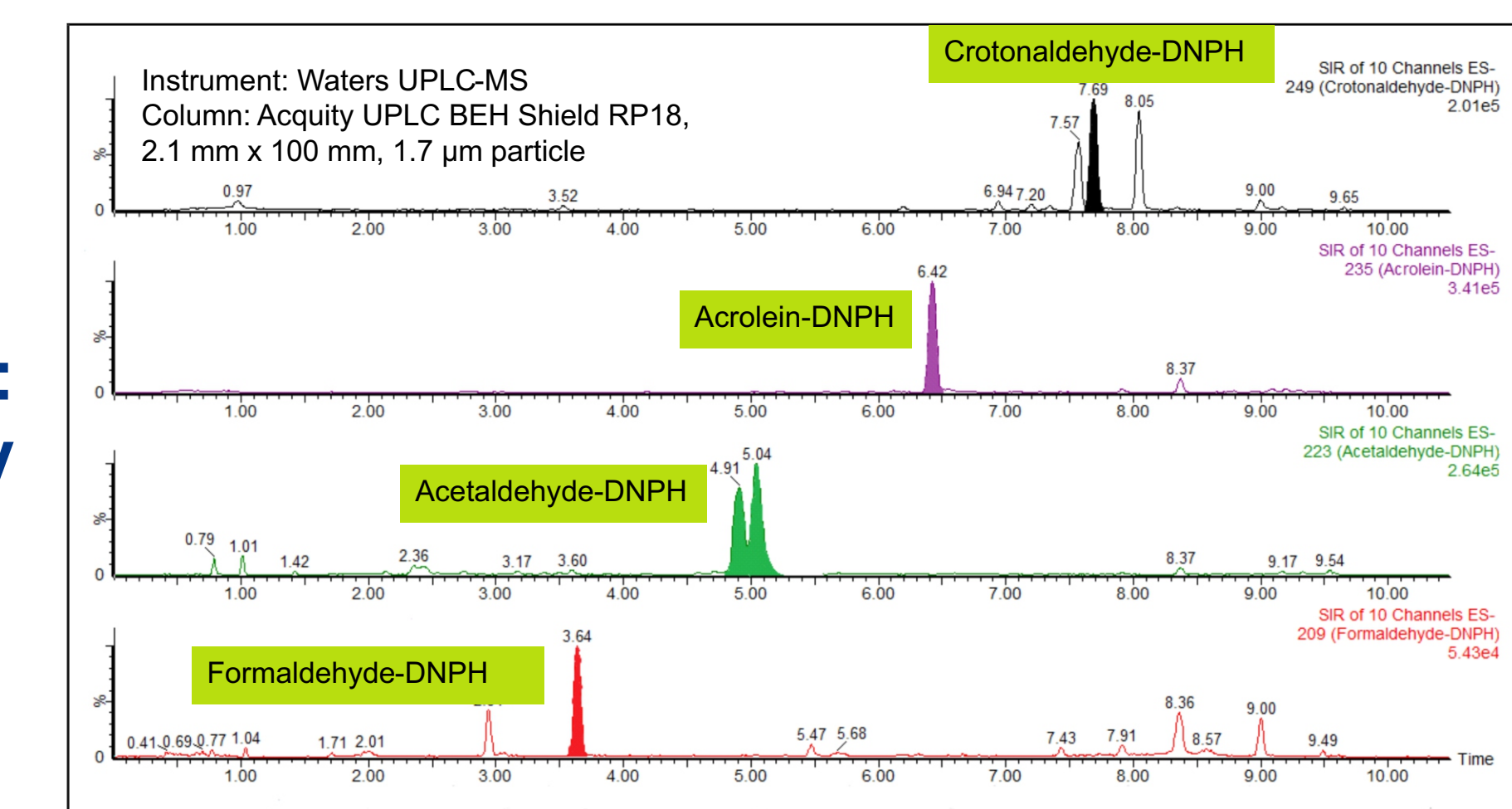
Significant chromatographic interferences were observed for formaldehyde and crotonaldehyde.

UPLC-UV: Conditions Listed



The UPLC-UV method afforded a shorter run time but still had unacceptable chromatographic resolution observed between formaldehyde and matrix component(s).

UPLC-MS: Negative Electropray



In addition to the shorter run times, selectivity is improved and matrix interferences are minimized using the UPLC-MS method.

Conclusions

1. Time studies evaluating the stability of the carbonyl-DNPH derivatives revealed no stability issues over a one-hour period for the two cigar brands tested. This indicates that the sample collection portion of the cigarette methods (CRM 74, T-104, and ISO/CD 21160:2017) may be fit for the collection of cigar smoke.
2. The level of cigar smoke interferences cause a significant bias in the accuracy of target carbonyl compounds by HPLC-UV which is used in CRM74, therefore CRM 74 under current method conditions is not fit for purpose for the analysis of cigars.
3. UPLC-UV offers shorter run times and better selectivity than HPLC-UV, but it also suffers from the same matrix interference issues as the HPLC-UV method.
4. The UPLC-MS method has a shorter run time and minimal matrix interference and may be better suited for the accurate reporting of carbonyl yields in cigar smoke.

References

1. Federal Food, Drug, and Cosmetic Act (the FD&C Act), as amended by the Family Smoking Prevention and Tobacco Control Act (Tobacco Control Act), May 2016. <https://www.gpo.gov/fdsys/pkg/FR-2016-05-10/pdf/2016-10685.pdf>
2. Health Canada method T-104: Determination of Selected Carbonyls in Mainstream Tobacco Smoke, 1999. <http://www.hc-sc.gc.ca/hc-ps/tobac-tabac/legislation/reg/indust/method/index-eng.php>
3. Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA).2014. CORESTA Recommended Method No. 74. Determination of selected carbonyls in mainstream smoke by HPLC. [http://www.coresta.org/recommended_methods/CRM_74-update\(July14\).pdf](http://www.coresta.org/recommended_methods/CRM_74-update(July14).pdf)
4. ISO/CD 21160:2017 Determination of selected carbonyls in the mainstream smoke of cigarettes -- Method using High Performance Liquid Chromatography. <https://www.iso.org/standard/69993.html?browse=tc>