Development and validation of a routine method for the determination of carbonyl compounds in heated tobacco products (HTP) by UPLC-MS

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Heated Tobacco Products (HTP)

- Current research in tobacco science aims at reducing potential exposure to HPHCs, including the introduction of heated tobacco products (HTP).
- Mainstream smoke (MS) is a complex mixture, containing >4800 compounds, including various carbonyls such as formaldehyde, acetaldehyde, acrolein, and crotonaldehyde.
- Rather than combustion, HTPs heat the tobacco to generate an aerosol, which is inhaled.
- HTPs typically reduce combustible HPHC yields by >90%.
- Due to lower aerosol/HPHC yields, traditional mainstream smoke methods (such as CRM-74) have limited use.
- Methods to analyze HTP require better sensitivity or improved stability to allow for the collection of more matrix.

Klupinski, *et al.* Chem. Res. Toxicol., 29, 162-168, https://doi.org/10.1021/acs.chemrestox.5b00371
Jaccard, G., *et al.,* Regul. Toxicol. Pharmacol. 90, 1–8. https://doi.org/https://doi.org/10.1016/j.yrtph.2017.08.006
Forster, M., *et al.,* Regul. Toxicol. Pharmacol. 93, 14–33. https://doi.org/https://doi.org/10.1016/j.yrtph.2017.10.006.

Challenges to Carbonyl Analysis

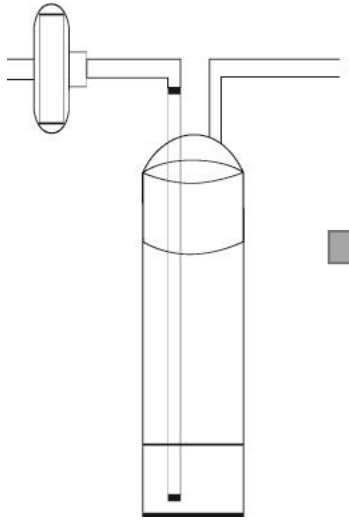
- A number of standardized methods for the analysis of carbonyls in mainstream smoke include CORESTA Recommended Method-74 (CRM-74).
- These methods trap the carbonyls in an acidic 2,4-dinitrophenylhydrazine (DNPH) solution to convert the carbonyls to their hydrazone adducts.
- Some DNPH adducts are known to be unstable under acidic conditions, such as acrolein-DNPH.
  - Poor acrolein recoveries are common.
- Traditional HPLC-UV methods can be long (>30 minutes) in order to achieve the desired selectivity
  - UPLC methods have improved upon instrument run times.
- Current, HPLC/UPLC-UV LOQs can be limited.

Cooperation Centre for Scientific Research Relative to Tobacco, 2018. CORESTA Recommended Method No. 74, Determination of Selected Carbonyls in Mainstream Cigarette Smoke by HPLC

Method Improvements

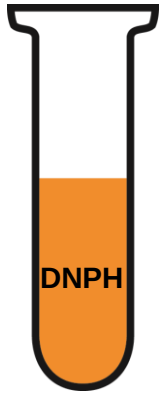
- Sample collection consists of passing aerosol through a Cambridge filter pad into a single impinger.
- Impinger trapping solution is 1:1 acetonitrile to isopropyl alcohol cooled to -35 °C
  - Improves stability of carbonyls by not collecting into acidic DNPH solution.
- Moved to using DNPH*HCl from recrystallized DNPH to minimize background levels of formaldehyde.
- Move analysis from HPLC/UPLC-UV to UPLC-MS to shorten run time and improve upon selectivity.
  - Selectivity is based upon both separation from liquid chromatography, and analyte mass-to-charge ratio (*m/z*).This also helps increase sensitivity, allowing for lower LOQs.
- With the increased selectivity, we are able to expand our analyte list to include12 carbonyls, while maintaining shorter run times.

Sample Collection to Analysis



Aliquot taken for sample prep

Excess extract available for use in additional analyses

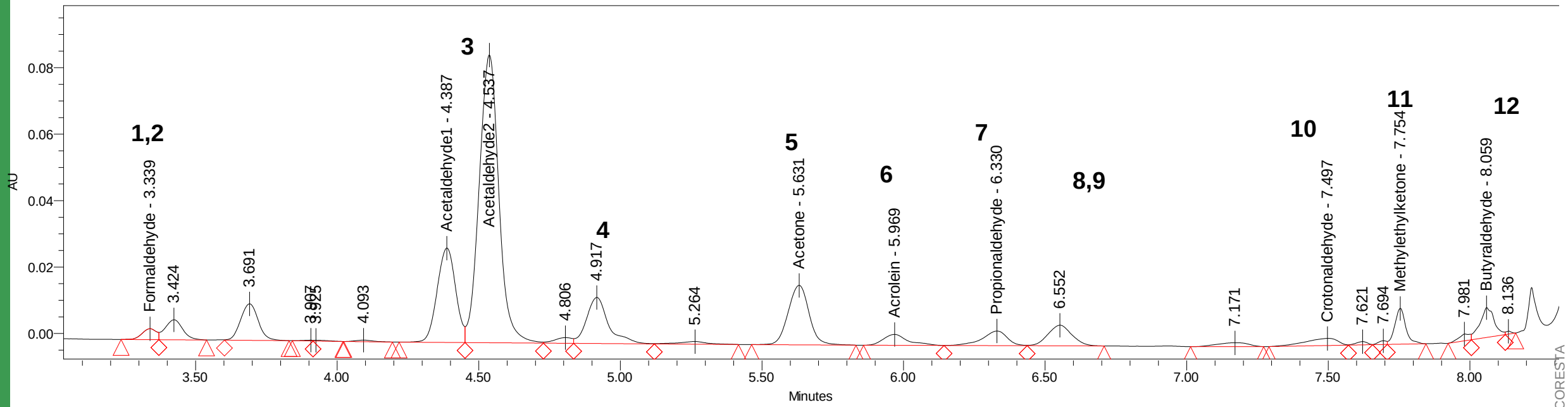


Collection **Extraction** **Derivatization** **Analysis**

Method Validation

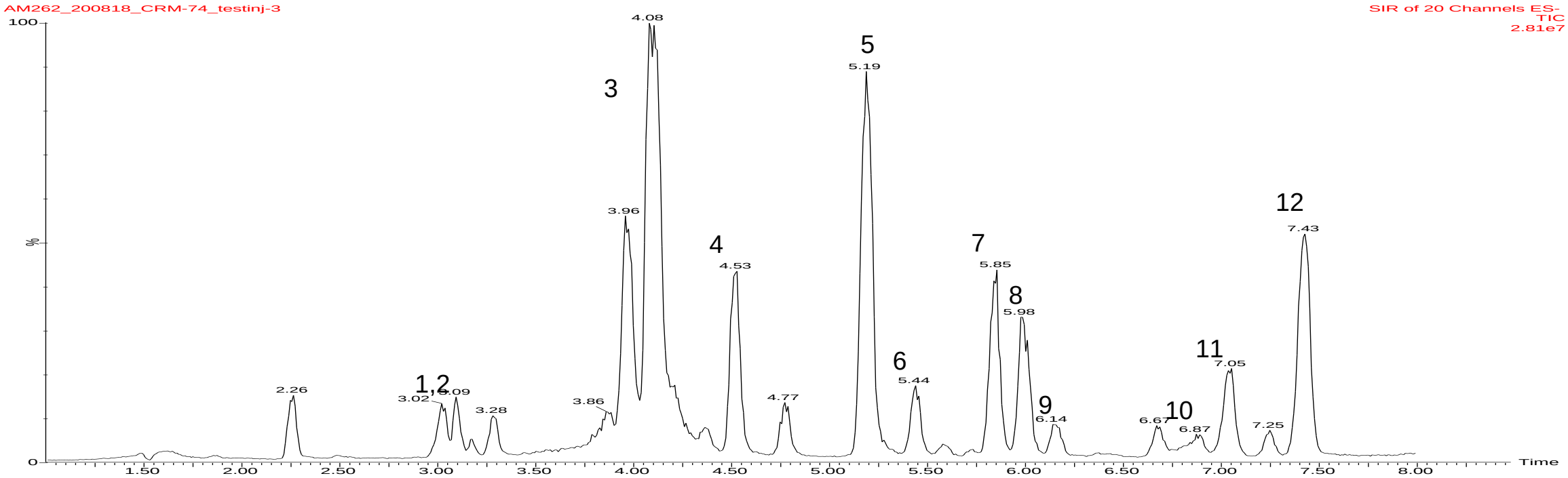
- Specificity/Selectivity
- Accuracy
- Precision
- Robustness
- Conformance to the calibration model
- Limit of Detection/Quantitation
- Trapping efficiency/Breakthrough
- Stability

UPLC-UV Selectivity



1. Formaldehyde
2. Acetoin
3. Acetaldehyde
4. Diacetyl
5. Acetone
6. Acrolein
7. Propanal
8. Furfural
9. Pentanedione
10. Crotonaldehyde
11. MEK
12. Butanal

UPLC-MS TIC



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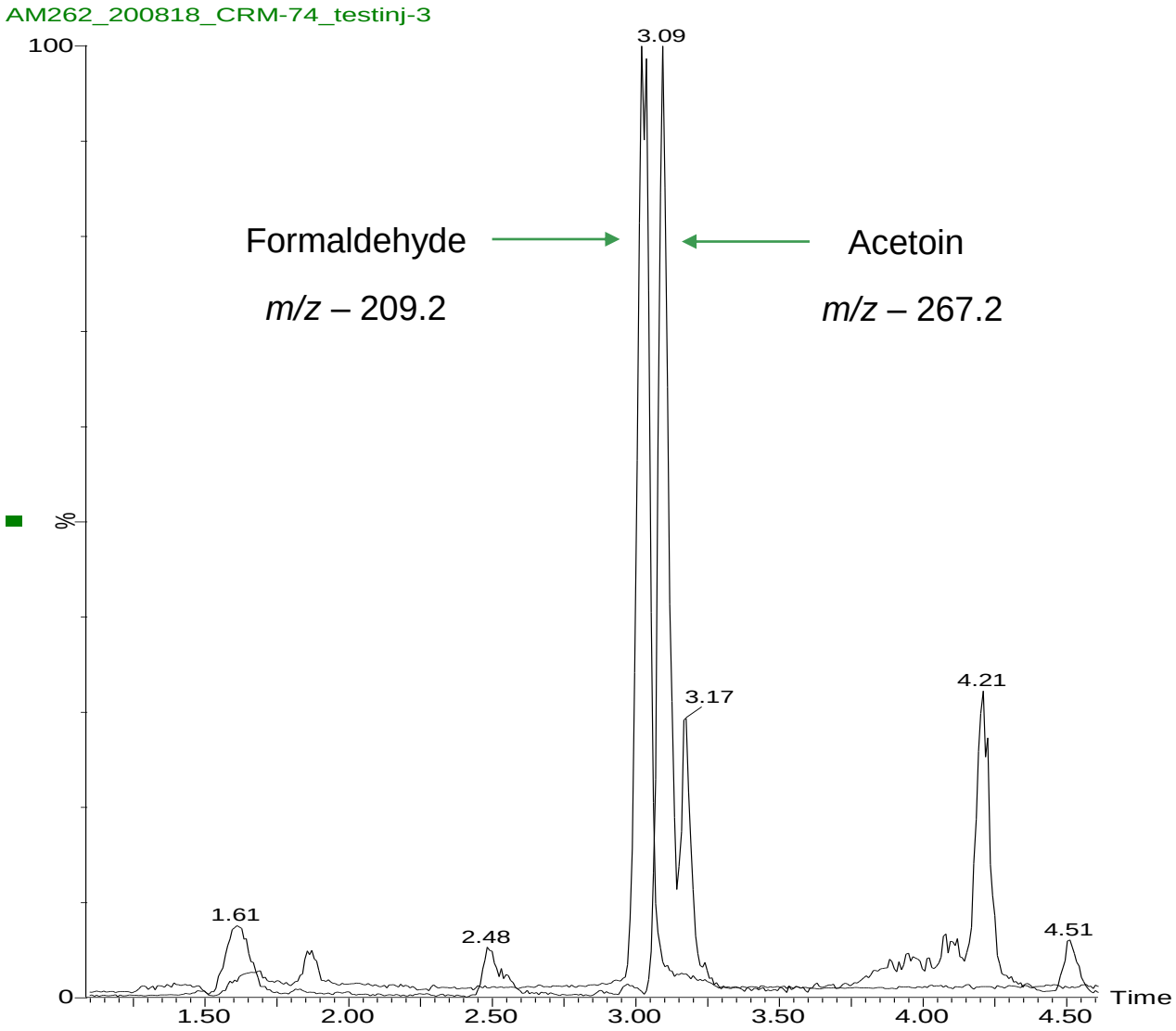
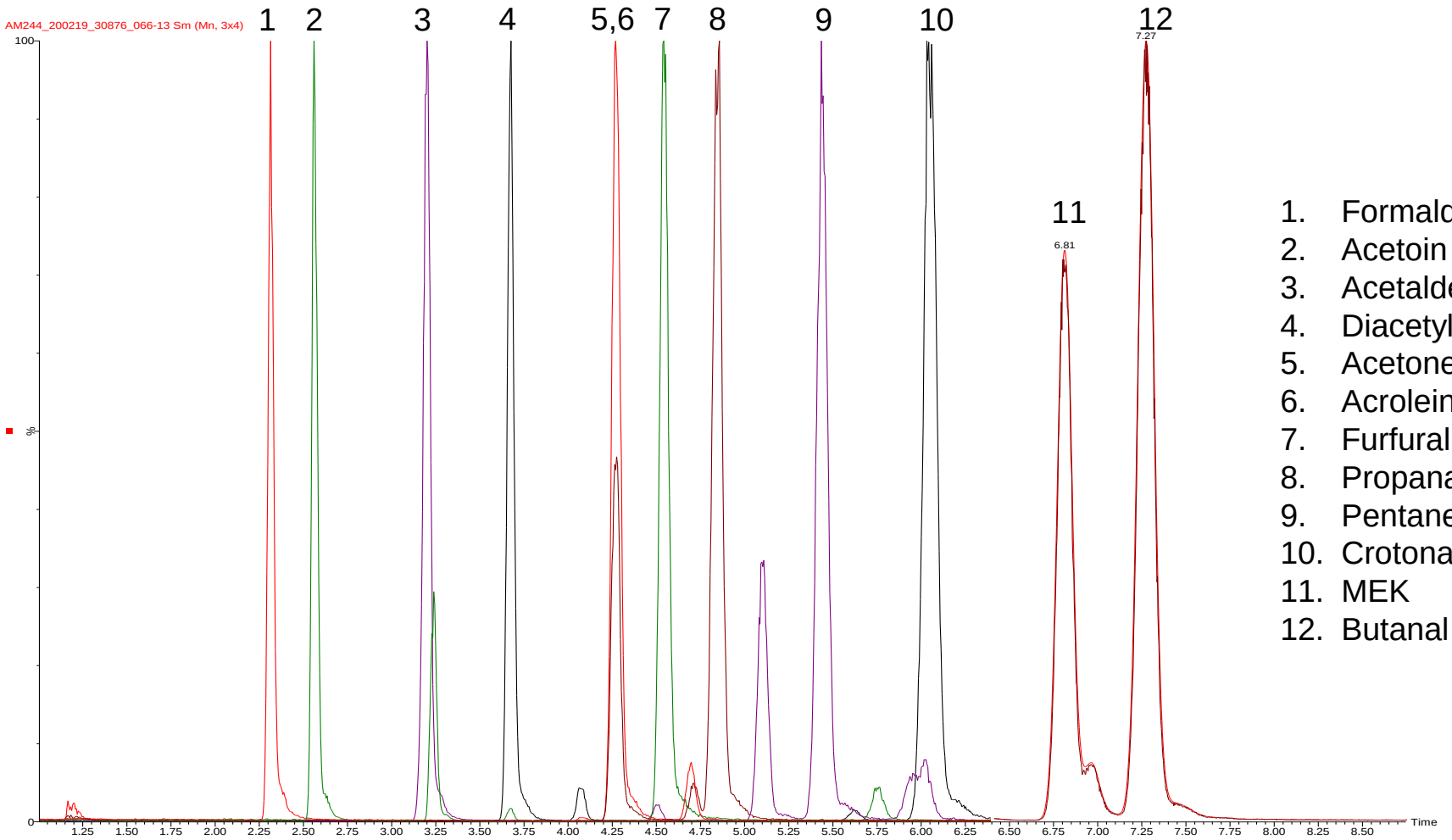
Formaldehyde and acetoin still overlap, but are able to be resolved with the added benefit of mass spec detection.



LCMS Selectivity



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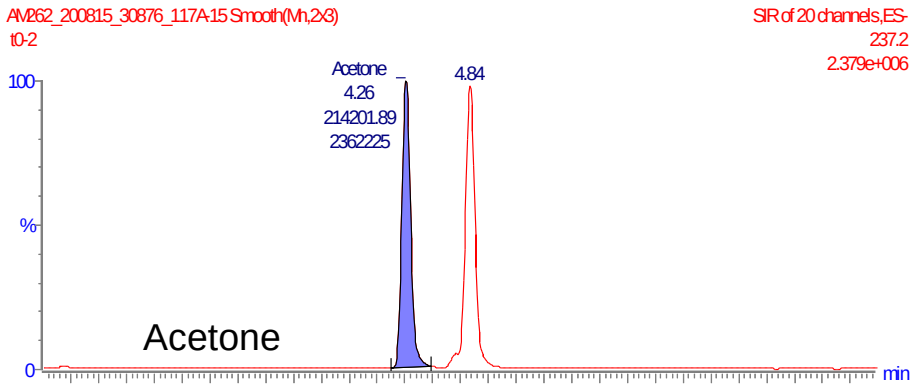
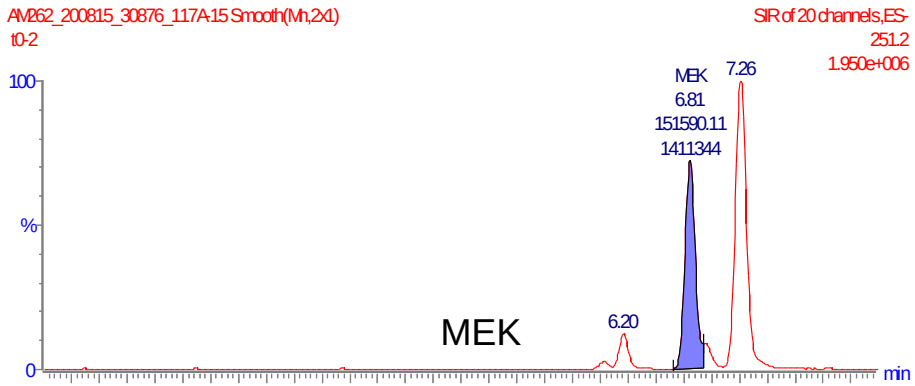
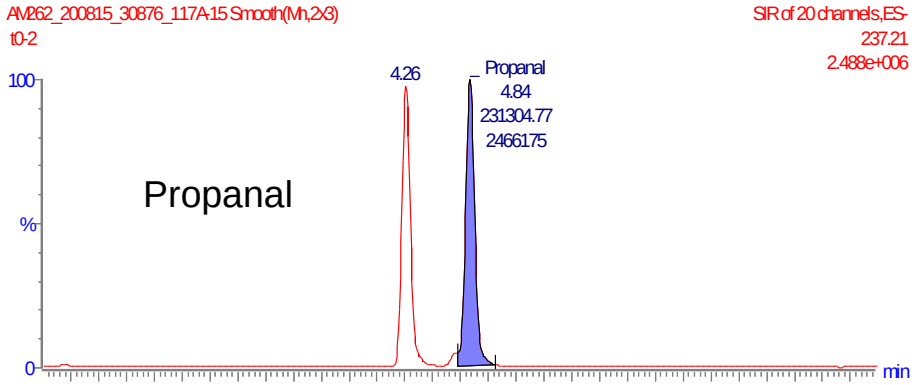
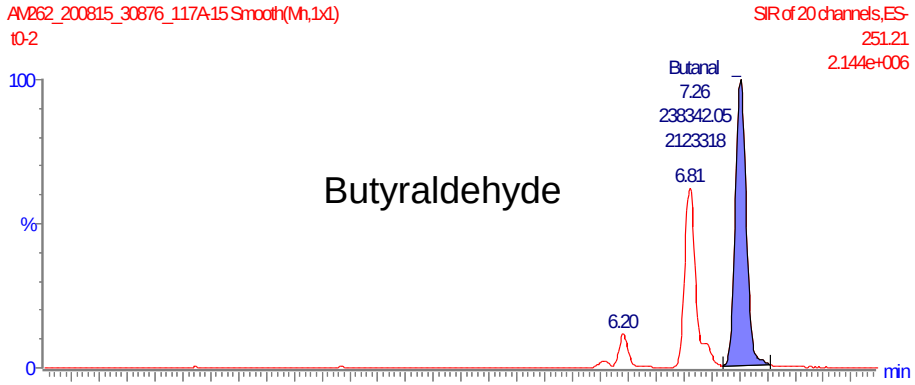




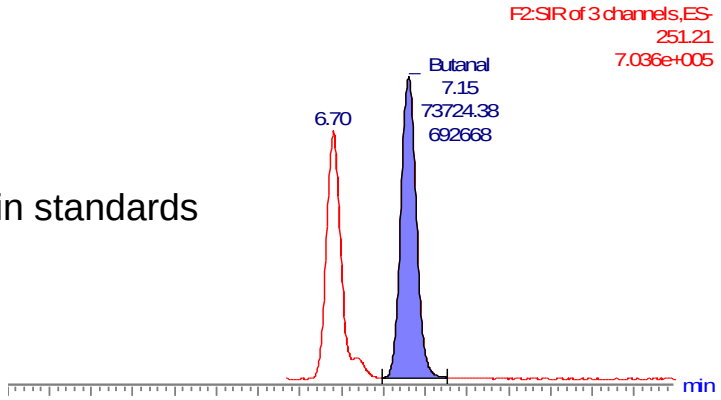


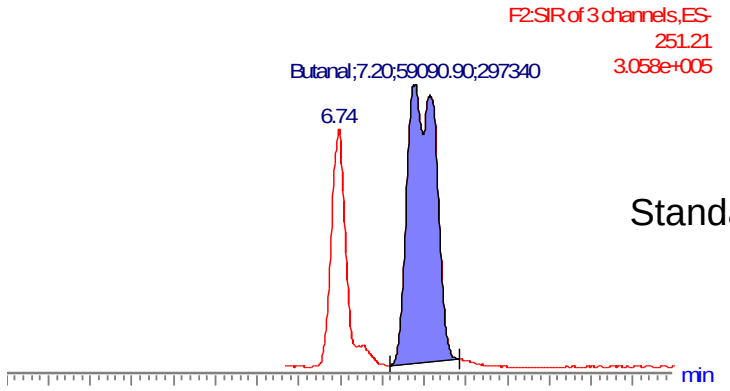


Easily able to achieve selectivity between compounds with the same m/z.

Butyraldehyde Selectivity

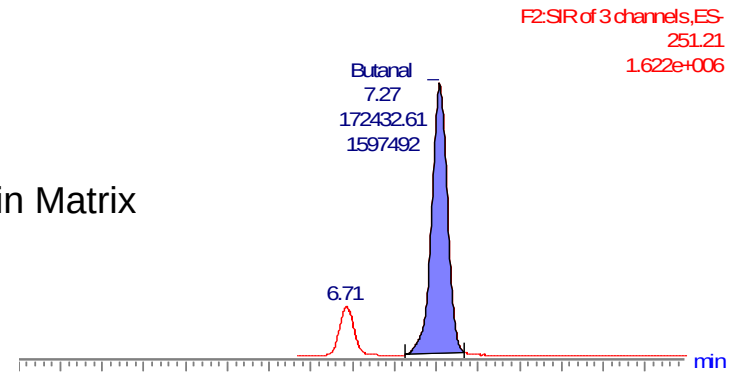
Butyraldehyde in standards

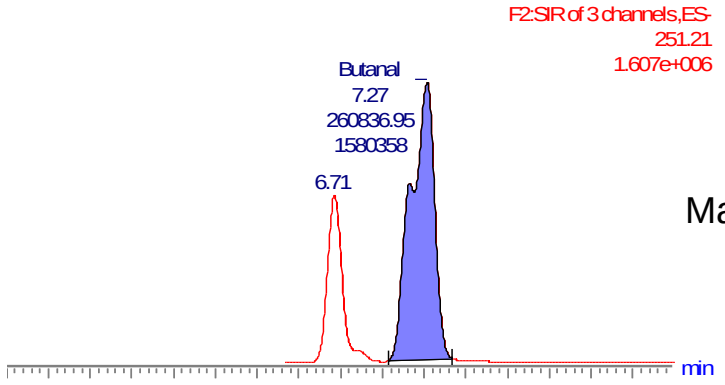




Standard fortified with iso-butyraldehyde

Butyraldehyde in Matrix

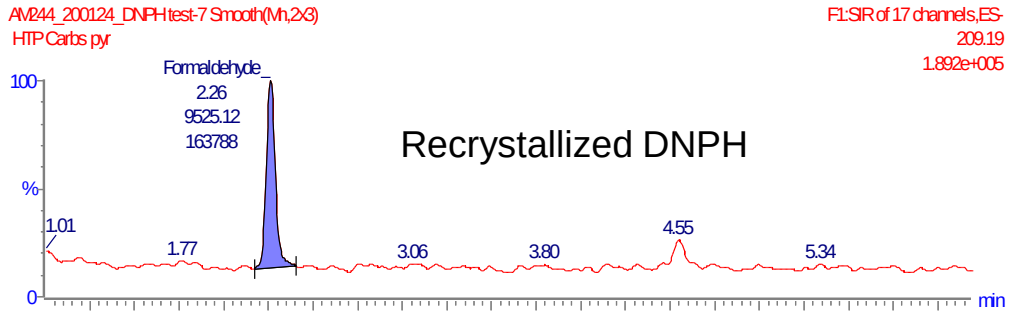


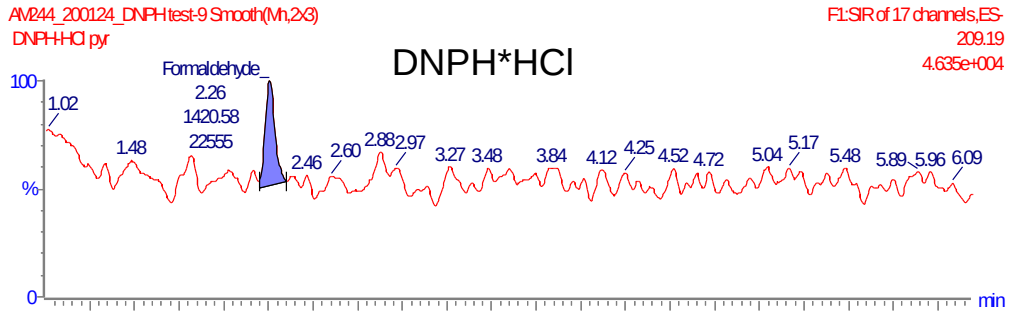


Matrix fortified with butyraldehyde

Formaldehyde Background

- Traditionally, DNPH can have high background levels of formaldehyde and may require recrystallization.
- Often, DNPH needs to be recrystallized multiple times to remove the excess formaldehyde.
- DNPH*HCl comes ready to use and background levels are substantially lower.
- In this example, there is almost 7 times the amount of formaldehyde in the recrystallized DNPH than in the DNPH*HCl.

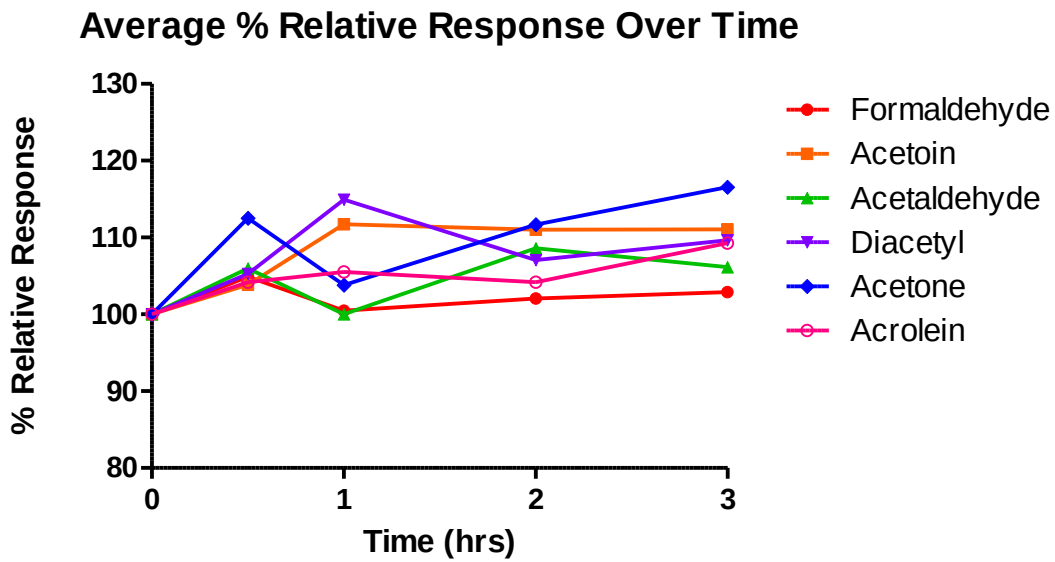


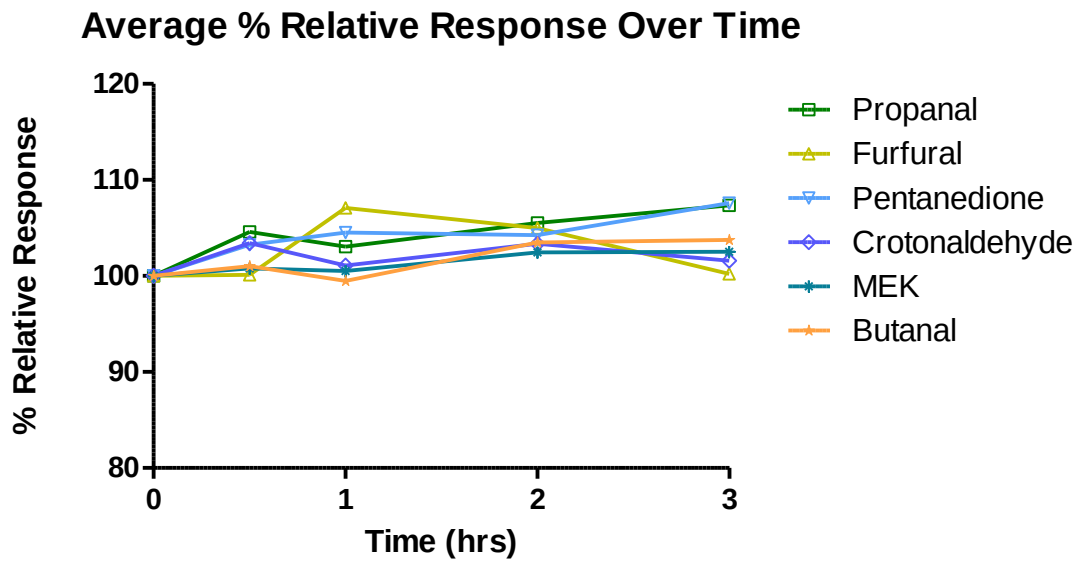


Method LOQs

Analyte	CRM-74	Health Canada T-104	New Method
	(µg/test piece)	(µg/test piece)	(µg/test piece)
Formaldehyde	2.53	1.20	0.38
Acetoin			0.38
Acetaldehyde	8.38	3.24	0.38
Diacetyl			0.38
Acetone	5.54	2.82	1.88
Acrolein	2.49	2.37	0.38
Propanal	3.05	3.33	0.38
Furfural			0.38
Pentanedione			0.38
Crotonaldehyde	3.54	3.29	0.38
MEK	3.34	3.66	0.38
Butyraldehyde	8.56	2.70	0.38

Stability in Trapping Solution





After two hours, less than 15% change in relative response for all analytes. After 3 hours, less than 20% change in relative response
Note: Samples were maintained at -35°C for the duration of sampling.

Trapping Efficiency

Analyte	% on pad	% in Impinger 1	% in Impinger 2
Formaldehyde	79.2	20.8	0.0
Acetoin	87.4	12.0	0.5
Acetaldehyde	0.3	98.5	1.2
Diacetyl	6.1	93.9	0.0
Acetone	1.3	98.3	0.4
Acrolein	2.3	97.7	0.1
Propanal	0.3	99.3	0.5
Furfural	40.7	59.3	0.0
Pentanedione	2.9	97.1	0.0
Crotonaldehyde	0.0	100.0	0.0
MEK	0.8	98.6	0.6
Butyraldehyde	0.0	100.0	0.0

≥ 98% Trapped between CFP and first impinger

Accuracy (Recovery from Matrix)

Analyte	Native (µg/mL)	Diluted (µg/mL)	Average Low Recovery (%)	Average Mid Recovery (%)	Average High Recovery (%)
Formaldehyde	0.168	0.043	**100.4**	**103.5**	**110.2**
Acetaldehyde	5.201	1.418	**91.8**	**86.3**	**102.9**
Acrolein	0.338	0.089	**107.2**	**110.1**	**106.9**
Crotonaldehyde	0.068	0.011	**102.1**	**101.2**	**101.3**

Note: Average recoveries for all other analytes ranged from 92.6% to 107.6%

Intermediate Precision

Analyte	Average (µg/test piece)	StDev	%RSD
Formaldehyde	4.81	0.49	10.1
Acetoin	11.4	0.9	8.0
Acetaldehyde	175	9	5.1
Diacetyl	47.5	3.4	7.1
Acetone	37.1	3.9	10.4
Acrolein	13.3	1.3	9.6
Propanal	11.9	0.8	6.3
Furfural	32.1	2.0	6.3
Pentanedione	11.2	0.7	5.9
Crotonaldehyde	2.33	0.18	7.8
MEK	6.92	0.44	6.3
Butyraldehyde	35.4	2.0	5.6

Notes:
- n=25
- Samples were prepared by three different analysts.
- Samples were injected and quantitated on three different days.

Conclusion

- A method for the determination of carbonyl compounds in heated tobacco products has been shown to be fit for purpose.
- Utilizing UPLC-MS, both selectivity and sensitivity were improved allowing for the quantitation of twelve carbonyl compounds.
- All 12 target compounds are stable in the 1:1 acetonitrile to isopropyl alcohol trapping solution for up to 3 hours without significant loss.
- Recoveries were improved for both acrolein and crotonaldehyde.
- Excellent precision was demonstrated over three days of analysis.

Thank you for your attention!

Contact: joseph.jablonski@enthalpy.com

Suggested DNPH Prep

Combine 9 g of DNPH*HCl with 500 mL of acetonitrile and 460 mL of 18.2 MΩ·cm water. Carefully add 40 mL of 10% H_3PO_4 in water and mix well. Solution should be filtered into an amber glass bottle prior to use.

Accuracy (Recovery from Matrix)

Analyte	Average Low Recovery (%)	Average Mid Recovery (%)	Average High Recovery (%)
Formaldehyde	**100.4**	**103.5**	**110.2**
Acetoin	**92.6**	**103.1**	**107.5**
Acetaldehyde	**91.8**	**86.3**	**102.9**
Diacetyl	**104.9**	**100.7**	**102.3**
Acetone	**99.6**	**105.9**	**-**
Acrolein	**107.2**	**110.1**	**106.9**
Propanal	**106.6**	**105.9**	**107.5**
Furfural	**107.0**	**103.2**	**100.5**
Pentanedione	**99.8**	**101.2**	**102.0**
Crotonaldehyde	**102.1**	**101.2**	**101.3**
MEK	**100.0**	**99.9**	**98.0**
Butyraldehyde	**94.0**	**101.8**	**107.6**