Evaluation of Methodologies for Determination of Carbonyls in Smokeless Tobacco Products

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INTRODUCTION

- Carbonyl compounds are listed as harmful and potentially harmful constituents (HPHCs) found in cigarette smoke and smokeless tobacco products (STPs).[1]
- CORESTA has recommended a method (CRM 74) for the determination of carbonyls in cigarette smoke.[2]
- STPs contain low levels of carbonyls relative to cigarette smoke.
- There is no standardized method for measuring carbonyls in STPs.

OBJECTIVE

- Evaluate two different methodologies for the determination of carbonyls in STPs.
  - UPLC-UV: ultra performance liquid chromatography coupled with ultraviolet detection adapted from CRM 74 for carbonyls in smoke[2]
  - GC-MS: gas chromatography with mass spectrometry adapted from the method presented by Labstat (Bao et al., 2013) at CORESTA[3]

METHOD: UPLC-UV

Adapted from CRM 74 for Determination of Carbonyls in Smoke[2]

1g tobacco + 10 mL of 17 mM DNPH with 0.36 M perchloric acid

Shake for 60 min

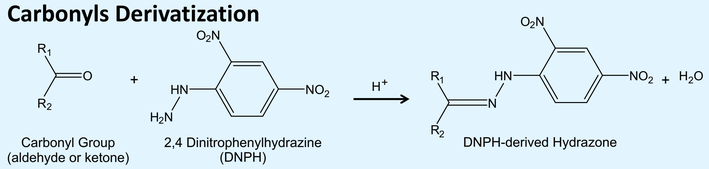
Remove 1 mL of sample extract + 50 µL of pyridine

Analyze sample by UPLC-UV

Instrument: Waters Acquity® UPLC
Column: Acquity® UPLC BEH Shield RP18, 2.1 mm × 100 mm, 1.7 µm particle
Injection volumn: 4µL
UV Detector: 365 nm
Gradient:

Time (min)	Flow (mL/min)	A (%)	B (%)	Curve
0.00	0.65	100	0	
6.00	0.65	80	20	6
27.00	0.65	63	37	6
28.00	0.65	0	100	6
30.00	0.65	0	100	6
30.50	0.65	100	0	6

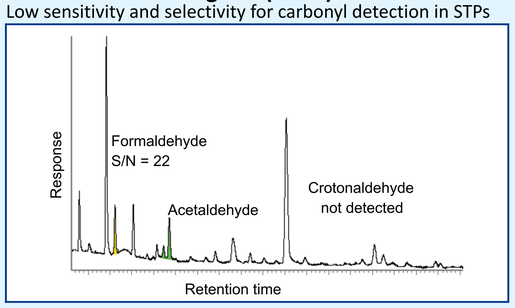
Carbonyls Derivatization



Carbonyl Group (aldehyde or ketone) | 2,4 Dinitrophenylhydrazine (DNPH) | DNPH-derived Hydrazone

UPLC-UV Chromatogram (CRP2)

Low sensitivity and selectivity for carbonyl detection in STPs



ABSTRACT

The carbonyls formaldehyde, acetaldehyde and crotonaldehyde are on the FDA list of harmful and potentially harmful constituents (HPHCs) found in tobacco products. As mandated by the Family Smoking Prevention and Tobacco Control Act, tobacco manufacturers and importers are required to report quantities of HPHCs to the United States Food and Drug Administration (FDA). Currently no standardized method exists for the determination of these carbonyls in smokeless tobacco products (STPs). The objective of this study was to compare two commonly used analytical platforms, gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography-ultraviolet detection (HPLC-UV), for the determination of these carbonyls in snus, moist smokeless tobacco (MST), dry snuff, and chewing tobacco. The GC-MS procedure used O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA) derivatization as compared to the 2,4-dinitrophenylhydrazine (DNPH) derivatization used with the HPLC-UV procedure. The extraction and derivatization steps were evaluated for stability of the carbonyl yields for CORESTA reference tobacco products CRP1, CRP2, CPR3, CRP4 as well as 3R4F tobacco filler samples. The GC-MS method had inherently greater selectivity due to mass spectrometry detection as well as greater sensitivity afforded by selected ion monitoring (SIM).

METHOD: GC-MS

Adapted from the Labstat (Bao et al., 2013) Method for Carbonyls in STPs[3]

1g tobacco + 10 mL water

100 µL Internal Standard + 100 µL PFBHA + 50 µL H_2SO_4 and 2 mL hexane

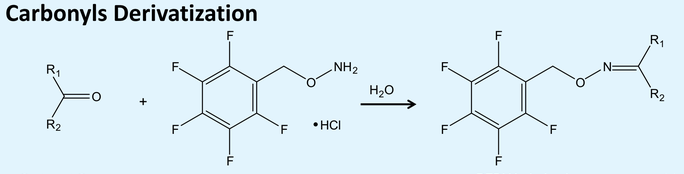
Shake for 60 min

Remove hexane layer for GC-MS

Instrument: Shimazdu GC-MS
Column: Agilent DB Wax (30 m × 0.25 mm ID × 0.25 µm)
Oven Temperature: 40 °C; hold 1 min; 15 °C/min to 205 °C; hold 3 min
Column Flow: 1 mL/min
Injection Mode: Split 10:1
MS Detector: SIM

PFBHA-Oximes	Quantitation Ion (m/z)
Acetaldehyde-C13	211
Methyl Ethyl Ketone MEK-D5	255
Formaldehyde	195
Acetaldehyde	209
Crotonaldehyde	250

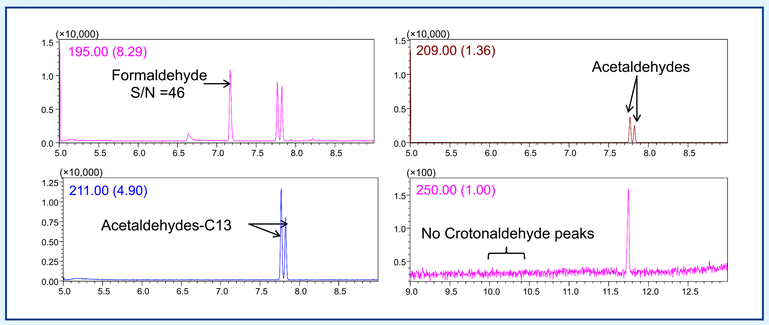
Carbonyls Derivatization



Carbonyl Group (aldehyde or ketone) | O-(2,3,4,5,6-Pentafluorobenzyl) hydroxylaminehydrochloride (PFBHA) | PFBHA-derived Oxime | + isomer(s)

GC-MS Chromatogram (CRP2)

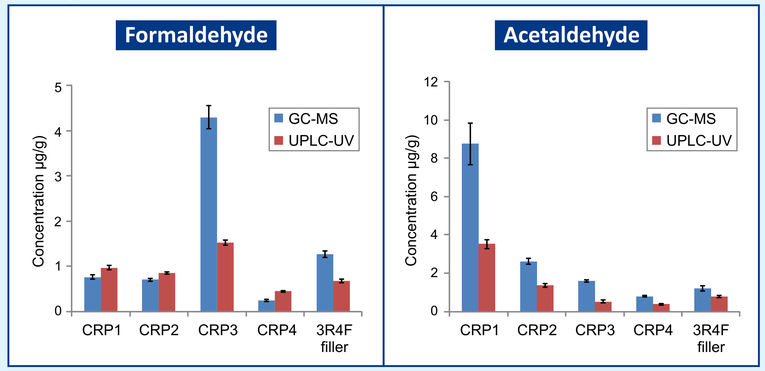
Improved sensitivity and selectivity compared with UPLC-UV



RESULTS

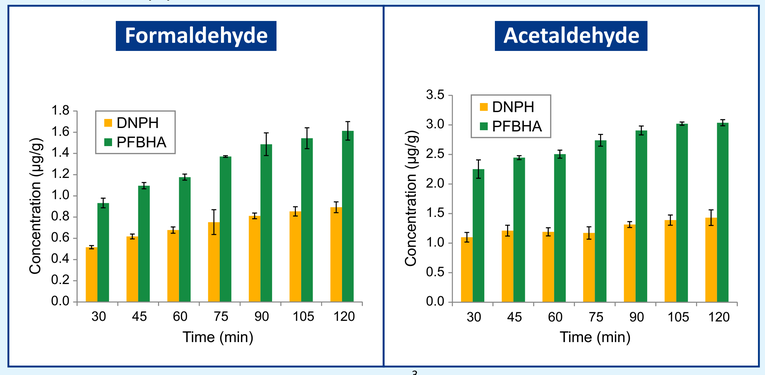
Comparison of Carbonyl Yields

Carbonyl yields are not consistent between the methods.



Derivatization Time/Carbonyl Stability for STPs

For CRP2, carbonyl yield increases with derivatization time for both DNPH and PFBHA.



Oximes are stable after back extraction in hexane.[3]

SUMMARY

- Mass spectrometry (MS)-based approach provides high selectivity and sensitivity required to measure carbonyls in STPs.
- The increase in carbonyl concentration during derivatization requires additional research.

REFERENCES

1. U.S. Food and Drug Administration (FDA). 2012. Draft guidance for industry: reporting harmful and potentially harmful constituents in tobacco products and tobacco smoke under section 904(a)(3) of the Federal Food, Drug, and Cosmetic Act. http://www.fda.gov/downloads/TobaccoProducts/GuidanceComplianceRegulatoryInformation/UCM297828.pdf.
2. Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA). 2014. CORESTA Recommended Method N° 74. Determination of selected carbonyls in mainstream cigarette smoke by HPLC. http://www.coresta.org/Recommended_Methods/CRM_74-update (July14).pdf.
3. Mingling Bao, Peter Joza, Andrew Masters, William Rickert. 2013. Analysis of selected carbonyl compounds in tobacco products by using PFBHA derivatization and GC-MS. 2013 CORESTA SSPT meeting, Seville, Spain.

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