Optimized Method for Determination of Selected Phenolic Compounds in Cigarette and Cigar Smoke by UPLC-FLD

Xiaohong Cathy Jin, Thomas J. Hurst and Karl A. Wagner
Tobacco Science Research Conference
Sept. 15th-18th 2019
Introduction

- Selected phenolic compounds are included in the FDA “Established List of HPHCs in Tobacco Products and Tobacco Smoke”.

- Analytical methods for phenols in tobacco and cigarette smoke:
  - Wu, J. et al. reported an HPLC-FLD method in 2012
  - CORESTA recommended method 78 (CRM 78), 34 min run time, developed in 2014

- No method for phenols in cigar smoke has been reported.

3. CRM 78, Determination of selected phenolic compounds in mainstream cigarette smoke by HPLC-FLD, December 2018
Objective

- To develop a rapid method suitable for analysis of phenols in both cigarette and cigar smoke using Ultra-High Pressure Liquid Chromatography (UPLC) with Fluorescence detector (FLD)
Selected phenolic compounds

- Catechol*
- Phenol*
- m-Cresol*
- o-Cresol*
- p-Cresol*
- Hydroquinone
- Resorcinol

* Included in FDA’s list of HPHCs
Experimental – Smoke collections

- Cigarette or cigar smoke was collected on a Cambridge filter pad (CFP) per smoking regime

<table>
<thead>
<tr>
<th>Samples</th>
<th>Smoking regime</th>
<th>Puff volume (mL)</th>
<th>Puff frequency (sec)</th>
<th>Puff duration (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarette</td>
<td>ISO (ISO 3308)</td>
<td>35</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Intense (ISO 20778)</td>
<td>55</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>Cigar</td>
<td>CORESTA¹</td>
<td>20²</td>
<td>40</td>
<td>1.5</td>
</tr>
</tbody>
</table>

1. CORESTA recommended method № 64, Routine analytical cigar-smoking machine – specifications, definitions and standard conditions, May 2018
2. For cigar diameter (d) > 12.0 mm, puff volume (mL) = 0.139* d²
Experimental – Sample preparations

1. Collect smoke sample on a CFP
   • Transfer pad to an amber vial

2. Add 40 mL of extraction solution (1% acetic acid)
   • Shake for 30 min at 350 RPM

3. Filter extract through 0.2 µm PVDF syringe filter
   • Inject 2 µL into UPLC-FLD
Column selection
-Chromatograms of standard 2

- Kinetex F5 column has better separation and was selected for UPLC method.

*PFP: pentafluorophenylpropyl
Analytical method
- Parameters

- Acquity I-Class UPLC system: binary pump, temperature controlled autosampler, fluorescence detector
- Kinetex® Core-shell column F5, 1.7µm, 100 x 2.1mm
- Autosampler temperature: 5 °C
- Injection volume: 2 µL
- Mobile phase, gradient elution (flow rate 0.35 mL/min)
  - A 1% acetic acid in Type 1 water
  - B 1% acetic acid in methanol
Analytical method

- Instrument settings

### Mobile phase gradients

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Analytes</th>
<th>Excitation wavelength (nm)</th>
<th>Emission wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 - 2.2</td>
<td>Hydroquinone, resorcinol, catechol</td>
<td>280</td>
<td>310</td>
</tr>
<tr>
<td>2.2 - 7.0</td>
<td>Phenol, (p, m, o) - cresol</td>
<td>274</td>
<td>298</td>
</tr>
</tbody>
</table>
Representative chromatograms of smoke samples

1R6F ISO

Cigar
## Method validation

### Calibration

<table>
<thead>
<tr>
<th>Elements</th>
<th>Analytes</th>
<th>Standards (µg/mL)</th>
<th>Conc. in smoke* (µg per cig. or cigar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration ranges</td>
<td>Hydroquinone, phenol, catechol</td>
<td>0.05 - 20</td>
<td>0.4 - 160 (cig. ISO) 0.7 - 267 (cig. Intense) 2 - 800 (cigar)</td>
</tr>
<tr>
<td></td>
<td>Resorcinol, p-cresol, m-cresol, o-cresol</td>
<td>0.01 - 4</td>
<td>0.08 - 32 (cig. ISO) 0.13 - 53 (cig Intense) 0.4 - 160 (cigar)</td>
</tr>
</tbody>
</table>

Coefficient of determination ($R^2$) > 0.999 for all 7 phenols

* Based on 40 mL extraction vol. and 5 cig. for ISO, 3 cig. for Intense, 1 cigar
## Method validation

### -Precision

<table>
<thead>
<tr>
<th>Samples</th>
<th>Smoking regime</th>
<th>TPM 3 days average (mg/cig)</th>
<th>Phenols intra-day precision (%RSD)</th>
<th>Phenols inter-day precision (%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3R4F</td>
<td>ISO</td>
<td>9.6</td>
<td>3.1 - 9.8</td>
<td>2.0 - 8.1</td>
</tr>
<tr>
<td></td>
<td>Intense</td>
<td>48.5</td>
<td>4.2 - 10.2</td>
<td>2.8 - 13.9</td>
</tr>
<tr>
<td>1R6F</td>
<td>ISO</td>
<td>10.4</td>
<td>2.4 - 6.5</td>
<td>4.1 - 6.8</td>
</tr>
<tr>
<td></td>
<td>Intense</td>
<td>48.0</td>
<td>2.3 - 10.4</td>
<td>3.3 - 8.9</td>
</tr>
<tr>
<td>CORESTA monitor 7</td>
<td>ISO</td>
<td>16.7</td>
<td>0.9 - 5.6</td>
<td>3.1 - 5.8</td>
</tr>
<tr>
<td>(CM7)</td>
<td>Intense</td>
<td>46.3</td>
<td>3.0 - 8.8</td>
<td>3.4 - 7.8</td>
</tr>
<tr>
<td>Cigar²</td>
<td>CORESTA</td>
<td>62.4</td>
<td>4.0 - 11.9</td>
<td>5.8 - 16.9</td>
</tr>
</tbody>
</table>

1. Intra-day: n=6 for cigarettes and n=4 for cigar; Inter-day: 3 days
2. Machine made, traditional dark cured tobacco, natural wrapper cigar
Method validation

- Phenols yields in validation samples (n=18 for cig. and n=12 for cigar)
# Method validation

- Recovery (2 fortification levels at 0.5x & 2x of native analytes conc., n=3)

<table>
<thead>
<tr>
<th>Fortification level</th>
<th>1R6F ISO</th>
<th>CM 7 ISO</th>
<th>Cigar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low level fortification ~0.5X of native conc.</td>
<td>74% - 96%</td>
<td>86% - 95%</td>
<td>75% - 83%</td>
</tr>
<tr>
<td>High level fortification ~2X of native conc.</td>
<td>75% - 96%</td>
<td>88% - 97%</td>
<td>77% - 97%</td>
</tr>
</tbody>
</table>
Compared to CRM 78

-Chromatograms of 3R4F ISO sample

UPLC method is 3 times faster than CRM 78.
Compared to CRM78
-Calibration ranges

<table>
<thead>
<tr>
<th></th>
<th>unit</th>
<th>UPLC</th>
<th>CRM 78</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroquinone, catechol, phenol</td>
<td>µg/mL</td>
<td>0.05 - 20</td>
<td>0.2 - 8</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>µg/mL</td>
<td>0.01 - 4</td>
<td>0.04 - 1.6</td>
</tr>
<tr>
<td>p-cresol, m-cresol, o-cresol</td>
<td>µg/mL</td>
<td>0.01 - 4</td>
<td>0.06 - 2.4</td>
</tr>
</tbody>
</table>

➤ UPLC method has wider dynamic calibration ranges than CRM 78.
Compared to CRM78
- Phenols yields in 3R4F and CM 7 smoke

Phenols yields in smoke samples using UPLC method are consistent with those reported in CRM 78.
Phenols yields in market cigarettes ISO smoke
-Commercially marketed American blended cigarettes (n=4)
Phenols yields and variability higher in cigar smoke (%RSD:2%-55%) than in cigarette smoke (%RSD:1%-8%)
Conclusions

- The UPLC-FLD method met the acceptable validation requirements.
- The UPLC method is 3 times faster and has wider dynamic calibration ranges compared to CRM 78.
- Phenols yields per stick in cigar smoke were higher and more variable than in cigarette smoke.

The UPLC-FLD method was found fit for purpose of analysis of selected phenolic compounds in both cigarette and cigar smoke.