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*
CORESTA SSPT CONFERENCE
OCT. 6th-10th 2019

Altria
Altria Client Services

Introduction

- Selected phenolic compounds are included in the FDA "Established List of HPHCs in Tobacco Products and Tobacco Smoke[1]".

- Analytical methods for phenols in tobacco and cigarette smoke:
  - Wu, J. et al. reported an HPLC-FLD method in 2012[2]
  - CORESTA recommended method 78 (CRM 78), 34 min run time, developed in 2014[3]

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

1. FDA Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke: Established List (2012)
2. Wu, J., et al. J. of chromatography A. 1264 (2012) 40-47
3. CRM 78, Determination of selected phenolic compounds in mainstream cigarette smoke by HPLC-FLD, December 2018
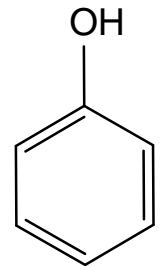
Objective

- 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)
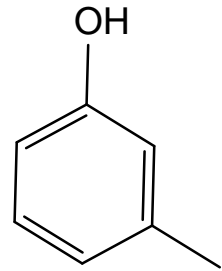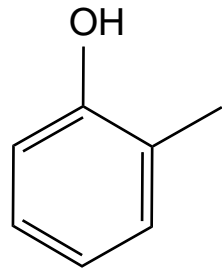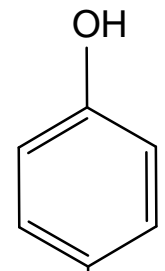
Altria
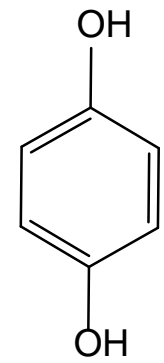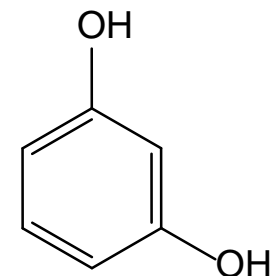Altria Client Services

Selected phenolic compounds
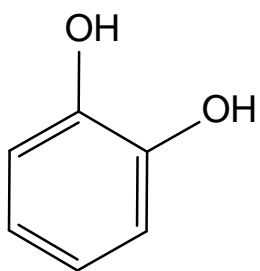
Catechol*

Phenol*

m-Cresol*

Hydroquinone

o-Cresol*

p-Cresol*

* Included in FDA's list of HPHCs

Resorcinol

Altria
Altria Client Services

Experimental – Smoke collections

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

Samples	Smoking regime	Puff volume (mL)	Puff frequency (sec)	Puff duration (sec)
Cigarette	ISO (ISO 3308)	35	60	2
	Intense (ISO 20778)	55	30	2
Cigar	CORESTA[1]	20^2	40	1.5

1. CORESTA recommended method N° 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* d^2

Altria
Altria Client Services

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)
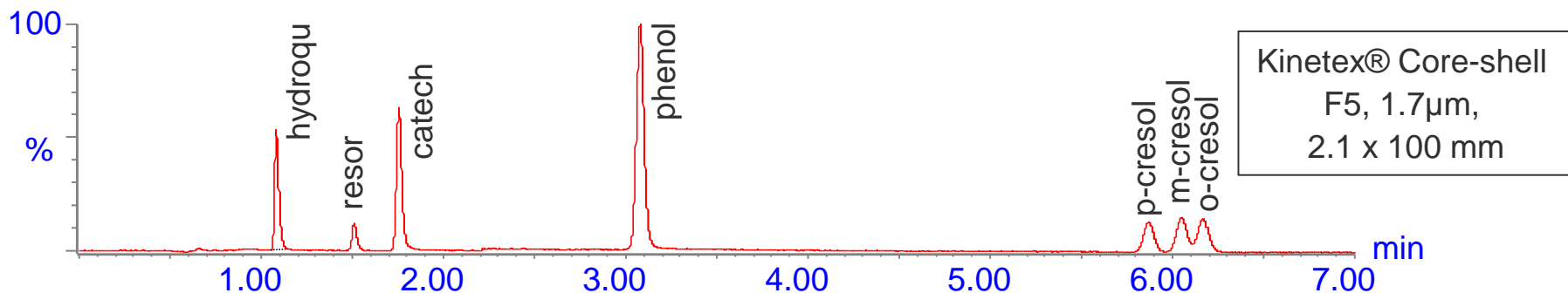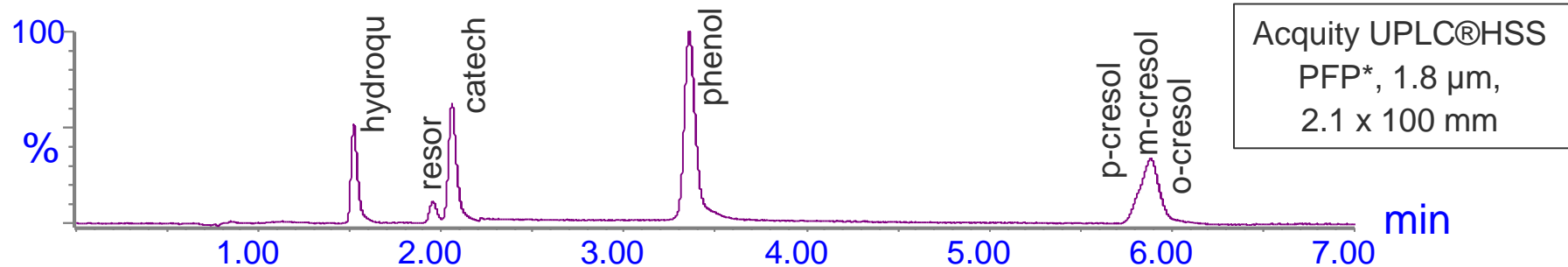- Shake for 30 min at 350 RPM

3
- Filter extract through 0.2 µm PVDF syringe filter
- Inject 2 µL into UPLC-FLD

Altria
Altria Client Services

Column selection

-Chromatograms of standard 2

Acquity UPLC®HSS PFP*, 1.8 µm, 2.1 x 100 mm

Kinetex® Core-shell F5, 1.7µm, 2.1 x 100 mm

Peaks labeled: hydroqu, resor, catech, phenol, p-cresol, m-cresol, o-cresol

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

*PFP: pentafluorophenylpropyl

Altria
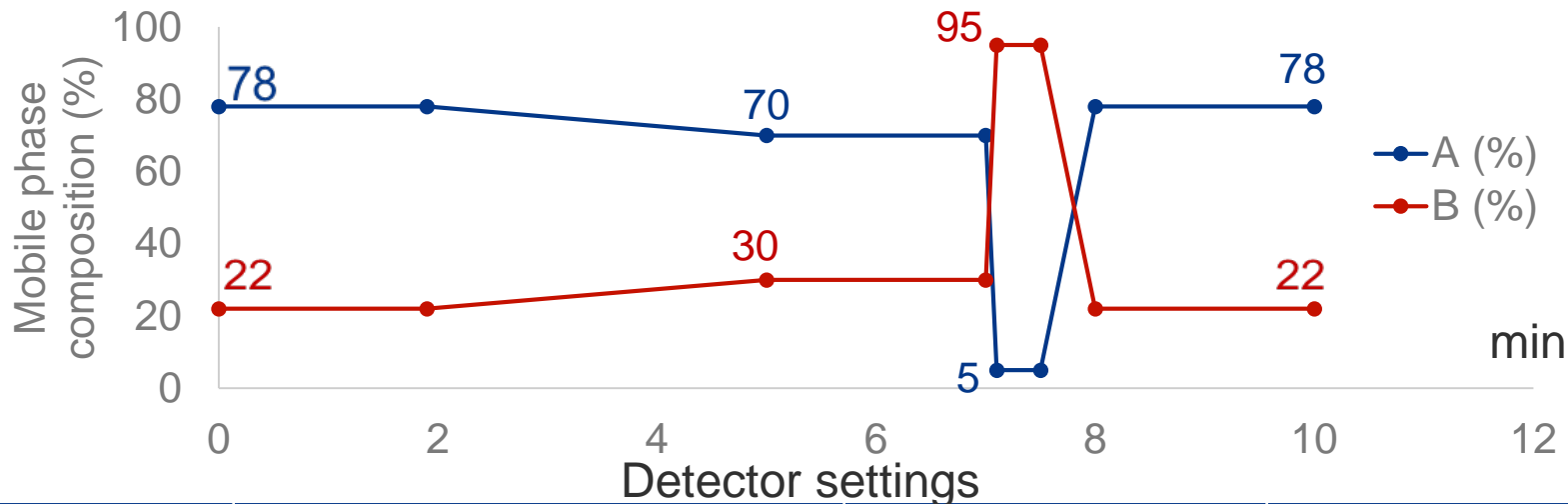Altria Client Services

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

Altria
Altria Client Services

Analytical method

-Instrument settings

Mobile phase gradients

Detector settings

Time (min)	Analytes	Excitation wavelength (nm)	Emission wavelength (nm)
0.0 - 2.2	Hydroquinone, resorcinol, catechol	280	310
2.2 - 7.0	Phenol, (p, m, o) - cresol	274	298

Altria
Altria Client Services

Representative chromatograms of smoke samples

Altria
Altria Client Services
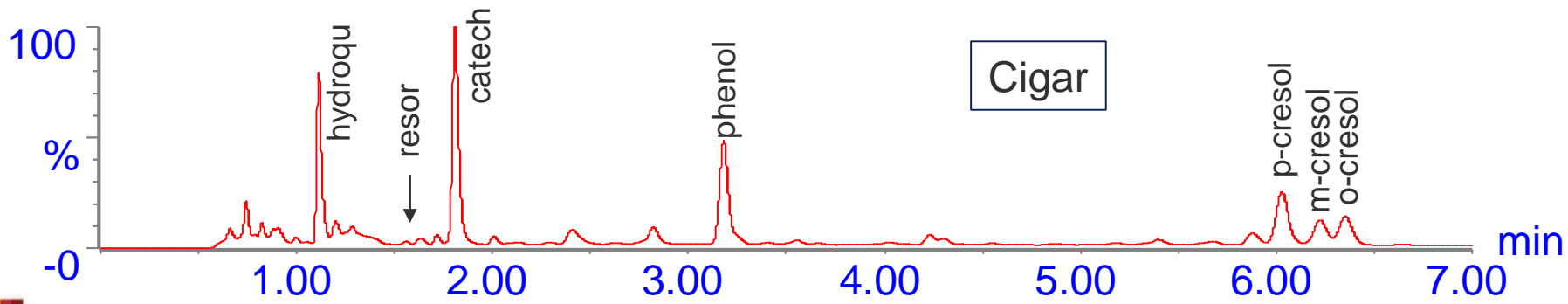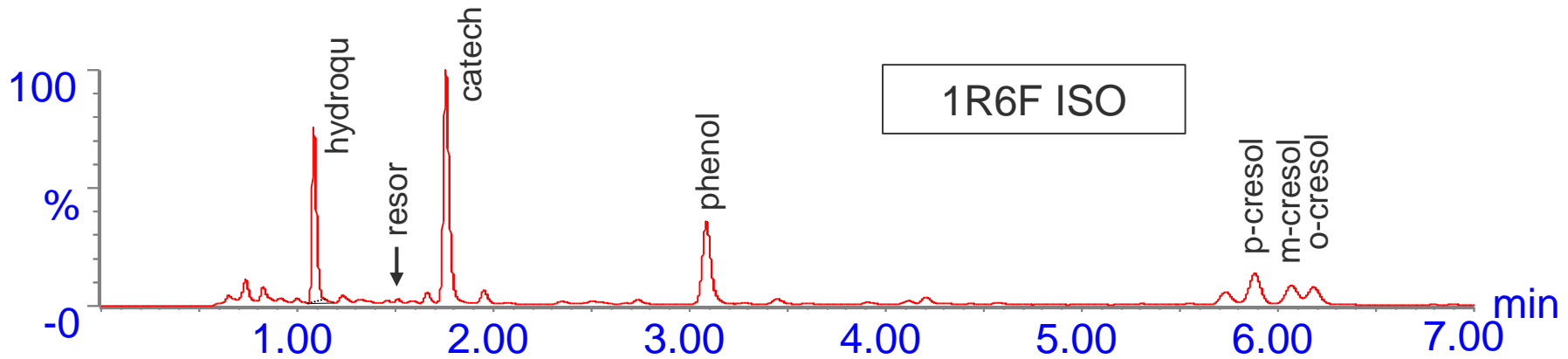
Method validation

-Calibration

Elements	Analytes	Standards (µg/mL)	Conc. in smoke* (µg per cig. or cigar)
Calibration ranges	Hydroquinone, phenol, catechol	0.05 - 20	0.4 - 160 (cig. ISO) 0.7 - 267 (cig. Intense) 2 - 800 (cigar)
	Resorcinol, p-cresol, m-cresol, o-cresol	0.01 - 4	0.08 - 32 (cig. ISO) 0.13 - 53 (cig Intense) 0.4 - 160 (cigar)
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[1]

Samples	Smoking regime	TPM 3 days average (mg/cig)	Phenols intra-day precision (%RSD)	Phenols inter-day precision (%RSD)
3R4F	ISO	9.6	3.1 - 9.8	2.0 - 8.1
	Intense	48.5	4.2 - 10.2	2.8 - 13.9
1R6F	ISO	10.4	2.4 - 6.5	4.1 - 6.8
	Intense	48.0	2.3 - 10.4	3.3 - 8.9
CORESTA monitor 7 (CM7)	ISO	16.7	0.9 - 5.6	3.1 - 5.8
	Intense	46.3	3.0 - 8.8	3.4 - 7.8
Cigar[2]	CORESTA	62.4	4.0 - 11.9	5.8 - 16.9

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
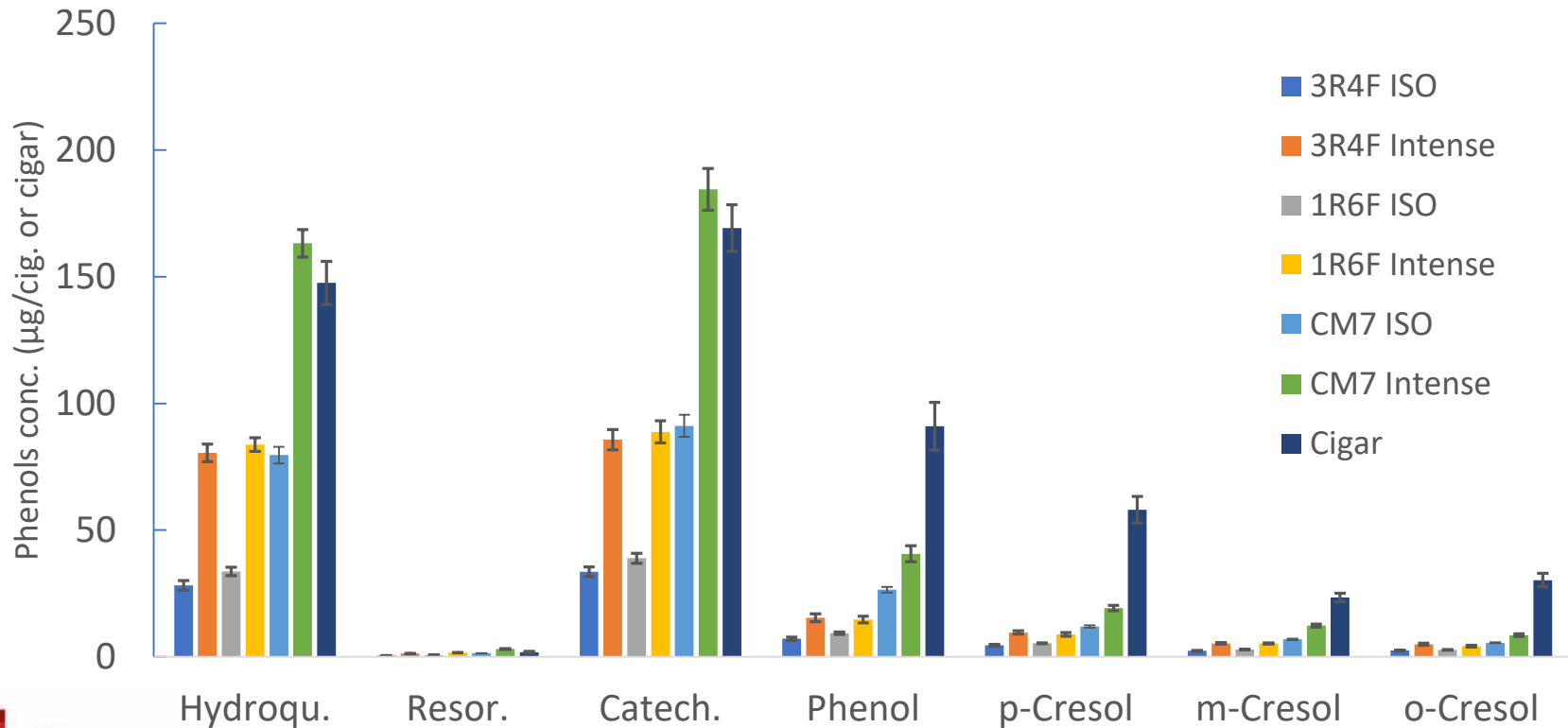
Altria
Altria Client Services

Method validation

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

Altria
Altria Client Services

Method validation

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

Fortification level	1R6F ISO	CM 7 ISO	Cigar
Low level fortification ~0.5X of native conc.	74% - 96%	86% - 95%	75% - 83%
High level fortification ~2X of native conc.	75% - 96%	88% - 97%	77% - 97%

Altria
Altria Client Services

Compared to CRM 78

-Chromatograms of 3R4F ISO sample
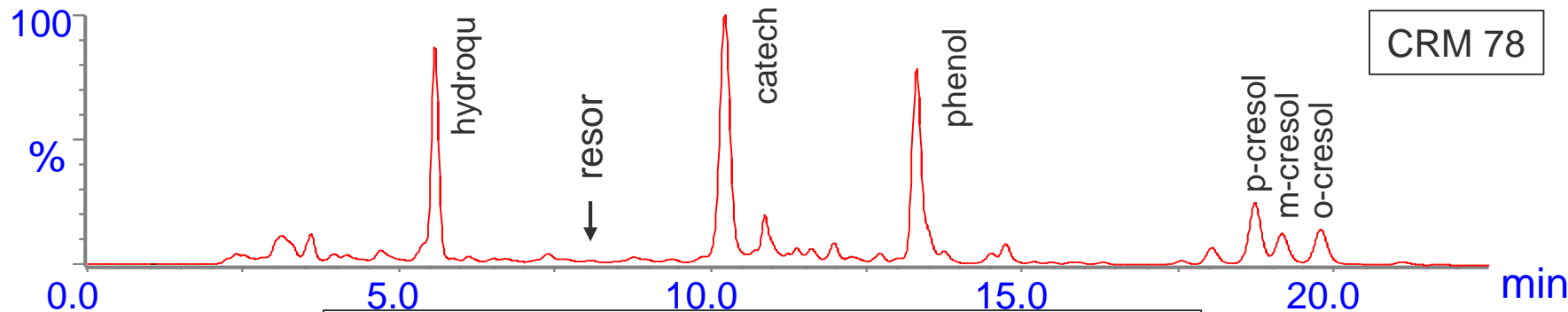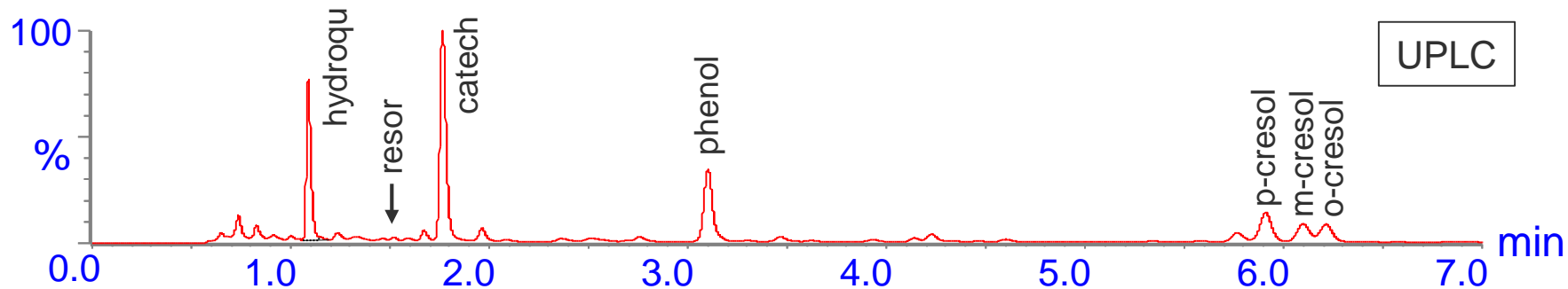
> ➤ UPLC method is 3 times faster than CRM 78.
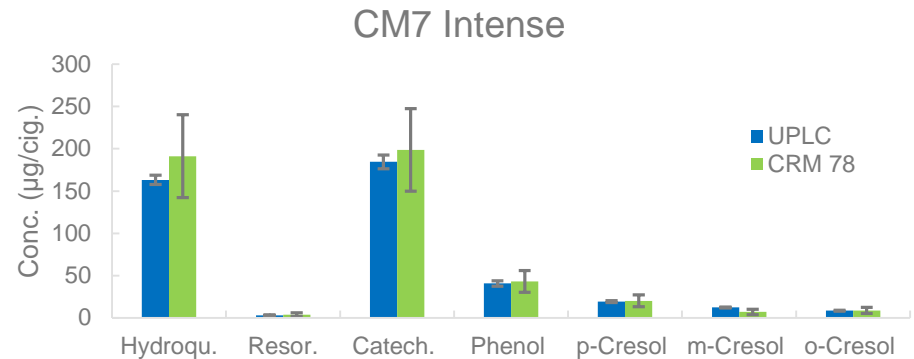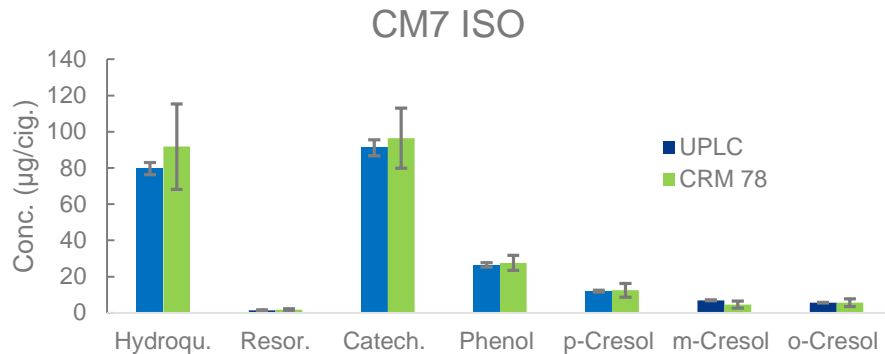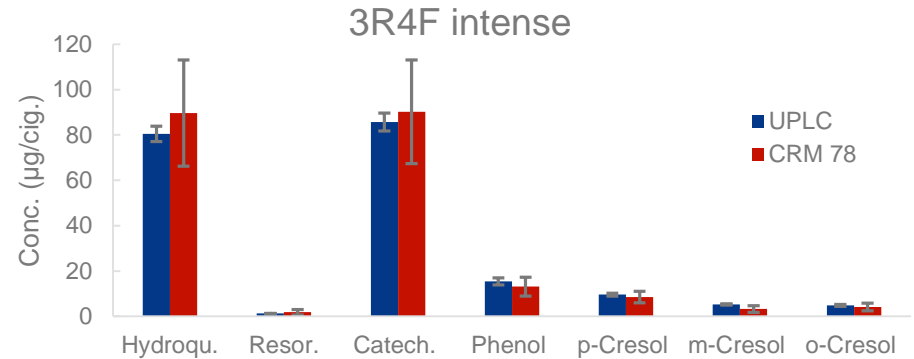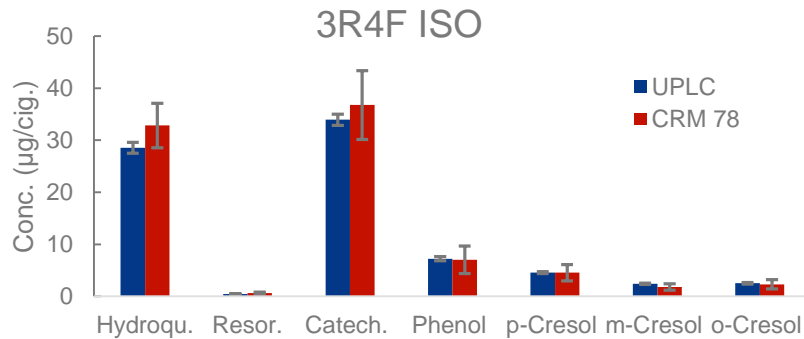
Compared to CRM78
-Calibration ranges

	unit	UPLC	CRM 78
Hydroquinone, catechol, phenol	µg/mL	0.05 - 20	0.2 - 8
Resorcinol	µg/mL	0.01 - 4	0.04 - 1.6
p-cresol, m-cresol, o-cresol	µg/mL	0.01 - 4	0.06 - 2.4

> ➢ UPLC method has wider dynamic calibration ranges than CRM 78.

Altria
Altria Client Services

Compared to CRM78

-Phenols yields in 3R4F and CM 7 smoke

3R4F ISO

3R4F intense
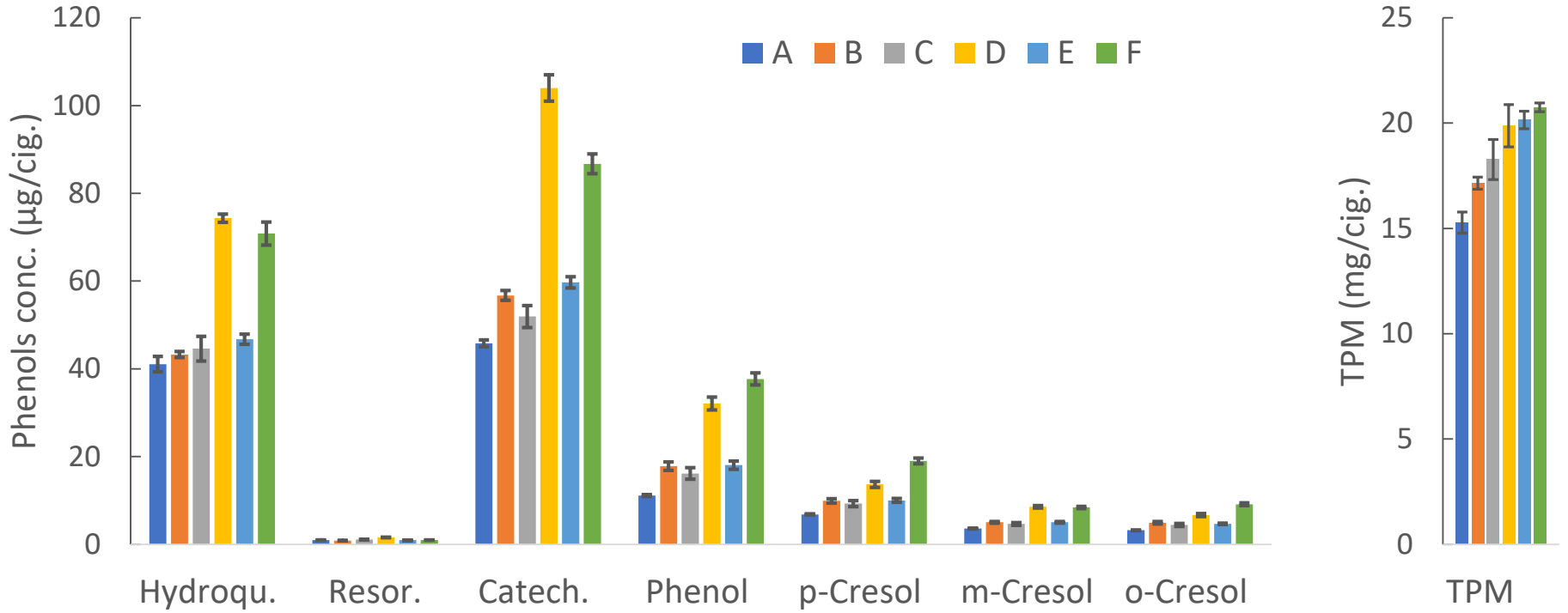
CM7 ISO

CM7 Intense

> ➢ 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)

Xiaohong Cathy Jin | Regulatory Affairs I Altria Client Services I CORESTA Oct 6th-10th I Final I

Phenols yields in machine-made cigar smoke
-Machine made cigars vs. 3R4F
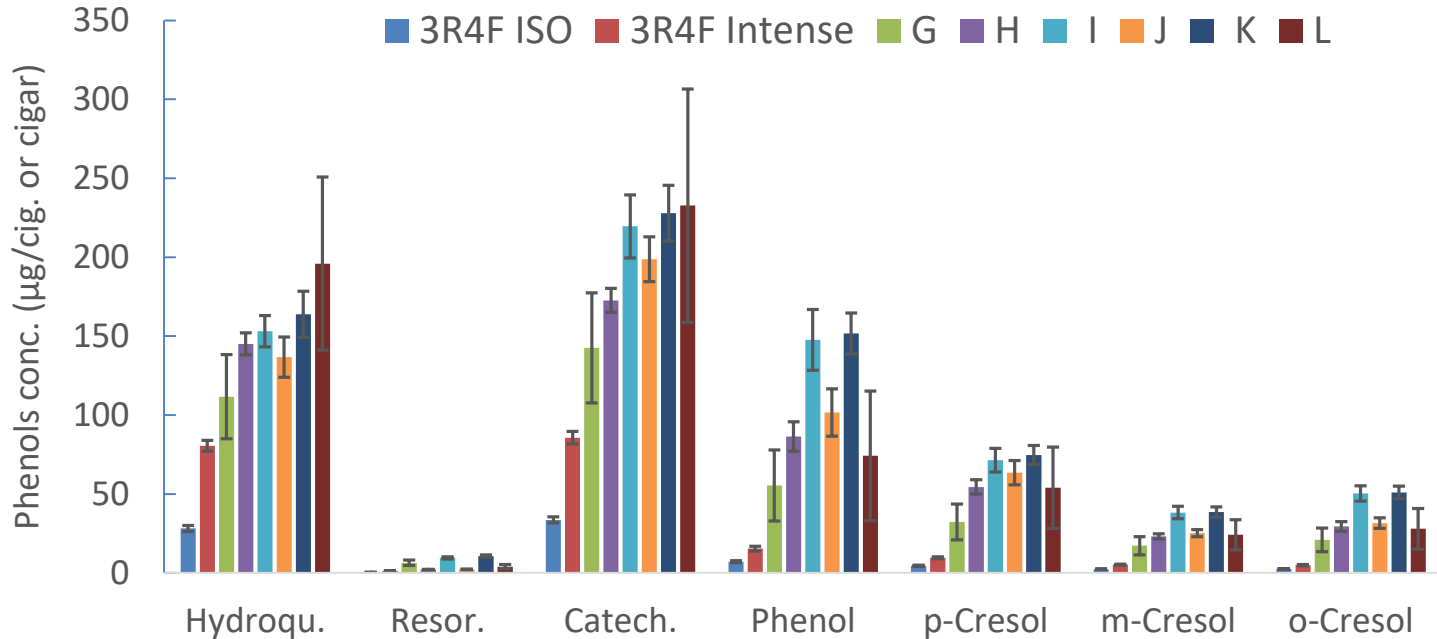
G H I J K L

> Phenols yields and variability higher in cigar smoke (%RSD:2%-55%) than in cigarette smoke (%RSD:1%-8%)

Altria
Altria Client Services

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.

Altria
Altria Client Services

Thank You

Questions?

For copies of this presentation visit the Altria's Science Website at
www.altria.com/alcs-science

Altria has a new Altria Science Twitter account
Follow us @AltriaScience