

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

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# Introduction

- Selected phenolic compounds are included in the FDA “Established List of HPHCs in Tobacco Products and Tobacco Smoke<sup>1</sup>”.
- Analytical methods for phenols in tobacco and cigarette smoke:
  - Wu, J. et al. reported an HPLC-FLD method in 2012<sup>2</sup>
  - CORESTA recommended method 78 (CRM 78), 34 min run time, developed in 2014<sup>3</sup>
- No method for phenols in cigar smoke has been reported.

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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

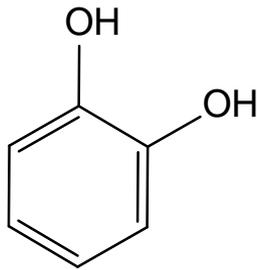
3. CRM 78, Determination of selected phenolic compounds in mainstream cigarette smoke by HPLC-FLD, December 2018



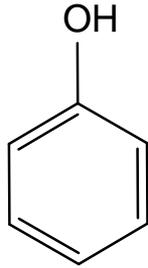
# Objective

- 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)

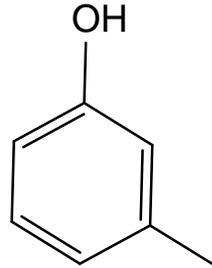
# Selected phenolic compounds



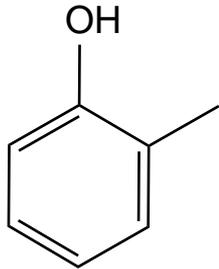
Catechol\*



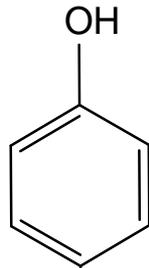
Phenol\*



m-Cresol\*

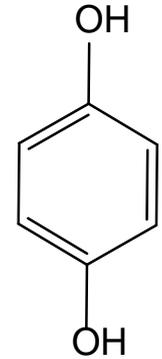


o-Cresol\*

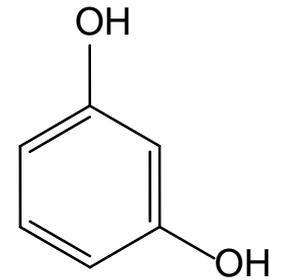


p-Cresol\*

\* Included in FDA's list  
of HPHCs



Hydroquinone



Resorcinol



# 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 <sup>1</sup>	20 <sup>2</sup>	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<sup>2</sup>



# 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

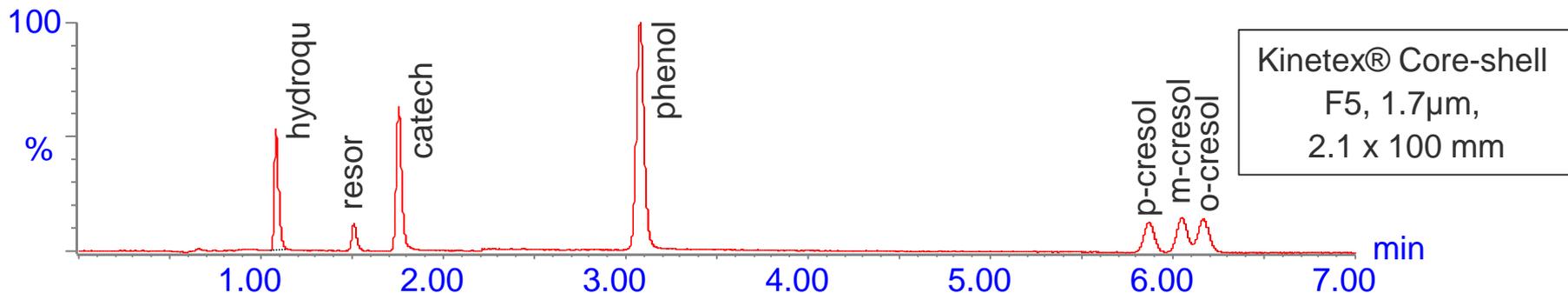
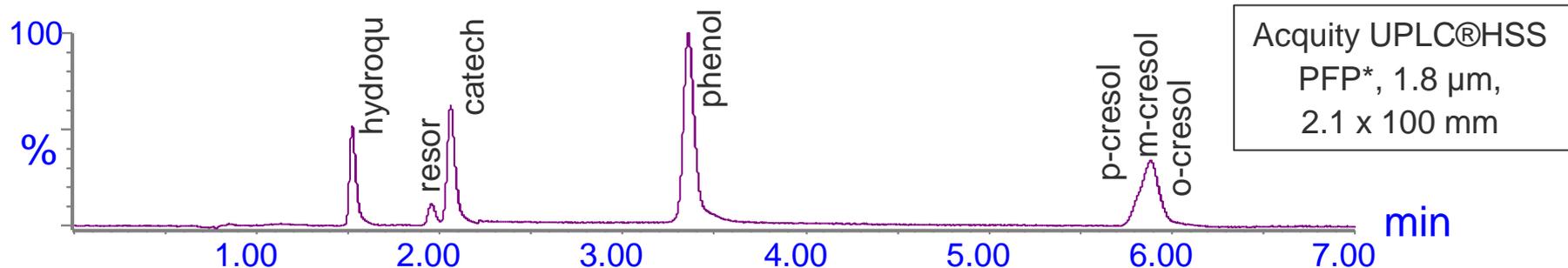
3

- Filter extract through 0.2  $\mu\text{m}$  PVDF syringe filter
- Inject 2  $\mu\text{L}$  into UPLC-FLD



# Column selection

## -Chromatograms of standard 2



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

\*PFP: pentafluorophenylpropyl



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# Analytical method

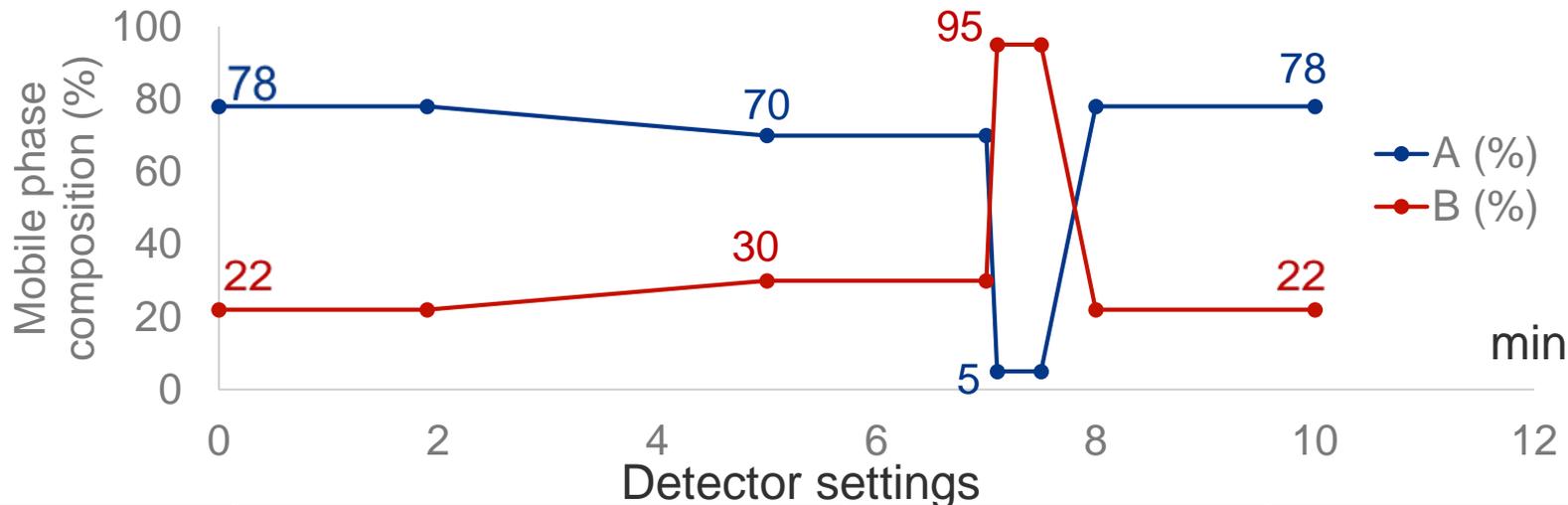
## -Parameters

- Acquity I-Class UPLC system: binary pump, temperature controlled autosampler, fluorescence detector
- Kinetex<sup>®</sup> Core-shell column F5, 1.7 $\mu$ m, 100 x 2.1mm
- Autosampler temperature: 5 °C
- Injection volume: 2  $\mu$ L
- 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

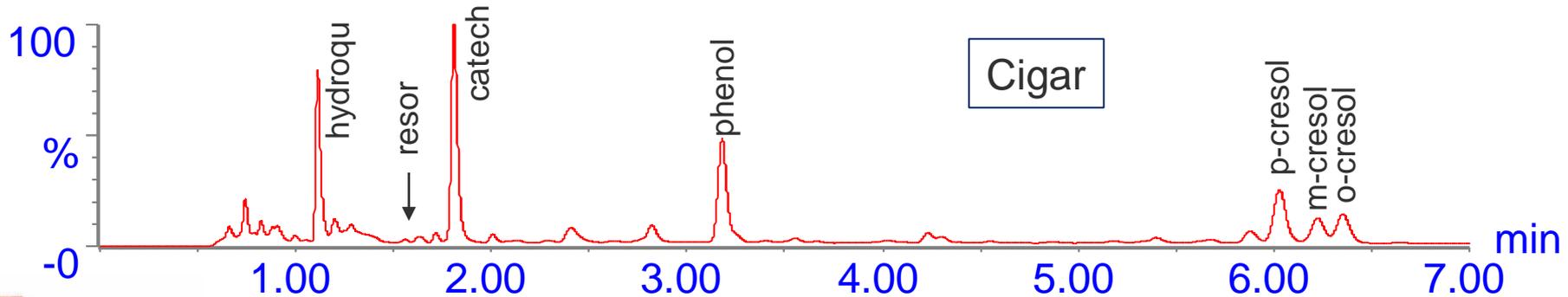
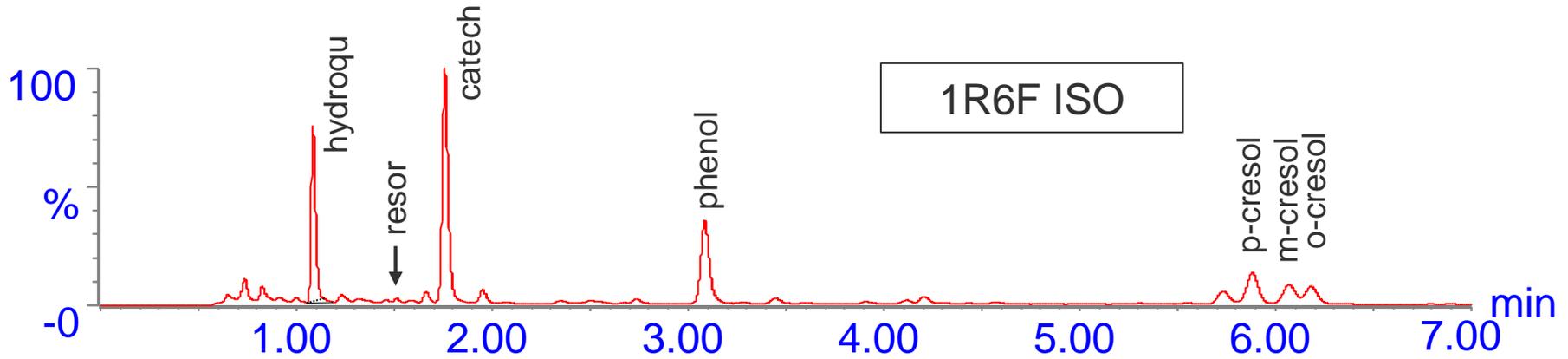
Mobile phase gradients



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



# Representative chromatograms of smoke samples



# 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 <sup>2</sup> )	> 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<sup>1</sup>

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 <sup>2</sup>	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

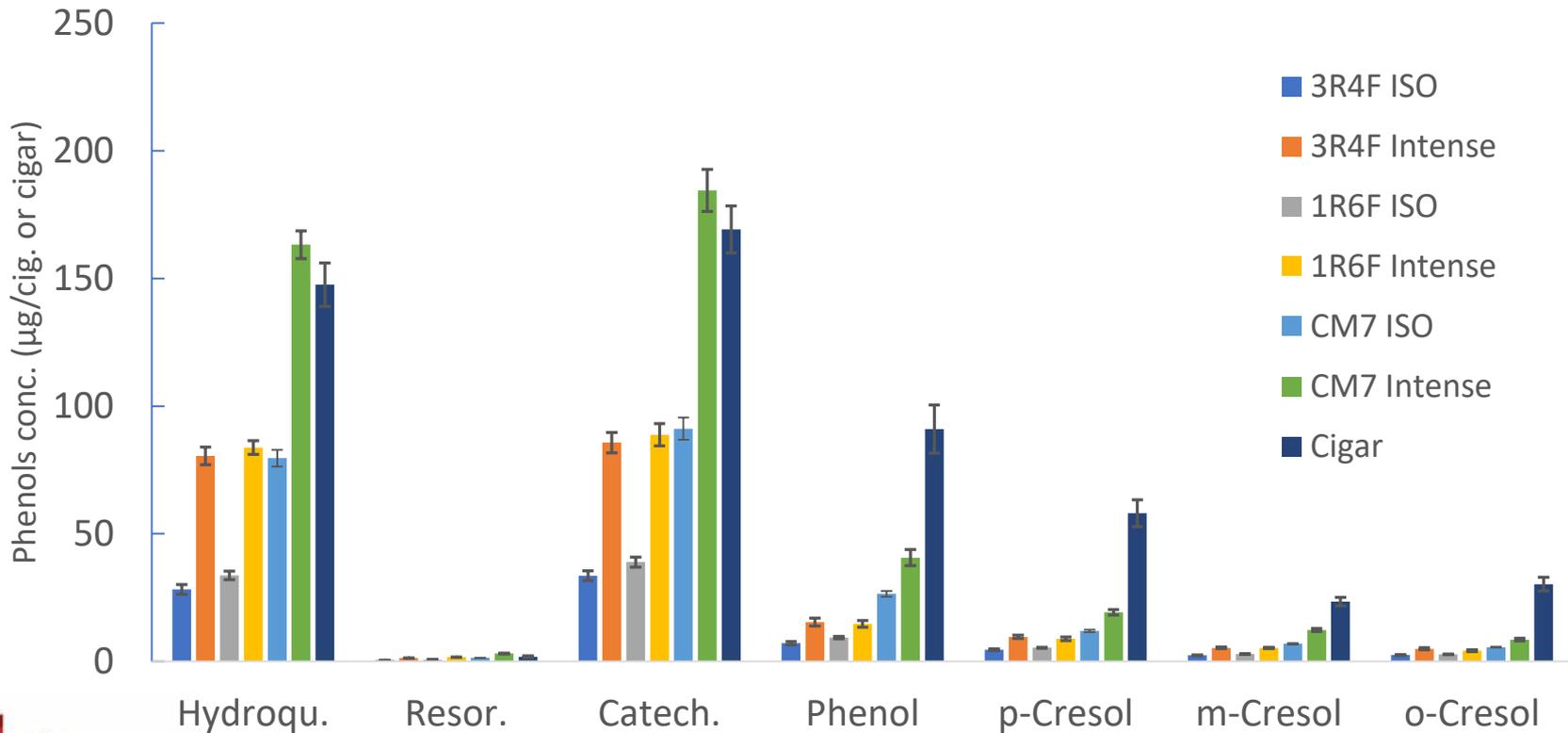


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# Method validation

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



# Method validation

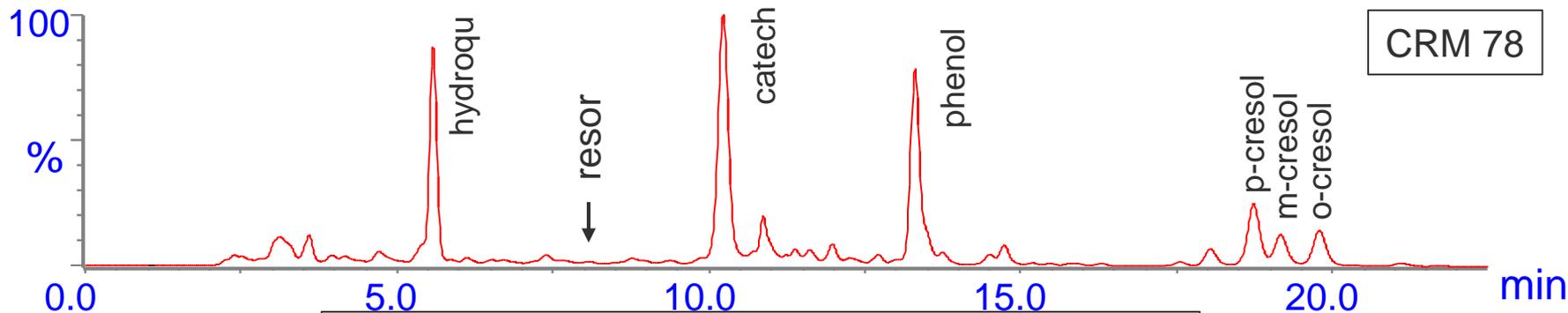
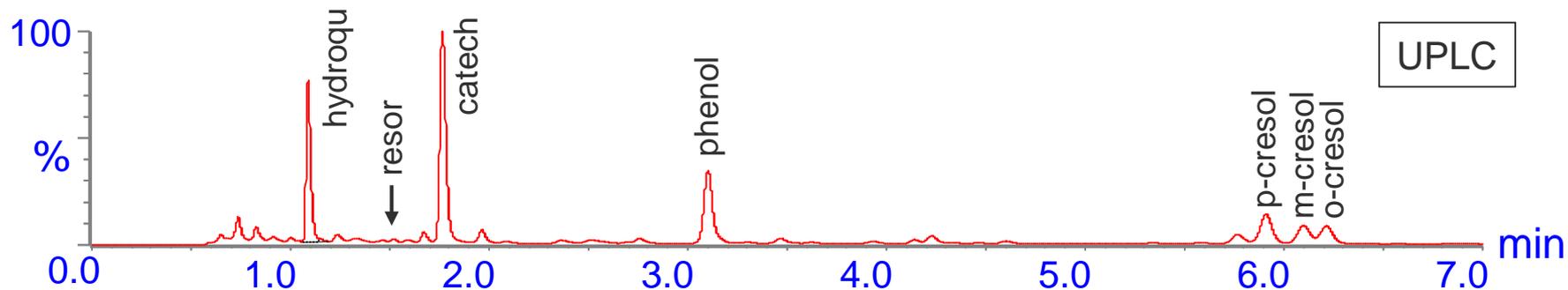
-Recovery (2 fortification levels at 0.5x & 2x of native analytes conc., n=3)

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%



# Compared to CRM 78

## -Chromatograms of 3R4F ISO sample



➤ UPLC method is 3 times faster than CRM 78.



# Compared to CRM78

## -Calibration ranges

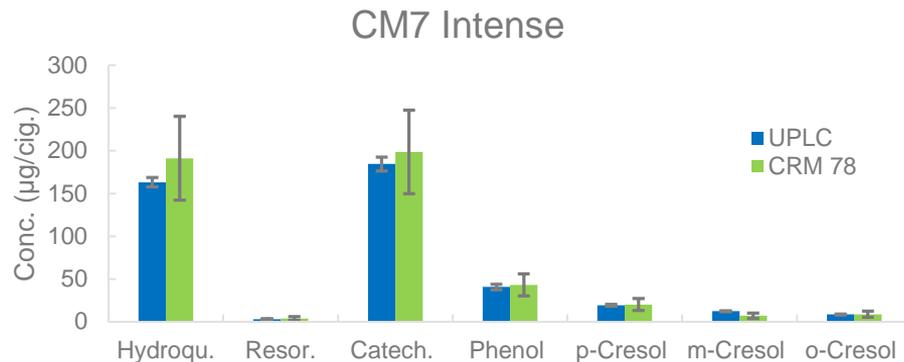
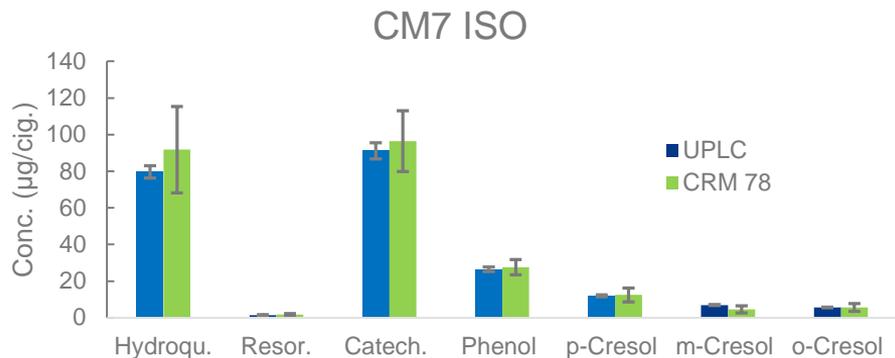
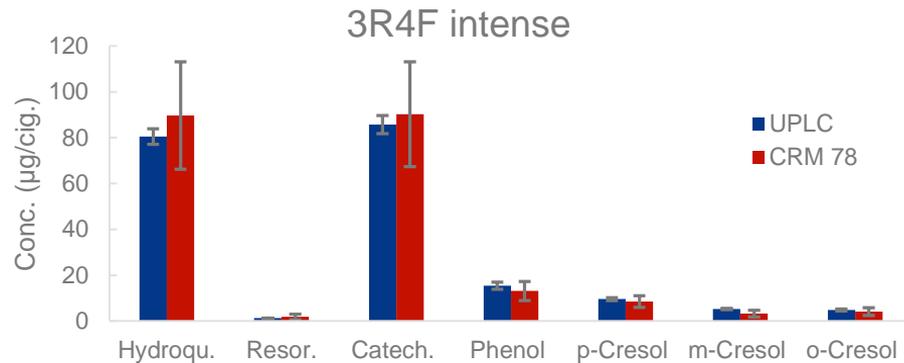
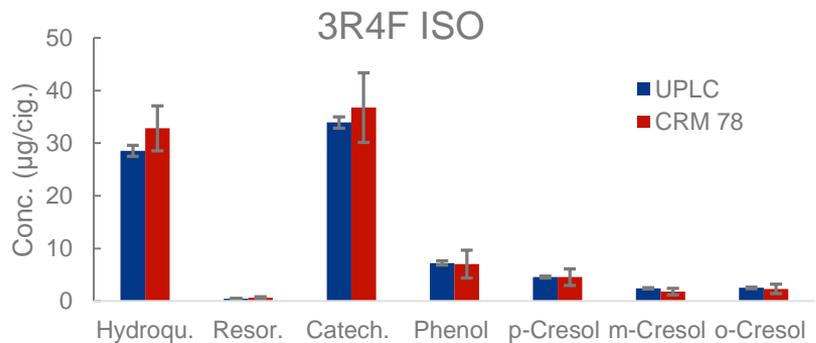
	unit	UPLC	CRM 78
Hydroquinone, catechol, phenol	µg/mL	0.05 - 20	0.2 - 8
Resorcinol	µg/mL	0.01 - 4	0.04 - 1.6
p-cresol, m-cresol, o-cresol	µg/mL	0.01 - 4	0.06 - 2.4

➤ UPLC method has wider dynamic calibration ranges than CRM 78.



# Compared to CRM78

## -Phenols yields in 3R4F and CM 7 smoke

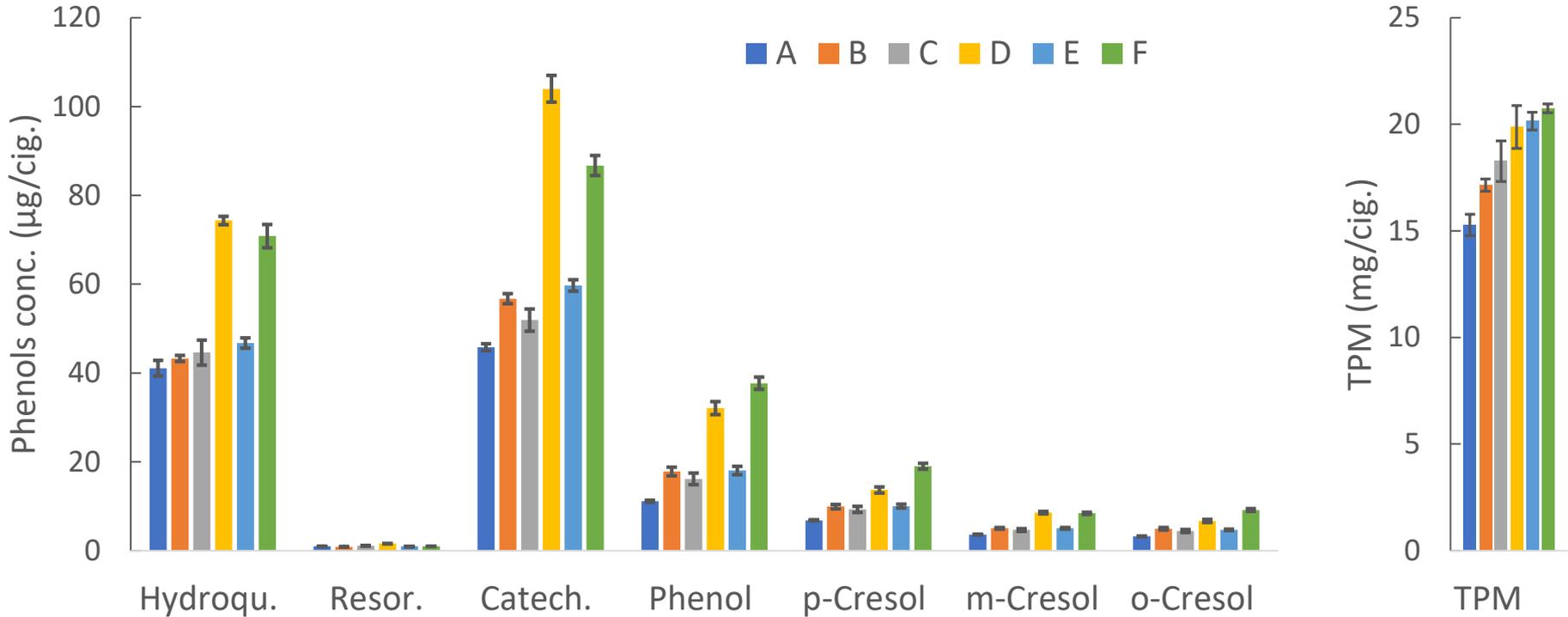


➤ Phenols yields in smoke samples using UPLC method are consistent with those reported in CRM 78.



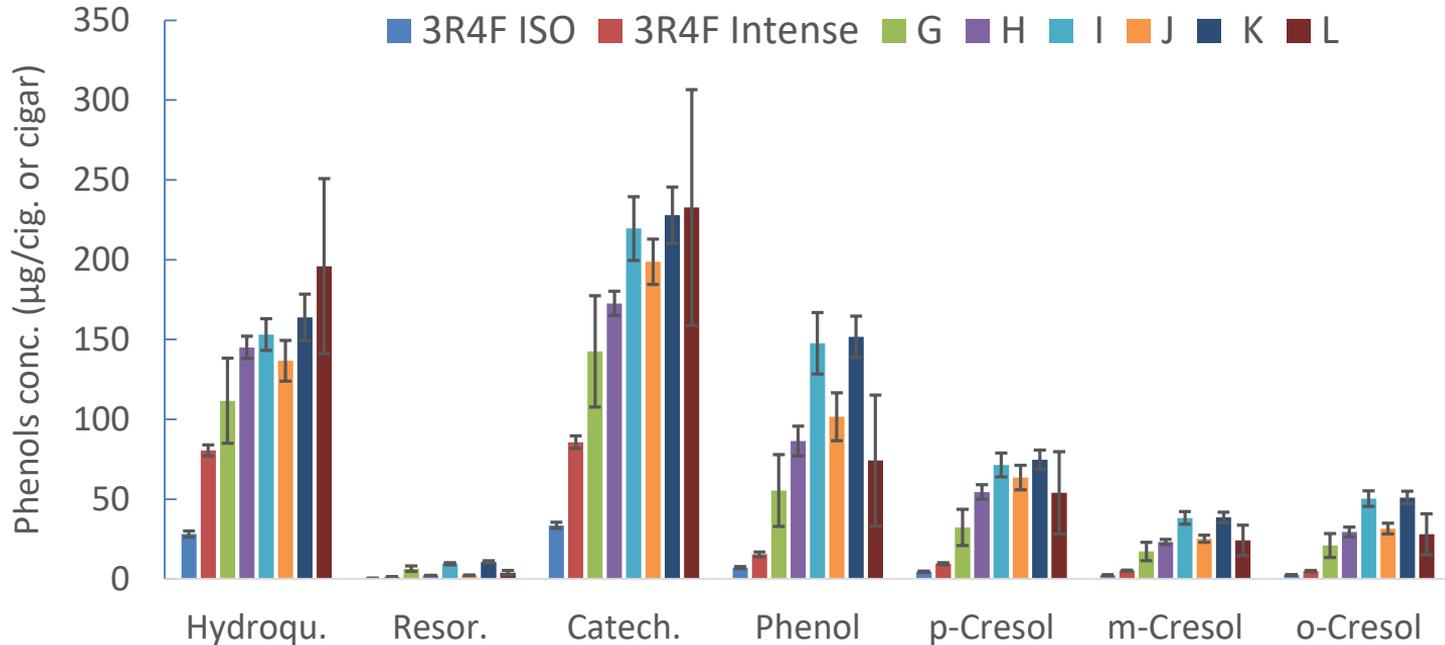
# Phenols yields in market cigarettes ISO smoke

-Commercially marketed American blended cigarettes (n=4)



# Phenols yields 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%)

# 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.