

# Determination of Benzo[a]pyrene in Smokeless Tobacco Products by GC-MS

Chorng B. Huang<sup>1</sup>, William Adams<sup>2</sup>,  
Karl Wagner<sup>1</sup> and **Naren Meruva**<sup>1</sup>

<sup>1</sup> Altria Client Services,

<sup>2</sup> Eurofins Lancaster Laboratories,  
601 East Jackson St, Richmond, VA 23219



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# Background

- The US FDA requires tobacco manufacturers to report quantities of harmful and potentially harmful constituents (HPHCs) in tobacco and tobacco smoke
- Standardized methods are essential for accurate and consistent measurements across the product testing laboratories

HPHCs in Smokeless Tobacco Products (STPs)*	CORESTA Recommended Method
Nicotine	N° 62
Tobacco Specific Nitrosamines (NNN and NNK)	N° 72
Ammonia	N° 73 <sup>#</sup>
<b>Benzo[a]Pyrene (B[a]P)</b>	
Carbonyls (Formaldehyde, Acetaldehyde & Crotonaldehyde)	
Metals (Arsenic and Cadmium)	

No  
standardized  
method exists  
currently

\*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” (Guidance for the Industry, March 2012).

<sup>#</sup> Under scope expansion



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# Objective

- Develop a rapid, sensitive and selective GC-MS method for the determination of B[a]P in STPs
  - Evaluate common workflow, instrument platform and conditions for the determination of B[a]P in various tobacco product types:
    - Cigarette smoke by GC-MS
    - Cigarette filler
    - Snus
    - Moist snuff
    - Dry snuff
    - Chewing tobacco
- } CRM-58/ISO 22634:2008
- } Wide Calibration Range  
High Sensitivity  
High Selectivity

GC-MS: Gas Chromatography Mass Spectrometry; CRM – CORESTA Recommended Method



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# Method Scope:

Product Type	Reference Products	Preparation
Snus pouch	CRP-1	cut the pouch open
Moist snuff	CRP-2	use as-is
Dry snuff	CRP-3	use as-is
Chewing tobacco	CRP-4	freeze ground using liquid nitrogen to <4mm particle size
Tobacco filler	3R4F	freeze ground using liquid nitrogen to <4mm particle size

CRP - CORESTA Reference Product

# Analytical Method

## Gas Chromatography (GC)

GC Column	DB-17MS (30 m x 0.25 mm ID x 0.25 $\mu$ m)
Oven Temperature Program	Initial 200 °C hold for 1.0 min Ramp 25 °C/min to 280 °C Ramp 40 °C/min to 325 °C, hold for 6.67 min
GC Run Time	12.0 min
Column Flow	1.0 mL/min
Inlet Temperature	300 °C
Injection Mode	Pulsed splitless, 25 psi until 0.95 min
Injection Volume	1 $\mu$ L



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# Analytical Method

## Mass Spectrometry (MS)

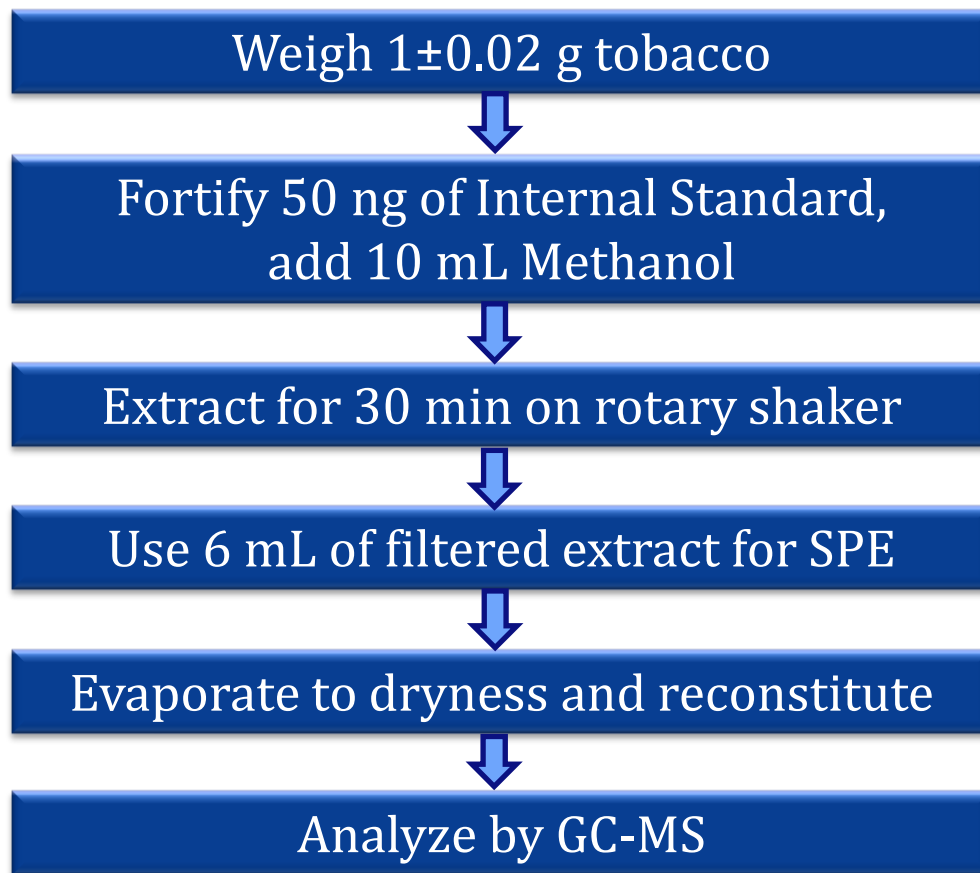
Acquisition	Selected Ion Monitoring mode 6 min solvent delay $m/z = 264/132$ amu for B[a]P-d <sub>12</sub> at 9.04 min $m/z = 252/126$ amu for B[a]P at 9.10 min
Transfer Line Temperature	315 °C
Quad/Source Temperatures	200 °C/250 °C



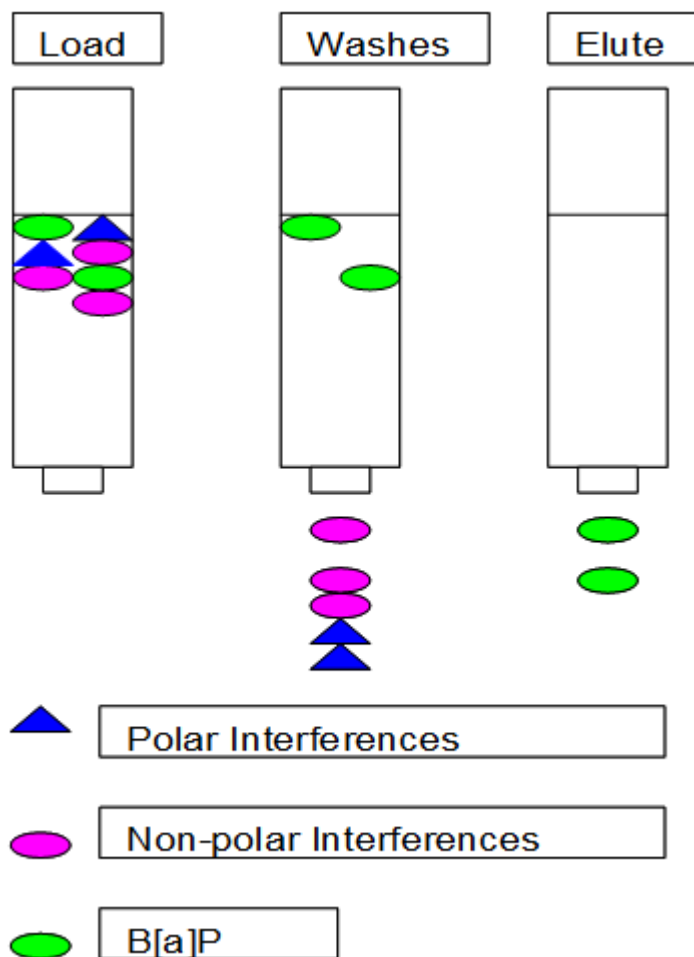
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# Method Workflow



# Solid Phase Extraction (SPE) for B[a]P\*



**SPE using Strata-X™ cartridges**  
(60 mg, 3 mL, 33µm reversed phase):

## Condition

- 3 mL methanol

**Load** 6 mL filtered methanol sample extract

## Wash

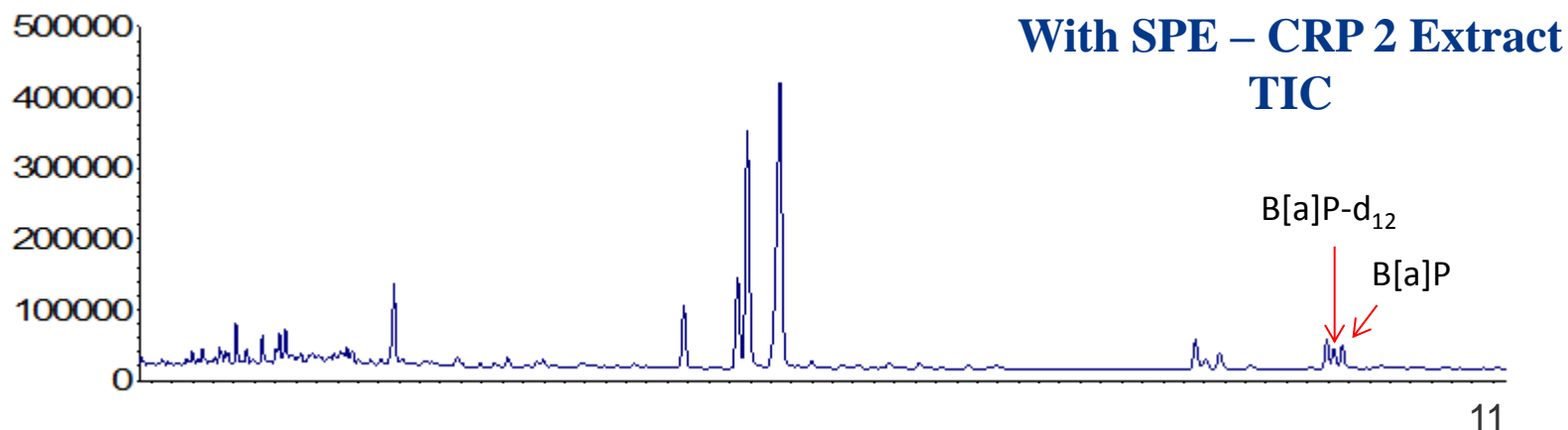
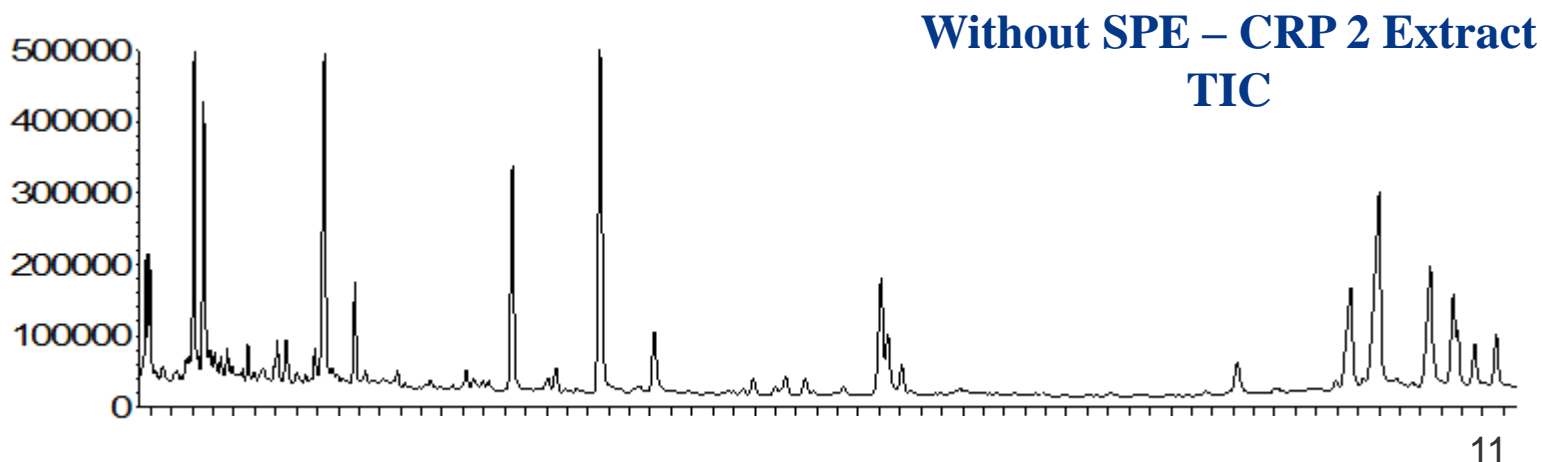
- 2 mL methanol:water (1:1)
- 2 mL isopropanol
- 0.3 mL hexane

**Elute** 3 mL toluene:iso-octane (1:1)

\* Reversed-Phase Sample Clean Up for Analysis of Benzo[a]pyrene by Gas Chromatography/Mass Spectrometry, Celeste Wilkinson and Craig Chwojdak Prepr. Pap.-Am. Chem. Soc., Div. Fuel Chem. 2007, 52 (1)



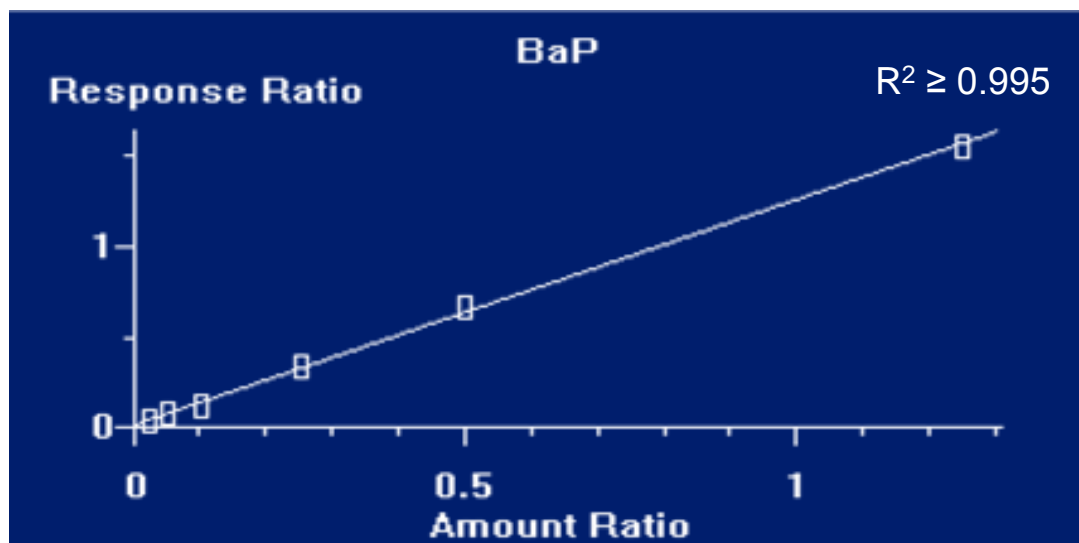
# Importance of SPE Cleanup



**SPE sample cleanup minimizes the matrix interferences  
for B[a]P detection**



# Calibration Range

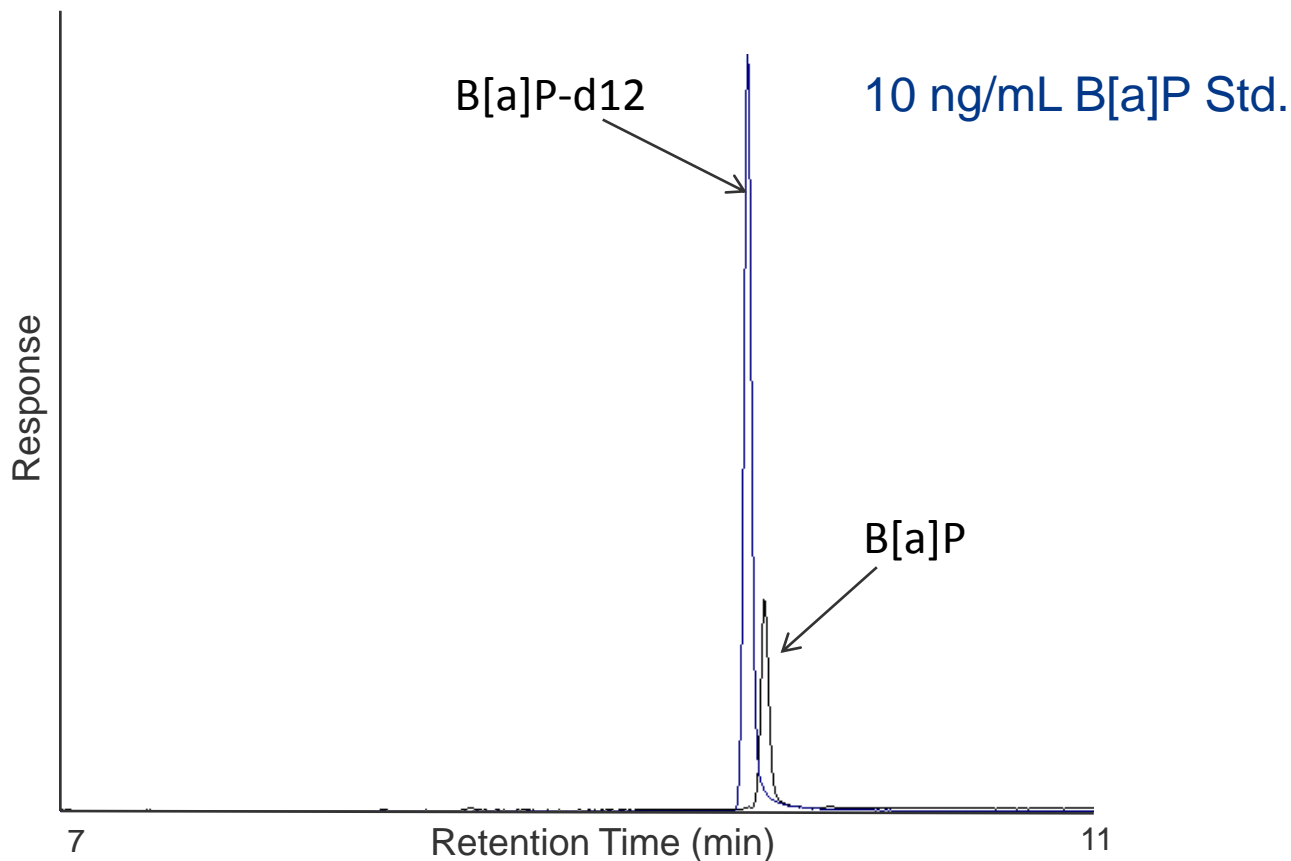


Level	B[a]P (ng/mL)
1	0.5
2	1.0
3	5.0
4	10.0
5	50.0
6	125.0

Extended calibration range covers B[a]P yields observed in various tobacco product types



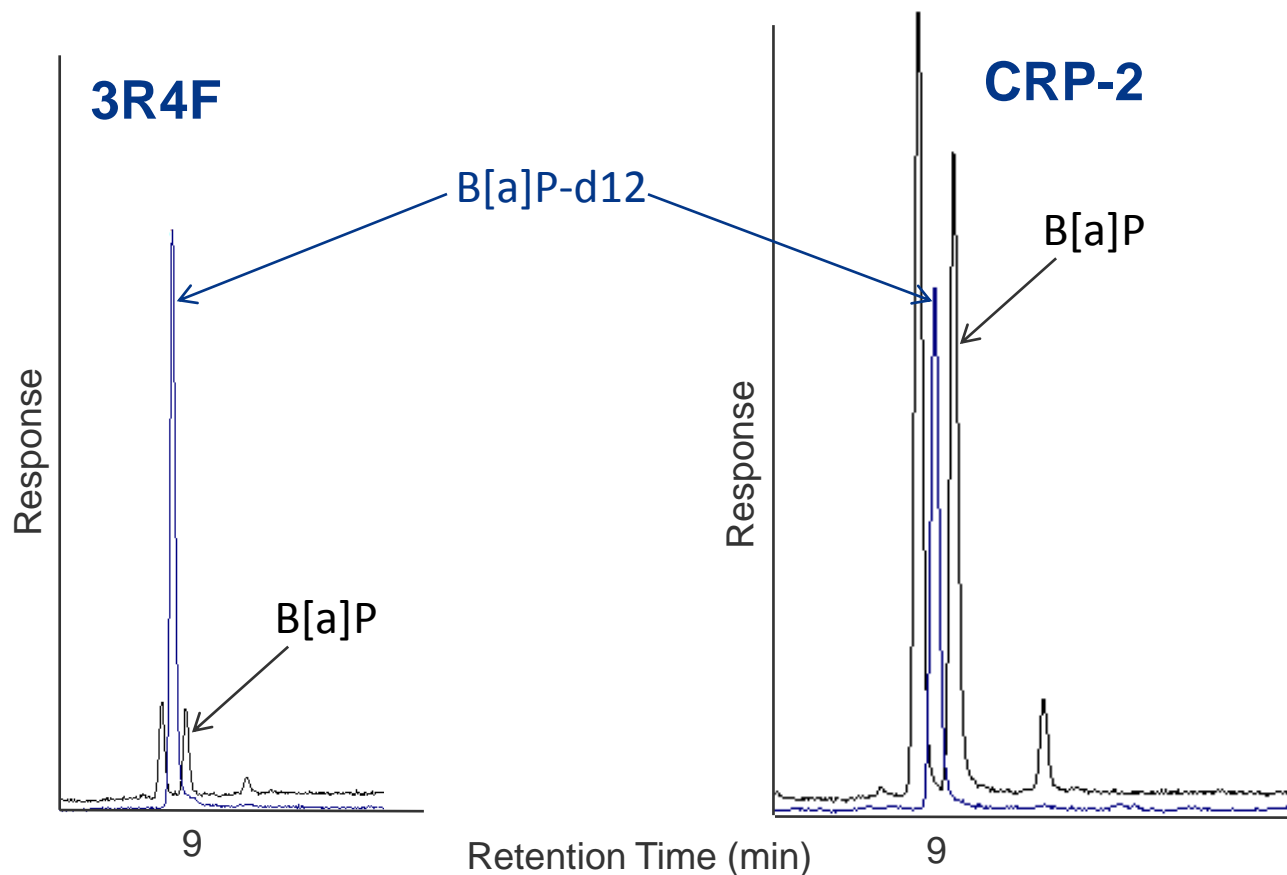
# B[a]P Chromatograms (Calibration Standard)



Selected ion monitoring (SIM) and use of labeled internal standard enables accurate B[a]P detection



# B[a]P Chromatograms (Tobacco Extracts)



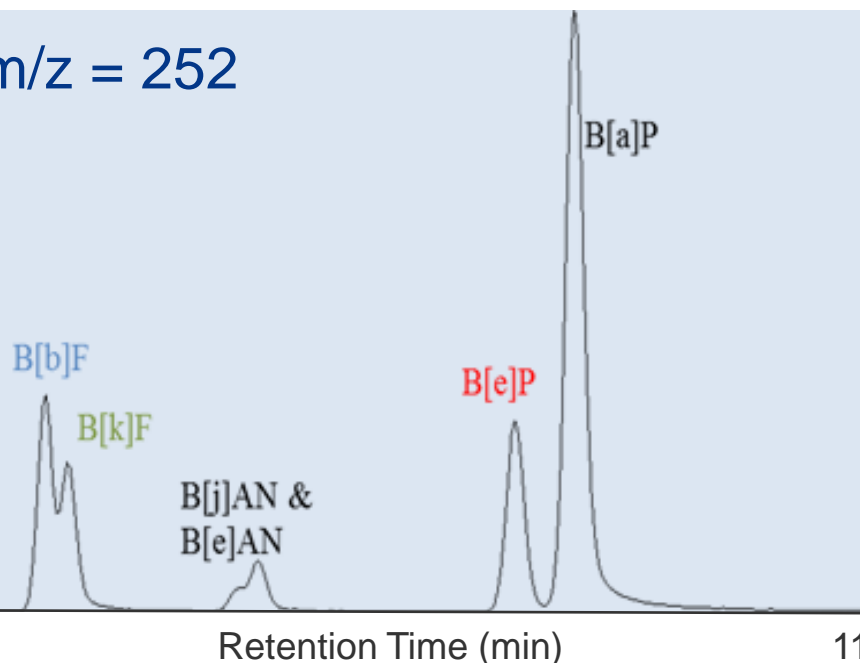
B[a]P levels vary significantly in different tobacco product types



# Selectivity

Sample – Mixture of polycyclic aromatic hydrocarbons (PAHs)

$m/z = 252$



PAH Analyte	$m/z$
B[b]F - Benzo[b]fluoranthene	252
B[k]F - Benzo[k]fluoranthene	252
Benz[j]AN - Benz[j]aceanthrylene	252
B[e]P - Benzo[e]pyrene	252
B[a]P - Benzo[a]pyrene	252

Current method optimized for B[a]P determination  
and could be extended for other PAHs

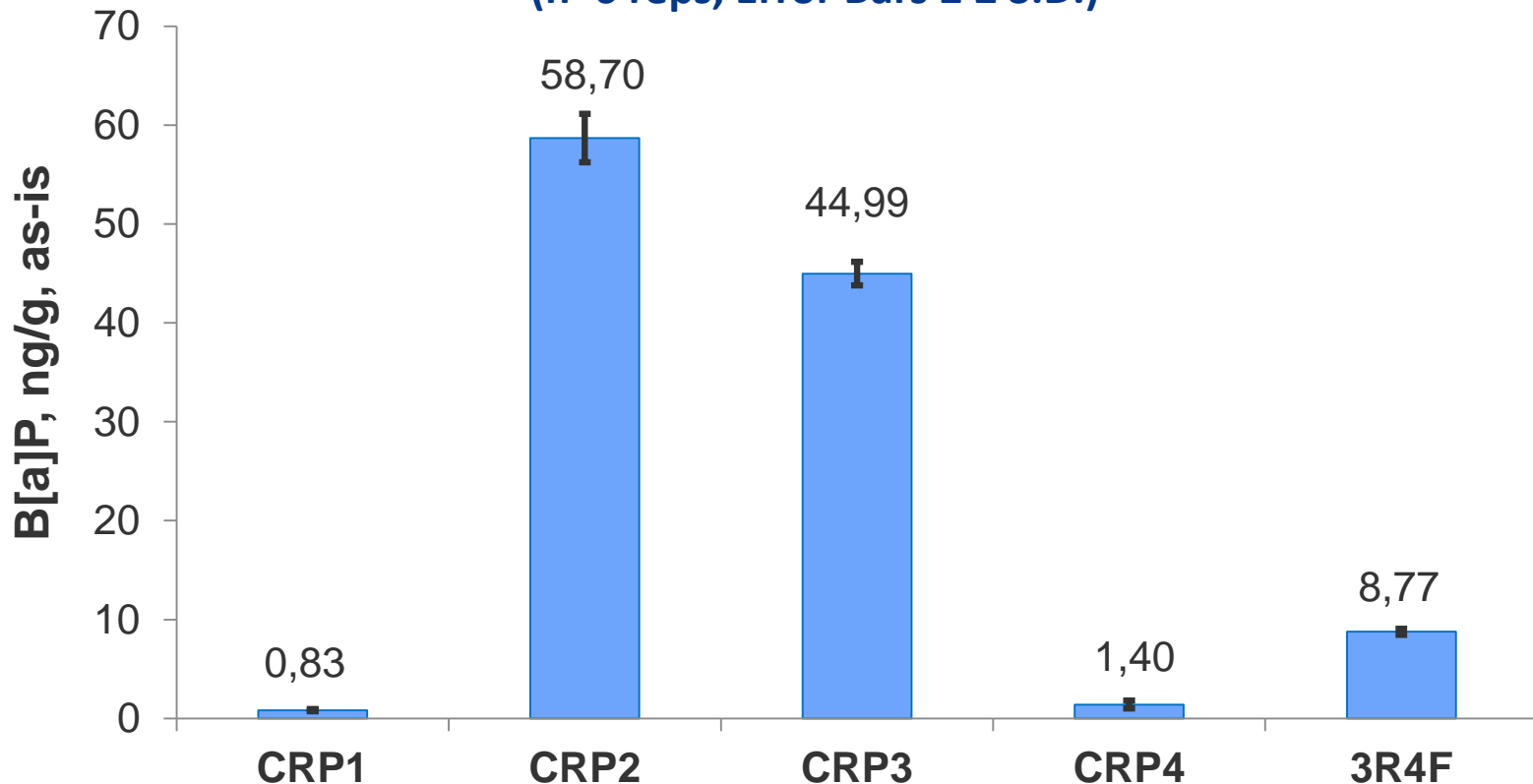
# B[a]P Validation Summary

Parameter	Summary
Calibration	$R^2 > 0.995$ , n=5 days
Accuracy	Average recovery 86.3% to 111.9%
Precision - Instrument	2.8% RSD (n=6)
Precision - Repeatability	3.4-7% RSD (n=6)
Precision – Intermediate	4.2-9.4% RSD (n=6, 3 days)
Selectivity	No interferences observed based on recovery results from standard addition studies
LOQ	0.5 ng/mL (Cal-1)



# Reference Product Data

## B[a]P in Smokeless Tobacco Products (n=6 reps, Error Bars $\pm 2$ S.D.)



# Summary

- Developed and validated a rapid, sensitive and selective GC-MS method for B[a]P in smokeless tobacco
- Suitable for B[a]P determination in snus, moist snuff, dry snuff, chewing tobacco and cigarette filler
- Method workflow consistent with BaP smoke method
- Currently being evaluated by CORESTA smokeless tobacco sub-group to determine inter-lab variability



