A Single-Step Solid-Phase Extraction Method for GC/MS Analysis of Aromatic Amines in Mainstream Cigarette Smoke

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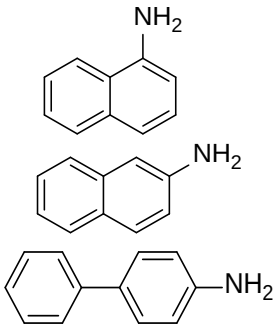
Objective

The purpose of this work is to develop a simple, sensitive, robust and automated SPE procedure for the analysis of aromatic amines (AAs) in mainstream cigarette smoke by gas chromatography and mass spectrometry (GC/MS).

- 1-aminonaphthalene (CAS 134-32-7)
- 2-aminonaphthalene (CAS 91-59-8)
- 4-aminobiphenyl (CAS 92-67-1)

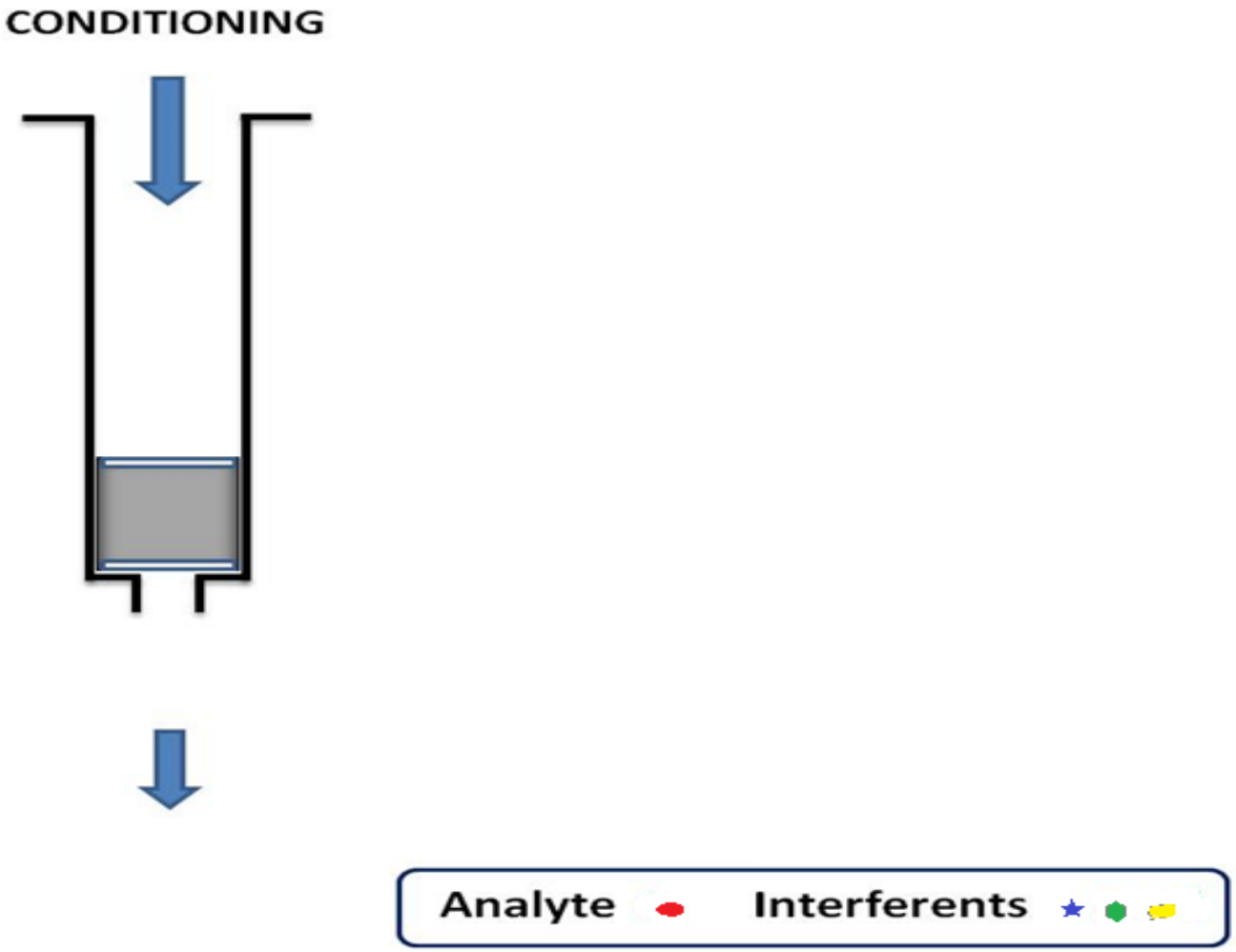


Background

- Cigarette smoke is an extremely complex matrix
- Aromatic Amines (AAs) are present at part-per-billion (ppb) levels in cigarette smoke
- AAs are on the FDA HPHC list*
- Traditional methods for AAs typically employ liquid-liquid extraction
- Solid-phase extraction (SPE) is a viable alternative and offers the following benefits:
  - Reduces matrix interference
  - Concentrates the sample
  - Easily automated

* "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).

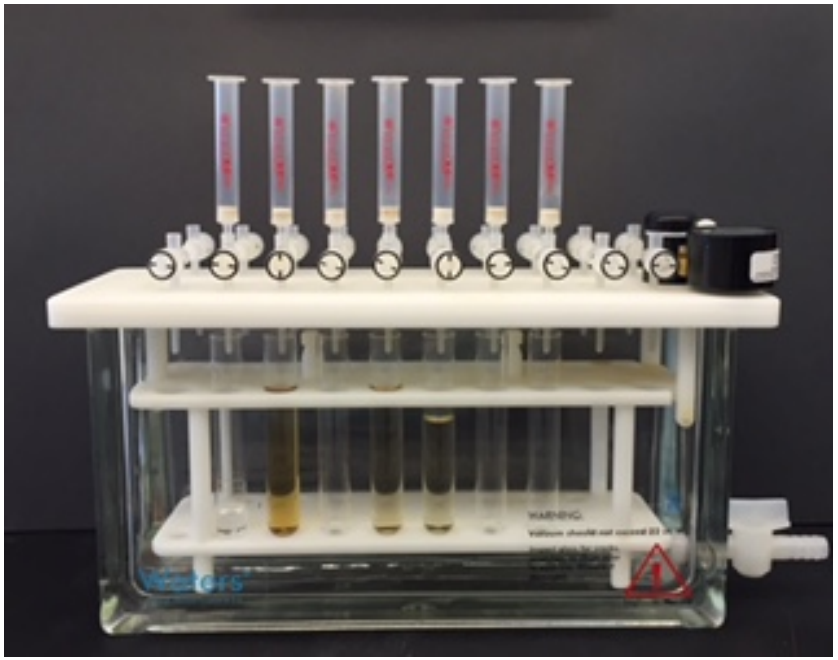
SPE Basics



SPE Equipment

RapidTrace®

Manifold



Published Methods Employing SPE for Measuring AAs in Cigarette Smoke

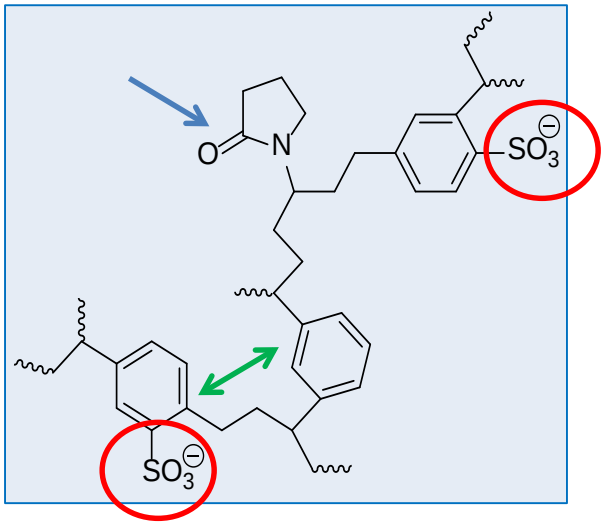
- **C. J. Smith, G.L. Dooly and S.C. Moldoveanu*, "*New Technology using Solid-Phase Extraction for the Analysis of Aromatic Amines in Mainstream Cigarette Smoke*". J. of Chromatography A, 991 (2003)**
- British American Tobacco Group Research & Development, "*Determination of Aromatic Amines in Mainstream Cigarette Smoke*", March 31, 2008
- S. S. Brown, "*Determination of Aromatic Amines through the use of Tandem Mass Spectrometry Coupled to Gas Phase Chromatography*", Enthalpy Analytical, 66th TSRC, 2012
- A. Martin, "*Selected Aromatic Amines by Gas Chromatography Mass Spectrometry: Challenges of Mainstream Cigarette Smoke*". Arista Laboratories, Inc., 67th TSRC, September 15-18, 2013

Chemistry – 2 SPE Cartridges

Moldoveanu *et al., J. of Chromatography A, 991 (2003)*

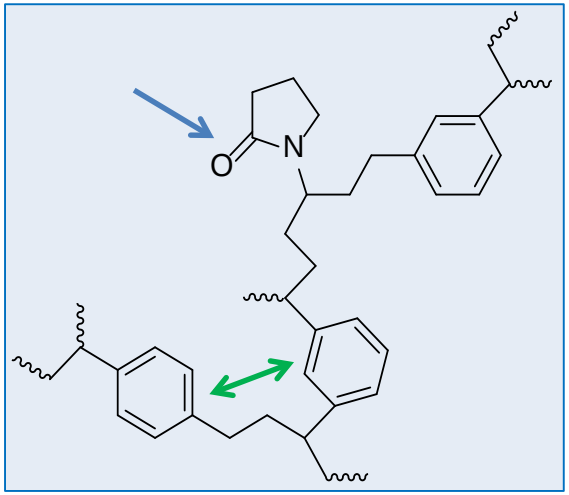
Step 1: Waters Oasis MCX

- Mixed mode reverse phase sorbent
- Polymeric phase
  - Hydrophilic
  - Lipophilic
  - Strong cation exchange
- Selective for bases



Step 2: Waters Oasis HLB

- All purpose reversed phase sorbent
- Polymeric phase
  - Hydrophilic
  - Lipophilic
- Ideal for acidic, basic and neutral species



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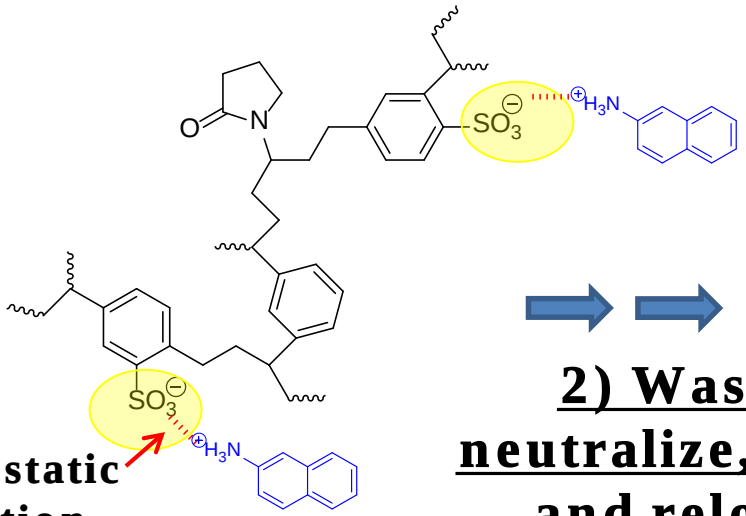
Published 2-Step SPE Retention Mechanism

Moldoveanu *et al., J. of Chromatography A, 991 (2003)*

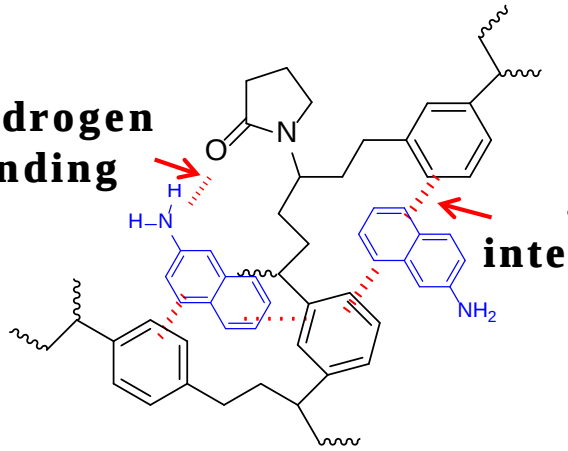
MCX SPE



Electrostatic interaction

2) Wash, neutralize, elute and reload

HLB SPE



Hydrogen bonding

π-π interaction

1) Load acidic smoke extract

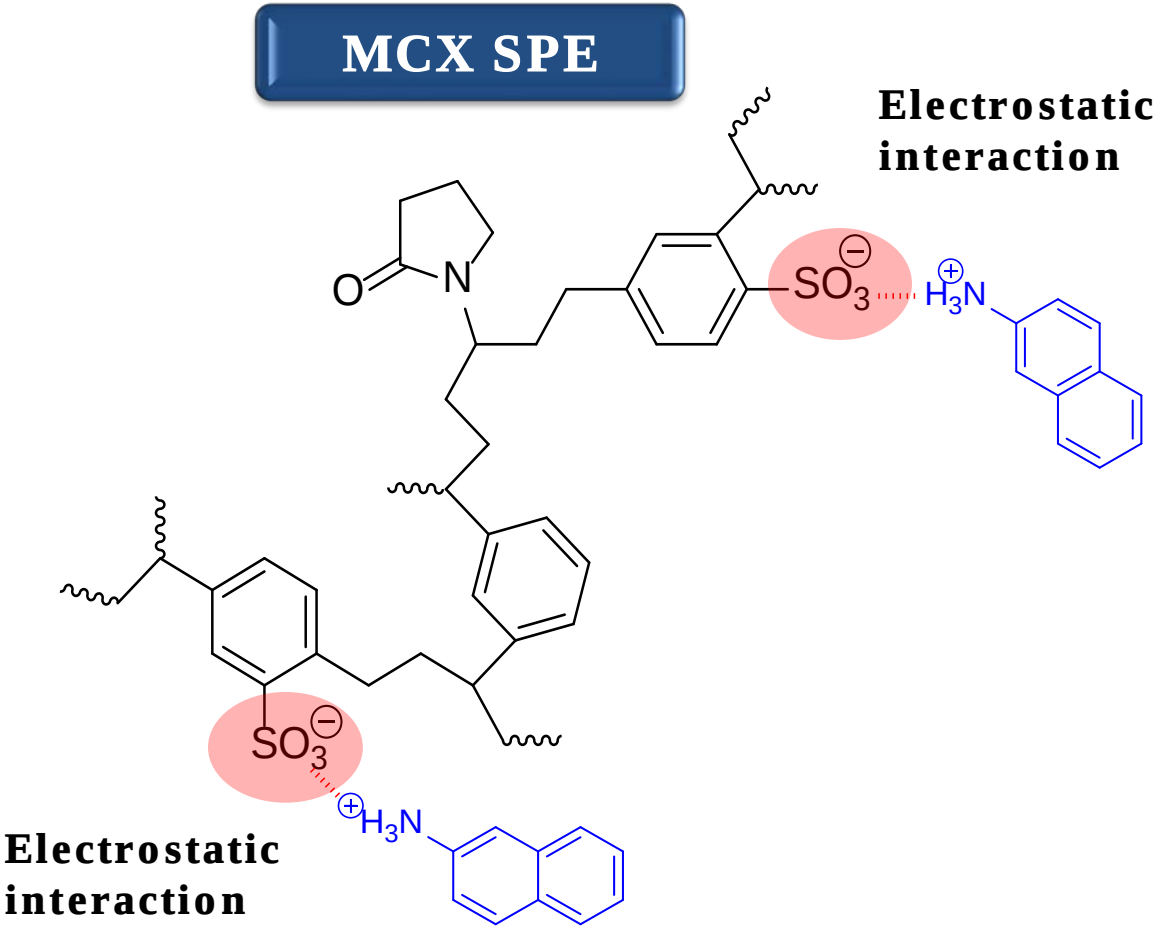
3) Elute from 2nd cartridge

The procedure requires 2 SPE cartridge and 7 reagents

Can This Method be Optimized?

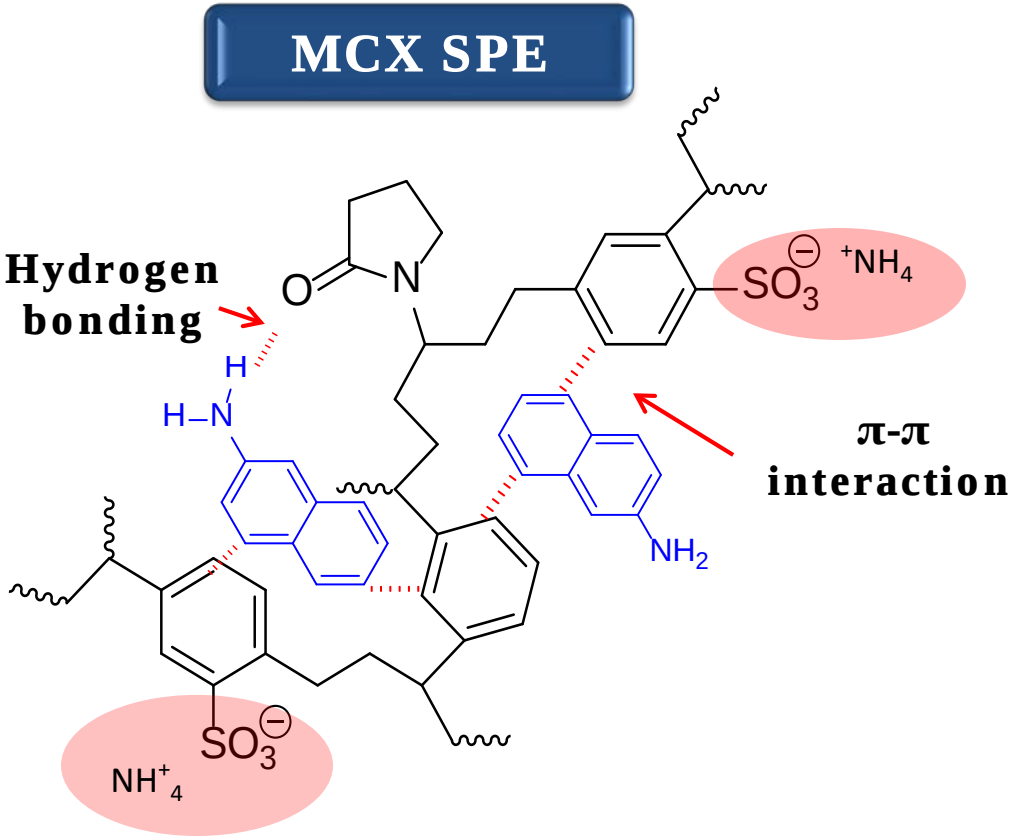
- The MCX and HLB cartridges are very similar
- The MCX has the three types of interaction sites necessary for the entire procedure
  - Strong cation exchange
  - Hydrophilic
  - Lipophilic
- The MCX SPE cartridge is sufficient to accomplish sample cleanup
  - One cartridge
  - Automatable

Proposed 1-Step MCX Retention Mechanism



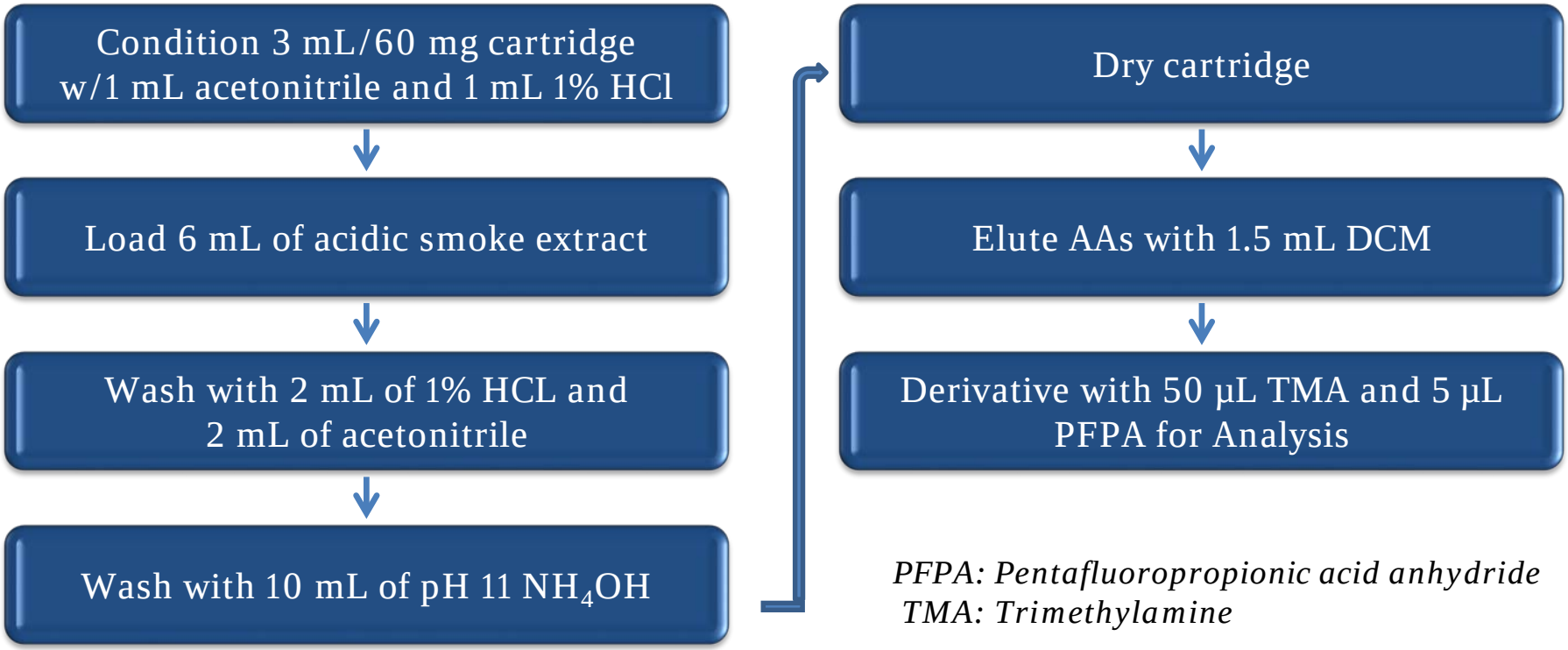
1) Load acidic smoke extract

Proposed 1-Step MCX Retention Mechanism



2) Wash, neutralize and elute from the same SPE cartridge

1-Step SPE Procedure – Oasis MCX Cartridge



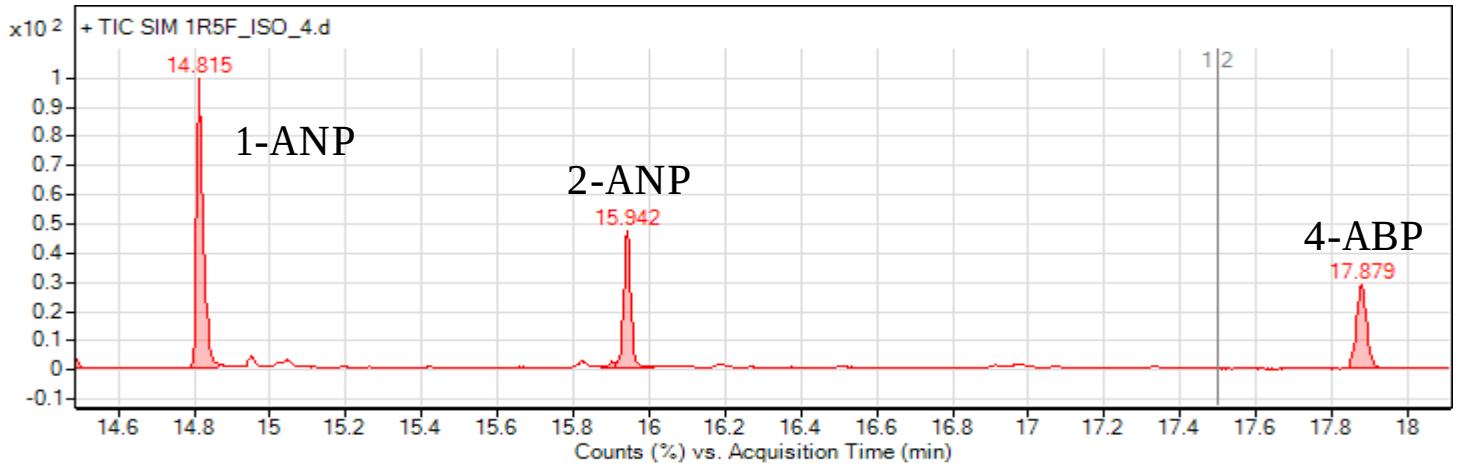
PFPA: Pentafluoropropionic acid anhydride
TMA: Trimethylamine

The procedure requires one SPE cartridge and 4 reagents

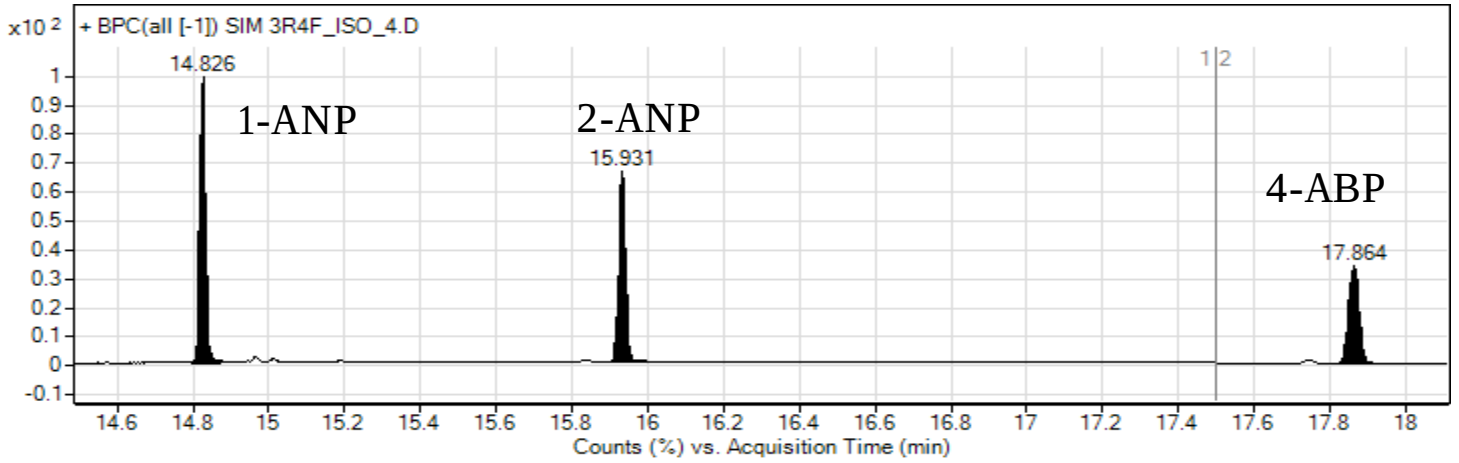
GC-MS Chromatograms

1-Step SPE

1R5F ISO



3R4F ISO



1-ANP = 1-Aminonaphthalene 2-ANP = 2-Aminonaphthalene

4-ABP = 4-Aminobiphenyl

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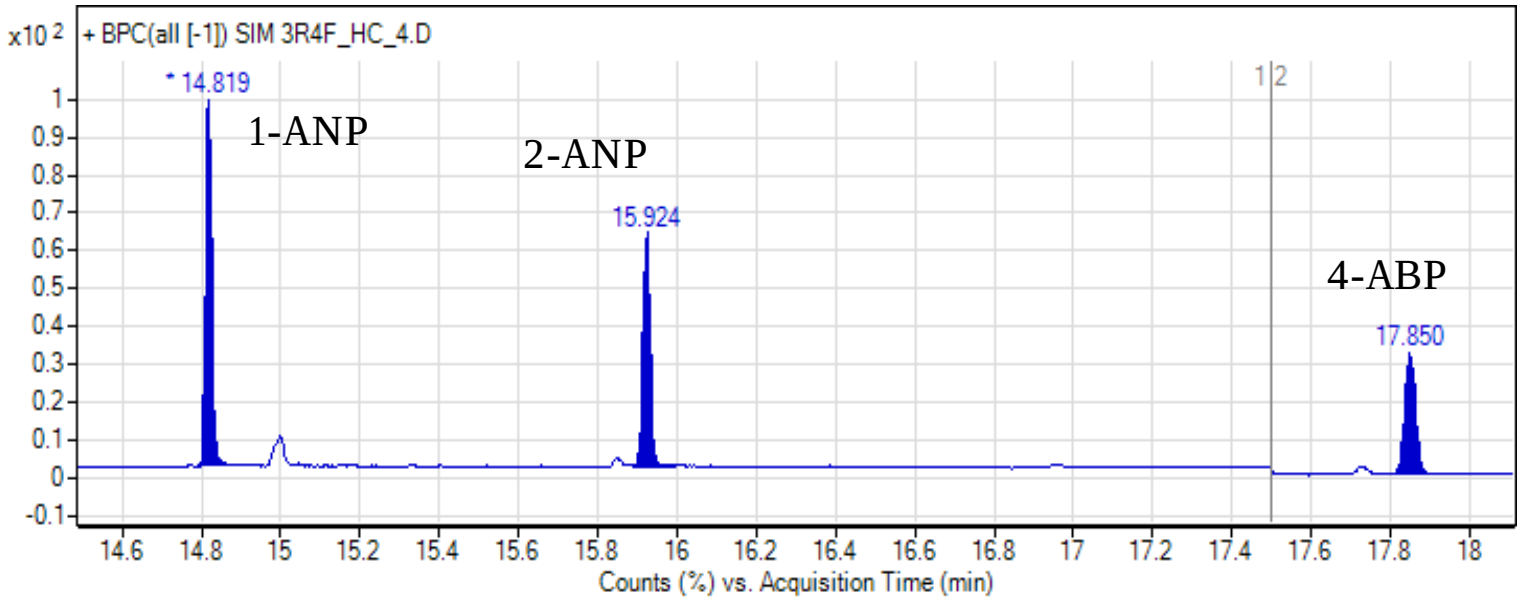
GC-MS Chromatograms

1-Step SPE

3R4F HC



1-ANP = 1-Aminonaphthalene
2-ANP = 2-Aminonaphthalene
4-ABP = 4-Aminobiphenyl
HC = Health Canada smoking regime

Minimal to no matrix interferences observed with reference products

SPE Breakthrough Study

1-Step SPE Method

	1R5F_ISO	3R4F_ISO	3R4F_HC
No. of cigarettes smoke/pad	5	5	**3**
TPM (mg)/Pad	10.5	51.5	**138.3**
TPM (mg) loaded on SPE cartridge	4.2	20.6	**55.3**

Initial waste fraction from the highest TPM sample 3R4F_HC was evaluated for SPE breakthrough

> 99% retention was observed for all analytes

Accuracy-Laboratory Fortified Matrix Spikes
1-Step SPE Method

3R4F ISO	1-ANP	2-ANP	4-ABP
Low (1X)	92.5%	98.8%	85.4%
Medium (5X)	101.9%	110.1%	98.0%
High (10X)	95.2%	106.4%	92.5%
Mean	97%	105%	92%

Mean Accuracies for the 3 AAs ranged from 92% to 105% for three concentrations of matrix spikes

Comparison of AAs Data in Smoke – Historical Data

3R4F_ISO	1-ANP (ng/cig)	2-ANP (ng/cig)	4-ABP (ng/cig)
1-Step SPE method	12.38	7.57	1.22
Liquid-Liquid + SPE *	13.7 (8.5-19.0)	9.1 (6.5-11.7)	1.5 (1.1-1.8)

3R4F_HC	1-ANP (ng/cig)	2-ANP (ng/cig)	4-ABP (ng/cig)
1-Step SPE method	22.75	15.04	3.02
Liquid-Liquid + SPE *	28.9 (22.2-35.6)	18.5 (12.5-24.5)	3.4 (2.6-4.1)

*Data, average (range), were acquired using liquid-liquid extraction followed by SPE cleanup and analysis by GC-MS collected over 3-years (n>148)

1-step SPE AAs data are comparable* to average values collected over 3 years using liquid-liquid extraction followed by SPE cleanup and GC-MS analysis

Previously Published Results

1. Michael Intorp and Steve Purkis “Analysis of Reference Cigarette Smoke Yield Data from 21 Laboratories for 28 Selected Analytes as a Guide to Selection of New CORESTA Recommended Methods”, Beiträge zur Tabakforschung International Contributions to Tobacco Research, Volume 26, No. 2, July 2014

2. Michael Intorp and Steve Purkis, “Determination of Aromatic Amines in Cigarette Mainstream Smoke. - The CORESTA 2007 Joint Experiment” Beiträge zur Tabakforschung International/Contributions to Tobacco Research Volume 24, No. 2, July 2010

Comparison of AAs Data in Smoke – Published Data

3R4F_ISO	1-ANP (ng/cig)	2-ANP (ng/cig)	4-ABP (ng/cig)
1-Step SPE method	12.38	7.57	1.22
Published Data 2014[1]	6.0 – 20.8	3.8 – 12.3	0.8 - 1.7

3R4F_HC	1-ANP (ng/cig)	2-ANP (ng/cig)	4-ABP (ng/cig)
1-Step SPE method	22.75	15.04	3.02
Published Data 2014[1]	16.2 – 32.8	10.2 – 23.4	2.2 – 4.1

1R5F_ISO	1-ANP (ng/cig)	2-ANP (ng/cig)	4-ABP (ng/cig)
1-Step SPE method	5.01	2.46	0.45
Published Data 2010[2]	1.7 – 5.3	1.0 – 2.9	0.2 – 1.2

1-step SPE AAs data are comparable* to published data

*values are within the measured range

Summary

- A 1-Step SPE method for GC/MS analysis of AAs in mainstream cigarette smoke has been developed and validated
- Mean accuracies for the 3 AAs ranged from 92% to 105%
- The automated 1-Step SPE method using the RapidTrace® system reduces laboratory resources, potential human errors, and cost while increasing sample throughput
- The AA results from 3R4F and 1R5F cigarette smoke using 1-step SPE method are comparable to published data

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