ANALYSIS OF AROMATIC AMINES IN MAINSTREAM CIGARETTE AND CIGAR SMOKE BY GC-MS

Jeff Zhu, Charles Brooks, Lee Pittaway
ITG Brands LLC, Greensboro, NC, USA

Abstract

Primary aromatic amines (PAAs) have been routinely tested for cigarette smoke for several years. Typical methods employed require complex sample extraction and clean up procedures, long run times, and extensive instrument maintenance. We sought to develop an improved method for analysis of 1 & 2-aminonaphthalene (1-AN & 2-AN) and 3 & 4-aminobiphenyl (3-AB & 4-AB) with application to cigarette and cigar smoke testing. Simplified sample preparation steps employed in the 2016 CORESTA Aromatic Amines collaborative study for seven PAAs using a method from BAT – Souza Cruz were adopted and modified for use with this method. Based on the referenced technique: steps for liquid-liquid extraction, neutralization, drying solvents and concentrating sample solutions steps were eliminated. For rapid analysis of the target compounds, GC column choice and parameters were optimized to allow for a short run time while improving selectivity and sensitivity over our previous internal method. The optimized method was demonstrated to be applicable to cigarette and machine made cigar smoke.

Challenges with Existing Method

Tedious sample prep process
Use of a range of chemicals, glassware and lab equipment
Frequent maintenance on GC inlet and MS source
Highly variable results

New Method

The smoke pad is extracted with a solution of 24 mL of dichloromethane (DCM) and 1 mL of ISTD Intermediate Solution 2 (see below) in a 40 mL vial using a shaker for 30 min @ 240 rpm. An aliquot of the extract is derivatized with heptafluorobutyric anhydride (HFBA), purified on a Florisil SPE and analyzed by GC/MS-NCI (Agilent 7890A / 5975C).

Standards

Nominal Concentrations	1-AN	2-AN	3-AB	4-AB	1-AN-d7	4-AB-d9	Solvent
Individual Stock Sol (µg/mL)	200	200	200	200	200	200	DCE
Mixed Interm Sol 1 (ng/mL)*	4000	200	800	400	10000	1000	DCE
Mixed Interm Sol 2 (ng/mL)*	50	25	10	5	50	5	DCE
Standard level 1 (ng/mL)	0.5	0.25	0.1	0.05	2	0.2	DCM
Standard level 2 (ng/mL)	1	0.5	0.2	0.1	2	0.2	DCM
Standard level 3 (ng/mL)	2	1	0.4	0.2	2	0.2	DCM
Standard level 4 (ng/mL)	5	2.5	1	0.5	2	0.2	DCM
Standard level 5 (ng/mL)	10	5	2	1	2	0.2	DCM
Standard level 6 (ng/mL)	20	10	4	2	2	0.2	DCM
Standard level 7 (ng/mL)	50	25	10	5	2	0.2	DCM
*: Separate mix for Stds and ISTD.							
DCE: 1,2-Dichloroethane							

Derivatization and SPE clean up

1) 2.5 mL of stds or sample solutions + 25 µL of HFBA, shake for 45 min @ 210 rpm.
2) Condition Florisil SPE cartridge 2 g (or 3 g)/12 mL with 10 mL of DCM.
3) Load derivatization solution, elute by gravity (~ 1 drop per second).
4) Add 5 mL of DCM, elute by gravity (~ 1 drop per second).
5) Combine the eluates, mix well, aliquot to GC vial.

GC Conditions

GC column:	DB-1701, 30 m × 0.25 mm × 1 µm
Carrier gas:	Helium at 1.5 mL/min constant flow
Septum Purge Flow:	3 mL/min
Inlet temperature:	250 °C
Injection mode:	Splitless, then purge at 0.5 min at 50 mL/min
Injection volume:	1 µL
Column temperature:	150 °C (0.1min), 15 °C/min to 260 °C (hold for 5 min) 50 °C/min to 280 °C (0 min)
Total runtime:	15.3 min

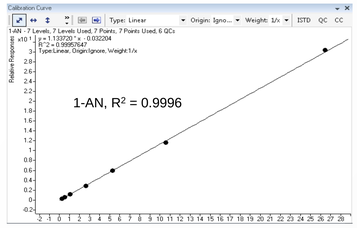
Mass Spectrometry Parameters

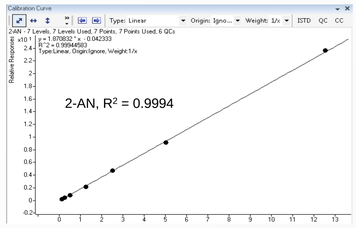
Transfer line temperature:	260 °C
Source temperature:	150 °C
Quadrupole temperature:	106 °C
MS Mode:	NCI/SIM.
Reagent gas:	Methane at 40% flow

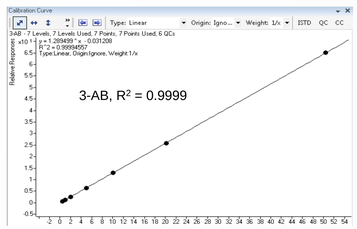
Name	TS	MZ	Type	ISTD Name	CF	CF Origin	CF Weight
1-AN	1	319	Target	1-AN-d7	Linear	Ignore	1/x
2-AN	1	319	Target	1-AN-d7	Linear	Ignore	1/x
1-AN-d7	1	326	ISTD	-	-	-	-
3-AB	2	345	Target	4-AB-d9	Linear	Ignore	1/x
4-AB	2	345	Target	4-AB-d9	Linear	Ignore	1/x
4-AB-d9	2	354	ISTD	-	-	-	-

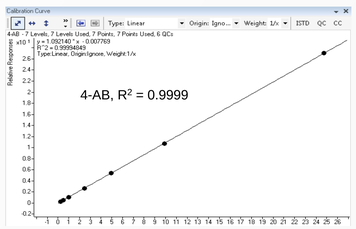
Results

Calibration Curves







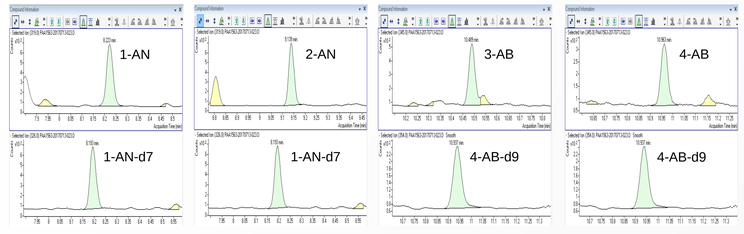


Recoveries

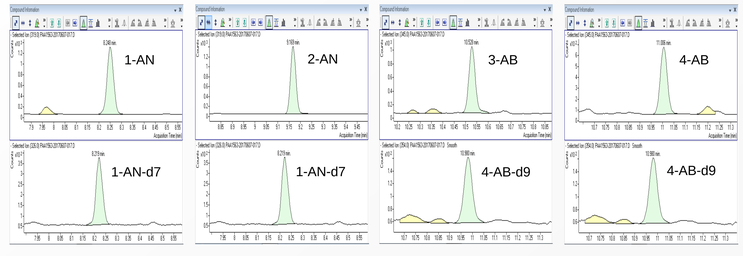
3R4F ISO	1-AN	2-AN	3-AB	4-AB	3R4F HCI	1-AN	2-AN	3-AB	4-AB
Low spike	98.3%	87.2%	92.7%	88.6%	Low spike	103.8%	89.1%	97.7%	106.3%
Mid spike	99.1%	88.2%	91.7%	91.8%	Mid spike	103.3%	90.6%	91.2%	89.6%
High spike	99.6%	87.0%	95.5%	97.3%	High spike	102.5%	86.5%	95.3%	98.7%

Machine Made Cigar	1-AN	2-AN	3-AB	4-AB	TPM (mg/cig)
Cigar Brand 1	106.0%	109.7%	100.4%	106.1%	18.8
Cigar Brand 2	98.5%	88.6%	102.4%	102.0%	51.7

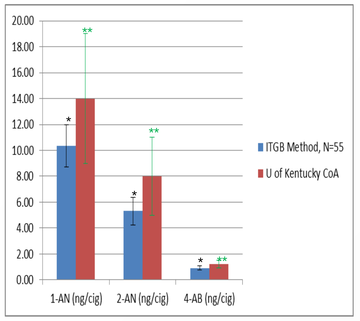
3R4F HCI (CAN Intense) Smoke Sample Chromatograms

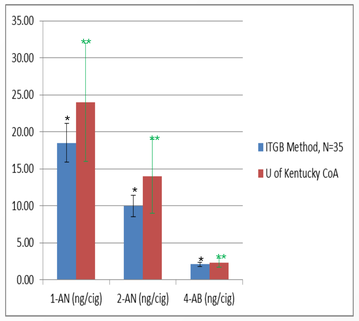


Cigar Brand 2 Smoke Sample Chromatograms



1R6F Data – Comparison to University of Kentucky CoA





*: Expanded uncertainties from 1 lab; **: Expanded uncertainties from 3 labs.
N = Multiple analysts, instruments and days

Conclusions

Summary of the New Method

Laboratory time after smoking reduced by 2/3
Safety: Eliminates the use of HCl, NaOH and **TMA**
Eliminates the use of common glassware such as separatory funnels and Erlenmeyer flask
Eliminates the use of TurboVap and oven
Much less maintenance on GC inlet and MS source required
Less variable results
1R6F results in good agreement with University of Kentucky CoA

Future Development

Further PAA analytes of interest may be added to this method as needed with GC column temperature gradient to be modified if necessary. A wide ranges of cigars (incl premium cigars) will be tested using the new method.