

DETERMINATION OF AROMATIC AMINES THROUGH THE USE OF TANDEM MASS SPECTROMETRY COUPLED TO GAS PHASE CHROMATOGRAPHY

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Methods

The number of compounds of regulatory interest in tobacco and tobacco smoke has increased in recent years. The US FDA Tobacco Products Scientific Advisory Committee (TPSAC) proposed list includes six aromatic amines. The established GC-MS methods for the analysis of aromatic amines involve complex sample preparation and encompass only a portion of the compounds of interest. We have developed a method using GC separation followed by MS/MS detection that is capable of measuring seven aromatic amines in tobacco smoke: *o*-Anisidine (ASD), *o*-Toluidine (TLD), 2,6-Dimethylaniline (DMAN), 1-Aminonaphthalene (1-ANP), 2-Aminonaphthalene (2-ANP), 3-Aminobiphenyl (3-ABP), and 4-Aminobiphenyl (4-ABP). Of these, all but 3-ABP are included in the full HPHC list. *o*-Anisidine-d7, *o*-Toluidine-d9, 2,6-Dimethylaniline-d11, 1-Aminonaphthalene-d7, 2-Aminonaphthalene-d7, and 4-Aminobiphenyl-d9 were used as internal standards.

Sample Preparation

Smoke samples were generated using a rotary smoking machine under either ISO (20 cigarettes/sample) or Canadian Intense (10 cigarettes/sample) conditions. Each sample was collected on 92 mm Cambridge filter pads. Pads were either extracted immediately or stored at < -70°C for up to 14 days.

The sample extraction procedure was adapted from the British American Tobacco (BAT) Group method for aromatic amines in mainstream cigarette smoke¹. In our method the sample extraction volume was reduced from 100 mL to 50 mL in order to increase processing throughput. Samples were extracted with aqueous acid, with the extracts subsequently processed through two solid phase extraction steps and derivatized with heptafluorobutyric anhydride (HFBA) prior to analysis. Separation was achieved using a dual-column GC system with post-run backflushing through the first column.

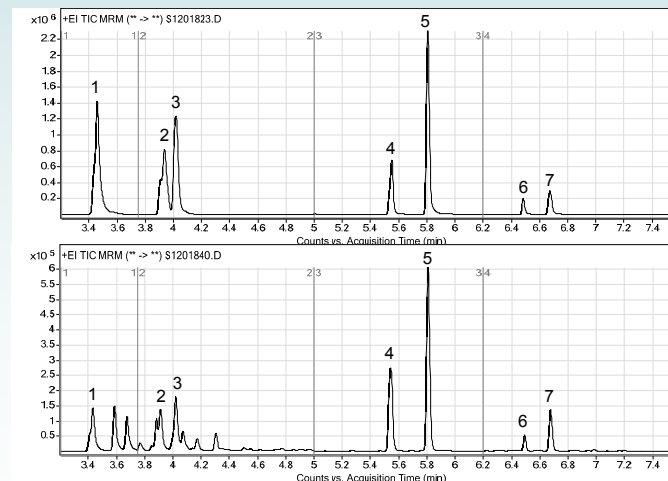
Instrument Conditions

System			Inlet Mode	Pulsed Splitless
Agilent 7890 GC and 7000 QQQ Triple Quad			Inlet Temperature	230 °C
Column			Injection Volume	1 µL
Two HP-5MS 15 m, 0.25 mm, 0.25 µm				
Oven Rate	Oven Temp	Ramp	Flow Rate (Column 1)	1 mL/min
°C/min	°C	minutes	Flow Rate (Column 2)	1.2 mL/min
Initial	100	0.5		
25	250	0	Transfer Line Temperature	280 °C
50	280	0		
Post-Run	280	0.5	Ionization Mode	EI
Backflush Initiation Temperature			Ion Source Temperature	250 °C

Compound Name	CAS #	Quantifier Transition ¹	Qualifier Transition ¹
TLD-d9	194423-7-7	310/141/20/12	310/113/20/30
TLD	95-53-4	303/134/20/12	303/106/20/30
ASD-d7	1219803-70-9	326/157/20/12	326/129/20/30
ASD	90-04-0	319/150.1/20/12	319/122/20/35
DMAN-d11	1092805-05-7	326/157/20/12	326/129/20/30
DMAN	87-62-7	317.1/148.1/20/12	317.1/120/20/30
1-ANP-d7	78832-53-8	346.7/149.1/20/20	346.1/122.1/20/30
1-ANP	134-32-7	339/142/20/30	339/170.1/20/18
2-ANP-d7	93951-94-1	346.7/149.1/20/20	346.1/122.1/20/30
2-ANP	91-59-8	339/142/20/30	339/170.1/20/18
4-ABP-d9	344298-96-0	374.1/177.1/20/20	374.1/205.1/20/20
4-ABP	92-67-1	365.1/168.1/20/30	365.1/196.1/20/155
3-ABP	2243-47-2	365.1/153/20/20	365.1/168.1/20/30
			365.1/196.1/20/15

¹The format is Q1 ion in amu/Q3 ion in amu/dwell in msec/collision energy in percent

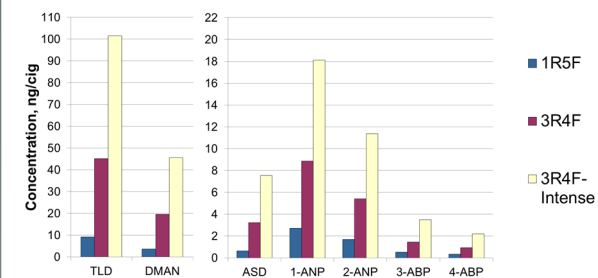
Replicate transitions within the same time segment (ASD-d7/DMAN-d11, 1-ANP/2-ANP, etc.) are monitored singly in the analysis method, then divided into separate analytes upon processing.



Peak ID	Analyte	Peak ID	Analyte	Peak ID	Analyte
1	TLD	4	1-ANP	6	3-ABP
2	DMAN	5	2-ANP	7	4-ABP
3	ASD				

Example Chromatograms – Mid-range Standard (top), 3R4F ISO conditions (bottom)

Aromatic Amines in Tobacco Smoke



Aromatic Amines yields

Compound Name	LOD ng/mL	LOD ng/cig (ISO)
TLD	0.72	0.036
ASD	0.39	0.020
DMAN	0.86	0.043
1-ANP	0.39	0.020
2-ANP	0.15	0.0076
4-ABP	0.12	0.0059
3-ABP	0.094	0.0047

Limits of Detection

Conclusion

We have developed a GC-MS/MS method that allows for the determination of seven aromatic amine compounds in cigarette smoke. This method has a short run time and low detection limits. Use of post-run column backflushing allows for reduced instrument downtime due to inlet and column maintenance, increasing productivity. Incorporation of tandem mass spectrometry produces a clean baseline and results in detection limits for the target aromatic amines was at or below 0.05 ng/cigarette.

References

1. British American Tobacco Group Research & Development Method: "Determination of aromatic amines in mainstream cigarette smoke." March 31, 2008.