A SCREENING METHOD BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY FOR QUANTITATION OF 33 AEROSOL CONSTITUENTS FROM A HEAT-NOT-BURN TOBACCO PRODUCT

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Introduction and Objectives

This screening method allows quantitation of 33 compounds (phenol, *o*-cresol, *m*-cresol, *p*-cresol, catechol, resorcinol, hydroquinone, 1,3-butadiene, isoprene, benzene, acrylonitrile, toluene, pyridine, styrene, propylene glycol, menthol, acrylamide, naphthalene, nicotine, acetamide, quinoline, triacetin, glycerin, carbon disulfide, furan, diacetyl, 2,3-pentanedione, acetol, glycidol, furfural, 2-furanmethanol, caprolactam, and 5-(hydroxymethyl) furfural) in the aerosol generated by an electronically heated tobacco system (PMI's Tobacco Heating System [THS] 2.4) with a single aerosol collection and a single analytical method, where the same extract is analyzed by using three different gas chromatography–mass spectrometry methods (GC–MS).

The aim of the method was to quantify a wide dynamic range of aerosol constituents in an optimized manner for use in routine mode for the development and characterization of heat-not-burn tobacco products.

Sample Preparation Procedure

The aerosol of the electronically heated tobacco, generated on a linear smoking machine (Cerulean SM450) under the Health Canada (HC) smoking regimen, was collected by using two micro-impingers containing a cooled solvent mixture and connected in series after a glass fiber Cambridge filter pad (CFP). After aerosol collection, the impinger solutions and CFP were used together for the extraction (Figure 1). Then, the same aerosol extract was split into three aliquots, where 21 compounds were analyzed on a DB-WAX capillary column (GC–MS system A), three on a DB-624 after derivatization with BSTFA (GC–MS system B), and nine on a DB-FFAP (GC–MS system C). As the quantitation process for carbon disulfide, diacetyl, 2,3-pentanedione, acetol, glycidol, caprolactam, and furanic compounds was developed and validated afterwards on GC–MS system C, 2-furanemethanol was analyzed on GC–MS system A and GC–MS system C (Table 1).

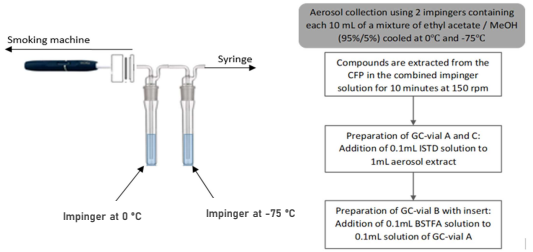


Figure 1: Schematic representation of the process

Method

Analyses are performed on three GC–MS systems (Agilent 7890B) equipped with Masshunter GC–MS Data Acquisition for data acquisition and Masshunter Workstation Software Quantitative Analysis for data treatment.

Table 1: GC conditions

Parameters	GC–MS system A	GC–MS system B	GC–MS system C
Capillary column	DBWAXUI, 30 m x 0.25 mm x 0.5 µm	DB-624, 30 m x 0.32 mm x 1.8 µm	DB-FFAP, 30 m x 0.25 mm x 0.25 µm
Pre-column	2 m, deactivated silica 0.25 µm	None	None
Injector temperature	240°C	220°C	250°C
Injection mode	Split, ratio 1:20	Pulsed splitless	Split, ratio 1:20
Injection volume	1.5 µL	1.0 µL	1.0 µL
Column flow	1.5 mL/min	3.2 mL/min	1.5 mL/min
Column oven:			
- Initial temp	40°C for 2.5 min	60°C for 1.0 min	35°C for 2.0 min
- Ramp 1	30°C/min to 150°C, 0 min	15°C/min to 130°C, 0 min	10°C/min to 200°C, 2 min
- Ramp 2	5°C/min to 200°C, 0 min	4°C/min to 180°C, 0 min	30°C/min to 250°C, 7 min
- Ramp 3	8°C/min to 220°C, 1 min	20°C/min to 250°C, 5 min	
- Ramp 4	30°C/min to 240°C, 8 min		
Total run time	28.333 min	26.667 min	29.167 min

Method

Table 2: MS conditions

Parameters	GC–MS system A	GC–MS system B	GC–MS system C
Ion source	Electron ionisation (EI)	EI	EI
Source temperature	230°C	230°C	230°C
MS quad temperature	150°C	150°C	150°C
Electro-multiplier (EM) settings	Delta electron multiplier voltage (EMV)	Delta EMV	Delta EMV
Acquisition mode	Selected ion monitoring (SIM), quantifier, qualifier ion m/z: 1,3-Butadiene, 54, 53 Isoprene, 67, 68 Benzene, 78, 77 Acrylonitrile, 53, 52 Toluene, 92, 91 Pyridine, 79, 51 Styrene, 104, 103 Propylene glycol (PG), 61, 45 2-Furanmethanol, 98, 97 Menthol, 138, 123 Acetamide, 59 Naphthalene, 128 Nicotine, 162, 161, 163 Acrylamide, 71, 55 Quinoline, 129 Phenol, 94, 95 *p*-Cresol, 108, 107 Triacetine, 145, 116 m-Cresol, 108, 107 p-Cresol, 108, 107 Glycerin, 61, 43	SIM, quantifier, qualifier ion m/z: Catechol, 254, 239 Resorcinol, 239, 254 Hydroquinone, 239, 254	SIM, quantifier, qualifier ion m/z: Carbon disulfide, 76, 78 Furan, 68, 39 Diacetyl, 86, 43 2,3-Pentanedione, 100, 57 Acetol, 74, 43 Glycidol, 44, 31 Furfural, 96, 95 2-Furanmethanol, 98, 97 Caprolactam, 113, 85 5-(Hydroxymethyl) furfural (HMF), 126, 97

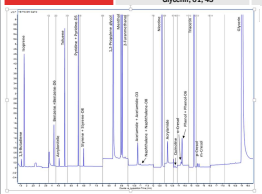


Figure 2: Zoomed-in view of the total ion chromatogram (TIC) from selected ion monitoring (SIM) for the highest calibration solution, analyzed on GC–MS system A.

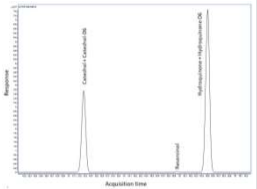


Figure 3: Total ion chromatogram (TIC) from selected ion monitoring (SIM) for the highest calibration solution, analyzed on GC–MS system B

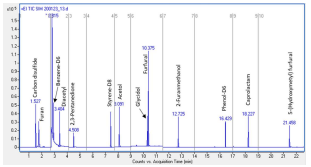


Figure 4: Total ion chromatogram (TIC) from selected ion monitoring (SIM) for the highest calibration solution, analyzed on GC–MS system C

The quantitation is performed by external calibration by using calibration curves with evenly distributed concentration levels and internal standardization for correction of instrumental drift and injection variability, covering a wide range of concentrations, which is compound-dependent (e.g., 1 ng/mL for quinoline and 210 µg/mL for glycerin for the lowest standard level).

Results

The method was validated in accordance with the guidelines of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) [1] and Association of Official Analytical Chemists[2].

Table 3: Summary of results of the evaluated parameters for 33 compounds in PMI's Tobacco Heating System (THS) 2.4) aerosol, collected under the Health Canada smoking regimen (12 puffs per item)

Compound	LOD (µg/item)	LLOQ (µg/item)	ULOQ (µg/item)	LWRL (µg/item)	UWRL (µg/item)	Nominal content (µg/item)	r (µg/item)	IP (µg/item)	$CD_{0.05}$ (µg/item)
1,3-Butadiene	0.0431	0.144	4.32	0.144	2.71	0.226	0.0703	0.0709	0.0365
Isoprene	0.0584	0.195	42.0	0.760	26.1	1.65	0.619	0.647	0.362
Benzene	0.0115	0.0382	4.80	0.235	3.17	0.529	0.151	0.207	0.160
Acrylonitrile	0.0147	0.0489	2.28	0.0760	1.45	0.133	0.0348	0.0374	0.0221
Toluene	0.0177	0.0590	10.0	0.695	6.85	1.52	0.522	0.662	0.484
Pyridine	0.0142	0.0475	16.0	3.53	13.9	7.28	2.01	2.82	2.22
Styrene	0.00210	0.00700	1.00	0.261	0.918	0.553	0.209	0.215	0.115
PG	4.71	15.7	1080	191	882	396	99.6	129	96.5
Menthol	5.46	18.2	3000	120	2040	NA	NA	NA	NA
2-Furanmethanol	0.138	0.458	80.0	15.4	66.8	32.6	10.3	14.7	11.7
Acetamide	0.182	0.605	24.00	1.65	16.4	3.42	0.950	0.950	0.358
Naphthalene	0.00107	0.00355	0.100	0.00596	0.0753	0.0116	0.00357	0.00522	0.00420
Nicotine	4.12	13.7	2250	644	1802	1252	279	288	156
Acrylamide	0.0415	0.138	12.0	0.757	8.12	1.57	0.381	0.429	0.274
Quinoline	0.00109	0.00364	0.120	0.00557	0.0758	0.00597	0.00203	0.00252	0.00181
o-Cresol	0.00161	0.00537	0.360	0.0459	0.272	0.0941	0.0472	0.0496	0.0282
Phenol	0.00443	0.0148	4.00	0.835	3.42	1.78	0.894	0.928	0.511
Triacetin	2.01	6.69	1500	257	1333	510	132	149	96.0
p-Cresol	0.00209	0.00697	0.360	0.0369	0.261	0.0864	0.0394	0.0485	0.0345
m-Cresol	0.00144	0.00479	0.360	0.0204	0.241	0.0425	0.0212	0.0225	0.0131
Glycerin	24.6	81.9	8400	2318	8709	4697	1358	1526	971
Catechol	0.0224	0.0746	32.0	7.08	27.8	14.7	3.94	3.94	1.87
Resorcinol	0.00057	0.00189	0.120	0.0178	0.094	0.0349	0.0113	0.0141	0.0101
Hydroquinone	0.0111	0.0369	16.0	3.49	13.9	7.02	2.63	2.63	1.26
Carbon disulfide	0,0854	0,285	156	1,17	11,7	<LOD	NA	NA	NA
Furan	0,171	0,570	156	2.57	16,9	5,26	0,567	0,573	0,296
Diacetyl	0,0336	0,112	156	5.53	67.0	57,8	6,65	7,07	4,10
Pentanedione	0,0515	0,172	156	1.70	24.3	13,0	1,40	1,40	0,357
Acetol	0,182	0,607	468	37.1	406	373	66,5	66,5	28,1
Glycidol	0,218	0,728	293	3.89	35.7	6,12	1,77	1,77	0,842
Furfural	0,0229	0,0763	156	3.44	46.1	34,8	3,50	4,29	3,04
2-Furanmethanol	0,0554	0,185	156	3.47	46.4	34,7	8,68	8,68	1,79
Caprolactam	0,0923	0,308	156	1,17	11,7	<LOD	NA	NA	NA
HMF	0,132	0,439	156	2.56	34.3	21,9	4,36	4,71	2,82

LOD - limit of detection
LLOQ - lower limit of quantitation
ULOQ - upper limit of quantitation
LWRL - lower working range limit
UWRL - upper working range limit

Discussion and Conclusion

The purpose of the developed method was to allow quantitation of 33 compounds in the aerosol generated by electronically heated tobacco by decreasing the numbers of aerosol collection methods from three to one and analytical methods from five to one (comprising three instrumental methods).

The method was successfully validated and shown to be selective, precise, and accurate over the tested concentration ranges for all compounds.

References

1. ICH Harmonized Tripartie Guideline: Validation of Analytical Procedures: Text and Methodology Q2(R1), 2005
2. AOAC official methods of analysis (2012) Guidelines for standard method performance requirements, Appendix F

Competing Financial Interest – The research described in this poster was sponsored by Philip Morris International

Introduction and Objectives

❖ **Screening method** for the quantitation of **33 compounds**

Parameters	GC–MS system A	GC–MS system B	GC–MS system C
Ion source	Electron ionisation (EI)	EI	EI
Source temperature	230°C	230°C	230°C
MS quad temperature	150°C	150°C	150°C
EM)settings	Delta EMV	Delta EMV	Delta EMV
Acquisition mode	(SIM), quantifier, qualifier ion m/z: 1,3-Butadiene, 54, 53 Isoprene, 67, 68 Benzene, 78, 77 Acrylonitrile, 53, 52 Toluene, 92, 91 Pyridine, 79, 51 Styrene, 104, 103 Propylene glycol (PG), 61, 45 2-Furanmethanol, 98, 97 Menthol, 138, 123 Acetamide, 59 Naphthalene, 128 Nicotine, 162, 161, 163 Acrylamide, 71, 55 Quinoline, 129 Phenol, 94, 95 *o*-Cresol, 108, 107 Triacetine, 145, 116 *m*-Cresol, 108, 107 *p*-Cresol, 108, 107 Glycerin, 61, 43	SIM, quantifier, qualifier ion m/z: Catechol, 254, 239 Resorcinol, 239, 254 Hydroquinone, 239, 254	SIM, quantifier, qualifier ion m/z: Carbon disulfide, 76, 78 Furan, 68, 39 Diacetyl, 86, 43 2,3-Pentanedione, 100, 57 Acetol, 74, 43 Glycidol, 44, 31 Furfural, 96, 95 2-Furanmethanol, 98, 97 Caprolactam, 113, 85 5-(Hydroxymethyl) furfural (HMF), 126, 97

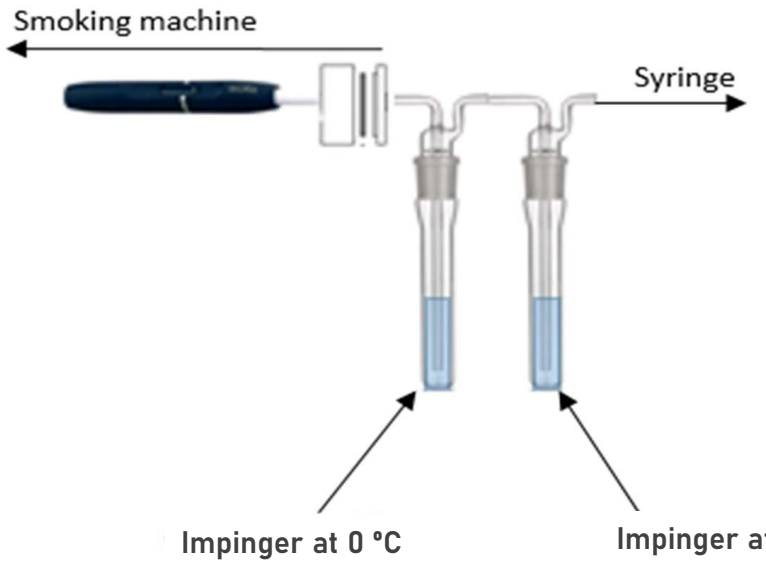
- in the aerosol generated by an electronically heated tobacco system (PMI's Tobacco Heating System (THS) 2.4
- one aerosol collection
- one analytical method

Same extract is analyzed by using three different gas chromatography–mass spectrometry methods (GC–MS)

** EM : Electro-multiplier*
** EMV : Electron multiplier voltage*
** SIM : Selected ion monitoring*

Sample Preparation Procedure

❑ Aerosol of the electronically heated tobacco ❑ Linear smoking machine (Cerulean SM450) ❑ Health Canada (HC) smoking regimen



Aerosol collection using 2 impingers containing each 10 mL of a mixture of ethyl acetate / MeOH (95%/5%) cooled at 0°C and -75°C

↓

Compounds are extracted from the CFP in the combined impinger solution for 10 minutes at 150 rpm

↓

Preparation of GC-vial A and C: Addition of 0.1mL ISTD solution to 1mL aerosol extract

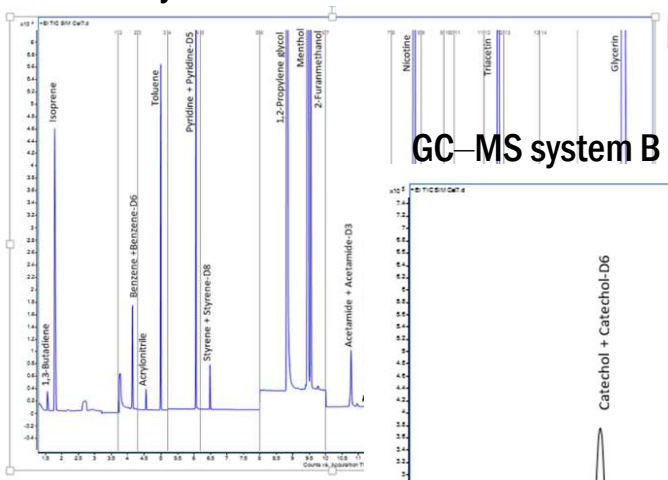
↓

Preparation of GC-vial B with insert: Addition of 0.1mL BSTFA solution to 0.1mL solution of GC-vial A

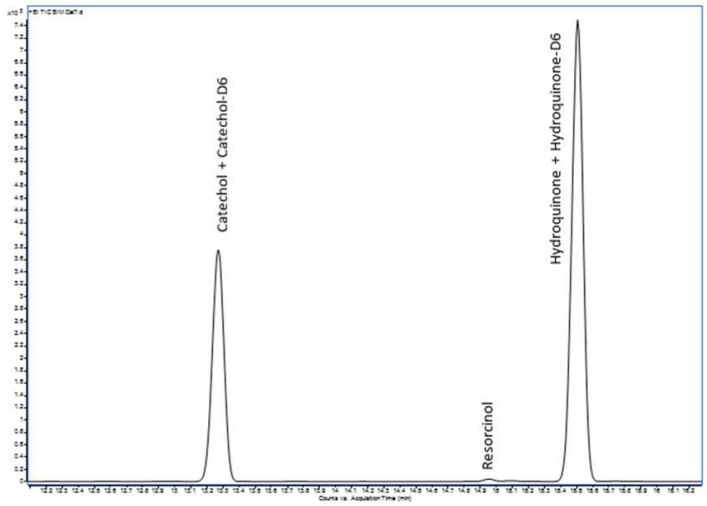
** BSTFA - N,O-Bis(trimethylsilyl)trifluoroacetamide*
** CFP – Cambridge Filter Pad*
** ISTD – Internal Standard*

Chromatography profile

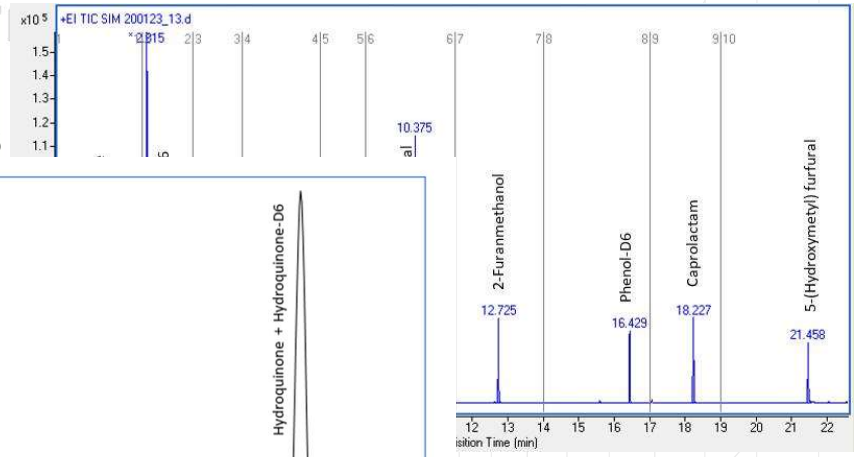
GC–MS system A



GC–MS system B



GC–MS system C



Results

Compound	LOD	LLOQ	ULOQ	LWRL	UWRL	Nominal content	r	IP	$CD_{0.95}$
	(µg/item)	(µg/item)	(µg/item)	(µg/item)	(µg/item)	(µg/item)	(µg/item)	(µg/item)	(µg/item)
Quinoline	0.00109	0.00364	0.120	0.00557	0.0758	0.00597	0.00203	0.00252	0.00181
Glycerin	24.6	81.9	8400	2318	8709	4697	1358	1526	971
1,3-Butadiene	0.0431	0.144	4.32	0.144	2.71	0.226	0.0703	0.0709	0.0365

Concentration range

Polarity

Volatility

The purpose of the developed method was to allow ***quantitation of 33 compounds*** in the aerosol generated by an electronically heated tobacco system by decreasing the numbers of aerosol collection methods from three to one and analytical methods from five to one, comprising of three instrumental methods.

The method was successfully validated and shown to be ***selective***, ***precise,*** and ***accurate*** over the tested concentration ranges for all compounds.