Determination of 2-Chloro-1,3-propanediol (2-MCPD) and 3-Chloro-1,2-propanediol (3-MCPD) in Emissions by Gas Chromatography-Mass Spectrometry (GC-MS)

Jiaming Wang, Angel Rodriguez-Lafuente and Peter Joza

LABSTAT INTERNATIONAL INC.

262 Manitou Drive, Kitchener, Ontario, Canada N2C 1L3

Phone: (519) 748-5409 Fax: (519) 748-1654 Web: www.labstat.com

74th TOBACCO SCIENCE RESEARCH CONFERENCE

Boston, Massachusetts USA

29th - 31st August 2021

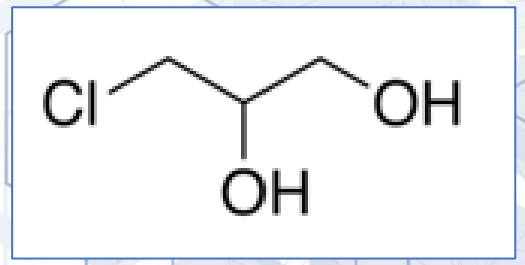
Labstat
A Certified Group Company

BACKGROUND

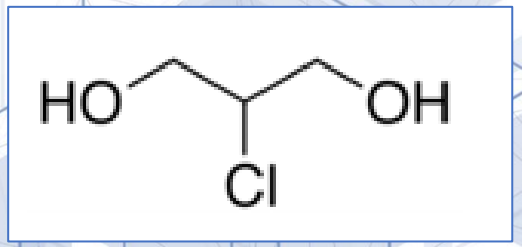
Monochloropropanediol (MCPD) and glycidol are thermally generated toxicants found in refined vegetable oils and glycerin.

- Both MCPD and glycidol are primarily produced when foods containing fats/oils and salts are heated.
- 3-MCPD has evidenced a carcinogenic effect in animal tests, in addition to other effects and is classified by the IARC as possibly carcinogenic to humans (category 2B).
- Regulatory limits include 0.1 mg/kg as per Regulation (EU) No. 231/2012 for the food additive glycerol.

3-MCPD



2-MCPD



Why is this important to nicotine containing products?

Labstat
A Certified Company

THE POTENTIAL OF MCPD IN TOBACCO PRODUCT EMISSIONS

Tobacco Filler – Combustible products

- Contain glycerol as a humectant.
- Contain inorganic chlorides and organic acids
- Heat – exposure to various temperatures

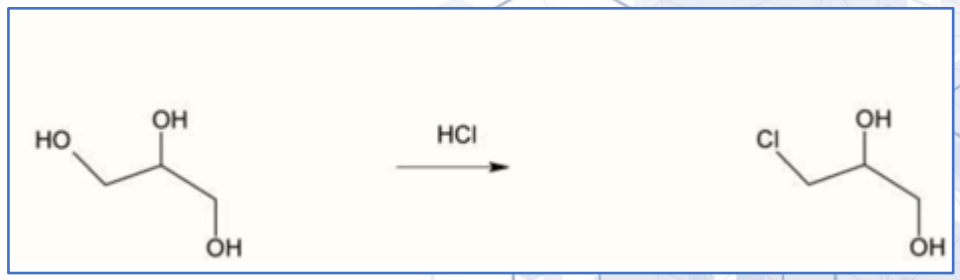
Heated Tobacco Products – Sticks / Test Pieces / Substrates

- Typically, higher glycerol content than combustibles
- May contain some inorganic chlorides and organic acids
- Controlled heating

Vaping Products - ENDS

- Predominantly a vegetable glycerin (VG) and propylene glycol (PG) matrix
- May contain some inorganic chlorides and organic acids (e.g. oxalic, glycolic, lactic, formic, acetic, etc.)
- Controlled heating

Simple Substitution?



Glycerol + Heat + Chlorides ⟶ MCPD ?

Labstat
A Certified Company

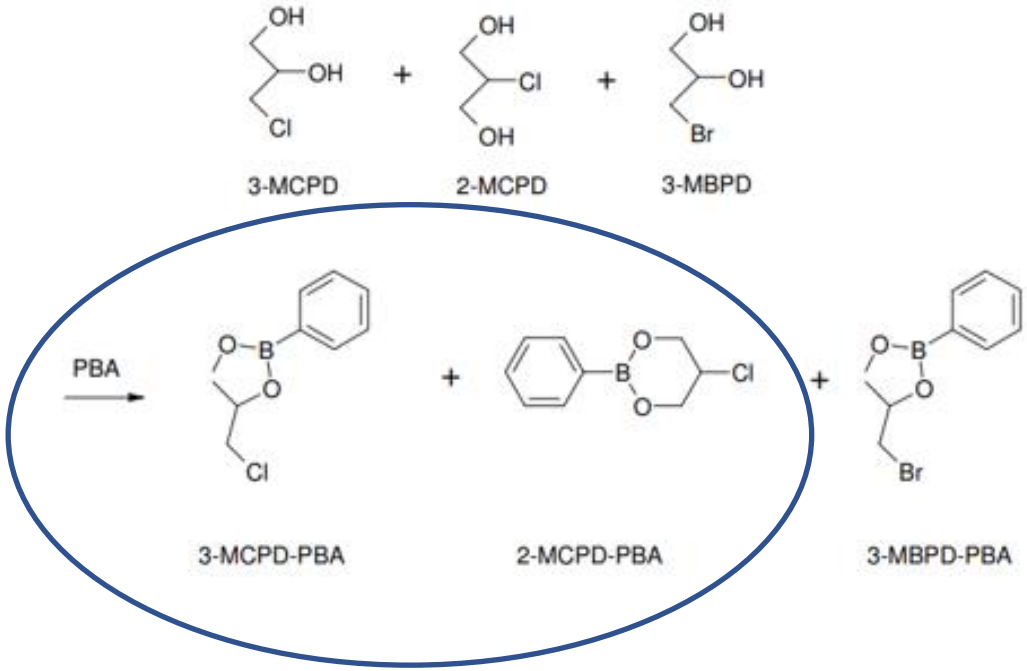
TRADITIONAL METHODOLOGY

Analysis of Foods

- AOAC Official Method Cd 29a-13

Test sample preparation (III):

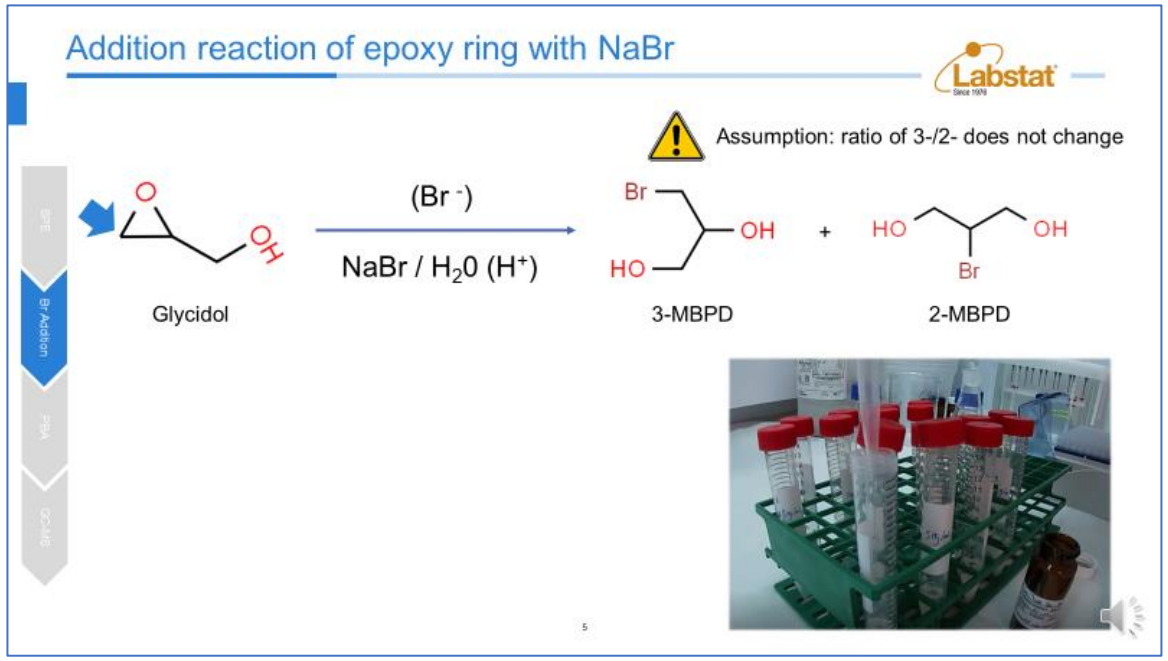
- phenylboronic acid, ultrasonic bath, 5 min
- extration of phenylboronic derivatives of 2- and 3-MCPD and 3-MBPD with n-heptane

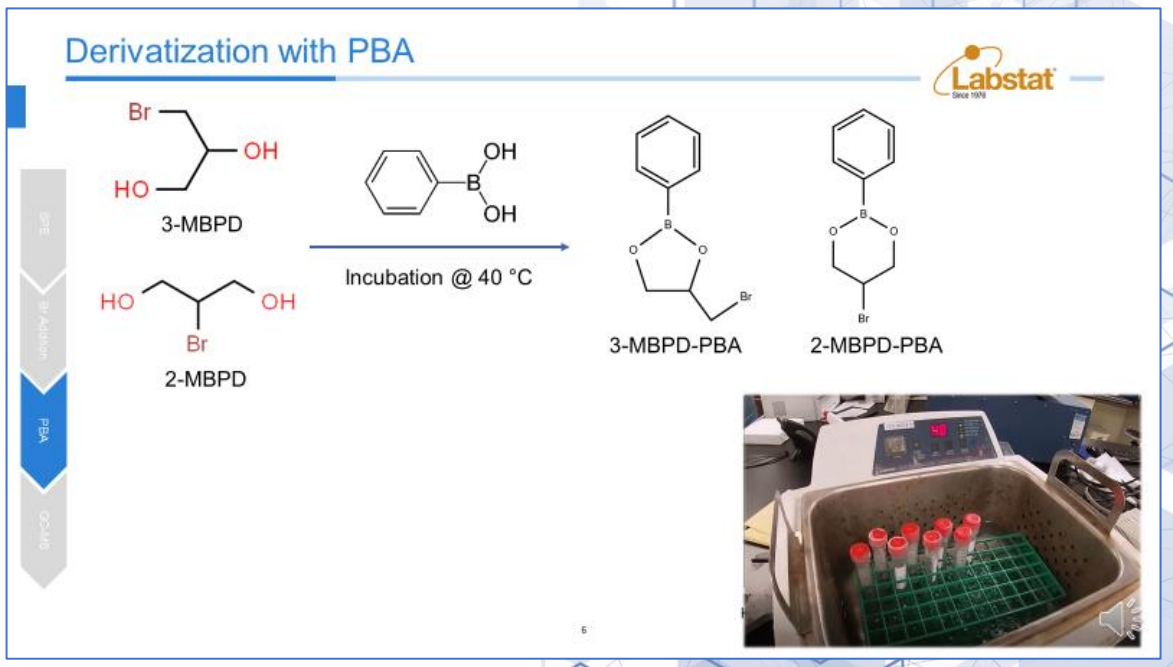


PBA DERIVITIZATION – USED IN TRACE LEVEL GLYCIDOL DETERMINATION

Determination of Glycidol in e-liquids and emissions from e-cigarettes

- Presented at Coresta Congress 2020 (Online)





METHOD DEVELOPMENT STRATEGY

Phase 1

- Evaluate/Optimize the PBA derivatization technique for 2- and 3-MCPD.
- Establish/Optimize chromatographic conditions and mass spectrometer(MS) parameters.
- Identify potential internal standards, quantifier and qualifier ions.

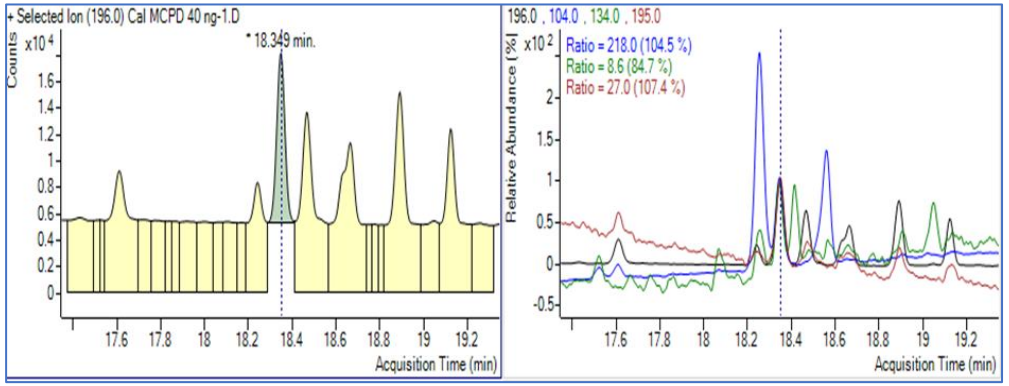
Phase 2

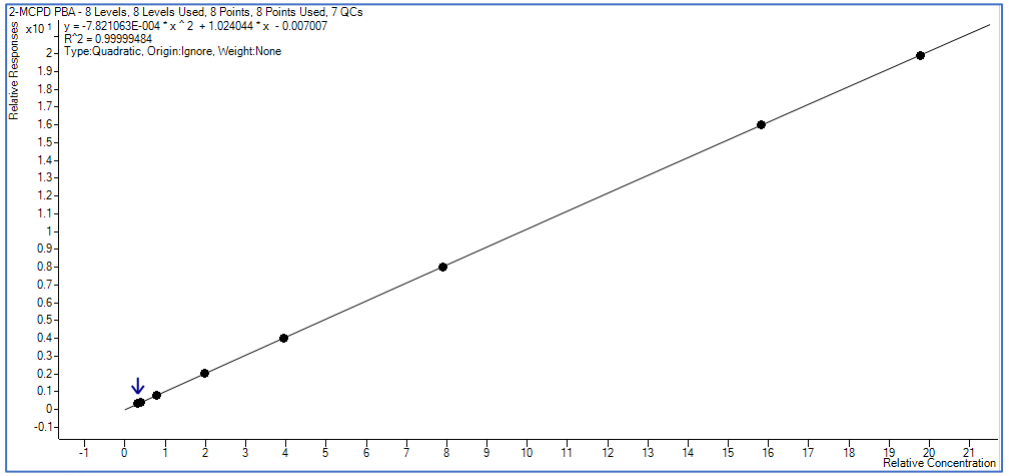
- Evaluate collection system requirements - aerosol distribution
- Establish range(s) of applicability

PHASE 1 METHOD DEVELOPMENT

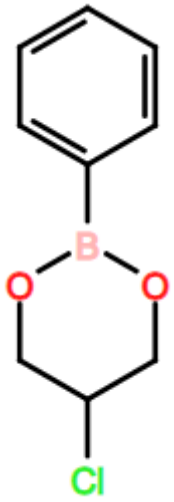
Optimization of Chromatographic and MS Conditions

- Analytical column: Agilent DB-5MS UI - 60 m x 250 µm x 0.25 µm





2-MCPD



PBA derivatization

- 40 mg/mL in diethyl ether
- 500 uL of PBA for 20 mins

2-MCPD-PBA （196, 104, 134, 195)

Internal standard:
2-MCPD-d5 (201, 203)

Calibration range: 40 to 2500 ng/mL
Calibration refits: ≥0.995

LRB (Cal 0 ng) concentration:

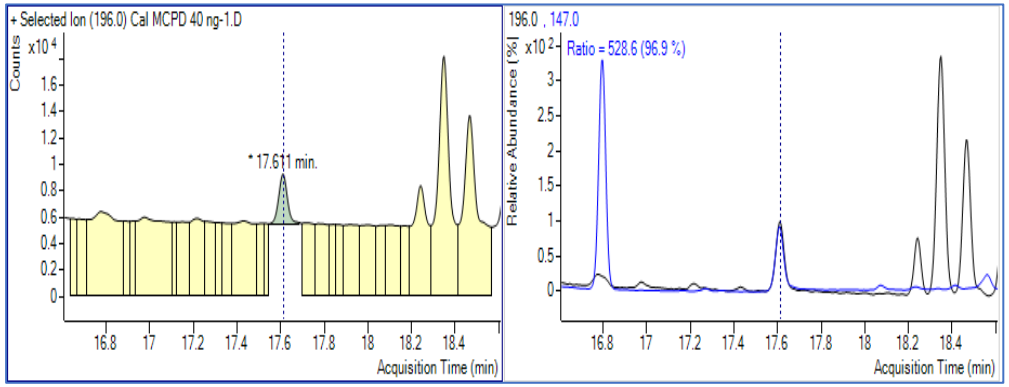
- 2-MCPD: 1.98 ng/mL

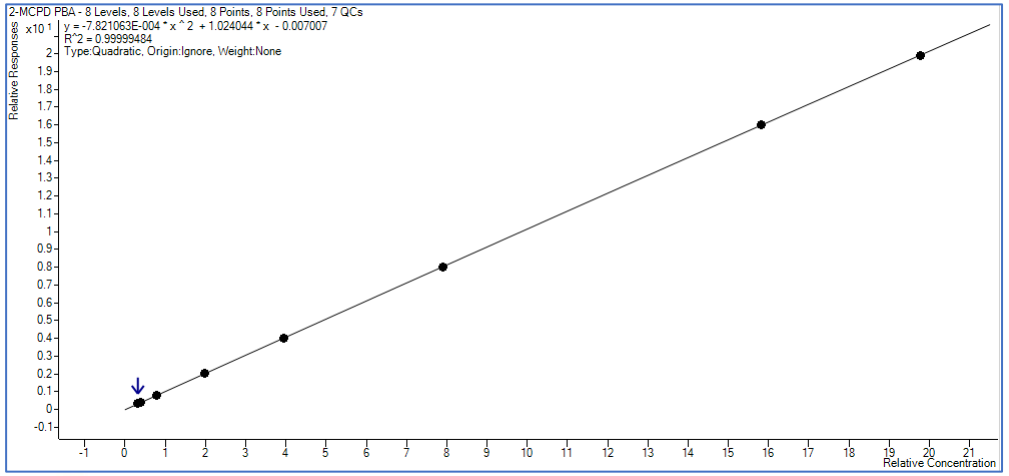
Labstat
A Certified Company

PHASE 1 METHOD DEVELOPMENT

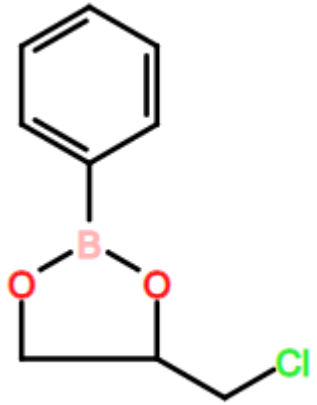
Optimization of Chromatographic and MS Conditions

- Analytical column: Agilent DB-5MS UI - 60 m x 250 µm x 0.25 µm





3-MCPD



PBA derivatization
- 40 mg/mL in diethyl ether
- 500 uL of PBA for 20 mins

3-MCPD-PBA (196, 147)

Internal standard:
3-MCPD-d5 (150, 201)

Calibration range: 40 to 2500 ng/mL
Calibration refits: ≥0.995

LRB (Cal 0 ng) concentration:
- 3-MCPD: 2.19 ng/mL

Labstat
A Certified Company

PHASE 2 – COLLECTION SYSTEM

Evaluation of a combustible Test Item

- Pad extract (IPA) – ISO puffing regimen

Under ISO standard, 5 cigs
318.6/5*20mL=1.27 µg/item

	sample ID	3-MCPD concentration	recovery(%)	2-MCPD concentration	recovery(%)
TNC unfortified	TNC unfortified PBA500-1.D	319.6		64.6	
	TNC unfortified PBA500-2.D	318.0		65.2	
	TNC unfortified PBA500-3.D	318.4		65.0	
	average	318.6		64.9	
TNC 100 ng fortified	TNC Low LFM PBA500-1.D	412.8	93.2	151.2	90.9
	TNC Low LFM PBA500-2.D	419.3	99.7	149.7	89.4
	TNC Low LFM PBA500-3.D	416.2	96.6	149.7	89.3
	average	416.1	96.5	150.2	89.9
TNC 250 ng fortified	TNC Med LFM PBA500-1.D	568.4	99.0	286.3	93.4
	TNC Med LFM PBA500-2.D	568.1	98.8	278.4	90.0
	TNC Med LFM PBA500-3.D	560.4	95.8	276.4	89.2
	average	565.6	97.9	280.4	90.8
TNC 2000 ng fortified	TNC High LFM PBA500-1.D	2352.3	100.7	1824.5	92.7
	TNC High LFM PBA500-2.D	2405.1	103.3	1821.8	92.6
	TNC High LFM PBA500-3.D	2329.2	99.6	1822.4	92.6
	average	2362.2	101.2	1822.9	92.7

Labstat
A Certified Company

PHASE 2 – COLLECTION SYSTEM

Evaluation of a 50:50 PGVG artificial e-liquid

- 200mg loading / 20mL IPA – Fortification level of 250ng/mL

	sample ID	3-MCPD concentration (ng/mL)	recovery(%)	2-MCPD concentration	recovery(%)
100 uL of PBA	unfortified PBA100-1.D	0.0		2.0	
	unfortified PBA100-2.D	0.0		1.4	
	unfortified PBA100-3.D	0.0		1.1	
	average	0.0		1.5	
	Med LFM PBA100-1.D	232.7	92.2	252.2	105.7
	Med LFM PBA100-2.D	239.3	94.8	246.3	103.2
	Med LFM PBA100-3.D	235.7	93.4	249.6	104.6
	average	235.9	93.5	249.3	104.5
500 ul of PBA	unfortified PBA500-1.D	1.8		2.2	
	unfortified PBA500-2.D	0.4		1.8	
	unfortified PBA500-3.D	0.8		2.0	
	average	1.0		2.0	
	Med LFM PBA500-1.D	252.2	99.9	238.1	99.8
	Med LFM PBA500-2.D	246.3	97.6	229.7	96.2
	Med LFM PBA500-3.D	249.6	98.9	224.7	94.1
	average	249.3	98.8	230.8	96.7

Labstat
A Certified Company

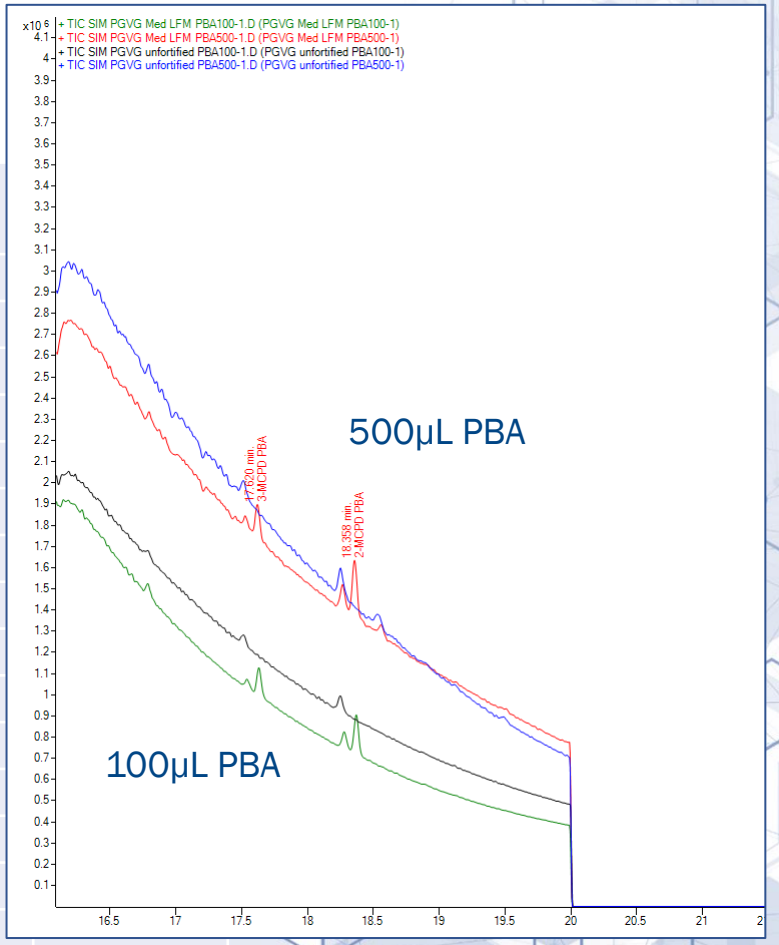
PHASE 2 – COLLECTION SYSTEM

Evaluation of a 50:50 PGVG artificial e-liquid

- 200mg loading / 20mL IPA – Fortification level of 250ng/mL

	sample ID	3-MCPD concentration (ng/mL)	recovery(%)	2-MCPD concentration	
100 uL of PBA	unfortified PBA100-1.D	0.0		2.0	
	unfortified PBA100-2.D	0.0		1.4	
	unfortified PBA100-3.D	0.0		1.1	
	average	0.0		1.5	
	Med LFM PBA100-1.D	232.7	92.2	252.2	
	Med LFM PBA100-2.D	239.3	94.8	246.3	
	Med LFM PBA100-3.D	235.7	93.4	249.6	
	average	235.9	93.5	249.3	
500 ul of PBA	unfortified PBA500-1.D	1.8		2.2	
	unfortified PBA500-2.D	0.4		1.8	
	unfortified PBA500-3.D	0.8		2.0	
	average	1.0		2.0	
	Med LFM PBA500-1.D	252.2	99.9	238.1	99.8
	Med LFM PBA500-2.D	246.3	97.6	229.7	96.2
	Med LFM PBA500-3.D	249.6	98.9	224.7	94.1
	average	249.3	98.8	230.8	96.7

Far below the estimated LOQ of 40ng/mL



Labstat
A Certified Company

PHASE 2 – COLLECTION SYSTEM

Trapping Efficiency – Breakthrough Study

- Combustible (KR 1R6F) and Heated Tobacco Product (eHTP)
  - Cambridge Pad + 2 X 20mL impingers in series
  - Two replicates each matrix type
- Intensive puffing regimen (55/2/30 – vent blocking where applicable)
- Additional extraction efficiency determination

Outcomes

- All 2-MCPD and 3-MCPD found in the particulate phase (Pad)
  - No impingers necessary in collection system
- No difference observed between 30 minute and 60 minute extraction times
  - Choose 30 minutes

PHASE 2 – COLLECTION SYSTEM

Trapping Efficiency – Breakthrough Study

- Combustible (KR 1R6F) and Heated Tobacco Product (eHTP)
  - Cambridge Pad + 2 X 20mL impingers in series
  - Two replicates each matrix type
- Intensive puffing regimen (55/2/30 – vent blocking where applicable)
- Additional extraction efficiency determination

Results

	3-MCPD (ng/mL)	2-MCPD (ng/mL)
THP PAD -1 30min-1.D	1883.40	303.00
THP PAD -2 30min-1.D	1800.35	288.47
Average	1841.87	295.73
KR 1R6F PAD -1 30min-1.D	301.59	33.58
KR 1R6F PAD -2 30min-1.D	305.51	34.91
Average	303.55	34.25

THP

- 5 test pieces/replicate
- 20mL extraction

3-MCPD = 7.37µg/item
2-MCPD = 1.18µg/item

Combustible (KR 1R6F)

- 3 cigarettes/replicate
- 20mL extraction

3-MCPD = 2.02µg/cig
2-MCPD = 0.23µg/cig

Labstat
A Certified Company

METHODOLOGY - FINALIZED

Sample Generation / Extraction

- Simple pad collection - transfer the pad into a 50 mL extraction bottle
- Add 200µL combined ISTD standard solution (10µg/mL)
- Add 20mL IPA
- Extract 30 minutes on platform shaker

Derivitization

- Transfer 0.5 mL of the extract to a 15 mL PP conical centrifuge tube.
- Add 0.5 mL of the 40 mg/mL PBA solution in diethyl ether and vortex
- Incubate solution at 40 °C for 20 min
- Concentrate samples to dryness under nitrogen at 40 °C
- Dissolve residue by adding 300 µL of isooctane, vortex for 5s to ensure that the residue is completely solubilized
- Carefully transfer the supernatant for GC-MS analysis

Labstat
A Certified Company

METHODOLOGY - FINALIZED

Sample Analysis – GC-MS Conditions (HES)

- Column: DB-5MS Ultra Inert (or equivalent) 60 m x 250 µm x 0.25 µm
  - Programmed: 100°C hold 3 minutes; 5°C/min to 200°C, then 40°C/min to 300°C; hold 3 minutes (≈ 26.5 minute run time)
  - Constant flow: 1mL/min He
- Injector: Splitless at 200°C; UI liner
- Injection Volume: 3µL

Compound (not derivatized)	Compound (derivatized)	SIM Group #	Expected RT DB-5	Quantifier Ion (m/z)	Qualifier Ion 1 (m/z)	Qualifier Ion 2 (m/z)
3-MCPD-d5 (ISTD)	3-MCPD-PBA-d5 (ISTD)	1	17.325	150	201	N/A
3-MCPD	3-MCPD-PBA	1	17.412	196	147	N/A
2-MCPD-d5 (ISTD)	2-MCPD-PBA-d5 (ISTD)	1	18.056	201	203	N/A
2-MCPD	2-MCPD-PBA	1	18.151	104	196	195

VALIDATION RESULTS

Calibration Curves and Linearity Range

- Seven levels of calibration standards were injected on separate days to build three calibration curves

Compounds	Calibration Range (ng/mL)	Correlation Coefficient (R^2)			Acceptance Criteria
		Cal 1	Cal 2	Cal 3	
3-MCPD	40 - 10000	0.999	0.999	1.000	≥ 0.995
2-MCPD	40 - 10000	1.000	1.000	1.000	

Labstat
A Certified Company

VALIDATION RESULTS

Calibration Curves and Linearity Range

- Seven levels of calibration standards were injected on separate days to build three calibration curves

Compound	Day	Cal std #1	Cal std #2	Cal std #3	Cal std #4	Cal std #5	Cal std #6	Cal std #7
		(%)	(%)	(%)	(%)	(%)	(%)	(%)
Nominal Standard Concentration		40 ng/mL	100 ng/mL	250 ng/mL	1000 ng/mL	2000 ng/mL	5000 ng/mL	10000 ng/mL
3-MCPD	Day 1	118	100	90.6	93.1	96.6	98.1	102
	Day 2	114	96.1	93.0	101	94.8	99.7	101
	Day 3	118	95.8	92.0	95.6	98.6	99.0	101
2-MCPD	Day 1	112	87.8	103	98.2	101	101	99.3
	Day 2	100	96.2	101	102	102	101	99.1
	Day 3	99.4	98.6	101	99.5	102	100	99.5
Acceptance Criteria (%):		Lowest standard recovery 100 ± 30%; All other levels 100 ± 20%						

Labstat
A Certified Company

VALIDATION RESULTS

Limits of Detection (LOD) and Limit of Quantification (LOQ)

- At least 10 replicate injections of the lowest calibration standard
- Signal-to-noise ratio (S/N) of the lowest calibration standard
- Assessment of refit of the lowest standard against the calibration curve

Calculated limits were lower than the lowest standard

Compounds	Instrument Limits		Mainstream ISO (5)		Mainstream HCI (3)	
	LOD [ng/mL]	LOQ [ng/mL]	LOD [ng/cig]	LOQ [ng/cig]	LOD [ng/cig]	LOQ [ng/cig]
3-MCPD	12	40	48	160	80	267
2-MCPD	12	40	48	160	80	267

Labstat
A Certified Company

VALIDATION RESULTS

Limits of Detection (LOD) and Limit of Quantification (LOQ)

- At least 10 replicate injections of the lowest calibration standard
- Signal-to-noise ratio (S/N) of the lowest calibration standard
- Assessment of refit of the lowest standard against the calibration curve

Calculated limits were lower than the lowest standard

Compounds	Instrument Limits		E-liquids (0.1g / 10mL)		THP Emissions (5)	
	LOD [ng/mL]	LOQ [ng/mL]	LOD [ng/g]	LOQ [ng/g]	LOD [ng/stick]	LOQ [ng/stick]
3-MCPD	12	40	1200	4000	48	160
2-MCPD	12	40	1200	4000	48	160

Labstat
A Certified Company

VALIDATION RESULTS

Accuracy and Precision

- KR1R6F Results

Method Development

	3-MCPD (ng/mL)
KR 1R6F PAD -1 30min-1.D	301.59
KR 1R6F PAD -2 30min-1.D	305.51
Average	303.55

Sample	TPM [mg/cig]	3-MCPD in extract [ng/mL]	2-MCPD in extract [ng/mL]
KR1R6F Day 1-1 HCI	50.5	282	65.5
KR1R6F Day 1-2 HCI	47.7	299	54.2
KR1R6F Day 2-1 HCI	49.8	283	45.0
KR1R6F Day 2-2 HCI	51.7	334	54.7
KR1R6F Day 3-1 HCI	49.5	346	60.1
KR1R6F Day 3-2 HCI	52.1	363	74.4
Average	50.2	318	59.0
Std. Dev.	1.60	34.6	10.2
Coeff. Var. (%)	3.19	10.9	17.2
KR1R6F Day 1-1 ISO	9.54	261	51.4
KR1R6F Day 1-2 ISO	10.4	275	50.1
KR1R6F Day 2-1 ISO	11.2	273	46.3
KR1R6F Day 2-2 ISO	10.8	238	42.8
KR1R6F Day 3-1 ISO	10.7	294	46.9
KR1R6F Day 3-2 ISO	10.3	248	57.4
Average	10.5	265	49.1
Std. Dev.	0.564	20.1	5.05
Coeff. Var. (%)	5.38	7.59	10.3

Labstat
A Certified Company

VALIDATION RESULTS

Accuracy and Precision

- Laboratory Fortified Blank (LFB) and Laboratory Fortified Matrix (LFM)
  - LFM Samples were prepared by fortification of collection pads (2 levels)

All results within acceptance criteria (70 – 130%)

Sample HCl Regime	3-MCPD Recovery [%]	2-MCPD Recovery [%]
KR1R6F Day 1-1 Low LFM	106	96.7
KR1R6F Day 1-2 Low LFM	106	87.0
KR1R6F Day 2-1 Low LFM	97.5	105
KR1R6F Day 2-2 Low LFM	99.6	104
KR1R6F Day 3-1 Low LFM	104	113
KR1R6F Day 3-2 Low LFM	109	112
Average	104	103
Std. Dev.	4.46	9.78
Coeff. Var. (%)	4.30	9.51
Acceptance Criteria - Recovery 70 - 130%		

Labstat
A Certified Company

VALIDATION RESULTS

Accuracy and Precision – High Level Fortification

Again - All results within acceptance criteria (70 – 130%)

Sample HCl Regime	3-MCPD Recovery [%]	2-MCPD Recovery [%]
KR1R6F Day 1-1 High LFM	105	94.2
KR1R6F Day 1-2 High LFM	105	88.8
KR1R6F Day 2-1 High LFM	97.8	103
KR1R6F Day 2-2 High LFM	99.7	99.7
KR1R6F Day 3-1 High LFM	104	116
KR1R6F Day 3-2 High LFM	102	107
Average	102	101
Std. Dev.	3.08	9.48
Coeff. Var. (%)	3.02	9.35
Acceptance Criteria - Recovery 70 - 130%		

Labstat
A Certified Company

TEST SAMPLE DATA – 3-MCPD

Combustible (KR 1R6F)

- Within day n=4
- Four days of collection (Total n = 16)

	Weight [mg/cig]	Puff Count [per cig]	MS TPM [mg/cig]	3-MCPD [µg/cig]
Day 1	956	9.6	53.5	2.42
Day 2	960	9.6	49.6	2.24
Day 3	961	9.4	51.6	2.38
Day 4	957	9.1	49.1	2.52
Overall				
Average	958	9.4	51.0	2.39
Std. Dev.	11	0.3	3.0	0.20
Coeff. Var.	1.1	3.5	5.9	8.6

Heated Tobacco Product (eHTP)

- Within day n=4
- Four days of collection (Total n = 16)

	Weight [mg/cig]	MS TPM [mg/cig]	3-MCPD [µg/cig]
Day 1	807	56.0	10.02
Day 2	809	55.7	10.95
Day 3	810	56.0	11.10
Day 4	807	55.1	9.62
Overall			
Average	808	55.7	10.4
Std. Dev.	2	1.7	1.1
Coeff. Var.	0.3	3.1	10.1

Labstat
A Certified Company

TEST SAMPLE DATA – 3-MCPD

Vaping Product A – 50 puff collection

- Within day n=4
- Four days of collection (Total n = 16)

	MS ACM [mg/coll]	Mass Loss [mg/coll]	3-MCPD [µg/coll]
Day 1	171	177	BDL
Day 2	182	190	NQ
Day 3	174	179	NQ
Day 4	176	181	NQ
Overall			
Average	176	182	NQ
Std. Dev.	10	11	NQ
Coeff. Var.	5.6	5.8	N/A

Vaping Product B – 50 puff collection

- Within day n=4
- Four days of collection (Total n = 16)

	MS ACM [mg/coll]	Mass Loss [mg/coll]	3-MCPD [µg/coll]
Day 1	173	179	BDL
Day 2	177	183	BDL
Day 3	173	180	BDL
Day 4	179	186	BDL
Overall			
Average	176	182	BDL
Std. Dev.	11	12	BDL
Coeff. Var.	6.4	6.5	N/A

Labstat
A Certified Company

SUMMARY / CONCLUSIONS

- ✓ A relatively simple method has been developed for the determination of MCPD in emissions from tobacco products
- ✓ The same methodology is effective in the quantitation of 3-MCPD in the emissions from combustible and heated tobacco products
  - ➢ For the products tested, 3-MCPD was ≈ 5X higher in the heated tobacco product than the combustible product on a "per stick" basis.
  - ➢ The two vapour products tested showed no quantifiable levels of 3-MCPD
    - ❖ It is suspected this may be due to low levels of chlorides present in the e-liquid.

ACKNOWLEDGEMENTS

Jiaming Wang
Angel Rodriguez-Lafuente

Labstat R&D team

- Cosmin Stoicoiu
- Hannah Felisa
- Jonathan Ly
- Sarah Hammond

Email: Angel Rodriguez (Arodrigu@labstat.com)

Email: Peter Joza (Pjoza@labstat.com)

Labstat: clientservices@labstat.com

Molecule figures in this presentation courtesy of Chemspider, Wikipedia and PubChem

Labstat
A Certified Company

YOUR BEST-IN-CLASS TESTING LABORATORY

PROVIDING ANALYTICAL, SCIENTIFIC AND RESEARCH SERVICES FOR NICOTINE AND CANNABIS CONTAINING PRODUCTS

Thank You for Your Attention!