Juul Labs Science

Determination of Glycidol in E-Liquid and Aerosol Samples from ENDS Products by GC-MS

David K. Cook, Beth Stump, Norman Fraley, Kathy Humphries, I. Gene Gillman, Brian Jameson

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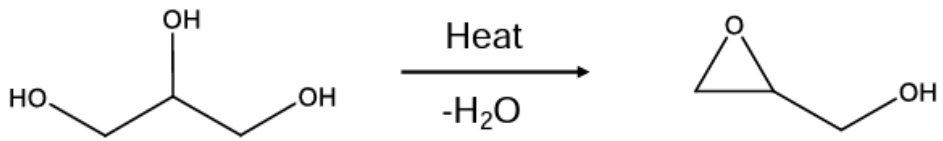
Introduction

Glycidol is detected in combustible cigarettes, heat-not-burn products, and Electronic Nicotine Delivery Systems (ENDS) as a thermal degradation byproduct of glycerin[1,2,3].

Listed as a probable carcinogen and constituent for consideration within FDA Premarket Tobacco Application for ENDS[4,5].

For the measurement of glycidol in ENDS, published or presented work uses a variety of GC-MS methods including direct injection, cool on column, thermal desorption, and derivatization for detection[1,2,3,6,7].



Challenges of GC-MS Glycidol Determination

Reactive molecule that is difficult to analyze due to chemical instability[2,3,6]

Direct injection GC-MS analysis can lead to *in situ* thermal degradation of glycerin to produce quantifiable levels of glycidol[2,3,9].

- Glycerin could convert to glycidol in a GC inlet at temperatures above 220℃
- Glycidol can form a glycidol dimer, starting at 100℃, and at elevated temperatures can convert to glycerin
- A comparison of direct injection and derivatization e-liquid results demonstrated that ~98% of the measured glycidol from direct injection GC-MS was a byproduct of the analytical method

At present, no standardized analytical methodology exists for the determination of glycidol in ENDS e-liquid and aerosol.

Objective

To develop and validate a stable, sensitive, and selective method for the determination of glycidol in e-liquid and aerosol samples utilizing a derivatization methodology via gas chromatography-mass spectrometry (GC-MS).

Review of Glycidol GC-MS Methods

Direct Injection:

- Artifactual formation of glycidol
- Susceptible to low molecular mass interferences

Cool On-column:

- Improvement from Direct Injection (mitigates artifactual formation)

Thermal Desorption:

- Requires specialized analytical equipment
- Difficult for analysis of e-liquids
- Limitations on aerosol collection (i.e. trapping capacity) and throughput

Derivatization:

- Stable, sensitive, and selective
- Unlikely to form artifactual glycidol during analysis
- Presented methods utilize complex sample prep (multi-step derivatization and/or SPE clean-up)

Proposed Methodology for ENDS Derivatization

Derivatives of Aliphatic Glycols[8]:

- Derivate Type: Acetonide
- Reagent: Acetone / *p*-toluenesulfonic acid (TsOH)
- Reaction: Specific protection group for 1,2 diols. The acetonide is a cyclic ketal formed by the reaction of an alcohol (OH) group with a carbonyl group (C=O) within the same molecule.
- Tosylate formation for better nucleophilic substitution

Advantages from presented derivatization methods:

- Removes need for Solid Phase Extraction (SPE) clean-up and reagents (lowers consumable costs)
- Reduces method complexities (i.e. sample preparation length, extensive training, etc.)

Chemicals and Reagents

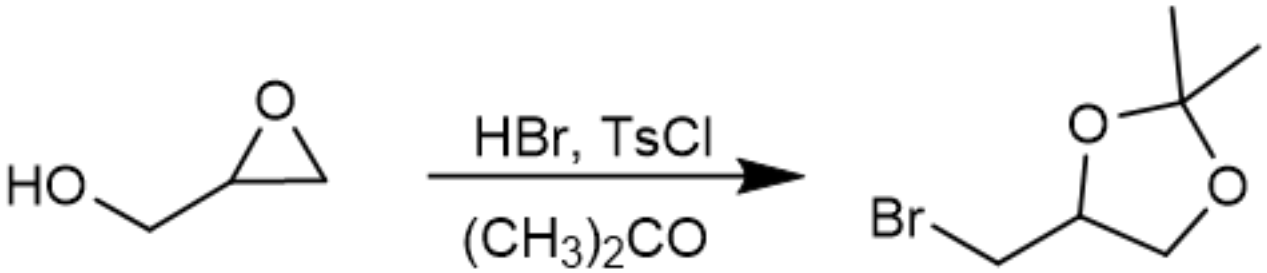
Reagent/Chemical	Grade/Purity
Glycidol	Custom standard in acetone (1000 ug/mL, or equivalent)
Glycidol d5	Custom standard in acetone (1000 ug/mL, or equivalent)
Acetone	Optima grade, or equivalent
Hexane	HPLC or Optima grade, or equivalent
Hydrogen Bromide	47-49%, or equivalent
p-Toluenesulfonyl chloride	99%, or equivalent
Water	in-house dionized (DI), or equivalent
Sodium Sulfate	Granular anhydrous
Sodium Bicarbonate	N/A

Solution Prep:

1. ISTD Extraction Solution: Acetone + Glycidol d5 @ 150 ng/mL
2. TsCl Solution: Acetone + p-toluenesulfonyl chloride @ 0.15 mg/mL
3. Sodium Bicarbonate solution: Add sodium bicarbonate to DI H2O until the solution appears saturated.

E-liquid Sample Preparation

1. Aliquot 100uL of e-liquid sample (target weight: 0.1 g).
2. Add 2mL ISTD Extraction solution.
3. Add 30 uL of 0.15 mg/mL TsCl Solution followed by 30uL concentrated Hydrogen Bromide (HBR). Cap a tumble for 15 minutes.
4. Add 6mL of D.I. Water and 2 mL of hexane. Cap and tumble for 15 minutes.
5. Decant top hexane layer and transfer to vial containing 0.2 to 1.0g of sodium sulfate. Mix well.
6. Transfer dried hexane to a 2 mL amber autosampler vial, cap, and analyze via GC-MS.



Aerosol Collection and Sample Preparation

Collections performed on a Cerulean SM450-e. Samples are vaped until end of life is reached (EOL). Each regime is puffed in blocks, starting with freshly charged devices, where a set number of puffs is reached before the device is replaced. Each set of devices will be recharged and rotated with fresh batteries during the vaping cycle until completed

Regime	Puff Volume	Duration (seconds)	Collections	Interval (seconds)	Typical Puff Block	Puff Profile
Non-Intense	55	3	1-EOL	30	50	Square Wave
Intense	110	6	1-EOL	30	20	Square Wave

Aerosol Sample Prep:

1. Following EOL collections, remove Cambridge filter pad (CFP) from its holder and wipe holder with the pad.
2. Insert CFP into 25 mL of ISTD Extraction Solution. Cap and tumble for 15 minutes.
3. Centrifuge (to remove CFP remnants) and aliquot 5 mL of sample with 50 uL of TsCl solution, quickly followed by 50uL of concentrated HBr. Cap and tumble.
4. Add 4 mL of sodium bicarbonate solution and 2 mL of hexane to each vial. Cap and vortex.
5. Decant top hexane layer and transfer to vial containing 0.2 to 1.0g of sodium sulfate. Mix well.
6. Transfer dried hexane to a 2 mL amber autosampler vial, cap, and analyze via GC-MS

Instrument Parameters

Parameter	Specification
Instrument	Agilent 8890/5977B MS
Column	Agilent DB-5MS UI
Detector	MS
Run Time	6.3 min
Carrier Gas	Helium
Oven Program	80℃, ramp 4℃/min to 90℃, ramp 50℃/min to 280℃
Injection Volume	2 uL
Injection Temperature	250℃
SIM Ions Glycidol	Primary 179, Secondary 181
SIM Ions Glycidol D5	Primary 184, Secondary 186

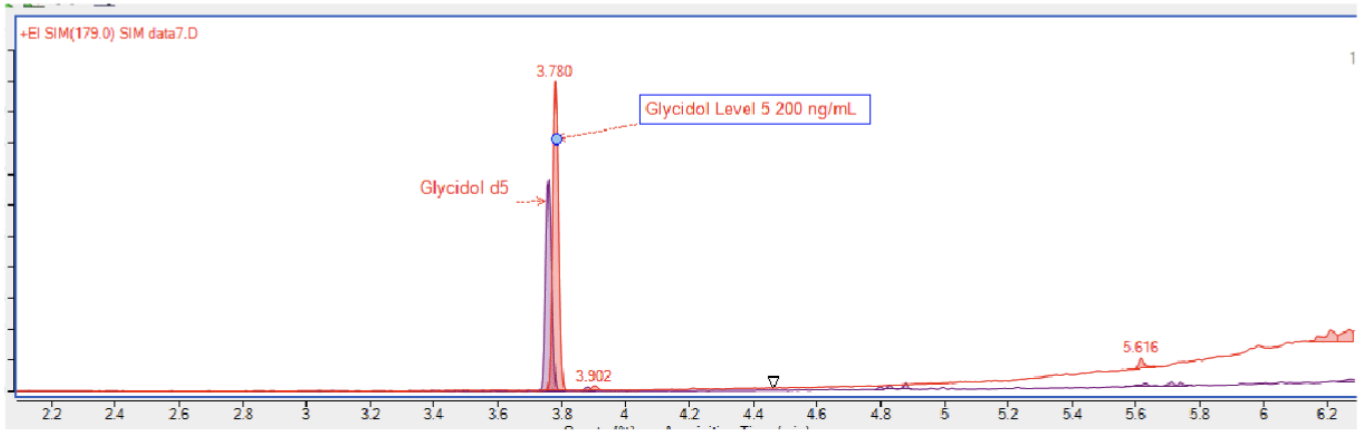
Validation Results

Test	E-liquid	Aerosol (Non-Intense & Intense)
Linearity/Range	R^2 ≥0.995; 10-800 ng/mL	
LOD (Instrument)	0.86 ng/mL	
LOQ (Instrument)	10 ng/mL	
LOD (Sample*)	16 ng/g	21.5 ng/collection
LOQ (Sample*)	180 ng/g	250 ng/collection
Specificity/Selectivity	Analytes were successfully determined and overlayed	
Accuracy	80% - 120% Recovery achieved	70%-130% Recovery achieved
Precision	<10% CV	<20% CV
Repeatability	<15%CV	<15%CV
Robustness**	Reported**	Reported**
Stability	Stable for 5 days @ Ambient & (-20℃)	Stable for 8 days @ Ambient &(-20℃)
Aerosol Breakthrough	N/A	Pad loading: 1200 mg ACM; Concentrations <10% on second pad

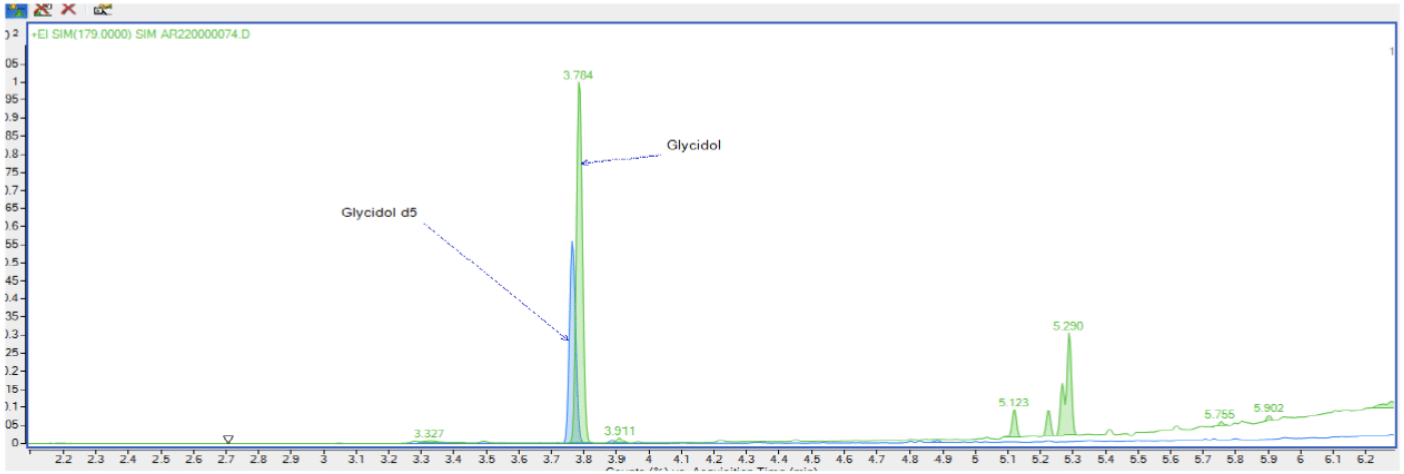
*Sample LOD/LOQ: (E-liquid) based on 0.1g sample aliquot and (Aerosol) 25 mL sample volume
**See Long Term Precision slide

Sample Chromatogram

Midpoint Standard:

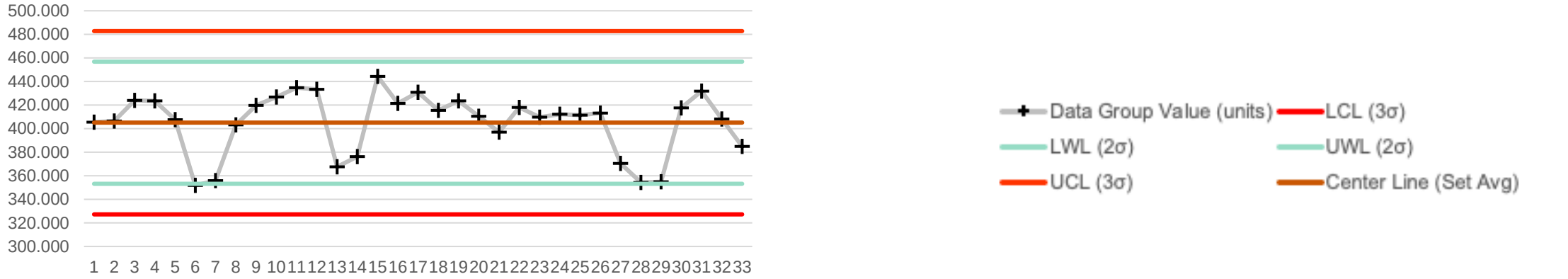


Sample Chromatogram:

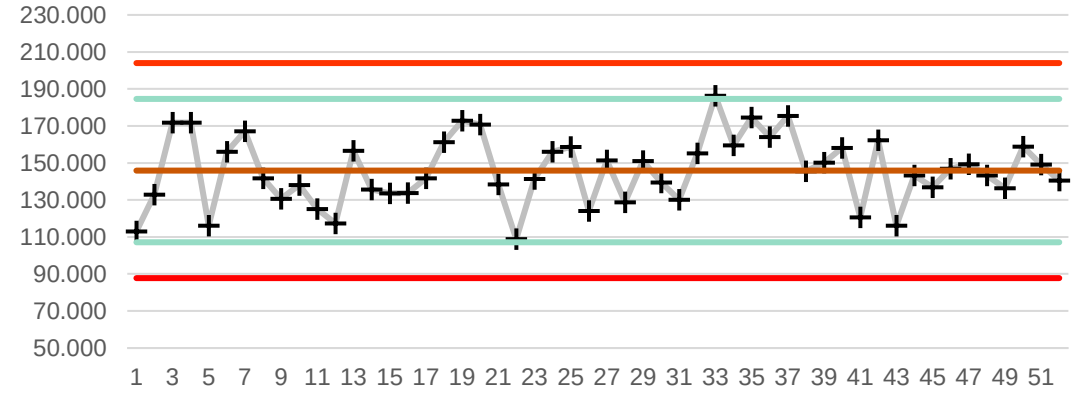


Long Term Precision

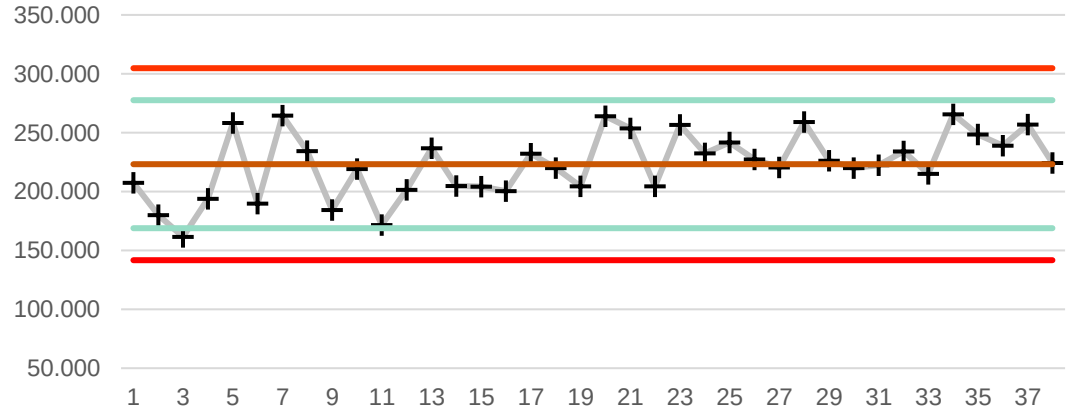
E-liquid



Aerosol (Non-Intense)



(Intense)



Method is ‘Fit-for-Purpose”

- Glycidol measurements conducted on nine commercially available disposable ENDS products under non-intense puffing.

Test	Aerosol (Non-Intense)
Product 1	LOQ
Product 2	6.55 ng/puff
Product 3	8.94 ng/puff
Product 4	12.3 ng/puff
Product 5	19.5 ng/puff
Product 7	47.6 ng/puff
Product 8	66.9 ng/puff
Product 9	329 ng/puff

Conclusion

- Presented is a high throughput derivatization methodology that reduces the potential artifactual formation at the injection port, and improves the stability, selectivity sensitivity for glycidol determination in ENDS aerosol and e-liquid.

- This method is deemed fit for purpose to accurately determine trace amounts of glycidol in both e-liquid and aerosol samples. All requirements for method validation were met.

References

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Acknowledgements and Questions