T3332

LC-MS/MS Quantification of Propylene Glycol

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Purpose

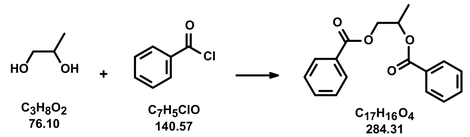
Electronic cigarettes (ecigs) have become increasingly popular in recent years. Promoted as a less harmful alternative to smoking or oral tobacco use, there are those that caution that ecigs may not be as safe as they might appear. While tobacco contains numerous and variable compounds as a result of the plant strain, the growing location, annual weather conditions, and possibly the fertilizers, pesticides, and herbicides used, ecig liquids consist of a fairly limited list of ingredients. The solvent is typically a mixture of propylene glycol (PG) and glycerol in a 70:30 or 50:50 ratio with 0.6 to 3.6% nicotine. In addition, the user (vaper) can select from an assortment of flavorings.

As PG is a major component in the ecig solvent, there has been interest in measuring the exposure to PG as a direct result of ecig use. The measurement of exposure to PG is complicated by the presence of PG in many consumer products, pharmaceuticals, and processed foods. To calculate exposure from ecigs, the PG from ecig use must be measurable in the presence of PG from other sources and measurements taken after ecig use must be corrected for concentrations present before ecig use. To simplify this problem, the use of stable-labeled PG, specifically $^{13}C_3$-PG (^{13}C-PG), in the ecig solvent was proposed and methods for both PG and ^{13}C-PG were developed.*

*Patent application pending

Methods

Sample Preparation
0.0500 mL sample (PG) or 0.100 mL sample (^{13}C-PG)
Add IS to samples or IS solvent to blanks and vortex to mix
Add pentane and vortex, then sonicate
Add NaOH and vortex
Add benzoyl chloride and vortex
Add pentane and vortex, followed by rotary mixing and centrifugation
Transfer a portion of the pentane phase to a clean vessel and evaporate to dryness
Reconstitute the residue with ACN:H_2O:HCOOH and sonicate



Instrumentation
Waters Acquity UPLC®
Waters CORTECS™ UPLC® C_{18+}, 90 Å, 50 x 2.1 mm, 1.6 µm
MPH A: 5:95:1 ACN:H_2O:HCOOH
MPH B: 95:5:1 ACN:H_2O:HCOOH
Flow rate: 0.8 mL/min
Injection volume: 5 µL
Ionization mode: Positive (TurbolonSpray)

Table 1. UPLC Gradient.

Total Time (min)	% MPH A
0.00	52
0.10	52
1.36	46
1.37	20
1.45	20
1.46	52

Table 2. MRM Transitions.

Analyte	MRM Transition	Dwell Time (msec)
PG	285.2 →163.3	10
$^{13}C_3$-PG	288.2 →166.3	130
$^{13}C_3d_3$-PG (IS)	291.2 →169.3	65

Figure 1. Blank - 0 ng/mL PG in Water (Black) and LLOQ Calibration Standard - 100 ng/mL PG in Water (Burgundy).

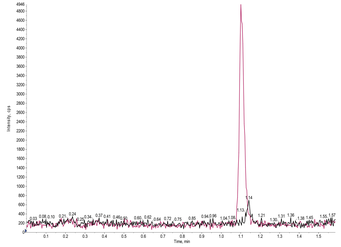
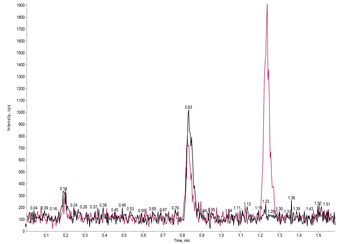


Figure 2. Blank - 0 ng/mL ^{13}C-PG in Plasma (Black) and LLOQ Calibration Standard - 5 ng/mL ^{13}C-PG in Plasma (Burgundy).



Results

Method Performance

Table 3. Intra- and Inter-Batch Precision and Accuracy of PG QC Samples.

		LLOQ QC 100	QC A 256	QC B 962	QC C 7495
		ng/mL			
Mean	PA 1	104	247	944	7300
%CV		3.4	4.3	3.6	3.1
%Bias		4.0	-3.5	-1.9	-2.6
Mean	PA 2	106	259	950	7240
%CV		9.1	3.7	4.6	2.7
%Bias		6.0	1.2	-1.2	-3.4
Mean	PA 3	101	251	930	7310
%CV		5.9	3.2	2.2	2.4
%Bias		1.0	-1.9	-3.3	-2.5
Mean	Inter-Batch	104	252	941	7282
%CV		6.6	4.2	3.6	2.7
%Bias		3.8	-1.4	-2.2	-2.8

Table 4. Intra- and Inter-Batch Precision and Accuracy of ^{13}C-PG Samples.

		LLOQ QC 5.00	QC A 15.0	QC B 75.0	QC C 750
		ng/mL			
Mean	PA 1	5.23	15.1	77.1	751
%CV		6.6	5.8	6.6	3.9
%Bias		4.6	0.4	2.8	0.1
Mean	PA 2	5.41	15.5	77.1	762
%CV		8.7	6.2	4.6	5.4
%Bias		8.2	3.2	2.8	1.7
Mean	PA 3	5.02	14.8	75.7	740
%CV		4.5	4.7	3.9	3.9
%Bias		0.3	-1.7	1.0	-1.3
Mean	Inter-Batch	5.24	15.2	76.6	752
%CV		6.6	5.6	4.8	4.2
%Bias		4.8	1.3	2.1	0.3

Table 4. Stability Testing Results.

	PG	^{13}C-PG
Long-Term Frozen Storage	21 days	5 days
Freeze-Thaw Cycles	5	5
Short-Term (Benchtop)	70 hr under uv-shielded lights at ambient temperature	70 hr under uv-shielded lights at ambient temperature
Post-Preparative (Extract)	100 hr	100 hr
Sample Collection	2 hr under white light at ambient temperature	2 hr under white light at ambient temperature

Application of the Method
- 6 subjects - 3 vapers and 3 nonvapers in a small room (21.5 m^3) for 2 hr
- 3 subjects vape for at least 3 sec every 30 sec for 30 inhalations from ecigs containing ^{13}C-PG
- Blood collections for 8 hr from all 6 subjects
- Measured PG and ^{13}C-PG in all subjects

Figure 3. PG Concentrations in Commercial Plasma Lots – Range ca. 40 to 4000 ng/mL.

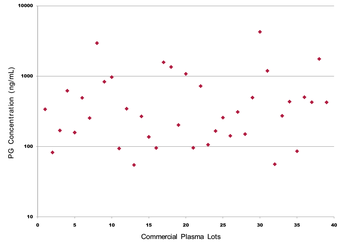


Figure 4. PG Concentrations vs. Time in Vaping and Nonvaping Subjects – Arrow Indicates the Time that Subjects Ate a Meal.

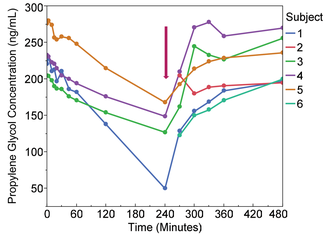


Figure 5. ^{13}C-PG Concentrations vs. Time in Vaping Subjects – Arrow Indicates the Time that Subjects Ate a Meal.

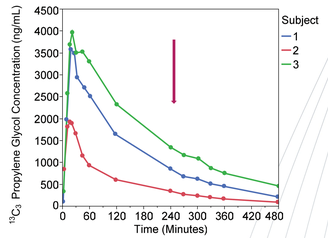
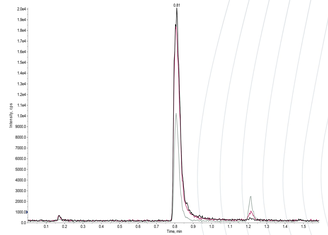


Figure 6. Chromatograms of ^{13}C-PG from a Nonvaping Subject's Pre-Exposure (Black) and Post-Exposure (Burgundy; ca. Cmax of Vapers) Samples with the LLOQ Standard (Gray) for Comparison.



Conclusions

- The methods developed for PG and ^{13}C-PG were adequately sensitive and selective for the accurate and precise quantitation of ubiquitous PG and ^{13}C-PG from ecig use
- Without the use of ^{13}C-PG, it would have been difficult to calculate exposure to propylene glycol that could be directly attributed to ecig use in vapers. It would have been impossible to detect secondary exposure to PG in non-vapers as the detection of ^{13}C-PG as a result of secondary exposure was below the limit of quantitation and approximately 0.05 to 5% of the PG concentrations in random plasma lots
- The use of stable-labeled analogs was an effective method of determining exposure to a ubiquitous analyte from a specific source without the safety concerns and licenses required for the use of radioactive isotopes

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