SUPPLEMENTARY INFORMATION

Quantifying PG:VG Ratio and Nicotine Content in Commercially Available E-Liquids Using Handheld Raman Spectroscopy

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Gas chromatography-mass spectrometry (GC-MS) samples preparation, acquisition and analysis parameters

For GC-MS analysis, a 30 mg·mL^{-1} base solution of nicotine in 50:50 PG:VG was created, and aliquots were further diluted in 50:50 PG:VG to make 20, 10, and 5 mg·mL^{-1} solutions, with aliquots of the 5 mg·mL^{-1} solution being further diluted to make 1, 0.75, and 0.5 mg·mL^{-1} solutions. A Kovats retention index solution was also created by dissolving 40 µL decane, dodecane and pentadecane, 30 mg nonadecane and 30 mg docosane in 10 mL hexane, then diluting 1 mL of this with 9 mL dichloromethane (DCM). One to two drops of each solution in the calibration curve was weighed into centrifuge tubes (25-50 mg) and a volume of 75:25 DCM/MeOH equal to 20 times the mass was added to each tube. These were all further diluted by a factor of 25 in DCM, and a 450 µL aliquot was added to GC vials alongside 50 µL retention index solution. The 1 mg·mL^{-1} and 20 mg·mL^{-1} nicotine samples were used as quality controls (QCs) and repeated every 9 samples. Experiments were performed on an Agilent 8890 GC instrument equipped with a VF-5ms capillary column (30 m x 0.25 mm) alongside an Agilent 7250 QTOF and a PAL RTC 120 injector (CTC Analytics). The following conditions were used: 1 µL injection volume; carrier gas: 1 mL·min^{-1} helium with a 50:1 split; injector temperature: 230 °C; temperature program: starting at 80 °C, increased at 25°C·min^{-1} until 205 °C is reached (5 min), then 120 °C·min^{-1} until 250 °C is reached (22 s), followed by a 1 min hold at 250 °C. MS data were collected in total ion current mode using a mass range of 20-500 *m*/*z*, electron ionisation energy of 70 eV, 20 Hz scan speed, 180°C source temperature and 300°C transfer line temperature.

GC-MS chromatograms were analysed using MassHunter MS Quantitative Analysis software (Agilent). Nicotine was quantified using a peak of *m/z* 84.1 and qualified with two peaks at *m/z* 133.1 and 162.1. Dodecane was chosen as an internal standard and quantified using a peak at *m/z* 57.1, as well as a peak at *m/z* 71.1 as a qualifier.

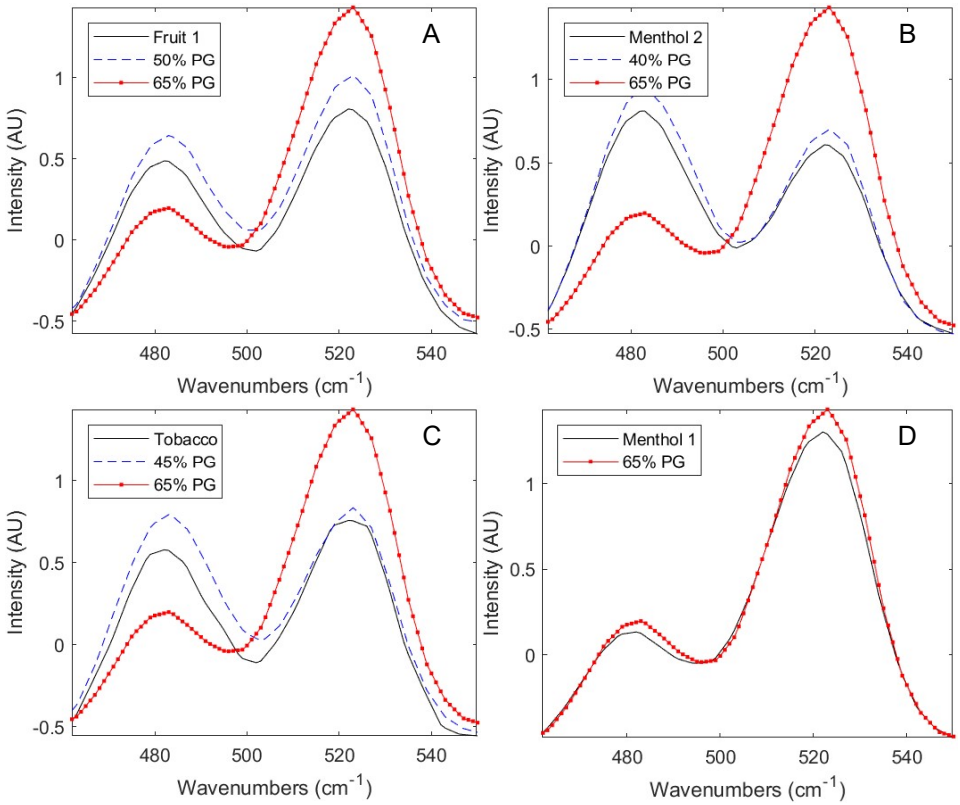


Figure S1: Typical Raman spectra from Producer B's Fruit 1 (S2A), Menthol 2 (S2B) and Tobacco (S2C) samples compared to both the stated and predicted PGVG ratio, alongside Menthol 1 (S2D) presented as a sample fitting the stated ratio. For each, the 470-550 cm^{-1} region containing the two subtracted peaks, is presented after baseline correction and normalisation, with the commercial sample shown in black, the closest PG:VG ratio in blue, and the stated 65% PG sample in red.

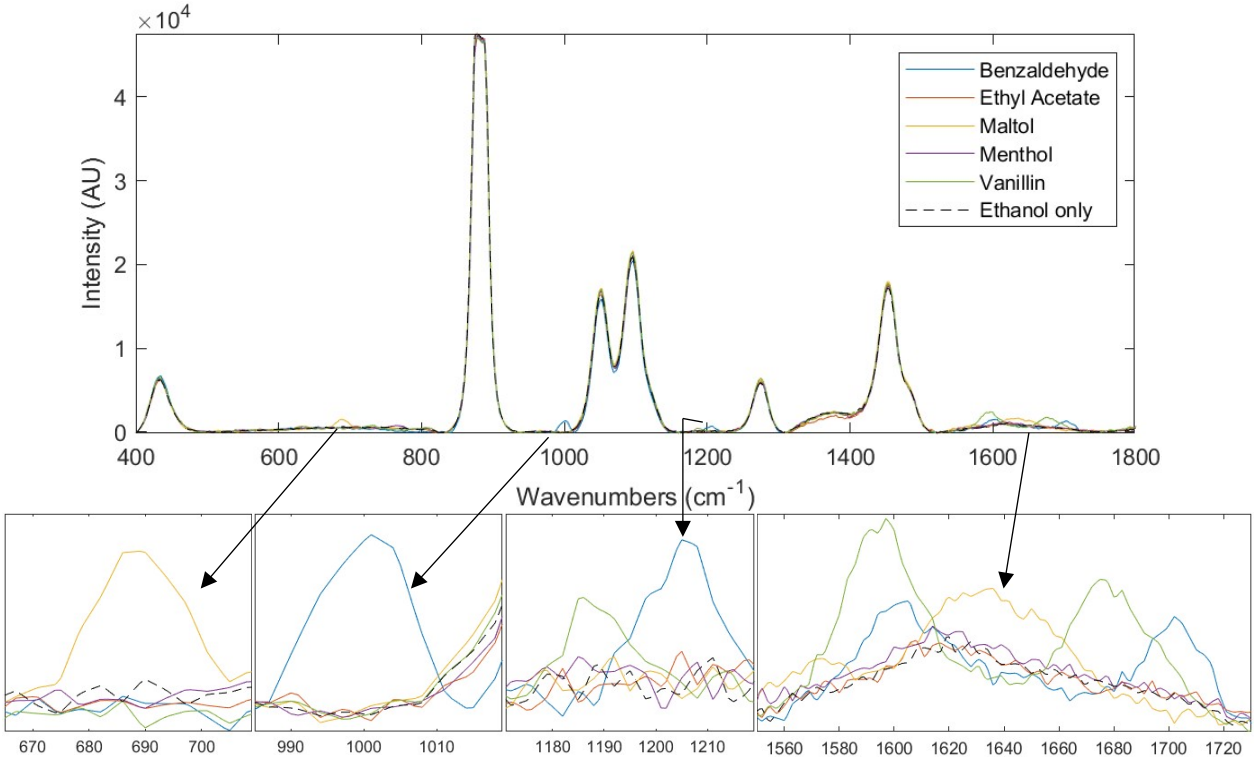


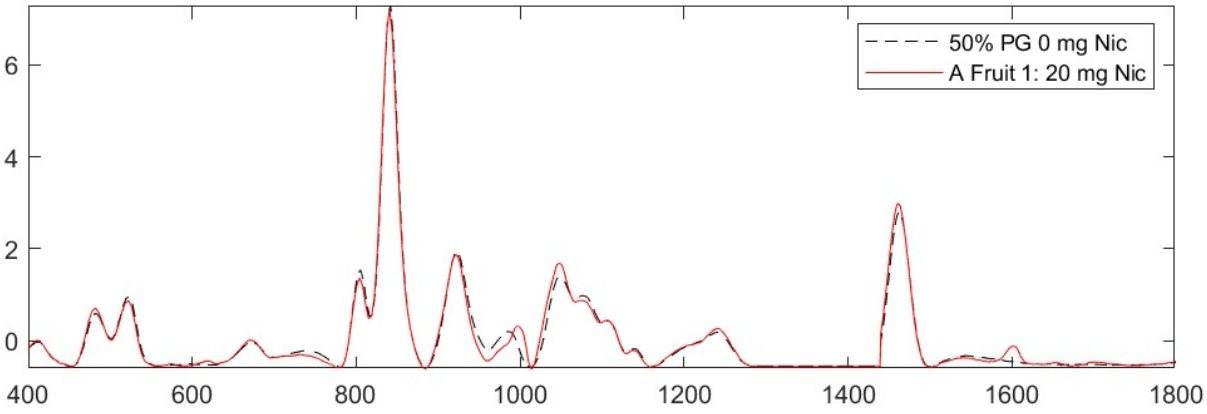
Figure S2 Baseline corrected Raman spectra of benzaldehyde (blue), ethyl acetate (red), maltol (yellow), menthol (purple) and vanillin (green) each at 5 mg·mL^{-1} in ethanol, with a representative spectrum of pure ethanol in black. In addition, four areas with peaks unique to each of the flavours are expanded.

Table S1: Tabulated form of Figure 4A & B. Values are predicted *minus* stated concentrations of nicotine in $mg \cdot g^{-1}$.

					Vials				Bottles			
		pH	Stated*	GC-MS	Trn only	With anchor	Plus sample	Sample used	Trn only	With anchor	Plus sample	Sample used
Producer A	Fruit 1	5	20	-0.53	13.54	-11.02	-4.63	F2: 18 mg	11.58	-12.50	-3.20	F2: 18 mg
	Menthol	9	3	0.24	20.80	1.19	0.08	M: 6 mg	19.51	1.41	0.10	F2: 1 mg
		9	6	0.36	22.02	2.02	0.67	M: 3 mg	21.20	0.48	0.00	F2: 1 mg
		9	11	0.49	14.20	-0.51	1.05	M: 16 mg	13.24	1.98	0.80	T: 1 mg
		9	16	-0.46	13.82	-1.43	0.11	M: 11 mg	12.23	-0.01	0.30	F2: 16 mg
		6	20	-1.49	18.38	-7.14	-0.74	T: 6 mg	20.05	-26.26	1.10	F2: 16 mg
	Fruit 2	5	1	0.13	15.80	0.03	0.10	F2: 3 mg	14.95	-1.21	-0.20	F2: 6 mg
		5	3	0.55	16.78	-0.12	-0.19	F2: 1 mg	15.54	-1.00	0.30	F2: 6 mg
		5	6	0.28	16.13	-0.53	-0.39	F2: 3 mg	15.26	-1.36	-0.58	F2: 3 mg
		7	11	-1.00	9.68	0.14	-0.17	F2: 3 mg	8.08	0.35	0.40	T: 6 mg
		7	16	2.07	9.00	-1.07	-0.62	F2: 6 mg	7.22	0.12	0.10	F2: 6 mg
		5	18	0.25	12.48	-9.08	-0.59	F1: 20 mg	13.30	-10.96	-1.80	F2: 16 mg
	Tobacco	6	1	0.45	27.50	8.83	-0.43	T: 3 mg	26.60	8.64	-0.60	T: 6 mg
		7	3	0.59	28.14	8.76	0.46	T: 1 mg	27.71	6.69	-0.20	T: 6 mg
		7	6	0.82	29.21	9.15	0.97	T: 3 mg	28.89	8.73	0.60	T: 1 mg
		8	11	3.69	19.85	10.98	2.15	T: 16 mg	19.29	11.28	0.50	T: 16 mg
		8	16	0.71	18.47	8.93	0.85	T: 6 mg	17.93	9.93	-0.38	T: 1 mg
Producer B	Menthol 1	8	9	-0.85	13.84	2.53	1.38	FB2: 9 mg				
	Fruit B1	5	0	0.01	47.97	41.56	-2.31	FB2: 9 mg				
	Fruit B2	8	9	0.89	15.86	6.81	2.65	M1: 9 mg				
	Fruit B3	9	9	-0.74	13.20	3.08	1.17	FB2: 9 mg				
	Menthol 2	6	0	0.01	24.40	6.41	0.32	T1: 18 mg				
	Tobacco 1	7	18	-1.22	21.57	1.08	-0.65	F1: 0 mg				

In the sample used column, F, M and T stand for Fruit, Menthol and Tobacco respectively.

* stated nicotine concentration on the packaging supplied with these commercial products.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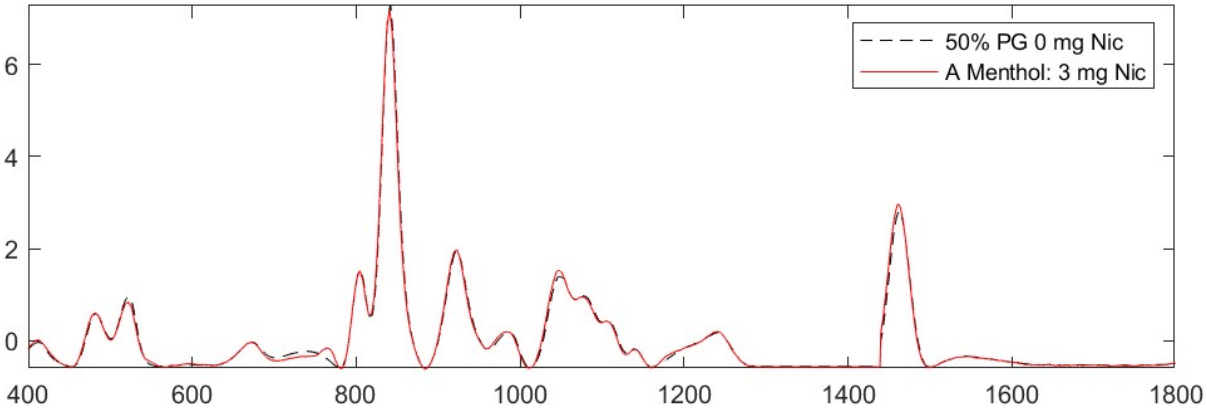
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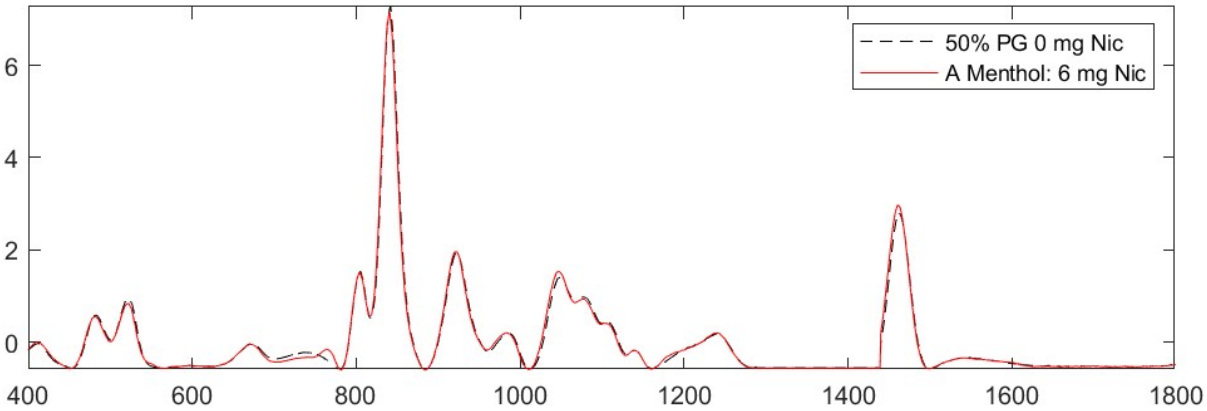
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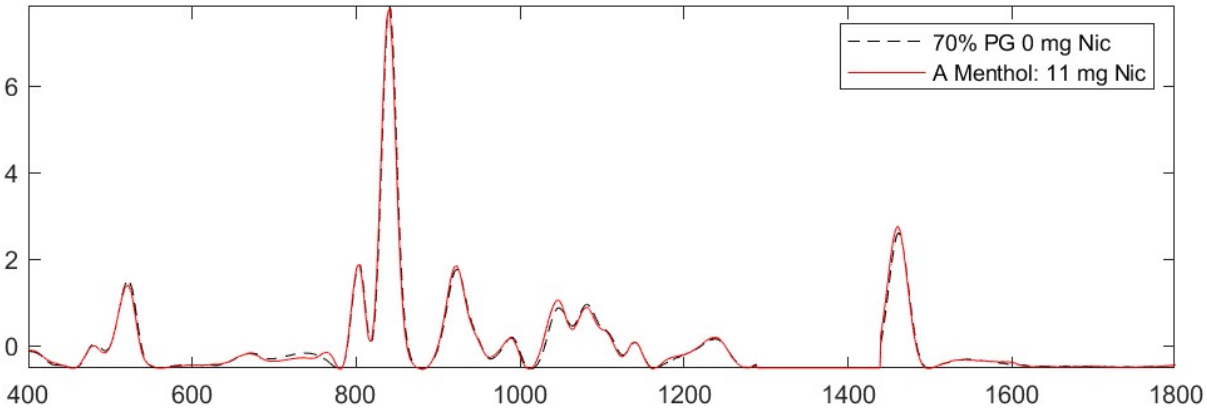
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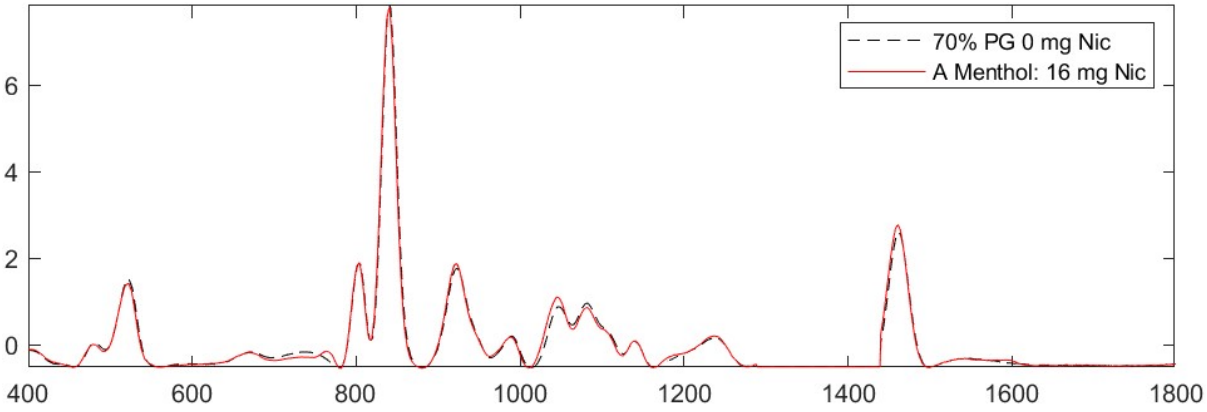
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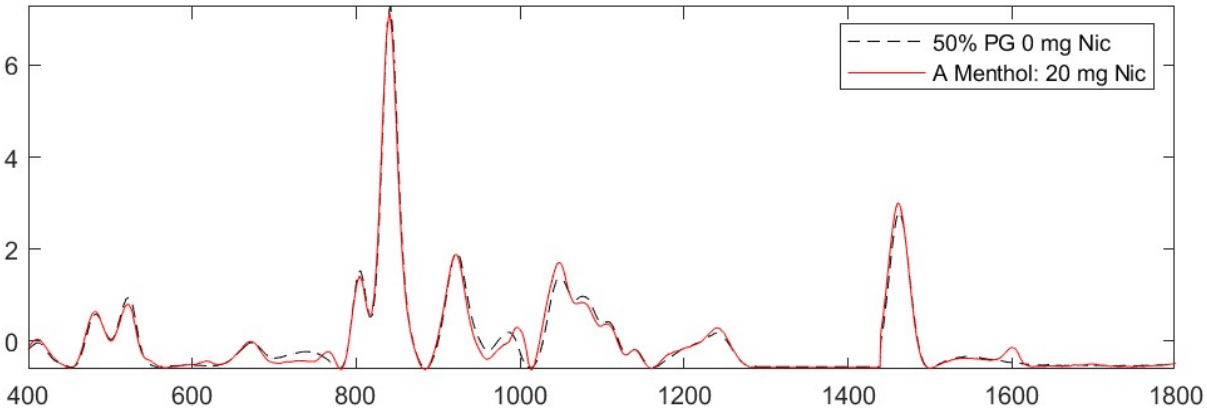
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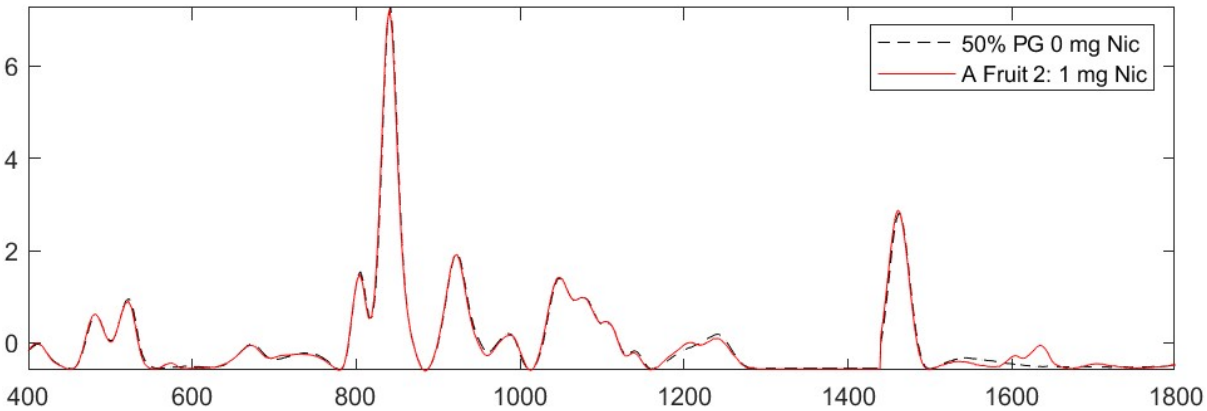
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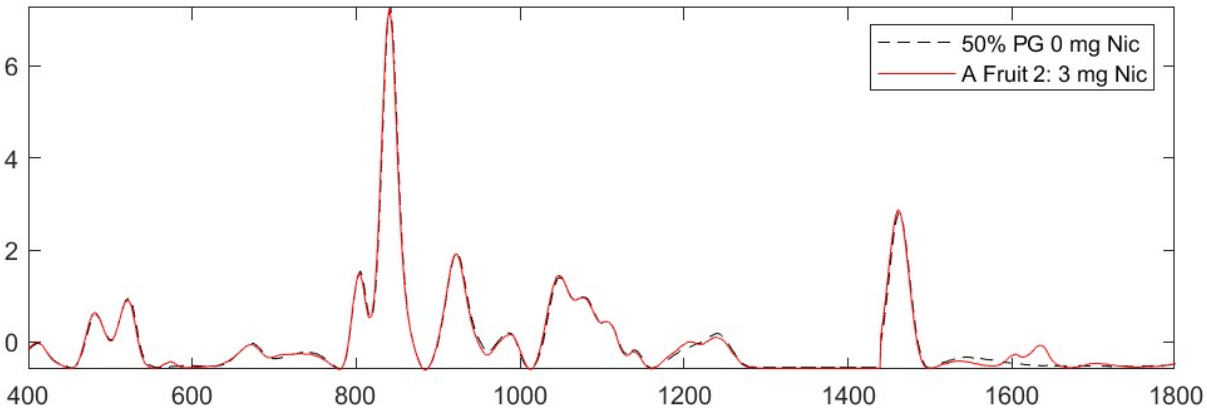
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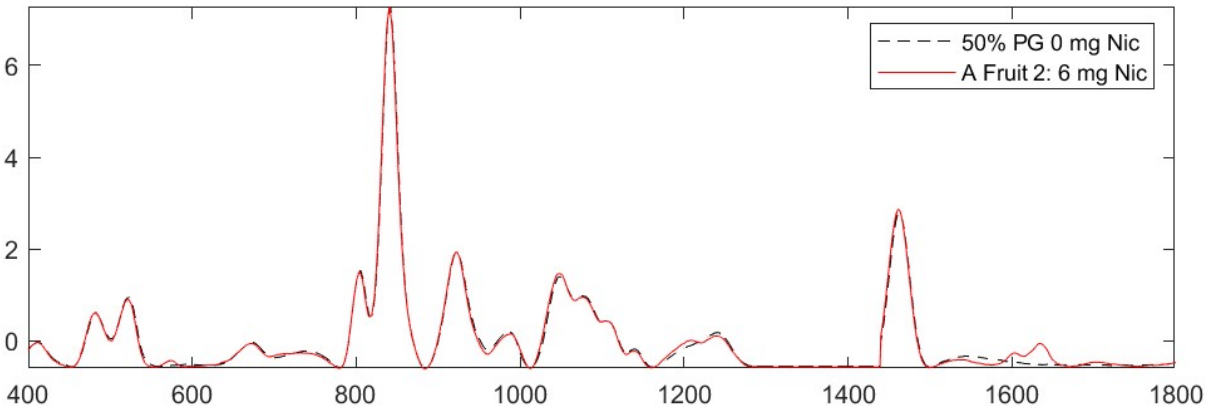
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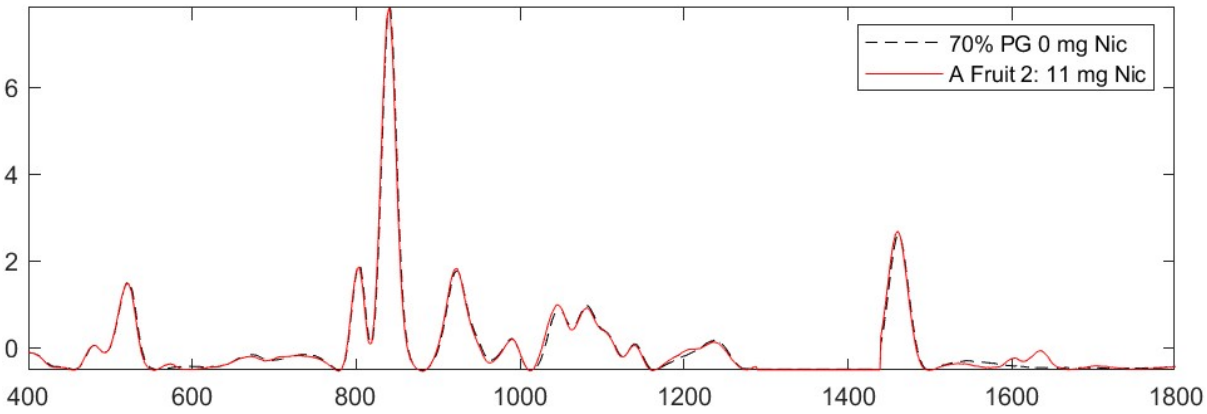
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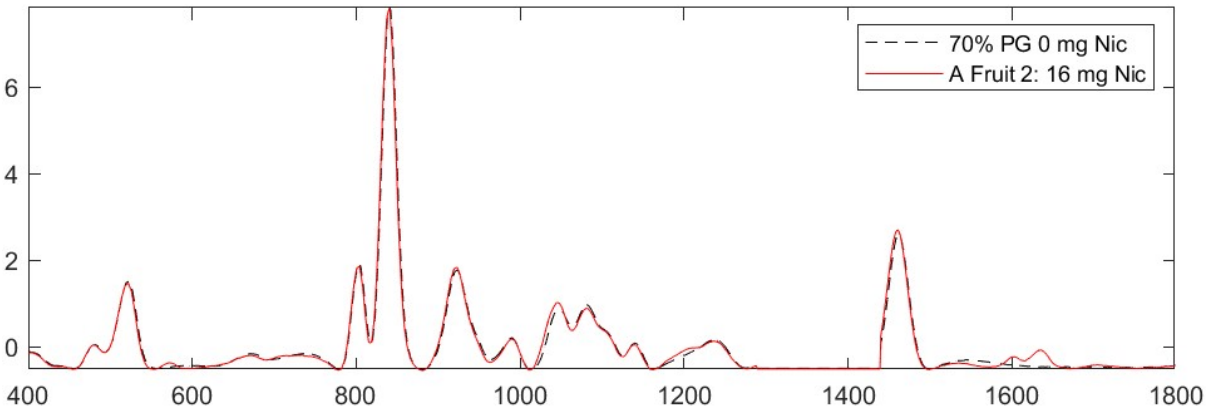
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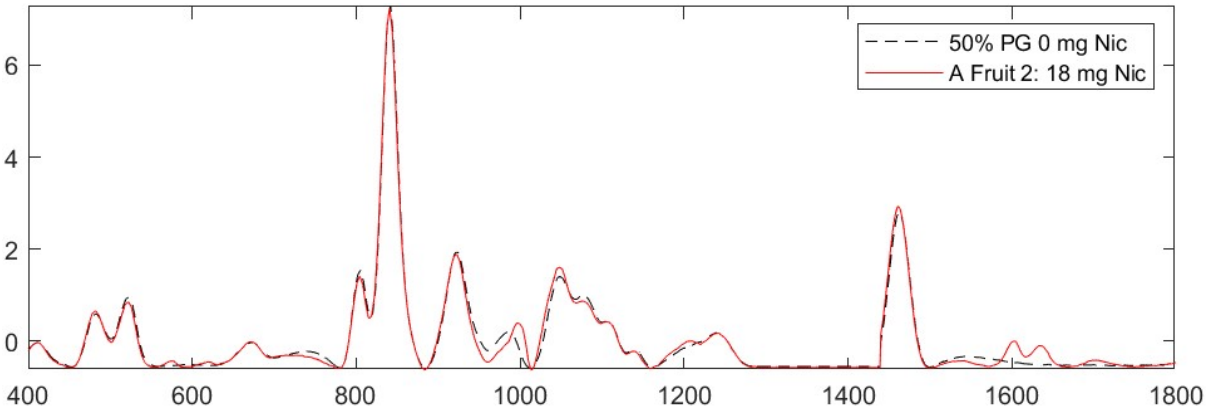
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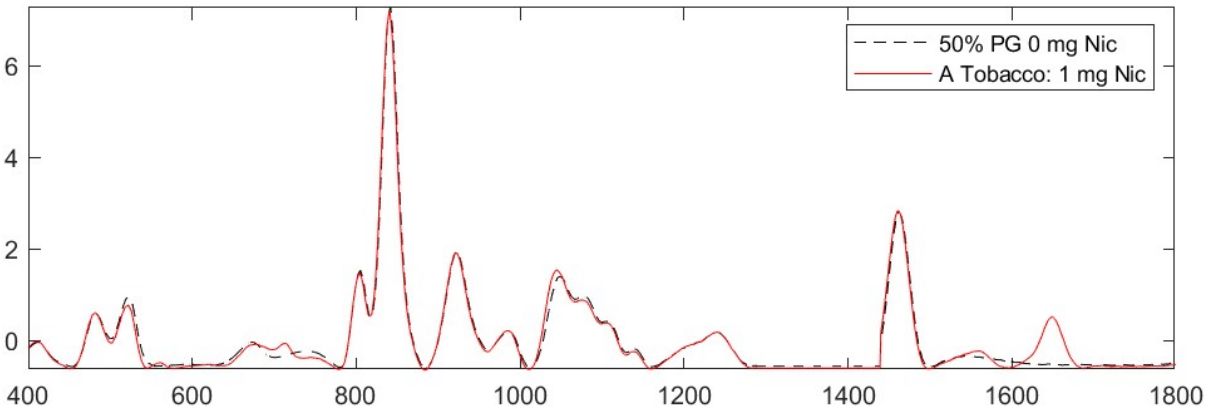
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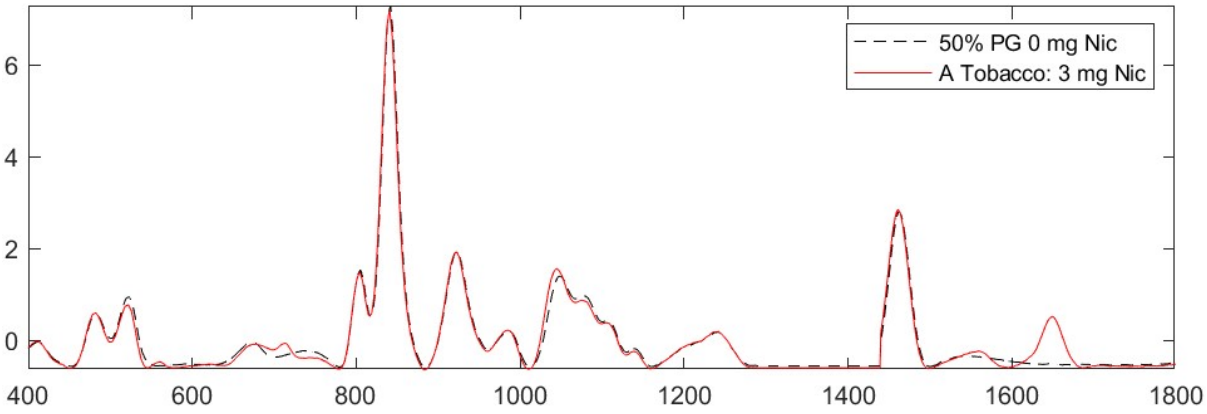
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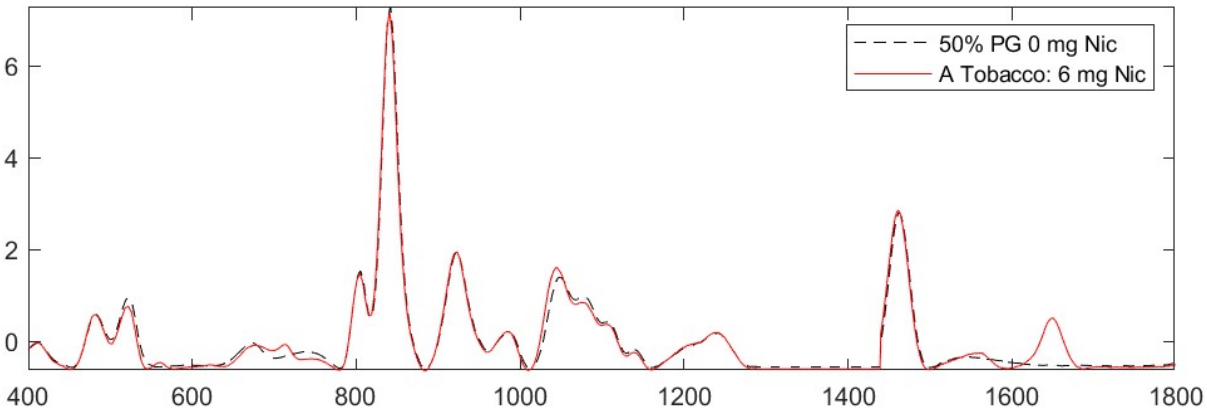
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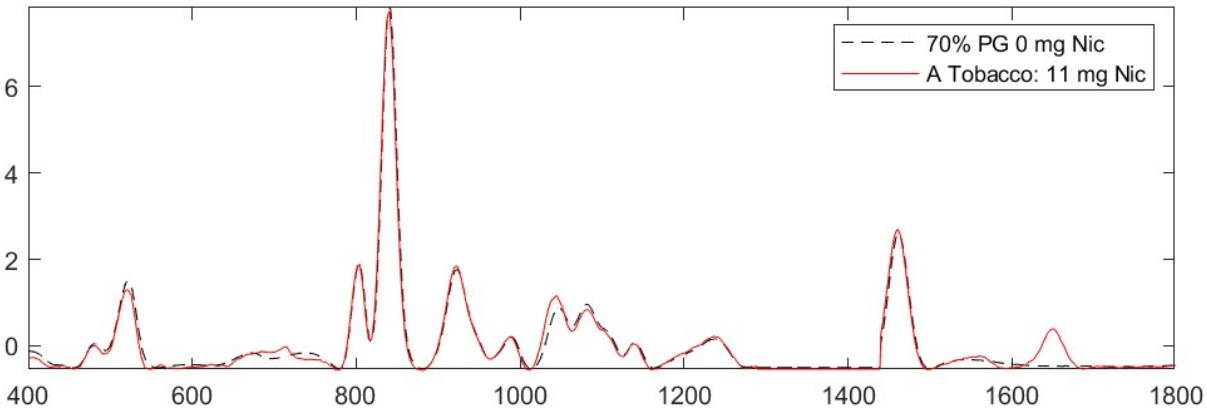
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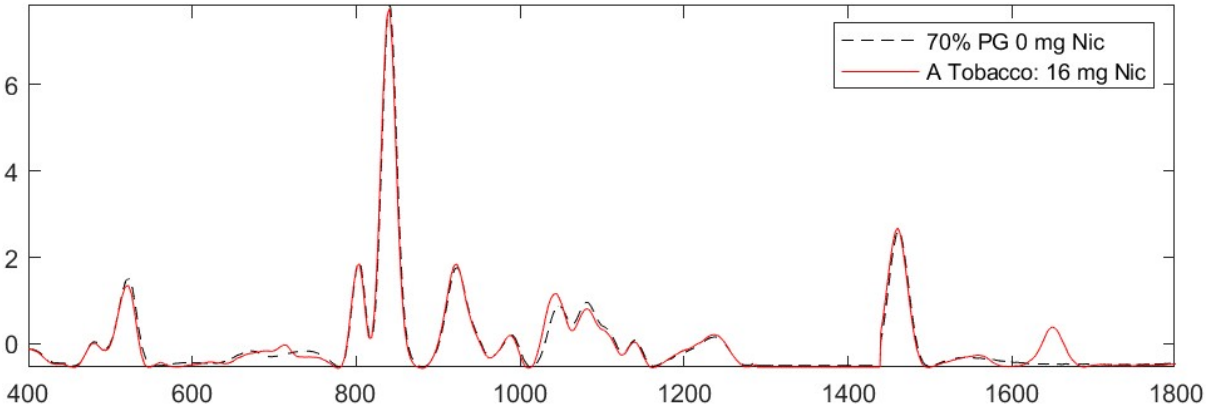
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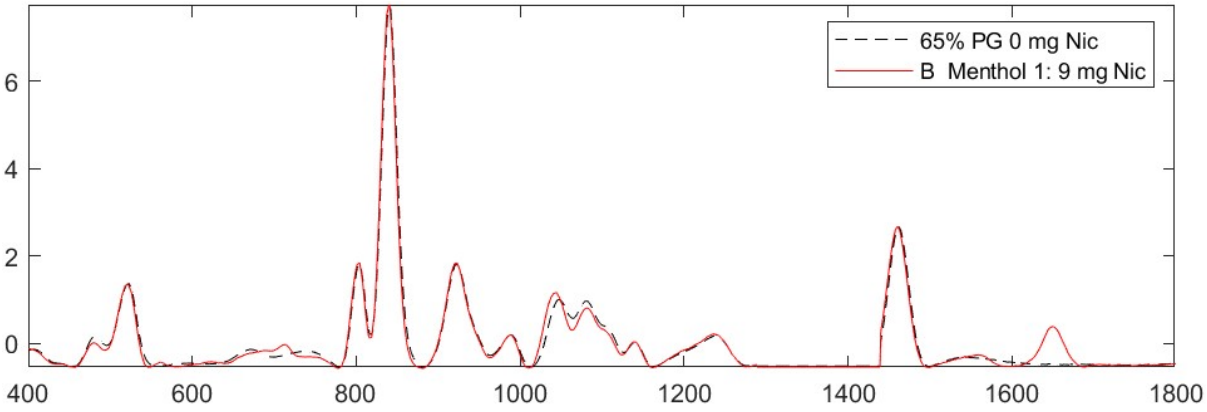
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B Menthol 1: 9 mg Nic
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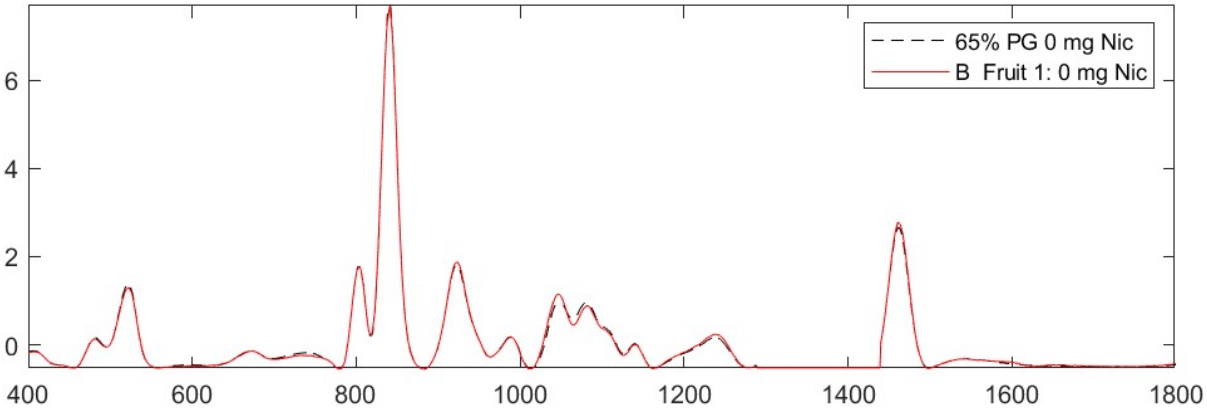
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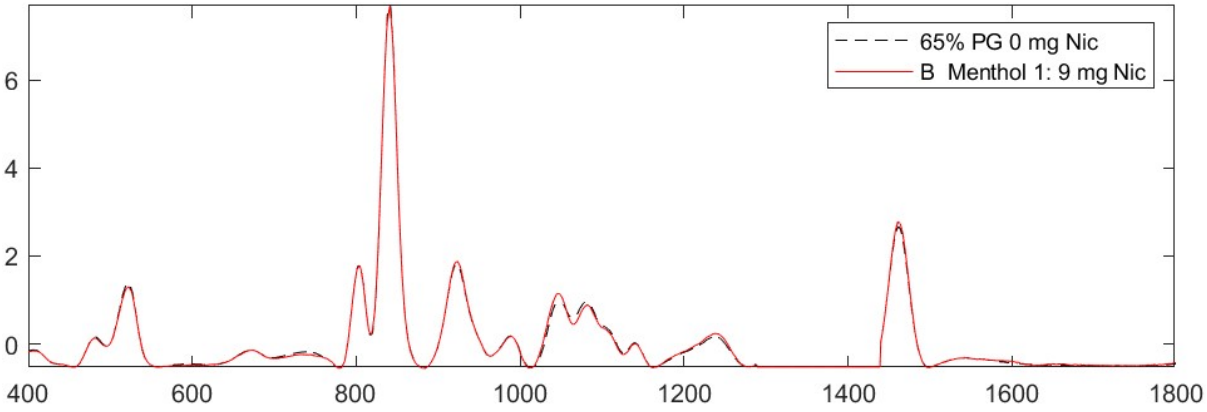
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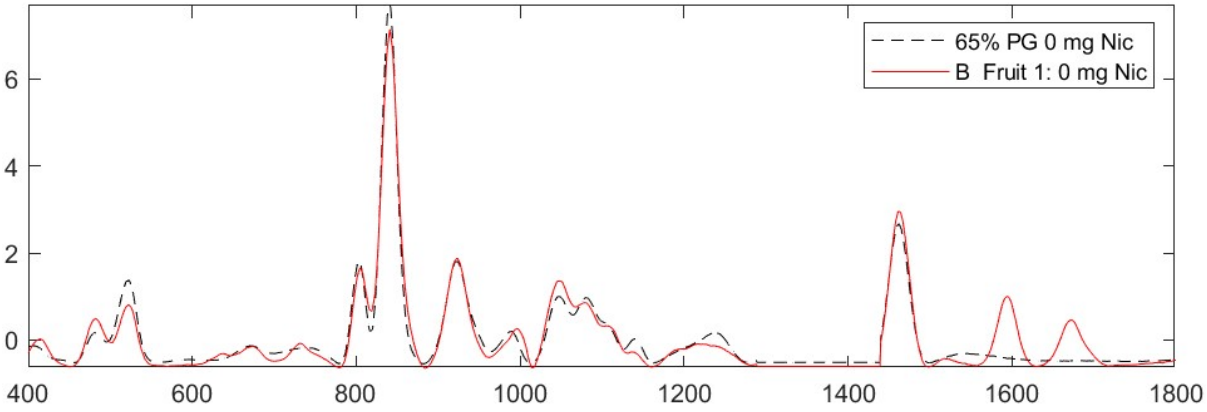
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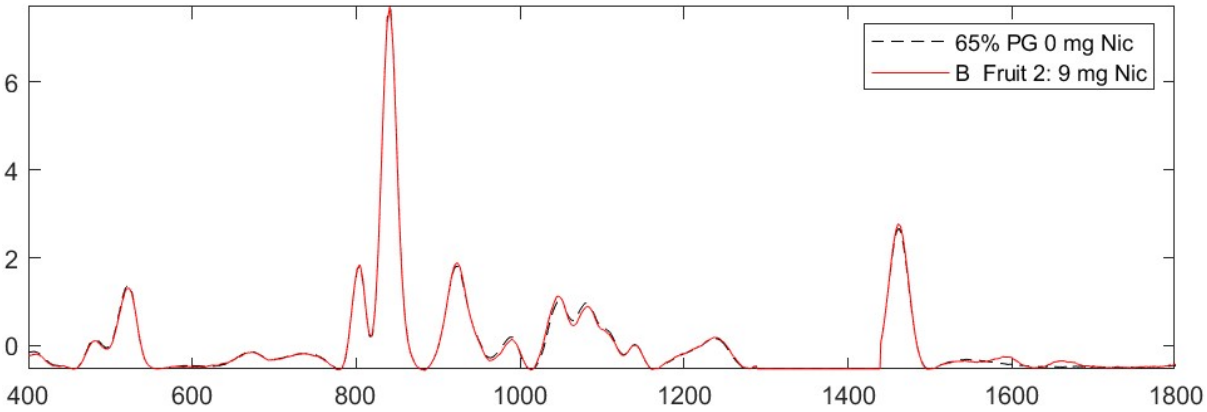
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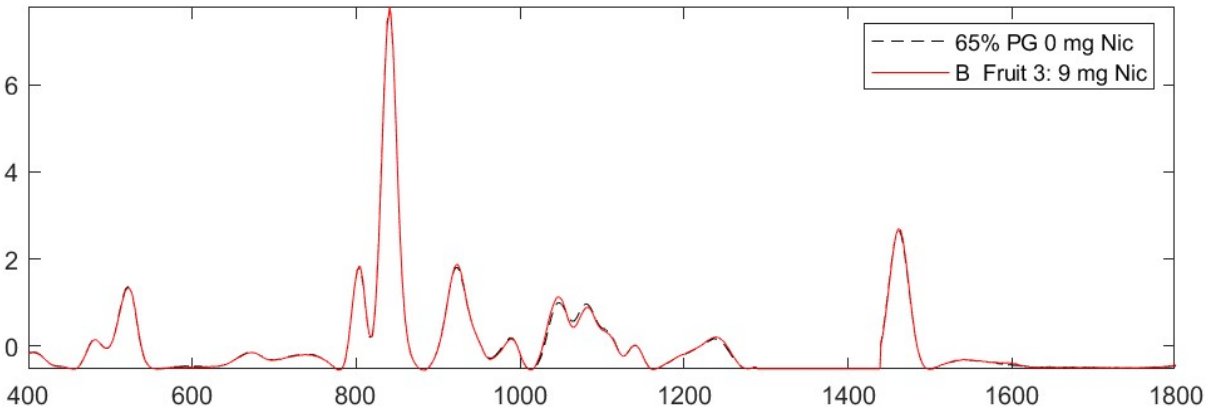
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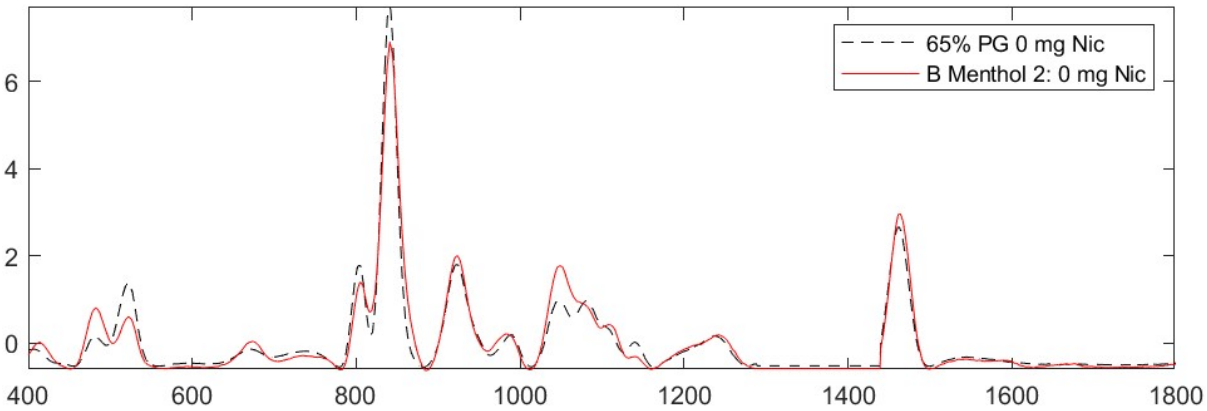
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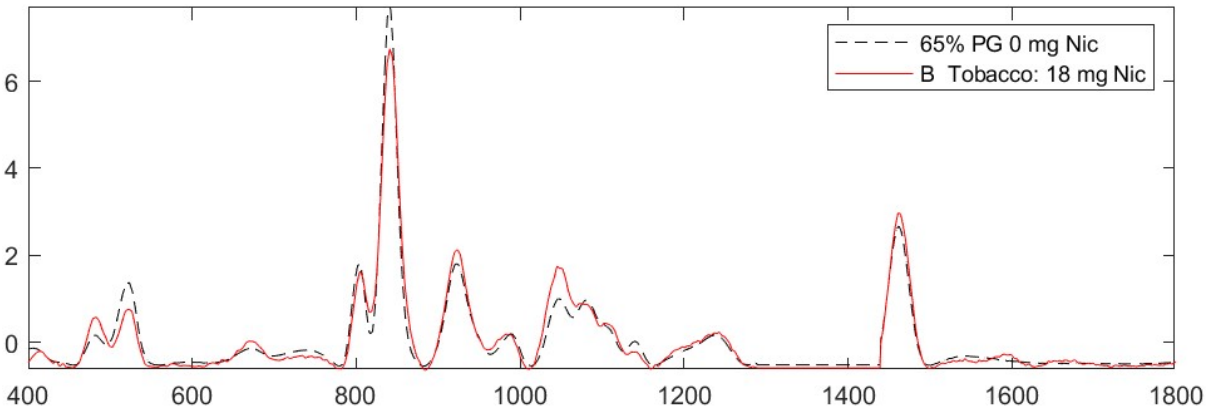

Figure S3: A series of Raman spectra of each commercial sample (red) alongside a spectrum of the stated PG:VG ratio (black) after baseline correction and normalisation.

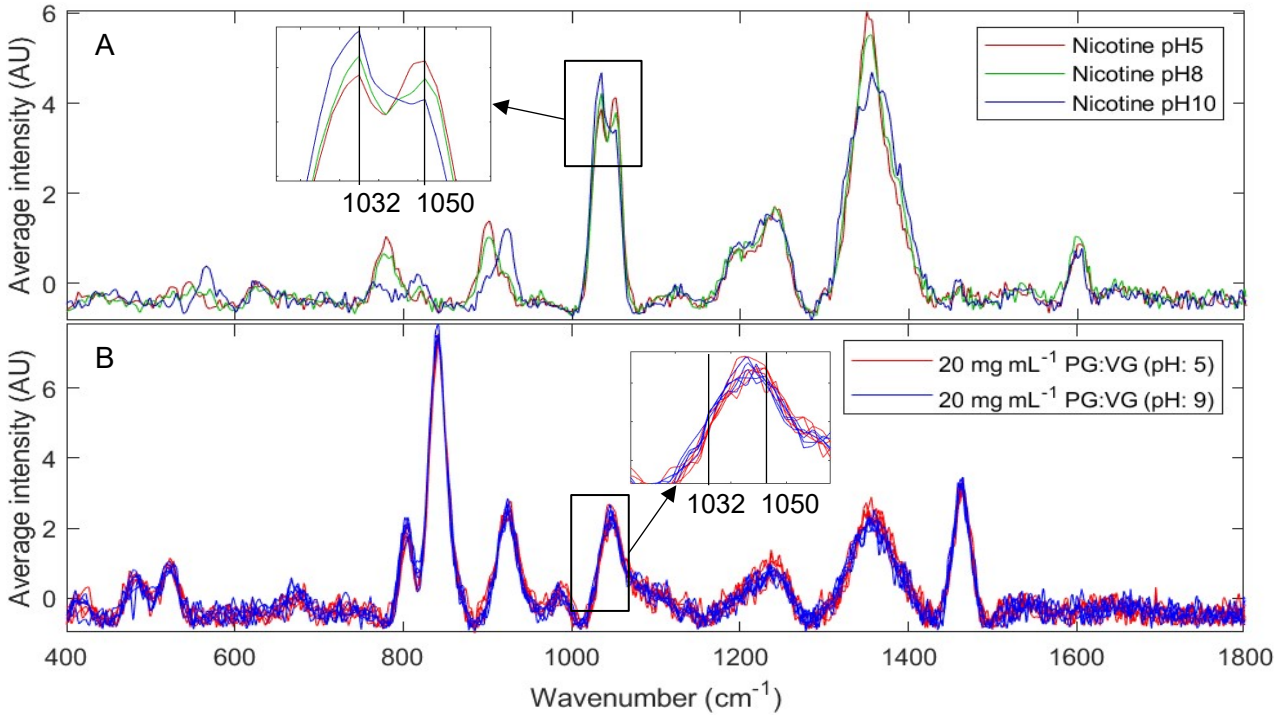

Figure S4: Raman spectra of 2% v/v aqueous nicotine at various pH levels (S4A), the pH was decreased using formic acid (red: pH 5, green: pH 8, blue: pH 10). The aqueous sample at pH 10 does not contain formic acid. In (S4B) 20 mg·mL^{-1} nicotine in 50:50 PG:VG, diluted 5:1 in deionised water and normalised to pH 5 (red) and pH 9 (blue) using formic acid. All spectra were baseline corrected and SNV normalised, and spectra in Figure S4A are the average of 5 acquisitions

Nicotine spiking

All prior experiments on commercial samples have used an imperfect training set. As stated prior, information on the compounds added for flavour and their concentrations is not only proprietary, but also differs between companies and even different nicotine concentrations of the same product. Indeed, our own GC-MS analyses showed some disparity between the actual levels estimated from GC-MS with those levels stated on the commercial e-liquid products. Prior experiments have aimed to reflect this imperfect picture to mimic the presumed conditions of regulatory screening. However, thanks to the relatively low costs of portable Raman spectrometers and their simplicity once the methods have been developed, there is a potential industrial outlet as a quality control tool. In this case, the flavour profile would be known and could easily be used within the training set, and this is what is done in other areas where one generates a database of samples that can be used within the PLSR calibration phase.

To replicate this, a sample of Producer A's 3 mg·g^{-1} tobacco flavoured e-liquid was spiked with increasing quantities of nicotine and analysed using Raman spectroscopy. The normalised peak intensity for the main nicotine peak 1045 cm^{-1} was then plotted against the amount of nicotine added and presented in Figure S5.

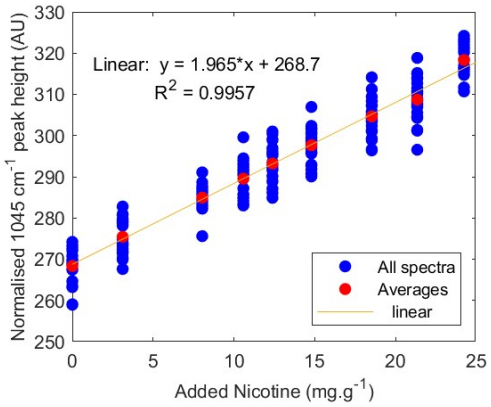

Figure S5. Relationship between the normalised peak height at 1045 cm^{-1} and the concentration of the nicotine spikes in mg·g^{-1}. Blue circles show the value for each individual spectrum, while red circles show the average of all 25 spectra. Linear regression was performed on the averages rather than individual points.

While each of the 25 individual spectra per concentration point show significant variance, it is clear from the average that, as long as the concentration of flavours remains constant, the relationship between the normalised 1045 cm^{-1} peak height and the concentration of nicotine is highly linear. Furthermore, it is worth considering that, in total, the acquisition of 25 spectra took slightly over 1 minute including the instrument's extra routines. However, while this method can be used to compare a new batch to a known control, it cannot be used to ascertain a sample's nicotine concentration through the standard addition method as described in[1,2]; the main visible peak at 1045 cm^{-1} is over a large PG:VG peak, and the secondary peak at 1640 cm^{-1} is not sufficiently pronounced to show a linear relationship over the noise.

1) Westley C, Xu Y, Thilaganathan B, Carnell AJ, Turner NJ, Goodacre R. Absolute Quantification of Uric Acid in Human Urine Using Surface Enhanced Raman Scattering with the Standard Addition Method. *Anal Chem*. 2017;89(4):2472-2477. doi:10.1021/acs.analchem.6b04588

2) Goodacre R, Graham D, Faulds K. Recent developments in quantitative SERS: Moving towards absolute quantification. *TrAC - Trends Anal Chem*. 2018;102:359-368. doi:10.1016/j.trac.2018.03.005