

## SUPPLEMENTARY MATERIAL TO

Geyik C., Gümüş Z.P. & Yararbaşı G. 2022. Composition analysis of e-liquids and their effects on healthy liver and pharyngeal carcinoma cell lines. *Trakya Univ J Nat Sci*, 24(1): xx-xx, DOI: 10.23902/trkjnat.1135237

### 1. Selected Parameters of Method Validation for Nicotine

#### 1.1. Linearity

Linearity criterion was measured by standard test solution at 6 different concentration levels. Linearity calculations were performed over the HPLC fields obtained at each concentration step. The obtained values are given in Table S1.

Table S1. Linearity Table of Nicotine

| Sample No | Concentration (µg / ml) | Peak Area (mAU * s) | Mean Peak Area (mAU * s) | % RSD |
|-----------|-------------------------|---------------------|--------------------------|-------|
| 1         | 1.00                    | 62                  | 58                       | 5.51  |
|           |                         | 57                  |                          |       |
|           |                         | 56                  |                          |       |
| 2         | 2.50                    | 103                 | 99                       | 4.04  |
|           |                         | 95                  |                          |       |
|           |                         | 99                  |                          |       |
| 3         | 5.00                    | 166                 | 170                      | 2.04  |
|           |                         | 172                 |                          |       |
|           |                         | 172                 |                          |       |
| 4         | 10.00                   | 318                 | 324                      | 1.70  |
|           |                         | 328                 |                          |       |
|           |                         | 327                 |                          |       |
| 5         | 25.00                   | 802                 | 801                      | 1.19  |
|           |                         | 791                 |                          |       |
|           |                         | 810                 |                          |       |
| 6         | 50.00                   | 1611                | 1632                     | 1.11  |
|           |                         | 1645                |                          |       |
|           |                         | 1639                |                          |       |

For the linearity criterion for nicotine, each concentration was injected 3 times and the calibration graph plotted over the mean values of the areas is given in Fig. S1.

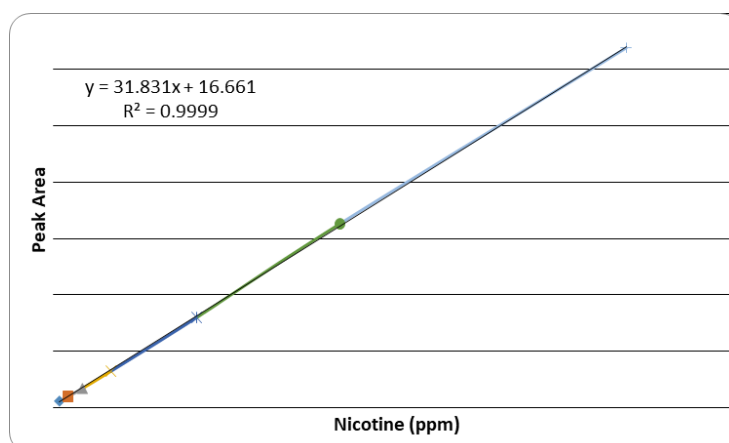
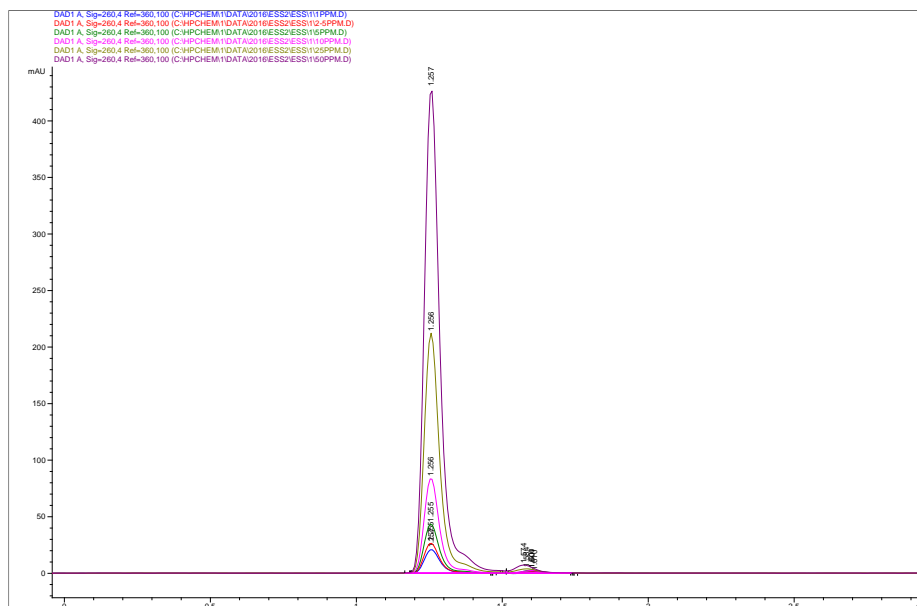


Fig. S1. Calibration graphs of nicotine

Fig. S2 shows the superimposed states of the chromatograms of the points used for linearity.



**Fig. S2.** Chromatograms of nicotine for each calibration point

According to chromatogram, it is also seen that there is no shift in the peak's retention times and there is good linearity.

### 1.2. Accuracy of method for nicotine

Test solutions prepared at low, medium and high concentrations were analyzed in order to determine how close the experimental values are to real values.

Electronic cigarette liquid with a zero nicotine ratio was used as a blank sample and the peak was not observed in the retention time where the nicotine peak.

Sample chromatograms for 3 concentrations selected for extraction reproducibility and recovery studies are given below. To calculate %recovery, following steps were used:

- i) Standard with known concentration was applied and mean area is measured.
- ii) From calibration plot, measured area is converted to concentration
- iii) % recovery =  $[(\text{Measured concentration}) / (\text{Known concentration})] \times 100$

Three parallel samples were run for each concentration level and the results obtained were calculated as mean recovery and % RSD. The results obtained are given in Table S2 below.

**Table S2.** Recovery of nicotine for accuracy of nicotine method.

| Sample No | Concentration (ug / ml) | Peak Area (mAU *s) | % Recovery | Mean Recovery (%) | % RSD |
|-----------|-------------------------|--------------------|------------|-------------------|-------|
| 1         | 2.5                     | 92                 | 92.34      | 93.88             | 1.15  |
|           |                         | 93                 |            |                   |       |
|           |                         | 94                 |            |                   |       |
| 2         | 2.5                     | 97                 | 96.11      |                   |       |
|           |                         | 96                 |            |                   |       |
|           |                         | 95                 |            |                   |       |
| 3         | 2.5                     | 93                 | 93.18      |                   |       |
|           |                         | 94                 |            |                   |       |
|           |                         | 94                 |            |                   |       |
| 4         | 5.0                     | 166                | 91.88      |                   |       |
|           |                         | 168                |            |                   |       |
|           |                         | 163                |            |                   |       |
| 5         | 5.0                     | 161                | 89.99      |                   |       |
|           |                         | 163                |            |                   |       |
|           |                         | 164                |            |                   |       |
| 6         | 5.0                     | 162                | 90.62      |                   |       |
|           |                         | 165                |            |                   |       |
|           |                         | 164                |            |                   |       |
| 7         | 10.0                    | 319                | 94.26      | 96.53             | 0.44  |
|           |                         | 321                |            |                   |       |
|           |                         | 318                |            |                   |       |
| 8         | 10.0                    | 326                | 96.36      |                   |       |
|           |                         | 325                |            |                   |       |
|           |                         | 327                |            |                   |       |
| 9         | 10.0                    | 336                | 98.98      |                   |       |
|           |                         | 333                |            |                   |       |
|           |                         | 334                |            |                   |       |

## 2. Selected Parameters of Method Validation for Propylene Glycol (PG) and Glycerin (GY)

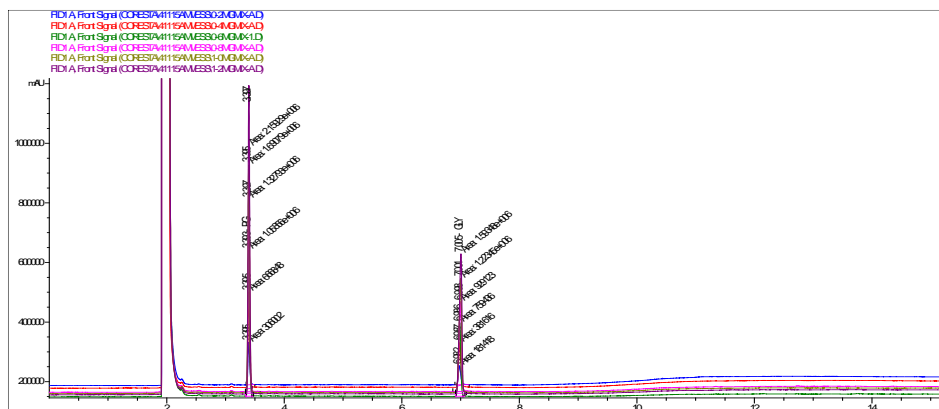
### 2.1. Linearity of PG and GLY

**Table S3.** Linearity values of PG

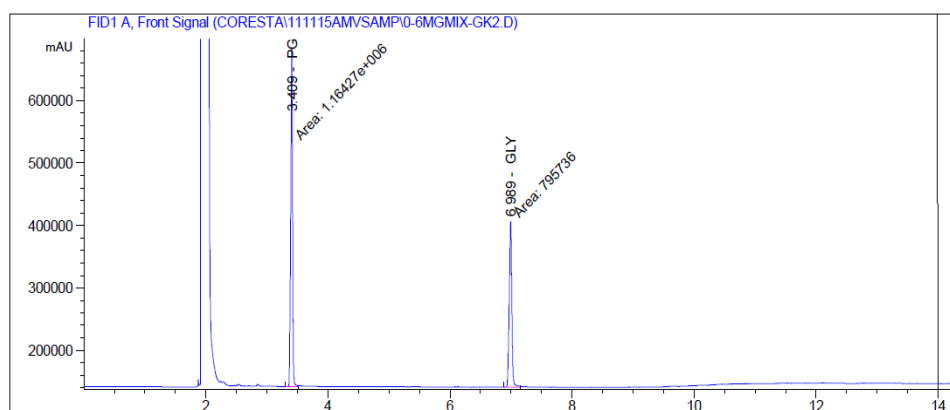
| PG Sample No | Concentration (mg / mL) | Peak Area (mAU * s) | Mean Peak Area (mAU * s) | % RSD |
|--------------|-------------------------|---------------------|--------------------------|-------|
| 1            | 0.2                     | 306002              | 306792                   | 3.89  |
|              |                         | 319095              |                          |       |
|              |                         | 295280              |                          |       |
| 2            | 0.4                     | 666848              | 676020                   | 1.21  |
|              |                         | 678921              |                          |       |
|              |                         | 682292              |                          |       |
| 3            | 0.6                     | 1044090             | 1026838                  | 3.16  |
|              |                         | 989375              |                          |       |
|              |                         | 1047050             |                          |       |
| 4            | 0.8                     | 1327930             | 1372223                  | 2.98  |
|              |                         | 1380020             |                          |       |
|              |                         | 1408720             |                          |       |
| 5            | 1.0                     | 1690790             | 1734583                  | 3.43  |
|              |                         | 1710720             |                          |       |
|              |                         | 1802240             |                          |       |
| 6            | 1.2                     | 2159290             | 2159550                  | 0.32  |
|              |                         | 2152720             |                          |       |
|              |                         | 2166640             |                          |       |

**Table S4.** Linearity values of GLY

| GLY Sample No | Concentration (mg / mL) | Peak Area (mAU * s) | Mean Peak Area (mAU * s) | % RSD |
|---------------|-------------------------|---------------------|--------------------------|-------|
| 1             | 0.2                     | 181418              | 197177                   | 13.02 |
|               |                         | 226792              |                          |       |
|               |                         | 183320              |                          |       |
| 2             | 0.4                     | 381616              | 417825                   | 7.69  |
|               |                         | 428808              |                          |       |
|               |                         | 443052              |                          |       |
| 3             | 0.6                     | 694399              | 695395                   | 3.80  |
|               |                         | 669475              |                          |       |
|               |                         | 722312              |                          |       |
| 4             | 0.8                     | 929123              | 986742                   | 5.34  |
|               |                         | 998594              |                          |       |
|               |                         | 1032510             |                          |       |
| 5             | 1.0                     | 1223450             | 1271243                  | 4.02  |
|               |                         | 1265160             |                          |       |
|               |                         | 1325120             |                          |       |
| 6             | 1.2                     | 1593480             | 1564967                  | 2.22  |
|               |                         | 1575240             |                          |       |
|               |                         | 1526180             |                          |       |



**Fig. S3.** Chromatograms of calibration point for PG and GLY



**Fig. S4.** Chromatogram of PG and GLY

**2.2. Accuracy of method for PG and GLY**

Recovery is calculated as described in 1.2

**Table S5.** Recoveries of PG and GLY for accuracy of method.

| Sample No | Concentration (mg / mL) | Peak Area (mAU *s) | % Recovery | Mean Peak Area(%) | % RSD |
|-----------|-------------------------|--------------------|------------|-------------------|-------|
| 1         | 0.3 (PG)                | 481789             | 91.27      | 94.65             | 3.85  |
|           |                         | 525230             | 98.51      |                   |       |
|           |                         | 499268             | 94.18      |                   |       |
| 2         | 0.6 (PG)                | 1134746            | 100.05     | 99.77             | 2.90  |
|           |                         | 1164270            | 102.51     |                   |       |
|           |                         | 1095106            | 96.74      |                   |       |
| 3         | 0.3 (GLY)               | 198169             | 103.88     | 104.14            | 2.23  |
|           |                         | 206241             | 106,58     |                   |       |
|           |                         | 192380             | 101.96     |                   |       |
| 4         | 0.6 (GLY)               | 478657             | 98.69      | 99.07             | 2.33  |
|           |                         | 495736             | 101.54     |                   |       |
|           |                         | 468327             | 96.97      |                   |       |

### 3. Volatile compound analysis by mass spectroscopy

Table S6. Volatile compounds identified from Wiley MS spectra database

|                               | A-CAR-0 | A-CAR-9 | A-CAR-18 | B-CAP-0 | B-CAR-0 | B-STR-0 | B*-STR-9 | B*-STR-18 | C-MIX-0 | C-MIX-6 | C-MIX-24 |
|-------------------------------|---------|---------|----------|---------|---------|---------|----------|-----------|---------|---------|----------|
| Ethyl n-butanoate             |         |         |          |         |         |         | *        | *         |         |         |          |
| Ethyl 2-methylbutanoate       |         |         |          |         |         |         | *        | *         |         |         |          |
| Methyl isobutyl ketone        | *       | *       | *        | *       | *       | *       |          |           | *       | *       | *        |
| Ethyl n-valerate              |         |         |          |         |         |         | *        | *         |         |         |          |
| Ethyl caproate                |         |         |          |         |         |         | *        |           |         |         |          |
| Ethylene glucol               | *       | *       | *        |         |         |         | *        |           |         |         |          |
| Amylcarbinol                  |         |         |          |         |         | *       |          |           |         |         |          |
| beta-gamma-hexenol            |         |         |          |         |         | *       | *        | *         |         |         |          |
| Thiocyanic acid, propyl ester | *       | *       | *        | *       |         |         |          |           |         |         |          |
| Caramel Furanone              | *       |         |          |         |         |         |          |           |         |         |          |
| Phenol, 2-methoxy-4-propyl-   | *       |         |          |         |         |         |          |           |         |         |          |
| 2,4-Dihydroxyacetophenon      | *       |         |          |         |         |         |          |           |         |         |          |
| Bourbonal                     | *       |         |          |         |         |         |          |           |         |         |          |
| Quantrovanil                  | *       |         |          |         |         |         |          |           |         |         |          |
| Ethovan                       | *       |         |          |         |         |         |          |           |         |         |          |
| 5-Methyl-2-furfural           |         |         |          | *       | *       |         |          |           |         |         |          |
| gamma-Valerolactone           |         |         |          | *       | *       |         |          |           |         |         |          |
| Hydrazine                     | *       |         |          |         |         |         |          |           |         |         |          |
| Gamma-N-Caprolactone          | *       | *       | *        |         |         |         |          |           |         |         |          |
| Caprylene                     |         |         |          |         |         |         |          |           | *       |         |          |
| Nicotine                      |         | *       | *        |         |         |         | *        | *         |         | *       | *        |
| Benzyl Alcohol                |         |         |          | *       | *       |         |          |           |         |         |          |
| Perhydroisoquinoline          |         |         |          |         |         |         |          |           | *       |         |          |
| Glycerol triacetate           |         |         |          | *       |         |         |          |           |         |         |          |
| Methyl cinnamate              |         |         |          |         |         |         | *        |           |         |         |          |
| Ethyl cinnamate               |         |         |          |         |         | *       |          |           |         |         |          |
| gamma-Decanolactone           |         |         |          | *       |         | *       |          |           |         |         |          |
| Ludoctal                      |         |         |          |         | *       |         | *        |           |         |         |          |
| Piperonal (Heliotropine)      | *       | *       | *        | *       | *       |         |          |           |         |         |          |