# Targeted and non-targeted analysis of cannabis-containing e-juice using nominal and accurate mass LC-MS/MS

***Using the SCIEX Triple Quad 4500 system and the SCIEX X500R QTOF system***

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The vaping crisis of 2019 and 2020, which involved nicotine and cannabis-based e-juice cartridges, contributed to 68 deaths and greater than 2,800 hospitalizations.[1] Laboratory findings showed a correlation between hospitalized patients and the presence of vitamin e acetate (VEA) in their e-juice.[2] As a result, many states have banned VEA from e-juice formulations and require testing to support this ban.

VEA is listed as a generally recognized as safe (GRAS) chemical in food commodities.[3] But the GRAS list does not consider high-temperature vaporization as a route of ingestion, nor does it explicitly consider the exposure of bronchiolar tissues to such food additives. Given the issues identified with VEA, there is a growing discussion that other GRAS additives in e-juice formulations warrant further study. This includes flavoring components, thickening agents, emulsifiers, medium-chain triglycerides (MCT), natural products such as phytol, and agricultural residues.[4] Myclobutanil, a commonly used fungicide, is an often-cited example of this, as one of its thermal degradants is hydrogen cyanide.

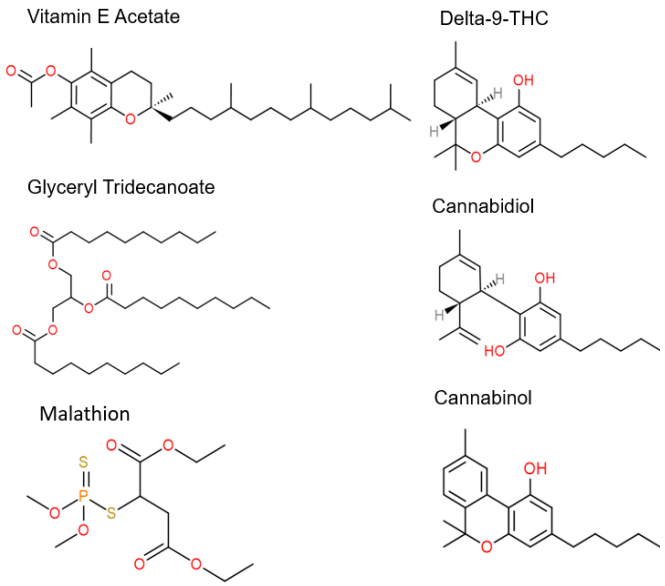


**Figure 1. Representative compounds in the targeted LC-MS/MS method.** Thickening agents and pesticides (left) and various cannabinoids (right) were targeted for quantitation.

In this note, a methodology for the accurate and precise quantitative assessment of e-juice components is described. These include cannabinoids, vitamin E, VEA, and MCT. This is accomplished via an easy-to-use and robust LC-MS/MS method using a SCIEX Triple Quad 4500 system coupled to an ExionLC AC system. In addition, a methodology to discover unknown contaminants and additives via accurate mass MS/MS library matching was explored. This was accomplished through the combined advantages of fast scanning using the SCIEX X500R QTOF system and streamlined data processing with SCIEX OS software.

## Key method features

- Simple sample preparation
- 10-minute optimized LC method with F5 column chemistry offered adequate separation, quickly, without carryover
- Fast quantitative data acquired on the SCIEX Triple Quad 4500 system and the SCIEX X500R system
- Simplified data management in SCIEX OS software for targeted and non-targeted analyses

## Methods

***Sample preparation:*** Ten target analytes and three corresponding internal standards (delta-9-THC $D_3$, delta-8-THC $D_3$, and vitamin E-$D_6$) were purchased from Cerilliant. A 10 µg/mL mix of target analyte was made in methanol alongside a separate 10 µg/mL mix of internal standards. These mixes were then diluted to two separate 1 µg/mL mixes for calibrator production in methanol. Triacylglyceryl decanoate (TAG10) was purchased neat and was diluted in isopropanol to 1 µg/mL before use. Solvent calibration curves were prepared from 1 ng/mL to 500 ng/mL. Depending on the analyte and its dilution, this corresponded to 0.0005% to 70% in sample. Each calibrator and sample contained 50 ng/mL of internal standards. E-juice formulations were diluted 1,000 times in methanol and subsequently fortified at 5 ng/mL, 10 ng/mL, and 20 ng/mL (in vial) in five replicates for each fortification level corresponding, after dilution, to 0.0005% to 0.002% in sample.

Twelve e-juice formulations containing cannabinoids were analyzed in this study. Seven (*1-7*) were from grey markets (not tested for regulatory compliance) and five were from green markets (*8-12*, previously tested for regulatory compliance). Approximately 50 mg of each sample was accurately weighed into a 1.5 mL conical centrifuge tube and methanol was added to bring the weight to 500 mg. Samples were vortexed and placed at -20 °C for two hours. The winterized samples were centrifuged and then filtered (0.2 µm, nylon) into an amber autosampler vial. The filtrate was further diluted with methanol and appropriate internal standards to give 100 x, 1,000 x, 100,000 x, and 10,000,000 x dilutions. The 100 x and 1,000 x dilutions were used for non-targeted analysis. The other dilutions were used for targeted quantification.

***Chromatography:*** For the targeted method HPLC separation was performed on an ExionLC system using a Phenomenex Kinetex F5 (100 × 4.6 mm, 2.6µm) using a 5 µL injection volume. The column temperature was held at 40 ºC while using a 10 minute gradient ramped from 20% organic to 100% organic at a 1.0 mL/min flow rate. Mobile phases consisted of either water or methanol with 0.1% formic acid and 5mM ammonium formate.

The non-targeted method employed a Phenomenex Luna Omega Polar C18 (150 × 3.0 mm, 3.0µm) using a 5 µL injection volume. 40 ºC was used as the column temperature while using a 15 minute gradient ramped from 20% organic to 100% organic at a 1.0 mL/min flow rate. Mobile phases were the same composition as used in the targeted method.

***Mass spectrometry:*** Targeted analysis was performed on the SCIEX Triple Quad 4500 system using positive polarity with electrospray ionization (ESI).[12] Source conditions were set to Curtain gas of 35, GS1 of 70, GS2 of 70, IS of 2500, and TEM of 500. Acquisition was performed using Analyst software 1.7.2. Scheduled MRM algorithm was used to optimize duty cycle to give greater than 12 cycles across the chromatographic peak. LLOQs and precision were calculated using n=5 replicate injections in 1000x diluted matrix (Figure 2). Calibration curves with the targeted method showed excellent linearity from 0.0005% to 70% in sample depending on the analyte and dilution. Linear or quadratic calibration functions displayed $R^2 \geq 0.99$ and accuracies were within 85-115% across all concentrations evaluated.

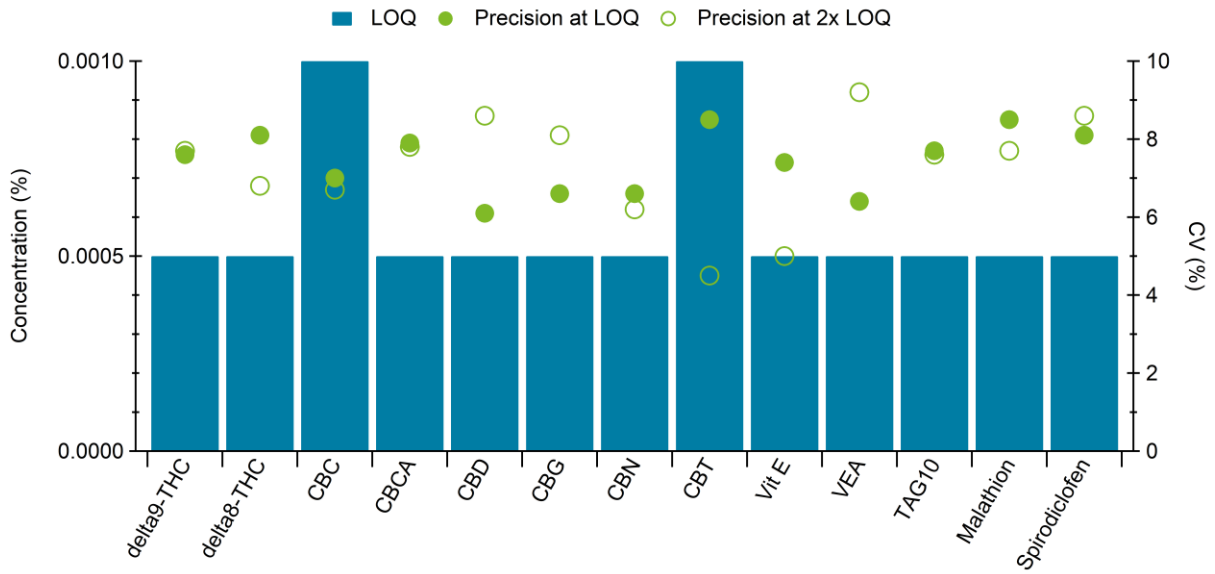


**Figure 2. Limit of quantitation (LOQ) and method precision (*n* = 5) for fortified e-juice formulations.** The 1,000x diluted matrix samples were fortified at the LOQ and 2 x LOQ levels for each analyte.

Non-targeted analysis was performed on the X500R QTOF system using independent data acquisition (IDA) with positive and negative ESI as well as positive APCI to maximize compound discovery. IDA MS/MS criteria were set to 10 candidate ions with a 300 cps intensity threshold, with exclusion time set for 12 seconds after 2 occurrences.

***Data processing:*** Data processing was performed with SCIEX OS software 2.1 for both targeted and non-targeted analyses. Non-targeted analysis was performed by filtering library hits with confidence over 90% by fit. Libraries searched included Forensic HR-MS/MS 2.1, HRAM All-in-One 1.1, Pesticides HR-MS/MS, Natural Products HR-MS/MS 2.0, Fluorochemical HR-MS/MS 2.0, Mycotoxin HR-MS/MS 1.0, TCM MS/MS 2.1, and NIST 2017 among others. Findings were then further scrutinized using the components list and flagging rules to show high mass accuracy and high confidence isotopic ratio vs expected chemical formulae.

## Results – targeted method

The recent vaping health crisis launched an interest by many regulatory bodies to better understand, control, and prevent the addition of potentially deleterious or illegal materials to e-juice formulations.[8] VEA was identified to be a main contributor to the health issues experience by e-juice consumers. In the targeted method, VEA and Vitamin E were not found at percent levels that would indicate addition as a cutting agent (their typical use in e-juice formulations).

MCT, like VEA, is on the GRAS list. There is, however, a growing concern with regulatory bodies on its usage in an inhaled product.[9] Ohio, Washington, and Colorado have banned the use of MCT in vaping products, for example, alongside VEA and polyethylene glycol.[10,11] In the targeted method, TAG10 (one component of MCT oil and used here as a marker) was found at concentrations that suggested it was added to the formulation (Table 1). These were found in most of the grey market e-juice, but not in the green market e-juice.

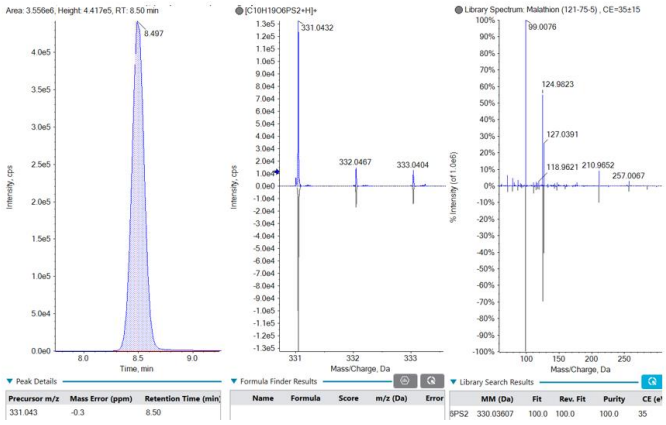


**Figure 3. Retention time, isotopic ratio, and library MS/MS matching for malathion.** 1,000x dilution of grey market e-juice containing cannabinoids revealed -0.3 ppm mass error, high confidence isotopic ratio, and 100% Fit and Purity in library matching by MS/MS.

Cannabinoid profiles were also tested in the targeted method, with particular interest towards d8-THC and d9-THC. Interest in d8-THC has recently exploded in many markets due to language in the U.S. farm bill that classifies this as a product of hemp, and thus legal for sale.[5] Many states have already banned or are working to ban the sales of e-juice containing d8-THC pending further study.[7] In this targeted method, most grey market vapes had both d8-THC and d9-THC (Table 1). In all of the green market vapes d9-THC was exclusively found with no evidence of d8-THC (Table 1). These findings highlight the inconsistency of label claims for products containing cannabis.

Green market samples come with certified label and the results obtained with this targeted method showed good agreement with the guaranteed reported concentration of cannabinoids.

## Results - non-targeted method

In the spirit of the original non-targeted search in e-juice that identified VEA, this study utilized spectral libraries on the SCIEX X500R QTOF system to investigate e-juice compositions (Table 1). Of most interest in the findings were the discovery in some of the grey market samples of pesticide contamination, phospholipids, and flavoring agents.

As an example, one of the grey market samples had several pesticides of concern in the finished product. Two of these, malathion and spirodiclofen, were confirmed using a targeted method and analytical standards. Figure 3 shows the library matching, mass accuracy, and isotopic ratio confidence for malathion seen in the non-targeted method.

The qualitative finding of two phospholipid species in one of the grey market vapes suggests that these compounds were added to the product, possibly as an emulsifying or cutting agent. Flavoring agents were found in several of the cartridges, usually of the citrus, vanilla, or cinnamon variety.

**Table 1. Representative targeted and non-targeted findings from grey and green market samples.** Vitamin E and VEA were found in many cases well below percentage levels such that it was likely naturally in the concentrate rather than added at levels suggestive of cutting agents and therefore were not included in the table. Results were reported in % due to the broad range in abundance expected and % in sample was calculated by multiplying the value found from the targeted acquisition by the dilution factor then converting to % assuming 1000 ppb = 0.0001%. In many cases the cannabinoids were diluted either 1 or 10 million times, due to the sensitivity of the mass spectrometer. All non-targeted results had < 5 ppm mass error. Conclusive identifications and quantifications require further confirmation with an analytical standard, as was done with *malathion* (Figure 3) and spirodiclofen in sample 3 using the targeted method.

| | Target method | | | | | | | | Non-targeted method | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample | d9-THC% | d8-THC% | CBD% | CBG% | CBN% | CBT% | MCT%* | Representative QTOF Findings (dilution factor) | Applicable Component Class |
| Grey market e-juice | 1 | 4.1 | 60 | ND | ND | 1.1 | ND | 0.6 | Bornyl acetate (100x) | Botanical flavoring agent |
| | 2 | 3.2 | 62 | ND | ND | 0.8 | ND | 0.5 | Germacrone (100x)<br>Costunolide (100x)<br>Cinnamaldehyde (1,000x) | Sesquiterpenoid<br>Sesquiterpene lactone<br>Botanical flavoring agent |
| | 3 | 59 | ND | 0.6 | 2.0 | 0.7 | ND | 0.5 | DEET (1,000x)<br>Malathion (1,000x)<br>Spirodiclofen (1,000x)<br>Thiabendazole (100x)<br>Paclobutrazol (100x) | Insect repellant<br>Organophosphate insecticide<br>Acaricide<br>Fungicide<br>Fungicide |
| | 4 | 9.8 | 35 | 2.8 | 0.2 | 1.6 | ND | 0.6 | Carvone (100x) | Monoterpene |
| | 5 | 4.1 | 52 | ND | ND | 0.9 | ND | 0.6 | Germacrone (100x)<br>16:0 PC (1,000x)<br>Glycerophosphocholine (1,000x) | Sesquiterpenoid<br>PC Phospholipid<br>Choline derivative |
| | 6 | 2.4 | 31 | ND | ND | 0.4 | ND | ND | Nobiletin (100x)<br>Tangeretin (100x)<br>Erianin (100x) | Citrus associated flavonoid<br>Citrus associated flavonoid<br>Botanical product |
| | 7 | 1.8 | 34 | ND | ND | 0.4 | ND | ND | Vanillin (1,000x)<br>Maltol (1,000x)<br>Cinnamaldehyde (1,000x) | Flavoring agent<br>Flavoring agent<br>Botanical flavoring agent |
| Green market e-juice | 8 | 51 | ND | 0.1 | 2.8 | 3.7 | ND | ND | Tanshinone II-A (100x)<br>Vitamin K1 (100x) | Diterpene quinone<br>Vitamin |
| | 9 | 63 | ND | 0.7 | 2.7 | 1.9 | ND | ND | Tanshinone II-A (100x)<br>Vitamin K1 (100x)<br>Polygodial (100x) | Diterpene quinone<br>Vitamin<br>Sesquiterpene dialdehyde |
| | 10 | 31 | ND | 37 | 2.1 | 1.0 | 1.2 | ND | Nobiletin (100x)<br>Nootkatone (100x)<br>Cuminaldehyde (100x)<br>Tangeretin (100x) | Citrus associated flavonoid<br>Sesquiterpenoid<br>Monoterpene<br>Citrus associated flavonoid |
| | 11 | 64 | ND | 0.2 | 2.8 | 0.6 | ND | ND | Carvone (100x)<br>Polygodial (100x) | Monoterpene<br>Sesquiterpene dialdehyde |
| | 12 | 64 | ND | 0.2 | 2.1 | 1.1 | ND | ND | Vitamin K1 (100x)<br>Carvone (100x) | Vitamin<br>Monoterpene |

*TAG10, a component in MCT oil, was monitored and is used as an indicator for the presence of MCT oil.

## Conclusions

As there is increased focus on the safety of e-juice, analytical methodology to both quantify known compounds of concern as well as profile potential future compounds can be helpful. Samples from both qualified sources and less qualified sources (green vs grey market samples) were used to test the capabilities of the two developed methods.

- Detection and quantitation of cannabinoids, e-juice components, and pesticides can precisely and accurately be achieved with the SCIEX Triple Quad 4500 system using the targeted approach
- The X500R QTOF system coupled with SCIEX OS software and accurate mass MS/MS libraries facilitates fast data acquisition and easy data processing to drive discovery of previously uncharacterized compounds in e-juice.

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