Evaluation of Presence or Absence of α -Tocopherol and α -Tocopheryl Acetate in Various E-Liquids*

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SUMMARY

The presence of α -tocopheryl acetate in e-liquids is linked to EVALI (Electronic Cigarette, or Vaping, product use-Associated Lung Injuries). Typical e-liquids containing flavors and nicotine have a glycerin/propylene glycol carrier and are highly unlikely to contain α -tocopherol or α -tocopheryl acetate. However, the e-liquids containing cannabinoids, most notably tetrahydrocannabinol (THC), may contain α -tocopherol or α -tocopheryl acetate for enhancing the solubility of THC which is a lipophilic compound. For the analysis of α -tocopherol and of α -tocopheryl acetate in e-liquids a new HPLC technique was adopted which has been developed for the analysis of these compounds in tobacco and cigarette smoke and was previously reported. Both UV and MS/MS (MRM mode) were used as detection procedure.

Thirty four e-liquids containing different levels of nicotine were evaluated using this method. None of the e-liquids contained either α -tocopherol or α -tocopheryl acetate. Also, three e-liquids containing cannabidiol (CBD) were analyzed. Two of the CBD containing liquids did not contain α -tocopherol or α -tocopheryl acetate. However, one of these e-liquids did contain α -tocopherol, but the level was low at about 10 $\mu\text{g/g}$, which is lower than the level of α -tocopherol per smoke of one cigarette (ISO smoking), and significantly lower than the level of α -tocopherol in tobacco. [Contrib. Tob. Nicotine Res. 30 (2021) 44–49]

KEYWORDS

α -Tocopherol, α -tocopheryl acetate, e-liquids, nicotine, cannabidiol.

ZUSAMMENFASSUNG

Das in E-Liquids enthaltene α -Tocopherylacetat wird mit Lungenschädigungen in Verbindung gebracht, die mit der Nutzung von E-Zigaretten oder Dampfprodukten assoziiert werden (EVALI, E-cigarette or Vaping product use-Associated Lung Injuries). Typische aroma- und nikotinhaltige E-Liquids verwenden Glycerin/Propylenglykol als Trägerstoff, und es ist sehr unwahrscheinlich, dass sie α -Tocopherol oder α -Tocopherylacetat enthalten. Jedoch können E-Liquids mit Cannabinoiden, insbesondere mit Tetrahydrocannabinol (THC), α -Tocopherol oder α -Tocopherylacetat enthalten, da es sich bei THC um eine lipophile Verbindung handelt, deren Löslichkeit hierdurch verbessert wird. Zur Analyse von α -Tocopherol und α -Tocopherylacetat in E-Liquids wurde eine neue HPLC-Methode verwendet, die für die Untersuchung dieser Verbindungen in Tabak und Zigarettenrauch entwickelt und bereits beschrieben wurde. Es wurden sowohl UV als auch MS/MS (MRM-Modus) als Detektionsverfahren verwendet. Mithilfe dieser Methode wurden 34 E-Liquids mit jeweils unterschiedlichem Nikotingehalt analysiert. Keines dieser E-Liquids enthielt α -Tocopherol oder α -Tocopherylacetat.

Zudem wurden drei E-Liquids mit Cannabidiol (CBD) analysiert. Zwei der CBD-haltigen Liquids enthielten weder α -Tocopherol noch α -Tocopherylacetat. Eines dieser E-Liquids enthielt zwar α -Tocopherol, allerdings in einer geringen Konzentration von ca. 10 $\mu\text{g/g}$. Diese Konzentration ist niedriger als die Konzentration von α -Tocopherol beim Abrauchen einer Zigarette gemäß ISO-Norm und signifikant niedriger als der α -Tocopherolgehalt in Tabak. [Contrib. Tob. Nicotine Res. 30 (2021) 44–49]

RESUME

La présence d'acétate de l' α -tocophéryle dans les liquides des dispositifs électroniques est associée à des lésions pulmonaires imputées à l'utilisation de produits de vapotage ou de cigarettes électroniques (EVALI en anglais). Les liquides habituels contenant des arômes et de la nicotine comportent de la glycérine et du propylène glycol comme excipients et sont très peu susceptibles de contenir l' α -tocophérol ou de l'acétate de l' α -tocophéryle. Toutefois, les liquides pour dispositifs électroniques comportant des cannabinoïdes, surtout du tétrahydrocannabinol (THC) notamment, peuvent contenir de l' α -tocophérol ou de l'acétate de l' α -tocophéryle afin d'accroître la solubilité du THC, qui est un composé lipophile. Dans le but d'analyser de l' α -tocophérol et de l'acétate de l' α -tocophéryle dans les liquides pour dispositifs électroniques, l'équipe a retenu une nouvelle technique CLHP, développée pour l'analyse de ces composés dans le tabac et la fumée de cigarette et précédemment décrite. Tant l'approche par les UV que la spectrométrie MS/MS (en mode MRM) furent intégrées à la procédure de détection.

Trente-quatre liquides pour dispositifs électroniques contenant des niveaux divers de nicotine furent analysés à l'aide de cette méthode. Aucun des liquides pour dispositifs électroniques ne s'avèrent contenir de l' α -tocophérol ou de l'acétate de l' α -tocophéryle. En outre, trois liquides pour dispositifs électroniques contenant du cannabidiol (CBD) furent analysés. Deux des liquides pour dispositifs électroniques contenant du CBD ne contenaient ni α -tocophérol ni acétate de l' α -tocophéryle. Cependant, un de ces liquides pour dispositifs électroniques contenait bel et bien de l' α -tocophérol mais la teneur en était faible et avoisinait les 10 $\mu\text{g/g}$, soit un niveau inférieur à la teneur en α -tocophérol par cigarette fumée (fumage ISO) et un niveau significativement inférieur à la teneur en α -tocophérol du tabac. [Contrib. Tob. Nicotine Res. 30 (2021) 44–49]

1. INTRODUCTION

The presence of α -tocopheryl acetate in e-liquids is linked to EVALI (E-cigarette or Vaping product use-Associated Lung Injuries) (1,2). Typical e-liquids containing flavors and nicotine have a glycerin/propylene glycol carrier and are highly unlikely to contain α -tocopherol or α -tocopheryl acetate. However, e-liquids containing cannabidiol (CBD) or even tetrahydrocannabinol (THC) may also contain α -tocopherol or α -tocopheryl acetate for enhancing the solubility of CBD or of THC which are lipophilic compounds. For this reason it was of interest to develop an

analytical procedure for the analysis of α -tocopherol and of α -tocopheryl acetate in e-liquids.

Various procedures for the analysis of tocopherols are reported in the literature (3–15). These methods include high performance liquid chromatography (HPLC) analysis with fluorescence detection (FD) (3), HPLC with ultra violet (UV) detection at various wavelengths (4–6), detection using tandem mass spectrometry (MS/MS) (10–12), as well as gas chromatography coupled with mass spectrometry (GC/MS) analysis (9). Depending on the matrix, and the desired sensitivity of the analysis, a specific procedure must be selected. However, the available methods were not found adequate for the analysis using both UV and MS/MS detection. The advantage of UV detection is its excellent reproducibility but it may suffer from poor selectivity. On the other hand, MS/MS detection is highly selective, but is frequently affected by comparatively larger reproducibility errors. The combination of the two techniques offers both good reproducibility and selectivity of the analysis. A technique for the analysis of α -tocopherol or α -tocopheryl acetate in tobacco and cigarette smoke using both UV and MS/MS detection with the elimination of potential interferences was recently reported in the literature (16). This method has been applied on 37 e-liquids, three of them containing cannabidiol (CBD), and the rest only nicotine. A summary description of the method and the results obtained on the e-liquid samples are reported in this study.

2. EXPERIMENTAL

2.1. Materials

α -Tocopherol, α -tocopheryl acetate, 2,5-bis(5-*tert*-butylbenzoxazol-2-yl)thiophene (Uvitex-OB), and 2,6-di-*tert*-butyl-4-methylphenol (BHT) as well as acetonitrile, formic acid, iso-propanol, and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). E-liquids were commercially available and were purchased from the U.S. market in the period from 2018–2020. The GC vials were 2-mL with screw top caps with PTFE lined septa.

2.2. Instrumentation

Two HPLC systems were used for the analysis. A1260-1290 HPLC system from Agilent (Wilmington, DE, USA) was used for UV detection. This HPLC system consisted of a binary pump, autosampler, column thermostatted compartment, and UV detector (diode array). The peak integration was performed with OpenLab ChemStation Edition. The other system was an Agilent 1200 HPLC binary system that consisted of a binary pump, an autosampler with cooling capability, and a column thermostatted compartment coupled with an API-5000 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA, USA) controlled using Analyst 1.6.2 software.

The peak integration was performed with MultiQuant 3.0.1 software. Both HPLC systems were equipped with a Zorbax Eclipse XDB-C18 column, 4.6 $\times$ 250 mm, 5 μm , also from Agilent. An ultrasonic bath 5510 from Branson (Danbury, CT, USA) from Thermo Fisher Scientific was also used.

2.3. Sample preparation

Prior the analysis the e-liquids were kept in a freezer at -20°C . For the analysis, 100 mg (± 10 mg) of e-liquid was precisely weighed in a 2-mL GC vial. 900 μL of methanol containing 560.4 $\mu\text{g/mL}$ BHT, and 20 μL internal standard (I.S.) was added to the vial. BHT was added as an antioxidant. The stock concentration of the Uvitex-OB that was used as I.S. was 523.5 $\mu\text{g/mL}$ in methanol. The sample was submitted for analysis by HPLC-UV and if a peak was detected at the corresponding retention time for α -tocopherol or of α -tocopheryl acetate, the same sample was submitted for analysis by HPLC-MS/MS.

2.4. Separation, detection, and quantitation

The HPLC separation was isocratic using as solvent a mixture of 15% isopropanol in acetonitrile. The injection volume for UV detection was 20 μL . The detection by UV absorption was performed at 210 nm. The flow rate was 1.5 mL/min. For the MS/MS detection electrospray ionisation (ESI) technique has been used with multiple reaction monitoring (MRM) detection in positive mode. The mobile phase for the MS/MS analysis was the same as for the UV analysis but the acetonitrile contained 0.1% formic acid, and because the flow rate of 1.5 mL/min was too high for MS/MS system the effluent from the chromatographic column was split and only 0.5 mL/min were introduced into the mass spectrometer. Also, the injection volume was 10 μL . The transitions utilized for the MS/MS detection were as follows: for α -tocopherol 431.4 $\rightarrow$ 165.1, for α -tocopheryl acetate 473.4 $\rightarrow$ 165.1, and for Uvitex-OB (I.S.) 431.5 $\rightarrow$ 415.3. The experimental conditions for the MS/MS detection were the same as described in reference (16).

Quantitation has been performed using the same procedure as described in reference (16), using a set of 10 standards with concentrations between 98.63 $\mu\text{g/mL}$ and 0.19 $\mu\text{g/mL}$ for α -tocopherol and between 96.47 $\mu\text{g/mL}$ and 0.19 $\mu\text{g/mL}$ for α -tocopheryl acetate. The calibration was linear for both UV detection and for MS/MS detection, with equations of the form:

$$Y = aX + b \quad [1]$$

For the UV detection, the values for Y were the concentrations of the analyte while the values for X were the peak areas necessary for the calculation of the concentrations of the unknown samples. The peak areas of the internal standard were used in case of UV detection only to monitor the reproducibility of each analysis. The coefficients a and b for the calibrations in case of UV detection are indicated in Table 1.

Table 1. Coefficients and R^2 for the calibration curves $Y = aX + b$ used in quantitation with UV detection.

Compound	Detection type	a	b	R^2
α -Tocopherol	UV	0.022401	-0.05633	1.00
α -Tocopheryl acetate	UV	0.022655	-0.05204	1.00

For the quantitation using the MS/MS data, an equation of the same form as given by expression [1] was used, but in this case Y remained the concentrations of the analyte while the values for X were the peak areas of the analyte normalized by the area of the internal standard. The stability of the MS/MS detector is lower than that of the UV detector, and the normalization generated more precise values. The coefficients a and b for the calibrations in case of MS/MS detection are indicated in Table 2.

Table 2. Coefficients and R^2 for the calibration curves $Y = aX + b$ used in quantitation with MS/MS detection.

Compound	Detection type	a	b	R^2
α -Tocopherol	MS/MS	34.2270	-1.00506	0.9997
α -Tocopheryl acetate	MS/MS	10.7472	-1.3916	0.9997

2.5. Brief method validation

Both LC-UV and LC-MS/MS methods for α -tocopherol and α -tocopheryl acetate analysis can be considered as having good specificity. For the UV detection, retention time is basically the only criterion for selectivity, but for the MS/MS detection, besides the retention time the masses of the ion for Q1 (the parent ion) and the ion for Q3 (the daughter ion) are also important.

Since the e-liquids are miscible with methanol, no analyte recovery was performed in this study. The limits of quantitation (LOQ) for both α -tocopherol and α -tocopheryl acetate was taken as equal to the concentration of the lowest calibration standard, and because the samples are prepared using a 1/10 dilution in methanol, the LOQ per g of sample was 10 times lower (LOQ = 1.9 $\mu\text{g/g}$ sample). The solutions of both α -tocopherol and α -tocopheryl acetate in the presence of BHT are stable up to two weeks when stored at -5°C .

3. RESULTS AND DISCUSSION

3.1. E-liquids containing nicotine

A variety of e-liquids containing nicotine were evaluated for the potential presence of α -tocopherol and/or α -tocopheryl acetate. The list of these e-liquids is given in Table 3. The table also indicates (when available) the nicotine level and content of glycerin and propylene glycol (PG). None of the e-liquids listed in Table 3 were found to contain either α -tocopherol or α -tocopheryl acetate at levels above limits of detection (LOD) of 0.6 $\mu\text{g/g}$.

3.2. E-liquids containing cannabidiol (CBD)

In addition to the e-liquids containing nicotine, three e-liquids containing cannabidiol (CBD) were also evaluated. The list of these three e-liquids is given in Table 4. The samples "Korent" and "Kore Organic" did not contain any α -tocopherol or α -tocopheryl acetate. However, in the

Table 3. List of e-liquids containing nicotine evaluated for the presence of α -tocopherol and/or α -tocopheryl acetate (nicotine level, and content of glycerin and PG indicated when available).

No.	Brand	Flavor	Nicotine mg/g	Glycerin % (w/w)	PG % (w/w)
1	Kilo	Dewberry Cream	8.57	61.72	29.53
2	Frequency Liquid	Boom Box	2.35	73.27	21.75
3	ZVR	Lemon Cheesecake	5.25	54.70	32.42
4	OKVMI	Haute Mocha	5.64	48.09	23.42
5	CRFT	Gravel Pit	3.22	53.10	40.18
6	Noca Elixirs	Bezoar	2.28	78.72	13.49
7	Cosmic Fog	The Shocker	2.25	73.55	15.50
8	Clancy's Riptide	—	9.28	69.97	21.96
9	AlfaLiquid	Noble Leaf	2.56	49.80	47.54
10	Virgin Vapor	Best Damn Tobacco	11.76	86.90	—
11	Series 8	Strawmilla	—	48.40	43.65
12	Five Pawns	Mixology Edition	2.45	53.97	36.63
13	Vype	Aniseed	10.83	50.78	30.88
14	BRV	Colonel's Custard	2.43	49.83	42.07
15	Taffy Man	TR4 BLU	0.17	77.93	18.06
16	Bad Drip	Bad Blood	2.24	69.47	20.69
17	Henley	Brighton Peach	1.86	57.14	36.46
18	Cuttwood Boss	Reserve	2.11	65.14	24.27
19	Kings Crest	Duchess	8.97	77.00	11.30
20	Haus	Berry	22.06	25.33	59.93
21	Haus	Ocean Mist	22.55	23.20	59.56
22	VaporFi	?	10.91	52.79	41.88
23	X2O	Natural Tobacco	17.77	60.93	35.39
24	Dewvy Bobba	—	4.60	72.24	20.72
25	Njoy	Para Mour	?	54.25	38.58
26	ALD Group	Mango (1)	50 mg/mL ^a	?	?
27	ALD Group	Mint (1)	50 mg/mL ^a	?	?
28	ALD Group	Crème (1)	50 mg/mL ^a	?	?
29	ALD Group	Mango (2)	50 mg/mL ^a	?	?
30	ALD Group	Mint (2)	50 mg/mL ^a	?	?
31	ALD Group	Crème (2)	50 mg/mL ^a	?	?
32	ALD Group	Fruit Medley	50 mg/mL ^a	?	?
33	Leap GO	Mint	50 mg/mL ^a	?	?
34	JUUL	Classic Tobacco	50 mg/mL ^a	?	?

^a 50 mg/mL as reported

? indicates: presence but level not reported

— indicates: presence unknown

LC-UV trace of the “Quanta” sample, a peak eluted at 11.904 min, a retention time very close to the retention time of α -tocopherol. No peak corresponded to the retention time of α -tocopheryl acetate. The HPLC chromatogram with UV detection for the “Quanta” sample is shown in Figure 1.

The retention time of the suspected α -tocopherol peak in the “Quanta” sample does not match exactly the retention time for α -tocopherol, and the peak shape is far from Gaussian. For this reason, it was suspected that an interference is responsible for the peak at 11.73 min in Figure 1.

Table 4. List of e-liquids containing CBD evaluated for the presence of α -tocopherol and/or α -tocopheryl acetate.

No.	Brand	Flavor	CBD mg/mL	Glycerin % (w/w)	PG % (w/w)
1	Korent	Fresh mint	10.0	?	?
2	Kore Organic	Blueberry mist	?	?	?
3	Quanta	Bubble mint	1.75/serv.	—	—

? indicates: presence but at unknown level

/serv. indicates: “per serving”

— indicates: not reported

Figure 1. The HPLC chromatogram with UV detection for the “Quanta” sample.

Figure 2. The ion trace for α -tocopherol in the LC-MS/MS chromatogram for the “Quanta” sample.

However, the sample was also analyzed by LC-MS/MS procedure. The extracted ion trace for α -tocopherol in the LC-MS/MS chromatogram for the “Quanta” sample is shown in Figure 2.

The result from Figure 2 indicated that α -tocopherol is indeed present in the “Quanta” sample. The quantitation based on the LC-MS/MS trace indicated that the level of α -tocopherol in the sample is 10.2 $\mu\text{g/g}$ (RSD% = 3.71%). The calculation based on the area count from the UV trace indicated a different, significantly higher value, indicating that the peak in the UV trace has an interference.

CONCLUSIONS

An HPLC separation using UV detection as well as MS/MS detection has been developed previously for the analysis of α -tocopherol and α -tocopheryl acetate. The method has been applied for the quantitation of these compounds in e-liquids. Thirty-four e-liquids containing nicotine were evaluated by the new methods and none of them was found to contain either α -tocopherol or α -tocopheryl acetate.

Three e-liquids containing CBD were analyzed. Two of the liquids did not contain α -tocopherol or α -tocopheryl acetate, but one of the liquids contained a low level of α -tocopherol.

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