Analysis of α -Tocopherol in Tobacco and Cigarette Smoke*

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SUMMARY

α -Tocopherol, a type of vitamin E, has been known to be present in tobacco for many years. The compound is an antioxidant protecting cell membranes from oxidants. α -Tocopherol is transferred from tobacco into cigarette smoke, where it is also present. Analysis of α -tocopherol has been reported in a number of studies and in various matrices including tobacco and tobacco smoke. However, no recent publication describes a method for quantitative analysis of tocopherol in tobacco and in cigarette smoke, and many methods reported from previous studies were not published and only presented at conferences or communicated in internal company publications.

The goal of this study was to quantitate α -tocopherol and, if present, α -tocopheryl acetate in tobacco and in tobacco smoke. For this analysis, an original HPLC technique was developed and is described in this report. Both UV and MS/MS (MRM mode) were used as detection procedure for the analysis. The results obtained using UV detection were in very good agreement with the results obtained using MS/MS detection. The method has been applied for the analysis of a number of tobaccos, as well as the total particulate matter (TPM) from cigarettes made with the same tobaccos. Depending on tobacco type, the levels of α -tocopherol vary in tobacco between about 200 $\mu\text{g/g}$ up to about 900 $\mu\text{g/g}$ ("dry weight basis"). For ISO type smoking, the levels of α -tocopherol vary in TPM between about 2 $\mu\text{g/mg}$ up to slightly above 4 $\mu\text{g/mg}$ of TPM. For a cigarette generating TPM of about 10 mg/cig , the α -tocopherol is between about 20 $\mu\text{g/cig}$ up to about 40 $\mu\text{g/cig}$. A relatively good correlation was obtained between the level of α -tocopherol in smoke (ISO type

smoking) and the level of the compound in tobacco. α -Tocopheryl acetate was absent in tobacco. [Beitr. Tabakforsch. Int. 29 (2020) 57–65]

KEYWORDS

α -Tocopherol; α -tocopheryl acetate; tobacco; cigarette smoke

ZUSAMMENFASSUNG

Das Vorkommen von α -Tocopherol, eine Form des Vitamin E, im Tabak ist seit Jahren bekannt. Diese Verbindung ist ein Antioxidans und schützt Zellmembranen vor der Schädigung durch oxidierende Substanzen. Beim Rauchen geht α -Tocopherol vom Tabak in den Rauch über und findet sich somit auch im Tabakrauch. In einer Vielzahl von Arbeiten wird der analytische Nachweis des α -Tocopherols in verschiedenen Matrices, unter anderem auch in Tabak und Tabakrauch, beschrieben. Keine der aktuelleren Publikationen beschreibt die quantitative Bestimmung von α -Tocopherol im Tabak oder Zigarettenrauch. Viele Methoden wurden nicht publiziert, sondern wurden nur bei Tagungen vorgestellt, bzw. finden sich ausschließlich in unternehmensinternen Dokumenten.

Das Ziel dieser Studie ist die Quantifizierung des α -Tocopherols und - wenn vorhanden - des α -Tocopherol-Azetats im Tabak und Tabakrauch. Die dafür entwickelte HPLC-Methode wird in dieser Publikation im Detail beschrieben. Für die Detektion dienten sowohl UV als auch

MS/MS (MRM-Mode). Die Ergebnisse, die durch die UV-Detektion erhalten wurden, stimmten mit den Daten, die mittels Massenspektrometrie gemessen wurden, gut überein. Das Verfahren wurde sowohl für die Analyse von α -Tocopherol in verschiedenen Tabaken als auch im Gesamtkondensat (TPM) im Hauptstromrauch von Zigaretten aus diesen Tabaken herangezogen. Abhängig von der Tabaksorte lagen die gemessenen Mengen an α -Tocopherol zwischen rund 200 $\mu\text{g/g}$ und 900 $\mu\text{g/g}$ Tabak, bezogen auf das Trockengewicht des Tabaks. Wenn das Gesamtkondensat der Zigaretten durch maschinelles Abrauchen nach ISO 3308 gewonnen wurde, fanden sich zwischen ca. 2 $\mu\text{g/mg}$ und geringfügig über 4 $\mu\text{g/mg}$ α -Tocopherol im TPM. Daraus ergeben sich für Zigaretten mit 10 mg Gesamtkondensat im Hauptstromrauch α -Tocopherol-Werte zwischen 20 und 40 $\mu\text{g/cig}$. Zwischen dem Gehalt an α -Tocopherol im Tabak und der jeweiligen gemessenen Menge im TPM des Hauptstromrauchs der aus ihnen gefertigten Zigaretten besteht eine gute Korrelation. α -Tocopherol-Azetat konnte nicht im Tabak gefunden werden. [Beitr. Tabakforsch. Int. 29 (2020) 57–65]

RESUME

La présence dans le tabac α -tocophérol, substance appartenant à la famille des vitamines E, est avérée depuis plusieurs années. Ce composé est un antioxydant et protège les membranes cellulaires des dommages causés par les substances oxydantes. Lors du fumage, α -tocophérol opère un transfert du tabac vers la fumée et est donc également détectable dans la fumée de tabac. Une multitude d'études décrivent la détection analytique de α -tocophérol dans un large éventail de matrices incluant, entre autres, le tabac et la fumée de tabac. Cependant, aucun des articles publiés à ce jour n'évoque la détermination quantitative de α -tocophérol dans le tabac ou dans la fumée de cigarette. Un grand nombre de ces méthodes ne fait l'objet d'aucune publication, n'a été présenté qu'à l'occasion de colloques ou n'apparaît exclusivement que dans des documents internes aux entreprises.

La présente étude entend explorer la quantification de α -tocophérol et, lorsqu'il est présent, de l'acétate d' α -tocophérol dans le tabac et la fumée de tabac. La méthode CLHP mise au point à cette fin est détaillée dans le présent article. Pour la détection, nous eûmes recours tant aux ultraviolets qu'à la spectrométrie de masse en tandem et mode MRM (contrôle des réactions multiples). Les résultats obtenus grâce à la méthode de détection par UV concordèrent bel et bien avec les données mesurées grâce à la spectrométrie de masse. Ce procédé analytique fut appliqué tant pour la détermination de α -tocophérol dans divers tabacs que pour sa quantification dans le condensat de fumée principale (matière particulaire totale- MPT) des cigarettes composées de ces mêmes tabacs. Selon la variété de tabac, les teneurs en α -tocophérol mesurées oscillaient entre environ 200 $\mu\text{g/g}$ et 900 $\mu\text{g/g}$, exprimées en poids sec de tabac. Après l'obtention du condensat de fumée principale (MPT) de cigarette par un fumage à la machine conforme à la norme

ISO 3308, nous observâmes des teneurs en MPT α -tocophérol oscillant entre environ 2 $\mu\text{g/mg}$ et un peu plus de 4 $\mu\text{g/mg}$ de MPT. Sur cette base, pour les cigarettes avec 10 mg de condensat sec, les teneurs en α -tocophérol dans la fumée principale oscillaient entre 20 et 40 $\mu\text{g/cigarette}$. Une bonne corrélation apparaît entre la teneur en α -tocophérol des tabacs et les mesures de MPT relevées dans la fumée principale desdits tabacs composant les cigarettes. Aucune présence d'acétate d' α -tocophérol ne put être observée dans le tabac. [Beitr. Tabakforsch. Int. 29 (2020) 57–65]

1. INTRODUCTION

The presence of α -tocopherol in tobacco seed oil was indicated in a report published as early as 1945 (1). Numerous other reports (2–15) indicate the presence of α -tocopherol in tobacco and in cigarette smoke, some of these were reporting qualitative results (1–12), others reported quantitative results (13–15). Unfortunately, most reports regarding quantitative analysis of α -tocopherol were not published but only presented at conferences or communicated in internal company publications. Also, various procedures for the analysis of tocopherols in a variety of matrices are reported in the literature (16–36). These methods include high performance liquid chromatography (HPLC) analysis with fluorescence detection (FD) (16), HPLC with ultra violet (UV) detection at various wavelengths (17–19), tandem mass spectrometry (MS/MS) detection (33–35), as well as gas chromatography coupled with mass spectrometry (GC/MS) analysis (22). 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 of both α -tocopherol and α -tocopheryl acetate in tobacco and in cigarette smoke due to using at the same time UV detection and MS/MS detection. The advantage of UV detection is its excellent reproducibility; a disadvantage is sometimes a lack of selectivity. On the other hand, MS/MS detection is highly selective, but frequently affected by larger reproducibility errors compared to UV analysis. The combination of the two techniques offer both good reproducibility and selectivity of the analysis. A technique using both UV and MS/MS detection is desired for the analysis of these compounds with the elimination of potential interferences from the tobacco or smoke matrix. The present study describes an original method adequate for this purpose and the results for tobacco and smoke. The importance of knowing the level of α -tocopherol in tobacco is related to the fact that the compound is one of the E vitamins, and that it has antioxidant properties. It was of interest to provide information regarding the level of α -tocopherol in tobacco and cigarette smoke as compared to a recommended dietary allowance for vitamin E of 15 mg/day (for an adult). In addition, α -tocopheryl acetate, was analyzed in the study but found absent in tobacco and cigarette smoke. This compound has been associated with EVALI (Electronic Cigarette, or Vaping, product use-Associated Lung Injury), and was reported in certain counterfeit products (37–39). However, there is no report of α -tocopheryl acetate or similar compounds in legitimate commercial e-vaping products (37).

Table 1. The list of standards in $\mu\text{g/mL}$ (the addition of 10 μL I.S. slightly changes the final concentrations from the values in the stock solution).

Compound	Standard in $\mu\text{g/mL}$									
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8	Level 9	Level 10
α -Tocopherol	98.63	49.31	24.66	12.33	6.16	3.08	1.54	0.77	0.39	0.19
α -Tocopherol acetate	96.47	48.24	24.12	12.06	6.03	3.01	1.51	0.75	0.38	0.19

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, isopropanol, and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The GC vials used were 2-mL with screw top caps with septa. Also 4-mL vials with screw top caps with septa were used. Polyvinylidene difluoride (PVDF) filters of 0.45- μm (Whatman Autovial, GE Healthcare, Little Chalfort, UK) were used for filtration.

2.2. Instrumentation

Two HPLC systems were used for the analysis. One system with UV detection was a 1260-1290 HPLC system from Agilent (Wilmington, DE, USA). 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. 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) was used. For smoking, a Cerulean SM 450 smoking machine (Cerulean, Linford Wood East, UK) has been used. Tobacco moisture was measured with halogen moisture analyzer HE53 (Mettler Toledo, Greifensee, Switzerland).

2.3. Standard preparation

Two initial standard stock solutions - one containing 98.4 $\mu\text{g/mL}$ α -tocopheryl acetate and the other 100.6 $\mu\text{g/mL}$ α -tocopherol - were prepared in methanol. Each stock solution also contained 5604.0 $\mu\text{g/mL}$ BHT. BHT is used as an antioxidant to prevent tocopherol and tocopheryl acetate oxidation. The stock solutions were also used as the highest standard.

The Level 2 of dilution was prepared by mixing equal volumes of the two stock solutions and the other standards were obtained by successive dilutions with methanol. 20 μL of Uvitex-OB internal standard (I.S.) were added to 1 mL of each standard and the resulting solution

concentrations for the calibration is given in Table 1. The concentration of the I.S. in each solution was 525.6 $\mu\text{g/mL}$.

2.4. Sample preparation

Before the analysis, the moisture of each tobacco sample was measured. The analysis of tobacco was performed on tobacco "as is", and 200 mg ($\pm$ 5 mg) tobacco was precisely weighed in a 4-mL vial. 2 mL methanol containing 560.4 $\mu\text{g/mL}$ BHT, and 40 μL internal standard were added to the vial. The capped vials were heated at 78 $^{\circ}\text{C}$ for 30 min with occasional agitation. After cooling, at room temperature the solutions were filtered through a 0.45- μm pore size PVDF filter and submitted for analysis.

For the analysis of cigarette smoke, smoking was performed using 35 mL puff volume, 2 s puff duration, and 60 s puff interval, with the cigarette filters not having the ventilation blocked (indicated as ISO (International Organization for Standardization)). The machine airflows were tuned for ISO conditions (40, 41). The smoking was performed at Eurofins/Lancaster Labs. Smoke from five cigarettes was collected in each run on a 44-mm diameter Cambridge pad. The pads were further extracted on a mechanical shaker for 30 min with 5 mL methanol containing 560.4 $\mu\text{g/mL}$ BHT, and 100 μL solution of internal standard (Uvitex-OB) were added. An aliquot from the extract was filtered through a 0.45- μm pore size PVDF filter and submitted for HPLC analysis.

2.5. HPLC separation and detection

The HPLC separation was performed using the gradient with solvent A being acetonitrile and solvent B isopropanol. For the MS/MS analysis the acetonitrile also contained 0.1% formic acid. The gradient timetable is described in Table 2.

Table 2. Timetable for the gradient HPLC separation.

Run time (min)	Solvent A %	Solvent B %	Detection
0.0	85.0	15.0	On
15.0	85.0	15.0	On
15.5	40.0	60.0	On
16.0	40.0	60.0	Off
18.5	40.0	60.0	Off
19.0	85.0	15.0	Off
25.0	85.0	15.0	Off

The detection by UV absorption was performed at 210 nm and the injection volume was 20 μL . The flow rate was 1.5 mL/min. For the MS/MS, because the flow rate of

1.5 mL/min was too high, 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 μ L.

The detection was kept on only for 16 min, the last part of the gradient being used to elute highly hydrophobic compounds from tobacco or smoke that have a long retention time in the separation. The chromatogram of standard Level 5 for the HPLC separation with UV detection is indicated in Figure 1.

Figure 1. Chromatogram of standard Level 5 (6.16 μ g/mL α -tocopherol and 6.03 μ g/mL α -tocopheryl acetate).

The MS/MS detection using electrospray ionisation (ESI) was performed in multiple reaction monitoring (MRM) positive mode. The transitions utilized for detection are indicated in Table 3. The table also indicates the collision energy that was different for each analyte.

Table 3. Parameters for the MS/MS detection.

Compound	Ion for Q1	Ion for Q3	Time (ms)	CE ³ (V)
α -Tocopherol	431.4	165.1	200	38.0
α -Tocopheryl acetate	473.4	165.1	200	52.0
Uvitex-OB (I.S.)	431.5	415.3	200	49.0

The other parameters for the MS/MS detection included:

Declustering potential DE = 150 V,
Entrance potential = 13 V,
Collision gas (N₂) CG = 6 mL/min,
Curtain gas CUR = 10 mL/min,
Ion spray voltage IS = 4000 V,
Temperature TE = 600 °C,
Ion source gas 1 GS1 = 35 mL/min,
Ion source gas 2 GS2 = 30 mL/min.

The chromatogram for the extracted ions of the analyzed compounds in Level 5 standard is shown in Figure 2.

2.6. Quantitation using UV detection

For UV detection, considering the stability of the UV response, the quantitation for the two analytes was performed using calibrations of amount of the analyte *versus* the peak area of the analyte (no normalization by the internal standard). The internal standard was used only to verify the repeatability of the chromatographic process. The analytes concentrations in standards were those indicated in Table 1.

Figure 2. Extracted ion chromatograms for the compounds in standard Level 5 (6.16 μ g/mL α -tocopherol and 6.03 μ g/mL α -tocopheryl acetate).

The calibration curves were linear and are shown in Figure 3 for α -tocopherol and in Figure 4 for α -tocopheryl acetate.

Figure 3. Calibration curve for α -tocopherol with UV detection.

Figure 4. Calibration curve for α -tocopheryl acetate with UV detection.

The coefficients a and b for the calibration curves of the form $Y = aX + b$, where Y is the concentration of the analyte and X is the peak area are given in Table 4.

Table 4. Coefficients for the calibration curves $Y = aX + b$ and of R^2 used in UV quantitation.

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

2.7. Quantitation using MS/MS detection

For the MS/MS detection, the quantitation for the two analytes was performed using calibrations of the amount of the analyte *versus* the normalized peak area of the analyte by the area of the internal standard. The calibration curves were linear and are shown in Figure 5 for α -tocopherol and in Figure 6 for α -tocopheryl acetate.

Figure 5. Calibration curve for α -tocopherol with MS/MS detection.

Figure 6. Calibration curve for α -tocopheryl acetate with MS/MS detection.

The coefficients a and b for the calibration curves of the form $Y = aX + b$, where Y is the concentration of the analyte and X is the peak area of the standard normalized by the area of the internal standard are given in Table 5.

Table 5. Coefficients for the calibration curves $Y = aX + b$ and of R^2 used in MS/MS quantitation.

Compound	a	b	R^2
α -Tocopherol	34.2270	-1.00506	0.9997
α -Tocopheryl acetate	10.7472	-1.39160	0.9997

2.8. 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 are the

masses of the ion for Q1 (for the parent ion) as the ion for Q3 (for the daughter ion).

The validation was further performed regarding limits of detection (LOD) and limits of quantitation (LOQ), linearity range, precision and intermediate precision, spike/recovery, accuracy, as well as solutions stability (see e.g. (42)).

The application of the procedure of considering $LOD = 3 \times SD$ (SD = standard deviation of the lowest standard) (42) would indicate from the SD values for α -tocopherol ($SD = 0.008 \mu\text{g/mL}$, three replicates) and for α -tocopheryl acetate ($SD = 0.001 \mu\text{g/mL}$, three replicates) that $LOD = 0.024 \mu\text{g/mL}$ for tocopherol and $LOD = 0.003 \mu\text{g/mL}$ for tocopheryl acetate (the LOQ values are three times higher). However, these values are not realistic since the true measurements are not feasible at these levels. For this reason, the limit of quantitation (LOQ) can be considered as equal with the lowest standard for both UV detection and MS/MS detection, and the values are indicated in Table 6.

Table 6. The values for LOQ for α -tocopherol and α -tocopheryl acetate in $\mu\text{g/mL}$ standard solution and per g of sample.

Compound	LOQ solution ($\mu\text{g/mL}$)	LOQ sample ($\mu\text{g/g}$)
α -Tocopherol	0.19	1.9
α -Tocopheryl acetate	0.19	1.9

Considering that the samples for tobacco analysis are prepared using a 1/10 dilution in methanol, the LOQ per g of sample is 10 times lower. The same LOQ values were considered for smoke analysis.

The precision of the method can be considered very good, based on the R^2 values of all the calibrations. However, samples were not injected a sufficient number of times to generate specific precision levels.

The recovery of α -tocopherol from the tobacco matrix was evaluated by performing a second extraction of the tobacco sample. For this reason, after the first extraction and the filtration of the liquid, the remaining solid tobacco was placed on a filter paper and, as much as possible, the remaining liquid was adsorbed on the paper. After that the extracted tobacco was allowed to dry. From this material, 100 mg were weighed in a 2-mL GC vial and 1 mL methanol containing BHT, plus 20 μL solution of the internal standard were added. The sample was heated at 78°C for 30 min with occasional agitation and after cooling the solutions were filtered through a $0.45\text{-}\mu\text{m}$ pore size PVDF filter and submitted for the HPLC analysis. The procedure was performed for four tobacco samples. The results are indicated in Table 7.

Table 7. Percent recovery of α -tocopherol in a second extraction from total in the sample.

Experiment	First extraction ($\mu\text{g/g}$)	Second extraction ($\mu\text{g/g}$)	% in second extraction
1	749.16	15.70	2.05
2	812.54	18.66	2.24
3	768.32	14.57	1.86
4	808.30	16.14	1.96

Table 8. List of tobaccos evaluated in this study, the moisture in % and the indication if cigarettes were available.

No.	Sample acronym ^a	Description	Moisture (%)	Cigarettes available
1	FC L (1)	Common blend of lower stalk flue-cured	6.44	No
2	FC U (1)	Common blend of upper stalk flue-cured	6.71	No
3	FC L (2)	Eastern NC belt, lower stalk (lug) flue-cured	6.84	Yes
4	FC U (2)	Eastern NC belt, upper stalk (leaf & some tips) flue-cured	7.35	Yes
5	FC L (3)	South Carolina belt, lower stalk (lug) flue-cured	7.86	Yes
6	FC U (3)	South Carolina belt, upper stalk (leaf & some tips) flue-cured	7.11	Yes
7	FC off L	Brazil, lower stalk (lugs & primings) flue-cured	8.13	Yes
8	FC off U	Brazil, upper stalk (leaf & tips) flue-cured	9.01	Yes
9	Bu L (1)	Common blend of lower stalk Burley	6.36	No
10	Bu U (1)	Common blend of upper stalk Burley	7.17	No
11	Bu L (2)	Kentucky & Tennessee, lower stalk (flyings & cutters) Burley	6.93	Yes
12	Bu U (2)	Kentucky & Tennessee, upper stalk (leaf) Burley	6.99	Yes
13	Bu L (3)	North Carolina & Virginia, lower stalk (flyings & cutters) Burley	8.09	Yes
14	Bu U (3)	North Carolina & Virginia, upper stalk (leaf) Burley	8.33	Yes
15	Bu off L	Malawi, lower stalk (flyings & cutters) Burley	8.19	Yes
16	Bu off U	Malawi, upper stalk (leaf) Burley	8.24	Yes
17	Or (1)	Common blend of Oriental	6.64	No
18	Or (2)	Common blend of Oriental	6.23	No
19	Or Sa U	Turkey, good quality middle to upper stalk, Samsun Oriental	8.40	Yes
20	Or Iz U	Turkey, good quality middle to upper stalk, Izmir Oriental	6.87	Yes
21	Commercial	Tobacco blend	7.21	Yes
22	3R4F	Tobacco blend	6.86	Yes

^a The number in parentheses indicates tobaccos of the same type but from different sources.

As indicated in Table 7, only a very small proportion of α -tocopherol was recovered in a second extraction, indicating a very good recovery of the analytical technique.

Based on literature reports (35) in the presence of BHT, the solutions are stable up to two weeks when stored at -5°C .

3. RESULTS AND DISCUSSION

3.1. Sample description

The list of tobaccos evaluated for the level of α -tocopherol and the potential presence of α -tocopheryl acetate is given in Table 8. The table also indicated whether cigarettes were made or not made from the corresponding tobacco.

The cigarettes were manufactured in the R.J. Reynolds Tobacco Co. Fabrication Laboratory to achieve a draft with perforated filter holes closed of about 155 mm H_2O and a draft with holes open of about 125 mm H_2O and different tobacco weights, with cigarette length: 83 mm, rod length: 56 mm, filter length: 27 mm, and circumference: 24.48 mm. The cigarettes had cellulose acetate filters with segment draft of about 100 mm H_2O and laser perforations.

3.2. Tobacco analysis

The analysis was performed for both α -tocopherol and for α -tocopheryl acetate. However, α -tocopheryl acetate was not detected in tobacco. The results of α -tocopherol as measured in the tobacco samples are given in Table 9.

The results from Table 9 indicate that flue-cured tobaccos have typically higher levels of α -tocopherol than Burley and Oriental tobaccos with the upper stalk leaves slightly

Table 9. Average α -tocopherol levels in $\mu\text{g/g}$ tobacco as measured using the UV detection.

Sample No.	Sample acronym ^a	($\mu\text{g/g}$) "as is"	($\mu\text{g/g}$) "dry base"	RSD (%)
1	FC L (1)	731.18	778.26	0.27
2	FC U (1)	857.27	914.80	1.92
3	FC L (2)	760.38	812.39	2.09
4	FC U (2)	823.72	884.26	1.92
5	FC L (3)	778.92	840.15	1.93
6	FC U (3)	823.06	881.57	2.53
7	FC off L	746.77	807.48	2.86
8	FC off U	764.48	833.36	2.65
9	Bu L (1)	231.93	246.68	0.24
10	Bu U (1)	336.85	361.00	0.81
11	Bu L (2)	396.24	423.70	0.54
12	Bu U (2)	599.52	641.43	2.80
13	Bu L (3)	267.59	289.24	2.54
14	Bu U (3)	284.40	308.09	0.56
15	Bu off L	327.57	354.40	0.92
16	Bu off U	255.28	276.32	0.37
17	Or (1)	296.70	316.40	0.65
18	Or (2)	267.31	283.97	0.08
19	Or Sa U	181.74	197.00	1.31
20	Or Iz U	427.01	456.35	0.37
21	Commercial	510.19	546.97	0.53
22	3R4F	404.30	432.03	0.31

^a The number in parentheses indicates tobaccos of the same type but from different sources.

higher in α -tocopherol compared to the lower stalk leaves. Oriental and Burley tobacco had about the same range of levels of the compound, with Samsun Oriental having the lowest level.

The results for the same analysis performed using the MS/MS detection generated very similar results. A graph showing the correlation between the UV data and the MS/MS data is given in Figure 7.

Figure 7. Correlation between the results for α-tocopherol analysis by UV detection and by mass spectrometry detection, expressed in µg/g tobacco.

3.3. Smoke analysis

The total particulate matter (TPM) results in g for five cigarettes smoked under ISO type conditions are indicated in Table 10.

The same table gives the average results for α-tocopherol in the collected TPM from the UV analysis.

Very similar results with those obtained using UV detection were obtained using MS/MS detection for α-tocopherol. A graph showing the correlation between the UV data and the MS/MS data for the smoke levels of α-tocopherol is shown in Figure 8.

As expected, the correlation between the UV and the MS/MS results is very good.

Figure 8. Correlation between the results for α-tocopherol in smoke analyzed by UV detection and by mass spectrometry detection, expressed in µg/mg TPM.

The results for α-tocopherol in smoke show a relatively good correlation with the level of the compound in the tobacco. In Figure 9 the correlation between the level of α-tocopherol in smoke as a function of the level of the compound in tobacco is shown. In the LC/MS smoke analysis, the trace of the transition $m/z = 473.4 \rightarrow 165.1$ corresponding to α-tocopheryl acetate shows a very small peak at the retention time of α-tocopheryl acetate. The peak area was below the quantitation limit, and it could not be determined if it belongs indeed to α-tocopheryl acetate, or it is an interference. The formation of even very low traces of α-tocopheryl acetate in cigarette smoke is not likely.

4. CONCLUSIONS

A HPLC separation using UV detection as well as MS/MS detection has been developed for the analysis of α-tocopherol and α-tocopheryl acetate. The method has

Table 10. The TPM in g generated from 5 cigarettes smoked under ISO type conditions and the average results for α-tocopherol in µg/mg in the collected TPM as obtained by the analysis with UV detection.

Sample No.	Sample acronym ^a	TPM, Repetition 1 (g)	TPM, Repetition 2 (g)	Average α-tocopherol in TPM (µg/mg)	RSD (%)
3	FC L (2)	0.0668	0.0639	3.35	0.38
4	FC U (2)	0.0794	0.0816	3.98	1.11
5	FC L (3)	0.0534	0.0559	4.17	3.60
6	FC U (3)	0.0818	0.0802	3.54	1.66
7	FC off L	0.0543	0.0561	4.08	1.99
8	FC off U	0.0714	0.0737	3.65	0.65
11	Bu L (2)	0.0586	0.0626	2.72	0.35
12	Bu U (2)	0.0704	0.0703	2.84	0.07
13	Bu L (3)	0.0679	0.0598	2.22	0.35
14	Bu U (3)	0.0650	0.0574	2.15	4.15
15	Bu off L	0.0701	0.0651	1.83	0.01
16	Bu off U	0.0669	0.0729	1.52	0.44
19	Or Sa U	0.0468	0.0463	1.68	1.13
20	Or Iz U	0.0504	0.0464	2.76	0.57
21	Commercial	0.0509	0.0421	2.95	0.67
22	3R4F	0.0643	0.0701	1.80	0.74

^a The number in parentheses indicates tobaccos of the same type but from different sources.

been applied for the quantitation of these compounds in a number of tobaccos, as well as in the TPM from cigarettes made with the same tobaccos. Depending on tobacco type, the levels of α -tocopherol varied between about 200 $\mu\text{g/g}$ up to about 900 $\mu\text{g/g}$ ("dry weight basis"). For ISO type smoking, the levels of α -tocopherol varied in TPM between about 2 $\mu\text{g/mg}$ up to slightly above 4 $\mu\text{g/mg}$. α -Tocopheryl acetate, which was associated with EVALI and reported in certain counterfeit products, was not detected in tobacco or in tobacco smoke.

Figure 9. Correlation between the level of α -tocopherol in smoke as a function of the level of the compound in tobacco.

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