Determination of (+)-α-Tocopherol in Environmental Tobacco Smoke

Charles H. Risner* and **Paul R. Nelson**
Bowman-Gray Technical Center, R. J. Reynolds Tobacco Company, Winston-Salem, NC 27102

Abstract

A high-performance liquid chromatographic method is described for the quantitation of (+)-α-tocopherol in the particulate phase of environmental tobacco smoke (ETS) collected on a 1-µm pore size Fluoropore membrane. A methanol (MeOH) extract of the membrane, which can be used for four other ETS procedures, is analyzed for (+)-α-tocopherol on a reversed-phase column with fluorescence detection at selective wavelengths of 280 nm excitation and 330 nm emission. A mobile phase of MeOH and water is used. The method is reproducible with a relative standard deviation (%) of about 12. Recovery is 88%, and the procedure is capable of detecting greater than 0.04 µg/m³ (+)-α-tocopherol in ETS. A comparison of the ETS from five commercially available cigarettes shows similar (+)-α-tocopherol concentrations. A cigarette that primarily heats tobacco yields about 6% of that amount of (+)-α-tocopherol found in ETS from tobacco-burning cigarettes. (+)-α-Tocopherol can be used as a marker for ETS respirable suspended particles (RSP) because it is found at a consistent amount in ETS RSP of 0.29%. However, sufficient amounts of RSP would have to be generated in order to detect (+)-α-tocopherol.

Introduction

(+)-α-Tocopherol has been reported in tobacco as early as 1958 (1). Rodgman et al. have reported the presence of (+)-α-tocopherol in mainstream cigarette smoke, the smoke formed at the unlit end of the cigarette when air is drawn through the tobacco rod (2,3). Large sample sizes were required; 30 kg was needed for determination in tobacco (1) and the mainstream wet total particulate matter (WTPM), which consists of tar, nicotine, and water, from at least 7000 cigarettes (3). The procedures for the extraction of tobacco and mainstream smoke to determine (+)-α-tocopherol were semiquantitative, employing extensive fractionation and isolation using gravity-flow silica and Florisil columns. A gas chromatography (GC) procedure has also been used to determine (+)-α-tocopherol in tobacco and mainstream cigarette smoke (4). This technique for tocopherols, however, has been almost entirely replaced by high-performance liquid chromatography (HPLC) (5).

* Author to whom correspondence should be addressed.

A reversed-phase, HPLC procedure for the determination of (+)-α-tocopherol in tobacco and mainstream WTPM has been previously reported by the principal author (6). This technique used methanol (MeOH) to extract as little as 0.5 g tobacco or the WTPM from five cigarettes. MeOH was also used as the extractant in this work, but the matrix was environmental tobacco smoke (ETS), which consists of the aged and diluted combination of exhaled mainstream and the smoldering sidestream cigarette smoke from the burning end of the cigarette. The sample was collected on a Fluoropore membrane that was used to determine the mass of respirable suspended particles (RSP), those particles larger than 1 µm (7). This same sample extract can be used for four other ETS particulate analyses: (*a*) ultraviolet particulate matter (UVPM) (7), (*b*) fluorescence particulate matter (FPM) (8), (*c*) solanesol (9), and (*d*) scopoletin (10). This reduced the number of samples required when all of these analyses were to be performed.

Because there is interest in particulate markers specific to ETS to distinguish cigarette smoke from other contributors to RSP, the procedure reported here may be used to complement the above methods. To our knowledge, this is the first reported procedure for the determination of (+)-α-tocopherol in ETS and the first application of a procedure for the determination of (+)-α-tocopherol levels in indoor air.

Experimental

Reagents

(+)-α-Tocopherol was obtained from Eastman Fine Chemicals (Rochester, NY). MeOH was obtained from Burdick and Jackson (Muskegon, MI). Water was obtained from a Nanopore system that consisted of a carbon resin cartridge, two mixed ion-exchange cartridges, another carbon resin cartridge, and a 0.2-µm filter (Barnstead, Dubuque, IA).

Cigarettes

Kentucky reference cigarettes 1R4F (K1R4F, 9.2 mg tar, 0.80 mg nicotine/cigarette) were purchased from the Tobacco and Health Research Institute (Lexington, KY) and were used for method validation. A full-flavor, "low-tar," 85-mm cigarette (FFLT85, 10.6 mg tar, 0.81 mg nicotine/cigarette), a full-flavor, "low-tar," 100-mm cigarette (FFLT100, 10.1 mg tar, 0.82 mg nicotine/cigarette), a full-flavor, 85-mm cigarette (FF, 16.0 mg

tar, 1.09 mg nicotine/cigarette), and an "ultra low-tar," 100-mm cigarette (ULT, 1.8 mg tar, 0.21 mg nicotine cigarette) were purchased locally. A cigarette that primarily heats tobacco (TOB-HT, 2.9 mg tar, 0.19 mg nicotine/cigarette) was manufactured by R. J. Reynolds and has been described elsewhere (11).

HPLC

The HPLC system consisted of two Waters 510 pumps, a 680 gradient controller, a 715 Ultra WISP, and a 474 fluorescent detector (Waters Chromatography, Milford, MA). Data were acquired on a VAX MULTICHROM chromatography data acquisition system (VG Instruments, Danvers, MA). Separations were accomplished on a Vydac 201TP104 (10-µm particle size) 250 × 4.6-mm column that was preceded by a Vydac high-performance guard column containing a reversed-phase C18 201TP (5-µm particle size) insert (The Separations Group, Hesperia, CA). The HPLC system for the solanesol analysis has been described elsewhere (9).

Sampling

Samples were collected in a 45-m^3 chamber at 75°F and 50% relative humidity with no air exchange. The collection device was a Fluoropore, 1-µm pore size, 37-mm membrane (Millipore, Bedford, MA) contained in a cassette (SKC, Eighty Four, PA) sealed with a 37-mm gasket (Sloan Valve, Franklin Park, IL) and connected by a nylon adapter (SKC) and a 4-cm length of 0.635-cm-i.d. tubing to the pump manifold. A model 224-PCXR7 pump (SKC) was used to draw air through the membrane.

Preparation of standard solutions

A stock standard was prepared by dissolving (+)-α-tocopherol in MeOH and diluting to the appropriate concentration with same. Standards were stored in the dark at –18°C.

Table I. Instrument Precision, Linearity, and Minimum Detectable Quantity

	Instrument precision*	Linearity (R^2)†	Minimum detectable quantity (ng)‡
Mean (µV)	26844818	0.9999	5
SD (µV)	557935		
RSD (%)	2.1		

* 200-µL injection volume of a 0.5 µg/mL standard (eight injections).
† Range: 0.1–1.5 µg/mL.
‡ Corresponds to 0.04 µg/m^3 when sampling for 2 h at a flow rate of 3.0 L/min.

Table II. Overall Precision

	Flow rate (L/min)	RSP (µg)	(+)-α-Tocopherol (µg)	(+)-α-Tocopherol (µg/m^3)	(+)-α-Tocopherol in RSP (wt%)
			Intraday (eight replicates)*†		
Mean	3.3	486	1.70	4.24	0.35
SD	0.1	26	0.06	0.17	0.01
RSD (%)	3.0	5.3	3.5	4.0	2.8
			Interday (20 replicates)*‡		
Mean	3.1	524	1.51	3.96	0.28
SD	0.06	42	0.18	0.49	0.02
RSD (%)	1.9	8.0	11.9	12.4	7.1

* 45-m^3 chamber, six human smokers, no air exchange, sampled for 2 h, 75°F, 50% relative humidity.
† Six K1R4F cigarettes per sample.
‡ Six FFLT85 cigarettes per sample.

Procedures

Chromatographic

Chromatographic separations were performed at room temperature under isocratic conditions of 88% MeOH with 12% H_2O for 30 min, after which a 5-min column wash of 100% MeOH was followed by a 5-min equilibration delay prior to the next injection. The total run time was 40 min at a flow rate of 3.0 mL/min. The injection volume was 200 µL. The excitation wavelength was 280 nm, and the emission wavelength was 330 nm. The emission slit was 40 nm, and the gain setting was X1000. Quantitative results were obtained by means of an external standard procedure using peak area response. The chromatographic conditions for the analysis of solanesol have been described elsewhere.

Sampling and sample preparation

The air sample collection device consisted of a preweighed Fluoropore membrane in a cassette. Flow was precalibrated with a soap film meter (The Gilibrator, Gilian Instrument Corp., Wayne, NJ) at about 3.2 L/min prior to sampling. Six

Table III. Recovery and Standard Addition

Sample	Amount added (µg)*	Total amount found (µg)	Recovery (%)	Amount added (µg)†	Total amount found (µg)	Recovery (%)
1	0.00	2.21		0.00	1.82‡	
2	0.86	2.72	59.3	1.00	2.68	86.0
3	1.70	2.34	7.6	1.50	3.07	83.3
4	3.40	2.83	18.2	5.00	6.49	93.4
Mean			28.4			87.6

* Added to membrane prior to smoking.
† Added to extract after smoking.
‡ Amount added by standard addition: 1.76 µg

cigarettes were smoked by human smokers in the chamber who smoked one cigarette each to a designated line 3 mm from the overwrap for tobacco-burning cigarettes or until there was a lack of aerosol for the TOB-HT cigarettes. After 20 min, the smokers left the chamber, and sampling was initiated. Sampling continued until a 120-min sample was obtained, after which time the sampling flow rate was rechecked.

The cassette was reweighed, and after use or storage at –18°C, the cassette was disassembled, and the membrane was transferred to a 4-mL autosampler vial. MeOH (3 mL) was added, and the membrane was extracted for 30 min using a wrist-action shaker. The extract containing the membrane was stored at –18°C prior to analysis by HPLC.

Results and Discussion

Precision, linearity, and minimum detectable quantity

Table I shows an instrument precision of about 2% relative standard deviation (RSD), a correlation coefficient to linearity (R^2) of 0.9999, and a minimum detectable quantity (MDQ) of 5 ng, which corresponds to 0.04 µg/m^3 when sampling for 2 h at 3.0 L/min.

Table II gives the overall precision results for intraday analysis using K1R4F cigarettes and interday analysis using FFLT85 cigarettes. Included are the RSDs of the sampling flow rates and RSP weights, both of which contribute to the variability of the concentration of (+)-α-tocopherol in ETS. The weight percentage of (+)-α-tocopherol in the RSP is also given. The intraday precision was acceptable at 4.0% RSD, but the interday study showed a variation of about 12%. This may be due to the higher variation in the RSP weight and because the sample membranes or the extracts of the sample membranes were at least one month old (UVPM, FPM, and solanesol analyses having been performed prior to that for (+)-α-tocopherol). The weight percent of (+)-α-tocopherol in the RSP appeared to be consistent at about 0.3 in both the intra- and interday studies, even using two different cigarettes but with similar FTC tar ranges. Figure 1A is a typical chromatogram obtained from the intraday precision study compared with a standard of similar concentration (Figure 1B).

Recovery and standard addition

Table III gives results for recovery obtained in two different manners: (*a*) addition of the standard to the Fluoropore membrane prior to sampling and (*b*) addition after sampling. The recovery when the standard was added prior to sampling was poor. This was not understood because the membranes were extracted immediately after sampling. However, similar results were found when the standards were added to filter pads prior to the mainstream smoking of the same cigarette (6). Some oxidative process may be occurring in the presence of cigarette smoke or when air is drawn across the Fluoropore membrane. When the standard was added to the extract after smoking, the recovery improved significantly (88%). The amount found by standard addition compared well with that found by external standard (1.76 versus 1.82 µg, respectively).

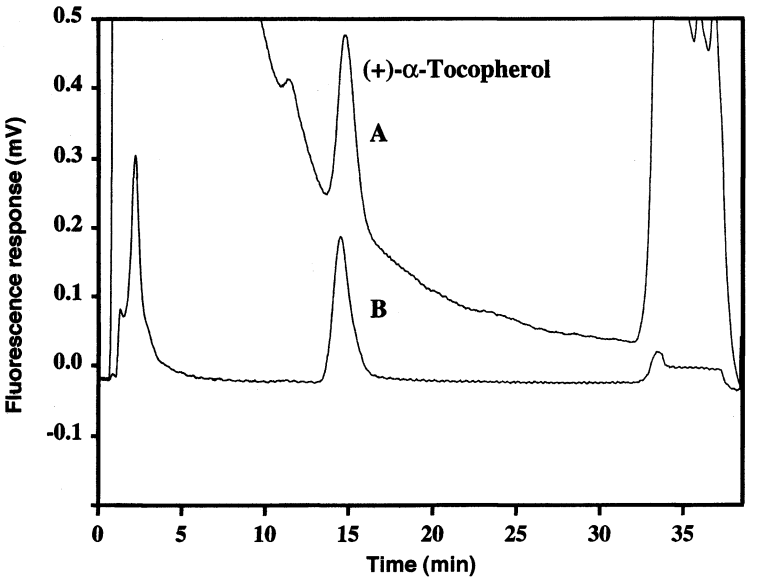

Figure 1. Chromatograms obtained during precision study: (A) MeOH extract of a Fluorophore membrane used to collect the ETS from six K1R4F cigarettes and (B) a 0.46 µg/mL (+)-α-tocopherol standard. Conditions are given in the Experimental section.

Table IV. Extract Stability

Sample	Initial value (µg)	Three weeks in lab* (µg)	Loss (%)	Two weeks at –18°C†	Loss (%)
1	1.55	1.24	20.0		
2	1.70	1.31	22.9		
3	1.72	1.35	21.5		
4	1.74	1.36	21.8		
5	1.72	1.37	20.3		
6	1.73	1.37	20.8		
7	1.75	1.40	20.0		
8	1.84			1.78	3.2
9	1.80			1.73	3.8

* In the light, at ambient temperature, and in the presence of a membrane.
† In the dark and in the presence of a membrane.

During this recovery and standard addition study, a second Fluoropore membrane was placed behind the first. No response for (+)-α-tocopherol was observed on the second membrane; the amount was below detection limits of 5 ng. This indicated that one Fluoropore membrane was sufficient for collection of ETS under these sampling conditions.

Extract stability

Table IV gives the results for the stability of the MeOH extract at ambient temperature in light and at –18°C in the dark. In both conditions, the membrane was left in the presence of the extract. As can be seen, the samples at ambient temperature in the light lost approximately 20% after three weeks, and those samples at –18°C in the dark lost a minimal amount of (+)-α-tocopherol, within experimental error.

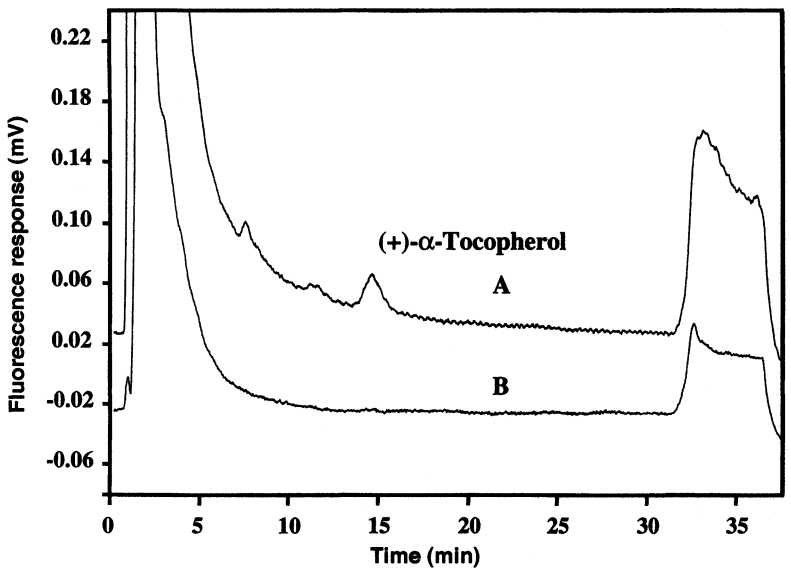

Figure 2. Chromatograms of the MeOH extract of a Fluorophore membrane used to collect the ETS from (A) TOB-HT and (B) a blank sample. Conditions are given in the Experimental section.

Table V. (+)-α-Tocopherol in the ETS of Cigarettes

Cigarette*	Number of replicates	Mean (μg/m³)	SD (μg/m³)	RSD (%)
K1R4F	10	4.32	0.22	5.09
FFLT85	20	3.96	0.49	12.4
FFLT100	10	5.12	0.46	9.0
ULT	10	3.50	0.63	18.0
FF	7	4.35	0.49	11.2
TOB-HT	8	0.26	0.12	46.2
Blank	18†	0.10	0.005	5.0

* 45-m³ chamber, six human smokers, no air exchange, sampled for 2 h, six cigarettes per sample, 75°F, 50% relative humidity.
† 14 values were below detection limits (< 0.04 μg/m³).

Table VI. Weight Percentage of (+)-α-Tocopherol and Solanesol in Environmental Tobacco Smoke RSP from Four Commercial Cigarettes

Cigarette	(+)-α-tocopherol (wt% of RSP)	SD	Solanesol (wt% of RSP)	SD	Number of replicates
FF	0.29	0.03	2.9	0.3	7
FFLT85	0.29	0.02	3.2	0.1	20
ULT	0.30	0.02	3.0	0.3	10
FFLT100	0.30	0.02	3.1	0.2	10
Overall	0.29	0.02	3.2	0.2	47

Applications

This procedure was applied to the determination of (+)-α-tocopherol in the ETS of six different cigarettes and under blank conditions in which no cigarettes were smoked (Table V). There appeared to be little difference in the amount of ETS (+)-α-tocopherol found for tobacco-burning cigarettes. The TOB-HT cigarette yielded about 6% of (+)-α-tocopherol concentration found for tobacco-burning cigarettes, and its value was within the range of the blank (see Figure 2). This represents at least a 90% reduction from the ETS for tobacco-burning cigarettes (see Table V and Figures 1A and 2A). The blank values found were in the range of the detection limits, and this background may have contributed to the ETS (+)-α-tocopherol found in the TOB-HT cigarette. This may also account for the high RSD of ETS (+)-α-tocopherol for these cigarettes.

Comparison of (+)-α-tocopherol with solanesol as an ETS–RSP marker

To examine the suitability of (+)-α-tocopherol as an ETS–RSP marker, it was compared with solanesol (a high-molecular-weight isoprenoid alcohol), currently the best available tobacco-specific marker for ETS–RSP (13,14). Concentrations of solanesol (9) and (+)-α-tocopherol were determined in RSP samples obtained from four different cigarettes. The cigarettes were the FF, FFLT85, ULT, and FFLT100 described in the Experimental section.

The fraction of solanesol and (+)-α-tocopherol in the ETS–RSP is shown in Table VI. Solanesol made up 3.2% of the mass of the ETS–RSP, whereas (+)-α-tocopherol contributed about 0.3% of the mass. The relative fraction of both components was essentially unchanged among the four cigarettes tested, despite differing ETS–RSP concentrations. Among the cigarettes tested, (+)-α-tocopherol appeared to be equivalent to solanesol in terms of a consistent ratio to ETS–RSP.

The quantitation limit for both solanesol and (+)-α-tocopherol was 5 ng/sample. For a 2-h sample taken at a flow rate of 3 L/min, that corresponds to a detection limit of 0.4 μg/m³ ETS–RSP for solanesol and approximately 5 μg/m³ ETS–RSP for (+)-α-tocopherol. The quantitation limit for (+)-α-tocopherol could be reduced further by increasing the injection volume or the sample concentration. If (+)-α-tocopherol were to be used in field studies for determining the contribution of ETS to indoor air RSP concentrations, it would be

best used in areas where smoking is known to take place or for longer (24 h) sampling periods. In this way, it is expected that ETS–RSP concentrations would be sufficiently elevated to attain reliable quantitation of low levels of (+)-α-tocopherol.

Conclusion

A method has been developed that is capable of detecting (+)-α-tocopherol in ETS from the MeOH extract of a Fluoropore membrane. This same extract can be used for other analyses. The procedure is capable of detecting (+)-α-tocopherol at 40 ng/m^3 and may find utility as a marker for the presence of ETS.

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