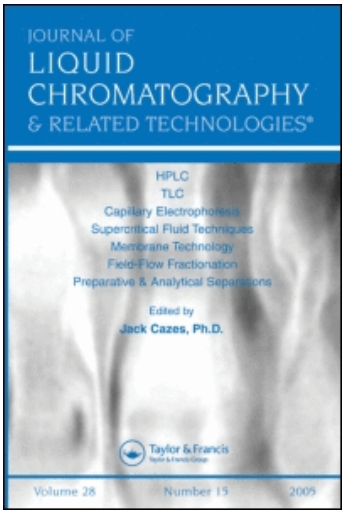

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

The Determination of Scopoletin in Environmental Tobacco Smoke by High-Performance Liquid Chromatography

Charles H. Risner[a]
[a] R. J. Reynolds Tobacco Company, Bowman Gray Technical Center, Winston-Salem, North Carolina

To cite this Article Risner, Charles H.(1994) 'The Determination of Scopoletin in Environmental Tobacco Smoke by High-Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 17: 12, 2723 — 2736
To link to this Article: DOI: 10.1080/10826079408013410
URL: http://dx.doi.org/10.1080/10826079408013410

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(12), 2723-2736 (1994)

THE DETERMINATION OF SCOPOLETIN IN ENVIRONMENTAL TOBACCO SMOKE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

CHARLES H. RISNER
R. J. Reynolds Tobacco Company
Bowman Gray Technical Center
Winston-Salem, North Carolina 27102

ABSTRACT

A high-performance liquid chromatographic (HPLC) method was developed for the quantification of scopoletin in environmental tobacco smoke (ETS) for use as an indicator of tobacco smoke in indoor air. Samples were collected on 1 μm pore size Fluoropore membrane filters and extracted with methanol (MeOH). This single extract was used for four analyses including scopoletin after dilution with 1% aqueous acetic acid (HAc). Scopoletin was determined on an octadecyl silane, silica-based column with fluorescence detection. A mobile phase gradient of 1% HAc and 99% acetonitrile + 1% glacial acetic acid was used. The method was reproducible with a relative standard deviation of 6.4%. Percent recovery was 98%. Analyses of smoke from oak leaves indicated the presence of scopoletin, but it was not detected in fireplace smoke. A short sampling time of one h was required and the procedure was capable of detecting < 3 ng m^{-3}.

INTRODUCTION

Scopoletin, a known constituent of tobacco, has been determined by using liquid chromatographic procedures (1,2). These procedures use semi-selective

absorbance detection as the endpoint to determine scopoletin in an aqueous methanol extract of tobacco. Previous work in our laboratory indicated that scopoletin is strongly fluorescing and is present in tobacco smoke (3). Although the reported technique was valid for other phenolic compounds in smoke, scopoletin suffered from a poor recovery from the collection device, a mixed cellulose ester membrane (MCEM).

The procedure reported here is based on the MeOH extract of the Fluoropore membrane used to determine respirable suspended particles (RSP) in indoor air (4). This same sample extract is being used for three other routine analyses in our laboratory: 1) ultraviolet particulate matter (UVPM) (4), 2) fluorescence particulate matter (FPM) (5) and 3) solanesol (6). This reduces the number of separate samples required for these analyses, an essential attribute when conducting field studies.

The method described in this work alleviates the recovery problem of scopoletin associated with earlier techniques (3) and validates its use as a particulate marker for ETS. This is the first reported, reliable procedure using selective fluorescence detection for the determination of scopoletin and the first application of the procedure for the determination of scopoletin levels in indoor air.

EXPERIMENTAL

Chemicals

Scopoletin was obtained from Aldrich (Milwaukee, WI, USA). Acetonitrile (ACN) and methanol (MeOH), "distilled in glass", were obtained

from Burdick and Jackson (Muskegon, MI, USA). Acetic acid (HAc) was reagent grade (Reagents, Inc., Charlotte, NC, USA). Water was obtained from a Nanopure system, which consisted of a carbon cartridge, two high-capacity mixed ion exchange cartridges and a 0.45-μm filter (Barnstead Co., Div. of Sybron Corp., Dubuque, IA, USA).

Equipment

HPLC

The HPLC system consisted of two ABI Spectroflow 400 pumps (Applied Biosystems, Inc., Foster City, CA, USA), an 878A autosampler fitted with a 200-μL sample loop and a Perkin-Elmer LS-4 Fluorescence Spectrometer (Perkin-Elmer Corp., Norwalk, CT, USA). The pumps and the autosampler were controlled by a DS-650 Data System and data were acquired on VAX MULTICHROM (VG Instruments, Danvers, MA, USA). Separations were accomplished on a Beckman Ultrasphere ODS, 4.6 X 150 mm, 5-μm particle size column (Beckman, San Ramon, CA, USA). A 30 X 4.6 mm Brownlee RP-18 Spheri-5 MPLC guard refill in a Brownlee 3-cm MPLC holder (Brownlee Labs, Inc., Santa Clara, CA, USA) was placed directly before the analytical column.

Sampling

The sampling device was a Fluoropore, 1-μm pore size, 37-mm membrane filter (Millipore Corp., Bedford, MA, USA) contained in a cassette (SKC Inc., Eighty Four, PA, USA) sealed with a 37-mm gasket (Sloan Valve Co., Franklin Park, IL, USA), connected by a nylon adaptor (SKC Inc., Eighty Four, PA, USA) and a 4-cm length of 0.635 cm I.D. tubing to the pump manifold. A

Dawson High Volume Air Sampler (Dawson Assoc., Inc., Lawrenceville, GA, USA) was used to draw air through the membrane.

Preparation of Standard Solutions

A stock standard was prepared by dissolving scopoletin in MeOH and diluting to the appropriate concentration with 8:2 (V/V), 1% HAc:MeOH.

Procedure

Chromatographic

All chromatographic separations were performed at room temperature with a mobile phase gradient of 1% HAc (A) and 99% ACN plus 1% glacial acetic acid (B) at a flow rate of 2.0 mL min^{-1}. Initial conditions were 100 % A followed by a linear change in B to 35 % over 10 min. A four min column wash of 100 % B and six min equilibrium delay of 100 % A prior to the next injection were included in the gradient program. The excitation wavelength was 342 nm and the emission wavelength was 464 nm. Quantitative results were obtained by means of an external standard procedure and a complete analysis took 20 min.

Sampling and Sample Preparation

The air sample collection device was precalibrated with a soap film flow meter (The Gilibrator, Gilian Instrument Corp., Wayne, NJ, USA) at about 2 L min^{-1} prior to sample collection. After sampling was complete (1h) the flow was rechecked. Following use, the cassette was disassembled and the membrane transferred to a 4-mL autosampler vial. MeOH (4 mL) was added and the membrane extracted for 30 min using a wrist-action shaker. One part of the MeOH extract was diluted with four parts 1% HAc prior to analysis.

RESULTS AND DISCUSSION

Recovery Re-evaluation

Since poor recovery (< 50%) was obtained in previous work when adding scopoletin to an MCEM (37 mm, 0.8 μm pore size, SKC Inc., Eighty Four, PA, USA) prior to sampling, it was decided to add scopoletin after sampling (3). The recovery results were still poor and even a blank MCEM (not used for sampling) yielded recoveries of only 38.6±7.2% (n=2).

The method of extraction, sonication for 10 min, was evaluated against wrist-action shaking for 30 min. The recovery from the MCEM did not improve.

A Teflon membrane (Fluoropore, described in the Experimental section) was then evaluated for collection of scopoletin. [The Teflon membrane had been evaluated previously versus the MCEM but was found not as efficient for the collection of catechol, a main component of interest under study (3).] This work showed that the Teflon membrane yielded more scopoletin than the MCEM under the same sampling conditions. It was decided to evaluate the recovery of scopoletin from the Teflon membrane.

A blank Teflon membrane, to which scopoletin was added, gave 103.4±0.0% (n=2) recovery, unlike the MCEM (see above). It can be concluded from these results that scopoletin either reacts with the MCEM or is irreversibly retained and cannot be completely extracted. The Teflon membrane has a more inert surface which enables quantitative extraction. Table 1 shows that the recoveries are acceptable and the sample results found by external standard quantitation are in agreement with those obtained by standard addition. The Teflon membrane was chosen as the device on which to collect scopoletin.

TABLE 1. Recovery/Standard Addition of Scopoletin Added to the Fluoropore Membrane[a]

Amount Added (ng)	Amount Found (ng total)	% Recovery
108	338	96.2
216	438	94.4
540	786	102.2
		$\bar{x}$ = 97.6
ng m^{-3}: standard addition = 1700, external standard = 1892		

[a] - amount added in MeOH after sampling the smoke of two cigarettes in an 18-m^3 chamber with no air exchange, sampled for one h at 2 L min^{-1}, n=2

Dilution of Sample Extract versus Decrease in Injection Volume

An objective of this work was to conduct different analyses from a single sample. The MeOH extract of the Teflon membrane used for the determination of RSP (4) was currently being used in our laboratory for UVPM (4), FPM (5) and solanesol (6) analyses. The determination of scopoletin from the same MeOH extract would eliminate the need for a separate sample.

It was found that the injection volume of the MeOH extract had to be reduced from 200 μL used in previous work (3) to 20 μL so that the peak shape of scopoletin remained symmetrical. This was due to the sample extract being in solvent (MeOH) which was much stronger than the initial mobile phase (1% HAc). Rather than reduce the injection volume, it was decided to dilute the MeOH initial extract with 1 % HAc in order to reduce the solvent strength. A 1:4, MeOH:1 % HAC, dilution resulted in a symmetrical peak for scopoletin when a 200 μL injection was used. The sensitivity using the 1 % HAc dilution

of the MeOH extract would amount to a 5-fold decrease versus a 10-fold decrease if reduction of the injection volume was used to improve the peak shape. Therefore, it was decided to dilute the initial MeOH extract of the Fluoropore filter. This dilution presented no problems since scopoletin in the samples was of sufficient quantity for detection.

Chromatographic Conditions

A previous method used for the analysis of six other phenolic compounds excluding scopoletin, required a two-step gradient using a 25 cm column and the analysis time was 1 h (3). A change from the 25 cm column to a 15 cm column reduced the retention time of scopoletin from 22 to 18 min, but it was still desired to reduce the runtime still further. Isocratic conditions were evaluated using the 15 cm column, but did not give a greater height response for scopoletin over that obtained using the original gradient when identical standards were evaluated (3). A gradient to 25% ACN+1% glacial acetic acid over 10 min gave increased response, so an increase in the amount of ACN+1% glacial acetic acid to 35% over 10 min was evaluated. This gave even greater response for scopoletin due to its elution in less mobile phase and thus, is the gradient used for analysis. Identical samples evaluated using these conditions yielded similar results, 566±25ng (n=2), to those obtained under previous conditions (3), indicating no coelution of other compounds with scopoletin when using the 15 cm column and 10 min gradient to 35% ACN+1% glacial acetic acid.

Extractant and Amounts

Table 2 shows that MeOH was as efficient as 1% HAc for removal of scopoletin from the Teflon membrane. A 2.5-fold increase in MeOH, 10 mL, did

TABLE 2. Extractant and Amount of Scopoletin Extracted[a]

Extractant (n)	mL	ng±SD
1% HAc (4)	4	256±5
MeOH (4)	4	251±9
MeOH (2)	10	245±11

[a]- two cigarettes smoked in an 18-m^3 chamber, no air exchange, sampled for one h at 2 L min^{-1}

not remove more scopoletin and, therefore, 4 mL MeOH was deemed to be sufficient for quantitative extraction.

Incremental Cigarette Smoking

Table 3 shows that the amount of scopoletin collected was essentially a linear function of the number of cigarettes smoked. It also shows the effect of dilution air when an air exchange rate of 2.5 air exchanges h^{-1} was used during sampling.

Precision

The instrument and overall precision were both found to be acceptable as seen in Table 4. Figure 1 is a typical chromatogram obtained in this overall precision study. The minimum detectable quantity was < 3 ng m^{-3} with an air volume of 0.12 m^3.

Linearity

The response for scopoletin was found to be linear over a 50-fold concentration range, $R^2 = 0.9999$, Table 5. The y-intercept was < 0.2 ng mL^{-1}.

TABLE 3. Environmental Tobacco Smoke Scopoletin from Two, Four and 16 Cigarettes[a]

No. Cigt. (n)	No. of Air Exchanges h^{-1}	ng Collected±SD	ng $cigt^{-1}$
2[b] (6)	0	212±12	106
4[b] (6)	0	496±16	124
16[c] (2)	2.5	654±31	102[d]

[a]- smoked in 18-m^3 chamber
[b]- sampled for one h at 2 L min^{-1}
[c]- sampled for four h at 2 L min^{-1}
[d]- corrected for air exchange

TABLE 4. Overall Precision of Scopoletin Collection (n = 6)

	Instrument[a] (mV)	Overall[b] (ng m^{-3})
	1036	1920
	1033	1578
	1032	1712
	1039	1789
	1039	1782
	1028	1750
$\bar{x}$	1034	1755
SD	4.3	112
% RSD	0.4	6.4
MDQ[c]	---------	2.8

[a]- 10.8 ng mL^{-1}, 200 μL injection volume
[b]- two cigarettes smoked in 18-m^3 chamber, no air exchange, sampled for 1 h at 2 L min^{-1}, Teflon membrane extracted with 4 mL MeOH, 1 mL MeOH extract diluted to 5 mL with 1% HAc
[c]- MDQ = minimum detectable quantity, at twice the signal-to-noise ratio

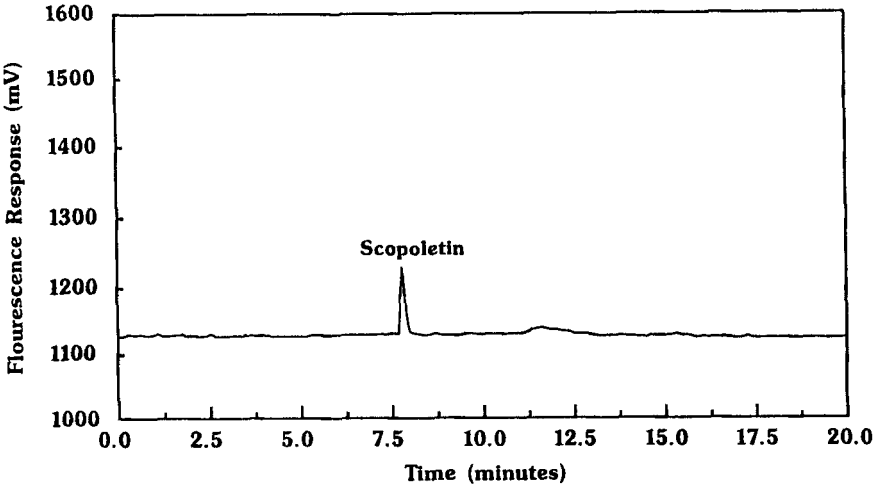

FIGURE 1. Chromatogram from overall precision study. Conditions: see text.

TABLE 5. Linearity of Scopoletin Standard Response

Concentration range (ng mL^{-1})	Height (mV), Fresh	Height (mV), Aged One Mo
1.08	117	140
5.40	536	624
10.80	1065	1253
16.20	1574	1852
32.40	3136	3688
43.20	4118	4810
54.00	5240	6126
	R^2 = 0.9999	R^2 = 0.9998
	Y - Intercept = 16 mV (0.14 ng mL^{-1})	Y - Intercept = 25 mV (0.19 ng mL^{-1})

A re-evaluation of the same set of standards after one month left in the laboratory in the light at room temperature showed $R^2 = 0.9998$ and the y-intercept again < 0.2 ng mL^{-1}. A peculiar difference between these fresh standards versus those that were aged one month was that the aged standards gave a response about 20% higher than the fresh standards. This was not understood but appeared to have no effect on sample results (see below, Storage).

Flow Dependence and Breakthrough

Table 6 gives the results for scopoletin after smoking 16 cigarettes and sampling at four different flow rates. As can be seen, the results in ng m^{-3} are equivalent. The apparent breakthrough at 1.0 L min^{-1} was probably caused by a small leak around the membrane in the first cassette. From these results, one Fluoropore membrane is sufficient for sample collection.

Storage

There was no problem with the storage of the MeOH extract in the presence or absence of the Fluoropore membrane either at 24°C or -2°C. The 1% HAc dilution of the MeOH extract was also stable at these temperatures for two weeks as seen in Table 7. Table 7 also gives the percentage change of scopoletin from samples when stored on the Fluoropore membrane contained in the cassette. There was a significant loss at 24°C after being stored in the laboratory. This loss may be the effect of light, since there was less loss when stored at -2°C in a dark freezer. As a result, samples should be stored in a freezer immediately after collection.

TABLE 6. Flow Dependence and Breakthrough of Scopoletin Collection[a]

Flow rate, L min^{-1}	Amount on 1st membrane, ng	Amount on 2nd membrane, ng	% on 2nd membrane	ng m^{-3}
0.5	156	BDL	------	1300
1.0	323	48	14.8	1346
2.0	649	BDL	------	1352
3.0	1002	BDL	------	1392

[a]- 16 cigarettes smoked in 18-m^3 chamber, sampled for four h, 2.5 air changes h^{-1}, Teflon membrane extracted in 4 mL MeOH, 1 mL MeOH extract diluted to 5 mL with 1% HAc, n=2
BDL = below detection limits

TABLE 7. Percentage Change of Amount of Scopoletin After Two Weeks Storage of Extract and Fluoropore Membrane[a]

	Condition	
Sample (n)	24°C, % Change±SD	-2°C, % Change±SD
MeOH Extract[b,c] (2)	-2.0±2.2	+1.4±1.4
MeOH Extract[c] (2)	+1.4±0.6	+4.4±3.6
MeOH Dilution[c] (4)	+1.4±1.0	+1.1±1.8
Fluoropore Membrane[c] (3)	-35.8±17.2	-10.2±4.7

[a]-two cigarettes smoked in 18-m^3 chamber, no air exchange, sampled for 1 h at 2 L min^{-1}
[b]-Fluoropore membrane left in extract
[c]-1 mL of 4 mL MeOH extract diluted to 5 mL with 1 % HAc

TABLE 8. Scopoletin in Smoke from Other Sources

Source (n)	Volume of Air Sampled (L)	ng $m^{-3} \pm SD$
Two Cigarettes[a] (6)	120	1755±112
Oak Leaves[a,b] (8)	120	527±20
Fireplace[c] (4)	120	BDL

[a]- smoked in 18-m^3 chamber, no air exchange, sampled for two h at 2 L min^{-1}, Teflon membrane extracted with 4 mL MeOH, 1 mL MeOH diluted to 5 mL with 1% HAc

[b]- 2 g burned

[c]- three feet from fireplace, Teflon membrane extracted with 3 mL MeOH, 1 mL MeOH diluted to 5 mL with 1% HAc

BDL = below detection limits, < 2.8 ng m^{-3}

Scopoletin from Other Sources

Besides tobacco smoke from a tobacco blend containing flue cured tobacco, a dominant source of polyphenols (1,2), smoke from oak leaves and a fireplace were also sampled for scopoletin (Table 8). Scopoletin has been reported in oak leaves (7) and was found in their smoke. Scopoletin was below detection limits in fireplace smoke.

CONCLUSIONS

A method has been developed for the determination of scopoletin in environmental tobacco smoke from a single MeOH extract which can be used for other analyses. Since amounts above 3 ng m^{-3} can be detected, the procedure is very sensitive to the presence of scopoletin.

REFERENCES

1. W. A. Court, J.Chromatogr., 130: 287-291 (1977).

2. M. E. Snook and O. T. Chortyk, Tob.Sci., 26: 25-29 (1982).

3. C. H. Risner, J.Liq.Chromatogr., 16: 4117-4140 (1993).

4. J. M. Conner, G. B. Oldaker and J. J. Murphy, Environ.Technol., 11: 189-196 (1990).

5. M. W. Ogden and K. C. Maiolo, Indoor Air '90, Precedings of the 5th International Conference on Indoor Air Quality and Climate, 2: 415-420 (1990).

6. M. W. Ogden and K. C. Maiolo, LC-GC, 10: 459-462 (1992).

7. H. Barn and G. Schwedt, Z. Lebensm-Unters-Forsch, 196: 370-374 (1993).

Received: January 12, 1994
Accepted: January 25, 1994