BIO-MEDICAL RESEARCH
DOCUMENT DESCRIPTION FORM

Duplicate in all cards: →

63 68	69 76	77 78
1980	0000147	VC
year as-1961-	File number [Right justify [Numeric only]	Sub-Index Code

R&S 107703

Author(s), as Last Name FS (No Punctuation) and coden for journal as JAMA preceeded by one blank space

1 20	21 40	41 60	61 62
Hoffmann D.			11
Garfinkotos C			12
Brunnemann KD			13

Title of Report; end with space-hyphen-hyphen-space. Follow with Index Terms, separated from each other with comma-space. Avoid other punctuation; do not abbreviate.

1 2 61 62

Chromatographic Determination of Vinyl Chloride in Tobacco Smoke -- Gas Pulsation Thermal Desmuroen Gas FPK 21 22 23 24

Source (Journal, Vol., Number, Pages, Date)

1 2 61 62

Analytical Chemistry, 48:1, pp. 47-50, Jan. 1976 31 32

Brief Summary

1 2 10 61 62

SUMMARY: 61 62 63 64

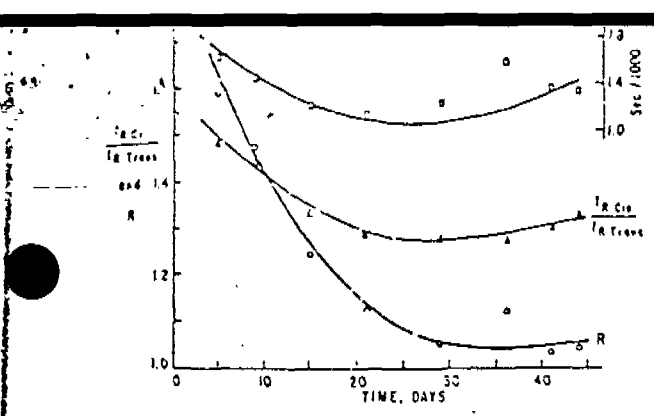

Figure 5. GC retention and resolution data for a mixture of *cis*- and *trans*-9-octadecenes on a 12.5% EGS polyester on silanized silver sulfobenzyl Porasil C column using 99.9999% helium carrier at a flow of 50 cm³/min. Column temperature, 180 °C

ence of H_2 in the carrier gas. Zero Gas grade helium contains about 1 ppm of H_2 (*12*) which reduced the Ag^+ to Ag^0 in 10 days of operation at 180 °C under the conditions of chromatography. The packing was removed from the column, extracted with CH_2Cl_2 and Ag^0 removed with 5 *N* HNO_3. About 70% of initial capacity of the ionogenic groups remained. In order to determine the effects of H_2 in the carrier, Airco No. 6 grade helium was utilized. Each day for 44 days, over a total period of 10 weeks, two samples of a mixture of the *cis*- and *trans*-9-octadecenes were injected into the column. Figure 5 summarizes the experiment and depicts an initial decrease in retention time, relative retention, and resolution although an adequate separation was achieved throughout the course of the study. When the column was operated above 180 °C, alkene retention times decreased, but bleeding of the EGS polyester increased rapidly with temperature and loss of resolution was accelerated. When this loss of resolution becomes excessive, the column packing may be removed and regenerated as the Ag^+ form.

The extent of interaction of solutes with the surface of the supposedly inert support in GLC is often debated. On face does interact as is evidenced by the effect of HMDS upon retention. In addition, the difference in interaction of AgSP with respect to the hydrogen-form support indicates that solutes penetrate the liquid coating and approach near to the surface. The surprising extent of coating penetration was apparent when *cis*- and *trans*-9-octadecenes were separated in 30 min at 183 °C with R = 1.45 on HMDS-treated AgSP at 15% loading of EGS. When a column with 20% EGS was used, a resolution of 0.57 at 180 °C was obtained. Thus, the approach described here has utility for analytical separations, provides a probe for the study of the migration of a solute through a liquid phase, and may be used to measure interactions of volatile solutes with surface-bonded groups.

ACKNOWLEDGMENT

The authors thank T. Foglia of our Center for the samples of *cis*- and *trans*-9-octadecenes, and M. A. Kaiser, M. O'Brien, and R. L. Grob, Villanova University, for determining the surface areas reported herein.

LITERATURE CITED

(1) O. K. Guha and J. Janák, *J. Chromatogr.*, 68, 325 (1972).
(2) J. J. Duffield and L. B. Rogers, *Anal. Chem.*, 34, 1193 (1962).
(3) R. F. Hirsch, H. C. Stober M. Kowblansky, F. N. Hubner, and A. W. O'Connell, *Anal. Chem.*, 45, 2101 (1973).
(4) J. J. Vollmer and S. A. Gordon, *Chemistry*, 47, 6 (1974).
(5) R. A. Barford, L. T. Olszewski, D. H. Saunders, P. Magidman, and H. L. Rothbart, *J. Chromatogr. Sci.*, 12, 555 (1974).
(6) K. Sisido, Y. Takeda, and Z. Kinugawa, *J. Am. Chem. Soc.*, 83, 538 (1961).
(7) D. H. Saunders, R. A. Barford, P. Magidman, L. T. Olszewski, and H. L. Rothbart, *Anal. Chem.*, 46, 834 (1974).
(8) D. Seyferth, R. Suzuki, C. J. Murphy, and C. R. Sabet, *J. Organomet. Chem.*, 2, 431 (1964).
(9) M. A. Kaiser, M. O'Brien, and R. L. Grob, *Amer. Lab.*, June–July (1975).
(10) K. Unger, *Angew. Chem., Int. Ed. Engl.*, 11, 267 (1972).
(11) V. Y. Davydov, L. T. Zhuralev, and A. V. Kieselev, *J. Phys. Chem., USSR*, 38, 1108 (1964).
(12) Private communication from Liquid Carbonic Corp., Dully's Lane, Burlington, N.J. (1974).

RECEIVED for review June 27, 1975. Accepted October 2, 1975. Mention of commercial products does not constitute an endorsement by the United States Department of Agriculture over others of a similar nature not mentioned.

R&S 107704

0000147

Chromatographic Determination of Vinyl Chloride in Tobacco Smoke

Dietrich Hoffmann,* Constantin Patrianakos, and Klaus D. Brunnemann

Division of Environmental Carcinogenesis, American Health Foundation, Valhalla, N.Y. 10595

Gio B. Gori

Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md. 20014

A chemical-analytical method has been developed for the quantitative determination of vinyl chloride (VC) in tobacco smoke. VC from the mainstream smoke is trapped on charcoal, extracted, and subsequently converted to 1,2-dibromo-1-chloroethane (DB-VC). The latter is enriched by column chromatography and determined by gas liquid chromatography using an electron capture detector with a high sensitivity for DB-VC. From the mainstream smoke of a popular 85-mm cigarette without filter tip, we isolated 12.2 ng of VC per cigarette. The VC content in the smoke of some domestic and foreign cigarettes and little cigars ranged from 5 to 27 ng, and that of a marijuana cigarette was 5.4 ng. The analytical data suggest that the total inorganic chloride in tobacco is a determining factor for the amount of VC in the smoke. VC may also be released into our respiratory environment during the burning of other chlorine-containing organic matter.

Vinyl chloride (VC) is causally associated with angiosarcoma of the liver in VC workers (*1*, *2*). Exposure of mice,

R&S 107705

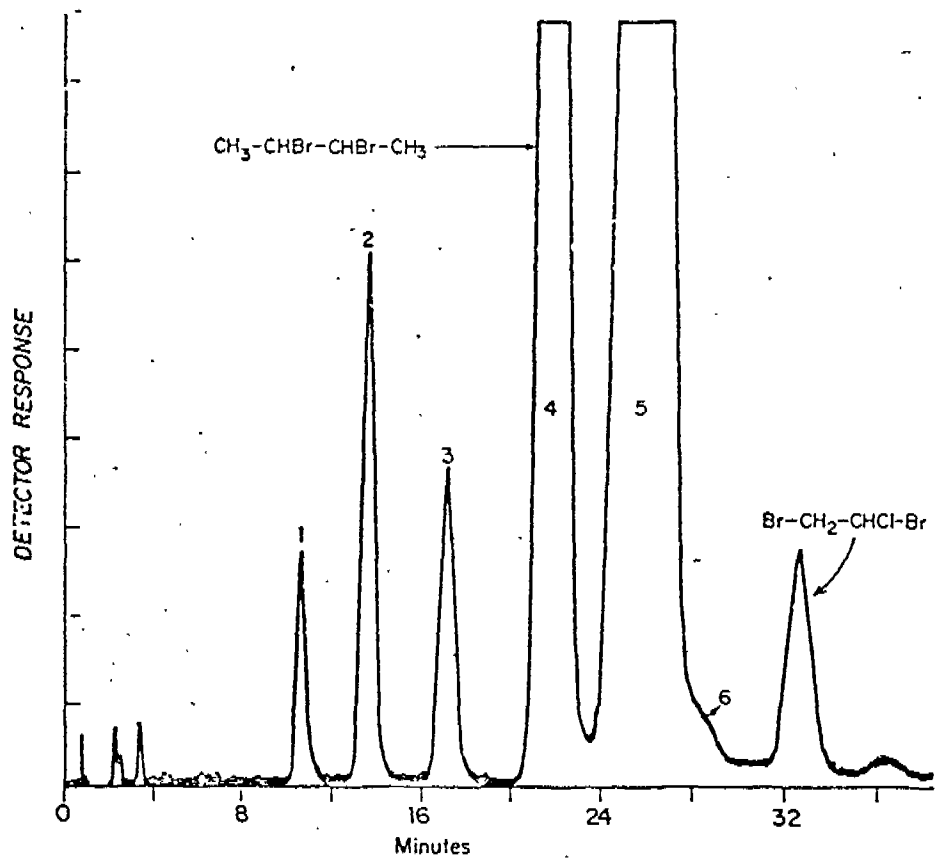

Figure 1. Gas chromatogram of a concentrate of vinyl chloride as 1,2-dibromo-1-chloroethane (DB-VC) from cigarette smoke

Peak 1 = a dibromopropane; 2 = a dibromopropene; 3 = a dibromobutane; 4 = 2,3-dibromo-*n*-butane; 5 = a dibromobutane and a dibromobutene; 6 (shoulder) = a dibromobutene; 7 (last peak) = 1,2-dibromo-1-chloroethane (DB-VC)

rats, and Syrian golden hamsters to VC in concentrations as low as 50 ppm leads to the induction of various types of tumors, including angiosarcoma of the liver (*1–3*). Until now, VC has been identified only in environments and materials related to vinyl chloride and poly(vinyl chloride) (*1, 2*).

The relatively high reactivity of VC, as well as its presumably low concentration in non-occupational respiratory environments, requires special analytical methods and sensitive detection techniques. This study reports a quantitative method for the determination of nanogram amounts of vinyl chloride in tobacco smoke.

EXPERIMENTAL

Apparatus. A Hewlett-Packard gas chromatograph Model 7260A with ^{63}Ni-ECD was used for the analysis. Cigarettes were smoked individually with a 30-port, automatic smoker (Heinrich Borgwaldt, Hamburg, Germany). Mass spectra were determined on a Hitachi/Perkin-Elmer RMU-6D instrument by the Mass Spectroscopy Laboratory of the Massachusetts Institute of Technology and on a Hewlett-Packard 5710-5980A GC/MS instrument. Conditions during analysis were: ion source, 185 °C; interface, 60 °C. NMR spectra were obtained with a Hitachi/Perkin-Elmer Model R-24 instrument. The elemental chlorine analysis was carried out by Galbraith Laboratories, Knoxville, Tenn.

Reagents. *n*-Hexane (spectrograde) was freshly distilled over Na_2SO_4 (anhydrous) prior to use. Bromine was extracted with concentrated H_2SO_4. Activated charcoal was obtained from the Pittsburgh Activated Carbon Co. (PCB – 12 × 30), silica gel (mesh 40-140) from J. T. Baker Chemical Co., OV-17 on Gas Chrom Q (mesh 80–100) from Applied Science Laboratories, pure vinyl chloride gas from Matheson Gas Products, and vinyl chloride mixture (49.5 ppm in air) from Scott Research Laboratories (Plumsteadville, Pa.). [All experiments with VC references were performed under well ventilated hoods].

Cigarettes. Domestic and foreign cigarettes were purchased on the open market in New York City and Westchester County, N.Y., during 1974–75. The cigarettes were selected by weight (±20 mg of average weight of 200 cigarettes) and by draw resistance (±5% of average value of 50 weight selected cigarettes). Before smoking, the cigarettes were stored at 22 ± 2 °C for at least 24 hr in a chamber with a relative humidity of 60 ± 3%.

1-Chloro-1,2-Dibromoethane (reference compound). Twenty ml of bromine were dissolved in 100 ml of *n*-hexane. Under magnetic stirring at −5 °C, pure vinyl chloride gas was bubbled slowly through the solution until the color disappeared. Most of the solvent was distilled at atmospheric pressure, and the remainder under reduced pressure. 1-Chloro-1,2-dibromoethane (dibromovinyl chloride, DB-VC) was distilled at 52 °C under 12 mm Hg pressure. It was sealed under dry nitrogen, covered with dark paper, and stored at 0 °C. Under these conditions, DB-VC remains unchanged for up to two weeks. The known DB-VC was characterized by NMR: 4.0 ppm, d, 2H; 5.6 ppm, t, 1H; and by MS (*m/e*; rel. int.): 145 (25), 143, (100), 141 (50), 105, 107; 79, 81 [The parent peaks (220, 222, 224, 226) of DB-VC are not observed under our conditions; see Figure 2]; the ratios of the major fragments are consistent with the natural abundance of Br and Cl isotopes. The purity of the compound was ascertained by gas chromatography.

Gas Chromatography. The most satisfactory separation of DB-VC from a cigarette smoke concentrate was obtained at 60 °C on a 12-ft by 2-mm i.d. glass column filled with 10% OV-17 on Gas Chrom Q (mesh 80–100). 5% Methane in argon was used as the carrier and purge gas with a flow rate of 22 ml/min. The retention time for DB-VC was 32.5 min. Using a ^{63}Ni-ECD at 200 °C, the detection limit was 15 pg (10^{-12}g) per injection. The detector response was linear in the range of 50–300 pg.

Model Studies. In model tests, it could be demonstrated that 1.0 g of activated charcoal packed in a 12-cm × 6-mm i.d. glass tube (ends closed with glass wool) retains VC quantitatively, at least up to 200 μg of VC from a stream of air (20 ml/sec) containing 49.5 ppm of VC. (VC remains unchanged on charcoal for at least 6 hr.)

The separation method was developed by blowing an air stream (flow rate 20 ml/sec) of 4 l. containing 200 μg of VC through a Cambridge filter (CM-113A) loaded with tar of 30 cigarettes (≃800 mg), and through 1.0 g of charcoal with the gas phase deposits from the cigarettes. After loading with VC, the charcoal was

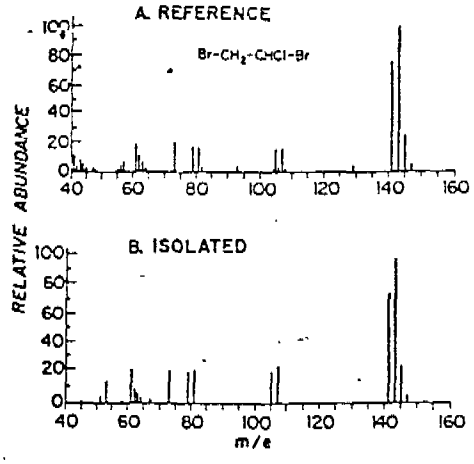

Figure 2. Mass spectra of 1,2-dibromo-1-chloroethane (DB-VC) (parent ions 220, 222, 224, 226 are unstable and not detected)

poured into a flask which was kept at −30 °C and which contained 0.5 ml of bromine and 11 ml of *n*-hexane. The determination of DB-VC by GLC from the solution after column chromatography gave recovery rates of more than 95%. Two control runs (as outlined under Procedure), one involving extraction with *n*-hexane of charcoal with smoke gas-phase deposits (no bromination) and the other involving bromination of charcoal without smoke (blank test), produced no signals at the retention time of DB-VC. (All equipment used was the same as under Procedure).

Procedure. *Isolation of Vinyl Chloride.* An amount of the concentrate from cigarette smoke sufficient (≃20 μg) for mass spectral identification of the 1,2-dibromo derivative of VC was collected by several runs following the procedure for quantitative analysis of VC (see below). Each successive run was quantitatively and qualitatively monitored by gas chromatography.

Quantitative Analysis. Thirty cigarettes (selected by weight and draw resistance) were smoked with a 30-port automatic smoking machine at a rate of one puff of 2-sec duration per min, a puff volume of 35 ml, to a butt length of 23 mm. The mainstream smoke was drawn through a Cambridge filter (CM-113A; 92-mm diameter) and subsequently through a glass tube (12-cm × 6-mm i.d.) containing 1.0 g of activated charcoal (glass wool at both ends of the tube). Immediately after smoking, the contents of two such tubes were poured into a flask [Reacti-flask with screw cap and Teflon-faced disk (Pierce Chemical Co.)] which was kept at −30 °C and contained 0.5 ml of bromine in 11 ml of *n*-hexane. The flask was sealed and covered with dark paper, and the contents were magnetically stirred for 7 min. An aliquot of the reaction mixture was chromatographed with *n*-hexane on silica gel (mesh 50–80) 1.0 × 60-cm at a column temperature of 0 °C. Fractions (average 7.3 ml) were collected every 2 min. Fractions 13–15 were combined and concentrated slowly under nitrogen at 45 °C to a 7-ml volume.

This solution was rechromatographed with *n*-hexane on silica gel mesh 60–80 (column 1.3 × 75-cm) at room temperature; 7.3-ml fractions were collected every 1.25 min. An aliquot of fraction 24 was analyzed by gas chromatography. (To ensure maximal recovery, neighboring fractions were also analyzed for DB-VC and, if needed, combined with fraction 24).

Quantitative Analysis of Inorganic Chloride in Tobacco. The technique employed was the Caldwell and Moyer modification of the Volhard method (4), with the further change of filtering the sample solution with Norite prior to titration. Cl^- was extracted from tobacco samples according to Yamazaki and Yamazaki (5).

RESULTS AND DISCUSSION

A gas chromatogram of a vinyl chloride (VC) concentrate from cigarette smoke as 1,2-dibromo derivative (DB-VC) is shown in Figure 1. The column effluent, which by retention time corresponded with DB-VC, was identified by its mass spectrum (Figure 2). The main peaks in the gas chromatogram (Figure 1) with retention times lower than that of DB-VC were identified by mass spectrometry as dibromopropanes and dibromobutanes; however, because of a lack of reference compounds, we were unable to assign structures with the exception of one case. Five analyses of 60 cigarettes each yielded an average of 12.2 ng VC isolated as DB-VC from the mainstream smoke of an 85-mm U.S. blended cigarette without filter tip (Table I). The recovery rate was found to be at least 85%. The minimum recovery rate was calculated by directing known amounts of VC in air through charcoal which was earlier loaded with the gaseous phase of the mainstream smoke of 10, 20, or 30 standard cigarettes, respectively. After completing the analysis, we calculated the recovery rate.

Table I. Vinyl Chloride in Cigarette Smoke, ng/cig.[a]

No. of analysis	Vinyl chloride isolated[b]
1	12.6
2	11.4
3	12.9
4	10.8
5	13.1
Average	12.2
Std dev	0.90
Dev coefficient	7.4 %

[a] Commercial, 85-mm, nonfilter cigarette. [b] Not corrected for losses.

Table II. Vinyl Chloride in Cigarette Smoke

Product[a]	Length, mm	Butt length, mm	Vinyl chloride, ng/cig.
Reference cigarette IRI			
Cigarette A NF	85	23	12.4
Commercial cigarettes			
Cigarette A F	85	23	5.6
Cigarette B F	85	23	14.1
Cigarette C F	85	23	11.4
Cigarette D NF	70	23	11.9
Cigarette E NF	70	23	15.8
Cigarette G F	85	23	10.9
Cigarette H NF	85	23	12.2
Commercial little cigars			
Little cigar A F	85	23	14.4
Little cigar B F	85	23	27.3
Charcoal filter cigarettes[b]			
Cigarette A with F	85	35	5.1
Cigarette A with F cut off	60	10	15.3
Cigarette B with F	85	35	1.3
Cigarette B with F cut off	60	10	4.0
Marijuana cigarette	85	23	5.4

[a] F: Filter; NF: nonfilter. [b] Cigarette column smoked: 50 mm.

In addition to the standard nonfilter cigarettes, we analyzed the mainstream smoke of some domestic and foreign cigarettes, of two little cigars, and a marijuana cigarette. The results are summarized in Table II and indicate significant differences in the concentration of VC in the mainstream smoke of commercial cigarettes. As expected, the smoke of cigarettes with charcoal filter tips contains significantly lower amounts of VC than does the smoke of cigarettes without this type of filter tip.

CH radicals are long known to be generated during the burning of tobacco products (6). Since many tobacco constituents give rise to CH radicals, a search for possible specific precursors for the C_2H_3 moiety of VC appears rather futile. On the other hand, tobacco contains traces of residues of chlorinated pesticides (7), some unknown, chlorinated organic compounds (6, 8), and up to 3% of inorganic chloride (9). It is, therefore, reasonable to assume that the inorganic chloride in tobacco contributes significantly to the concentration of VC in the smoke. This hypothesis is supported by the observation that the amount of methyl chloride in cigarette smoke is determined by the concentra-

R&S 107706

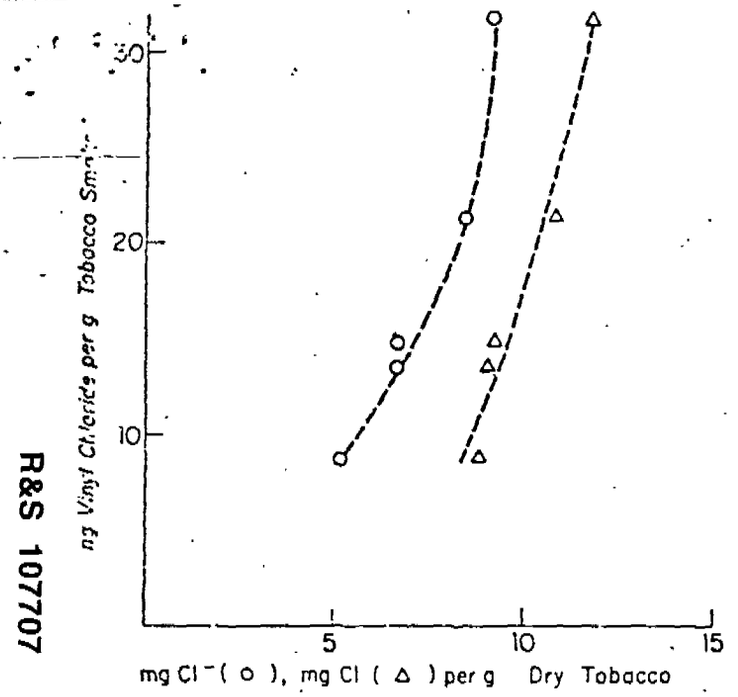

Figure 3. Correlation between inorganic chloride and total chlorine in tobacco and vinyl chloride in cigarette smoke

tion of inorganic chloride in tobacco (*10*). Figure 3 indicates that water soluble chloride, as well as total chlorine, in tobacco may contribute to the concentration of VC in the smoke. We realize that detailed studies are needed to ensure the nature of the major precursors in tobacco for the chlorine of VC in the smoke. Nevertheless, we should not disregard the possibility that also during the burning of other plant materials, and in fact of all organic matter which contains chlorine, vinyl chloride can be generated and can be released into our respiratory environment, as demonstrated for marijuana smoke.

This study has shown that smoke contains up to 16 ng of vinyl chloride per cigarette, and 27 ng of VC per little cigar. This amount corresponds to a concentration of about 30 ppb. Based on human data and results from animal studies, it appears to us that these minute amounts of VC will not contribute to a measurable degree to the carcinogenic activity of tobacco smoke. It should be realized, however, that we have directed our analysis only toward the identification and quantitative determination of vinyl chloride and the tobacco smoke may also contain other unsaturated, chlorinated hydrocarbons with carcinogenic activity. This may include the suspected human carcinogen 2-chlorobutadiene (chloroprene; *11, 12*) as well as chlorinated products of isoprene, the major unsatured hydrocarbon in tobacco smoke.

NOTE ADDED IN PROOF. This study was presented at the 29th Tobacco Chemists' Research Conference (College Park, Md., Oct. 8–10, 1975). Two questions arose. 1) How much VC is in ambient air? Using the above method, at different times we passed 20 l. of ambient laboratory air through charcoal. The extract of the charcoal was each time free of VC. 2) Is VC artificially formed from the ethylene of the smoke (trapped on charcoal) and the trace amounts of chlorine possibly present in the bromine? To answer this question, we led 400 ml of ethylene (CP-Matheson Gas Products) through charcoal and, using the same bromine, we processed it in the usual way. We did not observe a signal in the glc at the retention time of DBVC using the ECD at twice the usual sensitivity.

ACKNOWLEDGMENT

The authors thank C. Costello of the Mass Spectroscopy Laboratory, Massachusetts Institute of Technology, for her cooperation in obtaining and interpreting mass spectral data on DB-VC.

LITERATURE CITED

(1) International Agency for Research on Cancer, *Monogr.* **7**, 291 (1974).
(2) I. J. Selikoff and E. C. Hammond, "Toxicity of Vinyl Chloride–Polyvinyl Chloride", *Ann. N.Y. Acad. Sci.*, 246, 1 (1975).
(3) C. Maltoni, G. Lefemine, P. Checo, and D. Carretti, *Ospedaliidi, Bologna, Italy*, 1 (5–6), 1 (1974).
(4) J. R. Caldwell and H. V. Moyer, *Anal. Chem.*, **7**, 38 (1935).
(5) M. Yamazaki and J. Yamazaki, *Sci. Pap. Central Res. Inst. Jpn Monopoly Corp.*, 114, 45 (1972).
(6) E. L. Wynder and D. Hoffmann, "Tobacco and Tobacco Smoke, Studies in Experimental Carcinogenesis", Academic Press, New York, N.Y., 1967.
(7) F. F. Guthrie and P. G. Bowery, *Residue Rev.*, 19, 31 (1967).
(8) R. L. Stedman, *Chem. Rev.*, 68, 153 (1968).
(9) T. C. Tso, "Physiology and Biochemistry of Tobacco Plants", Dowden, Hutchinson, and Ross, Stroudsburg, Pa., 1972.
(10) R. R. Johnson and T. E. Smith, *Abstr. 24th Tobacco Chemists' Res. Conf.*, 24 (1970).
(11) E. A. Khackatryan, *Gig. Tr. Prof. Zabol.*, 18, 54 (1972).
(12) E. A. Khachatryan, *Publ. Oncol. USSR.*, 18, 85 (1972).

RECEIVED for review August 5, 1975. Accepted September 22, 1975. This is No. XXXVI of "Chemical Studies on Tobacco Smoke". It was supported in part by American Cancer Society Grant BC-56T and by Public Health Service Contract ECI-SHP-74-106.

Thin Layer Chromatography/Densitometry with Transferable Calibration Factors

H. Bethke and R. W. Frei*

Analytical Research and Development, Pharmaceutical Division, Sandoz Ltd., 4002 Basle, Switzerland

The principle of a transferable calibration technique is discussed and introduced with examples of dihydroergotamine. The quantitation of spots is carried out with direct UV reflectance spectrometric measurements of methylergobasine on different series of chromatoplates. The applicability of this same calibration transfer model to in situ fluorescence measurements which require a derivatizating reaction after separation is demonstrated with samples of digoxin. The reproducibility of the results obtained is comparable to individual calibration carried out for each plate (rel std dev about 2%). With the use of transferable calibration, 5 analyses can be carried out on one 20 × 20 cm plate which represents an increase in capacity of 60% compared to a previously discussed method.

R&S 107707