Multiple Headspace Extraction Gas Chromatography for the Determination of Volatile Halocarbon Compounds in Butter

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Multiple headspace extraction gas chromatography (MHEGC) is a discontinuous gas extraction technique that provides straightforward, rapid quantitation of volatile compounds from a wide variety of matrices. In this paper, the theory of MHE is summarized and an MHEGC procedure for the determination of volatile halocarbons (VHCs) in butter is described. Detection limits and quantitation limits, respectively, for six VHCs in butter were as follows (ppb): chloroform, 2, 10; 1,1,1-trichloroethane, 3, 10; carbon tetrachloride, 1, 5; trichloroethylene, 5, 15; bromodichloromethane, 3, 12; tetrachloroethylene, 2, 5. Over the concentration range 40–1500 ppb, average recovery (and standard deviation) for the six VHCs was 95 ± 3.2%.

Low molecular weight, volatile halocarbon compounds (VHCs) are ubiquitous environmental contaminants arising from various industrial sources (Bellar et al., 1974; Farr and Govelek, 1980; Novak et al., 1973) and as byproducts of water and wastewater chlorination (Rook, 1978; Helz and Hsu, 1978; Helz et al., 1985). The potential fate of VHCs in food has been recognized (Barcelona, 1979; Page and Charbonneau, 1978; Easley et al., 1981; Entz et al., 1982), and a number of analytical approaches have been used for the qualitative and quantitative analysis of foods for VHCs. These methods include dynamic headspace (Reinert et al., 1983), static headspace (Entz et al., 1982; Reinert et al., 1983; Entz and Hollifield, 1982), and solvent extraction schemes (Page and Charbonneau, 1978). In this paper, the use of multiple headspace extraction (MHE) for the rapid determination of selected VHCs in butter is described. We report here the separation and quantitation of chloroform, carbon tetrachloride, trichloroethylene, bromodichloromethane, 1,1,1-trichloroethane (methylchloroform or MC), and tetrachloroethylene (perchloroethylene or PCE).

Multiple headspace extraction gas chromatography (MHEGC) has several advantages over other techniques. Sample handling is minimized, since no fortification is necessary as with the method of standard additions (SA), greatly reducing the chance of accidental contamination. Considerably less test material is used, since MHE requires only one portion for the entire determination. Much less time is required for preparation of the test material, since no preliminary extraction or cleanup is required. The method is amenable to automation, an important consideration if high throughput is expected. Most important, matrix effects that often dramatically influence partitioning of the analyte between the test material and the headspace are compensated for by the method. Thus, for an array of different matrices, only one calibration run is required.

BACKGROUND

A number of authors have discussed MHEGC (Kolb et al., 1984; Kolb, 1982; Suzuki et al., 1970; Vitenberg and Reznik, 1984). An excellent review of the mathematical treatment of MHE data was given by Ettre et al. (1984). The MHE theory is discussed here briefly.

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MHE involves repeated withdrawal of headspace in equilibrium with a test portion, followed by a gas chromatographic (GC) separation of the components in the headspace. In principle, one could exhaustively extract a given analyte (analogous to liquid–liquid extraction) and compute the total amount of analyte present in the test portion by summing the peak areas of the corresponding chromatographic peaks over all the extractions. Unfortunately, this process would be very tedious and time consuming. However, an alternative method for interpreting MHE data exists. Repeated sampling of a headspace in equilibrium with a test portion reduces the concentration of the analyte in a first-order manner. The decrease in concentration of the analyte in the headspace (C) with the number of withdrawals (n) can be described by eq 1, where C_1 is the concentration of the analyte after

$$C_1 = C_0 e^{-kn} \tag{1}$$

the nth withdrawal, C_0 is the concentration of the analyte in the headspace before the first withdrawal, and k is a decay constant, containing both chemical and instrumental terms. Assuming that chromatographic peak area is

$$A_i = A_1 e^{-k(n-1)} \tag{2}$$

proportional to concentration, then eq 2 holds, where A_i is the peak area of the analyte after n withdrawals and A_1 is the peak area of the first withdrawal. Taking the logarithm of (2) leads to eq 3. Plotting $(n - 1)$ vs $\ln A_i$ yields

$$\ln A_i = -k(n - 1) + \ln A_1 \tag{3}$$

a straight line with slope $-k$. By using linear regression methods, the value of k can be determined from the MHE gas chromatographic data. The total peak area (A_T) can be represented by the geometric progression (4), which can be solved by (5). Thus, by obtaining the values of A_1 and k from the linear regression data, the value of A_T can be computed with eq 5.

$$A_T = A_1[1 + e^{-k} + e^{-2k} + e^{-3k} + \ldots + e^{-(n-1)k}] \tag{4}$$

$$A_T = A_1/(1 - e^{-k}) \tag{5}$$

Results are quantitated by converting total peak area to amount of analyte. This determination is performed on a gaseous mixture containing a known amount of analyte. The total peak area for a given amount of standard yields a response factor of amount per unit peak area. Test materials containing an unknown amount of analyte are determined under the same conditions as the standard, and

Table I. Multiple Headspace Extraction Calibration Data[a]

	ln (peak area)					
$(n-1)$	$CHCl_3$	MC	CCl_4	TCE	BDCM	PCE
0	11.919	12.724	13.957	11.194	13.485	12.822
1	11.214	11.985	13.088	10.467	12.516	12.087
2	10.503	11.235	12.130	9.738	11.614	11.314
3	9.789	10.505	11.157	8.976	10.774	10.529
4	9.106	9.787	10.304	8.348	9.983	9.769
r^2	0.99999	0.99997	0.9997	0.9998	0.9999	0.99996
$-k$	0.7041	0.7354	0.9237	0.7184	0.8474	0.7659
y – int	11.916	12.718	13.975	11.817	13.347	12.835
VHC added, ng	44.1	42.5	41.9	33.9	37.6	38.4
$A_T \times 10^{-6}$ [b]	0.5937	1.2887	3.8209	0.2837	2.194	1.384
$ng/A_T \times 10^3$	7.43	3.30	1.10	0.120	1.71	2.77

[a] Key: $CHCl_3$ = chloroform; MC = methylchloroform; CCl_4 = carbon tetrachloride; TCE = trichloroethylene; BDCM = bromodichloromethane; PCE = perchloroethylene. [b] Calculated from eq 5.

a value of A_T for the analyte is obtained. Simply multiplying this value by the response factor yields the amount of analyte in the test material.

EXPERIMENTAL SECTION

MHEGC experiments were carried out on a Perkin-Elmer Sigma 2000 gas chromatograph coupled with a Perkin-Elmer HS-100 automatic headspace sampler capable of automatic MHE programming. The IBM chromatography acquisition program CAP operating on an IBM 9000 data station was used for data acquisition and reduction. Postrun MHE data were analyzed with a separate BASIC computer program.

The gas chromatograph was fitted with a DB-5 capillary column, 30 m × 0.32 mm (i.d.), 1.0-μm film thickness (J & W Scientific, Inc., Rancho Cordova, CA). Headspace conditions were as follows: 30-min sample vial thermostating time at 60 °C; 9-s injection time (equilibration time for butter determined experimentally); transfer line temperature, 90 °C; injection split ratio, 5:1. GC conditions: carrier gas linear velocity, 32 cm/s; ^{63}Ni electron capture detector at 300 °C, using 95:5 argon–methane makeup gas at 28 mL/min. GC oven program: 40 °C for 11 min, then 20 °C/min to 150 °C, hold for 2 min. Figure 1 is a headspace gas chromatogram of a ca. 0.3-ng injection of a VHC standard mixture. VHCs are easily separated in less than 15 min.

Standard solutions of VHCs were prepared in methanol gravimetrically by the method of Entz and Hollifield (1982). Standards were stored in 1-mL screw-cap vials at 5 °C and checked periodically for integrity of composition and concentration. Headspace vials were stored in a 102 °C oven until used.

Selected butters were examined by the method of SA of Entz and Hollifield (1982). Six portions of ca. 1 g each were placed in 22-mL headspace vials and the vials sealed. Five of the vials were spiked with incremental amounts of the appropriate standard from a 5-μL syringe. VHCs were determined by GC, followed by SA data reduction.

Butters were obtained at local retail stores in suburban Washington, DC. In the laboratory, the butters were placed in a beaker, allowed to come to room temperature, and then homogenized by stirring with a spatula. Potential loss of VHCs during this mixing process was tested. Butters were fortified at 10 ppb with chloroform, MC, carbon tetrachloride, trichloroethylene, PCE, and bromodichloromethane. With the method of Entz and Hollifield (1982), analysis after mixing showed that, within experimental uncertainty, even the VHCs of lower molecular weight were completely recovered. The resulting composite was stored in a freezer until needed. The butter used in the fortification study (described below) was screened for VHCs by conventional headspace GC methods and determined to have no detectable VHCs except ca. 50 ppb of chloroform.

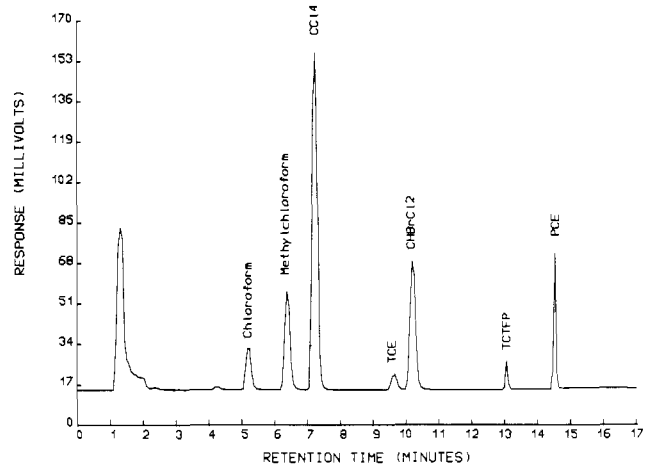

Figure 1. Headspace/capillary gas chromatogram of VHC standard representing ca. 0.3 ng of each component injected. Instrumental conditions are given in the text.

SPIKING EXPERIMENTS

A calibration standard was prepared by delivering a 2-μL standard solution of ca. 20 ng of VHC component/μL into a sealed headspace vial from a 5-μL syringe and immediately subjecting it to the procedure under headspace and GC conditions described above.

Butters were prepared by weighing 0.1–1 g of frozen, mixed butter into a pretared headspace vial. These portions were fortified by adding 2 μL of appropriate standard solution. One microliter of 20 ng of trichlorotrifluoropropane/μL was added as an internal standard.

RESULTS AND DISCUSSION

Response factors for the six analytes were determined by performing five-point MHE on the vial containing the calibration standard mixture. Plots of ln (peak area) vs $(n-1)$ were prepared, and a least-squares analysis was performed on the data. Values for the correlation coefficient, slope, total peak area (from eq 5), and response factors, in nanograms/A_T, are tabulated in Table I. Correlation coefficients of 0.999 or better were obtained in all cases. We have chosen $r^2 = 0.99$ as the correlation coefficient cutoff for analytical consideration. In a five-point linear regression plot, r^2 of 0.99 represents about a 3% uncertainty (standard error) in the slope (k). Thus, only data with r^2 of 0.99 or better are considered acceptable for quantitation. In examining butter by MHE, the major factors influencing r^2 are (1) too low an analyte level, which makes detection difficult or impossible, resulting in poor accuracy in peak area measurements, or (2) too large a test

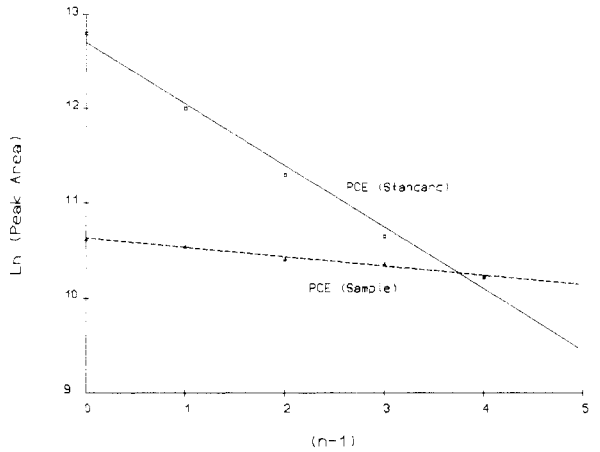

Figure 2. Multiple headspace extraction analyte decay curves for a PCE standard and PCE in butter.

Table II. Recovery of VHC from Butter by Multiple Headspace Extraction

compd[a]	added, ng	found, ng	std dev	RSD, %	rec, %
MC	4.25	4.66	0.08	1.7	110
CCl_4	4.19	4.34	0.16	3.8	104
TCE	3.39	3.76	0.21	4.4	111
BDCM	3.76	3.42	0.01	2.9	91
PCE	3.84	3.66	0.14	3.8	95
MC	21.3	20.8	1.10	5.5	98
CCl_4	21.0	18.1	0.70	3.5	82
TCE	17.0	16.5	0.53	3.2	95
BDCM	18.8	19.7	0.69	3.5	105
PCE	19.2	18.3	0.42	2.3	95
MC	170	168	7.1	4.5	99
CCl_4	168	163	2.5	1.5	97
TCE	136	139	1.7	1.6	102
BDCM	150	163	4.9	3.0	109
PCE	154	143	3.4	2.4	93

[a] See Table I.

portion, resulting in no measurable decrease in peak area over the five-step MHE. The detection limit for each compound is defined as that response giving a peak height signal-to-noise (S/N) of 3 (detector attenuation = 1). Quantitation limit is defined as that level yielding for the fifth peak in an MHE, a peak height S/N of 5. Calculated detection limits (ppb) for the VHCs in this study are as follows: chloroform, 2; carbon tetrachloride, 1; trichloroethylene, 5; bromodichloromethane, 3; MC, 3; PCE, 2. Calculated quantitation limits (ppb) are as follows: chloroform, 10; carbon tetrachloride, 5; trichloroethylene, 15; bromodichloromethane, 12; MC, 10; PCE, 5.

Butters analyzed in triplicate at three different fortification levels (about 50, 200, and 1500 ppb) were prepared and examined by five-point MHE. The GC data were handled as described above, and a value of A_T for each component in every material was computed. By using the response factor obtained from the standard run, the total amount of analyte in each test sample was calculated. Figure 2 shows a plot of ln (peak area) vs $(n - 1)$ for both standard and a test material. As is generally the case, the slope of the standard curve is steeper than that of the curve for the butter. This reflects the fact that the butter matrix, acting as an analyte reservoir, buffers the decay of the analyte from the headspace.

Table II is a summary of the data from the fortification experiment. Column 2 is nanograms of analyte added to each vial. Columns 3 and 4 are the average and standard deviation of the triplicate determinations, respectively; column 5 represents the repeatability of the method, expressed as percent relative standard deviation (% RSD). Column 6 is the calculated recovery of each analyte.

Table III. Comparison of Multiple Headspace Extraction and Standard Additions Methods[a]

butter	method	$CHCl_3$, ng/g	MC, ng/g	PCE, ng/g
1	SA	31.2 ± 5	1148 ± 180	2523 ± 410
	MHE	35.7 ± 1	1174 ± 50	2638 ± 300
2	SA	55.7 ± 6	151 ± 18	899 ± 140
	MHE	51.5 ± 3	136 ± 6	923 ± 130

[a] Data are the average of three measurements (±1 standard deviation).

The repeatability of the method ranged from 1.5 to 5.5% RSD, with an average of 3.2% RSD over all experiments. There was no discernible trend in precision over the three different fortification levels, suggesting that the variability was predominately associated with the injection system and the fortification technique.

Two different butters with known chloroform, MC, and PCE contamination (as determined by GC with electron capture detection and GC/mass spectrometry) were analyzed in triplicate by both MHE and SA. Table III shows the results. Values are reported in nanograms/gram ± 1 standard deviation. Good agreement between the two methods is evident; usually the mean values obtained between methods agreed within 10%. However, it can be seen that MHE (average RSD = 7.1%) is considerably more reproducible than SA (average RSD = 14.5%).

As a general observation, based on many determinations, we find that the repeatability of MHE is somewhat poorer with authentic butter test samples (generally in the ±15% range) than with the spiked butter test samples. This may reflect the inhomogeneity of analyte in the authentic samples or variability due to handling.

CONCLUSIONS

MHE is a rapid and reliable technique for the determination of VHCs in butter. The method is markedly less time consuming than alternative headspace techniques and yields analytical data with good precision and accuracy. In butter, quantitation of 20 ppb or less can be obtained for all the VHCs examined in this study when a ^{63}Ni electron capture detector is used.

Registry No. TCE, 79-01-6; PCE, 127-18-4; BDCM, 75-27-4; $CHCl_3$, 67-66-3; CCl_4, 56-23-5; 1,1,1-trichloroethane, 71-55-6.

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Colorimetric Determination of Cyanide in Enzyme-Hydrolyzed Extracts of Dried Sorghum Leaves

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The purpose of this study was to develop a simple and effective procedure for assaying large numbers of sorghum leaf samples for their potential to release cyanide. The procedure involves drying the tissue at 75 °C (this was accomplished without loss of the cyanogenic glucoside dhurrin), grinding the dry tissue, extracting with water, digesting the extract with an almond meal extract to release cyanide, and determining cyanide in the digest colorimetrically. Results obtained were comparable to those based on distillation of digested samples and potentiometric determination of cyanide in the distillates. The equipment and supplies required for the procedure are relatively inexpensive, and a skilled technician can conveniently assay 70–80 samples/day.

Cyanide occurs in the leaves of sudangrass [*Sorghum bicolor* (L.) Moench] and sorghum (also *S. bicolor*) plants as the cyanogenic glucoside dhurrin [*p*-hydroxy-(*S*)-mandelonitrile β-D-glucopyranoside]. Degradation of dhurrin yields equimolar amounts of HCN, glucose, and *p*-hydroxybenzaldehyde (*p*-HB). One of the objectives of sudangrass and sorghum breeding programs is reduction in the level of dhurrin and thus in the possibility that cyanide released from the plant tissues will be harmful to consuming livestock. Plant-breeding and genetics programs typically require the examination of large numbers of individual plants. The HCN potentials (HCN-p) of large numbers of young sorghum seedlings can be assayed conveniently by autoclaving seedling leaves in water to extract and hydrolyze the dhurrin, diluting the extract in alkali, and reading the absorbance at 330 nm, the absorbance maximum of *p*-HB in basic solution, as described by Gorz et al. (1977). However, this simple procedure is not satisfactory for mature sorghum leaves (Haskins et al., 1984), and assays of mature leaves are required when measurements on the forage actually consumed by animals are needed. Blaedel et al. (1971) described an assay for cyanide in sudangrass forage in which emulsin was used to hydrolyze dhurrin, and cyanide in the hydrolysate was then determined directly with a cyanide-selective electrode. In our experience, however, the electrode was affected adversely by constituents of the crude extracts; equilibration was slow, and misleading results were sometimes obtained.

The objective of this study was to adapt published procedures to provide for assay of the HCN-p of large numbers of samples with relatively inexpensive equipment and supplies. Specific information was sought on (1) the effect of tissue drying on HCN-p, (2) the feasibility of using an extract of almond meal to hydrolyze dhurrin in crude extracts of sorghum leaves, and (3) the suitability of the colorimetric procedure of Lambert et al. (1975) for determination of cyanide in these hydrolyzed extracts.

MATERIALS AND METHODS

Reagents. Succinimide, *N*-chlorosuccinimide, and barbituric acid for use in the procedure of Lambert et al. (1975) were obtained from Sigma Chemical Co., as was defatted almond meal. Other chemicals were obtained from customary sources.

Enzyme Preparation. Defatted almond meal was suspended in water (8 mg/mL), and the mixture was allowed to stand at room temperature for 3 h with occasional gentle shaking, after which it was filtered (Whatman No. 1 filter paper). The filtrate was used as the enzyme preparation. Such filtrates could be stored at 4 °C for at least 2 days without apparent loss in activity.

Plant Material. Week-old seedlings were grown in pans of a soil mixture in growth chambers at 27 °C under continuous cool white fluorescent light at about 150 μmol/m² per s, and shoots were excised just above the soil surface. For older plants from the field or greenhouse,

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