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ANALYSIS OF SHORT ALKYLSILANE BONDED SILICA GEL HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC STATIONARY PHASES USING HYDROFLUORIC ACID DIGESTION AND HEADSPACE ANALYSIS BY CAPILLARY GAS CHROMATOGRAPHY

THOMAS R. FLOYD*, NICHOLAS SAGLIANO Jr. and RICHARD A. HARTWICK*
Department of Chemistry, Rutgers University, Piscataway, NJ 08855-0939 (U.S.A..)

SUMMARY

A quantitative hydrofluoric acid digestion–headspace gas chromatography (GC) method was developed for the analysis of *n*-butyldimethylsilane and other short alkyl bonded silica gel high-performance liquid chromatographic stationary phases. The method requires only 10–30 mg of material and simple reaction vials. Digestion of the bonded silica requires 75 min, followed by a 4-min GC analysis. The average of the absolute value of the relative error for a range of alkyl bonded silica surface concentrations was 6.44% compared to elemental analysis, with an average precision of 1.55% (relative standard deviation). The method was used to identify and quantify both laboratory bonded and commercial stationary phases.

INTRODUCTION

Short alkylsilane bonded silica high-performance liquid chromatographic (HPLC) stationary phases are widely used in the separation of polypeptides[1], proteins[2,3] and oligonucleotides[4,5]. Although well suited for the separation of such compounds, these phases have been reported as being more susceptible to the hydrolytic loss of the bonded silanes than their longer chain counterparts[4]. Research in our laboratory regarding the acid stability of *n*-butyl phases employed for preparative separations of proteins required an analytical method by which the identity and surface concentration of these bonded alkylsilane phases could be determined. Such a method was needed (1) as a reliable means of measuring the extent of alkylsilane hydrolysis under accelerated hydrolytic conditions, (2) to determine the efficacy of various alkylsilane bonding reactions, and (3) to determine, quantitatively and qualitatively, multiple alkyl ligand bonded stationary phases.

Elemental analysis[6] has been the most popular means of quantifying bonded stationary phase concentrations due to the simplicity of sample preparation and its widespread availability. One problem with elemental analysis however, is its lack of

* Present address: Tennessee Eastman Co., Kingsport, TN, U.S.A.

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qualitative information and the inability to differentiate between multiple sources of carbon. Consequently, a number of other techniques have been useful in the analysis of bonded phases on silica gel supports. These include, spectroscopic techniques such as diffuse reflectance infrared Fourier transform spectroscopy and magic angle spinning ^{29}Si Nuclear magnetic resonance[7,8], surface techniques such as electron spectroscopy for chemical analysis[9] and fast atom bombardment mass spectroscopy (FAB-MS)[10], thermal methods[11], pyrolysis gas chromatography (GC)[12], and basic and acidic hydrolysis of phases followed by GC analysis[13,14]. One simple chemical means for determining bonded alkylsilane identity and quantity has been by the GC analysis of the fluorinated alkylsilanes derivatives generated by hydrofluoric acid digestion of the stationary phases. This method has been used for the analysis of a variety of bonded alkylsilanes[15–17].

During previous studies[16], it was determined that alkylfluorosilanes of less than about 7 carbon atoms were too volatile for hexane extraction, requiring instead a headspace sampling technique. Headspace GC was successfully used in this original work for the determination of trimethylsilane (TMS) concentrations following "capping" reactions. We wish to report further on the development of an expanded and refined headspace procedure for the analysis of various short alkylsilane bonded phases.

EXPERIMENTAL

Bonded phase synthesis

A single lot of silica gel was used throughout this study, CD-802 Lot 863-94, suplied by the PQ Corp. (Valey Forge, PA, U.S.A.). This was an earlier experimental lot of gel, which is currently marketed under the IMPAQ tradename. Chloroalkylsilanes were purchased from Petrarch Systems (Britol, PA, U.S.A.). Imidazole was purchased from Fisher Scientific (Springfield, NJ, U.S.A.).

The silylation procedure used is a modification to that described by Kinkel and Unger[20]. Silica gel (10 g) was weighed, transferred to a 1000-ml round-bottom flask, and dried at 150°C under vacuum for at least 24 h. Imidazole (10 g) was dissolved in 100 ml of dichloromethane. An approximate fourfold molar excess (10 ml) of *n*-butyldimethylchlorosilane was added to another 100 ml of dichloromethane. The imidazole solution, followed by the silane solution were transferred to the silica, which was then placed on a rotary evaporator. The reaction flask was rotated at moderate velocity for at least 24 h at room temperature. Following reaction, the bonded silica gel was washed with 100-ml volumes each of dichloromethane, acetone, methanol, water, methanol and acetone.

Hydrofluoric acid digestion and fluoroalkyl derivative generation

The hydrofluoric acid solutions were made up by volume percent of concentrated hydrofluoric acid (Fisher), distilled water, and an organic modifier, which in this study was either acetone or acetonitrile (Fisher). The digestion/derivatization step was carried out in 30 ml Tuf-Tainer PTFE/PFA vials (Pierce, Rockford, IL, U.S.A.) equipped with Tuf-Bond PTFE/silicone septa caps and PTFE-coated magnetic stir discs.

An amount of 10–30 mg (depending upon silane surface density) of bonded stationary phase was weighed out along with an approximately equivalent molar amount of internal standard. Ethyldimethylsilane or *n*-propyldimethylsilane bonded silicas were employed as internal standards. Hydrofluoric acid solution (10 ml) was added and then caps were securely tightened onto the vials. Vials were spun on a stir plate for 30 min allowing for digestion/derivatization of the silicas, at which time they were then transferred to a water bath (Precision Scientific, Chicago, IL, U.S.A.) maintained at 27.0°C for 45 min, to ensure thermal equilibration.

Gas chromatography of fluoroalkylsilane derivatives

A 500-μl side-hole gas-tight syringe (Hamilton, Reno, NV, U.S.A.) was used to sample the headspace of the sample vial. A 12 m × 250 μm I.D. SE-30 capillary column (Scientific Glass Engineering, Austin, TX, U.S.A.) was used in a HP 5890A gas chromatograph, equipped with a FID detector (Hewlett-Packard, Avondale, PA, U.S.A.). Split injection at a 50:1 ratio was used, and head pressure was set at 10 p.s.i. All GC runs were performed isothermally at 40°C. Chromatographic data werre collected by an HP 3392A recording intergrator.

RESULTS AND DISCUSSION

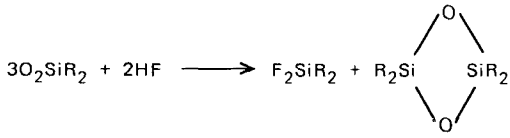
Reactions of silane with hydrofluoric acid

Aqueous fluorination of bonded alkylsilanes has been shown to proceed as

$$[\text{–Si–O–}]_{(4-n)}\text{–SiR}_n + \text{HF} \rightarrow \text{F}_{(4-n)}\text{–SiR}_n$$

where n represents the number of alkyl groups bonded to the silicon. Hydrolysis, followed by dimerization (or polymerization for mono- and dialkysilanes) of halosilanes in general is thermodynamically favored. However, for the silicon–fluorine bond, hydrolysis accounts for an insignificantly small percentage of the total fluorosilane starting material at equilibrium[18]. Under conditions of 7.2 *M* (25%) hydrofluoric acid, trialkylfluorosilanes have been shown to be chemically stable, allowing for quantitation[19].

Mono and dialkylsilanes have been less successfully quantitated due to polymerization side-reactions. In the case of a dialkylsilane



the siloxyl dimer can also be formed in significant quantities. In addition further complications arise when using alcohols in the digestion solution, since the alkoxysilane by-product will also occur. In general, it will be found that trialkylsilanes can be quantitatively converted to their fluoro derivatives, while dialkyl silanes may or may not yield 100% conversion, depending upon the particular silane and reaction conditions. Monoalkylsilanes under these conditions demonstrate a strong tendency to polymerize, and are generally not suited for quantitative hydrofluoric acid digestion and analysis, although they can often be qualitatively analyzed by this method.

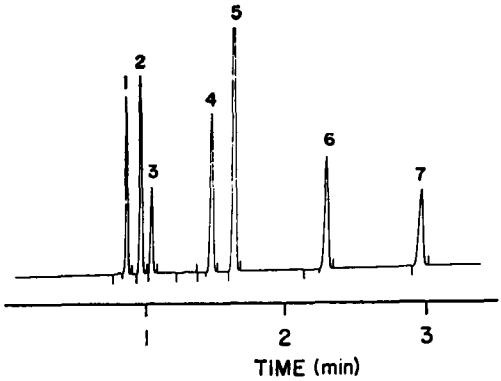


Fig. 1. Hydrofluoric acid digest of a number of fluorosilanes, analyzed by headspace GC. The compounds present: (1) trimethylfluorosilane, (2) *n*-butyltrifluorosilane, (3) ethyldimethylfluorosilane, (4) *n*-butylmethyldifluorosilane, (5) *tert.*-butyldimethylfluorosilane, (6) *n*-butyldimethylfluorosilane, and (7) triethylfluorosilane, were separated in less than 3 min isothermally at 40°C on a 12 m × 250 μm I.D. SE-30 capillary column. Bonded silanes were digested in 25% (v/v) aq. hydrofluoric acid.

Qualitative identification

Fig. 1 is the result of a 25% (v/v) aq. hydrofluoric acid digest of a number of pure alkylchlorosilanes standards, yielding the fluoro derivatives, determined by headspace GC. The total separation time was less than 3 min.

The retention times for *n*-butyltrifluorosilane, *n*-butylmethyldifluorosilane, and *n*-butyldimethylfluorosilane were 0.84, 1.54, and 2.32 min, respectively. Thus, unknown phases bonded with mono-, di- or trifunctional *n*-butyl silanes can be readily identified. Additionnally, co-bonded phases can also be quantitated for each of the alkyl ligands present provided that they partition into the gas phase.

During the course of this work, several commercially available butyl bonded phases were examined. One phase, Vydac C_4 214-TPB, produced fluorosilane peaks not observed in any of the other phases studied. Since this phase is widely employed for biopolymer separations, it was felt that further investigations using GC–MS were warranted. The mass spectrum indicated that the bonded silane species was a dibutylsilane. In addition the presence of the dioxo-bridge dibutylsiloxyl dimer, $(C_4H_9)_2SiO_2Si(C_4H_9)_2$, was also confirmed by GC–MS.

Distribution coefficients of fluorosilanes

In order to achieve reliable quantitative results using a static headspace sampling technique it was necessary to establish the conditions of linearity between the analyte concentration in the liquid phase and the gas phase, as well as the linearity of the GC detector response over the range investigated. The range over which a given distribution coefficient (K) is constant establishes the operating limits for bonded phase loadings to be determined, as well as the sensitivity of the method. Operation under linear conditions also permits the use of one point standardizations and allows for examining unknown phase concentrations more easily.

Assuming conditions of linearity, K is given by

$$K = \frac{C_l V_l}{C_g V_g} \tag{1}$$

where C_l and C_g are the concentrations of the solute in the liquid and gas phase and V_l and V_g are the volumes of the two respective phases.

The factors leading to non-linear effects acting on the concentration in the gas phase (C_g) have been well documented in the literature[21]. It has been reported that to prevent such effects, C_g should be kept small (*ca.* 0.01% of the vapor phase mole fraction)[22]. Since the combined mole fractions of the fluorosilane derivatives sample and internal standard are on the order of $1 \cdot 10^{-4}$ in the liquid phase and their values for K are typically in the range 1–10, a linear relationship[22] between C_l and C_g would be expected.

The presence of an organic modifier was observed to increase both the accuracy and precision of the assay. Acetonitrile and acetone were found to be chemically compatible with the hydrofluoric acid digestion step, and to elute in a non-interfering region of the gas chromatogram. Alcoholic modifiers were not used, since they interfere with the derivatization reaction.

Distribution coefficients for compounds 2, 3, 4, 6 (identified in Fig. 1) and for *n*-propyldimethylfluorosilane were determined by static headspace GC under a variety of liquid phase compositions[23]. Derivatization solvent systems were examined that consisted of 25% (v/v) hydrofluoric acid, containing various volume percents of water and either acetonitrile or acetone.

In general, the partition coefficients were found to be linear for the quantitation of *n*-butyldimethylsilane stationary phases over a range from 2–50 μmol (total) of alkylsilane present. Table I lists the slopes and correlation coefficients (R^2) using different fluorination solutions and internal standards for butyldimethylfluorosilane and butylmethyldifluorosilane. Examination of the residuals from the linear least squares fit indicated good linearity for the calibration curves over the reported ranges. The response slope was calculated from the ratio of the detector response to the ratio of the moles added for the butyl and ethyl fluorosilanes. This response slope represents a combination of factors. The slope is proportional to relative response factor of the sample and internal standard and their partition coefficients. The absolute values of K were increased by one to two orders of magnitude in the presence of 25% or 50% of either acetonitrile or acetone, as compared to their values in neat aqueous solutions. At the same time, the R.S.D. was decreased for the same samples. The conclusion is that the organic modifier can be used both to control the overall relative response factor and also to establish linear operating conditions for the headspace partition technique. Thus the selection of an organic modifier and the proportions used is critical to this method.

Quantitation

Having established the partition coefficients for the alkylfluorosilanes of interest, and establishing the required organic modifier conditions, the next goal was to find a suitable working internal standard for this system and to determine the linearity of responses and detection limits for the final analytical protocol. Since both the starting alkylsilanes and their fluoro derivatives are volatile, internal standards in the form of silanes bonded to silica gel were used. This allows the internal standard alkyl phase to be conveniently weighed and placed in the sample vial concurrently with analyte silica gel. *n*-Propyldimethylfluorosilane was chosen as the internal standard since the ethyldimethylfluorosilane eluted slightly behind the tailing acetone peak. It

TABLE I

LINEARITY OF HEADSPACE METHOD

Alkyl silane	*Internal standard*	*Derivatization solvent*	*Slope**	*Intercept*	r^2
n-Butyldimethyl	*n*-Ethyldimethyl	25% HF in water	1.664	0.091	0.9934
		25% HF/25% acetonitrile in water	1.336	0.101	0.9970
		25% HF/50% acetone in water	0.912	−0.042	0.9964
	n-Propyldimethyl	25% HF/50% acetone in water	0.797	−0.004	0.9977
n-Butylmethyl	*n*-Propyldimethyl	25% HF/50% acetone in water	0.466	0.003	0.9995

* The slope was calculated from a linear least square fit, the *X*-axis being the mole ratio of *n*-butyldimethylfluorosilane to ethyldimethylfluorosil *Y*-axis being the detector response ratio of *n*-butyldimethylfluorosilane to ethyldimethylfluorosilane.

TABLE II

ELEMENTAL PERCENT CARBON *VS.* GC HEADSPACE (25% HF/50% ACETONE)

Sample	*Elemental* carbon*	*GC Headspace**	*Difference (%)*
1	$8.81 \cdot 10^{-4}$	$9.19 \cdot 10^{-4}$	4.31
2	$7.56 \cdot 10^{-4}$	$7.33 \cdot 10^{-4}$	3.04
3	$6.59 \cdot 10^{-4}$	$6.62 \cdot 10^{-4}$	0.46
4	$3.98 \cdot 10^{-4}$	$3.79 \cdot 10^{-4}$	4.77
5	$2.60 \cdot 10^{-4}$	$2.09 \cdot 10^{-4}$	19.6
	Average difference	$0.27 \cdot 10^{-4}$	6.44

* Silane concentration recorded as weight-corrected mol per g bonded silica gel.

was not possible to separate the butyltrifluorosilane on the SE-30 capillary column under these conditions, since it co-eluted with the acetone peak. However, GC conditions can be modified if such analyses are required, at the expense of longer separation times.

Comparison to elemental analysis

Table II compares the relative accuracy of the headspace technique against elemental analysis for a series of of five *n*-butyldimethylsilane stationary phases. A relative response factor was determined using an internal standard and a known *n*-butyldimethylsilane phase standard. This standard was analyzed in triplicate for percent carbon by elemental analysis ($9.35 \cdot 10^{-4} \pm 0.12 \cdot 10^{-4}$ mol/g bonded silica). The day-to-day average response factor for the *n*-butyldimethylfluorosilane when using propyldimcthylfluorosilane as the internal standard was 1.295 ± 0.035 (2.68% R.S.D.). The average absolute difference between elemental analysis and the final recommended headspace technique was 6.44%. It should be noted that the elemental analysis is unreliable for low percent carbon coverages. This fact could explain the large absolute difference for sample 5 seen in Table II.

CONCLUSIONS

A qualitative and quantitative method has been described for the analysis of a variety of short aliphatic silane bonded stationary phases, particularly *n*-butyldimethylfluorosilane, by the use of a hydrofluoric acid digest–headspace GC method. This method provides a simple means of monitoring alkylsilane bonding procedures, of measuring the extent of bonded alkylsilane hydrolysis and identifying the type of silanes used in the bonding of commercial or unknown phases. An additional feature of this method is the suitability for determining, simultaneously, the surface concentrations of multiple alkyl ligands bonded to a silica surface.

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