Journal of Chromatography, 123 (1976) 335–345

CHROM. 9068

GAS–LIQUID CHROMATOGRAPHY OF NUCLEOSIDES

DERIVATIZATION AND CHROMATOGRAPHY

CHARLES W. GEHRKE and ANIL B. PATEL*
University of Missouri, Columbia, Mo. 65201 (U.S.A.)
(Received January 27th, 1976)

SUMMARY

The aims of this investigation were to establish the optimum reaction conditions for silylation of nucleosides with bis(trimethylsilyl)trifluoroacetamide (BSTFA) and to investigate the chromatographic properties of the following nucleosides: adenosine, guanosine, cytidine, thymine, inosine, xanthosine, and uridine. Closed tube silylations were performed with a 1000 molar excess of BSTFA at 25, 75, 120, 150, and 175° for 15, 30, 60, 120 and 240 min. The optimal time and temperature for derivatization were found to be 150° and 15 min. Using these reaction conditions, samples were then silylated with 50, 100, 200, 500, and 1000 molar excess of BSTFA; a molar excess of 225 was best. The stability of the nucleoside derivatives on standing at room temperature for 1–7 days was investigated. Quantitative gas–liquid chromatography of trimethylsilyl (TMS) nucleosides can be performed if samples are analyzed within 48 h, after which time the relative weight response for the nucleosides decreased somewhat.

Chromatographic column studies were made using various liquid phases and supports. With methylsiloxanes as the liquid phase, Supelcoport as support was found to be superior. Resolution of TMS guanosine from TMS cytidine was attempted at different column lengths using 3% (w/w) SE-30 on Supelcoport, different loadings (%, w/w) of SE-30 on Supelcoport, and different polarity liquid phases, 4% (w/w) OV-11 or 3% (w/w) OV-17 or 4% (w/w) Dexsil-300 on Supelcoport. A complete separation of the six ribonucleosides including guanosine and cytidine was obtained with 1 m × 4 mm I.D. glass columns of 4% (w/w) OV-11 and 3% (w/w) OV-17 on 100–120 mesh Supelcoport.

It was observed that with 2′-deoxycytidine, one obtains the TMS cytosine peak (retention temperature 150°) plus another peak with a retention temperature of 120°, under all derivatization conditions with BSTFA and bis(trimethylsilyl)-acetamide. Similar formation of bases from other 2′-deoxyribonucleosides occurred when the molar excess of BSTFA was greater than 500. The minimal detectable amounts obtained for all the nucleosides ranged from 5 to 10 ng injected with a signal-

* Present addres: Cooper Laboratories, Cedar Knolls, N. J. 07927, U.S.A.

to-noise ratio of 3. The relative standard deviations for all nucleosides and deoxynucleosides ranged from 1.2 to 4.8% on different methylsiloxanes on Supelcoport columns.

INTRODUCTION

Many methods for the detection of the nucleic acid components have been developed with varying degrees of quantitation obtained. These methods include ion-exchange chromatography by Cohn[1,2], paper chromatography by Vischer and Chargaff[3], paper electrophoresis by Gordon and Reichard[4], thin-layer chromatography by Randerath[5], paper chromatography and time-of-flight mass spectrometry by Studier *et al.*[6], and gas–liquid chromatography (GLC) by MacGee[7], Hashizume and Sasaki[8], Gehrke and Ruyle[9], Gehrke and Lakings[10], and Jacobson *et al.*[11].

Various derivatives of the purine and pyrimidine bases, and nucleosides have been studied at the macro level; these include the methyl derivatives by MacGee[7], the acetyl and isopropylidene derivatives by Miles and Fales[12], and the trimethylsilyl (TMS) derivative by Hashizume and Sasaki[8] and by Gehrke and Ruyle[9]. It appears from these investigations that the TMS derivative gave the best results when compared to other derivatives and is most likely to work for derivatization of the nucleosides. GLC of nucleosides was reported by Jacobson[11] using bis(trimethylsilyl)acetamide (BSA), but experiments delineating the best reaction conditions for derivatization were not reported.

The aims of this investigation were to establish the optimum reaction conditions for quantitative silylation of nucleosides with another silylation reagent bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and, in separation studies, to investigate the instrumental requirements and chromatographic properties of the silylated nucleosides.

EXPERIMENTAL

In the following sections the chemical derivatization method, chromatographic and instrumental conditions, and calculations are presented for the analysis of the TMS derivative of nucleosides.

Reagents and materials

Acetonitrile was obtained from Mallinckrodt (St. Louis, Mo., U.S.A.). BSTFA was obtained from Regis (Chicago, Ill., U.S.A.) and was refrigerated at 4°. All nucleosides were purchased from Mann Labs. (New York, N.Y., U.S.A.), and were "Mann Assayed" chromatographically pure. Phenanthrene and pyrene were obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.) and were of the highest purity available. The liquid phases SE-30, OV-11, OV-1, OV-101, OV-17, and Dexsil-300, and the support material Supelcoport (100–120 mesh) were obtained from Supelco (Bellefonte, Pa., U.S.A.). Other supports, Anakrom and Chromosorb G, were obtained from Analabs (Hamden, Conn., U.S.A.).

Apparatus and glassware

The oil-bath, in which the closed-tube silylation was conducted, consisted of a $3\frac{1}{2} \times 4 \times 6$ in. aluminum pan supported on a magnetic stirrer to maintain uniform temperature of the bath. Temperature control was achieved with two 100-W heaters and a Variac. A super D-21 safety shield obtained from Instruments for Research and Industry (I^2R; Cheltenham, Pa, U.S.A.) was used to provide protection from accidental breakage of the silylation reaction tube. The silylation reaction tube was a standard pyrex glass 16 × 75 mm screw-cap culture tube (Corning No. 9826) with a PTFE lined cap.

A Mark I Magnetic Stirring Unit with controlled hot plate (Cole-Parmer, Chicago, Ill., U.S.A.) was used as a dry heat bath for evaporation of solvents with a stream of filtered, dry nitrogen gas.

Syringes, Type 701N (10 μl), were purchased from Hamilton (Whittier, Calif., U.S.A.).

An ultrasonic cleaner (Branson Instruments, Stamford, Conn., U.S.A.) was used for mixing samples after the addition of silylating reagents.

Glass columns used in the gas chromatography (1 m × 4 mm I.D.) were obtained from the Glass Blowing Shop, Physics Department, University of Missouri. High temperature O-rings and silicone septa, and silanized spun glass wool were obtained from Analabs.

Instrumental and chromatographic conditions

A Micro-Tek Model MT 220 automatic sequential programmed-temperature gas chromatograph equipped with a four column oven, four flame ionization detectors (FIDs), and two differential electrometers were used for this investigation. A Varian Model 30 recorder was used for the chart presentation. The chromatograph was equipped with a Hewlett-Packard 3370 digital integrator and an Infotronics CRS-104 digital integrator for determination of peak areas for quantitative work.

Further conditions were as follows: columns, a variety of polysiloxanes on Supelcoport; column size, 1 m × 4 mm I.D.; initial temperature, 160°; program rate, 4 min hold, then 5°/min; carrier gas (nitrogen), 40 ml/min at 50 p.s.i.; air (to detector), 475 ml/min; hydrogen (to detector), 30 ml/min; temperature FID, 290°.

Internal standard method of calculation

This method was used to calculate the mole or weight percentage for each nucleoside. The calculation of the absolute amount of a nucleoside in a sample was accomplished by using pyrene as the internal standard (IS). Relative weight response (RWR) and/or relative molar response (RMR) values were calculated as follows:

$$\mathrm{RWR}_{N/P} = \frac{\mathrm{area}_N/\mathrm{grams}_N}{\mathrm{area}_P/\mathrm{grams}_P}; \text{ and } \mathrm{RMR}_{N/P} = \frac{\dfrac{\mathrm{area}_N}{\mathrm{grams}_N/\mathrm{GMW}_N}}{\dfrac{\mathrm{area}_P}{\mathrm{grams}_P/\mathrm{GMW}_P}}$$

where N = nucleoside, P = pyrene, GMW = gram molecular weight.

Preparation of chromatographic columns

A weighed amount of solid support (80–100 or 100–120 mesh) was placed in a

ribbed round-bottom flask, just covered with chloroform, then an appropriate amount of the desired substrate in chloroform was added to the flask. A number of different chromatographic columns were prepared as follows and are described in Results and discussion.

(a) 3% (w/w) SE-30 (methylsiloxane, M.W.: 1,000,000–2,500,000) on Supelcoport 100–120 mesh columns were prepared from 9.7 g solid support and 3 ml stock solution containing 100 mg liquid phase per ml chloroform.

(b) 4% (w/w) OV-11 (methylphenylsiloxane, 35% phenyl) on Supelcoport 100–120 mesh columns were prepared from 9.6 g solid support and 4 ml of stock solution containing 100 mg OV-11 per ml chloroform.

The solvent was slowly evaporated for 1–2 h at room temperature using a rotary evaporator until just damp. The flask was immersed in a 60° water bath under full vacuum until no odor of chloroform remained. While adding the packing to the column, gentle tapping was used to obtain a uniform distribution and to obviate fractionation of the packing. A $\frac{1}{4}$ in. plug of silanized spun glass wool was placed in both ends of the column to hold the packing in place. The column was flushed for 30 min with carrier gas and no-flow conditioned at 300° for 2–3 h, cooled to room temperature, and flow conditioned with 30–35 ml/min of nitrogen for 24–48 h as the oven was programmed at 5°/min to 300°, then allowed to remain undisturbed for at least 24–48 h. The FID flame was kept lit during the entire column conditioning procedure.

TMS derivatization method

An aqueous aliquot containing 250 μg (or 1 μmole of each in a mixture) of nucleosides and/or deoxynucleosides were added to a silylation reaction vial. The solution was evaporated to dryness by heating at 90° in a dry bath and passing a regulated stream of filtered nitrogen into the tube. To ensure complete azeotropic removal of water, 1 ml of benzene or methylene chloride was added and evaporated. An accurately measured amount of IS in acetonitrile and a known molar excess of BSTFA were added, then the tube was closed securely with a PTFE lined cap and heated.

Determination of optimum reaction conditions for silylation of nucleosides

Time and temperature. Standards of each nucleoside (100 μg/ml) were prepared in distilled water. The silylation reagent containing 2000 μmoles/ml was prepared by mixing 8 ml of BSTFA and 7 ml of acetonitrile just before derivatization. Each nucleoside was chromatographed separately and its retention temperature determined using a 3% (w/w) SE-30 on Supelcoport 100–120 mesh 1 m × 4 mm I.D. glass column. The results are presented in Table I.

Time and temperature conditions were studied using three sets of nucleoside standards. Aliquots of 2.5 ml of each nucleoside solution (250 μg) were pipetted to give solutions containing: (a) adenosine, thymidine, and cytidine, (b) uridine and xanthosine, (c) inosine and guanosine.

After drying the samples with nitrogen at 90°, 1.5 ml of silylation reagent were added to tube (a) and 1.0 ml to tubes (b) and (c). The final volume was made to 2.5 ml with acetonitrile, sonicated for 1 min to ensure mixing, then silylated at 25, 75, 150, and 175° for 15, 30, 60, 120, and 240 min. The derivatized samples were cooled and chromatographed.

TABLE I

RELATIVE WEIGHT RESPONSE (RWR) OF NUCLEOSIDES

RWR = (weight response of nucleoside)/(weight response of pyrene). RT = retention temp. Column: 3% SE-30 (methyl silicone, M.W. 1,000,000–2,500,000) on Supelcoport, 100/120 mesh. A, B and C: different batches of column packing from different lots. Each RWR value is a single determination on an independent sample. Silylation at optimal conditions: 150°, 15 min, 225 molar excess BSTFA. Chromatography as described in Experimental.

Nucleoside	*RT (°C)*	*RWR*			*Average RWR*	*Average RMR*	*S.D.*	*R.S.D.*
		A	*B*	*C*				
Thymidine	201	0.55	0.54	0.52	0.54	0.64	0.015	2.8
		0.55	0.53	0.56				
Uridine	203	0.98	0.98	0.95	0.96	1.15	0.017	1.8
		0.97	0.97	0.94				
Inosine	208	0.93	0.94	0.89	0.92	1.22	0.021	2.3
		0.94	0.92	0.90				
Adenosine	213	0.96	0.95	0.94	0.95	1.25	0.011	1.2
		0.93	0.95	0.95				
Xanthosine	219	0.89	0.90	0.92	0.89	1.41	0.015	1.7
		0.89	0.88	0.89				
Guanosine	224	0.89	0.88	0.86	0.88	1.23	0.019	2.2
		0.86	0.88	0.91				
Cytidine	225	0.48	0.53	0.49	0.51	0.61	0.021	4.1
		0.51	0.53	0.50				

Stability of TMS nucleosides at room temperature. The derivatizations were made at 150° (15 min) using a 1000 molar excess of BSTFA. The caps of the silylation tubes were opened for a few minutes, twice each day, and then closed tightly. Samples (5 μl) were chromatographed at intervals of 1, 2, 3, 4, and 7 days.

Molar excess of silylating reagent. Standard aqueous solutions containing 1 μmole of each nucleoside per ml were prepared and studied separately. The silylation reagent (8 ml BSTFA + 7 ml acetonitrile) was prepared just before derivatization. 2 ml of the nucleoside solutions were dried with nitrogen gas. Then, 1 ml of acetonitrile containing 500 μg pyrene, and 0.05, 0.10, 0.20, 0.50 and 1.00 ml of silylation reagent corresponding to 50, 100, 200, 500, and 1000 molar excess of BSTFA were added. The final reaction volume was adjusted to 2 ml with acetonitrile, sonicated for 1 min, then the tubes were closed and silylated at 150° for 15 min in an oil bath. After cooling to room temperature, 5 μl were chromatographed on a 3% (w/w) SE-30 on Supelcoport 100–120 mesh 1 m × 4 mm I.D. glass column. The RWR values were plotted as a function of the molar excess of silylation reagent.

Optimal TMS–thymidine derivatization. Experiments were carried out with thymidine in which the acetonitrile–BSTFA ratios were varied from 1:1, 3:1, 5:1, and 10:1 (v/v). Two μmoles of thymidine, 0.5 ml BSTFA, 0.5 ml acetonitrile containing 500 μg pyrene, and 0.0, 1.0, 2.0, and 4.5 ml acetonitrile were added. The silylations were made at 150° for 15 min, and 5-μl aliquots were chromatographed.

Minimum detectible amount (MDA). Standard solutions of TMS–ribonucleosides were diluted with acetonitrile to give samples containing 1 mg per 50 ml (20 ng per μl) and 1 mg per 200 ml (5 ng per μl) of derivative. These samples were analyzed by GLC using various size injections until a signal-to-noise ratio of 3:1 was obtained.

RESULTS AND DISCUSSION

The internal standard was selected after different polynuclear hydrocarbons were chromatographed on a 3% (w/w) SE-30 on Supelcoport 100–120 mesh column. When compared to the various TMS–ribonucleosides having retention temperatures from 200 to 230°, pyrene eluted at 190°, thus being the best resolved, it was selected as the IS.

Optimal derivatization condition

The reaction conditions required for the best silylation involved a study of time, temperature, and reagent concentration. The three groups of nucleoside standards, namely, uridine and xanthosine; inosine and guanosine; and thymidine, adenosine, and cytidine were silylated at 25, 75, 120, 150, and 175° for 15, 30, 60, 120, and 240 min. The RWR values were plotted as a function of time and temperature. A typical example for cytidine is presented in Fig. 1. In general, lower RWR values were obtained for all nucleosides when silylated at 175° for times of 15 min–4 h as compared with values for silylation at 150° for the same times.

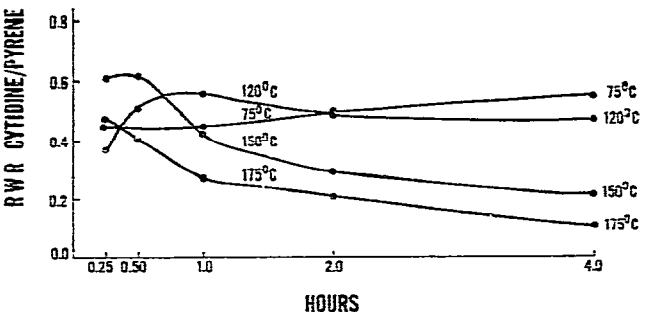

Fig. 1. Silylation of cytidine as a function of time and temperature.

Also, the multiple peaks observed for guanosine, cytidine, thymidine, and inosine on silylation at 175° may be attributed to thermal decomposition of more than one derivative of the nucleoside. An increase in the number of tube (cap) failures and leakage of sample also occurred at the higher silylation temperatures. The RWR values obtained on derivatization of thymidine and cytidine were significantly decreased if the silylation time was increased over 30 min at 150° and 175°. The cytidine response curves were markedly separated at different silylation temperatures with maximum response at 150° for 15 min. All of the other nucleosides were reproducibly derivatized under these conditions, and the relative standard deviation (R.S.D.) values ranged from 1.2 to 4.1% (Table I).

The derivatized nucleosides were chromatographed at intervals of 1, 2, 4, and 7 days, to ascertain stabilities of derivatives on standing at room temperature.

The $RWR_{N/IS}$ values of TMS–nucleosides on standing at room temperature were plotted against number of days, as shown in Fig. 2. It was observed that these derivatives were easily maintained and stable up to 48 h. On longer standing, inosine was comparatively most stable and cytidine and thymidine derivatives were least

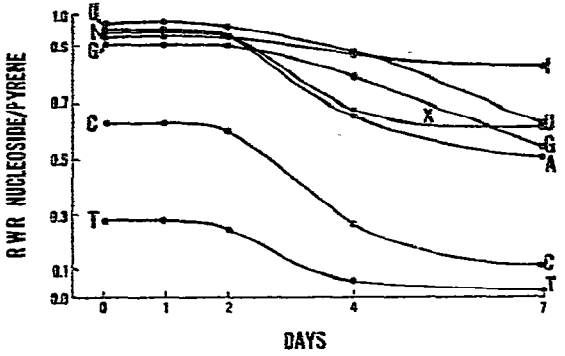

Fig. 2. Stability of nucleosides at room temperature. A = adenosine; C = cytidine, G = guanosine, I = inosine, T = thymidine, U = uridine and X = xanthosine.

stable. On standing 7 days, multiple peaks for guanosine, cytidine, thymidine, and inosine were observed. The peaks for the TMS–bases adenine, guanine, and thymine and other breakdown products were obtained from their respective nucleosides.

To obtain maximum yield of the TMS–nucleosides, molar excess studies were carried out with BSTFA. The $RWR_{N/P}$ values of various nucleosides at 50, 100, 200, 500, and 1000 molar excess of BSTFA are shown in Fig. 3. All of the nucleosides except thymidine showed linearity of response with a molar excess greater than 225 of BSTFA, thus this molar excess was selected for all later experiments. The decrease in the RWR value of thymidine with an increase in molar excess of BSTFA may be due to (a) breakdown of thymidine with the increased amount of BSTFA, or (b) a decrease in the solubility of thymidine as the BSTFA–acetonitrile ratio increases.

It was shown that TMS–thymine was formed on derivatization of thymidine when the molar excess of BSTFA was increased from 200 to 500 and above. The TMS–thymine peak was confirmed by spiking the derivatized sample with standard TMS–thymine. When other 2′-deoxynucleosides, *e.g.* 2′-deoxyuridine and 5-bromouracil 2′-deoxyribose were silylated with a large molar excess of BSTFA, peaks for the respective bases appeared when the molar excess of BSTFA was greater than 500. A similar observation was noted on the derivatization of 2′-deoxycytidine with the corresponding formation of the TMS–cytosine. To this time neither we nor any other

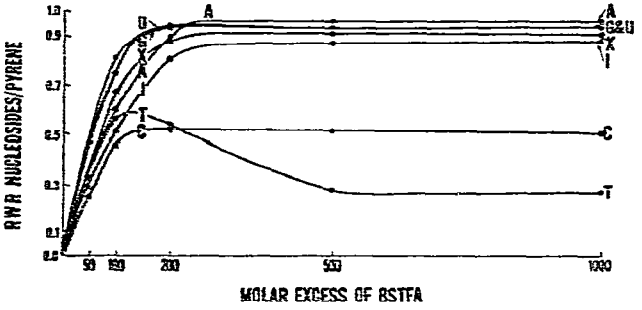

Fig. 3. Silylation of nucleosides as a function of molar excess of BSTFA. Explanation of symbols, see Fig. 2.

laboratories have obtained a single reproducible peak for 2'-deoxycytidine. One always obtains the TMS–cytosine peak (RT 150°) plus another peak with an RT of 120° under all derivatization conditions with BSTFA and BSA.

A study of solubility as a possible factor for incomplete silylation of thymidine was carried out by using 1000 molar excess BSTFA and varying the acetonitrile–BSTFA ratio from 1:1, 3:1, 5:1, and 10:1. The RWR values obtained for TMS–thymidine were 0.27, 0.26, 0.23, and 0.24, respectively. In all samples, the amount of internal standard pyrene, was held constant at 500 μg. With BSTFA alone, the average RWR value was 0.20. Since there was no significant change in the values with an increase in the volume ratios of acetonitrile–BSTFA, the solubility factor was ruled out.

The minimum detectible amounts were determined for the TMS–nucleosides on serial dilution of stock solutions containing 1 mg of derivatized nucleoside in 200 ml (5 ng per μl). Temperature programming was used in all experiments, and TMS–cytidine was also studied using an isothermal mode. The minimum detectable amounts obtained for all the nucleosides at a signal-to-noise ratio of 3:1 ranged from 5 to 10 ng injected (*i.e.*, 4×10^{-11} moles). In the different experiments reported in this paper, the amount of derivatized nucleoside injected was about 1.0 μg.

Chromatographic separation studies

To improve chromatographic separation and precision, a GLC column evaluation study was made. Experiments were made in which the support, column length, and polarity of the liquid phase were investigated. First, the TMS derivatives of the nucleosides were chromatographed on the following 1 m × 4 mm I.D. glass columns: (a) 0.2% (w/w) SE-30 on 100–120 mesh Corning glass beads (type 0201), (b) 2% SE-30 on 80–100 mesh Chromosorb G HP, (c) 2% SE-30 on 90–100 mesh Anakrom, (d) 4% SE-30 on 100–120 mesh Supelcoport.

Twice the loading was used with Supelcoport, which has approximately twice the surface area per unit weight. Very poor chromatographic peaks were obtained using glass beads as support. The Anakrom support gave peak shapes inferior to those for Supelcoport. When the TMS–nucleosides were chromatographed on the column with 4% SE-30 on Supelcoport, excellent chromatographic peak shapes were achieved. Thus, this support was selected for later experiments.

Experiments with 3% SE-30 (M.W. 1,000,000–2,500,000), OV-1 (M.W. 3,000,000–4,000,000), and OV-101 (M.W. 30,000) on Supelcoport columns showed that the RWR values were within experimental error. OV-101 is completely miscible in methylene chloride, and can be substituted for SE-30.

A 3% SE-30 on Supelcoport column showed difficulty in achieving resolution of TMS–cytidine from the TMS–guanosine (Fig. 4). Experiments were done next using different column lengths and loadings of SE-30 liquid phase on Supelcoport: (a) 3% SE-30 on Supelcoport 1, 1.5, 2, and 4 m × 4 mm I.D. columns and (b) 5 and 10% SE-30 on 1 m × 4 mm I.D. Supelcoport columns.

When either column lengths or loadings were increased, higher retention temperatures for the TMS–nucleosides were obtained, however, resolution of TMS–cytidine from TMS–guanosine was not achieved. Thus, more polar liquid phases were next investigated on 1 m × 4 mm I.D. glass columns: (a) 4% OV-11 (35% phenyl, 65% methylsiloxane) on Supelcoport (Table II), (b) 3% OV-17 (50% phenyl,

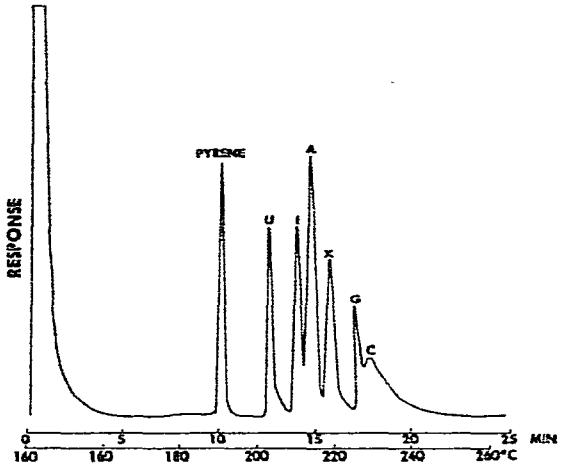

Fig. 4. GLC of TMS–ribonucleosides. Sample, 0.5 mg of nucleoside in 1.0 ml acetonitrile and 0.2 ml BSTFA (1% TMCS). Closed tube silylation, 150° for 15 min. Injected, 5 μl. Column, 3.0% (w/w) SE-30 on Supelcoport 100–120 mesh, 1 m × 4 mm I.D., glass. Initial temp., 160°, 4 min hold, then 5°/min. Attenuation, 4 × 100. Carrier gas, 40 ml/min at 50 p.s.i. FID, 290°. Similar chromatograms were obtained using OV-1 and OV-101 liquid phase. Peak identification, see Fig. 2.

50% methylsiloxane) on Supelcoport, (c) 4% Dexsil-300 (polycarborane siloxane) on Supelcoport (Table III).

A complete separation of the six ribonucleosides, including guanosine and cytidine, was obtained with 1 m × 4 mm I.D. glass columns of 4% OV-11 and 3% OV-17 on 100–120 mesh Supelcoport. See Fig. 5 showing a chromatogram of the TMS–ribonucleosides on the OV-11 column.

The nucleosides and deoxynucleosides were chromatographed singly and in mixtures on 3% SE-30, 4% OV-11, and 4% Dexsil-300 on 100–120 mesh Supelcoport, 1 m × 4 mm I.D. glass columns. Each observation represents a single determination on an independent sample containing single or a mixture of nucleosides at the same instrumental and chromatographic conditions, *i.e.*, initial temperature program rate, column detector temperature, etc. The relative standard deviations for the nucleosides and 2′-deoxyribonucleosides ranged from 1.2 to 4.8% on SE-30, OV-1, OV-101, and OV-11 on Supelcoport columns, and from 1.0 to 5.4% on Dexsil-300 on Supelcoport columns (Tables I–III).

CONCLUSIONS

From these experiments, it was concluded that the following reaction and chromatographic conditions were considered best for the silylation and separation of the TMS–nucleosides. Derivatization was made with a 225 molar excess of BSTFA at 150° for 15 min in a closed vial followed by chromatography on a 4% (w/w) OV-11 on 100–120 mesh Supelcoport and 1 m × 4 mm I.D. glass column. The derivatization

TABLE II

RELATIVE WEIGHT RESPONSE (RWR) OF NUCLEOSIDES AND DEOXYRIBONUCLEOSIDES

RWR = (weight response of nucleoside)/(weight response of pyrene). RT = retention temp. Column: 4% OV-11 (methyl phenyl silicone, 35% phenyl) on Supelcoport 100–120 mesh. A, B and C: different batches of column packing from different lots. Each RWR value is a single determination on an independent sample. Silylation at optimal conditions: 150°, 15 min, 225 molar excess BSTFA. Chromatography as described in Experimental.

Nucleoside or deoxyribonucleoside	*RT (°C)*	*RWR*			*Average RWR*	*Average RMR*	*R.S.D.*
		A	*B*	*C*			
Thymidine	214	0.50	0.50	0.54	0.52	0.62	4.0
		0.51	0.52	0.55			
Uridine	217	0.78	0.80	0.78	0.79	0.95	2.0
		0.80	0.81	0.77			
2-Deoxyuridine	216	0.87	0.84	0.85	0.85	0.90	1.8
		0.86	0.86	0.83			
Inosine	224	0.79	0.78	0.82	0.80	1.06	1.8
		0.80	0.80	0.79			
2-Deoxyinosine	223	0.86	0.81	0.83	0.84	1.05	2.1
		0.85	0.84	0.85			
Adenosine	228	1.00	0.99	0.95	0.97	1.28	2.1
		0.96	0.98	0.96			
2-Deoxyadenosine	229	0.92	0.94	0.92	0.93	1.15	1.7
		0.93	0.94	0.90			
Xanthosine	234	0.79	0.81	0.80	0.80	1.27	1.5
		0.80	0.82	0.81			
Guanosine	239	0.68	0.69	0.65	0.68	0.95	2.4
		0.69	0.67	0.67			
2-Deoxyguanosine	240	0.71	0.67	0.71	0.70	0.92	2.5
		0.72	0.69	0.70			
Cytidine	250	0.36	0.38	0.37	0.38	0.46	3.7
		0.39	0.38	0.40			
2-Deoxycytidine	120/153	0.54	0.57	0.56	0.54	0.61	4.8
		0.50	0.55	0.52			

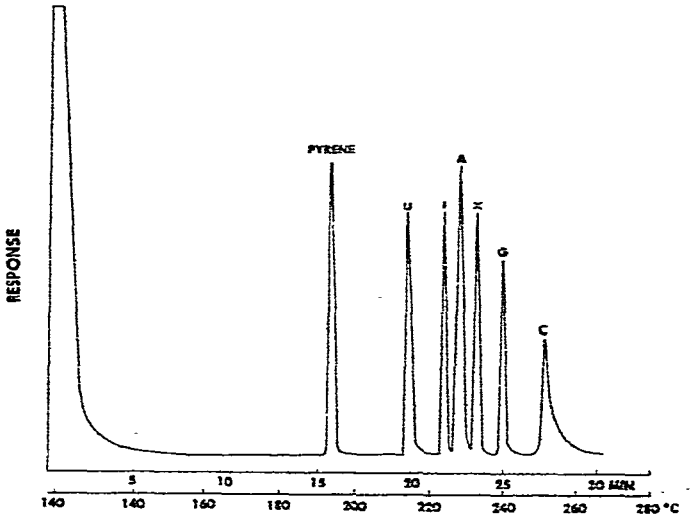

Fig. 5. GLC of TMS–ribonucleosides. Column, 4.0% (w/w) OV-11 on 100–120 mesh Supelcoport. Initial temperature, 140°, 5 min hold. then 5°/min. Other conditions, see Fig. 4. Peak identification, see Fig. 2.

TABLE III

RELATIVE WEIGHT RESPONSE (RWR) OF NUCLEOSIDES AND DEOXYNUCLEOSIDES

Column, 4 % Dexsil-300 on Supelcoport 100–120 mesh. RT = retention temperature. RWR = (weight response of nucleoside)/(weight response of pyrene). A and B: different batches of column packing from different lots. Each RWR value is a single determination on an independent sample. Silylation at optimal conditions 150°, 15 min, 225 molar excess BSTFA.

Nucleoside	*RT (°C)*	*RWR*				*Average RWR*	*Average RMR*	*R.S.D.*
		A	*A*	*B*	*B*			
Thymidine	214	0.40	0.43	0.38	0.39	0.40	0.48	5.4
Uridine	217	0.90	0.88	0.92	0.89	0.90	1.08	1.9
Deoxyuridine	218	0.85	0.87	0.83	0.84	0.85	0.90	2.0
Inosine	224	0.72	0.68	0.74	0.73	0.72	0.95	3.7
Deoxyinosine	225	0.65	0.63	0.62	0.61	0.63	0.78	2.7
Adenosine	226	0.84	0.84	0.85	0.83	1.11	0.84	1.0
Deoxyadenosine	227	0.77	0.76	0.78	0.77	0.77	0.96	1.1
Xanthosine	230	0.80	0.81	0.80	0.79	0.80	1.26	1.0
Guanosine	239	0.55	0.53	0.54	0.56	0.55	0.77	2.6
Deoxyguanosine	240	0.46	0.48	0.45	0.45	0.46	0.61	3.1
Cytidine	252	0.30	0.32	0.28	0.31	0.30	0.36	4.8
Deoxycytidine	150, 120	0.42	0.41	0.42	0.43	0.42	0.47	1.9

and chromatographic methods developed here will be useful in analyses of RNA and DNA, drugs, and in biologic markers programs for monitoring the course of cancer and effectiveness of chemotherapy.

REFERENCES

1 W. E. Cohn, *Chromatography*, Reinhold, New York, 2nd ed., 1966, Ch. 22.
2 W. E. Cohn, *Science*, 109 (1949) 377.
3 E. Vischer and E. Chargaff, *J. Biol. Chem.*, 168 (1947) 781.
4 A. H. Gordon and P. Reichard, *Biochem. J.*, 48 (1951) 569.
5 K. Randerath, *Thin-Layer Chromatography*, Academic Press, New York, 1963, pp. 185–199.
6 M. H. Studier, R. Hayats and K. Fuse, *Anal. Biochem.*, 26 (1968) 320.
7 J. MacGee, *Anal. Biochem.*, 14 (1966) 395.
8 T. Hashizume and Y. Sasaki, *Anal. Biochem.*, 21 (1967) 316.
9 C. W. Gehrke and C. D. Ruyle, *J. Chromatogr.*, 38 (1968) 473.
10 D. B. Lakings, C. W. Gehrke and T. P. Waalkes, *J. Chromatogr.*, 116 (1976) 69.
11 M. Jacobson, J. F. O'Brien and C. Hedgooth, *Anal. Biochem.*, 25 (1968) 363.
12 H. T. Miles and H. M. Fales, *Anal. Chem.*, 34 (1962) 860.