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DELINEATION OF BACKGROUND LABORATORY CONTAMINATION IN THE ANALYSIS OF TRACE CONCENTRATIONS OF EXTRACTABLE ORGANIC MATERIALS IN CRYSTALLINE ROCKS BY SOXHLET EXTRACTION, COLUMN CHROMOTOGRAPHY, AND GAS CHROMATOGRAPHY

By

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1.0 ABSTRACT

Contamination checks were carried out for sample preparation, Soxhlet extraction and the subsequent analyses of extracted bitumen, from high-grade metamorphic and igneous rocks, carbonaceous rocks from ore deposits, and other crystalline rocks. We examined possible contamination from solvents, laboratory apparatus, and from most of the analytical steps of the total procedure. The results of our examination were as follows: Once-distilled dichloromethane, twice distilled hexane, twice distilled benzene, and HPLC-grade methanol were pure enough to be used as solvents. No contaminants were leached from the septa of the cone vials used for the automated sampler on our gas chromatograph by hexane, benzene, or dichloromethane. Activated copper strips (used to remove sulfur during extraction) did not produce organic contamination by reacting with dichloromethane. However, new copper strips had to be rinsed with dichloromethane before their use, because the copper strips had a surficial wax coating from their manufacture. Saturated and aromatic hydrocarbon (HC) fractions from blank alumina/silica-gel column chromatography had only negligible peaks. Glass bottles with foil-lined caps should always be used for sample storage, because of possible wax contamination from cardboard products. Obviously, if samples are to be cut with a saw, it should be done in a pre-cleaned water-based system.

We compared the saturated- and aromatic-HC and resin gas chromatograms from a mantle xenolith to those from a blank procedural check, to determine indigenous organic materials in the mantle xenolith. This comparison demonstrated that we can quantitatively and qualitatively analyze organic materials at very low concentrations (0.1 to 0.5 ppm total bitumen by weight). This comparison also demonstrated that the contaminates in the saturated- and aromatic-HC fractions are negligible. Thus, as demonstrated by the results herein, our Soxhlet extraction procedure has been optimized for analysis of small concentrations of exotic indigenous organic compounds in very high-rank crystalline rocks.

2.0 INTRODUCTION

In organic geochemistry, we use many different organic solvents and different multi-step methodologies to quantitatively and qualitatively analyze organic compounds. For example, in extracting organic materials from oil-source rocks, we may use several solvents (chloroform, iso-octane, benzene, methanol, etc.) and several steps (crushing and grinding, extraction, evaporation, fraction-separation chromatography, gas chromatography, etc.). Some of these steps also use different organic solvents. The concentration of any organic contaminant that is in the organic solvents and is added to the sample extracts during any one of these steps, will increase with increasing volume of the solvents used in that procedure, since we can never make an absolutely pure mono-compound organic solvent. The possibility that the extract can become contaminated also increases with the increasing number of ancillary analytical methodologies. Thus, we cannot completely eliminate contamination from any given procedure, because we must use several different organic solvents and complex methodologies to carry out organic-geochemical analyses. However, for every sample there is a contaminant concentration which is acceptable as a maximum concentration for that sample, and that contaminant concentration depends on the concentration of indigenous organic materials in a sample. For example, normal petroleum-geochemical analyses involve relatively high concentrations of bitumen extracted from shales, or oils, and thus acceptable contaminant levels can be relatively high. On the other hand, the maximum contaminant concentration for analysis of our crystalline rock samples will, a priori, have to be very low, because high-grade metamorphic rocks, igneous rocks, and rocks from ore deposits contain only moderate (10-200 ppm by weight) to low (0.05 to 1 ppm by weight) concentrations of organic materials . To extract indigenous organic materials in those rocks and obtain useful quantitative and qualitative information about the

extracted organic materials, we must therefore use high-purity organic solvents, absolutely clean apparatus, and methodologies which exclude ambient contamination in the laboratory environment.

In this paper, we mainly tried to determine the highest-quality organic solvents possible, and to also delineate the degree of low-level contamination resulting from our Soxhlet extraction procedure. We thus researched the following three points in our procedure: 1) organic-solvent purity, 2) possible contamination resulting from the apparatus used, and 3) possible contamination resulting from the separate steps in our procedure. Finally, we illustrate the strengths and limitations of our procedure with a rock sample (a mantle xenolith) from the natural system that contains a very small concentration of extractable organic compounds.

In this paper we: 1) demonstrate the minimal levels of contamination which are associated with our analytical procedure; and 2) provide the details of our analytical procedure, so that other laboratories may replicate our results in the study of low concentrations of organic compounds in high-rank crystalline rocks. Because of the many possible pitfalls in such research, from many different possible sources of laboratory contamination, successfully carrying out these analyses is not a straight-forward task.

As an aside, we are not aware of any published study similar to this one, which has examined laboratory background contamination levels for the typical analyses normally carried out in petroleum geochemistry. Thus, this paper also serves to fill that void.

3.0 ANALYTICAL METHOD

In the study of metamorphic rocks, igneous rocks, and rocks from ore deposits, we use the following methodology: The surfaces of rock samples may, or may not, be cut off with a rock cutter, or a rock sample may, or may not, be cut with a cutting saw (water obviously being used as the cutting fluid). In any case, the original or trimmed rock is thoroughly rinsed with dichloromethane from a squirt bottle to remove any contaminants which the sample may have taken up from either laboratory or outcrop surroundings. The rinsed sample is crushed and ground with a jaw crusher and a ball mill, respectively. Both the jaw crusher and ball mill are thoroughly cleaned before use. The powdered sample is sieved to 100 or 200 mesh and the powder is stored in glass jars (previously baked at 400°C) with tin foil in their lids. For Soxhlet extraction, a weighed amount of powder is placed in a glass-fiber Soxhlet thimble which was previously baked at 450°C for 12 hours. The powder is extracted for 10 or 21 days using 1000 ml of dichloromethane. Boiling chips, and copper strips activated with hydrochloric acid, are placed in the round-bottom flask so that the solvent will boil smoothly and any free sulfur will be removed from the extract. If the volume of dichloromethane in the round bottom flask decreases, new dichloromethane is added to the system. We have found that wrapping all fritted-glass joints in the Soxhlet extractor with Teflon tape minimizes solvent loss during the extraction. This loss minimization, in turn, minimizes the need to add solvent during the long (10-21 days) extraction times. We have documented that the more solvent added to the system during extraction, the greater the introduction of low-boiling contaminants to the extract during the extraction process.

On completion of the extraction, the solvent and extract in the round-bottom flask are filtered with an all-glass filtering apparatus using GF/A silica-fiber paper over a fritted-glass filter. The filtered solution is caught in another round-bottom flask and from there is transferred to a 600 to 1000 ml heavy-walled beaker, for passive evaporation. All solvent transfers are quantitative. The filtering apparatus and the bank of 12 Soxhlet extraction units are dedicated only for extracts from crystalline rocks. Sedimentary rocks are extracted in another bank of Soxhlet extractors and processed in separate apparatus. Soxhlet

extractors and processing apparatus dedicated solely for use with rocks with very low concentrations of organic compounds are an absolute requirement to minimize contamination. All glassware that comes in contact with solvent and extract is baked at 400°-450°C for 12-24 hours and rinsed with dichloromethane just before use.

The filtered extract is passively evaporated (Price and Wenger, 1992) to 15-20 ml by leaving the beaker in a fume hood with the sash up and the fan off. When the solvent level reaches around 50 ml, the beakers are tilted on the vertical axis by putting props under the beakers, thus concentrating the solvent and extract to one side of the beaker. This prevents excessive loss of C_{12}- compounds from the extract during the last stages of solvent evaporation. When the solvent level reaches 20 ml, the solvent and extract are quantitatively transferred, with transfer pipettes, to an 8 dram vial. The solvent is then further passively-evaporated to 1 ml by leaving the 8 dram vial open in the fume hood (sash up, fan off). The dichloromethane is then replaced by hexane by adding 2 ml of hexane and passively evaporating the extract and solvent to 1 ml. This process is repeated thrice, causing the precipitation of small amounts of material insoluble in hexane (not asphaltenes in the usual petroleum-geochemical meaning). This material is filtered from the hexane using a small metal-filter assembly dedicated only for the crystalline rocks. The precipitate is mobilized from the filter with CH_2Cl_2 and caught in a tared vial, taken to dryness, and weighed. The previously-filtered bitumen is passively evaporated to 0.5 ml, and is thus ready for fraction separation by column chromatography.

The extract is separated by alumina/silica-gel column chromatography into three fractions; saturated hydrocarbons (HCS), aromatic HCS, and "resins". Each of the three fractions is split equally into two parts using 10 ml volumetric flasks and 5 ml pipettes. One 5 ml spilt is used for weighing the C_{12}+ organic materials in each of the three dried fractions to determine the concentrations of extracted organic materials. The other part is used for qualitative analysis of the C_8+ organic materials by flame ionization detection (FID) gas chromatography and/or mass spectrometry. The splits of each fraction are passively condensed to 500 µl, and 300 µl of the condensed 500 µl is subsequently evaporated to a volume dependent on the amount of organic material contained in the sample. The 300 µl split can be evaporated to as low as 5 µl when a rock sample has very low concentrations of organic materials.

The analytical conditions of the flame-ionization-detection gas chromatograph are as follows: One µl of sample is injected into the gas chromatograph inlet by the splitless method. The temperature of the inlet is 340°C. The column is a 60 m x 0.32 mm capillary column (Phenomex Zebron, ZB -1) coated with a 0.25 µm layer of 100% methyl polysiloxane. The flow rate of the helium carrier gas is 2.2 ml/min. The oven temperature-program is 50° to 330°C at 4.5°C/min., followed by 15 min. at 330°C. The detector temperature is at 350°C. Flow rates of H_2, air, and make up gas (He) at the FID are 40, 380, and 28.5 ml/min, respectively.

4.0 CONTAMINATION CHECKS

4.1 SOLVENTS

We again note that use of brand or trade names in this report is for descriptive purposes only and does not imply either endorsement or non-endorsement by the U.S. Geological Survey.

The purpose of the solvent checks was to determine: 1) the concentrations and compositions (if possible) of the different contaminants in the various solvents employed in our method; 2) the various

maximum concentrations which these contaminants are increased to, due to the various evaporative steps in our method; and 3) which solvents can be successively distilled for use in our studies of organic materials in various crystalline rocks. At the outset, we note that the purity of the different solvents we discuss herein is usually high enough for normal analytical procedures with extracts from sedimentary rocks. However, due to both the large amount of solvent we use (1000 to 3000 ml depending on the sample and the extraction times), and the low volumes to which one-half of the extract can be reduced for gas chromatography (500 to 1500 ml can be reduced as little as 20 µl for a 75,000 fold decrease in solvent volume in some cases), the differences in low-boiling contaminants between the various solvents becomes paramount.

In the following discussions, we have not measured, or even estimated, absolute concentrations of the different contaminants we found in our study, nor have we attempted to qualitatively identify most of the contaminant compounds. Both of these tasks would require substantial effort and would not contribute significantly to the paper. In this study, discussion of the relative concentrations of contaminant peaks among the different samples we have analyzed adequately serves our purposes. However, so that the reader may have some rough estimates of concentrations, we include the following: We normally perform gas chromatographic analyses of saturated-HC fractions at solvent concentrations of 8 mg/ml of C_{14}+ material, or about 8,000 ppm (see Figure 1g for an example). The resulting chromatograms of such analyses typically have full-scale detector responses of 250 to 550 mV, in the C_{14}+ range, depending on the sample characteristics, the amount of C_{14}- material in the sample, and other variables. In contrast, the detection limit for flame ionization detectors is generally about 0.05 ppm. Thus, the smallest peaks at our most sensitive detector settings, 40-60 mV, would represent that concentration.

4.11 Dichloromethane

In our study, on average, we use approximately 1200 ml of dichloromethane as the extraction solvent. In our procedure, one-half portion of the extract can be condensed to 20 µl when the concentration of organic materials is very low in the original sample. This process magnifies the concentration of any contaminants in the dichloromethane 600 ml/.020 ml, or 30,000 times. In our check of dichloromethane contamination, we used 1200 ml of dichloromethane put in a beaker and passively evaporated to 1 ml. A 200 µl sample was taken at each volume of 1200, 600, 200, 50, and 10 ml, and a 300 µl sample was taken at a CH_2Cl_2 volume of 1 ml. All these aliquots were taken by syringe and placed in a 300 µl cone vial used for our automatic sampler on the gas chromatograph. These six dichloromethanes were condensed to 20 µl and analyzed by FID gas chromatography. The levels of volume reduction of these six dichloromethanes are shown in Table 1.

We analyzed seven different dichloromethanes: D143-4 (HPLC by Fisher Scientific), D150-4 (HPLC by Fisher Scientific), D151-4 (OPTIMA by Fisher Scientific), D154-4 (GC Resolve by Fisher Scientific), once-distilled D143-4, once-distilled D-150-4 and once-distilled D154-4. Figure 1 shows all the gas chromatograms obtained from the different stages of evaporation of the D143-4 dichloromethane. A gas chromatogram of the saturated HCS from a typical oil is also included in Figure 1 (Fig. 1g) so that the reader may make some judgment of the approximate carbon number of the various contaminants discussed in this paper. Vita for the oil are in Price and LeFever (1994, their Table 1). Figure 2 shows gas chromatograms for the maximum volume reduction of the D143-4, D150-4, D151-4, D154-4, once-distilled D143-4, once-distilled D150-4, and once-distilled D154-4 dichloromethanes. The high scales of Figure 1 and Figure 2 are adjusted at 1000 and 100 mV, respectively.

Table 1. Levels of volume reduction of the different solvents used in our analytical procedure.

Solvent	Volume* (ml)	Concentrated rate (times)
Dichloromethane	1200	1.0 x 10
	600	2.0 x 10
	200	6.0 x 10
	50	2.4×10^2
	10	1.2×10^3
	1	1.8×10^4
Hexane	30	1.0 x 10
	15	2.0 x 10
	5	6.0 x 10
	1	4.5×10^2
Benzene	30	1.0 x 10
	15	2.0 x 10
	5	6.0 x 10
	1	4.5×10^2

*Volume at which a sample is taken for analysis.

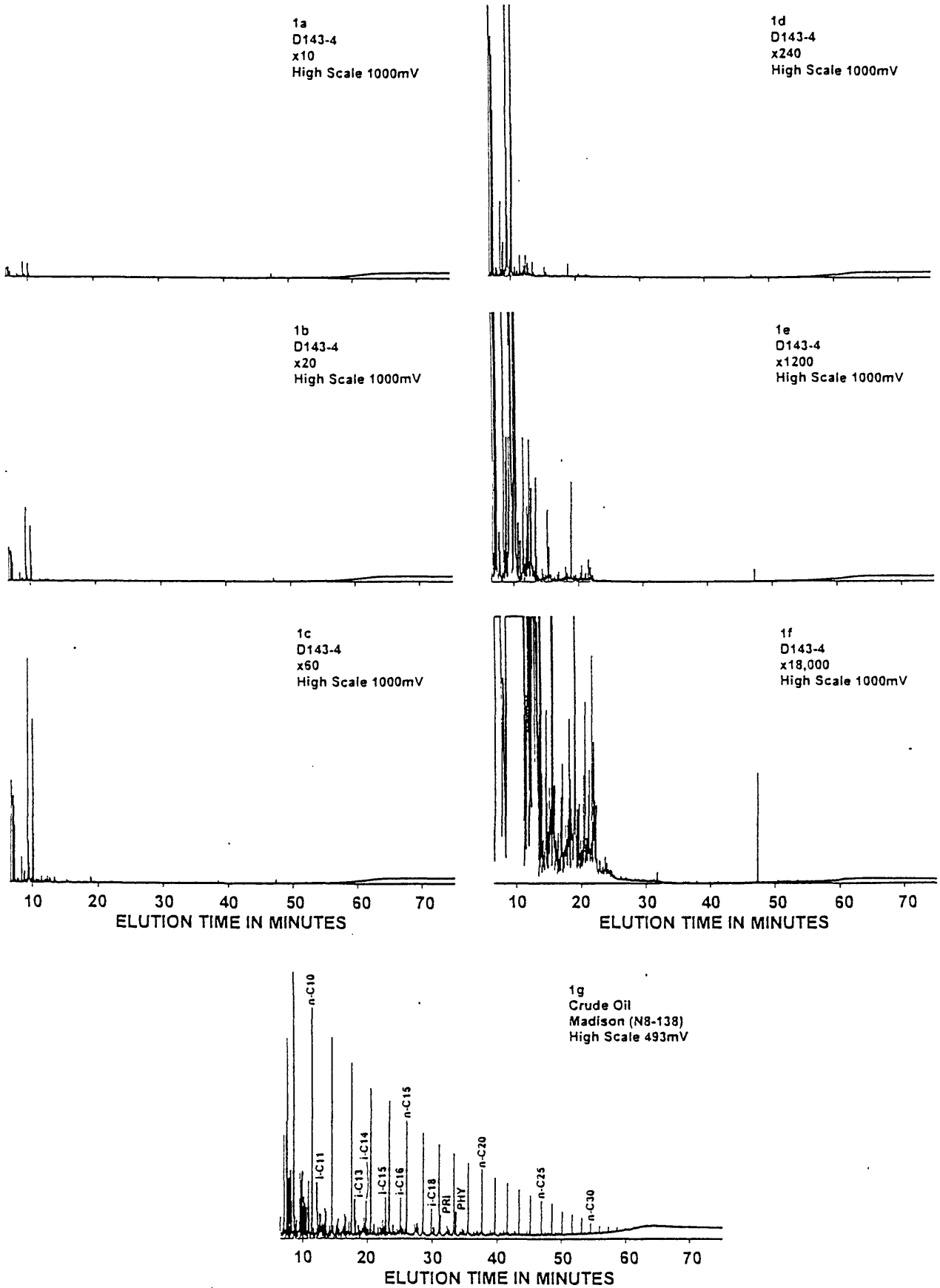


Figure 1. Gas chromatograms at an attenuation of 1000 mV demonstrating contamination in Fisher Scientific D143-4 dichloromethane progressively being concentrated by increasing solvent evaporation (1a to 1f). A gas chromatogram (1g) of the saturated HCS from a Williston Basin mid Madison crude oil is shown for calibration of carbon-number versus elution time.

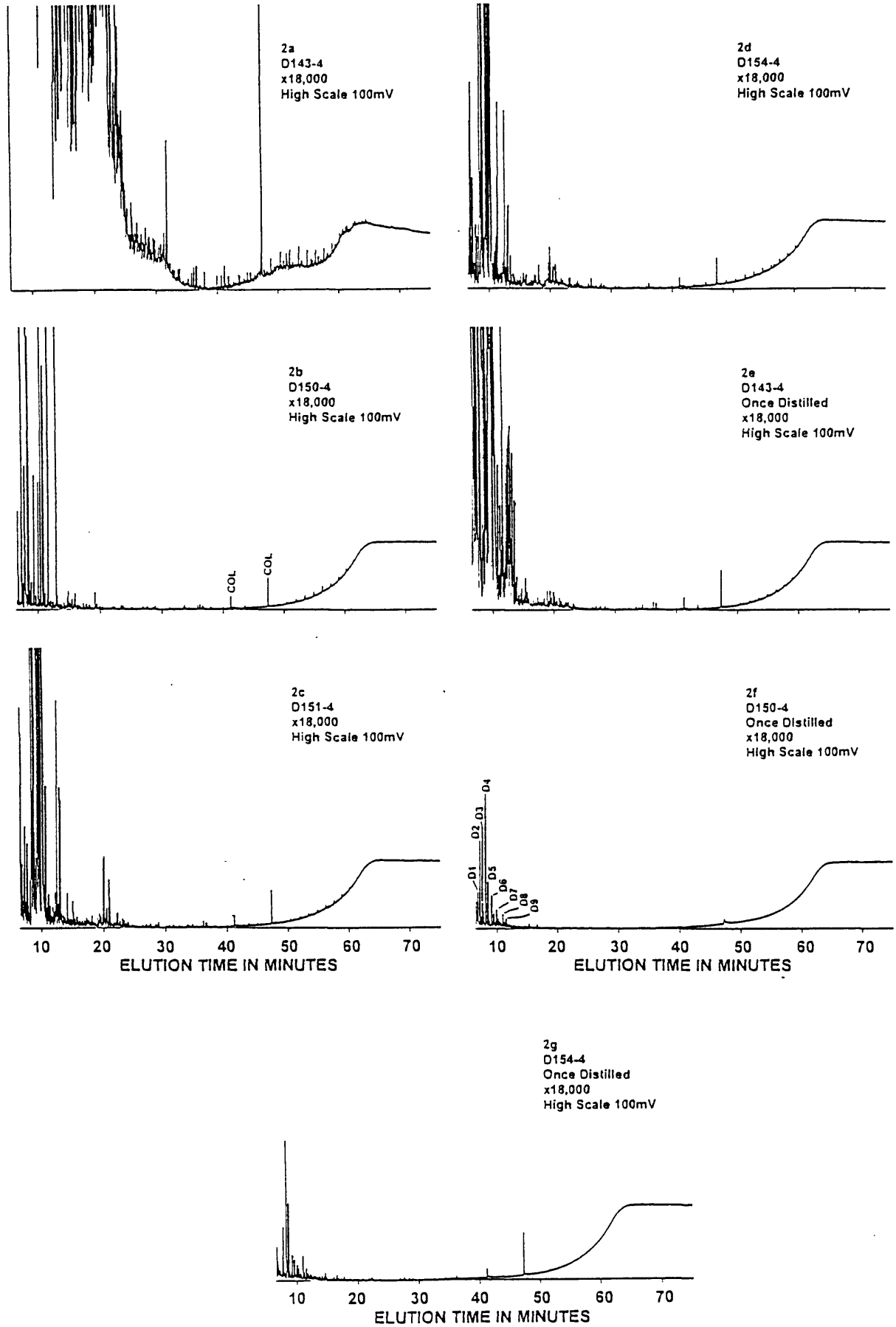


Figure 2. Gas chromatograms at an attenuation of 100 mV of four different dichloromethanes at our maximum level of evaporation (x 18,000; 2a to 2d) and of single distillations of three of the dichloromethanes (2c to 2g) at our maximum level of evaporation. Chromatogram labeling of 2f explained in text.

Many peaks appear on the gas chromatograms from the D143-4 solvent (Fig. 1), especially on the gas chromatograms of the highest levels of volume reduction (x 1200, Fig. 1e; and x 18,000, Fig. 1f). The peak distributions of all the chromatograms in Figure 1 are similar to each other, with the peak height increasing with increasing levels of volume reduction of the dichloromethane. This observation demonstrates that the organic materials resulting in these peaks are inherent in the dichloromethane (D143-4). The principal contaminant peaks in the D143-4 dichloromethane appear between 7 and 10 minutes retention time (Fig. 1). However, at the highest levels of volume reduction (x 1200, and x 18,000), high concentrations of contaminant peaks are present at retention times as high as 22.5 minutes, which corresponds to an elution time between the n-C_{13} and n-C_{14} paraffins. Clearly these contaminant levels are unacceptable for carrying out organic-geochemical analyses of samples with only very low concentrations or organic compounds, eg - the types of samples involved in our studies. In Figure 2, the other three dichloromethanes (D150-4, D151-4, and D154-4) had gas chromatograms similar to those of Figure 1, as the level of solvent-volume reduction increased. However, the series of chromatograms for those three dichloromethanes need not be shown here.

The gas chromatogram of D150-4 is markedly different from that of D143-4 both in peak pattern (Figs. 1d and 2b) and in the level of contaminant concentration (Figs. 2a and 2b). For example, D150-4 contains eight large peaks which appear over 7 to 13 minutes (Fig. 2b). In contrast, D143-4 contains a different complex of contaminant peaks eluting between 7.5 to 10 minutes (Fig. 1d). Please note that the level of solvent-volume reduction is markedly different between Figure 2b (x 18,000) and 1d (x 240). Thus, the concentration of contaminant peaks in D150-4 is very low compared to that of D143-4 (Figs. 2a and 2b). Therefore, where the two different dichloromethanes have had the same level of volume reduction (x 18,000), and are compared at the same full-scale peak height (eg, 100 mv), the contaminants in the D143-4 sample are off scale from 7.5 to 22.5 minutes. On the other hand, the D150-4 sample, has five peaks slightly off scale, and clearly has orders of magnitude less contaminant peaks than the D143-4 dichloromethane. This large difference in contaminant concentrations is at least partly due to stabilizers (amylene and cyclohexane) which are added to D143-4, but not to D150-4. Gas chromatograms of the D151-4 and D154-4 dichloromethanes with maximal reduced solvent volumes (x 18,000) are similar to each other (Figs. 2c and 2d), with contaminant concentrations intermediate to those of the D143-4 and D150-4 dichloromethanes.

Note that the two peaks labeled "COL" (for column) in Figure 2b at 41.2 and 47.3 minutes are contaminant peaks from the gas chromatograph column. These peaks occur sporadically, with variable peak heights, in most of our contamination checks and samples. The gas chromatograph which we used in this study is a multi-use instrument, used by different personnel in the USGS petroleum geochemistry group. Our study was performed over an 11 month time period, during which many other analyses of samples, other than our own, were performed on the gas chromatograph by other researchers. The two peaks under discussion likely were introduced onto the column by these other samples, and the peaks would then later appear in our analyses. Injection of blank benzene, benzene-methanol, or dichloromethane into the gas chromatograph would often cause the elution of these two peaks, which would decrease in peak height with successive blank injections. The presence of these peaks in our gas chromatograms will not be further discussed.

To increase the purity of all the solvents used in our procedure, we distilled all solvents at least once in moderately-large (4,000 ml) round bottoms in our laboratory. Figures 2e to 2g show the results of these distillations for the D143-4, D150-4, and D154-4 dichloromethanes, respectively. In all three cases, solvent volumes have been maximally reduced (x 18,000), and all 3 chromatograms are at 100 mv full

scale. The degree of contamination in the once-distilled D143-4 significantly decreases, compared to original D143-4 (compare Fig. 2a with Fig. 2e). However, the amount of contamination in the once-distilled D143-4 is still far too large for our purposes. Note that peaks with the higher retention times (15 minutes and greater) appear to have been preferentially reduced compared to the peak heights of the lower-boiling contaminants. This is, of course, expected, because organic materials with higher boiling temperatures are more easily removed than ones with lower boiling temperatures by distillation. The contaminant concentrations in the once-distilled D150-4 (Fig. 2f), and D154-4 (Fig. 2g) dichloromethanes become much lower compared to the original contaminant concentration levels (Figs. 2a to 2d).

Moreover, the gas chromatograms of the distilled dichloromethanes have much different peak patterns compared to the contaminant peak distributions in the original dichloromethanes. Furthermore, the maximum in the peak distribution is shifted from 10 to 7 minutes by distillation. The once-distilled D154-4 and D143-4 dichloromethanes contain slightly higher concentrations of contaminants with higher-boiling temperatures (elution times from 13 to 25 minutes) than does the once-distilled D150-4. We deemed the once-distilled D150-4 dichloromethane to be sufficient for our study. In a later section, we will discuss the peaks labeled D1 to D9 in the D150-4 gas chromatogram of the dichloromethane D150-4 (Fig. 2f).

4.12 Hexane

In our overall procedure, hexane is used for replacing dichloromethane in our extracts, for making silica-gel/alumina columns for fraction-separation column chromatography, and as the solvent to elute the saturated HCS during column chromatography. In all these steps, a total of 30 ml of hexane is employed. Because half of the 30 ml is condensed to 500 µl and is subsequently concentrated 15 times for samples with very low concentrations of extractable organic compounds, the maximum concentration of contaminant in hexane would be from a solvent volume reduction of 450 times. In this phase of our contamination check study, hexane was thus reduced in volume by 450 fold.

Thirty ml of hexane was thus put in a beaker and passively evaporated to 1 ml. Two hundred µl of a hexane aliquot were taken for a sample at a hexane volume of 30, 15, and 5 ml, and a 300 µl sample was taken at 1 ml hexane volume. All four samples were put into cone vials and condensed to 20 µl for analysis by FID gas chromatography. The concentrated rates of hexanes are listed in Table 1. We so evaluated six different hexanes; H302-4 (HPLC by Fisher Scientific), H303-4 (OPTIMA by Fisher Scientific), H334-4 (Spectranalyzed by Fisher Scientific), H217 (Non-Spectro by Burdick & Jackson), once-distilled H303-4, and twice-distilled H303-4. We show gas chromatograms from the H302-4 hexane at all four levels of solvent concentration (x 10, x 20, x 60, and x 450) in Figure 3 and gas chromatograms from the other hexanes at the solvent volume reduction level of 450 times in Figure 4. The high scales of Figure 3 and Figure 4 are adjusted at 1000 and 100 mV, respectively.

There are numerous large peaks on the H302 gas chromatograms with the greatest level of solvent reduction (Fig. 3d, x 450) with retention times between 6.5 and 22 minutes. The other hexanes that we examined, H217, H303-4, and H334-4, also contained high concentrations of contaminant peaks. However, the gas chromatograms from these hexanes for the four differentlevels of solvent reduction will not be shown here. The contaminant peak distribution of the H303-4 (Fig. 4c) and H334-4 (Fig. 4d) gas chromatograms, is remarkably similar to that of the H302-4 (Fig. 3d) gas chromatogram. However, this is not surprising, since these are all Fisher Scientific products which most probably are manufactured from the same starting product. However even the H217 gas chromatogram has a peak distribution (Fig. 4b)

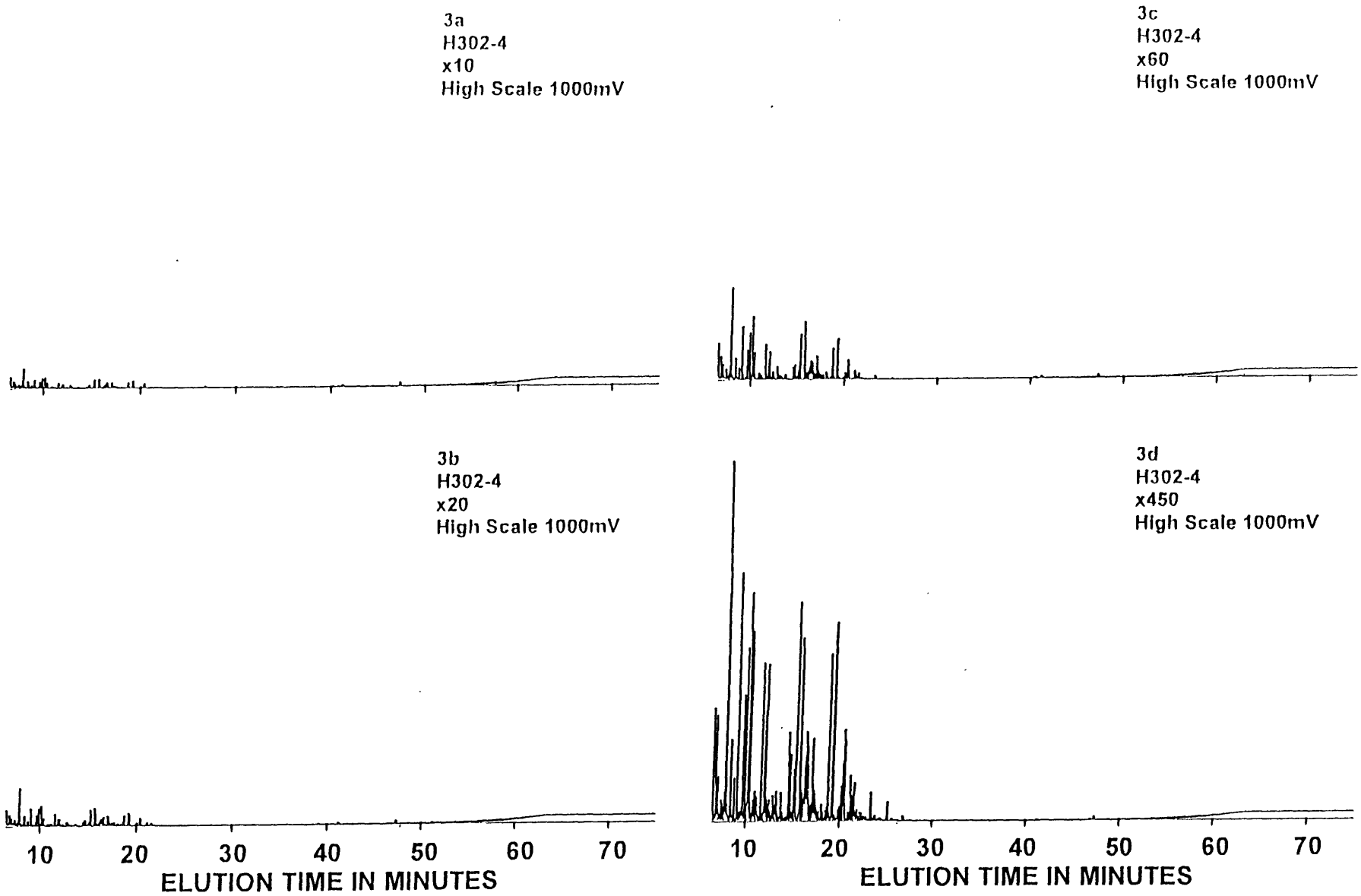


Figure 3. Gas chromatograms at an attenuation of 1000 mV demonstrating contamination in Fisher Scientific H302-4 hexane progressively being concentrated by increasing solvent evaporation.

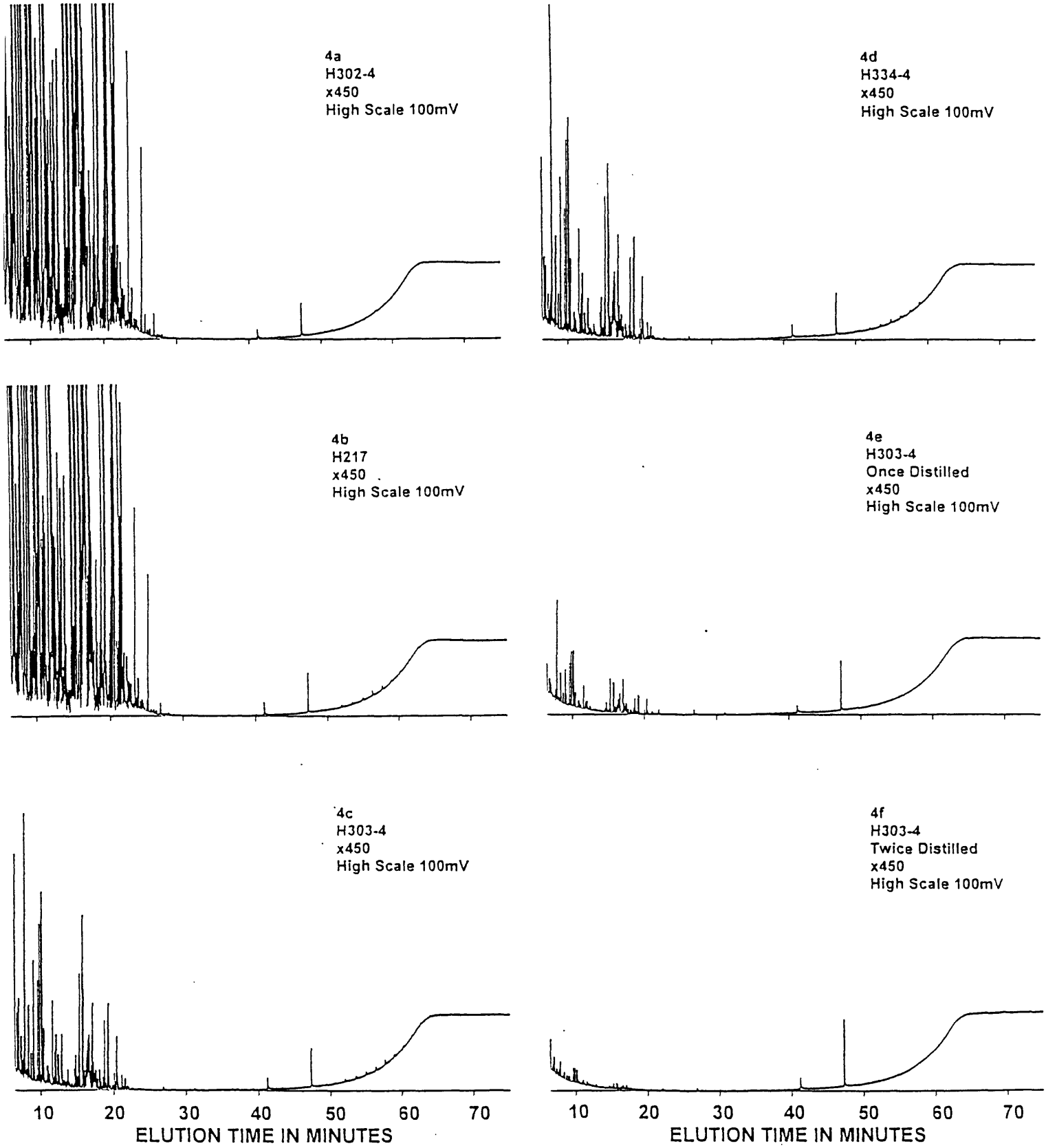


Figure 4. Gas chromatograms at an attenuation of 100 mV of four different hexanes at our maximum level of evaporation (x 450; 4a to 4d); and of once-distilled (4e) and twice distilled (4f) H303-4 hexane, also at our maximum level of evaporation.

somewhat similar to that of the other three hexanes (Figs. 4c, 4d, and 3d), even though the H217 hexane is made by a different manufacturer (Burdick & Jackson). This observation is probably due to the fact that all high-purity hexane solvents are originally derivatives from crude oil, all (or most) of which have the same C_6 to C_{10} isomers in them. Hence, we might expect to see the similar peak distributions that we do see, for these solvent hexanes derived from crude oils.

The original contaminant concentrations in the hexanes decrease in the order of H217=H302-4>>H334-4>H303-4 (Fig. 4). Note that the level of contaminants is much higher in H217 and H302-4 than in the other two hexanes (H334-4, and H303-4), which have about the same starting original contaminant concentrations. Of the solvent hexanes we examined, twice-distilled H303-4 (twice-distilled in our laboratory) proved to provide an adequate solvent for our research. The contaminant concentration in the H303-4 hexane dramatically decreased upon a single distillation (compare Fig. 4e (once-distilled) to original H303-4 hexane, Fig. 4c). However, contaminant peaks still remained too high for our purposes. A second distillation (Fig. 4f) yielded a product appropriate for our needs. We note that the H303-4 hexane, is actually a mixture of hexane isomers, and is not just n-C_6 (normal hexane). However, that H303-4 was an isomer mixture, rather than the single n-C_6 isomer, did not detract from its purity as a solvent.

4.13 Benzene

A total of 30 ml of benzene is used with the column chromatography in our procedure. Because half of the 30 ml of benzene is condensed to 500 µl and is subsequently concentrated 15 times for samples with the low concentrations of organic materials, the maximum concentration of contaminants in benzene would be 450 times. Thus, in our examination of contaminants in different benzenes, we decreased solvent volumes by 450 fold.

Thirty ml of benzene were put in a beaker and passively evaporated to 1 ml. Two hundred µl of benzene were withdrawn by syringe at benzene volumes of 30, 15, and 5 ml and, 300 µl were withdrawn at 1 ml and put into our 300 µL auto-sampler cone vials. These benzenes were condensed to 20 µl and were analyzed by FID gas chromatography. The concentrated rates of benzene are listed in Table 1. We checked eight different benzenes: B411-4 (Spectranalyzed by Fisher Scientific); JT9154-5 (BAKER ANALYZED Reagent by J. T. Baker); JT9256-3 (BAKER ULTRA RESI-ANALYZED by J. T. Baker); 33291 (Spectrophotometric Grade by Alfa Aesar); 27070 (HPLC grade by Sigma-Aldrich); EM-BX0212-1 (OMNISOLV grade by EM Science); once-distilled EM-BX0212-1; and twice-distilled EM-BX0212-1. We show gas chromatograms for the B411-4 benzene at the different levels of solvent concentration (Fig. 5) and gas chromatograms from all the other benzenes at the concentrated level of 450 times (Fig. 6). The high scales of Figure 5 and Figure 6 are adjusted at 1000 and 100 mV, respectively.

Numerous peaks appear between 7 and 12 minutes on the gas chromatogram of the B411-4 benzene at the 450 times level of solvent concentration (Fig. 5d). These peaks, and also other peaks from 12 to 25 minutes retention time, become much more prominent at the 100 mV response level (Fig. 6f). The lack of change in the peak patterns at the different levels of solvent concentration demonstrates that these peaks are contaminant compounds in the B411-4 benzene. The peak patterns and contaminant concentration levels are markedly different among the various benzenes (Figs. 6a to 6f), with some benzenes having very high concentrations of contaminant peaks. Moreover, the molecular-weight distribution of the contaminants also varies greatly between the different benzenes. For example, JT9154-5 (Fig. 6a) has significant levels of contaminant peaks over the entire molecular-weight range. On the other hand, the EM-BX0212-1 benzene has a more moderate concentration of lower-molecular weight material

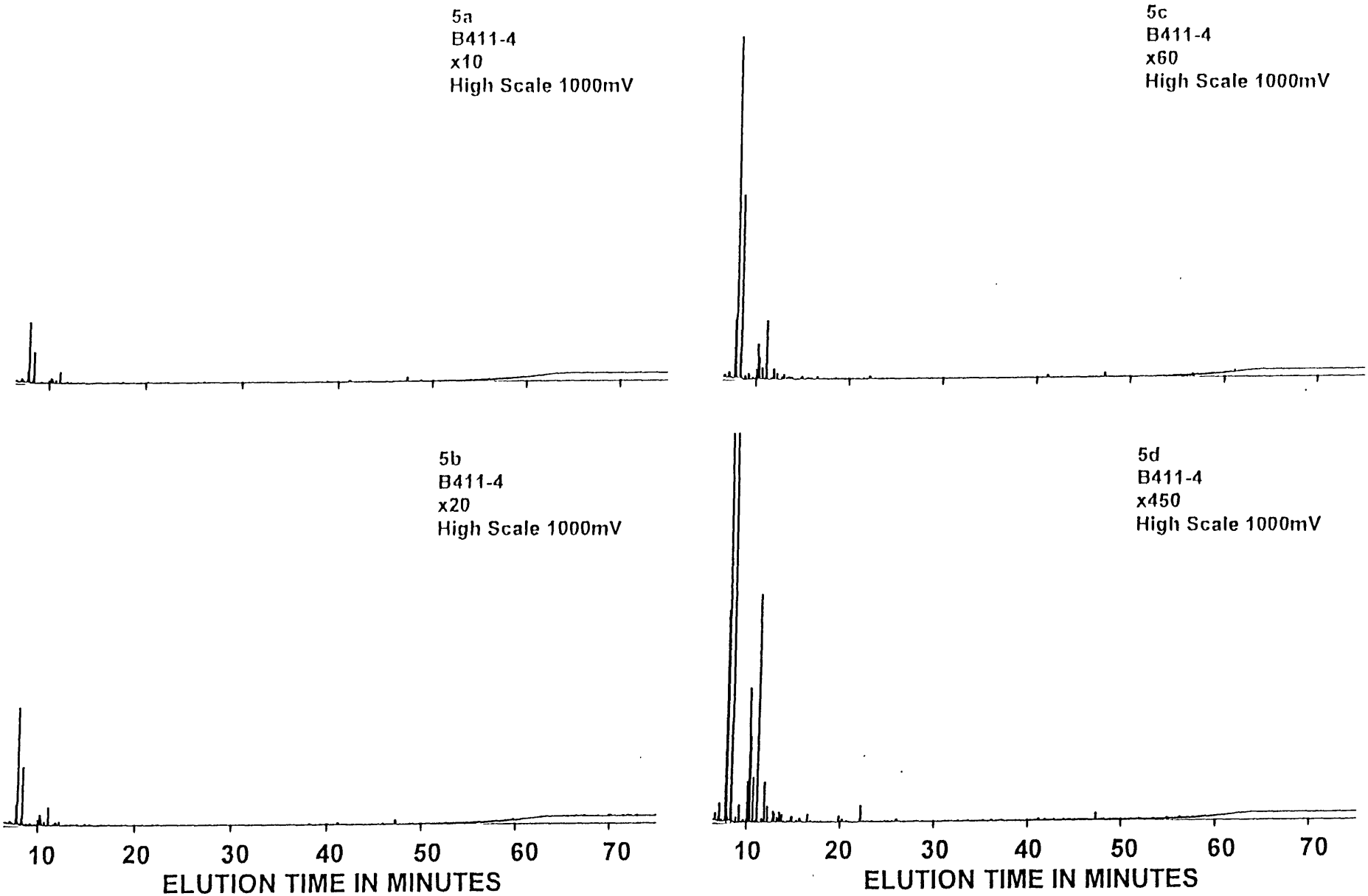


Figure 5. Gas chromatograms at an attenuation of 1000 mV demonstrating contamination in Fisher Scientific (B411-4) benzene being progressively concentrated by increasing solvent evaporation.

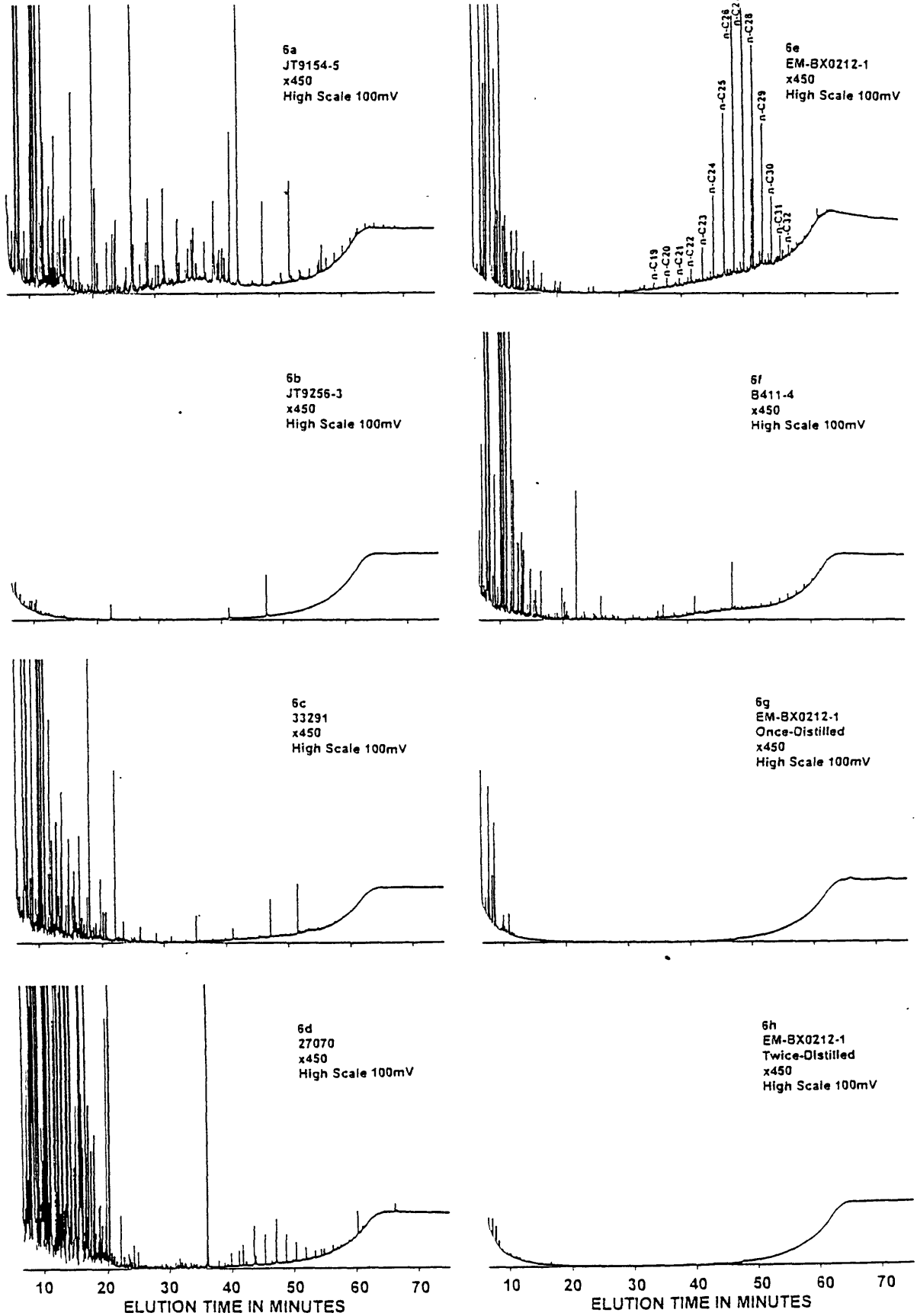


Figure 6. Gas chromatograms at an attenuation of 100 mV of six different benzenes at our maximum level of evaporation (x 450, 6a to 6f); and of once-distilled (6g) and twice-distilled (6h) EM-BX0212-1 benzene, also at our maximum level of evaporation.

(Fig. 6e). Moreover, the concentration of this lower-molecular weight material strongly decreases with increasing molecular weight, largely disappearing at a retention time of 20 minutes. Concurrently, the EM-BX0212-1 benzene also has high concentrations of what appear to be C_{23} to C_{31} n-paraffins centered around 50 minutes retention time (Fig. 6e). These C_{23} to C_{31} n-paraffins were so identified by retention time comparison with the saturated-HC gas chromatogram of Figure 1g. These n-paraffins most probably have resulted from wax contamination. The concentration of contaminants in the different benzenes decreases in the order of 27070 > JT9154-4 > 33291 > B411-4 > EM-BX0212-1 > JT9256-3. JT9256-3 is clearly the highest quality benzene and would be sufficient for our study. In fact, the JT9256-3 benzene was in use in our laboratory, before this large contamination-check study was carried out, for the analysis of the samples reported in Price et al. (1998a and b). However, after using almost all our remaining store of this benzene, we attempted to order more, only to find that the J. T. Baker Company had discontinued retailing any benzene products. Thus, we attempted to purify the EM-BX0212 benzene by distillation. However, the once-distilled EM-BX0212-1 benzene still had concentrations of contaminant peaks too high to be ignored (Fig. 6g), although the concentration of these contaminant peaks significantly decreased from that of the original EM-BX0212-1 benzene (Fig. 6e). Twice-distilled EM-BX0212-1 benzene (Fig. 6h) has even lower concentrations of contaminants than the JT9256-3 benzene (Fig. 6b). Thus, twice-distilled EM-BX0212-1 benzene clearly is adequate to be used as a solvent in our research program.

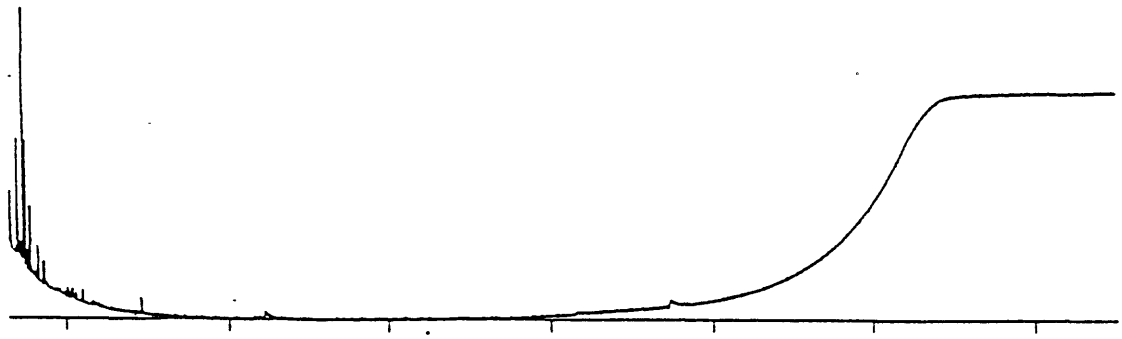
4.14 Benzene/Methanol

Five ml of a 1:1 benzene/methanol solution is used in the column-chromatography fraction-separation step of our procedure to elute the "resin" fraction of the extracted bitumen. Because only one half of the 5 ml is condensed to 500 µl and possibly subsequently concentrated 15 times for samples with low concentrations of indigenous organic compounds, the maximum contaminant concentration in the condensed benzene/methanol would be 75 times. Thus, in this step of our examination of solvent contamination, the benzene-methanol solution was concentrated 75 times and the contaminant concentrations were then analyzed.

Five ml of benzene/methanol was placed in a beaker and passively evaporated to 1 ml. Three hundred µl of this benzene/methanol solution was then evaporated to 20 µl and analyzed by FID gas chromatography. We checked two benzene/methanol solutions which were 1:1 mixtures of twice-distilled EM-BX0212-1 benzene and A452-4 (HPLC by Fisher Scientific) methanol or A454-4 (OPTIMA by Fisher Scientific) methanol. The two gas chromatograms from these mixtures, concentrated 75 times, are shown in Figure 7. The high scales of Figure 7 are adjusted to 100 mV.

There are several peaks before the 10 minute elution time on the gas chromatogram of the EM-BX0212-1 twiced-distilled benzene and A452-4 methanol 1:1 mixture (Fig. 7a); however, these peak heights are low. The A454-4 1:1 benzene/methanol mixture has an extremely low contaminant concentration (Fig. 7b), and thus this mixture is sufficiently pure to be used as an organic solvent in our research program.

7a
1:1 Benzene-Methanol
(A452-4 methanol)
x75
High Scale 100mV



7b
1:1 Benzene-Methanol
(A454-4 methanol)
x75
High Scale 100mV

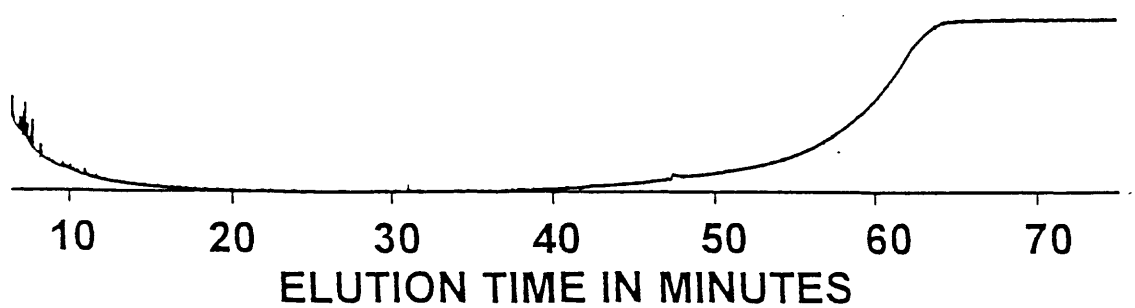


Figure 7. Gas chromatograms of 1:1 benzene-methanol mixtures using twice-distilled EM-BX0212-1 benzene and two different methanols, at our maximum level of evaporation (x75).

4.2 APPARATUS

4.21 Glassware

We bake most of our glassware (pipettes and collection vials for the column chromatography, vials and beakers used for evaporation, the thimble and flask of the Soxhlet-extraction assembly, etc.) at 450°C for 12 hours before use. Glassware which can not be baked because of size or fragility concerns (for example the Soxhlet extraction thimble holder and condenser) are thoroughly rinsed three times with dichloromethane (once-distilled D150-4).

The approach of baking glassware at 400°-450°C is commonly used in organic geochemistry, especially in the study of organic compounds thought to be indigenous in meteorites, to avoid organic contamination from laboratory procedures. Previous unpublished studies within our laboratory have demonstrated that this method completely eliminates organic contamination. A check carried out in our laboratory (discussed below) reconfirmed this conclusion.

4.22 Cone-Vial Cap

Samples ready for gas-chromatographic analysis are placed in 300 µl cone vials which are sealed with aluminum caps crimped over Teflon-coated silicone-based septa. Although Teflon and silicone-based septa are thought to be stable in exposure to most organic solvents, the possibility of contamination from the vial cap was nonetheless checked.

Three of the solvents we use (twice-distilled H303-4 hexane, twice-distilled EM-BX0212-1 benzene, and once-distilled D150-4 dichloromethane) were placed in three cone vials and capped. The capped vials sat sealed for two days and were then analyzed by FID gas chromatograpyhy (Fig. 8). The high scales of the three Figure 8 chromatograms are to 100 mV.

The three-Figure 8 gas chromatograms have no contaminant peaks except for a very small toluene peak (from the benzene solvent) in the Figure 8b benzene-leach chromatogram. Thus, the cone-vial septa can be used to seal our samples for analysis with no fear of contamination.

4.23 Rubber Bulb

We use disposable 1-2 ml transfer pipettes with rubber bulbs to transfer many of the liquids (organic solvents and extracts from samples) generated in our research study. Although we always keep those liquids from contacting the rubber bulbs, we cannot exclude a future possible accident in which solvents may contact the rubber bulbs. In such a case, we must know the characteristics of the materials dissolved from the rubber bulb, in order to differentiate those materials from indigenous organic compounds extracted from a rock sample. Thus, the rubber bulb was rinsed with dichloromethane (once distilled D150-4), which is the strongest and most polar solvent used in our research. After replacement of the dichloromethane in the bulb rinse with hexane (twice distilled H303-4), the replaced rinse was analyzed by FID gas chromatography. The resulting chromatogram is shown in Figure 9. Similar rinses of the transfer-pipette rubber bulbs were also carried out with our twice-distilled hexane and benzene. Similar results were obtained although the peak distributions were different depending on the solvent. Moreover, the amount of leached material decreased in the order: dichloromethane > benzene > hexane.

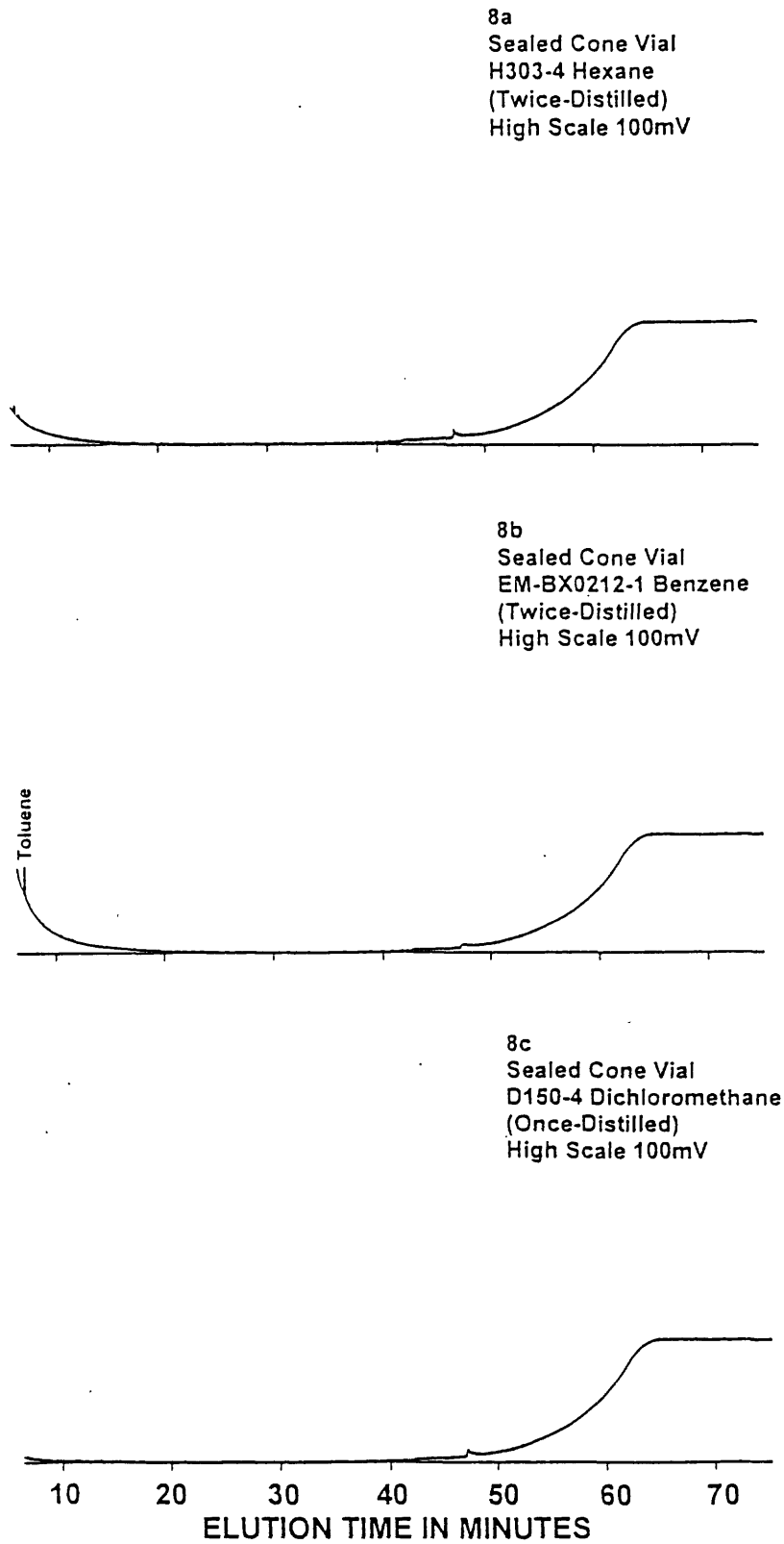


Figure 8. Gas chromatograms of three different solvents sealed in our auto sampler cone vials for two days with Teflon-backed septa.

The gas chromatogram from the rubber bulb rinse has seven principal peaks between 35 to 60 minutes retention time (Fig. 9) with some smaller peaks over 12 to 75 minutes retention time. If some solvent and/or sample had ever come in contact with the rubber bulb when transferring our solvent samples by pipette, then the transferred materials would contain organic compounds with peak patterns similar to that in Figure 9 (or similar to the hexane or benzene leaches). However, we never allowed solvent to contact the transfer-pipettes rubber bulb nor did we detect contamination from rubber bulbs in any of our samples.

4.24 Copper Strip

An activated copper strip, or as many as five strips in some cases, is/are used in the round- bottom flask of the Soxhlet extraction apparatus with the dichloromethane to remove free sulfur from the extract. (Free sulfur in the extract prevents correct measurement of the sample weight). Although one would suspect that organic contamination would be nonexistent, the strips were nonetheless checked as part of the overall contamination check.

Twenty-five new copper strips (15 x 75 mm) were activated by soaking them in hydrochloric acid (3N), immediately turning the color of the strips from copper to salmon-pink. The strips were then washed with tap water followed by methanol (A454-4), and then stored in a beaker with enough dichloromethane (once distilled D150-4) to cover the copper strips for 10 days. The dichloromethane was concentrated and analyzed by FID gas chromatography, the resulting gas chromatogram shown in Figure 10a. The copper strips were then reactivated in hydrochloric acid and treated again in the manner described above, except that they each were rinsed by dichloromethane before the dichloromethane soak. The second resulting gas chromatogram is shown in Figure 10b.

The extract obtained from 25 activated new copper strips contains high concentrations of normal alkanes ranging from C_{19} to C_{37}, with a maximum at C_{25} (Fig. 10a). On the other hand, the rinse from the original 25 copper strips after reactivation with hydrochloric acid following the original 10-day dichloromethane soak (and then soaked again in dichloromethane), does not contain detectable organic compounds (Fig. 10b). These two experiments demonstrate that although new copper strips are coated with wax, this wax is a surficial coating which can be rinsed off with organic solvents. High boiling waxy compounds are often found on the surfaces of new-rolled or machined metal products (1968 personal communication to Leigh Price from Robert Rex, then Professor of Geology, University of California at Riverside). Thus, rinsing the copper strips (whether new or used) before use prevents contamination from this source. These experiments also demonstrate that activated copper strips do not react with dichloromethane to produce organic contaminants. As long as we rinse these copper strips using dichloromethane before the use, we do not need to be concerned with possible contamination from activated copper strips.

4.25 Alumina / Silica-Gel Column

An alumina/silica-gel column is used in our procedure for fraction-separation chromatography. Alumina and silica gel may have absorbed organic materials during their manufacturing and/or packaging processes, because, after all, they are absorbents. It is thus important to determine the possible level of such contamination by performing a blank column separation in a manner which duplicates that used in our study of rock samples.

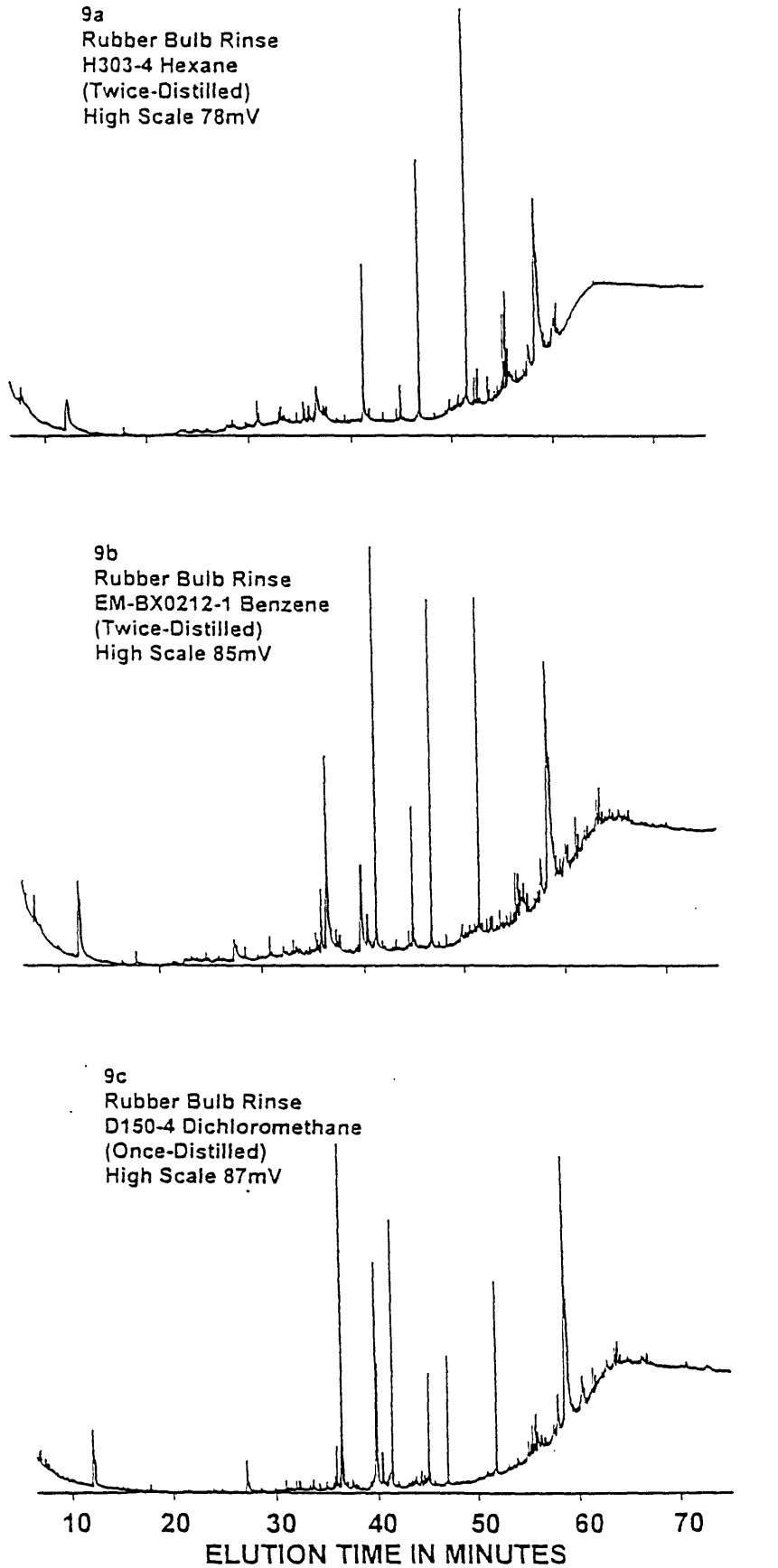


Figure 9. Gas chromatograms of rinses (using three different solvents) of the rubber transfer pipette bulbs used in our procedure.

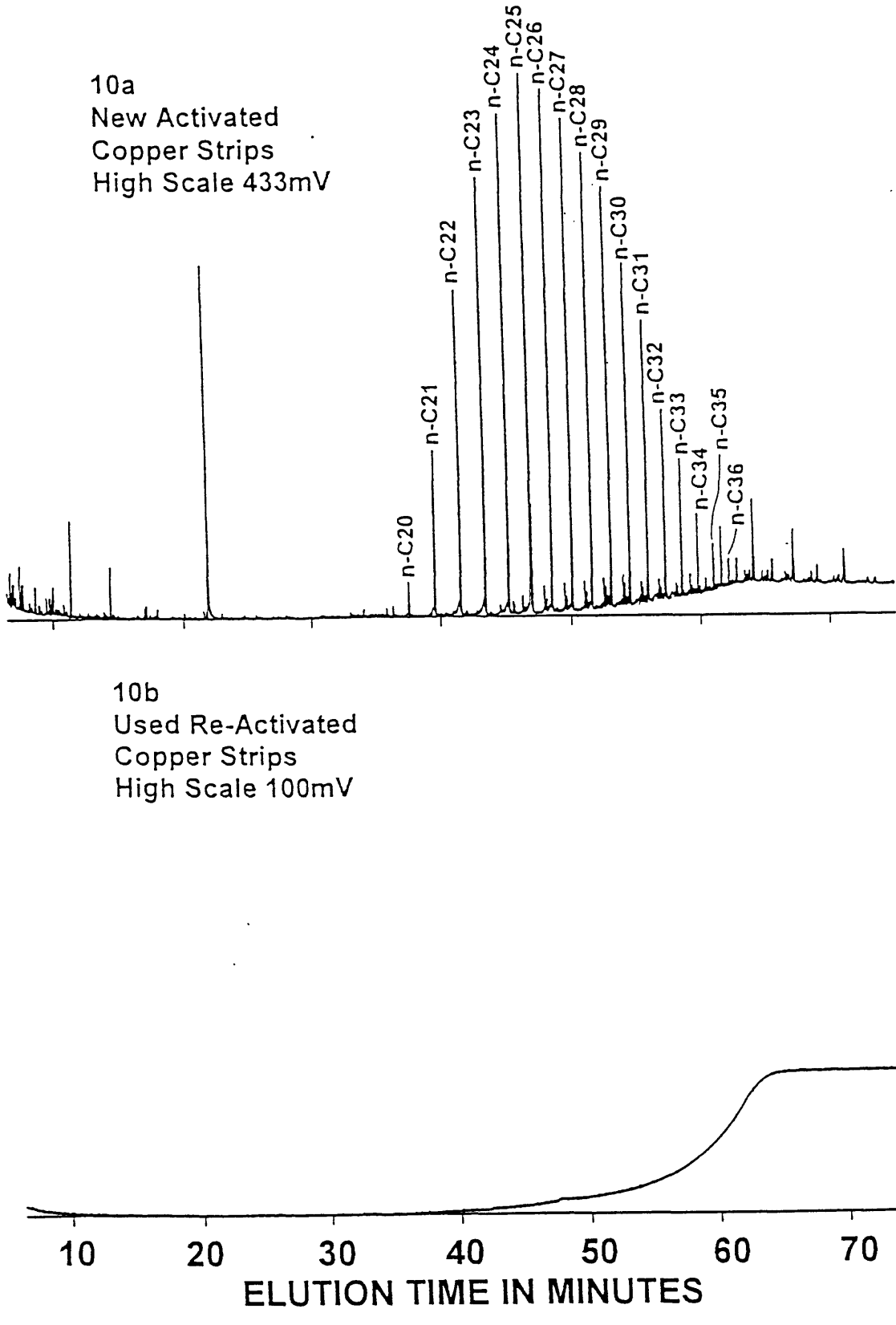


Figure 10. Gas chromatograms of a dichloromethane rinse of new activated copper strips (10a) and of used (previously rinsed) copper strips (10b).

The alumina/silica-gel column consists of fine silica gel (mesh size from 100 to 200, No. 923 by DAVISON CHEMICAL); coarse silica gel (mesh size from 60 to 100, No. 62 by DAVISON CHEMICAL); and aluminum oxide (BAKER ANALYZED reagent by J. T. Baker). Our first experiments with blank columns revealed that significant concentrations of contaminant peaks could be present in our saturated- and aromatic-HC and resin fractions which were eluted from the blank columns; however, gas chromatograms of those analyses will not be shown here. Thus, the absorbents appeared to be badly contaminated. Soxhlet extraction of the absorbents revealed this to be the case, as the solvent in the round-bottom (boiling) flask of the Soxhlet apparatus was colored bright yellow after extraction of the absorbents. As such, we elected to clean the absorbents by baking them at 400°C for 24 hours. The baked absorbents were then put in a dessicator with water for 10 days, such that they were completely deactivated. The three absorbents were then reactivated by baking them at 240°C for 24 hours. After packing silica glass wool (baked at 400°C for 24 hours) and a small cut piece of a GF/A silica-fiber filter into a disposable serological pipette (5 ml), 3 ml of a 923 silica-gel slurry of hexane (twice-distilled H303-4) is added to the pipette, 2 ml of 62 silica-gel slurry to is added, followed by 1 ml of alumina slurry. Three fractions are obtained in the order of: saturated HCS, aromatic HCS, and lastly resins. The saturated-HC fraction is collected by eluting it with 15 ml of twice-distilled H303-4 hexane, followed by initiating the benzene elution (20 ml of twice-distilled EM-BX0212-1 benzene). When the aromatic-HC elution front is 1.5 ml from the bottom of the column, the collection vial for the saturated HCS is removed and replaced by the collection vial for the aromatic HCS. After the 20 ml of benzene pass through the column, 5 ml of 1:1 benzene-methanol (twice-distilled EM-BX0212-1 benzene; A454-4 methanol) is added to the column, to commence the elution of the resins. When the resin elution front is 1.5 ml from the bottom of the column, the aromatic-HC collection vial is removed and replaced by the resin collection vial.

We performed the fraction-separation procedure described above, but using a blank silica-gel/alumina column wherein the absorbents had previously been baked at 400°C for 24 hours, as described above. The three collected fractions (saturated HCS, aromatic HCS, and resins) were all condensed by passive evaporation to 66 μL, which is the maximum solvent concentration level for the three fractions in our study. The three fractions were then analyzed by FID gas chromatography (Fig. 11). To compare the Figure 11 chromatograms with those from a rock sample (discussed below), the high scales of all three chromatograms were adjusted to 100 mv.

The gas chromatogram of the saturated-HC fraction (Fig. 11a) separated by the alumina/silica-gel column has no peaks except for a few small peaks on the tail of the solvent peak. The aromatic-HC fraction from the column chromatography has several minor peaks ranging from 6.5 to 17 minutes of retention time (Fig. 11b). From retention times using aromatic HC standards (see Price et al, 1998; or Price and Le Fever, 1994), peak B1 is most likely toluene; peak B2 ethylbenzene; peak B3 para and meta xylene; peak B4 orthoxylene; peak B5 1,2,4- and, 1,3,4-trimethylbenzene. Peak B6 is unidentified.

Note that the B1 to B6 peaks of Figure 11b are the same peaks as in Figure 6h, albeit larger. This suggests that these minor amounts of contaminant peaks have originated from the solvent, and that our second benzene distillation in the case of the Figure 11b solvent, was less efficient than in the case of the Figure 6h solvent. We have since rectified this problem by decreasing the distillation rate for the second benzene distillation.

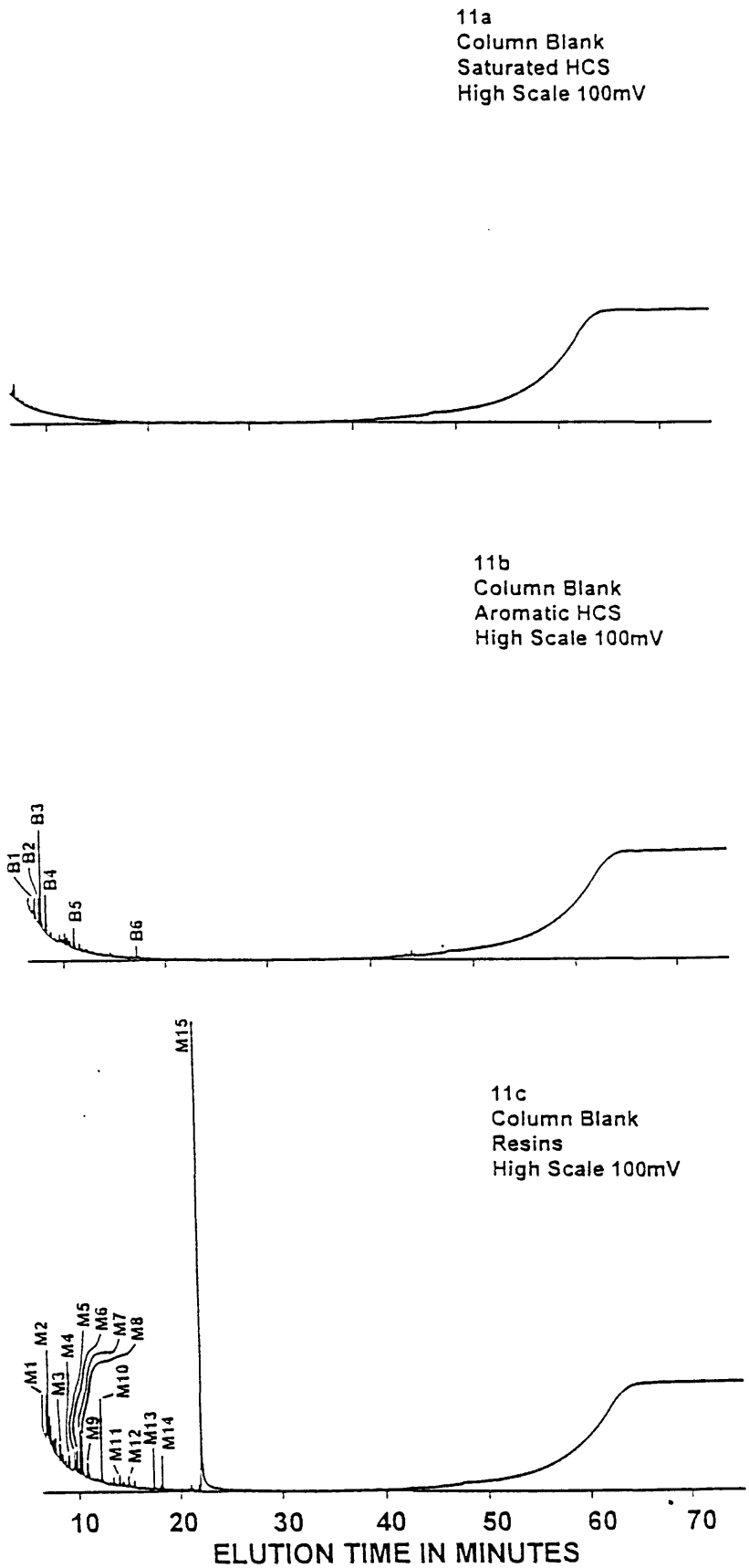


Figure 11. Gas chromatogram of the saturated HCS (11a), aromatic HCS (11b) and resins (11c) from a blank column elution. Chromatogram labeling explained in text.

The resin fraction eluted by the column chromatography has a prominent peak at a retention time of 22 minutes (peak "M15"), and a number of smaller peaks ranging from 7 to 18 minutes (Fig. 11c). The lowest-boiling compounds of Figure 11c (11 minutes and less) elute over the same retention times as the low-boiling peaks of Figure 7b. Thus, we attribute the peaks in 11c to less pure original methanol than that used in Figure 7b. (These two methanols had different manufacturer lot numbers). We have circumvented this problem by checking the purity of all new lots of methanol (and all other solvents we use). If the purity of new methanol is unsatisfactory, then we distill it once.

We now purchase all solvents, especially dichloromethane, in large quantities of single manufacturers lots, and analyze a sample of that lot for purity. If the solvent is found to be impure, the entire lot is returned and exchanged for another one. We have found that even with a single manufacturer's grade and type of solvent, there can be a substantial variation in original purity between different lots. To avoid solvent contamination contributions to our sample extracts, we have found that solvent purity must be constantly monitored.

The large peak in Figure 11c at 22 minutes retention time (peak M15) originates from the column chromatography procedure, because the peak never appears in any of the checks on solvent purity. However, in spite of repeated attempts along different lines, to isolate the cause for the origin of this peak, we were only able to identify its origin late in the study: from the 5 ml serological pipettes used for fraction separation by column chromatography. When these pipettes were baked at 450°C, the painted ml gradations on the pipettes flaked off. Since these gradations were necessary during several steps of both preparation of the columns and for the actual column chromatography, we elected to clean the columns by rinsing them with solvent rather than baking them. However, we used hexane as the rinse solvent, and hexane was not removing the contaminant responsible for the M15 peak from the inside of the serological pipettes. Late in the study, we began rinsing the serological pipettes with 1:1 benzene methanol, which did remove the contaminant causing the M15 peak in the resin gas chromatograms. Although we had been rinsing all other glassware, which was not baked at 450°, with dichloromethane, this experience drove home the necessity of rinsing unbaked glassware with a polar solvent.

Aside from the M15 peak in the resin fraction, none of the Figure 11 chromatograms have substantial contaminant peaks. The small low-boiling peaks that are present in Figures 11b and 11c have been further minimized by careful monitoring of original solvent purity and running our distillation units at lower distillation rates. Thus, for our research purposes, the fraction-separation column-chromatography procedure we use is more than adequate for our needs. We further discuss the labeled peaks B1 to B6 (Fig. 11b) and M1 to M15 (Fig. 11c) below.

4.26 Cardboard Storage Cartons

We normally store all our rock samples to be studied (either broken into large chunks or powdered to minus 100 or 200 mesh) into pre-baked glass jars with foil-lined lids. However, at one point early in our joint research project, we ran out of large glass jars and temporarily stored some crushed (but not powdered) samples in cylindrical white cardboard cartons ("Paper Cans" by FONDA). Gas chromatograms of these samples were substantially different than and obviously contaminated compared to those from other samples of the same set. The gas chromatograms in question contained a set of high-boiling waxes in the saturated HCS and other waxy appearing compounds in the aromatic HC and resin fractions. We isolated the source of contamination in these samples to a wax coating on the inside of cardboard cartons. Our early experience in this matter alerted us to the insidious nature of possible organic

contamination to our samples, in all steps of our analytical procedure, from sample collection to final gas chromatographic or mass spectrometric analysis. We here present the qualitative data from our "paper-carton experience" to illustrate possible problems which may arise in analyses of crystalline rocks which bear low quantities of indigenous organic compounds.

Once we noticed the contamination in the above-referenced samples, and isolated the source as possibly the wax coating on the FONDA cardboard storage cartons, we obtained an "extract" of these cartons by rinsing the inside of a carton with dichloromethane (once distilled D150-4). After the dichloromethane of the extract was replaced with hexane (twice distilled H303-4), the extract was separated into three fractions by alumina/silica-gel fraction-separation column chromatography. Gas chromatograms of the three fractions from FID gas chromatography are shown in Figure 12, along with gas chromatograms of the three fractions (saturated and aromatic HCS, and resins) from a badly carton-contaminated sample.

The gas chromatogram from the saturated-HC fraction of the carton rinse (Fig. 12a) contains n-C_{19} to n-C_{35} alkanes, with a maximum at the C_{24} n-paraffin. In other words, high concentrations of wax. The gas chromatogram of the aromatic HCS from the carton rinse (Fig. 12b) contains multiple families of periodic series of compounds: counting doublets, four different series with prominent peaks, and perhaps six more separate series with much smaller peak heights. We hypothesize that all these peaks in the aromatic HC fraction represent different homologous series of double-bond bearing aliphatic HCS, with the double bond, or double bonds, in different carbon positions for each series. However, no attempt was made to identify the compounds responsible for these different peaks. The gas chromatograms of the resin fraction from the carton rinse (Fig. 12c) contains perhaps three series of homologous periodic peaks, plus a large complex of peaks at 45 minutes retention time. The large peak at 22 minutes is the previously-identified contaminant peak (M-15, Fig. 11c) which originates from fraction-separation column chromatography. We have not tried to identify the compounds responsible for the different series of periodically-repeating peaks in Figure 12c. However, we suspect that they may be different series of aliphatic HCS with carboxylic acid or ester bonds, or alcohols, or aldehydes, all with or without double bonds. The ratio of the saturated and aromatic HCS, and resins obtained from the carton rinse by weight is 70:1:14, respectively. Thus, the principal components of the contamination from the carton are C_{21} to C_{30} n-paraffins.

Gas chromatograms from one of the most badly-contaminated samples (AS-607, a metamorphic rock from Sanbagawa, Japan) from the FONDA cartons are shown in Figure 12d to 12f, with the saturated HCS shown in Figure 12d at 100mV. To keep the largest peak in Figure 12d on of indigenous saturated HCS cannot be seen. Thus, the vertical scale of Figure 12d was substantially attenuated to 100mV. As is apparent, the low concentrations of indigenous saturated HCS in the AS-607 sample (Fig. 12d) are absolutely dwarfed by the wax contamination from the cardboard carton in which the crushed chips (~1/2", 1.27cm) of this rock were stored after crushing and before powdering. We know that the HCS marked indigenous in Figure 12d are just that, for two reasons: First, vertical expansion of the Figure 12a chromatogram, which is only the contaminating wax, to 100mV, does not reveal significant concentrations of n-C_{19}- paraffins. Second, analyses of Sanbagawa samples not contaminated by wax demonstrates saturated-HC distributions similar to those labeled indigenous in Figure 12d.

Figure 12e shows the wax-contaminated aromatic HCS extracted from the AS-607 sample. There is also a significant amount of contamination in the aromatic HCS; however, the relative level of contamination is much less than that of the saturated HCS (Fig. 12d). This is because, as stated above, the wax contamination is composed of 82.2% saturated HCS and only 1.2% aromatic HCS (with the resins making up the balance (16.6%) of the wax contamination).

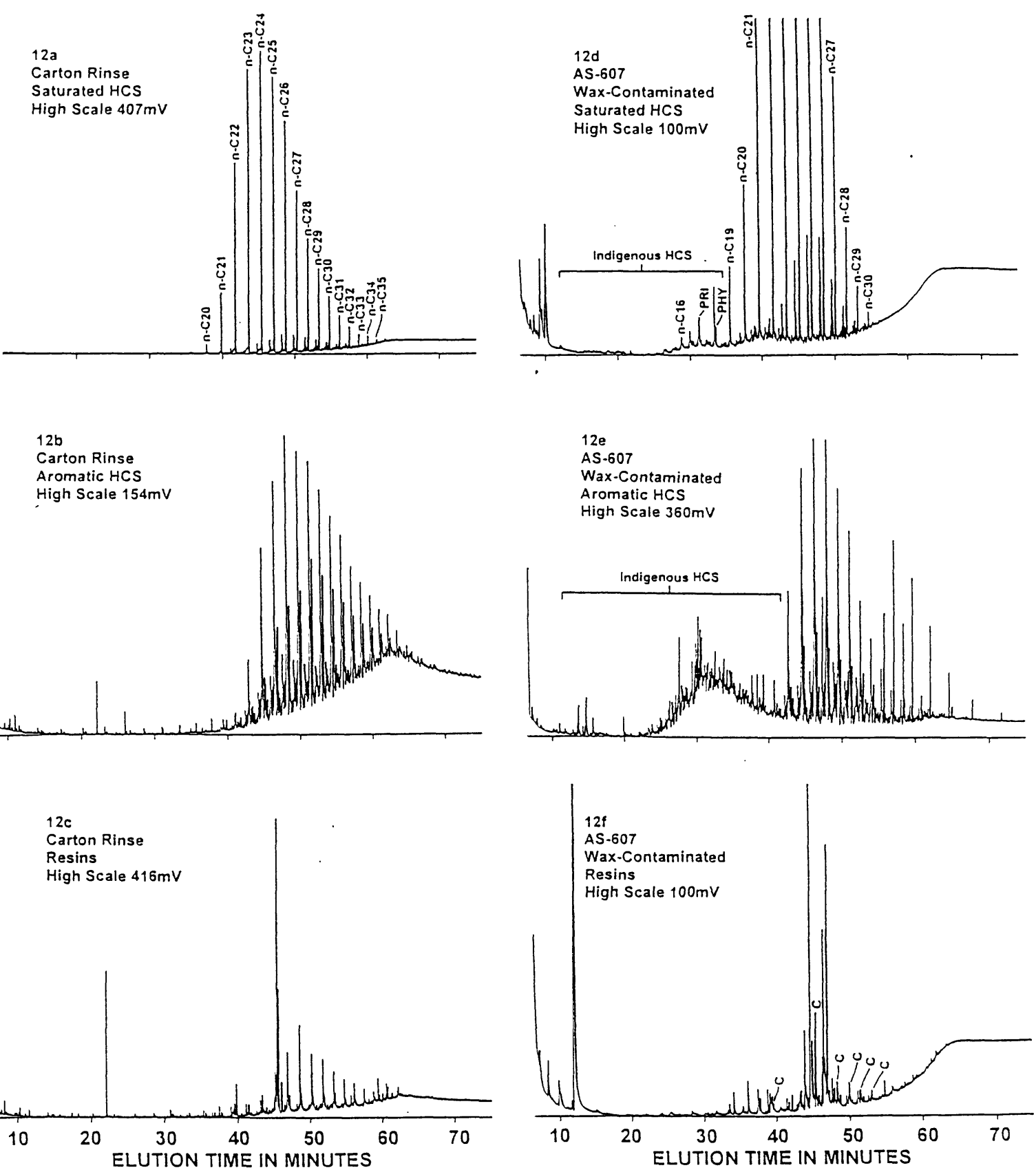


Figure 12. Gas chromatograms of the saturated HCS (12a) aromatic HCS (12b), and resins (12c) from a dichloromethane rinse of the wax-coated interior of a FONDA paper carton. Gas chromatograms from a sample contaminated by such a paper carbon are shown in 12d (saturated HCS), 12e (aromatic HCS), and 12f (resins). n-C and a number refers to the respective n-paraffin in 12a and 12d. PRI is pristane and PHY is phytane in 12d. "C" in 12f refers to contaminant peaks in the resin fraction from the AS-607 sample. All other peaks in the 12f chromatogram are indigenous to the sample.

The relative amount of wax contamination in the resin fraction (Fig. 12f) is even lower than that in the aromatic HCS (Fig. 12e), with the contaminant peaks labeled as "C" in Figure 12f. The reason for the even greater relative (apparent) decrease of contaminant peaks in the resin fraction is that, as discussed in Price et al. (1998, 1999), the resin fraction in carbonaceous high-rank rocks is the largest of the three fractions (by weight) resulting from column chromatography. In contrast, the aromatic-HC fraction is the smallest. The reasons for these observations are discussed in Price et al. (1988, 1999) and will not be detailed here.

The point of this discussion about contamination from the FONDA cardboard cartons is to demonstrate the magnitude of contamination possible, from a seemingly inconsequential step, in the analysis of extractable organic compounds which can be present in low concentrations in high-rank crystalline rocks. High levels of attention must be constantly focused to avoid significant contamination in the analysis of such samples.

4.27 Rock Crushing and Powdering

After thoroughly cleaning the rock surfaces, all rock samples must be crushed and ground to a powder (either to 100 mesh and less or to 200 mesh and less). Crushing is carried out via a large jaw crusher to a 1/2 inch (1.22 cm) size and less. Rocks are then powdered via steel ball mills. Both apparatuses are thoroughly cleaned before our use: The jaw crusher is wetted with water, scrubbed with a wire brush, rinsed with water and then rinsed with once-distilled D150-4 dichloromethane. The ball mills are cleaned by blowing them out with air, powdering a commercial-grade beach sand in them, blowing the powder out with compressed air, and then rinsing the mill cylinder and balls with the dichloromethane.

We hypothesized that the large contaminant peak in the resin fraction at 22.0 minutes (peak M15, Fig. 11c) may have originated from the rock-grinding process, and thus resolved to address the matter. Moreover, every step of the complete analytical process, including rock crushing and powdering, had to be checked as a possible contributor to analytically-derived contamination. This check was accomplished by subjecting a rock thought to be devoid of extractable-organic compounds (a sample blank) to every step of our analytical process, including crushing and grinding.

4.271 Vein ("Bull") Quartz

Vein ("bull") quartz (origin unknown), used as a xeriscape decorator rock outside our laboratory at the Denver Federal Center was originally chosen to be used as the sample blank. The rock was cleaned under running water, air dried, rinsed with once-distilled D150-4 dichloromethane, and then put through the jaw crusher, and finally ground to 100 mesh and less in our ball mills. The powdered bull quartz was then baked at 700°C for 5 days, and 500 grams of the baked quartz was Soxhlet extracted for 10 days. The resulting extract was subjected to our complete analytical workup, including fraction-separation column chromatography. The three fractions were then analyzed by gas chromatography. The gas chromatogram of the saturated-HC fraction is shown in Figure 13a. Solvent contamination is present from about 8 to 10 minutes. However, a series of small n-paraffin peaks (some of which are labeled with their carbon number) are also present from 35 to 55 minutes. The concentration level of these n-paraffins is much less than that found in even our leanest samples, thus the presence of these peaks was not of great concern to us. On the other hand, we were curious as to their point of origin.

Gas chromatograms of the aromatic-HC and resin fractions are shown in Figures 13b and 13c, respectively. Again the early-eluting peaks (at and before 10 minutes) in both chromatograms are due to

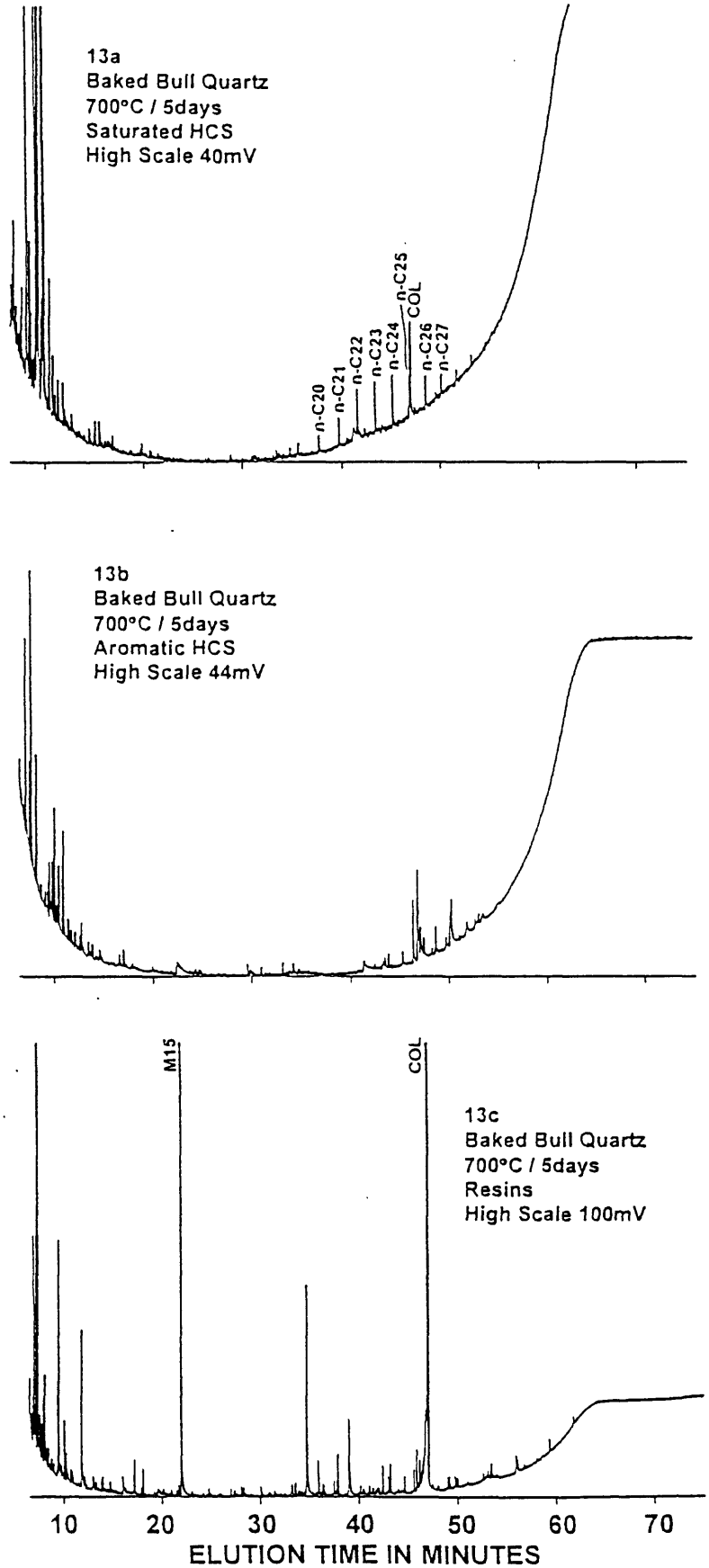


Figure 13. Gas chromatograms of the saturated HCS (13a), aromatic HCS (13b), and resins (13c) extracted from bull quartz. n-C and a number refers to the respective n-paraffin in 13a. "COL" in the saturated-HC (13a) and resin (13c) chromatograms is a contaminant peak from the gas chromatograph column. M15 is a contaminant from the seriological pipettes.

low-boiling contaminants in the solvents. However, there are still other numerous small peaks up to a 55 minute elution time in the aromatic-HC gas chromatogram, and up to about 63 minutes in the resin gas chromatogram. In both cases, the concentration levels of the contaminants were not high enough to be of major concern to us. However, again, in both cases, the origin of both sets of contaminant peaks was of interest to us. Note that the highest contaminant levels in the Figure 13 gas chromatograms was in the resin fraction (Fig. 13c).

4.272 Flint-Glass Bottle

As to the origin of the compounds in Figure 13, we hypothesized that they had not originated from the analytical procedure but instead may have been from fluid inclusions which may have been present in the bull quartz. To check this possibility, we elected to use flint-glass quart bottles as sample blanks. The bottles were cleaned, baked at 450°C for 5 days, crushed and powdered to 100 mesh and less, and subjected to the same analytical workup as the bull quartz. The saturated-HC gas chromatogram (Fig. 14a), to our surprise, still had noticeable, albeit small, concentrations of C_{14} to C_{24} n-paraffins and isoprenoid HCS. The concentrations of the aromatic HCS from 45 to 55 minutes noticeably decreased (Fig. 14b) compared to the bull quartz aromatic-HC gas chromatogram (Fig. 13b). The concentration of the resin peaks eluting after 20 minutes also decreased slightly in the case of the ground flint-glass bottle compared to the bull quartz (Fig. 14c versus Fig. 13c, respectively).

As it turned out, the n-paraffin contaminant peaks of Figures 13a and 14a were largely, and perhaps wholly, from copper-strip wax contamination. It was only later in our research, after we had made checks on the copper strips, that we realized we had put multiple new copper strips, of a different manufactured lot than that responsible for Figure 10a, in the round-bottom-extraction flasks for the bull-quartz and flint-glass extractions. Thus, most, if not all, of the contamination from our two sample "blanks" (bull quartz and flint glass) was from new copper strips.

However, there still is the possibility of other sources of contamination: Recall the differences in the aromatic HCS between the bull quartz (Fig. 13b) and flint glass (Fig. 14b) samples over 43 to 54 minutes. It is possible that these differences were due to the fact that copper strips from different manufacturers' lots were put in the bull quartz and flint glass round-bottom boiling flasks. However, careful perusal of the resin gas chromatograms (Fig. 13c and 14c) for these two samples reveals more significant differences in peak distribution. Moreover, the bull quartz sample has a slightly greater resin concentration than the flint-glass sample. This result would be expected if there were low concentrations of naturally-occurring indigenous extractable organic compounds in the vein bull quartz (Price et al. 1998 and 1999). Given the results of Price et al. (1998 and 1999), and other investigators (reviewed in Price et al. 1998 and 1999), as well as our (yet unpublished) results regarding the analyses of many different types of high-rank crystalline rocks, the presence of small concentrations of extractable-organic compounds in hydrothermally-deposited vein quartz would be expected. Thus, we conclude that very small concentrations of organic compounds may be present in fluid inclusions in the vein quartz. After heating the vein quartz at 700°C, some fraction of those inclusions which did not rupture during the heating might fracture on thermal contraction, thus exposing the inclusions, and the possible organic compounds therein to Soxhlet extraction.

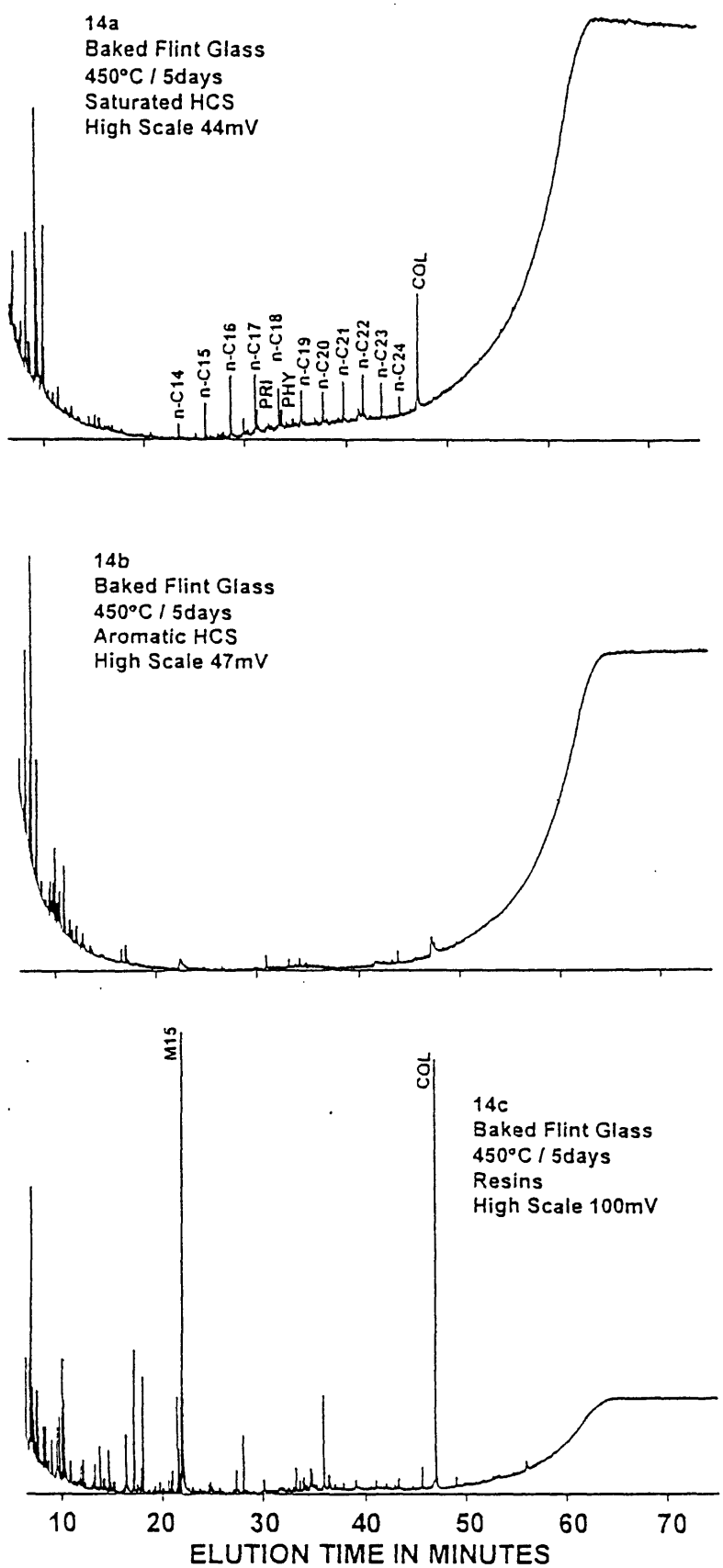


Figure 14. Gas chromatograms of the saturated HCS (Fig. 14a), aromatic HCS (14b), and resins (14c) extracted from a sample blank from a flint-glass bottle sample blank. "COL" in the saturated-HC (14a) and resin chromatograms (14c) is a contaminant peak from the gas chromatograph column; M-15 is a recurring contaminant peak from the seriological pipettes. n-C and a number refers to the respective n-paraffins in 14a.

4.273 Source-Rock Heating

One may argue that heating the quartz at 700°C for 5 days in an oxygen-rich atmosphere would have completely thermally destroyed any organic compounds present in the rock. This is not the case. We heated 500 grams of 60 mesh and smaller lower Cretaceous immature source rock previously used as a standard for ROCK-EVAL analysis in our laboratory at 500°C for 5 days in an oven open to the atmosphere. This shale has a total organic carbon (TOC) content of 4% and ROCK-EVAL hydrogen indices of around 400. After baking the source rock, it was powdered to 100 mesh and less and then Soxhlet extracted. Given the severity of the heating conditions and by all that is sacred in petroleum-geochemistry, all extractable-organic compounds should have been thermally destroyed. This was not the case (Fig. 15). In Figure 15, gas chromatograms are presented at both high and low mV settings. The saturated HCS are shown in Figures 15a and d, the aromatic HCS in Figures 15b and e, and the resins in Figures 15c and f. As is evident in the chromatograms of Figure 15, relatively large amounts of lower-molecular weight extractable material remain in the source rock, in spite of the severe heating conditions.

There clearly has been significant thermal decomposition of the extractable HCS in this rock as is demonstrated by the dominance of lower-molecular weight material in all three chromatograms. Moreover, the thermal destruction reactions have resulted in distributions typical of thermal cracking from laboratory experiments (Price, 1993). Also, only small concentrations of C_{15}+ material remained in the rock after baking as determined by weighing splits of the fractions obtained from column chromatography, to wit: saturated HCS, 0.478 ppm; aromatic HCS 0.132 ppm, and resins, 0.106 ppm. Be all that as it may, we clearly cannot assume that heating the bull-quartz at 700°C for 5 days would destroy all extractable-organic compounds in the closed-system, water-wet fluid inclusions of the quartz, if such compounds and inclusions were present.

4.28 The Cleaning Sand

Another source of contamination that could influence the procedural blanks (bull quartz, flint glass bottles), would be the sand used to clean the ball mill before (and after) powdering our rocks. With this in mind, we carried out three experiments: First, we powdered previously-crushed and baked (450°C for 5 days) flint-glass bottles in a ball mill that had been cleaned with the sand and then rinsed with dichloromethane. Second, we powdered splits of the same crushed and baked flint-glass bottles in a ball mill which had had multiple cleanings with the sand during the powdering of the flint-glass bottles, and no dichloromethane rinses. Third, we powdered 500 grams of the beach sand to 100 mesh and less.

Five hundred grams of powder from each of the above were Soxhlet extracted and subjected to our full analytical workup, including column chromatography. The saturated- and aromatic-HC, and resin gas chromatograms from these three samples are shown in Figure 16. Unfortunately, the resin fractions from these samples were lost during workup. The six chromatograms of Figure 16 result from a solvent-volume reduction of 18,000 fold. Figures 16a, 16b, 16d, and 16e demonstrate that there are no detectable HCS in the saturated- and aromatic-HC gas chromatograms for the two flint-glass samples. Figures 16b and 16e (multiple sand cleanings, no dichloromethane rinse) would be expected to demonstrate the presence of contamination, compared to Figures 16a and 16d (one sand cleaning and a dichloromethane rinse), if there were any from the sand. Thus, we may conclude that no detectable contamination within the saturated- and aromatic-HC fractions arises from use of the sand to clean the ball mills.

Figures 16c and 16f show saturated- and aromatic-HC gas chromatograms from the extraction of the ground beach sand. Significant amounts (relative to Figs. 16a and 16b) of saturated HCS (Fig. 16c)

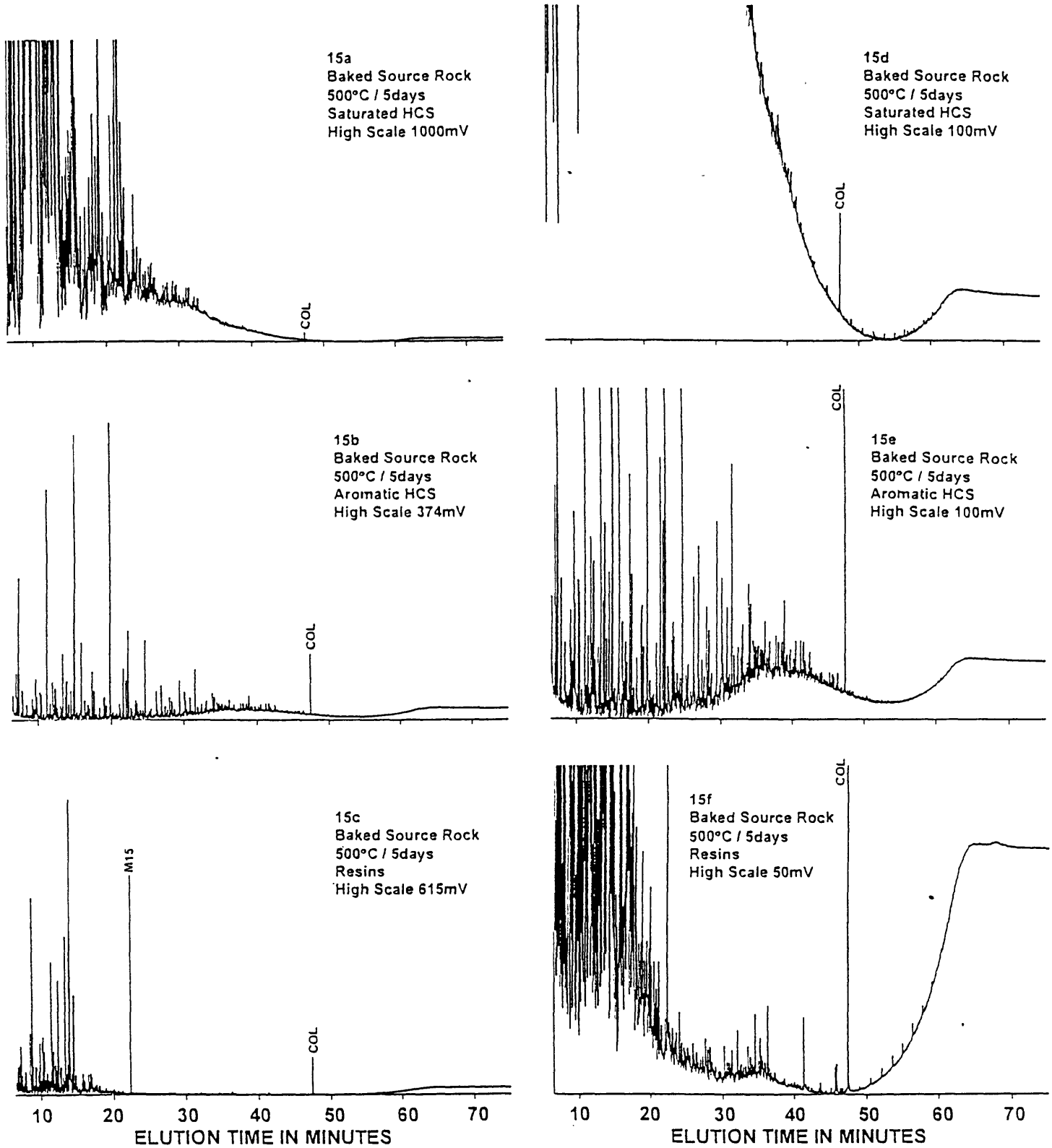


Figure 15. Saturated-HC gas chromatograms at two different attenuations (15a, 1000 mV; 15d 100 mV); aromatic-HC gas chromatograms at two different attenuations (15b, 374 mV; 15e, 100 mV); and resin gas chromatograms at two different attenuations (15c, 615 mV; 15f 50 mV), all from heating an organic rich (immature) shale at 500°C for 5 days in an oven open to the atmosphere.

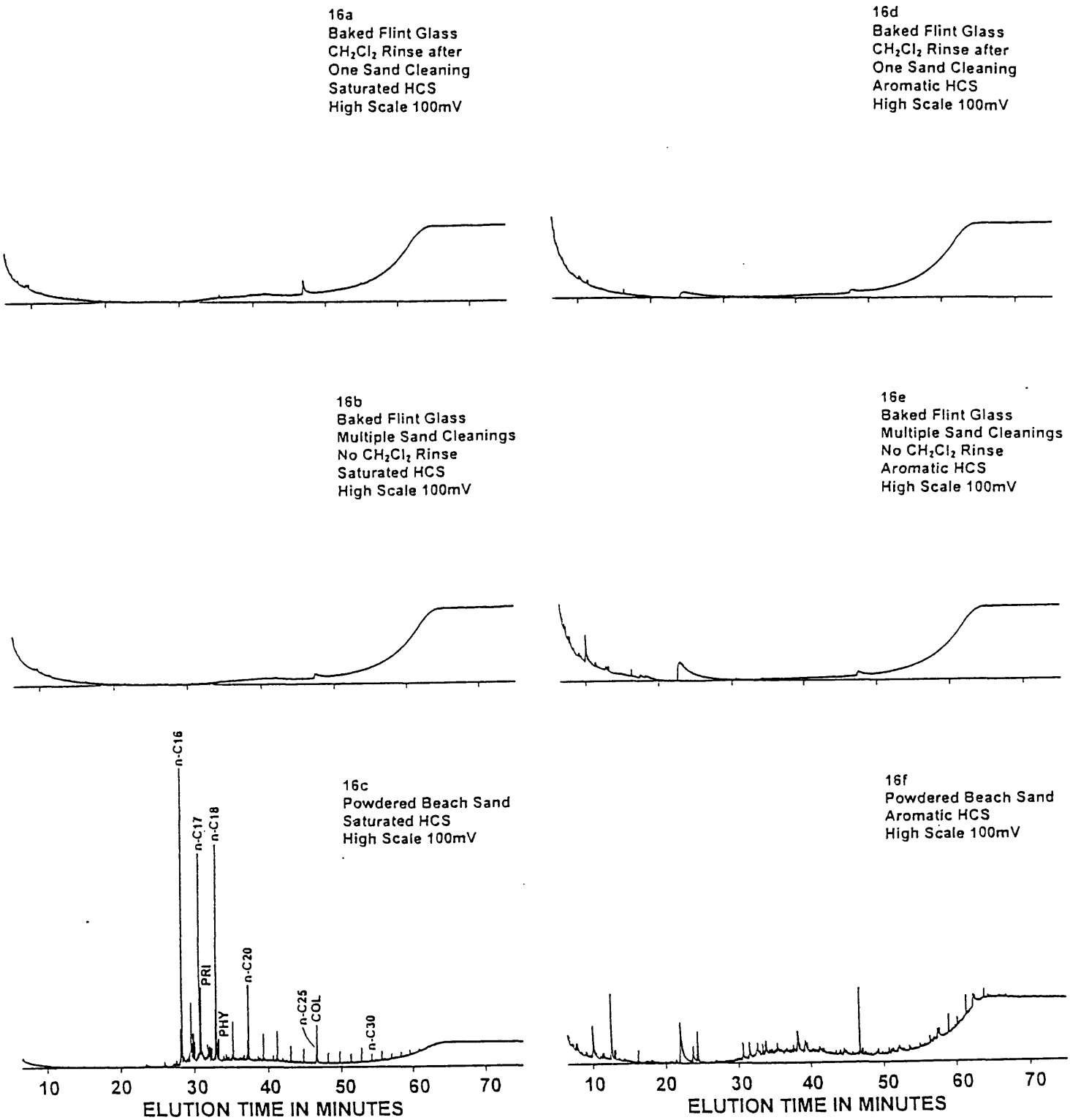


Figure 16. Saturated-HC gas chromatograms from flint-glass bottle sample blanks prepared by one sand cleaning before powdering the bottle with a dichloromethane rinse (16a), and by multiple sand cleanings while powdering the bottle, with no dichloromethane rinses (16b); and from an extract of the powdered sand used to clean the ball mills (16c). Aromatic-HC gas chromatograms from the same sample-blanks and sand are respectively shown by 16d to 16f. n-C and a number refers to the respective n-paraffin in 16c.

were present in the sand. However, the absolute amounts are very low, since Fig. 16c represents all the saturated HCS obtained from extracting 500 grams of beach sand. Be that as it may, because no sample split was taken for C_{15}+ weights for any of the beach-sand fractions from column chromatography, the actual amounts of saturated- and aromatic-HCS obtained from column chromatography are unknown, but we estimate that they would be in the range of 0.01 to 0.1 mg, if we were able to weigh them, or 0.02 to 0.2 ppm by weight.

Small amounts of aromatic HCS were also present in the beach sand (Fig. 16f). Although low concentrations of saturated- and aromatic HCS are present in the beach sand, these low concentrations did not contribute detectable contamination to our sample procedure. This is because Figures 16c and 16f result from extraction of 500 grams of sand, whereas only 10 to 20 grams of sand are used to clean the ball mill, and that 10 to 20 grams is disposed of, not extracted. This conclusion is obviously substantiated by Figures 16a, 16b, and 16d and 16e, which demonstrate no contamination from the beach sand.

4.29 Procedure Blanks

The saturated- and aromatic-HCS of Figure 16a, 16b, 16d, and 16e serve as contamination checks wherein blank (non-HC bearing) samples have been subjected to our full analytical workup. Those chromatograms demonstrate that no significant concentrations of organic compounds are introduced into our analytical procedure at any step of the total process, from sample-preparation to final analysis. However, we found that there could be some variance of the level of the background concentration of lower boiling materials dependent on solvent distillation rates. To illustrate this point, we here present the results from two other procedural blanks, one of which we also ran to further check the possibility of HC-bearing fluid inclusions in the bull quartz.

4.291 Bull Quartz

For the first procedure blank, we baked, cleaned and crushed (to roughly 1/2 inch, 1.27cm) pieces of bull quartz at 700°C for 5 days. The quartz was then powdered and 500 grams were then Soxhlet extracted (using previously-rinsed, reactivated copper strips) and put through our full analytical workup. The resulting gas chromatograms for the saturated and aromatic HCS and resins are shown in Figure 17a through 17c, respectively.

Significant solvent peaks (at this low attenuation, 44 mV) are present in the saturated-HC chromatogram (Fig. 17a) from 8 to 10 minutes and are labeled D4-D7, D9 and S1. Very small C_{20} to C_{25} n-paraffin peaks are also present, peaks which we can now attribute to fluid inclusions within the bull quartz. The aromatic HC gas chromatogram (Fig. 17b) also has a number of small solvent peaks from 6.5 to 16 minutes elution time. These peaks have been labeled D1, D2, B1-B6, and A1-A8. No significant contaminant peaks are present after 16 minutes.

A series of low-boiling solvent contamination peaks (M2-M15, labeling from Fig. 11c) are present in the bull-quartz resin chromatogram. Very small contaminant peaks are also present between the M15 peak and "COL" contaminant peaks. The presence of peaks in the resin gas chromatogram Fig. 17c) from the bull quartz and not in the aromatic HC gas chromatogram is expected if the contaminant peaks of Figures 17a and 17c originated from fluid inclusions in the quartz (Price et al. 1998 and 1999). However, the concentration of these compounds is far lower than that of the organic compounds in the high-rank crystalline rocks we study, as will be demonstrated in Section 5.0, below. Furthermore, the concentration of

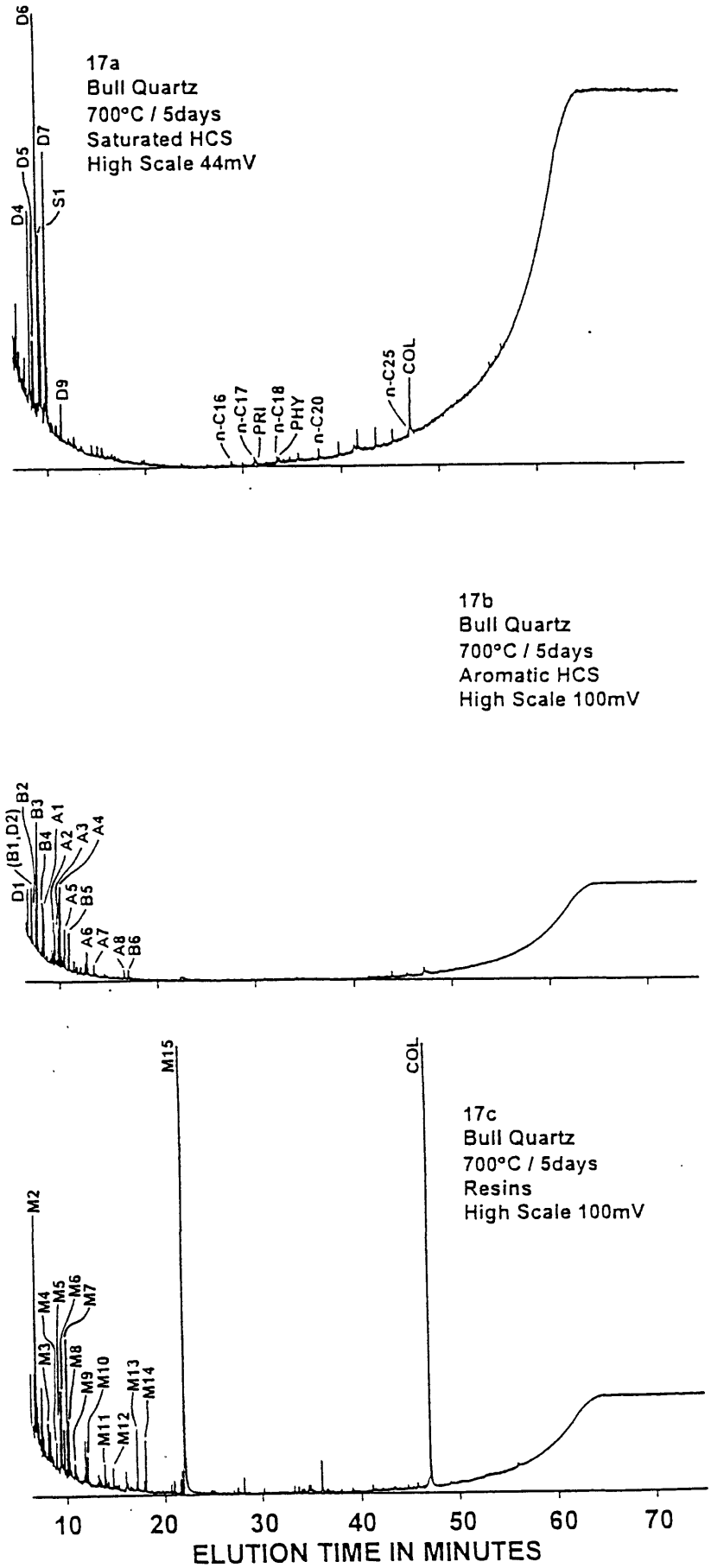


Figure 17. Gas chromatograms of saturated HCS (17a) aromatic HCS (17b), and resins (17c) extracted from a bull quartz sample blank. N-paraffins are shown by n-C and a number in 17a. Labeling of the low molecular weight peaks in all three chromatograms explained in text. Also see Figure 13 caption.

the low boiling compounds in Figures 17a to 17c is also low enough, such that in many of the samples we work with, only minor problems would result concerning interpretation of analytical results.

The solvent-derived peaks in the Figure 17 gas chromatograms were labeled as follows: The "D" peaks in the saturated-HC gas chromatogram (Fig. 17a) originate as solvent contamination from the once-distilled D150-4 dichloromethane (Fig. 2f). The S1 peak (Fig. 17a) is a newly detected contaminant peak in the saturated HCS. The D1 and D2 peaks of Figure 17b originate as solvent contamination from the once-distilled D150-4 dichloromethane (Fig. 2f). The peaks B1 to B6 (Fig. 17b) originate as solvent contamination from the twice-distilled EM-BX0212-1 benzene (Fig. 11b). The peaks A1 to A8 (Fig. 17b) are newly-detected aromatic HCS which most probably also originate as solvent contamination in the twice-distilled EM-BX0212-1 benzene. The peaks labeled M1-M14 (Fig. 17c) originate from solvent contamination (Fig. 11c). The origin of the M15 peak (Fig. 17c) is unknown.

4.292 Flint-Glass Bottle

As before, the flint-glass quart bottles were cleaned, baked at 450°C for 5 days, crushed and powdered to 100 mesh and less, and subjected to our full analytical workup from Soxhlet extraction to gas chromatographic analysis of the resulting fractions, the gas chromatograms of which are shown in Figure 18, the saturated-HC gas chromatogram being Figure 18a. Five small peaks labeled COL-1 to COL-5 were present in all six chromatograms of Figure 18. These peaks originated from contamination from the gas-chromatograph column, and not from our analytical work up. The saturated-HC gas chromatogram from the flint glass bottle (Fig. 18a) has no contaminant peaks originating from our analytical workup except for a very small series of peaks over roughly 27 to 44 minutes elution time.

The aromatic HC gas chromatogram has no contaminant peaks except for a relatively large peak at about 11 minutes elution time labeled "Fume Hood Peak" (Fig. 18b). This peak did not originate from our analytical workup either, but instead originated from cross-fume hood contamination. On the day we were allowing the aromatic HC fractions from the flint-glass bottle extraction, and the resins from a concurrently-run column blank, to passively evaporate in our fume hood, there was a chemical spill in a laboratory areally-continguous to ours. An odor from the materials used to contain the spill soon permeated the hallways. Eventually, we began smelling the odor in our fume hood and we realized this odor was a source of possible contamination. However, we elected to leave the 8-dram vials in the fume hood to test the possibility of contamination to our samples from these fumes. As is evident from Figures 18b and 18f, these fumes did result in contamination to our samples, again illustrating that at the highly-sensitive analytical level we are concerned with, contamination can result from the unlikiest of sources. Due to this experience (Figs. 18b and f), samples undergoing passive evaporation in our fume hoods are covered when an outside odor is detected in our hoods.

The gas chromatogram from the resin fraction of the flint-glass bottle exraction is shown in Figure 18c. The usual large peak from the gas chromatograph column (COL-47) is present. In addition, there are a series of small peaks from 21 to 46 minutes elution time. Of the three fractions from column chromatography (saturated and aromatic HCS, and resins), the resin fraction typically has the highest levels of contamination. However, the levels of contamination in Figure 18c are undectable in our resin analyses, because full scale resin gas chromatograms from our high-rank crystalline rock samples always are in the 500 to 1000mV range of attenuation.

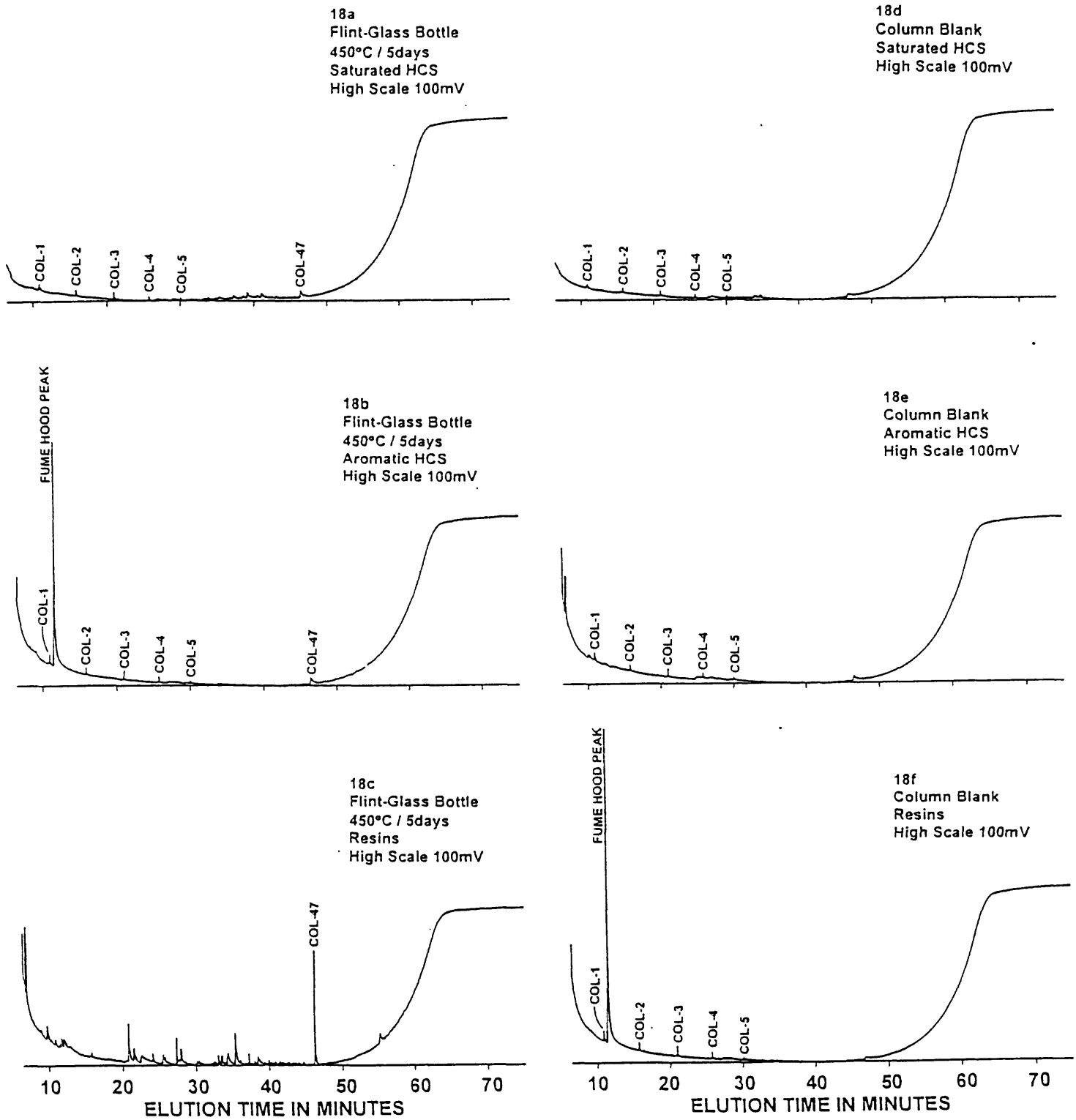


Figure 18. Gas chromatograms of saturated HCS (18a and 18d), aromatic HCS (18b and 18e), and resins (18c and 18f) from, respectively, a flint-glass bottle sample blank and blank elutions from a silica-gel/alumina column. Labeling of the chromatograms is explained in the text.

While analyzing the flint-glass bottle sample blank, column blanks were also run, wherein blank saturated-HC, aromatic-HC, and resin fractions were concurrently eluted, from a separate column, while the flint-glass bottle sample blank was being fractionated. In this manner, we would be able to assign any contamination which arose in the 6 eluted fractions to either the crushing-powdering step or the fractionation from column chromatography. The gas chromatograms of the three fractions from the column blank are shown in Figures 18d to 18f. No contaminant peaks are present in any of the three chromatograms, aside from the five peaks from the gas chromatograph column (peaks "COL-1 to COL-5") in all three chromatograms, and the contaminant peak from the fume hood odor ("Fume Hood Peak") in the resin gas chromatogram (Fig. 18f). These six peaks are all one-time peaks, which do not appear in other sample blanks.

From the lack of contaminant peaks in Figures 18d to 18f, we may conclude that the very minor contaminant peaks, over elution times of 27 to 44 minutes, in the saturated-HC gas chromatogram of the flint glass bottle (Fig. 18a) arises from the crushing and powdering step, as do the minor contaminant peaks in the gas chromatogram of the resin fraction from the flint-glass bottle (Fig. 18c). However, as stated, in neither case would the contaminant peaks even be detectable in the crystalline rock samples we analyze with even the lowest HC concentrations, as will be demonstrated in Section 5.0.

We stress two other points concerning Figure 18. Note that in the gas chromatograms of both resin fractions (Figs. 18c and 18f) the large contaminant peak at 21 minutes is missing. This peak ("M15") was present in most of our previous examples, and we were never able to identify its origin.

The peak eventually simply disappeared from our sample blanks (and samples), as in the chromatograms of Figure 18, and thus its origin remains a mystery. Secondly, note the absence of low molecular weight solvent peaks in all of the six chromatograms of Figure 18, especially compared to the chromatograms of Figures 1-17 and 19. We determined late in our contamination study that by reducing the distillation rates of the solvents we distilled, we were able to make very pure solvents with very low amounts of, or no, low-molecular weight contaminant peaks.

The chromatograms of Figure 18 thus serve as representative examples of the degree of contamination introduced by our analytical technique currently in use, e.g., very low amounts of analytically-derived contamination.

5.0 MANTLE XENOLITH: An Example from the Natural System

Most of the carbonaceous high-rank crystalline rocks we deal with have total "bitumen" contents on the order of 2 to 10 ppm by weight, although some concentrations can be much higher (up to 150 ppm, Price et al., 1998, 1999). Conversely, other rocks can have much lower concentrations. For example, the mantle xenoliths studied by Sugisaki and Mimura (1994) have total bitumen concentrations around 0.5 ppm. As an applied example of our analytical procedure, we present the results of an analysis of a mantle xenolith from the Hualalai Volcano, Hawaii, a principally olivine-bearing dunite (Fig. 19, sample HUA-2). The saturated HCS were at a concentration of 0.156 ppm in the rock, the aromatic HCS at 0.078 ppm, and the resins at 0.43 ppm.

The saturated-HC gas chromatogram (Fig. 19a) has a pronounced naphthenic hump with n-paraffins, isoprenoid HCS, and other compounds on top of the hump. The HUA-2 saturated-HC gas chromatogram replicates those reported by Sugisaki and Mimura (1994) from other mantle xenolith

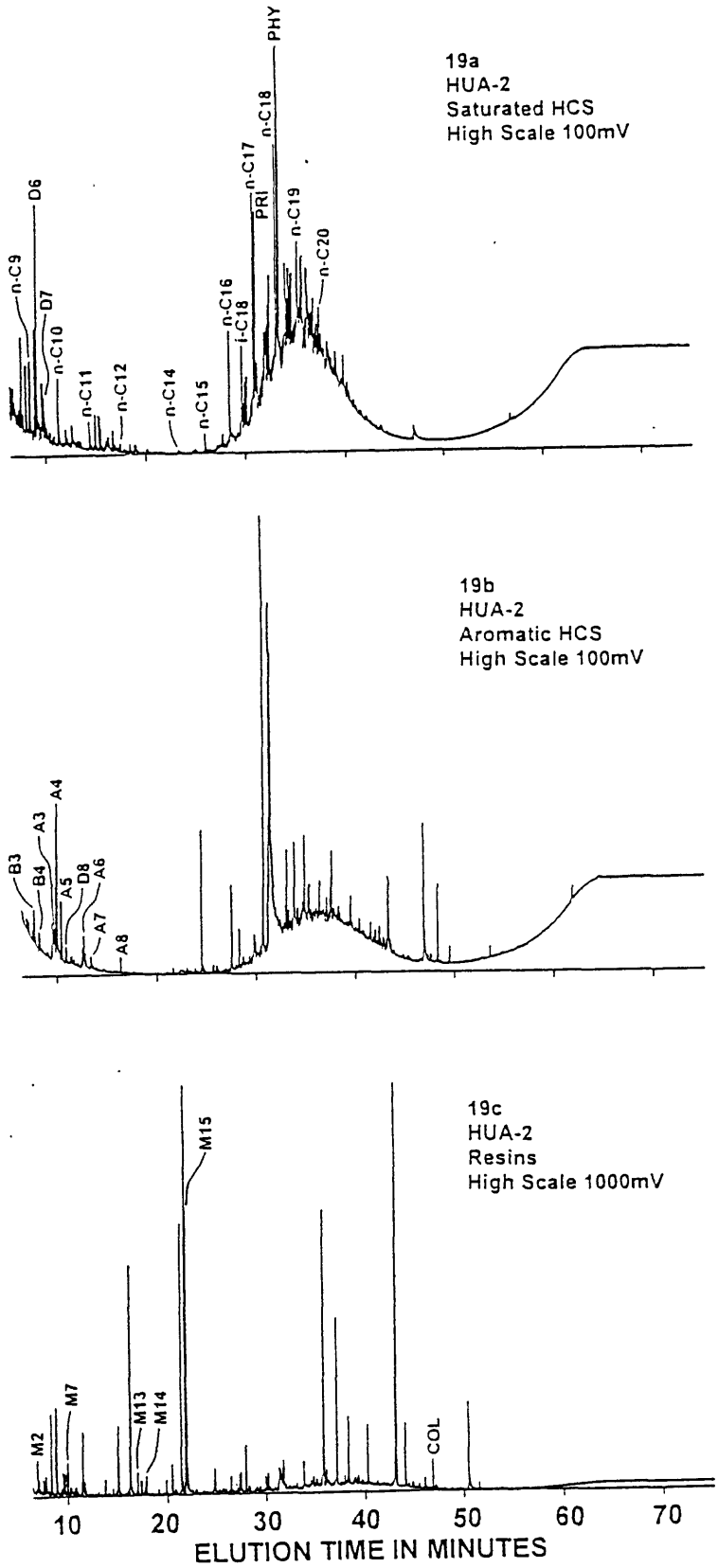


Figure 19. Gas chromatograms of saturated HCS (19a), aromatic HCS (19b), and resins (19c) extracted from a mantle xenolith. In 19a, n-paraffins are shown by n-C and their respective carbon number, PRI is pristane, PHY is phytane. Peak labeling is explained in the text.

samples. The aromatic-HC gas chromatogram (Fig. 19b) has a similar, but lower, hump, with numerous peaks on it. The gas chromatogram of the resin fraction (Fig. 19c) has a number of prominent peaks, two of which are offscale, and the chromatogram has no hump. Price et al. (1998, 1999), and unpublished work subsequent to those papers, have demonstrated that many of the peaks in gas chromatograms of the aromatic-HC and resin fractions from high-rank crystalline rocks are oxygen-bearing organic compounds. However, that topic will not be discussed in detail here.

The Figure 19 gas chromatograms allows four relevant conclusions. First, the distribution of peaks in all three chromatograms is much different than those arising from the much smaller concentrations of laboratory-induced contamination. Second, the low-boiling contaminant peaks in the saturated- and aromatic-HC gas chromatograms are the most prominent non-exogenous peaks. In many other samples of high-rank crystalline rocks, high concentrations of indigenous low-molecular weight compounds have often dwarfed the low-boiling contaminant peaks in the saturated and aromatic HCS. In fact, an indirect example of this is present in Figure 19c (resin gas chromatogram), wherein the low-molecular weight contaminant solvent peaks (labeled M-2, M-7, M-13, and M-14) are very small compared to the indigenous compounds in the resin fraction. Even the normally prominent M-15 and "COL" contaminant peaks of the resin gas chromatograms are smaller than the peaks from the compounds indigenous to the sample, and this is especially true for the column ("COL") contaminant peak.

Our third conclusion, is that the indigenous bitumen from the HUA-2 rock sample is in much greater concentrations than the higher-boiling small contaminant peaks which may arise from our analytical procedure (with the exception of the resin M-15 and "COL" contaminant peaks). Moreover, the Figure 19 gas chromatograms demonstrate that we could successfully analyze organic compounds present in high-rank crystalline rocks at concentration levels even lower than 0.1 to 0.5 ppm. The third conclusion allows our fourth, to wit: our analytical procedure is more than adequate for the analysis of very low concentrations of organic compounds in high-rank crystalline rocks. Of course, one may argue that the bitumen resulting in the Figure 19 gas chromatograms is contamination on or in the rock sample. However, both Sugisaki and Mimura (1994) and Price et al. (1998, 1999) have replied to this point, a point which does not concern us here.

Several other points should be made concerning the Figure 19 gas chromatograms. We processed the HUA-2 dunite before learning that we could lower our distillation rates such that the lowest molecular weight solvent peaks were greatly decreased or removed altogether (Fig. 18). However, the lower molecular weight contaminant peaks labeled in Figures 19a to 19c do not significantly detract from the results of our analysis. Observe that the M-15 contaminant peak (Fig. 19c) was still present in our analytical technique when the HUA-2 dunite was analyzed. Lastly, note that the HUA-2 sample represents the low-concentration end of the samples we analyze. However, the resin gas chromatogram is at 1000 mV full scale, with the two largest peaks still offscale. Thus, the small peaks in the resin fraction created by our analytical method (Fig. 18c) are undetectable in the resin fractions derived from our samples (Fig. 19c).

We conclude that the two principal points of this paper have been made: 1) to demonstrate that no significant levels of contamination are introduced from our laboratory procedures into the bitumen extracted from high-rank crystalline rock samples we study; and 2) to point out examples of the numerous possible pitfalls in the analysis of such rocks, so that other laboratories may replicate our results concerning the study of organic compounds in high-rank crystalline rocks.

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