GC/MS Detection of Paraffins in a Case of Lipoid Pneumonia Following Occupational Exposure to Oil Spray

M.C. Penes and **J.J. Vallon**
Laboratoire de Biochimie et Toxicologie, Hopital Edouard Herriot, 1 Place d'Arsonval-69374 Lyon and Laboratoire de Chimie Analytique III, Faculte de Pharmacie, 8 Avenue Rockefeller-69008 Lyon, France

J.F. Sabot
Laboratoire de Chimie Analytique II, Faculte de Pharmacie, 8 Avenue Rockefeller-69008 Lyon, France

C. Vallon
Clinique de Pneumologie, Hopital de la Croix-Rousse-69004 Lyon, France

Abstract

A case of lipoid pneumonia following occupational exposure to oil spray is described. Biological fluids (broncho-alveolar lavage and pleural fluid) are analyzed to determine if the compounds found in the industrial oils are present. The samples are purified using high-performance thin-layer chromatography, and after extraction, the compounds were submitted to infrared spectroscopy. Direct hexane extraction of biological fluids is also performed, followed by gas chromatography/mass spectrometry (GC/MS).

Introduction

Most lipoid pneumonia cases described in the literature (1–5) are caused by liquid paraffin inhalation during extended laxative treatments of patients with swallowing or pharynx and esophagus diseases. Chronic lipoid pneumonia following occupational exposures to mineral oils is very uncommon (6–7). In this case, a diffuse interstitial lung disease because of aerosol exposure to small-diameter (near 5-μm) oil particles is observed. Diagnosis of lipoid pneumonia is based on three elements: the history of the disease, bronchoalveolar lavage and a lung biopsy. Bronchopulmonary lavage has a dual purpose: It shows macrophages with lipoid inclusions, and extraction by organic solvents followed by thin-layer chromatography of silica gel leads to identification of an abnormal spot near the solvent front identical to that due to the mineral oil itself.

Case History

We report here a case of diffuse interstitial pneumonia that followed occupational exposure to oil spray in a 45-year-old patient who worked for 16 years on a machine tool. The patient was hospitalized after the discovery of an abnormal aspect of diffuse pneumonia by lung radiography during a labor medical inspection. We decided to perform a lung biopsy and biochemical analysis of bronchopulmonary lavage and pleural fluid because of the lung radiograph and patient information. He told us that he had been welding pieces of steel covered with cutting oil. A sample of this oil was used as a check sample.

Materials and Method

Extraction of biological samples. Brocho-alveolar lavage (BAL) and pleural fluid (PF) were extracted according to a method applied to analysis of pharyngeal fluid. An aliquot volume was extracted with two volumes of chloroform-methanol (2:1 v/v) mixture. Samples of industrial cutting or stripping oil were diluted in either hexane or carbon tetrachloride and used as reference compounds.

Purification. A purification step was accomplished by high-performance thin-layer chromatography (HPTLC) on silica gel 60 aluminium sheets (art 5547 Merck). The elution solvent was a mixture of chloroform-methanol-acetic acid-water (75:45:12:6 v/v) used for phospholipid separation.

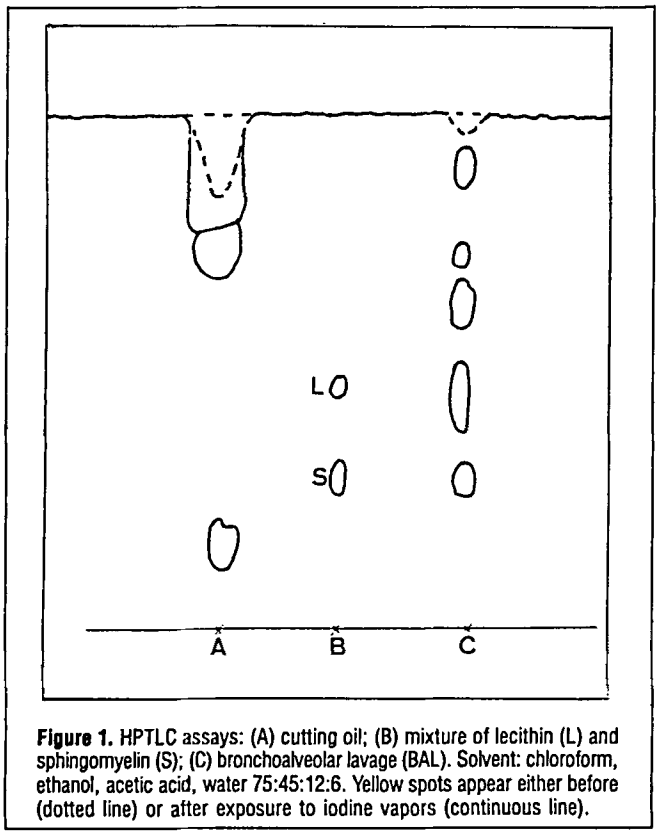

Figure 1. HPTLC assays: (A) cutting oil; (B) mixture of lecithin (L) and sphingomyelin (S); (C) bronchoalveolar lavage (BAL). Solvent: chloroform, ethanol, acetic acid, water 75:45:12:6. Yellow spots appear either before (dotted line) or after exposure to iodine vapors (continuous line).

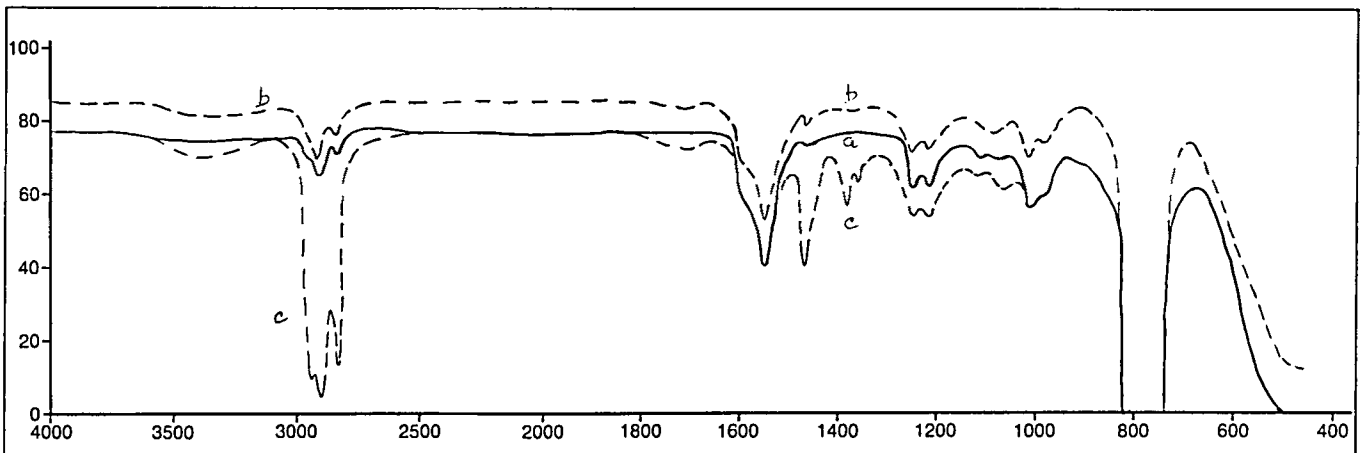

Figure 2. IR spectra: CCl_4 extract of (A) the silica layer; CCl_4 of (B) BAL chromatogram (solvent front); CCl_4 dilution of (C) cutting oil.

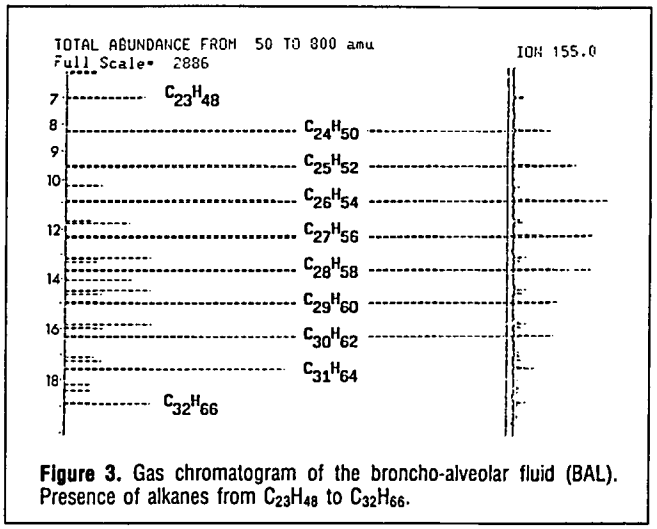

Figure 3. Gas chromatogram of the broncho-alveolar fluid (BAL). Presence of alkanes from $C_{23}H_{48}$ to $C_{32}H_{66}$.

Spots of interest, as indicated by reference samples, were eluted near the solvent front. Following their extraction from the silica gel by carbon tetrachloride, extracts were submitted to infrared spectroscopy. For gas chromatographic/mass spectrometric (GC/MS) assay, direct hexane extraction of BAL and PF or of chromatographic spots was used.

Infrared spectroscopy and gas chromatography/mass spectrometry. A Perkin-Elmer Model 457 IR spectrophotometer with NaCl cells was used for assays of carbon tetrachloride extracts between 4000 and 200/cm. A Hewlett-Packard 5790 gas chromatograph coupled with the HP 5790 A quadrupole mass filter was used. A fused-silica column (25 m × 0.32 mm i.d.) packed with a SE-54 bonded phase was used with a helium flow rate of 0.9 mL/min. The oven temperature program began at 200°C, increased to 290°C at 5°/min, and was held at this final temperature for 20 min.

For GC/MS, hydrocarbons (octadecane $C_{18}H_{38}$ to dotriacontane $C_{32}H_{66}$) were obtained from Fluka AG and Carlo Erba.

Results and Discussion

HPTLC assays. Chromatograms of the biological extracts and oils show yellow spots at the solvent front for BAL and the reference oil sample (Figure 1). In the case of pleural liquid, direct examination found a yellow green spot near the solvent front.

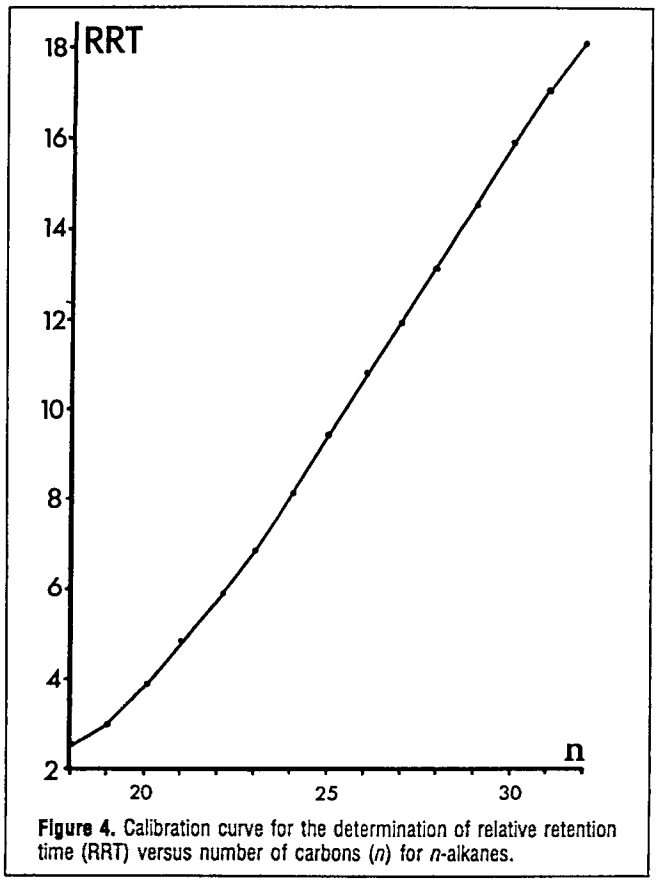

Figure 4. Calibration curve for the determination of relative retention time (RRT) versus number of carbons (n) for n-alkanes.

After iodine vapor exposure, a number of spots corresponding to phospholipids can be observed, especially lecithins along with sphingomyelin. Spots at the solvent front are not stained by iodine, indicating the lack of unsaturated structures.

Infrared spectroscopy. Different spectra for CCl_4 are shown in Figure 2. The CCl_4 extract of the silica layer as reference sample (a), the extract of the solvent front spot of the BAL chromatogram (b), and dilutions of oils in CCl_4 (c).

All spectra have features identical to the reference sample so that no identification can be achieved.

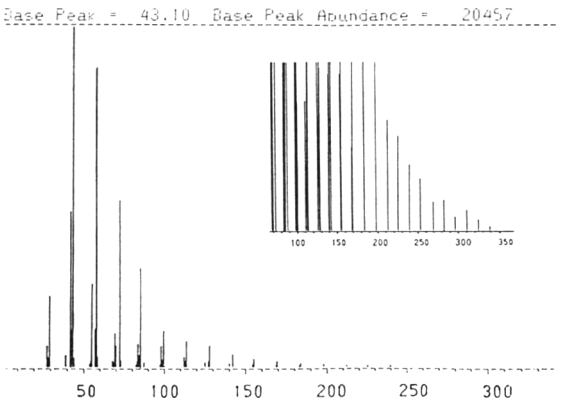

Figure 5. The electron-ionization mass spectra of a chromatographic peak. Note that no molecular ion peak is present. An expansion of the *m/z* 70–350 region is given as an insert.

Gas chromatography/mass spectrometry. Total ion-current chromatograms were obtained for the extracts of the two fluids and showed numerous, regularly spaced peaks (Figure 3).

The identification of hydrocarbons (HC) in our analyses was performed by comparison with the relative retention time (RRT) of standard hydrocarbons. Mixtures of "pure" HCs (from $C_{18}H_{38}$ to $C_{32}H_{66}$) were injected using the same protocol. The standard deviations of RRT did not exceed 5%, which we thought was a good result for a qualitative analysis. There were no interfering peaks in control samples.

Usually, *n*-alkanes are chosen for calculating the characteristic parameters of a column in gas chromatography. Relative retention times were plotted against the number of carbons (Figure 4). We used this methodology to verify the presence of hydrocarbons in the two studied fluids.

We admit that the BAL and PF contain compounds whose RRT correspond to those of *n*-alkanes. In the BAL, C_{23} to C_{32} HC were found and C_{18} to C_{32} HC were identified in the PF.

It was also necessary to confirm the presence of hydrocarbons in the studied fluids. For this purpose, we used the multiple ion detector of the mass spectrometer. Alkanes were identified by comparison of their spectra (Figure 5) with those of authentic standards, from *m/z* 30 to 350. Unfortunately, there were insufficient intensities of the highest peaks corresponding to the molecular ions of the hydrocarbons. Nevertheless, numerous peaks were obtained between *m/z* 57 and the highest *m/z* values. These fragments correspond to [M – 14] ions and [fragment – 14] ions, explaining the characteristic fragmentation of alkanes (i.e., losses of CH_2 groups).

Because the fragmentation pattern was similar to all the alkanes, we recorded ion currents at *m/z* 57, 71, 85, 99, 113, and 127. The detection of these characteristic ions was achieved by selected ion monitoring. This procedure is suitable for the specific detection of fragments issued from the studied hydrocarbons (Figure 6). Selected ion monitoring enabled the quantification of a number of different ions in a single analysis. Also, several ions from the one molecule or from identical compounds (alkanes) can be monitored equally well with consequent enhancement of the specificity of the analysis. The last method was applied to the two fluids and to the industrial oils (CO and SO). For the BAL and the PF, chromatograms and mass spectra obtained are virtually identical to those found for the analysis of standard solutions containing pure alkanes or extracts form cutting oil and stripping oil. Similar results were obtained from these four samples, allowing us to conclude that alkanes (oils, paraffins, and hydrocarbons) were present.

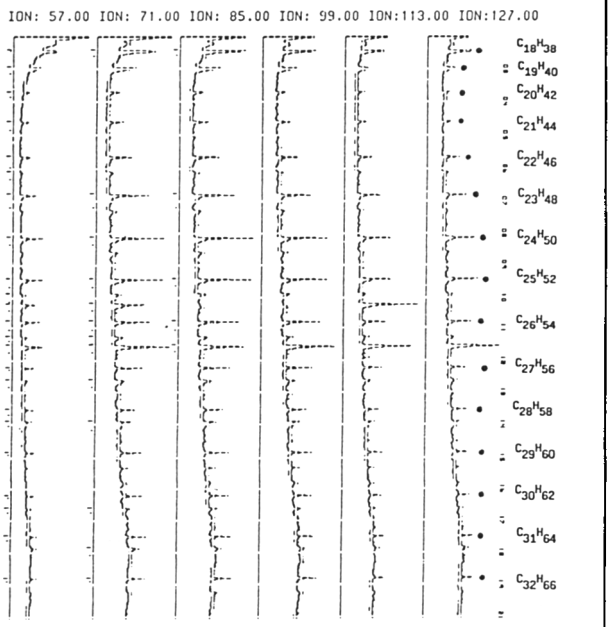

Figure 6. Selected ion profiles (*m/z* 57, 71, 85, 99, 113, and 127) obtained from the pleural fluid.

Conclusion

Direct body fluid extraction by hexane proved to be selective enough to remove impurities that interfered with thin-layer chromatography. The best results were obtained by direct injection of hexane extracts in the GC/MS. We conclude that the protocol used allows reliable qualitative research of alkanes in body fluids following exposure to oil spray.

References

1. J. Weiler and G. Roussel. Les pneumopathies lipidiques. *France Med.* **26:** 5–27 (1963).
2. F. Dongay, R. Levade, A. Caratero, R. Salvayre, D. Lauque, and P. Carles. Etude biochimique et cytologique du liquide de lavage alvéolaire dans 4 cas de pneumopathies huileuses. *Presse Méd.* **15:** 1863–68 (1986).
3. N.C. Hendricks, N.J. Linden, G.H. Collings, A.E. Dooley, J.Y. Garett, and J.B. Rather. A review of exposures to oil mist. *Arch. Env. Health* **4:** 139–45 (1962).
4. R.B. Foe and R.S. Bigham. Lipoid pneumonia following occupational exposure to oil spray. *J. Am. Med. Assoc.* **155:** 33–34 (1954).
5. M.R. Cullen, J.R. Bames, J.M. Robins, and G.J. Walker-Smith. Lipoid pneumonia caused by oil mist exposure from a steel rolling tandem mill. *Am. J. Ind. Med.* **2:** 51–58 (1981).
6. J.P. Proudfit, H.S.V. Orshand, and C.W. Miler. Chronic lipoid pneumonia following occupational exposure. *Arch. Ind. Hyg.* **1:** 105–111 (1950).
7. J.G. Jones. An investigation into the effects of exposure to an oil mist on workers in a mill for the cold reduction of steel strip. *Ann. Occup. Hyg.* **3:** 264–71 (1961).

Manuscript received September 20, 1989;
revision received March 21, 1990.