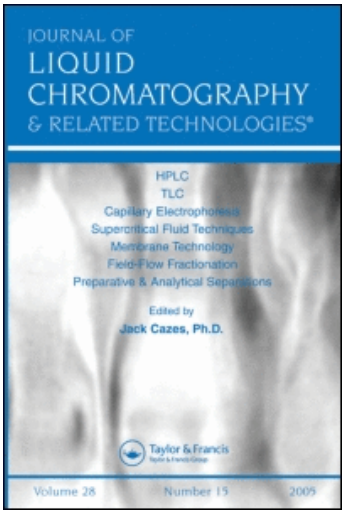

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Implication of On-Line Fourier Transform Infrared Detection in the Separation of Aromatic Compounds via Normal-Phase Liquid Chromatography

Sigrid Wachholz[a]; Heinz Geiβler[a]; Joachim Bleck[a]
[a] Central Institute of Physical Chemistry, Academy of Sciences of the German Democratic Republic, Berlin, German Democratic Republic

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IMPLICATION OF ON-LINE FOURIER TRANSFORM INFRARED DETECTION IN THE SEPARATION OF AROMATIC COMPOUNDS VIA NORMAL-PHASE LIQUID CHROMATOGRAPHY

Sigrid Wachholz, Heinz Geißler, Joachim Bleck

Central Institute of Physical Chemistry
Academy of Sciences of the German Democratic Republic
Rudower Chaussee 5
1199 Berlin, German Democratic Republic

ABSTRACT

Normal-phase liquid chromatography has been used to separate nitrogen heterocyclics and aromatics with polar groups which are known to be present together in solvent refined coal. The identification of the separated components was accomplished by on-line coupling of the Fourier transform infrared spectrometer as a chromatographic detector. The separation of these model compounds was carried out using Li Chrosorb NH_2 and CN in series. Chloroform was used as mobile phase. All the compounds could be identified in spite of imcomplete separation in some cases. A suitable interface adapted to the different methods of HPLC and FTIR allowed the detection of about 5 µg per injected compound.

INTRODUCTION

High-performance liquid chromatography (HPLC) with on-line coupling of Fourier transform infrared spectroscopy (FTIR) is a recommended

method in complex-mixture analysis. The identification of nonvolatile components in such mixtures requires an increased technological level of chromatography in order to reach the necessary resolution and a multiple, simultaneous, selective detection to obtain detailed information of the chromatographically unresolved components (1). The FTIR detection opens plenty of information not obtainable from typical detectors of the liquid chromatography. The recent developments in the normal-phase-HPLC-analysis of nonpolar and polar compounds in various coal-derived products are described (1-5). One fundamental paper has not only shown the advantage in sensitivity by applying a flow cell as interface for HPLC/FTIR, but also the difficulties caused by the infrared absorbance of the mobile phase (6). The use of chlorinated and deuterated solvents with good transparencies for FTIR is helpful in this field (4). The attainable detection limit below 40 ng of injected material (5) represents the high level in development of this hyphenated technique. Reversed-phase-HPLC/FTIR and an extraordinary possibility to eliminate the immiscible solvents are presented in (7). Numerous reports dealt with the separation of compounds present in complex mixtures as in coal-liquids. Normal-phase- and reversed-phase-HPLC for instance, were applied to the investigation of different stationary phases for the separation of polycyclic aromatic hydrocarbons from nitrogen heterocyclic compounds (8). The application of microbore HPLC/FTIR can enlarge the success of this method (1,3,9). In this paper preliminary results of the FTIR detection and identification of compounds not resolved chromatographically are reported.

EXPERIMENTAL

Instrumentation

High-performance liquid chromatograph: The pump and the valves enclosing an injector equipped with a 10 µl loop were home made by the

Centre of Scientific Instruments of the Academy of Sciences, GDR. A Laboratorny Pristroje (Prague, Czechoslovakia) refraction index detector was used.

The solvent delivery system operates at 0.2 ml/min. The columns (both 100 x 4 mm I.D.) were applied in series and self-filled. The first column contained Li Chrosorb NH_2, 10 µm particles, the second Li Chrosorb CN, 5 µm particles (Czechoslovakia). A very short (low dead-volume 0.25 mm I.D.) tube was used for the column connection with the infrared detection flow cell. The flow cell consisted of a home made cell holder with a 0.1 mm CaF_2 flow cell of 7.8 µl volume. Because of the adaption of the 10 mm diameter of the flow cell to the aperture of the spectrometer, the generally used beam condensor is abundant, and the whole radiation energy can be used for the measurements (see also 10).

Fourier transform infrared spectrometer: A Digilab FTS 20 equipped with TGS detector was used for IR detection of chromatographic peaks. The standard Digilab software package for GC/FTIR was used to collect 8 cm^{-1} resolution interferograms with a time resolution between interferograms (build up from 10 scans) of 8 sec. After the chromatographic run the interferograms were transformed to spectra and plotted after ratioing the sample files vs. backgroundfiles.

Materials

Chloroform was obtained from Laborchemie Apolda, GDR, containing ethanol for stabilization. The solvent was boiled before use every day. The model mixture (see table 1) of five nitrogen heterocyclic compounds, one polycyclic aromatic hydrocarbon, and two aromatic compounds with polar groups, respectively, was prepared by dissolving the compounds in the mobile phase. The mixture for injection contained approximately 50 µg per component, for testing the sensitivity of the IR detection only about 5 µg per component were used.

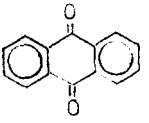
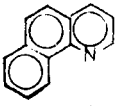
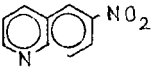
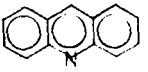
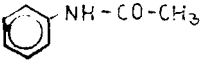
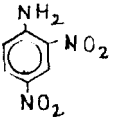
RESULTS AND DISCUSSION

Eight compounds (table 1) were investigated by HPLC/FTIR. They are predominantly basic polar compounds which are present in coal liquids.

We have decided on a normal-phase-HPLC, because reversed-phase systems, routinely applied for the separation of basic polar compounds, use aqueous or polar mobile phases showing very intense absorbances over a wide range of the IR region.

TABLE 1

Investigated Compounds, Retention Times (RT/min), and Injected Amounts (µg)

		RT	µg
1. anthraquinone		13.3	50.13
2. 7.8-benzoquinoline		13.5	47.60
3. 6-nitroquinoline		14.2	51.33
4. acridine		15.7	52.22
5. quinoline		15.8	53.45
6. pyridine		16.7	62.68
7. acetanilide		21.3	50.15
8. 2.4-dinitroaniline		23.2	33.00

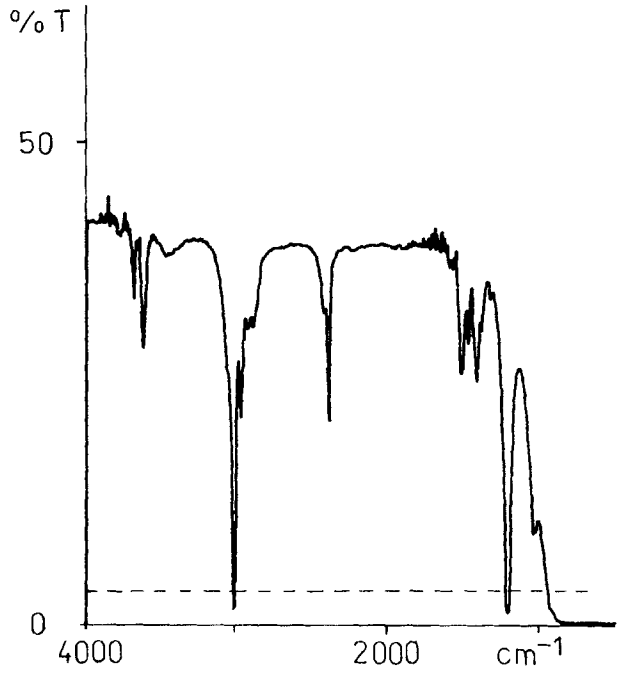


FIGURE 1 - FTIR-spectrum of chloroform, 0.1 mm CaF_2 cell during the LC run, plot against the empty beam

Chloroform shows large transparent IR regions presented in Fig.1. The broken line indicates the region of total IR absorbances, that means, for transparency <3.5 % the compensation was not possible during the HPLC run. The separation of the model mixture (compounds 1,3-8) is illustrated in Fig.2a. However the separation is not complete under these conditions. A poor chromatographic resolution between the polyaromatics is observed, but the sample loading was intentionally chosen very high in order to enable the identification of all of the compounds by FTIR detection. Fig.2b shows time resolved plots in the 2000-1250 cm^{-1} region for anthraquinone, 6-nitroquinoline, acridine, quinoline, pyridine, acetanilide, and 2.4-dinitroaniline. IR spectroscopy clearly distinguishes different po-

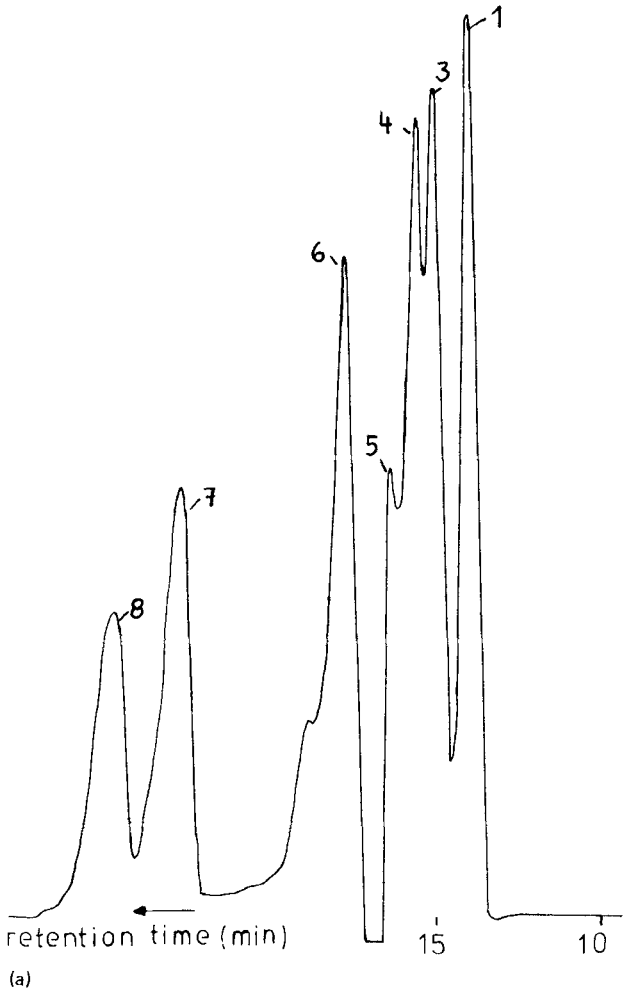


FIGURE 2 - HPLC/FTIR of the compounds No. 1,3-8; CaF_2 flow cell, 0.2 ml/min $CHCl_3$ elution; Li Chrosorb NH_2 and CN filled columns 100 x 4.0 mm I.D.
a) chromatogram
b) FTIR spectra; 10 scans coadded of the peak maxima, RES = 8 cm^{-1}

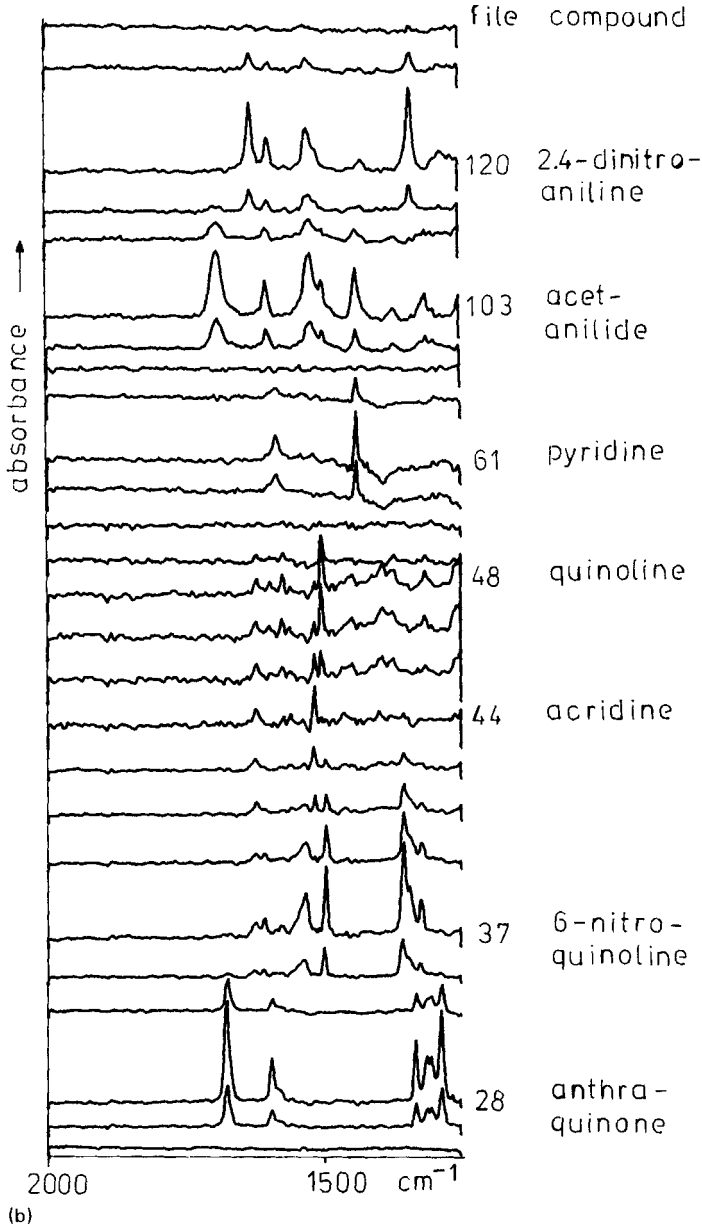


FIGURE 2 (continued)

lar groups. The spectra from the peak maxima of all compounds are identical with the spectra of the pure ones.

The separation changed for the worse by adding 7.8-benzoquinoline to the mixture. Fig.3 shows that the first five compounds are not completely separated. Fig.4 presents the FTIR spectra from the peak maxima. All but one (see Fig.3 and 4, marked by brackets) are identified excellently by comparison with the spectra of the pure substances. The descending slope of peak [2] of the chromatogram in Fig.3 does not only contain the main component 7.8-benzoquinoline but also anthraquinone, the first component.

This fact is recognized in Fig.5. The middle spectrum contains the mixture of the two compounds 7.8-benzoquinoline and anthraquinone. The spectra of the pure substances (10% in $CHCl_3$) are presented in the upper and the lower spectra respectively. The intensities of the bands of both components are similar in the middle spectrum, but the extinction indices of the strong absorption bands of anthraquinone are approximately five times larger than those of 7.8-benzoquinoline, as to be found out from the absorbance scale of Fig.5, spectrum A and B. Hence, a composition of 80% 7.8-benzoquinoline and 20% anthraquinone is estimated (Fig.3, position [2]). By means of the later used automated spectral subtraction routine the infrared spectrum of pure 7.8-benzoquinoline could be produced, as illustrated in Fig.6.

Finally, the sensitivity was tested by decreasing the eluated peak concentration. The injected amounts of the mixture were 4.4 µg anthraquinone, 6.7 µg quinoline, 6.7 µg pyridine, and 6.5 µg acetanilide. The columns were not overloaded. Results of the HPLC separation of the mixture using the refractive index detection are presented in Fig.7a. Fig. 7b - 7e represent the registrated IR spectra of the corresponding peak maxima. Whereas in the spectra of anthraquinone and acetanilide because of their high extinction indices a good signal/noise ration was observed, the detection limit is approximately obtained for quinoline and pyridine having weak absorptions.

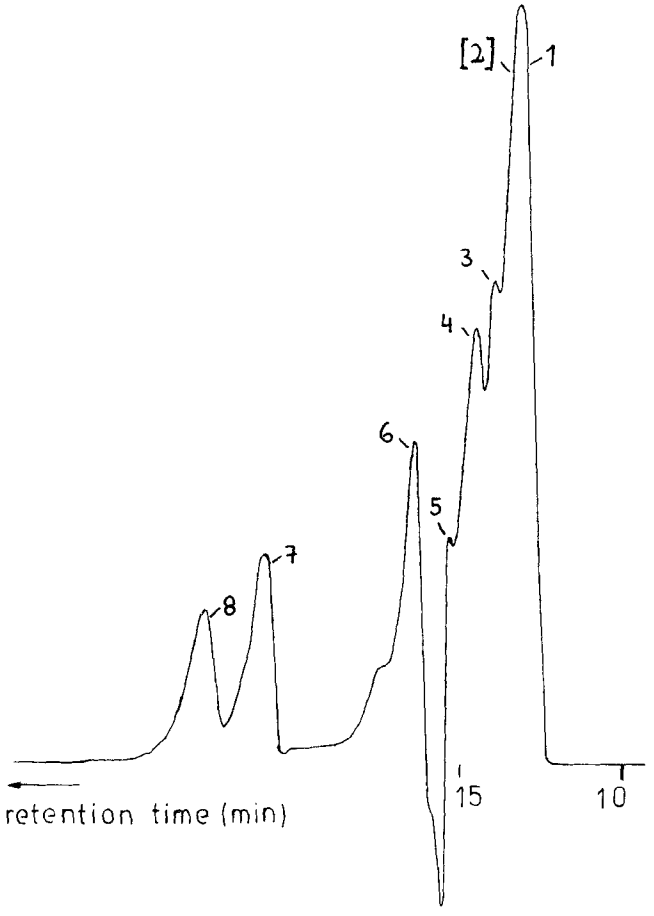


FIGURE 3 - HPLC chromatogram of 8 compounds (see table 1)
[2] = this part of the chromatogram contains worst separation

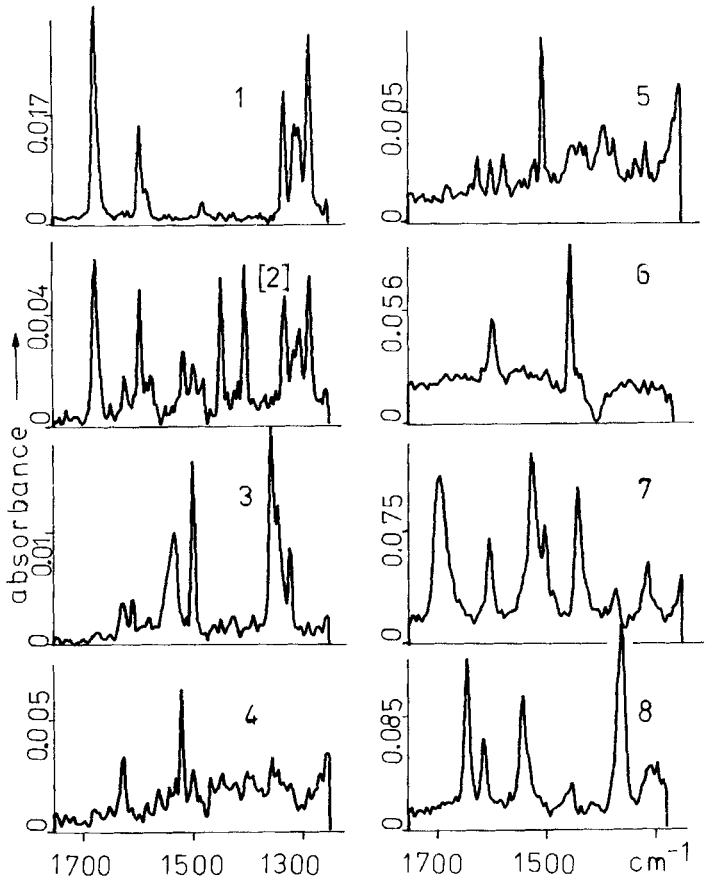


FIGURE 4 - Infrared spectra of the peak maxima obtained from mixture separation with chloroform:
1 anthraquinone, [2] 7.8-benzoquinoline (contains rest anthraquinone), 3 6-nitroquinoline, 4 acridine, 5 quinoline, 6 pyridine, 7 acetanilide, 8 2.4-dinitroaniline

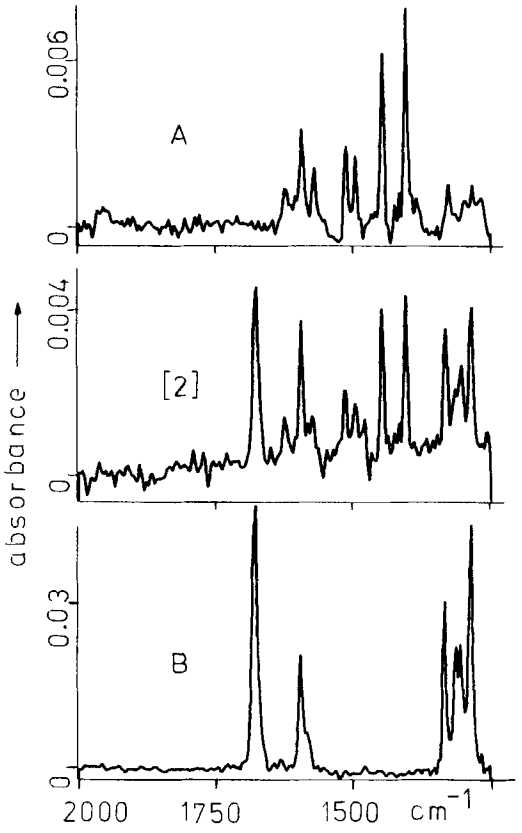


FIGURE 5 - Infrared spectrum [2] obtained from the unseparated peak (see Fig.3) in comparison with the pure substances (10% in $CHCl_3$):
A 7.8-benzoquinoline, B anthraquinone

In conclusion, our experiments demonstrate the usefulness of FTIR in specific detection of chromatographic peaks. An identification of the incompletely separated compounds was possible, and the chromatographic resolution was improved because of the possibility to distinguish IR spectroscopically two components in one peak. A normal-phase system using the combination of silica NH_2 - and CN stationary phases alone was not selective enough for a separa-

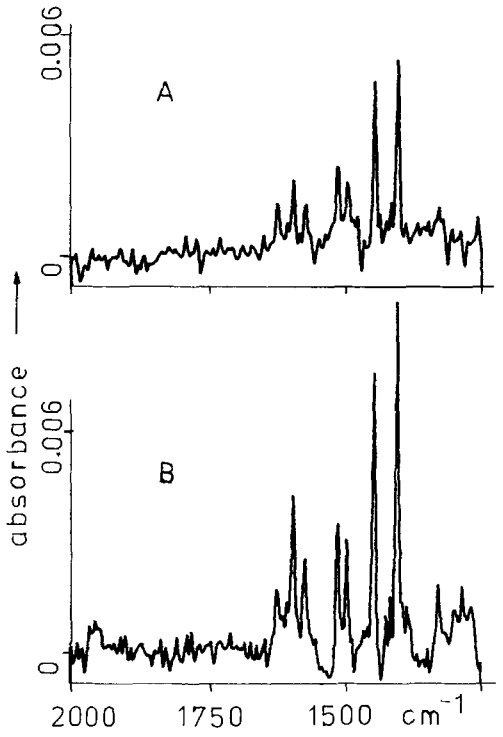


FIGURE 6 - Infrared spectrum of 7.8-benzoquinoline after automatic spectral subtraction.
A - subtraction of the spectra [2]-1:
[2] 7.8-benzoquinoline + anthraquinone
1 anthraquinone
B - 7.8-benzoquinoline (10% in $CHCl_3$)

tion of all of the used substances. Also the retention times were relatively large. The retention and the selectivity in normal-phase-HPLC systems are mainly determined by the interactions between the specific functional groups and the stationary phase. For further analytical work the selectivity of the separation and the sensitivity of the IR detection are to get improved by optimization of the whole equipment.

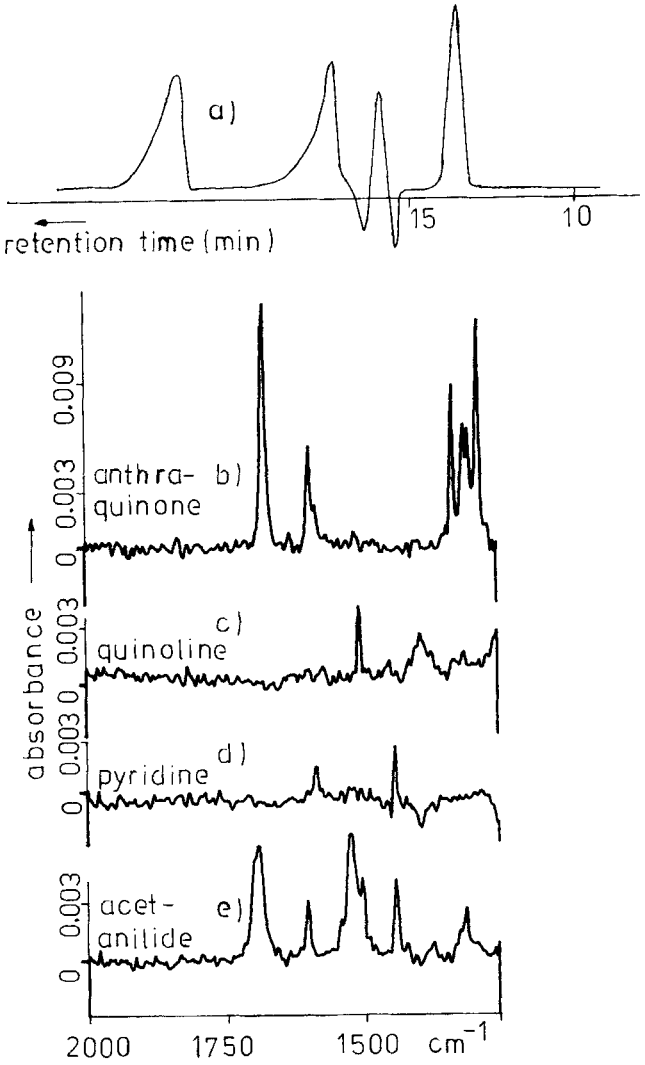


FIGURE 7 - Separation and identification by FTIR of a lower concentrated mixture:
a chromatogram
b-e infrared spectra corresponding to the peak maxima

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