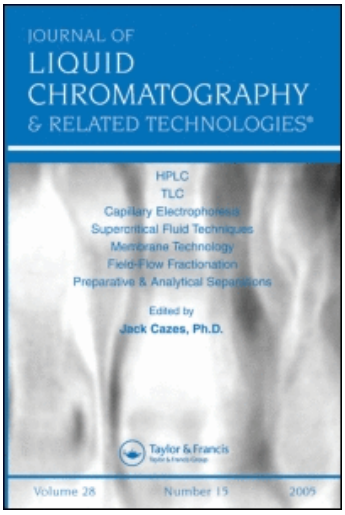

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Analysis of Benzoquinolines and Acridines in a Brazilian Diesel Oil by Particle Beam LC/MS and HPLC/UV

John Mao[ab]; Carlos R. Pacheco[ac]; Daniel D. Traficante[ad]; William Rosen[a]
[a] Department of Chemistry, University of Rhode Island, Kingston, Rhode Island [b] Springborn Laboratories, Inc, Massachusetts [c] Chemistry Division of Petrobras/Cenpes, Rio de Janeiro, Brazil [d] NMR Concepts University of Rhode Island, Kingston, Rhode Island

To cite this Article Mao, John , Pacheco, Carlos R. , Traficante, Daniel D. and Rosen, William(1995) 'Analysis of Benzoquinolines and Acridines in a Brazilian Diesel Oil by Particle Beam LC/MS and HPLC/UV', Journal of Liquid Chromatography & Related Technologies, 18: 5, 903 — 916
To link to this Article: DOI: 10.1080/10826079508010401
URL: http://dx.doi.org/10.1080/10826079508010401

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(5), 903-916 (1995)

ANALYSIS OF BENZOQUINOLINES AND ACRIDINES IN A BRAZILIAN DIESEL OIL BY PARTICLE BEAM LC/MS AND HPLC/UV

JOHN MAO[1,2]*, CARLOS R. PACHECO[1,3],
DANIEL D. TRAFICANTE[1,4], AND WILLIAM ROSEN[1]
[1]*Department of Chemistry*
University of Rhode Island
Kingston, Rhode Island 02881
[2]*Springborn Laboratories, Inc.*
790 Main Street
Wareham, Massachusetts 02571
[3]*Chemistry Division of Petrobras/Cenpes*
Rio de Janeiro, Brazil
[4]*NMR Concepts*
University of Rhode Island
Kingston, Rhode Island 02881

ABSTRACT

Nitrogen bases were isolated from a Brazilian diesel distillate by acid extraction. With minimum sample preparation and clean-up, nitrogen bases were analyzed by particle beam LC/MS and HPLC with photo diode-array detection using reversed phase chromatography. Benzoquinolines were identified as the major nitrogen containing compounds in this basic fraction. By using neutral mobile phases, benzoquinoline homologues were separated, enabling rapid class characterization as well as preparative HPLC isolation of individual benzoquinoline homologues. Acidified mobile phases, however, exhibited greater resolution for individual isomers. UV spectroscopy was used to differentiate various types of benzoquinolines (e.g. benzo[f]quinolines, benzo[h]quinolines, and acridines). Acridines can be easily distinguished from benzo[h]- or benzo[f]quinolines by its different UV absorption spectrum. Benzo[h]- and benzo[f]quinolines were differentiated by the differences in the first derivative of the absorbance ($dA/d\lambda$) vs. wavelength. To confirm the identification, nitrogen bases were also analyzed by conventional GC/MS methods.

* To whom correspondence should be addressed.

INTRODUCTION

Interest in understanding the organic nitrogen bases that are found in petroleum is associated with several of their undesirable physical and chemical properties[1]. The adverse effects of nitrogen bases in petroleum products are mainly in three areas: a) they poison the catalysts used in the cracking and hydrocracking reforming processes[2, 3], b) they contribute to the instability of fuels during storage[4 - 7], and c) they possess potentially carcinogenic and mutagenic activities[8]. The nitrogen bases found in petroleum generally occur as complex mixtures of alkylazaarenes (nitrogenated polyaromatic hydrocarbons) containing mainly one nitrogen atom[9, 10]. Alkyl-substituted quinolines and benzoquinolines have been reported as major basic nitrogen compounds in crude petroleum[7, 10]. Although much work has been done with regard to the characterization of these compounds in petroleum products, the determination of the precise location of the nitrogen atom in these polyaromatic molecules remains a challenge[10, 11]. It is also of importance to distinguish different classes of azaarenes and to study the distributions of individual isomers because of the information that can be gained relating to the geochemical formation pathways of petroleum[10]. For instance, it is known that the distribution of homologous alkyl-substituted aromatic nitrogen compounds differ widely in their origins and geochemical histories[10]. The toxicity of these nitrogen compounds can also be related to the location of the nitrogen atom[10]. In addition, many of these compounds represent key links in identifying the higher molecular weight nitrogen compounds that are invariably present in petroleum.

Previously, we reported the fractionation and characterization of basic and neutral nitrogen compounds from a Brazilian diesel oil sample[12]. We emphasize in this paper a more complete characterization of the basic nitrogen compounds through an analytical sequence involving complementary chromatographic and spectroscopic techniques. By a combination of LC/MS, GC/MS, and HPLC with online photo diode-array detection, we demonstrate an analytical approach that can be utilized for rapid characterization of organic nitrogen bases. Because of the extreme complexity of diesel oils, no single analytical technique can provide the identification of the position of the nitrogen atom in individual azaarene isomers. Although mass spectrometry is widely used as a structure elucidation tool for petroleum products, it is not very useful in determining the position of the nitrogen atom due to the lack of fragmentation patterns in the mass spectra of molecules having more than two fused aromatic rings[13, 14]. UV spectrometry has been used successfully to identify di- and triaromatic azaarenes[10] and is applied in this work to

differentiate various classes of benzoquinolines. Also discussed is the development of HPLC systems to separate these closely related nitrogen bases and the usefulness of the LC/MS technique as an alternative to conventional GC/MS methods.

MATERIALS AND METHODS

Materials:

The diesel oil sample was provided by Petrobras of Brazil. It was laboratory distilled at Petrobras and had a boiling point range of 200 - 400 °C. The total nitrogen content of this sample was approximately 700 ppm. Neutral aluminum oxide powder was obtained from J.T. Baker, Inc. and was of chromatography grade. Reference standards of benzo[h]quinoline and acridine were purchased from Aldrich Chemical Company and were analytical grade. Chemical structures of benzo[h]-, benzo[f]quinoline, and acridine are shown in Figure 1. All other chemicals were obtained from commercial sources and were at a minimum reagent grade. All solvents were HPLC grade.

Fractionation of basic nitrogen compounds:

Experimental procedures for the fractionation of basic nitrogen compounds in the Brazilian diesel sample are detailed in our previous work[12]. Briefly, approximately 500 mL of the diesel oil sample was dissolved in 500 mL of hexane and partitioned with 3 X 300 mL of 10% sulphuric acid and then with 300 mL of 20% sulphuric acid. The aqueous acid extracts were combined and washed with dichloromethane. After raising the pH to 12 - 13 with sodium hydroxide, the aqueous fraction was extracted with dichloromethane. The combined dichloromethane extracts were washed with water, dried over anhydrous sodium sulphate, and the solvent was removed by rotary evaporation. This sample was labeled as the basic fraction and an aliquot of it was dissolved in methanol for HPLC separations and/or LC/MS analysis.

High Performance Liquid Chromatography (HPLC):

HPLC instruments included a Hewlett Packard 1050 gradient solvent pump, a Hewlett Packard 1050 autosampler, a Kratos 757 UV detector, and a Hewlett Packard 3396A integrator. LC/MS analyses were conducted using a Hewlett Packard 5989A MS Engine equipped with a particle beam interface. Full scan electron impact (EI) mass spectra were obtained using 70 eV electron energy at a source temperature of 250 °C. HPLC-UV analyses were conducted using

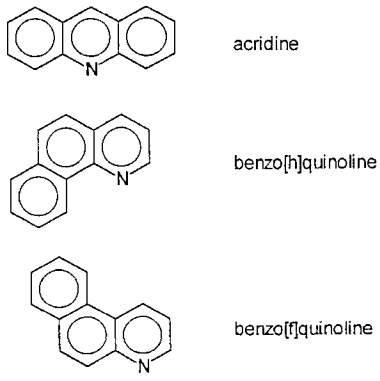

Figure 1. Chemical structures of acridine, benzo[h]quinoline, and benzo[f]quinoline.

online photo diode-array detection (Hewlett Packard 1040A). The UV scan range was 220 to 400 nm.

Two reversed phase chromatographic systems were developed for the separation of the basic fraction. Both systems utilized a MetaChem Nucleosil C18 (5 μm, 150 X 2.0 mm) column with gradient elution at 0.4 mL/minute. System I used neutral mobile phases (A: 40/60 acetonitrile/water; B: acetonitrile). The linear gradient program was: 0 min, 100A; 40 min, 50A/50B; 60 min, 100B. System II used acidified mobile phases (A: 10/90/0.2 acetonitrile/water/acetic acid; B: 80/20/0.1 acetonitrile/water/acetic acid). The linear gradient program was: 0 min, 100A; 60 min, 100B.

Capillary Gas Chromatography (GC):

GC/MS analysis was conducted using a Hewlett Packard model 5890 series II gas chromatograph coupled to a Hewlett Packard model 5971A mass selective detector (MSD). Experimental conditions were:

column:	Restek Rtx-1 (crossbonded 100% dimethyl polysiloxane, 30m x 0.25mm)
carrier gas:	helium
ionization:	electron impact (EI) at 70 eV
acquisition mode:	scan (60 - 300 amu)
temperature program:	initial - 70°C; initial time - 1 minutes; rate - 2°C/minute; final - 250°C; final time - 1 minutes

MSD was turned on 3 minutes after sample injection (solvent delay).

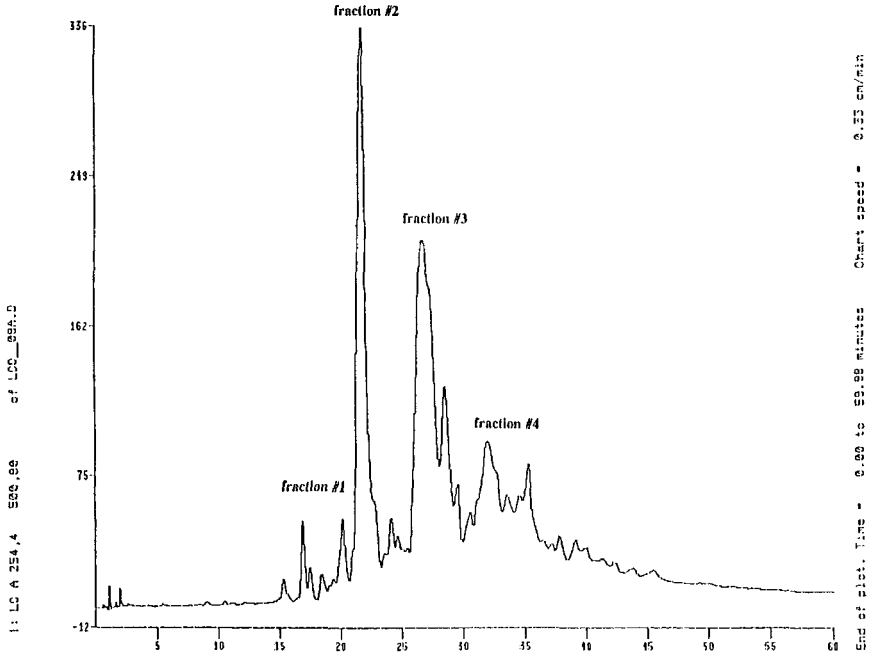

Figure 2. HPLC/UV (254 nm) chromatogram of the basic fraction using chromatographic system I. Also shown is the HPLC isolation scheme.

HPLC isolation of benzoquinoline homologues:

Major components of the basic fraction were isolated by HPLC using a semi-preparative HPLC column (Nucleosil C18, 5 μm, 250 x 10 mm). The isocratic mobile phase consisted of A: 40/60 acetonitrile/water and B: acetonitrile at a ratio of 50%A and 50%B. The flow rate was 5 mL/minute. The isolated fractions were concentrated to small volumes and then partitioned with ethyl acetate. Ethyl acetate extracts were separated, concentrated to dryness, and residues reconstituted in methanol. To check peak purity, the methanol solutions were reinjected onto an analytical C18 column using chromatographic system I.

RESULTS AND DISCUSSION

HPLC separation of benzoquinolines

We reported previously[12] that the basic nitrogen fraction of the Brazilian sample contained mostly alkyl substituted benzoquinoline homologues (C_2- to C_4- benzoquinolines). Similar to the

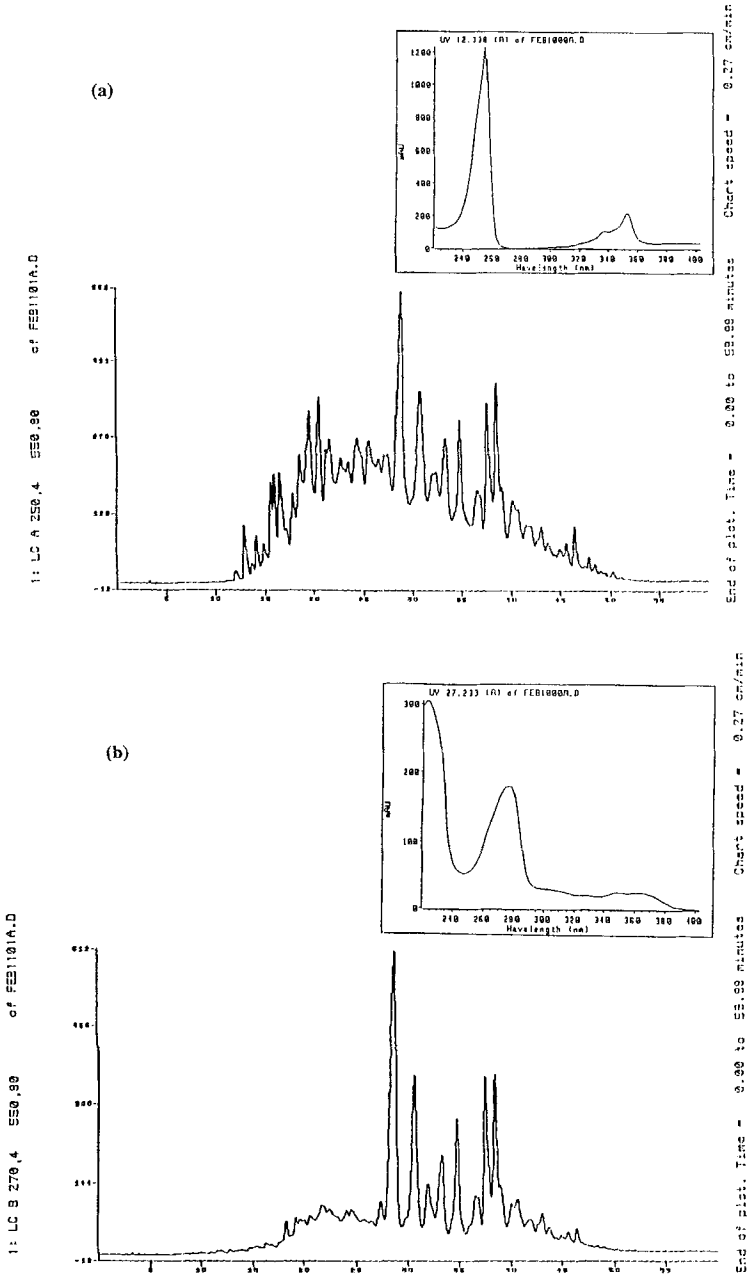

Figure 3. **HPLC separation of the basic fraction using acidified mobile phases (chromatographic system II). (a) - 250 nm, (b) - 270 nm.**
(insert to (a) - UV spectrum of acridine, insert to (b) - UV spectrum of benzo[h]quinoline)

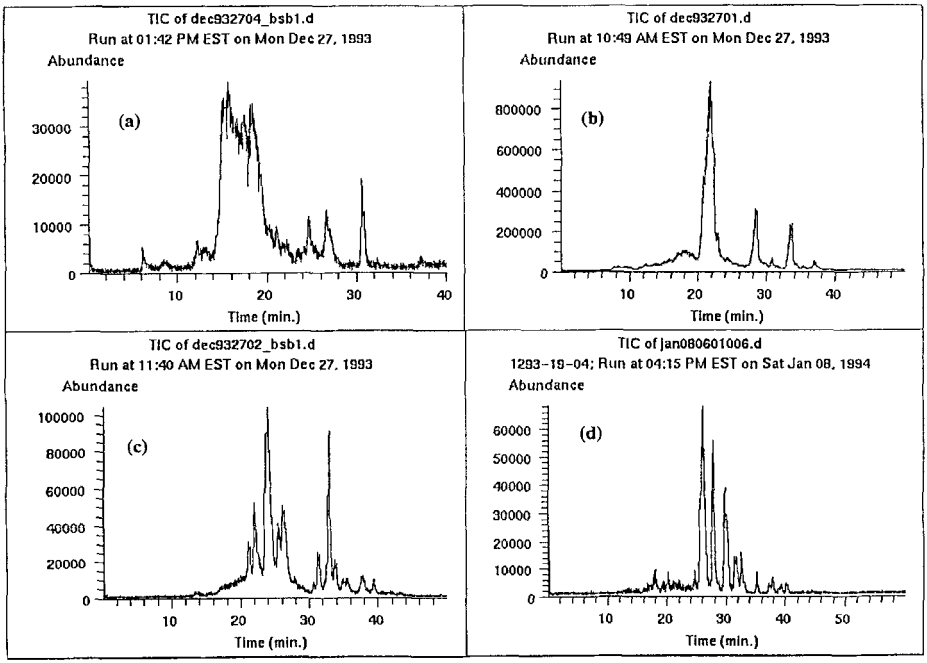

Figure 4. **LC/MS total ion chromatograms of (a) the isolated C_2- acridines (HPLC fraction isolated #1); (b) the isolated C_2- benzo[h]quinolines (HPLC isolated fraction #2); (c) the isolated C_3- benzo[h]quinolines (HPLC isolated fraction #3); and (d) the isolated C_4- benzo[h]quinolines (HPLC isolated fraction #4). Scan range: 70 - 300 amu.**

neutral nitrogen fraction (mostly carbazole homologues)[12], the HPLC separation of the basic fraction using neutral mobile phases was adequate for various benzoquinoline homologues. Using this condition, well resolved benzoquinoline homologues could be readily isolated preparatively for further characterization. However, the resolution of individual isomers using a neutral mobile phase was poor. By using acidified mobile phases, the resolution of individual isomers was greatly improved. Figures 2 and 3 illustrate the effect of acid on the separation of benzoquinolines. Based on the UV signal ratios at 250 and 270 nm (Figure 3), it was evident that this sample contained at least two classes of benzoquinolines - acridines and benzo[h]quinolines. (UV spectra of authentic standards of benzo[h]quinoline and acridine are shown in Figure 3.) The UV identification of benzo[h]quinolines and acridines was also supported by LC/MS. It should

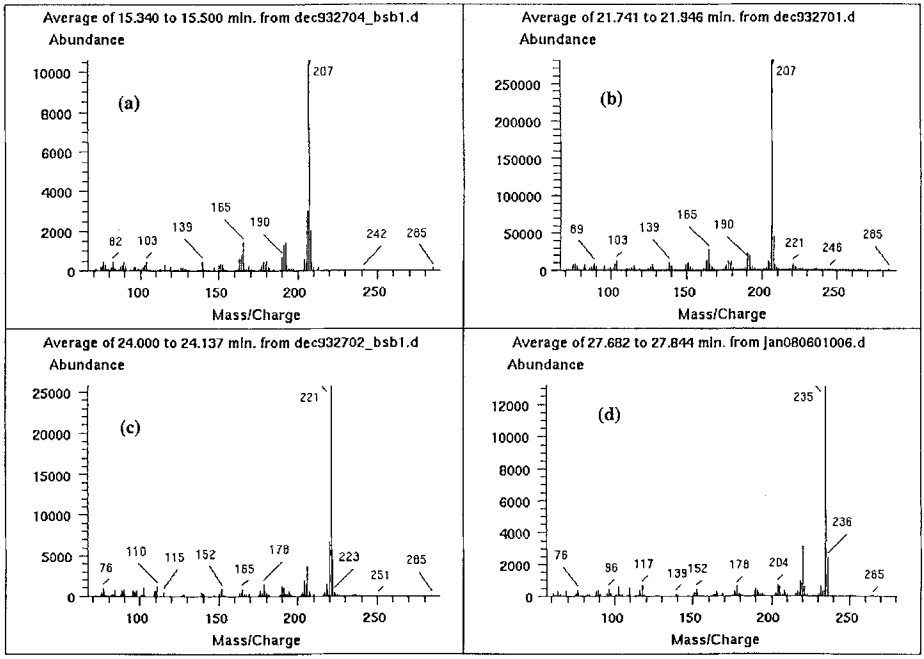

Figure 5. **LC/MS EI mass spectra of (a) C_2- acridine, (b) C_2- benzo[h]quinoline (c) C_3- benzo[h]quinoline, and (d) C_4- benzo[h]quinoline identified in HPLC isolated fractions #1, #2, #3, and #4, respectively.**

be noted that benzo[h]quinolines and acridines showed quite different chromatographic behaviors. Under acidic mobile phase conditions, acridines eluted much earlier than benzo[h]quinolines and showed significantly broader peaks. Since their mass spectra are nearly identical, benzo[h]quinolines and acridines are difficult to differentiate by conventional GC/MS methods. The combination of HPLC with photo diode-array detection and LC/MS enabled the identification of these two different classes of benzoquinolines.

Analysis of individual benzoquinoline homologues

One of our research interests is to study the distribution of individual nitrogen base isomers that are found in petroleum and to explore their geochemical formation pathways[15]. To investigate individual benzoquinoline homologues, major components of the basic fraction were isolated via repetitive HPLC injections on a semi-preparative column. The isolation scheme is shown in

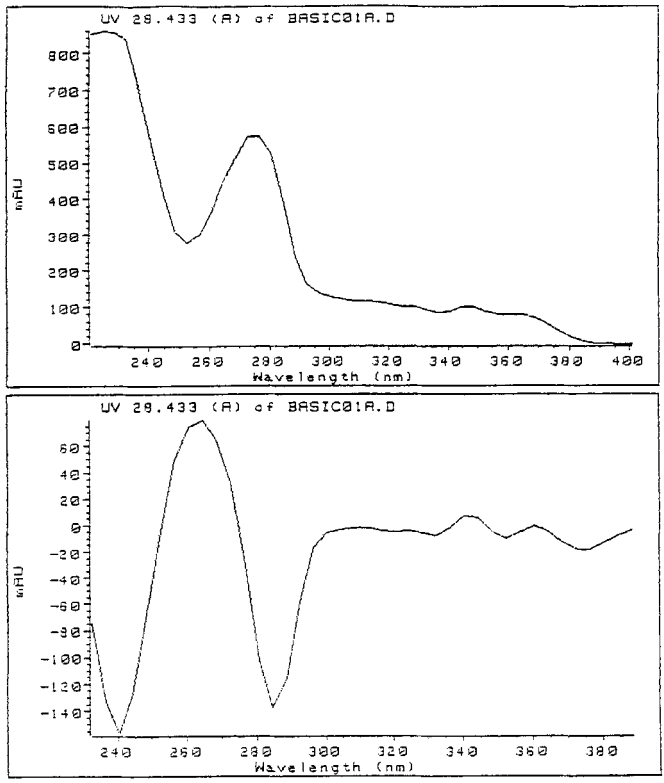

Figure 6. **Top - UV spectrum of a C_2- benzo[h]quinoline isomer identified in the HPLC fraction #2, and Bottom - the first derivative of the absorbance $dA/d\lambda$ plotted vs. the wavelength λ.**

Figure 2. The isolated fractions were concentrated and analyzed by particle beam LC/MS and HPLC/UV using HPLC system II. Full scan total ion chromatograms of these fractions are presented in Figure 4. Although the mass spectra of C_2- benzoquinoline and C_2- acridine were nearly identical, the fraction #1 (Figure 4a) was identified to be C_2- acridine (m/z 207) based on its UV characteristics. Fraction #2 (Figure 4b) contained three major isomers of C_2- benzoquinoline (m/z 207), while fraction #3 (Figure 4c) exhibited at least eight isomers of C_3- benzoquinoline (m/z 221). Fraction #4 (Figure 4d) was identified as the C_4- benzoquinolines having a similar number of isomers as that of the C_3- homologues. Full scan electron impact mass spectra of representative peaks in each fraction are shown in Figure 5. Based on the low values

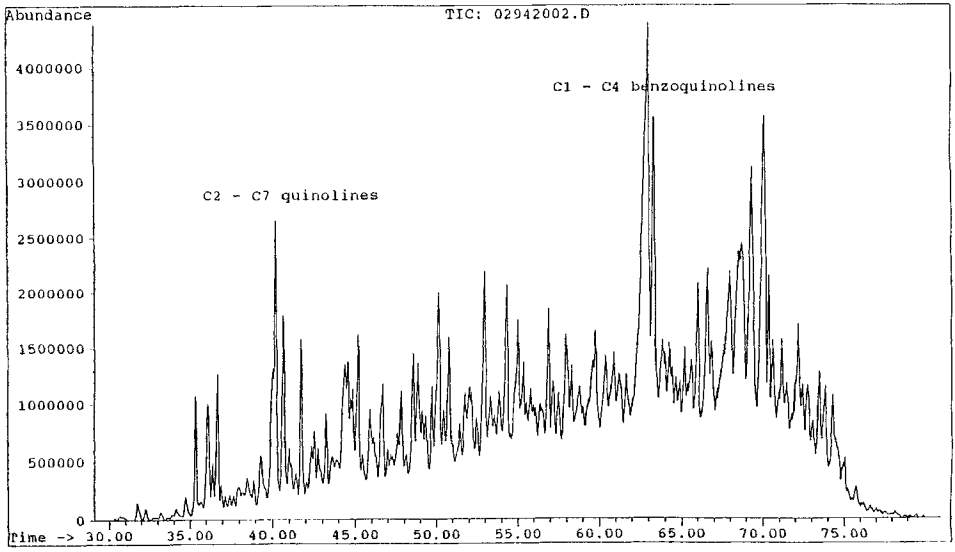

Figure 7. GC/MS total ion chromatogram of the basic fraction showing the separation of quinoline and benzoquinoline homologues. Scan range: 60 - 300 amu.

(e.g. < 0.4) of the two separate ratios of ions $(M\text{-}H)^+/M^+$ and $(M\text{-}CH_3)^+/M^{+1}$, as well as the absence of rearrangement ions, it is likely that the benzoquinolines are polymethylated (rather than ethyl, propyl, or butyl substituted). This conclusion is consistent with previous results[15-19].

The other possible classes of benzoquinolines, besides the benzo[h]quinolines, that might be present are the benzo[f]quinolines. Since their mass spectra as well as UV spectra are almost indistinguishable, the identification of benzo[h]- and benzo[f]quinolines presented significant difficulties. However, by plotting the first derivative of the absorbance ($dA/d\lambda$) vs. the wavelength, the two classes of compounds can be distinquished[20]. The ratio of the absorbance in the vicinity of 240 and 280 nm is close to 1 for benzo[h]quinolines and greater than 3 for benzo[f]quinolines[10]. Thus, this fingerprinting technique can significantly simplify the recognition patterns for isomeric types of azaarene molecules. The usefulness of this technique is illustrated in Figure 6. Based on similar UV data, it was concluded that benzo[h]quinolines were the major components in the HPLC isolated fractions #2 - #4.

GC/MS analysis of the basic fraction

To assist in the characterization scheme and to compare to the particle beam LC/MS technique, the basic fraction was also analyzed by GC/MS using full scan electron impact

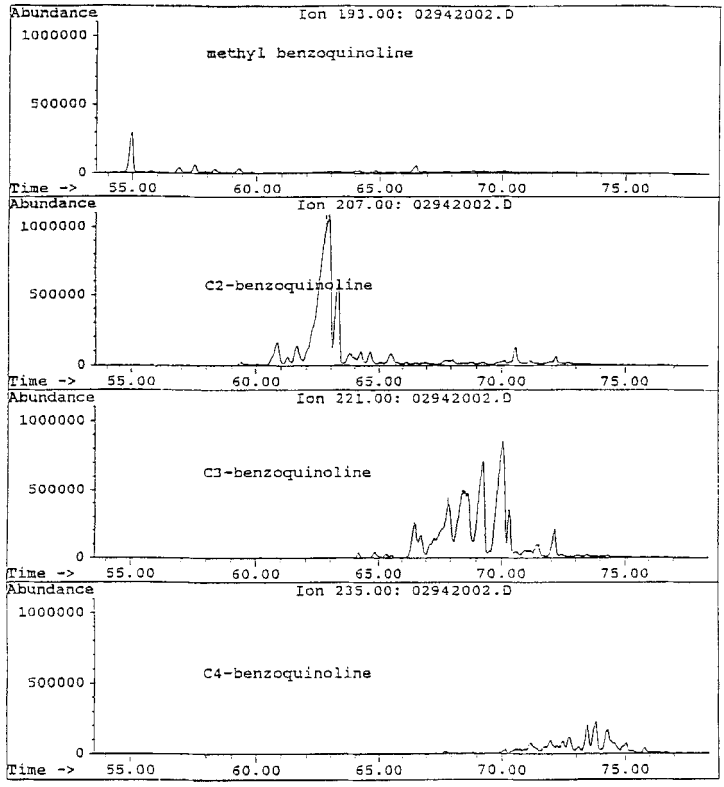

Figure 8. GC/MS extracted ion chromatograms of C_1- to C_4- benzoquinolines (m/z 193, 207, 221, and 235).

ionization. The total ion chromatogram of this sample is presented in Figure 7. Extracted ion chromatograms (m/z 193, 207, 221, and 235) are presented in Figure 8 showing benzoquinoline homologues and the separation of individual isomers. As shown, the separation obtained on a capillary GC column was significantly improved compared to HPLC. The other noticeable difference is the strong signals for the quinoline homologues (C_2- to C_7 -). Extracted ion chromatograms (m/z 157, 171, 185, 199, 213, and 227) are presented in Figure 9. Quinolines were also detected by particle beam LC/MS but at much lower signals compared to benzoquinolines. This was partially attributable to the higher volatility of quinolines which resulted in lower sensitivity by particle beam LC/MS. (Volatile compounds are often pumped away in the interface region prior to reaching the ion source.) Under reversed phase HPLC conditions, quinolines elute earlier than benzoquinolines. HPLC-UV analysis at both 250 and 270

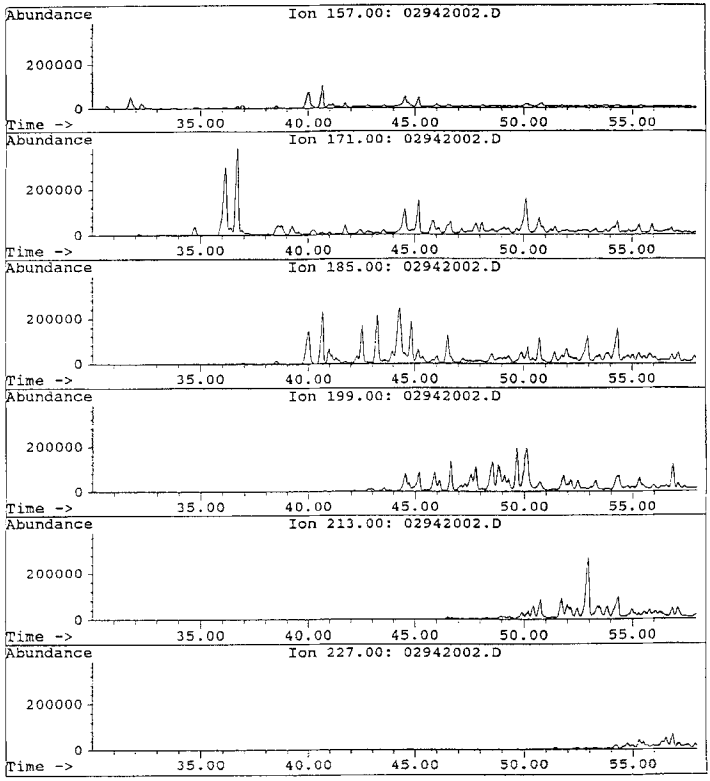

Figure 9. **GC/MS extracted ion chromatograms of C_2- to C_7- quinolines (m/z 157, 171, 185, 199, 213, and 227).**

nm showed that quinolines were minor components in the sample, which supported LC/MS data. On the other hand, GC/MS was much more sensitive to quinolines (because of their high volatilities) and intense signals were acquired. We should point out that under GC/MS conditions nitrogen species probably co-eluted with several polyaromatic hydrocarbons from the sample matrix. As a result, interpretations of some of the mass spectra were difficult. Nevertheless, both techniques (GC/MS and LC/MS) showed their effectiveness in characterizing complex samples such as diesel oils. Because of the direct correlation of LC/MS to HPLC/UV, difficult tasks such as distinguishing different types of alkylazaaerenes can be resolved. Such an advantage clearly places LC/MS among the attractive alternatives to conventional GC/MS methods as well as being a convenient method for direct analysis of some of these complex mixtures[20].

ACKNOWLEDGMENT

The authors would like to thank Petrobras for providing the diesel oil samples. This project was funded by Petrobras, Springborn Laboratories, Inc., and NMR Concepts as a cooperative venture.

REFERENCES

1. L. Chan, J. Ellis, P. T. Crisp, J. Chromatogr.,292: 355-368 (1984)
2. H. V. Drushel, A. L. Sommers, Anal. Chem., 38: 19-28 (1966)
3. E. Furimsky, Erdöl Kohle, 32: 383-390 (1979)
4. J. W. Frankenfeld, W. F. Taylor, Am. Chem. Soc., Div. Feul Chem., 23: 205-214 (1978)
5. C. D. Ford, S. A. Holmes, L. F. Thompson, D. R. Latham, Anal. Chem., 53: 831-836 (1981)
6. M. Dorbon, C. Bernasconi, Fuel, 68: 1067-1074 (1989)
7. J. F. McKay, J. H. Weber, D. R. Latham, Anal. Chem., 48: 891-898 (1976)
8. B. A. Tomkins, C. -h. Ho, Anal. Chem., 54: 91-96 (1982)
9. S. J. Marshman, Fuel, 70: 967-969 (1991)
10. J. M. Schmltter, H. Colin, J. L. Excoffler, P. Arpino, G. Gulochon, Anal. Chem., 54: 769-772 (1982)
11. M. Li, S. R. Larter, D. Stoddart, M. BjorØy, Anal. Chem., 64: 1337-1344 (1992)
12. J.Mao, C. R. Pacheco, D. D. Traficante, W. Rosen, Fuel, in press (1994)
13. J. M. Schmitter, I. Ignatiadis, P. J. Arpino, Geochimica et Cosmochimica Acta, 47: 1975-1984 (1983)
14. P. Burchill, A. A. Herod, E. Pritchard, J. Chromatogr., 246: 271-295 (1982)
15. J. M. Schmitter, Z. Vajta, P. J. Arpino, In Advances in Organic Geochemistry 1979, A. G. Douglas, J. R. Maxwell, eds., Pergamon Press, Oxford, 1980, pp. 67-76.
16. J. M. Schmitter, I. Ignatiadis, G. Guiochon, J. Chromatogr., 248: 203-216 (1982)

17. S. D. Sample, D. A. Lightner, O. Buchardt, C. Djerassi, J. Org. Chem., 32: 997-1005 (1967)

18. P. M. Draper, D. B. McLean, Can. J. Chem., 46: 1487-1497 (1968)

19. M. Novotny, R. Kump, F. Meril, L. Todd, J. Anal. Chem., 52: 401-406 (1980)

20. J. Mao, C. R. Pacheco, D. D. Traficante, W. Rosen, J.Chromatogr., in press (1994)

Received: August 26, 1994
Accepted: September 7, 1994